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Building the ecosystem that makes investigator-initiated clinical trials possible.

Habilitation thesis

(Collection of published scholarly works with commentary)

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Commentary

Investigator-initiated clinical trials (IICTs), also referred to as non-commercial clinical trials (NCCTs), have become an indispensable component of evidence-based medicine. By addressing clinically relevant questions that are often beyond the scope of commercially sponsored research, they generate independent evidence for optimizing existing treatments, improving healthcare delivery, and addressing unmet medical and public health needs. Consequently, academic clinical research plays a pivotal role in advancing patient-centred healthcare and informing clinical decision-making.

Over the past two decades, the environment in which NCCTs are conducted has evolved considerably. Increasing regulatory requirements, growing methodological complexity, advances in data science, and expanding expectations for patient and public involvement have fundamentally transformed the design, implementation, and evaluation of academic clinical research. At the same time, European research infrastructures and multinational research networks have created unprecedented opportunities to conduct high-quality IICTs capable of generating evidence with direct clinical and societal relevance. These developments have progressively shifted academic clinical research from the conduct of individual investigator-driven studies towards an interconnected research ecosystem in which scientific, methodological, regulatory, infrastructural, and societal components jointly determine whether clinically relevant questions can be translated into meaningful evidence and, ultimately, improvements in healthcare.

Unlike traditional habilitation theses that focus on a single disease area or therapeutic intervention, this work adopts a systems perspective on clinical research. Rather than examining individual clinical trials in isolation, it explores the interconnected components that determine their success, including methodological innovation, regulatory science, collaborative research infrastructures, investigator competence, patient engagement, and implementation into clinical practice. Collectively, these elements form the ecosystem through which IICTs translate unmet clinical needs and scientific questions into measurable improvements in patient care and public health.

This habilitation thesis is presented as a collection of the author's published scholarly works accompanied by an integrative commentary. The publications document the evolution of the author's research from clinical pharmacology and IICTs towards methodological research, European clinical research infrastructures, capacity building, patient engagement, and public health evaluation. Rather than representing separate research areas, these contributions address complementary components of the IICTs lifecycle. Together, they illustrate how high-quality academic clinical research extends beyond the conduct of individual studies to encompass the broader processes that enable the generation, implementation, and dissemination of clinically meaningful evidence. Their integration provides the basis for the conceptual framework developed in this habilitation thesis, linking clinical need, research

methodology, regulatory and infrastructural support, trial delivery, evidence generation, patient involvement, and implementation into clinical practice and public health.

The thesis is organised into three interconnected parts reflecting this scientific development:

- The first part introduces the methodological, regulatory, and organisational foundations of IICTs within the European context.

- The second part demonstrates these principles through selected IICTs and multinational collaborative research initiatives, illustrating their contribution to clinical innovation and public health.

- The third part examines broader system-level aspects of academic clinical research, including research capacity building, patient recruitment, inclusiveness, transparency, and patient and public involvement, highlighting their importance for the long-term sustainability and societal impact of NCCTs.

Each chapter combines an overview of the current state of knowledge with a critical discussion of the author's original scientific contributions. Taken together, the published works presented in this habilitation thesis support the central premise that the impact of IICTs depends not only on scientific excellence but also on the successful integration of robust methodology, supportive regulatory frameworks, collaborative research infrastructures, investigator competence, and meaningful patient engagement. This integrative perspective forms the central conceptual basis of the thesis and provides a framework for understanding how IICTs can progress from the identification of unmet clinical needs to the generation of evidence with measurable impact on patient care and public health.

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Abstract

Non-commercial clinical trials (NCCTs), including investigator-initiated clinical trials (IICTs), play an important role in addressing clinically relevant questions that are frequently beyond the scope or priorities of commercially sponsored research. They contribute to optimising existing therapies, comparative effectiveness research, drug repurposing, precision medicine, advanced therapy medicinal products, and other areas of high clinical and public health importance. However, their successful implementation and potential translation into healthcare benefit depend not only on scientific excellence but also on a supportive clinical research ecosystem.

This habilitation thesis analyses NCCTs from a systems perspective, integrating methodological, regulatory, infrastructural, organisational, educational, patient-related, and translational aspects of the European clinical research environment. Drawing on the author's published scientific work, it examines how clinically relevant research questions progress from the identification of clinical need through study design, regulatory and infrastructural support, trial delivery and evidence generation towards dissemination, implementation, and potential impact on clinical practice and public health. The included studies illustrate how NCCTs and IICTs can address unmet clinical needs across diverse clinical and methodological setting, while methodological and system-level analyses identify regulatory, organisational, financial, human capacity, and infrastructural factors that influence research feasibility, quality, and sustainability. The thesis further examines the complementary roles of research infrastructures, regulatory frameworks, multidisciplinary collaboration, investigator capacity building, patient and public involvement, and implementation strategies in supporting the clinical and societal value of NCCTs.

The principal original contribution of the thesis is the development of an integrated conceptual framework of the NCCTs´ ecosystem. The framework conceptualises research as a continuum in which the ability to generate clinically meaningful evidence depends on the interaction of successive stages of the research pathway with methodological, regulatory, infrastructural, organisational, and translational conditions rather than on methodological quality alone. Within this framework, clinical need and research questions constitute the starting point, while study design and delivery, evidence generation, patient and public involvement, dissemination, and implementation form the pathway towards evidence-informed healthcare. Cross-cutting enablers, including sustainable funding, capacity building, multidisciplinary collaboration, data and technology, and national and European research networks, support this process throughout the research lifecycle. Robust, transparent, clinically relevant, and patient-centred evidence, together with the infrastructures and effective implementation mechanisms required to translate into healthcare decision-making, is therefore central to realising the potential value of NCCTs. This systems perspective provides a comprehensive framework for understanding and further developing the European ecosystem of NCCTs.

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Glossary

ATMPs – Advanced Therapy Medicinal Products

CER – Comparative Effectiveness Research

CPR – CardioPulmonary Resuscitation

CRC – Clinical Research Coordinator

GCP – Good Clinical Practise

GMP – Good Manufacturing Practice

CTIS – Clinical Trials Information System

CTR – Clinical Trial Regulation

CZECRIN – Czech Clinical Research Infrastructure Network

DONET – Disease-Oriented Network

ECRIN – European Clinical Research Infrastructure Network

FAST-EU – Facilitating and Accelerating Strategic Clinical Trials

GDPR – General Data Protection Regulation

ICH E10 – International Committee of Harmonisation Effective 10

ICH GCP R3 – International Committee of Harmonisation Good Clinical Practise Revision 3

IICTs – Investigator-Initiated Clinical Trials

NCCTs – Non-Commercial Clinical Trials

PPI – Patient Public Involvement

RWD – Real World Data

Support-NCS – Support Non-Commercial Sponsors

1 Introduction

1.1 Background

Clinical trials represent the fundamental methodological framework for the generation of evidence-based knowledge in modern medicine. They provide the basis for evaluating the efficacy, safety, and effectiveness of therapeutic, diagnostic, and preventive interventions and directly influence healthcare policies, regulatory decision-making, and clinical practice[1]. Over recent decades, the landscape of clinical research in Europe has undergone substantial transformation, driven by increasing scientific complexity, regulatory harmonisation, technological innovation, and growing societal expectations regarding patient-centred healthcare[2,3]. Within this evolving environment, non-commercial clinical trials (NCCTs) have become as a critical component of the European clinical research ecosystem[4].

Historically, academic clinical trials were commonly referred to as investigator-initiated clinical trials (IICTs), reflecting the central role of individual academic investigators in their conception and initiation. More recently, the broader term, NCCTs, has increasingly been adopted to reflect the evolving nature of academic clinical research, which may involve universities, hospitals, research infrastructures, collaborative networks, and multinational consortia as sponsors or coordinating organisations. In this thesis, NCCTs is considered the preferred overarching terminology and is used throughout where appropriate. However, in specific sections, the term IICTs is deliberately retained when the intention is to emphasise that the clinical trial originated entirely within the academic environment and, in particular, to highlight the investigator's role as the originator of the research idea and the principal driver of its scientific development. IICTs is also retained where it reflects the terminology used in the original publications. NCCTs are clinical trials initiated, designed, and conducted primarily within the academic and NCCTs environment, including academic institutions, hospitals, universities, research organisations, and independent investigators, rather than being driven by the pharmaceutical or medical device industry. Unlike commercially sponsored trials, which are typically driven by regulatory approval pathways and medicinal product development objectives, NCCTs predominantly focus on addressing clinically relevant questions with high societal value, including optimisation of existing therapies, comparative effectiveness research, rare diseases, paediatric populations, public health interventions, and unmet medical needs. These studies frequently investigate areas where commercial incentives are insufficient or absent, despite significant potential benefits for patients and healthcare systems[5].

The distinction between investigator-initiated/non-commercial and industry-sponsored clinical research is therefore not solely related to funding mechanisms but also reflects differences in scientific priorities and expected outcomes. Industry-sponsored trials are indispensable for the development and registration of innovative medicinal products; however, their research priorities are necessarily shaped by product-development strategies,

regulatory requirements, intellectual property considerations, and market potential. In contrast, IICTs often pursue broader clinical and public health objectives, including optimisation of standard-of-care therapies, evaluation of treatment strategies in underrepresented populations, long-term effectiveness studies, or repurposing of existing drugs for rare and neglected diseases. Consequently, NCCTs play an essential role in complementing commercially driven research and ensuring that clinical evidence generation also addresses questions arising directly from clinical practice and unmet patient and healthcare-system needs[6].

The importance of NCCTs has become increasingly evident in the context of complex healthcare challenges such as ageing populations, antimicrobial resistance, emerging infectious diseases, and rare disorders. During the COVID-19 pandemic, investigator-initiated and publicly coordinated studies demonstrated exceptional flexibility and responsiveness in generating real-world evidence, supporting vaccination strategies, and rapidly implementing multinational collaborative research infrastructures. These developments highlighted both the scientific value and strategic importance of strong academic clinical research networks within Europe[7]. At the same time, the pandemic exposed persistent limitations in the ability to initiate, coordinate, and deliver academic clinical research rapidly and consistently across countries, illustrating that scientific expertise alone is insufficient without appropriate regulatory, organisational, financial, and infrastructural capacity.

At the European level, substantial efforts have been undertaken to strengthen the infrastructure supporting NCCTs and to reduce fragmentation in clinical research capacity. Among the most important initiatives is the European Clinical Research Infrastructure Network (ECRIN), which was established to facilitate multinational academic clinical trials through methodological support, regulatory guidance, data management, project management, monitoring, and quality assurance services. ECRIN significantly contributes to harmonisation of clinical research standards across Europe and enables academic investigators to conduct complex multinational studies that would otherwise exceed the capacities of individual institutions[8].

Similarly, the VACCELERATE consortium, established during the COVID-19 pandemic, created a pan-European infrastructure for vaccine trial coordination, participant recruitment, communication strategies, and rapid trial implementation. Through initiatives such as the VACCELERATE Site Network, Volunteer Registry, and communication toolkit, this infrastructure demonstrated how coordinated European collaboration can substantially improve the efficiency, inclusivity, and responsiveness of clinical research[9].

Together, these initiatives demonstrate that the success of IICTs depends on an interconnected ecosystem extending beyond individual investigators and study protocols to include methodological expertise, regulatory support, sustainable research infrastructures, trained research teams, effective recruitment strategies, patient and public involvement, appropriate data and technological capacity, and mechanisms for translating generated evidence into clinical practice.

Understanding how these components interact, and how their integration can enhance the feasibility, quality, efficiency, and societal impact of IICTs, therefore represents an increasingly important challenge for contemporary academic clinical research.

1.2 Current challenges

Despite their undeniable importance, NCCTs continue to face numerous barriers that limit their feasibility, sustainability, and clinical and societal impact. These barriers are predominantly structural and organisational rather than scientific in nature and include fragmented funding mechanisms, administrative burden, regulatory complexity, insufficient research infrastructure, limited methodological support, lack of specialised expertise, and inadequate training opportunities for investigators. Importantly, these challenges disproportionately affect academic and public-interest research compared with commercially sponsored trials, limiting the capacity of academic investigators and non-commercial sponsors to initiate, conduct, and sustain high-quality multinational clinical studies[10]. These barriers are closely interconnected: limitations in one component of the research ecosystem may amplify constraints in others, ultimately affecting whether a scientifically relevant research question can be translated into a successfully delivered clinical trial.

The author's research addressed these challenges progressively, moving from the analysis of national funding environments to the investigation of barriers affecting specific vulnerable areas of academic research, multinational drug-repurposing trials, and ultimately the competencies required to successfully design and deliver IICTs.

The funding issue was explored in **Annex 1**, *Investigator Initiated Clinical Trials (IICTs): A Systematic Search in Registries to Compare the Czech Republic and Portugal in Terms of Funding Policies and Scientific Outcomes*, which analysed how national funding policies were associated with the quantity and scientific output of IICTs. The study demonstrated substantial disparities between countries in terms of funding structures, institutional support, and publication outcomes, highlighting the importance of national research ecosystems in shaping clinical research productivity. The findings suggested that differences in scientific output are not necessarily caused by lack of expertise, but rather by unequal access to sustainable support mechanisms and infrastructure. The distribution of national and international funding sources identified in the two countries is summarised in Table 1, illustrating the different funding landscapes that underpin IICTs in the Czech Republic and Portugal.

Table 1: National and International Funding Agencies and Programmes in Czech Republic in and Portugal and Corresponding Number of Funded Clinical Trials. (Nº = Number of Clinical Trials Funded by the Respective Funding Organization/Program).

Funders		Czech Republic	Portugal
National Funding Agencies/ Funding Programs	Internal Grant Agency of the Czech Ministry of Health (IGA until 2014); Currently AZV (Czech Health Research Council)	61	–
	Charles University Research Fund	10	–
	Fundação para a Ciência e Tecnologia (FCT)	–	27
	National Structural funding (COMPETE, PRODEP)	–	2
	ARDITI – *Agência Regional para o Desenvolvimento da Investigação*	–	1
International Funding Agencies/ Funding Programs	European Commission (includes H2020, IMI, Erasmus Program)	18	17
	Canadian Institutes of Health Research (Canada)	7	0
	European Regional Development Fund	6	0
	Medical Research Council (UK)	7	2
	Australian National Health Service and Medical Research Council	0	5
	National Institutes of Health (NIH, USA)	0	3
	EEA Grants	0	1
	Carlos III Institute of Health (Spain)	0	2

Similarly, **Annex 2**, *The Current Status of European and National Financial Sources for Clinical Research and Their Impact on Paediatric Non-commercial Clinical Trials: A Case Study of the Czech Republic*, extended the analysis to paediatric clinical research, an area in which limited commercial incentives and specific methodological and regulatory requirements further increase dependence on sustainable public funding. The study identified fragmented and unstable funding structures as one of the principal limitations preventing the implementation of academically sponsored paediatric studies. The analysis further demonstrated that although European funding schemes provide important opportunities, they often fail to adequately address the specific needs of long-term, investigator-driven clinical research. Together, Annexes 1 and 2 therefore illustrate that funding is not merely a prerequisite for trial initiation but a structural determinant of which clinically relevant research questions can realistically progress into IICTs.

Moving beyond funding as an isolated barrier, **Annex 3,** *Identifying Obstacles Hindering the Conduct of Academic-Sponsored Trials for Drug Repurposing in Rare Diseases: An Analysis of Six Use Cases*, expanded the analysis to the broader set of practical and systemic challenges encountered during the implementation of multinational investigator-initiated drug repurposing trials. Based on six European case studies, the study identified numerous obstacles affecting the entire clinical trial pathway, including regulatory heterogeneity, complex administrative procedures, limited access to expertise, manufacturing challenges, difficulties in obtaining investigational medicinal products, fragmented ethical review processes, and insufficient coordination among stakeholders. The principal barriers identified through the systematic literature review are summarised in Table 2 and Table 3, illustrating that the challenges span scientific, regulatory, organisational, and operational domains rather than being confined to individual studies or countries.

The analysis demonstrated that successful drug repurposing in rare diseases requires not only sustainable funding but also coordinated regulatory support, specialised research infrastructures, multidisciplinary expertise, and greater harmonisation across European Member States. In this respect, **Annex 3** provides an important transition from viewing individual barriers separately towards understanding them as interacting components of the clinical research ecosystem. These findings further reinforced the conclusion that many of the major obstacles limiting NCCTs arise from deficiencies within the research ecosystem rather than from scientific quality or relevance of the underlying research questions.

Table 2: Major barriers to the conduct of randomized trials identified by systematic reviews

Major barriers to the conduct of randomized trials
Inadequate funding
Complex/not harmonized regulations
Excessive/non-focused monitoring
Over-restrictive interpretations of privacy laws without evidence of subject benefit/Lack of transparency
Inadequate understanding of methodology
Inadequate identification of the clinical research questions
Inadequate knowledge and understanding of clinical research
Inadequate knowledge and understanding of clinical trials
Inadequate infrastructures
Unsupportive administrative system
Competing demands
Difficult patient recruitment
Major barriers to the conduct of RCTs in rare diseases
Difficult to recruit patients due to rarity
Incomplete understanding of national history to inform trial design

Need for trial designs adapted to small population size and clinical heterogeneity
Organizational challenges as a consequence from the need for multinational randomized clinical trials
Need for more sensitive outcome measures to quantify disease
Need for involvement of all the stakeholders in the study design and conduct

Table 3: Key Barriers to Non-Commercial Clinical Trials and Corresponding Ecosystem Components

Barrier domain	Key barriers identified	Corresponding ecosystem component
Funding	Inadequate, fragmented or unsustainable funding	Sustainable funding
Regulatory and ethical	Regulatory heterogeneity, complex procedures, ethics and privacy requirements	Regulatory frameworks and harmonisation; Data governance and ethics
Organisational and infrastructural	Inadequate infrastructure, administrative burden, multinational coordination	Research infrastructures and networks
Methodological and competence-related	Limited methodological expertise, challenges in trial design, insufficient trial-specific knowledge	Investigator competence and capacity building
Recruitment and stakeholder-related	Difficult recruitment, rare populations, insufficient stakeholder involvement	Patient and public involvement

Finally, **Annex 4,** *Enhancing Pragmatic Competence in Investigator-Initiated Clinical Trials: Structure and Evaluation of the CONSCIOUS II Training Programme*, addressed another critical but frequently overlooked barrier to high-quality non-commercial clinical research—the shortage of adequately trained investigators and research professionals. While financial resources and regulatory support are fundamental prerequisites for IICTs, their successful implementation equally depends on the availability of personnel with sufficient methodological, regulatory, and operational expertise. The study described the development and evaluation of the CONSCIOUS II educational programme, an interdisciplinary training initiative designed to strengthen pragmatic competencies required throughout the lifecycle of IICTs. The programme combined theoretical instruction with case-based learning and practical exercises covering key aspects of clinical trial planning, methodology, regulatory requirements, project management, and multidisciplinary collaboration.

The evaluation demonstrated significant improvements in participants' self-reported competencies across multiple domains of clinical research and confirmed high levels of participant satisfaction with the educational approach. As these outcomes were based primarily on participant-reported assessment, they should be interpreted as evidence of perceived competency development rather than direct demonstration of improved trial performance. Importantly, the programme highlighted that effective capacity building extends beyond the transfer of theoretical knowledge and should foster critical thinking, interdisciplinary collaboration, and practical problem-solving skills directly applicable to IICTs.

Taken together, the studies presented in Annexes 1–4 identify four closely related determinants of sustainable IICTs: access to appropriate funding, supportive national and European research environments, coordinated multidisciplinary and infrastructural support, and adequately trained research personnel. Rather than acting independently, these determinants influence one another across the clinical trial lifecycle and collectively determine the capacity to translate relevant clinical questions into successfully delivered studies and usable evidence.

These findings support the growing recognition that sustainable clinical research infrastructures require continuous investment in human capital alongside investments in technological infrastructure and funding mechanisms. Consequently, education should be regarded as a strategic component of the clinical research ecosystem, strengthening the quality, efficiency, and long-term sustainability of IICTs. Within the conceptual framework proposed in this thesis, funding and capacity building therefore represent cross-cutting enablers that support multiple stages of the IICTs pathway rather than isolated prerequisites for individual trials.

1.3 Knowledge gap

Although methodological, regulatory, financial, and operational aspects of NCCTs have been extensively described individually, considerably less attention has been paid to understanding their interdependencies and how their integration across the research lifecycle influences the ability of academic clinical research to generate clinically meaningful evidence and ultimately contribute to patient care and public health. Existing literature predominantly focuses on isolated elements of the clinical research process, such as study design, regulatory compliance, funding mechanisms, statistical methodology, or operational management, whereas comparatively little emphasis has been placed on the clinical research ecosystem as an integrated system linking governance, infrastructure, funding, methodological expertise, patient engagement, evidence generation, and implementation into healthcare. This systems perspective has recently gained increasing recognition in international policy documents, particularly the WHO *Global Action Plan for Clinical Trial Ecosystem Strengthening*, but its application to the analysis of non-commercial academic clinical research lifecycle remains comparatively limited[11].

In practice, the successful translation of NCCTs finding into clinical benefit depends on the coordinated interaction of multiple components. High-quality methodology alone is insufficient without sustainable funding, appropriate regulatory support, specialised research infrastructures, multidisciplinary expertise, effective patient involvement, and mechanisms for translating evidence into clinical practice. Conversely, well-developed infrastructures or favourable regulatory frameworks cannot compensate for inadequate study design or limited methodological competence. These components should therefore not be viewed as independent prerequisites but as mutually dependent elements whose interaction may facilitate or constrain successive stages of the research process. Understanding these interdependencies is therefore essential for strengthening the contribution of IICTs to healthcare[4].

Furthermore, relatively few studies have examined NCCTs from the perspective of their entire translational pathway—from identifying unmet clinical needs and generating clinically relevant research questions, through trial design, regulatory and infrastructural support, study delivery, and evidence generation, to implementation and measurable impact on clinical decision-making, healthcare systems, and public health[12]. This systems perspective is particularly important in areas where commercial incentives are limited, including rare diseases, paediatric research, drug repurposing, comparative effectiveness research, and advanced therapy medicinal products. Consequently, a gap remains between knowledge generated about individual determinants of successful academic clinical research and an integrated understanding of how these determinants interact across the pathway from clinical need to healthcare impact.

The present habilitation thesis addresses this knowledge gap by integrated evidence from the author´s research across complementary domains of NCCTs and using body of work to develop an integrated framework that conceptualises NCTs within a broader clinical research ecosystem. Rather than examining methodological, regulatory, infrastructural, organisational, educational, patient-related, and translational aspects separately, the thesis explores how their interaction influences the feasibility and delivery of clinical studies, the generation of robust and clinically relevant evidence, and its subsequent translation into clinical practice and public health. The resulting framework therefore provides a structured synthesis linking clinical and scientific need, study design and delivery, evidence generation, patient and public involvement, implementation, and healthcare impact, while recognising the cross-cutting role of funding, research infrastructures, regulatory frameworks, investigator competence, data governance, and collaborative networks.

Despite substantial advances in individual domains of non-commercial clinical research, these components are frequently considered separately. Methodological quality, regulatory requirements, research infrastructures, funding, professional competencies, patient involvement, and implementation are often addressed as distinct challenges, although their interaction ultimately determines whether clinically relevant research questions can be translated into feasible studies and meaningful evidence.

What remains insufficiently integrated is a systems-level perspective linking these components across the entire research continuum—from the identification of clinical need

and formulation of research questions through study design and conduct, evidence generation and dissemination, to implementation in healthcare. Such an integrated perspective is particularly relevant to NCCTs, where successful trial delivery frequently depends on the coordinated interaction of multiple scientific, regulatory, organisational, infrastructural, and societal factors.

This habilitation thesis addresses this gap by integrating evidence from the author's clinical, methodological, and system-level research into a conceptual framework of NCCTs ecosystem.

1.4 Research question

Building on the identified knowledge gap, this habilitation thesis seeks to examine NCCTs from a systems perspective. Rather than focusing on individual methodological or regulatory components, it explores how multiple components of the research ecosystem interact across the research lifecycle to influence the generation of clinically relevant evidence, its implementation, and its ultimate impact on patient care and public health. The central research question is:

How do the methodological, regulatory, infrastructural, organisational, and translational components of the European NCCTs ecosystem interact to enable the generation and implementation of clinically relevant evidence and contribute to improve patient care and public health?

1.5 Aim of the Habilitation Thesis

The overarching aim of this habilitation thesis is to examine NCCTs from a systems perspective and to integrate the author's clinical, methodological, and system-level research into a conceptual framework describing how clinically relevant research questions progress through the research continuum towards the generation of robust evidence and its translation into evidence-informed healthcare.

Specifically, the thesis aims to:

- examine methodological approaches supporting the design and conduct of clinically relevant NCCTs;
- analyse regulatory, financial, infrastructural, organisational, and capacity-related factors influencing its feasibility and quality;
- demonstrate the contribution of NCCTs to comparative effectiveness research, optimisation of existing therapies, precision medicine, and advanced therapies;
- examine the roles of evidence transparency, patient and public involvement, and implementation in translating research findings into healthcare; and

- synthesise these dimensions into an integrated conceptual framework of the NCCTs ecosystem.

Despite substantial advances in individual domains of NCCTs, these components are frequently considered separately. Methodological quality, regulatory requirements, research infrastructures, funding, professional competencies, patient involvement, and implementation are often addressed as distinct challenges, although their interaction determines whether clinically relevant research questions can be translated into feasible studies, robust evidence, and ultimately improvements in healthcare.

What remains insufficiently integrated is a systems-level perspective linking these components across the entire research continuum—from the identification of clinical needs and formulation of research questions through study design and conduct, evidence generation and dissemination, to implementation in healthcare. Such a perspective is particularly relevant to NCCTs, where successful research delivery often depends on the coordinated interaction of multiple scientific, regulatory, organisational, infrastructural, and societal factors.

This habilitation thesis addresses this gap by examining these interdependencies across the research continuum and synthesising the findings into an integrated conceptual framework of the NCCTs ecosystem.

1.6 Hypothesis

1.6.1 Central Hypothesis

The central hypothesis of this habilitation thesis is that the successful translation of NCCTs into improvements in patient care and public health depends not only on scientific excellence but also on the effective integration of robust methodology, supportive regulatory frameworks, collaborative research infrastructures, sustainable funding, multidisciplinary expertise, investigator competence, appropriate data governance, and meaningful patient and public engagement. Together, these components constitute an interconnected ecosystem that influences the quality, feasibility, delivery, evidence generation, implementation, and societal impact of NCCTs.

1.6.2 Specific Hypotheses

The central hypothesis is explored through the following specific hypotheses:

1. NCCTs play an important role in addressing clinically relevant questions, unmet medical needs, and public health priorities that are insufficiently covered by commercially sponsored research.

2. The effectiveness, sustainability, and societal impact of NCCTs are influenced by the interaction of structural, regulatory, financial, organisational, methodological, and capacity-related factors, rather than by scientific quality alone.
3. European research infrastructures and multinational collaborative networks support the quality, efficiency, feasibility, and inclusiveness of NCCTs, by providing coordinated methodological, regulatory, operational, and collaborative support across the research lifecycle.
4. Investigator competence and multidisciplinary capacity building contribute to the quality and feasibility of NCCTs by strengthening methodological, regulatory, and operational capabilities required across the study lifecycle.
5. Meaningful patient and public involvement, together with effective dissemination and implementation strategies, can enhance the relevance, acceptability, and translation of research findings into clinical practice and public-health benefit.

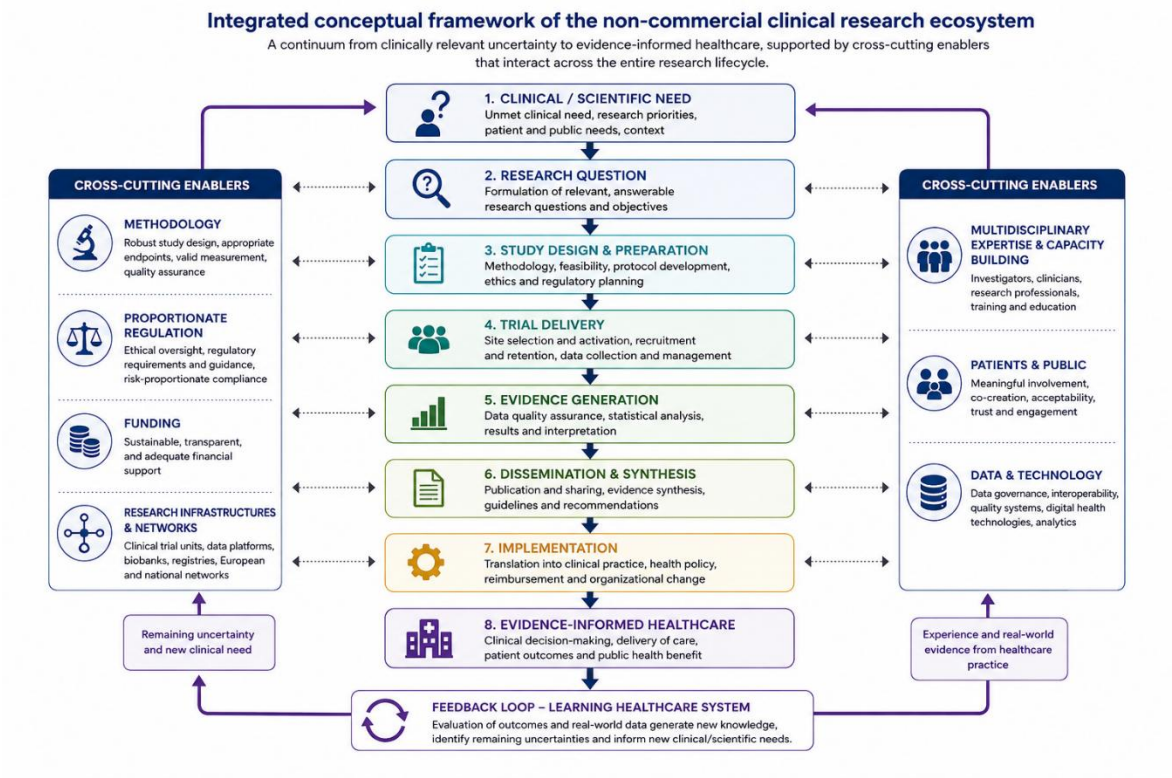
1.6.3 Original Scientific Contribution

The principal original contribution of this habilitation thesis is the development of an integrated conceptual framework of the NCCTs ecosystem, derived from the synthesis of the author's clinical, methodological, and system-level research.

The framework extends the conventional study-centred perspective on clinical trials by conceptualising NCCTs as a continuum connecting clinical and scientific need, research question formulation, study design and preparation, trial delivery, evidence generation, dissemination and synthesis, implementation, and evidence-informed healthcare. It further identifies the cross-cutting methodological, regulatory, infrastructural, financial, technological, professional, and patient-related factors that enable and support this continuum throughout the research lifecycle.

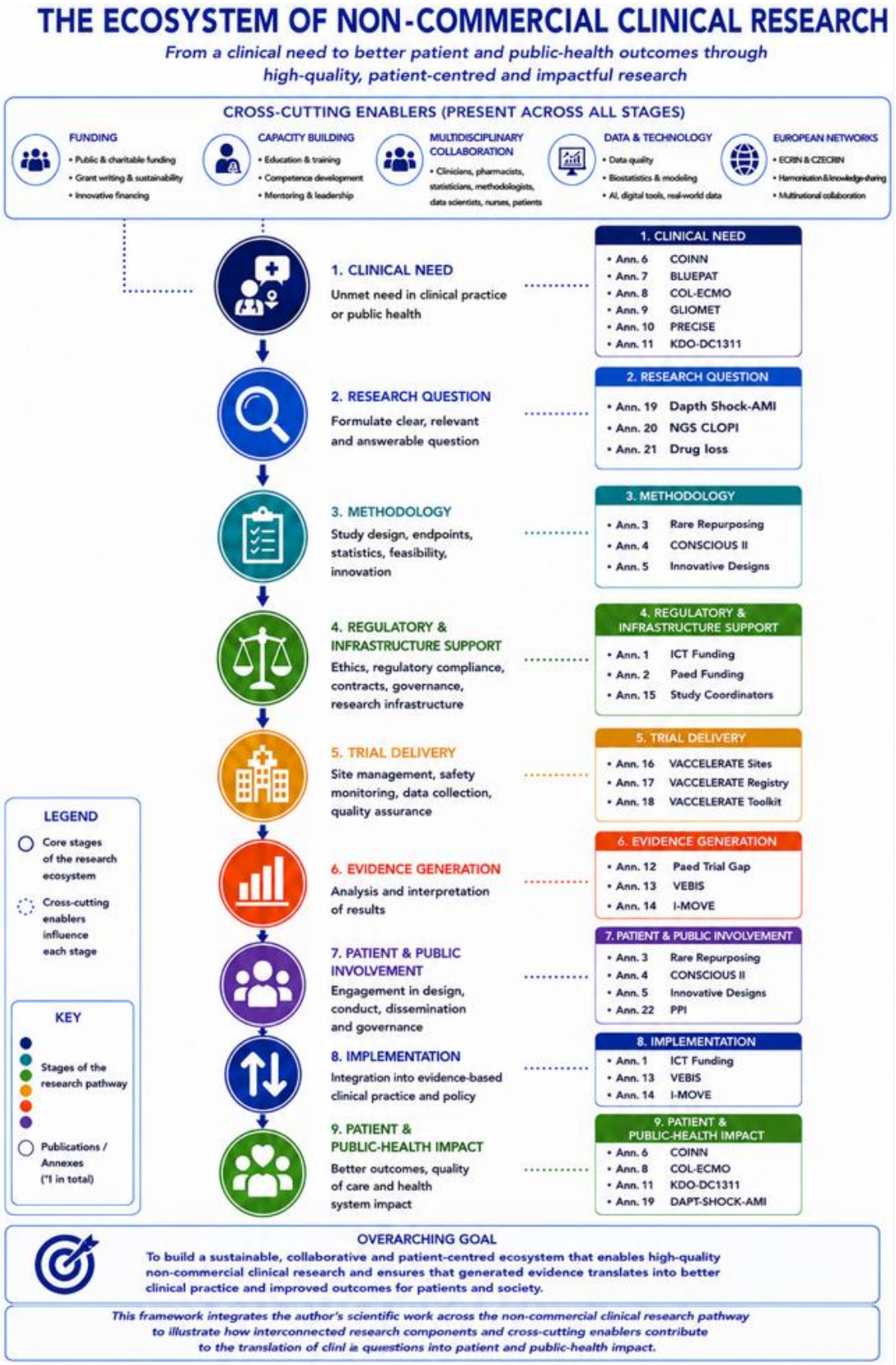
The framework conceptualises NCCTs as a continuum from clinical and scientific need and research question formulation through study design and preparation, trial delivery, evidence generation, dissemination and synthesis, and implementation to evidence-informed healthcare, presented in Figure 1. Cross-cutting enablers support the entire research lifecycle, while feedback from healthcare practice generates new evidence, identifies remaining uncertainties, and informs subsequent clinical and scientific needs.

Figure 1. Integrated conceptual framework of the non-commercial clinical research ecosystem.



The author's publications map onto complementary components of this framework, providing clinical, empirical, methodological, and system-level contributions across the research lifecycle—from comparative effectiveness and optimisation of existing therapies to precision medicine, research infrastructures, regulatory facilitation, capacity building, patient and public involvement, and evidence translation. Together, these studies illustrate how individual components of the non-commercial clinical research ecosystem interact across different stages of the research pathway. Their integration and mapping within a single systems perspective represent the principal integrative scientific contribution of this habilitation thesis. The Figure 2. maps the author's publications onto successive stages of the research continuum and its cross-cutting enablers, illustrating how the body of work included in this habilitation contributes to complementary components of the proposed systems perspective.

Figure 2. Mapping of the author's scientific contributions onto the integrated conceptual framework of the non-commercial clinical research ecosystem.


THE ECOSYSTEM OF NON-COMMERCIAL CLINICAL RESEARCH
From a clinical need to better patient and public-health outcomes through high-quality, patient-centred and impactful research
CROSS-CUTTING ENABLERS (PRESENT ACROSS ALL STAGES)
FUNDING
• Public & charitable funding
• Grant writing & sustainability
• Innovative financing
CAPACITY BUILDING
• Education & training
• Competence development
• Mentoring & leadership
MULTIDISCIPLINARY COLLABORATION
• Clinicians, pharmacists, statisticians, methodologists, data scientists, nurses, patients
DATA & TECHNOLOGY
• Data quality
• Biostatistics & modeling
• AI, digital tools, real-world data
EUROPEAN NETWORKS
• ECRIN & CZECRIN
• Harmonisation & knowledge-sharing
• Multinational collaboration
1. CLINICAL NEED
Unmet need in clinical practice or public health
1. CLINICAL NEED
• Ann. 6 COINN
• Ann. 7 BLUEPAT
• Ann. 8 COL-ECMO
• Ann. 9 GLIOMET
• Ann. 10 PRECISE
• Ann. 11 KDO-DC1311
2. RESEARCH QUESTION
Formulate clear, relevant and answerable question
2. RESEARCH QUESTION
• Ann. 19 Dapth Shock-AMI
• Ann. 20 NGS CLOPI
• Ann. 21 Drug loss
3. METHODOLOGY
Study design, endpoints, statistics, feasibility, innovation
3. METHODOLOGY
• Ann. 3 Rare Repurposing
• Ann. 4 CONSCIOUS II
• Ann. 5 Innovative Designs
4. REGULATORY & INFRASTRUCTURE SUPPORT
Ethics, regulatory compliance, contracts, governance, research infrastructure
4. REGULATORY & INFRASTRUCTURE SUPPORT
• Ann. 1 ICT Funding
• Ann. 2 Paed Funding
• Ann. 15 Study Coordinators
5. TRIAL DELIVERY
Site management, safety monitoring, data collection, quality assurance
5. TRIAL DELIVERY
• Ann. 16 VACCELERATE Sites
• Ann. 17 VACCELERATE Registry
• Ann. 18 VACCELERATE Toolkit
6. EVIDENCE GENERATION
Analysis and interpretation of results
6. EVIDENCE GENERATION
• Ann. 12 Paed Trial Gap
• Ann. 13 VEBIS
• Ann. 14 I-MOVE
7. PATIENT & PUBLIC INVOLVEMENT
Engagement in design, conduct, dissemination and governance
7. PATIENT & PUBLIC INVOLVEMENT
• Ann. 3 Rare Repurposing
• Ann. 4 CONSCIOUS II
• Ann. 5 Innovative Designs
• Ann. 22 PPI
8. IMPLEMENTATION
Integration into evidence-based clinical practice and policy
8. IMPLEMENTATION
• Ann. 1 ICT Funding
• Ann. 13 VEBIS
• Ann. 14 I-MOVE
9. PATIENT & PUBLIC-HEALTH IMPACT
Better outcomes, quality of care and health system impact
9. PATIENT & PUBLIC-HEALTH IMPACT
• Ann. 6 COINN
• Ann. 8 COL-ECMO
• Ann. 11 KDO-DC1311
• Ann. 19 DAPT-SHOCK-AMI
LEGEND
Core stages of the research ecosystem
Cross-cutting enablers influence each stage
KEY
Stages of the research pathway
Publications / Annexes (*1 in total)
OVERARCHING GOAL
To build a sustainable, collaborative and patient-centred ecosystem that enables high-quality non-commercial clinical research and ensures that generated evidence translates into better clinical practice and improved outcomes for patients and society.
This framework integrates the author's scientific work across the non-commercial clinical research pathway to illustrate how interconnected research components and cross-cutting enablers contribute to the translation of clinl ie questions into patient and public-health impact.

2 Building High-Quality Academic Clinical Trials

2.1 Methodology

2.1.1 Trial Design in Academic Settings

The methodological design of NCCTs may differ from that of commercially sponsored studies in terms of objectives, operational constraints, and scientific priorities. While industry-sponsored trials often focus on generating evidence to support the development and regulatory approval of medicinal products, IICTs (especially as a subgroup of NCCTs) frequently address clinically relevant questions for which commercial incentives may be limited, including optimisation of existing therapies, comparative effectiveness research, drug repurposing, patient-centred outcomes, and unmet medical needs[12]. As a result, academic trial methodology may place particular emphasis on clinical relevance, external validity, and applicability to routine healthcare settings, while maintaining the internal validity and methodological rigour required for reliable evidence generation. This emphasis has also support the adoption of innovative and pragmatic trial designs that seek to generate evidence directly applicable to clinical decision-making while maintaining methodological rigour[13].

Recent developments in clinical trial methodology further demonstrate that academic clinical research is increasingly adopting innovative and flexible study designs to address complex clinical questions efficiently. As discussed in **Annex 5,** *Innovative Clinical Trial Designs*, the author´s work examines the methodological principles, opportunities, and challenges associated with the transition beyond the traditional parallel-group randomised controlled trial towards approaches such as pragmatic, adaptive, platform, basket, umbrella and master protocol designs. These innovative methodologies aim to improve the efficiency, flexibility and patient-centredness of clinical research while maintaining scientific validity and regulatory acceptability. They are particularly relevant in areas such as rare diseases, precision medicine and advanced therapy medicinal products, where conventional trial designs may be challenged by small patient populations, biological heterogeneity and rapidly evolving scientific knowledge.

One of the defining characteristics of contemporary NCCT methodology is the increasing emphasis on pragmatic trial designs. Pragmatic clinical trials aim to evaluate interventions under routine clinical conditions and to generate evidence applicable to everyday clinical practice[14]. In contrast to explanatory trials, which are conducted under highly controlled settings to determine efficacy under more highly controlled conditions and primarily assess efficacy, pragmatic studies typically allow broader and more representative patient populations, greater flexibility in intervention delivery, and clinically meaningful outcomes reflecting routine care. This distinction is particularly relevant to academic clinical research which frequently seek to answer questions arising directly from clinical practice and to generate evidence of informing real-world clinical decission-maiking[15].

The examples presented below illustrate the methodological diversity of NCCTs represented in the author's research, ranging from pragmatic comparative effectiveness studies conducted under routine clinical conditions and procedural randomised trials to adaptive trial designs in critically ill populations, specialised pharmacokinetic investigations, and translational precision medicine studies integrating advanced diagnostics, predictive biomarkers, and personalised advanced therapies. Together with the methodological concepts discussed in **Annex 5**, these examples illustrate how methodological approaches can be adapted to the nature of the clinical question, patient population, intervention, and healthcare context. Collectively, the author's work demonstrates the breadth of methodological approaches required to address clinically relevant questions across different areas of NCCTs.

2.1.1.1 Pragmatic comparative effectiveness trials

The application of pragmatic trial methodology is illustrated by **Annex 6**, *Comparison of Continuous versus Intermittent Enteral Nutrition in Critically Ill Patients (COINN): Study Protocol for a Randomized Comparative Effectiveness Trial*. This IIT addressed a clinically relevant question in intensive care medicine regarding the optimal strategy for enteral nutrition administration in critically ill patients. The methodological approach was intentionally designed to reflect routine clinical practice rather than idealised experimental conditions, thereby enhancing the external validity and practical applicability of the generated evidence. By directly comparing two strategies used in clinical practice, the study illustrates how pragmatic NCCTs can address areas of therapeutic uncertainty where different approaches coexist despite limited comparative evidence. The study also illustrates an important role of NCCTs in evaluating and optimising established therapeutic strategies for which the clinical relevance may be substantial despite limited commercial incentives for comparative investigation.

In this context, methodological rigour is achieved not by maximising experimental control, but by embedding randomisation within a clinically representative care pathway while preserving predefined eligibility criteria, outcomes, and analytical procedures.

2.1.1.2 Pragmatic procedural and surgical trials

The methodological principles of pragmatic clinical research extend beyond pharmacological interventions to procedural and surgical studies. This is exemplified by **Annex 7**, *Labelling of Subpleural Pulmonary Nodules with Blue Dye and Contrast Agents under CT-Guided Control and Subsequent Video-Assisted Thoracoscopic Resection: the BLUEPAT Prospective Randomised Study.* With the increasing detection of small pulmonary nodules through lung cancer screening and incidental imaging, accurate preoperative localisation in an important prerequisite for effective minimally invasive thoracic resection.

However, comparative evidence on the relative performance of different localisation approaches remains limited. The BLUEPAT study was therefore designed as a prospective randomised IIT directly comparing two clinically applicable localisation strategies within the routine diagnostic and surgical pathway. By evaluating localisation success, procedural safety, technical feasibility, and perioperative outcomes, the study illustrates how randomised comparative methodology can be embedded within a procedural care pathway to generate clinically applicable evidence on interventions that are otherwise frequently evaluated through observational experience or single-centre practice. In this way, BLUEPAT extends the methodological spectrum of the author's work from pragmatic therapeutic comparisons to the evidence-based optimisation of diagnostic and surgical procedures.

2.1.1.3 Adaptive and innovative trial designs

Beyond pragmatic and conventional trial designs, contemporary NCCT methodology increasingly considers adaptive and master-protocol approaches that allow prespecified modifications of selected trial characteristics based on accumulating data while maintaining methodological validity. These designs may offer particular advantages in settings characterised by small patient populations, disease heterogeneity, multiple potential interventions, or rapidly evolving therapeutic strategies. At the same time, their implementation requires advanced statistical expertise, careful prospective planning, appropriate control of operating characteristics, and close alignment with regulatory requirements.

The methodological principles, opportunities, and limitations of these approaches are discussed in **Annex 5**, *Innovative Clinical Trial Designs*, which reviews adaptive designs together with platform, basket, umbrella, master-protocol, N-of-1, and SMART approaches. **Annex 5** further identifies limited methodological capacity, funding constraints, and the need for specialised education and research infrastructure as important barriers to the broader implementation of innovative trial designs.

A practical application of these methodological principles was explored during the development of the MEDIC4CAYA Horizon Europe proposal, in which an adaptive clinical trial concept was incorporated to address the combinational multi-drug therapeutic approach. Although the proposal was not selected for funding and the trial was therefore not implemented, its development illustrates the translation of methodological expertise in innovative trial design into the preparation of a complex multinational academic research project.

This example also highlights an important distinction between methodological innovation and successful trial implementation: the availability of an appropriate study design alone is insufficient without adequate funding, statistical and regulatory expertise, collaborative infrastructure, and operational capacity. In this respect, adaptive trial development provides a practical example of the broader ecosystem perspective proposed throughout this thesis.

2.1.1.4 Pharmacokinetic and pharmacometric studies

NCCTs may also employ specialised pharmacokinetic and pharmacometrics methodologies to optimise the use of established therapies in patient populations for whom dosing evidence remains insufficient. Such studies are particularly important in intensive care medicine, where p critical illness-related physiological changes and advanced organ-support technologies may substantially alter drug pharmacokinetics and contribute to uncertainty regarding optimal dosing[16].

This methodological approach is represented by **Annex 8**, *Changes of Colistin Pharmacokinetics in Critically Ill Patients due to Extracorporeal Membrane Oxygenation: Protocol for the COL-ECMO2022 Trial.* The study employed a prospective Phase IV pharmacokinetic design integrating intensive pharmacokinetic sampling with population pharmacokinetic modelling to evaluate the impact of extracorporeal membrane oxygenation on colistin disposition. By combining prospective clinical pharmacokinetic data collection, population pharmacokinetic modelling, and the complex clinical setting od extracorporeal life support, the study illustrates how specialised methodological approaches within NCCTs can address clinically relevant dosing uncertainties in critically ill populations. This model-informed approach enables variability in drug exposure to be characterised and provides a basis for evaluating whether established dosing strategies remain appropriate under substantially altered physiological and extracorporeal conditions.

2.1.1.5 Translational and biomarker-driven trial designs

A further methodological dimension of NCCTs is represented by translational and biomarker-driven studies that integrate advanced diagnostics, molecular or physiological biomarkers, personalised therapeutic approaches, and clinical evaluation withina single research framework. Such studies require multidisciplinary collaboration across clinical medicine, molecular biology, imaging, laboratory sciences, biostatistics, and regulatory science, thereby exemplifying the increasingly integrated nature of modern academic clinical research[17].

This approach is demonstrated by **Annex 9**, represented by the *GlioMET study*, and **Annex 10**, represented by the *PRECISE study*. The GlioMET trial integrates molecular imaging using ^11C-methionine PET into radiotherapy planning for patients with glioblastoma, with the aim of improving treatment precision through biomarker-guided therapeutic decision-making. In contrast, the PRECISE study prospectively validates an electroencephalography-based predictive model for vagal nerve stimulation in patients with drug-resistant epilepsy. By combining clinical, electrophysiological, and computational data, the PRECISE study illustrates an important step in biomarker translation: the transition from retrospective predictive model development to prospective multicentre clinical validation. Together, these studies demonstrate how NCCTs can support precision medicine by moving

candidate diagnostic and predictive tools towards prospective clinical validation and their potential integration into therapeutic decision-making.

A distinct form of translational and methodological integration is represented by **Annex 11**, *Dendritic Cell-Based Immunotherapy in Advanced Sarcoma and Neuroblastoma Paediatric Patients: Anti-cancer Treatment Preceding Monocyte Harvest Impairs the Immunostimulatory and Antigen-Presenting Behaviour of DCs and Manufacturing Process Outcome.* Unlike conventional clinical trials evaluating a fixed medicinal product, this translational study investigated autologous dendritic cell-based immunotherapy, in which the investigational product was individually manufactured from each patient's own monocytes under GMP conditions. Consequently, the study design extended far beyond clinical evaluation to encompass the entire translational pathway, including patient selection, leukapheresis, cell isolation, ex vivo differentiation and maturation, quality control, immunophenotypic characterisation, functional immunological assays, and clinical administration.

Importantly, the study demonstrated that preceding anticancer therapy significantly influenced the biological quality of the starting cellular material and subsequently affected dendritic-cell differentiation, antigen-presenting capacity, cytokine production, and manufacturing success. Rather than evaluating only clinical outcomes, the study incorporated laboratory biomarkers, manufacturing parameters, and product quality attributes as integral study endpoints. This multidimensional approach enabled the identification of patient- and treatment-related factors affecting the feasibility and biological quality of personalised ATMP manufacturing, thereby linking clinical history, starting-material characteristics, manufacturing performance, and product functionality.

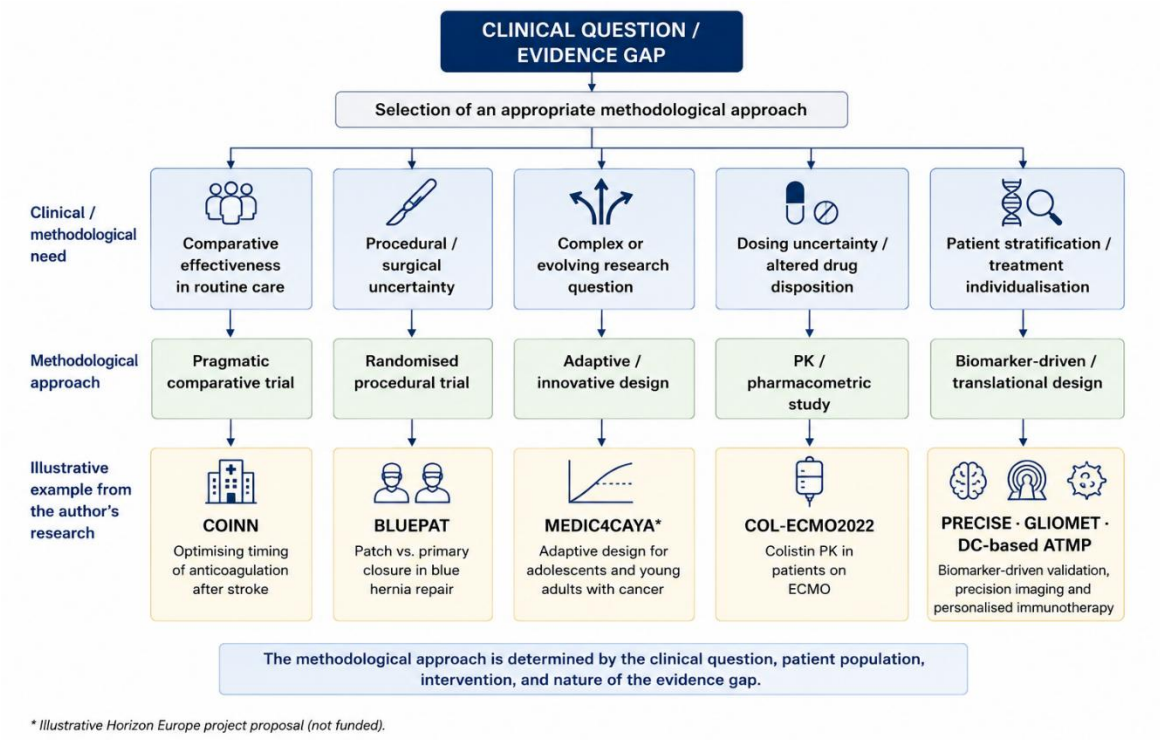
Methodologically, the study illustrates an integrated translational research model in which laboratory science, manufacturing, regulatory requirements, biomarker evaluation, and clinical investigation are inseparably linked. Such studies require close collaboration between clinicians, GMP manufacturing facilities, immunologists, laboratory scientists, pharmacologists, statisticians, and regulatory experts. The study therefore illustrates the methodological and organisational complexity associated with personalised ATMP research and the capacity of NCCTs to integrate clinical investigation with advanced manufacturing and translational science.

Collectively, these examples demonstrate the methodological diversity of NCCTs and illustrate how study design can be tailored to the nature of the clinical question, patient population, intervention, and evidence gap in the Figure 3. Across the author's work, this spectrum ranges from pragmatic comparative effectiveness and procedural randomised trials to innovative and adaptive methodological concepts, pharmacokinetic and pharmacometrics investigations, biomarker-driven prospective validation, and integrated translational studies involving personalised ATMPs. Rather than representing a hierarchy of methodological complexity, these approaches illustrate the need to select and adapt research methodology according to the scientific and clinical problem being addressed.

Taken together, the author's contributions demonstrate that methodological quality in NCCTs depends not on adherence to a single preferred trial model, but on the rigorous

selection and implementation of a design that is appropriate to the research question while remaining clinically relevant, operationally feasible, and scientifically valid. This methodological diversity enables NCCTs to generate complementary forms of evidence across a broad range of clinical and translational settings.

Figure 3: Methodological diversity of non-commercial clinical trials: selected examples from the author's research



2.1.2 Special Methodological Challenges

Despite their substantial scientific and societal value, NCCTs frequently address clinical questions in settings in which conventional trial methodology may be difficult to apply. Studies involving rare diseases, paediatric populations, critically ill patients, and public health interventions present methodological challenges that extend beyond statistical considerations and require careful integration of scientific rigor, ethical principles, operational feasibility, and available patient populations and resources. Consequently, methodological decision-making in NCCTs often involves adapting study design, recruitment strategies, analytical approaches, and collaborative models to generate reliable evidence under conditions in which conventional trial approaches may be difficult or infeasible[18].

The examples presented below illustrate how methodological approaches can be adapted to specific constraints related to population size, recruitment, disease rarity, and the

need for timely population-level evidence, while preserving scientific validity and clinical relevance.

2.1.2.1 Limited populations and recruitment challenges

One of the most fundamental methodological challenges concerns studies involving small or highly specific patient populations. Rare diseases and paediatric disorders frequently affect limited numbers of patients dispersed across multiple centres and countries, making recruitment one of the principal determinants of study feasibility. Under these circumstances, methodological quality depends not only on study design but also on international collaboration, specialised research networks, and efficient organisational support[19].

This challenge is illustrated by **Annex 12,** *Exploring the Publication Gap in Paediatric Randomized Clinical Trials: Completed versus Uncompleted Paediatric Clinical Trials.* The study demonstrated that a substantial proportion of paediatric clinical trials were not completed or did not result in publication, with insufficient patient recruitment identified as the most frequent reported reason for trial discontinuation. The principal reasons for clinical trial incompletion identified in the analysis are summarised in Table 3, demonstrating that recruitment failure occurs within a broader set of interconnected financial, methodological, organisational, regulatory, and operational challenges. Beyond reducing research efficiency, unsuccessful recruitment may compromise the ability to generate clinically meaningful evidence for vulnerable populations that are already underrepresented in clinical research. These findings reinforce the concept that recruitment is not merely an operational issue but an integral component of methodological feasibility and should therefore be considered prospectively during trial design, site selection, and feasibility assessment.

Table 4: Reported reasons for incompletion of paediatric clinical trials (Annex 13).

Uncompleted trials (*N* = 180)	**n**	**%**
Patient accrual	58	32.22
Nonreported or unclear	41	22.78
Company/business decision	22	12.22
Informative termination	21	11.67
Funding issue	13	7.22
Conduct problems	10	5.56
Regulatory issue	8	4.44
Principle investigator left	7	3.89

2.1.2.2 Rare diseases and methodological complexity

The methodological challenges associated with limited populations become even more pronounced in rare diseases, where small sample sizes are combined with disease heterogeneity, geographically dispersed patient populations, fragmented expertise, the frequent need for multinational trial conduct. These characteristics influence not only recruitment but also feasibility assessment, endpoint selection, statistical planning, operational design, and the choice of appropriate therapeutic development strategies. These challenges were systematically analysed in **Annex 3**, *Identifying Obstacles Hindering the Conduct of Academic-Sponsored Trials for Drug Repurposing in Rare Diseases: An Analysis of Six Use Cases.* As discussed earlier from a research-ecosystem perspective, the analysis identified regulatory, organisational, manufacturing, and infrastructural barriers affecting academic drug-repurposing trials. From a methodological perspective, these findings demonstrate that trial feasibility in rare diseases cannot be separated from the environment in which the study is designed and conducted. Small and geographically dispersed populations require early feasibility assessment, multinational collaboration, access to specialised expertise, and trial strategies capable of generating interpretable evidence from limited patient populations.

Thus, rare-disease research provides a particularly clear example of the interdependence between methodological design and the wider clinical research ecosystem: even scientifically robust protocols may remain unfeasible without coordinated regulatory, infrastructural, and operational support.

2.1.2.3 Real-world evidence and public health research

Another important methodological challenge concerns the evaluation of intervention effectiveness at the population level and under routine healthcare conditions, particularly when evidence needs to be generated repeatedly as epidemiological conditions, circulating pathogens, population immunity, or the interventions themselves evolve over time. Randomised trial alone cannot address all such questions, creating an important role for well-designed observational studies and real-world evidence. This approach is demonstrated by **Annex 13** and **Annex 14**, comprising the VEBIS and I-MOVE vaccine effectiveness studies. These multicentre observational studies employed the test-negative case-control design, an internationally recognised methodological framework for evaluating vaccine effectiveness during seasonal influenza epidemics and the COVID-19 pandemic. By integrating harmonised surveillance networks, standardised data collection, and statistical approaches addressing relevant confounding and bias, these studies generated timely evidence regarding vaccine effectiveness across different viral variants, age groups, and risk populations. They illustrate how harmonised multicentre observational methodology can generate comparable real-world evidence across countries and over time, complementing evidence from

randomised trials and supporting evidence-informed public health decision-making in a rapidly changing epidemiological context.

2.1.2.4 From methodological challenges to methodological innovation

Collectively, these examples demonstrate that methodological challenges in NCCTs arise not only from the scientific question itself but also from the characteristics of the population, disease prevalence, recruitment potential, clinical setting, and the type and timeliness of evidence required. Addressing these challenges therefore requires methodological approaches that are adapted to the specific research context while preserving scientific validity and interpretability.

Across the author's work, limited recruitment in paediatric trials, the complexity of multinational rare-disease research, and the need for rapidly generated vaccine-effectiveness evidence illustrate different manifestations of the same underlying principle: methodological feasibility is inseparable from operational feasibility and the surrounding research ecosystem. International research networks, harmonised methodologies, specialised infrastructures, multidisciplinary expertise, appropriate analytical approaches, regulatory support, and patient engagement are therefore not merely supportive components of NCCTs but important determinants of whether robust evidence can be generated at all[11]. Together, these elements contribute to a resilient European clinical research ecosystem capable of generating high-quality evidence, particularly for patient populations with substantial unmet medical needs[20].

This perspective extends the concept introduced in the preceding section: while the clinical question determines the appropriate methodological approach, the research context determines whether that methodology can be successfully implemented. Together, these dimensions link methodological rigour with the broader clinical research ecosystem developed throughout this thesis.

2.1.3 Capacity Building and Training

The successful design and implementation of NCCTs depend not only on methodological rigour but also on the competencies of the individuals, multidisciplinary Teams, and organisations responsible for their conduct[21]. While robust study design is fundamental to generating valid and clinically relevant evidence, high-quality academic clinical research also requires expertise in regulatory science, project management, pharmacovigilance, data management, quality assurance, ethics, patient involvement, and multidisciplinary collaboration[22]. Methodological quality therefore extends beyond the selection of an appropriate study design to the capacity to implement that design effectively throughout the clinical trial lifecycle[23]. In this context, capacity building represents a cross-cutting enabler of high-

quality NCCTs, supported by appropriately trained professionals, multidisciplinary research Teams, and sustainable organisational and infrastructural structures.

Compared with commercially sponsored research, non-commercial sponsors and academic investigators frequently operate with more constrained financial and human resources and may have a limited access to dedicated research personnel, specialised expertise, and structured educational programmes[6]. Such limitations can affect multiple stages of the trial lifecycle, including protocol development, regulatory compliance, study start-up, participant recruitment, data quality, trial management, and ultimately the reliability and usability of the evidence generated. Strengthening human and organisational capacity is therefore an important component of sustainable clinical research ecosystem and a strategic priority for IICTs across Europe[24].

2.1.3.1 Developing methodological competencies of investigators

Developing methodological competence among investigators represents a fundamental level of capacity building for NCCTs. Investigators must combine scientific and clinical expertise with practical knowledge of regulatory requirements, quality management, ethical principles, project coordination, patient involvement, and multidisciplinary collaboration. These competencies are particularly important in NCCTs, where investigators and academic teams may assume responsibilities that, in commercially sponsored trials, are distributed across specialised professional functions.

This challenge is addressed in **Annex 4**, *Enhancing Pragmatic Competence in Investigator-Initiated Clinical Trials: Structure and Evaluation of the CONSCIOUS II Training Programme*. The programme was developed as a multidisciplinary educational initiative designed to strengthen competencies required across the entire lifecycle. By combining e-learning, interactive workshops, and case-based learning, it provided practice-oriented training in protocol development, regulatory requirements, project management, quality assurance, patient involvement, and interdisciplinary collaboration.

Evaluation of the programme demonstrated significant improvements in participants' self-perceived competencies across multiple domains of clinical research indicating the potential value of combining theoretical knowledge with interactive and practice-oriented learning. Importantly, the programme reflected a broader concept of methodological competence: beyond understanding research methods, investigators need the ability to apply them effectively within the regulatory, organisational, ethical, and multidisciplinary context in which NCCTs are designed and conducted.

2.1.3.2 Strengthening multidisciplinary research teams

Modern clinical trials increasingly rely on multidisciplinary research Teams, within which study coordinators play a pivotal role. Although principal investigators retain overall scientific responsibility, effective trial conduct relies substantially on professional responsible for coordinating participant recruitment, study documentation, regulatory communication, data quality, monitoring activities, and day-to-day trial management. Strengthening the competencies and professional standing of these research professionals therefore represents an important component of institutional capacity for conducting high-quality NCCTs.

This aspect is explored in **Annex 15**, *The Role of the Clinical Research Coordinator in Healthcare Institutions – A Cinderella Among Clinical Trial Experts?* The national survey demonstrated substantial heterogeneity in the educational background, responsibilities, employment conditions, and institutional recognition of study coordinators across Czech healthcare institutions. Despite their central contribution to the successful conduct of increasingly complex clinical trials, study coordinators remain poorly defined within the Czech healthcare system and are not formally recognised as a distinct professional category within the national employment framework.

The study identified the need for harmonised competency frameworks, structured education, clearly defined career pathways, and formal professional recognition. These findings highlight that building methodological capacity requires investment not only in investigators but also in the multidisciplinary workforce responsible for translating study protocols and methodological requirements into high-quality trial conduct in routine research settings.

2.1.3.3 From national infrastructures to European collaboration

The complementary roles of national and European research infrastructures are essential for strengthening non-commercial clinical trials (NCCTs) and enabling their conduct across borders. At the European level, the **European Clinical Research Infrastructure Network (ECRIN)** provides a framework for multinational clinical research, facilitating cross-border coordination, methodological harmonisation, and access to distributed expertise and services across participating countries. However, effective multinational research depends fundamentally on strong national clinical research infrastructures capable of supporting study preparation and implementation within their respective healthcare and regulatory environments.

In the Czech Republic, this role is fulfilled by **CZECRIN**, the national clinical research infrastructure and the Czech scientific partner within ECRIN. CZECRIN provides coordinated methodological, regulatory, quality, monitoring, data management, pharmacovigilance, project management, and educational support for clinical research, with particular

relevance to investigator-initiated and other non-commercial clinical trials. By integrating expertise and research capacities at the national level, CZECRIN supports investigators and academic sponsors throughout the clinical trial lifecycle and facilitates their participation in multinational research.

The relationship between CZECRIN and ECRIN illustrates the **multilevel architecture required for sustainable academic clinical research in Europe**. National infrastructures provide the interface between investigators, healthcare institutions, academic sponsors, and the specific regulatory and organisational conditions of individual countries, while ECRIN connects these national capacities at the European level and enables their coordinated use in multinational studies. Neither level can fully substitute for the other: robust national capacity is a prerequisite for effective European collaboration, while European infrastructure enables national expertise and resources to be integrated into larger, cross-border research initiatives.

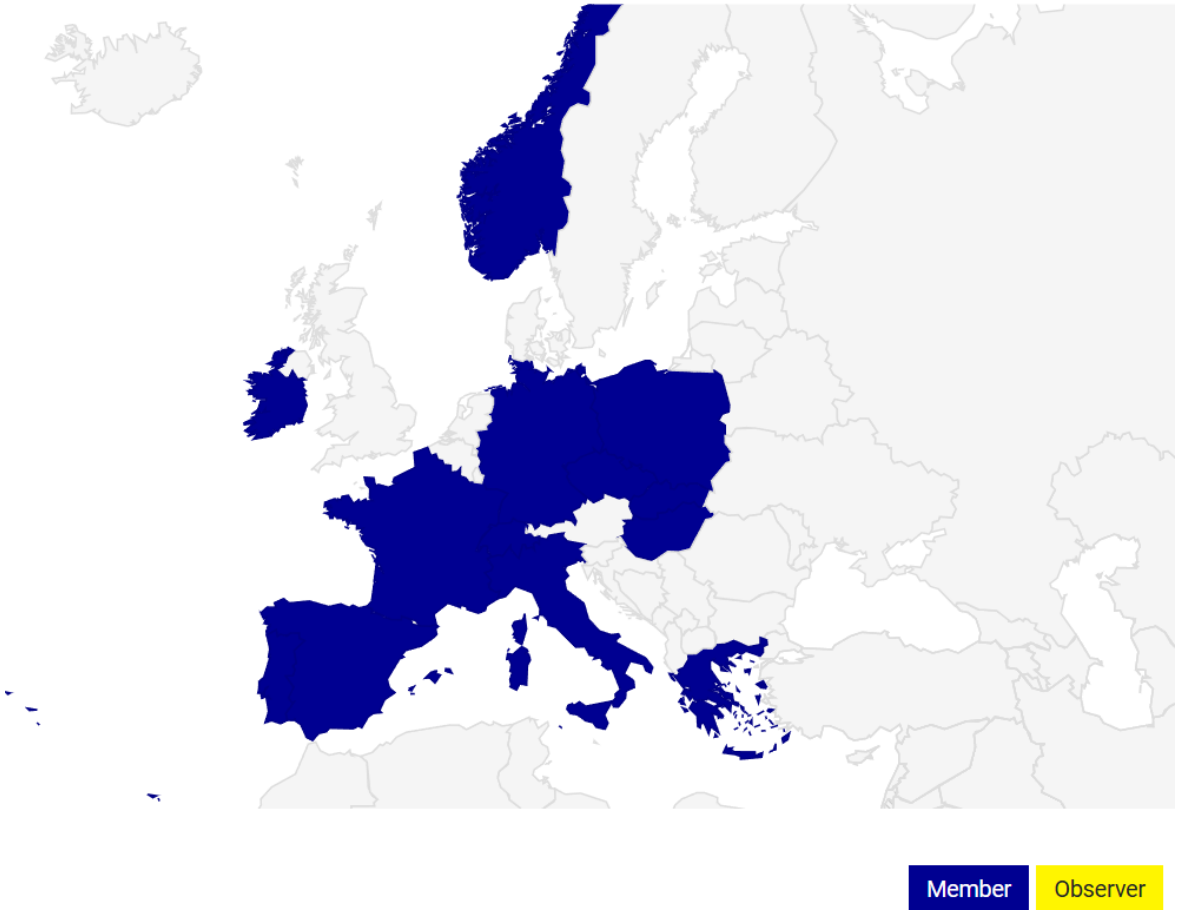
Together, **CZECRIN and ECRIN exemplify how interconnected national and European research infrastructures can transform individual academic research capacities into a coordinated clinical research ecosystem**, providing the methodological, operational, and organisational foundation required to initiate, conduct, and sustain high-quality multinational NCCTs.

2.1.3.4 Capacity building as a methodological prerequisite

Collectively, these examples demonstrate that capacity building operates at several interconnected levels: individual investigators, multidisciplinary research teams, disease-oriented collaborative networks, national clinical research infrastructures, and European research infrastructures. Methodological excellence in NCCTs therefore extends beyond the selection of appropriate study designs. It requires sustained investment in education, professional development, organisational support, and collaborative research networks that enable investigators and research teams to translate methodological principles into rigorous and effectively conducted clinical research.

This systems-based approach represents an important characteristic of contemporary NNCTs. The complementary functions of national infrastructures such as CZECRIN and the European Clinical Research Infrastructure Network (ECRIN) illustrate how capacity can be developed and connected across national and European levels, supporting the design and conduct of multinational NCCTs and the generation of robust, clinically relevant evidence. In this way, capacity building functions not merely as an educational activity, but as a methodological and infrastructural prerequisite for translating scientific questions into high-quality evidence and, ultimately, into improvements in patient care and public health. As shown in Figure 4, ECRIN connects Member and Observer countries within a European research infrastructure that supports multinational NCCTs and facilitates cross-border collaboration.

Figure 4: ECRIN´s Member or Observer country to the year 2026.



2.2 Regulation

The conduct of NCCTs in Europe is fundamentally influenced not only by methodological considerations but also by the regulatory environment within which these trials are designed and conducted. Although Europe represents one of the world's largest and scientifically strongest research regions, the European clinical trial landscape has historically been characterised by fragmented national procedures, heterogeneous regulatory interpretations, unequal research capacities, and substantial administrative complexity. These challenges have disproportionately affected non-commercial sponsors, who often have more limited access to dedicated financial, organisational, and regulatory resources than commercial sponsors.

Over the past two decades, the European Union has undertaken major efforts to transform this landscape through legislative harmonisation, digitalisation of regulatory processes, and the establishment of collaborative research infrastructures. While the introduction of the

Clinical Trials Regulation (CTR) has a created a more harmonised regulatory framework across Member States, experience has demonstrated that harmonised legislation alone is insufficient to ensure the efficient conduct of multinational academic clinical research[25]. Increasingly, European policy has evolved beyond regulatory harmonisation towards a broader concept of regulatory facilitation, recognising that legislation must be complemented by coordinated support structures, specialised expertise, and capacity-building initiatives that enable non-commercial sponsors and investigators to effectively navigate complex regulatory requirements.

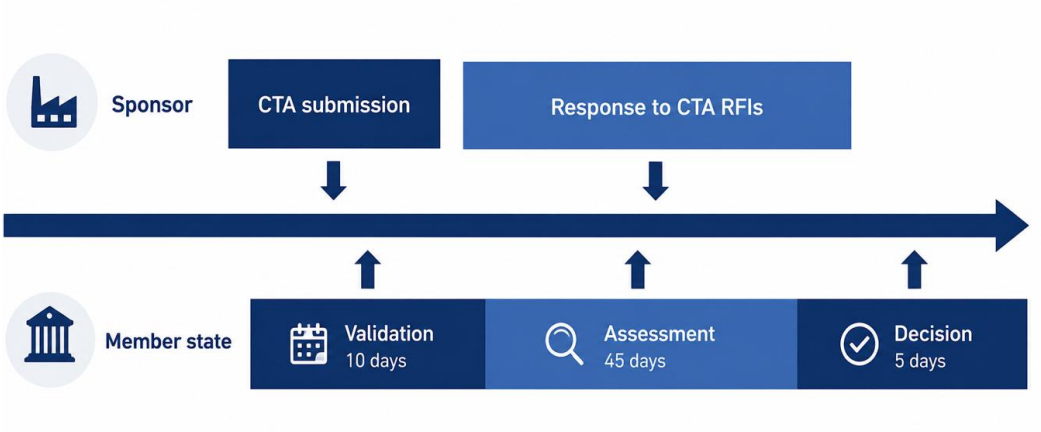
Consequently, regulation should no longer be viewed solely as a legal framework governing clinical trials. Rather, it represents an integral component of the European clinical research ecosystem, interacting with research infrastructures, regulatory authorities, ethics committees, national competence centres, and non-commercial sponsors to support the safe, efficient, and scientifically rigorous conduct of NCCTs, particularly in multinational settings.

2.2.1 European Regulatory Framework

The regulatory framework governing clinical trials in Europe has substantial transformation over the last two decades. A major milestone was the entry into application on 31 January 2022 of Regulation (EU) No 536/2014 on clinical trials (Clinical Trials Regulation, CTR), which replaced the previous Clinical Trials Directive 2001/20/EC. Although the Directive represented the first attempt to harmonise clinical trial regulation across the European Union, its implementation through national legislation resulted in multiple parallel national submissions, differences in regulatory procedures and interpretations, duplicated assessments, and substantial administrative burden. These shortcomings disproportionately affected multinational NCCTs and their sponsors, for whom the administrative and regulatory requirements associated with conducting trials across several countries represented a particular challenge.

The Clinical Trials Regulation was designed to overcome these limitations by introducing a harmonised legal framework supported by the Clinical Trials Information System (CTIS). CTIS enables a single electronic submission for multinational clinical trials, coordinated assessment by participating Member States, harmonised regulatory timelines, and increased transparency through public access to clinical trial information. Collectively, these measures aim to simplify regulatory procedures while maintaining high standards of participant protection and scientific quality. Figure 5 illustrates the CTIS assessment workflow and the timelines applicable to Member States under the Clinical Trials Regulation, highlighting the coordinated evaluation process and harmonised decision-making across participating countries[25].

Figure 5: Clinical Trials Information System (CTIS) workflow and timeline for Member State assessment.



For NCCTs, these developments represent an important opportunity to reduce fragmentation and facilitate multinational collaboration. At the same time, non-commercial sponsors remain responsible for fulfilling complex regulatory requirements, including pharmacovigilance, quality management, investigational medicinal product oversight, transparency obligations, and data governance. Consequently, regulatory harmonisation alone does not eliminate the need for specialised methodological and regulatory support.

An important recent development illustrating the continuing evolution of the European regulatory landscape is the FAST-EU (Facilitating and Accelerating Strategic Clinical Trials) pilot initiative. Launched in 2026 by the Clinical Trials Coordination Group under the Heads of Medicines Agencies, together with MedEthics EU, FAST-EU aims to accelerate the coordinated assessment and authorisation of selected multinational clinical trials while operating fully within the Clinical Trials Regulation and CTIS framework. The pilot introduces an accelerated coordinated assessment process, with an overall target of 70 calendar days from CTIS submission to final conclusion, including sponsor response times. Beyond shortening timelines, FAST-EU illustrates the broader evolution from regulatory harmonisation towards active regulatory facilitation, testing whether more streamlined and predictable assessment processes can be achieved within the existing European regulatory framework while maintaining scientific, safety, and ethical standards[26].

Ethical oversight remains a cornerstone of European clinical research regulation. NCCTs may address clinically important questions in vulnerable populations, including children, critically ill patients, and individuals living with rare diseases. These studies require careful evaluation of scientific justification, proportionality of risks and benefits, informed consent procedures, and patient protection. Ethical review therefore represents an integral component of responsible clinical research and participant protection, closely linked to methodological quality, rather than merely an administrative requirement.

Similarly, compliance with Good Clinical Practice (GCP) provides an internationally recognised framework for protecting trial participants and ensuring the reliability of clinical trial data. Although GCP principles apply equally to commercial and non-commercial research, implementation may be particularly demanding for academic institutions with limited access to dedicated clinical trial units and specialised quality management systems[20].

Data governance has emerged as another critical regulatory consideration following the implementation of the General Data Protection Regulation (GDPR). While GDPR significantly strengthened the protection of personal data, differences in national interpretation and institutional implementation may continue to complicate multinational data sharing, secondary use of research data, and informed consent procedures. These inconsistencies can contribute to administrative complexity for multinational IICTs[25].

Taken together, the European regulatory framework has evolved from a fragmented legislative environment towards an increasingly coordinated regulatory ecosystem. Nevertheless, successful implementation of harmonised legislation depends on complementary regulatory expertise, methodological support, and specialised clinical research infrastructures capable of supporting non-commercial sponsors and investigators throughout the clinical trial lifecycle.

2.2.2 National Disparities

Despite substantial progress in European regulatory harmonisation, important disparities persist among Member States regarding the conduct and support of NCCTs. These differences extend beyond legislation to encompass funding opportunities, administrative capacity, institutional infrastructure, regulatory expertise, and national research priorities. Consequently, investigators and non-commercial sponsors operating in countries with less developed support structures may face additional barriers to initiating and conducting clinical trials, irrespective of the scientific quality of the research questions[13].

Funding inequality represents one of the principal structural barriers affecting NCCTs. NCCTs frequently depend on public funding, institutional resources, or competitive European programmes, which may provide limited continuity across the clinical trial lifecycle. Unlike commercially sponsored trials NCCTs may lack dedicated funding mechanisms covering the full pathway from study preparation and regulatory submission through trial conduct, analysis, and dissemination[27].

This challenge was investigated in **Annex 2**, *The Current Status of European and National Financial Sources for Clinical Research and Their Impact on Paediatric Non-commercial Clinical Trials: A Case Study of the Czech Republic*. The study demonstrated that funding mechanisms supporting paediatric NCCTs were fragmented and frequently insufficient to sustain activities from protocol development to dissemination of results. While European funding programmes provide valuable opportunities, national funding mechanisms may not provide dedicated and continuous support across all stages of IICTs.

National differences were further explored in **Annex 1**, *Investigator-Initiated Clinical Trials (IICTs): A Systematic Search in Registries to Compare the Czech Republic and Portugal in Terms of Funding Policies and Scientific Outcomes*. This comparative analysis identified how different national funding and research environments may be associated with differences in clinical research activity and scientific output.

In addition to financial inequalities, non-commercial sponsors and investigators may face substantial administrative and operational requirements associated with ethics approvals, contractual negotiations, pharmacovigilance, insurance, data protection, and regulatory reporting. The relative burden of these requirements may be particularly relevant for low-risk pragmatic trials evaluating established or standard-of-care interventions, reinforcing the importance of proportionate regulatory approaches aligned with the actual risks and complexity of the trial[28].

These observations illustrate that harmonised legislation alone cannot eliminate disparities in the European clinical research landscape. Successful implementation of NCCTs requires national systems capable of translating European legislation into practical support for researchers[29].

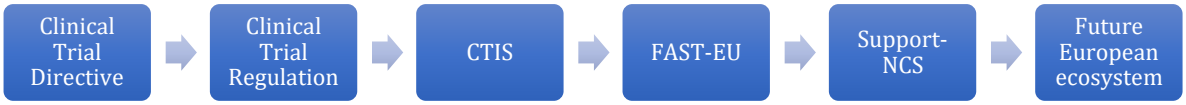
Recognising this need, the European Commission has supported collaborative initiatives aimed at strengthening the capacity available to non-commercial sponsors alongside regulatory harmonisation. An important example is the EU4Health Joint Action "Support to Non-Commercial Sponsors (Support-NCS)", coordinated by CZECRIN (Masaryk University)[30]. The Joint Action brings together national competent authorities, clinical research infrastructures, and national competence centres across Europe to strengthen support available to non-commercial sponsors, including through education and training, scientific and regulatory support, Exchange of expertise and good practise, and the development of shared approaches and guidance. By strengthening national competence while promoting collaboration across Member States, the initiative seeks to reduce disparities in regulatory expertise and improve the quality and efficiency of multinational NCCTs.

The Joint Action reflects an important evolution in European clinical research policy. Rather than focusing exclusively on harmonising legislation, current initiatives increasingly recognise that successful implementation depends equally on strengthening national competencies and creating sustainable support systems for investigators. This approach complements the role of European research infrastructures by ensuring that academic investigators receive practical methodological and regulatory support within their own national healthcare systems while remaining fully integrated into the broader European clinical research ecosystem[31].

Collectively, these findings demonstrate that effective regulation of NCCTs extends beyond legislative harmonisation. Sustainable NCCTs requires an integrated regulatory ecosystem combining harmonised European legislation, proportionate regulatory requirements, specialised national expertise and support structures, coordinated scientific advice, appropriate and sustainable funding mechanisms, and research infrastructures capable of supporting investigators throughout the entire clinical trial lifecycle. Recent developments—

including the Clinical Trials Regulation, CTIS, the FAST-EU pilot, and the EU4Health Joint Action Support-NCS—illustrate an evolution beyond regulatory harmonisation towards a broader model of regulatory facilitation, aimed at making the European regulatory framework more accessible and navigable for non-commercial sponsors while maintaining high standard of scientific quality, patient safety, and ethical integrity, is presented in Figure 6 [26].

Figure 6: Development of European Clinical Trial Ecosystem



2.3 Research Infrastructures as Enablers

The increasing complexity of contemporary clinical research has highlighted that high-quality NCCTs depend not only on scientific excellence but also on sustainable research infrastructures capable of supporting non-commercial sponsors, investigators, and research teams throughout the clinical trial lifecycle. Modern NCCTs require access to specialised expertise and services, including methodological consultation, regulatory guidance, quality management, monitoring, data management, education, patient and public involvement. The integration of these functions may extend beyond the capabilities of individual research groups and institutions, particularly in complex or multinational NCCTs, creating a need dedicated organisational structures and collaborative infrastructures at national and European levels[32].

Research infrastructures have consequently become important enabling components of the European clinical research ecosystem. Their roles include reducing fragmentation, supporting the harmonisation of research practices, enabling multinational collaboration, and providing non-commercial sponsors and investigators with access to the expertise and services required to design and conduct high-quality clinical trials[33]. By connecting methodological, regulatory, operational, and disease-specific expertise across institutions and countries, research infrastructures provide an organisational framework through which scientific questions can be translated into feasible and coordinated clinical research.

2.3.1 ECRIN as the European horizontal infrastructure

The European Clinical Research Infrastructure Network (ECRIN) represents a key European research infrastructure supporting multinational NCCTs across multiple therapeutic

areas. Unlike disease-specific research initiatives, ECRIN provides horizontal methodological and operational support that is independent of medical specialty or disease area. Its services include methodological consultation, regulatory support, monitoring, quality management, data management, biostatistics, trial management, and coordination of multinational studies[8].

By supporting harmonised approaches across participating countries and providing access to specialised expertise, ECRIN facilitates the design and conduct of complex multinational NCCTs by connecting investigators and sponsors with research capacity and expertise across national boundaries. Importantly, ECRIN does not replace national research infrastructures; rather, it connects national scientific partners within a coordinated European framework, combining national expertise and operational capacity to support complex multinational clinical research[34].

2.3.2 The role of national research infrastructures

Successful multinational clinical research relies on sufficient research capacity within participation countries. While ECRIN facilitates and coordinates cros-border collaboration, participating country require national capacity to support non-commercial sponsors and investigators, provide access to methodological and regulatory expertise, develop human resources, and coordinate research activities at the national level[8].

The Czech Clinical Research Infrastructure Network (CZECRIN) illustrates this complementary role. As the Czech national clinical research infrastructure and national scientific partner of ECRIN, CZECRIN provides comprehensive supporting NCCTs, including methodological consultation, regulatory guidance, monitoring, data management, education, quality management, and coordination of multicentre clinical studies. In addition to facilitating Czech participation in multinational ECRIN-supported trials, CZECRIN supports the development of national capacity through activities adapted to the Czech clinical research environment, including investigator and research-team training, support for clinical trial units, coordination of disease-oriented research networks, and broader capacity-building initiatives[35].

This complementary relationship between ECRIN and CZECRIN illustrates a multilevel model of European clinical research infrastructure. European and national infrastructures perform distinct but interconnected functions: European-level coordination facilitates cross-border collaboration, while national infrastructures provide locally accessible methodological, regulatory, operational, and organisational capacity. Together, these complementary functions connect national research environments with European collaboration and support the implementation of multinational NCCTs across participating countries.

2.3.3 Disease-oriented research networks

While horizontal infrastructures such as ECRIN and national infrastructures such as CZECRIN provide support across multiple areas of clinical research, certain scientific and

public health challenges benefit from additional disease-oriented or thematic- research networks. These networks are particularly valuable in areas characterised by rapidly evolving scientific knowledge, highly specialised expertise, urgent public health needs, or dispersed patient populations. Rather than replacing horizontal infrastructures, such networks complement them by bringing together domain-specific expertise, connecting specialised clinical sites and patient populations, supporting harmonised research approaches, and facilitating collaboration around defined clinical or public health priorities.

The strategic importance of such networks became particularly evident during the COVID-19 pandemic, when the need for rapid initiation and implementation of multinational vaccine trials required extensive cross-border coordination and rapid mobilisation of clinical research capacity. In response, the VACCELERATE consortium was established as a pan-European clinical research network focused on COVID-19 vaccine trials, bringing together clinical trial sites, expertise, participant-recruitment capacity, and public engagement activities across country[9]. VACCELERATE therefore provides an example of how a thematic European network can complement existing horizontal and national infrastructures by mobilising specialised research capacity around a specific and rapidly evolving public health need.

2.3.3.1 VACCELERATE as a model of thematic European research capacity

Several publications included in this habilitation illustrate how thematic research networks can develop complementary capacities that address challenges extending beyond the scope of individual clinical trials.

Annex 16, *VACCELERATE Site Network: Real-time Definition of Clinical Study Capacity in Europe*, describes the establishment of a continuously updated European network of clinical trial sites designed to support rapid assessment of study feasibility and identification of suitable sites for multinational vaccine trials. By systematically mapping site infrastructure, expertise, and potential recruitment capacity across Europe, the network created a mechanism for evidence-informed site identification and more efficient feasibility assessment during the preparation of multinational studies.

Complementing this initiative, **Annex 17**, *VACCELERATE Volunteer Registry: A European Study Participant Database to Facilitate Clinical Trial Enrolment*, presents the development of a centralised European volunteer registry enabling citizens to express interest in participating in vaccine trials. The registry established a cross-country mechanism for connecting potentially eligible volunteers with clinical studies, thereby supporting participant identification and recruitment across participating countries. Beyond its operational recruitment function, the registry also created a direct interface between the research infrastructure and citizens interested in contributing to vaccine research.

The importance of public engagement was further addressed in **Annex 18**, *Enhancing Public Health Communication Regarding Vaccine Trials: Design and Development of the Pan-European VACCELERATE Toolkit*. This initiative developed multilingual

communication resources designed to improve public understanding of vaccine research, address misinformation, and support informed participation in clinical trials. Together, the Site Network, Volunteer Registry, and communication toolkit illustrate how thematic research networks can integrate site preparedness, participant access, and public engagement within a coordinated research framework. They also demonstrate that enabling infrastructure for clinical research extends beyond methodological and operational trial support to include communication, trust-building, and meaningful engagement with patients and the public.

2.3.4 Research infrastructures as an integrated ecosystem

Collectively, these examples demonstrate that research infrastructures and collaborative research networks operate at multiple complementary levels. Horizontal infrastructures such as ECRIN support multinational NCCTs across therapeutic areas by providing cross-border coordination and access to specialised expertise. National infrastructures such as CZECRIN develop and provide access to the methodological, regulatory, operational, and organisational capacities required within individual countries and support their participation in multinational studies. Disease-oriented and thematic research networks complement these structures by mobilising specialised expertise, clinical sites, patient populations, and other research capacities around defined clinical, scientific, or public health priorities.

Rather than functioning independently, these structures interact within an integrated European clinical research ecosystem. Their complementary roles can help reduce fragmentation, strengthen methodological quality, support efficient trial preparation and conduct, facilitate patient recruitment, and enhance public engagement. This multi-level and interconnected model enable IICTs to address research questions that would be difficult or impossible for individual institutions to undertake alone.

The experience gained through ECRIN, CZECRIN, and VACCELERATE illustrates that a sustainable European ecosystem for NCCTs depends not only on scientific excellence and regulatory harmonisation, but also on research infrastructures and collaborative networks capable of connecting methodological expertise, operational support, national research capacity, disease- and theme-specific expertise, and multinational coordination. In this way, research infrastructures function as enabling structures that connect scientific questions with the expertise, capacity, and cross-border collaboration required to translate them into high-quality clinical research.

3 Investigator-Initiated Clinical Trials as Drivers of Clinical Innovation

3.1 Comparative Effectiveness Research

Comparative effectiveness research (CER) represents an important scientific contribution of NCCTs to evidence-based healthcare. Unlike registration trials designed primarily to demonstrate the efficacy and safety of novel medicinal products to support regulatory decision-making, comparative effectiveness studies seek to determine the relative benefits and risks of alternative therapeutic or healthcare strategies in conditions relevant to clinical practise. Their primary objective is therefore not the introduction of new technologies to the market, but the optimisation of clinical practice through the generation of evidence directly applicable to clinical and healthcare decision-making[36,37].

Historically, many medicinal products have been authorised on the basis of evidence generated in placebo-controlled superiority trials. Such studies have played a fundamental role in demonstrating efficacy and remain an important methodological otion when no proven therapy exists. The **Declaration of Helsinki** recognises placebo as an ethically acceptable comparator not only in the absence of an established intervention but also where compelling and scientifically sound methodological reasons justify its use and participants receiving placebo are not exposed to additional risks of serious or irreversible harm. Consequently, placebo-controlled studies continue to play an important role in clinical research and remain particularly common in commercially sponsored drug development programmes, where demonstrating superiority over placebo is often sufficient for regulatory approval[38].

However, once an effective standard of care has been established, the choice of comparator becomes both an ethical and a scientific consideration. In such settings, the appropriateness of placebo use depends on the research question, clinical context, availability of an established treatment, and the potential risks associated with withholding effective therapy. This principle is reflected not only in the Declaration of Helsinki but also in the ICH E10 Guideline on Choice of Control Group and Related Issues in Clinical Trials, which provides a methodological framework for selecting appropriate comparators according to the scientific objective, ethical acceptability, and clinical context. Active-controlled superiority or non-inferiority trials may therefore be particularly relevant in therapeutic areas, particularly in cardiovascular medicine, infectious diseases, oncology, and chronic diseases, where effective treatments are already available[39].

From the perspective of evidence-based medicine, active-comparator trials provide information that is often directly relevant to routine clinical decision-making than placebo-controlled studies. While placebo comparisons can establish the efficacy of an intervention relative to placebo, head-to-head comparisons can determine the relative benefits and risks

of alternative therapeutic strategies, including differences in effectiveness, safety, convenience, other outcomes relevant to patients and healthcare systems. Such evidence is important not only by clinicians but also by health technology assessment bodies, healthcare payers, and clinical guideline developers[40].

For NCCTs, active-comparator designs are particularly important. Whereas commercially development programmes necessarily address evidence requirement related to marketing authorisation, NCCTs frequently address questions arising from clinical practise that may remain in answered after regulatory approval, including optimisation of existing therapeutic strategies, compare standards of care, evaluate treatment pathways, and answer questions arising directly from clinical practice. Consequently, comparative effectiveness research represents an important area of NCCTs[13,41].

This distinction reflects not only different scientific priorities but also important practical considerations. For non-commercial sponsors, the development and manufacturing of matching placebo medicinal products may represent substantial financial, logistical, and regulatory challenges that frequently exceed the resources available to non-commercial sponsors. Manufacturing placebo medicinal products may involve Good Manufacturing Practice (GMP)-compliant production, quality control, packaging, labelling, stability testing, and regulatory documentation, increasing study costs and operational complexity[25]. In settings where an established standard of care exists and an active comparator appropriately addresses the research question, placebo-controlled designs may therefore be neither necessary nor the most clinically informative option. Comparative studies of established therapeutic strategies or standard-of-care interventions can instead generate evidence with direct relevance to everyday healthcare [37–39]

The author's research presented in this habilitation thesis illustrates several complementary applications of comparative effectiveness research. The COINN trial**, Annex 6,** investigated continuous versus intermittent enteral nutrition in critically ill patients, addressing an important clinical question concerning the optimisation of supportive care using a pragmatic comparative design. Rather than evaluating a novel medicinal product, the study compared two routinely used treatment strategies to generate evidence relevant to their use in clinical practice.

Similarly, the DAPT-SHOCK-AMI trial**, Annex 19,** compared intravenous cangrelor with crushed ticagrelor in patients with acute myocardial infarction complicated by cardiogenic shock. This investigator-initiated randomised trial addressed a therapeutic question in a critically ill and clinically complex in whom treatment evidence is limited. By directly comparing two established antiplatelet strategies under emergency conditions, the study exemplifies the ability of NCCTs to address clinically relevant comparative questions in specialised patient populations. The concept of comparative effectiveness research extends beyond pharmacological interventions. The BLUEPAT study, **Annex 7,** demonstrates how investigator-initiated research can evaluate innovative patient-centred approaches within routine healthcare delivery, expanding comparative effectiveness research beyond therapeutic interventions to organisational and healthcare-delivery strategies. Such studies illustrate that

the optimisation of patient care frequently depends not only on the selection of medicinal products but also on improvements in healthcare processes and clinical pathways.

Looking ahead, the concept of the comparator in clinical research is beyond therapeutic interventions to organisational and healthcare-delivery strategies. The recently adopted **ICH E6(R3) Good Clinical Practice Guideline**, particularly Annex 2, formally recognises the increasing role of real-world data (RWD), registries, and external control cohorts may complement conventional randomised comparisons in appropriate designed studies, particularly where conventional randomisation is difficult or where additional contextual evidence is required. Contemporary clinical research frameworks increasingly recognise the use of diverse data sources and trial designs, provided that their fitness for purpose, data quality, and methodological validity are adequately addressed, especially in rare disease, ATMPs and other specific situations [20].

Beyond these approaches, AI-driven technologies have the potential to further transform comparative clinical research. Advanced computational models, including virtual patients, digital twins, and virtual control arms, may in the future support modelling of disease progression and treatment response by integrating clinical, molecular, imaging, and real-world data. Although these approaches are still under active development and require further methodological validation and regulatory acceptance, they may eventually contribute to reducing recruitment requirements and improving the feasibility of research in small or vulnerable populations[42].

European initiatives such as **CERTAINTY**, which is developing patient-specific virtual twins to support personalised decision-making in CAR-T cell therapy, illustrate the development of AI-enabled computational modelling approaches in healthcare. While the primary objective of CERTAINTY is clinical decision support rather than clinical trial design, projects of this type establish the scientific and technological foundations that may ultimately enable more sophisticated data-driven comparative methodologies in future clinical research[43,44].

Collectively, the studies presented in **Annexes 6, 7, and 19** demonstrate that investigator-initiated comparative effectiveness research extends beyond conventional comparisons of medicinal products. Through pharmacological, supportive-care, and healthcare-delivery interventions, these studies illustrate how NCCTs can address comparative questions arising directly from clinical practice and generate evidence relevant to therapeutic choices, care processes, and patient-centred healthcare. More broadly, comparative effectiveness research is itself evolving as conventional randomised comparisons are increasingly complemented, where methodologically appropriate, by real-world data, external controls, and emerging computational approaches. Together, these developments illustrate the continuing adaptation of comparative clinical research to changing scientific, clinical, and methodological possibilities while preserving its central objective: generating evidence that supports better-informed patient care.

3.2 Optimising Existing Therapies

The optimisation of established therapeutic interventions represents an important contribution of NCCTs to evidence-based medicine. Beyond establishing whether an intervention is effective, NCCTs frequently address a complementary and clinically relevant question: how can existing therapies be used more effectively, safely, and appropriately in routine clinical practice?

Many medicinal products continue to be used for many years after marketing authorisation. During this period, new evidence may emerge regarding their pharmacokinetic and pharmacodynamic properties, optimal dosing strategies, effectiveness in previously understudied populations, drug-drug interactions, long-term safety, and potential use in new clinical indications. Some of these questions may remain insufficiently addressed by conventional drug-development programmes, particularly where additional evidence is unlikely to generate substantial commercial return despite its clinical relevance for patients and healthcare systems[45].

Consequently, optimisation of existing therapies represents an important area of NCCTs. These studies frequently investigate approved medicinal products in populations, clinical contexts, or therapeutic strategies that were not comprehensively evaluated during their original development. Typical examples include critically ill patients, children, patients with rare diseases, individuals receiving extracorporeal organ support, or populations with multiple comorbidities. NCCTs can therefore plays a crucial role in extending the evidence base beyond the populations and clinical settings originally investigated during drug development[46].

From a methodological perspective, optimisation studies occupy an important position throughout the lifecycle of medicinal products. Rather than generating evidence primarily to support initial market authorisation, they contribute to the continuous refinement of therapeutic use after medicines enter c clinical practice. Their findings may inform dosing recommendations, treatment guidelines, prescribing practices, and potentially reimbursement of other healthcare decision-making, thereby contribution to improved treatment effectiveness and patient safety[47].

The author's publications presented in this section illustrate three complementary approaches through which NCCTs can contribute to the optimisation of existing therapies:

- improving pharmacotherapy in highly specialised clinical settings,
- generating pharmacokinetic evidence in complex patient populations, and
- identifying new therapeutic applications for already authorised medicinal products through drug repurposing.

3.2.1 Clinical Pharmacology and Optimisation in Critically Ill Patients

One of the major challenges in clinical pharmacology is the use of established medicinal products in patients whose physiological conditions differ substantially from those

represented in the populations studied during pre-authorisation development. Critical illness can substantially alter drug absorption, distribution, metabolism, and elimination, making extrapolation from standard dosing recommendations uncertain. NCCTs and investigator-initiated pharmacological studies can therefore generate evidence needed to refine therapeutic strategies in these complex patient populations and supports the safer and more effective use of existing medicines.

The author's early research focused on understanding the pharmacokinetic and pharmacodynamic factors influencing drug effectiveness in critically ill patients. **Annex 20**, *Impaired bioavailability and antiplatelet effect of high-dose clopidogrel in patients after cardiopulmonary resuscitation (CPR),* demonstrated that despite administration of high-dose clopidogrel, patients following successful cardiopulmonary resuscitation exhibited markedly reduced bioavailability and insufficient platelet inhibition. The study highlighted the potential impact of critical illness on drug absorption and therapeutic response, demonstrating that dosing strategies established in more stable patient populations may not necessarily achieve comparable drug exposure or pharmacodynamic effects in critically ill patients.

The optimisation of pharmacotherapy in patients with altered routes of drug administration was further investigated in **Annex 21**, *Quantitative analysis of drug losses administered via nasogastric tube – An in vitro study.* This work quantified drug losses occurring during administration through nasogastric tubes and demonstrated that the administration process itself may substantially influence the delivered dose. The findings identified practical factors relevant to medication administration in intensive care settings and demonstrated how targeted experimental studies can address clinically relevant uncertainties in the safe and effective delivery of established medicines.

These early studies established the scientific foundation for the author's subsequent IICTs aimed at optimising pharmacotherapy in highly specialised clinical settings. This research trajectory was subsequently extended in **Annex 8**, *Changes of colistin pharmacokinetics in critically ill patients due to extracorporeal membrane oxygenation: protocol for the COL-ECMO2022 trial.* Building upon previous work investigating altered drug disposition in critically ill patients, this prospective investigator-initiated Phase IV pharmacokinetic study was designed to evaluate the influence of extracorporeal membrane oxygenation on colistin pharmacokinetics. By integrating prospective pharmacokinetic sampling with population pharmacokinetic in critically ill patients receiving ECMO, the study aimed to characterise changes in colistin disposition and generate evidence to inform dosing in a clinical setting where pharmacokinetic uncertainty may have important therapeutic consequences.

Taken together, these studies demonstrate a progressive development of the author's research in clinical pharmacology, from identifying factors affecting drug exposure and administration in critically ill patients to the design of a prospective pharmacokinetic NCCT addressing drug disposition during advanced organ support. They illustrate how IICTs research can complement evidence generated during medicinal product development by addressing pharmacological uncertainties that emerge when established therapies are used in complex patient populations and clinical settings. More broadly, they exemplify an

important contribution of NCCTs and related IICTs: extending and refining the evidence base for medicinal products throughout their lifecycle to support their safe, effective, and context-appropriate use in clinical practice.

4 Translational and Precision Medicine

4.1 Introduction

The transition from conventional "one-size-fits-all" medicine towards precision medicine represents a major transformation in contemporary healthcare. Advances in genomics, molecular biology, advanced imaging, computational modelling, artificial intelligence, and cell-based therapies have created new opportunities to tailor diagnosis, prognosis, and treatment to individual patients and disease characteristics. However, scientific discoveries alone do not necessarily translate into improve patient outcomes. Their successful implementation requires rigorous translation and clinical research to establish analytical and clinical validity, evaluate diagnostic and therapeutic approaches, optimise treatment strategies, and demonstrate clinical utility in relevant patient populations and healthcare setting[48].

As discussed in the previous chapters, IICTs complements commercially sponsored drug development by addressing clinically important questions that may arise throughout the therapeutic development and implementation pathway. Whereas commercial clinical development necessarily includes studies required to establish the efficacy, safety, and quality of medicinal products for regulatory approval, NCCTs can address complementary questions concerning how diagnostic and therapeutic innovations should be selected, adapted, and implemented in clinical practice. These questions include identifying patients most likely to benefit from treatment, validating predictive and prognostic biomarkers, optimising treatment delivery, refining treatment sequencing, investigating determinants of treatment delivery, improving manufacturing processes, and integrating advanced diagnostics into clinical decision-making[49].

Consequently, NCCTs can play a unique and important role in the translational pathway by generating evidence that connects biomedical discoveries and technological innovations with their evaluation and implementation in patient care. In precision medicine, this may include the prospective validation of biomarkers, evaluation of biomarker-guided therapeutic strategies, optimisation of treatment delivery, and investigation of patient-specific factors influencing the feasibility or effectiveness of complex therapies.

The studies included in this habilitation illustrate different stages and dimensions of this translational continuum—from biomarker development and clinical validation, through personalised treatment selection and optimisation of treatment delivery, to the development and manufacture of personalised Advanced Therapy Medicinal Products (ATMPs). Together, they demonstrate that precision medicine extends beyond molecularly targeted therapies to encompass biomarker-guided treatment selection, functional and prognostic assessment, precision in treatment delivery, and the integration of patient-specific biological characteristics into the development and manufacturing of complex medicinal products.

4.2 Biomarkers for personalised treatment selection

A fundamental objective of precision medicine is to identify patients who are most likely to benefit from a particular therapeutic intervention, while minimising ineffective or unnecessary treatment in those unlikely to benefit. Biomarkers therefore have the potential to support therapeutic decision-making by informing treatment selection, stratification, or prediction of treatment response.

Importantly, biomarkers used in precision medicine are highly heterogeneous. Depending on the clinical question, they may include molecular characteristics, imaging findings, electrophysiological signals, pharmacological measurements, or functional characteristics of patient-derived biological material. Clinical research plays an essential role in translating such candidate biomarkers from biological or technological observations into clinically validated tools by establishing their predictive or prognostic performance and evaluating their potential clinical utility. NCCTs can contribute particularly where biomarker validation is embedded within clinically driven questions of treatment selection or optimisation.

This concept is illustrated by the **Annex 9**, the *GlioMET study*, which translates advances in tumour metabolism into a clinical investigation evaluating metformin as a metabolism-targeting therapeutic strategy for glioblastoma. By integrating molecular tumour characteristics, advanced metabolic imaging, and clinical outcomes, the study provides a framework for investigating whether biological and metabolic tumour characteristics are associated with treatment response and may contribute to future patient stratification. Rather than evaluating a novel medicinal product, *GlioMET* investigates the therapeutic potential of an existing medicine in relation to the biological characteristics of the tumour, linking drug repurposing with a precision-medicine approach.

A complementary strategy is represented by the **Annex 10,** the *PRECISE study*, addressing personalised treatment selection in patients with drug-resistant epilepsy undergoing vagus nerve stimulation. Since only a proportion of patients experience meaningful clinical benefit, *PRECISE* is designed to prospectively validate an electroencephalography-based predictive model for identifying patients more likely to respond to VNS before implantation. Unlike genomic biomarkers, this approach relies on electrophysiological characteristics derived from EEG, demonstrating that precision medicine is not restricted to molecular profiling but may also incorporate functional biomarkers generated using clinically accessible technologies.

Together, these studies illustrate complementary routes towards more personalised therapeutic decision-making. *GlioMET* investigates the relationship between tumour biology, metabolic characteristics, and therapeutic response, whereas *PRECISE* prospectively evaluates a functional biomarker for prediction of response to an established therapeutic intervention. Both illustrate how clinically oriented research can move beyond average treatment effects towards understanding interindividual variability in treatment response and generating evidence that may ultimately support more targeted use of healthcare interventions.

4.3 Optimisation of therapeutic delivery and clinical management

Precision medicine extends beyond treatment selection and patient stratification. It also encompasses the optimisation of how therapeutic interventions are delivered, with the aim of reducing avoidable variability in treatment exposure and ensuring that intended therapeutic strategies are implemented as accurately and consistently as possible in clinical practice.

This concept is illustrated by the **Annex 21**, *Quantitative Analysis of Drug Losses Administered via Nasogastric Tube*, which quantified drug losses associated with the administration of medicines through nasogastric tubes and demonstrated that the administration process itself may influence the amount of drug ultimately delivered to critically ill patients. Using quantitative analytical methods, the study identified sources of variability arising during drug preparation and administration and provided evidence relevant to optimising medication delivery in patients requiring enteral administration. Although this approach does not involve molecular or predictive biomarkers, it represents another dimension of precision medicine: reducing treatment variability by ensuring more accurate and reproducible delivery of the intended therapy.

This study therefore extends the concept of precision medicine from identifying the appropriate treatment for an individual patient to optimising the delivery of that treatment in clinical practice. It illustrates how translational research can address practical sources of therapeutic variability and generate evidence supporting more precise and context-appropriate use of established medicines.

4.4 Personalised Advanced Therapy Medicinal Products

The complexity of precision medicine is particularly evident in the field of ATMPs, especially in autologous cell-based therapies, where the characteristics of the medicinal product may be intrinsically linked to patient-derived starting material and individual manufacturing processes. Unlike conventional medicinal products, the development of such therapies may require coordinated optimisation of patient selection, biological material quality, manufacturing procedures, quality control, release criteria, and clinical administration. For personalised cell-based therapies, clinical and manufacturing considerations are therefore closely interconnected throughout the translational pathway.

This concept is illustrated by the **Annex 11**, *Dendritic Cell-Based Immunotherapy in Advanced Sarcoma and Neuroblastoma Paediatric Patients*, which investigated patient- and treatment-related determinants affecting the generation and functional characteristics of autologous dendritic-cell vaccines. The study demonstrated that prior anticancer treatment was associated with characteristics of the monocyte starting material and subsequent dendritic-cell differentiation and function, including antigen-presenting capacity and cytokine production. These findings indicate that variability in the resulting cell-based medicinal

product may arise not only from the manufacturing process itself but also from the biological characteristics and treatment history of the individual patient from whom the starting material is obtained.

Methodologically, this study represents a highly integrated translational investigations included in this habilitation. It combines clinical oncology, immunology, laboratory medicine, GMP manufacturing, quality control, and assessment of functional cellular characteristics within an IICTs setting. Rather than considering the therapeutic intervention solely in terms of clinical outcomes, it incorporates manufacturing quality attributes, functional immunological parameters, and biological characteristics of the starting material as interconnected determinants relevant to the development of an autologous cell-based therapy.

This perspective extends precision medicine beyond the selection or delivery of a therapeutic intervention. In autologous cell-based therapies, patient-specific biological characteristics may influence not only potential treatment response but also the properties and potentially the manufacturability of the medicinal product itself. Investigator-initiated translational research can therefore contribute to understanding and optimising the continuum from patient selection and collection of biological material through GMP manufacturing and quality control to clinical administration. This example also illustrates the contribution that academic and IICTs can make to ATMP development by investigating questions at the interface between patient biology, manufacturing, and clinical care. Understanding how characteristics of the starting material, previous treatment exposure, manufacturing processes, and clinical timing interact is particularly important for the development of personalised cell-based therapies and may inform strategies to improve manufacturing consistency and their subsequent clinical evaluation.

4.5 From biomarker innovation to precision clinical care

Despite the rapid expansion of biomedical research, translation of promising biomarkers, technologies, and therapeutic concepts into clinically useful interventions remains a complex multistep process. Successful translation requires analytical validation, clinical validation, demonstration of clinical utility, integration into healthcare pathways, and prospective evaluation within appropriately designed clinical studies. NCCTs plays a pivotal role in this process by addressing clinically relevant questions that frequently extend beyond the objectives of commercial product development.

The studies presented in this chapter illustrate different stages and dimensions of this translational continuum. **Annex 9**, *GlioMET* translates advances in tumour biology into a clinical investigation linking biological and metabolic tumour characteristics with therapeutic response and potential future patient stratification. **Annex 10**, *PRECISE*, is designed to prospectively validate an electrophysiological predictor of treatment response. **Annex 21** extends the concept of precision from treatment selection to treatment delivery by

identifying and quantifying sources of variability during nasogastric drug administration. Finally, **Annex 11** extends personalisation to the medicinal product itself, demonstrating how patient- and treatment-related characteristics of biological starting material may influence the generation and functional properties of an autologous cell-based therapy.

Collectively, these studies demonstrate that precision medicine is not defined solely by molecular diagnostics or targeted therapies. Rather, it encompasses multiple interconnected dimensions of clinical translation—from understanding interindividual variability and identifying predictors of treatment response, through precise delivery of therapeutic interventions, to consideration of patient-specific biological factors in the manufacture of personalised medicinal products. In this context, IICTs can complement other forms of biomedical and clinical research by generating evidence on for whom an intervention may be most appropriate, how it can be delivered more precisely, and how patient-specific characteristics may affect the development of complex personalised therapies.

Ultimately, these examples illustrate one of the central themes of this habilitation:

IICTs provides the essential bridge between biomedical innovation and routine clinical practice.

By evaluating candidate biomarkers and predictors of treatment response, refining therapeutic strategies, optimising treatment delivery, and investigating patient-specific determinants relevant to personalised ATMPs, NCCTs and related IICTs contribute to the translation of scientific and technological advances into clinically actionable evidence that improves patient outcomes and supports the implementation of precision medicine within modern healthcare systems.

5 From Evidence to Public Health

5.1 Evidence-Informed Healthcare

The generation of high-quality scientific evidence represents only one stage of the clinical research continuum. The ultimate objective of IICTs is to generate clinically relevant evidence capable of improving healthcare by informing clinical decision-making, supporting evidence-based guidelines, optimising therapeutic strategies, and addressing unmet medical needs that may receive limited attention within commercially driven research programmes[11]. While commercially sponsored clinical development necessarily generates evidence required for regulatory approval and subsequent development of medicinal products, NCCTs can address complementary questions arising throughout the therapeutic lifecycle, including those related to comparative effectiveness, optimisation of established interventions, and areas where commercial incentives are limited[12]. Evidence generated by NCCTs can therefore contribute to evidence-informed healthcare by informing the refinement of standards of care, comparing alternative therapeutic strategies, evaluating treatment pathways, and optimising the use of established interventions in routine clinical conditions.

However, evidence-informed healthcare depends not only on the quality of individual clinical trials but also on the completeness and transparency of the available evidence[41]. Clinical decisions, systematic reviews, meta-analyses, and clinical practice guidelines rely substantially on the accessible evidence base. When completed clinical trials remain unpublished, particularly those with negative, neutral, or inconclusive findings, the available evidence may become systematically biased, leading to publication bias and potentially distorted estimates of intervention effects and safety[50].

This challenge is illustrated by the **Annex 12**, which demonstrated that a substantial proportion of paediatric randomized clinical trials remain unpublished or are discontinued before completion. While completed studies are considerably more likely to result in publication, unpublished studies and trials discontinued before completion contribute to gaps in the available evidence base. Their absence may limit the completeness of systematic reviews and evidence synthesis and may consequently affect clinical decision-making when conclusions are based on incomplete evidence. The study therefore highlights that transparency and dissemination of research results—including negative, neutral, and inconclusive findings—are important components of responsible clinical research and evidence-informed healthcare and represent an ethical responsibility towards research participants and future patients.

The studies presented in this habilitation illustrate several complementary pathways through which IICTs can contribute to evidence-informed healthcare. Comparative effectiveness studies generate evidence comparing therapeutic strategies, optimisation studies address the safer and more effective use of established medicines, while translational investigations support the development and evaluation of more personalised approaches to

treatment selection, delivery, and advanced therapies. Together, these studies demonstrate that the societal value of academic clinical research lies not only in generating new knowledge but also in contributing to an evidence base that is clinically relevant, transparent, and sufficiently complete to support healthcare decision-making. The availability of results irrespective of the direction or statistical significance of findings is fundamental to reliable evidence synthesis and, subsequently, to the development of clinical guidelines and informed healthcare decisions.

Importantly, evidence alone rarely changes clinical practice. Its translation into healthcare depends on subsequent processes including incorporation into clinical guidelines, professional education, health technology assessment, reimbursement decisions, and organisational processes within healthcare systems. NCCTs therefore form part of a broader evidence-to-practise ecosystem in which research findings are evaluated, synthesised, interpreted, and ultimately translated into decisions at both the individual-patient and population levels.

5.2 Patient Engagement as a Driver of Research Translation

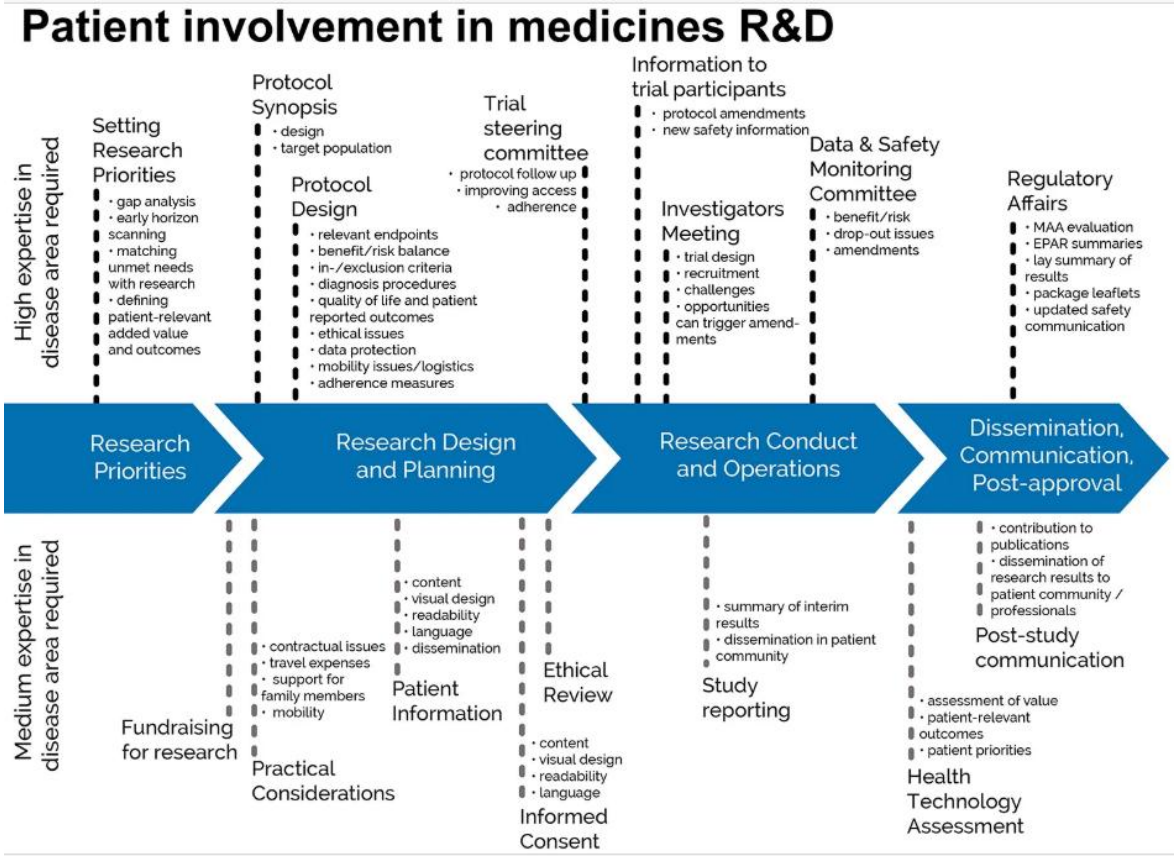
Scientific excellence alone is insufficient to ensure that research addresses the needs of patients or that its findings can be meaningfully translated into healthcare. Increasingly, patients are recognised not merely as research participants but as contributors and partners at different stages of the clinical research lifecycle. Meaningful patient involvement can enhance the relevance, feasibility, acceptability, and potential societal value of IICTs[51,52].

This evolution reflects a broader transformation in clinical research. As discussed in the **Annex 22**, *Patient and Public Involvement in Clinical Research: From Regulatory Requirements to Partnerships with Patients*, patient engagement has progressed from a predominantly consultative or voluntary activity towards a more systematic component of contemporary clinical research. This development is consistent with the increasing emphasis of contemporary ethical, regulatory, and methodological frameworks on participant-centred trials design, proportionality, and the incorporation of patient perspectives. The revised ICH Good Clinical Practice (ICH E6(R3)) and its Quality by Design principles emphasise the importance of designing trials around factors critical to participant protection and the reliability of trial results, while the latest revision of the Declaration of Helsinki further reinforces engagement with patients and communities in medical research, increasingly recognise meaningful Patient and Public Involvement (PPI) [21]. In parallel, dedicated PPI frameworks and major public research programmes increasingly encourage or require meaningful involvement of patients and the public in the development and conduct of research, reflecting a broader recognition that research benefits from being shaped together with those whom it is intended to serve[53].

Patient engagement may begin a clinical study is designed and continue throughout its lifecycle. Patients can contribute to identifying research priorities, defining clinically

meaningful research questions and endpoints, improving study protocols, enhancing the readability of participant information and informed consent documents, and identifying aspects of study design that may create unnecessary participant burden. Their perspectives can help researchers identify outcomes that matter to patients and improve the acceptability and feasibility of study procedures. Patient advocates and patient organisations can further facilitate dialogue between researchers and patient communities and support structured involvement throughout the research process. The broad spectrum of patient involvement across the medicines research and development lifecycle is illustrated in Figure 7, demonstrating that meaningful patient engagement may extend from the identification of unmet needs and study design through clinical development, regulatory decision-making, and dissemination of research outcomes[54].

Figure 7: Patient involvement in medicines Research and Development.



The experience gained through European collaborative initiatives further illustrates how research infrastructures can create mechanisms for broader patient and public engagement. Within the VACCELERATE programme, patient-centred communication strategies, multilingual educational resources, and the Volunteer Registry were developed to improve access to understandable information about vaccine research, facilitate engagement with

potential participants, and support recruitment into multinational vaccine studies. These initiatives illustrate that successful clinical research depends not only on methodological excellence but also on transparent communication, accessibility of information, trust, and meaningful dialogue with patients and the wider public[9].

Patient engagement can also continue beyond the conduct of a clinical trial. Patients may contribute to interpreting research findings, preparing lay summaries, supporting dissemination activities, and informing approaches to subsequent implementation[55]. In addition, patient experience measures and patient-reported outcomes provide complementary evidence that captures dimensions of health, treatment burden, and care that may not be reflected in conventional clinical endpoints, thereby supporting more patient-centred evaluation and clinical decision-making. By incorporating the patient perspective throughout the translational pathway, research can become more relevant and understandable to those whom i tis intended to benefit and potentially more responsive to the reality of clinical care[52].

Consequently, patient engagement should not be regarded merely as an additional activity alongside clinical research, but as an increasingly important component of a translational research approach linking scientific discovery with clinically relevant and patient-centred evidence. By informing research priorities and study design, strengthening communication, supporting recruitment and retention, and contributing to dissemination and implementation, meaningful patient involvement can enhance the relevance, feasibility, and societal value of NCCTs. The transition from conducting research **for** patients to conducting research **with** patients represents an important evolution in contemporary clinical research and supports the development of evidence-informed, patient-centred healthcare[51,56].

5.3 Implementation into Clinical Practice and Health Systems

The successful completion of a clinical trial does not automatically translate into changes in clinical practice or improvements in patient outcomes. The possible impact of NCCTs depends on only on the generation of robust evidence but also on its subsequent interpretation, synthesis, dissemination, and implementation within healthcare systems. This process is inherently complex and requires coordinated interaction among researchers, healthcare professionals, patients, professional societies, regulatory authorities, health technology assessment bodies, payers, and healthcare policymakers[57].

Implementation is a multidimensional process extending beyond publication in scientific journals. Evidence generated by IICTs informs clinical practice guidelines, therapeutic recommendations, diagnostic algorithms, reimbursement decisions, and organisational changes within healthcare systems. At the same time, implementation in routine practise can generate clinical experience and real-world data reveal residual uncertainties, identify new knowledge gaps, and generate questions for subsequent clinical research[58].

This continuous interaction underpins the concept of a learning healthcare system, in which evidence generation and healthcare delivery are connected through iterative cycles of

research, implementation, evaluation, and refinement. Within such a system, clinically relevant questions emerging from routine care can inform new research, while evidence generated through clinical studies can, after appropriate evaluation and implementation, contribute to the continuous improvement of healthcare delivery[59].

The different areas of research presented in this habilitation illustrate several points along this evidence-to-practice continuum. Precision-medicine approaches require the translation of biological, diagnostic, and therapeutic insights into clinically applicable strategies through collaboration among researchers, clinicians, diagnostic laboratories, manufacturers, regulatory authorities, and patients. Comparative effectiveness research and studies optimising established therapies address questions arising directly from clinical practice and generate evidence that may inform therapeutic choices, treatment pathways, and subsequent clinical recommendations. Drug repurposing provides another example of how clinically driven research can generate new evidence for the use of established medicinal products in different therapeutic contexts[60–62].

Future developments may further strengthen the integration of research and healthcare delivery. Increasing use of real-world data and evidence, disease registries, artificial intelligence, and digital health technologies may complement evidence generated in clinical trials and enable longitudinal evaluation of interventions in broader patient populations and routine healthcare settings. NCCTs can contribute to this evolving research environment by addressing clinically driven questions and integrating complementary data sources and methodological approaches where scientifically appropriate[63].

Ultimately, the research and methodological perspectives presented throughout this habilitation demonstrate that the value of IICTs extends beyond the generation of scientific publications. Its contribution lies in transforming clinically relevant uncertainties into researchable questions, generating robust and clinically meaningful evidence, and supporting the subsequent translation of that evidence into healthcare decision-making and practice. The potential of this research to contribute to better patient care and public health depends on an interconnected clinical research ecosystem combining methodological rigour, proportionate regulation, sustainable research infrastructures, multidisciplinary expertise, meaningful patient involvement, transparent dissemination, and effective implementation.

6 Conclusion

This habilitation thesis examined IICTs from a system perspective, extending beyond the traditional focus on individual studies, methodological approaches, or regulatory requirements. Across the different areas addressed, it shows that the value and translational potential of NCCTs depend not solely on scientific excellence, but on the interaction of rigorous methodology, proportionate regulation, sustainable research infrastructures, multidisciplinary expertise, meaningful patient involvement, transparent evidence generation and dissemination, and effective pathways for implementation into healthcare.

The publications included in this thesis illustrate the broad spectrum of contributions made by NCCTs. They demonstrate how NCCTs and related concrete IICTs can contribute to the optimisation of existing therapies, comparative evaluation of therapeutic strategies, development of validation of approaches support precision medicine, investigation of ATMPs, and generation of evidence addressing clinically relevant questions arising from routine healthcare. Beyond individual clinical studies, the work presented also addresses the conditions necessary for such research to be conducted effectively, including research capacity, regulatory support, collaborative infrastructures, and patient involvement. Together, these contributions illustrate the complementary role of IICTs within the wider clinical research landscape, particularly in addressing questions driven by clinical need and public-health relevance.

A central perspective emerging from this thesis is an integrated view of IICTs within the broader European clinical research ecosystem. Rather than viewing methodology, regulation, research infrastructures, professional competencies, evidence transparency, patient engagement, and implementation as independent components, the work presented demonstrates their interdependence across the research continuum – from the formulation of clinically relevant questions and generation of robust evidence to its interpretation, dissemination, and potential implementation in healthcare. This systems perspective shifts the focus from success of an individual clinical trial towards the conditions required for clinically relevant research to generate sustainable value beyond the study itself.

An important implication of this perspective is that the generation of scientifically robust evidence represents a necessary, but not sufficient, condition for research impact. Evidence must also be accessible and transparent, sufficiently relevant to patients and clinical practice, and capable of being interpreted and integrated within healthcare decision-making. Strengthening NCCTs therefore requires investment not only in individual studies, but in the ecosystem that enables them: methodological and regulatory expertise, multidisciplinary research teams, national and European research infrastructures, professional education and capacity building, meaningful patient involvement, and mechanisms supporting evidence translation and implementation. As clinical research continues to evolve through precision medicine, advanced therapies, real-world data and evidence, artificial intelligence, and increasing integration between research and healthcare delivery, IICTs will continue to have an important

complementary role within the evolving research landscape. Its close connection to clinical practice enables clinically driven questions to be investigated across the therapeutic lifecycle, including questions of comparative effectiveness, optimisation of established interventions, treatment individualisation, and areas in which evidence gaps persist despite limited commercial incentives. At the same time, emerging methodological and technological approaches create opportunities to connect clinical trials more closely with data generated in routine care and to support iterative cycles of evidence generation, evaluation, and refinement.

Ultimately, this thesis supports the recognition of IICTs not as a collection of isolated research activities, but as an integral component of a continuously learning clinical research and healthcare ecosystem.

Its contribution begins with clinically relevant uncertainty: transforming unanswered questions from clinical practice into methodologically rigorous research, generating transparent and clinically meaningful evidence, and creating the conditions for that evidence to inform subsequent healthcare decisions. Realising this potential requires the interaction of investigators, patients, healthcare professionals, research infrastructures, regulators, funders, and healthcare systems across national and European levels.

Within such an ecosystem, the value of IICTs extends beyond the individual trial. It lies in the capacity to connect clinical need with evidence generation and evidence with practice, while continuously returning new uncertainties from healthcare to research. Strengthening this cycle is essential for a sustainable, responsive, and patient-centred clinical research environment capable of addressing questions that matter to patients, healthcare professionals, and society.

7 Annexes - Collection of author´s published scholarly works with commentary

7.1 Annex 1

Madeira C, Hořavová L, Dos Santos F, Batuca JR, Nebeska K, Součková L, Kubiak C, Demotes J, Demlová R, Monteiro EC. Investigator Initiated Clinical Trials (IICTs): A Systematic Search in Registries to Compare the Czech Republic and Portugal in Terms of Funding Policies and Scientific Outcomes. Ther Innov Regul Sci. 2021 Sep;55(5):966-978. doi: 10.1007/s43441-021-00293-w. Epub 2021 May 18. PMID: 34003473; PMCID: PMC8332583.

Summary:
Purpose:
The aim of this study was to analyse the landscape of investigator-initiated clinical trials (IICTs) in two European countries—Czech Republic and Portugal—and to evaluate how differences in funding policies influence clinical research output. The study specifically focused on identifying structural determinants affecting the quantity and quality of academic clinical trials.

Methodology:
A retrospective systematic search of registered IICTs conducted between 2004 and 2017 was performed using four major clinical trial registries. Each identified trial was analysed in terms of sponsor type, funding source, intervention characteristics, and subsequent publication status after completion.

Results:
The study revealed substantial differences between the two countries in the number of IICTs conducted, funding structures, and publication outputs. These disparities were linked to national funding models and institutional support, with more structured funding systems associated with higher research productivity and better dissemination of results.

Conclusion:
The findings demonstrate that the success and impact of IICTs are strongly influenced by systemic and policy-level factors rather than scientific capability alone. Strengthening national and European funding frameworks and improving support mechanisms for academic trials are essential to enhance the effectiveness and output of NCCTs.

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DIA

ORIGINAL RESEARCH

Investigator Initiated Clinical Trials (IICTs): A Systematic Search in Registries to Compare the Czech Republic and Portugal in Terms of Funding Policies and Scientific Outcomes

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Abstract

Objectives Clinical trials provide one of the highest levels of evidence to support medical practice. Investigator initiated clinical trials (IICTs) answer relevant questions in clinical practice that may not be addressed by industry. For the first time, two European Countries are compared in terms of IICTs, respective funders and publications, envisaging to inspire others to use similar indicators to assess clinical research outcomes.

Methods A retrospective systematic search of registered IICTs from 2004 to 2017, using four clinical trials registries was carried out in two European countries with similar population, *GDP, HDI* and medical schools but with different governmental models to fund clinical research. Each IICT was screened for sponsors, funders, type of intervention and associated publications, once completed.

Results IICTs involving the Czech Republic and Portugal were $n=439$ (42% with hospitals as sponsors) and $n=328$ (47% with universities as sponsors), respectively. The Czech Republic and Portuguese funding agencies supported respectively 61 and 27 IICTs. Among these, trials with medicinal products represent 52% in Czech Republic and 4% in Portugal. In the first, a higher percentage of IICTs' publications in high impact factor journals with national investigators as authors was observed, when compared to Portugal (75% vs 15%).

Conclusion The better performance in clinical research by Czech Republic might be related to the existence of specific and periodic funding for clinical research, although further data are still needed to confirm this relationship. In upcoming years, the indicators used herein might be useful to tracking clinical research outcomes in these and other European countries.

Keywords Investigator initiated clinical trials · Funding · Clinical trials registry · Clinical research · Clinical trial · Clinical research outcome

C. Madeira and L. Hořavová have contributed equally to this work.

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Abbreviations

AICIB	*Agência para a Investigação Clínica e Investigação Biomédica*
AIFA	Italian Medicines Agency
ANZCTR	Australian New Zealand Clinical Trials Registry
AZV	Czech health research council
CAC	Clinical Academic Centers
CLIC	Clinical Investigator Certificate
CTR	Clinical Trial Registry
CzeCRIN	Czech Clinical Research Infrastructure Network
ECRIN-ERIC	European Clinical Research Infrastructure Network-European Research Infrastructure Consortium
EU-CTR	European Clinical Trials Registry
FCT	*Fundação para a Ciência e Tecnologia*
GDP	Gross Domestic Product
IICTs	Investigator Initiated Clinical Trials
IMP	Investigational Medicinal Product
ISRCTN	International Standard Randomised Controlled Trial Number
NIH	U.S. National Institutes of Health
PtCRIN	Portuguese Clinical Research Infrastructure Network
PTCSRT	Portugal Clinical Scholars Research Training

Introduction

In the last decades, clinical research played a crucial role on increasing the medical knowledge for the prevention, diagnostic, treatment and cure of diseases. Clinical trials provide one of the highest levels of evidence to support medical practice and might be initiated by the industry or by clinical investigators. The latter, commonly called investigator-initiated clinical trials (IICTs), are interventional studies using medicinal products, medical devices, surgery techniques, behavior testing or several types of procedures. These trials are pivotal to generate relevant and unbiased data decisive for the implementation of new therapies and better-informed decisions by health regulators [1]. However, the importance and relevance of IICTs rely heavily on its independence and impartiality, which in turn is directly linked to the funding source. Some IICTs are funded by industry, which can introduce a bias in the outcome of these studies. In fact, a systematic review of 1140 studies demonstrated that industry-sponsored clinical trials were significantly more likely to reach conclusions favorable to the sponsors than those who were not industry-sponsored trials [2].

Therefore, public national funding agencies have a critical role on supporting fully independent clinical research with the sole purpose of benefiting the large patients´ population through the improvement of medical knowledge. Public funding of IICTs can also play an important role on addressing important clinical questions that remain unresolved because they are not addressed in industry-funded trials. Crowe and colleagues showed that there is an important mismatch on clinical research priorities identified by the patients and the clinicians, which in part is affected by the commercial aspect of clinical research [3].

The unbalanced ratio between private and public funding of IICTs (and its impact on national health systems through the definition of public policies for the treatment of diseases) is particularly relevant in countries with negligible expenditure in this area, which is usually associated to limited funds for the promotion of clinical research. It is important to generate significant scientific data to be able to provide better and informed guidance for the funding of IICTs by the national public funding agencies.

The Czech Republic and Portugal have similar population numbers (10.6 million and 10.3 million, respectively), GDP per capita (€14.8 thousand and €16.9 thousand, respectively), Human Development Index (2017: 0.896 and 0.858, respectively) and number of Medical Schools ($n=9$ and $n=8$, respectively), which makes them comparable countries for such study. In a previous publication, and considering only clinical trial registries in the European Clinical Trials Registry (EU-CTR) it was shown that Czech Republic and Portugal are both below the average among all European countries in what regards the percentage of IICT/Total CT (5% and 8%, respectively) [4].

Both countries are members of the European Union and thus have access to the same funding schemes and also operating under to the same health regulatory environment (supervised by the European Medicines Agency). Additionally, both countries are members of the European Clinical Research Infrastructure Network (ECRIN-ERIC), which is a public, non-profit organisation committed in supporting and facilitating multinational independent clinical trials in Europe.

Currently, the Czech Clinical Research Infrastructure Network (CZECRIN) aims its activities to bring together academic research institutes, scientists and policymakers in discussion on issues related, particularly public funding of the clinical research in the Czech Republic. CZECRIN's specific goal is to promote and support academic research on the national basis and the integration of national investigators in multinational clinical studies.

The Portuguese Clinical Research Infrastructure Network (PtCRIN) has been developing several national studies where the status of IICTs in Portugal were assessed [4, 5]. Despite the relevance of such data to define national strategies for this area, the impact of such analysis can be maximized through a comprehensive benchmarking with other

European countries, particularly the ones with similar characteristics. Therefore, intending to perform a comprehensive assessment of the current situation, this study analyzed the type of registered IICTs in the Czech Republic and in Portugal and for the first time unveiled the differences in terms of funding policies and scientific outcome.

Methods

We identified past or current IICTs registered and starting in two European countries (Czech Republic and Portugal) from 01/01/2004 to 31/12/2017 to identify detailed studies´ characteristics such as the funding sources and the respective scientific impact. This period of time starts in the year after which the International Committee of Medical Journal Editors' implementation (ICMJE), policy required the registration of clinical trials as a prerequisite for consideration for publication (2004) until the end of 2017. A separate search in four clinical trial registries (CTRs) was performed for each country: ClinicalTrials.gov, European Clinical Trials Registry (EU-CTR), International Standard Randomised Controlled Trial Number (ISRCTN) registry, and the Australian New Zealand Clinical Trials Registry (ANZCTR) by selecting trials registered and with recruitment sites in Czech Republic or Portugal. These CTRs encompass 81% of the registrations uploaded to the International Clinical Trials Registry Platform (ICTRP) from WHO, according to the information provided by others [6]. Furthermore, we collected and compared the national public policies implemented by the Portuguese and Czech governments to promote clinical research during this timeframe.

Search Methodology for Clinical Trials Identification in Each Database

The inclusion criteria were: clinical trial starting from 01/01/2004 until 31/12/2017; non-commercial sponsor; sponsor or recruiting sites in Portugal or in Czech Republic. The following exclusion criteria were used: industry sponsored trials; starting date before 01/01/2004; sponsor or recruitment site not involving the Czech Republic or Portugal; non-intervention trials.

Step 1: The search in the EU-CTR allowed to include as general term "non-commercial" therefore eliminating the commercial studies. The timeframe selected was from 01/01/2004 until 31/12/2017 as well as the countries, the Czech Republic or Portugal. No other requirement in the advanced search field was selected.

Step 2: As the discrimination between commercial and non-commercial clinical trial is not possible at the ClinicalTrials.gov platform, an advanced search was performed. The IICTs were extracted by selecting the predefined options: "Interventional studies", the above-mentioned time period, each one of the countries, restricting to: NIH (U.S. National Institutes of Health), Other U.S. Federal agency, all others (individuals, universities, organizations) and Industry were chosen.

Step 3: Registrations in the BiomedCentral CTR are associated with ISRCTN. In this CTR it was not possible to refine the search with the exception of selecting the Czech Republic or Portugal as the recruiting country. Trials initiated before or after the referred timeframe were discarded.

Step 4: In the ANZCTR, we performed the search in the selected timeframe, using the Czech Republic or Portugal as the recruiting country. Registrations in this CTR have the initial code ACTRN.

The search results were reviewed individually by two independent experts in each national team to confirm the compliance with the inclusion and exclusion criteria.

Data Extraction, Duplicates and Complementary Information

Step 5: Information about trial identification (Trial ID) number (main and secondary), recruitment status, sponsor name and country, trial title, trial phase (when applicable), type of intervention, therapeutic area, type of funding and publications of the completed studies, were extracted manually and independently from the registered records (from 1/03/2018 to 31/08/2018), gathered and organized in an Excel sheet for each country. No restrictions on each of the referred information was applied. Forty percent of the records were double-checked by two individuals from each team and discussed until a consensus was reached.

Step 6: Duplicates were identified in each database, when secondary IDs were provided or when the sponsor and the study title were the same. The same study registered in different CTRs was considered duplicated and removed at this point of the search. When complementary information was provided in different registrations about the same study it was added to the respective entry in the working Excel sheet. None of the studies identified had recruitment sites in both countries, so no clinical trial is considered in both databases.

Sponsors were coded as: Disease-Specific Organization (Disease associations or research institutes dedicated to a specific therapeutic area), Foundation (Research Institutes or Health Institutions legally constituted as foundations), Hospital, Research Institutes (non-specific therapeutic area), University, and others (Private health clinic, Funder Agency, acting as sponsor). In the four CTRs, the funding source was identified in different fields. Both in ISRCTN and ANZCTR there is a specific field entitled "Funder". In Clinicaltrials.gov CTR the funders were identified from the field "Sponsor and Collaborators". In EU-CTR the funder was identified in the field "sources of monetary

support ". All the funders identified in these CTRs were classified as: (i) Public organizations (non-profit organization such as public institutions, funding agencies, disease specific organizations); (ii) Private organization (for-profit organization—Industry); (iii) Both, when the funding is provided by the industry and one or more public organizations; (iv) Not Indicated, when the information was not provided in any registry or in the publication.

When only the sponsor was mentioned in the ClinicalTrials.gov registry, we considered it as the funder. The support of funding agencies was perceived through secondary IDs where the code of the grant agreement is in some cases added. Exclusive funding for PhD or Post-Doctoral grants was not considered.

Search of Publications of Completed IICTs

Step 7: Information regarding the publications of the registered and completed IICTs were manually and independently screened. The Trial ID was used to search abstracts of journals indexed in Medline (using PubMed) as well as in the four CTRs. By the principal investigator´s name was possible to identify publications, when no paper was found with the previous strategies. In some publications, the Trial ID was not included in the abstract, which rendered the search more difficult. All completed trials and published until 31/12/2017 were considered for further analysis. Subsequently, screening of each publication was performed to complete the information about the funding sources of IICTs to identify any other relevant information that was not complete in CTRs. The journal's impact factor was obtained from Web of Science, Research Gate or Bioxbio.com, considering this order when different impact factors for the year of publication were found for the same journal. The citation number since the year of publication up to 1 year after the paper was published was assessed through Pubmed metrics. Around 40% of the data were mutually exchanged between Czech and Portuguese team and double checked by two independent reviewers.

Results

A total of 3496 and 1427 registrations were found in the four CTRs involving the Czech Republic and Portuguese institutions, respectively: ClinicalTrials.gov, EU-CTR, ISRCTN, and ANZCTR. Clinical trials were screened to isolate those with a non-commercial sponsor, recruiting sites in Czech Republic or Portugal, and a start date between 01/01/2004 and 31/12/2017.

Identification of Non-commercial Studies

The total number of trials for Czech Republic and Portugal, identified in all databases is respectively 3496 and 1427 as shown in Fig. 1. After discarding industry sponsored trials, non-interventional studies, or those with no recruiting sites in the Czech Republic or Portugal, 485 and 378 non-commercial trials (i.e. IICTs) were respectively considered eligible from all the screened databases. The same trial registered in different CTRs was separated and considered as duplicate. After discarding duplicates, a total number of 439 IICTs were identified involving the Czech Republic whereas for Portugal 328 trials were found. Forty-one percent (n = 180/439) and 44% (n = 144/328) of the studies were already completed and from those 41% (n = 73/180) and 55% (n = 79/144) were published, in the Czech Republic and Portugal, respectively.

Characteristics of IICTs in the Czech Republic and in Portugal

In both countries, most of IICTs has a national sponsor and were performed in the respective country (Fig. 2a). These IICTs, with national sponsors are those initiated by investigators from each of these countries. Only a low percentage of IICTs with national sponsor are also multinational trials (implemented in other countries)—6% in the Czech Republic (n = 15/257) and 4% in Portugal (n = 8/193).

Regarding the international trials which are those initiated by investigators from other countries and conducted in The Czech Republic or in Portugal, both countries have also the same percentage (41%; n = 182/439 and n = 135/328) (Fig. 2a).

In the Czech Republic, most trials´ sponsors (42%; n = 183/439) were hospitals while in Portugal, universities assume the sponsorship in higher number of trials when compared to the other types of organizations (47%; n = 153/328) (Fig. 2b).

Figure 2c shows that trials with hospitals as the sponsor were testing investigational medicinal products (IMPs) (46% (n = 84/183), and 45% (n = 32/71), respectively in the Czech Republic and Portugal), medical devices (16% (n = 30/183), and 20% (n = 14/71), respectively in Czech Republic and Portugal) and behavior (2% (n = 4/183), and 13% (n = 9/71), respectively in the Czech Republic and Portugal). Regarding all trials sponsored by universities, 62% (n = 94/150) test IMPs in the Czech Republic, whereas in Portugal, the largest group by intervention type, 49% (n = 75/153), focuses on behavior (Fig. 2b, c). The percentage of trials with IMPs in phase III trials is quite similar in both countries: 32%, (n = 82/258) in the Czech Republic and 39% (n = 54/136) in Portugal (Supplementary Information 1 (S1), Fig. 1a, a1). Additionally, the two most focused therapeutic areas of

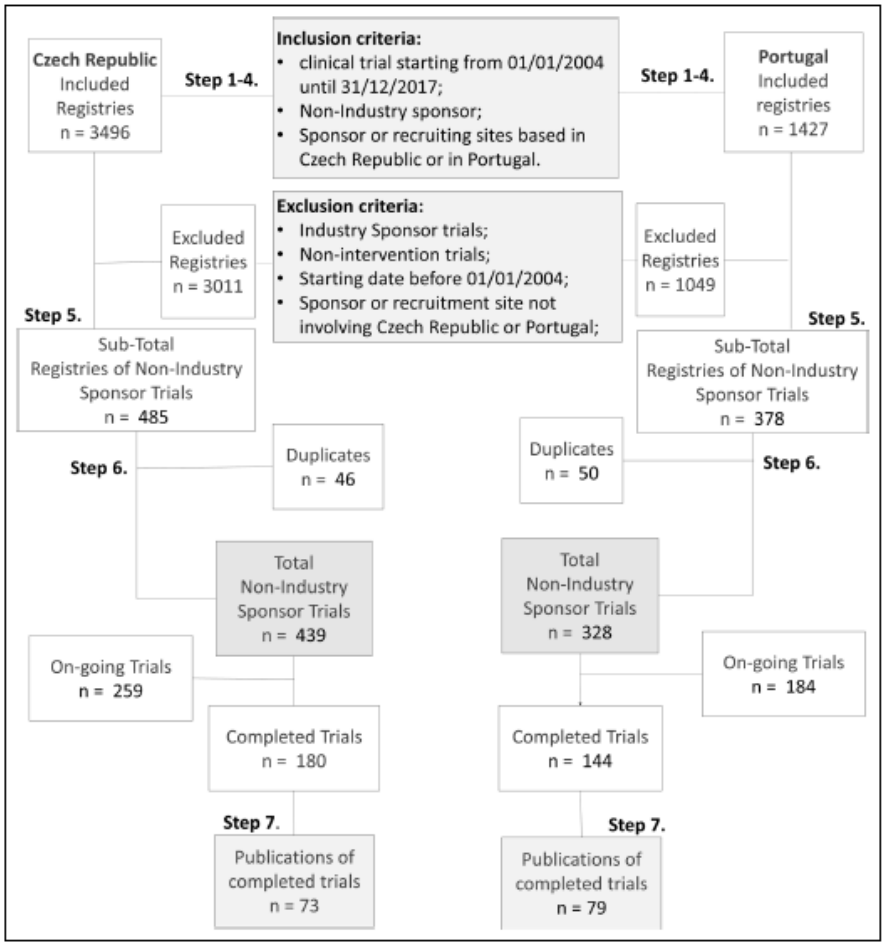


Figure 1 Flowchart Showing the Systematic Search of Non-commercial Clinical Trials (i.e. IICTs), Involving the Czech Republic and Portuguese Institutions Recruiting Participants. The search was performed in four clinical trial registries, CTRs (EU-CTR, Clinicaltrials.gov, ISRCTN and ANZCTR). Studies starting from 01/01/2004 until 31/12/2017 were identified in each of the databases separately (Steps 1–4). After discarding commercial trials all remaining studies were gathered in one Excel sheet for each country (Step 5). Duplicate studies were also discarded, and the final number of trials was cleaned and harmonized (Step 6). Further details were collected from all the databases, including the identification of completed studies. Publications published until December 2017, with results from completed studies were identified (Step 7).

the interventional studies are the same: oncology and cardiovascular diseases, followed by gastroenterology in the Czech Republic and musculoskeletal disorders in Portugal (S1, Fig. 1b, c).

IICTs in both countries are mainly funded by public organizations (Fig. 2d) such as universities, hospitals, disease specific organizations, foundations and funding agencies: 78% ($n = 343/439$) in the Czech Republic and 69% ($n = 226/328$) in Portugal. Considering all types of interventions funded by public organizations, 50% ($n = 173/343$) are trials with IMPs in the Czech Republic

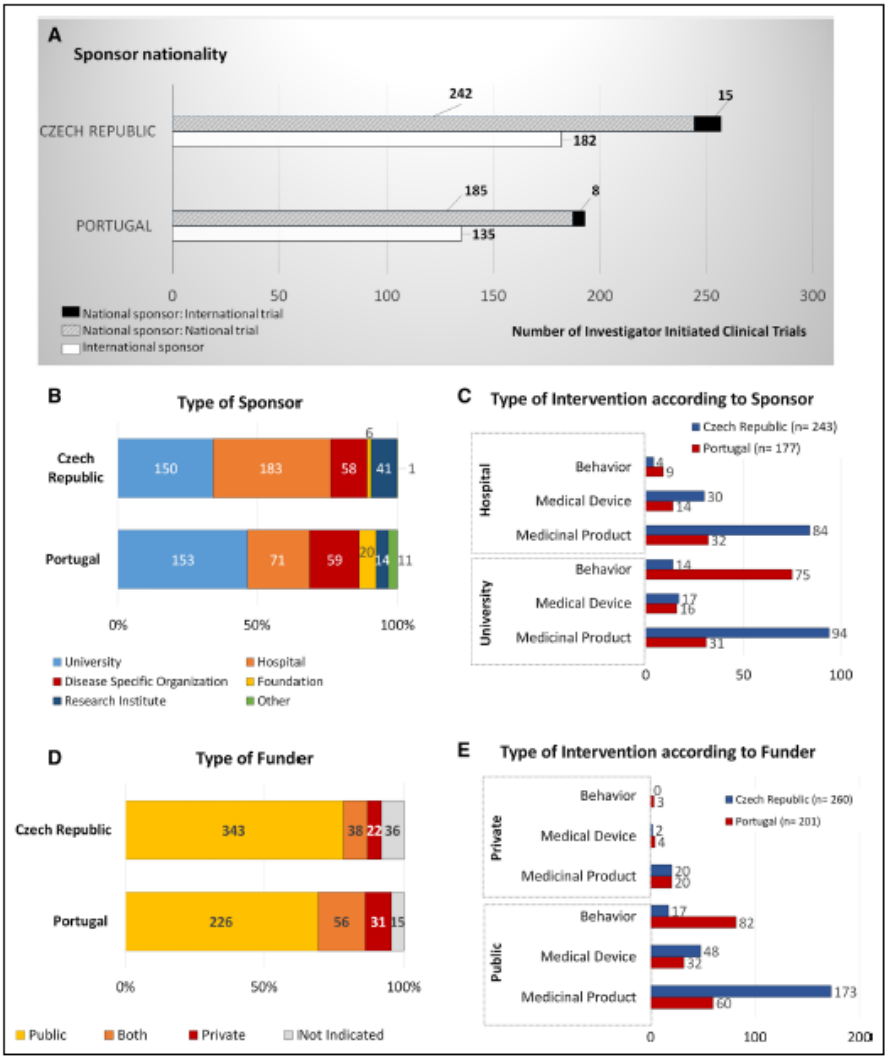


Figure 2 Characteristics of IICTs in the Czech Republic and in Portugal. Number of trials according to the sponsor (national vs international) and to the involvement of other countries in national sponsored trials (national vs multinational trial) (**a**); Percentage and number of trials according to the type of sponsor organization (**b**) and number of trials according to the type of most frequent interventions sponsored by universities or hospitals ($n=243$ out of 333 for the Czech Republic and $n=177$ out of 224 for Portugal) (**c**); Percentage and number of trials according to the type of funder (**d**) and number of trials according to the type of most frequent intervention funded by public or private organizations ($n=260$ out of 365 for the Czech Republic and $n=201$ out of 257 for Portugal) (**e**).

and only 27% ($n=60/226$) in Portugal. In the latter, most trials funded by public organizations, 36% ($n=82/226$), are behavior-based (Fig. 2d, e). Details about each registry are provided as Supplementary Information 2 (S2).

Clinical Trials Funded by National and International Funding Agencies

One of the greatest public funders of the IICTs in both countries were funding agencies (national and international) corresponding to 29% (n = 109/381) and 21% (n = 60/282) of the public funding in the Czech Republic and in Portugal, respectively. The total number of trials indicated above (381 and 282) refers to those funded by public organizations alone or together with private institutions (Fig. 2d: Public and Both). From the 109 IICTS trials funded by governmental agencies in the Czech Republic 65% (n = 71/109) of the trials were funded by national funding grant agencies while the remaining 35% (n = 38/109) IICTs were funded by international counterparts (Fig. 3a). Figure 3a shows that in this country both national and international agencies mainly fund trials with IMPs. Among the 61 trials funded by the Czech Health Research Council (Table 1), 32 (52%, n = 32/61) are testing medicinal products (data not shown).

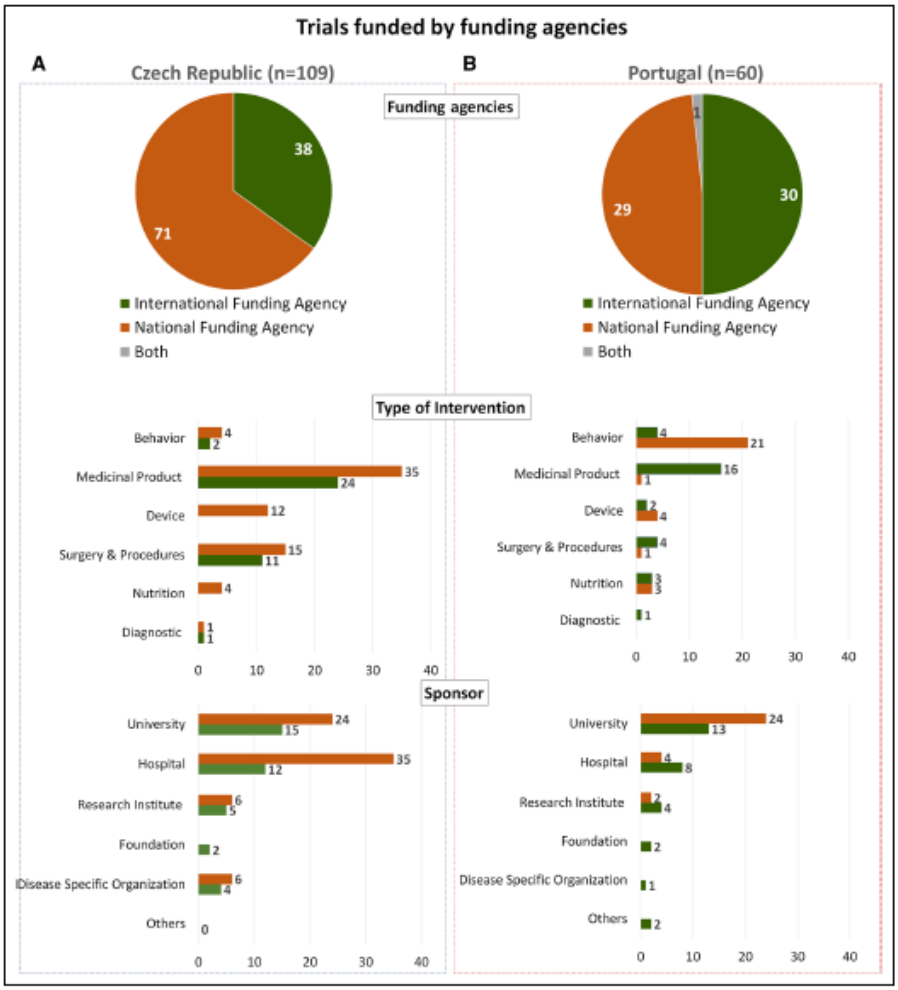


Figure 3 Characteristics of IICTs Funded by Funding Agencies. Number of trials funded with national or international funding agencies in the Czech Republic (**a**) and in Portugal (**b**), according to the type of intervention and sponsor.

Table 1 National and International Funding Agencies and Programs in Czech Republic in and Portugal and Respective Number of Funded Trials. (N°= Number of Clinical Trials Funded by the Respective Funding Organization/Program).

Funders		Czech Republic	Portugal
National Funding Agencies/Funding Programs	Internal Grant Agency of the Czech Ministry of Health (IGA until 2014); Currently AZV (Czech Health Research Council)	61	–
	Charles University Research Fund	10	–
	Fundação para a Ciência e Tecnologia (FCT)	–	27
	National Structural funding (COMPETE, PRODEP)	–	2
	ARDITI – *Agência Regional para o Desenvolvimento da Investigação*	–	1
International Funding Agencies/ Funding Programs	European Commission (includes H2020, IMI, Erasmus Program)	18	17
	Canadian Institutes of Health Research (Canada)	7	0
	European Regional Development Fund	6	0
	Medical Research Council (UK)	7	2
	Australian National Health Service and Medical Research Council	0	5
	National Institutes of Health (NIH, USA)	0	3
	EEA Grants	0	1
	Carlos III Institute of Health (Spain)	0	2

On the other hand, among the 60 IICTs in Portugal receiving financial support from funding grant agencies, only half of these (50%, $n = 30/60$) were funded by national funding grant agencies or funding programs (Fig. 3b) while the other half was funded by international funding agencies. Among the 27 trials funded by *Fundação para a Ciência e Tecnologia* (Table 1) only one (4%, $n = 1/27$) is testing medicinal products (data not shown). During the time period studied in the present work, FCT launched only one call, in 2007, specifically focused in clinical research. The Ministry of Health had a financial contribution to this call and a total amount of 9 M € were used to fund 63 projects [7]. However, none of these funded studies were found in our search, probably because most of them were not interventional studies or interventional without IMPs which registration is desirable but not mandatory. Nevertheless, screening the information provided by FCT we found at least one interventional study with IMP that was not properly classified, consequently not registered in a database by the investigators [8].

The level of participation of Czech Republic and Portugal in international grants was similar ($n = 38$ and $n = 30$, respectively), most of these involving trials with IMPs in both cases (Fig. 3a, b). The organizations receiving national funds, thus acting as sponsor, in higher number of trials in the Czech Republic were hospitals (32%: $n = 35/109$) and universities (22%: $n = 24/109$). Whereas in Portugal were the universities (40%: $n = 24/60$) that sponsored more trials funded by national funding agencies followed by hospitals (7%: $n = 4/60$). Portuguese hospitals sponsored more trials funded by international funding grants then with national funding grant agencies ($n = 8$ vs $n = 4$).

Table 1 shows all the funding grant agencies identified both in the registries and in the publications for each completed and published clinical trial. In the Czech Republic there were a higher number of trials funded by national funding agencies and the number of registrations is gradually growing every year since 2004 (S1, Fig. 1d–e). When compared to the Czech Republic, a lower number of funded IICTs by public funding agencies were identified in Portugal, however, the national investigators have been involved in trials with more diverse funding opportunities, especially international ones.

In the Czech Republic, University hospitals are pressured to apply to grant support and carry out clinical research, because most physicians working in the hospital are university employees at the same time and need to build up their career as researchers. Moreover, there is a lack of experienced grant offices in the Czech Republic which would help the investigators to prepare an application for international grant call, e.g. H2020, IMI2. However, the mandatory insurance to initiate a clinical trial with medicines is not eligible in these grants´ call which might hamper the initiation of more trials. In theory, most Hospitals in Portugal have an office dedicated to support clinical research (as a government decision from 2015) [9]—but only a few can support physicians in the design or implementation of more complex clinical trials involving IMPs or medical devices, or even provide support to prepare proposals for national or international funding. The concept of academic clinical trial units (CTU) introduced in these two countries by ECRIN has been valuable to support clinical investigators and multinational IICTs [9], in the conduct of IICTs, especially in IMPs and/or medical devices trials, during all phases of the process

(from the idea until the close out). Universities and biomedical research institutes in Portugal detected earlier the need of professional supporting offices to prepare and manage national and international research projects.

National Policies for Clinical Research

Both countries present national public policies implemented (or not) for clinical research, in particularly IICTs, which are summarized in Table 2. The data were collected from national legal decisions, analysis of the call's eligibility criteria for funding national infrastructures and projects as well as higher education institutions in each country.

There have been legal decisions to establish clinical research as a priority that included the membership of ECRIN-ERIC and the definition of the clinical investigator status in both countries [10–15]. Since 2015, ten trials were initiated by ECRIN and conducted in the Czech Republic in this time period: POEM_H2020, FAIRPARK II_H2020, NISCI_H2020, SECURE_H2020, EuroHYP_7FP, PrOOf_H2020, VISION_DMD_H2020, DISCHARGE_FP7, PopART_H2020, TENSION_H2020. Some of these projects were also conducted in Portugal (FAIR PARK II, DISCHARGE and POPART). Besides these, the projects MACUSTAR_IMI, BETA3LVH_H2020 and HCQ4Surfedefect_ERA-NET, were also conducted in Portugal making a total of 6 trials initiated in the country through ECRIN. In both countries, none of these trials were from the initiative of national investigators.

The Internal Grant Agency (IGA) of the Ministry of Health supporting medical research and development was established in 1990 in Czech Republic and has been replaced by Czech health research council, AZV, founded on 01/04/2014 [19, 20]. This council, launch calls each year and supports the selected applications of clinical research in the different medicine areas. The establishing the AZV and defining the national priorities of oriented research, experimental development and innovations are the long-term strategies of the Czech government to support the clinical research in medicine. Nevertheless, to our best knowledge in the Czech Republic, the higher education courses specific to train clinical trials professionals are being prepared. In Portugal, the national government supported and invested in specific training for medical doctors[14] and research team professionals from hospitals [16].

The investment of the Portuguese government, public and private universities in post-graduation courses, including the Portugal Clinical Scholars Research Training (PTCSRT) [17] and the Clinical Investigator Certificate, CLIC [16] is an asset to trigger a high-quality clinical investigation. However, strategies to allocate research time to clinical investigators still need to be implemented [11].

To share knowledge and resources in 2016 a Portuguese government decision created the concept of Clinical Academic Centers (CAC)—joining hospitals, universities and research institutes around a medical school [13]. Currently, 9 CACs are officially established in Portugal, however, they are still deciding on the respective organizational strategy. In line with what is established in the Czech Republic since 2004, in Portugal a new agency to fund exclusively clinical research studies: *Agência para a Investigação Clínica e Investigação Biomédica* (AICIB), focusing on translational medicine products trials, was launched in 2018 but is not yet operational [18].

Clinical Trials Published in Peer-Reviewed Scientific Journals

In the Czech Republic, there are 41% of published trials among the 180 completed ($n=73/180$) while in Portugal, from the 144 completed trials, 55% ($n=79/144$) are published in peer-reviewed journals (Fig. 1, step 7). In the Czech Republic, a higher percentage of published studies focus on IMPs (44%, $n=32/73$), followed by surgery and procedures (32%, $n=23/73$). On the other hand, in Portugal,

Table 2 National Public Policies Implemented by the Portuguese and Czech Governments Involved in or to Promote Clinical Research.

Public Policies	Czech Republic	Portugal
Establish clinical research as a priority	Yes [10]	Yes [11–13]
Be member of ECRIN-ERIC	Yes [14]	Yes [13]
Define a legal specific status for clinical investigators	Yes [15]	Yes [11]
Investment in public infrastructures to promote/support IICTs	Yes	No
Specific and periodic funding for IICT	Yes	No
National funding agency specific for health research	Yes	No[a]
National public initiatives for clinical research team's capacitation	No[b]	Yes [16, 17]

ECRIN-ERIC European Clinical Research Infrastructure Network-European Research Infrastructure Consortium

[a]A national agency (AICIB) for clinical research funding is now implemented in Portugal [18]

[b]A course dedicated to clinical trials management is under preparation

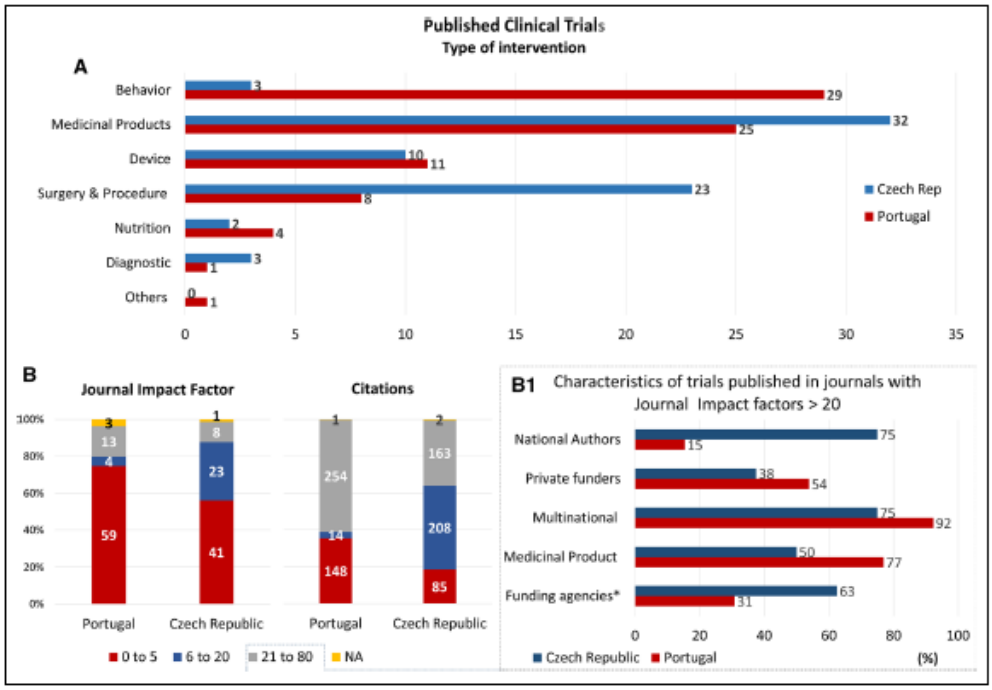


Figure 4 IICTs Published in Peer-Reviewed Journals According to the Type of Intervention (**a**) and Impact Factor of the Journals and Paper Citations Up to 1 Year After Publication (**b**). Papers published in 2018 were not considered. Inset of B (B1): clinical trials published in journals with impact factor higher than 21. * Includes national and international funding agencies.

most publications focus on behavior studies (37%, $n=29/79$) followed by IMPs (32%, $n=25/79$) (Fig. 4a).

The impact factor (IF) of the journal where these IICTs were published varies widely between 0 to 72 as shown in Fig. 4b. Thirty percent (30%, $n=23/73$) of the trials performed in the Czech Republic with publications were published in journals with IF between 6 to 20. Around 75% ($n=59/79$) of published papers with trials involving Portuguese organizations, have an IF below 5 and only 16% ($n=13/79$) were published in journals with IF above 20. The average IF of all these journals is similar for both countries and is around 9. The total number of citations of papers published in Czech Republic and in Portugal up to 1 year after publication were, 458 and 417, respectively (Fig. 4b).

Portuguese investigators are authors/co-authors of 15% ($n=2/13$) of the papers published in journals with IF > 20, conversely to what is observed with Czech investigators who authored/co-authored of 75% ($n=6/8$) of the papers published in high impact factor journals (Inset B1, Fig. 4b). Furthermore, international funding agencies funded 50% ($n=4/8$) of trials in the Czech Republic and only 31% ($n=4/13$) in Portugal that were published in journals with IF > 21 (Inset B1, Fig. 4b). None of these published trials in high impact journals was funded by the Portuguese national funding agency while in the Czech Republic, one of these trials was funded by the national funding agency. Around half of the trials published in journals with IF > 21 are funded by private funders in Portugal (54%, $n=7/13$) and around 38% ($n=3/8$) in the Czech Republic. Additionally, most of these published trials were multinational and focused in IMPs, in both countries.

According to our results the Czech Republic might have had a better return of the investment in this field, publishing a higher number of publications in journals with IF above 6 compared with Portugal ($n=32$ vs $n=20$), with a higher number of citations when compared to those published in Portugal. When compared to the Czech Republic, Portugal has slightly a higher number of published completed trials and a higher number of publications in journals with IF greater than 21 ($n=13$ vs $n=8$), but the participation of Portuguese recruiting sites does not correspond to more authorships of Portuguese investigators in the publication since

only 15% of these publications have Portuguese authors, whereas 75% of publications with the involvement of the Czech Republic recruiting sites have national investigators as authors. The values above mentioned might be underestimated because in this work, only studies registered in CTR and the first publication with results of each completed IICTs were considered.

Discussion

In this study, a systematic search of IICTs registered in four CTRs was conducted with the main goal of comparing the type of studies, main funders and scientific output in the Czech Republic and in Portugal. The methodology used in this work, allowed us for the first time to have a picture of IICTs in two European countries with similar GDP and population, HDI and number of medical schools, collecting information both from CTRs and publications.

Globally we found a better performance of the Czech Republic in terms of higher number of IICTs, including those with IMPs, of papers published in higher impact factor's journals, citations, and publication with more national authorships, when compared with Portugal. These findings suggest that a funding agency more focused in clinical research as the Czech Republic funding agency, may facilitate the authorship of national investigators in clinical research studies with higher quality.

In both countries, only a small percentage of the national organizations were sponsoring multinational IICTs in the Czech Republic (6%) and in Portugal (4%), in line with previous reports, where only 3% of all IICTs in the International Clinical Trials Registry Platform (ICTRP) are international [21]. The existence of national funding grant agencies is of major relevance to implement pilot IICTs that later could be escalated to the European level.

The national governments and European Commission investment in clinical research infrastructures have been critical for the development of this area [22, 23]. Both, the Czech Republic and Portugal governments have been investing in European infrastructures of clinical research such as ECRIN-ERIC. However, while in the Czech Republic the government also invested in the national network of CTUs, in Portugal the investment was limited to the ECRIN-ERIC membership fee. Recently a positive discrimination for clinical research has been introduced in the Portuguese infrastructures' roadmap national strategy [24]. As referred in a previous publication by the same authors, Germany, France, and the UK are good examples to show the increase in the number of IICTs after the investment of respective governments in both the funding of IICTs and supporting infrastructures [4].

When compared to other European countries, Czech Republic and Portugal are below the average both in the number of IICTs/million of citizens and % IICT/total CT [4]. Considering the recent governmental strategies it is clear that this is a work in progress, worthwhile to follow and assess in upcoming years. It would be worth following the example of countries like Denmark (Innovation Fund Denmark) [25] or the UK [26] who establish new rules to support the funding of multinational clinical trials by their national grant agencies. On the other hand, the recent governmental initiatives in Czech Republic and in Portugal to stimulate clinical research might serve as an example for those countries with lower number of IICTs identified in previous publications such as Slovakia and Poland [4, 21]. Additionally, if other countries publish their report assessment on clinical research performance it would facilitate the comparison and extrapolation among European countries policies and outcomes.

The relevance and impact of IICTs for the continuous improvement of therapies and the development of new public policies rely on the results' independence from the interests of any other stakeholders [27]. Therefore, it is critical for the countries to promote IICTs through a comprehensive and stimulating set of public policies and funding. For example, Italian government decided to invest on IICTs funding, through the Italian Medicines Agency (AIFA) that resulted in an increase of the number of independent clinical research and scientific outputs [28, 29]. This initiative started in 2005 and an amount of 40 M€/year have been provided by the industry to AIFA, to fund competitive IICTs projects developed by Italian physicians working in the public/non-profit sector [29].

As a limitation of this study, the number of IICTs with IMPs in both countries might be underestimated due to lack of registration and/or misclassification, although being mandatory [30, 31]. Similar underestimation might also have occurred in the registration of the other type of interventional studies since these are not mandatory in both countries. Nevertheless, with this work, we expect to reinforce the need of registration of all types of clinical studies, in particularly those funded by national funding agencies. This would facilitate the tracking of the studies in which a huge investment was made, as well as the assessment of the outcomes in terms of publications, guidelines, etc.

In our view, the development of clinical research relies in a national strategy that gather health authorities with science, innovation and economy stakeholders. A change in the way health care units' value clinical research outcomes and how their performance is evaluated and rewarded, requires a new paradigm. We expect that this work has unveiled the need to explore further details on the IICT quality and funding in other European countries and to learn with those that might have implemented effective indicators to monitor their performance in this particular type of research.

Conclusions

For the first time, two European Countries were compared in terms of the number of IICTs, funders and the respective scientific outcome. Our results showed that in the same timeframe, the Czech Republic has higher number of registered IICTs and higher number of publications with Czech authors in top journals (impact factors > 21) when compared to Portugal. These findings might be related to the existence of specific and periodic funding for clinical research in the Czech Republic, although the factors behind this difference are difficult to unravel with the methodological approach in this study. We anticipate using the results of this study as a baseline to the better appraisal of IICTs evolution in the upcoming years and inspire other countries to do the same type of evaluation.

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Author Contributions
ECM, CM and LH conceived and designed the study. CM and LH, contributed equally to this work and carried out the search of the registrations and publications independently. Both CM and LH double checked 40% of the information gathered in the registrations and publications. All authors analyzed and interpreted data, drafted and performed a critical review of the manuscript. All authors have approved the submitted version of the manuscript.

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Data Availability Statement
All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Conflict of Interest
The authors declare no competing interests.

Ethics Statement
The data used in this work was obtained from public clinical trials databases. There was no use of human or animal subjects and/or tissue.

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Supplementary Information
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References

1. Konwar M, Bose D, Gogtay NJ, Thatte UM. Investigator-initiated studies: challenges and solutions. *Perspect Clin Res.* 2018;9(4):179–83. https://doi.org/10.4103/picr.PICR_106_18.
2. Bekelman JE, Li Y, Gross CP. Scope and impact of financial conflicts of interest in biomedical research—a systematic review. *JAMA.* 2003;289(4):454–65. https://doi.org/10.1001/jama.289.4.454.
3. Crowe S, Fenton M, Hall M, Cowan K, Chalmers I. Patients', clinicians' and the research communities' priorities for treatment research: there is an important mismatch. *Res Involv Engagem.* 2015;1:2. https://doi.org/10.1186/s40900-015-0003-x.
4. Madeira C, Pais A, Kubiak C, Demotes J, Monteiro EC. Investigator-initiated clinical trials conducted by the Portuguese Clinical Research Infrastructure Network (PtCRIN). *Contemp Clin Trials Commun.* 2016;4:141–8. https://doi.org/10.1016/j.conctc.2016.08.002.
5. Madeira C, Santos F, Kubiak C, Demotes J, Monteiro EC. Transparency and accuracy in funding investigator initiated clinical trials: a systematic search in clinical trials databases. *BMJ Open.* 2019;9:e023394. https://doi.org/10.1136/bmjopen-2018-023394.
6. Zarin DA, Tse T, Williams RJ, Rajakannan T. Update on trial registration 11 years after the ICMJE policy was established. *NEJM.* 2017;376(4):383–91. https://doi.org/10.1056/NEJMsr1601330.
7. *Fundação para a ciência e Tecnologia, Concurso de Projectos de I&D em Investigação Clínica.* https://www.fct.pt/apoios/projectos/concursos/invclinica/. Accessed Oct 2019.
8. Almeida N, Romãozinho JM, Donato MM, Luxo C, Cardoso O, Cipriano MA, Marinho C, Sofia C. Triple therapy with high-dose proton-pump inhibitor, amoxicillin, and doxycycline is useless for

Helicobacter pylori eradication: a proof-of-concept study. *Helicobacter*. 2014;19(2):90–7. https://doi.org/10.1111/hel.12106.
9. *European Clinical Research Infrastructure Network (ECRIN)*. https://www.ecrin.org/activities/trial-management. Assessed Oct 2019.
10. *Roadmap for Large Research, Development and Innovation Infrastructures in the Czech Republic—Update 2011*. https://www.vyzkumne-infrastruktury.cz/wp-content/uploads/2019/06/Cestovni-mapa-velkých-výzkumných-infrastruktur-ČR_2011_EN.pdf. Accessed July 2019.
11. *Resolução do Conselho de Ministros (RCM) nº 18/2015, Diário da República, 1.ª série — N.º 67 — 7 de abril de 2015*. https://dre.pt/web/guest/pesquisa/-/search/66902772/details/maximized. Accessed Sept 2019.
12. *Despacho n.º 7216/2015, Diário da República, 2.ª série — N.º 126 — 1 de julho de 2015*. https://dre.pt/home/-/dre/67644371/details/maximized. Accessed Oct 2019.
13. *Portaria n.º 237/2014, Gabinetes do Secretário de Estado Adjunto e do Orçamento e da Secretária de Estado da Ciência - Diário da República, 2.ª série — N.º 60 — 26 de março de 2014*. https://www.acm.gov.pt/documents/10181/43818/Aviso+n.%C2%BA+4107-2014.pdf/aa89bcc8-4e1a-4110-a92d-0a8f9eb6b182. Accessed Sept 2019.
14. Decision Ref. No. :MSMT-3495/2014.
15. Act. No. 378/2007 Coll., Decree No. 226/2008 Coll.
16. *Clinical Investigator Certificate*. http://clic.pharmaceutical-medicine.pt/. Accessed Oct 2019.
17. *Portugal Clinical Scholars Research Training*. https://postgraduateeducation.hms.harvard.edu/certificate-programs/custom-programs/portugal-clinical-scholars-research-training. Assessed Oct 2019.
18. *Resolução do Conselho de Ministros (RCM) n.º 20/2016, Diário da República, 1.ª série — N.º 70 — 11 de abril de 2016*. https://data.dre.pt/eli/resolconsmin/20/2016/04/11/p/dre/pt/html. Accessed Sept 2019.
19. *Czech Health Research Council (AZV)*. http://www.azvcr.cz/en. Assessed Oct 2019.
20. *Czech Publication by AZV Director About the Establishment of the Czech Health Research Council*. https://www.tribune.cz/clanek/40308-agentura-pro-zdravotnicky-vyzkum-ceske-republiky. Assessed Oct 2019.
21. Atal I, Trinquart L, Porcher R, Ravaud P. Differential globalization of industry- and non industry-sponsored clinical trials. *PLoS ONE*. 2015;10(12):e0145122. https://doi.org/10.1371/journal.pone.0145122.
22. von Niederhäusern B, Magnin A, Pauli-Magnus C. The impact of clinical trial units on the value of clinical research in Switzerland. *Swiss Med Wkly*. 2018;148:w14615. https://doi.org/10.4414/smw.2018.14615.
23. Maier-Lenz H. *Academic strength in Germany, Applied Clinical Trials 2011*. http://www.appliedclinicaltrialsonline.com/academic-strength-germany. Accessed Oct 2019.
24. *Despacho n.º 4157/2019; Diário da República, 2.ª série — N.º 75 — 16 de abril de 2019*. https://dre.pt/home/-/dre/122109185/details/maximized. Accessed Oct 2019.
25. *Innovation Fund Denmark*. https://innovationsfonden.dk/en. Accessed Oct 2019.
26. Fox L, Toms C, Kernaghan S, Snowdon C, Bliss JM. Conducting non-commercial international clinical trials: the ICR-CTSU experience. *Trials*. 2017;18:440. https://doi.org/10.1186/s13063-017-2176-0.
27. Neyt M, Christiaens T, Demotes J, Walley T, Hulstaert F. Publicly funded practice-oriented clinical trials: of importance for healthcare payers. *J Comp Eff Res*. 2016;5(6):551–60. https://doi.org/10.2217/cer-2016-0018.
28. Neyt M, Christiaens T, Demotes J, Hulstaert F. Publicly funded Practice-oriented Clinical Trials. Health Services Research (HSR) Brussels: Belgian Health Care Knowledge Centre (KCE). KCE Reports 2015; 246. D/2015/10.273/53.
29. Italian Medicines Agency R, Development Working Group. Feasibility and challenges of independent research on drugs: the Italian medicines agency (AIFA) experience. Eur J Clin Investig. 2010. https://doi.org/10.1111/j.1365-2362.2009.02226.x.
30. *Clinical Trials Directive 2001/20/EC, European Commission*. https://ec.europa.eu/health/human-use/clinical-trials/directive_pt. Accessed Oct 2019.
31. *EudraCT Public Home Page*. https://eudract.ema.europa.eu/. Accessed Sept 2019.

7.2 Annex 2

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Summary

Purpose:

The aim of this study was to analyse the availability and structure of European and national funding sources for clinical research, with a specific focus on paediatric NCCTs in the Czech Republic. The study sought to identify key financial barriers and evaluate how funding mechanisms influence the feasibility and sustainability of academic clinical research in paediatrics.

Methodology:

A comprehensive analysis of funding programmes at both European and national levels was conducted, including public grants, institutional support, and other financial instruments relevant to clinical research. The study combined policy analysis with a case study approach, evaluating the accessibility, continuity, and adequacy of funding schemes for paediatric NCCTs.

Results:

The analysis revealed a fragmented and insufficient funding landscape, characterised by limited long-term sustainability and gaps in dedicated support for paediatric clinical research. European funding mechanisms were found to be competitive and not always aligned with national needs, while national funding sources lacked stability and targeted support for NCCTs.

Conclusion:

The study demonstrates that inadequate and poorly coordinated funding structures represent a major barrier to the development and implementation of paediatric NCCTs. Strengthening targeted funding strategies and improving alignment between European and national funding frameworks are essential to support high-quality academic clinical research and ensure better outcomes for paediatric patients.

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DIA

ORIGINAL RESEARCH

The Current Status of European and National Financial Sources for Clinical Research and Their Impact on Paediatric Non-commercial Clinical Trials: A Case Study of the Czech Republic

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Abstract

Introduction Paediatric non-commercial interventional clinical trials (NICTs) are crucial for healthcare provision. In spite of the fact that current regulations and initiatives try to enhance the quantity and quality of paediatric NICTs, there are still shortcomings that need to be addressed in order to accelerate the conduct of relevant clinical trials in children. To improve the current landscape of paediatric clinical research, it is necessary to identify and analyse the main trends and shortcomings, along with their impact on national performance in paediatric NICTs and this is the aim of this work.

Method A retrospective systematic search of paediatric NICTs was performed on four international clinical trials registries. Entries were filtered by date from 01/01/2004 to 31/12/2017. Each identified paediatric NICT was screened and analysed for sponsors, funders, type of intervention, therapeutic area, design characteristics and associated publications.

Results The search identified 439 unique NICTs. When stratifying the trials by enrolment ages, 86 trials were found involving the paediatric population. Most trials investigated the use of medicinal products and were focused on cancer or cardiovascular diseases. The most common sources of the funding were non-profit organizations. Furthermore, from the total number of completed trials, only half of them already published their results.

Conclusion The main shortcomings—specifically, ethical, methodological and, in particular, economic obstacles were identified. There is a continual need for greater support and collaboration between all major stakeholders including health policymakers, grant agencies, research institutions, pharmaceutical industries and healthcare providers at the national and international level.

Keywords Non-commercial Clinical Trials · Paediatric clinical research · Financial sources · International Clinical Trials Registries · Infrastructure

Abbreviations

NICTs	Non-commercial Interventional Clinical Trials
ANZCTR	Australia and New Zealand Clinical Trials Registry
CR	Czech Republic

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CROs	Clinical Research Organizations
CTs	Clinical Trials
CZECRIN	Czech Clinical Research Infrastructure Network
ECRIN	European Clinical Research Infrastructure Network
EMA	European Medicines Agency
EUCTR	European Union Clinical Trials Register
ICMJ	International Committee of Medical Journal Editors
ID	Identification Number
ISCTRN	International Standard Randomized Controlled Trial Number Registry
NIH	National Institutes of Health
PedCRIN	Paediatric Clinical Research Infrastructure Network
WHO-ICTRP	World Health Organization's International Clinical Trials Registry Platform

Introduction

Although children are our future and they should have the same options and conditions of treatment as adult patients, it is not the current reality [1]. The most common situation in paediatrics is treatment using medical products authorized for adults with an adjustment of dose or drug dosage form [2]. The majority of drugs for children are prescribed empirically, based on physician's experiences on an "off-label" regime and the authorization for children is missing [3]. In comparison with adult medicine, it is striking because adult patients are treated exclusively with drugs developed, authorized and confirmed in clinical trials designed for the adult population. Herein lies the challenge, a child is not just a small adult and in the absence of specific trial-based data on children, clinicians are forced to extrapolate from results of trials on adults [4]. This extrapolation is often inappropriate because children have a different range of diseases and the drugs hold different pharmacokinetic and pharmacodynamic characteristics in children, resulting in responses to treatment that are unpredictably different to adults and can be endangering [5]. The development of new drugs specially for children is rare, and therefore, there is no choice but to use adult medicines for children; still, these drugs should have their viability confirmed in paediatric clinical trials [6]. However, paediatric clinical trials have been largely neglected over the long term which has led to a lack of available data of efficacy and safety even of drugs which we have been administering for decades [7].

The off-label use of adult medicines is a result of the fact that conducting paediatric clinical trials is more challenging than those conducted on adults [8]. The pharmaceutical companies are not interested in conducting clinical trials on the paediatric population [9]. For pharmaceutical companies, clinical trials are a basic tool to get authorization and approval for marketing of their new medicinal products [10]. The available market of medicines for children is of a much lower quantity and quality as for the adult population [11]. The conducting of costly and time-consuming paediatric clinical trials tends to be pointless for pharmaceutical companies [12]. It will not increase their sales through more prescribed drugs because they are already prescribed in an "off-label" regime [13]. The non-refundable costs embedded in paediatric clinical trials represent an investment that many companies cannot afford nowadays [14].

The regulatory authorities throughout Europe react to this to create smoother conditions and supporting initiatives for conducting paediatric clinical trials. To address the paucity of paediatric research, many regulations have been launched with the primary objective being a shift in mindset from "protecting children from research" to "protecting children through research" [15]. In practice, the development of medicinal products for the paediatric population within Europe is now obligatory. For instance, the European Paediatric Regulation requires applications for marketing authorizations to be accompanied by either a product-specific waiver or a paediatric investigation plan, to be approved by the European Medicines Agency (EMA) [16]. In return, the patent is protected for an additional 6-month period via a patent extension, for drugs used in orphan diseases from 10 to 12 years and medical products must be authorized in all EU member states. These rules apply for new medicines and new pharmaceutical forms, new methods of administration of existing authorized products [17–19]. Although regulators have implemented these measures to increase the number of paediatric clinical trials and, consequently, marketed new medicines for children, the impact on the number of clinical trials performed remains modest and new drugs authorized for the paediatric population are not accruing [20].

The pharmaceutical companies tend to initiate paediatric trials only if a sufficient return on investment is likely. As a consequence, the pharmaceutical industry will most likely focus on a very limited number of potentially profitable products [12]. From this perspective, non-commercial clinical trials represent a crucial tool for developing and updating the guidelines for clinical practice. Although NICTs usually provide little direct potential economic benefit as newly marketed drugs, the added value of paediatric NICTs lies in a high scientific benefit with transmission to clinical practice, appearing e.g. at the level of updated, optimized guidelines [21].

Generally, NICTs (also named academic) are focused on patient approach and an attempt to answer relevant questions from clinical practice. These trials include testing of currently authorized drugs for adults but in the paediatric population, comparisons between effectiveness and safety

trials, or providing evidence of novel indications for registered drugs, including rare diseases. NICTs could also facilitate the accessibility of a new treatment in paediatric patients. Besides drugs, other interventions such as medical devices, nutrition, behaviour or therapeutic, diagnostic and surgical procedures are included in NICTs. Above all, NICTs provide robust evidence to improve therapeutic guidelines, support clinical decisions and enable policy makers to make sustainable policy decisions on public health [22].

In this regard, it is possible to define the level of quality of applied biomedicine research in the country using NICTs as a measurement, not only the number of ongoing studies but also the quality of the results publications from the finished trials. The main objective of this work is to identify and analyse the main trends and shortcomings, along with their impact on national performance in paediatric NICTs.

Methods

In accordance with the main goal of this work—investigating paediatric NICTs to discover exactly how these types of trials are currently being conducted. We identified all paediatric NICTs which were registered between the dates 01/01/2004 to 31/12/2017 from recruitment sites in the Czech Republic.

This work represents a unique overview of the current landscape of paediatric NICTs in the Czech Republic.

Search Methodology

Data related to all non-commercial interventional clinical trials from 4 international clinical trials databases were collected. It was comprised of information about trials that had been registered between the dates 01/01/2004 to 31/12/2017and marked as "Non-commercial" and "Interventional" trials.

The selection of the databases used for the search was based on previous research undertaken by Madeira et al. [23], namely:

- ClinicalTrials.gov
- The European Union Clinical Trials Register (EUCTR)
- BioMed Central International Standard Randomized Controlled Trial Number Registry (ISCTRN)
- Australia and New Zealand Clinical Trials Registry (ANZCTR)

The search strategy was different in each of the databases noted above, therefore the input of search keywords was varied. The detailed search methodology is specified in Table 1. The database searches covered the period from 01/01/2004 until 31/12/2017. If possible, the terms "Non-commercial", "Interventional" trial and the name of the country "Czech Republic" were used in the general search field in combination with an individual inspection.

The search of the EU Clinical Trials Register (www.clinicaltrialsregister.eu) was performed using the time interval

Table 1. The Detailed Search Methodology.

	Display in the Search Field as			
Keys Terms	ClinicalTrials.gov	EUCTR	ISCTRN	ANZCTR
Interventional	Trial type: interventional	Not applicable (NA) This database contains only information on interventional clinical trials	NA	Trial type: interventional
Non-commercial	Additional Criteria Funder Type: National Institutes of Health, All others, etc	General search field: Non-commercial	NA	Primary sponsor type: Government body, Hospital, etc. besides Commercial sector/ industry)
Country	Locations: Country	Select Country	Countries of recruitment:	Countries of recruitment
Timeframe from 01/01/2004 until 31/12/2017	Additional Criteria: Start date from–to	Select Date Range: from–to	Date applied: from–to	Trial start date: from–to
Population	Child (birth–17)	Adolescent; Children; In utero; Infant and Toddler; Newborn; Preterm new born infants; Under 18	Neonate; Child	Child (under 18 years)
Link	www.clinicaltrials.gov	www.clinicaltrialsregister.eu	https://www.isrctn.com/	www.anzctr.org.au

EUCTR The European Union Clinical Trials Register, *ISCTRN* BioMed Central International Standard Randomized Controlled Trial Number Registry, *ANZCTR* Australia and New Zealand Clinical Trials Registry.

between two dates (from 01/01/2004 until 31/12/2017) and with the terms "CZECH REPUBLIC and NON-COMMERCIAL" in the general search field.

At ClinicalTrials.gov it was not possible to distinguish between commercial and non-commercial trials therefore an advanced search was performed. The trials were extracted by selecting the options "Interventional", the limited time period, the name of the country and the applied filter for the funder type: NIH (U.S. National Institutes of Health), Other U.S. Federal agency, All others (individuals, universities, organizations) and Industry.

At ANZCTR an advanced search was performed by selecting the terms "Interventional", the name of the county and the referred timeframe in the relevant fields.

The ISCTRN registry is associated with the BiomedCentral database. In this database it was not possible to refine the search except by selecting the time period and the name of the country.

Extraction and Complementary Information

The following information was manually extracted from the registries for each of the NICTs found and is grouped by clinical specialties within each database. The data elements of interest were: Trial Identification number (Trial ID)—main and secondary, population, recruitment status, sponsor name and country, trial phase (when applicable), intervention, purpose, therapeutic area, design characteristics, type of funding and, publications of the completed trials.

Finally, manual review was used to confirm the compliance with the inclusion and exclusion parameters.

Exclusion parameters were:

- Industry sponsored trials
- Starting before 01/01/2014
- Participant recruitment beyond the Czech Republic
- Non-intervention trials

To categorize the non-commercial sponsors the following categories were created: Disease-Specific Organization (Disease associations or research institutes dedicated to a specific therapeutic area), Foundation, Hospital, Others (e.g. Individual investigators acting as sponsors), University and Research Institutes (non-specific therapeutic area).

NICTs can be funded by one or more sources, e.g. a secondary sponsor or collaborator. All these sources of funding found in the searches have been assigned to the predefined categories:

- Not-for-Profit organization (such as public institutions, funding agencies, disease-specific organizations)
- For Profit Organizations, when a private company is identified (Industry)
- Both, when the funding is provided from industry and another not-for-profit organization
- Not clear, when the information is not provided in any registry.

Because clinicaltrials.gov does not require funding information, the funding source was delivered on the basis of the "collaborator" fields and the secondary IDs in some cases. The support of funding agencies was perceived through secondary IDs where the code of the grant agreement was added in some cases.

The field "Source(s) of Monetary Support" in the WHO-ICTRP database (World Health Organization's International Clinical Trials Registry Platform) is fed by the information from the field "Sponsor and collaborators" from ClinicalTrials.gov. Therefore, when the funder was unclear on ClinicalTrials.gov and only the sponsor was listed under 'sponsor', those mentioned under WHO-ICTRP in the section "Source(s) of Monetary Support", were considered as funders also.

Identification of Duplicate Records

Some NICTs are registered in more than one register. A search allowing the identification of replicated trials was performed with the use of the excel function to identify duplicates comparing titles, sponsor and the main and secondary trial identification number public search function. The duplicates were excluded and not considered for the total number.

Searching Publications of Completed NICTs

To determine whether trials had published manuscripts in scientific literature, the characteristics of the trial (such as the titles, ID, names of the investigators and acronyms associated with the trial) were used to search both the PubMed database and Google.

Searches of results reporting were restricted to completed trials until the end of 2017.

Additionally, manual review was undertaken to complete information about the funding sources of all published trials to identify any other relevant information that may have been missed by registry searches.

Identification of Paediatric NICTs

A later step was focused on selecting trials that involved the paediatric population. Trials with young participants were identified by manual review according to the trial characteristic "population" in our database. The records were considered acceptable if the participant age range was specified (0–18 years) (for more details, see Table 1).

Finally, around 40% of the data were checked by an independent expert for appropriate content and consistency, ensuring that entries were relevant and correct. The evaluation of the results was restricted to paediatric trials only. The descriptive statistic has been used.

Results

Identification of Non-commercial Paediatric Trials

A total of 3496 interventional trials were identified in the four international clinical trials databases and were screened individually to isolate those that had a non-commercial sponsor, mention the Czech Republic as a country of the sponsor or recruiting site, involved the paediatric population and started between the dates of 01/01/2004 to 31/12/2017.

In Fig. 1 the number of registered trials found in each database is shown. From all the screened trials, 2971 were found to have a commercial sponsor. Although marked as non-commercial trials, 46 were recognized as duplicates and the other 40 were also discarded because they did not meet the inclusion criteria (the start of the trial was beyond the time period or they were only observational). Those all were removed and not considered in the total number.

Removing these from the original total of 3496, the search identified 439 unique NICTs with non-commercial sponsors conducted in the Czech Republic. When stratifying these trials by enrolment age, 86 trials were found to involve the paediatric population.

Within the referred timeframe, between the dates of 01/01/2004 to 31/12/2017, a slightly increasing trend with some upward or downward deflection can be observed. The greatest number of trials were started in the years 2013 and 2015. By contrast, in the year 2004 only one trial was started and a similar situation can be seen in the year 2008 ($N=2$) and 2011 ($N=3$). The sharpest rise came in the year 2005, when the number increased to 6, which was largely attributable to the implementation of the ICMJE (International Committee of Medical Journal Editors [21]) policy. In the remaining years of the period the number of the trials was stable (Fig. 2).

As shown in Table 2, hospitals ($N=35$), universities ($N=33$) and disease-specific organizations ($N=14$) are the most frequent sponsors of non-commercial paediatric trials.

The focus of paediatric non-commercial trials is most commonly oncology ($N=29$), cardiology ($N=7$) and metabolic disease ($N=5$), in the centre of this spectrum are haematology, neurology, infectious disease and gastroenterology ($N=4$). Less commonly involved are therapeutic areas

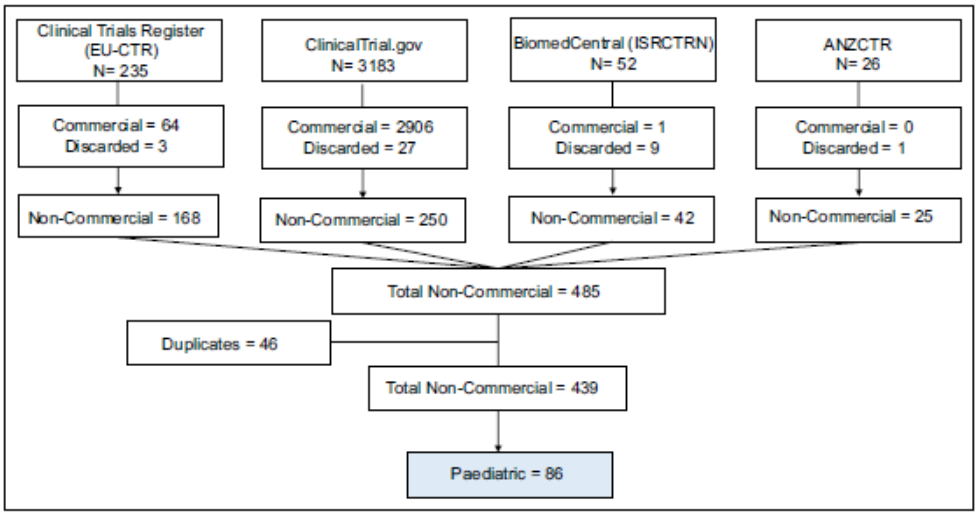


Figure 1. Flowchart Depicting the Systematic Search of Paediatric NICTs in Each Database. The Search was Performed on Four International Clinical Trials Registries (EUCTR, Clinicaltrials.gov, ISRCTN and ANZCTR). Clinical Trials Starting from 01/01/2004 until 31/12/2017 were Identified in Each of the Database Registries. After Discarding Commercial Trials, All Remaining CTs were Gathered into One Excel Sheet. Duplicate CTs were also Discarded. Paediatric CTs were Identified via Manual REVIEW According to the Trial Characteristic "Population".

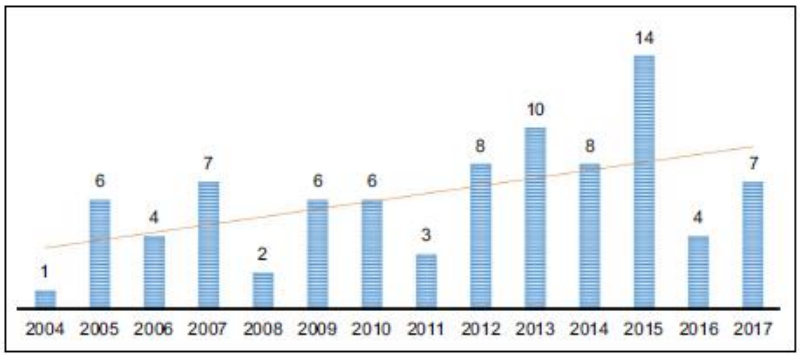


Figure 2. The Number of Paediatric NICTs Started in Each Year During the Time Period 01/01/2004 to 31/12/2017 ($N = 86$).

Table 2. The Number of Paediatric NICTs According to the Sponsor ($N = 86$).

Sponsor	Total Number
Hospital	35
University	33
Disease Specific Organization	14
Research Institute	3
Foundation	1

Table 3. The Number of Paediatric NICTs According to the Therapeutic Area ($N = 86$).

Therapeutic Area	Total Number
Oncology	29
Cardiology	7
Metabolic Diseases	5
Otolaryngologists	4
Gastroenterology	4
Immunology	4
Infectious Diseases	4
Neonatology	4
Neurology	4
Haematology	4
Others	3
Anaesthesiology	3
Traumatology	3
Pain	2
Dermatology	2
Respiratory Diseases	2
Ophthalmology	1
Musculoskeletal System Diseases	1

such as musculoskeletal system disease ($N = 1$) and ophthalmology ($N = 1$; Table 3).

The majority of the trials ($N = 60$) were focused on medicinal products versus other interventions as a procedure ($N = 6$), surgery ($N = 5$), diagnostic method ($N = 4$) or medical device ($N = 4$; Table 4). Most trials testing medicinal products are in phase III ($N = 22$), followed by phase II and IV ($N = 60$; Table 5).

The majority of the trials had an international sponsor ($N = 57$; Table 6A). A small percentage of the paediatric trials with national sponsors were conducted as international ($N = 3$, 10%; Table 6B).

In total, 48 trials (56%) were monocentric and 23 (27%) trials were conducted as a multicentre (Table 7A). Most of the trials were randomized ($N = 53$, 62%; Table 7B).

The analysis of the funding sources showed that more than two-thirds of trials were funded by a public organization ($N = 65$, 76%); generally, grant agencies, government bodies or other non-commercial organizations. Of those trials, a total of 12 received financial support from international funding agencies such as the European Commission and 5 from national funding agencies such as the Internal Grant Agency of the Czech Ministry of Health. Industry was identified as the funding source for 4 trials

Table 4. The Number of Paediatric NICTs According to the Type of Intervention ($N = 86$).

Type of Intervention	Total Number
Medicinal product	60
Procedure	6
Surgery	5
Behaviour	4
Medical device	4
Diagnostic method	4
Nutrition	3

Table 5. The Number of Paediatric NICTs with Medicinal Products Divided According to the Type of Phase ($N = 60$)

Type of Phase	Total Number
Bioequivalence	1
I	1
I/II	3
II	11
II/III	4
III	22
III/IV	1
IV	7
Not specified	10

(5%), while 9 trials (10%) were funded by both private and public organizations. No information regarding the funder was reported for 9% of the trials (Fig. 3A).

At the moment, more than half of the trials ($N = 53$, 62%) are active and are recruiting patients at the time of writing. The total number of completed paediatric trials was 24 (28%); only 12 of them have already published the results, which amount to 50% (Fig. 3B). Most of the published trials were focused on the testing of medicinal products ($N = 7$) and were published in journals with an impact factor lower than 5 ($N = 6$). In 83% ($N = 10$) of the published trials the funding source was from public organizations (Table 8).

Discussion

For the first time, this work provides a unique overview of the facts about paediatric investigator-initiated clinical trials in the CR between 01/01/2004 and 31/12/2017. This period includes the time after 2004, the year of the International Committee of Medical Journal Editors' implementation (ICMJE), policy requiring the registration of clinical trials as a prerequisite for consideration for publication [21].

The work examined the data collected from four clinical trial public databases to describe the current status of paediatric NICTs in the CR with the aim of defining trends and shortcomings, knowledge of which would help to increase the level and quality of independent non-commercial clinical research in the CR. The type of sponsor, intervention, phase, therapeutic area, design characteristics, and type of funding were reviewed and analysed.

In practice, conducting NICTs is extremely time-consuming and requires good logistics and management services. Limited financial and human resources hamper the organization of NICTs which have to comply with the same regulatory requirements as big pharmaceutical companies investigating a novel therapy [24]. The limitation of funding from national grants is the cost eligibility. Only national costs are eligible, which means to only national trial can be conducted. However, to get a powerful sample, you need multinational trials. For university hospital physicians with an enormous workload of teaching and ensuring medical

Table 6. Distribution of NICTs According to the Origin of Sponsor (A) International vs National and Involvement of Other Countries in CTs with National Sponsors (B) National vs International Trial

(A) Origin of Sponsor	Total number
International sponsor paediatric NICTs	57
National sponsor paediatric NICTs	29
(B) Type of Trial with National Sponsor	**Total number**
National trial	26
International trial	3

Table 7. The Number of Paediatric NICTs According to Type of Clinical Trial (A) Monocentric vs Multicentre and (B) Randomized vs Non-randomized ($N = 86$).

(A) Type of Clinical Trial	Total Number
Monocentric	48
Multicentre	23
Not specified	15
(B) Type of Clinical Trial	**Total Number**
Randomized	53
Non-randomized	21
Not specified	12

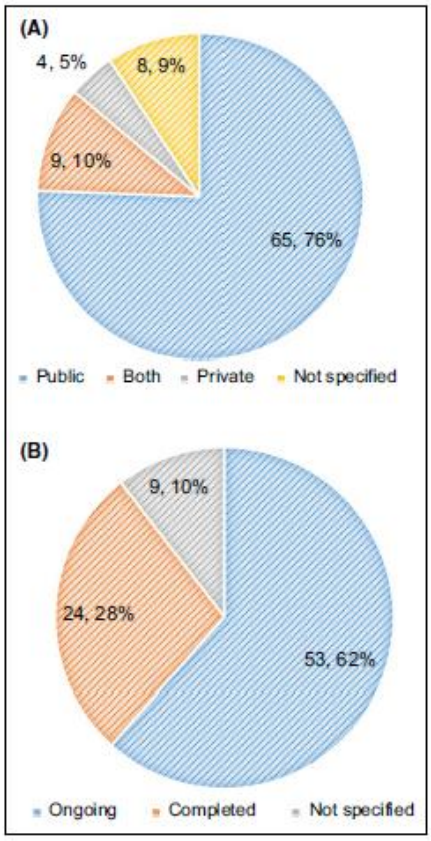


Figure 3. **a** The Percentage and Number of Paediatric NICTs According to the Type of Funder ($N = 86$). **b** The Percentage and Number of the Paediatric NICTs According to the Status ($N = 86$).

care, preparing, initiating and enacting a multinational clinical trial is challenging [25]. The competition involved in obtaining financial resources from a European grant takes great effort, time and expertise. Physicians are not generally well trained in the application process for the grant funding. Therefore, the support of specialists in this area is crucial [26]. Furthermore, the long delay between application and funding all the way to approval can lead to the failure of even starting NICTs.

The limitation of these funds is usually a short timeframe for patient recruitment and the fact that they generally do not allow subcontracting with for-profit Clinical Research Organizations (CROs) and involve other centres in different European countries.

In some cases, NICTs can also be funded by other not-for-profit organizations such as patient associations, governmental organizations and foundations [27]. Private sources of funding for NICTs are increasing over recent years. Participation of private companies may also be in the form of drug product supply, financial resources or both. With this type of collaboration between pharmaceutical companies and an individual non-commercial investigator, there may be a risk that the company struggles to persuade the investigator to work on defined priorities rather than their own research aims [28]. In the face of this, these trials should not be truly considered in the sense of independent non-commercial research.

Thus, the continuous funding and commitment of governments is an important step, as is mentioned in the upcoming Clinical Trial Regulation (Regulation (EU) No 536/2014)—*In order to maximize the valuable contribution of such non-commercial sponsors and to further stimulate their research but without compromising the quality of clinical trials, measures should be taken by Member States to encourage clinical trials conducted by those sponsors* [29].

During the last decade several EU-level guidelines came into force requiring the conduct of more paediatric clinical trials in order to force the development and accessibility of medical products for children [30, 31]. In line with this there has been a gradual shift to the current consensus that children deserve the same level of healthcare as any other age group [32]. It was acknowledged that this initiative had a positive impact and led to an increase in the number of non-commercial trials [33, 34]. The implementation of Directive 2001/20/EC certainly improves the quality of clinical trials and assures the safety and well-being of trial subjects [35]. Nevertheless, at the same time, performing an NICT according to the new requirements is nearly impossible for clinicians and non-commercial researchers without cooperating with expensive specialized experts,

Table 8. Characteristic of the Published Paediatric NICTs ($N = 12$).

Intervention		Impact Factor		Funders	
Behaviour	2	0 to 5	6	Non-profit organization	10
Device	1	6 to 20	5	Both	2
Medicinal Product	7	21 and higher	1		
Surgery	2				

such as project managers, statisticians, data managers, pharmacists and monitors [36]. This area also presents a lot of challenges mainly due to differences in national law and practice; without industry sponsorships, conducting paediatric NICTs seems almost unthinkable. Indeed, the investigators spend a lot of time and resources on planning and preparing study documentation in order to successfully submit to the relevant regulatory authorities and ethics committees.

Also, Fig. 2 supports the conclusion that regulatory policy has had a slight effect on paediatric trials practice in the CR. The number of paediatric NICTs conducted in CR grew between 2004 and 2017. On the other hand, these NICTs seem to have been registered in order to comply with ICMJE policy [21]. In 2016, there was a significant decrease in the number of NICTs, which could be explained as an impact of the termination of the Internal Grant Agencies under the Ministry of Health in the CR in 2014 and the establishment of the new Agency for Health Research a year later [37]. Researchers had limited opportunities to gain resources to support NICTs, which could have reduced the number of registered NICTs.

Over half of the trials have international sponsors, which is a good indicator of CR's attractiveness. Despite this, only a small number of NICTs initiated by a national sponsor were conducted as multinational and multicentric [38]. This can likely be explained by the condition of national grants that do not allow the provision of financial support to foreign partners. By contrast, Belgium and Denmark, for example, have the different rules [39, 40]. Public national funds in those countries can also be used for the involvement of foreign partners. This fact fosters the implementation of multinational, multicentre trials and also boosts patient recruitment and often leads to an improved collaboration.

Furthermore, there are other impediments when conducting multinational trials including regulatory fragmentation in participating countries' centres (such as an informed consent/assent, ethical review, pharmacovigilance, data monitoring, language and insurance) [41].

The analysis of therapeutic areas shows that NICTs cover a wide range, with oncology and cardiology at the forefront. Next in line are metabolic diseases, haematology, neurology, infectious diseases/immunology and gastroenterology, but no particular area dominates. Generally, this is a good sign as it demonstrates that paediatric research is covering a wide range of diseases. Most paediatric NICTs are randomized and most trials test drugs in Phase III which corresponds to the fact that non-commercial trials typically test already licensed drugs for a new indication, different route of administration, or new dose/dosage form.

Overall, paediatric research has gained a boost in all of Europe as is shown in the EMA report. The proportion of clinical trials in the European clinical trial database EudraCT that includes children has increased by 50% in 2007–2016 from 8.25 to 12.4% [30].

Indeed, CR is below the European average in regards to conducting non-commercial clinical trials. Only 4% of all registered clinical trials are initiated by investigators without industry sponsorship, while the European average is 17% [42].

The registration of trials and provision of basic information about a sponsor, protocol and results in a publicly accessible registry is the first step to promoting transparency in clinical trials research and it is also one of ethical principles of the Declaration of Helsinki [43]. The primary responsibility for registration and providing data lands with the sponsors, principal investigators and in some instances other persons/organizations authorized by the sponsor. The provided data should be current and complete, although in some cases information was not available in the screened registries. This is probably due to the fact that completion of some items is now optional. Future improvement could be to make full completion of the individual trial in the registry, before its approval, mandatory, including the fields referring to a funder and ownership of the results. According to the presented results, most of the paediatric NICTs in CR (76%, $N=65$) are funded by non-commercial organizations, 15% of the NICTs received support from the private sector. This support could be financial, the supply of medical products/devices or both; when the trial is funded or supported by a private company it is often not clear who will be the owner of the obtained data. If the owner is a private company, the trial should not be considered as independent non-commercial research. According to the screened registries, it is not possible to find out who is ownership of the results. Nowadays, some journals have introduced a requirement of the full disclosure of funders [23].

From the total number of completed non-commercial trials, ($N=24$) only half of them have already published the results, despite the duty stated in the Declaration of Helsinki. Additionally, in the case of clinical trials on paediatric subjects the results should be publicly available 6 months after the end of the trial (generally the obligation for public results of clinical trials is one year, [17, 43]). This corresponds to the cohort study published in The Lancet where compliance with legal requirement to report clinical trial results on ClinicalTrials.gov was followed. Only 1722 trials from the 4209 registered in our particular timeframe on ClinicalTrials.gov reported results within a 1-year period [44].

Conclusion

Generally, non-commercial clinical trials are the basis of a country's clinical research strength [45, 46]. They have a crucial importance for healthcare provision, as they answer

questions which are highly relevant but often disregarded by the pharmaceutical industry, especially in the case of the paediatric population due to its very low profitability [47]. However, those trials still represent a relatively small percentage compared to the commercial ones [48].

One potential way to support paediatric clinical trials could be via European infrastructures such as the ECRIN [49], particularly the PedCRIN project which is focused on developing the capacity for the management of multinational paediatric non-commercial clinical trials [50]. At this time CR is a full member of the ECRIN infrastructure and a national network named Czech Clinical Research Infrastructure Network (CZECRIN) was established at the end of 2014 [51, 52]. The aim of CZECRIN is to create a sustainable research environment and help researchers with the planning and conducting of NICTs [53]. The offered support encompasses, for instance, submission procedure to regulatory authorities, trial management, pharmacovigilance, monitoring or data management. CZECRIN has established a network of Clinical Trial Centres at Czech medical faculties/university hospitals with the aim to deliver these scientific services across this network [51].

One of the most pressing issues is public funds. There is a need to simplify and refine the financing rules for the future which include all the stakeholders. From the healthcare providers, to the government, grant agencies, research institutions and industry, we need to make international costs more affordable, where the sponsor comes from the CR but NICTs must be multinational for the aforementioned reasons. New concepts of funding should be set up, for example specific grants of the European Union or their member states. Furthermore, pharmaceutical companies and all stakeholders involved in paediatric clinical trials must realize that the small financial contributions of the past are not sufficient any more to perform high quality paediatric IITs according to Directive 2001/20/EC [35].

Another important step in order to follow future evaluations and comparison of NICTs performance is improving the accuracy of clinical trials' registrations. A legal mandate for trial registration, not just for medical products, including disclosure of the funder and respective ownership of the results could also have to impact on the transparency of clinical research. A comparison of other European countries could be a useful scientific indicator; however, it is difficult because publications allocated to this topic are not commonly available.

Author Contributions

LH: Conceptualization, Methodology, Formal analysis, Investigation, Writing—Original Draft. KN: Conceptualization, Methodology, Formal analysis, Investigation, Writing—Original Draft. LS: Conceptualization, Validation, Writing—Review & Editing, Supervision. RD: Validation, Funding acquisition. PB: Validation, Supervision. All authors approved the finalized version of the manuscript.

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Data Availability

The datasets collected and analysed during the searches are available as supplemental file.

Compliance with Ethical Standards

Conflict of Interest

All named authors have no conflict of interest to declare.

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s43441-020-00173-9) contains supplementary material, which is available to authorized users.

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References

1. Convention on the Rights of the Child. UN General Assembly. 1989. https://www.refworld.org/docid/3ae6b38f0.html. Accessed 9 May 2020
2. Hawcutt DB, Cooney L, Oni L, Pirmohamed M. Precision dosing in children. *Expert Rev Precis Med Drug Dev*. 2016;1:69–78.
3. Corny J, Bailey B, Lebel D, Bussières J-F. Unlicensed and off-label drug use in paediatrics in a mother-child tertiary care hospital. *Paediatr Child Health*. 2016;21:83–7.
4. Ferro A. Paediatric prescribing: why children are not small adults. *Br J Clin Pharmacol*. 2015;79:351–3.

5. Stephenson T. How children's responses to drugs differ from adults. *Br J Clin Pharmacol*. 2005;59:670–3.
6. Mulugeta Y, Zajicek A, Barrett J, Sachs HC, McCune S, Sinha V, Yao L. Development of drug therapies for newborns and children. *Pediatr Clin N Am*. 2017;64:1185–96.
7. Wimmer S, Neubert A, Rascher W. The safety of drug therapy in children. *Dtsch Aerzteblatt*. 2015. https://doi.org/10.3238/arztebl.2015.0781.
8. Evens RP, editor. *Drug and Biological Development*. Boston: Springer; 2007.
9. Park MS. Regulatory innovation for expansion of indications and pediatric drug development. *Transl Clin Pharmacol*. 2018;26:155.
10. Tamimi NAM, Ellis P. Drug development: from concept to marketing! *Nephron Clin Pract*. 2009;113:c125–c131131.
11. Bavdekar S. Pediatric clinical trials. *Perspect Clin Res*. 2013;4:89.
12. Li JS, Eisenstein EL, Grabowski HG, Reid ED, Mangum B, Schulman KA, Goldsmith JV, Murphy MD, Califf RM, Benjamin DK. Economic return of clinical trials performed under the pediatric exclusivity program. *JAMA*. 2007;297:480.
13. Cavalla D. *Off-Label Prescribing: Justifying Unapproved Medicine*. Chichester: Wiley; 2015.
14. Collier J. Paediatric prescribing: using unlicensed drugs and medicines outside their licensed indications. *Br J Clin Pharmacol*. 1999;48:5–8.
15. Lavan M, Byrn SR, Knipp G. Pediatric formulations: knowledge gaps limiting the expedited preclinical to clinical translation in children. *AAPS PharmSciTech*. 2019;20:73.
16. Khan-Boluki J, Hundt F. Children in clinical trials: survey on the current situation in paediatric university clinics in Germany. *Ger Med Sci GMS E-J*. 2008;6:Doc01.
17. European Union. Regulation (EC) No 1901/2006 of the European Parliament and of the Council of 12 December 2006 on medicinal products for paediatric use and amending Regulation (EEC) No 1768/92, Directive 2001/20/EC, Directive 2001/83/EC and Regulation (EC) No 726/2004. 26 January 2007.
18. Regulation (EC) No 1902/2006 of the European Parliament and of the Council of 12 December 2006 amending Regulation 1901/2006 on medicinal products for paediatric use.
19. Penkov D, Tomasi P, Eichler I, Murphy D, Yao LP, Temeck J. Pediatric medicine development: an overview and comparison of regulatory processes in the European Union and United States. *Ther Innov Regul Sci*. 2017;51:360–71.
20. Permanand G, Mossialos E, McKee M. The EU's new paediatric medicines legislation: serving children's needs? *Arch Dis Child*. 2007;92:808–11.
21. De Angelis C, Drazen JM, Frizelle FA, et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. *N Engl J Med*. 2004;351:1250–1.
22. Bero L, Oostvogel F, Bacchetti P, Lee K. Factors associated with findings of published trials of drug–drug comparisons: why some statins appear more efficacious than others. *PLoS Med*. 2007;4:e184.
23. Madeira C, Santos F, Kubiak C, Demotes J, Monteiro EC. Transparency and accuracy in funding investigator-initiated clinical trials: a systematic search in clinical trials databases. *BMJ Open*. 2019;9:e023394.
24. Hartmann M, Hartmann-Vareilles F. The clinical trials directive: how is it affecting Europe's Noncommercial Research. *PLoS Clin Trials*. 2006;1:e13.
25. Minisman G, Bhanushali M, Conwit R, Wolfe GI, Aban I, Kaminski HJ, Cutter G. Implementing clinical trials on an international platform: challenges and perspectives. *J Neurol Sci*. 2012;313:1–6.
26. Konwar M, Bose D, Gogtay NJ, Thatte UM. Investigator-initiated studies: challenges and solutions. *Perspect Clin Res*. 2018;9:179–83.
27. Ravinetto R, De Nys K, Boelaert M, Diro E, Meintjes G, Adoke Y, Tagbor H, Casteels M. Sponsorship in non-commercial clinical trials: definitions, challenges and the role of Good Clinical Practices guidelines. *BMC Int Health Hum Rights*. 2015. https://doi.org/10.1186/s12914-015-0073-8.
28. Janiaud P, Cristea I-A, Ioannidis JPA. Industry-funded versus nonprofit-funded critical care research: a meta-epidemiological overview. *Intensive Care Med*. 2018;44:1613–27.
29. European Commission, Clinical trials - Regulation EU No 536/2014. https://ec.europa.eu/health/human-use/clinical-trials/regulation_en.
30. State of Paediatric Medicines in the EU 10 years of the EU Paediatric Regulation. Commission to the European Parliament and the Council. 2017. https://ec.europa.eu/health/sites/health/files/files/paediatrics/docs/2017_childrensmedicines_report_en.pdf. Accessed 24 Jan 2020
31. European Parliament resolution of 15 December 2016 on the regulation on paediatric medicines. https://www.europarl.europa.eu/doceo/document/TA-8-2016-0511_EN.html.
32. Better Medicines for Children From Concept to Reality, General Report on experience acquired as a result of the application of Regulation (EC) n° 1901/2006 on medicinal products for paediatric use. 2013. https://ec.europa.eu/health/sites/health/files/files/paediatrics/2013_com443/paediatric_report-com%282013%29443_en.pdf. Accessed 9 May 2020.
33. -year Report to the European Commission. European Medicines Agency and its Paediatric Committee. 2016. https://ec.europa.eu/health/sites/health/files/files/paediatrics/2016_pc_report_2017/ema_10_year_report_for_consultation.pdf. Accessed 9 May 2020
34. Study on the economic impact of the Paediatric Regulation, including its rewards and incentives. Technopolis group Ecorys Nederland BV empirica GmbH. 2016. https://ec.europa.eu/health/sites/health/files/files/paediatrics/docs/paediatrics_10_years_economic_study.pdf. Accessed 5 May 2020
35. Clinical Trials Directive 2001/20/EC, European Commission.
36. Welzing L, Harnischmacher U, Weyersberg A, Roth B. Consequences of Directive 2001/20/EC for investigator-initiated trials in the paediatric population—a field report. *Eur J Pediatr*. 2007;166:1169–76.
37. Czech health research council. https://www.azvcr.cz/en.
38. Lee K-H. The conceptualization of country attractiveness: a review of research. *Int Rev Adm Sci*. 2016;82:807–26.
39. The European & Developing Countries Clinical Trials Partnership. https://www.edctp.org/funding-opportunities/.
40. Innovation Fund Denmark. https://innovationsfonden.dk/en.
41. Lepola P, Needham A, Mendum J, Sallabank P, Neubauer D, de Wildt S. Informed consent for paediatric clinical trials in Europe. *Arch Dis Child*. 2016;101:1017–25.
42. Madeira C, Pais A, Kubiak C, Demotes J, Monteiro EC. Investigator-initiated clinical trials conducted by the Portuguese Clinical Research Infrastructure Network (PtCRIN). *Contemp Clin Trials Commun*. 2016;4:141–8.
43. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. *JAMA*. 2013;310:2191.
44. DeVito NJ, Bacon S, Goldacre B. Compliance with legal requirement to report clinical trial results on ClinicalTrials.gov: a cohort study. *Lancet*. 2020;395:361–9.
45. Investigator-driven clinical trials forward look. European Science Foundation, Strasbourg. Fondation européenne de la science. 2009. https://archives.esf.org/fileadmin/Public_documents/Publications/IDCT.pdf. Accessed 5 May 2020.
46. Alemayehu C, Mitchell G, Nikles J. Barriers for conducting clinical trials in developing countries—a systematic review. *Int J Equity Health*. 2018;17:37.
47. Intini A, Bonifazi D, Migliaccio G. Challenges and new frontiers in the paediatric drug discovery and development. *Drug Discov Dev*. 2020. https://doi.org/10.5772/intechopen.85635.
48. Nevens H, Harrison J, Vrijens F, Verleye L, Stocquart N, Marynen E, Hulstaert F. Budgeting of non-commercial clinical trials:

development of a budget tool by a public funding agency. *Trials*. 2019;20:714.
49. The European Clinical Research Infrastructure Network. https://www.ecrin.org/.
50. Paediatric Clinical Research Infrastructure Network. https://www.ecrin.org/projects/pedcrin.
51. Czech Clinical Research Infrastructure Network. https://www.czecrin.cz/czecrin-en.
52. TA ČR Starfos. https://starfos.tacr.cz/en/project/LM2013034.
53. Large research infrastructures supported by the Ministry of Education, Youth and Sports of the Czech Republic. https://www.vyzkumne-infrastruktury.cz/en/biomedical-sciences/czecrin/.

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7.3 Annex 3

Del Álamo M, Bührer C, Fisher D, Griese M, Lingor P, Palladini G, Sireau N, Hivert V, Sangiorgi L, Guillot F, Halftermeyer J, Soucková L, Nosková K, Demlová R. Identifying obstacles hindering the conduct of academic-sponsored trials for drug repurposing on rare-diseases: an analysis of six use cases. Trials. 2022 Sep 15;23(1):783. doi: 10.1186/s13063-022-06713-y. PMID: 36109818; PMCID: PMC9479412.

Summary

Purpose:

The aim of this study was to identify and systematically analyse the key barriers affecting the initiation and conduct of academic-sponsored clinical trials focused on drug repurposing in rare diseases. The study addresses a critical gap, as drug repurposing represents a highly relevant strategy for rare diseases, yet remains underutilised due to structural and regulatory challenges.

Methodology:

The study was based on an in-depth analysis of six multinational clinical trial “use cases” within a European research framework, examining real-world experiences from trial design to implementation. Data were collected from coordinating investigators and analysed to identify methodological, regulatory, financial, and operational obstacles encountered during trial setup and execution.

Results:

The analysis revealed multiple interrelated barriers, including complex and heterogeneous regulatory requirements across countries, insufficient and fragmented funding mechanisms, and challenges in trial coordination and infrastructure. These obstacles led to significant delays, increased administrative burden, and in some cases hindered trial initiation or completion.

Conclusion:

The study demonstrates that the feasibility of academic-sponsored trials in rare diseases is strongly limited by systemic and organisational factors rather than scientific challenges. Addressing regulatory harmonisation, improving funding structures, and strengthening research infrastructures are essential to enable efficient implementation of NCCTs in this field.

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Trials

RESEARCH Open Access

Identifying obstacles hindering the conduct of academic-sponsored trials for drug repurposing on rare-diseases: an analysis of six use cases

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Abstract

Background: Academic-sponsored trials for rare diseases face many challenges; the present paper identifies hurdles in the set-up of six multinational clinical trials for drug repurposing, as use cases.

Methods: Six academic-sponsored multinational trials aiming to generate knowledge on rare diseases drug repurposing were used as examples to identify problems in their set-up. Coordinating investigators leading these trials provided feedback on hurdles linked to study, country, and site set up, on the basis of pre-identified categories established through the analysis of previous peer-reviewed publications.

Results: Administrative burden and lack of harmonization for trial-site agreements were deemed as a major hurdle. Other main identified obstacles included the following: (1) complexity and restriction on the use of public funding, especially in a multinational set up, (2) drug supply, including procurement tendering rules and country-specific requirements for drug stability, and (3) lack of harmonization on regulatory requirements to get trial approvals.

Conclusion: A better knowledge of the non-commercial clinical research landscape and its challenges and requirements is needed to make drugs—especially those with less commercial gain—accessible to rare diseases patients. Better information about existing resources like research infrastructures, clinical research programs, and counseling mechanisms is needed to support and guide clinicians through the many challenges associated to the set-up of academic-sponsored multinational trials.

Keywords: Randomized clinical trials, Rare diseases, Drug repurposing, Academic-sponsored, Barriers, Challenges

Background

Many barriers exist to advancing knowledge and treatment for rare diseases. The most acknowledged challenge is the small number of eligible participants for a given study, with a major impact in the design and coordination of randomized clinical trials (RCTs), which are still the basis for the evaluation of interventions and clinical guidelines. In this set up, multinational, multicenter clinical trials are required to achieve sufficient recruitment. International collaboration is nevertheless constrained by scientific, ethical, economic and regulatory considerations.

In 2008, Duley and collaborators [1] identified major barriers to conduct of RCTs for all diseases areas through

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a systematic literature review. More recently similar analysis has been published for all disease areas [2], rare diseases [3], and developing countries [4]. Although rare diseases may present unique clinical problems, some of the methodological and operational challenges to studying health outcomes are common for other diseases areas. Indeed, all these publications have similarly concluded that the barriers to the conduct of multinational RCTs are significant, and although none of these reviews make a clear distinction between industry-sponsored trials and academic/investigator-initiated trials, most of the identified constraints might affect especially the latter [5].

Commercially sponsored clinical trials are responsible for bringing most of the new drugs to the market. However, these clinical trials assess the safety and efficacy of drugs that are chosen by a commercial entity that funds the entire process. Non-commercial or academic trials have their own additional specific objectives, often focusing on refining or getting new indications of available treatments (drug repurposing) and optimizing therapeutic strategies that do not have as much financial gain for the pharmaceutical industry. Drug repurposing has especially been coined as a possible relevant strategy for development of medicines for rare diseases. Indeed, a recent analysis [6] showed that 75% of the developments of repurposed orphan drugs authorized during the 2016–2020 period were started in academia. Pharmaceutical companies may find a commercial interest in pursuing clinical development to repurpose drugs that are still on patent. However, they are less likely to carry out such a development for out-of-patent medicines. Academic institutions would be willing to explore repurposing options despite the fact that academic-sponsored trials face a high number of challenges, including (a) lack of funding or long ranging financial support covering unexpected issues during the trial (b) inadequate infrastructure to plan, implement, and execute the trial including a working quality management system (c) necessary structured processes to facilitate academic collaborations, and a lack of platforms to discuss and solve issues related to academic trials that results in difficulties to access the right partner assisting on the operational management, usually provided by contract research organizations (CROs) on industry-sponsored trials.

Academic sponsors and investigators end-up getting involved not only in the scientific aspects of the research, but also having to navigate the operational coordination and management themselves.

Because drug repurposing has the potential to deliver treatments faster to patients with rare diseases, in this study, we address the following research question—what are the hurdles than hinder the conduct of clinical trials on drug repurposing for rare diseases initiated by academia? This paper describes outcomes of a use cases analysis of the methodological and operational challenges faced by coordinating investigators leading 6 investigator-initiated trials for drug repurposing on rare diseases over the last five years, most of them funded through a "virtual common pot" mechanism, i.e., through the combination of national funding sources, without cross-border transfer of funds.

Methods

This work is based on topics discussed in two meetings, the mid-term monitoring meeting of E-Rare3 JTC2016 (organized in 2020) and the European Joint Program Networking Support Scheme (EJP NSS) workshop "Identifying systematic obstacles hindering the development of academic-sponsored trials for drug repurposing on rare-diseases." The E-rare3 2020 mid-term Monitoring Meeting followed the development and advances of the projects granted on the E-Rare3-Joint Translational Call 2016 ("Clinical research for new therapeutic uses of already existing molecules-repurposing-in Rare Diseases"). This was the first time that a Rare Diseases Joint Transnational Call funded clinical trials and 8 projects were selected. Five of the 8 investigators awarded on this call, who presented the progress of their funded trials in 2020, were later invited to participate in the EJP NSS workshop, aiming to discuss and identify operational hurdles linked to the development of multinational academic-sponsored trials for rare diseases. Additionally, the NSS event included the communication of the DevelopAKUre drug development program. This program was not funded through the E-Rare3-JTC2016 but comprised three multinational academic-sponsored trials (SONIA1, SONIA2 and SOFIA) for drug repurposing partly funded by the EU (FP7 program) and was considered of high interest to reach the goals of the NSS event. Thus, six academic-sponsored multinational RCTs for rare diseases were selected as use cases to describe operational hurdles (Table 1). The 3 trials integrating the DevelopAKUre program were considered all together as one "use-case," provided that they were all coordinated by the same team and had a similar operational set up.

All 6 use cases were selected based on the following criteria:

- Multinational clinical trial for rare diseases
- Investigator-initiated trial
- Drug repurposing

and availability of their coordinators to participate in the EJP NSS workshop.

Table 1 Summary of the 6 academic-sponsored multinational RCTs for rare diseases selected as use cases to describe operational hurdles

Project	Trial/ClinicalTrials.gov (NCT)	Protocol/results
HCQ4SurfDefect	Hydroxychloroquine (HCQ on Paediatric Interstitial Lung Disease (ILD) (HCQ-chILD-EU) NCT02615938	Prospective evaluation of hydroxychloroquine in pediatric interstitial lung diseases: Study protocol for an investigator-initiated, randomized controlled, parallel-group clinical trial [7] Randomized controlled phase 2 trial of hydroxychloroquine in childhood interstitial lung disease [8]
Redox	A trial of Doxycycline vs. Standard Supportive Therapy in Newly-diagnosed Cardiac AL Amyloidosis Patients Undergoing Bortezomib-based Therapy NCT03474458	Protocol not published
ROPROP	Oral propranolol for prevention of threshold retinopathy of prematurity (ROPROP) NCT03083431	Oral propranolol for prevention of threshold retinopathy of prematurity (ROPROP): protocol of a randomized controlled trial [9]
ROCK ALS	Inhibition of Rho Kinase (ROCK) with fasudil as disease-modifying treatment for ALS (ROCK-ALS) NCT03792490	ROCK-ALS: Protocol for a Randomized, placebo-controlled, double-blind Phase IIa trial of safety, tolerability and efficacy of the Rho kinase (ROCK) inhibitor fasudil in Amyotrophic Lateral Sclerosis [10]
TAM DMD	Tamoxifen in Duchenne Muscular Dystrophy (TAMDMD) NCT03354039	Tamoxifen in Duchenne muscular dystrophy (TAMDMD): study protocol for a multicenter, randomized, placebo-controlled, double-blind phase 3 trial [11]
DevelopAKUre Program SONIA 1	Dose response study of nitisinone in Alkaptonuria (SONIA 1) NCT01828463	Suitability of nitisinone in alkaptonuria 1 (SONIA 1): an international, multicenter, randomized, open-label, no-treatment controlled, parallel-group, dose-response study to investigate the effect of once daily nitisinone on 24-h urinary homogentisic acid excretion in patients with alkaptonuria after 4 weeks of treatment [12]
DevelopAKUre Program SONIA2	Suitability of nitisinone in alkaptonuria 2 (SONIA 2) NCT01916382	Efficacy and safety of once-daily nitisinone for patients with alkaptonuria (SONIA 2): an international, multicenter, open-label, randomized controlled trial [13]
DevelopAKUre Program SOFIA	SOFIA	Subclinical ochronosis features in Alkaptonuria: A cross-sectional study [14]

Coordinating investigators were asked to provide feedback regarding main obstacles hindering the development of the trials according to the following categories and sub-categories:

1) Study set up

- Study design
- Funding and funding mechanism
- Sponsorship
- Drug procurement
- Project management
- Data management
- Vigilance
- Monitoring

2) Country set up

- Country selection
- National Competent Authority (NCA)/Ethics Committee (EC) approval
- Insurance
- GCP requirements

3) Site set up

- Site agreements
- Site compliance

This categories' classification is based on main pre-identified barriers described in previous publications:

- Articles describing systematic barriers, opportunities, and lessons learned in the set-up of academic-sponsored international multi-center clinical trials for rare diseases [15–17]
- Systematic reviews aiming to identify barriers to the conduct of RCT (Table 2)

Table 2 Major barriers to the conduct of randomized trials identified by systematic reviews

Major barriers to the conduct of randomized trials	**Source**
Inadequate funding	Duley et al. (2008) [1], Djurisic et al. (2017) [2], Alemayehu et al. (2018) [4]
Complex/not harmonized regulations	Duley et al. (2008) [1], Djurisic et al. (2017) [2], Alemayehu et al. (2018) [4]
Excessive/non-focused monitoring	Duley et al. (2008) [1], Djurisic et al. (2017) [2]
Over-restrictive interpretations of privacy laws without evidence of subject benefit/Lack of transparency	Duley et al. (2008) [1], Djurisic et al. (2017) [2]
Inadequate understanding of methodology	Duley et al. (2008) [1]
Inadequate identification of the clinical research questions	Djurisic et al. (2017) [2]
Inadequate knowledge and understanding of clinical research	Djurisic et al. (2017) [2]
Inadequate knowledge and understanding of clinical trials	Djurisic et al. (2017) [2]
Inadequate infrastructures	Djurisic et al. (2017) [2], Alemayehu et al. (2018) [4]
Unsupportive administrative system	Alemayehu et al. (2018) [4]
Competing demands	Alemayehu et al. (2018) [4]
Difficult patient recruitment	Alemayehu et al. (2018) [4]
Major barriers to the conduct of RCTs in rare diseases	**Source**
Difficult to recruit patients due to rarity	Rath et al. (2017) [3]
Incomplete understanding of national history to inform trial design	Rath et al. (2017) [3]
Need for trial designs adapted to small population size and clinical heterogeneity	Rath et al. (2017) [3]
Organizational challenges as a consequence from the need for multinational randomized clinical trials	Rath et al. (2017) [3]
Need for more sensitive outcome measures to quantify disease	Rath et al. (2017) [3]
Need for involvement of all the stakeholders in the study design and conduct	Rath et al. (2017) [3]

Barriers identified by these 7 sources were all compiled. All listed barriers were asked to be considered for the "use cases" analysis.

Data were collected from investigator's presentations during the NSS meeting and the discussions held after the "use cases" presentations. Presentations were structured as per the listed categories. Information was structured as shown in Table 3, which was fully reviewed by all investigators during the manuscript's preparation, to ensure accuracy.

Investigators were also asked to provide feedback regarding any possible additional barrier not previously identified.

Results and discussion

Table 3 summarizes the main findings on the analysis of the 6 RCTs presented and discussed during the 2020 E-Rare3 and EJP RD NSS meetings.

Study set up

Study design

Lack of natural history studies to inform about trial design, lack of registries and patient reported outcome measures are some of the possible obstacles to choose an appropriate study design [3, 17]. Patient groups are very small and heterogeneous and the risk of being underpowered to test efficacy is high. The need to develop cost-efficient and novel trial designs and analysis to study efficacy in heterogeneous, small populations has led to the development of specific research programs funded by the European Commission to work on these topics [18–20], and the International Rare Diseases Research Consortium (IRDiRC) has published recommendations for the design of small population clinical trials [21]. Nevertheless, implementation of novel methodologies might still be suffering of "methodology aversion" [22] that jeopardizes the regulators' acceptance of useful methods that might not be fully understood by stakeholders not involved in its development. As an attempt to improve the dialogue between academia and regulators the STARS project [23] aims to reach out to medicine innovators in academia to bridge the regulatory knowledge gap which might prevent the access of patients to these innovations.

Only one of the coordinating investigators of the RCTs analyzed in this work reported the study design as an obstacle. This perception might reflect that this challenge prevents investigators to opt for innovative trial designs and/or embark on setting up the trial, even from applying for funding. Thus, this topic might have been disregarded as a constraint by the investigators involved in these studies. It is worth noting that two of the studies

Table 3 Major barriers to the conduct of randomized trials for rare diseases identified by use cases on RCT for drug repurposing

STUDY SET UP	Comments/rational	Identified as a hurdle (trial) (number of trials)
Study design	Lack of natural history studies to inform about trial design Lack of registries validated surrogate outcomes and patient reported outcome measures to choose an appropriated study design Small and heterogeneous patients groups (risk of being underpowered to test efficacy)	HCQ4SurfDefect (1/6)
Funding and funding mechanism	Non-commercial sponsors often face budgetary problems due to lack of public funding opportunities and/or poor flexibility of the external funding	ROP ROP, Redox, DevelopAKUre, ROCK-ALS, TAM DMD, HCQ4SurfDefect (6/6)
Sponsorship	Lack of experience and/or insufficient knowledge of academic organizations about sponsor responsibilities in a multinational setting Lack of trial-specific legal support Co-sponsorship not yet possible in Europe	Redox, ROCK-ALS, HCQ4SurfDefect (3/6)
Drug procurement	Tendering process to select drug supplier Custom restrictions Country-specify requirements for data on drug stability Lack of placebo suppliers	Redox, ROCK-ALS, HCQ4SurfDefect (3/6)
Project management	Lack of experience in multinational trials in legal affairs department	ROP ROP (1/6)
Data management	Lack of harmonization among countries Lack of experience on multinational trials from selected data centers	Not reported
Vigilance (PV)	Lack of harmonization among countries Lack of experience on multinational trials from selected PV center	Redox (1/6)
Monitoring	Lack of harmonization on monitoring plan requirements	Not reported
COUNTRY SET UP		
Country selection	Scarcity of patients leads to large-scale studies, i.e., multicenter/multinational set-ups	Not reported
NCA/EC approval/regulatory	Timing/unexpected delays Lack of harmonization	ROP ROP, ROCK-ALS, TAM DMD (3/6)
Insurance	Lack of harmonization on requirements, minimum coverage varies among EU countries	ROCK-ALS (1/6)
Good Clinical Practice (GCP) requirements	Lack of harmonization on GCP training content/extent	ROP ROP (1/6)
SITE SET UP		
Site agreements	Lack of harmonization on site agreements templates even within the same country Country-specific terminology/interpretation (legal responsibilities, intellectual/industrial property, GDPR) included in site contracts, reference to the national, legal and regulatory framework (liability and insurance), templates tailored to industry sponsored trials, need to align with Consortium/Grant Agreements	ROP ROP, Redox, TAM DMD, HCQ4SurfDefect (4/6)
Site compliance	Lack of personnel experienced in clinical research Lack of personnel involved in academic trials Personnel's turnover due to trial's extensions	Not reported

(DevelopAKUre program studies SONIA 1, SONIA 2 and SOFIA and TAM DMD trials) looked for scientific advice (EMA and/or National Competent Authority), taking advantage of the several opportunities opened by EMA and National Competent Authorities (NCA) to support investigators in protocol development, with special focus

on academic and SMEs-initiated trials, rare diseases trials, and trials on drug repurposing. EMA charges a fee for scientific advice, which varies depending on the scope of the advice.

Reductions apply for certain types of medicines and applicants, including a 75% fee reduction for medicines for orphan medicines and a 90% fee reduction for SMEs. Since 2020, academia is eligible for EMA protocol assistance (as a special form of scientific advice) free of charge for the development of orphan medicines. Some national competent authorities in Europe (for instance AEMPs, Spain) support informal consultations free of charge for investigator-initiated trials. More recently, in October 2021, a coordinated pilot program between EMA and HMA (Heads of Medicines Agencies) has been launched to support the repurposing of medicines [24]. The aim of this initiative is to support not-for-profit organizations and academia to gather or generate sufficient evidence on the use of an established medicine in a new indication with the view to have this new use formally authorized by a regulatory authority.

Funding and funding mechanism

While this may be a less pertinent problem for industry-funded trials, funding has been identified as a main challenge for all trials (Table 3). In the rare diseases area, the small patient population can dampen commercial interest, making this challenge more relevant for non-commercial RTC for rare diseases.

Five out of the six studies analyzed in this work were mainly funded through the E-Rare-3 JTC2016, based on a multinational funding scheme by national funding agencies (a "virtual common pot" mechanism, whereby each national funding body funds the national component of the trial, with no cross-border funding), while the DevelopAKUre program were granted under a central EU funding mechanism.

Some trials were funded through additional sources. HCQ4surfdefect trial was supported by three different funding schemes, in part consecutively, in part in parallel in full mutual recognition of the funders. For ROCK-ALS, investigators collected more than 100.00 EUR of donations to reimburse participants for travel to and from the trial site as well as for hotel costs. TAM DMD was supported also for two patients' associations and the DevelopAKUre program got both European (FP7) and national (NHS) funding.

Restrictions on the use of national funds for cross-cutting services and tendering rules were identified as important hurdles. Investigators funded through the multinational funding scheme agreed on the need of a funding scheme that allows for a mechanism (a real common pot, or a co-funding from the EU central budget, or any other funding source not restricted to a single country) to account for crosscutting activities and to allow flexibility in the budget allocation, reducing the administrative constraints of working with country-specific budgets. This administrative burden, the lack of flexibility to move transnationally funds, upper limits in the amount, and the duration of the funding hinder trial management, which often requires fast and frequent adaptation to changes in the recruitment rates, changes on exchange rates, different value added taxes (VATs), and other reasons out beyond control of investigators. This will have to be taken into consideration in the design of the ERA4HEALTH partnership, where a pillar will be dedicated to the funding (with an EU cofund) of multinational investigator-initiated clinical studies. Even though some sponsors underestimate the budget, in reality, many cost items may be impossible to foresee in advance. Poor awareness of Good Clinical Practice (GCP) requirements may lead sponsors to request insufficient financial resources from the funding agency, but in other cases, the budget awarded by the funding agency is much lower than the initially requested one and may be insufficient to meet all the cost required for full GCP compliance, i.e., adequate external monitoring or data management set up. Another cause for underestimation of the budget by a sponsor may also be the perception that a lower budget would decrease the likelihood of rejection. "Inadequate funding" was already identified as a barrier by several systematic reviews, but these sources referred mainly to the total cost estimation and did not specifically point out the lack of flexibility of the "funding mechanism" linked to public funding as a key obstacle.

Sponsorship

For non-industry sponsored trials, the sponsor is usually the institution to which the investigator is affiliated, a research foundation, and/or patient's organization. Some of these academic/public institutions have previous experience and specific personnel to work as Sponsor's representatives, but in other cases, the coordinating investigator assumes the sponsor's role.

Three out of the six studies reported challenges linked to sponsor's responsibilities: insufficient legal support from academic institutions acting as sponsors (Redox), no possible co-sponsorship (ROCK-ALS), and insufficient experience in quality assurance requirements (HCQ4SurfDefect) were identified as major hurdles.

For academic sponsored trials, generally one agency (or more in the case of the E-Rare funding scheme, which supported five of the six analyzed studies) provides the funding and another organization (usually the one that hosts the coordinating investigator) designs and conduct the trial in collaboration with research partners.

The set-up of a complex multi-institutional partnership may sometimes be at odds with the concept of "single sponsor" which was developed for ensuring the protection of participants in the context of commercial trials. Indeed, single sponsorship is rooted in the need to clearly identify the legal responsibility and does not hinge upon the funding aspect. The ambiguity between the terms "funder" and "sponsor" [25] might contribute to the poor awareness of some inexperienced sponsors, especially in the non-commercial sector, of the scope of their own responsibilities. The legal sponsor is ultimately responsible for the scientific, ethical, regulatory, and legal aspects of the trial and also for the financial aspects (i.e., if an external funder withdraws, the sponsor will be responsible to look for funds to complete the trial). It is therefore its primary role and responsibility to ensure that sufficient resources are planned for full compliance with ethical and GCP requirements.

Interestingly, none of the previous systematic reviews had identified aspects linked to sponsorship as a main challenge, probably due to the fact that these reviews did not make a distinction between academic and industry sponsored trials. According to the provisions of the 536/2014 Clinical Trial Regulation, co-sponsorship should be an option to facilitate the sharing of tasks and responsibilities in multinational investigator-initiated trials.

Drug procurement

Drug procurement is generally perceived as a hurdle (four out of six investigators reported it as a challenge) due to (a) the lack of harmonization among countries on the requirements for Quality assurance, i.e., stability of the drug after re-conditioning, re-formulation, (b) need for a tendering process, and (c) the high cost of drug distribution. The ROPROP and the HCQ4SurDef studies were both requested to present additional stability data for repurposed drugs only in some countries. Redox's trial coordinator perceived the tendering process as especially cumbersome, and the ROCK-ALS coordinator described as a special challenge the procedure to import the drug from a non-European country.

A recent meta-research study [26] describes a series of challenges in obtaining placebo for investigator-initiated drug trials, including the high cost for production and packaging and the unwillingness of manufactures to provide placebos for RCTs without previous assessment and approval of protocols [27]. Five of the six trials analyzed in this work were placebo-controlled. Two of them received placebo from the drug manufacturer, while for the other three, a hospital pharmacy was involved in the manufacturing and distribution. One investigator reported unwillingness/unavailability of the manufacturer to provide the study drug.

Project management

As reflected by the many aspects linked to the lack of harmonization among countries described in this and previous works, management of multinational trials requires experienced academic clinical trial units (CTUs). In many cases, CTUs, publicly funded at national level to support in-house investigators, have limited experience supporting multi-country studies. Indeed, differential globalization of industry and non-industry sponsored trials has been reported [5] as academic-sponsored trials are mainly conducted in a single country.

Investigators relied in both local academic clinical trial units/research units (four out of six studies) and CROs (two out of six studies) for global project management. Only one coordinating investigator reported project management as a challenge linked to the limited previous experience of the CTU on multinational management. Fortunately, the reported increase in the number of academic-sponsored trials over the last years [5] should lead to an improvement on the investment to promote the important role of academic clinical trial units and international research infrastructures (ref www.ecrin.org) on international clinical trials management.

Data management

Managed by both CTUs and CROS for the analyzed RCTs, only one of the trial coordinators reported data management as a main hurdle. This might reflect sponsor's awareness on the need to maintain a good quality control system, as data management was identified as one of the top critical GCP findings [28] during EMA inspections. Most of the critical findings are related to insufficient quality control (e.g., edit checks) performed on the data captured in the database considering that relevant inconsistencies in the data were not recognized and not followed up.

Vigilance and monitoring

Pharmacovigilance and monitoring was generally not perceived as a hurdle. Only one trial's coordinator described the lack of experience of the initially selected CTU to perform pharmacovigilance at multinational level.

Country set up

Country selection

Country selection was not perceived as a hurdle as such, but some investigators pointed out that the funding scheme might favor the selection of participating countries based on the funding rules (specially for co-fund programs) instead of being driven by a feasibility analysis of adequate country sites, investigators, and patients.

NCA/EC approvals

Timing and lack of harmonization is generally perceived as a major hurdle. The Voluntary Harmonization Procedure (VHP) was conceived to harmonize and coordinate procedures and to streamline the review process of competent authority applications for multi-center/multinational clinical trials, while the implementation of the 2014 CTR (Clinical Trials Regulation) [29] was delayed. However, there is no legal obligation for the EU countries to take part in this process. Investigators were interrogated about the use of VHP for regulatory submission. Interestingly, none of the studies applied for approval through VHP. Moreover, two studies reported having withdrawn VHP as it was perceived as more time consuming and less flexible that single country-per-country applications even though the procedure has received increasing acceptance since its introduction in 2009 [30]. Published sponsor's experience on the use of VHP is scarce, and while the first commercial sponsor to use VHP reported a positive experience [31], others did not report special benefit over the use of multiple national applications [32]

Insurance coverage

Insurance or indemnity to cover the liability of the investigator and sponsor must be secured before the study commences. The "obligatory insurance" was introduced in Europe with the implementation of Directive 2001/20/EC. This insurance was meant to cover the sponsor in case of liability for injury or death to participants and the terms depend on the individual risk and number of patients. In the USA, institutions are usually covered by their institution liability insurance. Cost and discordant requirements for insurance and indemnification in Europe has been previously reported to challenge the RCT set up at country level [15, 16] leading to recommendations to better inform investigators and sponsors about the rational for the requirements in each country [33]. Interestingly, with the 2014 Clinical Trials Regulation [29], national indemnification is not applicable to low-intervention clinical trials. While this might favor especially implementation of academic-sponsored RTC on drug repurposing, the definition of "low-intervention trial" still requires a determination as to which trials pose "minimal additional risk or burden." For now, the term seems open to interpretation, which might hinder the financial benefit of "insurance exemption".

Insurance handling was nevertheless not perceived as a hurdle. E-Rare-3 funded studies planned insurance handling as a national coordinator's responsibility, facilitating country-specific contracting and preventing the sponsor from having to deal with not-harmonized rules in terms of coverage requirements.

Good clinical practice (GCP) training

One study highlighted lack of harmonization on requirements/content of GCP training. While European Clinical Trials legislation requires all clinical trials being conducted in accordance with the principles of GCP, personnel GCP training is required to get the study approved and/or initiated in all countries. Nevertheless, the content and extent of GCP training, the site personnel requiring the training, and the requirements to obtain GCP certificates are not harmonized, varying from country to country [34]. Indeed, while most commercial sponsors have developed sponsor-specific GCP training based on industry-agreed standards (Transcelerate mutual recognition) [35], ethics committees might require and/or recommend for both commercial and academic trials, specific training adapted to local specificities and in local language.

Site set up

Site agreements

Generally perceived as a hurdle linked to multiple aspects as:

- Lack of harmonization on site agreement templates, even within the same country
- Country-specific terminology and interpretation of legal responsibilities, intellectual/industrial property, GDPR (General Data Protection Regulation)
- Reference to the national legal and regulatory framework (liability and insurance)
- Cost variability (applicable taxes/overheads)
- Translations
- Templates tailored to industry-sponsored trials, not aligned with Consortium and Grant Agreements linked to publicly funded studies

Site agreement management challenges especially academic sponsor's legal departments due to the lack of experienced personnel and/or limited resources.

Four out the six trials highlighted site contracting as a main operational constraint. Local site agreements often create the greatest time delay, and pragmatic, sensible legal, and administrative solutions are required. In this sense, the development of a pan-European site agreement template has been proposed [32]. Acceptance of this template by implementing site/institutions could be an eligibility criterion for publicly funded/multinational trials in the EU.

Site compliance

Many academic researchers at academic institutions struggle with short-term employment contracts and the

pressure to publish rapidly. Engagement in long-term strategic clinical research might be considered as counterproductive for the individual clinician because results might remain unpublishable for many years. Lack of personnel to work in academic-sponsored trials was not considered a hurdle for the analyzed use cases.

Conclusions

Multinational clinical trials for drug repurposing in rare diseases are mostly initiated by academia [6], but sponsors and investigators leading these trials face a wide range of challenges than hinder patient's access to drugs. Main hurdles are more operational than scientific, ranging from regulatory submission to site contracting. Sponsorship requirements as well as "funding mechanism" have not been pointed out before as an important challenge for academia. Limited funding had been previously described as an obstacle but it is actually the public funding mechanism, without cross-border budget flexibility, more than the total amount, what is perceived as an important hurdle. There are no main clinical trials' academic-sponsor advocacy groups in Europe and academy have very little presence in clinical trials' European forums, which has led to the development of regulations and guidelines that might suit better pharmaceutical industry's needs. A better knowledge of the non-commercial clinical research landscape and its challenges and requirements is needed to achieve RD academic clinical research's main goal, which is making drugs—especially those with less commercial gain—accessible to rare diseases patients. Better information about existing resources like research infrastructures, clinical research programs, and counseling mechanisms is needed to support and guide clinicians through the many challenges associated to the set-up of academic-sponsored multinational trials.

Abbreviations

AEMPS: Agencia Española de Medicamentos y Productos Sanitarios (Spanish drug and medical devices agency); CRO: Clinical Research Organization; CTU : Clinical trial unit; EC: Ethics Committee; EJP: European Joint Programme on Rare Diseases; EMA: European Medicines Agency; EU: European Union; FP7: 7th Framework Program; GCP: Good Clinical Practice; GDPR: General Data Protection Regulation; HMA: Heads of Medicines Agencies; NCA: National Competent Authority; NCT: National clinical trial; NHS: National Health Service; RCT: Randomized clinical trial; SME: Small and medium-sized enterprises; VHP: Voluntary Harmonization Procedure.

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Authors' contributions

CB, DF, MG, PL, GP, and NS analyzed and summarized the barriers to conduct the RTCs that they coordinated. MdA, LS, and KN consolidated, analyzed, and summarized the results. MdA wrote the manuscript. All authors participated in the discussions, read and critically revised the manuscript for important intellectual content, and approved the final version for publication.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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References

1. Duley L, Antman K, Arena J, Avezum A, Blumenthal M, Bosch J, et al. Specific barriers to the conduct of randomized trials. Clin Trials. 2008;5(1):40–8.
2. Djurisic S, Rath A, Gaber S, Garattini S, Bertele V, Ngwabyt SN, et al. Barriers to the conduct of randomised clinical trials within all disease areas. Trials. 2017;18(1):360.
3. Rath A, Salamon V, Peixoto S, Hivert V, Laville M, Segrestin B, et al. A systematic literature review of evidence-based clinical practice for rare diseases: what are the perceived and real barriers for improving the evidence and how can they be overcome? Trials. 2017;18(1):556.
4. Alemayehu C, Mitchell G, Nikles J. Barriers for conducting clinical trials in developing countries- a systematic review. Int J Equity Health. 2018;17(1):37.
5. Atal I, Trinquart L, Porcher R, Ravaud P. Differential globalization of industry- and non-industry–sponsored clinical trials. Lexchin J, editor. PLoS One. 2015;10(12):e0145122.
6. van den Berg S, de Visser S, Leufkens HGM, Hollak CEM. Drug repurposing for rare diseases: a role for academia. Front Pharmacol. 2021;12:746987.
7. Griese M, Köhler M, Witt S, Sebah D, Kappler M, Wetzke M, et al. Prospective evaluation of hydroxychloroquine in pediatric interstitial lung diseases: study protocol for an investigator-initiated, randomized controlled, parallel-group clinical trial. Trials. 2020;21(1):307.
8. Griese M, Kappler M, Stehling F, Schulze J, Baden W, Koerner-Rettberg C, et al. Randomized controlled phase 2 trial of hydroxychloroquine in childhood interstitial lung disease. Orphanet J Rare Dis. 2022;17(1):289.
9. Bührer C, Erdeve Ö, Bassler D, Bar-Oz B. Oral propranolol for prevention of threshold retinopathy of prematurity (ROPROP): protocol of a randomised controlled trial. BMJ Open. 2018;8(7):e021749.
10. Lingor P, Weber M, Camu W, Friede T, Hilgers R, Leha A, et al. ROCK-ALS: protocol for a randomized, placebo-controlled, double-blind phase IIa trial of safety, tolerability and efficacy of the rho kinase (ROCK) inhibitor fasudil in amyotrophic lateral sclerosis. Front Neurol. 2019;10:293.
11. Nagy S, Hafner P, Schmidt S, Rubino-Nacht D, Schädelin S, Bieri O, et al. Tamoxifen in Duchenne muscular dystrophy (TAMDMD): study protocol for a multicenter, randomized, placebo-controlled, double-blind phase 3 trial. Trials. 2019;20(1):637.
12. Ranganath LR, Milan AM, Hughes AT, Dutton JJ, Fitzgerald R, Briggs MC, et al. Suitability of Nitisinone in Alkaptonuria 1 (SONIA 1): an international, multicentre, randomised, open-label, no-treatment controlled, parallel-group, dose-response study to investigate the effect of once daily nitisinone on 24-h urinary homogentisic acid excretion in patients with alkaptonuria after 4 weeks of treatment. Ann Rheum Dis. 2016;75(2):362.
13. Ranganath LR, Psarelli EE, Arnoux JB, Braconi D, Briggs M, Bröjersén A, et al. Efficacy and safety of once-daily nitisinone for patients with alkaptonuria (SONIA 2): an international, multicentre, open-label, randomised controlled trial. Lancet Diab Endocrinol. 2020;8(9):762–72.
14. Cox T, Psarelli EE, Taylor S, Shepherd HR, Robinson M, Barton G, et al. Subclinical ochronosis features in alkaptonuria: a cross-sectional study. BMJ Innov. 2019;5(2–3):82.
15. Crow RA, Hart KA, McDermott MP, Tawil R, Martens WB, Herr BE, et al. A checklist for clinical trials in rare disease: obstacles and anticipatory actions—lessons learned from the FOR-DMD trial. Trials. 2018;19(1):291.
16. Lingor P, Koch JC, Statland JM, Hussain S, Hennecke C, Wuu J, et al. Challenges and opportunities for multi-National Investigator-Initiated clinical trials for ALS: European and United States collaborations. Amyotrop Lateral Sclerosis Frontotemp Degen. 2021;22(5–6):419–25.
17. Griggs RC, Batshaw M, Dunkle M, Gopal-Srivastava R, Kaye E, Krischer J, et al. Clinical research for rare disease: opportunities, challenges, and solutions. Mol Genet Metab. 2009;96(1):20–6.
18. Hilgers RD, Bogdan M, Burman CF, Dette H, Karlsson M, König F, et al. Lessons learned from IDeAl — 33 recommendations from the IDeAl-net about design and analysis of small population clinical trials. Orphanet J Rare Dis. 2018;13(1):77.
19. Gagne JJ, Thompson L, O'Keefe K, Kesselheim AS. Innovative research methods for studying treatments for rare diseases: methodological review. BMJ. 2014;349(nov24 15):g6802.
20. Friede T, Posch M, Zohar S, Alberti C, Benda N, Comets E, et al. Recent advances in methodology for clinical trials in small populations: the InSPiRe project. Orphanet J Rare Dis. 2018;13(1):186.
21. Day S, Jonker AH, Lau LPL, Hilgers RD, Irony I, Larsson K, et al. Recommendations for the design of small population clinical trials. Orphanet J Rare Dis. 2018;13(1):195.
22. Bauer P, König F. The risks of methodology aversion in drug regulation. Nat Rev Drug Discov. 2014;13(5):317–8.
23. Starokozhko V, Kallio M, Howell ÅK, Salmi AM, Andrew-Nielsen G, Goldammer M, et al. Strengthening regulatory science in academia: STARS, an EU initiative to bridge the translational gap. Drug Discov Today. 2021;26(2):283–8.
24. Asker-Hagelberg C, Boran T, Bouygues C, Eskola SM, Helmle L, Hernández C, et al. Repurposing of medicines in the EU: launch of a pilot framework. Front Med. 2022;8:817663.
25. Ravinetto R, De Nys K, Boelaert M, Diro E, Meintjes G, Adoke Y, et al. Sponsorship in non-commercial clinical trials: definitions, challenges and the role of good clinical practices guidelines. BMC Int Health Hum Rights. 2015;15(1):34.
26. Speich B, Logullo P, Deuster S, Marian IR, Moschandreas J, Taji Heravi A, et al. A meta-research study revealed several challenges in obtaining placebos for investigator-initiated drug trials. J Clin Epidemiol. 2021;131:70–8.
27. Christensen M, Knop FK. The unobtainable placebo: control of independent clinical research by industry? Lancet. 2012;379(9810):30.
28. Classification and analysis of the GCP inspection findings of GCP inspections conducted at the request of the CHMP. 2014 INS/GCP/46309/2012 Compliance and Inspections.
29. The European Parlament and the European Union. Regulation (EU) No 536/2014 of the European parliament and of the council of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC. 2014;
30. Krafft H, Bélorgey C, Szalay G. Experience and further development with the voluntary harmonization procedure for multinational clinical trials in the European Union. Nat Rev Drug Discov. 2012;11(5):419.
31. Amexis G, Schmitt E. A sponsor's experience with the voluntary harmonization procedure for clinical trial applications in the European Union. Nat Rev Drug Discov. 2011;10(5):393.
32. Diallo A, Traseid M, Simensen VC, Boston A, Demotes J, Olsen IC, et al. Accelerating clinical trial implementation in the context of the COVID-19 pandemic: challenges, lessons learned and recommendations from DisCoVeRy and the EU-SolidAct EU response group. Clin Microbiol Infect. 2022;28(1):1–5.
33. Neaton JD, Babiker A, Bohnhorst M, Darbyshire J, Denning E, Frishman A, et al. Regulatory impediments jeopardizing the conduct of clinical trials in Europe funded by the National Institutes of Health. Clin Trials. 2010;7(6):705–18.
34. Magnin A, Iversen VC, Calvo G, Čečetková B, Dale O, Demlova R, et al. European survey on national training activities in clinical research. Trials. 2019;20(1):616.
35. Minimum Criteria for ICH E6 (R2) GCP Investigator Site Personnel Training (Excerpt from TransCelerate BioPharma Operating Principles). Version 2.0 [Internet]. 2017. Available from: https://www.transceleratebiopharmainc.com/wp-content/uploads/2017/03/GCP-MR-Minimum-Criteria-R2_FINAL.pdf

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7.4 Annex 4

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Summary

Purpose:

The aim of this study was to develop and evaluate a structured educational programme (CONSCIOUS II) designed to enhance the competencies required for conducting high-quality investigator-initiated clinical trials (IICTs). The study addresses a significant gap in clinical research education, particularly for PhD students and early-career researchers who often lack formal, practice-oriented training in trial methodology and management.

Methodology:

The programme was developed as a multidisciplinary, competency-based curriculum consisting of 12 core modules covering key aspects of clinical trials, including design, methodology, regulatory affairs, pharmacovigilance, and leadership. It employed a blended learning design, combining asynchronous e-learning materials with a structured international pilot course and interactive sessions; the programme was subsequently evaluated using participant questionnaires and external expert assessment of the educational materials.

Results:

The study resulted in a comprehensive and freely accessible curriculum, supported by an online platform that enables dissemination and adaptation across institutions. Evaluation outcomes demonstrated consistently positive feedback from participants and stakeholders, indicating improved understanding of clinical trial processes, enhanced practical skills, and high perceived relevance of the training content.

Conclusion:

The CONSCIOUS II programme effectively addresses the educational gap in training future clinical trialists by providing a scalable, interdisciplinary, and practice-oriented learning model. Its implementation has the potential to improve the quality, feasibility, and overall impact of NCCTs by strengthening investigator competence and supporting the development of skilled research teams.

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BMC Medical Education

RESEARCH Open Access

Enhancing pragmatic competence in investigator-initiated clinical trials: structure and evaluation of the CONSCIOUS II training programme

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Abstract

Introduction Investigator-initiated trials (IITs) bridge the gap between applied clinical research and everyday clinical practice. However, they require the skilled multidisciplinary teams from different backgrounds but all with clinical trial training to ensure trials are designed, conducted and reported according to best practice and regulatory standards. The availability of trainings to fulfil these needs is limited. The CONSCIOUS II project facilitated to expand the supply of such programmes. The objective is to describe the curriculum designed for PhD students and early-career researchers, and evaluate participants' perceptions and feedback after completion of the training.

Methods The curriculum was developed according to key principles that underpin building of competencies relevant to quality IITs and transdisciplinary skills. A multidisciplinary team created the curriculum, elaborated a comprehensive set of study materials, including the training platform. This team also conducted an international, collaborative pilot course. The effectiveness of the educational materials for the target audience was assessed through questionnaires administered after the pilot course. Additionally, all learning materials, including the video recordings of the pilot course, were externally evaluated.

Results A 12-chapter thoroughly revised curriculum was developed for asynchronous preparation and served as a pre-class reading for a 3-month pilot course. The chapters, along with supplementary materials, and recordings of the pilot course are freely accessible on the CONSCIOUS II training platform. This platform facilitates the dissemination and implementation in the existing curricula. The feedback from both the pilot course participants and the stakeholders was uniformly positive across all survey aspects.

Conclusion This remote programme which combines asynchronous and synchronous components with international and interprofessional collaboration effectively addresses the gap in developing core competencies for

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the 21st -century clinical researchers. The implementation of this curriculum has the potential to improve the quality of IITs.

Keywords Investigator-initiated trials, Curriculum, Early-career researchers, Practice-oriented

Introduction

Investigator-initiated trials (IITs) are essential for patient-centered research, bridging the knowledge gap between clinical trials and real-world scenarios [1–4]. These studies, typically driven by clinical practice or academia interest, address the challenges and needs of everyday practice, often focusing on underserved populations or therapeutic specific areas. In a wider context, IITs contribute significantly to improving clinical practices. Their value lies in providing unbiased evaluations free from commercial interests, which helps in developing accurate clinical guidelines, formulating population-specific policies and repurposing existing drugs [1–4]. This approach enables the adoption of impartial and sustainable health policies, offering both direct and indirect benefits to patients and enhance the efficiency of the healthcare system. However, for such medical research, it is necessary to educate the trial leaders of the future [3]. In IITs, the role of the principal investigator (PI) extends beyond orientation in the field and critical evaluation of available literature to design quality trials. They typically assume the role of the sponsor and take responsibility for the coordination and management of the entire study through their parent organization [5].

Education in clinical research is not systematic compared to medical education and often relies on the mentor-mentee relationship or experience gained as a sub-investigator [4, 6–8]. Unfortunately, mentoring in IITs is constrained by dramatic changes in the clinical trial landscape, regulatory framework, and ever-evolving quality demands. Repetitive, one-size-fits-all training like the GCP certificate, a standard mandatory qualification requirement for PIs and any staff of the team in a clinical trial, is often perceived as a necessity with little added value due to its generality and didacticism [7]. There is a general consensus on the ideal direction for developing clinical investigator training: it should be individualized and flexible, based on real-world applications of GCP guidelines and real-world problems. Training should take into account the diverse experiences of participants and focus on building practical skills, including non-traditional training modalities [3, 7, 9, 10]. At the same time, it is important to reflect that for many investigators, research is somewhat secondary to their own clinical work, and therefore, the goal should be to maximize the content-, time-, and cost-effectiveness of training [9]. The format of the training is also important, especially with innovative approaches (e.g., learning by doing, peer-to-peer training) significantly enhancing training effectiveness [7].

The CONSCIOUS II project facilitates a practice-oriented, and pragmatic learning program applying innovative teaching methods to the widest possible academic and non-academic stakeholders community. The curriculum provides support for designing, implementing, conducting, managing and reporting IITs. A broad consortium of partners with extensive expertise in IITs management, clinical research methodology and IITs design, data science, clinical pharmacology, teaching, and research in medical education formulated the CONSCIOUS II curriculum. This dynamic set of skills enabled the creation of a unique curriculum in terms of content and format. The objective of this paper was to (1) describe the developed curriculum, (2) summarize participant's perceptions after completion, and (3) review stakeholders' feedback.

Methods

Curriculum design

The development of the CONSCIOUS II curriculum began with the conceptualization of key principles and ideas based on the project objectives and the target population. This included a careful selection of chapter topics focused on building general competencies applicable to all clinical trials, specific types of clinical trials, and transdisciplinary skills. The primary target population includes PhD students, early-career researchers as potential IITs PIs or members of project or study teams, as well as teachers and lecturers. At first, an outline was created for each chapter with an executive summary, aims, learning outcomes, and complementarity of the new materials with those already available from the previous CONSCIOUS project [11]. These outlines now accompany each chapter, providing a clear framework and context. The second step was to define the structure of each chapter: core content, additional/advanced content (explanatory, expanding information, providing further context), and the activities (exercises, case reports, discussion boards, quizzes to practice theoretical knowledge or skills). The third step focused on the teaching methods implemented in the chapters, the requirements for the training platform to support interactivity and communication between participants, and the techniques used in the pilot course. The next step was implementing the graphic design, including promotional materials (leaflets and interview videos with the primary authors). In

addition, the individual chapters and video-recordings of the pilot lessons are licensed under CC-BY-SA 4.0.

All materials underwent multiple revisions. The first outputs were outlines. The outlines were first discussed within the consortium. A revised version was submitted to European Clinical Research Infrastructure Network (ECRIN) experts for review [12]. Based on the comments, the first official version of each chapter outline was issued. The chapter was then prepared according to this proposal. It went through the same review procedure. Once the chapter was completed, the outline was modified where necessary. This revised material served as training material for the participants of the pilot course. After the pilot course, the chapters and outlines were again revised and aligned.

An educational platform was created to consolidate all materials in one place, enhance the digital competencies of the target population, and facilitate the implementation of the curriculum into existing programs. Moreover, such a platform preserves the interactivity of the content, allows interaction between participants with each other or between lecturers and participants (discussion boards) regardless of their geographical location.

The pilot course

The pilot course was designed as an international, interdisciplinary, and collaborative course. The course was intended as a flipped classroom where participants were required to independently review the core content of the relevant chapter and complete the exercises in the week preceding the session. This independent preparation was followed by an online lesson, led by the chapter's primary author. Each online lesson was recorded and an edited version– excluding parts of the teamwork in breakout rooms, time for technical problem solving– became a project output, serving both learning material, and a teacher's guide. The participants obtained a certificate upon fulfilling the conditions of attending more than 60% of lessons and being present more than 60% of the overall time of the lesson.

Curriculum evaluation, stakeholders' feedback

The curriculum evaluation took place at two levels– feedback from participants of the pilot course (i.e., the target population) and feedback from stakeholders (i.e., the knowledgeable population– experienced clinical trialist, university teachers guaranteeing subjects on drug development and clinical research, IITs project managers, national agencies', clinical research organizations', and ECRIN representatives; nobody was involved in preparation of the curriculum). An online questionnaire consisting of 32 and 20 questions, respectively, was developed for both groups. The paticipant questionnaire was divided into five sections– (1) Participant Profile, (2) General Satisfaction, (3) Platform, (4) Pilot Teaching, and (5) Course Improvement; the stakeholder questionnaire was similar but modified in the first (Stakeholder Profile) and fourth sections (Pilot Teaching Records). A five-point Likert-type scale was used for the questions in Sects. 2, 3, and 4 (with range: 1 as "strongly disagree" to 5 "strongly agree"). Two questions were open-ended. The questionnaire was distributed to the pilot course participants immediately after the course, followed by two reminders and a total of 8 weeks to complete it. The stakeholders recieved the questionnaire approx. 3 months later, after all materials and pilot lessons recordings had been completed and uploaded to the training platform, with one-follow-up reminder issued. As a result, participants' feedback covered both asynchronous and synchronous components of the curriculum, while stakeholders' feedback reflected all asynchronous parts, i.e. including video-recordings of synchronous sessions.

Another type of feedback was obtained during three face-to-face dissemination meetings [13–15]. In these meetings, participants were given a general overview of the project and the curriculum. They were then asked to share their views via a questionnaire on the extent to which a similarly focused curriculum was available or established at their institution, its need, and the appropriateness of the chosen topics and format. The answers are scored on a five-point Likert-type scale, with 1 as "strongly disagree" to 5 as "strongly agree".

Results

The curriculum consists of 12 multiply revised chapters divided into three thematic clusters, encompassing approximately 70 h of individual training (see Table 1 for details). The learning materials for the asynchronous work are hosted on the Moodle learning platform (version 3.0) and are also available in a printable format on the project websites (www.conscious2.eu) [16]. The platform is freely accessible, protected by credentials provided upon request by the CONSCIOUS II project contact persons. The chapters are visibly structured in core content, additional/advanced content (highligthed in yellow boxes), and activities (interactive elements in the training platform and blue boxes in the printable version). Each chapter is part of a comprehensive package of materials on the topic that includes: the chapter, the outline, an interview video with the primary author, a video-recording from the pilot course session. These packages are available on both the training platform and the project websites (here, except for the video-recordings from the pilot course). To help navigate the curriculum, learning materials, and organization of the pilot lesson, an introductory session preceded the pilot course and was published on the platform.

Table 1 Curriculum structure and time allocation

Thematic cluster	Chapter	Time required to complete the chapter (in hours)			Duration of the pilot lesson recording (in hours)
		Core content	Additional/ advanced content	Activities/ practical exercises	
General competencies	Clinical Trial Design	2.0	3.0	0.5	1.5
	Trial Methodology Research	0.6	1.5	2.75	1.5
	Trial Management	3.0	1.5	0.5	1.4
	Quality and Regulatory Affairs	0.9	0.3	1.25	1.2
	Pharmacovigilance and Study Medication	1.2	0.3	1.0	1.0
	Data Management and Statistical Analysis	1.0	0.5	–	1.5
Specific types of trials	Early Phase Trials	0.8	1.2	0.7	1.3
	Pediatric Clinical Trials	1.0	0.25	1.0	1.4
	Clinical Evaluation and Clinical Investigation of Medical Devices	1.5	0.5	1.5	0.9
Transdisciplinary skills	Leadership for 21st -Century Trialists	4.0	2.0	0.5	1.4
	Scientific Communication	3.0	1.5	0.5	1.5
	Training the Trainers of the 21st Century	3.0	1.5	0.5	1.3
Average time required per chapter part		1.8	3.0	0.9	1.3
Total time required to complete the chapter part		22.0	36.5	10.7	15.9
Time required to complete all the curriculum materials		85.1			

Table 2 Structure of registered participants according to countries

Country	*n*	%
Czech Republic	84	32.6
Ireland	63	24.4
Portugal	63	24.4
United Kingdom	24	9.3
outside Europe*	8	3.1
Netherlands	4	1.6
Hungary	3	1.2
Slovakia	3	1.2
Serbia	2	0.8
Belgium	1	0.4
Spain	1	0.4
Greece	1	0.4
Italy	1	0.4

* Angola, Brazil, Kenya, Malaysia, Peru, South Africa, Taiwan, USA

Table 3 Structure of registered participants according to the position/role

Position/role	*n*	%
PhD student	114	44.2
Early-career researcher	59	22.9
Clinical trial position*	54	20.9
Teacher/lecturer/supervisor	15	5.8
Undergraduate student	9	3.5
Other	7	2.7

* Project Manager, Data Manager, Pharmacovigilance Specialist, Clinical Research Associate, Investigator, Study Coordinator

The pilot course ran from January to April 2024 comprising 12 sessions that corresponded with the 12 chapters, each approximately 90 min long, held at weekly intervals. A total of 258 participants registered for the pilot course (see Table 2 for geographic distribution and Table 3 for the distribution according to the main position/role). Adherence to the flipped classroom format is summarized in Table S1 (see Supplementary materials). During the pilot course lessons, hands-on activities were implemented in breakout rooms, polls, and quiz questions with subsequent discussion, and moderated interviews with experts as activating didactic approaches. In total, 108 certificates were issued to participants who met the attendance and participation criteria.

Forty-eight of the 108 certified pilot course participants (44.4%) provided the feedback (Table S2 and Table S3 provide details on their structure according to nationality, current level of studies and experience in clinical trials before the course). They expressed consistent satisfaction with the course (mean score 4.40 out of 5), ranked the skills acquired as applicable to their professional practice (mean score 4.25 out of 5), and found the content as easy to understand (mean score 4.27 out of 5). The chapters on Clinical Trial Design, Trial Management, and Leadership for 21st -Century Trialists were rated as the most interesting. Conversely, the chapters on Quality and Regulatory Affairs, Data Management and Statistical Analysis, Clinical Evaluation and Clinical Investigation of Medical Devices were identified as the most challenging to understand. The lowest average rating (3.69 out of 5) was for the adequacy of the time required to complete

all platform materials. Participants also strongly agreed that the pilot course helped them to understand the topic better than the pre-class reading alone (4.50/5). Further details from the participant feedback are in Figure S1 (see Supplementary materials).

The curriculum achieved similar ratings in the knowledgeable stakeholder population: overall satisfaction (4.33/5), the expected applicability of skills to professional practice (4.83/5), and the understandability of content (4.50/5). A better rating was achieved in the time estimate for completing all platform materials (4.50/5). Stakeholders suggested a more unified approach to each lesson of the pilot course and the structuring of activities from the familiar to the new and application within breakout rooms, hand in hand with the assignment of a group leader. The stakeholders strongly agreed that the pilot course improved their understanding of the topics better than pre-class reading alone (4.17/5) and anticipated the same for the participants (4.67/5).

Thirty-three people completed the survey as part of multiplier events, 81.8% working at a higher education institution (HEIs). Ten individuals (30.3%) reported that their institution had a multi-year clinical trialist program in place for more than 2 years, and 12 (36.3%) participants' institutions have a multi-week or semester course of the same focus. Sixteen (48.4%) respondents do not have a similar course at their institutions. Respondents agreed on the need for such a curriculum (mean 4.27/5). There was also agreement on the choice of topics, format, and willingness to implement the materials in their teaching (4.39/5, 4.18/5, and 4.09/5, respectively). Furthemore, there was also agreement on whether teachers could benefit from the curriculum (4.64/5).

Discussion

Training future researchers in clinical research is a burning issue, especially in the context of IITs and patient-centered research. A plethora of training courses was identified earlier that vary in terms of target group, overall scope, required background knowledge, and the expertise of the trainers [4, 8–10]. However, Boeynaems et al. conclude that these courses tend to be local, spontaneous, and often reactive activities [10]. The CONSCIOUS II training program primarily targets PhD students and early career researchers, i.e., less experienced researchers today but potential PIs of the future. As the results of our survey show, the availability of courses building skills in designing and conducting clinical trials, as well as interdisciplinary curricula, is limited. In our small sample, almost 50% of HEIs did not offer even short-term training. Fortunately, the situation is improving as at least the CONSCIOUS II consortium partners have already implemented the curriculum in postgraduate programmes and microcredentials.

Bechtel et al. suggest that, unlike experienced investigators, these less experienced trialists may benefit from more comprehensive, time-intensive types of training [7]. Markman et al. and Boeynames et al. also emphasize the time demands since academic research is usually secondary to the primary clinical activities [8, 10]. This is also true for the target audience of this training program. A flipped classroom format was thus chosen for the curriculum, where synchronous online sessions are preceded by individual, time-flexible asynchronous preparation. The combination of asynchronous and synchronous components is also advantageous in the context of achieving learning outcomes– while the individual work is a transferr of knowledge through self-paced learning, is more didactic but encourages deeper exploration and independent problem solving, the synchronous part focuses on practice, building skills, and is essential to reduce misconceptions and refine understanding through direct communication with and insight from the teacher and peers. Despite this arrangement, the time estimate for completing the pre-class materials ranked lowest in the feedback (3.69/5). This might be explained by a combination of the high time density of the course, the inconsistent anticipated length of preparation for each lesson, and, in some cases, being too demanding for the target group. The value of the synchronous part was confirmed by the feedback of both the participants in the pilot course and knowledgeable stakeholders, who strongly agreed that the pilot course helped them to understand the topic better than the pre-class reading alone. The participants were awarded certificates upon course completion. However, the certificate was not contingent on creating and submitting elaborated case studies or practical exercises. Participants did contribute to case studies, by making a short submission in advance of the synchronous session which resulted in a discussion point (learning by doing) rather than final evaluation and certification criteria. Similarly, the course did not conclude with a final test. This is a limitation because the entire evaluation of the course relies on subjective feedback rather than an objective assessment or a pre- and post-course comparison.

The curriculum has several advantages, including a low entry threshold with no prerequisites such as prior clinical research experience or initial level of knowledge. While the lack of validation of initial knowledge, such as in the pilot course, might contribute to the high ratings of content clarity, this aligns with the target group of less-experienced PhD students and early-career researchers. For this group, it can be expected that the variability of knowledge will not be as high as for experienced researchers, for whom the CONSCIOUS II course would probably take on the character of a one-size-fits-all training course. Notably, the curriculum emphasizes

information-seeking skills, offers guidance on where to go for consultation, and presents practical tools. This contributes to the curriculum degree of sustainability, even though it covers the highly variable area of clinical trials. ECRIN is an associate partner of the project, and most of the curriculum content authors are associated with ECRIN. This provides students with valuable contacts on whom to refer to if they become IITs PIs possibly.

A wide range of experts contributed to the curriculum, including project managers, monitors, data managers, regulatory specialists, clinical research methodologists, teachers, and researchers in medical education. This breadth of expertise differentiates the curriculum from purely academic courses, which may lack real-world context. However, all the partners working on the curriculum are based in the European region and work with European legislation daily. This partly limits the transferability of the curriculum to other areas with different regulatory frameworks for clinical research but our funder was the EU and this was the reason for this focus. However, the materials are available to any country in the world where they may add country and regional specific examples to the existing foundation materials.

Transferability was one of the primary considerations in designing the curriculum, consistent with one of the key principles articulated by Clinical Trials Transformation Initiative (CTTI) for improving the quality, conduct and efficiency of clinical trials: the PI must be supported by an effective study team and infrastructure [7]. All curriculum materials are freely available on the training platform, ensuring accessibility and interlinking with existing curricula. Since the pilot sessions usually used pre-class materials, the video-recordings can serve as a teacher's guide. This mitigates any limitations arising from the local trainer's experience and supports the wide implementation of the curriculum. The second idea stemming from the principle cited above is interdisciplinary collaboration. The training platform itself supports this through discussion boards connecting all platform users. It was also supported by the wide dissemination and inclusiveness of the pilot course, with PhD students and early career researchers making up 67.1% and experienced clinical trialists almost 21% of the attendees. On the other hand, not all professionals involved in clinical research need to acquire the same level of competence in clinical trial performance, and this curriculum primarily focuses on potential PIs [10]. The curriculum, or parts of it, can be used as supplementary material for biomedical students, lifelong education, or training programs for patient organizations. It can also serve as a basis for broader courses or degree programmes. The license used, the focus on providing guidance rather than bare facts, the platform supporting adaptability and transferability, the automatic assessments, and the availability of teacher's guides support reuse in areas such as those mentioned above.

The curriculum developed within the CONSCIOUS II project follows previously proposed recommendations for future investigator training [3, 7, 10]. It has the potential to fill a gap in the education of clinical trialists, especially PIs, and thus support the development of quality patient-centered research focused on underserved therapeutic areas and populations, as well as real-world issues.

Conclusion

The CONSCIOUS II project aims to support quality patient-centered research by developing a training program for the PIs of the future. The pragmatic curriculum reflects the previously proposed recommendations for the clinical trialists' education programs, fills the existing gap in this area, and extends the range of voluntary training activities individualized for early-career researchers and PhD students. The subjective perception of the curriculum of both the graduates and the knowledgeable stakeholders is more than positive regarding the content, format, and applicability to clinical trial practice. However, only an objectively measurable change in the PI's skills will demonstrate the actual value of the developed curriculum.

Abbreviations

CTTI	Clinical Trials Transformation Initiative
ECRIN	European Clinical Research Infrastructure Network
HEIs	Higher education institutions
IITs	Investigator-initiated trials
PI	Principal investigator

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12909-025-07054-5.

Supplementary Material 1

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Author contributions

JR, ZC, VN, LS, StM, GLK, JB, SaM, FS– conceptualization, ZC, KN, TO, MP– formal analysis, JR, VN, LS, StM, GLK, CK, ET, MP, ECM, JB, FS - funding acquisition, JR, ZC, VN, KN, LS, StM, GLK, CK, ET, TO, MP, ECM, SaM, JB, FS– investigation, JR, LS, VN, SaM, JB, FS– methodology, JR– project administration, TO, MP– software, JR, ZC, LS, JB, FS– writing– original draft, VN, KN, StM, GLK, CK, ET, ECM, SaM– writing– review and editing.

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Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics committee approval
The CONSCIOUS II project was approved by the Review Board of the Faculty of Medicine, Masaryk University. Following a request by the project investigator, PharmDr. Jitka Rychlíčková, Ph.D., a certificate has been issued that confirms that the CONSCIOUS II project does not require approval by the Ethics Committee of the Faculty of Medicine, Masaryk University. The certificate is provided to the editor. As a result, it was not necessary for the participants to sign the informed consent form for their involvement in the CONSCIOUS II project activities. Their participation in the pilot course and in the feedback collection was entirely voluntary. The research carried here is in compliance with the Declaration of Helsinki principles.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Pingali U. Investigator initiatives for academic trials: dilemmas and difficulties. Perspect Clin Res. 2023;14:159–60.
2. Konwar M, Bose D, Gogtay NJ, Thatte UM. Investigator-initiated studies: challenges and solutions. Perspect Clin Res. 2018;9:179–83.
3. Thompson P, Fenton J, Cotterill L, Neilson JP. Increasing support for the next generation of clinical trials leaders. Pilot Feasibility Stud. 2016;2:31.
4. Magnin A, Iversen VC, Calvo G, Čečetková B, Dale O, Demlova R, et al. European survey on National training activities in clinical research. Trials. 2019;20:616.
5. del Álamo M, Bührer C, Fisher D, Griese M, Lingor P, Palladini G, et al. Identifying Obstacles hindering the conduct of academic-sponsored trials for drug repurposing on rare-diseases: an analysis of six use cases. Trials. 2022;23:783.
6. Sambunjak D, Straus SE, Marušić A. Mentoring in academic medicinea systematic review. JAMA. 2006;296:1103–15.
7. Bechtel J, Chuck T, Forrest A, Hildebrand C, Panhuis J, Pattee SR, et al. Improving the quality conduct and efficiency of clinical trials with training: recommendations for preparedness and qualification of investigators and delegates. Contemp Clin Trials. 2020;89:105918.
8. Markman KM, Brewer SK, Magnuson B. Developing an engaging and accessible clinical research training program for new investigators. J Clin Transl Sci. 2023;7:e53.
9. Saleh M, Naik G, Jester P, Joiner C, Westfall E, Kimberlin DW, et al. Clinical investigator training program (CITP)– A practical and pragmatic approach to conveying clinical investigator competencies and training to busy clinicians. Contemp Clin Trials Commun. 2020;19:100589.
10. Boeynaems J-M, Canivet C, Chan A, Clarke MJ, Cornu C, Daemen E, et al. A European approach to clinical investigator training. Front Pharmacol. 2013;4:112.
11. CONSCIOUS Curriculum Development of Human Clinical Trials for the Next Generation Biomedical Students. http://conscious.novaims.unl.pt/login/index.php. Accessed 12 Aug 2024.
12. ECRIN European Clinical Research Infrastructure Network. https://ecrin.org/.
13. Fostering Research Culture in Clinical Pharmacology Services Agenda. Portuguese Society of Pharmacology. https://spfarmacologia.pt/uploads/FosterareasearchcultureinClinicalPharmacologyServicesAgenda_final.pdf
14. IDEAL Teacher training seminars. IDEALITER. https://ideal.u-paris.fr/teacher-training-seminars/
15. I. společná konference farmakologických společností. https://www.spolecna-konference-farma.cz/
16. CONSCIOUS II Curriculum Development of Human Clinical Trials. https://conscious2.eu/

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7.5 Annex 5

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Summary

Purpose:

The aim of this review was to describe the evolution of innovative clinical trial designs and to discuss their role in addressing the growing complexity of modern clinical research, particularly in precision medicine, rare diseases, and advanced therapy medicinal products (ATMPs). The article highlights the methodological limitations of conventional randomized controlled trials and presents innovative approaches that enable more efficient, flexible, and patient-centred evaluation of therapeutic interventions.

Methodology:

This publication is a narrative methodological review synthesising current regulatory guidance and contemporary literature on innovative clinical trial designs. It provides a structured overview of adaptive trial designs, master protocols (umbrella, basket, and platform trials), as well as emerging approaches including N-of-1 studies, single-arm trials, and SMART designs. The review discusses their methodological principles, advantages, limitations, statistical considerations, and potential clinical applications.

Results:

The review demonstrates that innovative trial designs offer greater flexibility and efficiency than traditional randomized controlled trials by allowing prospective adaptations, simultaneous evaluation of multiple interventions, and more efficient use of available data and resources. These methodologies are particularly valuable in settings characterised by limited patient populations, molecularly defined diseases, and rapidly evolving therapeutic approaches. However, their successful implementation requires careful statistical planning, robust methodological expertise, and close alignment with regulatory expectations. The article further identifies important barriers to implementation in the Czech Republic, including limited methodological capacity, insufficient funding, and the need for specialised education and research infrastructure.

Conclusion:

This review demonstrates that innovative clinical trial designs represent a key methodological advancement in contemporary clinical research. It emphasises that selecting the study design according to the clinical question—rather than relying exclusively on traditional randomized controlled trials—can improve the efficiency, flexibility, and translational potential of investigator-initiated clinical research. The publication also highlights the need for continued capacity building, interdisciplinary collaboration, and specialised methodological support to facilitate the wider adoption of innovative trial designs in academic clinical research.

Inovativní designy klinických studií

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Inovativní designy klinických studií představují významný posun v metodologii klinického výzkumu, který reflektuje rostoucí komplexitu onemocnění, rozvoj personalizované medicíny a nástup léčivých přípravků pro moderní terapii. Vedle tradičních randomizovaných kontrolovaných studií se stále častěji uplatňují adaptivní designy, master protokoly či jednoramenné studie, které umožňují flexibilnější a cílenější hodnocení terapeutických intervencí. Tyto přístupy mohou zrychlit identifikaci účinných terapií a efektivněji využívat dostupné zdroje, současně však přinášejí metodologické a organizační výzvy, zejména v oblasti statistického plánování, kontroly zkreslení a interpretace výsledků.

V českém prostředí zůstává implementace inovativních designů zatím omezená, především v důsledku nedostatku metodologické expertizy, omezených finančních zdrojů v akademickém sektoru a nedostatečného systematického vzdělávání v oblasti klinických studií. Iniciativy zaměřené na budování kapacit mohou přispět k posílení odborného zázemí a podpořit aktivnější zapojení českých institucí do mezinárodního výzkumného prostoru.

Inovativní designy představují klíčový nástroj moderního klinického výzkumu a jejich další rozvoj bude záviset na systematické metodologické podpoře a mezioborové spolupráci.

Klíčová slova: inovativní designy, klinický výzkum, adaptivní designy, metodologie klinických studií.

Innovative clinical trial designs

Innovative clinical trial designs represent an important shift in the methodology of clinical research, reflecting the growing complexity of diseases, the development of personalized medicine, and the emergence of advanced therapy medicinal products. In addition to traditional randomized controlled trials, approaches such as adaptive designs, master protocols or single-arm studies are increasingly being used, enabling more flexible and targeted evaluation of new therapeutic interventions. They may accelerate the identification of effective therapies and improve the efficiency of resource utilization; however, they also introduce methodological and organizational challenges, particularly in relation to statistical planning, control of bias, and interpretation of results.

In the Czech Republic, the implementation of innovative trial designs remains limited, mainly due to insufficient methodological expertise, limited funding in the academic sector, and the lack of systematic education in clinical trial methodology. Capacity-building initiatives may help strengthen the methodological background and support a more active involvement of Czech institutions in the international research landscape.

DECLARATIONS:

Declaration of originality:
The manuscript is original and has not been published or submitted elsewhere.

Ethical principles compliance:
The authors attest that their study was approved by the local Ethical Committee and is in compliance with human studies and animal welfare regulations of the authors' institutions as well as with the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects adopted by the 18th WMA General Assembly in Helsinki, Finland, in June 1964, with subsequent amendments, as well as with the ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, updated in December 2018, including patient consent where appropriate.

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Innovative trial designs represent a key tool of modern clinical research, and their further development will depend on systematic methodological support and interdisciplinary collaboration.

Key words: innovative designs, clinical research, adaptive designs, clinical trial methodology.

Úvod

Randomizované kontrolované studie (RCTs) s paralelním uspořádáním představují zlatý standard v klinickém výzkumu. Tento design je schopen poskytnout robustní data o bezpečnosti a účinnosti testovaných léčiv díky fixní randomizaci a rigidním protokolům (1). Nicméně nepružnost protokolu má v současném výzkumném prostředí řadu nedostatků: potřeba velkého počtu účastníků, dlouhá doba sledování, omezená statistická síla pro hodnocení účinnosti v důležitých podskupinách pacientů nebo vysoké náklady na studii (2).

Dalším limitem tradičních designů, včetně RCTs, je jejich omezená použitelnost pro hodnocení cílených (tj. terapie zaměřené na specifické molekulární cíle) či moderních terapií (ATMP, advanced therapy medicinal product), včetně genové terapie nebo somatobuněčných terapií. Cílené terapie naráží na zaužívané designy klinických hodnocení „jeden lék, jedna diagnóza", zatímco u ATMPs je použití tradičních RCTs limitované malým počtem pacientů, etickými omezeními randomizace nebo heterogenitou populace. Výše popsaná omezení vytvořila prostor pro inovativní designy, které umožňují větší pružnost a efektivnější využití dostupných dat a zdrojů při zachování vědecké validity a integrity studie (3).

Na potřebu modernizovat designy klinických studií reagovaly i regulační autority. Jak Evropská léková agentura (EMA), tak americký Úřad pro kontrolu potravin a léčiv (FDA) vydaly své metodické pokyny, které popisují podmínky přijatelnosti inovativních designů. V pokynech je zdůrazněna nezbytnost předchozího vědeckého dialogu, transparentnosti statistických metod a zajištění ochrany proti systematickému zkreslení (4, 5). V červnu roku 2025 byla navíc vydána Mezinárodní radou pro harmonizaci směrnice ICH E20: Guideline on Adaptive Designs for Clinical Trials upravující pravidla pro návrh a hodnocení adaptivních designů v klinických studiích (6).

Inovativní designy klinických studií tudíž nepředstavují náhradu tradičních metod, ale jejich rozšíření a adaptaci na současné potřeby a pokroky medicíny. V kontextu rychlého rozvoje moderních terapií a rostoucích požadavků na efektivitu zdravotnických systémů lze předpokládat, že jejich význam bude nadále narůstat a postupně se stanou nedílnou součástí standardního klinického výzkumu.

Adaptivní designy

Adaptivní designy nejsou novinkou – již v 70. letech 20. století byl zaveden koncept adaptivní randomizace (7). Jejich praktické využití ale bylo omezené, zejména z důvodu metodologických, regulačních a operačních nejasností spojených s jejich implementací a interpretací výsledků. Vyhodnocení studií aplikujících adaptivní design totiž vyžaduje složitější analýzu za použití validovaných softwarů a robustních statistických metod zajišťujících kontrolu chyby typu I (nesprávné zamítnutí nulové hypotézy), multiplicity a zachování integrity studie. Současně existuje obava výrobců léčiv, zda budou výsledky takových studií dostatečné pro HTA (Health Technology Assessment; hodnocení zdravotnických technologií) analýzu a přijaty regulačními autoritami. Tato nejistota obecně souvisela s omezenými zkušenostmi s komplexními adaptivními postupy a s potřebou transparentního předdefinování adaptačních pravidel. Navíc provádění studií s adaptivním designem je i organizačně mnohem náročnější. Situace se postupně začala zlepšovat díky vzniku odborných skupin jako je ADSWG (Adaptive Design Scientific Working Group) organizace DIA (Drug Information Association) (8), dostupnosti specializovaných softwarových řešení a také zveřejnění výše zmíněných doporučení EMA (4) a FDA (5) k adaptivním designům.

EMA definuje „adaptivní design" jako takový, který umožňuje upravit prvky studie (např. velikost souboru, poměr randomizace) na základě interim analýz za současné kontroly chyby typu I. Tyto postupy doporučuje zejména v případech, kdy je provedení studií s tradičním designem složité, například z etických důvodů (4). Současně EMA zdůrazňuje nutnost zachování validity a interpretovatelnosti výsledků, zejména při použití komplexních adaptačních strategií. Doporučení FDA se na rozdíl od EMA zaměřuje i na praktické aspekty hodnocení a statistické analýzy dokončených studií s adaptivním designem (5).

Typy adaptací

Adaptace je definována jako modifikace jednoho nebo více prvků designu studie na základě průběžně sbíraných dat (7). Všechny potenciální adaptace a jejich podmínky musí být prospektivně naplánované a řádně zdokumentované v protokolu klinické studie. Je důležité zdůraznit, že adaptace nepředstavují *ad hoc* změny nebo dodatky protokolů, ale jsou integrální součástí designu studie (5). Přehled nejčastějších adaptací je uveden v tabulce 1.

Master protokoly

Master protokoly jsou definovány jako integrované rámce klinického hodnocení umožňující současné nebo sekvenční zodpovězení více výzkumných otázek v rámci jednotné infrastruktury studie (10). Dělí se do tří hlavních kategorií podle toho, zda sdružují různá onemocnění s jedním léčivem (tzv. basket design), jedno onemocnění s různými léčivy (tzv. umbrella design), nebo kombinují oba přístupy v rámci průběžně adaptované platformy (tzv. platformové studie) (11).

Umbrella

Umbrella studie hodnotí účinnost několika cílených terapeutických intervencí u jedné nemoci, která je rozdělena do několika molekulárně definovaných podskupin. Pacienti se společnou diagnózou (obvykle vymezenou histologicky) podstupují molekulární profilování za účelem identifikace prediktivních a terapeuticky využitelných biomarkerů (12). Následně jsou přiřazeni do specifických terapeutických ramen podle přítomnosti těchto molekulárních alterací. Tento přístup umožňuje efektivní hodnocení více cílených intervencí

Tab. 1. *Typy nejčastějších adaptací v průběhu interim analýz (4, 5, 9)*

Typ adaptace	Popis	Výhody	Problémy/Výzvy
Adaptace velikosti studijní populace	Přehodnocení velikosti studijní populace, aby bylo zajištěno, že studie bude dostatečně průkazná.	Snižuje pravděpodobnost negativní studie u léčiva/intervence se smysluplným účinkem.	Vypočtená velikost studijní populace může být nereálná, neproveditelná.
Předčasné ukončení z důvodu zbytečnosti/marnosti **Předčasné ukončení z důvodu prokázání účinnosti**	Zastavení klinické studie, kdy je zřejmé, že studie nemá šanci na úspěch. Zastavení klinické studie, kdy je zřejmé, že testovaná intervence je účinnější než kontrolní rameno.	Jedná se o etický přístup, který chrání pacienty a šetří zdroje, které mohou být realokovány. Rychlejší přístup pacientů k efektivní terapii, snížení nákladů, minimalizace zátěže účastníků.	Časné ukončení studie může vést ke snížení statistické síly, pokud skutečný účinek existuje. Toto riziko lze omezit konzervativním nastavením hranic, přičemž je nutné vyvážit ztrátu síly a možnost včas odhalit marnost/účinnost.
Adaptace studijní populace	Umožňuje během studie upravit cílovou populaci pacientů. Data získaná před i po této adaptaci lze kombinovat pro hodnocení intervence jak v cílové skupině, tak i v celkové populaci.	Adaptace umožňuje upravit kritéria pro zařazení tak, aby byly do studie zahrnuty podskupiny pacientů s nejvyšší pravděpodobností přínosu z dané intervence.	Podskupiny pacientů mohou být velmi malé, například u vzácných onemocnění nebo u populací, které je obtížné do studie zařadit, což ztěžuje hodnocení přínosu léčby.
Adaptivní randomizace	Rozlišujeme dva typy: ▪ **covariate-adaptive randomization**: přiřazení léčby je upravováno tak, aby byly skupiny vyvážené podle prognostických faktorů. ▪ **response-adaptive randomization**: pravděpodobnost přiřazení do jednotlivých ramen se mění podle kumulujících se dat o účinnosti.	Zlepšení statistické efektivity studie, snížení variability odhadů a v některých situacích umožnění menší velikosti vzorku nebo kratší trvání studie. Response-adaptive randomizace může mít také etický přínos tím, že více pacientů dostane účinnější léčbu.	Covariate-adaptive přístupy mohou zvýšit predikovatelnost přiřazení léčby, což vyžaduje dodatečné náhodné prvky k zachování integrity randomizace, a oba typy adaptací vyžadují statistické metody, které správně zohlední design studie při analýze dat.
Adaptace léčebných ramen	Adaptace umožňuje změny v léčebných ramenech, typicky přidávání nových ramen nebo ukončení neperspektivních ramen.	U dose-range* studií adaptace léčebných ramen umožňuje rychleji identifikovat optimální dávku pro konfirmační studie. U konfirmačních studií lze díky adaptaci ukončit omezeně účinné rameno dříve a soustředit zdroje na perspektivnější rameno. To dělá klinickou studii etičtější.	Podobně jako u response-adaptivní randomizace je důležité umožnit adaptaci až po shromáždění dostatečného množství dat. Vzhledem k nevratnosti rozhodnutí o vyřazení studijního ramene je tento postup obvykle nastaven konzervativněji než response-adaptivní randomizace.
Adaptace výběru endpointů (cílových ukazatelů)	Možnost zvolit jeden cílový ukazatel z několika potenciálních primárních endpointů v situaci, kdy při průběžné analýze panuje nejistota ohledně velikosti účinku u jednotlivých sledovaných výsledků.	Primární cílový ukazatel může být změněn i v průběhu studie.	EMA zdůrazňuje, že klinický význam endpointu by se v průběhu studie neměl měnit a změna je jen těžce obhajitelná. FDA doporučuje nejdřív přezkoumání, rozsáhlou diskuzi a vyžaduje konzultaci s příslušnou hodnotící sekcí FDA.
Přechod z non-inferiorní studie na superiorní	Studie s aktivním komparátorem může být plánována jako non-inferiorní, s možností přechodu k testování superiority podle průběžných výsledků.	Umožňuje prokázání superiority, pokud je léčba skutečně účinnější než aktivní komparátor.	Interpretace výsledků může být složitá, zejména pokud se průběžné a finální výsledky liší. Regulační autority často preferují potvrzení výsledků v nezávislé studii místo pokračování jedné studie k prokázání superiority.

**hodnocení účinku při různých dávkách léčiva; zjištění rozsahu dávky*

současně při zachování biologické homogenity jednotlivých substudií.

Koncept umbrella studií vychází z poznání, že to, co bylo historicky považováno za jedno onemocnění, ve skutečnosti představuje soubor molekulárně odlišných podtypů, které vyžadují individualizované terapeutické přístupy. Typickým příkladem je nemalobuněčný karcinom plic, který byl dříve léčen jako homogenní onemocnění, avšak dnes je chápán jako skupina mnoha různých molekulárních subtypů definovaných různými mutacemi EGFR a dalšími „driver" alteracemi (ALK, MET, RET, HER2 atd.), z nichž každá může vyžadovat specifickou cílenou léčbu (13). Umbrella design tak reflektuje posun od histologicky definované klasifikace onemocnění k biologicky a molekulárně založenému konceptu precision medicine.

Basket

Basket trial je typ master protokolu, ve kterém se jedno zkoušené léčivo (nebo kombinace léčiv) testuje u různých populací, definovaných sdílenou biologickou charakteristikou, nejčastěji specifickým molekulárním biomarkerem, bez ohledu na histologický typ onemocnění. Studie je strukturovaná do paralelních substudií (tzv. košů) v rámci jednoho společného protokolu (11, 14). Každý „koš" představuje relativně homogenní subpopulaci, ve které je hodnocena účinnost a bezpečnost dané intervence, zatímco sdílená infrastruktura studie umožňuje efektivnější generování důkazů.

V onkologii se basket studie rozšířily i na studie fáze 1b/2, kde se v rámci jedné studie zkoumá více různých nádorových diagnóz, aby se získal důkaz o účinnosti (proof of concept) předtím, než se léčivo bude dále hodnotit v konfirmačních studiích. Konfirmační basket studie obvykle vybírají účastníky podle jednoho specifického biomarkeru, tedy určité genetické nebo biologické charakteristiky nádoru, která má předpovídat účinek léčiva (15). Například mutace BRAF V600E se vyskytují u melanomu, kolorektálního karcinomu, karcinomu štítné žlázy a dalších ma-

lignit (16). To pochopitelně vyvolává otázku, zda mohou inhibitory BRAF vykazovat srovnatelnou účinnost i u histologicky rozdílných nádorů nesoucích tuto mutaci. Naproti tomu proof of concept basket studie nemusí být založeny na biomarkerech a mohou zahrnovat širší spektrum nádorů, aby se zjistilo, zda léčivo vykazuje aktivitu napříč různými indikacemi (15). Tento přístup však přináší metodologické výzvy spojené s heterogenitou populací a interpretací výsledků mezi jednotlivými „koši".

Platformové studie

Platformové studie představují typ klinického hodnocení založený na dlouhodobě fungující studijní infrastruktuře, která umožňuje systematické hodnocení více terapeutických intervencí v rámci jednoho onemocnění. Na rozdíl od tradičních klinických studií nejsou platformové studie koncipovány jako jednorázové projekty s pevně stanoveným koncem, ale jako průběžný výzkumný rámec umožňující flexibilní generování důkazů v čase (17).

V rámci jedné platformy mohou být léčebná ramena přidávána nebo ukončována na základě předem definovaných rozhodovacích pravidel a aktuálně dostupných dat. V odůvodněných případech lze dokonce pacienta převést z jednoho ramena studie do jiného ramene (17, 18). Platformové studie často využívají sdílená kontrolní ramena a jednotnou studijní infrastrukturu, což zvyšuje efektivitu využití zdrojů a umožňuje rychlejší identifikaci účinných terapií ve srovnání s tradičními studiemi zaměřenými na hodnocení jediné intervence (19).

Platformové studie mohou, ale nemusí být randomizované nebo adaptivní. V praxi však moderní platformové studie často kombinují randomizaci, adaptivní prvky a/nebo pokročilé statistické metody.

Platformové studie se uplatnily zejména v oblasti onkologie a infekčních onemocnění a jejich popularita vzrostla během pandemie covidu-19, kdy umožnily rychlé zhodnocení více terapeutických strategií v dynamicky se vyvíjejícím klinickém prostředí (20, 21). Platformové studie nacházejí využití i v dalších oblastech, například u chřipky (22), respiračních infekcí (23), neurodegenerativních onemocnění (24), nebo eboly (25).

Navzdory svým výhodám představují platformové studie metodologicky i organizačně náročný přístup. Jejich dlouhodobý charakter vyžaduje stabilní koordinaci, financování a spolupráci více výzkumných týmů se sponzory (11). Z metodologického hlediska je nad rámec výše uvedených požadavků třeba pečlivě plánovat statistické analýzy, kontrolovat multiplicitu testování, řídit sdílená kontrolní ramena a minimalizovat riziko zkreslení při srovnávání skupin zařazených v různých obdobích. Tyto aspekty jsou klíčové pro zachování validity a interpretovatelnosti výsledků (9, 11, 17).

Další inovativní designy

N-of-1 studie

N-of-1 design je zaměřený na hodnocení účinnosti léčby u jednotlivého pacienta.

Metodologicky se jedná typicky o zaslepenou studii s opakovaným střídáním intervence a kontrolní léčby v několika časových obdobích (cross-over sekvence) s dostatečnými wash-out periodami. Pacient slouží jako vlastní kontrola, což minimalizuje vliv interindividuální variability. Tento design je zvláště vhodný pro chronická onemocnění se stabilním průběhem (diabetes mellitus 2. typu, chronická bolest, chronická plicní onemocnění), kde je cílem optimalizovat terapii pro konkrétního pacienta (26).

Single arm design

Single arm (jednoramenné) designy pracují pouze s experimentálním ramenem, bez zahrnutí paralelního kontrolního ramene do studie. Jednoramenné studie se typicky uplatňují v časných fázích klinického vývoje nebo v situacích, kdy etické, praktické či epidemiologické okolnosti znemožňují použití paralelní kontrolní skupiny (27). Vzhledem k absenci paralelní kontrolní skupiny je však obtížné kontrolovat řadu zavádějících (confounding) faktorů, a výsledky mohou být hůře interpretovatelné. Z tohoto důvodu jsou jednoramenné studie zpravidla využívány v situacích, kdy lze očekávat výrazný léčebný efekt, který je možné interpretovat i bez přímého srovnání s kontrolní skupinou.

Aby se jednoramenné studie přiblížily konceptu RCTs, využívají často jako kontrolu historická data (28). V současné metodologii se tato oblast významně posouvá i směrem k využití externích komparátorových kohort (external comparator cohorts) a syntetických kontrolních ramen (synthetic control arms) odvozených z dat z reálné klinické praxe (RWD – real-world data). Tyto pokročilé přístupy využívají registry pacientů, elektronickou zdravotní dokumentaci nebo data z předchozích klinických studií k tomu, aby pomocí pokročilých statistických metod co nejpřesněji nasimulovaly chybějící kontrolní skupinu (29, 30). Výběr těchto externích kontrol musí být proveden pečlivě, s důrazem na kvalitu zdrojových dat, minimalizaci selekčního zkreslení a vhodnost pro danou studii (28, 29).

V současnosti se jednoramenné studie využívají především ve studiích fáze I a II v klinickém výzkumu nádorových onemocnění, vzácných onemocnění a u léčiv, které jsou vysoce žádané pro naplnění nenaplněných léčebných potřeb (unmet medical needs) (28, 31). V těchto specifických oblastech nabývá na významu také využití důkazů z reálné praxe (RWE – real-world evidence) regulačními autoritami při rozhodování o registraci a vstupu moderních terapií na trh (32–34). Navzdory praktickému významu jednoramenných studií však tyto studie poskytují omezenou úroveň důkazů ve srovnání s randomizovanými designy a jejich interpretace vyžaduje zvýšenou opatrnost.

SMART design

Zajímavým podtypem adaptivních designů je SMART design (sequential multiple assignment randomized trial). Ve studiích se SMART designem jsou účastníci na začátku randomizováni do jedné z intervenčních skupin, podobně jako je to v případě tradiční RCT. Po určité době je vyhodnocena jejich odpověď na léčbu a účastníci s nedostatečnou odpovědí mohou být znovu randomizováni do jiné intervenční skupiny. Tento proces může probíhat ve více po sobě následujících fázích, čímž vznikají sekvenční léčebné strategie (dynamic treatment regimens), které lze následně mezi sebou porovnávat (3). Tento přístup je zvláště vhodný u chronických onemocnění, kde je běžné léčbu modifikovat podle průběžné odpovědi pacienta. SMART designy tak v podstatě systematicky modelují standardní klinickou praxi založenou na postupném přizpůsobování léčby individuální odpovědi pacienta (3, 35).

Možnosti a bariéry implementace v českém prostředí

Zkušenosti s adaptivními designy a master protokoly jsou v České republice zatím omezené a jsou soustředěné v klinických pracovištích, která participují např. v mezinárodních platformových studiích. Role českých institucí jako iniciátorů studií s adaptivním designem však zůstává spíše výjimečná. Důvodem je zejména nedostatečná kapacita kvalifikovaných statistiků s expertizou v adaptivních metodách, omezené financování akademického klinického výzkumu a administrativní náročnost při schvalování protokolů s adaptivními prvky jak na straně etických komisí, tak na straně regulačních autorit. Posun v budování expertizy může přinést projekt TRIANGLE, připravený v rámci programu švýcarsko-české spolupráce výzkumnými infrastrukturami CZECRIN (Czech Clinical Research Infrastructure Network) a SCTO (Swiss Clinical Trial Organisation).

Závěr

Inovativní designy klinických studií představují významný posun v metodologii klinického výzkumu. Jejich správné použití přináší řadu výhod: rychlejší, efektivnější a mnohdy i etičtější hodnocení nových léčiv a intervencí, což je spojeno s přímým benefitem pro pacienty a efektivnějším využitím zdrojů.

Na druhé straně je však nutno otevřeně diskutovat i o limitech. Komplexita adaptivních designů zvyšuje riziko metodologických chyb při jejich nesprávné implementaci. Nedostatečně transparentní plánování nebo analýza těchto studií může vést k ohrožení validity a interpretovatelnosti výsledků. Selektivní publikování a nekompletní prezentace výsledků adaptivních studií mohou systematicky zkreslit dostupnou vědeckou evidenci. Proto je důležité dodržování mezinárodních doporučení a metodologických standardů, které upravují plánování, provádění a interpretaci studií využívajících inovativní designy.

Závěrem lze říci, že inovativní designy nejsou módním trendem, ale přirozeným vývojem metodologie klinických studií reagujícím na výzvy moderní medicíny. Jejich další širší využití však bude záviset na rozvoji metodologického know-how, mezioborové spolupráci a systematickém budování kapacit v oblasti klinického výzkumu.

LITERATURA

1. Bothwell LE, Avorn J, Khan NF, et al. Adaptive design clinical trials: a review of the literature and ClinicalTrials.gov. BMJ Open. 2018;8:e018320.
2. Bhatt DL, Mehta C. Adaptive Designs for Clinical Trials. N Engl J Med. 2016;375:65-74.
3. Kaizer AM, Belli HM, Ma Z, et al. Recent innovations in adaptive trial designs: a review of design opportunities in translational research. J Clin Transl Sci. 2023;7:e125.
4. European Medicines Agency. Methodological issues in confirmatory clinical trials planned with an adaptive design – Scientific guideline [Internet]. European Medicines Agency; 2007 [cited 2026-04-15]. Available from: https://www.ema.europa.eu/en/methodological-issues-confirmatory-clinical-trials-planned-adaptive-design-scientific-guideline.
5. Center for Drug Evaluation and Research. Adaptive Design Clinical Trials for Drugs and Biologics Guidance for Industry [Internet]. U.S. Food and Drug Administration; 2020 [cited 2026-04-15]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/adaptive-design-clinical-trials-drugs-and-biologics-guidance-industry.
6. European Medicines Agency. ICHE20 adaptive designs for clinical trials - Scientific guideline [Internet]. European Medicines Agency; 2025 [cited 2026-04-13]. Available from: https://www.ema.europa.eu/en/ich-e20-adaptive-designs-clinical-trials-scientific-guideline.
7. Chow SC, Chang M. Adaptive design methods in clinical trials – a review. Orphanet J Rare Dis. 2008;3:11.
8. Miller E, Gallo P, He W, et al. DIA's Adaptive Design Scientific Working Group (ADSWG): Best Practices Case Studies for 'Less Well-understood' Adaptive Designs. Ther Innov Regul Sci. 2017;51:77-88.
9. Kaizer AM, Belli HM, Ma Z, et al. Recent innovations in adaptive trial designs: a review of design opportunities in translational research. J Clin Transl Sci. 2023;7:e125.
10. Woodcock J, LaVange LM. Master Protocols to Study Multiple Therapies, Multiple Diseases, or Both. N Engl J Med. 2017;377:62-70.
11. Bogin V. Master protocols: New directions in drug discovery. Contemp Clin Trials Commun. 2020;18:100568.
12. Park JJH, Siden E, Zoratti MJ, et al. Systematic review of basket trials, umbrella trials, and platform trials: a landscape analysis of master protocols. Trials. 2019;20:572.
13. Wang Y, Zhai H, Wang J, et al. Study protocol of an open-label prospective phase II umbrella study of precise neoadjuvant therapy for patients with stage II-IIIB resectable non-small cell lung cancer (PURPOSE). Front Oncol. 2022;12:1052774.
14. Center for Drug Evaluation and Research. Master Protocols: Efficient Clinical Trial Design Strategies to Expedite Development of Oncology Drugs and Biologics Guidance for Industry [Internet]. U.S. Food and Drug Administration; 2024 [cited 2026-04-18]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/master-protocols-efficient-clinical-trial-design-strategies-expedite-development-oncology-drugs-and.
15. Lu C, Li X, Broglio K, et al. Practical Considerations and Recommendations for Master Protocol Framework: Basket, Umbrella and Platform Trials. Ther Innov Regul Sci. 2021;55:1145-1154.
16. Cohn AL, Day B-M, Abhyankar S, et al. BRAFV600 mutations in solid tumors, other than metastatic melanoma and papillary thyroid cancer, or multiple myeloma: a screening study. Onco Targets Ther. 2017;10:965-971.
17. Roustit M, Demarcq O, Laporte S, et al. Platform trials. Therapies. 2023;78:29-38.
18. Burki T. Platform trials: the future of medical research? Lancet Respir Med. 2023;11:232-233.
19. Saville BR, Berry SM. Efficiencies of platform clinical trials: A vision of the future. Clin Trials. 2016;13:358-366.
20. RECOVERY Collaborative Group, et al. Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med. 2021;384:693-704.
21. Hobbs FDR, Dorward J, Hayward G, et al. The PRINCIPLE randomised controlled open label platform trial of hydroxychloroquine for treating COVID19 in community based patients at high risk. Sci Rep. 2025;15:23850.
22. Waite AAC, Peto L, Gordon AC, et al. Platform Trials to Assess Therapeutics in Patients Hospitalized With Influenza. J Infect Dis. 2025;232:S254-S261.
23. Angus DC, Berry S, Lewis RJ, et al. The REMAP-CAP (Randomized Embedded Multifactorial Adaptive Platform for Community-acquired Pneumonia) Study. Rationale and Design. Ann Am Thorac Soc. 2020;17:879-891.
24. UCSF. UCSF Alzheimer's Disease Trial: Alzheimer's Tau Platform. Master Protocol [Internet]. University of California San Francisco; 2025 [cited 2026-03-14]. Available from: https://clinicaltrials.ucsf.edu/trial/NCT06957418.
25. Berry SM, Petzold EA, Dull P, et al. A response adaptive randomization platform trial for efficient evaluation of Ebola virus treatments: A model for pandemic response. Clin Trials. 2016;13:22-30.
26. Lillie EO, Patay B, Diamant J, et al. The n-of-1 clinical trial: the ultimate strategy for individualizing medicine? Per Med. 2011;8:161-173.
27. Wang M, Ma H, Shi Y, et al. Single-arm clinical trials: design, ethics, principles. BMJ Support Palliat Care. 2024;15:e004984.
28. Yao S, Shang Q, Ouyang M, et al. Designing Single-Arm Clinical Trials: Principles, Applications, and Methodological Considerations. Ann Clin Epidemiol. 2025;7:90-98.
29. Thorlund K, Dron L, Park JJH, et al. Synthetic and External Controls in Clinical Trials - A Primer for Researchers. Clin Epidemiol. 2020;12:457-467.
30. Pignatti F, El-Galaly TC, Kaiser M, et al. Assessing Overall Survival Benefits in Advanced Cancers: The Role of External Comparator Cohort Studies with Real-World Data. Clin Pharmacol Ther. 2026;119:1080-1087.
31. European Medicines Agency. Single-arm trials as pivotal evidence for the authorisation of medicines in the EU [Internet]. European Medicines Agency; 2023 [cited 2026-04-22]. Available from: https://www.ema.europa.eu/en/news/single-arm-trials-pivotal-evidence-authorisation-medicines-eu.
32. Center for Drug Evaluation and Research. Considerations for the Use of Real-World Data and Real-World Evidence To Support Regulatory Decision-Making for Drug and Biological Products [Internet]. U.S. Food and Drug Administration; 2023 [cited 2026-06-03]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/considerations-use-real-world-data-and-real-world-evidence-support-regulatory-decision-making-drug.
33. Center for Drug Evaluation and Research. Real-World Data: Assessing Electronic Health Records and Medical Claims Data To Support Regulatory Decision-Making for Drug and Biological Products [Internet]. U.S. Food and Drug Administration; 2025 [cited 2026-06-03]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/real-world-data-assessing-electronic-health-records-and-medical-claims-data-support-regulatory.
34. European Medicines Agency. Use of real-world evidence in regulatory decision making – EMA publishes review of its studies [Internet]. European Medicines Agency; 2023 [cited 2026-06-03]. Available from: https://www.ema.europa.eu/en/news/use-real-world-evidence-regulatory-decision-making-ema-publishes-review-its-studies.
35. Almirall D, Nahum-Shani I, Sherwood NE, et al. Introduction to SMART designs for the development of adaptive interventions: with application to weight loss research. Transl Behav Med. 2014;4:260-274.

7.6 Annex 6

Hrdy O, Vrbica K, Strazevska E, Suk P, Souckova L, Stepanova R, Sas I, Gal R. Comparison of continuous versus intermittent enteral nutrition in critically ill patients (COINN): study protocol for a randomized comparative effectiveness trial. Trials. 2020 Nov 23;21(1):955. doi: 10.1186/s13063-020-04866-2. PMID: 33228772; PMCID: PMC7682133.

Summary
Purpose:
The aim of this study was to investigate whether the mode of enteral nutrition administration (continuous versus intermittent) influences the time required to reach nutritional targets, as well as the occurrence of intolerance and complications in critically ill patients. This research addresses a clinically relevant but unresolved question in intensive care, where existing evidence remains inconsistent.

Methodology:
The study was designed as a prospective, randomized, monocentric clinical trial involving critically ill adult patients requiring enteral nutrition. Participants were randomly assigned to either continuous or intermittent feeding, with nutritional advancement guided by tolerance indicators such as gastric residual volume; primary and secondary endpoints included nutritional targets, complications, and clinical outcomes.

Results:
As a protocol publication, the study primarily defines a rigorous methodological framework, including clearly specified endpoints and standardized criteria for assessing tolerance and complications. It highlights anticipated challenges such as variability in patient.

Conclusion:
This study provides a robust methodological basis for evaluating enteral nutrition strategies in critically ill patients and aims to generate evidence to inform clinical practice. It also exemplifies how investigator-initiated trials can address practical clinical questions that are not typically prioritized in commercial research.

Hrdy et al. Trials (2020) 21:955
https://doi.org/10.1186/s13063-020-04866-2

Trials

STUDY PROTOCOL Open Access

Comparison of continuous versus intermittent enteral nutrition in critically ill patients (COINN): study protocol for a randomized comparative effectiveness trial

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Abstract

Background: Enteral nutrition is part of the treatment of critically ill patients. Administration of enteral nutrition may be associated with signs of intolerance, such as high gastric residual volumes, diarrhea, and vomiting. Clinical trials regarding the effects of the mode of administration of enteral nutrition on the occurrence of these complications have yielded conflicting results. This trial aims to investigate whether the mode of administration of enteral nutrition affects the time to reach nutritional targets, intolerance, and complications.

Methods: COINN is a randomized, monocentric study for critically ill adult patients receiving enteral nutrition. Patients will be randomly assigned to two groups receiving (1) continuous or (2) intermittent administration of enteral nutrition. Enhancement of enteral nutrition will depend on signs of tolerance, mainly the gastric residual volume. The primary outcome will be the time to reach the energetic target. Secondary outcomes will be the time to reach the protein target, tolerance, complications, hospital and ICU lengths of stay, and 28-day mortality.

Discussion: This trial aims to evaluate whether the mode of application of enteral nutrition affects the time to reach nutritional targets, signs of intolerance, and complications.

Trial registration: ClinicalTrials.gov NCT03573453. Registered on 29 June 2018

Keywords: Critical care, Enteral nutrition, Intensive care unit, Gastric residual volume, Diarrhea

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Administrative information

Title {1}	Comparison of Continuous Versus Intermittent Enteral Nutrition in Critically Ill Patients (COINN): study protocol for a randomized comparative effectiveness trial.
Trial registration {2a and 2b}.	ClinicalTrials.gov: NCT03573453
Protocol version {3}	Protocol version 2.0, dated May 28th, 2019.
Funding {4}	This study is not funded.
Author details {5a}	OH[3], KV[1], ES[1], PS[1], LS[2], RS[2], IS[1], RG[1] [1] Department of Anesthesiology and Intensive Care Medicine, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic. [2] Department of Pharmacology, Faculty of Medicine, Masaryk University, VVI CZECRIN
Name and contact information for the trial sponsor {5b}	**Trial Sponsor**: Masaryk University Brno **Contact name**: prof. MUDr. Martin Smrcka, Ph.D., MBA **Address**: Jihlavska 20, 625 00 Brno **Telephone**: +420532232884 **Email**: smrcka.martin@fnbrno.cz
Role of sponsor {5c}	The sponsor has no role in the design of this trial and will not have any role during its execution, analyses, interpretation of the data, or the decision to submit results.

Introduction

Background and rationale {6a}

Gastric enteral nutrition (EN) is commonly used in critically ill patients who cannot be fed orally [1, 2]. It can be performed with a continuous infusion or the administration of several boluses via a gastric tube. Continuous feeding consists of nutritional infusion at a constant rate. Intermittent nutrition involves administration of a bolus volume delivered over 15–40 min multiple times per day [3]. The rate of continuous infusion or the volume of the bolus is set to meet the daily energetic target. EN begins with the administration of a small amount of nutrition. If the EN is tolerated well, the amount of administered nutrition is increased until the target energy is reached. When signs of intolerance are recognized, the amount of administered nutrition is decreased; thus, the tolerance toward EN determines the time to reach the estimated energetic target—the primary outcome of this study. Both methods have advantages and disadvantages. Continuous administration of nutrients is unphysiologic, does not promote anabolism, and is associated with metabolic complications [4]. However, continuous administration is also associated with fewer gastrointestinal or respiratory complications, and meeting the nutritional targets earlier [5]. Intermittent feeding is physiologic and can promote proteosynthesis. However, it leads to high gastric residual volumes (GRVs) and higher occurrence of diarrhea and aspiration [6]. Studies investigating the effects of the mode of administration on the occurrence of complications and other outcomes have yielded conflicting results [6–9]. The authors of a systematic review of the effectiveness of continuous versus intermittent enteral nutrition have been unable to affirm recommendations for one method over the other [10]. The new guidelines of the European Society for Parenteral and Enteral Nutrition (ESPEN) on clinical nutrition in intensive care units recommend the use of continuous rather than bolus EN. However, the authors assumed that evidence on this issue is very limited, that bolus and continuous feeding are similar in achievement of nutritional targets without either increasing the rate of adverse effects, and that bolus feeding may be a greater stimulus for protein synthesis [11]. On the basis of current knowledge, a clear recommendation for a preferred method of administration of enteral feeding cannot be made [12, 13]. The aim of this study is to determine whether any difference exists in the time to reach the energetic target between two tolerance-driven modes of administration of EN in critically ill adult patients.

Objectives {7}

The primary study objective is to compare continuous and intermittent modes of application of enteral feeding on the time to reach an energetic target in critically ill adult patients.

Key secondary objectives are to determine the effects of the mode of administration of enteral feeding on the time to reach the protein target, the gastrointestinal tolerance (on the basis of assessment of GRV), vomiting, diarrhea, and the need for postpyloric nutrition. Other secondary objectives are complications of enteral feeding, as assessed by the incidence of aspiration and ventilator-associated pneumonia (VAP).

Trial design {8}

The COINN trial is designed as a prospective monocentric explorative trial with two parallel arms. The primary endpoint is the time to reach the energetic target in critically ill adult patients on enteral feeding. Block randomization with a 1:1 allocation will be performed.

Methods: participants, interventions, and outcomes

Study setting {9}

The COINN trial is currently ongoing in four intensive care units (ICUs) at one academic hospital in the Czech Republic.

Eligibility criteria {10}

All patients admitted to the ICU are screened for the inclusion criteria. After informed consent is obtained, each patient is randomized to one of the trial arms. The randomization occurs before EN is started.

The inclusion criteria are as follows:

1. Age 18–80 years
2. Indication of EN with a nasogastric or an orogastric tube
3. Nutrition Risk in the Critically Ill (mNUTRIC) score ≥ 5
4. Body mass index 18–50
5. Mechanical ventilation expected for at least 72 h

Patients meeting any of the following criteria are excluded:

1. Upper gastrointestinal tract surgery in previous medical history
2. Bowel obstruction
3. Bowel ischemia
4. Acute pancreatitis
5. Severe diarrhea (> 1 L/24 h)
6. Gastrointestinal bleeding
7. Short bowel syndrome
8. Malabsorption syndrome in previous medical history
9. Ongoing EN on admission to ICU

Who will take informed consent? {26a}

A trained study investigator will describe the study to patients or to authorized surrogates if applicable. Patients (or authorized surrogates) will also receive information sheets. The study investigator will discuss the study with patients (or authorized surrogates) in light of the information provided in the information sheets. The study investigator will obtain written consent from patients (or authorized surrogates) willing to participate in the trial. In the case of patients' inability to consent because of a medical condition, the ability to participate in the trial will be assessed by a medical council consisting of one independent physician informed about the study details and one study investigator.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Not applicable. Ancillary studies using participant data and biological specimens are not planned.

Interventions

Explanation for the choice of comparators {6b}

EN can be administered as separate bolus doses several times per day or as a continuous infusion over a long period of time. Continuous administration is favored by current ESPEN guidelines and will serve as the comparator for the intervention of intermittent feeding.

Intervention description {11a}

The position of the gastric tube will be verified by measurement of gastric pH or abdominal X-ray. Commercial isocaloric formula with a high content of whey-based protein will be used. To eliminate the effects of formula composition on primary and secondary outcomes of the study, the same enteral formula will be used in all patients included in both study arms. Energetic and protein targets will be estimated according to current guidelines, on the basis of body mass index (BMI) and actual body weight (ABW), as summarized in Table 1 [1]. The energy and protein targets will be determined by the study investigator. The study investigator will provide information to the nurse and attending physician regarding the arm of the study to which the patient was assigned, the time of initiation of EN, and the target rate and bolus volume.

Administration of EN begins no earlier than 6 am and no later than 6 pm if signs of intolerance are absent (see below) and the GRV is below 500 mL. Otherwise, the start of EN will be postponed to the time of the next GRV measurement.

EN will be administered in the first arm (CONT) with continuous infusion via an enteral pump with an infusion time of 18 h per day. During the infusion, the patient will be in a 30–40° head-up position. The initial rate of infusion will be set to 25 mL/h and gradually increased every 4 h according to clinical signs of intolerance and GRV measurements. Signs of intolerance will be considered if abdominal distension or pain, vomiting, or diarrhea occur, and if the GRV exceeds 500 mL.

The rate of infusion will be increased according to tolerance and GRV. If there is no sign of intolerance, and the GRV is < 250 mL in two consecutive measurements, the rate will be increased by 25 mL/h until the target rate is reached. If signs of intolerance are absent, and the GRV is 250–500 mL, the current rate will remain unchanged. If intolerance signs are observed, or the GRV is > 500 mL, the rate will be decreased to half until the next assessment of tolerance and GRV.

The daily dose of EN will be divided in the second arm (INT) into six portions administered in 4-h intervals via an enteral pump. The rate of the enteral pump will

Table 1 Energy and protein targets

BMI	Energy	BMI	Protein
< 30	25–30 kcal/kg of ABW/24 h	< 30	1.2–2 g/kg ABW/24 h
30–50	11–14 kcal/kg of ABW/24 h	30–40	2 g/kg ABW/24 h
–	–	> 40	2–2.5 g/kg ABW/24 h

depend on the volume of the bolus. To avoid sudden gastric distension, the rate will increase with increasing bolus volume [4]. The rates of the enteral pump for particular volumes of EN are summarized in Table 2.

During the infusion of EN, the patient will be in a 30–40° head-up position. After the administration is finished, the gastric tube will be rinsed with 20 mL of water. The initial volume of the bolus will be 80 mL and will be gradually increased every 4 h according to clinical signs of intolerance and GRV measurements. Signs of intolerance will be considered as abdominal distension or pain, vomiting, diarrhea, and a GRV exceeding 500 mL.

The rate of infusion will be increased according to tolerance and GRV. If there is no sign of intolerance and the GRV is < 250 mL in two consecutive measurements, the volume will be increased by 80 mL/h until the target volume is reached. If intolerance signs are absent and the GRV is 250–500 mL, the current volume of the bolus will remain unchanged. If intolerance signs or a GRV > 500 mL is observed, the volume will be decreased to half until the next assessment of tolerance and GRV.

Measurement of GRV will be performed throughout the entire study period (days 1–5) in 4-h intervals with a 150-mL syringe used for aspiration of gastric content. Volumes lower than 150 mL will be returned to the stomach, and larger amounts will be discarded.

Criteria for discontinuing or modifying allocated interventions {11b}

Not applicable. Modification and discontinuation of study interventions is described in the previous section.

Strategies to improve adherence to interventions {11c}

Administration of EN will be provided by nurses who will receive training before the study begins. During the training, the principal investigator or another responsible member of the study team will present the objectives of the study, the methods of measuring patient height and weight, the protocol of administration of EN in both study arms, the method of measuring GRV, and how other study variables will be assessed and documented in the patient medical records. After the study commencement, re-training will occur every 6 months until the trial ends.

Table 2 Volumes of boluses and rates of the enteral pump

Bolus volume (mL)	Rate of enteral pump (mL/h)	Time of administration (min)
80	160	30
160	320	30
240	320	45
320	320	60
400	400	60
> 400	400	Until administered

Relevant concomitant care permitted or prohibited during the trial {11d}

Prokinetics of the upper gastrointestinal tract

Prokinetics will be administered if an increase in the rate or bolus volume is not reached in three consecutive assessments of tolerance and GRV measurements, respectively. If the rate or bolus volume is lowered in two consecutive assessments of tolerance and GRV measurements, the administration of prokinetics will also start. The choice and dose of prokinetic agent will be made by the attending physician. The effects of prokinetics will be assessed after 24 h of administration. If intolerance persists and the GRV is below 250 mL, EN will continue on a fixed rate of 25 mL/h in both arms. If intolerance and GRV > 250 mL persist, a postpyloric tube will be inserted, and postpyloric nutrition will commence.

Parenteral nutrition

The administration of parenteral nutrition will be associated with institutional standards of care. Parenteral nutrition may be considered if less than 60% of the nutritional goal on days 7–10 is reached.

Prokinetics of the lower gastrointestinal tract

The prokinetics of the lower gastrointestinal tract will be indicated by the attending physician any time during the study period in the absence of signs of bowel movements. The choice of agent, dosage, and route of administration will be made by the attending physician.

Provisions for post-trial care {30}

Not applicable.

The trial period includes the first 5–7 days of critical illness. Administration of EN in this trial will follow current guidelines [1, 11], and participation in the trial carries no additional risks beyond those arising from the critical illness itself.

Outcomes {12}

The primary outcome of the trial is the time from the beginning of the administration of EN to the time at which at least 80% of the target energy is delivered. This will be reported as the day of EN administration. The Kaplan-Meier time-to-event analysis will be used for the analysis of the primary outcome.

Secondary outcomes:

1. Time from the beginning of the administration of EN to the time at which at least 80% of the target

protein dose is delivered. This will be reported as the day of EN administration. The Kaplan-Meier time-to-event method of analysis will be used for the analysis of this secondary outcome.
2. Achievement of the energetic target. This outcome will be aggregated as the proportion of patients who achieve the energetic target during the study period.
3. Tolerance of EN GRV will be aggregated as the proportion with GRV < 250 mL, 250–500 mL, and > 500 mL. Episodes of vomiting will be aggregated as the proportion of patients with any number of episodes of vomiting. Diarrhea stools will be aggregated as the proportion of patients with diarrhea stool > 1 L/day. The need for postpyloric nutrition will be aggregated as the proportion of patients with this need.
4. Incidence of complications of EN. The number of aspiration episodes will be aggregated as the proportion of patients with any number of episodes of aspiration. VAP will be aggregated as the proportion of patients with confirmed VAP.
5. Total serum protein, albumin, and prealbumin concentrations on days 1 and 5. Serum concentrations of protein, albumin, and prealbumin will be aggregated as the mean.
6. ICU and hospital length of stay. ICU and hospital length of stay will be aggregated as the mean. The day of admission and day of discharge will be counted as 1 day.
7. The 28-day mortality will be evaluated with a Kaplan-Meier curve.

Participant timeline {13}
The schedule of enrolment, interventions, and visits is summarized in Table 3.

Sample size {14}
Studies focused on the primary target of our study are lacking. However, we have identified several studies comparing bolus and continuous administration of enteral feeding. These studies included small numbers of patients, were conducted in different patient populations, and did not assess the time to reach the nutritional targets as a primary outcome. However, for calculation of the sample size, we hypothesized a difference of 1 day between groups (target being reached on day 2 and day 3, respectively). The alpha was set to 5%, and the power was set to 80%. The estimated sample size was 122 patients per arm. To account for potential drop-out, we increased the sample size to 300 participants in total, with 150 patients in each study arm.

Recruitment {15}
Recruitment of patients began in November 2018. During the first 12 months of the study, 787 patients were screened, among whom 100 were allocated to one of the two study arms. Allocation of the last patient is anticipated to occur in November 2021.

Assignment of interventions: allocation

Sequence generation {16a}
COINN is a single-center study. The randomization procedure is implemented in an electronic case report form

Table 3 Participant timeline

	Study period							Follow-up
Time point	Screening	D0	D1	D2	D3	D4	D5	D28
Number of visits	1	2	3	4	5	6	7	8
Eligibility criteria	x							
Informed consent	x							
Allocation	x							
Demographic data		x						
APACHE II		x						
SOFA score		x						
Laboratory tests (total serum protein, albumin, prealbumin)		x					x	
Estimation of energy and protein target		x						
Administration of enteral nutrition			x	x	x	x	x	
Assessment of tolerance (diarrhea, vomiting, GRV, postpyloric nutrition)			x	x	x	x	x	
Assessment of complications			x	x	x	x	x	
Assessment of energetic and protein targets			x	x	x	x	x	
Assessment of hospital LOS, ICU LOS, and mortality								x

(eCRF) with a randomization ratio of 1:1. The randomization list was generated by the study statistician and implemented in the eCRF by the study data manager.

Concealment mechanism {16b}

The web-based randomization system ensures allocation concealment.

Implementation {16c}

The allocation sequence generation is embedded in the trial web site. The study investigator enrolls participants and randomizes them by using the web-based randomization system.

Assignment of interventions: blinding

Who will be blinded {17a}

The study staff members and treating physicians and other staff will not be blinded, because of the impossibility of blinding while performing the study intervention. The data management group and statisticians will work with a pseudonymized data set.

Procedure for unblinding if needed {17b}

Not applicable.

This trial is not blinded.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Demographic and baseline data

The study will collect demographic and baseline characteristics from medical records and electronic medical records, including age, sex, type of admission, and baseline characteristics. Patient weight will be measured on admission in a standard manner by using a bed-integrated scale; patient height will be measured on admission through a standardized protocol with a tape measure. The patient will lie flat in a supine position. A tape measure will be placed perpendicular to the patient, and the distance from the patient's heels to the head will be measured.

Conventional scoring systems validated for critically ill patients will be used. Estimation of APACHE II, SOFA, and mNUTRIC scores will be performed by the attending physician and documented in the medical records. Results of laboratory tests will be documented in the electronic medical records and entered by the trial investigator in the eCRF.

Primary outcome

The time to reach the energetic target will be defined as the number of days from the beginning of the administration of EN until the day at which at least 80% of the planned EN is administered. The trial investigator will estimate the amount of EN administered by analyzing medical records in which the volume of EN is documented according to institutional standards.

Secondary outcomes

The time to reach the protein target will be defined as the number of days from the beginning of the administration of EN to the day at which at least 80% of the planned protein administration has occurred. The trial investigator will estimate that day by analyzing the medical records.

GRVs, episodes of vomiting, the number and volume of diarrhea stools, and the need for postpyloric nutrition will be documented in the medical records and entered in the eCRF by the trial investigator. Diarrhea will be defined as passing of loose stools three or more times per day. The number and volume of stools will be documented in the medical records. Volumes of diarrhea stools exceeding 1 L/day will be reported.

Aspiration of EN is defined as the presence of EN in the airway of the patient. It will be assessed by a nurse during standard airway care and documented in the medical records. The number of aspiration episodes and the incidence of VAP will be entered in the eCRF by the study investigator. Total serum protein, albumin, and prealbumin concentrations on days 1 and 5 will be obtained from the electronic medical records and entered in the eCRF by the study investigator. The ICU and hospital length of stay will be entered in the eCRF by the study investigator after analysis of the medical records. The information on whether the patient is alive or dead on day 28 will be obtained by telephone.

Plans to promote participant retention and complete follow-up {18b}

After a participant is enrolled or randomized, the study site will make every reasonable effort to follow the participant for the entire study period. Study site staff members will be responsible for developing and implementing local standard operating procedures to achieve maximal follow-up.

Data management {19}

The study data will be entered online in an Internet-based database and collected from medical records. The study staff members will have access to the medical records of the patients. Investigators will be responsible for (1) screening of patients, (2) obtaining informed consent, (3) randomization, and (4) collecting study data and entering the data in the eCRF. The statistician will analyze the study data in cooperation with the principal investigator. The data will be stored for 15 years after completion of the study and then destroyed.

To promote data quality, the eCRFs of each participant will be reviewed by another member of the study team.

Confidentiality {27}
All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with limited access. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to additional identifying information will be stored in a separate locked file in an area with limited access.

Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}
Not applicable.

In this study, no biological specimens for genetic or molecular analysis will be collected.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}
For analyses of study data, the intention-to-treat principle will be used.

For analyses of the primary outcome, the Kaplan-Meier curve, log-rank, or Wilcoxon test will be used. Standard descriptive statistics (i.e., number of available values, mean, SD, median, 25% and 75% quartiles, minimum and maximum) will be calculated for continuous variables. Categorical variables will be described by using tables of frequency. Secondary outcome measures will be compared between study groups with the *t* test or Mann-Whitney test as applicable, and the chi-square test will be used for categorical data. Differences in laboratory tests between different days of measurement will be analyzed with paired tests (paired *t* test or non-parametric Wilcoxon test). A Kaplan-Meier curve will be used to demonstrate 28-day mortality. A significance level of 5% will be used for all statistical tests. Confidence intervals will be presented as appropriate.

The data analyses will be performed after completion of the study. Interim analysis is not planned.

Interim analyses {21b}
Not applicable.

Interim analysis is not planned.

Methods for additional analyses (e.g., subgroup analyses) {20b}
Not applicable.

Additional analysis is not planned.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}
No analysis populations are defined for this study. Protocol non-adherence will be assessed by the principal investigator case by case. Patients with major deviations from the study protocol will be excluded from the analysis.

Missing data are not planned to be imputed. However, in the event of substantial missing data for any parameter, a sensitivity analysis using any method of imputation could also be used.

Plans to give access to the full protocol, participant level-data, and statistical code {31c}
Data sharing statement:

No later than 3 years after the collection of the 1-year postrandomization, we will deliver a completely deidentified data set to an appropriate data archive for sharing purposes.

Statistical codes will be archived in accordance with SOPs.

Oversight and monitoring

Composition of the coordinating center and trial steering committee {5d}
No coordinating center or a trial steering committee will be established.

Composition of the data monitoring committee, its role, and reporting structure {21a}
Not applicable.

No data monitoring committee will be established. The study intervention is part of the standard care of critical care patients, and both modes of application of EN can be used interchangeably.

Adverse event reporting and harms {22}
The formula of EN used in this study is approved for use in critically ill patients, and no severe adverse events relating to the components of this formula are expected.

Serious adverse events have been reported according to standard rules and standard operating procedures of the coordinating unit. All study participants will be monitored for potential adverse events associated with the administration of EN.

We expect the following adverse events to be associated with the administration of EV.

1. Tube malposition. Strategies to prevent gastric tube malposition are described in the "Intervention description {11a}" section of the protocol.
2. Gastrointestinal complications (high GRV, diarrhea, or vomiting). Management of high GRV is described in the "Intervention description {11a}"

section of the protocol. These complications are considered signs of EV intolerance, which result in a decreased rate/volume of EN administered.

3. Refeeding syndrome. Strategies for prevention and management of refeeding syndrome (RFS) are part of the standard care in participating units. They include daily clinical and laboratory screening for RFS and standard medical management if RFS is diagnosed.

Frequency and plans for auditing trial conduct (23)

Trial conduct will be audited by the Institutional Ethics Committee every 6 months during the study. The process of auditing will be independent of the investigator and sponsor.

Plans for communicating important protocol amendments to relevant parties (e.g., trial participants, ethical committees) (25)

Any modifications to the protocol that may affect the conduct of the study, the potential benefits to the patient, or patient safety, including changes in study objectives, study design, patient population, sample sizes, study procedures, or significant administrative aspects, will require a formal amendment to the protocol. Any amendments will be agreed on and approved by the ethics committee before implementation, and the health authorities will be notified, in accordance with local regulations. The protocol amendment will be sent to all members of the study team by e-mail. All attending physicians and nurses of the study units will be acquainted with the amendment at a meeting. The invitation to the meeting will be sent by e-mail. The protocol of the study and its amendments will be available at each study unit.

Dissemination plans (31a)

We intend to submit the results of the study to be published in a peer-reviewed international medical journal.

Discussion

EN has been used in the treatment of critically ill patients for decades. Guidelines for its provision have been developed by several international societies [1, 11, 14] and are periodically updated. Although the body of evidence is growing, several controversies and uncertainties remain. Recent guidelines of the American Society for Parenteral and Enteral Nutrition (ASPEN) suggest that in high-risk patients and those shown to be intolerant to bolus gastric EN, the delivery of EN should be switched to continuous infusion [1]. This recommendation is based on expert consensus but is supported by very limited evidence. The studies in the analysis were small and performed on different patient populations [6, 8, 9, 15–18].

After the commencement of this study, new ESPEN guidelines on clinical nutrition in the intensive care unit were published. One recommendation is that continuous rather than bolus EN should be used. However, the authors assumed that the evidence on this issue is very limited. In a randomized clinical trial, intermittent feeding in comparison with continuous feeding has been found to result in no statistically significant difference in vomiting, abdominal distension, or diarrhea [19].. No significant differences in high GRV, abdominal distention, diarrhea, and vomiting in critically ill patients who received EF by bolus versus the continuous method have been reported [2]. A review of continuous and intermittent feeding has summarized the benefits and drawbacks of both feeding methods and found no evidence favoring either method with regard to metabolic, gastrointestinal, and organizational concerns [20].

Our protocol uses clinical signs of tolerance and GRV to determine the administration rate of EN. We use a volume of GRV > 500 mL as a sign of intolerance. In some studies, smaller volumes have been considered markers of intolerance [2]. Limitation or interruption of the administration of EN on the basis of lower GRV has been shown to lead to inadequate nutrition intake, and EN cessation is not recommended for GRV < 500 mL [1, 21, 22].

To promote EN tolerance, prokinetics will be used under the circumstances described in the "Relevant concomitant care permitted or prohibited during the trial (11d)" section of this protocol. Erythromycin or metoclopramide can be used. Both drugs are effective in decreasing feeding intolerance without increasing the rates of diarrhea and significant arrhythmias [23]. Metoclopramide has the same effectiveness regardless of the mode of administration, and the choice of a particular agent and mode of administration will be the responsibility of the attending physician [24]..

Regarding the primary outcome, the time to reach the energy target has been compared in two studies [6, 8]. After a detailed review of the study protocols, we have found that in both these studies, the increments of EN in the intermittent and continuous arm were set differently. In the case of optimal tolerance of EN, the energy target would have been reached at different time points, although the EN would have been started simultaneously in the bolus or continuous arm. Therefore, comparison of EN administration modes is problematic. In our study, we set the increments of EN so that the energy target is reached at the same time point regardless of whether EN is administered continuously or as a bolus. Therefore, both modes of EN

administration are comparable regarding the primary outcome.

Aspiration of EN is a complication of EN administration. Objectively estimating aspiration is difficult. The method of coloring EN has been used in some studies. However, this method is currently not recommended for identification of aspiration because of potential adverse metabolic effects of the dye [25–27].

Aspiration of EN will be identified and noted in the medical record by nursing staff during standard airway care. This method of identification of EN aspiration is highly subjective and may be a potential source of bias.

In conclusion, we believe that this randomized trial will provide additional information regarding whether continuous or bolus feeding is associated with any differences in the tolerance and complication rate.

Trial status

Protocol version 2.0, dated 28 May 2019. The recruitment began on 25 November 2018. One hundred patients had been randomized by 1 December 2019. The randomization of the final 300 patients is anticipated by November 2021. Completion of the follow-up will end in December 2021.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13063-020-04866-2.

Additional file 1. Ethical committee approval

Abbreviations

ABW: Actual body weight; APACHE I: Acute Physiology and Chronic Health Evaluation; BMI: Body mass index; eCRF: Electronic case report form; EN: Enteral nutrition; ESPEN: European Society for Parenteral and Enteral Nutrition; GRV: Gastric residual volume; ICU: Intensive care unit; mNUTRIC: Modified Nutrition Risk in the Critically Ill; SOFA score: Sequential Organ Failure Assessment score; VAP: Ventilator-associated pneumonia

Acknowledgements

We would like to thank all clinical staff at the study site.

Authors' contributions (31b)

OH, KV, ES, PS, LS, RS, IS, and RG: study design and preparation of the protocol. RS: statistical plan. OH, ES, and PS: collection of the data. OH, ES, and KV: data interpretation. RG and IS: critical revision of the manuscript. All authors have read and approved the final manuscript.

Competing interests (28)

The authors declare that they have no competing interests.

Funding (4)

Not applicable. This study is not funded.

Availability of data and materials (29)

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication (32)

Not applicable. Publication of any data associated with an individual person is not planned. An example consent form is available from the corresponding author on reasonable request.

Ethics approval and consent to participate (24)

This protocol and the template informed consent form were reviewed and approved by the Ethical Committee of the University Hospital Brno (ref. number: 02-130618) with respect to scientific content and compliance with applicable research and human subject regulations. Ethical committee approval is contained in an additional file.
Informed consent will be obtained from all participants prior to enrollment in the study.

Authors' information

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References

1. McClave SA, Taylor BE, Martindale RG, et al. Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). J Parenter Enter Nutr. 2016;40:159–211.
2. Serpa LF, Kimura M, Faintuch J, Ceconello I. Effects of continuous versus bolus infusion of enteral nutrition in critical patients. Rev Hosp Clin Fac Med S Paul. 2003;58(1):9–14.
3. Patel JJ, Rosenthal MD, Heyland DK. Intermittent versus continuous feeding in critically ill adults. Curr Opin Clin Nutr Metab Care. 2018;21(2):116–20.
4. Marik PE. Feeding critically ill patients the right 'whey': thinking outside of the box. A personal view. Ann Intensive Care. 2015;5:51.
5. Ichimaru S, Intermittent AT. Bolus methods of feeding in critical care. In: Rajendram R, Preedy V, Patel V, editors. Diet and nutrition in critical care. New York: Springer; 2014. p. 1–17.
6. Nasiri M, Fani Z, Ahangari M, Dadgari F. Comparison of intermittent and bolus enteral feeding methods on enteral feeding intolerance of patients with sepsis: a triple-blind controlled trial in intensive care units. Middle East J Dig Dis. 2017;9(4):218–27.
7. Stevens EC, Lipscomb AF, Poole GV, Sacks GS. Comparison of continuous vs intermittent nasogastric enteral feeding in trauma patients: perceptions and practice. Nutr Clin Pract. 2002;17:18–22.
8. MacLeod JB, Lefton J, Houghton D, Roland C, Doherty J, Cohn SM, et al. Prospective randomized control trial of intermittent versus continuous gastric feeds for critically ill trauma patients. J Trauma. 2007;63:57–61.
9. Bonten MJ, Gaillard CA, van der Hulst R, de Leeuw PW, van der Geest S, Stobberingh EE, et al. Intermittent enteral feeding: the influence on respiratory and digestive tract colonization in mechanically ventilated intensive-care-unit patients. Am J Respir Crit Care Med. 1996;154(2 Pt 1):394–9.
10. Aguilera-Martínez R, Ramis-Ortega E, Carratalá-Munuera C, Fernández-Medina JM, Saiz-Vinuesa MD, Barrado-Narvión MJ. Effectiveness of continuous enteral nutrition versus intermittent enteral nutrition in intensive care patients: a systematic review. JBI Database System Rev Implement Rep. 2014;12(1):281–317.
11. Singer P, Blaser AR, Berger MM, Alhazzani W, Calder PC, Casaer MP, Hiesmayr M, Mayer K, Montejo JC, Pichard C, Preiser JC, van Zanten ARH, Oczkowski S, Szczeklik W, Bischoff SC. ESPEN guideline on clinical nutrition in the intensive care unit. Clin Nutr. 2019;38(1):48–79.
12. Marik PE, Rice TW. Feeding the critically ill: more questions than answers! Am J Respir Crit Care Med. 2017;195(5):555–6.
13. Di Girolamo FG, Situlin R, Fiotti N, Biolo G. Intermittent vs. continuous enteral feeding to prevent catabolism in acutely ill adult and pediatric patients. Curr Opin Clin Nutr Metab Care. 2017;20(5):390–5.
14. Heyland DK, Dhaliwal R, et al. Canadian clinical practice guidelines for nutrition support in mechanically ventilated, critically ill adult patients. JPEN J Parenter Enteral Nutr. 2003;27(5):355–73.
15. Ibrahim EH, Mehringer L, Prentice D, et al. Early versus late enteral feeding of mechanically ventilated patients: results of a clinical trial. JPEN J Parenter Enteral Nutr. 2002;26(3):174–81.

16. Hiebert JM, Brown A, Anderson RG, Halface S, Rodeheaver GT, Edlich RF. Comparison of continuous vs intermittent tube feedings in adult burn patients. JPEN J Parenter Enteral Nutr. 1981;5(1):73–5.
17. Kocan MJ, Hicklsch SM. A comparison of continuous and intermittent enteral nutrition in NICU patients. J Neurosci Nurs. 1986;18(6):333–7.
18. Ciocon JO, Galindo-Ciocon DJ, Tiessen C, Galindo D. Continuous compared with intermittent tube feeding in the elderly. JPEN J Parenter Enteral Nutr. 1992;16(6):525–8.
19. Tavares de Araujo VM, Gomes PC, Caporossi C. Enteral nutrition in critical patients; should the administration be continuous or intermittent? Nutr Hosp. 2014;29(3):563–7.
20. Bear DE, Hart N, Puthucheary Z. Continuous or intermittent feeding: pros and cons. Curr Opin Crit Care. 2018;24(4):256–61.
21. Montejo JC, Minambres E, Bordeje L, et al. Gastric residual volume dur-ing enteral nutrition in ICU patients: the REGANE study. Intensive Care Med. 2010;36(8):1386–93.
22. Pinilla JC, Samphire J, Arnold C, Liu L, Thiessen B. Comparison of gastrointestinal tolerance to two enteral feeding protocols in critically ill patients: a prospective, randomized controlled trial. JPEN J Parenter Enteral Nutr. 2001;25(2):81–6.
23. Lewis K, Alqahtani Z, McIntyre L, et al. The efficacy and safety of prokinetic agents in critically ill patients receiving enteral nutrition: a systematic review and meta-analysis of randomized trials. Crit Care. 2016;20(1):259.
24. Malekolkottab M, Khalili H, Mohammadi M, Ramezani M, Nourian A. Metoclopramide as intermittent and continuous infusions in critically ill patients: a pilot randomized clinical trial. J Comp Eff Res. 2017;6(2):127–36.
25. Maloney J, Metheny N. Controversy in using blue dye in enteral tube feeding as a method of detecting pulmonary aspiration. Crit Care Nurse. 2002;22(5):84–5.
26. Maloney JP, Ryan TA. Detection of aspiration in enterally fed patients: a requiem for bedside monitors of aspiration. JPEN J Parenter Enteral Nutr. 2002;26(6):534–41.
27. Koln-Keeth C, Frankel EH. Taking blue dye out of tube feedings. Nursing. 2004;34(2):14.

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7.7 Annex 7

Chovanec, Z., Červeňák, V., Čundrle, I. *et al.* Labelling of subpleural pulmonary nodules with blue dye and contrast agents under CT-guided control and subsequent video-assisted thoracoscopic resection: the BLUEPAT prospective randomised study. *Trials* (2026). https://doi.org/10.1186/s13063-026-09748-7

Summary
Purpose:
The aim of this study was to evaluate the feasibility, safety, and clinical utility of CT-guided colour marking of small subpleural pulmonary nodules prior to video-assisted thoracoscopic surgery (VATS). The study addresses a clinically important challenge in thoracic surgery, as small or poorly palpable nodules may be difficult to localise during minimally invasive procedures, potentially leading to prolonged surgery or incomplete resection.

Methodology:
The BLUEPAT trial was designed as a prospective, monocentric, open-label, randomized clinical study including 72 patients requiring histological verification of subpleural lung nodules. Participants were randomized in a 1:1 ratio either to standard VATS wedge resection or to a preoperative CT-guided localisation procedure using Patent Blue V dye combined with iohexol contrast agent before VATS resection; safety and feasibility outcomes were systematically monitored during a 30-day follow-up period.

Results:
As a study protocol publication, the article primarily establishes a detailed methodological framework for evaluating the novel localisation technique, including randomisation strategy, perioperative workflow, and safety monitoring. The protocol highlights the expected advantages of colour-guided localisation, including improved intraoperative identification of small nodules, reduction of unnecessary tissue resection, and optimisation of minimally invasive surgical procedures.

Conclusion:
The BLUEPAT study represents an innovative investigator-initiated surgical trial aimed at improving precision and safety in thoracic surgery through integration of CT-guided localisation techniques with minimally invasive VATS procedures. The study illustrates how NCCTs can support translational surgical innovation and contribute to optimisation of diagnostic and therapeutic pathways in patients with focal lung disease.

Chovanec et al. Trials (2026) 27:428
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Trials

STUDY PROTOCOL Open Access

Labelling of subpleural pulmonary nodules with blue dye and contrast agents under CT-guided control and subsequent video-assisted thoracoscopic resection: the BLUEPAT prospective randomised study

Zdeněk Chovanec[1], Vladimír Červeňák[1,2], Ivan Čundrle[2,3], Michal Šitina[2,3], Jiří Vaníček[1], Alena Berková[1], Tomáš Hanslík[1], Igor Penka[1] and Lenka Součková[3,4*]

Abstract

Background Colour marking of indicated nodules is an innovative, accurate, and gentle method that allows subsequent wedge resection of the marked tissue using a mini-invasive, video-assisted thoracoscopic (VATS) method with all its benefits. One of the expected benefits of this clinical trial is introducing and optimising the evaluated way into routine management in diagnosing and treating focal lung diseases. The BLUEPAT clinical trial aims to assess the technique of colour marking of subpleural deposited small lung nodules with the subsequent performance of VATS wedge resection in surgical practice.

Methods The BLUEPAT clinical trial is a prospective, monocentric, open-label, randomised study that will include 72 participants with subpleural deposited nodules with the need for additional histological diagnostics to optimise subsequent treatment. These patients will be randomised into two arms in a 1:1 ratio. The interventional arm will undergo VATS wedge resection of lung nodules marked with Patent Blue V and contrast agent iohexol under CT-guided control. The control arm will undergo VATS resection without prior colour coding of the lung nodules. The feasibility and safety of both methods will be assessed based on the evaluation of the parameters of the primary and secondary objectives of the study. The safety of the methods will be monitored by recording all related adverse events. All enrolled patients will undergo a 30-day follow-up period within the framework of clinical trial.

Results This article reports the clinical trial protocol; outcome data will be published upon study completion.

Discussion The BLUEPAT clinical trial offers a novel assessment of this innovative approach for the diagnosis and treatment of focal lung diseases. The use of patent blue dye has been well established in diagnostic procedures for breast cancer and this study extends its potential application to pulmonary indications.

Trial registration EudraCT Number 2021-001122-22. Registered on March 18, 2021.

Keywords Diagnosis and treatment of focal lung diseases, Video-thoracoscopic resection, Patent blue, Contrast agent iohexol, CT-guided

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Introduction

Background and rationale {6a}

Epidemiology and clinical relevance of subpleural pulmonary nodules

Focal lung nodules are a common on thoracic computed tomography (CT). The primary diagnostic challenge lies in determining whether these nodules are benign or malignant. In patients with a history of cancer, the possibility of metastatic disease must be prioritised in the differential diagnosis. By contrast, in patients without signs of an oncological diagnosis, incidentally detected lung nodules are referred to as *incidentalomas*—have a broad spectrum of possible aetiology. These are most frequently post-inflammatory granulomas after a healed infection, scars, intraparenchymal lymph nodes, etc.

Currently, there are no universally binding criteria for managing these incidental nodules. However, data from large lung cancer screening trials have provided a foundation for evidence-based recommendations, aiming to improve the early diagnosis of lung cancer, increase survival, and reduce the socio-economic burden of advanced disease management. To support consistent evaluation, our study applies the Lung-RADS v1.1 criteria [1], which incorporate lesion morphology, density, borders, calcifications, and dynamic growth, while also stratifying patients into low- and high-risk categories.

Challenges of traditional surgical approaches

Subpleural nodules Subpleural nodules are often invisible during surgery and can typically be detected only by careful palpation. This requirement traditionally necessitates an open thoracotomy, usually 10–20 cm in length, to allow the surgeon to locate and excise the lesion for histological assessment. While effective, thoracotomy is associated with substantial morbidity, including severe postoperative pain, frequent rib fractures, and complications related to epidural analgesia. Hospital stays and recovery periods are consequently prolonged, and the risk of postoperative complications is increased [2].

(VATS) minimally invasive alternative, performed Video-assisted thoracoscopic surgery (VATS) provides a minimally invasive alternative through one to three small (1–3 cm) incisions using a thoracoscope and endoscopic instruments.

VATS metastasectomy has demonstrated oncological outcomes comparable to thoracotomy [3], particularly for small, peripherally located lesions (≤3 cm) [4]. However, VATS is limited in its ability to detect nodules ≤10 mm in diameter located more than 5 mm beneath the pleural surface. In such cases, intraoperative visualisation is often impossible without additional localisation methods, as even a marker placed only 1 mm subpleural may remain invisible without colour coding. If a small pulmonary focal lesion (≤10 mm) is located more than 5 mm from the nearest pleural surface, it is recommended to use the method of colour marking the lesion [5].

Localisation and labelling techniques

The localisation and labelling of subpleural lung nodules is an established surgical technique that has been successfully utilised for many years [6]. To address these challenges, various localisation techniques have been developed and validated. Established methods include the use of metal coils, hook wires, and radio-opaque markers applied under CT or bronchoscopy guidance. Fluorescent and radioactive tracers have also been employed, though these require specialised imaging equipment for intraoperative detection [7].

A widely available and cost-effective solution is the use of vital dyes, particularly methylene blue or Patent Blue V, often combined with a radiological contrast agent. These dyes diffuse rapidly into tissue, creating a clearly demarcated area that can be easily identified intraoperatively, even in altered lungs [8, 9]. The addition of a contrast medium enables CT-based verification of correct dye placement and diffusion around the lesion, ensuring precise and safe resection.

Methods

Trial design {8}

The BLUEPAT trial is an investigator-initiated, prospective, single-centre, open-label, randomised clinical study with a superiority framework, scheduled to commence in March 2026. A total 72 participants with subpleural pulmonary lesions requiring additional histological evaluation to guide subsequent treatment will be enrolled. The eligible patients will be randomised in a 1:1 ratio into one of two arms.

- Intervention arm (PBV marking): Video-assisted thoracoscopic surgery (VATS) wedge resection of lung nodules marked preoperatively with a combination of patent blue dye and iohexol contrast agent under computed tomography (CT) guidance
- Control arm: VATS wedge resection of lung nodules without preoperative colour marking

The study's primary objective is to assess whether preoperative lung nodule marking with patent blue and iohexol improves surgical feasibility and safety compared with standard VATS resection without marking.

Safety will be evaluated by recording all related adverse events and serious adverse events. Secondary objectives include an assessment of perioperative

Table 1 Participant timeline

Assessment	Day 0 (admission)	Day 1 (surgery)	Day 2	Day 3	Day 10	Day 30
Informed consent form	X					
Pregnancy test[a]	X					
CT (interventional arm with colour labelling nodule)		X				
Colour marking and resection of nodule		X				
Characterisation of nodule[b]		X				
Assessment of methodology feasibility[c]		X				
RTG chest[d]		X	(X)	(X)	(X)	
Blood sample–blood gases monitoring[e]		X	(X)	(X)		
Histological examination of tissue		X[s]			X[s]	
Clinical examination of participant's status	X	X	X	X	X	(X)
Analgesic consumption, monitoring[f]		X	X	X	X	X
Report of intra operative complication[g]		X				
Postoperative algesia (VAS)		X	X	X	X	(X)
Report of postoperative complication[h]						X
Adverse effect		X	X	X	X	X
Length of ICU stay (days)						X
Length of hospitalisation (days)						X
Report of 30 days mortality						X
Thoracic drainage insertion time (days)						X
Report of the need for further surgery						X
Questionnaire postoperative quality of life SF-36	X					X

[a] Analysis of hCG from blood or urine in women of childbearing potential

[b] Typing according to Lung-RADS criteria version 1.1, localisation in the lung parenchyma (designation of the lung segment), size, and subpleural depth [10]

[c] Total time of radiolabelling of the lesion from arrival at CT, total time from radiologist to visualisation of the lesion by the surgeon, presence of staining substance outside the marked area (parietal pleura, mediastinum, free thoracic cavity), comparison of volume of marked area after resection with size of solid lesion on CT

[d] Two hours after the surgery, at the time of the reduction of the chest suction to the 8 cm water column, 2 h after the extraction of the chest drain, at the time of the suture extraction during the outpatient examination (7th–14th postoperative day)

[e] In the postoperative period, blood will be taken a total of 3 times to analyse blood gases

[f] Preparation, dose, dosing interval, duration of administration

[g] CRF record from medical records—yes/no; if yes: description of complication, duration, treatment

[h] CRF record from medical records—yes/no; if yes: description of complication, duration, treatment

[s] Tissue sampling; [R] - Results of tissue histology

parameters, complication rates, and procedural efficacy. Participants will be followed for 30 days post-procedure. All data will be collected prospectively, and the trial will be conducted in compliance with Good Clinical Practice (GCP) guidelines and the Declaration of Helsinki.

Study setting {9}

The BLUEPAT study is not yet recruiting. It is planned to initiate the study in the Czech Republic in March 2026.

University Hospital St. Anne's Brno, the coordinating centre for the study, is responsible for the education and training of research staff, tracking participants' enrolment, mathematical analysis, monitoring, pharmacovigilance, data management, and reporting of the study.

Participant timeline {13}

The enrolment, intervention, and visits schedule are summarised in Table 1. The duration of the study participation in a given patient is 30 days.

Eligibility criteria {10}

Inclusion criteria

A patient may be included in the clinical trial if they meet all of the following inclusion criteria:

1. Age ≥ 18 years

2. Subpleural deposited pulmonary nodules with the need for further histological diagnosis, which is:

   a) Localised at a depth of 0 to 30 mm below the pleura
   b) Less than 40 mm in length
   c) Subsolid with a solid component more significant than 6 mm
   d) With growth progression according to the Lung-RADS criteria (version 1.1) [1]
   e) Clearly not infiltrating the parietal pleura according to CT examination

3. The patient must have sufficient cognitive capacity and overall health status to fully comprehend the information about clinical trial and voluntarily provide informed consent.
4. Participants of childbearing potential must agree to the use approved contraceptive methods for the entire duration of the clinical trial:

   a) Women of childbearing potential

      Must commit to one of the following highly reliable methods of contraception, used consistently and correctly:

      – Hormonal contraception:

– Combined hormonal contraception (oral, vaginal, or transdermal dosage form)
– Gestagen hormonal contraceptives associated with ovulation inhibition (oral or injectable dosage form)

      – Intrauterine devices:

– Non-hormonal intrauterine device
– Hormone-releasing intrauterine device

      – Permanent methods:

– Bilateral tubal occlusion
– Partner's confirmed vasectomy

      – Other:
      – Complete and persistent sexual abstinence

   b) Men
      Must commit to one of the following:

      – Permanent sexual abstinence
      – Barrier contraceptions
      – Correct use of a condom in any case of sexual intercourse
         – Additional note: If the partner is not using a highly reliable method of contraception, condom use is mandatory throughout the trial.

Exclusion criteria

A patient will be excluded from participation in the clinical trial if they meet any of the following exclusion criteria:

1. Pregnancy or breast-feeding
2. History of hypersensitivity/allergy to patent blue or triphenylmethane dyes contained in medicinal products, food, or cosmetics
3. Hypersensitivity/allergy or history of severe adverse reactions to iodine or iodine contrast agents (e.g. severe renal impairment after previous administration of iodine contrast agent)
4. Hypersensitivity/allergy to excipients in PBV or iodine contrast agent
5. Hypersensitivity/allergy to trimecaine or other local anaesthetics
6. Manifest hyperthyroidism or thyrotoxicosis
7. Scheduled thyroid scan, thyroid function test, or radioiodine treatment

Recruitment {15}

Recruitment of the patients is planned to commence in March 2026. During the first 2 years, all planned patients shall be recruited. The patients will be referred within cooperating centres in the Czech Republic and recruited at University Hospital St. Anne Brno.

The patient will be informed about the study before the surgery or by telephone, during which initial eligibility for the survey will be determined, and the research staff will explain the study protocol and design.

Who will take informed consent? {26a}

The study protocol, including the informed consent, and patient information sheet were reviewed and approved by the Institutional Review Board/Ethics Committee of the participating site and by the National Regulatory Authority—The State Institute of Drug Control. Only trained and qualified study investigators are authorised to obtain informed consent form. The investigator will provide potential participants with comprehensive verbal and written information sheets about the study, using the approved patient information sheet. Ample time will

be given to review the materials and ask questions before any decision is made.

Eligibility and process:

- In accordance with the inclusion criteria, only patients with sufficient cognitive ability and decision-making capacity to provide informed consent may be enrolled.
- The investigator will discuss the study in detail with the patient, ensuring full understanding of the study purpose, procedures, potential risks, benefits, and alternatives.
- Written informed consent will be obtained before any study-specific procedures are initiated.

All procedures will comply with the principles of the International Council for Harmonisation Good Clinical Practice (ICH-GCP E6), the Declaration of Helsinki, and applicable national legal and ethical frameworks.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

A part of the informed consent will be dedicated to reusing the data obtained in the proposed study to improve the current clinical practice and to disseminate evidence-based medicine. The study conforms to the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Objectives {7}

We hypothesise that the use of Patent Blue V (PBV) for preoperative marking of subpleural pulmonary nodule, followed by VATS wedge resection, will improve surgical outcomes compared with standard VATS resection without prior PBV staining. Specifically, we expect PBV (Patent Blue Sodium 2.5%) and contrast agent iohexol (Omnipaque 350 mg I/ml) under CT-guided control labelling to increase the accuracy of intraoperative localisation, reduce perioperative morbidity, and improve early postoperative recovery.

This is a superiority trial comparing VATS wedge resection with and without PBV-based labelling.

To evaluate whether PBV-guided labelling of subpleural lung nodules improves surgical feasibility and safety compared with standard VATS resection without labelling:

Composite primary objective (assessed within 30 days post-surgery):

- Feasibility—successful intraoperative localisation of the target lesion (visualisation of the PBV-marked area by the surgeon following CT-guided marking)
- Safety—incidence of predefined adverse events and reactions of special interest (AESIs)

The composite endpoint will be analysed as the proportion of patients in whom both conditions are met. A two-sided significance level of 0.05 will be applied. Multiplicity arising from separate components of the composite will be adjusted using Bonferroni correction in sensitivity analyses.

Secondary objectives:

1) Clinical outcomes

- Incidence of intraoperative complications
- Incidence of postoperative pulmonary and cardiovascular complications
- 30-day all-cause mortality
- Length of stay (hospital and ICU)
- Postoperative pain (VAS) and analgesic consumption [11]

2) Feasibility and procedural performance

- Radiologist time for CT-guided marking
- Interval from completion of marking to intraoperative visualisation
- Accuracy of marking (comparison of resected specimen with CT-defined lesion size and location)
- Occurrence of dye spread outside the intended lesion area

3) Diagnostic yield

- Nodule typing according to Lung-RADS v1.1 [1] and correlation with histopathology
- Proportion of benign versus malignant lesions
- Nodule characteristics (size, subpleural depth, anatomical segment)

4) Additional safety and recovery parameters

- Postoperative chest X-ray findings
- Chest drain duration
- Total length of hospitalisation

Outcomes {12}

This is a superiority trial comparing VATS wedge resection with and without PBV-based labelling.

The aim is to evaluate whether PBV-guided labelling of subpleural lung nodules improves surgical feasibility and safety compared with standard VATS resection.

Composite primary endpoint (within 30 days post-surgery):

- Feasibility—successful intraoperative localisation of the target lesion (visualisation of the PBV-marked area by the surgeon following CT-guided marking)
    - Outcomes: concordance of radiologist CT-visibility and surgeon intraoperative visibility (radiologist YES/surgeon YES; radiologist YES/surgeon NO); percentage of resections performed in sano
- Safety—incidence of predefined adverse events of special interest (AESIs)
    - Outcomes:
        - Mild/moderate AESIs—pneumothorax <2 cm not requiring drainage, non-surgical bleeding, mild allergic reaction (rash)
        - Severe/life-threatening AESIs—pneumothorax >2 cm requiring drainage, bleeding requiring surgery, pulmonary embolism, anaphylaxis

The composite endpoint will be analysed as the proportion of patients in whom both feasibility and safety criteria are met. A two-sided significance level of 0.05 will be applied. Multiplicity from separate components will be adjusted in sensitivity analyses using Bonferroni correction.

Secondary endpoints:

1) Clinical outcomes

- Incidence of intraoperative complications (inability to identify lesion, bleeding, lung parenchymal injury, intrathoracic injury, conversion to thoracotomy)
- Incidence of postoperative pulmonary and cardiovascular complications (reoperation, re-drainage, secretion volume from thoracic drain in 24 h, postoperative air leak)
- 30-day all-cause mortality
- Length of stay (hospital and ICU)
- Postoperative pain (VAS 0–10) and analgesic consumption (drug, dose, interval, duration)

2) Feasibility and procedural performance

- Radiologist time for CT-guided marking (minutes)
- Interval between completion of marking and intraoperative visualisation (minutes)
- Accuracy of marking (comparison of resected specimen volume with CT-defined lesion size and location)
- Occurrence of dye spread outside the intended lesion area (pleura, mediastinum, thoracic cavity)

3) Diagnostic yield

- Nodule classification according to Lung-RADS v1.1 and correlation with histopathology
- Proportion of benign vs. malignant nodules
- Nodule characteristics: anatomical segment, size/volume (cm^3), subpleural depth (mm)

4) Additional safety and recovery parameters

- Postoperative chest X-ray findings (immediate and follow-up as per protocol)
- Duration of chest drain placement (days)
- Total length of hospitalisation (days)
- Postoperative quality of life (SF-36 questionnaire)

Assignment of interventions: allocation

Explanation for the choice of comparators {6b}

This study includes an active comparator arm to evaluate the added value of preoperative colour coding of lung nodules in patients undergoing video-assisted thoracoscopic surgery (VATS) wedge resection. Patients will be randomised in a 1:1 ratio into two arms:

- Interventional arm: VATS wedge resection of lung nodules marked preoperatively with patent blue dye and the contrast agent iohexol under CT-guided control
- Control arm: VATS wedge resection without prior colour coding of lung nodules

The comparator was selected to represent the current standard surgical practice in cases where preoperative localisation is not performed. This design enables the direct assessment of whether colour coding improves the accuracy, safety, and efficiency of nodule localisation and resection, while controlling for other surgical and perioperative factors.

Sequence generation {16a}

Randomisation will be performed in a ratio of 1:1 allocating participants to either the interventional arm (patent blue dye marking, PBV) or the control arm (no preoperative lung nodule staining). The allocation sequence will be computer-generated using the REDCap randomisation module hosted on the secure institutional server. The randomisation list will be prepared in advance by an

independent statistician who is not involved in patient recruitment or clinical management. The sequence will be generated using permuted blocks of varying sizes to maintain allocation concealment and prevent predictability.

Concealment mechanism {16b}
The allocation sequence will be stored in the REDCap randomisation database with restricted access. Randomisation will be performed only after the patient's eligibility has been confirmed and informed consent obtained. Upon entry of the required baseline data into REDCap, the system will automatically assign the participant to one of the two study arms, ensuring immediate, concealed allocation. The use of REDCap's secure web-based platform ensures that neither investigators nor clinical staff can access or modify the allocation sequence prior to randomisation.

Implementation {16c}
The principal investigator at clinical site will enrol eligible participants and enter baseline data into REDCap. Once eligibility is confirmed and informed consent signed, the REDCap randomisation module will be activated to assign the patient to the interventional arm or the control arm. In the interventional arm (PBV marking), patients will undergo CT-guided preoperative lung nodule staining before VATS wedge resection. In the control arm, patients will proceed directly to VATS wedge resection without staining.

The principal investigator is responsible for:

- Screening patients according to inclusion and exclusion criteria
- Ensuring compliance with study procedures
- Documenting intraoperative and postoperative complications according to the study protocol

The sponsor, University Hospital St. Anne's Brno, will oversee randomisation process adherence, perform periodic audits, and verify that all allocations are generated and stored exclusively through the REDCap platform.

Assignment of interventions: blinding

Who will be blinded {17a}
Blinding of surgeons and procedural staff is not feasible because the intervention (CT-guided dye marking with Patent Blue V) produces an immediately visible intraoperative signal that necessarily informs localisation and resection; therefore, the operating team would be unavoidably unblinded. Participants are under general anaesthesia during surgery, and perioperative workflows (radiology marking vs. no marking) make patient blinding impracticable in routine care. Given these constraints, outcome-assessor blinding will be implemented wherever possible to minimise bias: (i) predefined, objective endpoints (AESIs, 30-day events, ICU/hospital LOS, re-interventions) will be extracted from source records by staff blinded to allocation; (ii) imaging (postoperative chest X-rays/CT) will undergo blinded central review; (iii) pain management and discharge criteria will follow standardised protocols; (iv) data analysts will be blinded to group codes until the primary analysis is locked. These measures mitigate detection and performance bias in the absence of surgical blinding.

Procedure for unblinding if needed {17b}
Not applicable. This study is not blinded.

Interventions

Intervention description {11a}
Patient selection phase
Patients' suspicious pulmonary lesions are routinely evaluated during multidisciplinary lung board meetings at St. Anne's University Hospital Brno, a tertiary referral centre with extensive experience in the diagnosis and management of thoracic malignancies. The lung board includes board-certified thoracic surgeons, oncologists, radiologists, and pulmonologists with documented expertise in the treatment of pulmonary tumours and related conditions. All specialists involved have long-standing clinical experience in this field and regularly manage complex oncological cases according to national and international guidelines.

The activities undertaken by physicians during the clinical decision meetings will be performed within the framework of routine clinical practice and are independent of the conduct of this clinical trial. Patients meeting the indication criteria will then be ordered for admission to the 1st Department of Surgery, Faculty of Medicine, Masaryk University, University Hospital St. Anne's Brno. From these patients, the investigator will select individuals with potential for inclusion in the study according to the inclusion and exclusion criteria.

Hospital admission—day 0
On the day of admission to the 1st day of the entrance, the patient will be offered participation in the clinical trial and interviewed by examining physicians (interventional radiologist and surgeon) who will clearly explain the method of marking the lung nodules and surgery. Further aspects of participation in the clinical trial and its potential risks and benefits will be presented. If the patient agrees to participate, written informed consent will be obtained.

All patients enrolled in the trial will receive treatment in accordance with current standard clinical practice and established guidelines for the management of suspicious pulmonary nodules, irrespective of randomisation arm. Participation in the trial does not replace or delay indicated diagnostic or therapeutic procedures but evaluates two accepted approaches to lesion localisation prior to standard surgical resection.

A pregnancy blood or urine test will be performed on women of childbearing potential. On the day of admission, patients will be randomised into one of two arms (1st interventional arm—PBV marking, 2nd control arm—undergoing lesion staining and subsequent resection without PBV marking) using the randomizer.org web software. All patients will complete the SF-36 questionnaire on the day of admission.

Scheduled surgery day—day 1

On the day of the planned surgery, patients from the control arm will be transported directly to the operating room, where they will undergo surgery under general anaesthesia with selective ventilation in the lateral position.

In all patients in the interventional arm with colour-coded lung nodules, a chest CT will be performed on the day of the planned operation, during which the monitored lesions will be re-examined. In the case of multiple nodules, the most suspicious and optimally localised nodules will be selected by both the radiologist and the surgeon for both marking and subsequent resection.

The patient will be positioned on a mobile CT table. The patient's position (e.g. lateral decubitus, prone, or supine) will be determined based on the prior CT scan to minimise the radiation exposure to the implanted markers and to optimise the safety and suitability of the puncture access trajectory. After alignment using coordinate points on the CT scan, the injection site point will be precisely selected, and a route to the selected subpleural deposited area or district for subsequent resection will be planned. The procedure occurs under aseptic cautery after skin disinfection, sterile masking of the operating field, and local anaesthesia with trimecaine (mesocain 1% inj. sol). The patient will have a peripheral vein secured by an intravenous cannula throughout the procedure.

Preparation of marking mixture and its application After applying a local anaesthetic, the radiologist prepares an examination marking mixture on CT under aseptic conditions. This is followed by withdrawing 1 ml of Omnipaque 350 mg I/ml solution for injection into a sterile syringe. He then changes the needle, takes 2 ml of Patent Blue V Sodium Injection 2.5% from the ampoule, and mixes the mixture in a syringe.

Subsequently, a thin needle will be inserted into the preselected part of the lungs under CT control. The needle is gradually introduced on inspiration by the step-by-step method, emphasising the most accurate placement of the needle tip to the observed lesion. We will pass the needle through the pleura only when the direction of the needle is in the intended trajectory so that the pleural leaf is broken by only one penetration. When the needle is in a suitable location, about 0.5 ml of the prepared mixture will be instilled into the pulmonary area.

A common and natural reaction to applying the mixture is a cough. Therefore, after application of the marking mixture, the needle will be immediately withdrawn on inspiration to prevent lung injury from coughing. At the end of the marking procedure, a control CT scan will be performed on a short scale to clarify the distribution of the mixture and possible complications (bleeding, pneumothorax). The patient will then be transported directly to the operating room accompanied by the surgeon for their operation. A radiologist and surgeon will supervise the patient throughout the procedure at the Radiology Department. All acquired CT scans will be stored in the PACS system.

Nodule resection The blue dye serves as an exact indication of the location of the nodule. The operator visually identifies the region marked in this way. The contrast agent in the substance performs the task of verifying the distribution and exact instillation of the colour mixture on the CT image, i.e. as close as possible to the site of the observed pathology. For the control arm, the lesion identification will be performed palpably through surgical instruments or by a surgeon through the surgical port.

In the operating theatre, the patient will undergo surgery under general anaesthesia with selective ventilation in the lateral position; the operated lung will not be ventilated during the operation. First, a thoracic camera will be introduced into the thoracic cavity through a port (entrance) of about 1 cm, followed by an aspect of the thoracic cavity with visual verification of the marked area. The marked/selected area will be wedge-shaped (extra anatomically) and resected using endo staplers through one or two established working ports. The operation will be completed by checking the operating field and introducing a thoracic drain used to detect leakage (tightness of the lung parenchyma), restore vacuum in the thoracic cavity, and control the amount and nature of secretion from the thoracic cavity. Postoperatively, the patient will be transferred to the ICU, where he will have a first lung X-ray 2 h after the operation to verify the position of the chest drain and check the lung parenchyma.

Postoperative phase—days 2–30

The patient will be hospitalised in the ICU, where a series of control lung X-rays will be performed. The 2nd X-ray will be performed at the time of the reduction of the chest suction to the 8 cm water column. The 3rd X-ray will be performed after the extraction of the thoracic drain. The 4th image will be taken during the inspection in the outpatient clinic during suture extraction (7th–14th postoperative day). The patients will be hospitalised according to standard discharge criteria (afebrile ≥24 h; SpO_2 on room air or baseline; pain controlled on oral meds; drain removed or defined threshold; mobilising independently; no unresolved complications).

The result of histological examination of the marked area is usually available within 10 days of the operation. It will be available and stored in the patient's medical records. After discharge from the hospital by day 30, the patient will be monitored at the clinical trial centre by the examining physicians. Day 30 is the date of termination of the patient's participation in the clinical trial.

Following completion of study participation, patients will continue to be monitored in thoracic, pneumological, or oncological outpatient clinics as appropriate.

Provisions for post-trial care (30)

The BLUEPAT clinical trial investigates the use of colour marking followed by VATS resection of lung nodules, with the aim of reducing hospital stay, decreasing analgesic requirements, enhancing patient comfort, and in some cases accelerating diagnosis. Compared with conventional thoracotomy, VATS preserves pulmonary function during and after the procedure, is associated with significantly less postoperative pain, and avoids atrophy of the latissimus dorsi muscle [7]. The method is generally well tolerated and not associated with substantial additional risks beyond standard VATS procedures. In the control arm, patients will undergo minimally invasive VATS resection without prior staining.

Potential risks

Possible risks for participants include:

- Allergic reactions to the staining agent (patent blue), the iodine-based contrast agent (iohexol), or local anaesthetics.
- Increased procedural pain despite the minimally invasive approach; this can be managed with analgesics.
- Bleeding into the lung parenchyma, pleural cavity, or chest wall following subcutaneous puncture; such events are generally not life-threatening and can be detected via immediate post-procedure CT verification.
- Pneumothorax, managed according to severity by manual aspiration or chest drainage [12].
- Rare but serious complications such as pulmonary embolism [13].
- Technical complications such as insufficient lesion staining, dye leakage into the pleural space, or excessive tissue staining; in such cases, conversion to open thoracotomy may be necessary.
- Coughing of blue dye following intrabronchial administration.
- Increased radiation exposure due to CT-guided localisation.
- Rare occurrences of infection or pneumonia. All possible adverse reactions to the investigational medicinal product are listed in the respective Summary Product of Characteristics for patent blue and contrast agent iohexol.

All participants are covered by a dedicated clinical trial insurance policy. In the event that a participant experiences trial-related harm, they will be entitled to appropriate medical treatment and financial compensation in accordance with applicable legal and regulatory requirements.

Post-trial care

All participants will continue to receive standard postoperative follow-up according to the clinical site's routine practice for thoracic surgery patients, including monitoring for procedure-related complications, wound care, pain management, and pulmonary function assessment. Any adverse events related to the study intervention will be treated promptly and in accordance with Good Clinical Practice (GCP) and the Declaration of Helsinki. No study-specific long-term interventions are planned after trial completion; however, patients will be referred back to their treating physician for ongoing management of their underlying condition. Any significant findings during follow-up will be communicated to the patient and their primary care or specialist team.

Strategies to improve adherence to interventions (11c)

Training before initialisation will ensure compliance during the whole study. If there are doubts, a chief investigator is designated to address them.

Relevant concomitant care permitted or prohibited during the trial (11d)

Due to the intrapulmonary administration of the study treatment, there are no restrictions on the concomitant treatment of chronic diseases, acute conditions, or other pharmacotherapy administered during the study.

Criteria for discontinuing or modifying allocated interventions {11b}

The trial participant has the right to terminate participation in this clinical trial at any time, for any reason, or without stating it.

The investigator must:

- Instruct the evaluation body on the right to early termination of participation
- Inform them that the termination of participation would not affect the physician's approach, the method of further treatment, or its quality
- Ask them to discuss this decision with the investigator in advance
- Enter in the documentation the date of early termination of the entity's participation and the reason, if known

Other reasons for early termination of the entity's participation include:

- Allergic reaction to investigational medicinal products
- Non-marking of the patent blue dye due to technical complications
- The examiner's decision that further patient involvement is not in the patient's best interests
- Persistent or repeated non-cooperation of the patient, or loss of contact with the patient
- Pregnancy of a patient participating in the clinical trial
- Termination of the study by a decision of the sponsor or the regulatory authority

Data collection and management

Data management {19}

The study data will be entered online in an Internet-based database (REDCap®) and collected from medical records. The study staff members will have access to the patients' medical records. The investigators will be responsible for screening patients, obtaining informed consent, collecting study data, and entering it into the eCRF. The statistician will analyse the study data in cooperation with the principal investigator. The data will be stored for 25 years after completion of the study and then destroyed. To promote data quality, the eCRFs of each participant will be reviewed by another member of the study team as a monitor.

Plans for assessment and collection of outcomes {18a}

The study will collect demographic and baseline characteristics from medical and electronic medical records, including age, sex, type of admission, and baseline characteristics such as body weight, body height, patient history, and pharmacological history. CT scans and physical examination results will be documented in the electronic medical records and entered by the trial investigator in the electronic case report form (eCRF).

Plans to promote participant retention and complete follow-up {18b}

All participants will remain hospitalised after surgery until they meet predefined standardised discharge criteria (e.g. adequate pain control with oral medication, absence of fever for ≥24 h, sufficient respiratory function on room air or baseline support, removal or management of thoracic drains, independent mobilisation, and absence of unresolved surgical or medical complications). Because this is a monocentric trial, in-hospital follow-up is ensured for all participants until discharge. After discharge, study staff will conduct scheduled assessments according to the protocol, either during outpatient visits to capture any late postoperative events. The study site will maintain complete follow-up of all participants through these measures.

To ensure maximal follow-up, site staff will:

- Maintain an up-to-date contact list for each participant (including secondary contacts such as family members, if consented)
- Schedule all follow-up visits in advance and provide written and telephone reminders
- Use national health records, hospital information systems, and/or cancer registries (where permitted) to obtain outcome data when direct contact is not possible

Local standard operating procedures (SOPs) will be developed and implemented at each site to ensure consistency and compliance with these follow-up requirements.

Confidentiality {27}

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with limited access. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to add identifying information will be stored in a separate locked file in an area with limited access.

Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}
Not applicable. In this study, no biological specimens for genetic or molecular analysis will be collected.

Statistical methods

Sample size {14}
Design and driver endpoint. This is a two-arm, parallel-group superiority trial (PBV labelling vs. no labelling). Although the primary analysis is a composite feasibility–safety endpoint at 30 days, the sample size is driven by hospital length of stay (LOS), a key secondary endpoint for which reliable pilot data exist and which is clinically meaningful and sensitive to the intervention.

Assumptions (two-sample comparison of means, equal allocation) are:

- Expected mean LOS: 6.0 days (control) vs. 4.0 days (PBV)
- Mean difference (Δ): −2.0 days
- Common SD (σ): 2.0 days (pilot retrospective cohort)
- Type I error (two-sided α): 0.01
- Power ($1-\beta$): 0.90
- Standardised effect size (Cohen's d): 1.0 ($\Delta/\sigma = 2/2$)

Computation: Using a two-sample t-test framework (conservative for planning; analysis will use models as specified), the required sample is $n = 30$ per group (total $N = 60$).

Attrition: Allowing for 10–20% attrition (withdrawal, missing primary 30-day assessment, protocol violations precluding analysis), the planned recruitment target is $N = 72$ participants.

Rationale: Powering on LOS provides a feasible and precise estimate anchored in observed variance, while the composite primary endpoint—analysed at $\alpha = 0.05$ with pre-specified sensitivity and multiplicity control—remains the principal confirmatory analysis.

Sensitivity scenarios (planning): If the true SD were larger (e.g. $\sigma = 2.5$ days), power at $N = 60$ would be ~84% for $\Delta = 2.0$ days at $\alpha = 0.01$; conversely, for $\Delta = 1.5$ days with $\sigma = 2.0$, power would be ~73% at $N = 60$ (≥80% with $N \approx 76$). These scenarios support the prudence of the $N = 72$ recruitment target.

Analysis populations: The primary inference will use the intention-to-treat set; per-protocol analyses will be performed as sensitivity checks.

Software: Calculations were performed using standard formulae for two-sample comparisons and will be reproducible in R/SAS; full code will be provided in the statistical analysis repository.

Sample size re-estimation: No blinded or unblinded sample size re-estimation is planned during the trial.

Statistical methods for primary and secondary outcomes {20a}
The primary outcome is a composite endpoint comprising (i) successful intraoperative localisation of the PBV-marked lesion and (ii) incidence of predefined adverse events of special interest (AESIs) within 30 days post-surgery. The composite will be analysed as the proportion of patients meeting both criteria, compared between groups using the chi-square test (or Fisher's exact test if expected counts are low). Sensitivity analyses will separately assess feasibility and safety components, with multiplicity adjusted using Bonferroni correction.

Secondary outcomes include intraoperative and postoperative complications, postoperative pain (VAS) and analgesic consumption, ICU and hospital length of stay, postoperative quality of life, and 30-day all-cause mortality. Continuous variables will be assessed for normality using the Shapiro–Wilk test and compared using Student's t-test. Categorical variables will be analysed using chi-square or Fisher's exact tests, as appropriate. Data will be reported as mean ± standard deviation (SD) for continuous variables and as counts and percentages for categorical variables. A two-sided p value <0.05 will be considered statistically significant.

All analyses will be performed according to the intention-to-treat (ITT) principle, including all randomised patients in the groups to which they were assigned.

Statistical analyses will be conducted using Statistica software version 12.0 (StatSoft Inc., Prague, Czech Republic). Randomisation will be performed using a validated web-based tool (e.g. randomizer.org).

Interim analyses {21b}
No interim analysis is planned.

Methods for additional analyses (e.g. subgroup analyses) {20b}
No subgroup or additional analyses are planned.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

No analysis populations are defined for this study. Protocol non-adherence will be assessed by the principal investigator case by case. Patients with major protocol deviations will be excluded from the analysis.

Missing data are not planned to be imputed. However, in the event of substantial missing data for any parameter, a sensitivity analysis using any method of imputation could also be used.

Plans to give access to the full protocol, participant-level data, and statistical code {31c}

In compliance with Regulation (EU) No. 536/2014 on clinical trials, all clinical trial data will be stored for a minimum of 25 years after trial completion. A fully anonymised dataset, together with the statistical code, will be deposited in the Institutional Repository of St. Anne's University Hospital Brno. Archiving and access will follow national legislation, Good Clinical Practice (GCP), and institutional SOPs.

Access to anonymised participant-level data, the statistical code, and the full protocol will be made available for non-commercial scientific research purposes upon justified request. Interested researchers may submit a formal application to the corresponding author and sponsor. All requests will be reviewed by the trial steering committee to ensure compliance with ethical standards, applicable regulations, and data protection requirements.

Oversight and monitoring

Composition of the coordinating centre and trial steering committee {5d}

Not applicable. No coordinating centre or a trial steering committee will be established.

Composition of the data monitoring committee, its role and reporting structure {21a}

The on-site monitoring of the study conduct is regularly performed throughout the study period to assess the accuracy and completeness of the data. The study site may be subject to a quality assurance visit. If so, the site will be contacted in advance to organise a monitoring visit. The investigator will ensure direct access to all study documents for quality assurance monitors.

Adverse event reporting and harms {22}

Definition

Adverse event (AE): Any untoward medical occurrence in a trial participant, irrespective of whether it has a causal relationship with the intervention. This includes new symptoms, signs, illnesses, or clinically significant abnormal laboratory findings.

Serious adverse event (SAE): An AE that results in death, is life-threatening, requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability/incapacity, or any other medically important condition that may require intervention to prevent one of the above outcomes.

Importantly, any adverse events occurring intraoperatively will be immediately managed according to current standard clinical practice and institutional surgical and anesthesiological protocols, ensuring patient safety at all times. Participation in the trial does not modify or delay the implementation of established emergency or therapeutic procedures.

Adverse events of special interest (AESIs)

Given the intervention, specific safety events are predefined as AESIs:

- Moderate AESIs: pneumothorax <2 cm not requiring drainage, non-surgical bleeding, mild allergic reaction (rash)
- Severe/life-threatening AESIs: pneumothorax >2 cm requiring drainage, bleeding requiring surgery, pulmonary embolism, anaphylaxis

Recording and reporting procedures

All AEs and SAEs will be documented in the electronic case report form (eCRF), including onset, duration, severity, seriousness, outcome, and assessment of causality in relation to the investigational intervention.

SAEs and AESIs must be reported by the investigator to the sponsor within 24 h of becoming aware of the event.

Non-serious AEs will be recorded in the eCRF and summarised in aggregated form in the clinical study report.

The sponsor will be responsible for reporting suspected unexpected serious adverse reactions (SUSARs) via the Clinical Trials Information System (CTIS) in accordance with EU Clinical Trial Regulation (EU No. 536/2014) requirements (fatal/life-threatening SUSARs within 7 days; other SUSARs within 15 days).

All participants experiencing an AE/SAE will be followed until resolution, stabilisation, or determination of a non-study related cause.

Analysis of adverse events

Safety analyses will be conducted on all randomised patients who undergo at least one study-related procedure (safety population). AEs will be coded using the

latest version of the Medical Dictionary for Regulatory Activities (MedDRA).

Adverse events will be summarised by:

- Treatment arm
- System organ class and preferred term
- Severity (mild, moderate, severe)
- Relationship to study intervention (related, possibly related, unrelated)
- Seriousness

The incidence proportion of patients experiencing at least one AE, SAE, or AESI will be compared descriptively between study arms. Where appropriate, comparative analyses (e.g. chi-square or Fisher's exact test) will be performed to evaluate differences in safety outcomes between groups. No formal hypothesis testing for safety endpoints is planned unless otherwise specified in the statistical analysis plan.

Reporting and handling of deaths

All deaths occurring during the study period, regardless of causality, will be recorded as SAEs.

For each death, the following will be documented:

- Date of death
- Primary cause of death
- Contributing factors
- Assessment of relationship to the investigational intervention
- Availability of supporting documentation (e.g. medical records, imaging, autopsy report if applicable)

If a death qualifies as a suspected unexpected serious adverse reaction (SUSAR), it will be reported via CTIS within 7 days in accordance with EU Clinical Trial Regulation (EU No. 536/2014), with follow-up information submitted within an additional 8 days.

Deaths will be included in the safety analysis and reported transparently in the final clinical study report, including a narrative summary when appropriate.

Oversight

The trial is monocentric, with hospitalised patients monitored daily during the perioperative period until discharge (minimum 10 days post-surgery). This setup minimises the risk of missed safety events. The principal investigator has primary responsibility for SAE assessment, while the sponsor ensures compliance with Good Clinical Practice (GCP), safety reporting regulations, and archiving requirements.

Frequency and plans for auditing trial conduct (23)

Trial conduct will be subject to independent auditing to ensure compliance with Good Clinical Practice (GCP), the approved protocol, and applicable regulatory requirements.

- Scope: Audits will include verification of data integrity (source data verification, consistency between source and eCRF), informed consent procedures, adherence to eligibility criteria, SAE/SUSAR reporting, and overall trial conduct.
- Institutional audit: The Institutional Ethics Committee will audit the study annually throughout its duration. These audits are fully independent of the investigator and sponsor.
- Sponsor audit: In addition, the sponsor will organise at least one internal audit per study to verify adherence to protocol-defined procedures and data management standards.

Documentation: All audit findings will be documented in formal audit reports, and corrective/preventive actions will be tracked until resolution.

Research ethics approval (24)

This protocol and the template informed consent form were reviewed and approved by the Ethical Committee of the University Hospital St. Anne's Brno (February 2, 2019) with respect to the scientific content and compliance with applicable research and human subject regulations.

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) (25)

Any modifications to the study protocol that may impact trial conduct, participant safety, or the scientific validity of the results will be formally documented as protocol amendments. All amendments will be submitted for review and approval to the Institutional Ethics Committee and the National Drug Agency prior to implementation. In addition, any approved changes will be updated in the relevant clinical trial registry in accordance with regulatory requirements.

Dissemination plans (31a)

The results of this clinical trial will be submitted for publication in a peer-reviewed international medical journal, regardless of the outcome. Findings will also be presented at relevant scientific and clinical conferences. In accordance with Regulation (EU) No. 536/2014, summary results including lay summary for participants and public

will be uploaded to the EU Clinical Trials Information System (CTIS) within the required timelines.

Discussion

Low-dose CT screening of high-risk adults (55–74 years with a heavy smoking history), as established by the US National Lung Screening Trial (NLST), reduces lung cancer mortality by 20% versus chest radiography and is associated with a high rate of positive screens (24.2% across three rounds), most of which are false positives (96.4%) [14]. The introduction of a national pilot screening program using low-dose CT in the Czech Republic for individuals aged 55–74 years with a history of smoking has markedly increased the detection of small subpleural pulmonary nodules requiring histological verification. In our cohort, 66.2% of nodules measured <10 mm, and 21.6% were classified as subsolid or pure ground-glass opacities (GGOs)—lesion types that are particularly difficult to identify intraoperatively [15]. While CT or positron emission tomography (PET) CT remain the primary tools for malignancy risk assessment, using established criteria such as the Fleischner classification (size, margins, density, and growth dynamics), imaging alone cannot provide a definitive diagnosis [16]. Consequently, reliable and minimally invasive localisation methods prior to resection are essential to ensure both diagnostic accuracy and surgical precision [17].

Intraoperative challenges

Subpleural nodules are frequently invisible and non-palpable during minimally invasive surgery [18]. In our series, 63% of nodules would not have been detectable intraoperatively without marking, underscoring the limited value of tactile feedback during VATS [15]. GGOs and subsolid lesions, owing to their similar consistency to normal parenchyma, are particularly challenging. Historically, these difficulties often necessitated conversion to open thoracotomy, thereby compromising the advantages of minimally invasive surgery.

VATS and the need for localisation

VATS has become a well-established alternative to thoracotomy, offering reduced postoperative pain, shorter hospitalisation, and improved cosmetic outcomes [19, 20]. However, its success critically depends on the preoperative localisation of small or deep nodules. Without image-guided marking, conversion rates to thoracotomy have been reported as high as 63% [5]. In our previous institutional experience, retrospectively collected data from a pilot cohort suggested a low conversion rate (3.3%) in patients undergoing preoperative localisation [15].

However, these findings were derived from a non-randomised, retrospective analysis and are therefore subject to inherent methodological limitations and potential selection bias. The present randomised controlled trial has been designed to prospectively evaluate and confirm whether preoperative marking strategies reduce conversion rates and improve surgical outcomes compared with standard localisation approaches under controlled conditions.

Alternative localisation techniques

Numerous localisation methods have been explored, yet none is ideal. Compared with PBV, intraoperative ultrasound provides real-time visualisation but is technically demanding and less reproducible in a collapsed lung, making it less feasible in routine VATS practice [21]. CT-guided wire or coil placement achieves similar localisation accuracy to PBV but with a higher incidence of pneumothorax and marker dislodgement, and requires more complex coordination with radiology teams [7, 10, 22, 23]. While radiotracer and fluorescent dye techniques provide comparable precision, they are less practical than PBV due to dependence on nuclear medicine infrastructure and specialised detection devices [24]. Comparative evidence among these techniques is limited, and direct head-to-head analyses remain scarce, leaving the optimal approach undetermined.

PBV localisation

PBV dye marking has recently emerged as a simple, inexpensive, and visually effective alternative [25–27]. Unlike metallic markers, PBV avoids mechanical complications, and unlike radiotracers or fluorescent agents, it requires no specialised detection devices [28, 29]. In our previous institutional experience, retrospectively collected data from a pilot cohort suggested a high localisation success rate (96.7%) with a low complication rate (8.3%). These findings were consistent with retrospective series reporting success rates of 94–100% [25–27, 30, 31] but, in contrast to those studies, our prospective design provides stronger evidence by minimising reporting bias and systematically capturing complications. However, such studies were predominantly retrospective and may have been influenced by selection and reporting biases, including the exclusion of technically challenging nodules and inconsistent documentation of complications [15].

The present prospective randomised trial has been designed to rigorously evaluate the feasibility, safety, and clinical impact of PBV marking under controlled conditions. By systematically capturing consecutive cases, including subsolid and deep lesions, and prospectively recording complications and downstream surgical outcomes, this study aims to provide higher-quality evidence regarding localisation success, conversion rates, margin

status, and procedural workflow compared with standard approaches.

Clinical implications

Reliable preoperative localisation has several key clinical implications: it may facilitate complete resection with negative margins, reduce conversion rates, and streamline surgical workflow. In our previous institutional experience, retrospectively collected pilot data indicated a 0% rate of positive resection margins in patients undergoing PBV marking. These findings were consistent with prior retrospective PBV studies reporting positive margin rates of 0–3%, and appeared favourable compared with coil- or wire-based techniques, which have reported positive margins in up to 10% of cases. However, these comparisons are based primarily on non-randomised data and may be influenced by selection and reporting biases.

Therefore, the present prospective randomised trial has been designed to formally evaluate whether PBV localisation improves surgical outcomes, including margin status and conversion rates, compared with standard localisation approaches. PBV represents a potentially pragmatic balance between accuracy, safety, and procedural simplicity. Unlike wire- or coil-based localisation, PBV does not require fluoroscopy or hardware removal, which may reduce procedural complexity and associated risks. Compared with ultrasound or radiotracer-based techniques, PBV may be simpler to implement and more accessible to centres without advanced imaging or nuclear medicine facilities. Its relatively low cost and minimal equipment requirements make it a potentially attractive option for broader clinical adoption, pending confirmation through high-quality prospective evidence.

Future directions

Despite these encouraging results, evidence supporting PBV remains limited to retrospective cohort analysis from one hospital experiences. The absence of large, prospective trials restricts definitive conclusions regarding its generalisability. Future research should focus on standardising the injection protocol (volume, timing, and depth), assessing inter-operator variability, and evaluating long-term outcomes such as recurrence rates, surgical efficiency, and cost-effectiveness compared with alternative localisation strategies.

Trial status

Protocol version 1.0, dated 15 May 2021. The recruitment is planned from March 2026. Seventy-two patients have to be recruited by the end of December 2026. Completion of the follow-up is anticipated in July 2027.

Abbreviations

AE	Adverse event
AESI	Adverse event of special interest
CT	Computed tomography
eCRF	Electronic case report form
GGO	Ground-glass opacity
GCP	Good Clinical Practise
ICF	Informed consent form
ICU	Intensive care unit
ID	Identification number
IMP	Investigational medicinal product
LOS	Length of stay
PACS	Picture archiving and communicating system
PBV	Patent Blue V
PET	Positron emission tomography
SD	Standard deviation
SOP	Standard operational procedure
VAS	Visual analogue scale
VATS	Video-assisted thoracoscopic surgery

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Access to data (29)

The final trial dataset will be accessible to all investigators participating in this study.

Ancillary and post-trial care (30)

The clinical trial BLUEPAT is conducted in full accordance with the principles of the Declaration of Helsinki (in the latest valid version), in accordance with GCP requirements, and in accordance with applicable national and European legislation. BLUEPAT was discussed and approved by the local ethics committee and State Institute for Drug Control before the start.
All patients were informed about the possible risks of their participation in this study and about the possibility of ending their participation in the study at any time without consequences. The patients are also informed about the measures that will be taken for the protection of personal data and about the fact that, in the event of damage to health as a result of the implementation of the study, liability insurance for the examiner and the contracting authority has been arranged in accordance with the applicable legislation, through which the possible compensation of assessment participants.

Authors' contributions (31b)

VČ—design of the study, protocol writing and revision, design of the surgery part, the first author, ZCH—principal investigator, he conceived the study, led the proposal and protocol writing and revision, design of the surgery part; MŠ—statistician, statistical analysis; IČ—design of the study, protocol revision; LS—design of the study, protocol writing and revision, corresponding author; AB—design of the study, design of the imaging part; TH—design of the study, design of the surgery part; IP—design of the study, design of the imaging part; JV—design of the study, design of the imaging part.

Funding (4)

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Data availability (29)

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate (24)
This protocol and the template informed consent formThis protocol and the template informed consent form were reviewed and approved by the Ethical Committee of the University Hospital St. Anne's Brno (in August 2021), with respect to the scientific content and compliance with applicable research and human subject regulations. Informed consent will be obtained from all participants prior to enrolment in the study.

Consent for publication (32)
Not applicable. Publication of any data associated with an individual person is not planned. An example consent form is available from the corresponding author on reasonable request.

Competing interests (28)
The authors declare that they have no competing interests.

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References

1. Dyer SC, Bartholmai BJ, Koo CW. Implications of the updated Lung CT Screening Reporting and Data System (Lung-RADS version 1.1) for lung cancer screening. J Thorac Dis. 2020;12(11):6966–77. https://doi.org/10.21037/jtd-2019-cptn-02. PMID: 33282402; PMCID: PMC7711402.
2. Mutsaerts EL, Zoetmulder FA, Meijer S, et al. Long term survival of thoracoscopic metastasectomy vs metastasectomy by thoracotomy in patients with a solitary pulmonary lesion. Eur J Surg Oncol. 2002;28:864–8.
3. Nakas A, et al. Video-assisted versus open pulmonary metastasectomy: the surgeon's finger or the radiologist's eye? Eur J Cardiothorac Surg. 2009;36:469.
4. Mutsaerts EL, et al. Outcome of thoracoscopic pulmonary metastasectomy evaluated by confirmatory thoracotomy. Ann Thorac Surg. 2001;72(1):230–3.
5. Suzuki K, Nagai K, Yoshida J, et al. Video-assisted thoracoscopic surgery for small indeterminate pulmonary nodules: indications for preoperative marking. Chest. 1999;115(2):563–8. https://doi.org/10.1378/chest.115.2.563. PMID: 10027460.
6. Lenglinger FX, Schwarz CD, Artmann W. Localization of pulmonary nodules before thoracoscopic surgery: value of percutaneous staining with methylene blue. Am J Roentgenol. 1994;163:297–300.
7. Yoshida Y, Inoh S, Murakawa T, et al. Preoperative localization of small peripheral pulmonary nodules by percutaneous marking under computed tomography guidance. Interact Cardiovasc Thorac Surg. 2011;13(1):25–8. https://doi.org/10.1510/icvts.2011.266932. Epub 2011 Apr 11 PMID: 21486758.
8. Vieira T, Antoine M, Ruppert AM, et al. Blood vessel invasion is a major feature and a factor of poor prognosis in sarcomatoid carcinoma of the lung. Lung Cancer. 2014;85:276–28. https://doi.org/10.1016/j.lungcan.2014.06.004.
9. Stephenson JA, et al. A simple and safe technique for CT guided lung nodule marking prior to video assisted thoracoscopic surgical resection revisited. Lung Cancer Int. 2015;1:235720. https://doi.org/10.1155/2015/235720.
10. Hwang S, Kim TG, Song YG. Comparison of hook wire versus coil localization for video-assisted thoracoscopic surgery. Thorac Cancer. 2018;9(3):384–9. https://doi.org/10.1111/1759-7714.12589. Epub 2018 Jan 11. PMID: 29322646; PMCID: PMC5832470.
11. Hawker GA, et al. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (Cpgs), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). Arthritis Care Res. 2011;63(S11):S240–52.
12. Yamagami T, Nakamura T, Iida S, et al. Management of pneumothorax after percutaneous CT-guided lung biopsy. Chest. 2002;121(4):1159–64.
13. Freund MC, Petersen J, Goder KC, et al. Systemic air embolism during percutaneous core needle biopsy of the lung: frequency and risk factors. BMC Pulm Med. 2012;12(1):2.
14. National Lung Screening Trial Research Team, Aberle DR, Berg CD, Black WC, et al. The National Lung Screening Trial: overview and study design. Radiology. 2011;258(1):243–53. https://doi.org/10.1148/radiol.10091808.
15. Červeňák V, Chovanec Z, Berková A, et al. Subpleural pulmonary nodule marking with patent blue V dye prior to surgical resection. Front Oncol. 2024;14:1–9. https://doi.org/10.3389/fonc.2024.1392398. LAUSANNE: FRONTIERS MEDIA SA.
16. Bueno J, Landeras L, Chung JH. Updated Fleischner Society guidelines for managing incidental pulmonary nodules: common questions and challenging scenarios. Radiographics. 2018;38(5):1337–50. https://doi.org/10.1148/rg.2018180017.
17. Naidich DP, Bankier AA, MacMahon H, et al. Recommendations for the management of subsolid pulmonary nodules detected at CT: a statement from the Fleischner Society. Radiology. 2013;266(1):304–17. https://doi.org/10.1148/radiol.12120628. Epub 2012 Oct 15. PMID: 23070270.
18. Mack MJ, Hazelrigg SR, Landreneau RJ, et al. Thoracoscopy for the diagnosis of the indeterminate solitary pulmonary nodule. Ann Thorac Surg. 1993;56(4):825–30. https://doi.org/10.1016/0003-4975(93)90339-j. discussion 830-2.
19. Wolf A, Liu B, Leoncini E, et al. Outcomes for thoracoscopy versus thoracotomy not just technique dependent: a study of 9,787 patients. Ann Thorac Surg. 2018;105(3):886–91. https://doi.org/10.1016/j.athoracsur.2017.09.059. Epub 2018 Feb 1. PMID: 29397101.
20. Salati M, Brunelli A, Rocco G. Uniportal video-assisted thoracic surgery for diagnosis and treatment of intrathoracic conditions. Thorac Surg Clin. 2008;18(3):305–10. https://doi.org/10.1016/j.thorsurg.2008.04.005. PMID: 18831508.
21. Huang YH, Chen KC, Chen JS. Ultrasound for intraoperative localization of lung nodules during thoracoscopic surgery. Ann Transl Med. 2019;7(2):37. https://doi.org/10.21037/atm.2019.01.41. PMID: 30854390; PMCID: PMC6381266.
22. Miyoshi K, Toyooka S, Gobara H, et al. Clinical outcomes of short hook wire and suture marking system in thoracoscopic resection for pulmonary nodules. European Journal of Cardio-Thoracic Surgery. 2009;36(2):378–82. https://doi.org/10.1016/j.ejcts.2009.03.039.
23. Finley RJ, Mayo JR, Grant K, et al. Preoperative computed tomography-guided microcoil localization of small peripheral pulmonary nodules: a prospective randomized controlled trial. J Thorac Cardiovasc Surg. 2015;149(1):26–31. https://doi.org/10.1016/j.jtcvs.2014.08.055. Epub 2014 Sep 16. PMID: 25293355.
24. Chella A, Lucchi M, Ambrogi MC, et al. A pilot study of the role of TC-99 radionuclide in localization of pulmonary nodular lesions for thoracoscopic resection. Eur J Cardiothorac Surg. 2000;18(1):17–21. https://doi.org/10.1016/s1010-7940(00)00411-5. PMID: 10869935.
25. Lin MW, Tseng YH, Lee YF, et al. Computed tomography-guided patent blue vital dye localization of pulmonary nodules in uniportal thoracoscopy. J Thorac Cardiovasc Surg. 2016;152(2):535-544.e2. https://doi.org/10.1016/j.jtcvs.2016.04.052. Epub 2016 Apr 25. PMID: 27189890.
26. Tseng YH, Lee YF, Hsieh MS, et al. Preoperative computed tomography-guided dye injection to localize multiple lung nodules for video-assisted thoracoscopic surgery. J Thorac Dis. 2016;8(Suppl 9):S666–71. https://doi.org/10.21037/jtd.2016.09.46. PMID: 28066667; PMCID: PMC5179341.
27. Chen JR, Tseng YH, Lin MW, et al. Safety and efficacy of computed tomography-guided dye localization using Patent Blue V for single lung nodule for video-assisted thoracoscopic surgery: a retrospective study.

Ann Transl Med. 2019;7(2):28. https://doi.org/10.21037/atm.2019.01.27. PMID: 30854381; PMCID: PMC6381270.
28. Vieira SC, Alves VC, Oliveira TC, et al. Patent blue and air as an alternative for resection of nonpalpable breast lesions: a case series. Sao Paulo Med J. 2014;132(1):10–4. https://doi.org/10.1590/1516-3180.2014.1321559. PMID: 24474074.
29. Stephenson JA, Mahfouz A, Rathinam S, et al. A simple and safe technique for CT guided lung nodule marking prior to video assisted thoracoscopic surgical resection revisited. Lung Cancer International. 2015;2015:1–3.
30. Ko KH, Huang TW, Lee SC, et al. A simple and efficient method to perform preoperative pulmonary nodule localization: CT-guided patent blue dye injection. Clin Imaging. 2019;58:74–9. https://doi.org/10.1016/j.clinimag.2019.06.015. Epub 2019 Jun 27. PMID: 31279987.
31. Lin CW, Ko HJ, Yang SM, et al. Computed tomography-guided dual localization with microcoil and Patent Blue vital dye for deep-seated pulmonary nodules in thoracoscopic surgery. J Formos Med Assoc. 2019;118(6):979–85. https://doi.org/10.1016/j.jfma.2019.02.005.

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7.8 Annex 8

Suk P, Rychlíčková J, Součková L, Kubíčková V, Urbánek K. Changes of colistin pharmacokinetics in critically ill patients due to the extracorporeal membrane oxygenation: protocol for the COL-ECMO2022 trial - a prospective, non-randomised, open-label phase IV pharmacokinetic clinical trial. BMJ Open. 2023 Jul 30;13(7):e071649. doi: 10.1136/bmjopen-2023-071649. PMID: 37518089; PMCID: PMC10387630.

Summary
Purpose:
The aim of this study is to evaluate the impact of extracorporeal membrane oxygenation (ECMO) on the pharmacokinetics of colistin and its prodrug colistimethanesulfonate in critically ill patients. This research addresses a clinically important issue, as colistin is often used as a last-resort antibiotic for multidrug-resistant infections, and ECMO may significantly alter drug disposition and therapeutic effectiveness.

Methodology:
The study is designed as a prospective, non-randomised, single-centre phase IV pharmacokinetic clinical trial, enrolling up to 30 critically ill adult patients receiving intravenous colistin. Patients are stratified based on the presence or absence of ECMO, and plasma concentrations of colistin and its prodrug are measured using high-performance liquid chromatography–mass spectrometry, followed by population pharmacokinetic modelling to assess ECMO-related effects.

Results:
As a protocol publication, the study primarily defines a detailed methodological framework, including sampling strategy, pharmacokinetic endpoints, and modelling approaches. It highlights anticipated challenges such as high interindividual variability, altered drug distribution due to ECMO circuits, and the need to define clinically significant differences in drug exposure.

Conclusion:
The COL-ECMO2022 trial is expected to provide important evidence on optimal dosing strategies of colistin in critically ill patients undergoing ECMO support. The study exemplifies how investigator-initiated trials can generate essential pharmacological data in complex clinical settings that are not typically addressed by commercial research.

Open access Protocol

BMJ Open

Changes of colistin pharmacokinetics in critically ill patients due to the extracorporeal membrane oxygenation: protocol for the COL-ECMO2022 trial – a prospective, non-randomised, open-label phase IV pharmacokinetic clinical trial

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ABSTRACT

Introduction Colistin is a lipopeptide antibiotic administered as an inactive prodrug—colistin methanesulfonate (CMS). Colistin is a drug with a narrow therapeutic window; the limiting factors are mainly nephrotoxicity and neurotoxicity, dependent on plasma concentrations. The number of patients with infections caused by multidrug-resistant Gram-negative bacteria sensitive only to colistin and the number of patients requiring extracorporeal membrane oxygenation (ECMO) support for severe respiratory failure increased significantly in association with COVID-19-induced infections. ECMO can generally affect the pharmacokinetics of drugs by creating a new compartment.

Methods and analysis The COL-ECMO2022 study is a prospective, non-randomised, single-centre, phase IV pharmacokinetic clinical trial designed to assess the influence of ECMO on the pharmacokinetics of colistin and CMS. Up to 30 patients treated with colistin will be included in the study and assigned to one of two arms, depending on the presence/absence of ECMO. All study participants will receive standard CMS dose intravenously. The plasma concentrations of colistin and CMS taken at defined intervals will be assessed by high-performance liquid chromatography-mass spectrometry. Patients will participate in the clinical trial for a maximum of three monitored dosing intervals. A population pharmacokinetic model will be developed to assess the influence of ECMO on pharmacokinetics. A difference greater than 25% is considered clinically significant.

Ethics and dissemination The study has been approved by the Ethics Committee of St. Anne's University Hospital Brno (Number 10ML/2022-AM). Related manuscripts will be submitted to peer-review journals.

Trial registration numbers EudraCT Number 2022-000291-19; NCT05542446.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- → COL-ECMO2022 is a prospective, non-randomised, open-label phase IV pharmacokinetic study.
- → Only critically ill patients with and without extracorporeal membrane oxygenation (ECMO) will be recruited to provide information on this population, for which data on colistin pharmacokinetics is limited.
- → A maximum of three monitored dosing intervals are set for each subject to monitor trends in plasma concentrations over a more extended period of time and to limit the burden on subjects.
- → The primary outcome is to assess the impact of ECMO on colistin pharmacokinetics.
- → A limited sampling strategy of four samples per interval is a limitation of this study.

INTRODUCTION

The number of patients requiring extracorporeal membrane oxygenation (ECMO) for severe respiratory failure increased significantly during the COVID-19 pandemic. Most of these patients require long-term intensive care with the need for repeated antibiotic treatment. These factors lead to a higher frequency of infections with multidrug-resistant Gram-negative bacteria sensitive only to colistin.

ECMO circuits generally represent an additional pharmacokinetic compartment that increases the volume of distribution of drugs and may also affect their clearance by adsorption of the drug on the circuit surface.[1] This phenomenon is determined by the lipophilicity of the drug and its binding to plasma proteins, but the material of the

ECMO circuit components or their surface modification also have an influence.

Based on their chemical characteristics, the adsorption of colistin, or its prodrug, to the surface of the ECMO circuit seems unlikely. There are very limited data on the actual effect of ECMO on colistin pharmacokinetics.[2 3] Nevertheless, colistin adsorption on polysulfone dialysis membranes has been previously published.[4]

Colistin is a lipopeptide antibiotic administered as an inactive prodrug—colistin methanesulfonate (CMS). Bioactivation of the prodrug is non-enzymatic by hydrolysis. Colistin is an antimicrobial agent with a concentration-dependent and time-dependent effect, where the target pharmacokinetic-pharmacodynamic parameter is the area under the plasma concentration curve (AUC). The target 24-hour steady-state AUC ($AUC_{ss,24h}$) is approximately 50 mg×hour/L, which corresponds to a steady-state mean plasma concentration of approximately ($C_{avg,ss}$) 2 mg/L.[5] Colistin is a drug with a narrow therapeutic window. The limiting factors are mainly nephrotoxicity and neurotoxicity, both dependent on plasma concentrations. Sorlí *et al* reported that the minimum plasma concentration (c_{min}) of 2.42 mg/L at the end of therapy, respectively, 3.33 mg/L on day 7 of colistin therapy is predictive for the development of acute renal failure.[6]

The estimation of the colistin AUC is complicated by several factors. The first is the rate of bioactivation of the prodrug, which can vary interindividually, between products of various manufacturers, or even in batches of one manufacturer.[7] The second is the variability in the time to maximum plasma concentration (T_{max}) of colistin, which depends not only on the rate of conversion of CMS to colistin but also on the rate of elimination of CMS. Clearance of CMS is dependent on creatinine clearance. When creatinine clearance decreases, more CMS is converted to colistin. The excretion of colistin is not fully described; however, significant tubular resorption was reported. The pharmacokinetics of colistin in critically ill patients have been described in detail in several studies.[4 8–14]

Because of the considerable heterogeneity of the pharmacokinetic parameters of colistin and the lack of a reliable population kinetic model, it is appropriate to use multiple samplings during a single dosing interval to calculate the AUC. This, together with the determination of the plasma concentration of the prodrug at the corresponding times, will give a better idea of the plasma concentration profile of both substances and improve the estimation of the colistin AUC. By comparing the achieved plasma concentrations of colistin and CMS in patients with and without ECMO, we can then estimate the presence and quantify the extent of adsorption of both agents to the ECMO circuit material or other processes induced by the ECMO circuit.

STUDY OBJECTIVES

The objective of this phase IV pharmacokinetic trial is to assess the influence of ECMO on the pharmacokinetics of colistin and CMS in critically ill adults and to propose dosing guidance.

METHODS

Trial design

The COL-ECMO2022 is an investigator-initiated trial. The COL-ECMO2022 clinical trial is a prospective, non-randomised, open-label, single-centre, phase IV pharmacokinetic clinical trial that has been started in September 2022. The COL-ECMO2022 study plans to include 30 participants with or without ECMO support receiving CMS as part of the standard medical care. The indication, dosage and duration of colistin administration are, therefore, entirely within the discretion of the attending physician. These study participants will be consecutively assigned to two groups until the planned number of participants: ECMO and non-ECMO, in a 1:1 ratio depending on their actual need for ECMO support. Both groups are treated by intravenous CMS at standard, approved dosing. The influence of ECMO on CMS and colistin pharmacokinetics will be assessed by comparing the pharmacokinetic endpoints of the study. Study subjects will participate in the clinical trial for up to three monitored CMS dosing intervals

Study setting

The principal investigator and St. Anne's University Hospital Brno, the coordinating and only recruiting centre for the study, are responsible for the education and training of research staff, tracking participants' enrolment, mathematical analysis, monitoring, pharmacovigilance, data management and reporting of the study.

Department of Pharmacology, Faculty of Medicine and Dentistry, Palacky University in Olomouc, is responsible for a qualitative and quantitative analysis of colistin and CMS in blood samples by liquid chromatography and mass spectrometry (LC-MS) determination.

Creatinine clearance from urine collection reflecting the observed interval(s) after colistin administration will be determined by the Department of Clinical Biochemistry, St. Anne's University Hospital Brno.

Study population

Inclusion criteria

All consecutive patients will be included in the clinical trial if they meet all of the following criteria:

1. Age ≥18 years.
2. Men and women (with a negative pregnancy test prior to study enrolment in women of childbearing potential).
3. Hospitalised at the Department of Anaesthesiology and Intensive Care, St. Anne's University Hospital Brno.
4. Indication for parenteral colistin (administered as CMS) as a part of standard medical care, that is, in patients with severe bacterial infection.

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5. Informed consent was given. In unconscious patients, the study investigator will decide whether to include the patient in the study considering all individual risks.

Additional inclusion criterion

For some patients (15 participants expected), in addition to all the criteria listed in the Inclusion criteria section, the following inclusion criterion is provided:

1. ECMO support is needed as part of standard therapy for severe respiratory failure.

Exclusion criteria

A patient must not be included in the clinical trial if he/she meets any of the following criteria:

1. Pregnancy.
2. Breast feeding.
3. Refusal to give informed consent (primarily or after regaining consciousness).

Who will take informed consent?

The study protocol, including the informed consent, was reviewed and approved by the Institutional Review Board of the site (Ethics Committee of St. Anne's University Hospital Brno) and by the National Regulatory Authority (NRA) (The State Institute of Drug Control). A trained study investigator describes the study to patients. Patients will receive written information about the study as well (see online data supplemental 1). The investigator will discuss with patients the design and characteristics of the study, potential benefits, and risks based on the written information provided to the participant. The investigator obtains written informed consent from patients willing to participate in the trial. If a patient is unable to give informed consent due to his/her medical condition (eg, unconsciousness), his/her ability to participate in the study will be assessed by the investigator. In this case, the Ethics Committee of St. Anne's University Hospital Brno will be informed of the patient's inclusion. The investigator will ask study participants in whom good quality consciousness is restored to give subsequent informed consent without unreasonable delay.

Sample size

We plan to include 15 study participants in each group (ECMO and non-ECMO).

The sample size is difficult to calculate due to the significant variation of pharmacokinetic parameters among published studies, the unknown effect of ECMO on pharmacokinetics, and unknown interindividual variability.

If, at the time of completion of the planned recruitment, a large number of patients has been monitored for only one interval or there is a high frequency of deviations from the sampling protocol, or sample loss/damage is detected, the plan is to increase the sample size or even extend the overall study duration. On the other hand, colistin is a reserve and relatively toxic antibiotic. Therefore, there is only a limited number of suitable patients.

Interventions

The commencement of colistin therapy is the responsibility of an attending physician. Colistin will be given as CMS intravenously via the central venous catheter at the approved dosage: a loading dose of 9 million international units (MIU) over 30 min followed by a maintenance dose of 4.5 MIU over 30 min every 12 hours. There is a protocol for dose reduction in patients with impaired renal function, including patients treated with intermittent haemodialysis. The dosing interval is reduced to 8 hours, and the loading dose is increased to 12 MIU in patients requiring continuous renal replacement therapy (CRRT). The dosage strictly follows the local guideline proposed on the international guidelines.[5 15] The protocol for colistin dosage applicable at the study site is in online data supplemental 2: Colistin dosage local protocol.

The diagram presenting the course of the study is depicted in figure 1.

Visit 1: screening: patient selection phase

Patients meeting the inclusion criteria will be screened if not meeting any of the exclusion criteria. A urine pregnancy test will be performed on women of childbearing potential. The patient will be offered participation in the clinical trial and interviewed by the investigator, who clearly explains the purpose, design, benefits and risks of the trial. If the patient agrees to participate in the clinical trial, he/she will give informed consent. After signing the informed consent form, the patients will be recruited for this study and distributed to ECMO or non-ECMO groups according to their actual need for ECMO support. For patients unable to give informed consent, an interim informed consent will be signed by the investigator.

Visit 2 (visits 3 and 4 if colistin therapy continues): pharmacokinetics (blood samples collection)

A total of four (three in case of CRRT) blood samples will be taken during each monitored dosing interval. Based on the blood sample volume of 5 mL, up to 20 mL of blood will be drawn from the patient per monitored dosing interval. The timing of blood samples is summarised in figure 2.

Blood samples will be taken from the arterial catheter, routinely placed in critically ill patients in intensive care unit. This will minimise blood loss and requires no further interference with the physical integrity of enrolled patients. The sample timing is precisely defined according to the start of the CMS infusion. A 12-hour urine collection will be performed during the monitored dosing interval to determine the creatinine clearance.

Blood samples will be preanalytically processed (centrifugation, freezing and storing separated plasma at −80°C).

The last blood sample collection in the third monitored interval is the last intervention in the study for an individual patient and is the time of termination of the patient's participation in the clinical trial. This last intervention corresponds with blood sampling at 7 hours and 50 min after starting CMS infusion in CRRT patients and

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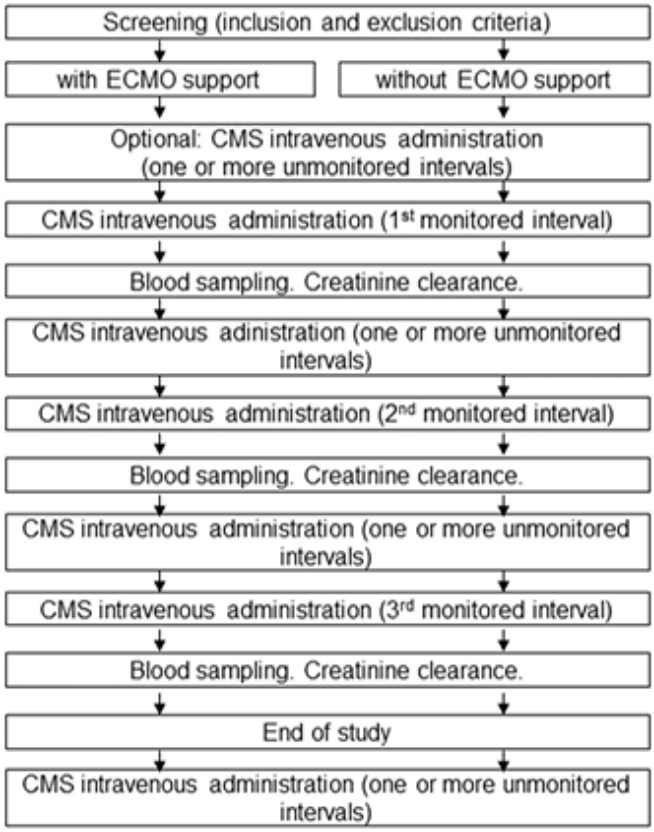


Figure 1 Trial schema for COL-ECMO2022 study. CMS, colistin methanesulfonate; ECMO, extracorporeal membrane oxygenation.

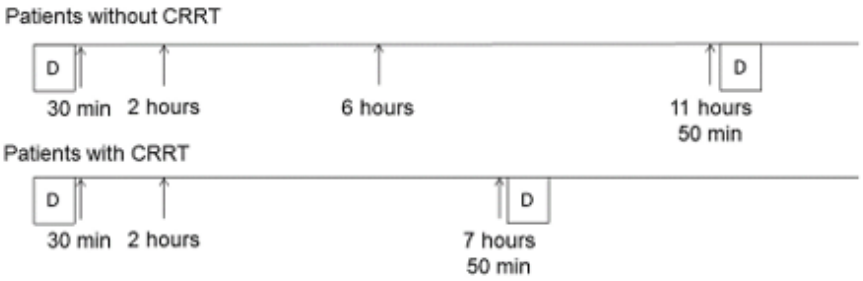


Figure 2 Blood sampling schedule for COL-ECMO2022 study. Arrows indicate blood sampling time points. CRRT, continuous renal replacement therapy; D, administration of CMS dose as a 30 min intravenous infusion.

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Table 1 Participant timeline for COL-ECMO2022 study

	Visit 1	Visit 2	Visit 3*	Visit 4*
	Screening	First monitored interval	Second monitored interval	Third monitored interval
Inclusion/exclusion criteria	X			
Urine pregnancy test in women of childbearing potential	X			
Informed consent	X			
CMS intravenous administration over 30 min		X	X	X
Pharmacokinetic blood samples		X	X	X
12-hour urine collection for creatinine clearance†		X	X	X

*Only if CMS therapy continues (optional visit), maximum of three dosing intervals per subject.
†Only in a patient with urine output of more than 500 mL per day.
CMS, colistin methanesulfonate.

at 11 hours and 50 min after starting CMS infusion in patients without CRRT. CMS therapy can continue.

Participant timeline

The enrolment, intervention and visits schedule is summarised in table 1. The duration of the study participation for each subject is a maximum of three monitored dosing intervals.

Criteria for discontinuing or modifying allocated interventions

The trial participant has the right to terminate participation in this clinical trial at any time, for any reason or without stating it.

The investigator must:

- Instruct the participant on the right to early termination of participation.
- Inform him/her that the termination of participation would not affect the physician's approach, the method of further treatment or its quality.
- Ask him/her to discuss this decision with the investigator in advance.
- State in the records the date of early termination of the patient's participation and the reason, if known.

Other reasons for the early termination of the subject's participation include:

- The investigator's decision that further patient involvement is not in the patient's best interests.
- Pregnancy of a patient participating in the clinical trial.
- Termination of the study by a decision of the sponsor or the regulatory authority.
- Discontinuation of colistin therapy at the discretion of the attending physician before completion of the third monitored dosing interval.

Outcomes

Since the efficacy of colistin is dependent on the AUC, colistin average steady-state concentration ($C_{ss,avg}$) is the main objective. A difference between ECMO and non-ECMO patients larger than 25% is considered clinically significant. The toxicity correlates with colistin minimum plasma concentration. The endpoints are the following pharmacokinetic parameters: C_{max}, T_{max}, C_{trough}, AUC, V_d, clearance and intercompartment clearance of CMS and colistin.

Patient and public involvement

None.

Data collection and management

Plans for assessment and collection of outcomes

The study will collect demographic and baseline characteristics from medical records and electronic medical records, including age, gender, ECMO support and baseline characteristics such as body weight, body height. Concomitant inhalation CMS and laboratory results will be documented in the paper medical records and entered by the trial investigator in the paper case report form (CRF).

Data management

The study data will be collected from medical records and manually entered into the paper CRF. The study team members will have access to the patients' medical records. The investigators will be responsible for screening patients, obtaining informed consent, collecting study data and entering it into the CRF. Once recruitment is complete and the last intervention in the study is performed, pseudonymised data from the paper CRFs will be digitised into an Excel worksheet and formatted for use in pharmacokinetic software. Data will be digitally submitted for statistical processing as well. The statistician will analyse the study data in cooperation with the principal investigator. The data will be stored for 15 years after the completion of the study and then destroyed. To promote data quality, the CRFs of each participant will be reviewed by another member of the study team as a monitor.

Confidentiality

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with limited access. Forms, lists, logbooks, appointment books and any other listings

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that link participant ID numbers to add identifying information will be stored in a separate locked file in an area with limited access.

STATISTICAL METHODS

A population pharmacokinetic model will be developed based on measured plasma levels of CMS and colistin. A non-linear mixed-effects model will be used for the calculation of population pharmacokinetic parameters. To improve the model fit, the following variables will be evaluated as covariates: ECMO therapy, creatinine clearance, renal replacement therapy, actual or adjusted body weight, and age. A difference of at least 25% between the ECMO and non-ECMO groups will be considered clinically significant.

The Shapiro-Wilk test will be used to evaluate the normal distribution. Student's t-test or Mann-Whitney U test will be used for comparisons as appropriate. Data will be presented as means±SD or median (IQR); p values <0.05 will be considered statistically significant. Statistica software V.14.0 (TIBCO Software, U.S.A.) will be used for statistical analysis.

ETHICS AND DISSEMINATION

This protocol and the template informed consent form were reviewed and approved by the Ethical Committee of the University Hospital St. Anne's Brno (Number 10ML./2022-AM on 13 July 2022) with respect to the scientific content and compliance with applicable research and human subject regulations. Any new protocol modifications will be sent for review by the ethics committee and will be amended at the clinical trial registry. Informed consent will be obtained from all participants prior to enrolment in the study. In unconscious patients, the study investigator will decide whether to include the patient in the study after considering all individual risks. In this case, the Ethics Committee of St. Anne's University Hospital Brno will be informed of the patient's inclusion. The investigator will ask study participants in whom good quality consciousness is restored to give subsequent informed consent without unreasonable delay.

We intend to submit the results of the study to be published in a peer-reviewed international medical journal and disseminate them to academic and healthcare professionals via presentations at conferences.

EudraCT Number of the study is 2022-000291-19 registered on 21 June 2022. Study registered at the Clinical Trials register https://clinicaltrials.gov/ct2/show/NCT05542446 on 15 September 2022.

DISCUSSION

The COL-ECMO2022 trial is, as far as we know, the first of its kind. Its results will provide new pharmacokinetic data and a population pharmacokinetic model for critically ill patients and refine colistin dosage in patients on ECMO. However, several non-standard approaches in the methodology deserve more detailed justification. The first is the definition of inclusion criteria. It is not usual for inclusion criteria to be the logical opposite of exclusion criteria and vice versa. In this protocol, pregnancy fulfils this condition and the reason for the wording is a requirement of the NRA and national legislation. According to current national legislation, pregnant women can only be enrolled in clinical trials of a therapeutic or preventive nature directly aimed at the pregnancy or the unborn fetus. This is also why pregnancy has been established as an exclusion criterion despite being a standard-of-care trial. The second relevant reason is that it is a pharmacokinetic study in a small sample of critically ill patients. Pregnancy leads to significant changes in drug pharmacokinetics, which would lead to undesirable bias in the parameters obtained.

Another unusual practice is the inclusion by the investigator of a patient unable to consent to participate in the trial. The inclusion of unconscious patients in the trial is a touchy ethical issue on which the responsible institutional review board and the NRA had difficulty finding consensus when reviewing this trial. The usual practice of obtaining consent from a legally authorised representative, a close person or a pair of an independent physician with an independent witness was unacceptable. A compromise acceptable to both institutions was the procedure described above, whereby the principal investigator decides independently on the inclusion of the patient and informs the institutional review board ex post. Given the nature of the study, a waiver of informed consent would have been possible in many cases. And this is perhaps the reason why the procedure described above was finally acceptable. Notably, the process would likely be different if the study had been submitted under the new regulation; however, the first submission was made before the entry into force of Reg. (EU) 536/2014.

The next questions concern patients on CRRT. The first is the differentiation of colistin adsorption to the ECMO circuit and the CRRT circuit in ECMO+CRRT patients. Adsorption to the haemofilter is a known phenomenon that we consider in the dosing frequency. Thus, non-ECMO+CRRT patients will be the most appropriate comparison group to quantify adsorption to ECMO in those patients. For the other patient groups (ECMO+CRRT vs ECMO+non CRRT vs non-ECMO+non CRRT), the predictive value will only be in comparing trough concentrations between groups and concentrations taken at 30 min after the start of the infusion in particular groups. However, CRRT will be a binary covariate for the pharmacokinetic analysis, similar to ECMO. Hence, we should be able to distinguish the effect of either extracorporeal method on colistin pharmacokinetics. The second issue related to patients on CRRT is how creatinine clearance is determined. If patients are oliguric, we do not plan to collect urine (see note in table 1), given the limited significance of this value. Creatinine clearance, necessary for the construction of

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the population pharmacokinetic model, will be set to an average value of 30 mL/min, recognising the slight variability depending on the individual CRRT setting. We do not expect a high proportion of patients on CRRT in this study. However, we did not primarily want to exclude them from the study, even at the risk of bias that their pharmacokinetic parameters may introduce.

Several methodological limitations of this study can affect the outcomes negatively: anticipated sample size, implemented limited sampling strategy, CMS administration per standard medical care which the investigator cannot modify. First, the sample size was estimated based on pilot data to make the study feasible. The pilot data were collected during the COVID-19 pandemic when the patient profile differed from the routine. Following this, we chose a more conservative and still feasible target number of subjects. Second, since this is a standard-of-care trial, we implemented a limited sampling strategy to minimise patient burden and interference between study procedures and routine care. We also took into account the data published by Kim *et al*.[16] The trough and concentration at 2 hours after initiation of the 30 min infusion showed the highest correlation with AUC. Third, although colistin dosing is not a study procedure and is subject to routine medical care, it respects local and international guidelines. The actual dosage is recorded in the CRF. As a result, a reliable and transferable population pharmacokinetic model can be constructed.

TRIAL STATUS

Trial sponsor: St. Anne's University Hospital in Brno, Pekařská 664/53, 656 91 Brno, Czech Republic

Protocol version 1.2, dated 25 May 2022. The recruitment was started in September 2022. Thirty patients are planned to be recruited by the end of December 2023. Completion of the study with data analysis is anticipated in December 2023.

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Contributors PS: design of the study, protocol writing, first author, principal investigator, statistical analysis. JR: design of the study, protocol writing. LS: design of the study, protocol revision. VK: protocol revision, design of the laboratory analytic part. KU: design of the study, design of the laboratory analytical part.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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REFERENCES

1 Shekar K, Fraser JF, Smith MT, *et al*. Pharmacokinetic changes in patients receiving Extracorporeal membrane oxygenation. *J Crit Care* 2012;27:741.
2 Suk P, Rychlíčková J. Pharmacokinetics of Colistin during Extracorporeal membrane oxygenation. *J Antimicrob Chemother* 2022;77:2298–300.
3 Peng D, Zhang F, Lv P, *et al*. Plasma concentrations of Colistin sulfate in a patient with septic shock on Extracorporeal membrane oxygenation and continuous renal replacement therapy: a case report. *Ann Transl Med* 2022;10:614.
4 Markou N, Fousteri M, Markantonis SL, *et al*. Colistin pharmacokinetics in intensive care unit patients on continuous Venovenous Haemodiafiltration: an observational study. *J Antimicrob Chemother* 2012;67:2459–62.
5 Harigaya Y, Bulitta JB, Forrest A, *et al*. Pharmacodynamics of vancomycin at simulated epithelial lining fluid concentrations against Methicillin-resistant Staphylococcus aureus (Mrsa): implications for dosing in Mrsa pneumonia. *Antimicrob Agents Chemother* 2009;53:3894–901.
6 Sorli L, Luque S, Grau S, *et al*. Trough Colistin plasma level is an independent risk factor for nephrotoxicity: a prospective observational cohort study. *BMC Infect Dis* 2013;13:380.
7 Nation RL, Velkov T, Li J. Colistin and Polymyxin B: peas in a pod, or chalk and cheese *Clin Infect Dis* 2014;59:88–94.
8 Plachouras D, Karvanen M, Friberg LE, *et al*. Population pharmacokinetic analysis of Colistin Methanesulfonate and Colistin after intravenous administration in critically ill patients with infections caused by gram-negative bacteria. *Antimicrob Agents Chemother* 2009;53:3430–6.
9 Garonzik SM, Li J, Thamlikitkul V, *et al*. Population pharmacokinetics of Colistin Methanesulfonate and formed Colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. *Antimicrob Agents Chemother* 2011;55:3284–94.
10 Mohamed AF, Karaiskos I, Plachouras D, *et al*. Application of a loading dose of Colistin Methanesulfonate in critically ill patients: population pharmacokinetics, protein binding, and prediction of bacterial kill. *Antimicrob Agents Chemother* 2012;56:4241–9.
11 Grégoire N, Mimoz O, Mégarbane B, *et al*. New Colistin population pharmacokinetic data in critically ill patients suggesting an alternative loading dose rationale. *Antimicrob Agents Chemother* 2014;58:7324–30.
12 Karaiskos I, Friberg LE, Pontikis K, *et al*. Colistin population pharmacokinetics after application of a loading dose of 9 MU Colistin Methanesulfonate in critically ill patients. *Antimicrob Agents Chemother* 2015;59:7240–8.

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13 Markou N, Markantonis SL, Dimitrakis E, *et al*. Colistin serum concentrations after intravenous administration in critically ill patients with serious multidrug-resistant, gram-negative Bacilli infections: a prospective, open-label, uncontrolled study. *Clin Ther* 2008;30:143–51.
14 Menna P, Salvatorelli E, Mattei A, *et al*. Modified Colistin regimen for critically ill patients with acute renal impairment and continuous renal replacement therapy. *Chemotherapy* 2018;63:35–8.
15 Nation RL, Garonzik SM, Thamlikitkul V, *et al*. Dosing guidance for intravenous Colistin in critically-ill patients. *Clin Infect Dis* 2017;64:565–71.
16 Kim EJ, Oh J, Lee K, *et al*. Pharmacokinetic characteristics and limited sampling strategies for therapeutic drug monitoring of Colistin in patients with multidrug-resistant gram-negative bacterial infections. *Ther Drug Monit* 2019;41:102–6.

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7.9 Annex 9

Lakomý, R., Lojová, M., Souckova, L. et al. 11C-methionine in the diagnostics and management of glioblastoma patients with rapid early progression: nonrandomized, open label, prospective clinical trial (GlioMET). BMC Cancer 24, 736 (2024). https://doi.org/10.1186/s12885-024-12469-2

Summary
Purpose:
The aim of this study was to evaluate the role of ^{11}C-methionine PET/CT imaging in improving diagnostic accuracy and optimizing radiotherapy planning in glioblastoma patients with rapid early progression (REP). This subgroup of patients has a particularly poor prognosis, and standard MRI-based planning may fail to identify all areas of aggressive tumour growth.

Methodology:
The study was designed as a prospective, monocentric, nonrandomized, open-label clinical trial with parallel comparison to a historical cohort. Patients with glioblastoma and REP underwent both conventional MRI and ^{11}C-methionine PET/CT, and radiotherapy target volumes were defined using combined imaging, with progression-free survival assessed as the primary endpoint.

Results:
As a clinical trial protocol, the publication primarily establishes a detailed methodological framework, including imaging procedures, patient selection, and outcome definitions. It highlights the expected advantage of PET-based imaging in identifying tumour regions that may not be visible on MRI, potentially enabling more precise targeting of radiotherapy.

Conclusion:
The GlioMET study represents one of the first prospective efforts to integrate advanced molecular imaging into radiotherapy planning for glioblastoma patients with REP. The study is expected to demonstrate that incorporating PET imaging may improve treatment precision and clinical outcomes, illustrating the role of investigator-initiated trials in advancing personalised oncology.

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^{11}C-methionine in the diagnostics and management of glioblastoma patients with rapid early progression: nonrandomized, open label, prospective clinical trial (GlioMET)

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Abstract

Background Glioblastoma (GBM) is the most common and aggressive primary brain cancer. The treatment of GBM consists of a combination of surgery and subsequent oncological therapy, i.e., radiotherapy, chemotherapy, or their combination. If postoperative oncological therapy involves irradiation, magnetic resonance imaging (MRI) is used for radiotherapy treatment planning. Unfortunately, in some cases, a very early worsening (progression) or return (recurrence) of the disease is observed several weeks after the surgery and is called rapid early progression (REP). Radiotherapy planning is currently based on MRI for target volumes definitions in many radiotherapy facilities. However, patients with REP may benefit from targeting radiotherapy with other imaging modalities. The purpose of the presented clinical trial is to evaluate the utility of ^{11}C-methionine in optimizing radiotherapy for glioblastoma patients with REP.

Methods This study is a nonrandomized, open-label, parallel-setting, prospective, monocentric clinical trial. The main aim of this study was to refine the diagnosis in patients with GBM with REP and to optimize subsequent radiotherapy planning.

Glioblastoma patients who develop REP within approximately 6 weeks after surgery will undergo ^{11}C-methionine positron emission tomography (PET/CT) examinations. Target volumes for radiotherapy are defined using both standard planning T1-weighted contrast-enhanced MRI and PET/CT. The primary outcome is progression-free survival defined using RANO criteria and compared to a historical cohort with REP treated without PET/CT optimization of radiotherapy.

Discussion PET is one of the most modern methods of molecular imaging. ^{11}C-Methionine is an example of a radiolabelled (carbon 11) amino acid commonly used in the diagnosis of brain tumors and in the evaluation of response to treatment. Optimized radiotherapy may also have the potential to cover those regions with a high risk

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of subsequent progression, which would not be identified using standard-of-care MRI for radiotherapy planning. This is one of the first study focused on radiotherapy optimization for subgroup of patinets with REP.

Trial Registration NCT05608395, registered on 8.11.2022 in clinicaltrials.gov; EudraCT Number: 2020–000640-64, registered on 26.5.2020 in clinicaltrialsregister.eu. Protocol ID: MOU-2020–01, version 3.2, date 18.09.2020.

Keywords Glioblastoma, Rapid early progression, Radiopharmaceutical, ^{11}C-methionine, Clinical trial, Positron emission tomography, Radiotherapy

Introduction

Background and rationale {6a}

Compared to other oncological diagnoses, primary brain tumours are less common, with an incidence of 8/100,000 population. Unfortunately, the most aggressive tumors, glioblastomas (GBM), account for approximately half of all primary malignant brain tumors in adults. Due to their biological behaviour, these tumours are ranked among the most difficult-to-treat diseases and therefore represent a serious health problem despite the relatively low incidence. Despite advances in the complex oncological treatment of gliomas, treatment results remain unsatisfactory [1, 2].

The reported median survival of 14–17 months with a five-year survival of 10% is observed mainly in patients with favourable prognostic factors who undergo complete adjuvant oncological therapy [3, 4]. Despite all efforts, the median overall survival has only increased by a few months over the past thirty years. For this reason, further research and development of new therapeutic procedures are necessary with the aim of ensuring better disease control and prolonging overall survival [5].

Clinical studies evaluating the role of modern targeted therapy and immunotherapy have not yet demonstrated higher effectiveness of this treatment strategy, and the results of GBM treatment have thus not fundamentally changed. The only small advance in the last 15 years has been treatment using alternating electric fields emitted from electrodes taped to the scalp (Optune). However, the broader availability of this method is limited by the enormously high cost, patient motivation, and some debatable issues in the clinical trials performed [6, 7].

The current standard of care for GBM is based on a multimodal approach combining the maximum possible and safe surgery, postoperative radiotherapy (RT), and chemotherapy (CHT) with alkylating cytostatic temozolomide (TMZ). Adjuvant therapy is usually started within 4 to 6 weeks after surgery, during which the patient recovers from surgery, and all necessary procedures (multidisciplinary indication committee, preparation of radiation plan, etc.) take place before further oncological treatment. The standard postoperative management of newly diagnosed patients with GBM has remained unchanged since the published results of the EORTC 26981–22981/NCIC CE3 trial (Stupp regimen), which completed patient recruitment as early as 2002 and published the findings in 2005. In this protocol, TMZ (75 mg/m^2) is administered on days 1 to 42 concurrently with RT (60 Gy), followed by TMZ alone (150 to 200 mg/m^2) on days 1–5 in six consecutive 4-week cycles. Coadministration of RT and TMZ improved survival from 12.1 months (with RT alone) to 14.6 months (with the addition of TMZ) (3). Due to a certain stagnation in the treatment of GBM in recent years, intensive research at all levels is necessary to improve patients' perspectives on this unfavourable diagnosis.

The Perry accelerated regimen [8] is a treatment option for patients aged ≥ 65 years and those for whom long conventional RT (60 Gy in six weeks) combined with chemotherapy is considered unsuitable by their treating physicians. The total RT dose for this accelerated regimen is 40.05 Gy, administered in 15-day fractions over three weeks.

Concomitant TMZ is administered here at a dose of 75 mg/m^2 per day for 21 consecutive days from the start of RT until its termination. As with Stupp's regimen, adjuvant TMZ alone follows at 150 to 200 mg/m^2 daily for five consecutive days of a 28-day cycle, up to 12 cycles or until disease progression. For patients who are not candidates for concomitant chemoradiotherapy (regardless of age), individual adjuvant treatment is indicated as standard, usually accelerated RT alone (total dose 30–50 Gy in a 10–20-day dose, for example, fractionation regimens of 10×3.0 Gy, 10×3.4 Gy, 15×2.67 Gy, 20×2.5 Gy and others), followed in some patients by TMZ palliative chemotherapy based on the actual overall condition of the patient.

Glioblastoma as a progressive disease

In connection with the greater availability of magnetic resonance (MR), it is possible to optimize the planning of radiotherapy according to the findings of the so-called planning MR for an increasing number of patients. It is an MR study performed immediately (approximately a few days) before the start of radiotherapy. Following the performance of this MR study (typically approx. 4–5 weeks after surgery), a new phenomenon called very rapid

progression (REP, rapid early progression) is described. The REP diagnosis is based on comparing early postoperative MR findings (usually within 48–72 h after surgery) and planning MR before radiotherapy (preRT).

Retrospectively comparing early postoperative MR with preRT MR performed approximately 30 days later, Farace et al. described 30% of preRT MR with signs strongly suggestive of tumour progression during this period [9]. An even more significant proportion of patients with documented REP was described by Palmer et al., where up to 52% progressed before starting adjuvant therapy without any effect of waiting time to start RT (median time from resection to RT was 32.5 vs. 33 days in patients with REP vs. without REP ($p=0.337$) [10]. These results are consistent with our retrospective analysis of 90 patients with GBM treated between 2014 and 2017, where 51% of patients had suspected REP at planning preRT MR [11]. These patients represent a subset with particularly aggressive GBM requiring further intensive research and possibly treatment modification.

According to these and several other retrospective analyses, it was confirmed that the presence of early progression on planning MR was associated with a more aggressive form of GBM and worse overall survival [12–14]. A higher risk can, of course, be expected in patients after nonradical resections [14]. It is still unclear what further influences the prognosis of patients with early progression. Palmer et al. even described significantly worse survival in patients with unmethylated MGMT (O6-methylguanine-DNA methyl-transferase), the methylation of MGMT is considered a marker of higher chemosensitivity [10]. Analysis of other potential biomarkers still needs to be improved. Due to a large number of patients with REP and the retrospective nature of the studies carried out thus far, it is essential to analyse this more aggressive subgroup of tumors prospectively and try to influence the negative course of the disease more clinically.

The optimal treatment approach for patients with REP needs to be determined. It is unclear whether it is better to indicate repeat surgery for recurrence, choose accelerated RT regimens with or without concurrent chemotherapy, or directly administer more aggressive and intensive chemotherapy with a combination of alkylating cytostatics if MGMT methylation is present [15]. Treatment of these patients with REP currently does not differ from that of patients without REP; if so, it is a purely individual approach. These patients distorted the results of previous clinical trials where routine MR examination was not performed before RT. Currently, these patients are usually excluded from clinical trials.

Moreover, current clinical trials often randomize patients until after concomitant chemoradiotherapy if there is no progression on follow-up MR after chemoradiotherapy. REP on planning MR is a significant negative prognostic factor that should be a stratification factor in future clinical trials. In general, the aggressive character of GBM is manifested by its microenvironment, the molecular background of glioma cells, or the miRNA profile, which may be essential in GBM oncogenic signalling and has the potential to serve as a biomarker of this disease and a new therapeutic target in oncology [16–22].

Radiotherapy in the treatment of glioblastoma

Due to the almost zero risk of developing distant metastases, and on the contrary, due to the nearly 100% risk of developing local recurrence in the brain, in the case of GBM, great emphasis is placed on local treatment methods, i.e., surgery and radiation. Radiotherapy has experienced a stormy development in the last decade due to improved computer technology, greater availability of imaging methods, and more advanced radiotherapy systems [7, 23, 24]. A crucial part of radiotherapy treatment is correctly determining the target area for the prescribed radiation dose. In the case of brain tumours, the gold standard for defining target volumes is MR imaging. Recently, the importance of additional imaging methods, especially PET imaging, for the closer characterization of gliomas has been emphasized [24, 25].

^{11}C-Methionine PET in the imaging of glioblastoma

Positron emission tomography is currently the most dynamically developing area for functional brain imaging. It is used both pretreatment and as part of the follow-up of GBM patients after treatment. The development of PET diagnostics is aimed not only at constructing more sensitive and robust PET scanners but also at introducing new radiopharmaceuticals, especially in centres with their own cyclotron.

Fluorodeoxyglucose (2-deoxy-2-[18F]fluoro-D-glucose, FDG) is the most commonly used radiopharmaceutical in PET/CT diagnostics in general. However, it may not provide optimal results for brain examination mainly due to the high metabolic activity in the physiological brain tissue, the image background. That is why other radiopharmaceuticals are used in the targeted diagnosis of brain tumors, most often different amino acid derivatives. Of these, [18F]-O-(2-fluoroethyl)-L-tyrosine (fluoroethyltyrosine, FET) and L-(S-methyl-[^{11}C])-methionine (methionine, MET) play the most important roles. Carbon ^{11}-labelled methionine is historically the most frequently used drug for glioma imaging.

Amino acid (AA) tracers, including FET and MET, naturally cross the blood–brain barrier through neutral amino acid transport mechanisms. Unlike naturally occurring methionine, the artificially prepared amino

acid FET is not subsequently incorporated into protein fractions [26, 27]. Their transport and incorporation into proteins are increased in tumour tissue. However, it is still being determined which of these mechanisms is more critical for PET imaging. Methionine is also incorporated into fats and nucleic acids; this incorporation is less significant for imaging brain tumours. Studies have confirmed the correlation between the intensity of MET accumulation, tumour grading according to WHO and proliferative activity assessed by the Ki-67 index [28] and the density of microvascularization [29]. Methionine is incorporated to an increased extent even in low-grade gliomas [30], while its accumulation is low in the background of normal brain tissue. Since the transport of AA tracers does not depend on the blood–brain barrier violation, the accumulation of AA tracers can be detected even in tumours that do not show contrast saturation and enhancement during MR examination.

MET PET is more suited than MR to accurately delineate the target volume for radiotherapy and determine the extent of viable tumours. Conversely, MR shows the overall extent, including pathological changes associated with the tumour, such as oedema in the immediate vicinity. The widespread use of MET PET is most hindered by its short half-life, so the use of MET is limited only to centres with their cyclotron and the possibility of its production. In patients with REP glioblastoma, it is necessary to start oncological treatment as quickly as possible. It is, therefore, unethical to wait for the delivery of other commercially available radiopharmaceuticals labelled with fluorine 18 (18F-FET, 18F-FLT, 18F-DOPA, etc.), which have a longer half-life. They are also available in PET centres without a cyclotron, but only on predetermined and planned production days. In complex neuro-oncology centres, the possibility of individual preparation of MET is thus an opportunity to benefit patients with REP, the extraordinarily aggressive glioblastomas.

In addition to the subjective assessment, the evaluation of the PET image is refined by quantitative measurement. For this quantitative measurement in clinical practice, drawing a suitable area of interest and software evaluation of the SUVmax parameter ("maximum standardized intensity of accumulation") are usually used. The assessment of the SUVavg ("average value of the standardized intensity of accumulation") is chosen less often. Nevertheless, it is influenced by the specific location of the area of interest, with more significant variability between evaluators. Therefore, SUVavg is less suitable for standardized assessment in studies.

The area of interest can be either circular in the plane for one selected slice, 2D-ROI, or spherical in space, 3D—VOI. The two-dimensional region of interest is also very strongly influenced by the choice of a particular slice for assessment and is also burdened by low reproducibility and high interrater variability. For our evaluation, the measurement of SUVmax in a spatial 3D-VOI defined with a size of 1.5 cm was chosen to minimize variability.

The ratio of the radiopharmaceutical accumulation in the tumour relative to the background ("tumour-to-background" ratio, T/B) is used to differentiate the areas of the physiological and pathological intensity of accumulation. There is no definite recommendation on how to choose the background, and even any recommendations may be influenced by the possibilities of a specific patient. There are also no exact values of the T/B ratio defined as a threshold for distinguishing tumour tissue from physiological tissue. According to available literature data, values between 1.3 and 1.7 are usually chosen.

Explanation for choice of comparator {6b}

This study is an open-label prospective clinical trial. The experimental drug ^{11}C-methionine PET/CT will be administered to all patients recruited to this study and compared to the cohort consisting of a historical group of GBM patients with REP collected in the period 2014–2018.

Objectives {7}

Patients diagnosed with GBM who develop REP before starting adjuvant radiotherapy have an abysmal prognosis. The optimal treatment for these patients has yet to be discovered, and no prospective clinical evaluation has been performed. We assume that the lesion detected on MR is not the only area of REP development. It may be MET PET that can reveal other areas of the aggressive part of the tumour, and the subsequent RT can then be better planned to increase the time to progression in such a cohort of patients. Therefore, the purpose of this study was to refine the diagnosis in patients with GBM with proven REP and subsequent optimized planning of the following treatment procedure. Chemotherapy will be administered as standard treatment to all patients.

Trial design {8}

The GlioMET study is an investigator-initiated study. This clinical trial is a prospective, monocentric, open-label, nonrandomized clinical study with a parallel assignment initiated in June 2020.

The main aim of the clinical trial is to improve the progression-free survival of patients with GBM and REP via optimizing of diagnostic and treatment procedures. The experimental drug ^{11}C-methionine PET/CT will be administered to all patients recruited to this study and compared to the cohort consisting of a historical group of patients collected in the period 2014–2018. The setting is

a confirmatory framework against the historical cohort. A schematic overview of the design of the study is presented in Fig. 1.

Methods: participants, interventions, and outcomes

Study setting {9}

The GlioMET study is a monocentric study. The recruitment period began in October 2020. Patient recruitment in the Czech Republic is planned to be completed in February 2024.

Masaryk Memorial Cancer Institute in the Czech Republic, the coordinating centre for the study, is responsible for educating and training research staff, tracking participants' enrolment, mathematical analysis, monitoring, pharmacovigilance, data management, and reporting of the study. The control cohort consisted of a historical group of patients collected in the period 2014–2018. Data are collected only from the Czech Republic.

Eligibility criteria {10}

A patient may be included in the clinical trial if they meet all of the following criteria:

1. The participant is a person with a histologically proven diagnosis of glioblastoma (GBM) according to WHO 2016.
2. The participants are male or female and aged 18 years or older.
3. Performance status (PS) according to ECOG (Eastern Cooperative Oncology Group) 0–2.
4. Healed operation wound.
5. Postoperative MR up to 72 h.
6. Indication for adjuvant chemoradiotherapy.
7. The participants must express his/her informed consent and sign the form before the screening period.
8. Rapid early progression was detected.
9. The participants must achieve the following values of laboratory parameters in the peripheral blood during the screening period:

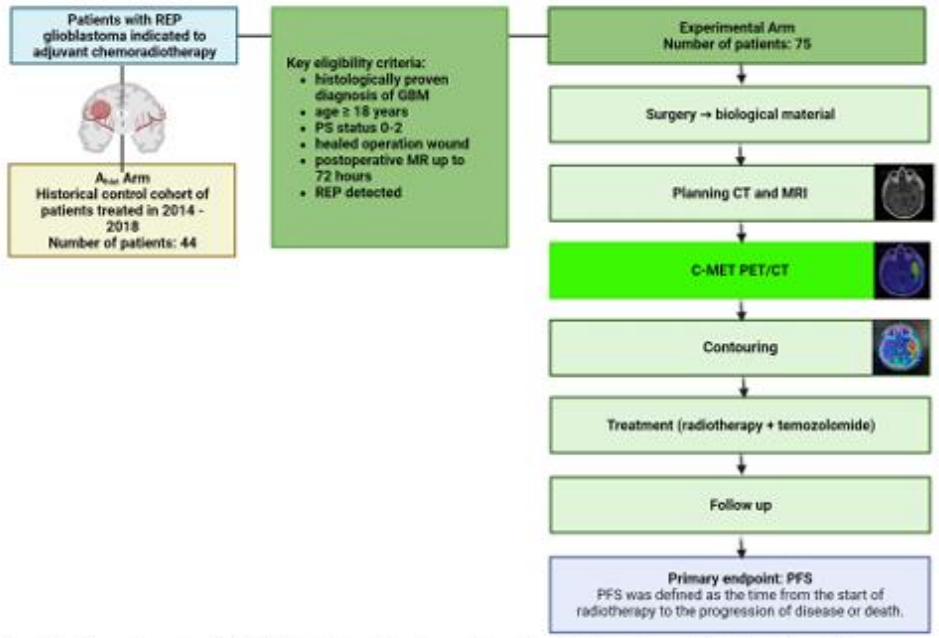


Fig. 1 The Schematic overview of the GlioMET study design. A_{hist} Arm – a historical arm. C-MET—carbon radiolabelled methionine. CT – computer tomography. GBM—glioblastoma multiforme. MRI – magnetic resonance imaging. PET/CT—positron emission tomography/computer tomography. PFS – progression-free survival. PS – performance status. REP—rapid early progression

- neutrophils (total count) ≥ 1500/mm.3
- platelets (total count) ≥ 100,000/mm.3
- hemoglobin ≥ 9.0 g/dL
- serum creatinine ≤ 1.5 × of upper limit of normal (ULN)
- total bilirubin ≤ 1.5 × ULN, unless documented Gilbert's syndrome, for which bilirubin ≤ 3 × ULN is permitted
- AST/ALT ≤ 3 × ULN

The exclusion criteria were as follows:

1. Prior brain surgery.
2. Prior brain radiotherapy.
3. The history of active/currently treated cancer (solid tumor) was as follows: nonmelanoma skin cancer, in situ bladder carcinoma, in situ gastric cancer, in situ colorectal carcinoma, in situ cervical carcinoma, and in situ breast cancer.
4. Any systemic disease or health condition that might pose a risk for anticancer therapy and imaging techniques (MRI, MET PET).
5. Patients must not have substance abuse disorders that would interfere with cooperation with the requirements of the trial.
6. Patients must not have any evidence of ongoing (active) infection (HIV, hepatitis A, B, C).
7. Pregnant and/or breastfeeding women.
8. Patients who disagreed and refused to sign an informed consent form.

Intervention description {11a}

The intervention is the radiopharmaceutical application performing ^{11}C-methionine PET examination. The activity of the ^{11}C-methionine applied for each patient will be 300 MBq (± 50 MBq) of ^{11}C-methionine. The preparation of ^{11}C-methionine for a specific patient is provided by a lab worker only be performed with written consent. ^{11}C-MET will be sufficiently hydrated, meaning that they have not consumed any food for at least 4 h prior to application. To obtain the best quality images and to reduce radiation exposure to the bladder, the patient will be asked to drink plenty of fluids and void before the PET scan. After a PET scan, the bladder should be emptied as often as possible. If patient sedation is needed, it will be started approximately 20–60 min before the start of the examination. The application of the radiopharmaceutical will be carried out after ensuring venous access with an intravenous cannula. Finish the application by rinsing with 20 ml of physiological solution. The patient will be monitored for 2 h after the application of the radiopharmaceutical due to the possibility of a hypersensitivity reaction.

Criteria for discontinuing or modifying allocated interventions {11b}

The clinical trial will be interrupted or prematurely terminated for the following reasons:

1. In the case of serious adverse events, the resulting benefit-risk ratio is unacceptable.
2. In the event that other comparable clinical evaluations reveal an unacceptable level of risk for the evaluation subjects. The sponsor reserves the right to interrupt or prematurely end the clinical trial at the centre in the event that:

   (i) the safety of the subjects would be significantly compromised,
   (ii) sufficient recruitment would not be achieved,
   (iii) there would be repeated deviations from the protocol on the part of the investigating centre.

Strategies to improve adherence to interventions {11c}

Training before initialization ensures compliance during the whole study. If there are doubts, a chief investigator is designated to address them.

Relevant concomitant care permitted or prohibited during the trial {11d}

The modification consists of changing the irradiation plan. Two chemoradiotherapy regimens of concomitant chemoradiotherapy with temozolomide are permissible at the discretion of the attending physician [31–33]:

Regime according to Stupp:

RT 60 Gy/30 fr. each 2.0 Gy (6 weeks, Mon-Fri) + temozolomide 75 mg/m^2 D1-42 including weekends, then adjuvant temozolomide 150–200 mg/m^2 D1-5, 28-day interval, six cycles with the possibility of extension to a total of 12 cycles (if no more than low-grade adverse effects occur and if a benefit is demonstrated) or until progression or toxicity.

Perry's regimen

RT 40.05 Gy/15 fr. each 2.67 Gy (3 weeks, Mon-Fri) + temozolomide 75 mg/m^2 D1-21 including weekends then adjuvant temozolomide 150–200 mg/m^2 D1-5, interval 28 days, max. 12 cycles or until progression or toxicity.

The choice of regimen is at the discretion of the attending physician. In general, the Perry regimen is used for frail and elderly patients, and these patients would probably not be able to handle the classic long Stupp regimen. It is a modified Stupp regimen, where there is a shorter (3-week) concomitant CHT/RT with 40.05 Gy instead of 6 weeks with 60 Gy, followed by classical adjuvant 150–200 mg/m^2, but up to 12 cycles of temozolomide.

Outcomes {12}

Primary Outcome Measures of the clinical trial are to demonstrate the progression-free survival (PFS) in the 11C-methionine PET/CT arm as Experimental arm vs. PFS in the historical patient cohort as an arm Ahist. PFS was defined as the time from the start of radiotherapy to the progression of disease or death [Time Frame: 44 months].

The secondary outcomes are the following:

1) Rapid-Early-Progression incidence [Time Frame: 38 months]

   Prospective evaluation of the incidence of Rapid-Early-Progression (REP) on planning MR in patients with GBM indicated for adjuvant chemoradiotherapy.
2) Overall survival (OS) in the Experimental Arm vs. Arm Ahist [Time Frame: 44 months]
   OS in the Experimental Arm compared to the historical retrospective group (arm Ahist). OS = time from surgery to death related to glioblastoma multiforme.
3) Biomarkers I [Time Frame: 38 months].
   Immunohistochemical analysis of GFAP, Ki-67, IDH1, ATRX, and PDL1. GFAP: positive/negative/focal positive Ki-67, IDH1: positive/negative, ATRX loss of expression: positive/negative, PDL1 protein expression: positive/negative.
4) Biomarker II [Time Frame: 38 months]
   Mutational status of TERT and IDH2: mutated/wild-type.
5) Biomarkers III [Time Frame: 38 months]
   Sanger sequencing of IDH1 in IDH1 IHC-positive patients: mutated/wild-type.
6) Biomarkers IV [Time Frame: 38 months]
   MGMT promoter methylation status: ≥ 25% = methylated/ < 25% = unmethylated.
7) Biomarkers V [Time Frame: 38 months]
   1 p/19 q codeletion: positive/negative
8) Pattern-of-failure analysis [Time Frame: 44 months]
   Evaluation of spatial patterns of failure (PoF; central (V95% ≥ 95%)/in-field (80% ≤ V95%)/marginal (20% ≤ V95% < 80%)/distant (V95% < 20%)) in patients with REP (the Experimental Arm) compared to the historical retrospective group (Arm Ahist). In the case of multifocal progression, each PD is evaluated independently, i.e., the patient could have one central and one distal PD. In subsequent statistical analysis, each PD was evaluated independently. PoF could be performed even in deceased subjects if MR describing the progression is available.
9) Quality of Life assessment using the standardized European Organization for Research and Treatment of Cancer (EORTC) questionnaire QLQ-C30 [Time Frame: 44 months]
   Quality of life will be assessed using a standardized questionnaire: the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C30 v.3.0 during the screening period, visits 2, 4, 6, 8, 10, and 12 (End-Of-Treatment) and during the follow-up period every 3 months (± 1 week) up to progression of the disease.
10) Quality of Life assessment using the standardized European Organization for Research and Treatment of Cancer (EORTC) Quality of Life questionnaire-BN20 [Time Frame: 44 months]
    Quality of life will be assessed using a standardized questionnaire: additional module Brain Cancer European Organization for Research and Treatment of Cancer Quality of Life questionnaire BN20 during the screening period, visits 2, 4, 6, 8, 10, and 12 (End-Of-Treatment) and during the follow-up period every 3 months (+/- 1 week) up to the disease.

Participant timeline {13}

The schedule of enrolment, intervention and visits is summarized in Tables 1 and 2. The duration of the study participation is 44 months (recruitment 38 months + 6 months follow-up).

The modification consists of changing the irradiation plan.

Two chemoradiotherapy regimens of concomitant chemoradiotherapy with temozolomide are permissible at the discretion of the attending physician.

Stupp's regimen can be extended to 12 cycles of adjuvant temozolomide due to the unification of the possible length of administration of adjuvant temozolomide itself and thus minimizing the occurrence of bias in the length of adjuvant treatment between younger and older/frail groups in the clinical study.

Since 2005, when temozolomide was registered based on the study with 6 cycles of adjuvant temozolomide, a number of phase 3 studies were conducted, where it was possible to extend this adjuvant period up to 12 cycles. On the other hand, in patients without REP, extension of adjuvant chemotherapy up to 12 cycles is currently not recommended [2].

Table 1 Participant timeline for the regimen according to Stupp

4.3.1 Stupp regimen	Screening	CHEMO RADIO THERAPY	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 (EoT)	Follow-up until PD	EoS Visit
Days / Months	**max. 14 D**	**D1-D42 (M 1–2)**	**D42 + 1 M (± 1 tweek)**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**Every 3 M (± 1 tweek)**	**PD**
Inclusion/exclusion criteria	X															
Informed consent	X															
Anamnesis[a]	X															
Pregnancy test (serum/urine)	x*	xx*	x	x	x	x	X	x	x	x	x	x	x			
HIV, HBV, HCV test	X**															
Physical and laboratory examination[b]	X**	x***	x	x	x	x	x	x	x	x	x	x	x	x		
ECOG Performance status	X	x	x	x	x	x	x	x	x	x	x	x	x	x		
Extent of surgery[c]	x															
^{11}C-MET PET/CT[d]	x															
Quality of life questionnaires[e]	x			x		x		x		x		x		x	x	
MR and RANO evaluation[f]				x (á 3 months)												
Molecular markers[g]	*To be performed during the patient's participation in the study*															
Radiotherapy (**start max. 8 weeks since surgery and max. 2 weeks after planning MR**)		x														

Table 1 (continued)

4.3.1.Stupp regimen	Screening	CHEMO RADIO THERAPY	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 (EoT)	Follow-up until PD	EoS Visit
Days / Months	max. 14 D	D1-D42 (M 1–2)	D42 + 1 M (± 1 tweek)	+1 M	+1 M	+1 M	+1 M	+1 M	+1 M	+1 M	+1 M	+1 M	+1 M	+1 M	Every 3 M (± 1 tweek)	PD
Chemotherapy (**start max. 8 weeks since surgery and max. 2 weeks after planning MR**)	x		x	x	x	x	x	X	x	x	x	x	x	x		
Adverse events	x		x	x	x	x	x	X	x	x	x	x	x	x	x	x
PoF analysis[h]																x

The description of performed examinations:

[a] The pregnancy test will be performed at the center (1. As part of the screening phase before 11C-methionine applications and 2. Before the 1st application of concomitant chemoradiotherapy)

[b] Laboratory analysis incl. tests for HIV, HBV and HCV will be performed in the screening phase if they have not been performed in the previous 5 days

[c] Laboratory analysis (blood count + differential + biochemistry) will be performed every week as part of CHRT. Physical examination will be performed on D1 and D21 (if not performed in the previous 5 days)

[d] Anamnesis

Collection of anamnestic data will take place following standard clinical practice. Taking a family history will focus on the familial occurrence of malignancies and genetic syndromes. As part of the personal anamnesis, pre-existing severe diseases will be recorded even at the time of diagnosis; a pharmacological anamnesis and information on allergies will automatically be included. Data will also be collected regarding substance abuse and social and work anamnesis

[e] Physical and laboratory examination

Physical examination + neurological examination + height/weight/vital functions: BSA calculation, blood pressure, pulse, respiratory rate, body temperature;

Performance status: according to ECOG 0–2; Laboratory examination: blood count including differential count, Na, K, Cl, urea, creatinine, glucose, total bilirubin, ALT, AST, ALP, amylase, LD, albumin, total protein, CRP

[f] Scope of operation

Evaluation according to postoperative MR (performed within 72 h after surgery):

Gross Total Resection: complete macroscopic resection

Subtotal Resection: resection of ≥ 90% of the tumor

Partial Resection: resection of < 90% of tumor (biopsy only = exclusion criterion)

[g][1] C-MET PET/CT

[1] carbon radiolabelled methionine positron emission tomography/computer tomography

[g] Quality of life questionnaire

These are standardized questionnaires assessing the quality of life of oncology patients, according to the EORTC. Official versions of the questionnaires in Czech are available. These questionnaires can be completed at home. The basic questionnaire (EORTC QLQ-C30/Czech) contains 30 questions with the possibility of answering a degree of four points or a seven-point Likart scale. Part of the quality of life questionnaires is also a module of an additional 20 questions optimized to assess the quality of life of neuro-oncology patients

[f] Magnetic Resonance Imaging and assessment of RANO criteria

[g] Molecular markers

Immunohistochemical labelling of GFAP, Ki-67, IDH1 and ATRX, PDL1 protein expression by immunohistochemistry, mutational status of TERT and IDH2 genes and then IDH1 in immunohistochemically positive patients by Sanger sequencing, methylation status of the MGMT promotor and 1p/19q codeletion

[h] PoF analysis

Spatial evaluation of recurrence in relation to the area irradiated with a high dose of radiation (patterns of failure, PoF) is performed by a radiotherapist. MR defining progression according to RANO (MRRANO) criteria is used to analyze PoF, specifically T1-weighted sequence with contrast medium administration

Standard image fusion is performed in the radiotherapy planning system. MRRANO is registered to the original planning CT. The fusion is focused on the calf area (preferential choice of Hounsfield HU units in the bone band)

Table 2 Participant timeline for the regimen according to Perry

4.3.2. Perry regimen	Screening	CHEMO RADIO THERAPY	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 (EoT)	Follow-up until PD	EoS Visit
Days / Months	**max. 14 D**	**D1-D21 (M 1–2)**	**D21+1 M (± 1 week)**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**Every 3 M (± 1 week)**	**PD**
Inclusion/exclusion criteria	x															
Informed consent	x															
Anamnesis[a]	x															
Pregnancy test (serum/urine)	x*	x*	x	x	x	x	x	x	x	x	x	x	x			
HIV, HBV, HCV test	X**															
Physical and laboratory examination[b]	X**	x***	x	x	x	x	x	x	x	x	x	x	x	x		
ECOG Performance status	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Extent of surgery[c]	x															
^{11}C-MET PET/CT[d]	x															
Quality of life questionnaires[e]	x			x		x		x		x		x		x	x	
MR and RANO evaluation[f]					x (á 3 months)											
Molecular markers[g]	*To be performed during the patient's participation in the study*															
Radiotherapy/ **(start max. 8 weeks since surgery and max. 2 weeks after planning MR)**		x														

Table 2 (continued)

4.3.2. Perry regimen	Screening	CHEMO RADIO THERAPY	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 (EoT)	Follow-up until PD	EoS Visit
Days / Months	**max. 14 D**	**D1-D21 (M 1–2)**	**D21 + 1 M (± 1 week)**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**+1 M**	**Every 3 M (± 1 week)**	**PD**
Chemotherapy **(start max. 8 weeks since surgery and max. 2 weeks after planning MR)**	x		x	x	x	x	x	x	x	x	x	x	x	x		
Adverse events	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
PoF analysis[h]																x

The description of performed examinations:

[a] The pregnancy test will be performed at the center (1. As part of the screening phase before 11C-methionine applications and 2. Before the 1st application of concomitant chemoradiotherapy)

[b] Laboratory analysis incl. tests for HIV, HBV and HCV will be performed in the screening phase if they have not been performed in the previous 5 days

[c] Laboratory analysis (blood count + differential + biochemistry) will be performed every week as part of CHRT. Physical examination will be performed on D1 and D21 (if not performed in the previous 5 days)

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[e] Physical and laboratory examination

Physical examination + neurological examination + height/weight/vital functions: BSA calculation, blood pressure, pulse, respiratory rate, body temperature;

Performance status: according to ECOG 0–2; Laboratory examination: blood count including differential count, Na, K, Cl, urea, creatinine, glucose, total bilirubin, ALT, AST, ALP, amylase, LD, albumin, total protein, CRP

[f] Scope of operation

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Partial Resection: resection of < 90% of tumor (biopsy only = exclusion criterion)

[g,i] C-MET PET/CT

[i] carbon radiolabelled methionine positron emission tomography/computer tomography

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[f] Magnetic Resonance imaging and assessment of RANO criteria

[g] Molecular markers

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[h] PoF analysis

Spatial evaluation of recurrence in relation to the area irradiated with a high dose of radiation (patterns of failure, PoF) is performed by a radiotherapist. MR defining progression according to RANO (MRRANO) criteria is used to analyze PoF, specifically T1 -weighted sequence with contrast medium administration

Standard image fusion is performed in the radiotherapy planning system. MRRANO is registered to the original planning CT. The fusion is focused on the calf area (preferential choice of Hounsfield HU units in the bone band)

Sample size {14}
A historical retrospective cohort included 44 patients with REP who were indicated for adjuvant chemoradiotherapy (Arm Ahist). The median PFS in this cohort was 4.9 months. Assuming that the time distribution in the Experimental Arm follows a proportional hazard model, a significance level of 5%, and a power of 80%, 65 events are needed to detect a hazard ratio of 1.65 (i.e., median PFS in the Experimental Arm of 8 months). With an assumed recruitment of 38 months and a follow-up duration of six months, 67 patients are needed based on the historical cohort. With the expected screening failure rate of 5% and a dropout rate of 5%, approximately 75 patients with confirmed REP should be enrolled.

Recruitment {15}
Recruitment of the patients has been carried out since October 2020. During the first four years, all planned patients will be recruited. The patients were recruited only at Masaryk Memorial Cancer Institute in Brno, Czech Republic.

The patient will be informed about the study during the visit, during which initial eligibility for the study will be determined, and the research staff will explain the study protocol and design.

Methods: assignment of interventions: allocation

Sequence generation {16a}, Concealment mechanism {16b}
The GlioMET study is nonrandomized, and the assignment of interventions is not applicable.

Implementation {16c}
The study investigator will enroll the participants. All study visits will be scheduled to coincide with the regimen according to Stupp or Perry.

Blinding (masking) {17a}
Not applicable. This study is not blinded; it is open-label.

Methods: data collection, management and analysis

Data collection methods {18a}
The study collected demographic and baseline characteristics from medical records and electronic medical records, including age, sex, type of admission, and baseline characteristics such as body weight, body height, patient history, and pharmacological history. The results of PET/CT scans and physical examinations are documented in the electronic medical records and entered by the trial investigator in the electronic Case Report Form (eCRF).

Plans to promote participant retention and complete follow-up {18b}
The study site will make every reasonable effort to follow the participant for the entire study period. Study site staff members will be responsible for developing and implementing local standard operating procedures to achieve maximal follow-up.

Data management {19}
The study data are entered online in an internet-based database (RedCap®) and collected from medical records. The study staff members had access to the medical records of the patients. The investigators will be responsible for screening patients, obtaining informed consent, collecting study data, and entering it into the eCRF. The statistician will analyse the study data in cooperation with the principal investigator. The data will be stored for 15 years after completion of the study and then destroyed. To promote data quality, the eCRFs of each participant will be reviewed by another member of the study team as a monitor.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}
All data will be described for each arm separately and for the entire group of patients. The appropriate descriptive statistics will be used, i.e., counts and percentages for categorical characteristics and median and interquartile range (IQR) or mean and standard deviation (SD) for continuous characteristics. Depending on the data type, common statistical tests such as Fisher's exact test or chi-square test for comparing categorical variables and nonparametric Mann–Whitney test or Kruskal–Wallis test for comparing continuous variables or their parametric alternatives if the assumption of data normality is met will be used for comparison of patient characteristics between arms.

The survival parameters that will be considered are progression-free survival (PFS) and overall survival (OS). PFS is considered the time from the start of radiotherapy to the occurrence of progression or death. OS is considered the time from surgery to death (in relation to GBM). The Kaplan–Meier method will be used to estimate the probability of survival. Survival comparisons between groups will be made using the log-rank or Gehan-Wilcoxon test. The Cox proportional hazards model will be used to calculate the hazard ratio in univariable and multivariable analyses.

All statistical tests and confidence intervals will be calculated at a significance level of 5%.

Methods for any additional analyses (20b)
Not applicable. The subgroup and adjusted analyses are not planned.

Definition of analyses population relating to protocol nonadherence and any statistical methods to handle missing data (multiple imputation) (20c)
Protocol nonadherence will be assessed by the principal investigator case by case. Patients with major protocol deviations will be excluded from the analysis. Missing data are not planned to be imputed. However, in the event of substantial missing data for any parameter, a sensitivity analysis using any method of imputation could also be used.

The data will be analysed for the individual groups below:

- Full Analysis Set (FAS)

  All patients who successfully completed recruitment met the inclusion/exclusion criteria.
- Per Protocol Set (PPS)
  FAS subgroup with such patients who undergo trial without significant deviations from the protocol.
- Safety Set
  Includes all patients who were administered a medicinal product within the framework of the GlioMET study.

Methods: monitoring

Data monitoring (21a)
The on-site monitoring of the study conduct is regularly performed throughout the study period to assess the accuracy and completeness of the data. The study site may be subject to a quality assurance visit. If so, the site will be contacted in advance to organize a monitoring visit. The investigator will ensure direct access to all study documents for quality assurance monitors.

Interim analyses (21b)
Not applicable. An interim analysis is not planned.

Harms (22)
Serious adverse events will be reported according to standard rules and standard operating procedures of the coordinating unit. All study participants will be monitored for potential adverse events.

Auditing (23)
Trial conduct will be audited by the Institutional Projects Evaluation Committee every year during the study. The process of auditing will be independent of the investigator and sponsor.

Research ethics approval (24)
This protocol and the template informed consent form were reviewed and approved by the Ethical Committee of the Masaryk Memorial Cancer Institute (in September 2020) with respect to the scientific content and compliance with applicable research and human subject regulations. Study protocol was evaluated by institutional Project Steering Committee and institutional Ethics Committee No. 2020/1206/MOU.

Protocol Amendments (25)
Any new protocol modifications will be sent for review by the ethics committee and a national Drug Agency and will be amended at the clinical trial registry.

Consent and Assent (26a)
Informed consent will be obtained from all participants prior to enrolment in the study.

A trained study investigator will describe the study to patients or authorized surrogates if applicable. Patients will also receive information sheets. The investigator will discuss the study with patients in the sense of the information provided in the information sheets. The investigator will obtain written informed consent from patients willing to participate in the trial. In the case of patients who are unable to consent because of a medical condition, their ability to participate in the study will be assessed by a medical council consisting of at least one independent physician informed about the study details and one study investigator.

Confidentiality (27)
All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with limited access. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to add identifying information will be stored in a separate locked file in an area with limited access.

Declaration of interest (28)
The authors declare that they have no competing interests.

Access to data (29)
The final trial dataset will be accessible to all investigators participating in this study.

Ancillary and posttrial care (30)

The clinical trial GlioMET is conducted in full accordance with the principles of the Declaration of Helsinki (in the latest valid version), in accordance with GCP requirements and in accordance with applicable national and European legislation. GlioMET was discussed and approved by the local ethics committee and State Institute for Drug Control before the start.

All patients were informed about the possible risks of their participation in this study and about the possibility of ending their participation in the study at any time without consequences. The patients are also informed about the measures that will be taken for the protection of personal data and about the fact that, in the event of damage to health as a result of the implementation of the study, liability insurance for the examiner and the contracting authority has been arranged in accordance with the applicable legislation, through which the possible compensation of assessment subjects.

Dissemination policy (31a)

We intend to submit the results of the study to be published in a peer-reviewed international medical journal and disseminate them to academic and healthcare professionals via presentations at conferences.

Abbreviations

2D-ROI	Two-dimensional region of interest
3D-ROI	Three-dimensional volume of interest
^{11}C-methionine	11 Carbon radiolabelled methionine
^{18}F-FET	18 Fluorine radiolabelled fluoroethyltyrosine
^{18}F-FLT	18 Fluorine radiolabelled fluorothymidine
^{18}F-DOPA	18 Fluorine radiolabelled dihydroxyphenylalanine
AA	Aminoacid
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATRX	Alpha-thalassemia X
CHT	Chemotherapy
D	Day
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EORTC	European Organization for Research and Treatment of Cancer
FAS	Full analysis set
FDG	2-Deoxy-2-[18F] fluoro-D-glucose
FET	Fluoroethyltyrosine
Fri	Friday
GBM	Glioblastoma multiforme
GCP	Good clinical practice
GFAP	Glial fibrillary acidic protein
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
ID	Identificational data
IDH1	Isocitrate dehydrogenase 1
IDH2	Isocitrate dehydrogenase 2
IHC	Immunohistochemistry
IQR	Interquartile range
Ki-67 index	Marker of proliferation Kiel 67
M	Month
MET	Methionine
MEYS	Ministry of education, youth and spo
MGMT	O^6-methylguanine-DNA methyl-transferase
miRNA	Micro-ribonucleic acid
MMCI	Masaryk Memorial Cancer Institute
Mon	Monday
MR	Magnetic resonance
MRI	Magnetic resonance imaging
OP	Overall survival
PD	Progressive disease
PDL1	Programmed Cell Death Ligand 1
PET/CT	Positron emission tomography/computer tomography
PFS	Progression-free survival
PoF	Patterns of failure
PPS	Per Protocol Set
preRT	Preoparative radiotherapy
PS	Performance status
QLQ	Quality of Life Questionnaire
RANO	Response Assessment in Neuro-Oncology
REP	Rapid early progression
RT	Radiotherapy
SUVavg	Average value of standardized uptake value
SUVmax	Maximum value of standardized uptake value
SD	Standard deviation
T/B ratio	Tumour to background ratio
TERT	Telomerase reverse transcriptase
TMZ	Temozolomide
ULN	Upper limit of normal
WHO	World Health Organization

Acknowledgements

Not applicable

Authors' contributions (31b)

RL: design of the study, protocol writing, first author, principal investigator, statistical analysis; ML: design of the study, protocol writing and revision; LS: design of the study, protocol writing and revision; LH: design of the study, protocol writing, design of the radiology part; KP: design of the study, protocol writing, design of the radiology part; JV: design of the study, protocol writing and revision, design of the imaging and PET part; RD: design of the study, protocol writing; AP: protocol revision, design of the laboratory analytic part; JS: design of the study, design of the neurosurgery part; TP: design of the study, design of the laboratory analytical part; MS: JS: design of the study, design of the neurosurgery part; PF: design of the study, protocol writing and revision, design of the neurosurgery part; RJ: design of the study, protocol writing and revision; IS: design of the study, protocol writing, revision, statistical part; RB: design of the study, protocol writing, revision, design of the radiology part; PS: design of the study, protocol writing, revision, design of the radiology part; PP: design of the study, protocol writing, design of the imiging part. TK: design of the study, protocol writing, corresponding author.

Funding (31c)

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The study received partial funding from Czech Health Research Council within Czech Ministry of Health (reg. number NU20-03–00148). This is one-stage public tender in research, experimental development and innovation for special purpose support in the Applied Health Research Support. We were successful in the 2019 competition and received funding of cca €400,000 for the period 1.5.2020–31.12.2023. This support was used mostly to cover cost of 11C-methionin examination and personal costs. Other costs (patient insurance, open access publication fees, etc.) were covered by the hospital's own money dedicated for research funding (Masaryk Memorial Cancer Institute). Some activities connected with study preparation are created in collaboration with MED MUNI through the CZECRIN project (LM2023049), supported by the national budget through MEYS and from the European Regional Development Fund—project CZECRIN_4 PATIENTS (CZ.02.1.01/0.0/0.0/16_013/0001826). Projects submitted to public tender under Czech Health Research Council within Czech Ministry of Health undergo 4-stepts peer review and the standard process of project scientific evaluation.

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This protocol and the template informed consent form were reviewed and approved by the Ethical Committee of the Masaryk Memorial Cancer Institute (in September 2020) with respect to the scientific content and compliance with applicable research and human subject regulations. Study protocol was evaluated by institutional Project Steering Committee (institutional review board) and institutional Ethics Committee No. 2020/1206/MOU. Moreover, peer review was also conducted by State Institute for Drug Control before the start of patients enrollment.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Wen PY, Weller M, Lee EQ, et al. Glioblastoma in adults: a Society for Neuro-Oncology (SNO) and European Society of Neuro-Oncology (EANO) consensus review on current management and future directions. Neuro Oncol. 2020;22(8):1073–113. https://doi.org/10.1093/neuonc/noaa106.
2. Weller M, van den Bent M, Preusser M, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood [published correction appears in Nat Rev Clin Oncol. 2022 May;19(5):357-358]. Nat Rev Clin Oncol. 2021;18(3):170–86. https://doi.org/10.1038/s41571-020-00447-z.
3. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352(10):987–96. https://doi.org/10.1056/NEJMoa043330.
4. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009;10(5):459–66. https://doi.org/10.1016/S1470-2045(09)70025-7.
5. Lakomy R, Kazda T, Selingerova I, et al. Real-world evidence in glioblastoma: stupp's regimen after a decade. Front Oncol. 2020;10:840. https://doi.org/10.3389/fonc.2020.00840. Published 2020 Jul 3.
6. Stupp R, Taillibert S, Kanner A, et al. Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. JAMA. 2017;318(23):2306–16. https://doi.org/10.1001/jama.2017.18718.
7. Kazda T, Dziacky A, Burkon P, et al. Radiotherapy of Glioblastoma 15 Years after the Landmark Stupp's Trial: more controversies than standards? Radiol Oncol. 2018;52(2):121–8. https://doi.org/10.2478/raon-2018-0023.
8. Perry JR, Laperriere N, O'Callaghan CJ, et al. Short-course radiation plus temozolomide in elderly patients with glioblastoma. N Engl J Med. 2017;376(11):1027–37. https://doi.org/10.1056/NEJMoa1611977.
9. Farace P, Amelio D, Ricciardi GK, et al. Early MRI changes in glioblastoma in the period between surgery and adjuvant therapy. J Neurooncol. 2013;111(2):177–85. https://doi.org/10.1007/s11060-012-0997-y.
10. Palmer JD, Bhamidipati D, Shukla G, et al. Rapid Early Tumor Progression is Prognostic in Glioblastoma Patients. Am J Clin Oncol. 2019;42(5):481–6. https://doi.org/10.1097/COC.0000000000000537.
11. Lakomy R, Kazda T, Selingerova I, et al. Pre-radiotherapy progression after surgery of newly diagnosed glioblastoma: corroboration of new prognostic variable. Diagnostics (Basel). 2020;10(9):676. https://doi.org/10.3390/diagnostics10090676. Published 2020 Sep 5.
12. Wee CW, Kim E, Kim TM, et al. Impact of interim progression during the surgery-to-radiotherapy interval and its predictors in glioblastoma treated with temozolomide-based radiochemotherapy. J Neurooncol. 2017;134(1):169–75. https://doi.org/10.1007/s11060-017-2505-x.
13. Merkel A, Soeldner D, Wendl C, et al. Early postoperative tumor progression predicts clinical outcome in glioblastoma-implication for clinical trials. J Neurooncol. 2017;132(2):249–54. https://doi.org/10.1007/s11060-016-2362-z.
14. Villanueva-Meyer JE, Han SJ, Cha S, Butowski NA. Early tumor growth between initial resection and radiotherapy of glioblastoma: incidence and impact on clinical outcomes. J Neurooncol. 2017;134(1):213–9. https://doi.org/10.1007/s11060-017-2511-z.
15. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. New Engl J Med. 2005;352:997–1003. https://doi.org/10.1056/NEJMoa043331.
16. Sana J, Hajduch M, Michalek J, et al. MicroRNAs and glioblastoma: Roles in core signalling pathways and potential clinical implications. J Cell Mol Med. 2011;15(8):1636–44. https://doi.org/10.1111/j.1582-4934.2011.01317.x.
17. Ondracek J, Fadrus P, Sana J, et al. Global microRNA expression profiling identifies unique microrna pattern of radioresistant glioblastoma cells. Anticancer Res. 2017;37(3):1099–104.
18. Kren L, Slaby O, Muckova K, et al. Expression of immune-modulatory molecules HLA-G and HLA-E by tumor cells in glioblastomas: an unexpected prognostic significance? Neuropathology. 2011;31(2):129–34. https://doi.org/10.1111/j.1440-1789.2010.01149.x.
19. Zhao H, Wang S, Song C, et al. The prognostic value of MGMT promoter status by pyrosequencing assay for glioblastoma patients' survival: a meta-analysis. World J Surg Oncol. 2016;14(1):261.
20. Karsy M, Neil JA, Guan J, et al. A practical review of prognostic correlations of molecular biomarkers in glioblastoma. Neurosurg Focus. 2015;38(3):E4. https://doi.org/10.3171/2015.1.FOCUS14755.
21. Piwecka M, Rolle K, Belter A, et al. Comprehensive analysis of microRNA expression profile in malignant glioma tissues. Mol Oncol. 2015;9(7):1324–40. https://doi.org/10.1016/j.molonc.2015.03.007.
22. Lakomy R, Sana J, Hankeova S, et al. MiR-195, miR-196b, miR-181c, miR-21 expression levels and O-6-methylguanine-DNA methyltransferase methylation status are associated with clinical outcome in glioblastoma patients. Cancer Sci. 2011;102(12):2186–90. https://doi.org/10.1111/j.1349-7006.2011.02092.x.
23. Niyazi M, Andratschke N, Bendszus M, et al. ESTRO-EANO guideline on target delineation and radiotherapy details for glioblastoma. Radiother Oncol. 2023;184:109663. https://doi.org/10.1016/j.radonc.2023.109663.
24. Galldiks N, Niyazi M, Grosu AL, et al. Contribution of PET imaging to radiotherapy planning and monitoring in glioma patients - a report of the PET/RANO group. Neuro Oncol. 2021;23(6):881–93. https://doi.org/10.1093/neuonc/noab013.
25. Albert NL, Weller M, Suchorska B, et al. Response Assessment in Neuro-Oncology working group and European Association for Neuro-Oncology recommendations for the clinical use of PET imaging in gliomas. Neuro Oncol. 2016;18(9):1199–208. https://doi.org/10.1093/neuonc/now058.
26. Heiss P, Mayer S, Herz M, et al. Investigation of transport mechanism and uptake kinetics of O-(2-[18F]fluoroethyl)-L-tyrosine in vitro and in vivo. J Nucl Med. 1999;40:1367–73 PMID: 10450690.
27. Okubo S, Zhen HN, Kawai N, et al. Correlation of l-methyl- 11 C-methionine (MET) uptake with l-type amino acid transporter 1 in human

gliomas. J Neurooncol. 2010;99(2):217–25. https://doi.org/10.1007/s11060-010-0117-9.
28. Nariai T, Tanaka Y, Wakimoto H, et al. Usefulness of L-[methyl- 11 C] methionine-positron emission tomography as a biological monitoring tool in the treatment of glioma. J Neurosurg. 2005;103(3):498–507.
29. Kracht LW, Friese M, Herholz K, et al. Methyl-[11C]- l-methionine uptake as measured by positron emission tomography correlates to microvessel density in patients with glioma. Eur J Nucl Med Mol Imaging. 2003;30(6):868–73. https://doi.org/10.1007/s00259-003-1148-7.
30. Herholz K, Holzer T, Bauer B, et al. 11C-methionine PET for differential diagnosis of low-grade gliomas. Neurology. 1998;50:1316–22. https://doi.org/10.1212/wnl.50.5.1316.
31. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. J Clin Oncol. 2010;28(11):1963–72. https://doi.org/10.1200/JCO.2009.26.3541.
32. Niyazi M, Brada M, Chalmers A.J, et al. ESTRO-ACROP guideline "target delineation of glioblastomas." Radiother Oncol. 2016;118(1):35–42. https://doi.org/10.1016/j.radonc.2015.12.003.
33. Scoccianti S, Detti B, Gadda D, et al. Organs at risk in the brain and their dose-constraints in adults and in children: a radiation oncologist's guide for delineation in everyday practice. Radiother Oncol. 2015;114(2):230–8. https://doi.org/10.1016/j.radonc.2015.01.016.

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7.10 Annex 10

Dolezalova I, Koritakova E, Souckova L, Chrastina J, Chladek J, Stepanova R and Brazdil M (2022) Prediction of Vagal Nerve Stimulation Efficacy in Drug-Resistant Epilepsy (PRECISE): Prospective Study for Pre-implantation Prediction/Study Design. Front. Neurol. 13:839163. doi: 10.3389/fneur.2022.839163

Summary
Purpose:
The PRECISE study aimed to develop and validate a multimodal predictive model for identifying patients with drug-resistant epilepsy who are most likely to benefit from vagal nerve stimulation (VNS). Despite the established efficacy of VNS, treatment response remains highly variable, and reliable pre-implantation predictors are lacking. The study addresses an important unmet clinical need by supporting more personalised patient selection and improving treatment decision-making.

Methodology:
The publication presents the protocol of a prospective, investigator-initiated, multicentre observational clinical study. Adult patients with drug-resistant epilepsy scheduled for VNS implantation undergo comprehensive preoperative assessment, including advanced magnetic resonance imaging (MRI), electroencephalography (EEG), neuropsychological evaluation, autonomic nervous system assessment, and clinical phenotyping. These multimodal data are integrated to identify biomarkers predictive of treatment response. The primary endpoint is seizure reduction following VNS implantation, with secondary outcomes including quality of life, cognitive function, and other clinically relevant measures.

Results:
As a study protocol, the publication establishes a rigorous methodological framework for the prospective identification and validation of predictive biomarkers for VNS efficacy. It describes standardised patient assessment, harmonised data collection, predefined clinical outcomes, and a multimodal analytical approach designed to integrate neuroimaging, electrophysiological, autonomic, and clinical data. The study exemplifies a precision medicine approach by aiming to individualise treatment selection based on patient-specific biological characteristics rather than relying solely on conventional clinical criteria.

Conclusion:
The PRECISE study provides a robust methodological framework for developing predictive biomarkers to optimise patient selection for vagal nerve stimulation in drug-resistant epilepsy. It illustrates how investigator-initiated clinical research can integrate advanced diagnostics, multimodal biomarkers, and personalised medicine approaches to improve clinical decision-making. The study further demonstrates the role of academic clinical research in translating innovative diagnostic strategies into evidence that supports more effective and individualised patient care.

frontiers in Neurology

STUDY PROTOCOL
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Prediction of Vagal Nerve Stimulation Efficacy in Drug-Resistant Epilepsy (PRECISE): Prospective Study for Pre-implantation Prediction/Study Design

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Background: Vagal nerve stimulation (VNS) can be indicated in patients with drug-resistant epilepsy, who are not eligible for resective epilepsy surgery. In VNS therapy, the responder rate (i.e., percentage of subjects experiencing ≥50% seizure reduction) is ~50%. At the moment, there is no widely-accepted possibility to predict VNS efficacy in a particular patient based on pre-implantation data, which can lead to unnecessary surgery and improper allocation of financial resources. The principal aim of PRediction of vagal nerve stimulation EfficaCy In drug-reSistant Epilepsy (PRECISE) study is to verify the predictability of VNS efficacy by analysis of pre-implantation routine electroencephalogram (EEG).

Methods: PRECISE is designed as a prospective multicentric study in which patients indicated to VNS therapy will be recruited. Patients will be classified as predicted responders vs. predicted non-responders using pre-implantation EEG analyses. After the first and second year of the study, the real-life outcome (responder vs. non-responder) will be determined. The real-life outcome and predicted outcome will be compared in terms of accuracy, specificity, and sensitivity. In the meantime, the patients will be managed according to the best clinical practice to obtain the best therapeutic response. The primary endpoint will be the accuracy of the statistical model for prediction of response to VNS therapy in terms of responders and non-responders. The secondary endpoint will be the quantification of differences in EEG power spectra (Relative Mean Power, %) between real-life responders and real-life non-responders to VNS therapy in drug-resistant epilepsy and the sensitivity and specificity of the model.

Discussion: PRECISE relies on the results of our previous work, through which we developed a statistical classifier for VNS response (responders vs. non-responders) based on differences in EEG power spectra dynamics (Pre-X-Stim).

Trial Registration: www.ClinicalTrials.gov, identifier: NCT04935567.

Keywords: drug-resistant epilepsy, vagal nerve stimulation, efficacy prediction, statistical model, study design

INTRODUCTION

Background and Rationale

The main goal of epilepsy treatment is long-term seizure cessation. In two-thirds of patients, seizure-freedom can be established with antiseizure medication (ASM) intake (1). Seizures persist despite ASM treatment in one-third of patients, and these are labeled as drug-resistant or refractory epilepsy. Resective surgery should be considered in all drug-resistant cases. If the patient is not eligible for resection, neurostimulation can be indicated as a therapeutic option.

Vagal nerve stimulation (VNS) is the most commonly used neurostimulation method in the treatment of patients with epilepsy. More than 100,000 patients have already been implanted world-wide. The therapy with VNS provides a high chance for a substantial seizure reduction. Approximately 50% of implanted patients are responders (seizure reduction ≥50%) after 1 year of VNS, and about 5 % of implanted patients reach complete seizure freedom. However, there is a group of implanted patients, non-responders (seizure reduction <50%), who do not significantly benefit from VNS (2–5).

Currently, we are not able to reliably predict the response to VNS based on patients' pre-implantation data. This prediction is crucial for proper selection of VNS candidates, and reduction of unnecessary surgery and costs associated with the implantation. Moreover, the ability to predict VNS efficacy seems to be essential for VNS use in resource-limited countries.

Our group developed and validated a statistical model (Pre-X-Stim) for the prediction of VNS efficacy (6). This algorithm is based on the analysis of routine scalp electroencephalogram (EEG) data, which is the main advantage of our approach because it is an inexpensive, easily-accessible method with minimal burden on the patient. When summarizing the procedure in brief, we retrospectively identified pre-implantation routine scalp EEG recordings in patients treated with VNS for drug-resistant epilepsy. The patients were characterized as responders or non-responders based on the long-term outcomes. The EEG recordings were segmented into several time intervals. In each interval, the changes of dynamics in EEG power spectra were calculated. Based on these changes, the statistical classifier for a prediction of VNS efficacy was developed (Patent No. EP 3437692). The validity of the model was verified on an independent data set, in which it was able to predict the response to VNS with 86% accuracy, 83% sensitivity, and 90% specificity. The usability of this model with different EEG systems was subsequently proven (7). Our previous studies were, however, retrospective and monocentric, and these are the crucial limitations that we would like to overcome with the proposed project, PRECISE (PRediction of vagal nerve stimulation EfficaCy In drug-reSistant Epilepsy).

Objectives

The study is designed to determine the availability of VNS prediction based on pre-implantation data. The main goal is to validate Pre-X-Stim in a real-life prospective study.

The primary aim of our study is to evaluate whether we are able to predict VNS efficacy in an individual patient based on the pre-implantation scalp EEG. We defined two hypotheses:

1. We hypothesize that there are differences in the dynamics of EEG power spectra in response to activation methods (eye-opening/closing, photic stimulation, hyperventilation) between responders and non-responders to VNS therapy.
2. We hypothesize that we can predict the patient's response to VNS therapy in terms of responder vs. non-responder by our statistical model based on the changes in the dynamics of EEG power spectra.

Based on these hypotheses the primary and secondary endpoints were established. Primary endpoint involves the accuracy of the statistical model for prediction of response to VNS therapy in terms of responders and non-responders in drug-resistant epilepsy, evaluated after 2 years. Secondary endpoints include (1) the quantification of differences in EEG power spectra (Relative Mean Power, %) between real-life responders and real-life non-responders to VNS therapy in drug-resistant epilepsy; (2) prediction of patients' response to VNS therapy in terms of responders (≥50% seizure reduction from baseline) and non-responders (<50% seizure reduction from baseline). Each endpoint will be evaluated 2 years after recruitment.

Trial Design

PRECISE is an investigator-initiated study. It is a prospective multicentric, multinational clinical study that will be started in January 2022. The patients with drug-resistant epilepsy will undergo standard VNS implantation based on a clinical decision. Before implantation, a scalp EEG, according to the study protocol, will be recorded. The EEG will be mathematically processed, and the patient's predicted outcome in terms of predicted responder vs. predicted non-responders will be statistically determined. The stimulation parameters and ASM therapy will be modified according to the best clinical practice. The real-life outcome will be measured by the percent of seizure reduction. Subsequently, the real-life and predicted outcomes will be compared in terms of accuracy, sensitivity, and specificity.

The proposed study is based on the results of our previous work, through which a statistical model was developed for the prediction of VNS efficacy based on a mathematical and statistical analysis of scalp EEG data (6).

METHODS AND ANALYSIS

Study Setting

The PRECISE study has not yet been recruited. The study is planned to be initiated in nine European countries (Czech Republic, France, Germany, UK, Spain, Austria, Portugal, Italy, and Belgium) in 2022.

Brno Epilepsy Center, the coordinating center for the study, is responsible for education and training of research staff, tracking participants' enrolment, mathematical analysis, including statistics of scalp EEG, and reporting of the study.

The other collaboration centers are responsible for patient selection according to eligibility criteria, adherence to all criteria of the study as described below, and adjustment of ASM and VNS therapy according to the best clinical practice.

Eligibility Criteria

Inclusion criteria include a diagnosis of drug-resistant epilepsy based on the definition of the International League against Epilepsy (8). The patient has to be indicated to VNS therapy based on a clinical decision.

All adult patients with drug-resistant epilepsy indicated to VNS will be screened in participating centers for inclusion criteria. Written informed consent for each study participant will be obtained prior to any data collection.

Inclusion criteria are as follows:

1. Drug-resistant epilepsy indicated to VNS based on clinical decision. Drug-resistant epilepsy is defined as a failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapy or in combination) to achieve sustained seizure freedom (8).
2. Age ≥18 years.
3. Availability to record 20 min EEG with photic stimulation, hyperventilation, and eye-opening/closing according to the pre-defined protocol.
4. Availability of seizure diaries at least 3 months before VNS implantation OR reliable information about seizure frequency at least 3 months before VNS implantation.
5. The ability of a patient/family member/caregiver to record seizures precisely into seizure diaries OR the ability of a patient/family member/caregiver to report seizures precisely in other ways.
6. In cases with very high seizure frequency (several seizures per day), it is acceptable to report only the days without any seizures.

Patients meeting any of the following criteria are excluded:

1. The indication and planning of resective brain surgery as a treatment option for drug-resistant epilepsy. If a patient clearly demonstrates refusal of resective surgery, the patient can be included in the study.
2. The presence of psychogenic non-epileptic seizures which cannot be reliably distinguished from epileptic seizures by a patient/family member/caregiver.
3. The presence of another condition that can resemble epileptic seizures and which cannot be reliably distinguished from epileptic seizures by a patient/family member/caregiver.
4. Metabolic condition or other diseases, in which an increase of seizure frequency can be expected.
5. The inability of a patient to make regular visits.
6. Life expectancy shorter than 2 years.

Who Will Take Informed Consent?

The study protocol including the informed consent will be reviewed and approved by the Institutional Review Board at each of the sites. A trained study investigator will describe the study to patients or to authorized surrogates if applicable. Patients will also receive information sheets. The study investigator will discuss the study with patients based on the information provided in the information sheets. The study investigator will obtain the written informed consent from patients willing to participate in the trial. In the case of patients who are unable to give consent because of a medical condition, their ability to participate in the study will be assessed by a medical council consisting of at least one independent physician, who is informed about the study details, and one study investigator.

Additional Consent Provisions for Collection and Use of Participant Data and Biological Specimens

A part of informed consent will be dedicated to reusing the data obtained in the proposed study to improve the proposed statistical model or the development of a novel predictive statistical model. The study conforms to the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Interventions

Explanation for the Choice of Comparators

Not applicable. The comparator is not used in this study.

Intervention Description

Assessment 0 includes screening for eligibility criteria and obtaining baseline data on subjects participating in the study. After signing the informed consent, the patient will be given a unique code with which all data will be labeled. The demographic information, health history, including psychiatric disease and type of epilepsy, concomitant medication (ASM and other drugs), and seizure frequency will be collected. The seizure frequency will be analyzed based on the patient diary. Standard EEG will be recorded according to the protocol described below. Patients will be asked to fill in a questionnaire focusing on the quality of life.

Protocol for EEG Recording

The EEG will be recorded on the EEG recording system with a sampling frequency of 250 Hz or higher, with electrodes placed on the scalp according to the 10–20 system (with the following

TABLE 1 | Predefined segments ordering in the course of EEG recording.

Segment 1	Rest 1	duration at least 2 min
Segment 2	Eyes opening/closing	duration at least 10 s
Segment 3	Rest 2	duration at least 10 s; immediately after eye closure
Segment 4	Photic stimulation	stimulation frequencies: 5 Hz (10 s)—10 Hz (10 s)—15 Hz (10 s)—20 Hz (10 s)—25 Hz (10 s)—30 Hz (10 s)—25 Hz (5 s)—20 Hz (5 s)—15 Hz (5 s)—10 Hz (5 s)—5 Hz (5 s); light intensity at least 0.7 Joule
Segment 5	Hyperventilation	duration at least 4 min (2 min hyperventilation by nose + 2 min hyperventilation by mouth)
Segment 6	Eyes opening/closing	duration at least 10 s
Segment 7	Rest 3	duration at least 10 s, immediately after eye closure
Segment 8	Rest 4	duration at least 2 min

electrodes: Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, and O2). Different electrode names are allowed, but their position must correspond with the position of the previously defined electrodes. Additional electrodes are also allowed, but will not be evaluated within the study. EEGs with fewer or differently-positioned electrodes are not allowed.

The EEG is recorded in a supine position with eyes closed, except for periods with opening of eyes. The recorded EEG will have a duration of 20 min or more and must contain defined segments in the following order as in **Table 1**.

The recorded EEG will be anonymized and labeled by a unique code previously attributed to a patient. In the next step, the EEG will be sent for mathematical/statistical processing. Based on this processing, the patients' predicted response to VNS will be determined in terms of predicted responders (seizure reduction ≥50%) vs. predicted non-responders (seizure reduction <50%). Neither the patient nor the physician will be informed about the classification results.

Two assessments are designed for patients' follow-up:

Assessment 1—1 year after stimulation initialization, and

Assessment 2—2 years after stimulation initialization.

The patients will be evaluated 1 and 2 years after the start of VNS therapy. The patient will be requested to report any changes in health history, including psychiatric disease, and specific requests will be focused on changes in medication (ASM and other drugs), VNS parameters settings, and seizures. The patients will be asked whether unexpected events influenced their clinical response. If these unexpected events are regarded as "clinically highly important" (such as non-compliance or other newly-diagnosed disease worsening epilepsy), the patient can be excluded based on clinical decision. The data on seizure frequency will be collected by reviewing the patient's diary. The reported seizure frequency will be compared with pre-implantation seizure frequency, and real-life response will be calculated in terms of real-life responders (seizure reduction ≥50%) or real-life non-responder (seizure reduction <50%). The patients will fill in a questionnaire focusing on the quality of life. If the patient does not tolerate at least the minimal VNS settings (0.75 mA) because of serious adverse events, they will be excluded from further analysis (the patient's data will be reported separately). The patients included in our study will be managed according to the best clinical praxis, i.e., the change of ASM and VNS parameters will be done based on the clinicians' decision to reflect the real-life response.

The information collected in Assessments 1 and 2, including real-life response to VNS, will be labeled with the patient's unique code and sent to the coordinating center, where it will be compared with the patient's predicted response.

At the end of Assessment 2, patients will be asked if they are interested in the predicted response to VNS. If the answer is yes, the study physician will contact the coordinating center, where the predicted response for a given patient will be conveyed. The patient will be informed about the predicted response by telephone or during a regular visit (not a part of the study protocol, described below).

Criteria for Discontinuing or Modifying Allocated Interventions

If a patient does not tolerate at least the minimal VNS setting (0.75 mA) because of serious adverse events, the patient will be excluded from further analysis (the patient's data will be reported separately).

Strategies to Improve Adherence to Interventions

Training before initialization will ensure the fidelity during the whole study. If there are doubts, the coordinating center will solve them.

Relevant Concomitant Care Permitted or Prohibited During the Trial

Regular visits—not part of the study protocol.

There are regular visits between Assessments 0, 1, and 2. The regular visits are not part of the study protocol. The physician will adjust the VNS parameters and change concomitant ASM according to the best clinical practice to obtain the best response with minimal side-effects. The regular visits will be scheduled according to the individual habits of cooperating centers. The change of ASM (types and dosing) is allowed within the whole study.

Outcomes

The primary outcome of the study is the accuracy of the statistical model for prediction of response to VNS therapy in drug-resistant epilepsy, expressed as a percentage of correct predictions (responders vs. non-responders), evaluated after 2 years.

The secondary outcomes of the study are the quantification of differences in EEG power spectra (Relative Mean Power, %) between real-life responders and real-life non-responders to VNS therapy in drug-resistant epilepsy and the prediction of patients' response to VNS therapy in terms of responders, expressed as sensitivity of the model, and non-responders expressed as specificity of the model. If possible, these data can be used for further improvement of the statistic model.

TABLE 2 | Participant timeline.

	Assessment 0	Assessment 1	Assessment 2
	Day 0	Year 1	Year 2
Screening—inclusion/exclusion criteria	X		
ICF	X		
Demographic information	X	X	X
Health history	X	X	X
Concomitant medication	X	X	X
Seizure frequency	X	X	X
EEG	X		

Between assessment 0 and Assessment 1, VNS will be performed, but it is not a part of this study.

The study is concerned with the verification of the real-life validity of our statistical classifier for the prediction of VNS response based on pre-implantation EEG data. The patients will be classified based on their predicted response as predicted responders (≥50% seizure reduction) and predicted non-responders (<50% seizure reduction). In Assessments 1 and 2, respectively, the patients' real-life response will be determined: real-life responders (≥50% seizure reduction) vs. real-life non-responder (<50% seizure-reduction). The predicted and real-life responses will be statistically compared in terms of accuracy, sensitivity, and specificity.

The individual patients' groups (predicted responders vs. predicted non-responders, real-life responders vs. real-life non-responders) will be compared based on their demographic data, health history, ASM, and VNS parameters. A suitable statistical test will be chosen concerning the characteristics of the data (Pearson's Chi-squared test for categorical variables, and Wilcoxon-Mann-Whitney test for continuous variables).

Participant Timeline

The schedule of enrolment, intervention and visits is summarized in **Table 2**. The study is focused on patients indicated to VNS therapy as their standard for clinical care and is concerned with the prediction of VNS efficacy based on pre-implantation EEG. The duration of the study participation in a given patient is 2 years.

Sample Size

The sample size was estimated for the primary endpoint, which was defined as the accuracy of the statistical model for prediction of response to VNS therapy in terms of responders and non-responders in drug-resistant epilepsy. During the verification of the validity on the independent dataset, the model was able to predict the response to VNS with 86% accuracy. The minimal clinically significant accuracy was set to 70%. The sample size is estimated for the following hypothesis:

Hypothesis H0: $p = 0.7$ vs. Hypothesis H1: $p\text{-}value > 0.7$.

At the level of significance $\alpha = 0.05$ and with a power of 90%, the number of patients needed for the analysis of the primary endpoint is 57. Assuming a drop-out rate of 50%, a total number of 114 patients should be enrolled.

Recruitment

Recruitment of the patients is planned from January 2022. During the first 2 years, the total planned number of 140 patients will be recruited. The patients will be recruited within cooperating epilepsy centers. The recruitment limited to cooperating centers will ensure the correct indication for VNS therapy and the adjustment of VNS parameters according to the best medical practice, which are not part of the study protocol itself, but are crucial for the study results.

The VNS will be indicated in a particular patient based on a clinical decision as a standard of the patient's therapy (i.e., the indication of VNS is not a part of the proposed project). The patient will be informed about the study on a pre-implantation visit or by a telephone call, during which initial eligibility for the study will be determined, and the study protocol and design will be explained by the research staff.

Assignment of Interventions: Allocation

Sequence Generation

Not applicable. This study is not randomized.

Concealment Mechanism

Not applicable. This study is not randomized.

Implementation

The study investigator enrolls the participants. The other collaboration centers are responsible for patients' selection according to eligibility criteria, adherence to all criteria of study as described below, and adjustment of ASM and VNS therapy according to the best clinical practice, and enrolling the participants.

Assignment of Interventions: Blinding

Who Will Be Blinded?

Not applicable. This study is not blinded.

Procedure for Unblinding If Needed

Not applicable. This study is not blinded.

Data Collection and Management

Plans for Assessment and Collection of Outcomes

The study will collect demographic and baseline characteristics from medical records and electronic medical records, including age, sex, type of admission, and baseline characteristics such as body weight, body height, patient history, and pharmacological history. Results of EEG tests and neurological assessment will be documented in the electronic medical records and entered by the trial investigator in the electronic case report form (eCRF).

Plans to Promote Participant Retention and Complete Follow-Up

The study site will make every reasonable effort to follow the participant for the entire study period. Study site staff members will be responsible for developing and implementing local standard operating procedures to achieve maximal follow-up.

Data Management
The study data will be entered online in an Internet-based database (RedCap®) and collected from medical records. The study staff members will have access to the medical records of the patients. Investigators will be responsible for screening the patients, obtaining informed consent, collecting study data, and entering the data in the eCRF. A statistician will analyse the study data in cooperation with a principal investigator. The data will be stored for 15 years after completion of the study and then destroyed. To promote data quality, the eCRFs of each participant will be reviewed by another member of the study team as monitor.

Confidentiality
All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with limited access. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to additional identifying information will be stored in a separate locked file in an area with limited access.

Plans for Collection, Laboratory Evaluation, and Storage of Biological Specimens for Genetic or Molecular Analysis in This Trial/Future Use
Not applicable. In this study, no biological specimens for genetic or molecular analysis will be collected.

Statistical Methods
Statistical Methods for Primary and Secondary Outcomes
Subjects will be classified using logistic regression or another classifier (such as support vector machines or linear discriminant analysis) as predicted responders or non-responders. The predicted outcomes will be compared with real-life outcomes to determine the accuracy, sensitivity, and specificity of our statistical model. The accuracy will be calculated as the proportion of correctly predicted responders and correctly predicted non-responders within the whole analyzed population. The minimum clinically significant accuracy was set to 70%. The sensitivity of the model will be expressed as the proportion of predicted responders within all real-life responders. The specificity of the model will be expressed as the proportion of predicted non-responders within all real-life non-responders.

Differences in the EEG power spectra between real-world/predicted responders vs. non-responders will be examined using two-sample t-test or Mann-Whitney test, as applicable.

As a level of statistical significance, $\alpha = 0.05$ will be used for all statistical tests.

Interim Analyses
Not applicable. Interim analysis is not planned.

Methods for Additional Analyses (e.g. Subgroup Analyses)
Not applicable. Additional analysis is not planned.

Methods in Analysis to Handle Protocol Non-adherence and Any Statistical Methods to Handle Missing Data
No analysis populations are defined for this study. Protocol non-adherence will be assessed by the principal investigator case by case. Patients with major protocol deviations will be excluded from the analysis.

Missing data are not planned to be imputed. However, in the event of substantial missing data for any parameter, a sensitivity analysis using any method of imputation could also be used.

Plans to Give Access to the Full Protocol, Participant Level-Data, and Statistical Code
Data sharing statement: No later than three years after the collection of the second year after the last patient's last visit, we will deliver a completely anonymized data set to an appropriate data archive for sharing purposes. Statistical codes will be archived following GCP and SOPs.

Oversight and Monitoring
Composition of the Coordinating Center and Trial Steering Committee
Not applicable. No coordinating center or a trial steering committee will be established.

Composition of the Data Monitoring Committee, Its Role and Reporting Structure
Not applicable. No data monitoring committee will be established. The study interventions as VNS or EEG are parts of the standard care of patients with drug-resistant epilepsy. The studied tool is a statistical mathematical model predicting the responsiveness to VNS.

Adverse Event Reporting
Serious adverse events have been reported according to the standard rules and standard operating procedures of the coordinating unit. All study participants will be monitored for potential adverse events. We do not expect any adverse event to be associated with the Pre-X-Stim because it is a statistical mathematical model evaluated as a tool able to predict the response to VNS.

Frequency and Plans for Auditing Trial Conduct
Trial conduct will be audited by the Institutional Ethics Committee every year during the study. The process of auditing will be independent of the investigator and sponsor.

DISCUSSION
In the population of patients with drug-resistant epilepsy, VNS represents a well-established treatment option to standard ASM and resective surgery. ASM fails to abolish seizures in one-third of patients (1). Resective surgery represents a gold standard for the treatment of these patients with a high probability of complete seizure remission (9). However, there is a large group of patients who are not eligible for resective surgery because of several reasons, namely, our inability to form a rational hypothesis on the

precise localization of the epileptogenic zone even with the use of invasive EEG recording, multifocal epilepsy, severe intellectual disability, and personal reasons. In all these cases, VNS can represent a method of choice with a relatively low risk of surgical and post-surgical complications (10).

We have relatively precise knowledge about VNS efficacy in drug-resistant epilepsy based on our experience with a large group of already implanted patients (2–5, 11). An individual patient has a chance of about 50% to become a responder (≥50% seizure reduction) in the first year, and this chance increases to ~60% in the fifth year. The VNS has proved its superiority with respect to seizure-reduction and quality of life, even in a prospective randomized controlled trial (12).

It is widely known that better response to VNS is present in children, in patients with generalized or post-traumatic epilepsy or patients with tuberous sclerosis. However, we are not able to predict the efficacy of an individual patient based on his/her pre-implantation data. In this situation, it is clear that some patients, family members, caregivers, and physicians do not have confidence in this treatment option. It seems crucial to reliably identify responders to VNS based on their clinical data pre-operatively, to minimize unnecessary surgery and additional costs.

At the moment, four studies have focussed on the prediction of VNS efficacy based on pre-implantation data (see below). Remarkably, all works share a similar theoretical concept, specifically the functional mechanism of VNS action. The mechanism of VNS action, despite not being fully understood, relies on the afferent projections of the vagal nerve to the nucleus tractus solitarius (NTS). NTS has a dense connection with other brainstem structures, thalamus, and limbic structures, and just the modulation of thalamocortical circuits is suspected to be responsible for VNS action (13–16).

Our group developed the statistical classifier based on standard EEG records. Moreover, we managed to prove its validity on an independent patient data set. On the other hand, the most important limitation associated with our previous results is the retrospective and monocentric nature of our study. We aim to overcome these limitations through the proposed project PRECISE.

PRECISE will be a prospective multicentric study that will be conducted in co-operation with several well-established and experienced epilepsy centers in Europe. These centers were chosen to be able to correctly identify suitable candidates for VNS therapy, adjust ASM or VNS parameters to obtain the best response, and minimalize side-effects. We decided on this "best clinical practice" model because this approach reflects the regular daily practice that is essential for further applicability of our statistical classifier. This classifier is based on analyzing routine scalp EEG, which we supposed to be its main advantage. The scalp EEG is a painless, non-distressing method that can be recorded everywhere with minimal costs for purchasing the device. Above all, routine EEG recording is an obligatory investigation performed in all patients suffering from drug-resistant epilepsy. Thus, the design of our study is uncomplicated, which is essential for patient recruitment and study fidelity. The patients have to make regular visits for VNS and ASM adjustments. We added three additional assesments—one before and two after stimulation initialization (year one and two). During these visits, the primary clinical information and seizure frequency will be evaluated. The most problematic and discussable part of our protocol is the evaluation of seizure frequency. This activity is standardly done by a review of patients' seizure diaries, which is the practice that is currently used in all clinical studies determining the capability of therapeutic intervention to reduce seizure frequency. We are very much aware that the information in seizure diaries could be influenced both unconsciously (i.e., postictal amnesia) and consciously (i.e., rental tendencies). However, there is currently no available possibility to determine the precise seizure frequency over the course of 2 years. We included only adult patients (i.e. older than 18 years). If we prove the utility of our statistical classifier in this population, we would like to conduct a similar study for children as well, because they represent a population with a high benefit from correctly indicated VNS and form approximately one-third of all implanted patients.

In the text: We also see the limitations of our study. We primarily focused on seizure frequency and seizure reduction. On the other side, it would be helpful to analyse the impact of VNS on other aspects of patients' life, i.e., mood, cognition, and memory. However, these analyses require more time for both physicians and patients. We tried to keep our study as simple as possible because we needed to include a relatively large proportion of those implanted to reach the results in a realistic period of 2–3 years. This limitation is conditioned by the fact that each center implants only a few (5–10) adult patients per year, which was the information obtained in our survey among European epilepsy centers.

As mentioned above, three other studies predicted VNS efficacy on pre-implantation data with similar accuracy (17–19). Ibrahim et al. (17) published a prediction model based on the analysis of resting-state functional magnetic resonance imaging (fMRI) in a group of 21 children with drug-resistant epilepsy. They demonstrated that thalamocortical connectivity to the anterior cingulate and insular cortices is stronger in responders than in non-responders (17). The second study published by Babajani-Feremi et al. (18) predicts VNS efficacy by analysis of resting-state magnetoencephalography (MEG). The authors calculated three global graph measures on recorded resting-state MEG, which include modularity, transitivity, and characteristic path length, in which they found statistically significant differences between responders and non-responders (18). The third study by Mithani et al. (19) proved the differences in white matter microstructures in diffusion tensor imaging (DTI) between responders and non-responders. These differences were subsequently used for the construction of a classifier for VNS response prediction (19).

To conclude, there are several studies published recently that promise to predict VNS response based on available

electrophysiological and neuroimaging data. We formulated the statistical model working as a classifier for VNS prediction in retrospective data sets. To determine the validity of this classifier, it needs to be evaluated in a prospective multicentric study. We believe this approach has the potential to significantly improve clinical management of drug-resistant epilepsy patients.

ETHICS AND DISSEMINATION

Ethics Approval and Consent to Participate

This protocol and the template of informed consent form were reviewed and approved by the Ethical Committee of the University Hospital St. Anne's Brno, with respect to scientific content and compliance with applicable research and human subject regulations. Informed consent will be obtained from all participants prior to enrolment in the study.

Plans for Communicating Important Protocol Amendments to Relevant Parties (e.g., Trial Participants, Ethical Committees)

Any modification of the protocol that may affect the conduct of the study, the potential benefits to the patient, or patient safety, including changes in study objectives, study design, patient population, sample sizes, study procedures, and significant administrative aspects, will require a formal amendment of the protocol. Any amendment will be agreed on and approved by the ethics committee before implementation, and the health authorities will be notified, in accordance with local regulations. The protocol amendment will be sent to all members of the study team by e-mail. All attending investigators and study nurses of the study units will be acquainted with the amendment at a meeting. The invitation to the meeting will be sent by e-mail. The protocol of the study and its amendments will be available at each study unit.

Dissemination Plans

We intend to submit the results of the study to be published in a peer-reviewed international medical journal.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethic Committee St. Anne's University Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ID and LS: design of the study, protocol preparation, and manuscript preparation. EK and RS: statistical analysis. JChr: design of the study and protocol. JChl: mathematical analysis. MB: conceived the study and led the proposal and protocol development. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Kwan P, Brodie MJ. Early identification of refractory epilepsy. *N Engl J Med.* (2000) 342:314–9. doi: 10.1056/NEJM200002033420503
2. Kuba R, Brazdil M, Kalina M, Prochazka T, Hovorka J, Nezadal T. Vagus nerve stimulation: longitudinal follow-up of patients treated for 5 years. *Seizure.* (2009) 18:269–74. doi: 10.1016/j.seizure.2008.10.012
3. Englot DJ, Chang EF, Auguste KI. Vagus nerve stimulation for epilepsy: a meta-analysis of efficacy and predictors of response. *J Neurosurg.* (2011) 115:1248–55. doi: 10.3171/2011.7.JNS11977
4. Englot DJ, Rolston JD, Wang DD, Hassnain KH, Gordon CM, Chang EF. Efficacy of vagus nerve stimulation in posttraumatic versus nontraumatic epilepsy. *J Neurosurg.* (2012) 117:970–7. doi: 10.3171/2012.8.JNS122
5. Englot DJ, Rolston JD, Wright CW, Hassnain KH, Chang EF. Rates and predictors of seizure freedom with vagus nerve stimulation for intractable epilepsy. *Neurosurgery.* (2016) 79:345–53. doi: 10.1227/NEU.0000000000001165
6. Brázdil M, Doležalová I, Koritáková E, Chládek J, Roman R, Pail M. EEG reactivity predicts individual efficacy of vagal nerve stimulation in intractable epileptics. *Front Neurol.* (2019) 10:392. doi: 10.3389/fneur.2019.00392
7. Koritakova E, Dolezalova I, Chladek J, Jurkova T, Chrastina J, Plesinger F. A novel statistical model for predicting the efficacy of vagal nerve stimulation in patients with epilepsy (Pre-X-Stim) is applicable to different EEG systems. *Front Neurosci.* (2021) 15:635787. doi: 10.3389/fnins.2021.635787
8. Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Hauser WA, Mathern G. Definition of drug resistant epilepsy: consensus proposal by the *ad hoc* Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia.* (2010) 51:1069–77. doi: 10.1111/j.1528-1167.2009.02397.x
9. Jobst BC, Cascino GD. Resective epilepsy surgery for drug-resistant focal epilepsy: a review. *JAMA.* (2015) 313:285–93. doi: 10.1001/jama.2014.17426
10. Giordano F, Zicca A, Barba C, Guerrini R, Genitori L. Vagus nerve stimulation: surgical technique of implantation and revision and related morbidity. *Epilepsia.* (2017) 58(Suppl. 1):85–90. doi: 10.1111/epi.13678
11. Chrastina J, Novak Z, Zeman T, Kocvarova J, Pail M, Dolezalova I. Single-center long-term results of vagus nerve stimulation for epilepsy: a 10-17 year follow-up study. *Seizure.* (2018) 59:41–7. doi: 10.1016/j.seizure.2018.04.022
12. Ryvlin P, Gilliam FG, Nguyen DK, Colicchio G, Iudice A, Tinuper P. The long-term effect of vagus nerve stimulation on quality of life in patients with pharmacoresistant focal epilepsy: the PuLsE (Open Prospective Randomized Long-term Effectiveness) trial. *Epilepsia.* (2014) 55:893–900. doi: 10.1111/epi.12611
13. Beckstead RM, Morse JR, Norgren R. The nucleus of the solitary tract in the monkey: projections to the thalamus and brain stem nuclei. *J Comp Neurol.* (1980) 190:259–82. doi: 10.1002/cne.901900205
14. Rutecki P. Anatomical, physiological, and theoretical basis for the antiepileptic effect of vagus nerve stimulation. *Epilepsia.* (1990) 31(Suppl. 2):S1–6. doi: 10.1111/j.1528-1157.1990.tb05843.x

15. Vonck K, De Herdt V, Bosman T, Dedeurwaerdere S, Van Laere K, Boon P, et al. Thalamic and limbic involvement in the mechanism of action of vagus nerve stimulation, a SPECT study. *Seizure.* (2008) 17:699–706. doi: 10.1016/j.seizure.2008.05.001
16. Hachem LD, Wong SM, Ibrahim GM. The vagus afferent network: emerging role in translational connectomics. *Neurosurgical Focus.* (2018) 45:E2. doi: 10.3171/2018.6.FOCUS18216
17. Ibrahim GM, Sharma P, Hyslop A, Guillen MR, Morgan BR, Wong S. Presurgical thalamocortical connectivity is associated with response to vagus nerve stimulation in children with intractable epilepsy. *Neuroimage Clin.* (2017) 16:634–42. doi: 10.1016/j.nicl.2017.09.015
18. Babajani-Feremi A, Noorizadeh N, Mudigoudar B, Wheless JW. Predicting seizure outcome of vagus nerve stimulation using MEG-based network topology. *Neuroimage Clinl.* (2018) 19:990–9. doi: 10.1016/j.nicl.2018.06.017
19. Mithani K, Mikhail M, Morgan BR, Wong S, Weil AG, Deschenes S. Connectomic profiling identifies responders to vagus nerve stimulation. *Ann Neurol.* (2019) 86:743–53. doi: 10.1002/ana.25574

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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7.11 Annex 11

Hlavackova E, Pilatova K, Cerna D, Selingerova I, Mudry P, Mazanek P, Fedorova L, Merhautova J, Jureckova L, Semerad L, Pacasova R, Flajsarova L, Souckova L, Demlova R, Sterba J, Valik D and Zdrazilova-Dubska L (2019) Dendritic Cell-Based Immunotherapy in Advanced Sarcoma and Neuroblastoma Pediatric Patients: Anti-cancer Treatment Preceding Monocyte Harvest Impairs the Immunostimulatory and Antigen-Presenting Behavior of DCs and Manufacturing Process Outcome. Front. Oncol. 9:1034. doi: 10.3389/fonc.2019.01034

Summary

Purpose:

The study aimed to evaluate the impact of anti-cancer treatment administered prior to monocyte collection on the manufacturing process and functional quality of dendritic cell (DC)-based vaccines in paediatric patients with advanced sarcoma and neuroblastoma. The research addressed an important translational challenge in cell-based immunotherapy by investigating whether previous chemotherapy influences the feasibility and immunological potency of autologous dendritic cell products.

Methodology:

This investigator-initiated translational study analysed monocytes collected from paediatric patients enrolled in a dendritic cell immunotherapy programme. The manufacturing process of dendritic cell vaccines was evaluated according to Good Manufacturing Practice (GMP) standards, while functional analyses assessed dendritic cell maturation, antigen-presenting capacity, cytokine production, and T-cell stimulatory potential. The effects of different anti-cancer treatment regimens on manufacturing success and biological function were compared with those of healthy donor-derived dendritic cells.

Results:

The study demonstrated that anti-cancer therapy administered before leukapheresis significantly impaired both the manufacturing process and the biological properties of dendritic cells. Monocyte yield and differentiation capacity were reduced, resulting in lower vaccine manufacturing success and diminished antigen-presenting and immunostimulatory functions. These findings indicate that previous chemotherapy may compromise the quality and therapeutic potential of autologous dendritic cell vaccines. The study also identified important considerations for optimising the timing of cell collection within ATMP development and clinical trial protocols.

Conclusion:

This study provides important translational evidence supporting the optimisation of manufacturing strategies for dendritic cell-based advanced therapy medicinal products (ATMPs). It demonstrates how investigator-initiated translational research can bridge laboratory findings and clinical application by identifying critical factors influencing cell therapy manufacturing and product quality. The results contributed to improving the design of subsequent clinical studies and manufacturing processes for personalised dendritic cell immunotherapy in paediatric oncology, representing an important step towards the successful clinical implementation of ATMPs.

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ORIGINAL RESEARCH
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Dendritic Cell-Based Immunotherapy in Advanced Sarcoma and Neuroblastoma Pediatric Patients: Anti-cancer Treatment Preceding Monocyte Harvest Impairs the Immunostimulatory and Antigen-Presenting Behavior of DCs and Manufacturing Process Outcome

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Despite efforts to develop novel treatment strategies, refractory and relapsing sarcoma, and high-risk neuroblastoma continue to have poor prognoses and limited overall survival. Monocyte-derived dendritic cell (DC)-based anti-cancer immunotherapy represents a promising treatment modality in these neoplasias. A DC-based anti-cancer vaccine was evaluated for safety in an academic phase-I/II clinical trial for children, adolescents, and young adults with progressive, recurrent, or primarily metastatic high-risk tumors, mainly sarcomas and neuroblastomas. The DC vaccine was loaded with self-tumor antigens obtained from patient tumor tissue. DC vaccine quality was assessed in terms of DC yield, viability, immunophenotype, production of IL-12 and IL-10, and stimulation of allogenic donor T-cells and autologous T-cells in allo-MLR and auto-MLR, respectively. Here, we show that the outcome of the manufacture of DC-based vaccine is highly variable in terms of both DC yield and DC immunostimulatory properties. In 30% of cases, manufacturing resulted in a product that failed to meet medicinal product specifications and therefore was not released for administration to a patient. Focusing on the isolation of monocytes and the pharmacotherapy preceding monocyte harvest, we show that isolation of monocytes by elutriation is not superior to adherence on plastic in terms of DC yield, viability, or immunostimulatory capacity. Trial patients having undergone monocyte-interfering pharmacotherapy prior to monocyte harvest was associated with an impaired DC-based immunotherapy product outcome.

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Certain combinations of anti-cancer treatment resulted in a similar pattern of inadequate DC parameters, namely, a combination of temozolomide with irinotecan was associated with DCs showing poor maturation and decreased immunostimulatory features, and a combination of pazopanib, topotecan, and MTD-based cyclophosphamide was associated with poor monocyte differentiation and decreased DC immunostimulatory parameters. Searching for a surrogate marker predicting an adverse outcome of DC manufacture in the peripheral blood complete blood count prior to monocyte harvest, we observed an association between an increased number of immature granulocytes in peripheral blood and decreased potency of the DC-based product as quantified by allo-MLR. We conclude that the DC-manufacturing yield and the immunostimulatory quality of anti-cancer DC-based vaccines generated from the monocytes of patients were not influenced by the monocyte isolation modality but were detrimentally affected by the specific combination of anti-cancer agents used prior to monocyte harvest.

Keywords: dendritic cells, anti-cancer medications, sarcoma, neuroblastoma, cell-based medicinal products, investigator-initiated clinical trial, manufacturing outcome variability

INTRODUCTION

Several progressive and relapsing malignancies in pediatric patients have dismal life prognosis. Refractory neuroblastoma and refractory or metastatic sarcoma have an especially poor prognosis, with no consistently curative treatments available. Oberlin et al. (1) published a meta-analysis of North American and European studies on primary metastatic sarcomas and well-defined risk factors that—where two or more are present at presentation—distribute patients into a subgroup with only a 14% event-free and overall survival probability at 3 years from diagnosis. Patients over 10 years of age with limb primary or "other site" primary tumors with the alveolar subtype of rhabdomyosarcoma, bone marrow or bone involvements, and more than three metastatic sites are defined as having markers for a worse prognosis (1). Similar results were published in a study of relapsed rhabdomyosarsomas, with the prognosis for survival being < 10% at 5 years (2). In high-risk neuroblastoma, survival after relapse is poor, and the usual life expectancy is < 6 months. Based on our experience, patients with neuroblastomas with a high MIBG score after induction therapy have very poor 2-year survival (3). High-risk rhabdomyosarcomas are treated according to several globally accepted protocols with a combination of chemotherapy, surgery, and radiotherapy. Chemotherapy regimens consist of the alkylating agent ifosfamide or cyclophosphamide and vinca alkaloids combined with either etoposide or doxorubicin and actinomycin D. The cytotoxic chemotherapy regimens for relapsed and refractory neuroblastoma typically use a combination of camptothecins, topotecan, and irinotecan with agents such as cyclophosphamide and temozolomide, and achieve objective tumor responses but poor long-term outcomes. For such poor-prognosis patients, treatments with innovative and metronomic therapies (e.g., COMBAT, METRO) (4, 5), cell-based immunotherapies (6, 7), and novel molecularly targeted agents (8) are justified and are also effective in many cases, although their long-term effect has yet to be demonstrated.

DCs are essential antigen-presenting cells for the initiation, maintenance, and regulation of immune response (9). Active cancer immunotherapy directs the immune system to attack tumor cells by targeting tumor-associated antigens. We manufacture a fully personalized monocyte-derived dendritic cell-based vaccine that was evaluated in the investigator-initiated clinical trial "Combined antitumor therapy with *ex vivo* manipulated dendritic cells producing interleukin-12 in children, adolescents, and young adults with progressive, recurrent, or primarily metastatic high-risk tumors" (EudraCT number 2014-003388-39). The primary endpoint of the trial was an assessment of safety by analysis of the frequency of occurrence of AESI (adverse events of special interest). Vaccines that meet quality control (QC) requirements are registered for use and applied intradermally every 2–4 weeks for up to 35 doses.

Dendritic cell-based medical products are mostly manufactured through derivation from monocytes. Autologous monocytes are readily accessible and can be obtained from peripheral blood in sufficient amounts to prepare 10^7-10^8 DCs. Monocytes arise from hematological precursors in bone marrow, with a maturation time of 50–60 h (10), and enter the bloodstream for several days until their recruitment into tissues, where they possess the property to mature into tissue macrophages (11). Specifically, the classical CD14++ CD16− subpopulation representing 80–95% of circulating monocytes has a 1-day lifespan in circulation, the intermediate CD14+ CD16+ subpopulation (2–8% of circulating monocytes) has a 4-day lifespan, and the non-classical CD14+ CD16++ subpopulation (2–11% of circulating monocytes) has a 7-day lifespan in circulation (12–14). Monocyte count and function are influenced by various anti-cancer agents. Nevertheless, the published data on the impact of particular anti-cancer agents on the development and function of monocytes are scarce in comparison with those on hematologic toxicity

toward neutrophils and lymphocytes. As most anti-cancer agents target DNA, they interfere with dividing cells including hematopoetic cells. Also, tyrosine kinase inhibitors (regorafenib, sunitinib, sorafenib) are associated with adverse events including hematological toxicities (15). Regorafenib hematological toxicity has been explained by the TK inhibition of FMS like tyrosine kinase 3 (FLT-3) and stem cell factor (c-KIT ligand), which represent hematopoietic growth receptors (15, 16). Reduction in the circulating monocyte count after sunitinib has been shown (17). Monocytes are also highly sensitive to the methylating agent temozolomide (TMZ) (18, 19). Cisplatin and carboplatin have been shown to alter monocyte differentiation to favor the generation of IL-10-producing M2 macrophages (20).

Various chemotherapeutics affect cell differentiation and the antigen presentation of DCs when treated *in vitro* during the differentiation process (21). Data are lacking on the potential *in vivo* impact of hematotoxic agents on the properties of medicinal products from monocyte-derived DCs. During the manufacture of DC-based anti-cancer immunotherapy under stringent GMP-compliant conditions, we experienced highly variable final product parameters in terms of both DC yield and immunostimulatory properties, and we hypothesized that hematotoxic anti-cancer therapy preceding monocyte harvest may influence the quality of DC-based medicinal products. The issue of the effect of pharmacotherapy on the quality of human monocyte-derived DCs cannot be reliably assessed in mimicked conditions by *in vitro* pretreatment of monocytes by anti-cancer agents. Thus, data addressing this issue can only be gathered retrospectively from real-life clinical conditions, such as our clinical trial, though with a limited number of patients included. Here, the Phase-I/II clinical trial protocol designed for heavily pre-treated cancer patients with heterogenic anti-cancer therapeutic protocols allows us to observe and analyze the effect of pharmacotherapy on the quality and presumably also on the anti-cancer action of *ex vivo*-manufactured DCs.

Therefore, our primary aims were to analyze the impact of (i) cytotoxic and targeted anti-cancer therapy preceding monocyte harvest and (ii) variability in the complete blood count on the quality of DC-based anti-cancer immunotherapy in high-risk sarcoma and neuroblastoma patients, representing the two main diagnoses in the DC clinical trial. A secondary aim was to reveal whether monocyte isolation by elutriation is superior to the isolation of monocytes through their adherence to plastic cultivation flasks.

METHODS

Patients and Clinical Trial

Clinical Trial Eligibility and Allowed Medication

Patient eligibility/inclusion criteria for the clinical trial included being 1–25 years old male/female with histologically confirmed refractory, relapsing, or primarily metastatic high-risk tumors and having a performance status (Karnofsky or Lansky score) $\geq$ 50 and a life expectancy of longer than 10 weeks. Patients had to be clinically eligible for the surgical procedure to harvest tumor tissue for histological verification and tumor antigen extraction. Female patients had to have had a negative pregnancy test. All patients had to have adequate bone marrow, kidney, liver, and heart function, defined as absolute neutrophil count (ANC) $\geq$ 0.75 $\times$ 10^9/L, thrombocytes $\geq$ 75 $\times$ 10^9/L, hemoglobin 80 g/L, estimated glomerular filtration rate (eGFR) $\geq$ 70 mL/min/1.73 m^2, serum creatinine $\leq$ 1.5-fold the upper limit for the appropriate age, bilirubin $\leq$ 1.5-fold the upper limit for the appropriate age, AST and ALT $\leq$ 2.5-fold the upper limit for the appropriate age, ejection fraction $\geq$ 50%, and fractional shortening $\geq$ 27% as assessed by echocardiography. In the case of bone marrow infiltration, the allowable ANC was $\geq$ 0.5 $\times$ 10^9/L and blood platelets 40 $\times$ 10^9/L. In case of liver metastases, AST and ALT had to be $\leq$ 5-fold the upper limit for the appropriate age. The exclusion criteria were as follows: seropositivity to HIV1,2, *Treponema pallidum*, hepatitis B or C, known hypersensitivity to the study medication, autoimmune disease that was not adequately treated, uncontrolled psychiatric disease, or uncontrolled hypertension defined as systolic and diastolic blood pressure over the 95th percentile for the appropriate age and height (patients $\leq$ 17 years old) or $\geq$ 160/90 mmHg or diastolic blood pressure $\geq$ 90 mmHg (patients $\geq$ 17 years old). Patients previously treated with dendritic cells or participating in another clinical trial during the 30 days before enrollment were not eligible to enter this clinical trial.

The allowed medication prior to monocyte harvest (leukapheresis) was as follows: metronomic chemotherapy, immune checkpoint inhibitors, and anti-CD20 antibodies were allowed as concomitant medication for any time before leukapheresis. Monoclonal antibodies (except anti-CD20), high-dose chemotherapy, and high-dose corticoids had to have been withdrawn at least 3 weeks prior to leukapheresis with the exception of corticoid treatment of brain edema, which was allowed. Since November 2017, an amendment has been made to the procedure for monocyte harvest, and tyrosine kinase inhibitors have to be withdrawn according to their half-life: drugs with a short half-life of 3–14 h must be withdrawn at least 2 days before leukapheresis (axitinib, dabrafenib, dasatinib, ibrutinib, idelalisib, nintedanib, ruxolitinib, and trametinib), drugs with a medium half-life of 15–35 h at least 7 days before leukapheresis (alectinib, bosutinib, lapatinib, lenvatinib, nilotinib, osimertinib, pazopanib, ponatinib, regorafenib, and non-TKI everolimus), and drugs with a long half-life of 36–60 h at least 12 days before leukapheresis (afatinib, ceritinib, erlotinib, gefitinib, imatinib, cabozantinib, crizotinib, sorafenib, sunitinib, vemurafenib, and non-TKI temsirolimus). Myelopoietic growth factors have to be withdrawn at least 7 days before leukapheresis/monocyte harvest.

Evaluation of Preceding and Concomitant Therapy

A precise analysis was performed of preceding and/or concomitant therapy 60 days before monocyte harvest for clinical trial subjects with neuroblastoma and sarcoma diagnoses. Data were mined from the clinical trial electronic case report form and the subjects' medical records. We particularly focused on therapeutic agents with a potential impact on the generation of DCs from monocytes and on DC immunostimulatory properties. These agents and the reports on their role in monocyte biology are summarized in **Supplementary Table 1**.

DC Manufacture and Quality Control

Dendritic cell vaccine manufacture encompassed two phases—(i) preparation of tumor lysate as a source of the patient's tumor antigens and (ii) preparation of monocyte-derived DCs and their loading with tumor lysate. Quality control tests evaluated safety (negativity for pathogens), identity (cell immunophenotype), viability, and functions (cytokine production, stimulation of T-cells). The flow and decision tree of the manufacturing process is shown in **Supplementary Figure 1**.

Self-Tumor Antigen Extraction

Tumor lysate was prepared from the tumor tissue obtained from the patient during curative surgery or extended biopsy. In Clean Rooms, necrotic areas and connective tissue were removed from the tumor tissue with a surgical scalpel, keeping the specimen immersed in buffered solution. The remaining tissue was sliced into fragments of about 0.5 mm with a scalpel and forceps and then further crushed with the back of a syringe. Each suspension of tumor fragments and cells in HBSS was lysed through repeated (5 times) freezing in liquid nitrogen and thawing at 37°C. The crude tumor lysate was centrifuged at 450 g/7 min/4°C to remove particulate components. The tumor lysate was released for DC manufacture if the following criteria were met: (i) presence of viable tumor cells reported by a histopathologist, (ii) protein concentration, and (iii) microbiological sterility.

Peripheral Mononuclear Cell Collection

Monocytes were harvested as part of the mononuclear white blood cell (WBC) fraction. Mononuclear cells were collected from the peripheral blood of the patient using the Terumo BCT Spectra Optia Apheresis System. For collection, we used either an intermittent or continuous leukapheresis system. Due to its superior collection efficacy and easier procedure settings, we have preferred the continuous leukapheresis system since April 2018. A citrate dextrose solution, solution A (ACD-A), was used as an anticoagulant. In patients with a body weight of < 20 kg, anticoagulation with heparin was used to prevent citrate toxicity. The requirement for the minimal WBC count was 3×10^9/L before the initiation of leukapheresis. To prevent risk of bleeding or ischemic complications during and after the procedure, hemoglobin of at least 80 g/L and platelets of at least 30×10^9/L were required. In case of a patient with a body weight of < 20 kg, the leukapheresis set was pre-filled with donor erythrocytes. The aim of the leukapheresis was to obtain 60–80 mL of concentrate of mononuclear cells with a content of at least 0.5×10^9 monocytes. Subsequent addition of 5% human albumin to the minimum required volume of 80 mL for further processing was allowed.

DC Manufacture in Clean Rooms

The numbers of WBCs, B-cells and T-cells, monocytes, and granulocytes in the leukapheretic product were evaluated using a hematology analyzer (XT-4000i, Sysmex) and flow cytometer (FC-500, Beckman Coulter) with staining for CD3 (clone UCHT1, Beckman Coulter) and CD19 (clone J3-119, Beckman Coulter). Monocytes for DC manufacture were separated from the leukapheresis product by either elutriation or adherence to a plastic surface. During elutriation (using an Elutra cell separator, Gambro BCT), blood cells were separated on the basis of sedimentation velocity into six fractions, where the last fraction rich in monocytes was used for DC manufacture. Contaminating cells after elutriation were mainly granulocytes with similar sedimentation velocity to monocytes. Five hundred million monocytes adhered for 2–4 h in three 175-cm^2 tissue culture flasks with 35 mL of CellGenix® GMP DC Medium at 37°C/5% CO_2 and were then washed with HBSS and processed further. Monocytes seeded from the elutriation product or attached by plastic adherence were then cultivated in three 175-cm^2 tissue culture flasks with 70 mL of CellGenix® GMP DC medium supplemented with GM-CSF (1000 U/mL, CellGenix®) and IL-4 (320 U/mL, CellGenix®) at 37°C/5% CO_2/6 days. On day 3, a fresh 70 mL of medium supplemented with the same concentration of GM-CSF and IL-4 was added to the culture. On day 6, immature DCs were exposed to autologous tumor lysate antigens (10 μg/mL) with added keyhole limpet haemocyanin (KLH, 1 μg/mL), IL-4 (320 U/mL), and GM-CSF (1000 U/mL) at 37°C/5% CO_2/for 1.5–2 h. Maturation was induced by lipopolysaccharide (200 U/mL) and interferon-γ (50 ng/mL) for an additional 6 h at 37°C/5% CO_2. Finally, cells were collected using accutase (Accutase®, Corning), counted in a Bürker cell chamber and frozen in aliquots of 2×10^6 DCs in 100 μL of freezing medium CryoStor® CS2 at -80°C. All doses of the DC-based investigational medical product (IMP) named "MyDendrix®" were stored at -150°C until administration to the patient.

Quality Control of DC-Based Investigational Medicinal Product

DC characteristics were evaluated as a part of the quality control process of IMP from an aliquot of manufactured DC from each batch. The cryotube with DC was removed from a deep freezing box (-150°C) into a laminar flow box, quickly and gently thawed in hand while avoiding shaking, 1 mL of cold (2–8°C) DC medium (CellGenix® GMP-grade) was slowly added to the thawed DCs, and the DC suspension was transferred into 2 mL of cold DC medium. The DC suspension was handled at room temperature and processed immediately. DCs (8×10^5 cells) were seeded into 1 well of a 6-well culture plate for sensitive adherent cells (Sarstedt, TC Plate 6-well, Cell+, growth area 8.87 cm^2) and cultured in 3 mL of DC medium for 2 days (37°C/5% CO_2) to obtain (i) medium containing cytokines produced by DCs during cultivation and (ii) mature DCs for phenotypic evaluation after 2 days of post-thaw cultivation. A 0.5 mL volume of medium containing DC-produced cytokines was collected after 23–25 h upon DC seeding and was centrifuged (10 min/410 g/4°C), and the supernatant was stored at -25°C for no longer than 30 days prior to analysis. For immunophenotypic evaluation of mature DCs, both detached and adherent DCs were harvested 47–49 h after DC seeding. The culture medium was collected and pooled with DCs harvested by accutase (0.5 mL/well 8.87 cm^2/37°C) and centrifuged (5 min/410 g/20°C). The pellet was resuspended in 800 μL HBSS with 0.25% human albumin (Grifols) and processed immediately for immunophenotypic evaluation. Viability quantification was

performed by propidium iodide (PI) exclusion assay. Briefly, 10^5 DCs were stained with 10 µL of 1% PI in HBSS followed by immediate flow cytometric (Cytomics FC500) analysis of PI-positive events (= non-viable cells). The immunophenotype of DCs was evaluated in post-thaw DCs and in post-cultivation mature DCs. For the detection of each surface molecule, 0.5 × 10^5 DCs were incubated for 20 min in the dark with the following antibodies: CD80-PC7 (clone MAB104, 10 µL), CD83-FITC (clone HB15e, 10 µL), CD86-PE (clone HA5.2B7, 10 µL), CD197-PE (clone G043H7, 10 µL), HLA-DR-PC5 (clone Immu357, 10 µL), CD14-PE (clone RMO52, 10 µL), or isotype controls IgG-PC5 (clone 679.1Mc7, 10 µL), IgG-PC7 (clone 679.1Mc7, 10 µL), IgG2a-FITC (clone 7T4-1F5, 10 µL), or IgG2a-PE (7T4-1F5, 10 µL), all from Beckman Coulter. Flow cytometric analysis was performed using a Cytomics FC500 with CXP software by manual gating on individual parameters, and the discrimination by appropriate isotype control was used to gate and quantify positive events. The concentrations of IL-12 and IL-10 in the DC culture medium were measured by flow cytometric bead assay (BD Biosciences) using internal quality controls (Quantikine® Immunoassay Control Group 1, R&D Systems). Absolute production of IL-12 or IL-10 per 10^6 DC and the IL-12/IL-10 ratio were calculated. The allogenic (allo) and autologous (auto) stimulatory properties of DCs were examined by mixed lymphocyte reaction (MLR). In allo-MLR, the target cells were the peripheral blood mononuclear cells (PBMCs) obtained from pooled buffy coats from healthy donors. In auto-MLR, the target cells were the patient's lymphocytes separated by centrifugation in a density gradient using Histopaque-1077 (SigmaAldrich, density 1,077 g/mL) from the leukapheresis product obtained for DC manufacture. These pre-vaccination lymphocytes were cryopreserved using CryoStor CS5 medium (BioLife solutions) at -150°C and thawed prior to auto-MLR seeding. A sample of 10^7 target lymphocytes were stained with 250 µL 10 µM carboxyfluorescein succidimidyl ester (CFSE, SigmaAldrich) and seeded into a sterile 96-well culture plate (Sarstedt, TC Plate 96-well, Suspension, F) at 10^5 cells/well in 200 µL of complete X-vivo 10 medium (Lonza) containing 5% inactivated human male AB serum (SigmaAldrich) for the following: (i) 10^4 DC/well in 10:1 target:effector MLR, (ii) positive control (PC) with phytohemagglutinin (PHA, SigmaAldrich) at a final concentration of 10 µg/mL, or (iii) negative control (NC) with complete X-vivo medium only. MLR experiments were seeded in triplicate and cultured for 6 days at 37°C/5% CO_2. 2 × 10^4 cells from each well were stained with CD3-PC7 (clone UCHT1, 10 µL/test, Beckmann Coulter) for flow cytometric detection of CFSE fluorescence on CD3+ T cells. Discrimination for dividing cells was set up using NC. T-cell proliferation was calculated as follows: [(average % of dividing T-cells in 10:1 MLR) − (average % of dividing T-cells in NC)] × 100/[(average % of dividing T-cells in PC) − (average % of dividing T-cells in NC)].

Statistical Analysis

The Spearman correlation coefficient with a significance test was used to measure the strength of the relationship between patient CBC prior to leukapheresis, the parameters of the leukapheresis product, the DC yield, and the quality control parameters. Differences in parameter values between groups were assessed by the non-parametric Mann-Whitney or Kruskal-Wallis test. Hierarchical clustering analyses were performed using the complete linkage method with the distance based on the Spearman correlation coefficient. The Spearman correlation distance was used for clustering of batches, and the absolute Spearman correlation distance was used for clustering DC parameters. For clustering analyses, DC parameters were centered and scaled (Z-score of parameters). $P < 0.05$ were considered statistically significant. All statistical analyses were performed with R 3.5.3 software (22).

RESULTS

Clinical Trial Accrual and Course

As of May 2019, 47 subjects were enrolled in the clinical trial, and the manufacturing process of DC-based vaccine was performed in 31 cases. Of these 31, the most common diagnoses were sarcoma, with 19 cases (61%), and high-risk neuroblastoma, with 4 cases (**Table 1**). In this group of 23 patients, we performed analysis of the manufacturing issues presented here. Sarcomas were specifically: seven Ewing sarcomas (36% of sarcoma pts), five (26%) osteosarcoma, two (11%) alveolar rhabdomyosarcoma, two (11%) embryonal rhabdomyosarcoma, and three (16%) synovial sarcoma (**Table 1**). The median enrollment age of the clinical trial was 14 years; 15 years for sarcoma patients and 5 years for neuroblastoma patients (**Table 1**). All 23 study subjects, i.e., 19 with sarcoma and four with neuroblastoma, underwent initial surgery to obtain tumor tissue for the tumor lysate-manufacturing process, and tumor lysates were manufactured without any tumor antigen extraction failure. Monocyte harvest and the subsequent manufacturing of DC-based IMP were performed for all 23 subjects. Out of the 23, 16 DC-based IMPs successfully passed through the manufacturing process and met the quality control criteria for administration to the patients. DC-based IMPs from seven subjects (six sarcoma, one neuroblastoma) were not manufactured or failed to pass quality control due to inadequate immunostimulatory properties (**Table 1**). The basic patient characteristics are described in **Table 1**, and the detailed clinical course is summarized in **Supplementary Table 2**.

Dendritic Cell Manufacturing, Its Yield, and DC Quality Including Immunostimulatory Properties

We achieved DC yields ranging from 0 to 43.6%, with a mean of 17.2% and an s.d. of 12.7% in this specific cohort. A DC yield equal to 0 represented a manufacturing process that was unsuccessful, with all DCs detached from the flasks. The quality control parameters involved microbial sterility and *Mycoplasma* spp. negativity, the viability and phenotype of thawed DCs, the phenotype of thawed DCs after 2-day cultivation, the production of IL-12 and IL-10 during 24-h cultivation of thawed DCs, and 6-day allo-MLR and auto-MLR. All batches of DCs fulfilled the microbiological criteria of QC and the criteria

TABLE 1 | DC-based vaccine-manufacturing outcome, basic patient characteristics, therapy preceding monocyte harvest.

Primary diagnosis	Date of study enrollment/Age in years at study enrollment/Pt No	Treatment line prior to monocyte harvest/Treatment and its duration/Date of monocyte harvest	DC-based vaccine-manufacturing outcome
EWING SARCOMA			
Ewing sarcoma of the mandible	09/2015; 14; KDO-0101	2nd; VCR/Irino + pazopanib, 09/2015–04/2016; 01/2016	Passed QC
Localized Ewing sarcoma of the left femur	02/2016; 12; KDO-0109	3rd; ARST08P1 + sunitinib, 03/2016–06/2016; 03/2016	Did not pass QC
Localized Ewing sarcoma of the left distal humerus	02/2016; 12; KDO-0111	2nd; AEWS1031 + pazopanib, 02/2016–08/2016; 05/2016	Did not pass QC
Localized Ewing sarcoma of the spine C5-Th2, extradural, and intraspinal involvement	08/2016; 24; KDO-0118	2nd; AEWS1031, 08/2016–02/2017, 2 cycles VTC, 2 cycles VCR/Irino; 01/2017	Passed QC
Ewing sarcoma of the pelvis	12/2016; 14; KDO-0121	1st; Euro Ewing 2008, 11/2016–05/2017; 06/2017	Did not pass QC
Ewing sarcoma of the left proximal tibia	12/2016; 15; KDO-0122	2nd; VTC cycles, 01/2017–05/2017; 03/2017	Did not pass QC
Localized Ewing sarcoma of the left tibia	08/2018; 22; KDO-0144	2nd; 2x TMZ/Irino, 08/2018–10/2018; 10/2018	Did not pass QC
OSTEOSARCOMA			
Localized high-grade osteosarcoma of the right distal femur	09/2015; 10; KDO-0102	4th; VCR/Irino + pazopanib; 12/2015	Passed QC
High grade osteoblastic osteosarcoma of the left distal femur	10/2016; 8; KDO-0120	1st; AOST 0331, 10/2016–07/2017; 03/2017	Not manufactured
Localized osteoblastic osteosarcoma of the right proximal tibia	01/2017; 18; KDO-0124	3rd; AOST 1321 + VBL + CPM, 02/2017–10/2017; 3/2017	Passed QC
Localized osteosarcoma of the right proximal femur	02/2018; 25; KDO-0133	2nd; COMBAT III, 04/2018–12/2018; 04/2018	Passed QC
High-grade osteoblastic osteosarcoma of the left distal femur	05/2018; 22; KDO-0139	2nd; AOST0331 – cycle IE 07/2018; 09/2018	Passed QC
ALVEOLAR RHABDOMYOSARCOMA			
Alveolar rhabdomyosarcoma of the right calf	10/2015; 14; KDO-0103	2nd; ARST 0921 + TEM, 11/2015–01/2016; 12/2015	Passed QC
Alveolar rhabomyosarcoma, primum ignotum	10/2016; 12; KDO-0119	1st; ARST08P1 + TEM, 10/2016–05/2018; 04/2017	Passed QC
EMBRYONAL RHABDOMYOSARCOMA			
Embryonal rhabomyosarcoma of the pelvis	09/2017; 18; KDO-0131	1st; EpSSG RMS 2005, 09/2017–06/2018; 01/2017	Passed QC
Localized embryonal rhabomyosarcoma of the pelvis	07/2018; 15; KDO-0143	3rd; - rEECur - Topo/CYC, 08/2018–12/2018; 09/2018	Passed QC

(Continued)

TABLE 1 | Continued

Primary diagnosis	Date of study enrollment/Age in years at study enrollment/Pt No	Treatment line prior to monocyte harvest/Treatment and its duration/Date of monocyte harvest	DC-based vaccine-manufacturing outcome
SYNOVIALOSARCOMA			
Synovial sarcoma of the left thigh	04/2016; 14; KDO-0114	1st followed by COMBAT II 05/2015–12/2016; 12/2016	Passed QC
Localized synovial sarcoma of the neck	04/2018; 17; KDO-0137	2nd; Modified COMBAT III from 04/2018 + pazopanib from 08/2018; 06/2018	Passed QC
Localized synovial sarcoma of the left calf	06/2018; 21; KDO-0141	2nd; COMBAT III modified, 08/2018–02/2019; 10/2018	Passed QC
NEUROBLASTOMA			
Neuroblastoma in the retroperitoneum	04/2016; 12; KDO-0115	2nd; METRO-NB2012, 05/2016–10/2016; 07/2016	Passed QC
High-risk neuroblastoma in the left glandula suprarenalis	02/2018; 4; KDO-0135	1st followed by dinutuximab + retinoic acid, 11/2018–02/2019; 02/2019	Passed QC
Neuroblastoma in the right retroperitoneum	07/2018; 3; KDO-0142	2nd; ANBL 1221 - 3 cycles TMZ/Irino + dinutuximab, 08/2018–11/2018; 08/2018	Did not pass QC
Neuroblastoma in the right glandula suprarenalis	10/2018; 6; KDO-0147	4th; METRO-NB2012, 05/2017–12/2018; 11/2018	Passed QC

CPM, cyclophosphamide; Irino, irinotecan; TEM, temsirolimus; TMZ, temozolomide; Topo, topotecan; VBL, vinblastine; VCR, vincristine; IE, ifosfamide etoposid; VTC, vincristine, topotecan, cyclophosphamide; Pt. No., patient number; QC, quality control. Chemotherapy protocols: AEWS1031 (Ewing sarcoma)—vincristine, doxorubicin, cyclophosphamide, ifosfamide, etoposide; AOST0331 (osteosarcoma)—cisplatin, doxorubicine, methotrexate; AOST1321 (osteosarcoma)—danosumab; ARST0921 (refractory or relapsed rhabdomyosarcoma)—bevacizumab, vinorelbine, cyclophosphamide and temsirolimus; ARST1321 (non-rhabdomyosarcoma soft tissue sarcomas)—ifosfamide, doxorubicin, pazopanib; COMBAT II (metronomic)—celecoxib, etoposide, temozolomide, fenofibrate, ergocalciferol, bevacizumab, vinorelbine, cis-retinoic acid; EpSSG RMS 2005 (rhabdomyosarcoma)—ifosfamide, vincristine, actinomycin, doxorubicin; Euro Ewing (Ewing sarcoma)—vincristine, ifosfamide, doxorubicin, etoposide, actinomycin, cyclophosphamide; METRO-NBL2012 (metronomic treatment for neuroblastoma)—etoposide, celecoxib, propranolol, cyclophosphamide, vinblastine; rEECur protocol (relapsed soft tissue sarcoma)—topotecan, cyclophosphamide, irinotecan, temozolomide. Details on anti-cancer therapy dosing are summarized in **Supplementary Table 2**.

of viability, ranging from 85 to 100% with a mean of 95%. Their variability in phenotype and immunostimulatory property is shown in **Supplementary Table 3**. The mean phenotype of the manufactured DCs immediately after thawing for selected parameters was as follows: CD8019 (range: 2–86%), CD86 91% (76–100%), CD83 21% (0–86%), CD14 20% (1–69%), and CD197 90% (73–99%). The mean phenotype of thawed DCs after 2-day cultivation for selected parameters was as follows: CD80 77% (range: 25–97%), CD86 99% (95–100%), CD83 61% (12–89%), and MHC II 93% (63–100%). Mean cytokine production was as follows: IL-12 8,327 pg/10^6 DC (range: 9–80,824 pg/10^6 DC), IL-10 280 pg/10^6 DC (6–1,731 pg/10^6 DC), and IL-12/IL-10 ratio 35 (1–246). The mean *in vitro* proliferation of T-cells stimulated by manufactured DCs was 67% (29–98%) in allo-MLR and 9% (−3–37%) in auto-MLR. Due to inappropriate results for the immunostimulatory parameters of QC (phenotype, cytokine production, MLR), six out of 22 (27%) of the manufactured batches of DCs were not released for use in the clinical trial. The parameter values of the manufactured batches of DCs are shown in **Supplementary Table 3**.

Isolation of Monocytes by Adherence vs. Elutriation and Its Impact on Manufacturing Process Yield and the Immunostimulatory Parameters of DCs

Isolation of monocytes for DC manufacture was performed by elutriation in 14 cases and by plastic adherence in nine (39%) cases based on the real-world situation. Until March 2017, we performed elutriation of the leukapheresis product in all cases (11 cases: KDO-0101, -0102, -0103, -0109, -0111, -0114, -0115, -0118, -0120, -0122, -0124). Between April and September 2018, we performed elutriation in cases KDO-0121, -0137, and -0139, and adherence to plastic in cases KDO-0133, -0142, and -0144 due to there being > 10% neutrophils in the leukapheresis product or technical issues with the Elutra device for KDO-0119 and -0131. After October 2018, we isolated monocytes exclusively by adherence to the plastic surface in all cases: KDO-0135, -0141, -0144, and -0147.

Addressing the issue of whether the elutriation process is superior to adherence to plastic retrospectively, we compared the proportions of batches passing QC and their DC yield and phenotypic and immunostimulatory properties under the

two methods. Adherence to plastic resulted in two (22%) batches not being released, and elutriation resulted in five (36%) batches not being released (four did not pass QC and one was not manufactured). The OR (odds ratio) for passing QC in the plastic-adherence modality was 1.94 (95% CI: 0.29–13.19). The DC yield, viability, phenotype, and immunostimulatory properties (IL-12, IL-10, the IL-12/IL-10 ratio, allo-MLR, auto-MLR) in adherence to plastic vs. elutriation are summarized in **Figure 1**. A statistically significant difference was observed between QC results and monocyte isolation modality for the following post-thaw parameters (i) DC expression of CD86 on day 0 that was higher in the manufacturing process with plastic adherence, and (ii) borderline significant expression of CD14 on day 0 that was higher with elutriation. The values of both parameters were in favor of adherence to plastic. It is of note here that the subgroup with isolation of monocytes by the adherence to plastic was not biased by including a higher proportion of cases without potentially monocyte-interfering pharmacotherapy ("m" vs. "0" as described later; $p = 0.643$). Thus, we conclude that the isolation of monocytes by adherence to plastic is comparable to a manufacturing process with monocyte elutriation.

Parameters of CBC Prior to Monocyte Harvest, and Parameters of the Leukapheresis Product and Their Impact on Manufacturing Process Yield and the Immunostimulatory Properties of DCs

With the aim of identifying the CBC parameters (shown for each batch in **Supplementary Table 3**) associated with adequate DC characteristics and thus predicting whether the DC-manufacturing process would pass QC, we analyzed CBC prior to monocyte harvest in the context of batches that fail to pass QC and DC yield, phenotype, and immunostimulatory properties. The presence of immature granulocytes in CBC was associated with unsuccessful manufacturing ($p = 0.046$). DC yield was not associated with any single parameter of CBC. Expression of CD14 on manufactured cells was negatively correlated with relative lymphocyte count in CBC ($p = 0.001$) (**Figure 2**). The level of allogenic MLR was negatively associated with both the presence of immature granulocytes ($p = 0.010$) and NRBC ($p = 0.018$)

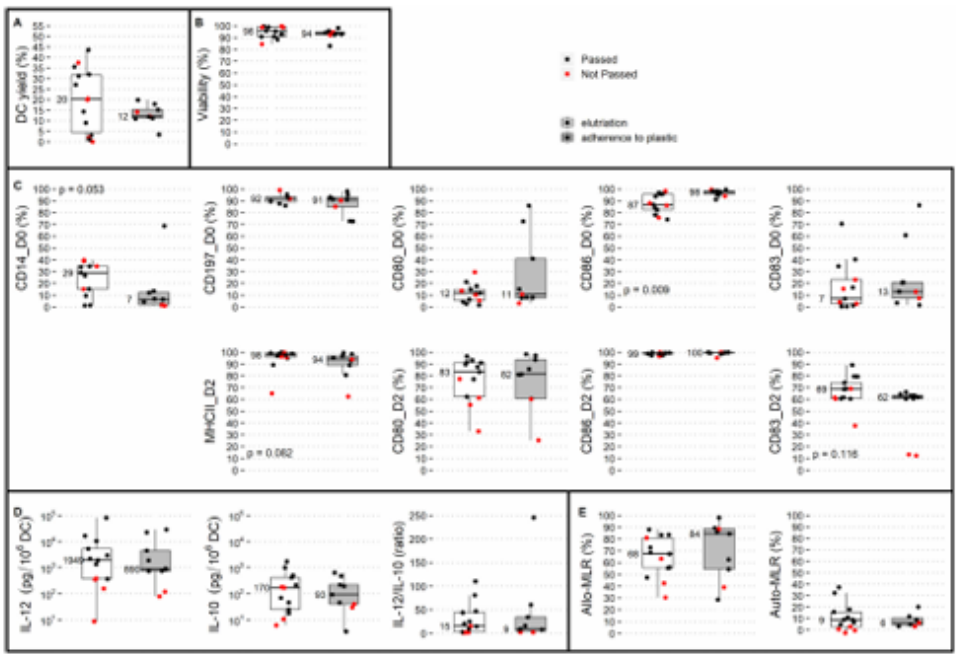


FIGURE 1 | Comparison of two monocyte isolation modalities with respect to dendritic cell (DC) production. Elutriation (white box plots) and adherence to plastic (gray box plots) were compared based on QC parameters: **(A)** DC yield, and post-thaw: **(B)** viability, **(C)** DC phenotype on day 0: CD14, CD197, CD80, CD86, and CD83 and on day 2: MHC II, CD80, CD86, and CD83, and immunostimulatory properties presented by **(D)** IL-12 production, IL-10 production, and IL-12/IL-10 production ratio, **(E)** allo-MLR and auto-MLR. Median values are shown for each parameter for each monocyte isolation modality. Black dots show QC results of manufactured DCs that passed quality control, and red dots show results of manufactured DCs that did not pass quality control.

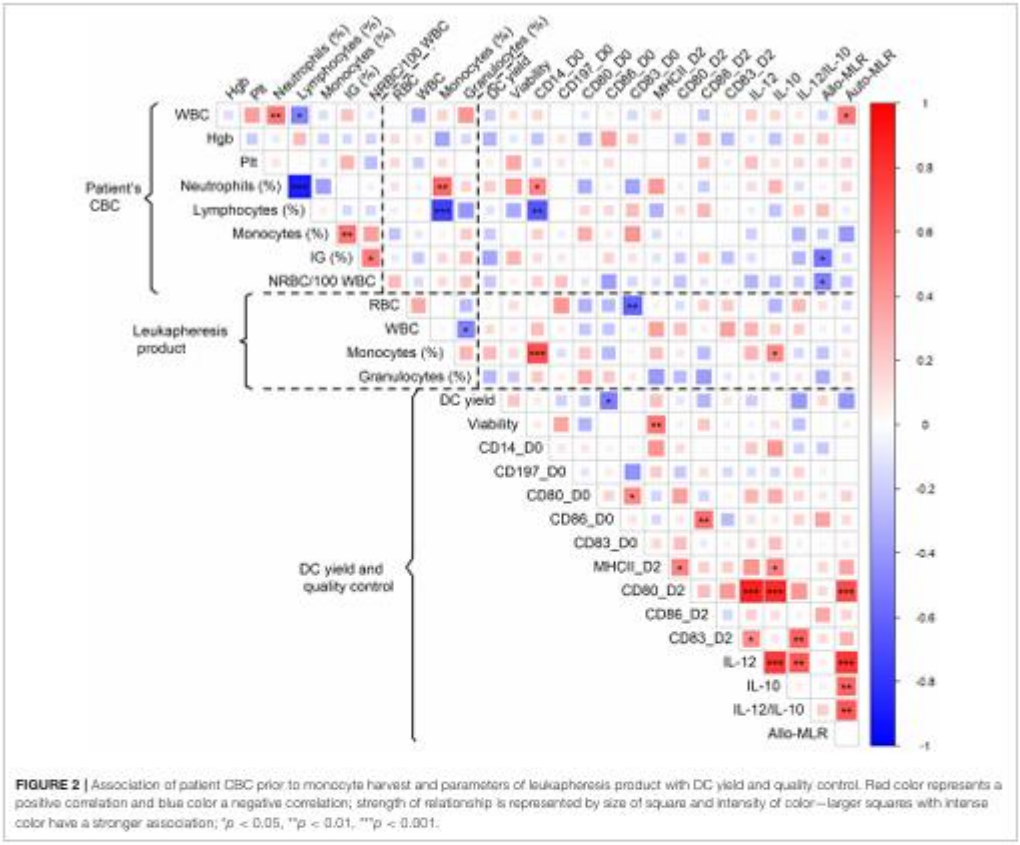


FIGURE 2 | Association of patient CBC prior to monocyte harvest and parameters of leukapheresis product with DC yield and quality control. Red color represents a positive correlation and blue color a negative correlation; strength of relationship is represented by size of square and intensity of color—larger squares with intense color have a stronger association; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

in pre-leukapheresis CBC (**Figure 2**). The level of autologous MLR was positively associated with absolute leukocyte count ($p = 0.016$) (**Figure 2**). Similarly, a high proportion of monocytes ($p < 0.001$) and low proportion of T-cells ($p = 0.001$) in the leukapheresis product were associated with increased expression of CD14 on manufactured cells (**Figure 2**). A high proportion of monocytes in the leukapheresis product was associated with increased production of IL-10 by manufactured cells ($p = 0.027$) (**Figure 2**).

Therapy Preceding and/or Concomitant With Monocyte Harvest and Its Association With Manufacturing Process Yield and the Immunostimulatory Properties of DCs

The patient history of anti-cancer treatment and the outcome of DC manufacture were evaluated for an association between DC parameters and lines of therapy classified as 1st, 2nd, and 3rd or subsequent lines that were followed by monocyte harvest for DCs. The history of anti-cancer treatment had no observed impact on the quality of manufactured DCs (**Supplementary Figure 2**). Pharmacotherapeutics 60 days prior to and/or concomitant to monocyte harvest were classified into two groups and designated as follows (i) "m" ($n = 17$) for administration of therapy potentially interfering with monocyte viability and/or differentiation, namely TKI, mTOR inhibitors, chemotherapy in cell biology-interfering doses, i.e., MTD-based dose, anti-RANKL mAb, retinoic acid, and/or G-CSF (**Supplementary Table 1**) < 60 days prior to monocyte harvest, (ii) "0" ($n = 6$) for metronomic therapy/chemotherapy or no potentially monocyte-interfering therapy concomitantly or < 60 days prior to monocyte harvest. All batches from the "0" category passed QC, whereas seven out of 17 (41%) monocyte-derived DCs from the "m" category failed to be released for patient administration. The OR for passing QC in category "0" was 9.3 (95% CI: 0.5–191). DC yield, DC immunophenotype on day 0 and day 2, and production of IL-10 did not differ between

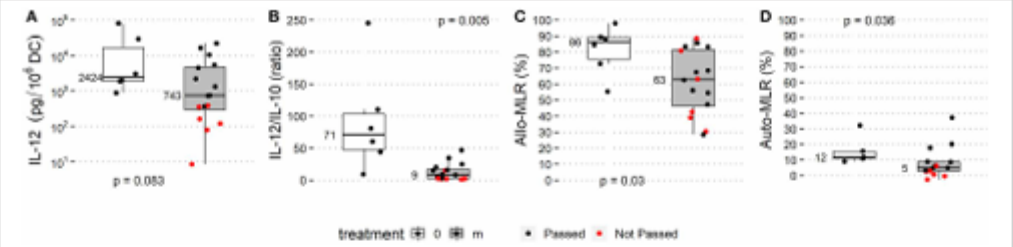


FIGURE 3 | Treatment prior to monocyte harvest and immunostimulatory properties of manufactured DCs. Manufacturing subgroup from monocytes harvested after MTD-based therapy potentially interfering with monocyte biology (listed in **Supplementary Table 1**; "m" treatment, gray box plots) and manufacturing subgroup from monocytes from untreated patients or after non-interfering treatment ("0" treatment, white box plots) were compared based on QC parameters: **(A)** IL-12 production, **(B)** IL-12/IL-10 production ratio, **(C)** allo-MLR and **(D)** auto-MLR. Median values are shown for each parameter for each treatment subgroup. Black dots show QC results of manufactured DCs that passed quality control, and red dots show results of manufactured DCs that did not pass quality control.

the "0" and "m" categories (**Supplementary Figure 3**). Median IL-12 production was 2,424 pg/10^6 DCs in the "0" category and 743 pg/10^6 DCs in category "m" ($p = 0.083$). The median IL-12/IL-10 ratio was 71 in the "0" category and 9 in the "m" category ($p = 0.002$). The median T-cell proliferation in allo-MLR was 86% in the "0" category and 63% in the "m" category ($p = 0.027$), and the in auto-MLR was 12% in the category "0" and 5% in category "m" ($p = 0.036$) (**Figure 3**).

In the analyzed study cohort, therapeutic regimens were heterogenic, with patients often treated with a combination of various compounds prior to monocyte harvest, and thus further categorization into single agent-defined subgroups and their analysis were impossible. Therefore, we performed cluster analysis of DC parameters in the context of therapy prior to monocyte harvest (**Figure 4**). Here we observed a cluster defined mainly by a superior IL-12/IL-10 ratio but low DC yield comprising batches KDO-0133 without any anti-cancer treatment, KDO-0137 treated with metronomic modified COMBAT with celecoxib, fenofibrate, low-dose cyclophosphamide, and low-dose vinblastine, and KDO-0115 treated with metronomic therapy with low-dose vinblastine, celecoxib, low-dose cyclophosphamide, and propranolol (see **Supplementary Table 2** for details on the treatment schedule and dosing). Furthermore, we observed a very similar pattern in DC properties in two batches, KDO-0142 and KDO-0144, that were manufactured from monocytes obtained from patients treated with temozolomide and irinotecan. These batches exhibited robust monocyte differentiation, as represented by their low CD14 expression, but failed to produce IL-12 or an immunostimulatory phenotype when matured, as represented by CD80 on post-cultivation DCs on day 2, and therefore did not meet the QC criteria. A pattern of relatively low DC yield, high production of IL-12, and notable monocyte differentiation and DC immunostimulatory phenotype and function was observed for batches KDO-0147, generated from monocytes from patients treated with celecoxib, and KDO-0141, from patients pretreated with combined metronomic therapy with low-dose vinblastine, low-dose etoposide, celecoxib, cholecalciferol, and fenofibrate. Batches KDO-0103 and KDO-0122 similarly exhibited poor yield, poor monocyte differentiation, a rather low IL-12/IL-10 ratio, and very low immunostimulatory functions toward donor T-cells. Monocytes from both batches were pretreated with an MTD-based combination of topoisomerase inhibitor and alkylating agent, with last administration from day 21 to 17, namely etoposide and ifosfamide in KDO-0103 and topotecan and cyclophosphamide in KDO-0122. This was followed in both cases by 9 days of administration of G-CSF filgrastim up to 7 days prior to monocyte harvest. High DC yield and viability but low markers of differentiation, immunostimulatory phenotype and IL-12/IL-10 ratio were similarly observed for batches KDO-0111 and KDO-0109 treated with topotecan, cyclophosphamide, and pazopanib. Based on features such as good DC yield and viability but low monocyte differentiation and a below-average IL-12/IL-10 ratio, these two batches clustered with KDO-0139 (treated with etoposide, ifosfamide, and filgrastim), KDO-0121 (etoposide, ifosfamide, and filgrastim), KDO-0118 (irinotecan and sunitinib), and KDO-0119 (cyclophosphamide, temsirolimus, and filgrastim). Notably, monocytes affected by retinoic acid (KDO-0135) or anti-RANKL denosumab (KDO-0124) produced DCs of average quality. In summary, monocyte-interfering MTD-based treatment of the clinical trial patients prior to monocyte harvest was associated with an impaired DC-based immunotherapy manufacturing process outcome. Certain combinations of anti-cancer treatments elicited a similar pattern of inadequate DC parameters. Namely, a combination of temozolomide and irinotecan was associated with poor DC maturation and immunostimulatory features, and a combination of pazopanib, topotecan, and MTD-based cyclophosphamide was associated with poor DC differentiation maturation and immunostimulatory parameters.

DISCUSSION

Here we show that despite strict adherence to the validated manufacturing protocol, the outcome of the manufacture of

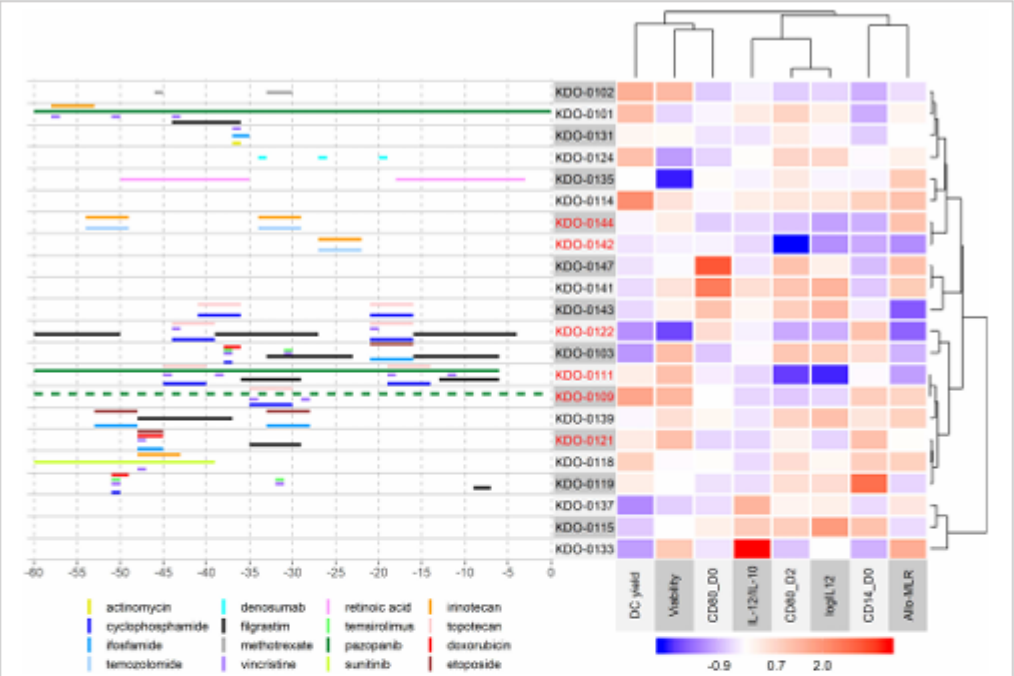


FIGURE 4 | Cluster analysis of DC parameters in the context of therapy prior to monocyte harvest. The heatmap on the right shows the immunostimulatory properties of manufactured DCs centered and scaled in the column direction (Z-score of parameters). Clusters are based on correlations. For clustering of DC parameters, but not batches, an equal meaning to positive and negative correlations was considered, and therefore strongly correlated parameters in the positive or negative manner clustered together. The left panel shows the treatment administered within 60 days of monocyte harvest. The day of the mononuclear harvest was set as day 0. An interactive version of the left panel with a detailed description of treatment including dosing is provided in **Supplementary Material 1**. Metronomic doses of chemotherapeutic drugs and supportive therapy such as vitamins and probiotics are not shown here but are summarized in **Supplementary Table 2**. Batches that did not pass quality control are indicated in red.

the medicinal product with monocyte-derived DCs is highly variable in terms of both DC yield and immunostimulatory properties. Moreover, in 30% of cases, manufacture of DC-based immunotherapy for advanced sarcoma and high-risk neuroblastoma patients resulted in a product that did not meet the specifications for the medicinal product and therefore was not released for application. This product failure rate was higher than in published studies (23, 24). Thus, in an attempt to improve the manufacturing process, to predict DC-manufacturing outcome, and, subsequently, to avoid laborious and costly DC manufacture that would not meet QC specifications, we addressed key variables in the manufacturing process. Namely, we focused on the issues of (i) monocyte isolation from the mononuclear leukapheresis product, (ii) parameters of the patient's CBC prior to monocyte harvest and parameters of the leukapheresis product, and (iii) anti-cancer therapy preceding monocyte harvest that may interfere with the ability of monocytes to differentiate into immunostimulatory DCs.

Regarding the method of monocyte isolation, we assessed whether monocyte extraction by a simple method of adherence to a plastic surface is comparable to the elaborate method of elutriation. During elutriation, monocytes can be contaminated with granulocytes with a similar sedimentation velocity to monocytes. Based on this observation, we validated the DC-manufacturing process with isolation of monocytes by adherence to plastic (25) to avoid contaminants that may interfere with DC differentiation by altering the levels of pro-differentiation cytokines and/or the formation of a suppressing microenvironment through generating decay products during cultivation. By comparative analysis of DC yield and immunostimulatory properties from the manufacturing processes of isolation of monocytes by elutriation vs. adherence to plastic, we conclude that the adherence method is comparable to the elutriation method. The method of adherence to plastic is simple in terms of the equipment, material, and manufacturing steps required and therefore is less costly, less prone to errors,

and more GMP-friendly than the elutriation process. In healthy adult volunteers, monocyte-derived DC yield with monocyte elutriation has been shown to be superior to adherence to plastic (26); this was not observed under our manufacturing conditions of heavily pretreated pediatric sarcoma and neuroblastoma patients.

With regards to the pharmacotherapy preceding monocyte harvest, we observed that therapy with agents interfering with the biology of monocytes 60 days prior to monocyte harvest was associated with reduced production of IL-12 and deficient functional immunostimulatory properties of the manufactured DC-based vaccine and subsequently often resulted in QC failure. It is of note here that failures in DC production occurred more often prior to the implementation of stricter criteria for non-allowed pharmacotherapy preceding monocyte harvest. Specifically, we observed impaired monocyte differentiation and, subsequently, inadequate immunostimulatory features in monocytes pretreated with a combination of an MTD-based dose of the alkylating agent cyclophosphamide, topoisomerase I inhibitor topotecan, and TKI pazopanib. We have previously shown that TKI pazopanib *in vitro* impairs the immunostimulatory properties of monocytes, including up-regulation of the immunoinhibitory surface molecule ILT-3 and decreased capability to up-regulate MHC II in response to LPS (27). Interestingly, however, pretreatment of monocytes *in vivo* with pazopanib without any other immediate treatment (KDO-0101) did not result in attenuated DC vaccine quality. Topotecan has been shown to partially activate monocyte-derived DCs but to prevent the full maturation of DCs stimulated with a cocktail of proinflammatory mediators (28). A different pattern was observed for DCs from cases treated with a combination of the alkylating agent temozolomide (TMZ) and the topoisomerase I inhibitor irinotecan (iri), and we observed monocyte differentiation but not DC immunostimulatory properties, resulting in a medicinal product that did not pass QC and was not administered. It is of note that one case was a sarcoma and one a neuroblastoma patient. Moreover, we also observed a similar pattern of poor DC parameters in a case of synovialosarcoma with TMZ/iri therapy in a cohort of patients outside this clinical trial. It has been shown that monocytes are particularly sensitive to the methylating agent temozolomide, undergoing apoptosis, while monocyte-derived DCs and macrophages are resistant to TMZ (19). Briegert and Kaina and Bauer et al. showed that monocytes accumulated single-strand DNA breaks due to failure of the re-ligation step in base excision repair and showed a lack of DNA repair protein expression (18, 19). Following TMZ treatment, monocytes demonstrated an unbalanced expression of DNA repair proteins, impairing base excision repair and the accumulation of double-stranded breaks (18, 19). *In vitro* studies of TMZ/iri cytotoxicity to neuroblastoma cells have revealed single- or double-stranded DNA damage to be mostly due to SN-38 (the active metabolite of irinotecan) and to be further enhanced through the addition of TMZ (29). Thus, we hypothesize that DNA damage caused by the combination of irinotecan and TMZ in the context of particular hypersensitivity of monocytes to temozolomide may underlie the unfavorable effect of anti-cancer therapy with TMZ/iri on the monocyte-derived immunostimulatory DC-manufacturing process. Monocytes from a patient treated with methotrexate, doxorubicin, and cisplatin failed to produce viable dendritic cells, but monocytes from another patient treated with methotrexate did not fail to produce DC vaccine. Methotrexate has reportedly inducedl apoptosis, reduced viability, induced differentiation, and reduced inflammatory properties of monocytes (30–33), and we may speculate, although based on anecdotal observation, that if combined with cisplatin, thereby shifting monocyte differentiation into an immunosuppressive phenotype (20), methotrexate may result in failure of monocyte-derived DC generation.

Regarding the composition of pre-leukapheresis CBC and the derived leukapheresis product and the outcome of DC manufacture, we observed that three interconnected features, i.e., (i) a low relative lymphocyte count, (ii) a high relative neutrophil count in CBC, and (iii) a high proportion of monocytes in the leukapheresis product, were associated with unfavorably high expression of CD14 on the manufactured cell product. Moreover, the presence of an increased number of immature granulocytes was associated with decreased potency of the DC-based product as quantified by allo-MLR. These observations may be underlain by emergency myelopoesis stimulated by G-CSF, which leads to a quantitative and qualitative change in all circulating myeloid cell types including neutrophils, monocytes, and myeloid-derived suppressor cells (34, 35). While fostering granulocyte effector functions, G-CSF also seems to promote immunosuppressive and tolerogenic properties in monocytes and monocyte-derived cells including increased production of IL-10 (36–39). In this context, it is of note that six out of seven cases treated with G-CSF within 60 days prior to monocyte harvest exhibited donor T-cell stimulation below the average and that the level of T-cell stimulation decreased with the intensity of G-CSF prior to monocyte harvest. Although the effect of G-CSF treatment on the DC-manufacturing process in our study cannot be dissected from the effect of preceding chemotherapy and targeted therapy, the tentative interpretation is that stimulation of myelopoesis with growth factors of granulocytes may have a rather negative impact on the outcome of the DC-based vaccine-manufacturing process.

Here, we show that treatment of patients with certain anti-cancer agents in MTD-based doses prior to monocyte harvest often leads to failure of manufacture of the immunostimulatory DC-based vaccine. We propose that the optimal time for monocyte harvest for generating DCs is prior to a cell-interfering treatment. With respect to the DC-manufacturing workflow, this would mean, in a majority of cancer patients, the implementation of DC manufacture from cryopreserved monocytes. Several studies have investigated the effect of cryopreservation on monocyte differentiation into DCs, but results have been conflicting. Some studies observed cryopreservation to have no effect on monocyte-derived DC production (40, 41). On the other hand, Silveira et al. showed that, when compared to fresh monocytes, cryopreserved monocytes exhibited impaired differentiation into dendritic cells, with lower rates of maturation and cytokine production in response to LPS and lower lymphocyte proliferation in allo-MLR (42). Thus, the cryopreservation of monocytes for

DC generation may decrease the quality of manufactured DCs, and the level of this decrease needs to be specified for a particular manufacturing protocol. In case of a minor drop in DC maturation and immunostimulatory parameters and function due to the cryopreservation of monocytes, this manufacturing modality should be considered, as it would allow harvesting of therapy-naïve monocytes and avoid a potentially detrimental effect of certain anti-cancer and supportive treatment on the quality of DC-based anti-cancer immunotherapy.

Another issue in the context of the concurrence of anti-cancer treatment and monocyte-derived DC manufacture is the length of the pharmacotherapy-free period prior to monocyte harvest. From our real-life experience gained on this study group, we conclude that a 30-day interval without treatment is not sufficient for the combination of temozolomide and irinotecan to sufficiently wash out the monocyte biology-interfering effect of this combination. However, the issue of a safe therapy-free window is not likely to be addressable through the establishment of a wash-out period for a particular drug. The fitness of monocytes and their capacity to differentiate and mature into DCs with high antigen-presenting effect is a matter of their biological function in the context of iatrogenic affection, which is complexly shaped by the need for immediate treatments, their combinations, their cumulative doses, and the long-term history of treatment. Therefore, identifying a marker revealed from a patient's peripheral blood that predicts the outcome of DC-generation would help to avoid an unproductive anti-cancer DC-manufacturing process. Here we show that a high monocyte count in CBC is not predictive of an efficacious outcome for DC generation. Nevertheless, we find that the presence of immature granulocytes in CBC may predict decreased immunostimulation elicited by DCs and, subsequently, unsuccessful preparation of DC-based IMP. However, closer evaluation of monocyte function prior to their collection for DC generation may be considered. A surrogate marker for the immunostimulatory capacity of monocytes may be evaluated in (i) their phenotype, e.g., the level of HLA-DR or ILT-3 expression on monocytes or the proportion of particular monocyte subsets according to CD14 and CD16 expression, or (ii) their ability to produce pro-inflammatory cytokines upon TLR stimulation (27).

In summary, monocytes represent a key starting material for anti-cancer DC-based vaccine manufacture. Therefore, monocyte conditions have an impact on the manufacturing yield, the differentiation into DCs, and the level of maturation and subsequent immunostimulatory functions. For DC manufacture from heavily pretreated pediatric patients with high-risk sarcomas and neuroblastoma, we conclude that the manufacturing yield and immunostimulatory quality of anti-cancer DC-based vaccine generated from patient's monocytes were not influenced by the monocyte isolation modality but were detrimentally affected by certain combinations of anti-cancer agents. Thus, the combination of chemotherapy or targeted therapy with DC-based immunotherapy needs to be scheduled not only with respect to the likely beneficial role of anti-cancer agents on the immunogenicity of tumor antigens for both *in vitro* DC generation via induction of immunogenic cell death and *in vivo* for effector response of DC-activated T-cells but also with respect to optimal monocyte immunostimulatory functions. Finally, these findings may also have implications for the general pharmacology of anticancer treatment. As our model of *ex vivo*-activated DC preparation generally parallels the *in vivo* differentiation pathways of monocytes to the antigen-presenting cells, we may imply that drug combinations at doses used clinically may result in an impairment of patient DCs and possibly immune competence in general. In conclusion, these findings may stimulate further research on dose and mechanism-of-action-based drug combination in patient-centered trials to optimize the treatment modalities currently available in clinical oncology.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the manuscript/**Supplementary Files**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee, University Hospital Brno. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

EH contributed to the trial design, contributed to the study design, participated in clinical data acquisition and analysis, contributed to Supplementary Material preparation, and drafted the manuscript. KP supervised IMP manufacture, contributed to laboratory data acquisition and analysis, contributed to data interpretation, and drafted the manuscript. DC participated in clinical data acquisition, contributed to Supplementary Material preparation, and revised the manuscript. IS performed statistical analysis, contributed to figure preparation and data interpretation, and drafted the manuscript. PMu contributed to the trial design, performed patient enrollment and treatment, contributed to data interpretation, and drafted the manuscript. PMa contributed to the trial design, participated in patient treatment, and drafted the manuscript. LFe contributed to laboratory data acquisition and analysis, contributed to Supplementary Material preparation, and drafted the manuscript. JM contributed to the trial design and drafted the manuscript. LJ participated in IMP manufacturing and revised the manuscript. LSe contributed to IMP manufacturing—monocyte harvest and drafted the manuscript. RP contributed to IMP manufacturing—starting material harvest and revised the manuscript. LFl contributed to IMP manufacturing—certification and revised the manuscript. LSo contributed to the trial design and revised the manuscript. RD, JS, and DV contributed to the trial design, contributed to data interpretation, and revised the manuscript. LZ-D conceived the study design, designed and supervised laboratory data acquisition and analysis, contributed to data analysis and interpretation, and drafted and finalized the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.01034/full#supplementary-material

Supplementary Figure 1 | Decision tree for DC-IMP manufacturing workflow including in-process and quality controls.

Supplementary Figure 2 | Number of anti-cancer therapy lines preceding monocyte harvest were compared based on QC parameters: **(A)** DC yield, and post-thaw: **(B)** viability, **(C)** DC phenotype on day 0: CD14, CD197, CD80, CD86, CD83 and on day 2: MHC II, CD80, CD86, CD83 and immunostimulatory properties presented by **(D)** IL-12 production, IL-10 production and IL-12/IL-10 production ratio, **(E)** allo-MLR and auto-MLR.

Supplementary Figure 3 | Manufacturing subgroup from monocytes harvested after MTD-based therapy potentially interfering with monocyte biology and manufacturing subgroup from monocytes from untreated patients or after non-interfering treatment compared based on QC parameters: **(A)** DC yield, and post-thaw: **(B)** viability, **(C)** DC phenotype on day 0: CD14, CD197, CD80, CD86, CD83 and on day 2: MHC II, CD80, CD86, CD83 and immunostimulatory properties presented by **(D)** IL-12 production, IL-10 production and IL-12/IL-10 production ratio, **(E)** allo-MLR and auto-MLR.

Supplementary Table 1 | Monocyte biology-interfering medications.

Supplementary Table 2 | Study patient characteristics, disease course, and therapy.

Supplementary Table 3 | Source data: CBC parameters, manufacturing details, and QC parameters.

Supplementary Material 1 | html. Interactive—medications 60 days prior to monocyte harvest.

REFERENCES

1. Oberlin O, Rey A, Lyden E, Bisogno G, Stevens MC, Meyer WH, et al. Prognostic factors in metastatic rhabdomyosarcomas: results of a pooled analysis from United States and European cooperative groups. *J Clin Oncol.* (2008) 26:2384–9. doi: 10.1200/JCO.2007.14.7207
2. Pappo AS, Anderson JR, Crist WM, Wharam MD, Breitfeld PP, Hawkins D, et al. Survival after relapse in children and adolescents with rhabdomyosarcoma: a report from the Intergroup Rhabdomyosarcoma Study Group. *J Clin Oncol.* (1999) 17:3487–93. doi: 10.1200/JCO.1999.17.11.3487
3. Sharp SE, Trout AT, Weiss BD, Gelfand MJ. MIBG in neuroblastoma diagnostic imaging and therapy. *Radiographics.* (2016) 36:258–78. doi: 10.1148/rg.2016150099
4. Zapletalova D, Andre N, Deak L, Kyr M, Bajciova V, Mudry P, et al. Metronomic chemotherapy with the COMBAT regimen in advanced pediatric malignancies: a multicenter experience. *Oncology.* (2012) 82:249–60. doi: 10.1159/000336483
5. Berthold F, Homberg M, Proleskovskaya I, Mazanek P, Belogurova M, Ernst A, et al. Metronomic therapy has low toxicity and is as effective as current standard treatment for recurrent high-risk neuroblastoma. *Pediatr Hematol Oncol.* (2017) 34:308–19. doi: 10.1080/08880018.2017.1373314
6. Krishnadas DK, Shusterman S, Bai F, Diller L, Sullivan JE, Cheerva AC, et al. A phase I trial combining decitabine/dendritic cell vaccine targeting MAGE-A1, MAGE-A3 and NY-ESO-1 for children with relapsed or therapy-refractory neuroblastoma and sarcoma. *Cancer Immunol Immunother.* (2015) 64:1251–60. doi: 10.1007/s00262-015-1731-3
7. Merchant MS, Bernstein D, Amoako M, Baird K, Fleisher TA, Morre M, et al. Adjuvant immunotherapy to improve outcome in high-risk pediatric sarcomas. *Clin Cancer Res.* (2016) 22:3182–91. doi: 10.1158/1078-0432.CCR-15-2550
8. Klement GL, Arkun K, Valik D, Roffidal T, Hashemi A, Klement C, et al. Future paradigms for precision oncology. *Oncotarget.* (2016) 7:46813–31. doi: 10.18632/oncotarget.9488
9. Rossi M, Young JW. Human dendritic cells: potent antigen-presenting cells at the crossroads of innate and adaptive immunity. *J Immunol.* (2005) 175:1373–81. doi: 10.4049/jimmunol.175.3.1373
10. Hoffman R, Benz EJ Jr, Shattil S, Furie B, Cohen H. *Hematology: Basic Principles and Practice.* Churchill Livingstone (2005).
11. Jakubzick CV, Randolph GJ, Henson PM. Monocyte differentiation and antigen-presenting functions. *Nat Rev Immunol.* (2017) 17:349–62. doi: 10.1038/nri.2017.28
12. Boyette LB, Macedo C, Hadi K, Elinoff BD, Walters JT, Ramaswami B, et al. Phenotype, function, and differentiation potential of human monocyte subsets. *PLoS ONE.* (2017) 12:e0176460. doi: 10.1371/journal.pone.0176460
13. Patel AA, Zhang Y, Fullerton JN, Boelen L, Rongvaux A, Maini AA, et al. The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. *J Exp Med.* (2017) 214:1913–23. doi: 10.1084/jem.20170355
14. Sampath P, Moideen K, Ranganathan UD, Bethunaickan R. Monocyte subsets: phenotypes and function in tuberculosis infection. *Front Immunol.* (2018) 9:1726. doi: 10.3389/fimmu.2018.01726
15. Zhou JX, Feng LJ, Zhang X. Risk of severe hematologic toxicities in cancer patients treated with PARP inhibitors: a meta-analysis of randomized controlled trials. *Drug Des Devel Ther.* (2017) 11:3009–17. doi: 10.2147/DDDT.S147726
16. Rusten LS, Lyman SD, Veiby OP, Jacobsen SE. The FLT3 ligand is a direct and potent stimulator of the growth of primitive and committed human CD34+ bone marrow progenitor cells *in vitro*. *Blood.* (1996) 87:1317–25.
17. Kao J, Timmins J, Ozao-Choy J, Packer S. Effects of combined sunitinib and extracranial stereotactic radiotherapy on bone marrow hematopoiesis. *Oncol Lett.* (2016) 12:2139–44. doi: 10.3892/ol.2016.4851
18. Briegert M, Kaina B. Human monocytes, but not dendritic cells derived from them, are defective in base excision repair and hypersensitive to methylating agents. *Cancer Res.* (2007) 67:26–31. doi: 10.1158/0008-5472.CAN-06-3712
19. Bauer M, Goldstein M, Heylmann D, Kaina B. Human monocytes undergo excessive apoptosis following temozolomide activating the ATM/ATR pathway while dendritic cells and macrophages are resistant. *PLoS ONE.* (2012) 7:e39956. doi: 10.1371/journal.pone.0039956
20. Dijkgraaf EM, Heusinkveld M, Tummers B, Vogelpoel LT, Goedemans R, Jha V, et al. Chemotherapy alters monocyte differentiation to favor generation of cancer-supporting M2 macrophages in the tumor microenvironment. *Cancer Res.* (2013) 73:2480–92. doi: 10.1158/0008-5472.CAN-12-3542
21. Hu J, Kinn J, Zirakzadeh AA, Sherif A, Norstedt G, Wikstrom AC, et al. The effects of chemotherapeutic drugs on human monocyte-derived dendritic cell differentiation and antigen presentation. *Clin Exp Immunol.* (2013) 172:490–9. doi: 10.1111/cei.12060
22. R Core Team. *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna (2019).
23. Ge C, Li R, Song H, Geng T, Yang J, Tan Q, et al. Phase I clinical trial of a novel autologous modified-DC vaccine in patients with resected NSCLC. *BMC Cancer.* (2017) 17:884. doi: 10.1186/s12885-017-3859-3
24. Rodriguez J, Castanon E, Perez-Gracia JL, Rodriguez I, Viudez A, Alfaro C, et al. A randomized phase II clinical trial of dendritic cell vaccination

following complete resection of colon cancer liver metastasis. *J Immunother Cancer.* (2018) 6:96. doi: 10.1186/s40425-018-0405-z

25. Repnik U, Knezevic M, Jeras M. Simple and cost-effective isolation of monocytes from buffy coats. *J Immunol Methods.* (2003) 278:283–92. doi: 10.1016/S0022-1759(03)00231-X
26. Dohnal AM, Graffi S, Witt V, Eichstill C, Wagner D, Ul-Haq S, et al. Comparative evaluation of techniques for the manufacturing of dendritic cell-based cancer vaccines. *J Cell Mol Med.* (2009) 13:125–35. doi: 10.1111/j.1582-4934.2008.00304.x
27. Zdrazilova Dubska L, Fedorova L, Pilatova K, Mudry P, Hlavackova E, Matoulkova E, et al. TKI pazopanib impaires immunostimulatory properties of monocytes: implication for monocyte-derived DC-based anti-cancer vaccine preparation. *Ann Oncol.* (2016) 27:18P. doi: 10.1093/annonc/mdw525.18
28. Trojandt S, Knies D, Pektor S, Ritz S, Mailander V, Grabbe S, et al. The chemotherapeutic agent topotecan differentially modulates the phenotype and function of dendritic cells. *Cancer Immunol Immunother.* (2013) 62:1315–26. doi: 10.1007/s00262-013-1431-9
29. Cai W, Maldonado NV, Cui W, Harutyunyan N, Ji L, Sposto R, et al. Activity of irinotecan and temozolomide in the presence of O6-methylguanine-DNA methyltransferase inhibition in neuroblastoma pre-clinical models. *Br J Cancer.* (2010) 103:1369–79. doi: 10.1038/sj.bjc.6605927
30. Seitz M, Zwicker M, Loetscher P. Effects of methotrexate on differentiation of monocytes and production of cytokine inhibitors by monocytes. *Arthritis Rheum.* (1998) 41:2032–8. doi: 10.1002/1529-0131(199811)41:11<2032::AID-ART19>3.0.CO;2-J
31. Cutolo M, Sulli A, Craviotto C, Felli L, Pizzorni C, Seriolo B, et al. Antiproliferative-antiinflammatory effects of methotrexate and sex hormones on cultured differentiating myeloid monocytic cells (THP-1). *Ann N Y Acad Sci.* (2002) 966:232–7. doi: 10.1111/j.1749-6632.2002.tb04220.x
32. Moller B, Kukoc-Zivojnov N, Okamgba S, Kessler U, Puccetti E, Ottmann OG, et al. Folinic acid antagonizes methotrexate-induced differentiation of monocyte progenitors. *Rheumatol Int.* (2002) 22:60–7. doi: 10.1007/s00296-002-0188-9
33. Perpetuo IP, Caetano-Lopes J, Rodrigues AM, Campanilho-Marques R, Ponte C, Canhao H, et al. Methotrexate and low-dose prednisolone downregulate osteoclast function by decreasing receptor activator of nuclear factor-kappabeta expression in monocytes from patients with early rheumatoid arthritis. *RMD Open.* (2017) 3:e000365. doi: 10.1136/rmdopen-2016-000365
34. Manz MG, Boettcher S. Emergency granulopoiesis. *Nat Rev Immunol.* (2014) 14:302–14. doi: 10.1038/nri3660
35. Pilatova K, Bencsikova B, Demlova R, Valik D, Zdrazilova-Dubska L. Myeloid-derived suppressor cells (MDSCs) in patients with solid tumors: considerations for granulocyte colony-stimulating factor treatment. *Cancer Immunol Immunother.* (2018) 67:1919–29. doi: 10.1007/s00262-018-2166-4
36. Hartung T, Docke WD, Gantner F, Krieger G, Sauer A, Stevens P, et al. Effect of granulocyte colony-stimulating factor treatment on *ex vivo* blood cytokine response in human volunteers. *Blood.* (1995) 85:2482–9.
37. Mielcarek M, Graf L, Johnson G, Torok-Storb B. Production of interleukin-10 by granulocyte colony-stimulating factor-mobilized blood products: a mechanism for monocyte-mediated suppression of T-cell proliferation. *Blood.* (1998) 92:215–22.
38. Boneberg EM, Hareng L, Gantner F, Wendel A, Hartung T. Human monocytes express functional receptors for granulocyte colony-stimulating factor that mediate suppression of monokines and interferon-gamma. *Blood.* (2000) 95:270–6.
39. Saito M, Kiyokawa N, Taguchi T, Suzuki K, Sekino T, Mimori K, et al. Granulocyte colony-stimulating factor directly affects human monocytes and modulates cytokine secretion. *Exp Hematol.* (2002) 30:1115–23. doi: 10.1016/S0301-472X(02)00889-5
40. Hori S, Heike Y, Takei M, Maruyama M, Inoue Y, Lee JJ, et al. Freeze-thawing procedures have no influence on the phenotypic and functional development of dendritic cells generated from peripheral blood CD14+ monocytes. *J Immunother.* (2004) 27:27–35. doi: 10.1097/00002371-200401000-00003
41. Ghanekar SA, Bhatia S, Ruitenberg JJ, Dela Rosa C, Disis ML, Maino VC, et al. Phenotype and *in vitro* function of mature MDDC generated from cryopreserved PBMC of cancer patients are equivalent to those from healthy donors. *J Immune Based Ther Vaccines.* (2007) 5:7. doi: 10.1186/1476-8518-5-7
42. Silveira GF, Wowk PF, Machado AM, Duarte Dos Santos CN, Bordignon J. Immature dendritic cells generated from cryopreserved human monocytes show impaired ability to respond to LPS and to induce allogeneic lymphocyte proliferation. *PLoS ONE.* (2013) 8:e71291. doi: 10.1371/journal.pone.0071291

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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7.12 Annex 12

Nebeska K, Souckova L, Stepanova R, Demlova R. Exploring the publication gap in pediatric randomized clinical trials: completed vs. uncompleted pediatric clinical trials. Front Med (Lausanne). 2025 May 30;12:1590125. doi: 10.3389/fmed.2025.1590125. PMID: 40520805; PMCID: PMC12163232.

Summary
Purpose:
The aim of this study was to analyse the extent of incompletion and non-publication of pediatric randomized clinical trials and to identify factors associated with these inefficiencies. The study addresses a critical issue in pediatric research, where incomplete or unpublished trials may lead to biased evidence and negatively impact clinical decision-making and patient safety.

Methodology:
A cross-sectional analysis of randomized pediatric clinical trials registered on ClinicalTrials.gov between 2011 and 2013 was conducted, with follow-up assessment of completion status by 2017. Trial characteristics, funding sources, and publication outcomes were systematically evaluated, and statistical analyses were performed to identify predictors of trial incompletion and delayed or missing dissemination.

Results:
Out of 1,088 eligible pediatric randomized clinical trials, approximately 16.5% were not completed, most commonly due to insufficient patient recruitment. Among completed trials, only about 58% reported results in registries and 70% were published in peer-reviewed journals, with notable delays in dissemination influenced by trial phase, funding, and sample size.

Conclusion:
The study demonstrates that pediatric randomized trials are affected by substantial inefficiencies, including high incompletion rates and delayed reporting of results. Strengthening regulatory oversight, improving trial feasibility planning, and ensuring timely dissemination are essential to maximise the impact of NCCTs and to safeguard evidence-based pediatric care.

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Exploring the publication gap in pediatric randomized clinical trials: completed vs. uncompleted pediatric clinical trials

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Background: Randomized clinical trials (RCTs) are essential for advancing medical knowledge, especially in pediatrics, where they provide critical evidence for safe and effective treatments. Regulatory frameworks like the Pediatric Regulation in the EU and the Best Pharmaceuticals for Children Act in the U.S. have spurred growth in pediatric RCTs. However, these clinical trials face unique challenges, including ethical complexities, recruitment difficulties, and funding limitations, which can hinder their completion. The publication of RCTs' results is equally crucial, yet not all completed RCTs share their findings, contributing to publication bias. This bias, especially in pediatric clinical trials, can distort medical evidence, impact clinical decisions, and potentially compromise patient safety.

Methods: We conducted a cross-sectional analysis of pediatric RCTs registered on ClinicalTrials.gov between 2011 and 2013, with completion status by 2017. Inclusion criteria included clinical trials with drug interventions in participants aged 0-17 and randomization. Data on RCTs characteristics, including phase, funding source, participant age, and enrollment, were extracted. RCTs completion status was assessed, and reasons for incompletion were categorized. Publication status was evaluated through registry and manual searches on PubMed and Google Scholar. Statistical analyses, including logistic regression, were performed to identify factors associated with trial incompletion and non-publication.

Results: Out of 2875 pediatric clinical trials reviewed, 1088 met the inclusion criteria. Among these, 16.54% were uncompleted, primarily due to patient accrual issues (32.22%). Academic sponsors funded 48.53% of trials, and industrial sponsors funded 45.13%. Of 908 completed RCTs, 58.48% posted results in registries, while 70% had results published in peer-reviewed journals. Industrially funded RCTs were more likely to post results, but academic RCTs had a higher scientific publication rate. The median time to first result publication in registries was 21 months, with significant delays linked to the trial phase, funding, and participant enrolment size.

Conclusion: Our study highlights significant challenges in pediatric RCTs, including high incompletion rates and delays in result reporting. Ethical,

regulatory, and logistical barriers hinder progress, impacting evidence transparency. Strengthened regulatory oversight and enhanced compliance are essential to improve pediatric research outcomes and ensure timely dissemination of RCTs results.

KEYWORDS

registry, clinical trials, pediatrics, results, publication bias

1 Introduction

Randomized clinical trials (RCTs) are pivotal in medical research and healthcare as their rigorous design minimizes bias and delivers high-quality evidence on the safety and efficacy of new medicines. The same prerequisites apply to RCTs in pediatrics. In most cases, new pediatric medicines are launched to the market only after thorough evaluation through RCTs, which remain the gold standard for the Regulatory Agencies.

Complementing new drug applications, pediatric RCTs also form the cornerstone of evidence-based medicine (EBM) when professional societies, medical practitioners, and policymakers rely on the results of well-conducted RCTs to set up or update guidelines, make informed decisions about patient care, and adjust healthcare policies. Investigator-initiated trials (IITs) aim to answer unresolved questions from clinical practice by conducting RCTs to gather more robust evidence on frequent off-label drug use in pediatrics. This approach is particularly beneficial in pediatrics, where IITs are strongly represented.

To launch the new medicine for pediatric patients to the market, to optimize the standard pediatric health care, and to adjust the clinical guidelines appropriately, the first assumption is (i) the sufficient number of pediatric clinical trials, the second prerequisite is (ii) their completion and the third crucial condition is the (iii) open access to their results.

1.1 Trends in pediatric RCTs

There was a significant increase in pediatric research in Europe after introducing the Pediatric Regulation (EC) No 1901/2006 (1). The proportion of clinical trials that include children increased by 50% between 2007 and 2016, from 8.25 to 12.4%, leading to more evidence-based information on medicines used in children (2).

In the U.S., the Best Pharmaceuticals for Children Act (BPCA) of 2002 and the Pediatric Research Equity Act (PREA) of 2003 established incentives and mandates for pediatric drug development, respectively. Since 2007, FDA has issued priority review vouchers for tropical diseases, medical countermeasures, and rare pediatric diseases, and this voucher program has been extended for rare disease product applications with a rare pediatric disease designation through September 30, 2024 (3).

1.2 Proportion of the uncompleted pediatric RCTs

Despite regulatory support, initiating and conducting trials in the pediatric population is still more difficult than in adults because of multifaceted ethical barriers, the overall low burden of disease, unfavorable market incentives, and lack of funding (4). Examining the number of pediatric RCTs conducted over a specific period would provide valuable insights.

Ethically, enrolling minors in clinical research demands strict protective measures, parental consent, and assent depending on age, which can complicate participant recruitment and retention (5). Moreover, the smaller size of pediatric populations for certain conditions makes reaching adequate sample sizes difficult, affecting the clinical trial's statistical power and potential completion. Some of these barriers may lead to delay or incompletion of clinical trials and thus to waste unnecessarily included pediatric patients.

Previous work has demonstrated that 11% of interventional pediatric RCTs registered in ClinicalTrials.gov during the years 2007-2020 were prematurely discontinued, with poor participant recruitment as a predominant reason for trial incompletion (4). This issue could have ethical, social, health and economic implications: patients consent to participate in the trial with the expectation that the clinical findings will foster new medical knowledge. Therefore, trial incompletion may undermine patients' trust in clinical research and represent a waste of financial (public) and human resources (6).

1.3 Release of the RCT's results

According to the Declaration of Helsinki, it is the responsibility of the researchers and sponsors to disseminate the trial results involving human participants, regardless of the findings. In the EU, according to EC guideline 2012/c302/03, the sponsor must disclose the results of the registered trial in EUCTR to EMA within 12 months of the trial completion. In the case of a pediatric clinical trial, the timeline is shortened to 6 months (7). In addition, Regulation (EU) No 536/2014 mandates that a summary of the results for the lay public accompanies the clinical trial results.

For trials with at least one U.S. site, a 2007 FDA amendment requires that results be posted on ClinicalTrials.gov within 1 year of clinical trial completion. Moreover, in September 2016, the U.S. Department of Health and Human Services issued a Final Rule for Clinical Trials Registration and Results Information Submission (42 CFR Part 11) that clarifies and expands the regulatory requirements and procedures for submitting registration and summary results information of clinical trials on ClinicalTrials.gov, under FDAAA 801. Trial results published in registries should include a summary of participants' information, protocol and changes, summary results for pre-specified primary and secondary endpoints, details of adverse events, and statistical analyses (8).

1.4 Risk and implications of publication bias in pediatric RCTs

Although the abovementioned regulations have been in force for a long time, their compliance by clinical trial sponsors is not fully respected (9, 10). The non-publication of results from pediatric clinical trials is a critical issue with multiple underlying causes and significant consequences. One of these consequences is the phenomenon of publication bias, where trials with negative or inconclusive results are less frequently published, and this phenomenon is well documented in medical research (11). In pediatrics, the risk of publication bias is elevated due to the unique dependency on clinical trials for determining appropriate treatments for children. Many medications used in pediatric care do not have specific approval for use in children and are instead prescribed off-label (12). This practice makes the accurate and comprehensive reporting of clinical trial results crucial, as it directly influences the safety and efficacy of medicines administered to this vulnerable population. The lack of publication of completed and unbiased clinical trial results may lead to an incomplete understanding of a medication's risk profile, potentially resulting in adverse effects that could have been mitigated or avoided.

The impact of non-publication of clinical trial results extends beyond academic research and has implications for clinical decision-making and policy-making (6). By understanding and addressing the causes of clinical trial incompletion and non-publication, researchers and policymakers can enhance the reliability and accessibility of medical evidence, ensuring that the benefits of pediatric clinical trials are fully realized and contribute to evidence-based medicine.

2 Materials and methods

The aim of this research work was firstly to map the current trends of a number of pediatric clinical trials, their rate of completion, and their results availability. As a second aim, this cross-sectional, data-based analysis focuses on exploring the factors leading to the high rates of incompletion and non-publication of pediatric clinical trial results and offering insights that could potentially improve future research practices and policy interventions.

2.1 Data source

We systematically searched randomized pediatric clinical trials, which were registered on ClinicalTrials.gov. ClinicalTrials.gov is an online database of clinical research studies that contains more than 490,000 studies from 222 countries, making it the most comprehensive WHO-recognized registry (13). The clinical trials' selection was done in this database according to predefined inclusion criteria:

1. RCT,
2. pediatric (the age of participants was limited from birth to 17 years),
3. with the drug intervention,
4. registered between 1.1.2011 and 31.12.2013,
5. completed or uncompleted by 31.12.2017.

The recruitment status of clinical trial participants has, therefore, been narrowed down to completed, withdrawn, suspended, and terminated according to ClinicalTrials.gov Glossary Terms (14). According to this glossary, a trial was considered completed if it had ended normally and participants were no longer being examined or treated. If the trial stopped early, before the first participant was enrolled, we refer to it as withdrawn. The trial was classified as suspended if it had stopped early, but could start again. The trial was given a terminated status if it was stopped prematurely and not restarted. Participants were no longer examined or treated. The content we searched was downloaded in JSON format on the same day as our search for detailed analysis, ensuring the file's immutability.

The exclusion criteria follow:

1. non-randomized,
2. non-pediatric,
3. without drug intervention,
4. without final confirmed status after December 31, 2017,
5. trial registration was done > 60 days after the clinical trial start date (15).

The trial was included in our analysis if it fulfilled all the inclusion criteria and did not meet any of the exclusion criteria. This research is an independent follow-up survey to the work of Harvard Medical School researchers Pica and Bourgeois (16), whose work was completed with pediatric RCTs registered by the end of 2010. The timeframe chosen for the pediatric clinical trials included in the registry is therefore linked to Pica's research, while allowing sufficient time for the trials to be completed and the results to be published.

The EU Clinical Trials Register (EUCTR) was consulted to identify the trial identifier (EudraCT number) and to determine whether trial results had been published in the registry. This procedure has only been used for studies taking place in the EU. PubMed and Google Scholar were also systematically searched to identify relevant publications in peer-reviewed journals.

2.2 Clinical trial characteristics

We collected the following trials' specific data from the ClinicalTrial.gov downloaded content to provide pediatric RCTs characteristics:

a) Year of RCT registration;

– 2011, 2012, 2013

b) Age of participant;

– Clinical trial participants were categorized by age into "preterm, newborn, infant," "toddler and preschool," "school age," "adolescent," "mixed ages," and "combined." While the "mixed ages" clinical trials include children of different ages, the "combined" clinical trials include children and adults.

c) Funding source (academic, industry, other);

– ClinicalTrial.gov describes the funder as an organization that provides funding and support for a clinical trial (including activities

such as design, data analysis, and reporting). Both sponsors and collaborators are considered as funders according to the registry. ClinicalTrials.gov distinguishes funding types as industry, the National Institutes of Health (NIH), and government agencies (other than NIH) and other. We have categorized the funding source based on sponsor and collaborator, similar to what has been described in other trials (4, 16, 17). When only the sponsor was mentioned in the ClinicalTrials.gov registry, we considered it the funder. If more than one sponsors were listed, we determined the lead sponsor to be the primary funder (16).

– Subsequently, we used three funder categories: academic, industry, and other. While trials where hospitals, universities, and foundations were listed as sponsors, we classified them as academic funders, trials with sponsors from the industry (pharmaceutical companies, any profit organization) were assigned to industry funders. Category other included all government-funded trials.

d) Masking;

– Open-label, single, double, triple, quadruple

e) Actual enrollment;

– ClinicalTrials.gov allows for the identification of the number of patients planned for the trial and the number of patients recruited.

f) Sample size;

– For each of these patients' groups, we created 5 sample sizes: 0-50, 51-100, 101-500, 501-1000, and > 1000.

g) trial phase;

– I, II, III, IV

h) medical condition;

– Medical conditions were classified according to the Internal Classification of Diseases (ICD-10) (18).

i) the justification for an incompletion;

– In the case of uncompleted clinical trials, we ascertained the reason for termination, which was stated directly in the ClinicalTrials.gov registry or the published article, if any. Reasons for termination were categorized as follows: patient accrual, company/business decision, informative termination, funding or regulatory issue, conduct problems, principle investigator left and non-reported or unclear reason.

2.3 Publication related to pediatric RCTs' results search

The search for publications of pediatric RCT results in peer-reviewed scientific journals followed a structured approach:

1st step: Registry Search

j) We first searched for publications directly in the registry for each included pediatric RCT. Any publications found were reviewed to assess their relevance to the clinical trial. A publication was considered relevant if it mentioned the ClinicalTrials.gov Identifier (NCT) of the completed or terminated pediatric RCT in either the article's abstract or full text, and if it was published after the clinical trial's completion.

2nd step: Manual Search

k) For pediatric RCTs with no publications or non-relevant publications listed in the registry, two independent researchers (KN and LS) conducted additional searches on PubMed and Google Scholar. The search was based on the trial's NCT or EudraCT number, trial title, author names, institutions, and clinical trial keywords.

3rd step: Publication Linkage

l) Articles were linked to the respective trial by comparing trial data from the registry with information in the abstract or full manuscript when needed. In cases where multiple publications were identified for a single trial, we selected the earliest publication.

2.3.1 Time to publication

We decided to search pediatric RCTs completed by December 31, 2017, allowing investigators at least 2 years to analyze data, draft a manuscript, and complete the necessary steps for publication in peer-reviewed journals (19). This extended timeframe was intended to provide sponsors and researchers sufficient time to publish their trial results.

m) The time to publication was calculated by determining the number of months between the trials's completion and the publication date. The trial completion date was obtained from ClinicalTrials.gov, while the publication date was taken from the first available version, either in print or electronic format.

2.4 Release of pediatric RCTs results search

n) The ClinicalTrials.gov registry was used to search for the results of completed trials that met the inclusion criteria. Trials without published results in ClinicalTrials.gov were searched in the EU Clinical Trial Register (EUCTR). If the trials' results were published in the EUCTR, we added them to the trials with found results.

2.5 Data quality control checking

An independent researcher (RS) controlled each 10th trial. Each data point for all included RCTs was verified against the source information in the registry. In case of discrepancies or missing data, the researchers were informed, and any discrepancies were resolved through discussion and mutual agreement. Corrections were made where necessary following consensus.

2.6 Statistical analysis

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, United States). Descriptive statistics were used to summarize both categorical and continuous variables. For categorical variables, data were presented as counts (n) and percentages (%), with graphical representations where appropriate. For continuous variables, results were reported using the median, along with the minimum and maximum values. A chi-square test was applied to compare categorical variables, and *P*-values were reported to indicate statistical significance. A *P*-value of < 0.05 was considered statistically significant.

To find factors influencing a publication gap, a two-step approach was employed to build the final multivariate logistic regression model. Univariate logistic regression was performed

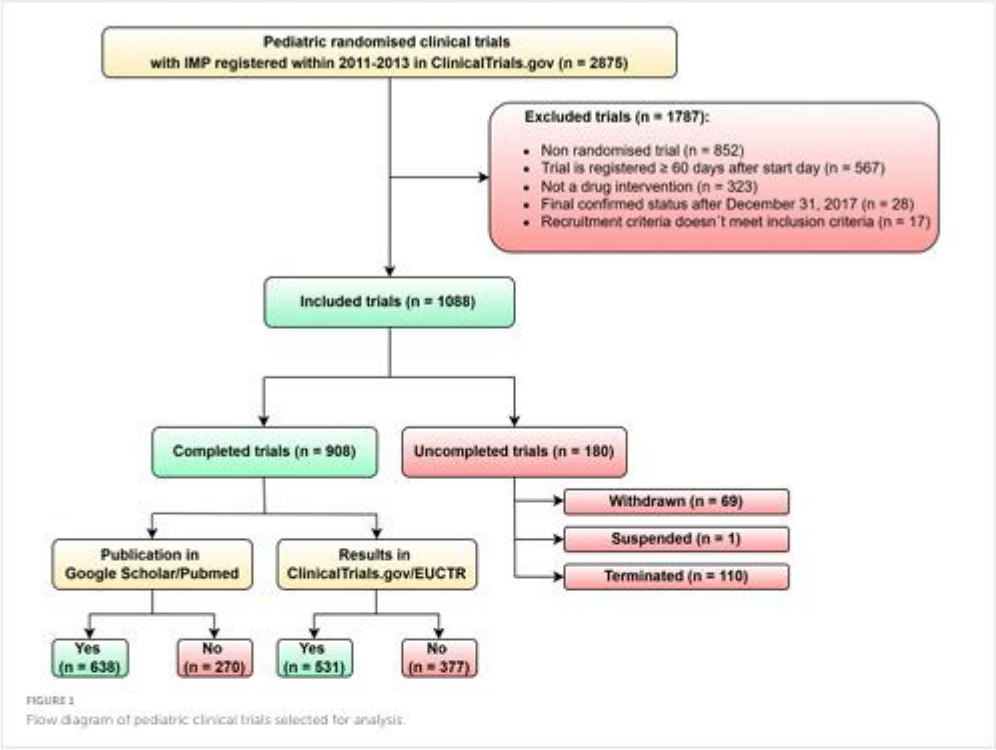


FIGURE 1
Flow diagram of pediatric clinical trials selected for analysis.

for each independent variable to assess its association with the dependent variable. All variables with a $P < 0.1$ in the univariate analysis were selected for inclusion in the multivariate logistic regression model. Only the significant factors (i.e., parameters with $P < 0.05$) from multivariate models are presented in tables. The results of the logistic regression are presented as odds ratios (OR), along with 95% confidence intervals (CI) and P-values.

3 Results

3.1 Trial characteristics

The dataset contains data from 2875 pediatric clinical trials. We identified 1787 pediatric clinical trials that did not meet the inclusion criteria for our analysis and 1088 that fulfilled inclusion criteria and were included in the analysis. As shown in Figure 1, there were five reasons for excluding trials. The most frequent reason was that the trial was not randomized (n = 852; 47.68%). Other reasons why trials were excluded: the trial was registered more than 60 days after the trial start day (n = 567; 37.73%), the trial was not a drug interventional trial (n = 323; 18.07%), final confirmed status was after December 31, 2013 (n = 28; 1.57%) and the last reason was that recruitment criteria did not meet inclusion criteria (n = 17; 0.95%). This last exclusion criterion was selected if the start date was earlier than 1.1.2011 or multiple exclusion criteria were present in one trial. The number of clinical trials that were included in our analysis is 1088.

3.2 Characteristics of the included pediatric RCTs

Of all included trials, 528 (48.53%) had an academic primary sponsor, and 491 (45.13%) trials had an industrial primary sponsor. The most represented age groups were combined (children + adult) (n = 558; 51.29%) and mixed age (n = 354; 32.54%) groups, followed by the preterm/newborn/infant group (n = 99; 9.10%) (Table 1). Certain infectious and parasitic diseases (17.10%) and diseases of the respiratory system were among the most common medical areas of interest (15.90%) (Table 2).

3.2.1 Uncompleted pediatric RCTs

One hundred eighty (16.54%) of included clinical trials (n = 1088) were uncompleted, with 61.11% (n = 110) terminated, 38.33% (n = 69) withdrawn, and one trial suspended.

The most common reasons for incompletion were patient accrual (32.22%), company decision (12.22%), and informative decision (11.67%) (Table 3), e.g., safety and efficacy reasons or interim analysis revealed a negative effect. Many clinical trials did

TABLE 1 Characteristics of completed and uncompleted pediatric RCTs.

	All included trials (*N* = 1,088)		Completed trials (*N* = 908)		Uncompleted trials (*N* = 180)		
	n	%	n	%	n	%	*p*-value
Year registered							0.4839
2011	397	36.49	338	37.22	59	32.78	
2012	394	36.21	327	36.01	67	37.22	
2013	297	27.30	243	26.76	54	30.00	
All	1,088	100.00	908	100.00	180	100.00	
Age of participant							0.2294
Preterm, newborn, infant	99	9.10	77	8.48	22	12.22	
Toddler and preschool	26	2.39	24	2.64	2	1.11	
School age	26	2.39	23	2.53	3	1.67	
Adolescent	25	2.30	18	1.98	7	3.89	
Mixed age	354	32.54	296	32.60	58	32.22	
Combined[a]	558	51.29	470	51.76	88	48.89	
All	1,088	100.00	908	100.00	180	100.00	
Funding							0.0441
Academic	528	48.53	427	47.03	101	56.11	
Industry	491	45.13	425	46.81	66	36.67	
Other[b]	69	6.34	56	6.17	13	7.22	
All	1,088	100.00	908	100.00	180	100.00	
Masking							0.3138
Open Label	254	23.35	206	22.69	48	26.67	
Single	85	7.81	69	7.60	16	8.89	
Double	173	15.90	145	15.97	28	15.56	
Triple	178	16.36	144	15.86	34	18.89	
Quadruple	398	36.58	344	37.89	54	30.00	
All	1,088	100.00	908	100.00	180	100.00	
Patients enrolled							< 0.0001
<50	356	32.72	210	23.13	146	81.11	
51–100	216	19.85	202	22.25	14	7.78	
101–500	338	31.07	322	35.46	16	8.89	
501–1,000	97	8.92	94	10.35	3	1.67	
>1,000	81	7.44	80	8.81	1	0.56	
All	1,088	100.00	908	100.00	180	100.00	
Planned sample size							0.0022
<50	238	21.88	182	20.04	56	31.11	
51–100	236	21.69	201	22.14	35	19.44	
101–500	422	38.79	349	38.44	73	40.56	
501–1,000	102	9.38	93	10.24	9	5.00	
>1,000	86	7.90	79	8.70	7	3.89	
UNK	4	0.37	4	0.44			
All	1,088	100.00	908	100.00	180	100.00	

(Continued)

TABLE 1 (Continued)

	All included trials (*N* = 1,088)		Completed trials (*N* = 908)		Uncompleted trials (*N* = 180)		
	n	%	n	%	n	%	*p*-value
Phase							0.0077
I	58	5.33	47	5.18	11	6.11	
II	291	26.75	231	25.44	60	33.33	
III	417	38.33	370	40.75	47	26.11	
IV	183	16.82	148	16.30	35	19.44	
UNK	139	12.78	112	12.33	27	15.00	
All	1,088	100.00	908	100.00	180	100.00	

[a]Children and adults. [b]Government-funded trials.

not have a reason for their incompletion stated in the registry, and the reason was not traceable even in publications (22.78%).

Combined age, mixed age, and preterm/newborn/infant age groups were most often represented (Table 1) in uncompleted clinical trials.

The relationship between reasons for trial incompletion and the number of enrolled patients is well illustrated in Table 4. Out of a total of 180 uncompleted trials, 92 of them were not published anywhere. The number of patients enrolled in these studies was 3267.

Statistically significant was also a difference in funding ($p = 0.0441$) (Table 1), with 101 (56.11%) uncompleted clinical trials receiving funding from an academic source, 66 (36.67%) uncompleted trials from industry, and 13 (7.22%) trials receiving funds from another source (e.g., government-funded studies).

The number of pediatric patients enrolled in uncompleted clinical trials was 9,904. This number was counted for terminated and suspended clinical trials only as no patients were recruited in the clinical trials with withdrawn status.

A statistically significant difference was found between completed and uncompleted trials in planned sample size ($p = 0.0022$), the number of patients enrolled ($p < 0.0001$), and the phase of the clinical trial ($p = 0.0077$).

3.3 Access to the results in completed pediatric RCTs

Overall, we have identified 908 completed pediatric RCTs in our research, where we searched for published results of these clinical trials. We focused on results recorded directly in clinical trial registries (ClinicalTrials.gov and EUCTR) and publications with clinical trial results in peer-reviewed journals. We have identified 531 (58.48%) completed pediatric RCTs that have posted results in ClinicalTrials.gov + EUCTR and 377 (41.52%) completed pediatric RCTs with no results in registers. The number of completed pediatric RCTs with trial results published in peer-reviewed journals found in Google Scholar/PubMed and ClinicalTrials.gov/EUCTR as well was 640 (70%) (Figure 2).

Table 5 presents an analysis of the relationship between the posting of clinical trial results on ClinicalTrials.gov and EUCTR (the European Clinical Trials Registry) and the presence of corresponding scientific publications identified in ClinicalTrials.gov, EUCTR, Google Scholar, or PubMed.

The data are divided into categories based on whether results were posted in the registries and whether a corresponding scientific publication was found. These findings are dependent parameters, as the Chi-Square test indicates (p-value is 0.0427):

1. Results Not Posted, No Scientific Publication: 125 clinical trials (13.77%) neither had results posted in ClinicalTrials.gov or EUCTR nor had any scientific publication associated with them.
2. Results Not Posted, Scientific Publication Found: 252 clinical trials (27.75%) did not have results posted in the registries but were found to have a corresponding scientific publication.
3. Results Posted, No Scientific Publication: 143 clinical trials (15.75%) had their results posted in the registries but did not have a corresponding scientific publication.
4. Results Posted, Scientific Publication Found: 388 clinical trials (42.73%) had both their results posted in the registries and a corresponding scientific publication.

For 504 (55.51%) completed clinical trials, the registry included a reference to a scientific publication with the clinical trials' results. The remaining 404 (44.49%) completed clinical trials were without any reference to scientific publication. Table 6 shows the relevance of the publications found in the registry to the clinical trial's outcomes, with 86.9% being relevant and 13.1% being classified as non-relevant.

The age of the participants ($p = 0.0255$), funding ($p < 0.0001$), masking ($p = 0.0234$), the patient enrolled ($p = 0.0096$), and phase ($p < 0.0001$) were found to be significant determinants of (not)posting results in ClinicalTrials.gov and EUCTR (Supplementary Table S1). In multivariate analysis, evaluating all factors together, funding, phase, and patient enrollment remained the factors associated with posting results in ClinicalTrials.gov and EUCTR (Table 7). Pediatric RCTs with industrial funding have a 3.3 times greater chance of being posted results than academic RCTs. Phase I clinical trials have a 78% lower chance of having published trial results in ClinicalTrials.gov/EUCTR compared to Phase III clinical trials. Clinical trials with more than 1,000 enrolled patients have a 63% lower chance of being posted in ClinicalTrial.gov and EUCTR compared to clinical trials with < 50 patients.

TABLE 2 Disease categories addressed in 1,088 included pediatric RCTs.

Condition category	n	%
Certain infectious and parasitic diseases	186	17.10
Diseases of the respiratory system	173	15.90
Endocrine, nutritional, and metabolic diseases	108	9.93
Mental and behavioral disorders	84	7.72
Symptoms, signs, and abnormal clinical and laboratory findings, not elsewhere classified	67	6.16
Diseases of the skin and subcutaneous tissue	51	4.69
Diseases of the nervous system	50	4.60
Diseases of the eye and adnexa	43	3.95
Diseases of the digestive system	41	3.77
Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism	35	3.22
Congenital malformations, deformations, and chromosomal abnormalities	33	3.03
Injury, poisoning and certain other consequences of external causes	33	3.03
Neoplasms	33	3.03
Certain conditions originating in the perinatal period	32	2.94
Diseases of the circulatory system	24	2.21
Diseases of the musculoskeletal system and connective tissue	22	2.02
Pregnancy, childbirth, and the puerperium	21	1.93
Diseases of the genitourinary system	18	1.65
External causes of morbidity and mortality	11	1.01
Diseases of the ear and mastoid process	10	0.92
Factors influencing health status and contact with health services	7	0.64
Metabolic disorders	2	0.18
Unknown	2	0.18
Diseases of oral cavity, salivary glands and jaws	1	0.09
Other and unspecified disorders of the circulatory system	1	0.09

TABLE 3 Reasons for clinical trial incompletion.

Uncompleted trials (N = 180)	n	%
Patient accrual	58	32.22
Nonreported or unclear	41	22.78
Company/business decision	22	12.22
Informative termination[a]	21	11.67
Funding issue	13	7.22
Conduct problems[b]	10	5.56
Regulatory issue[c]	8	4.44
Principle investigator left	7	3.89

[a]Includes changes in standard of care and safety or efficacy findings or interim analysis revealed a negative effect. [b]Includes technical difficulties and logistical issues. [c]Includes issues with institutional review board or other regulatory body.

There is a statistically significant difference between completed clinical trials with and without scientific publication found in the age of the participants (p = 0.0096), the patient enrolled ($p < 0.0001$) and planned sample size ($p < 0.0001$) (Supplementary Table S2). Funding was evaluated as probably not statistically significant in this case. In Supplementary Table S2, we can see a higher percentage of scientific publications in academic pediatric RCT in comparison with industry sponsors.

In multivariate analysis, masking, participant age, and patient enrollment were significant factors in evaluating all factors together (Table 8). Pediatric RCTs with more than 1000 enrolled patients have a 4.8x higher chance of publishing scientific publications than clinical trials with < 50 patients. RCTs involving school-age participants have a 75% lower chance of having found their scientific publication compared to RCTs involving only preterm, newborn, or infant participants.

3.3.1 Time to publication of results in the completed pediatric RCTs

For completed clinical trials, the median time from the end of the trial to the first publication of results in ClinicalTrials.gov/EUCTR was 21 months (min/max: −35.93/119.77). There was no statistically significant difference between parameters in completed clinical trials that posted results within 12 months or after 12 months in ClinicalTrials.gov/EUCTR.

The median time from the end of the clinical trial to the publication of the results in peer-reviewed journals was almost 27 months (min/max: −17.97/108.79) for completed clinical trials). After focusing on statistically significant differences in some parameters of completed studies, in multivariate analysis, funding, phase, and patient enrollment remained the significant factors associated with publication within 12 months (Table 9). Trials with industrial funding have a 79% lower chance of being published within 12 months than academic clinical trials. In some cases, results or publications in peer-reviewed journals were released before the clinical trial was completed. Reasons for that might be that the sponsor or investigator had submitted the results from interim analysis into registries or to a journal.

3.3.2 Access to the results in uncompleted pediatric RCTs

Reporting of results of uncompleted pediatric RCTs was analyzed, and 62 (56.36%) of 110 terminated RCTs reported the results in registries. Almost 23% (n = 25) of uncompleted RCTs had a relevant publication with clinical trial results found in a peer-reviewed journal. Three publications did not mention that the clinical trial was terminated early, yet they reported the results without any warning. In contrast, the remaining articles consistently highlighted the limitations.

TABLE 4 Reasons for trial incompletion and number of enrolled patients in terminated and suspended trials.

Terminated and suspended (N = 111)[a]	Number of enrolled patients				
Reason for a trial incompletion	<50	51–100	101–500	501–1,000	>1,000
	n (%)	n (%)	n (%)	n (%)	n (%)
Patient accrual	40 (36.04)	4 (3.60)	1 (0.90)	1 (0.90)	–
Informative termination	9 (8.11)	5 (4.50)	5 (4.50)	–	–
Nonreported or unclear	9 (8.11)	2 (1.80)	4 (3.60)	–	1 (0.90)
Company/business decision	6 (5.41)	1 (0.90)	4 (3.60)	1 (0.90)	–
Conduct problems	4 (3.60)	1 (0.90)	1 (0.90)	1 (0.90)	–
Principle investigator left	3 (2.70)	1 (0.90)	–	–	–
Regulatory issue	3 (2.70)	–	1 (0.90)	–	–
Funding issue	3 (2.70)	–	–	–	–

[a]Definition for a withdrawn trial is: a clinical trial stopped early, before enrolling its first participant. This is a reason for not including them in this table.

4 Discussion

Our analysis focused on RCTs in the pediatric population with drug intervention completed or finished by the end of year 2017.

Despite efforts to raise awareness of this issue through previously published work (4), the number of uncompleted RCTs in the pediatric population is increasing. Pica et al. (16) found that 10% of RCTs were not completed in the previous review period. According to our findings, the number of incomplete RCTs actually increased to 16.5% in the subsequent follow-up period. The most common reason for incompletion was insufficient patient recruitment, which remains the same identified factor in the previous period by Pica and Bourgeois (16). In more than half of the discontinued trials, a total of ten thousand patients were recruited. Despite the short duration of the clinical trials we analyzed, this figure is quite alarming. It is evident, that the conduct of pediatric clinical trials deserves increased attention. Although the current legal framework states the necessity of publishing results, just over half of the completed clinical trials had their results listed in registries (ClinicalTrials.gov and EUCTR), and only 20% had results published in registries within 12 months of clinical trial completion. A significant proportion of trials had results registered four years or more after the clinical trial completion date (17%). Interestingly, 70% of completed clinical trials had results traceable to peer-reviewed journals. Only 15% of pediatric RCTs had published results in scientific articles within 12 months. Similar to results published in registries, we found a high number of clinical trials (20%) with the first publication of results in scientific journals four years or more after clinical trial completion.

Although our analysis categorized pediatric trials consistently with previous research, it is important to recognize that the pediatric population encompasses a wide range of developmental stages, from pre-term neonates and infants to adolescents. These different age groups can vary substantially in terms of enrollment feasibility, consent and assent requirements, pharmacokinetics, pharmacodynamics, and disease characteristics. Such differences may influence both trial design and outcomes, including recruitment speed, treatment efficacy, and safety profiles. Due to limited age-specific detail in registry data, further subgroup analysis was not feasible, but we highlight this as a relevant consideration for future studies (3.1 Trial Characteristics). We

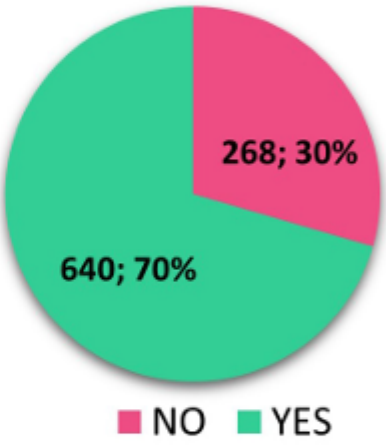


FIGURE 2
Scientific publication found in ClinicalTrials.gov/EUCTR and/or in Google Scholar/PubMed.

decided to use the register ClinicalTrials.gov as the source data for our analysis. This register includes a vast number of clinical trials from around the world, not just from the United States, making it more comprehensive and globally relevant compared to the EUCTR register, which is focused only on clinical trials within the European Union. This broader scope provided a wider data set for our research. The data structure and presentation are detailed, the register's environment is user-friendly, and the choice of filters allowed us to obtain the data set that we needed for our analyses.

Failure to complete a clinical trial always causes significant financial, time, and ethical losses. The reasons for early termination can be heterogeneous (3.2.1 Uncompleted pediatric RCTs). The most frequently cited reason in registries was low patient recruitment. This reason has remained unchanged for many years and has been mentioned in previous trials as a major challenge

TABLE 5 Results posted in ClinicalTrials.gov + EUCTR and Scientific publications found in ClinicalTrials.gov + EUCTR and/or in Google Scholar/PubMed.

Results posted in ClinicalTrials.gov + EUC	Scientific publication found in ClinicalTrials.gov + EUCTR and/or in Google Scholar/PubMed		
	No	Yes	Total
No	125 13.77%	252 27.75%	377 41.52%
Yes	143 15.75%	388 42.73%	531 58.48%
Total	268 29.52%	640 70.48%	908 100.00%

in pediatric clinical trials (4, 16, 20, 21). Compared to the adult population, this particular barrier may be explained by the low number of pediatric patients for a given disease and the process of obtaining informed consent, which has been more complex in children (22, 23). Although new legislation in the EU is already fully in place, allowing a child to be enrolled in a trial on the basis of consent from only one parent, in some countries, both parents still need to give informed consent, which can have a negative impact on overall trial recruitment. Public funding agencies, institutional review boards, and ethics committees should also require investigators to provide empirical evidence to support the feasibility of achieving the target sample size within an acceptable recruitment period: published or at least registered pilot clinical trials that include obtaining informed consent from participants are likely to provide the best evidence of the feasibility of the clinical trial protocol and provide the most realistic estimates for participant recruitment (24).

There is also increasing recognition of the importance of involving children and families in recruitment, consent, and study design (3, 25). This approach can strongly contribute to the feasibility and clinical trial completion.

To overcome the problem of small sample sizes in children, significant advances have been made in the development of statistical methods and in the collaboration of dedicated international groups and networks of pediatric clinical trials that pool their data and resources. Including the European Clinical Research Infrastructure Network (ECRIN) in the preparation and conduct of multicenter and multinational clinical trials, particularly those involving pediatric populations, represents a significant advantage. All these factors can improve the feasibility of the clinical trial.

Commercial RCTs are more likely to be completed. This is consistent with previous research performed by Pica and Bourgeois (16) And Rees et al. (26). One of the factors could be the interest of the pharmaceutical company to complete the clinical trial and get their product on the market. Financial considerations may play an important role here when in commercial clinical trials, the investigators are used to get fees for their scientific work, which increases the motivation of the trialists. Moreover, pharmaceutical companies are more familiar with involving patient advocates in the planning and organizing of clinical trials and usually have more developed technical infrastructure. On the other hand, with academic clinical trials, research is often funded by public funds and foundations, which have time and financial limits that can be challenging to meet.

TABLE 6 References to publications listed in the ClinicalTrials.gov registry and publication's relevance.

	n	%
No links to publications	404	44.49
Links to publications presented	504	55.51
Relevant	438	86.90
Not relevant	66	13.10

TABLE 7 Significant factors associated with posting results in ClinicalTrials.gov + EUCTR.

Parameter	OR (95% CI)	*p*-value
Funding industry vs. academic	3.332 (2.344; 4.737)	<0.0001
Phase I vs. III	0.223 (0.109; 0.456)	<0.0001
Patient enrolled > 1,000 vs. <50	0.369 (0.204; 0.669)	0.0010

A previous research study published in Pediatrics that reviewed interventional pediatric clinical trials registered with ClinicalTrials.gov between 2008 and 2010 indicates that 29.8% of completed trials remained unpublished in peer-reviewed journals (16). In our research, which loosely followed the above study with an analysis of completed trials registered in the same registry between 2011 and 2013, 30% of pediatric RCTs had no published results in scientific journals and 42% in registries (3.3 Access to the results in completed pediatric RCTs). Similar findings are reported in another study where 50% of the trials registered in EUCTR as completed or terminated by 2017 had published results in the registry (9). The same collective of authors from The Bennett Institute for Applied Data Science at the University of Oxford further addressed this topic and created websites (27) with tools that clearly show the number of published and unpublished clinical trial results by sponsors in the registries. In other words, these monthly updated trackers monitor the compliance of every individual sponsor from the ranks of pharmaceutical companies, universities, hospitals or foundations with FDAAA 2007 (FDAAA TrialsTracker) (28) and EU rules (EU Trials Tracker) (29). Brewster and colleagues found that only 23.5% of completed pediatric clinical trials registered between 2007 and 2020 reported results on the ClinicalTrials.gov registry, and 38.8% of the clinical trials were published in a peer-reviewed journal (4).

TABLE 8 Significant factors associated with having a scientific publication found in ClinicalTrials.gov + EUCTR and/or in Google Scholar/PubMed.

Parameter	OR (95% CI)	*p*-value
Masking double vs. open label	0.596 (0.368; 0.965)	0.0354
Patient enrolled 51–100 vs. < 50	1.929 (1.266; 2.939)	0.0022
Patient enrolled 101–500 vs. < 50	2.354 (1.580; 3.509)	<0.0001
Patient enrolled 501–1,000 vs. < 50	2.481 (1.388; 4.432)	0.0022
Patient enrolled > 1,000 vs. < 50	4.847 (2.351; 9.990)	<0.0001
Age of participant combined vs. preterm, newborn, infant	0.361 (0.185; 0.704)	0.0028
Age of participant school age vs. preterm, newborn, infant	0.245 (0.085; 0.706)	0.0092

TABLE 9 Significant factors associated with the trial's result publishing within 12 months.

Parameter	OR (95% CI)	*p*-value
Funding industry vs. academic	0.210 (0.119; 0.372)	<0.0001
Patient enrolled > 1000 vs. < 50	2.675 (1.229; 5.824)	0.0132
Phase II vs. I	0.312 (0.120; 0.806)	0.0162
Phase IV vs. I	0.157 (0.055; 0.451)	0.0006

Our analysis also shows that industry-funded trials were more likely to have their results published in registries and less likely to be published in peer-reviewed journals than academic clinical trials. The disparity in the publication of results between non-commercial and commercial sponsors is highlighted in the Joint Letter by the European Commission, EMA, and HMA from April 2019. The letter first points to the overall compliance with the publication rules, with 68% of completed RCTs publishing their results. Further evaluation of this group by the sponsor shows a disproportion between industry sponsors and academic sponsors in reporting compliance (77% for commercial sponsors vs. 23% for academic sponsors) (30).

It is likely that academic sponsors are unaware of their obligations or do not have administrative procedures or sources in place to highlight non-compliance. To be compliant with the legislative requirements and good clinical practice, the sponsor is responsible for reporting the clinical trial results to the registry. However, in practice, in the case of hospitals acting as sponsors of an academic clinical trial, the principal investigator often becomes responsible and may not be sufficiently informed of the reporting obligation (9). Pfizer has reported that the preparation of results summaries requires 4–60 h, and it is possible that the academic sponsor doesn't have administrative sources available to ensure reporting on time (31). Another significant reason could be the incentive structure. Commercial sponsors, such as pharmaceutical companies, are often motivated to publish results due to the financial implications of drug development and market approval. Regulatory bodies (FDA or EMA) enforce strict guidelines, and failure to comply may lead to penalties or delays in product approval. Furthermore, academic researchers may focus on publishing in high-impact journals to advance their careers or secure further funding. As a result, registry submissions, which may not carry the same academic recognition, are sometimes deprioritized. Caution should be exercised when interpreting the results of prematurely terminated and, therefore, underpowered pediatric RCTs. Nevertheless, their results can still be considered valid and could potentially contribute valuable pilot data for future studies. In addition, these findings could influence systematic reviews and meta-analyses (24).

There is no doubt that it is necessary to report the results of studies in registries according to the instructions and requirements of each registry. On the other hand, we believe that publishing results in peer-reviewed journals is more readable and accessible to the scientific community. But for the patient community, it is hardly accessible, mainly because of non-open access articles or the requirement to be a health professional. Ideally, the clinical trial sponsor would share the positive or negative results of the trial with the scientific and lay public community in both forms (results in the registry + scientific publication). However, almost 14% of completed pediatric RCTs did not publish their results in either a registry or a scientific journal. This completely contradicts the ethical commitment to participants who agreed to contribute to scientific progress in the expectation that their participation would produce publicly available knowledge (19). Specifically, academic and other academic sponsors should be encouraged to publish the results of their trials in registries in order to maximize their valuable contribution to meeting public health needs and to the advancement of clinical research, especially where there are fewer commercial interests (30).

As mentioned previously, timely reporting of clinical trial results is also a legal requirement. Our results show a median time for results publication of 21 months (3.3.1 Time to publication of the result in completed pediatric RCTs). Anderson's study focusing on compliance with result reporting at ClinicalTrials.gov describes a median time of 17 months (31). While prompt dissemination of clinical trial data ensures that researchers, healthcare providers, and policy-makers can access the most current evidence, enabling informed decision-making and fostering evidence-based clinical practices. In addition, timely reporting may reduce the risk of redundant research (32).

We believe that the public ranking of sponsors' performance in registry reporting, carried out through the work of the Bennett Institute's team of experts, and the dissemination of information related to clinical trial registration and publication of clinical trial results provided by the AllTrials.net initiative (33) is meaningful. This may represent one way to encourage sponsors (e.g., to avoid reputational damage) not to underestimate the reporting of study results. Another approach to improving compliance with EU and US regulations on reporting study results would be for national regulatory authorities to conduct open public audits of compliance and regularly fine or withhold funding from those violating these ethical and legal rules.

In summary, our analysis maps the current trends of a number of pediatric clinical trials, highlighting significant challenges in their completion and publication of their results related to this vulnerable population. The unique ethical, regulatory, and logistical hurdles associated with pediatric research—such as recruitment difficulties, ethical considerations related to informed consent, and the complexity of pediatric clinical trial design—contribute to a higher rate of uncompleted RCTs compared to adult RCTs. Furthermore, our findings indicate substantial delays or outright omissions in the reporting of pediatric RCT results, both in clinical trial registries and in peer-reviewed journals. This underreporting compromises the transparency and availability of evidence necessary to make a reasonable decision in clinical practice and policy in pediatric care.

The discrepancies in reporting, particularly among academic sponsors, suggest that further incentives or enforcement mechanisms may be required to ensure the timely dissemination of clinical trial results. Addressing these barriers is crucial to improving pediatric research outcomes, fostering greater trust in the clinical trial process, and ensuring that children benefit from advancements in medical science. Future efforts should focus on enhancing regulatory oversight, streamlining clinical trial processes, and encouraging broader compliance with reporting rules to advance the quality and impact of pediatric clinical research.

Data availability statement

The original contributions presented in this study are included in this article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

KN: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. LS: Conceptualization, Methodology, Supervision, Writing – review & editing. RS: Data curation, Formal Analysis, Methodology, Software, Writing – review & editing. RD: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2025.1590125/full#supplementary-material

SUPPLEMENTARY TABLE S1
Posting of results in ClinicalTrials.gov + EUCTR.

SUPPLEMENTARY TABLE S2
Scientific publication found in ClinicalTrials.gov/EUCTR and/or in Google Scholar/PubMed.

SUPPLEMENTARY TABLE S3
Protocol. Prospective protocol for data extraction, classification, and analysis.

References

1. European Commission. *2017 Commission Report on the Paediatric Regulation*. (2024). Available online at: https://health.ec.europa.eu/medicinal-products/medicines-children/2017-commission-report-paediatric-regulation_en (accessed May 27, 2024).

2. European Commission. *State of Children's Medicines in the EU*. (2017). Available online at: https://ec.europa.eu/commission/presscorner/detail/en/ip_17_4121 (accessed September 17, 2024).

3. McCune S, Portman R. Accelerating pediatric drug development: A 2022 special issue of therapeutic innovation & regulatory science. *Ther Innov Regul Sci*. (2022) 56:869–72. doi: 10.1007/s43441-022-00398-w

4. Brewster R, Wong M, Magnani C, Gunningham H, Hoffer M, Showalter S, et al. Early discontinuation, results reporting, and publication of pediatric clinical trials. *Pediatrics*. (2022) 149:e2021052557. doi: 10.1542/peds.2021-052557

5. Alotaibi T. Ethical challenges with the informed consent process in pediatric research studies. *Med Arch.* (2024) 78:65–7. doi: 10.5455/medarh.2024.78.65-67

6. Schrier L, Illy K, Valialis A, Wyder C, Stiris T. EAP viewpoint on unpublished data from paediatric clinical trials. *Eur J Pediatr.* (2018) 177:275–7. doi: 10.1007/s00431-017-3005-x

7. Official Journal of the European Union. *Commission Guideline — Guidance on Posting and Publication of Result-Related Information on Clinical Trials in Relation to the Implementation of Article 57(2) of Regulation (EC) No 726/2004 and Article 41(2) of Regulation (EC) No 1901/2006.* (2012). Available online at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52012XC1006(01) (accessed October 6, 2012).

8. ClinicalTrials.gov. *F 801 and the Final Rule.* (2024). Available from: https://clinicaltrials.gov/policy/fdaaa-801-final-rule (accessed Sep 3, 2024).

9. Goldacre B, DeVito N, Heneghan C, Irving F, Bacon S, Fleminger J, et al. Compliance with requirement to report results on the EU Clinical Trials Register: Cohort study and web resource. *BMJ.* (2018) 362:k3218. doi: 10.1136/bmj.k3218

10. DeVito N, Bacon S, Goldacre B. Compliance with legal requirement to report clinical trial results on ClinicalTrials.gov: A cohort study. *Lancet.* (2020) 395:361–9. doi: 10.1016/S0140-6736(19)33220-9

11. Benjamin D, Smith P, Murphy M, Roberts R, Mathis L, Avant D, et al. Peer-reviewed publication of clinical trials completed for pediatric exclusivity. *JAMA J Am Med Assoc.* (2006) 296:1266–73. doi: 10.1001/jama.296.10.1266

12. Wan M, Alessandrini E, Brogan P, Eleftheriou D, Warris A, Brüggemann R, et al. Risk-proportionate approach to paediatric clinical trials: The legal requirements, challenges, and the way forward under the European Union Clinical Trials Regulation. *Clin Trials.* (2022) 19:573–8. doi: 10.1177/17407745221093812

13. ClinicalTrials.gov. *Trends and Charts on Registered Studies.* (2024). Available online at: https://clinicaltrials.gov/about-site/trends-charts (accessed May 27, 2024).

14. ClinicalTrials.gov. *ClinicalTrials.gov Glossary Terms.* (2024). Available online at: https://clinicaltrials.gov/study-basics/glossary (accessed May 27, 2024).

15. Jones C, Handler L, Crowell K, Keil L, Weaver M, Platts-Mills T. Non-publication of large randomized clinical trials: Cross sectional analysis. *BMJ.* (2013) 347:f6104. doi: 10.1136/bmj.f6104

16. Pica N, Bourgeois F. Discontinuation and nonpublication of randomized clinical trials conducted in children. *Pediatrics.* (2016) 138:e20160223. doi: 10.1542/peds.2016-0223

17. Madeira, C, Hořavová L, dos Santos F, Batuca J, Nebeska K, Součková L, et al. Investigator initiated clinical trials (IICTs): A systematic search in registries to compare the Czech Republic and Portugal in terms of funding policies and scientific outcomes. *Ther Innov Regul Sci.* (2021) 55:966–78. doi: 10.1007/s43441-021-00293-w

18. ICD-10 Version:2019. *International Statistical Classification of Diseases and Related Health Problems 10th Revision.* (2023). Available online at: https://icd.who.int/browse10/2019/en (accessed October 3, 2023).

19. Ross J, Mocanu M, Lampropulos J, Tse T, Krumholz H. Time to publication among completed clinical trials. *JAMA Intern Med.* (2013) 173:825–8. doi: 10.1001/jamainternmed.2013.136

20. Chaudhuri N, Ravi R, Goglay N, Thatte U. Recruitment and retention of the participants in clinical trials: Challenges and solutions. *Perspect Clin Res.* (2020) 11:64–9. doi: 10.4103/picr.PICR_206_19

21. Probstfield J, Frye R. Strategies for recruitment and retention of participants in clinical trials. *JAMA.* (2011) 306:1798–9. doi: 10.1001/jama.2011.1544

22. Laventhal N, Tarini B, Lantos J. Ethical issues in neonatal and pediatric clinical trials. *Pediatr Clin North Am.* (2012) 59:1205–20. doi: 10.1016/j.pcl.2012.07.007

23. Ungar D, Joffe S, Kodish E. Children are not small adults: Documentation of assent for research involving children. *J Pediatr.* (2006) 149(1 Suppl):S31–3. doi: 10.1016/j.jpeds.2006.04.048

24. Amstutz A, Schandelmaier S, Frei R, Surina J, Agarwal A, Olu K, et al. Discontinuation and non-publication of randomised clinical trials supported by the main public funding body in Switzerland: A retrospective cohort study. *BMJ Open.* (2017) 7:e016216. doi: 10.1136/bmjopen-2017-016216

25. Joseph P, Craig J, Caldwell P. Clinical trials in children. *Br J Clin Pharmacol.* (2015) 79:357–69. doi: 10.1111/bcp.12305

26. Rees C, Pica N, Monuteaux M, Bourgeois F. Noncompletion and nonpublication of trials studying rare diseases: A cross-sectional analysis. *PLoS Med.* (2019) 16:e1002966. doi: 10.1371/journal.pmed.1002966

27. AllTrials.net. *The TrialsTracker Project.* (2024). Available online at: https://www.trialstracker.net/ (accessed May 16, 2024).

28. FDAAA Tracker. *Who's Sharing their Clinical Trial Results?.* (2024). Available online at: https://fdaaa.trialstracker.net/ (accessed August 30, 2024).

29. Eu Trials Tracker. *Who's not Sharing Clinical Trial Results?.* (2024). Available online at: http://eu.trialstracker.net/ (accessed August 30, 2024).

30. EMA and HMA. *Joint Letter by the European Commission, EMA and HMA.* (2019). Available online at: https://eudract.ema.europa.eu/docs/guidance/1R%20-%20Joint%20letter%20signed%20by%20EC,%20EMA,%20HMA_en.pdf (accessed June 20, 2019).

31. Anderson M, Chiswell K, Peterson E, Tasneem A, Topping J, Califf R. Compliance with results reporting at ClinicalTrials.gov. *N Engl J Med.* (2015) 372:1031–9. doi: 10.1056/NEJMsa1409364

32. Prayle A, Hurley M, Smyth A. Compliance with mandatory reporting of clinical trial results on ClinicalTrials.gov: Cross sectional study. *BMJ.* (2012) 344:d7373. doi: 10.1136/bmj.d7373

33. AllTrials. *All Trials Registered. All Results Reported.* (2024). Available online at: https://www.alltrials.net/ (accessed August 30, 2024).

7.13 Annex 13

Rose AM, Nicolay N, Sandonis Martín V, Mazagatos C, Petrović G, Niessen FA, Machado A, Launay O, Denayer S, Seyler L, Baruch J, Burgui C, Loghin II, Domegan L, Vaikutytė R, Husa P, Panagiotakopoulos G, Aouali N, Dürrwald R, Howard J, Pozo F, Sastre-Palou B, Nonković D, Knol MJ, Kislaya I, Luong Nguyen LB, Bossuyt N, Demuyser T, Džiugytė A, Martínez-Baz I, Popescu C, Duffy R, Kuliešė M, Součková L, Michelaki S, Simon M, Reiche J, Otero-Barrós MT, Lovrić Makarić Z, Bruijning-Verhagen PC, Gomez V, Lesieur Z, Barbezange C, Van Nedervelde E, Borg ML, Castilla J, Lazar M, O'Donnell J, Jonikaitė I, Demlová R, Amerali M, Wirtz G, Tolksdorf K, Valenciano M, Bacci S, Kissling E; I-MOVE-COVID-19 hospital study team; VEBIS hospital study team; Members of the I-MOVE-COVID-19 and VEBIS hospital study teams (in addition to the named authors). Vaccine effectiveness against COVID-19 hospitalisation in adults (≥ 20 years) during Alpha- and Delta-dominant circulation: I-MOVE-COVID-19 and VEBIS SARI VE networks, Europe, 2021. Euro Surveill. 2023 Nov;28(47):2300186. doi: 10.2807/1560-7917.ES.2023.28.47.2300186. PMID: 37997666; PMCID: PMC10668259.

Summary
Purpose:
The aim of this study was to estimate the effectiveness of COVID-19 vaccines in preventing hospitalisation among adults during periods dominated by Alpha and Delta SARS-CoV-2 variants in Europe. The study also sought to generate robust real-world evidence to inform vaccination strategies and public health decision-making during evolving pandemic phases.

Methodology:
The study used a multicentre, multinational test-negative case-control design, conducted within the I-MOVE and VEBIS hospital surveillance networks across multiple European countries. Adult patients hospitalised with severe acute respiratory infection (SARI) were systematically tested for SARS-CoV-2, with positive cases compared to test-negative controls; vaccine effectiveness was estimated using adjusted logistic regression models accounting for confounders such as age, comorbidities, study site, and calendar time.

Results:
The analysis included several thousand hospitalised patients and demonstrated that COVID-19 vaccination provided high protection against hospitalisation, particularly after completion of the primary vaccination schedule. Vaccine effectiveness was generally higher during Alpha circulation and slightly reduced during Delta dominance, but remained substantial across age groups and settings.

Conclusion:
The study confirms that COVID-19 vaccines are highly effective in preventing severe disease requiring hospitalisation in real-world European populations, even during variant transitions. It also highlights the robustness of the test-negative design within coordinated

surveillance networks as a key methodological approach for generating timely, policy-relevant evidence in NCCTs.

RESEARCH

Vaccine effectiveness against COVID-19 hospitalisation in adults (≥ 20 years) during Alpha- and Delta-dominant circulation: I-MOVE-COVID-19 and VEBIS SARI VE networks, Europe, 2021

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Introduction: Two large multicentre European hospital networks have estimated vaccine effectiveness (VE) against COVID-19 since 2021. **Aim:** We aimed to measure VE against PCR-confirmed SARS-CoV-2 in hospitalised severe acute respiratory illness (SARI) patients ≥ 20 years, combining data from these networks during Alpha (March–June)- and Delta (June–December)-dominant periods, 2021. **Methods:** Forty-six participating hospitals across 14 countries follow a similar generic protocol using the test-negative case–control design. We defined complete primary series vaccination (PSV) as two doses of a two-dose or one of a single-dose vaccine ≥ 14 days before onset.

Results: We included 1,087 cases (538 controls) and 1,669 cases (1,442 controls) in the Alpha- and Delta-dominant periods, respectively. During the Alpha period, VE against hospitalisation with SARS-CoV2 for complete Comirnaty PSV was 85% (95% CI: 69–92) overall and 75% (95% CI: 42–90) in those aged ≥ 80 years. During the Delta period, among SARI patients ≥ 20 years with symptom onset ≥ 150 days from last PSV dose, VE for complete Comirnaty PSV was 54% (95% CI: 18–74). Among those receiving Comirnaty PSV and mRNA booster (any product) ≥ 150 days after last PSV dose, VE was 91% (95% CI: 57–98). In time-since-vaccination analysis, complete all-product PSV

VE was > 90% in those with their last dose < 90 days before onset; ≥ 70% in those 90–179 days before onset. Conclusions: Our results from this EU multi-country hospital setting showed that VE for complete PSV alone was higher in the Alpha- than the Delta-dominant period, and addition of a first booster dose during the latter period increased VE to over 90%.

Introduction

COVID-19 has caused considerable morbidity and mortality in Europe since March 2020. International collaboration accelerated COVID-19 vaccine development and within the European Union (EU)/European Economic Area (EEA), by the end of December 2021, there were five COVID-19 vaccines authorised for use with conditional marketing [1,2]: two are spike protein-based mRNA vaccines: Comirnaty (BNT162b2; Pfizer-BioNTech) and Spikevax (mRNA-1273; Moderna); two are spike-protein-based adenoviral vector vaccines: Vaxzevria (ChAdOx1; AstraZeneca) and Jcovden (Ad26.COV 2.5; Johnson & Johnson); one is a subunit vaccine: Nuvaxovid (NVX-CoV2373; Novavax).

Earlier in 2021, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Alpha variant was dominant in Europe, superseded by the Delta variant during the summer months. High efficacy was observed in randomised controlled trials of COVID-19 vaccines carried out before Alpha or Delta circulation [3-6]. However, evaluating the real-world COVID-19 vaccine performance is critical for understanding and evaluating vaccination programmes [7,8].

The I-MOVE (Influenza – Monitoring Vaccine Effectiveness in Europe) network expanded in 2020 to include monitoring of COVID-19 vaccine effectiveness (VE) and became the I-MOVE-COVID-19 network. The I-MOVE-COVID-19 and Vaccine Effectiveness, Burden and Impact Studies (VEBIS) networks in Europe have carried out hospital-based COVID-19 VE studies since 2021. Both networks use common generic protocols [9,10] and the test-negative case–control design.

We estimated VE against hospitalisation with PCR-confirmed SARS-CoV-2. In the absence of complete genetic sequencing information, we aimed to investigate VE during the Alpha and Delta periods between March and December 2021.

Methods

Setting and study period

There were 15 I-MOVE-COVID-19 and VEBIS hospital VE study sites from 14 European countries participating in the two networks during the study period from 2 March to 25 December 2021 (Figure 1), including 46 hospitals.

We defined the Alpha- and Delta-dominant periods as weeks where ≥ 80% of variants sequenced were Alpha or Delta, respectively, in each country using GISAID data [11]. We included data only from these weeks: 3 March to 11 June for Alpha, and 21 June to 25 December for Delta dominance.

Study design and case definitions

We used the test-negative case–control design [12], in which we defined cases as SARI patients testing SARS-CoV-2-positive by RT-PCR within 48 h of hospital admission or within the previous 14 days. Controls were SARI patients testing PCR-negative for SARS-CoV-2 within 48 h of admission. We used the ECDC definition for a possible COVID-19 case to define our study population of SARI patients (individuals hospitalised for at least 24 h, presenting with fever or cough or shortness of breath or sudden onset anosmia, ageusia, or dysgeusia) [13].

Study sites adapted their I-MOVE-COVID-19 or VEBIS VE network hospital study protocols [9,10] to their country-specific setting, as appropriate. Eleven sites included all SARI patients admitted to participating hospitals. The participating hospital in Belgium, the two in Lithuania and the over 20 hospitals in participating Spanish regions (except Navarra) included all SARI patients admitted on either 1 or 2 days each week. One site (the Netherlands) included a maximum of 10 SARI patients per week.

Study site teams collected demographic data (age, sex), clinical data (chronic conditions) and COVID-19 vaccination information via questionnaire, electronic medical records, vaccine registries or patient interview.

Inclusion and exclusion criteria

We included patients aged 20 years and over who belonged to the age-specific target group for COVID-19 vaccination of their country at time of swab. We excluded patients with missing/erroneous key variables (age, sex, key dates, vaccination information). Study sites excluded patients with contraindications for vaccination.

Definitions of vaccination status

We defined SARI patients as partially vaccinated with primary series vaccination (PSV) 14 days after receiving one of two recommended doses of a two-dose vaccine; as completely vaccinated with PSV 14 days after receiving either the second of two recommended doses of a two-dose vaccine, or a single dose of JCovden; as having received a complete PSV plus booster dose 14 days after receiving the first mRNA booster; as unvaccinated if they did not receive any COVID-19 vaccine or were vaccinated with the first dose of a two-dose vaccine (or a single dose of JCovden) on or after the date of symptom onset. Those vaccinated with the second dose of a two-dose vaccine on or after their date of onset were recoded as partially vaccinated. Anyone vaccinated 1–14 days before onset was excluded. All analyses used unvaccinated as the comparison unexposed group (Table 1).

KEY PUBLIC HEALTH MESSAGE

What did you want to address in this study?
To understand how well the COVID-19 vaccine was performing in Europe against hospitalisation during SARS-CoV-2 Alpha and Delta variant periods, we present vaccine effectiveness results from a multi-country study of complete and booster dose COVID-19 vaccination among adults (aged 20 years and over).

What have we learnt from this study?
Between March and June 2021 (Alpha period), vaccine effectiveness against hospitalisation with laboratory-confirmed SARS-CoV-2 was 43% for partial vaccination and 86% for complete vaccination. For June to December 2021 (Delta period), vaccine effectiveness for complete vaccination was lower (52%) but with addition of an mRNA booster dose, effectiveness reached 91%, and remained >90% up to 119 days after the booster dose.

What are the implications of your findings for public health?
In Europe in 2021, COVID-19 vaccine effectiveness results for the Alpha period indicated an excellent benefit for preventing hospitalisation after complete vaccination. During Delta variant circulation, however, a booster dose was required to achieve this level of effectiveness, and this was maintained for up to 4 months post booster.

For this analysis, we excluded those who had heterologous primary series doses or at least one unknown primary series product and we only included patients receiving the same product for both PSV doses (homologous PSV).

Statistical analysis

We compared the odds of vaccination between cases and controls using logistic regression, calculating VE as 1 minus the OR of vaccination among cases and controls (expressed as a percentage). We included study site (as a fixed effect) and date of swab (modelled as a spline or categorical variable, with the best functional form designated by the Akaike information criterion) in all VE analyses.

We further adjusted the OR for age, sex and presence of at least one of the four commonly collected and COVID-19-relevant chronic conditions (asthma, diabetes, heart disease and lung disease).

For the age-specific analyses, we stratified the data into three age groups: 20–59, 60–79 and ≥ 80 years. We also performed stratified analyses by sex and by presence of at least one chronic condition (vs no chronic conditions). In time-since-vaccination analyses for the Delta period, we measured VE by days since last PSV dose (for those with complete PSV) and by days since mRNA booster dose (for those with complete PSV plus first booster), for 30-, 60- and 90-day periods.

During the Alpha period, we estimated VE against hospitalisation with COVID-19 in SARI patients for partial and complete PSV (Table 1; Analysis 1).

During the Delta period, we estimated VE against hospitalisation in SARI patients with COVID-19, restricting most analyses to patients eligible for a booster dose (i.e. who were in the age-specified booster dose target group based on national recommendations; Table 1). We estimated VE in all those with complete PSV without booster dose who had received their last PSV dose at least 150 days before onset (Group A; Analysis 2), and in those with a first mRNA booster, who had ≥ 150 days between their last PSV dose and the booster dose (Group B; Analysis 2). The 150-day restriction in Group A was used to facilitate comparison with Group B (on average, participating countries recommended first booster dose to be 5 months after last PSV dose). This analysis was conducted for the Delta period because the long duration of circulation resulted in longer delays from last PSV dose to onset.

In Analysis 3, we estimated VE for those receiving PSV only, in different vaccination target groups: the PSV target group (Group C), and the country-specific booster dose target group (BDTG; Group D). Time since last PSV was not taken into account (Table 1).

In Analysis 4, we estimated VE for complete PSV without and with booster by time since vaccination (using 30-, 60- and 90-day periods) for all vaccine products combined.

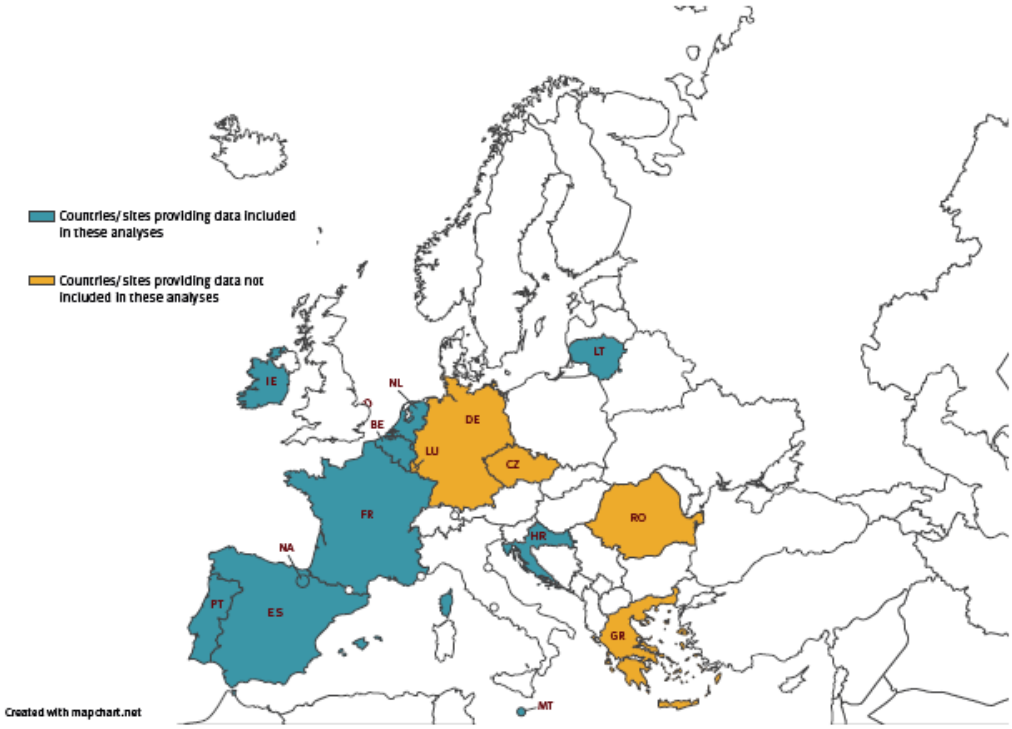
In Analysis 5, we estimated VE for those with complete PSV in a country-specific BDTG with symptom onset less than 150 days from their last PSV dose (Table 1).

Sensitivity analyses

We conducted sensitivity analyses excluding all SARI patients with known prior infection, and another

FIGURE 1

Countries and study sites participating in I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, by provision of data for this analysis, Europe, 2021



I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

Fifteen participating sites: Belgium (BE), Croatia (HR), Czechia (CZ), France (FR), Germany (DE), Greece (GR), Ireland (IE), Lithuania (LT), Luxembourg (LU), Malta (MT), the Netherlands (NL), Portugal (PT), Romania (RO), Spain 11 regions (ES), Spain Navarra region (NA). Ten included in this analysis: BE, ES, FR, HR, IE, LT, MT, NA, NL, PT.

restriction included only SARI patients with severe outcomes (either admission to an intensive care unit (ICU) or death in hospital). Where the number of cases or controls per parameter was < 10, a sensitivity analysis was conducted using Firth's method of penalised logistic regression (PLR) to assess small sample bias [14,15], which we considered to be present if there was a difference of 10 percentage points between the PLR and original VE estimate. Any estimates meeting this criterion for small sample bias were not included, also we do not present any VE estimates in any stratum for which the total number of vaccinated cases and controls was < 20.

Results

Entire Alpha and Delta period (March to December 2021)

Fifteen sites submitted data on 8,616 SARI patients swabbed between 2 March and 25 December 2021 (Figure 1, Figure 2). Data from four sites were not eligible for inclusion in analysis from the outset, as their data included fewer than five cases (one site) or controls (two sites), or their participating hospitals had been designated 'COVID only' earlier in the pandemic and SARI patients were almost exclusively SARS-CoV-2-positive (one site). After exclusions, a further four sites were excluded from the Alpha period and one more from the Delta period (Figure 1) for having fewer than five cases or controls.

Alpha period (3 March to 11 June 2021)

Descriptive analysis

Seven sites submitted data eligible for inclusion in this period (Table 2). After applying the study and analysis exclusion criteria (Figure 2), we included 1,087 cases and 538 controls who had been swabbed during the Alpha-dominant period. Most cases (n = 838; 77%) were younger than 80 years, while 320 controls (60%)

TABLE 1

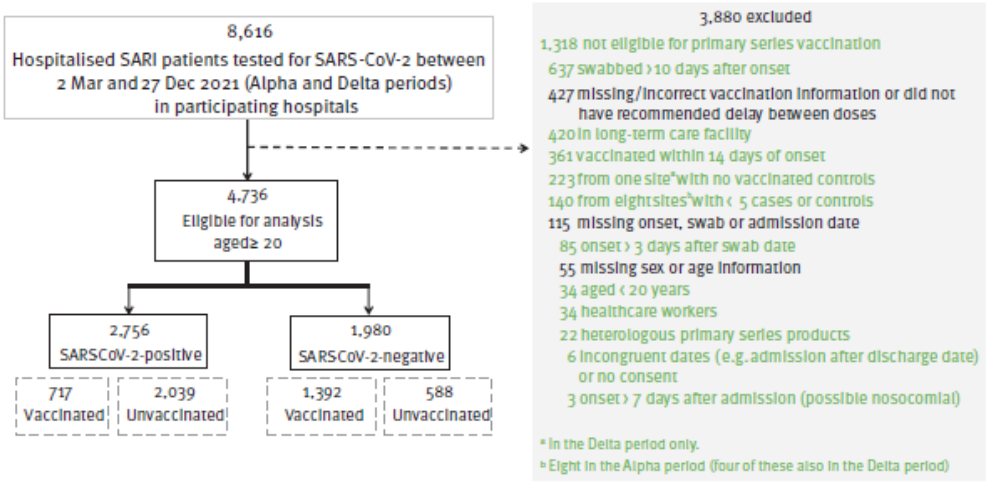
Vaccination and analysis definitions for SARS-CoV-2 Alpha (3 March–11 June 2021) and Delta (21 June–25 December 2021) periods, I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies

Period	Alpha	Alpha, Delta	Delta						
Vaccination or group	Partial PSV	Complete PSV	Booster	Group A	Group B	Group C	Group D	Time since vaccination	Group E
Definition	14 days after receiving one of two recommended doses of a two-dose vaccine	14 days after receiving either: - the second of two recommended doses of a two-dose vaccine or - a single dose of JCovden	14 days after receiving the first mRNA booster	Complete PSV, with: - no booster, but eligible for a booster dose i.e. in the age-specified BDTG based on national recommendations - last PSV dose received ≥ 150 days before onset	Complete PSV+first mRNA booster in BDTG, with: - ≥ 150 days between the last PSV dose and the booster dose	Complete PSV only, for: - all in PSV target group regardless of time since last dose	Complete PSV only, for: - all in BDTG regardless of time since last dose	Complete PSV without and with booster for all vaccine products combined, for: - all in BDTG - by time since vaccination (30-, 60- and 90-day periods)	Complete PSV, in BDTG, with - symptom onset < 150 days from last PSV dose
Analysis	**Analysis 1:** Alpha period VE for partial and complete PSV		**Analysis 2:** Delta period VE for complete PSV ± booster, with last PSV dose ≥ 150 days before onset (complete PSV only), or ≥ 150 days before booster (complete PSV + booster)			**Analysis 3:** VE for complete PSV only for those in different vaccine target groups, regardless of time since last PSV dose		**Analysis 4:** VE for those in BDTG, complete PSV ± booster, over time	**Analysis 5:** VE for complete PSV only, with last PSV dose < 150 days before onset

BDTG: booster dose target group; I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; PSV: primary series vaccination; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VE: vaccine effectiveness; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

FIGURE 2

Exclusions for I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, Europe, 2021 (n = 8,616)



I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

Included data are from 42 hospitals in 10 participating sites.

were in this age group (Table 2). Fifty-seven per cent of cases (n = 622) and 54% of controls (n = 292) were male. Fifty-six per cent of cases (n = 606) and 74% of controls (n = 399) had one or more of the four commonly collected chronic conditions.

There were 62 (6%) partially and 12 (1%) completely vaccinated cases, 94 (18%) partially and 90 (17%) completely vaccinated controls. Vaccination rollout continued as cases and controls were selected (Figure 3).

All 12 completely vaccinated cases had received Comirnaty. Among the 90 completely vaccinated controls, 82 (91%) had received two doses of Comirnaty, seven (8%) had received Spikevax and one (‹1%) had received a single dose of JCovden (Table 2).

A higher proportion of cases than controls were admitted to ICU or died in hospital (22% vs 4% and 19% vs 9%, respectively; Table 1). Most (› 90%) cases and controls were swabbed between weeks 10–18 (Figure 3).

Vaccine effectiveness estimates

Analysis 1: Vaccine effectiveness in patients ≥ 20 years for partial and complete primary series vaccination

Partial vaccination VE among those aged ≥ 20 years (all vaccine products combined) was 43% (95% confidence interval (CI): 13–62); 45% (95% CI: 8–67) for Comirnaty and 31% (95% CI: –46 to 67) for Vaxzevria. Sensitivity analyses excluding SARI patients with known prior infection are appended in Supplementary Table S1, where the VE was within 2 percentage points for partial VE. For SARI patients with severe outcomes during the Alpha period, numbers were too small to estimate VE.

Complete PSV (all products) VE during this period was 86% (95% CI: 71–93); 85% (95% CI: 69–92) for Comirnaty (Table 3). There was insufficient sample size to estimate complete PSV VE for any other vaccine product during this period. The point estimate of VE for sensitivity analyses (prior infection) were identical for complete PSV; these results are made available in Supplementary Table S1. Again, there was not sufficient sample size for the sensitivity analysis for severe outcomes.

Partial VE was ‹ 60% in those aged 60–79 years and ‹ 30% in those ≥ 80 years; there was only sufficient sample size to estimate complete PSV VE in the oldest age group (≥75%); for partial and complete PSV results among adults (≥20 years) in the PSV target group, overall and in those eligible for a booster dose, see Supplementary Table S2. During this period, few individuals aged 20–59 years had been targeted for vaccination, so we could not provide estimates for this age group.

TABLE 2A

Characteristic of cases and controls, I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, Europe, SARS-CoV-2 Alpha and Delta periods March–December 2021 (n = 4,736)

Patient characteristic	Alpha period: January to June 2021 (n = 1,625)				Delta period: July to December 2021 (n = 3,111)			
	SARS-CoV-2 cases (n = 1,087)		Test-negative controls (n = 538)		SARS-CoV-2 cases (n = 1,669)		Test-negative controls (n = 1,442)	
	Number	%	Number	%	Number	%	Number	%
Median age (years)	69		76		63		72	
Age groups (years)								
20–59	247	22.7	69	12.8	695	41.6	348	24.1
60–79	591	54.4	251	46.7	644	38.6	641	44.5
≥ 80	249	22.9	218	40.5	330	19.8	453	31.4
Sex								
Male	622	57.2	292	54.3	984	59.0	815	56.5
Female	465	42.8	246	45.7	685	41.0	627	43.5
At least one chronic condition[a]								
No	481	44.3	139	25.8	929	55.7	449	31.1
Yes	606	55.7	399	74.2	740	44.3	993	68.9
COVID-19 vaccination status								
Unvaccinated	1,013	93.2	354	65.8	1,026	61.5	234	16.2
Partial vaccination only[b]	62	5.7	94	17.5	35	2.1	68	4.7
Complete PSV[c]	12	1.1	90	16.7	598	35.8	1,043	72.3
Complete PSV + first booster[d]	NA				10	0.6	97	6.7
Vaccine product among vaccinated: first dose								
Comirnaty	45	61.6	145	80.6	392	61.0	886	73.4
Vaxzevria	21	28.8	19	10.6	156	24.3	178	14.7
Spikevax	7	9.6	15	8.3	22	3.4	90	7.5
JCovden	0	0.0	1	0.6	72	11.2	47	3.9
Other/unknown[e]	1	NC	4	NC	1	NC	7	NC
Vaccine product among vaccinated: second dose								
Comirnaty	12	100	82	92.1	381	71.1	852	78.0
Vaxzevria	0	0	0	0.0	135	25.2	155	14.2
Spikevax	0	0	7	7.9	20	3.7	81	7.4
Other/unknown[e]	0	NC	0	NC	0	NC	5	NC
Vaccine product among vaccinated: first booster								
Comirnaty	NA				10	100	89	91.8
Spikevax					0	NC	7	7.2
Other/unknown[e]					0	NC	1	1.0

ICU: intensive care unit; I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; NA: not applicable; NC: not calculated; NI: no data included; PSV: primary series vaccination; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

[a] At least one of four commonly collected conditions (diabetes, heart disease, lung disease, asthma).

[b] Partially vaccinated: any SARI patient who received only one of two recommended doses of a two-dose vaccine.

[c] Completely vaccinated: any SARI patient who received either both of two recommended doses of a two-dose vaccine, or a single dose of Jcovden.

[d] Vaccinated with first booster: any SARI patient who received either both of two recommended doses of a two-dose vaccine, or a single dose of Jcovden, plus one booster dose.

[e] Missing totals not included in percentages.

TABLE 2B

Characteristic of cases and controls, I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, Europe, SARS-CoV-2 Alpha and Delta periods March–December 2021 (n = 4,736)

Patient characteristic	Alpha period: January to June 2021 (n = 1,625)				Delta period: July to December 2021 (n = 3,111)			
	SARS-CoV-2 cases (n = 1,087)		Test-negative controls (n = 538)		SARS-CoV-2 cases (n = 1,669)		Test-negative controls (n = 1,442)	
	Number	%	Number	%	Number	%	Number	%
Severe outcomes								
Hospitalisation	1,087	100	538	100	1,669	100	1,442	100
ICU admission	231	21.7	20	4.0	330	20.0	119	8.4
No ICU admission	835	78.3	482	96.0	1,317	80.0	1,295	91.6
Missing*	21	NC	36	NC	22	NC	28	NC
In-hospital death	181	19.0	27	8.7	195	12.6	92	7.3
Discharged alive	744	78.2	242	77.5	1,286	83.0	1,082	85.9
Still in hospital/transferred	27	2.8	43	13.8	69	4.4	85	6.8
Missing*	135	NC	226	NC	119	NC	183	NC
Study site and country[r]								
Belgium	87	8.0	68	12.6	115	6.9	88	6.1
Czechia	NI				NI			
Spain	236	21.7	110	20.5	379	22.7	400	27.7
France	39	3.6	14	2.6	197	11.8	136	9.4
Germany	NI				NI			
Greece	NI				NI			
Croatia	579	53.3	74	13.8	433	25.9	92	6.4
Ireland	NI				75	4.5	53	3.7
Lithuania	24	2.2	6	1.1	34	2.0	22	1.5
Luxembourg	NI				NI			
Malta	NI				95	5.7	225	15.6
Navarra, Spain	37	3.4	85	15.8	42	2.5	109	7.6
the Netherlands	85	7.8	181	33.6	77	4.6	149	10.3
Portugal	NI				222	13.3	168	11.7
Romania	NI				NI			
All sites	1,087	100	538	100	1,669	100	1,442	100

ICU: intensive care unit; I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; NA: not applicable; NC: not calculated; NI: no data included; PSV: primary series vaccination; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

* Missing totals not included in percentages.

[r] Eight sites submitting data during the Alpha and five during the Delta period did not have sufficient eligible SARI patients for inclusion in these analyses (this table gives final numbers by site, after exclusions).

Delta period (21 June–25 December 2021)

Descriptive analysis

Ten sites submitted data eligible for inclusion in this period (Table 2). After applying the study and analysis exclusion criteria (Figure 2), we included 1,669 cases and 1,442 controls. Most cases (n = 1,339; 80%) were aged‹80 years, while 989 controls (69%) were in this age group (Table 1). Fifty-nine per cent of cases (n = 984) and 57% of controls (n = 815) were male. Forty-four per cent of cases (n = 740) and 69% of controls (n = 993) had one or more of the four commonly collected chronic conditions.

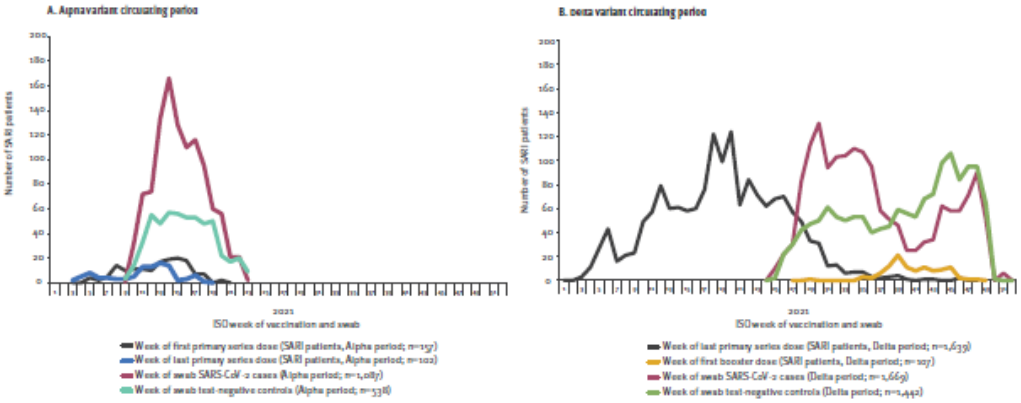
There were 35 (2%) partially and 598 (36%) completely vaccinated cases, 68 (5%) partially and 1,043 (72%) completely vaccinated controls (Table 2). Ten cases (1%) and 97 controls (7%) had received a first booster. Vaccination rollout continued as cases and controls were selected (Figure 3).

Of the 598 completely vaccinated cases, 391 (65%) had received an mRNA vaccine product (371 Comirnaty and 20 Spikevax), 135 had received Vaxzevria (23%) and 72 (12%) had received JCovden. Among the 1,043 completely vaccinated controls, 838 (80%) had received an mRNA product (759 Comirnaty and 79 Spikevax), 153 (15%) had received Vaxzevria and 47 (5%) had received JCovden (Table 2).

A higher proportion of cases than controls were admitted to ICU or died in hospital (20% vs 8% and 13% vs 7%; Table 2). Most cases (n = 1,095; 66%) were swabbed in weeks 28–39, while most controls (n = 895; 62%) were swabbed in weeks 38–49 (Figure 3).

FIGURE 3

Number of SARI patients by case status and week of COVID-19 vaccination (second and booster doses) or swab, I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, Europe, 2021 (n = 4,736)



I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; SARI: severe acute respiratory infection; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

Vaccine effectiveness estimates

Analysis 2: Vaccine effectiveness in patients ≥ 20 years and eligible for a booster dose

The VE for complete PSV alone (all products combined) was 52% (95% CI: 18–71); 91% (95% CI: 57–98) in those with complete PSV and an mRNA booster dose. For Comirnaty, these estimates were 54% (95% CI: 18–74) and 91% (95% CI: 57–98), respectively. Insufficient sample size prevented estimation of VE for other vaccine products (Table 3). The VE in sensitivity analysis (severe outcomes) was 32 percentage points higher for complete PSV; small sample size precluded sensitivity analysis for those with booster dose. Excluding those with known prior infection gave VE within 4 percentage points of this main analysis for those with booster; complete sensitivity analysis data are accessible in Supplementary Table S1.

Analysis 3: Vaccine effectiveness for complete primary series vaccination only, for those in different vaccination target groups

For SARI patients in Group C (PSV target group), complete PSV VE was 79% (95% CI: 74–83) for all products combined and 82% (95% CI: 77–86) for Comirnaty. For Group D (patients in BDTG), complete PSV was 69% (95% CI: 51–80) for all products and 74% (95% CI: 57–84) for Comirnaty. Sample size was small and confidence intervals wide for other products, particularly for stratified estimates (Table 3).

Analysis 4: Vaccine effectiveness by time since vaccination for those in a booster dose target group

There was insufficient sample size to estimate VE for complete PSV only in those with onset < 60 days since vaccination, and in those who had received a first mRNA booster dose for any period ≥ 120 days since last booster dose.

For complete PSV without booster, among those who received their last PSV dose 60–119 and ≥ 120 days before onset, VE was 79% (95% CI: 57–90) and 66% (95% CI: 45–78), respectively. The VE in this group was highest in those receiving their last PSV dose 14–89 days before onset, at 93% (95% CI: 77–98). For those with onset between 90 and 179 days after their last PSV dose, VE remained at or above 70%, but dropped to 33% (95% CI: −27 to 64) in those with last PSV dose ≥ 180 days before onset. Among those with a first mRNA booster, VE was 92% (95% CI: 67–98) if the booster dose was received 14–59 days before onset and remained > 90% up to 119 days. The VE in sensitivity analysis (severe outcomes) was 11 percentage points higher for complete PSV among those vaccinated ≥ 14 days before onset; the full sensitivity analysis is accessible in Supplementary Table S1.

Analysis 5: Vaccine effectiveness for complete primary series vaccination for those aged ≥ 20 years in a booster dose target group (but without booster dose) with last primary series vaccination dose < 150 days before onset

Complete PSV VE was 76% (95% CI: 57–87). Age stratification showed the highest VE in the youngest age group (20–59 years), at 92% (95% CI: 75–98) (Table 3).

Discussion

In our study, during the Alpha period, Comirnaty VE against COVID-19 hospitalisation in adults aged ≥ 20 years was higher for complete PSV (86%) than for partial vaccination (43%). During Delta-dominant circulation, for SARI patients with their last PSV or mRNA booster dose at least 150 days before onset, VE for complete PSV was 52%, rising to 91% on addition of the mRNA

TABLE 3A

Effectiveness of COVID-19 partial and complete primary series vaccination against hospitalisation among adults (≥ 20 years) with complete primary vaccination, by dose, age group and vaccine product, I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, Europe, Alpha and Delta periods March–December 2021 (n = 4,736)

PSV vaccine product	Vaccinated/unvaccinated cases; vaccinated /unvaccinated controls		VE[a] (95% CI)	
Analysis 1: VE of partial and complete PSV, Alpha period (March–June 2021; n = 1,625)				
	Partial PSV	Complete PSV	Partial PSV	Complete PSV
All products combined	**7 sites[b]; n = 1,523[c]**	**7 sites[b]; n = 1,469[d]**		
All ≥ 20 years	62/1,013; 94/354	12/1,013; 90/354	43 (13 to 62)	86 (71 to 93)
Comirnaty PSV	**7 sites[b]; n = 1,463**	**6 sites[e]; n = 1,434**		
All ≥ 20 years	33/1,013; 63/354	12/991; 82/349	45 (8 to 67)	85 (69 to 92)
Vaxzevria PSV	**6 sites[e]; n = 1,358**	**0 sites[f]; n = 0**		
All ≥ 20	21/975; 19/343	NA	31 (–46 to 67)	NA
Analysis 2: VE in patients eligible for a booster dose. Group A: those who received complete PSV only, with last PSV dose administered ≥ 150 days before onset. Group B: those who received PSV plus an mRNA booster dose administered ≥ 150 days after last PSV dose. Delta period (June–December 2021; n = 3,111)				
	Complete PSV (Group A)	Complete PSV + mRNA booster (Group B)	Complete PSV (Group A)	Complete PSV + mRNA booster (Group B)
All products combined	**7 sites[g]; n = 524[h]**	**2 sites[i]; n = 88[j]**		
All ≥ 20 years	103/102; 268/51	4/39; 21/24	52 (18 to 71)	91 (57 to 98)
Comirnaty PSV	**6 sites[k]; n = 435**	**2 sites[l]; n = 88**		
All ≥ 20 years	80/75; 233/47	4/39; 21/24	54 (18 to 74)	91 (57 to 98)
Vaxzevria PSV	**2 sites[l]; n = 107**	**0 sites[m]**		
All ≥ 20 years	6/65; 2/34	ND	ND[n]	NA
JCovden PSV	**3 sites[l]; n = 141**	**0 sites[m]**		
All ≥ 20 years	5/92; 6/38	ND	ND[n]	NA
Spikevax PSV	**2 sites[m]; n = 112**	**0 sites[m]**		
All ≥ 20 years	4/65; 9/34	ND	ND[n]	NA

BDTG: booster dose target group; CI: confidence interval; ICU: intensive care unit; I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; NA: not applicable; ND: not done; PSTG: primary series target group; PSV: primary series vaccination; VE: vaccine effectiveness; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

[a] Odds ratio adjusted by study site as a fixed effect, time (restricted cubic spline of swab date, or swab month as a categorical term, depending on model), age or 5-year age group (linear or categorical term depending on model), sex, and presence of at least one of four chronic conditions (asthma, diabetes, heart disease, lung disease).

[b] Seven study sites: Belgium, Croatia, France, Lithuania, Navarra, the Netherlands and Spain.

[c] n = 1,523 after dropping 102 records from patients with complete vaccination.

[d] n = 1,469 after dropping 156 records from patients with partial vaccination.

[e] Six study sites: Belgium, Croatia, France, Navarra, the Netherlands and Spain.

[f] None of the participating sites with patients receiving complete vaccination had used Vaxzevria during the Alpha-dominant period.

[g] Seven study sites: Belgium, France, Malta, Navarra, the Netherlands, Portugal and Spain.

[h] n = 524 after dropping 2,195 records from patients who were not yet in the target group for booster dose vaccination, 211 from those vaccinated < 150 days before onset, 101 from those with a booster dose, 213 from patients with partial vaccination, and 59 from sites with fewer than five 5 cases/controls (or a total of cases and controls < 20).

[i] Two study sites: Belgium and Spain.

[j] n = 88 after dropping 2,195 records from patients who were not yet in the target group for booster dose vaccination, 634 from those with partial vaccination or complete vaccination without booster, 114 from sites with fewer than five cases/controls (or a total of cases and controls < 20) and 80 from one site with no vaccinated cases.

[k] Six study sites: France, Malta, Navarra, the Netherlands, Portugal and Spain.

[l] Two study sites: France and Spain.

[m] None of the participating sites recruited patients who had received a booster dose after having a complete primary course of Vaxzevria, JCovden or Spikevax.

[n] Numbers are too small to provide robust VE estimates when there are ≤ 20 vaccinated cases and controls in total.

TABLE 3B

Effectiveness of COVID-19 partial and complete primary series vaccination against hospitalisation among adults (≥ 20 years) with complete primary vaccination, by dose, age group and vaccine product, I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, Europe, Alpha and Delta periods March–December 2021 (n = 4,736)

PSV vaccine product	Vaccinated/unvaccinated cases; vaccinated /unvaccinated controls		VE[a] (95% CI)	
Analysis 3: VE in patients vaccinated≥ 14 days before onset for those with PSV only, for those in the PSTG (Group C) and those in the BDTG (Group D); Delta period (June–December 2021; n = 3,111)				
	Complete PSV, in PSTG (Group C)	Complete PSV, in BDTG (Group D)	Complete PSV, in PSTG (Group C)	Complete PSV, in BDTG (Group D)
All products combined	**10 sites[c]; n = 2,901[f]**	**7 sites[g]; n = 735[q]**		
All ≥ 20 years	598/1,026; 1,043/234	168/102; 414/51	79 (74 to 83)	69 (51 to 80)
20–59 years	124/558; 223/92	21/45; 57/14	86 (80 to 91)	86 (62 to 95)
60–79 years	271/350; 479/92	87/41; 193/24	80 (72 to 85)	71 (44 to 85)
≥ 80 years	203/118; 341/50	60/16; 164/13	38 (–4 to 62)	40 (–64 to 78)
No chronic condition[r]	209/701; 286/107	39/48; 78/13	86 (80 to 90)	79 (47 to 91)
Any chronic condition[r]	389/325; 757/127	129/54; 336/38	71 (61 to 78)	65 (40 to 79)
Comirnaty PSV	**10 sites[c]; n = 2,390**	**7 sites[g]; n = 560**		
All ≥ 20 years	371/1,026; 759/234	96/102; 311/51	82 (77 to 86)	74 (57 to 84)
20–59 years	57/558; 140/92	9/45; 35/14	91 (86 to 94)	92 (73 to 98)
60–79 years	140/350; 310/92	38/41; 127/24	82 (74 to 88)	79 (54 to 90)
≥ 80 years	174/118; 309/50	49/16; 149/13	49 (11 to 71)	56 (–31 to 85)
No chronic condition[r]	37/661; 16/98	6/43; 2/7	55 (7 to 78)	ND[n]
Any chronic condition[r]	263/325; 565/127	81/54; 256/38	74 (64 to 81)	69 (46 to 82)
Vaxzevria PSV	**10 sites[c]; n = 1,548**	**3 sites[s]; n = 203**		
All ≥ 20 years	135/1,026; 153/234	36/92; 37/38	69 (57 to 78)	50 (–4 to 75)
20–59 years	31/558; 29/92	4/44; 2/12	59 (18 to 79)	ND[n]
60–79 years	85/350; 118/92	26/36; 34/18	81 (69 to 88)	63 (10 to 85)
≥ 80 years	19/118; 6/50	6/12; 1/8	11 (–183 to 72)	ND[n]
No chronic condition[r]	50/701; 53/107	12/43; 9/7	79 (65 to 88)	74 (–14 to 94)
Any chronic condition[r]	85/325; 100/127	24/49; 28/31	63 (43 to 76)	37 (–38 to 72)
Covden PSV	**8 sites[t]; n = 1,309**	**3 sites[s]; n = 162**		
All ≥ 20 years	65/976; 47/221	16/92; 16/38	60 (37 to 75)	60 (1 to 84)
20–59 years	30/525; 26/84	5/44; 7/12	79 (58 to 90)	ND[n]
60–79 years	30/335; 14/88	8/36; 6/18	21 (–76 to 65)	
≥ 80 years	5/116; 7/49	3/12; 3/8	ND[n]	
No chronic condition[r]	37/661; 16/98	6/43; 2/7	55 (7 to 78)	
Any chronic condition[r]	28/315; 31/123	10/49; 14/31	60 (26 to 78)	64 (–4 to 87)
Spikevax PSV	**5 sites[u]; n = 1,117**	**1 site[v]; n = 53**		

BDTG: booster dose target group; CI: confidence interval; ICU: intensive care unit; I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; NA: not applicable; ND: not done; PSTG: primary series target group; PSV: primary series vaccination; VE: vaccine effectiveness; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

[a] Odds ratio adjusted by study site as a fixed effect, time (restricted cubic spline of swab date, or swab month as a categorical term, depending on model), age or 5-year age group (linear or categorical term depending on model), sex, and presence of at least one of four chronic conditions (asthma, diabetes, heart disease, lung disease).

[c] Seven study sites: Belgium, France, Malta, Navarra, the Netherlands, Portugal and Spain.

[n] Numbers are too small to provide robust VE estimates when there are ≤ 20 vaccinated cases and controls in total.

[s] Ten study sites: Belgium, Croatia, France, Ireland, Lithuania, Malta, Navarra, the Netherlands, Portugal and Spain.

[f] n = 2,901 after dropping 107 from those with a booster dose and 103 from those with partial vaccination only.

[q] n = 735 after dropping 2,195 records from patients who were not yet in the target group for booster dose vaccination, 101 from those with a booster dose, 21 from patients with partial vaccination, and 59 from sites with fewer than five cases or controls (or a total of cases and controls < 20).

[r] In this analysis stratified by chronic condition, the adjustment for presence of at least one chronic condition was removed.

[g] Three study sites: Belgium, France and Spain.

[t] Eight study sites: Belgium, Croatia, France, Malta, Navarra, the Netherlands, Portugal and Spain.

[u] Five study sites: Croatia, France, the Netherlands, Portugal and Spain.

[v] One study site: Spain.

TABLE 3C

Effectiveness of COVID-19 partial and complete primary series vaccination against hospitalisation among adults (≥ 20 years) with complete primary vaccination, by dose, age group and vaccine product, I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, Europe, Alpha and Delta periods March–December 2021 (n = 4,736)

PSV vaccine product	Vaccinated/unvaccinated cases; vaccinated /unvaccinated controls		VE[a] (95% CI)	
All ≥ 20 years	20/849; 61/187	ND[e]	89 (81 to 94)	ND[e]
20–59 years	3/439; 21/60		97 (90 to 99)	
60–79 years	12/306; 28/80		82 (60 to 72)	
≥ 80 years	5/104; 12/47		ND[e]	
No chronic condition[f]	8/566; 19/75		94 (84 to 98)	
Any chronic condition[f]	12/283; 42/112		83 (64 to 92)	
Analysis 4: Time-since-vaccination analysis. VE in patients vaccinated ≥ 14 days before onset who are in a BDTG, with complete PSV and those with complete PSV plus mRNA booster. Delta period (June–December 2021; n = 3,111)				
	Complete PSV only	Complete PSV + mRNA booster	Complete PSV only	Complete PSV + mRNA booster
All products combined	**7 sites[d]; n = 735[g]**	**3 sites[h]; n = 168[i]**		
All ≥ 20 years	168/102; 414/51	4/92; 34/38	69 (52 to 80)	94 (77 to 98)
Time since last dose (days)				
14–29	0/102; 6/51	1/92; 16/38	ND[e]	ND[e]
30–59	1/102; 7/51	3/92; 12/38	ND[e]	ND[e]
≥ 60	167/102; 401/51	0/92; 6/38	68 (50 to 80)	ND[e]
14–59	1/102; 13/51	4/92; 28/38	ND[e]	92 (67 to 98)
60–119	27/102; 55/51	0/92; 6/38	78 (55 to 90)	ND[e]
≥ 120	140/102; 346/51	0/92; 0/38	66 (45 to 78)	ND[e]
14–89	6/102; 35/51	4/92; 33/38	93 (76 to 98)	93 (75 to 98)
90–119	22/102; 33/51	0/92; 1/38	73 (37 to 88)	ND[e]
14–119	28/102; 68/51	4/92; 34/38	81 (62 to 91)	94 (76 to 98)
120–149	37/102; 78/51	0/92; 0/38	73 (48 to 86)	ND[e]
150–179	30/102; 102/51	0/92; 0/38	70 (43 to 85)	ND[e]
≥ 180	73/102; 166/51	0/92; 0/38	25 (−44 to 61)	ND[e]
Analysis 5: VE of complete PSV among patients without booster dose but in BDTG and vaccinated 14–149 days before onset; Delta period (June–December 2021; n = 3,111)				
All products combined	**Three sites[h]; n = 308[j]**			
All ≥ 20	53/92; 125/38		76 (57 to 87)	
20–59 years	9/44; 40/12		92 (75 to 98)	
60–79 years	34/36; 73/18		70 (31 to 87)	
80 years	10/12; 12/8		ND[k]	
No chronic condition[f]	17/43; 21/7		77 (24 to 93)	
Any chronic condition[f]	36/49; 104/31		76 (54 to 88)	

BDTG: booster dose target group; CI: confidence interval; ICU: intensive care unit; I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; NA: not applicable; ND: not done; PSTG: primary series target group; PSV: primary series vaccination; VE: vaccine effectiveness; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

[a] Odds ratio adjusted by study site as a fixed effect, time (restricted cubic spline of swab date, or swab month as a categorical term, depending on model), age or 5-year age group (linear or categorical term depending on model), sex, and presence of at least one of four chronic conditions (asthma, diabetes, heart disease, lung disease).

[e] Numbers are too small to provide robust VE estimates when there are ≤ 20 vaccinated cases and controls in total.

[g] n = 735 after dropping 2,195 records from patients who were not yet in the target group for booster dose vaccination, 101 from those with a booster dose, 21 from patients with partial vaccination, and 59 from sites with fewer than five cases or controls (or a total of cases and controls < 20).

[f] In this analysis stratified by chronic condition, the adjustment for presence of at least one chronic condition was removed.

[d] Three study sites: Belgium, France and Spain.

[h] Pooled analysis not possible with just one site.

[j] n = 308 after dropping 2,195 records from patients who were not yet in the target group for booster dose vaccination, 371 from those who were vaccinated ≥ 150 days before onset, 115 from sites with fewer than five cases/controls (or a total of cases and controls < 20), 101 from patients with a booster dose, and 21 from those with partial vaccination only.

[k] Evidence of small sample bias so VE estimate not shown.

booster dose. For those having mRNA booster dose administered within 120 days before onset of symptoms, VE was at least 90%. Sample size precluded any booster dose VE estimates after this time.

Looking at time since vaccination for those with complete PSV only, VE was 93% for those with last dose within 90 days of onset, at least 70% for those with last dose 90–179 days before onset, and 25% for those with their last dose≥ 180 days before onset. This may indicate that the most important criterion for effectiveness is the time which has elapsed since receipt of last dose of any vaccine, regardless of number of doses (we observed a VE of 93% in those with complete PSV alone and in those with mRNA booster, when last dose was received 14–89 days before onset). However, in the longer period 14–119 days from last dose to onset, we observed higher VE in those with booster dose (94% vs 81%). More analyses with greater sample size need to be carried out to investigate this further. Importantly, we observed during the Delta period that VE in patients‹ 80 years remained over 60% in those without a booster dose 150 days from their last PSV dose, and was over 50% in the oldest age group, with the median time since vaccination being considerably shorter in the younger vs the older age groups (176 vs 195 days, respectively). In addition, albeit with overlapping confidence intervals, we observed a ca 40 percentage point greater VE in those with chronic conditions in a BDTG on receipt of a booster dose.

Our study had some important limitations. We sought to mitigate the heterogeneity inherent in a multi-country observational study by the common use of a generic protocol in all sites and the adjustment of VE estimates by site. In addition, the test-negative design should reduce heterogeneity between study sites; conducted in a hospital setting, this design should limit the presence of any healthcare-seeking bias.

The ECDC case definition for possible COVID-19 patients [13] was developed to respond to the broader symptom range of COVID-19 and includes a wider range of symptoms than those used in the World Health Organization SARI case definition [16]. Using this more sensitive SARI case definition could have resulted in the inclusion of more patients hospitalised with, rather than because of, COVID-19; i.e. potentially vaccinated patients with milder COVID-19, hospitalised for another cause. This concern was highlighted during circulation of the milder Omicron variant [17,18]; it would result in a higher proportion of included vaccinated cases, underestimating the VE. In sensitivity analyses using more specific severe outcomes (ICU admission or death), we found considerably higher VE in those with complete PSV during the Delta, but not during the Alpha period.

Prior SARS-CoV-2 infection can provide some immunity to unvaccinated SARI patients. In some participating countries in our study (e.g. France, Spain), known prior infection resulted in a delay of the next scheduled dose. In other countries, some individuals may also have delayed vaccination if they had a known infection. This could result in milder disease in unvaccinated cases being discovered when a SARI patient is hospitalised with (not because of) COVID-19, as described above. In sensitivity analyses estimating VE after excluding those with prior infection, we found almost identical VE estimates, whether for partial and complete PSV (Alpha) or those with and without booster (Delta), although this information was missing in almost one-third of patients (data not shown).

Earlier in 2021, the SARS-CoV-2 Alpha variant was dominant in Europe, superseded by the Delta variant during the summer months. In participating countries, vaccine rollout was initially limited to vulnerable populations [19] (e.g. older adults, those in long-term care or those with co-morbidities; for a list of target groups for primary course and first booster dose vaccination by participating countries, see Supplementary Tables S3 and S4) with more susceptibility to COVID-19. In particular, the Alpha-dominant circulating period coincided with the early phase of the vaccine roll-out in the EU/EEA. This was based on an age-staggered approach that prioritised older adults and those at risk of severe outcome, as well as healthcare workers. Therefore, recruitment of study participants in this period would have mainly been among those initial target groups, and we could not provide VE estimates in all age groups during the Alpha-dominant period. Similarly, estimates could only be calculated for partial PSV for one other product than Comirnaty (Vaxzevria), while complete PSV VE could only be provided for Comirnaty, as this was the main COVID-19 vaccine product distributed in the EU/EEA at this time [20].

Despite these limitations, our all-product, complete PSV VE against COVID-19 hospitalisation in those aged at least 20 years during the Alpha-dominant period (86%; 95% CI: 71–93) was similar to or slightly lower than some other published estimates. For example, studies in those aged≥ 18 years showed VE of 87% (95% CI: 81–91) in the United States (US) [21] and 93% (95% CI: 89–96) in a systematic review [22]. Other studies in Canada, the United Kingdom (UK), Israel and Denmark have shown somewhat higher estimates during this period [23-26]. Comparisons across studies should bear in mind differences in study design, minimal hospitalisation time, age groups (≥ 16 or≥ 18 years vs our≥ 20 years), potentially different severity criteria, and pooling of results for different severe outcomes (e.g. combining hospitalisation and death).

Similar to our findings, other studies have reported Comirnaty VE estimates for partial PSV to be lower than for complete PSV, and VE in the oldest age group to be lower than in younger age groups [25,27,28] during the Alpha period.

Our PSV VE estimates during the Delta-dominant circulating period (from 52% to 79% overall) were slightly

lower than those published elsewhere (by product, overall and by age group). For example, other studies have reported PSV VE estimates against COVID-19 hospitalisation of 67–99% (Norway; combined vaccine products; cohort study [29]), 74–84% (Hungary; Comirnaty, Spikevax and Vaxzevria; cohort study [30]), 75–80% (US; Comirnaty; cohort study [31]), ≥ 95% (Canada; Comirnaty, Spikevax and Vaxzevria; test-negative design [32]) and ≥ 96% (UK; Comirnaty; test-negative design [24,26]). This could be due to our more sensitive SARI case definition, described above, as well as variations in time since vaccination between studies.

It is well documented that the VE against COVID-19 hospitalisation, including groups at higher risk for severe disease, declines with increasing time since vaccination [29,33-35], particularly in older adults [34,35]. A systematic review indicated that, as in our study, other studies had only small (9–10 percentage points) changes in VE ≤ 180 days from last primary series dose, with VE remaining ≥ 70% up to this time [35]. The temporal evolution of VE varies by age, vaccine product, circulating virus variant and region. This highlights the importance of multi-country studies using a similar generic protocol in an attempt to standardise the methodological approach and provide robust pooled results.

Conclusions

Our results from an EU multi-country hospital setting add to the evidence already available from other settings. Our study indicates that the VE point estimate of complete PSV was higher during the period of Alpha than during Delta-dominant circulation (overall and for those vaccinated 14–89 days since last PSV dose), and that addition of a first booster dose during Delta-dominant circulation increased VE to over 90%. Finally, during the Delta-dominant period, we observed declining effectiveness over time, likely due to waning immunity. Despite this, VE was 93% 14–89 days after the last booster or PSV dose and remained 70% or greater 90–179 days from last PSV dose (in those without booster).

Ethical statement

The planning, conduct and reporting of the studies was in line with the Declaration of Helsinki. Official ethical approval was not required if studies were classified as being part of routine care/surveillance: Spain, Ireland, Malta. For the Netherlands, the study was not subject to the Dutch Medical Research with Human Subjects Law (Wet Medisch-wetenschappelijk onderzoek met mensen, WMO) as it is non-interventional, uses routine clinical data only and data were collected retrospectively. In Belgium and Germany, VE is included in SARI surveillance. For Belgium, this protocol was approved by the central Ethical Committee (CHU St Pierre, Bruxelles) and the local ethical committees of each participating hospital in 2011 (AK/12-02-11/4111), updated in 2014 (B.U.N. 143201215671). The German SARI surveillance was approved by the Charité-Universitätsmedizin Berlin Ethical Board (Reference EA2/218/19). Other study sites obtained local ethical approval from a national review board (Croatia: approved 24 May 2021 and 26 January 2022, Ethics committee of the Croatian Institute of Public Health, Klasa:030-02/21-01/1, Ur.broj:381-15-21-7; Klasa:030-02/21-01/1, Ur.broj:381-15-22-14; France: eighth amendment approved 28 May 2021 by the French Data Protection Agency, and the French ethics research committee 'Comité de Protection des Personnes'; Lithuania: the protocol approved by the Lithuanian Biomedical Research Ethics Committee No. V06-V01LTU issued 29 June, 2020; updated on 11 May 2021 No. 6B-21-85; Navarra: approved by the Navarra Ethical Committee for Clinical Research (PI2020/45); Portugal: approved 19 January 2021 by the Ethics Committee of Instituto Nacional de Saúde Doutor Ricardo Jorge, no registration number given; Romania: approved by the Ethics Committee of the Ministerul Apărării Naționale Institutul Național de Cercetare pentru Dezvoltare Medico-Militară „Cantacuzino" for the period 2022–2023, No. CE199/2022).

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Participating laboratories submitted their sequences to GISAID (www.gisaid.org) for easy sharing with the central laboratory in Madrid.

Conflict of interest

None declared.

Authors' contributions

AMCR was involved in the original methodological design of the study (generic protocol). She contributed to the coordination of the I-MOVE-COVID-19 and VEBIS hospital networks and undertook the statistical analysis on which the research article is based. She led the manuscript writing, interpreted results and approved the final version of the manuscript.

JH contributed to the coordination of the VEBIS hospital network, contributed to analysis, helped interpret results, contributed to manuscript writing and approved the final version of the manuscript.

VSM and FP coordinated the I-MOVE virological analysis of the I-MOVE-COVID-19 and VEBIS hospital networks, helped interpret results, and read, contributed to and approved the final version of the manuscript.

MV initiated the original methodological design of the study and coordinated the I-MOVE-COVID-19 network. EK contributed to the coordination of the I-MOVE-COVID-19 network and MV and EK interpreted results, contributed to manuscript writing and approved the final version of the manuscript. NN and SB were involved in study design, interpretation of results, review of the manuscript and approval of the final version of the manuscript.

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References

1. Harder T, Koch J, Vygen-Bonnet S, Külper-Schiek W, Pilic A, Reda S, et al. Efficacy and effectiveness of COVID-19 vaccines against SARS-CoV-2 infection: interim results of a living systematic review, 1 January to 14 May 2021. Euro Surveill. 2021;26(28):2100563. https://doi.org/10.2807/1560-7917.ES.2021.26.28.2100563 PMID: 34269175
2. European Medicines Agency (EMA). COVID-19 medicines. Amsterdam: EMA. [Accessed: 28 Mar 2021]. Available from: https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/treatments-vaccines-covid-19-authorised-medicines
3. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021;384(5):403-16. https://doi.org/10.1056/NEJMoa2035389 PMID: 33378609
4. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 2020;383(27):2603-15. https://doi.org/10.1056/NEJMoa2034577 PMID: 33301246
5. Sadoff J, Gray G, Vandebosch A, Cárdenas V, Shukarev G, Grinsztejn B, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. N Engl J Med. 2021;384(23):2187-201. https://doi.org/10.1056/NEJMoa2101544 PMID: 33882225
6. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet. 2021;397(10269):99-111. https://doi.org/10.1016/S0140-6736(20)32661-1 PMID: 33306989
7. World Health Organization WHO). Evaluation of COVID-19 vaccine effectiveness. Geneva: WHO; 2021. Available from: https://www.who.int/publications-detail-redirect/WHO-2019-nCoV-vaccine_effectiveness-measurement-2021.1
8. Tregoning JS, Flight KE, Higham SL, Wang Z, Pierce BF. Progress of the COVID-19 vaccine effort: viruses, vaccines and variants versus efficacy, effectiveness and escape. Nat Rev Immunol. 2021;21(10):626-36. https://doi.org/10.1038/s41577-021-00592-1 PMID: 34373623
9. Epiconcept. European study of COVID-19 vaccine effectiveness against hospitalised SARI patients laboratory-confirmed with SARS-CoV-2. Draft generic protocol. Paris: Epiconcept; 2021. Available from: https://www.imoveflu.org/wp-content/uploads/2021/03/08feb2021_draft_generic_VE_protocol_hospital-based_COVID-19_v07.pdf
10. European Centre for Disease Prevention and Control (ECDC). Core protocol for ECDC studies of COVID-19 vaccine effectiveness against hospitalisation with Severe Acute Respiratory Infection laboratory-confirmed with SARS-CoV-2, version 1.0. Stockholm: ECDC; 2021. Available from: https://www.ecdc.europa.eu/en/publications-data/core-protocol-ecdc-studies-covid-19-vaccine-effectiveness-against-hospitalisation
11. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. Euro Surveill. 2017;22(13):30494. https://doi.org/10.2807/1560-7917.ES.2017.22.13.30494 PMID: 28382917
12. Jackson ML, Nelson JC. The test-negative design for estimating influenza vaccine effectiveness. Vaccine. 2013;31(17):2165-8. https://doi.org/10.1016/j.vaccine.2013.02.053 PMID: 23499601
13. Peralta-Santos A. Assessment of COVID-19 surveillance case definitions and data reporting in the European Union. Briefing requested by the ENVI committee. Brussels: European Parliament; July 2020. Available from: https://www.europarl.europa.eu/RegData/etudes/BRIE/2020/652725/IPOL_BRI(2020)652725_EN.pdf
14. Peduzzi P, Concato J, Feinstein AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis. II. Accuracy and precision of regression estimates. J Clin Epidemiol. 1995;48(12):1503-10. https://doi.org/10.1016/0895-4356(95)00048-8 PMID: 8543964
15. Covenay J. FIRTHLOGIT: Stata module to calculate bias reduction in logistic regression. Boston: Boston College Department of Economics; 2008. [Accessed: 3 Feb 2020]. Available from: https://econpapers.repec.org/software/bocbocode/s456948.htm
16. World Health Organization (WHO). WHO surveillance case definitions for ILI and SARI. Geneva: WHO; 2014. Available from: https://www.who.int/teams/global-influenza-programme/surveillance-and-monitoring/case-definitions-for-ili-and-sari
17. Feikin DR, Abu-Raddad LJ, Andrews N, Davies MA, Higdon MM, Orenstein WA, et al. Assessing vaccine effectiveness against severe COVID-19 disease caused by omicron variant. Report from a meeting of the World Health Organization. Vaccine. 2022;40(26):3516-27. https://doi.org/10.1016/j.vaccine.2022.04.069 PMID: 35595662
18. Stowe J, Andrews N, Kirsebom F, Ramsay M, Bernal JL. Effectiveness of COVID-19 vaccines against Omicron and Delta hospitalisation, a test negative case-control study. Nat Commun. 2022;13(1):5736. https://doi.org/10.1038/s41467-022-33378-7 PMID: 36180428
19. European Centre for Disease Prevention and Control (ECDC). Overview of the implementation of COVID-19 vaccination strategies and vaccine deployment plans in the EU/EEA. Stockholm: ECDC; 2021 [Accessed: 28 Mar 2021]. Available from: https://www.ecdc.europa.eu/en/publications-data/overview-implementation-covid-19-vaccination-strategies-and-vaccine-deployment
20. European Centre for Disease Prevention and Control (ECDC). COVID-19 vaccine tracker. Stockholm: ECDC. [Accessed: 28 Mar 2021]. Available from: https://vaccinetracker.ecdc.europa.eu/public/extensions/COVID-19/vaccine-tracker.html#uptake-tab
21. Tenforde MW, Patel MM, Ginde AA, Douin DJ, Talbot HK, Casey JD, et al. . Effectiveness of severe acute respiratory syndrome coronavirus 2 messenger rna vaccines for preventing coronavirus disease 2019 hospitalizations in the United States. Clin Infect Dis. 2022;74(9):1515-24. https://doi.org/10.1093/cid/ciab687 PMID: 34358310
22. Liu Q, Qin C, Liu M, Liu J. Effectiveness and safety of SARS-CoV-2 vaccine in real-world studies: a systematic review and meta-analysis. Infect Dis Poverty. 2021;10(1):132. https://doi.org/10.1186/s40249-021-00915-3 PMID: 34776011

23. Chung H, He S, Nasreen S, Sundaram ME, Buchan SA, Wilson SE, et al. Effectiveness of BNT162b2 and mRNA-1273 covid-19 vaccines against symptomatic SARS-CoV-2 infection and severe covid-19 outcomes in Ontario, Canada: test negative design study. BMJ. 2021;374:n1943. https://doi.org/10.1136/bmj.n1943 PMID: 34417165

24. Stowe J, Andrews N, Gower C, Gallagher E, Utsi L, Simmons R, et al. Effectiveness of COVID-19 vaccines against hospital admission with the Delta (B.1.617.2) variant. Public library. London: UK Health Security Agency. [Accessed: 1 Jan 2023]. Preprint. Available from: https://khub.net/web/phe-national/public-library/-/document_library/v2WsRK3ZIEig/view_file/479607329?_com_liferay_document_library_web_portlet_DLPortlet_INSTANCE_v2WsRK3ZIEig_redirect=https%3A%2F%2Fkhub.net%3A443%2Fweb%2Fphe-national%2Fpublic-library%2F-%2Fdocument_library%2Fv2WsRK3ZIEig%2Fview%2F479607266

25. Glatman-Freedman A, Bromberg M, Dichtiar R, Hershkovitz Y, Keinan-Boker L. The BNT162b2 vaccine effectiveness against new COVID-19 cases and complications of breakthrough cases: A nation-wide retrospective longitudinal multiple cohort analysis using individualised data. EBioMedicine. 2021;72:103574. https://doi.org/10.1016/j.ebiom.2021.103574 PMID: 34537449

26. Gram MA, Emborg HD, Schelde AB, Friis NU, Nielsen KF, Moustsen-Helms IR, et al. Vaccine effectiveness against SARS-CoV-2 infection or COVID-19 hospitalization with the Alpha, Delta, or Omicron SARS-CoV-2 variant: A nationwide Danish cohort study. PLoS Med. 2022;19(9):e1003992. https://doi.org/10.1371/journal.pmed.1003992 PMID: 36048766

27. Skowronski DM, Setayeshgar S, Zou M, Prystajecky N, Tyson JR, Galanis E, et al. Single-dose mRNA vaccine effectiveness against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), including Alpha and Gamma variants: a test-negative design in adults 70 years and older in British Columbia, Canada. Clin Infect Dis. 2022;74:(7):1158-65. PMID: 34244723

28. Skowronski DM, Setayeshgar S, Zou M, Prystajecky N, Tyson JR, Sbihi H, et al. Comparative single-dose mRNA and ChAdOx1 vaccine effectiveness against severe acute respiratory syndrome coronavirus 2, including variants of concern: test-negative design, British Columbia, Canada. J Infect Dis. 2022;226(1):485-96. https://doi.org/10.1093/infdis/jiac023 PMID: 35084500

29. Starrfelt J, Danielsen AS, Buanes EA, Juvet LK, Lyngstad TM, Rø GØI, et al. Age and product dependent vaccine effectiveness against SARS-CoV-2 infection and hospitalisation among adults in Norway: a national cohort study, July-November 2021. BMC Med. 2022;20(1):278. https://doi.org/10.1186/s12916-022-02480-4 PMID: 36050718

30. Vokó Z, Kiss Z, Surján G, Surján O, Barcza Z, Wittmann I, et al. Effectiveness and waning of protection with different SARS-CoV-2 primary and booster vaccines during the Delta pandemic wave in 2021 in Hungary (HUN-VE 3 study). Front Immunol. 2022;13:919408. https://doi.org/10.3389/fimmu.2022.919408 PMID: 35935993

31. Puranik A, Lenehan PJ, Silvert E, Niesen MJM, Corchado-Garcia J, O'Horo JC, et al. Comparison of two highly-effective mRNA vaccines for COVID-19 during periods of Alpha and Delta variant prevalence. medRxiv; 2021.08.06.21261707. https://doi.org/http://dx.doi.org/10.1101/2021.08.06

32. Skowronski DM, Febriani Y, Ouakki M, Setayeshgar S, El Adam S, Zou M, et al. Two-dose severe acute respiratory syndrome coronavirus 2 vaccine effectiveness with mixed schedules and extended dosing intervals: test-negative design studies from British Columbia and Quebec, Canada. Clin Infect Dis. 2022;75(11):1980-92. https://doi.org/10.1093/cid/ciac290 PMID: 35438175

33. Goldberg Y, Mandel M, Bar-On YM, Bodenheimer O, Freedman L, Haas EJ, et al. Waning immunity after the BNT162b2 vaccine in Israel. N Engl J Med. 2021;385(24):e85. https://doi.org/10.1056/NEJMoa2114228 PMID: 34706170

34. Andrews N, Tessier E, Stowe J, Gower C, Kirsebom F, Simmons R, et al. Duration of protection against mild and severe disease by Covid-19 vaccines. N Engl J Med. 2022;386(4):340-50. https://doi.org/10.1056/NEJMoa2115481 PMID: 35021002

35. Feikin DR, Higdon MM, Abu-Raddad LJ, Andrews N, Araos R, Goldberg Y, et al. Duration of effectiveness of vaccines against SARS-CoV-2 infection and COVID-19 disease: results of a systematic review and meta-regression. Lancet. 2022;399(10328):924-44. https://doi.org/10.1016/S0140-6736(22)00152-0 PMID: 35202601

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7.14 Annex 14

Rose AM, Nicolay N, Sandonis Martín V, Mazagatos C, Petrović G, Baruch J, Denayer S, Seyler L, Domegan L, Launay O, Machado A, Burgui C, Vaikutyte R, Niessen FA, Loghin II, Husa P, Aouali N, Panagiotakopoulos G, Tolksdorf K, Horváth JK, Howard J, Pozo F, Gallardo V, Nonković D, Džiugytė A, Bossuyt N, Demuyser T, Duffy R, Luong Nguyen LB, Kislaya I, Martínez-Baz I, Gefenaite G, Knol MJ, Popescu C, Součková L, Simon M, Michelaki S, Reiche J, Ferenczi A, Delgado-Sanz C, Lovrić Makarić Z, Cauchi JP, Barbezange C, Van Nedervelde E, O'Donnell J, Durier C, Guiomar R, Castilla J, Jonikaite I, Bruijning-Verhagen PC, Lazar M, Demlová R, Wirtz G, Amerali M, Dürrwald R, Kunstár MP, Kissling E, Bacci S, Valenciano M; I-MOVE-COVID-19 hospital study team; VEBIS hospital study team; Members of the I-MOVE-COVID-19 and VEBIS hospital study teams (in addition to authors above).
Vaccine effectiveness against COVID-19 hospitalisation in adults (≥ 20 years) during Omicron-dominant circulation: I-MOVE-COVID-19 and VEBIS SARI VE networks, Europe, 2021 to 2022. Euro Surveill. 2023 Nov;28(47):2300187. doi: 10.2807/1560-7917.ES.2023.28.47.2300187. PMID: 37997665; PMCID: PMC10668256.

Summary
Purpose:
The aim of this study was to estimate the effectiveness of COVID-19 vaccines in preventing hospitalisation among adults during the Omicron-dominant period in Europe. The study also aimed to assess the impact of booster doses and to provide real-world evidence to guide vaccination strategies during a phase characterised by immune escape and high transmission.

Methodology:
The study was conducted using a multicentre, multinational test-negative case-control design within the I-MOVE-COVID-19 and VEBIS hospital networks, including 46 hospitals across 13 European countries. Adults hospitalised with severe acute respiratory infection (SARI) were systematically tested for SARS-CoV-2, with PCR-positive patients classified as cases and PCR-negative patients as controls; vaccine effectiveness was estimated using adjusted logistic regression models accounting for confounders such as age, comorbidities, site, and calendar time.

Results:
The analysis included several thousand hospitalised patients and demonstrated that COVID-19 vaccines continued to provide protection against severe disease during the Omicron period, although effectiveness was lower compared to earlier variants. Booster doses

significantly improved protection, particularly in older adults and high-risk populations, highlighting the importance of additional vaccine doses in maintaining immunity.

Conclusion:
The study confirms that COVID-19 vaccination, especially with booster doses, remains effective in reducing hospitalisation risk during Omicron-dominant circulation despite reduced effectiveness compared to previous variants. It also demonstrates the strength of harmonised European surveillance networks and the test-negative design as a robust methodological framework for generating timely, policy-relevant real-world evidence in NCCTs.

RESEARCH

Vaccine effectiveness against COVID-19 hospitalisation in adults (≥ 20 years) during Omicron-dominant circulation: I-MOVE-COVID-19 and VEBIS SARI VE networks, Europe, 2021 to 2022

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Introduction: The I-MOVE-COVID-19 and VEBIS hospital networks have been measuring COVID-19 vaccine effectiveness (VE) in participating European countries since early 2021. **Aim:** We aimed to measure VE against PCR-confirmed SARS-CoV-2 in patients ≥ 20 years hospitalised with severe acute respiratory infection (SARI) from December 2021 to July 2022 (Omicron-dominant period). **Methods:** In both networks, 46 hospitals (13 countries) follow a similar test-negative case–control protocol. We defined complete primary series vaccination (PSV) and first booster dose vaccination as last dose of either vaccine received ≥ 14 days before symptom onset (stratifying first booster into received < 150 and ≥ 150 days after last PSV dose). We measured VE overall, by vaccine category/product, age group and time since first mRNA booster dose, adjusting by site as a fixed effect, and by swab date, age, sex, and presence/absence of at least one commonly collected chronic condition. **Results:** We included 2,779 cases and 2,362 controls. The VE of all vaccine products combined against hospitalisation for laboratory-confirmed SARS-CoV-2 was 43% (95% CI: 29–54) for complete PSV (with last dose received ≥ 150 days before onset), while it was 59% (95% CI: 51–66) after addition of one booster dose. The VE was 85% (95% CI: 78–89), 70% (95% CI: 61–77) and 36% (95% CI: 17–51) for those with

KEY PUBLIC HEALTH MESSAGE

What did you want to address in this study?
In order to understand how well the COVID-19 vaccine is performing in Europe against hospitalisation during the period when the SARS-CoV-2 Omicron variant was circulating, we investigated vaccine effectiveness using data from a multi-country study of complete and booster-dose COVID-19 vaccination among adults aged 20 years and over.

What have we learnt from this study?
Between December 2021 and July 2022, vaccine effectiveness against hospitalisation with laboratory-confirmed SARS-CoV-2 was 43% for complete vaccination. With addition of an mRNA booster dose, effectiveness was 59% overall. It was higher when onset of illness was close to the date of the last vaccination, at 85% when last booster dose was 14–59 days before onset, at 70% for 60–119 days, and falling below 40% for 120–179 days.

What are the implications of your findings for public health?
In European hospital settings in 2022, during the Omicron period, COVID-19 mRNA booster vaccine provided an improved benefit for preventing hospitalisation, particularly if disease onset was within 4 months of receiving the booster dose.

onset 14–59 days, 60–119 days and 120–179 days after booster vaccination, respectively. Conclusions: Our results suggest that, during the Omicron period, observed VE against SARI hospitalisation improved with first mRNA booster dose, particularly for those having symptom onset<120 days after first booster dose.

Introduction

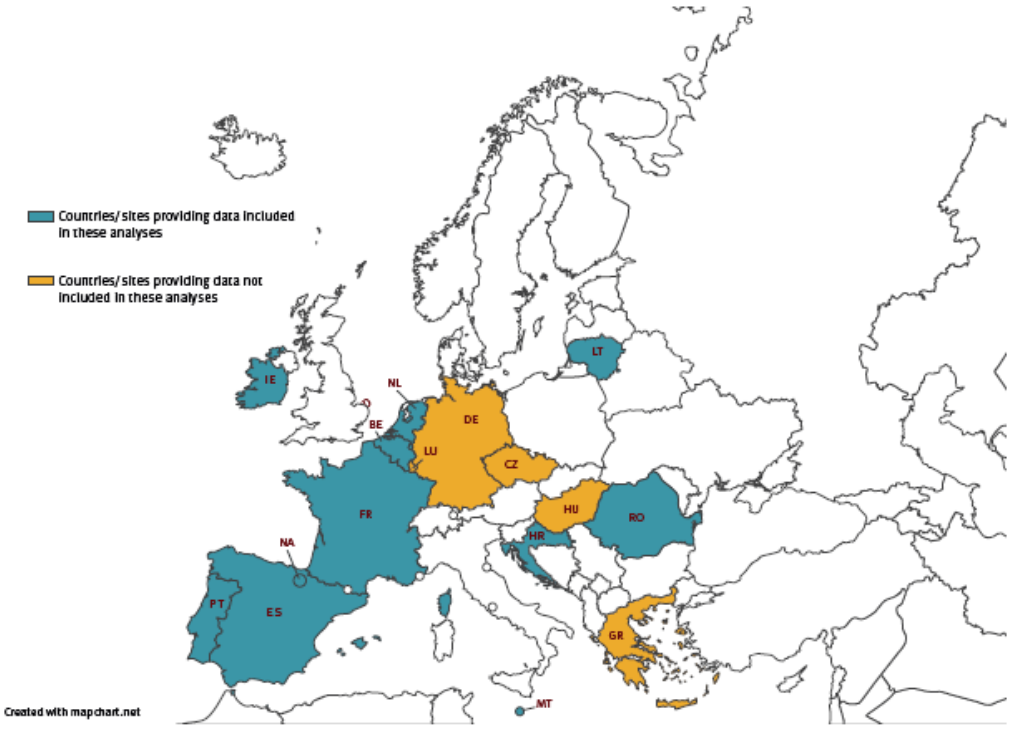
Safe and effective COVID-19 vaccines were a powerful tool for the control of the COVID-19 pandemic, along with non-pharmaceutical interventions to reduce transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). By the end of 2021 in the European Union (EU)/European Economic Area (EEA), one subunit and four spike protein-based COVID-19 vaccines had a conditional marketing authorisation for use [1]: the mRNA vaccines Comirnaty (BNT162b2; Pfizer-BioNTech) and Spikevax (mRNA-1273; Moderna), the adenoviral vector vaccines Vaxzevria (AZD1222; AstraZeneca) and Jcovden (Ad26.COV 2.5; Johnson & Johnson), and Nuvaxovid (NVX-CoV2373; Novavax), a subunit vaccine. All vaccines deployed in the early phase of the EU vaccination campaign were reported to be highly efficacious in randomised clinical trials [2-4]. The effectiveness of complete primary series vaccination (PSV) in real-life, post-authorisation studies was high [5-8]. Hospitalisation rates in patients ≥ 80 years in the EU/EEA declined by between 46% and 78% (depending on the level of the country's vaccine uptake) after vaccine deployment [9], which also averted› 450,000 deaths in those aged 60 years and over [10].

Since the beginning of its circulation, SARS-CoV-2 has been evolving, with new variants emerging. The initial real-life effectiveness studies were performed in 2021 during the circulation of the Alpha and Delta variants of concern. The emergence of the Omicron variant late in 2021 emphasised the indication of a first booster dose [11,12]. The rationale behind this was from evidence of waning of neutralising vaccine antibodies, which could cause a loss of protection against SARS-CoV-2 [13,14], shown by a decline in vaccine effectiveness (VE) over time against infection and hospitalisation [15,16]. Although the objective of PSV was to prevent or reduce the occurrence of all COVID-19 outcomes [5,17,18], the administration of a booster dose aimed to restore the protection against severe outcomes conferred by primary vaccination at time of Delta predominance [19]. By December 2021, all EU/EEA countries recommended a booster dose after complete PSV of authorised vaccines for adults [20], with many recommending a delay of 150–180 days between last PSV dose and booster [21]. Priority groups for the administration of a booster dose included individuals at risk of severe COVID-19 outcomes based on presence of underlying medical condition or age with an age-based staggered approach from older to younger age groups. By 30 June 2022, the vaccination coverage rates for first booster dose had reached 83% in≥ 80-year-olds compared with 39% in 18–24-year-olds in the EU/EEA countries [22].

Monitoring of VE against specific sub-lineages is vital to inform public health guidance for COVID-19 vaccination. In Europe, multicentre hospital-based studies have been implemented to monitor VE over time [23]. We aimed to estimate COVID-19 VE using pooled data from the Vaccine Effectiveness, Burden and Impact (VEBIS) hospital severe acute respiratory infection (SARI) VE network [24] and the I-MOVE-COVID-19 network [25]. At the end of 2021, the SARS-CoV-2 Omicron variant began to dominate in countries participating in these networks.

FIGURE 1

Countries and study sites participating in I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, by provision of data for this analysis, Europe, 2021–2022



I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

Sixteen participating sites: Belgium (BE), Croatia (HR), Czechia (CZ), France (FR), Germany (DE), Greece (GR), Hungary (HU), Ireland (IE), Lithuania (LT), Luxembourg (LU), Malta (MT), the Netherlands (NL), Portugal (PT), Romania (RO), Spain 11 regions (ES), Spain Navarra region (NA). Eleven included in this analysis: BE, ES, FR, HR, IE, LT, MT, NA, NL, PT, RO.

We aimed to estimate VE of complete PSV and first booster dose against hospitalisation with PCR-confirmed SARS-CoV-2 among patients aged≥ 20 years with SARI swabbed between 13 December 2021 and 31 July 2022 (Omicron-dominant period).

Methods

Setting

The two hospital networks (I-MOVE-COVID-19 and ECDC VEBIS) include 48 hospitals across 16 sites in 15 countries (with two sites in Spain; Figure 1).

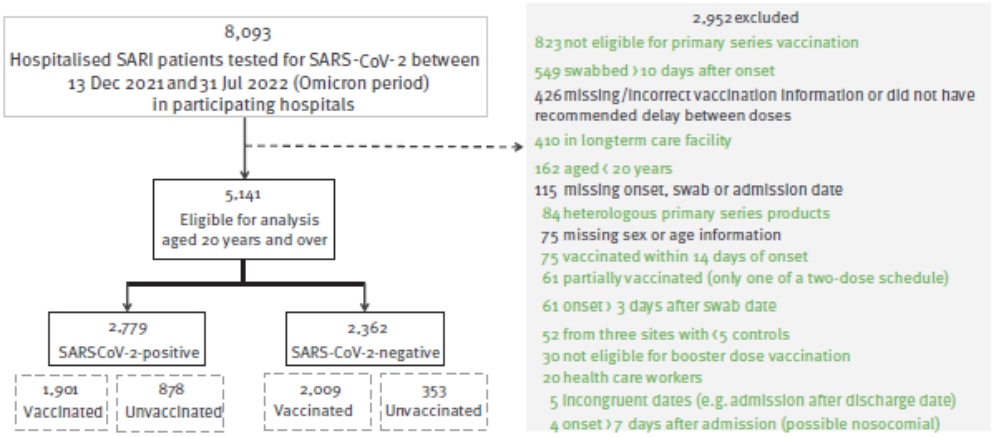
Study design and case definitions

Hospitals participating in both networks share similar generic protocols [24,25] and use the test-negative case–control design [26]. Demographic and clinical data and COVID-19 vaccination information were collected via questionnaire, electronic medical records or patient interview, depending on the site. Study sites collected information on all, or a systematic sample of, hospitalised SARI patients (for details see [27]).

We defined a SARI patient as an individual hospitalised for≥ 24 h with at least one symptom (fever, cough, shortness of breath, sudden onset anosmia, ageusia or dysgeusia) using the ECDC definition for a possible COVID-19 case [28]. Cases were SARI patients PCR-positive for SARS-CoV-2 within 48 h of admission or in the 14 days before hospital admission. Controls were SARI patients PCR-negative at or within 48 h of admission. Controls with known SARS-CoV-2 infection confirmed by PCR within 14 days of onset were recoded as cases. We used time of swab as a proxy for start of Omicron and Omicron sub-lineage dominance,

FIGURE 2

Exclusions, I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, Europe, 2021–2022 (n = 8,093)



I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

Included data are from 40 hospitals in 11 participating sites.

defined as the first day of the country-specific week in which ≥ 80% of all samples available in GISAID [29,30] were identified as B.1.1.529 (or Omicron BA.1, BA.2 and BA.4/5), up to the end of the study period (31 July 2022).

Inclusion and exclusion criteria

We excluded patients with missing/erroneous key variables (age, sex, key dates, vaccination information). We also excluded (i) patients not in each country's age-specific booster dose target group (BDTG) at the time of swab, (ii) those with non-homologous PSV (for two-dose schedules; counting Comirnaty and Spikevax as 'homologous' as both are mRNA vaccines) and (iii) those with mRNA booster vaccination who had received this dose < 150 days after their last PSV dose. We did not include in the analysis data from sites with fewer than five cases/controls or fewer than 20 total cases and controls.

Definitions of vaccination status

We defined complete PSV as two doses of a two-dose vaccine schedule (or three for immunocompromised individuals) or one dose of Jcovden, and booster vaccination as complete PSV plus first mRNA booster dose. Vaccination was considered valid only if vaccines were received ≥ 14 days before symptom onset and with recommended delays between doses (those vaccinated < 14 days before symptom onset, and those with non-recommended delays between doses were excluded).

Statistical analysis

We compared the odds of vaccination between cases and controls using logistic regression, calculating VE as 1 minus the odds ratio (OR) of vaccination among cases and controls (expressed as a percentage). We included study site (as a fixed effect) and date of swab (modelled as a spline or categorical variable, with the best functional form designated by the Akaike information criterion) in all VE analyses. We further adjusted the OR by age (modelled as a spline, a linear term, or as a categorical age group variable, with the best functional form designated by the Akaike information criterion), by sex and by presence of at least one of four commonly collected chronic conditions (asthma, diabetes, heart disease, lung disease) or absence of all four.

We estimated absolute VE during the Omicron period for complete PSV only and for complete PSV plus first mRNA booster dose, vs no COVID-19 vaccination. For complete PSV analyses, we included vaccinated patients in two main groups: those not yet eligible for booster, having received their last PSV dose < 150 days prior (Analyses 1–2), and those eligible for booster, with last PSV dose ≥ 150 days before onset of symptoms (Analyses 3–5). The 150-day period was selected as, in general, the recommendation in participating countries was for booster to be administered 5 months after last PSV dose.

TABLE 1A

Characteristics of cases and controls, I-MOVE-COVID-19 and VEBIS vaccine effectiveness hospital networks, Europe, Omicron period December 2021–July 2022 (n = 5,141)

Patient characteristic	SARS-CoV-2 cases (n = 2,779)		Test negative controls (n = 2,362)	
	Number	%	Number	%
Median age (years)	77		75	
Age groups (years)				
20–59	420	15.1	439	18.6
60–79	1,147	41.3	1,051	44.5
≥ 80	1,212	43.6	872	36.9
Sex				
Male	1,592	57.3	1,294	54.8
Female	1,187	42.7	1,068	45.2
At least one chronic condition[a]				
No	895	32.2	589	24.9
Yes	1,884	67.8	1,773	75.1
Omicron sub-lineage periods[b]				
Swab date 13 December 2021–20 February 2022 (BA.1)	1,067	38.4	503	21.3
Swab date 7 March–29 May 2022 (BA.2)	554	19.9	755	32.0
Swab date 10 June–31 July 2022 (BA.4/5)	399	14.4	207	8.8
Sub-lineage period undefined[c]	759	27.3	897	38.0
COVID-19 vaccination status				
Unvaccinated	878	31.6	353	14.9
Complete PSV only	502	18.1	395	16.7
Complete PSV + first booster only	1,389	50.0	1,598	67.7
Complete PSV + two boosters	10	0.4	16	0.7
Vaccine product among vaccinated: first dose				
Comirnaty	1,480	77.9	1,508	75.1
Vaxzevria	200	10.5	256	12.7
Spikevax	158	8.3	185	9.2
Janssen	62	3.3	53	2.6
Other/unknown	1	0.1	7	0.3
Vaccine product among vaccinated: second dose				
Comirnaty	1,482	80.6	1,497	76.5
Vaxzevria	200	10.9	256	13.1
Spikevax	156	8.5	196	10.0
Other/unknown	1	0.1	7	0.4

ICU: intensive care unit; I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; IQR: inter-quartile range; NI: no data included; PSV: primary series vaccination; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

[a] At least one of four commonly collected chronic conditions: asthma, diabetes, heart disease, lung disease.

[b] Omicron sub-lineage period defined using proxy dates in each study country to mark the week in which the proportion of samples sequenced and reported to GISAID reached ≥ 80% for the start of the period, and the end of the week before the one in which < 80% of samples sequenced were from a particular sub-lineage (BA.1, BA.2 and either BA.4 or BA.5).

[c] Sub-lineage period undefined means all those SARI patients with onset dates that did not fall within any of the defined sub-lineage periods above.

TABLE 1B

Characteristics of cases and controls, I-MOVE-COVID-19 and VEBIS vaccine effectiveness hospital networks, Europe, Omicron period December 2021–July 2022 (n = 5,141)

Patient characteristic	SARS-CoV-2 cases (n = 2,779)		Test negative controls (n = 2,362)	
	Number	%	Number	%
Vaccine product among vaccinated: first booster dose				
Comirnaty	1,096	78.3	1,155	71.6
Spikevax	299	21.4	446	27.6
Other/unknown	4	0.2	13	0.8
Vaccine product among vaccinated: second booster dose				
Comirnaty	8	80.0	12	75.0
Spikevax	2	20.0	3	18.8
Other/unknown	0	0.0	1	6.3
Study site and country[d]				
Belgium	161	5.8	223	9.4
Croatia	515	18.5	167	7.1
Czechia	NI			
France	112	4.0	91	3.9
Germany	NI			
Greece	NI			
Hungary	NI			
Ireland	96	3.5	109	4.6
Lithuania	23	0.8	49	2.1
Luxembourg	NI			
Malta	185	6.7	261	11.0
the Netherlands	104	3.7	166	7.0
Portugal	78	2.8	58	2.5
Romania	83	3.0	5	0.2
Spain (Navarra)	111	4.0	84	3.6
Spain (11 regions)	1,311	47.2	1,149	48.6
Days from vaccination (any vaccine product)	Median	IQR	Median	IQR
Last PSV dose to onset (patients with PSV only; including those with last dose< 150 days from symptom onset)	240	183–303	244	190–310
Last PSV dose to onset (patients with PSV only; excluding those with last dose< 150 days from onset)	254	208–314	253	209–319
First booster dose to symptom onset	159	98–208	127	81–176
Last PSV dose to first booster dose*	209	189–231	206	188–229

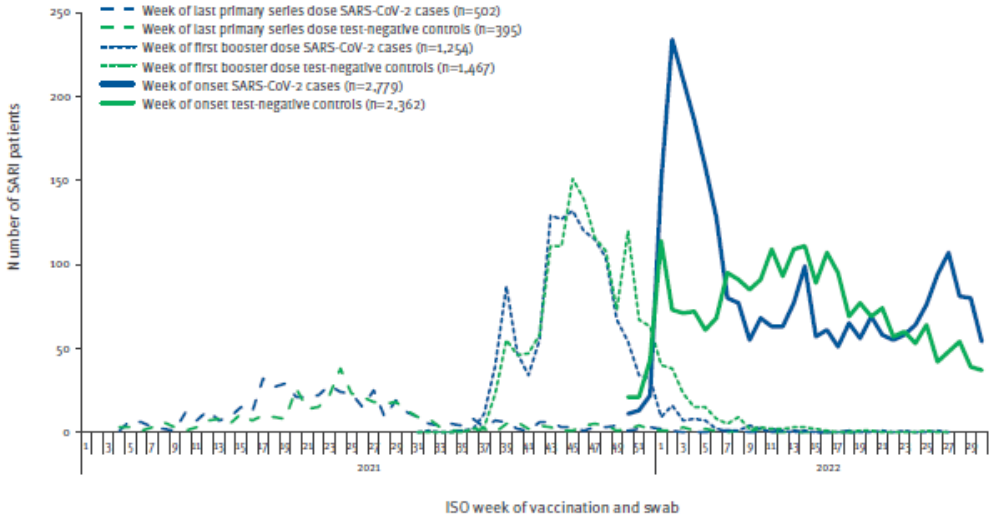
ICU: intensive care unit; I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; IQR: inter-quartile range; NI: no data included; PSV: primary series vaccination; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

[d] Three sites submitting data during the Omicron period did not have a sufficient number of eligible SARI patients for inclusion in these analyses, and two sites did not submit data for this period (this table gives final numbers by site, after exclusions).

* Note that patients with booster dose< 150 days from last PSV dose were excluded from analyses.

FIGURE 3

Number of SARI patients by case status and week of COVID-19 vaccination or swab (complete primary series and booster doses), by vaccine product, I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, Europe, 2021–2022 (n = 5,141)



I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; SARI: severe acute respiratory infection; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

In Analysis 1, we estimated VE for all products combined in those who had received complete PSV only with symptom onset < 150 days of the last PSV dose. In Analysis 2, we estimated booster dose VE for those with at least 150 days between last PSV dose and booster dose. For comparison, we estimated VE in those who had not received a booster dose, but who had received their last PSV dose ≥ 150 days before onset. We estimated VE overall, by age group and by PSV product.

In Analysis 3, we estimated VE by time since vaccination for those receiving a first mRNA booster dose for all ages combined, using the intervals 14–59, 60–119, 120–179 and ≥ 120 days from receipt of the booster (overall and by PSV product). In Analysis 4 we also estimated VE by time since vaccination, for the same periods as Analysis 3, but stratified into three age groups (20–59, 60–79 and ≥ 80 years), for all PSV products combined. In both of these analyses, we only included vaccinated SARI patients for whom the delay between last PSV dose and booster dose was ≥ 150 days.

In supplementary analyses, we estimated VE by time since last PSV dose for each of three periods (14–59, 60–119 and 120–149 days). Further, we measured VE for the periods of dominance of each of the Omicron variant sub-lineages BA.1, BA.2 and BA.4/5, overall and by vaccine category/product, age group, presence of at least one chronic condition and time since booster dose vaccination (< 60 days, 60–119 days, ≥ 120 days).

Sensitivity analyses

We performed sensitivity analyses (i) excluding all SARI patients with known prior infection > 14 days before onset and (ii) including those with severe outcomes only (admission to an intensive care unit (ICU) or in-hospital death). Where the number of cases or controls per parameter was < 10, we conducted sensitivity analysis using Firth's method of penalised logistic regression (PLR) to assess small sample bias [31,32]. We do not show estimates with a difference between the PLR and original VE estimate > 10 percentage points.

Results

Descriptive analysis

After applying exclusion criteria (Figure 2), we included 2,779 cases and 2,362 controls aged ≥ 20 years from 40 of 48 European hospitals in 11 of 16 participating study sites providing sufficient data (at least five cases and controls) for the study period (Figure 1).

The highest proportion of cases by age group were those ≥ 80 years (1,212; 44% vs 872 controls; 37%); 1,187 (43%) cases and 1,068 (45%) controls were female; 1,884 (68%) cases and 1,773 (75%) controls had at least one of the four listed chronic conditions

TABLE 2A

Effectiveness of COVID-19 complete primary series vaccination and booster vaccination against hospitalisation among adults (≥ 20 years) by age group and vaccine product, I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, Europe, 13 December 2021–31 July 2022 (n = 5,115[a])

PSV vaccine product	Vaccinated/unvaccinated cases; Vaccinated/unvaccinated controls		VE[b] (95% CI)	
Analysis 1: VE< 150 days from receipt of last PSV dose to symptom onset in those not eligible for booster dose				
Any PSV vaccine product (eight sites[c]; n = 1,245[d])				
All 14–149 days	67/791; 48/339		51 (21 to 69)	
Analysis 2: VE in those eligible for first booster dose. Vaccination ≥ 150 days before symptom onset for patients with PSV only and ≥ 150 days between last PSV and booster dose (for those with PSV + mRNA booster)				
	Complete PSV only	Complete PSV plus mRNA booster	Complete PSV only	Complete PSV plus mRNA booster
Any PSV product	**11 sites[e]; n = 2,007[f]**	**10 sites[g]; n = 3,876[h]**		
All ≥ 20	430/878; 346/353	1,249/811; 1,467/349	43 (29 to 54)	59 (51 to 66)
Age group				
20–59	103/179; 137/97	83/165; 161/94	65 (46 to 77)	66 (47 to 78)
60–79	193/382; 143/155	460/349; 643/154	38 (13 to 56)	58 (45 to 68)
≥ 80	134/317; 66/101	706/297; 663/101	29 (–7 to 53)	58 (42 to 69)
Chronic condition[i]				
No	146/374; 121/119	292/346; 279/116	60 (43 to 72)	64 (48 to 75)
Yes	284/504; 225/234	957/465; 1,188/233	32 (11 to 48)	56 (45 to 64)
Comirnaty PSV	**10 sites[g]; n = 1,670**	**10 sites[g]; n = 3,401**		
All ≥ 20	282/802; 240/346	1,079/811; 1,162/349	49 (34 to 60)	54 (45 to 62)
Age group				
20–59	79/165; 89/93	56/165; 101/94	58 (33 to 73)	59 (34 to 74)
60–79	104/342; 98/153	371/349; 461/154	53 (29 to 68)	52 (36 to 64)
≥ 80	99/295; 53/100	652/297; 600/101	27 (–16 to 54)	54 (37 to 66)
Chronic condition[i]				
No	90/341; 82/114	248/346; 205/116	64 (45 to 76)	60 (41 to 72)
Yes	192/461; 158/232	831/465; 957/233	34 (11 to 52)	52 (39 to 61)
Spikevax PSV	**Eight sites[c]; n = 1,275**	**10 sites[g]; n = 1,382**		
All ≥ 20	42/855; 34/344	92/811; 130/349	50 (14 to 71)	60 (44 to 71)
Age group				
20–59	7/174; 17/91	12/165; 31/94	76 (32 to 92)	69 (32 to 86)
60–79	25/370; 12/154	38/349; 52/154	23 (–77 to 66)	54 (21 to 73)
≥ 80	10/311; 5/99	42/297; 47/101	NC	58 (26 to 76)
Chronic condition[i]				
No	14/360; 10/116	17/346; 29/116	64 (5 to 86)	75 (49 to 88)
Yes	28/495; 24/228	75/465; 101/233	39 (–17 to 68)	50 (27 to 66)
Vaxzevria PSV	**Nine sites[j]; n = 1,263**	**Nine sites[j]; n = 1,355**		
All ≥ 20	72/802; 43/346	60/802; 147/346	30 (–10 to 56)	69 (54 to 79)

CI: confidence interval; ICU: intensive care unit; I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; PSTG: primary series target group; PSV: primary series vaccination; VE: vaccine effectiveness; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

[a] n = 5,115 after dropping 26 with a second booster (sample size too small to analyse second booster).

[b] Odds ratio adjusted by country, time (restricted cubic spline of swab date or swab month as categorical variable, depending on model), age (restricted cubic spline or age as linear variable, depending on model), sex, presence/absence of chronic condition (asthma, diabetes, heart disease, lung disease).

[c] Eight sites: Belgium, Croatia, France, Ireland, Malta, the Netherlands, Portugal and Spain.

[d] n = 1,245 after dropping 2,987 records of patients who received a first mRNA booster dose, 776 with last primary series vaccination dose ≥ 150 days from onset and 107 from three sites with fewer than five controls or fewer than 20 SARI patients.

[e] Eleven sites: Belgium, Croatia, France, Ireland, Lithuania, Malta, Navarra, the Netherlands, Portugal, Romania and Spain.

[f] n = 2,007 after dropping 2,987 records of patients who received a first mRNA booster dose, and 266 with last primary series vaccination dose< 150 days from onset.

[g] Ten sites: Belgium, Croatia, France, Ireland, Lithuania, Malta, Navarra, the Netherlands, Portugal and Spain.

[h] n = 3,876 after dropping 897 records from patients who had received primary series vaccination only (no booster), 262 who had received their booster dose< 150 days before symptom onset, and 76 from one site with fewer than five controls.

[i] In analyses stratified by chronic condition, the adjustment for presence/absence of chronic condition was removed.

[j] Nine sites: Belgium, Croatia, France, Ireland, Lithuania, Malta, the Netherlands, Portugal and Spain.

TABLE 2B

Effectiveness of COVID-19 complete primary series vaccination and booster vaccination against hospitalisation among adults (≥ 20 years) by age group and vaccine product, I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, Europe, 13 December 2021–31 July 2022 (n = 5,115[a])

PSV vaccine product	Vaccinated/unvaccinated cases; Vaccinated/unvaccinated controls		VE[b] (95% CI)	
Age group				
20–59	5/165; 12/93	11/165; 23/93	NC	71 (25 to 89)
60–79	51/342; 25/153	39/342; 110/153	−5 (−95 to 44)	68 (47 to 81)
≥ 80	16/295; 6/100	10/295; 14/100	42 (-68 to 80)	51 (−54 to 84)
Chronic condition[j]				
No	26/341; 17/114	21/341; 40/114	58 (13 to 80)	71 (43 to 86)
Yes	46/461; 26/232	39/461; 107/232	15 (−52 to 53)	69 (51 to 80)
Analysis 3: VE by time (number of days) since receipt of first mRNA booster dose (14–59, 60–119, 120–179, ≥ 180 days from receipt of booster to symptom onset) by PSV product				
All PSV products combined (10 sites[g]; n = 3,876[k])				
All ≥ 14 days from first mRNA booster dose	1,249/811; 1,467/349		59 (51 to 66)	
Days from first mRNA booster dose to onset (among vaccinated cases and controls)				
14–59	115/811; 212/349		85 (78 to 89)	
60–119	304/811; 464/349		70 (61 to 77)	
120–179	328/811; 444/349		36 (17 to 51)	
≥ 180	502/811; 347/349		−3 (−37 to 23)	
Comirnaty PSV (10 sites[g]; n = 3,401)				
All ≥ 14 days from first mRNA booster dose	1,079/811; 1,162/349		54 (45 to 62)	
Days from first mRNA booster dose to onset				
14–59	89/811; 164/349		86 (79 to 90)	
60–119	260/811; 340/349		66 (56 to 74)	
120–179	280/811; 358/349		34 (13 to 50)	
≥ 180	450/811; 300/349		−8 (−45 to 20)	
Spikevax PSV (10 sites[g]; n = 1,382)				
All ≥ 14 days from first mRNA booster dose	92/811; 130/349		60 (44 to 71)	
Days from first mRNA booster dose to onset				
14–59	10/811; 22/349		85 (65 to 93)	
60–119	20/811; 40/349		79 (61 to 89)	
120–179	29/811; 37/349		27 (−28 to 58)	
≥ 180	33/811; 31/349		27 (−29 to 59)	
Vaxzevria PSV (nine sites[i]; n = 1,355)				
All ≥ 14 days from first mRNA booster dose	60/802; 147/346		69 (54 to 79)	

CI: confidence interval; ICU: intensive care unit; I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; PSTG: primary series target group; PSV: primary series vaccination; VE: vaccine effectiveness; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

[a] n = 5,115 after dropping 26 with a second booster (sample size too small to analyse second booster).

[b] Odds ratio adjusted by country, time (restricted cubic spline of swab date or swab month as categorical variable, depending on model), age (restricted cubic spline or age as linear variable, depending on model), sex, presence/absence of chronic condition (asthma, diabetes, heart disease, lung disease).

[c] Eight sites: Belgium, Croatia, France, Ireland, Malta, the Netherlands, Portugal and Spain.

[d] n = 1,245 after dropping 2,987 records of patients who received a first mRNA booster dose, 776 with last primary series vaccination dose ≥ 150 days from onset and 107 from three sites with fewer than five controls or fewer than 20 SARI patients.

[e] Eleven sites: Belgium, Croatia, France, Ireland, Lithuania, Malta, Navarra, the Netherlands, Portugal, Romania and Spain.

[f] n = 2,007 after dropping 2,987 records of patients who received a first mRNA booster dose, and 266 with last primary series vaccination dose < 150 days from onset.

[g] Ten sites: Belgium, Croatia, France, Ireland, Lithuania, Malta, Navarra, the Netherlands, Portugal and Spain.

[k] n = 3,876 after dropping 897 records from patients who had received primary series vaccination only (no booster), 262 who had received their booster dose < 150 days before symptom onset, and 76 from one site with fewer than five controls.

[j] In analyses stratified by chronic condition, the adjustment for presence/absence of chronic condition was removed.

[i] Nine sites: Belgium, Croatia, France, Ireland, Lithuania, Malta, the Netherlands, Portugal and Spain.

TABLE 2C

Effectiveness of COVID-19 complete primary series vaccination and booster vaccination against hospitalisation among adults (≥ 20 years) by age group and vaccine product, I-MOVE-COVID-19 and VEBIS hospital vaccine effectiveness studies, Europe, 13 December 2021–31 July 2022 (n = 5,115[a])

PSV vaccine product	Vaccinated/unvaccinated cases; Vaccinated/unvaccinated controls	VE[b] (95% CI)
Days from first mRNA booster dose to onset		
14–59	14/802; 20/346	75 (45 to 89)
60–119	18/802; 77/346	78 (60 to 88)
120–179	16/802; 36/346	43 (–11 to 71)
≥ 180	12/802; 14/346	27 (–74 to 70)
Analysis 4: VE by time (number of days) since receipt of mRNA booster dose (14–59, 60–119, 120–179, ≥ 180 days from receipt of booster dose to symptom onset) for all PSV products combined, by age group (20–59, 60–79, ≥ 80 years)		
Aged 20–59 years (10 sites[c]; n = 503)		
All ≥ 14 days from first mRNA booster dose	83/165; 161/94	66 (47 to 78)
Days from first mRNA booster dose to onset (among vaccinated cases and controls)		
14–59	23/165; 47/94	81 (62 to 91)
60–119	27/165; 69/94	59 (25 to 78)
120–179	17/165; 30/94	38 (–33 to 71)
≥ 180	16/165; 15/94	–35 (–257 to 49)
Aged 60–79 years (10 sites[d]; n = 1,606)		
All ≥ 14 days from first mRNA booster dose	460/349; 643/154	58 (45 to 68)
Days from first mRNA booster dose to onset (among vaccinated cases and controls)		
14–59	56/349; 105/154	85 (75 to 91)
60–119	113/349; 209/154	70 (55 to 80)
120–179	135/349; 200/154	28 (–7 to 52)
≥ 180	156/349; 129/154	2 (–59 to 40)
Aged ≥ 80 years (10 sites[e]; n = 1,767)		
All ≥ 14 days from first mRNA booster dose	706/297; 663/101	58 (42 to 69)
Days from first mRNA booster dose to onset (among vaccinated cases and controls)		
14–59	36/297; 60/101	86 (73 to 93)
60–119	164/297; 186/101	76 (63 to 85)
120–179	176/297; 214/101	38 (5 to 59)
≥ 180	330/297; 203/101	–4 (–59 to 32)

CI: confidence interval; ICU: intensive care unit; I-MOVE: Influenza – Monitoring Vaccine Effectiveness in Europe; PSTG: primary series target group; PSV: primary series vaccination; VE: vaccine effectiveness; VEBIS: Vaccine Effectiveness, Burden and Impact Studies.

[a] n = 5,115 after dropping 26 with a second booster (sample size too small to analyse second booster).

[b] Odds ratio adjusted by country, time (restricted cubic spline of swab date or swab month as categorical variable, depending on model), age (restricted cubic spline or age as linear variable, depending on model), sex, presence/absence of chronic condition (asthma, diabetes, heart disease, lung disease).

[c] Eight sites: Belgium, Croatia, France, Ireland, Malta, the Netherlands, Portugal and Spain.

[d] n = 1,245 after dropping 2,987 records of patients who received a first mRNA booster dose, 776 with last primary series vaccination dose ≥ 150 days from onset and 107 from three sites with fewer than five controls or fewer than 20 SARI patients.

[e] Eleven sites: Belgium, Croatia, France, Ireland, Lithuania, Malta, Navarra, the Netherlands, Portugal, Romania and Spain.

[f] n = 2,007 after dropping 2,987 records of patients who received a first mRNA booster dose, and 266 with last primary series vaccination dose < 150 days from onset.

[g] Ten sites: Belgium, Croatia, France, Ireland, Lithuania, Malta, Navarra, the Netherlands, Portugal and Spain.

[h] n = 3,876 after dropping 897 records from patients who had received primary series vaccination only (no booster), 262 who had received their booster dose < 150 days before symptom onset, and 76 from one site with fewer than five controls.

[i] In analyses stratified by chronic condition, the adjustment for presence/absence of chronic condition was removed.

[j] Nine sites: Belgium, Croatia, France, Ireland, Lithuania, Malta, the Netherlands, Portugal and Spain.

(Table 1). For this analysis, 1,570 SARI patients (31%) were swabbed during the Omicron BA.1 sub-lineage period (13 December 2021–20 February 2022), 1,309 (25%) during the BA.2 period (7 March–29 May 2022) and 606 (12%) in the BA.4/5 period (10 June–31 July 2022). The remaining 1,656 (32%) were swabbed during a period undefined by these sub-lineages (Table 1).

At the time of swab, 878 cases (32%) and 353 controls (15%) were unvaccinated. Half of the cases (n = 1,389) and 1,598 controls (68%) had received both complete PSV and first booster doses; 10 cases (0.4%) and 16 controls (0.7%) had a second booster dose (Table 1). Seventy-eight per cent of cases and 75% of controls were vaccinated with Comirnaty for their first dose; 81% and 77% for the second dose, respectively.

SARI patients received their last PSV dose between week 4, 2021 and week 18, 2022 (Figure 3). The median time from last PSV dose to onset of symptoms for patients with complete PSV only was 240 days (interquartile range (IQR): 183–303) in cases and 244 days (IQR: 190–310) in controls (Table 1). Excluding those with last PSV dose < 150 days of onset, these medians were 254 days (IQR: 208–314) and 253 days (IQR: 209–319), respectively. SARI patients received their first booster doses between week 21, 2021 and week 26, 2022. The median time from first booster dose to onset was 160 days (IQR: 101–211) for cases and 132 days (IQR: 86–181) for controls. The median time between last PSV dose and first booster dose (in those with booster dose≥150 days after last complete PSV dose) was 209 days (IQR: 189–231) for cases, 206 days (IQR: 188–229) for controls.

Analysis 1: Vaccine effectiveness for primary series vaccination in individuals not yet eligible for a booster dose

We estimated VE among individuals who had received their last PSV dose <150 days prior to symptom onset. Among this group, VE against hospitalisation with COVID-19 for complete PSV was 51% (95% confidence interval (CI): 21–69) for all PSV products combined (Table 2). For age-stratified and time-since-vaccination results for this group, see Supplementary Table S1.

Analysis 2: Vaccine effectiveness of primary series vaccination with or without first mRNA booster

We estimated VE among individuals eligible for booster and with ≥150 days between last PSV and symptom onset (for PSV alone) or booster dose (for PSV plus booster). For all PSV products combined, VE in those who received complete PSV alone was 43% (95% CI: 29–54). In those receiving complete PSV and an mRNA booster dose, VE was 59% (95% CI: 51–66). With Comirnaty as PSV, the VE for any mRNA booster was 54% (95% CI: 45–62); it was 60% (95% CI: 41–72) with Spikevax as PSV and 69% (95% CI: 54–79) with Vaxzevria as PSV. For those aged 20–59, 60–79 and≥80 years, mRNA booster dose VE for all PSV products combined was 66% (95% CI: 47–78), 58% (95% CI: 45–68) and 56% (95% CI: 45–64), respectively. The VE for all PSV products combined in those without any of the four commonly collected chronic conditions was 60% (95% CI: 43–72) without and 64% (95% CI: 48–75) with mRNA booster dose. For those with at least one of these chronic conditions, these VE were 32% (95% CI: 11–48) and 56% (95% CI: 45–64), respectively (Table 2).

Analysis 3: Vaccine effectiveness by time since mRNA booster dose

We estimated VE among those receiving a first booster dose for the intervals 14–59, 60–119, 120–179 and≥180 days between first booster dose and symptom onset. For all PSV products combined, VE was 85% (95% CI: 78–89) in those receiving the first mRNA booster dose 14–59 days before symptom onset. For those with mRNA booster 120–179 days before onset, VE was 36% (95% CI: 17–51). For Comirnaty and Spikevax PSV, followed by first mRNA booster dose 14–59 days before symptom onset, VE point estimates were≥85%; for Vaxzevria PSV plus mRNA booster 14–59 days before onset, VE was 75% (95% CI: 45–89). For those receiving Vaxzevria, Comirnaty and Spikevax as PSV followed by a first mRNA booster dose 120–179 days before symptom onset, VE was 43% (95% CI: −11 to 71), 34% (95% CI: 13–50) and 27% (95% CI: −28 to 58), respectively. Our results showed no protection ≥ 180 days post booster for all products combined (Table 2).

Analysis 4: Vaccine effectiveness by age group, overall and by time since mRNA booster dose

We estimated VE among those who had received a first booster dose for the intervals 14–59, 60–119, 120–179 and ≥ 180 days from first booster dose to symptom onset. For all PSV products combined, VE after receipt of first mRNA booster dose 14–59 days before onset was at least 81% in each age group (20–59, 60–79 and≥80 years). In the two older age groups (≥60 years), the VE for first mRNA booster received 60–119 days before symptom onset was at least 70%, while in the youngest age group (20–59 years) mRNA booster dose VE was 59%. For those vaccinated 120–179 days before onset, the VE point estimates for all age groups were≤40%, with no protection observed for any age group when the booster was received≥180 days before onset (Table 2).

Supplementary analyses

We estimated the VE of mRNA booster dose by Omicron sub-lineage (BA.1, BA.2, BA.4/5) dominance periods, estimated using country-specific week of swab as proxy. The VE for mRNA booster dose (all PSV products combined) for Omicron sub-lineage BA.1 was>75% overall and across all age groups and was highest in those aged ≥ 80 years. For BA.2 and BA.4/5, the VE was 25% and 13% overall, respectively. Sample size was very low for BA.4/5, resulting in very wide confidence intervals and precluding some stratified analyses. The VE by time since vaccination for these

sub-lineages were greater in those whose last vaccination was<60 days before onset than in those with vaccination≥60 days, and the VE at<60 days was lower for the later sub-lineages (87% and 41% for BA.1 and BA.2, respectively; BA.4/5 sample size was too small to complete this analysis as most vaccinated patients were vaccinated ≥120 days before onset). For the details of these results by sublineage, we refer to Supplementary Tables S2 and S3.

Sensitivity analyses

Excluding 463 cases and 281 controls with known prior (PCR-confirmed) infection in those receiving complete PSV alone produced slightly greater VE for all products combined: from 1 (PSV only) to 5 percentage points higher for those with booster dose vaccination. The VE point estimates by age group for those who had only received complete PSV ranged from 13 percentage points lower (for those 20–59 years old) to 2 percentage points higher (in those aged 60–79 years) and 12 percentage points higher (in those aged≥80 years). Booster dose VE in those without known prior infection ranged from 1 percentage point higher in the youngest age group to 7 percentage points higher in those aged 60–79 years. The data for these sensitivity analyses are accessible in Supplementary Table S4 (Analysis 1). In the group who were either admitted to ICU or died in hospital (severe outcomes), and were in the BDTG, the VE for complete PSV in those≥150 days from their last PSV dose was 14 percentage points higher than in the main analysis for this group. For those aged≥80 years, although VE was 43 percentage points higher than in the main analysis, numbers were small and 95% CIs wide (sample size was too small to estimate VE for the younger age groups). For those receiving a booster dose≥150 days from their last PSV dose, the VE was 3–5 percentage points different from the main analysis, overall and for each age group. For the detailed analyses we refer to Supplementary Table S4 (Analysis 2).

Discussion

Pooled results from this multi-country European study of VE against hospitalisation with COVID-19 showed that the overall VE was≤50% for all PSV products among hospitalised SARI patients aged≥20 years who were eligible for but not receiving an mRNA booster dose during the Omicron circulating period (December 2021–July 2022) and who received their last PSV dose≥150 days before symptom onset. In those who received an mRNA booster dose, VE increased to 54% (95% CI: 45–62), 60% (95% CI: 41–72) and 69% (95% CI: 54–79), respectively, for Comirnaty, Spikevax and Vaxzevria as PSV. Analysis by time since vaccination with mRNA booster (three 60-day periods from last booster dose to onset) for all PSV products combined indicated that the VE for the first mRNA booster dose decreased in each 60-day period, from 85% and 70% in the first two periods, to a much reduced 36% in the period 120–179 days between mRNA booster dose and symptom onset. The VE in those with onset 14–59 days after their first mRNA booster was≥75% regardless of individual PSV product but was<30% for those with onset≥180 days after their first mRNA booster dose. We observed only small differences in VE point estimates (with overlapping 95% CIs) for those who received mRNA PSV and booster products vs those with non-mRNA PSV product followed by mRNA booster. Our study showed that for those SARI patients who were eligible for a booster but had not yet received this, several months (median>200 days for both cases and controls) had passed between their last PSV dose and symptom onset. The lower VE in these individuals is likely to reflect waning immunity in the absence of a booster dose.

Our results should be interpreted with the following strengths and limitations in mind. Firstly, as with any multi-country study using pooled data, heterogeneity across countries is expected, from vaccine roll-out timing, coverage and product use, and SARS-CoV-2 variant distribution, to healthcare-seeking behaviour and case management strategies. To counter some of these, an adapted core protocol was in use, with homogeneous methodology applied across participating countries. Although residual and unmeasured confounding may be present and hence unaccounted for, most sites collected data prospectively, documenting important information on key confounders. For those receiving complete PSV alone, we estimated VE for those with onset<150 days and≥150 days from last PSV dose, to allow better comparability with those receiving a booster, as a booster dose was recommended in participating countries on average ca 150 days from last PSV dose. Product-specific VE estimates showed overlapping confidence intervals and the results did not demonstrate significant differences in protection against hospitalisation with PCR-confirmed SARS-CoV-2.

Combining individual-level data from several countries permitted a larger sample size to provide robust results for continued monitoring of VE against severe disease across Europe. However, sample sizes were low by individual vaccine product, limiting the precision of some estimates and preventing subgroup analyses among those aged 20–59 years and those with no chronic conditions.

We did not account for previous infection in either cases or controls (although sensitivity analyses showed 1–13 percentage point higher VE when those with known prior infection were excluded). However, as not all sites collect this information, and as some patients will have had mild or even asymptomatic prior infection not captured by our study, it is difficult to fully interpret the results. Measuring VE without knowledge of prior infection is a major challenge. In some participating countries, individuals with a SARS-CoV-2 infection have their subsequent COVID-19 vaccination dose delayed. Self-testing for SARS-CoV-2 has become common in many countries; it is difficult for hospital staff to document the number of prior infections, with dates, for each included patient (some of whom may

have had more than one prior infection during 2022 alone). Of the 463 cases and 281 controls in our study with known, PCR-confirmed prior infection, the proportion unvaccinated was similar to those with no known, PCR-confirmed prior infection for cases (32% vs 31%, respectively) but was lower for controls (20% vs 14%, respectively). Further sensitivity analyses adjusting by known prior infection are planned for the future, on larger datasets with fewer missing this information. As sequencing results were not available for most SARI patients, we used proxy dates for when ≥ 80% samples sequenced by a country were Omicron; a process which has been used in other studies [33,34].

Other studies have also shown a positive effect of booster doses in preventing COVID-19 hospitalisation, although our results were lower than most (e.g. we found an all-product VE of 70% at 60–119 days, 27% ≥ 120 days from booster dose, vs 89% within 60 days and 66% at ≥ 120 days [35]). Another multi-country study during the Omicron period with a similar test-negative design pooled data from several Latin American countries. They showed Comirnaty VE of 25% without booster among those ≥ 65 years [36]. Our Comirnaty VE for those ≥ 65 years is potentially higher, as we show VE of 53% and 27% for those 60–79 and ≥ 80 years, respectively. With booster, the Latin American study had more comparable Comirnaty VE results to ours, however, at 56% among those ≥ 65 years vs 52% in 60–79 and 54% in ≥ 80-year-olds in our study [36]. There are several reasons which could explain our low observed VE. One could be our use of a sensitive SARI case definition [28], potentially leading to more cases with milder disease being recruited. This could result in inclusion of hospitalised patients 'with' COVID-19 rather than only those hospitalised 'due to' COVID-19. This also explains the lower VE found overall in our study compared with others, as our VE estimates were more similar to those found against infection than against hospitalisation [37]. Our sensitivity analysis using more severe hospitalised outcomes (ICU or death in hospital) was difficult to interpret due to low sample size, possibly due to the milder effect of the Omicron variant, which is known to have caused fewer hospitalisations [38,39]. Booster-vaccinated patients in our study had all received an mRNA booster dose (Comirnaty or Spikevax), and most patients with PSV had also received an mRNA vaccination; our results therefore reflect mainly receipt of homologous mRNA PSV and booster vaccination. Despite the lower level of effectiveness, however, our study (like others) shows that the booster dose improves VE and hence has the potential to decrease hospital burden and improve outcomes [35,40-42].

Evolving evidence based on early VE data and analysis of antibody levels after the first booster dose suggest that there is gradual waning of immunity against the Omicron variant. Single-country studies in the United States (US) [35,43-45], Denmark [46] and Canada [47] undertaken during the early phase of Omicron emergence with short follow-up periods reported high VE of the booster dose (> 80%) against severe outcomes of Omicron infection. Other studies (some with the same test-negative design as ours) confirmed these early findings. For example, VE against COVID-19 hospitalisation and death was in the range of 70–80% any time after the second PSV dose and > 90% after the first booster dose in Qatar [48]. In the US, Tartof et al. reported VE after three doses of Comirnaty of 85% (95% CI: 80–89) against hospital admission due to the Omicron variant within 90 days, falling to 55% (95% CI: 28–71) ≥ 90 days [15]. Other study results indicated rapid waning of this effect with increasing time from vaccination [37,46,47,49-51], starting from 90 days after booster administration. Our results showing decreasing VE over time during the Omicron period, with no protection observed from 6 months after the booster dose, underscore the importance of monitoring VE against hospitalisation with this variant. This has policy implications for European countries in terms of recommendations for timing of booster doses.

This study provides some evidence of waning effectiveness, but the lower VE observed in patients with onset from 120 days and particularly from 180 days after first mRNA booster dose than in those with first mRNA booster dose < 60 days from onset could also be due to the different sub-lineages circulating at the time of their illness. In supplementary analyses, VE was significantly higher in those receiving an initial booster dose for the much earlier BA.1 period (from the study start in December 2021 to February 2022) than the BA.2 (from March to May 2022) or BA.4/5 (from June to study end in July 2022) variants. Our study also showed that VE was lower with greater time since last booster dose within each sub-lineage period.

Measuring only overall VE against Omicron may mask differential VE in different sub-lineage periods and over time, with the low overall VE due in part to many patients having long lags since their last booster dose (particularly for older individuals, who would have been vaccinated earlier) and the emergence of sub-lineages with greater ability for immune escape. The emergence of Omicron sub-lineages BA.5 and, to a lesser extent, BA.4, did raise concern about high transmissibility [52] and partial escape of natural and vaccine-induced immunity [53-55]. These variants have rapidly become dominant in the EU/EEA. For countries participating in our study, BA.4/5 became dominant only towards the end of our study period, from 10 June 2022. A higher sample size for BA.2 and BA.4/5 is needed to better disentangle the effects of time since vaccination and VE against Omicron sub-lineages.

Countries have progressively introduced additional booster doses in vulnerable groups, and EU/EEA countries recommended a second booster in immunocompetent individuals aged 60 years and older in late 2022 [21], when vaccination campaigns focused on administering adapted bivalent booster vaccines. Although

subsequent boosters may partly restore the immunity and increase protection conferred by the earlier doses of the vaccine [56,57], the speed of waning immunity needs further study, along with attempts to disentangle the competing effects of age, variant sub-lineage, prior infection and time since vaccination.

Conclusion

Our results suggest that, during dominant circulation of the Omicron variant in participating countries, among adults aged≥ 20 years, overall VE for complete PSV without booster, ≥150 days after last PSV dose, was≤50% for the three main vaccine products in use. Higher VE after administration of a booster dose suggests beneficial effect of booster vaccination during this period, particularly for those with onset within 120 days of their booster dose. Although the policy implications of these findings are potentially substantial, there is a need for confirmatory findings from research groups in other countries.

Ethical statement

The planning, conduct and reporting of the studies was in line with the Declaration of Helsinki. Official ethical approval was not required if studies were classified as being part of routine care/surveillance (Spain, Ireland, Malta); for the Netherlands, the study was not subject to the Dutch Medical Research with Human Subjects Law (Wet Medisch-wetenschappelijk onderzoek met mensen, WMO) as it is non-interventional, uses routine clinical data only and data were collected retrospectively. In Belgium and Germany, VE estimation is included in SARI surveillance. For Belgium, the protocol was approved by the central Ethical Committee (CHU ST Pierre, Bruxelles) and each participating hospital's local ethical committees in 2011 (AK/12-02-11/4111), updated in 2014 (B.U.N. 143201215671). The German SARI surveillance was approved by the Charité-Universitätsmedizin Berlin Ethical Board (Reference EA2/218/19). Other study sites obtained local ethical approval from a national review board (Croatia: approved 24 May 2021 and 26 January 2022, Ethics committee of the Croatian Institute of Public Health, Klasa:030-02/21-01/1, Ur.broj:381-15-21-7; Klasa:030-02/21-01/1, Ur.broj:381-15-22-14; France: eighth amendment approved 28 May 2021 by the French Data Protection Agency, and the French ethics research committee 'Comitê de Protection des Personnes'; Hungary: approved in March 2021 by the National Scientific and Ethical Committee for the period 01 September 2021–01 September 2024 (IV/1885-5/2021/EKU); Lithuania: approved 11 May 2021 by Lithuanian Biomedical Research Ethics Committee, No. 6B-21-85; Navarra: PI2020/45; Portugal: approved 19 January 2021 by the Ethics Committee of Instituto Nacional de Saúde Doutor Ricardo Jorge, no registration number given; Romania: approved by the Ethics Committee of the Ministerul Apărării Naționale Institutul Național de Cercetare pentru Dezvoltare Medico-Militară „Cantacuzino" for the period 2022–2023, No. CE199/2022).

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Conflict of interest

None declared.

Authors' contributions

AMCR was involved in the original methodological design of the study (generic protocol). She contributed to the coordination of the I-MOVE-COVID-19 and ECDC SARI VE networks and undertook the statistical analysis on which the research article is based. She led the manuscript writing, interpreted results and approved the final version of the manuscript.

JH contributed to the coordination of the ECDC SARI VE network, contributed to analysis, helped interpret results, contributed to manuscript writing and approved the final version of the manuscript.

VSM and FP coordinated the I-MOVE virological analysis of the I-MOVE-COVID-19 and ECDC networks, helped interpret results, and read, contributed to and approved the final version of the manuscript.

MV initiated the original methodological design of the study and coordinates the I-MOVE-COVID-19 network. EK contributed to the coordination of the I-MOVE-COVID-19 network and MV and EK interpreted results, contributed to manuscript writing and approved the final version of the manuscript. NN and SB were involved in study design, interpretation of results, review of the manuscript and approval of the final version of the manuscript.

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References

1. European Medicines Agency (EMA). COVID-19 medicines. Amsterdam: EMA. [Accessed: 7 Mar 2023]. Available from: https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/treatments-vaccines-covid-19-authorised-medicines
2. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021;384(5):403-16. https://doi.org/10.1056/NEJMoa2035389 PMID: 33378609
3. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 2020;383(27):2603-15. https://doi.org/10.1056/NEJMoa2034577 PMID: 33301246
4. Sadoff J, Gray G, Vandebosch A, Cárdenas V, Shukarev G, Grinsztejn B, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. N Engl J Med. 2021;384(23):2187-201. https://doi.org/10.1056/NEJMoa2101544 PMID: 33882225
5. Dagan N, Barda N, Kepten E, Miron O, Perchik S, Katz MA, et al. BNT162b2 mRNA Covid-19 vaccine in a nationwide mass vaccination setting. N Engl J Med. 2021;384(15):1412-23. https://doi.org/10.1056/NEJMoa2101765 PMID: 33626250
6. Haas EJ, Angulo FJ, McLaughlin JM, Anis E, Singer SR, Khan F, et al. Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. Lancet. 2021;397(10287):1819-29. https://doi.org/10.1016/S0140-6736(21)00947-8 PMID: 33964222
7. Lopez Bernal J, Andrews N, Gower C, Robertson C, Stowe J, Tessier E, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. BMJ. 2021;373(1088):n1088. https://doi.org/10.1136/bmj.n1088 PMID: 33985964
8. Vasileiou E, Simpson CR, Shi T, Kerr S, Agrawal U, Akbari A, et al. Interim findings from first-dose mass COVID-19 vaccination roll-out and COVID-19 hospital admissions in Scotland: a national prospective cohort study. Lancet. 2021;397(10285):1646-57. https://doi.org/10.1016/S0140-6736(21)00677-2 PMID: 33901420
9. Nicolay N, Innocenti F, Beauté J, Učakar V, Grgič Vitek M, Poukka E, et al. Initial assessment of the COVID-19 vaccination's impact on case numbers, hospitalisations and deaths in people aged 80 years and older, 15 EU/EEA countries, December 2020 to May 2021. Euro Surveill. 2021;26(48):2101030. https://doi.org/10.2807/1560-7917.ES.2021.26.48.2101030 PMID: 34857068
10. Meslé MM, Brown J, Mook P, Hagan J, Pastore R, Bundle N, et al. Estimated number of deaths directly averted in people 60 years and older as a result of COVID-19 vaccination in the WHO European Region, December 2020 to November 2021. Euro Surveill. 2021;26(47):2101021. https://doi.org/10.2807/1560-7917.ES.2021.26.47.2101021 PMID: 34823641
11. Burki TK. Omicron variant and booster COVID-19 vaccines. Lancet Respir Med. 2022;10(2):e17. https://doi.org/10.1016/S2213-2600(21)00559-2 PMID: 34929158
12. Mohapatra RK, El-Shall NA, Tiwari R, Nainu F, Kandi V, Sarangi AK, et al. Need of booster vaccine doses to counteract the emergence of SARS-CoV-2 variants in the context of the Omicron variant and increasing COVID-19 cases: An update. Hum Vaccin Immunother. 2022;18(5):2065824. https://doi.org/10.1080/21645515.2022.2065824 PMID: 35594528
13. Pegu A, O'Connell SE, Schmidt SD, O'Dell S, Talana CA, Lai L, et al. Durability of mRNA-1273 vaccine-induced antibodies against SARS-CoV-2 variants. Science. 2021;373(6561):1372-7. https://doi.org/10.1126/science.abj4176 PMID: 34385356
14. Müller L, Andrée M, Moskorz W, Drexler I, Walotka L, Grothmann R, et al. Age-dependent immune response to the Biontech/Pfizer BNT162b2 coronavirus disease 2019 vaccination. Clin Infect Dis. 2021;73(11):2065-72. https://doi.org/10.1093/cid/ciab381 PMID: 33906236
15. Tartof SY, Slezak JM, Puzniak L, Hong V, Xie F, Ackerson BK, et al. Durability of BNT162b2 vaccine against hospital and emergency department admissions due to the omicron and delta variants in a large health system in the USA: a test-negative case-control study. Lancet Respir Med. 2022;10(7):689-99. https://doi.org/10.1016/S2213-2600(22)00101-1 PMID: 35468336
16. Goldberg Y, Mandel M, Bar-On YM, Bodenheimer O, Freedman L, Haas EJ, et al. Waning immunity after the BNT162b2 vaccine in Israel. N Engl J Med. 2021;385(24):e85. https://doi.org/10.1056/NEJMoa2114228 PMID: 34706170
17. Barda N, Dagan N, Cohen C, Hernán MA, Lipsitch M, Kohane IS, et al. Effectiveness of a third dose of the BNT162b2 mRNA COVID-19 vaccine for preventing severe outcomes in Israel: an observational study. Lancet. 2021;398(10316):2093-100. https://doi.org/10.1016/S0140-6736(21)02249-2 PMID: 34756184
18. Bar-On YM, Goldberg Y, Mandel M, Bodenheimer O, Freedman L, Kalkstein N, et al. Protection of BNT162b2 vaccine booster against Covid-19 in Israel. N Engl J Med. 2021;385(15):1393-400. https://doi.org/10.1056/NEJMoa2114255 PMID: 34525275
19. European Centre for Disease Prevention and Control (ECDC). Interim public health considerations for the provision of additional COVID-19 vaccine doses. Stockholm: ECDC; 2021. Available from: https://www.ecdc.europa.eu/en/publications-data/covid-19-public-health-considerations-additional-vaccine-doses
20. European Centre for Disease Prevention and Control (ECDC). Overview of the implementation of COVID-19 vaccination strategies and deployment plans in the EU/EEA. Stockholm: ECDC; 2022. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Overview-of-the-implementation-of-COVID-19-vaccination-strategies-and-deployment-plans-in-the-EU-EEA-April-2022.pdf
21. European Centre for Disease Prevention and Control (ECEC). Overview of the implementation of COVID-19 vaccination strategies and deployment plans in the EU/EEA. Stockholm: ECDC; 2023. Available from: https://www.ecdc.europa.eu/en/publications-data/overview-implementation-covid-19-vaccination-strategies-and-vaccine-deployment
22. European Centre for Disease Prevention and Control (ECDC). COVID-19 vaccine tracker. Stockholm: ECDC. [Accessed: 28 Mar 2023]. Available from: https://vaccinetracker.ecdc.europa.eu/public/extensions/COVID-19/vaccine-tracker.html#uptake-tab
23. European Centre for Disease Prevention and Control (ECDC). Interim analysis of COVID-19 vaccine effectiveness against severe acute respiratory infection due to laboratory-confirmed SARS-CoV-2 among individuals aged 30 years and older, ECDC multi-country study – second update. Stockholm: ECDC; 2022. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-vaccine-effectiveness-interim-analysis-second-update_0.pdf
24. European Centre for Disease Prevention and Control (ECDC). Core protocol for ECDC studies of COVID-19 vaccine effectiveness against hospitalisation with Severe Acute Respiratory Infection laboratory-confirmed with SARS-CoV-2, version 1.0. Stockholm: ECDC; 2021. Available from: https://www.ecdc.europa.eu/en/publications-data/core-protocol-ecdc-studies-covid-19-vaccine-effectiveness-against-hospitalisation

25. I-MOVE influenza and COVID-19 networks. European study of COVID-19 vaccine effectiveness against hospitalised SARI patients laboratory-confirmed with SARS-CoV-2. Draft generic protocol. Paris: Epiconcept; 2021. Available from: https://www.imoveflu.org/wp-content/uploads/2021/03/08feb2021_draft_generic_VE_protocol_hospital-based_COVID-19_v07.pdf
26. Jackson ML, Nelson JC. The test-negative design for estimating influenza vaccine effectiveness. Vaccine. 2013;31(17):2165-8. https://doi.org/10.1016/j.vaccine.2013.02.053 PMID: 23499601
27. Rose AMC, Nicolay N, Sandonis Martín V, Mazagatos C, Petrović G, Niessen FA, et al. Vaccine effectiveness against COVID-19 hospitalisation in adults (≥ 20 years) during Alpha- and Delta-dominant circulation: I-MOVE-COVID-19 and ECDC-VEBIS SARI VE networks, Europe, 2021. Euro Surveill. 2023;42(47):2300187.
28. Peralta-Santos A. Assessment of COVID-19 surveillance case definitions and data reporting in the European Union. Briefing requested by the ENVI committee. Brussels: European Parliament; 2020. [Accessed: 28 May 2022]. Available from: https://www.europarl.europa.eu/RegData/etudes/BRIE/2020/652725/IPOL_BRI(2020)652725_EN.pdf
29. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. Euro Surveill. 2017;22(13):30494. https://doi.org/10.2807/1560-7917.ES.2017.22.13.30494 PMID: 28382917
30. European Centre for Disease Prevention and Control (ECDC). Data on SARS-CoV-2 variants in the EU/EEA. Stockholm: ECDC. [Accessed: 5 Dec 2022]. Available from: https://www.ecdc.europa.eu/en/publications-data/data-virus-variants-covid-19-eueea
31. Peduzzi P, Concato J, Feinstein AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis. II. Accuracy and precision of regression estimates. J Clin Epidemiol. 1995;48(12):1503-10. https://doi.org/10.1016/0895-4356(95)00048-8 PMID: 8543964
32. Covenay J. FIRTHLOGIT: Stata module to calculate bias reduction in logistic regression. Boston: Boston College Department of Economics; 2008. [Accessed: 3 Feb 2020]. Available from: https://econpapers.repec.org/software/bocbocode/s456948.htm
33. Gonçalves BP, Hall M, Jassat W, Balan V, Murthy S, Kartsonaki C, et al. An international observational study to assess the impact of the Omicron variant emergence on the clinical epidemiology of COVID-19 in hospitalised patients. eLife. 2022;11:e80556. https://doi.org/10.7554/eLife.80556 PMID: 36197074
34. Andrews N, Tessier E, Stowe J, Gower C, Kirsebom F, Simmons R, et al. Duration of protection against mild and severe disease by Covid-19 vaccines. N Engl J Med. 2022;386(4):340-50. https://doi.org/10.1056/NEJMoa2115481 PMID: 35021002
35. Ferdinands JM, Rao S, Dixon BE, Mitchell PK, DeSilva MB, Irving SA, et al. Waning of vaccine effectiveness against moderate and severe covid-19 among adults in the US from the VISION network: test negative, case-control study. BMJ. 2022;379:e072141. https://doi.org/10.1136/bmj-2022-072141 PMID: 36191948
36. Nogareda F, Regan AK, Couto P, Fowlkes AL, Gharpure R, Loayza S, et al. Effectiveness of COVID-19 vaccines against hospitalisation in Latin America during three pandemic waves, 2021–2022: a test-negative case-control design. Lancet Reg Health Am. 2023;27:100626. https://doi.org/10.1016/j.lana.2023.100626
37. Stowe J, Andrews N, Kirsebom F, Ramsay M, Bernal JL. Effectiveness of COVID-19 vaccines against Omicron and Delta hospitalisation, a test negative case-control study. Nat Commun. 2022;13(1):5736. https://doi.org/10.1038/s41467-022-33378-7 PMID: 36180428
38. Shao W, Zhang W, Fang X, Yu D, Wang X. Challenges of SARS-CoV-2 Omicron variant and appropriate countermeasures. J Microbiol Immunol Infect. 2022;55(3):387-94. https://doi.org/10.1016/j.jmii.2022.03.007 PMID: 35501267
39. Ding K, Jiang W, Xiong C, Lei M. Turning point: A new global COVID-19 wave or a signal of the beginning of the end of the global COVID-19 pandemic? Immun Inflamm Dis. 2022;10(4):e606. https://doi.org/10.1002/iid3.606 PMID: 35349754
40. Ventura MI, Azizian A, Evans SE, Velasquez S, Arguello JC, Warburton K. Vaccine breakthrough infections with SARS-CoV-2: Why older adults need booster vaccinations. Public Health Pract (Oxf). 2022;4:100307. https://doi.org/10.1016/j.puhip.2022.100307 PMID: 36092529
41. Mallah N, Pardo-Seco J, López-Pérez LR, González-Pérez JM, Rosón B, Otero-Barrós MT, et al. Effectiveness of COVID-19 vaccine booster in the general population and in subjects with comorbidities. A population-based study in Spain. Environ Res. 2022;215(Pt 2):114252. https://doi.org/10.1016/j.envres.2022.114252 PMID: 36096168
42. Tan CY, Chiew CJ, Pang D, Lee VJ, Ong B, Lye DC, et al. Vaccine effectiveness against Delta, Omicron BA.1, and BA.2 in a highly vaccinated Asian setting: a test-negative design study. Clin Microbiol Infect. 2023;29(1):101-6. https://doi.org/10.1016/j.cmi.2022.08.002 PMID: 36028091
43. Sharma A, Oda G, Holodniy M. Effectiveness of messenger RNA-based vaccines during the emergence of the severe acute respiratory syndrome coronavirus 2 Omicron variant. Clin Infect Dis. 2022;75(12):2186-92. https://doi.org/10.1093/cid/ciac325 PMID: 35475889
44. Thompson MG, Natarajan K, Irving SA, Rowley EA, Griggs EP, Gaglani M, et al. Effectiveness of a Third Dose of mRNA Vaccines Against COVID-19-Associated Emergency Department and Urgent Care Encounters and Hospitalizations Among Adults During Periods of Delta and Omicron Variant Predominance - VISION Network, 10 States, August 2021-January 2022. MMWR Morb Mortal Wkly Rep. 2022;71(4):139-45. https://doi.org/10.15585/mmwr.mm7104e3 PMID: 35085224
45. Tseng HF, Ackerson BK, Luo Y, Sy LS, Talarico CA, Tian Y, et al. Effectiveness of mRNA-1273 against SARS-CoV-2 Omicron and Delta variants. Nat Med. 2022;28(5):1063-71. https://doi.org/10.1038/s41591-022-01753-y PMID: 35189624
46. Hansen CH, Schelde AB, Moustsen-Helm IR, Emborg HD, Krause TG, Mølbak K, et al. Vaccine effectiveness against SARS-CoV-2 infection with the Omicron or Delta variants following a two-dose or booster BNT162b2 or mRNA-1273 vaccination series: A Danish cohort study. medRxiv. 2021.12.20.21267966 . https://doi.org/10.1101/2021.12.20.21267966
47. Buchan SA, Chung H, Brown KA, Austin PC, Fell DB, Gubbay JB, et al. Estimated effectiveness of COVID-19 vaccines against Omicron or Delta symptomatic infection and severe outcomes. JAMA Netw Open. 2022;5(9):e2232760. https://doi.org/10.1001/jamanetworkopen.2022.32760 PMID: 36136332
48. Chemaitelly H, Ayoub HH, AlMukdad S, Coyle P, Tang P, Yassine HM, et al. Duration of mRNA vaccine protection against SARS-CoV-2 Omicron BA.1 and BA.2 subvariants in Qatar. Nat Commun. 2022;13(1):3082. https://doi.org/10.1038/s41467-022-30895-3 PMID: 35654888
49. Andrews N, Stowe J, Kirsebom F, Toffa S, Rickeard T, Gallagher E, et al. Covid-19 vaccine effectiveness against the Omicron (B.1.1.529) variant. N Engl J Med. 2022;386(16):1532-46. https://doi.org/10.1056/NEJMoa2119451 PMID: 35249272
50. Hall V, Foulkes S, Insalata F, Kirwan P, Saei A, Atti A, et al. Protection against SARS-CoV-2 after Covid-19 vaccination and previous infection. N Engl J Med. 2022;386(13):1207-20. https://doi.org/10.1056/NEJMoa2118691 PMID: 35172051
51. Gram MA, Emborg HD, Schelde AB, Friis NU, Nielsen KF, Moustsen-Helms IR, et al. Vaccine effectiveness against SARS-CoV-2 infection or COVID-19 hospitalization with the Alpha, Delta, or Omicron SARS-CoV-2 variant: A nationwide Danish cohort study. PLoS Med. 2022;19(9):e1003992. https://doi.org/10.1371/journal.pmed.1003992 PMID: 36048766
52. Tegally H, Moir M, Everatt J, Giovanetti M, Scheepers C, Wilkinson E, et al. Emergence of SARS-CoV-2 Omicron lineages BA.4 and BA.5 in South Africa. Nat Med. 2022;28(9):1785-90. https://doi.org/10.1038/s41591-022-01911-2 PMID: 35760080
53. Ai J, Zhang H, Zhang Y, Lin K, Zhang Y, Wu J, et al. Omicron variant showed lower neutralizing sensitivity than other SARS-CoV-2 variants to immune sera elicited by vaccines after boost. Emerg Microbes Infect. 2022;11(1):337-43. https://doi.org/10.1080/22221751.2021.2022440 PMID: 34935594
54. Hachmann NP, Miller J, Collier AY, Ventura JD, Yu J, Rowe M, et al. Neutralization escape by SARS-CoV-2 Omicron subvariants BA.2.12.1, BA.4, and BA.5. N Engl J Med. 2022;387(1):86-8. https://doi.org/10.1056/NEJMc2206576 PMID: 35731894
55. Cao Y, Yisimayi A, Jian F, Song W, Xiao T, Wang L, et al. BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by Omicron infection. Nature. 2022;608(7923):593-602. https://doi.org/10.1038/s41586-022-04980-y PMID: 35714668
56. Arbel R, Sergienko R, Friger M, Peretz A, Beckenstein T, Yaron S, et al. Effectiveness of a second BNT162b2 booster vaccine against hospitalization and death from COVID-19 in adults aged over 60years. Nat Med. 2022;28(7):1486-90. https://doi.org/10.1038/s41591-022-01832-0 PMID: 35468276
57. Adams K, Rhoads JP, Surie D, Gaglani M, Ginde AA, McNeal T, et al. Vaccine effectiveness of primary series and booster doses against covid-19 associated hospital admissions in the United States: living test negative design study. BMJ. 2022;379:e072065. https://doi.org/10.1136/bmj-2022-072065 PMID: 36220174

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7.15 Annex 15

Selvekerová Š, Hanáková M, Demlová R, Nebeská K, Krist H, Dušek M, Piuškařová N, Nevolová P, Brandejsová P, Doležalová H, Cviklíková K, Krempová J, Součková L. Role studijního koordinátora v klinickém výzkumu ve zdravotnických zařízeních – popelka mezi experty v klinických studiích? *Klinicka Farmakologie a Farmacie*, 2024, **38**(3), 124–131. ISSN 1212-7973.

Summary
Purpose:

The article aimed to map the current position and role of clinical research coordinators (CRCs) within healthcare institutions in the Czech Republic, increase awareness of the activities they perform in clinical research, and identify their educational and professional-development needs. Particular attention was given to the organisational position of CRCs within healthcare institutions and to the demand for a formal educational programme supporting the development and professionalisation of this increasingly important role in clinical research.

Methodology:
The publication combines an overview of the role and responsibilities of clinical research coordinators with the results of two questionnaire surveys mapping the situation in the Czech Republic. The surveys addressed two complementary perspectives: healthcare institutions involved in clinical research and individual clinical research coordinators. In total, 42 healthcare institutions and 120 clinical research coordinators participated, providing information on the organisational embedding of clinical research coordination, professional and employment characteristics of coordinators, activities performed within clinical trials, and existing and perceived educational needs.

Results:
The findings demonstrated considerable heterogeneity in how the role of the clinical research coordinator is organised and formally recognised across Czech healthcare institutions. Only 45% of participating institutions had a formalised Clinical Trials Unit, indicating substantial differences in the institutional structures supporting clinical research. Approximately 40% of clinical research coordinators were classified in non-healthcare professional categories, complicating appropriate recognition and formal employment classification of their role. The surveys further demonstrated that CRCs undertake a broad range of activities essential to the conduct and coordination of clinical trials, while a comprehensive and standardised system of undergraduate and postgraduate education for this profession is currently lacking. The findings therefore identified both a need and demand for more structured education and professional development of clinical research coordinators.

Conclusion:

The publication highlights clinical research coordinators as an important professional group supporting the conduct of clinical trials in healthcare institutions, while demonstrating that their organisational position, professional recognition, and educational pathways remain insufficiently formalised in the Czech Republic. The findings support the need to formalise the CRC role, develop structured educational opportunities, and establish a professional organisation capable of harmonising methodological guidance and strengthening institutional support for coordinators

Role studijního koordinátora v klinickém výzkumu ve zdravotnických zařízeních – popelka mezi experty v klinických studiích?

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Článek se zaměřuje na zmapování současného postavení studijního koordinátora ve zdravotnických zařízeních v České republice. Hlavním cílem je zvýšit povědomí o činnostech, které studijní koordinátoři vykonávají, a identifikovat potřeby jejich vzdělávání. Studie rovněž zkoumá poptávku po formálním vzdělávacím programu, který by podpořil profesní rozvoj této klíčové role v klinickém výzkumu. Výsledky dvou dotazníkových průzkumů, kterých se aktivně zúčastnilo 42 zdravotnických zařízení a 120 studijních koordinátorů, ukazují, že pouze 45 % zařízení má formalizované oddělení klinických studií. Přibližně 40 % studijních koordinátorů je zařazeno do nezdravotnických kategorií, což komplikuje jejich odpovídající pracovní zařazení. Studijním koordinátorům chybí ucelený systém pregraduálního i postgraduálního vzdělávání. Závěry zdůrazňují potřebu formalizace této pracovní pozice včetně vytvoření profesní organizace, která by sjednocovala metodické pokyny a posílila institucionální podporu studijních koordinátorů.

Klíčová slova: studijní koordinátor, klinické studie, vzdělávání, zdravotnická zařízení.

The Role of the Clinical Research Coordinator in Healthcare Institutions – A Cinderella Among Clinical Trial Experts?

The article focuses on mapping the current status of clinical research coordinators in healthcare institutions in the Czech Republic. The main objective is to raise awareness of the tasks performed by clinical research coordinators and to identify their educational needs. The study also examines the demand for a formal educational program that would support the professional development of this crucial role in clinical research. The results of two surveys, involving 42 healthcare institutions and 120 clinical research coordinators, show that only 45% of institutions have a formal Clinical Trials Unit. Approximately 40% of clinical research coordinators are classified in non-healthcare categories, complicating their appropriate job classification. Coordinators lack a comprehensive system for both undergraduate and postgraduate education. The conclusions emphasize the need for formalizing this position, including the establishment of a professional organisation to unify guidelines and strengthen institutional support for clinical research coordinators.

Key words: clinical research coordinator, clinical trials, education, healthcare institutions.

DECLARATIONS:

Declaration of originality:
The manuscript is original and has not been published or submitted elsewhere.

Ethical principles compliance:
The authors attest that their study was approved by the local Ethical Committee and is in compliance with human studies and animal welfare regulations of the authors' institutions as well as with the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects adopted by the 18th WMA General Assembly in Helsinki, Finland, in June 1964, with subsequent amendments, as well as with the ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, updated in December 2018, including patient consent where appropriate.

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Snahou této práce je:

- zmapovat současné postavení studijního koordinátora ve zdravotnických zařízeních,
- zvýšit povědomí o činnostech zastávaných studijním koordinátorem,
- zjistit poptávku po vzdělávání pro roli studijního koordinátora.

Studijní koordinátoři hrají klíčovou roli v realizaci klinických studií, přičemž jejich pracovní náplň a zařazení se výrazně liší napříč zdravotnickými zařízeními v České republice. Jejich role a odpovědnosti stále nejsou jasně definovány a formální uznání jako odborníka v České republice stále chybí, přestože zajišťují důležité úkoly při provádění klinických studií a podílí se na dodržování standardů kvality. Cílem tohoto výzkumu bylo detailně zmapovat rozdíly v institucionálním zařazení, popisu pracovních pozic a vzdělávacích potřebách studijních koordinátorů.

Za tímto účelem byly provedeny dva typy dotazníkového šetření v období 06/2023 – 02/2024. První dotazník, tzv. institucionální, byl rozeslán do 170 zdravotnických zařízení v České republice. Jeho návratnost byla 35,5 %. Druhý, tzv. individuální dotazník, obdrželo 200 studijních koordinátorů a návratnost byla 60 %. Data z obou dotazníků byla porovnána a agregována tak, abychom získali co nejkomplexnější údaje.

Z dotazníků vyplynulo, že ze 42 zdravotnických zařízení, která jsou zapojena do provádění klinických studií, má pouze 29 zdravotnických zařízení formalizované oddělení klinických studií (OKS). Zejména se jedná o fakultní nemocnice.

Jedním z hlavních zjištění individuálních dotazníků je fakt, že u studijních koordinátorů převažuje zdravotnické vzdělání, a to celkem v 59 % případů. Přesto je 40 % studijních koordinátorů zařazeno dle katalogu prací do nezdravotnických kategorií, nejčastěji na pozici technickohospodářského pracovníka (THP). Pracovní agenda vystihující nejlépe pracovní pozici studijních koordinátorů byla uváděna s následující četností, s možností volby více odpovědí. Studijní koordinátor/ka (administrativní a koordinátorská činnost) – 51 % (102), data manažer/ka (zadávání dat do databáze zadavatele) – 30,5 % (61), start-up koordinátor/ka (agenda přípravy klinických studií a administrativa) – 20 (40 %), studijní/výzkumná sestra (přímá péče o pacienta) – 11,5 % (23), manažer klinických studií (vedoucí pracovník) – 9,5 % (19). Z dotazníkového šetření vyplynula výrazná potřeba inovativního a přizpůsobeného vzdělávacího programu pro studijní koordinátory.

Výsledky naznačují potřebu vyšší míru začlenění studijních koordinátorů do struktur zdravotnických zařízení a rozvoje specifických vzdělávacích programů, které by lépe reflektovaly jejich klíčovou roli v klinickém výzkumu léčiv nebo zdravotnických prostředků. Důraz na kontinuální profesní vzdělávání a uznání různorodosti rolí by mohl podstatně přispět k zefektivnění realizace klinických hodnocení a zkoušek.

Současný stav

V posledních dekádách dochází k výraznému nárůstu počtu a složitosti klinických hodnocení a zkoušek (dále jen klinické studie), což klade stále vyšší nároky na kvalitu a efektivitu jejich provádění. Realizace klinických studií je stále komplikovanější, náročnější a vysoce kompetitivní (1). Zároveň se zpřísňují i legislativní a regulační požadavky, které jsou nezbytné pro zajištění bezpečnosti pacientů a integrity dat (2, 3). V tomto kontextu představují studijní koordinátoři klíčové články v řetězci klinického výzkumu, neboť zajišťují koordinaci klinických studií, komunikaci s účastníky a management dat (4, 5).

Ačkoli se role studijního koordinátora mezi různými státy liší, ve většině případů zajišťuje studijní koordinátor péči o účastníky klinických studií a komunikaci s nimi, organizaci klinické studie na pracovišti včetně zadávání dat, dohlíží na splnění etických standardů, včetně požadavků správné klinické praxe a požadavků na kvalitu (6, 7), asistuje zkoušejícímu při realizaci klinické studie. Studijní koordinátor svou prací významně přispívá k proveditelnosti klinické studie na daném centru a s tím souvisejícím výsledkům klinického výzkumu. V návaznosti na to, se zasazuje o zlepšení péče o pacienty prostřednictvím medicíny založené na důkazech (8).

Z předchozích průzkumů vyplývá, že studijní koordinátor se v průměru stará o 6 klinických studií současně a 80 účastníků za rok (8–10).

Z výzkumu hodnotícího kvalitu klinických studií jednoznačně vyplynulo, že klinické studie, na jejichž realizaci se podílí studijní koordinátor, jsou kvalitněji zpracované, koherentnější, s méně nedostatky ve srovnání se studiemi, na kterých se studijní koordinátor nepodílel (11).

Roztříštěnost v názvu, postavení a činnosti studijního koordinátora/sestry

V České republice je situace studijních koordinátorů komplikovaná zejména nejednotností v pojmenování pozic a jejich rozdílným zařazením v rámci zdravotnických zařízení. V dnešní době se můžeme setkat s různým označením pro studijního koordinátora v klinických studiích – studijní sestra, výzkumná sestra, studijní koordinátor, start-up koordinátor, manažer klinických studií, případně nesprávně označovaný data manažer. Pro zjednodušení budeme v tomto článku všeobecně používat označení studijní koordinátor, který bude zastřešovat všechny výše zmíněné role.

Stejně tak jako neexistuje jednotnost v pojmenování pracovní pozice, neexistuje dosud ani harmonizace v postavení studijního koordinátora ve zdravotnickém zařízení. Existují tři základní způsoby vedení klinické studie ve zdravotnickém zařízení, z čehož pak vyplývá způsob zařazení studijního koordinátora:

1. Hlavní zkoušející si organizuje a vede klinickou studii sám. Studijní koordinátoři jsou přímými zaměstnanci soukromých ambulancí nebo klinik a pracují přímo pod vedením zkoušejícího.
2. Studijní koordinátor je zaměstnán soukromou společností či samostatně jako externista (např. jako osoba samostatně výdělečně činná) a je najatý zdravotnickým zařízením k práci na jedné či více klinických studiích.
3. Ve zdravotnickém zařízení je zřízeno oddělení klinických studií (OKS), které administruje právní a finanční zajištění studie. Studijní koordinátor je veden pod OKS. Pokud vyvstane požadavek od zkoušejícího, studijní koordinátor je na danou klinickou studii alokován.

Tento nedostatek a zejména nejednotnost organizovaného prostředí může snížit efektivitu a naopak, zvýšit počet rizik spojených s realizací studií.

Za poslední desetiletí se role studijního koordinátora značně změnila a vyvinula (5). V určitých ohledech můžeme o studijních

koordinátorech mluvit jako o expertních manažerech klinického výzkumu.

Rozsah jejich činností zdaleka přesahuje administrativu klinických studií (12). Jednou z klíčových oblastí práce studijního koordinátora je zadávání dat do elektronických Záznamových listů pacienta (eCRF). Management klinických dat představuje v dnešní době vysoce odbornou činnost, a to jak z pohledu obsahového, tak i technického. Součástí této agendy je i několikastupňová kontrola a zodpovídání zpětných dotazů. Navíc je potřeba zdůraznit, že i v českém prostředí je značná část komunikace vedena v anglickém jazyce.

Bylo definováno celkem 5 oblastí, které tvoří náplň práce studijního koordinátora (8, 13):

1. klinická práce (zpracování biologických vzorků včetně uchovávání a zaslání vzorků do centrální laboratoře, zadávání dat včetně dotazů, podpora náboru, podíl na udržení pacientů ve studii – retention),
2. koordinační práce (naplánování návštěv a jednotlivých vyšetření, kontrola zásob/objednání hodnocených léčivých přípravků či komparátorů, laboratorních kitů, administrativa (včetně investigátorské dokumentace), zajištění certifikátů a životopisů členů studijního týmu),
3. řízení klinické studie (účast na monitorovacích návštěvách, komunikace se studijním týmem a zástupci zadavatele, projektový management – reporting, sledování termínů),
4. ochrana účastníků klinické studie (komunikace s pacientem, znalost protokolu, edukace pacienta, hlášení nežádoucích událostí),
5. přispívání k vědecko-výzkumným činnostem (účast na konferencích, odborné konzultace k protokolu, podíl na odborných publikacích).

Činnosti studijních koordinátorů se liší napříč jednotlivými klinikami, studijními centry, městy, ale i státy. V některých zemích jsou studijní koordinátoři více zapojeni do dalších odborných činností např. i do získávání informovaných souhlasů.

Vzdělávání

Základním předpokladem pro výkon práce studijního koordinátora je znalost Správné klinické praxe (SKP či GCP), kterou lze získat na školeních různého rozsahu a formy (14). Školení bývají organizována Státním ústavem pro kontrolu léčiv (SÚKL), smluvními výzkumnými organizacemi (CRO), samotnými farmaceutickými společnostmi nebo např. zdravotnickými zařízeními, která si obvykle najmou na tuto činnost externí společnost. Další možností je kurz „Správné klinické praxe pro zkoušející a členy studijních týmů: GCP a specifika provádění klinických studií v českých zdravotnických zařízeních", který pořádá Asociace inovativního farmaceutického průmyslu ve spolupráci (AIFP) s Asociací smluvních výzkumných organizací (ACRO-CZ), Karlovou univerzitou a Masarykovou univerzitou (MUNI). Tento kurz je dvoudenní, je zakončen testem a úspěšní absolventi získají certifikát (15). Školení mohou mít prezenční nebo distanční/on-line formu a samotným rozsahem se také liší. Některá je možné absolvovat do 2 hodin, jiná jsou třeba i dvoudenní. Některá školení jsou ukončena testem, u jiných účastník získá certifikát o účasti. Doklady o znalosti SKP (certifikáty) toto částečně reflektují a podle toho se liší i jejich platnost. U kratších školení je obvykle certifikát platný 2 roky, u komplexních školení až 3 roky. U zkušených pracovníků v klinickém výzkumu, je možné se po skončení platnosti certifikátu účastnit kratšího školení, tzv. refresher kurzu. Bohužel školení SKP určené pro potřeby studijních koordinátorů, které by bylo dostupné kdykoli, a ještě v českém jazyce, zde stále chybí.

V současné době nejsou pro studijního koordinátora či studijní sestru v oblasti klinického hodnocení definovány požadavky na odborné vzdělávání mimo zmíněné SKP.

Jediným uceleným vzděláváním, cíleným na studijní koordinátory, je třídenní certifikovaný kurz Koordinátor klinického hodnocení léčiv, který Národní centrum ošetřovatelství a nelékařských zdravotnických oborů (NCO NZO) v Brně nabízí od roku 2013, a jehož teoretickou a praktickou částí prošlo již několik stovek koordinátorů. Individuální stáže dlouhodobě nabízí také Masarykův onkologický ústav (MOÚ). Další možnosti, kde získat aktuální informace a novinky z oblasti klinických studií cílené na potřeby studijních koordinátorů, je účast na konferenci studijních sester a koordinátorů, která se každoročně pořádá ve spolupráci s MOÚ, MUNI, Velkou výzkumnou infrastrukturou CZECRIN a pod záštitou nadačního fondu PharmAround (16).

Ostatní vzdělávací příležitosti v oblasti klinických studií bývají velmi obecné nebo zaměřené buď na potřeby zkoušejících, či naopak na potřeby zástupců zadavatele, ať už projektových manažerů či monitorů klinických studií.

Celkově v České republice chybí ucelené komplexní pregraduální vzdělání v oblasti klinického výzkumu určené pro studijní koordinátory. Tento vzdělávací program je přitom na zahraničních univerzitách dostupný pro bakaláře nebo magistry, limitací však zůstává anglický jazyk, nutnost cestování a také značné finanční náklady. Asociace profesionálů v klinickém výzkumu (ACRP) zajišťuje odborné přezkoušení studijních koordinátorů v 15 evropských zemích, Spojených státech amerických a Asii, bohužel do této iniciativy Česká republika zapojena není (17).

Chybějící systém vzdělávání tak musí často zajišťovat sami zaměstnavatelé – zdravotnická zařízení. Zaškolování nových studijních koordinátorů je proto časově a organizačně velmi náročné a k jejich plnohodnotnému zapojení do klinických studií dochází až v řádu měsíců po nástupu.

V návaznosti na potřebu zvýšit povědomí o roli studijního koordinátora, zlepšit jejich postavení a zmapovat jejich potřebu speciálního vzdělávání, byla ustanovena pracovní skupina složená se zástupců akademické sféry (CZECRIN) a zástupců průmyslové sféry ACRO-CZ a AIFP. Tato pracovní skupina vnímá důležitou roli, avšak současně nelehké postavení studijních koordinátorů, a tak společně vytvořili institucionální a individuální dotazníky, distribuovali je mezi respondenty a následně analyzovali odpovědi. Tento dotazníkový průzkum slouží jako podkladová analýza pro další jednání s klíčovými partnery v oblasti klinických studií. Svým společným úsilím se jednak pracovní skupina snaží tuto problematiku zviditelnit, sjednotit postavení studijního koordinátora, zlepšit možnosti vzdělávání, vytvořit platformu pro setkávání a vzájemné sdílení zkušeností, ale především vytvořit podmínky pro usnadnění práce těchto důležitých členů studijního týmu, kteří ovšem stojí neprávem v pozadí.

Metodika

Výše zmíněnou pracovní skupinou byly vytvořeny dva anonymní dotazníky v Google forms v českém jazyce. Institucionální dotazník byl určený pro zdravotnická zařízení a druhý individuální dotazník byl určen jednotlivcům (studijním koordinátorům). Dotazy byly navrženy a revidovány všemi členy pracovní skupiny, dokud se nedosáhlo společného konsenzu tak, aby se předešlo nedorozumění a jakýmkoli pochybnostem při porozumění otázkám. Oba dotazníky tvoří přílohu č. 1 (institucionální) a č. 2 (individuální) tohoto článku.

Institucionální dotazníky byly e-mailem rozeslány na všechna zdravotnická zařízení na seznamu MZ ČR a po dvou týdnech byla zaslána upomínka tam, odkud dosud scházela odpověď.

Individuální dotazník byl distribuován na konferenci studijních sester a koordinátorů v roce 2023, a prostřednictvím QR kódu bylo možné vyplnit dotazník on-line, dále byl zaslán e-mailem s žádostí o jeho rozeslání na další studijní koordinátory.

Sběr dat probíhal v období 9/2023 – 02/2024.

Data byla kontrolována dvěma nezávislými odborníky, jak v individuálních, tak institucionálních dotaznicích a byly odstraněny duplicity.

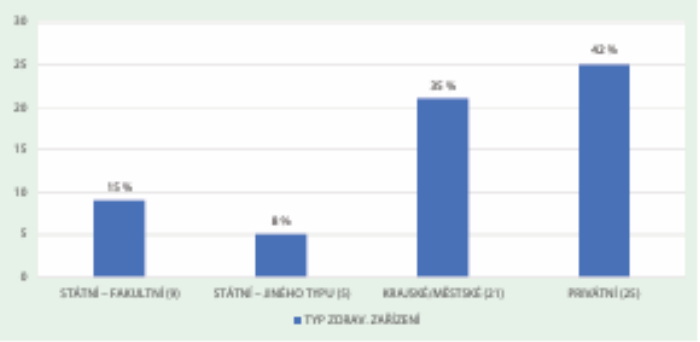
Data byla zpracována prostřednictvím MS Excel pro Windows a SAS 9.4 softwaru. Jednotlivé proměnné byly vyjádřeny číselnou hodnotou a procenty. Výsledky byly diskutovány na uzavřených jednání pracovní skupiny s cílem zvýšení jejich srozumitelnosti.

Výsledky

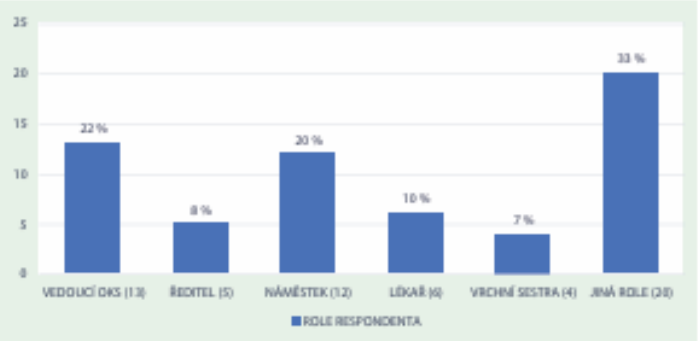
Z celkově 170 oslovených zdravotnických zařízení, vyplnilo institucionální dotazník celkem 60 zdravotnických zařízení (35,3 %), z nichž 42 % tvořily privátní nemocnice (25), 35 % krajské či městské nemocnice (21) a 23 % státní nemocnice (14), z nichž bylo 9 fakultních nemocnic, 5 státních nemocnic jiného typu (Graf č. 1).

Institucionální dotazník byl vyplněn ve 22 % vedoucími pracovníky oddělení klinických studií (13), ve 20 % náměstky a náměstkyněmi pro ošetřovatelskou péči (12). V 10 % vyplňovali dotazník lékaři (6), v 8 % ředitel nemocnice (5), v 7 % vrchními sestrami (4). Z 33 % respondenti dotazníku měli jinou roli ve zdravotnickém zařízení (personalista, management nemocnice aj.) (20) (Graf č. 2.).

Graf 1. *Rozdělení zdravotnických zařízení dle typu dle institucionálního dotazníku*



Graf 2. *Role respondenta u institucionálního dotazníku*



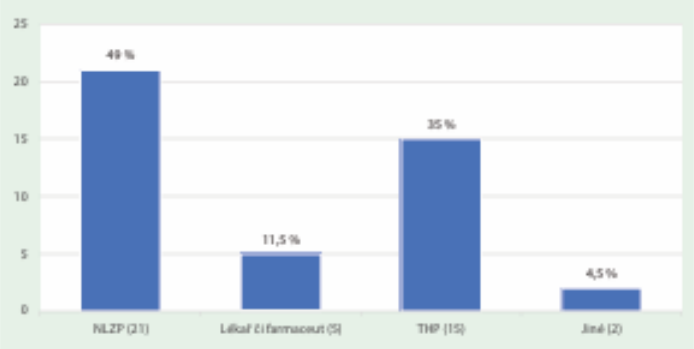
Tab. 1. *Rozdělení zdravotnických zařízení, kde je ustanoveno OKS*

Soukromé zdravotnické zařízení	Fakultní nemocnice	Státní zdravotnické zařízení jiného typu
Agel	FN Brno	IKEM
CCR Prague	FN Bulovka	MOÚ
G-CENTRUM Olomouc s.r.o.	FN Hradec Králové	Nemocnice Milosrdných sester sv. Karla Boromejského v Praze
Gyneko spol. s r. o.	FTN	NÚDZ
Kardiologie Vinohrady s.r.o.	FN KV	Revmatologický ústav Praha
Krajská nemocnice Liberec, a.s.	FN Olomouc	ÚHKT
Krajská zdravotní a.s.	FN Ostrava	
MediTrial s.r.o	FN Plzeň	
Multiscan s.r.o.	FNUSA	
Oblastní nemocnice Kladno a.s.	VFN	
Ordinace Hradební s.r.o.	FN Motol	
Pratia Czech Republic		

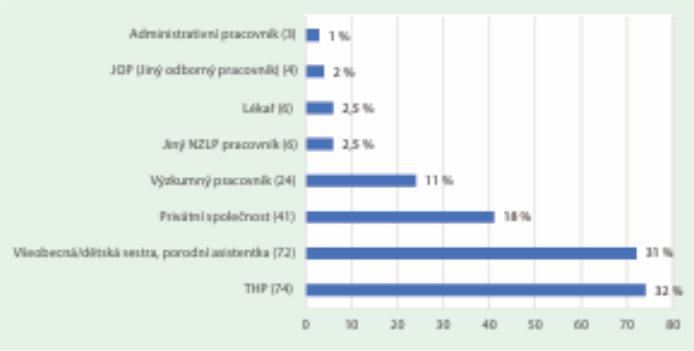
Ze zdravotnických zařízení, která vyplnila dotazník (n = 60), klinické studie realizují celkem ve 42 nemocnicích (70 %), z nichž oddělení pro klinické studie je zavedeno pouze v 19 (45 %), zejména ve fakultních nemocnicích.

Individuální dotazník vyplnilo celkem 120 respondentů z celkem 38 zdravotnických zařízení, z nichž většina (77 %) uvádí, že ve zdravotnickém zařazení, kde působí, je ustanoveno oddělení klinických studií. Z individuálního dotazníku vyplývá, že OKS je zavedeno u 21 poskytovatelů zdravotní péče. Z institucionálního dotazníku vyplývá, že OKS je založeno u 19 poskytovatelů zdravotní péče. Po agregaci dat z institucionálních a individuálních dotazníků vyplývá, že Oddělení klinických studií je založeno celkem ve 29 zdravotnických zařízení. Tabulka č. 1 uvádí všechna zdravotnická zařízení, ve kterých se nachází Oddělení klinických studií.

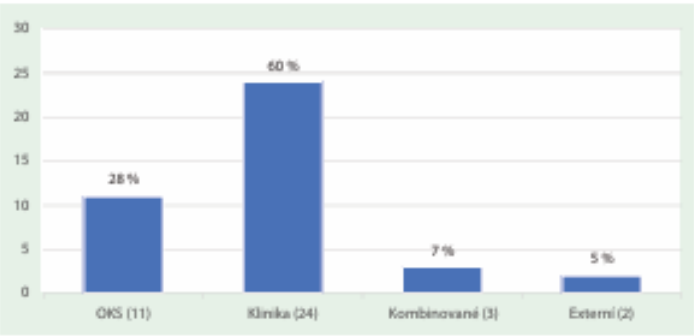
Graf 3. *Pozice studijního koordinátora zaměstnaného ve zdravotnickém zařízení dle institucionálního dotazníku*



Graf 4. *Pozice studijního koordinátora zaměstnaného ve zdravotnickém zařízení dle individuálního dotazníku*



Graf 5. *Zařazení studijního koordinátora ve zdravotnickém zařízení dle institucionálního dotazníku*



Zařazení a pozice studijního koordinátora ve zdravotnickém zařízení

Z institucionálního dotazníku vyplynulo, že studijní koordinátor je ve 49 % případů zaměstnaný jako NLZP (sestra, jiný NLZP pracovník) (20), ve 35 % má pozici technickohospodářského pracovníka (THP) (15), zahmující pozici administrátora či výzkumníka, téměř v 12 % tuto roli zastával lékař či farmaceut (5) a v 5 % případů (2) šlo o jiné než výše uvedené zařazení dle Katalogu prací, což reflektuje Graf č. 3.

Data z individuálního dotazníků ukazují, že ve většině případů jsou studijní koordinátoři zaměstnáni na pozici THP (32 %) a dále pak jako nelékařští zdravotničtí pracovníci (31 %). Ostatní respondenti jsou vedeni jako výzkumní pracovníci (11 %), nebo pracují na pozici lékaře, který vykonává současně práci studijního koordinátora (2,5 %), nebo se jedná o zaměstnance soukromých společností (18 %).

Z dat z institucionálního dotazníku je patrné, že studijní koordinátor je zařazen v 60 % pod kliniku (24), kde se provádí klinické studie. Pouze ve 28 % je zaměstnancem oddělení klinických studií (14), v 7 % má kombinovaný úvazek pod kliniku a oddělením klinických studií současně (3) a v 5 % si nemocnice najímá externí studijní koordinátory, poskytované soukromými společnostmi (2) (Graf č. 5).

V individuálním dotazníku, studijní koordinátoři uváděli, že jsou v 39 % zaměstnanci klinik, v 38 % zaměstnanci oddělení klinických studií, v 10 % mají kombinované zařazení, ve 13 % jsou zaměstnanci soukromých společností, což je uvedeno v Grafu č. 6.

O přijetí studijního koordinátora rozhoduje ve 42 % případech vedení nemocnice, ve 34 % vedení kliniky, ve 4 procentech samotný zkoušející, lékař a ve 20 % vedoucí oddělení klinických studií.

Z odpovědí institucionálního dotazníku vyplynulo, že v daných zdravotnických zařízeních pracuje celkem 213 studijních koordinátorů bez ohledu na výši úvazku. V případě, že ve zdravotnickém zařízení není ustanovena role studijního koordinátora, jeho práci vykonává ve většině případů přidělená sestra, v ojedinělých případech zajišťují vše zkoušející lékaři sami.

V individuálním dotazníku, studijní koordinátoři uváděli jako nejčastější agendu své práce administraci a koordinaci klinických studií (42 %). U čtvrtiny případu převažuje zadávání dat. V 16 % případů jsou koordinátoři zapojeni do přípravy klinické studie či jsou přímo manažery klinické studie (8 %). U 9 % studijních koordinátorů převažuje péče o pacienty, což zmiňuje Graf č. 7.

Vzdělání studijního koordinátora

Nejčastější forma vzdělání studijního koordinátora dle institucionálního dotazníku, je zdravotnické vzdělání (sestra, farmaceut, laborant, lékař), a to v 59 % případů, ve 21 % případů se jedná o přírodovědné vzdělání,

v 10 % má studijní koordinátor jiné vysokoškolské vzdělání a v dalších 10 % dosáhl jiného středoškolského vzdělání než zdravotnického, znázorněno v Grafu č. 8.

Nejčastější forma vzdělání studijního koordinátora dle individuálního dotazníku je zdravotnické vzdělání (sestra, laborant, farmaceut, lékař), a to v 50 % případů, v 18 % případů se jedná o přírodovědné vzdělání, v 15 % má studijní koordinátor jiné vysokoškolské vzdělání než zdravotnické vzdělání, v 7 % dosáhl jiného středoškolského vzdělání než zdravotnického a ve 10 % má vzdělání ekonomické, zobrazeno v Grafu č. 9.

Z individuálního dotazníků vzešla jasný zájem o další vzdělávání v oboru klinických studií na pozici studijního koordinátora. Graf č. 10. zobrazuje, že 99 % respondentů má zájem o další vzdělávání v oboru klinických studií přizpůsobené roli a potřebám studijních koordinátorů, včetně zavedení vysokoškolského vzdělání v oblasti klinických studií.

Z nejčastějších překážek v oblasti klinického hodnocení zdravotnická zařízení uváději nedostatek koordinátorů, nedostatek zkušených uchazečů, nízkou odbornost a neznalost prostředí klinických studií. Dále je zmíněna významná náročnost samotné práce spojená nejen s intenzivní administrativou, ale i zodpovědností, znalost anglického jazyka a obecně vysoká kompetence studijního koordinátora. Z finančních aspektů nemocnice uváděly omezenou kapacitu úvazků na klinikách či na oddělení klinických studií, ale současně zmiňují i nízké platové ohodnocení vycházející ze zařazení studijního koordinátora do neodpovídající kategorie v katalogu prací.

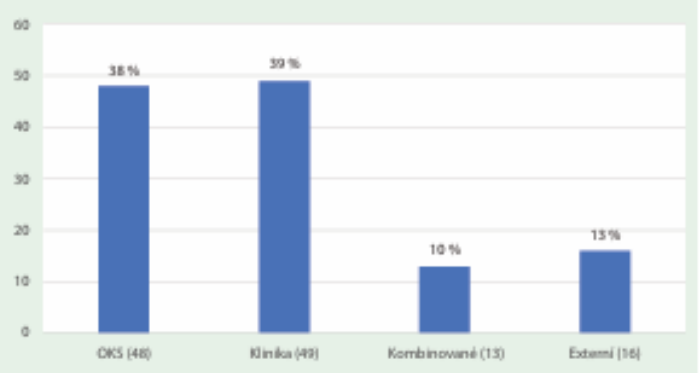
Diskuze

Podle nám dostupných informací se jedná o první dotazníkový průzkum mezi poskytovateli zdravotní péče mapující postavení studijních koordinátorů a jejich zařazení ve zdravotnických zařízeních napříč celou Českou republikou.

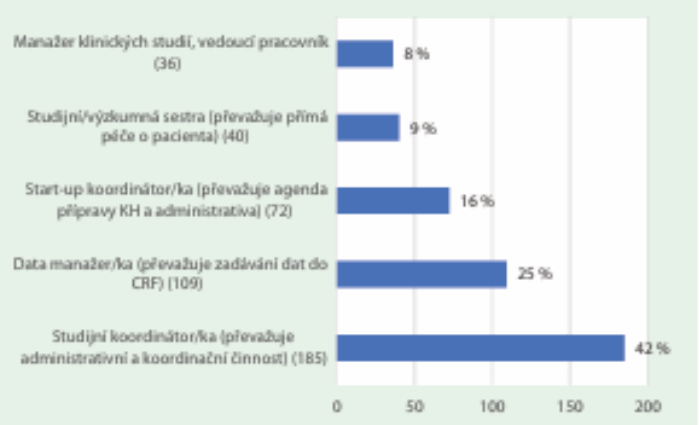
Míra návratnosti odpovědí u individuálního dotazníku, 35,5 %, je porovnatelná s obdobnými dotazníkovými studiemi, které byly provedeny v Itálii (35,9 %) či Švýcarsku (43,4 %) mezi poskytovateli zdravotní péče [18, 19].

Jedním z hlavních zjištění dotazníkového průzkumu mezi zdravotnickými zařízeními je, že oddělení klinických studií je založeno pouze u 29 z 42 poskytovatelů zdravotní péče, kteří se věnují realizaci klinických studií, což vnímáme jako velký prostor pro zlepšení. Primárně se jedná o fakultní nemocnice a některá státní zdravotnická zařízení, u 7 z nich, se jedná o soukromá zdravotnická zařízení, která vnímají důležitost, ale i finanční profit z realizace klinických studií.

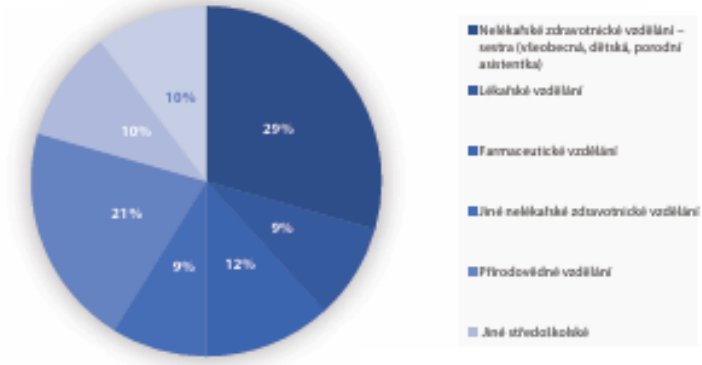
Graf 6. *Zařazení studijního koordinátora ve zdravotnickém zařízení dle individuálního dotazníku*



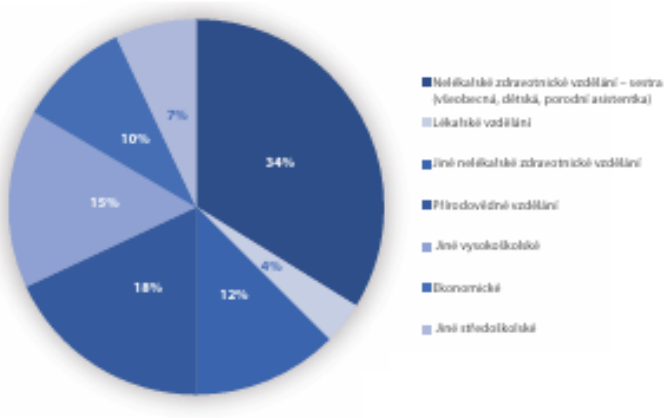
Graf 7. *Agenda studijního koordinátora dle individuálního dotazníku*



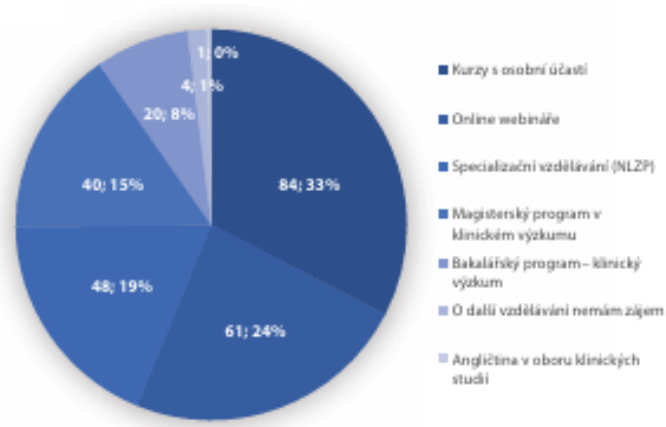
Graf 8. *Forma vzdělání studijního koordinátora dle institucionálního dotazníku*



Graf 9. *Forma vzdělání studijního koordinátora dle individuálního dotazníku*



Graf 10. *Potřeba dalšího vzdělávání v oboru*



Z podobné práce, zaměřené na onkologická centra, jednoznačně vyplynula souvislost mezi přítomností oddělení klinických studií ve zdravotnickém zařízení a vyšším počtem realizovaných klinických studií a současně přítomnosti studijního koordinátora. V nemocnicích, kde probíhalo více jak padesát klinických studií v dané době, byly přítomni alespoň tři studijní koordinátoři (19).

Studijní koordinátor je přímým zaměstnancem oddělení klinických studií v 38 % případů a v 39 % případů je zaměstnancem kliniky, kde klinická studie probíhá. Současný trend umožňuje optimalizaci distribuce práce mezi koordinátory a dalšími členy studijního týmu. OKS představuje místo, kde mohou studijní koordinátoři sdílet své zkušenosti a prohlubovat své znalosti oproti případům, kdy je koordinátor zaměstnaný klinikou, často v ojedinělé roli, kde může sdílet své zkušenosti či získat radu či návrhy na zlepšení své praxe od kolegů (20) jen stěží.

Přítomnost studijního koordinátora ve studijním týmu má přitom významný vliv na dodržování etických, regulačních a právních požadavků včetně etických principů (21).

Studijní koordinátor jako klíčový člen studijního týmu je nejčastěji v kontaktu s pacienty, účastníky klinické studie, a tak může jako první odhalit případná rizika, která mohou ohrozit jejich bezpečí či celkové blaho (22).

Rozvoj role studijního koordinátora v České republice však omezuje mnoho faktorů, např. dosud nezavedené povolání v Katalogu prací, a tudíž nejednoznačně uchopené zařazení personalisty ve zdravotnických zařízeních. Dalším nedostatkem je chybějící vzdělání zaměřené na potřeby a specifika práce studijního koordinátora, stejně tak nejednotnost v popisu základní pracovní náplně, kterou většina studijních koordinátorů zajišťuje. Vysoké nároky, náročnost práce, stresové prostředí a nedostatečná odměna vedou k častým změnám studijních koordinátorů. Vysoká fluktuace na této pozici, která v kombinaci se současně časově velmi náročným zaškolením vede k nedostatku studijních koordinátorů. Velká fluktuace na pozici studijního koordinátora přitom může negativně ovlivnit provedení, kvalitu, ale i výsledky klinické studie.

Studijní koordinátor plní podstatnou roli nejen při ochraně účastníků, ale i jejich edukaci a informovanosti, a tak zajišťují vysoký etický standard v klinických studiích (6).

Jedním z benefitů práce studijního koordinátora je její různorodost a pestrost. Fakt, který potvrdí asi všichni studijní koordinátoři je, že každá studie je jiná a den co den se objeví něco nového, s čím je potřeba si poradit, což přispívá k získávání dalších zkušenosti a neustálému zvyšování odbornosti. Tím je také práce studijního koordinátora zajímavá a pestrá. O to důležitější je potřeba vzájemného sdílení zkušenosti a vytvoření optimálních podmínek. Řešením může být zmíněná platforma pro sdílení a pravidelné setkávání (20).

Závěr

Systematicky jsme analyzovali současné postavení, ale i potřeby studijních koordinátorů, jak na individuální, tak institucionální úrovni.

Vzhledem k rostoucí důležitosti a reálné potřebě činností studijního koordinátora, doporučujeme, aby tato pozice byla zavedena do katalogu prací, měla tak zajištěnou stabilnější a ukotvenou pracovní pozici ve zdravotnických zařízeních. Zvláště se to týká těch koordinátorů, kteří jsou v současné době zařazeni za nejméně odpovídajících a vhodných podmínek, tedy jako THP. Koordinátoři s nelékařským zdravotnickým vzděláním, kteří v rámci své činnosti v klinických studiích poskytují částečně i odbornou zdravotnickou činnost, by měli být současně zařazeni ideálně dle své kvalifikace, tedy jako NZLP.

Z našich zjištění vyplývá, že dosavadní dostupné vzdělávání v národním jazyce není dostačující a je zájem o vytvoření komplexního vzdělávacího programu.

Doporučujeme, aby bylo zavedeno kontinuální, komplexní, a především přizpůsobené vzdělávání pro studijní koordinátory v oblasti klinického výzkumu.

Porozumění roli a potřebě studijního koordinátora v klinických studiích, zlepšení jeho postavení ve zdravotnických zařízeních a nastavení zásad dobré praxe studijních koordinátorů je klíčové pro zvyšování kvality provádění klinických studií v České republice a přispívá k zajištění ochrany účastníků klinických studií a zlepšení jejich compliance.

LITERATURA

1. Markey N, Howitt B, El-Mansouri I, et al. Clinical trials are becoming more complex: a machine learning analysis of data from over 16,000 trials. Sci Rep. 2024;14:3514. doi:10.1038/s41598-024-53211-z.
2. Gribben J, Macintyre E, Sonneveld P, et al. Reducing Bureaucracy in Clinical Research: A Call for Action. HemaSphere. 2020;4(2):e352. doi:10.1097/HS9.0000000000000352.
3. Perez-Gracia JL, Penel N, Calvo E, et al. Streamlining clinical research: an ESMO awareness call to improve sponsoring and monitoring of clinical trials. Ann Oncol. 2023;34(1):70-77. doi:10.1016/j.annonc.2022.09.162.
4. Davis AM, Hull SC, Grady C, et al. The Invisible Hand in Clinical Research: The Study Coordinator's Critical Role in Human Subjects Protection. J Law Med Ethics J Am Soc Law Med Ethics. 2002;30(3):411-419.
5. Mora V, Colantuono S, Fanali C, et al. Clinical research coordinators: Key components of an efficient clinical trial unit. Contemp Clin Trials Commun. 2023;32:101057. doi:10.1016/j.conctc.2023.101057.
6. Peng Z, Ma Y, Wang J, et al. Awareness of Clinical Research Coordinators Toward Ethics and Protection of Clinical Trial Patients. Ther Innov Regul Sci. 2023;57(3):581-589. doi:10.1007/s43441-022-00488-9.
7. Brinkman-Clenney S. An international comparison of the clinical trials nurse role. 2013;20(8):32-40. doi: 10.7748/nm2013.12.20.8.32.e1060.
8. Jones CT, Hastings C, Wilson LL. Research nurse manager perceptions about research activities performed by non-nurse clinical research coordinators. Nurs Outlook. 2015;63(4):474-483. doi:10.1016/j.outlook.2015.02.002.
9. Getz KA. Impact of In-Pharmacy Education on Patients' Knowledge and Attitudes About Clinical Trials. Ther Innov Regul Sci. 2013;47(3):336-340. doi:10.1177/2168479013478953.
10. Getz KA. Are CRCs Reaching Their Tipping Point? Applied Clinical Trials. Appl Clin Trials. 2012;21(11):28-30.
11. Fan J, Liu X, Li Y, et al. Quality problems of clinical trials in China: evidence from quality related studies. Trials. 2022;23. doi:10.1186/s13063-022-06281-1.
12. Behar-Horenstein L, Potter J, Prikhidko A, et al. Training Impact on Novice and Experienced Research Coordinators. Qual Rep. 2017;22(12):3118-3138. doi:10.46743/2160-3715/2017.3192.
13. Castro K, Bevans M, Miller-Davis C, et al. Validating the Clinical Research Nursing Domain of Practice. Oncol Nurs Forum. 2011;38(2):E72-E80. doi:10.1188/11.ONF.E72-E80.
14. DuBois JM, Mozersky JT, Antes AL, et al. Assessing clinical research coordinator knowledge of good clinical practice: An evaluation of the state of the art and a test validation study. J Clin Transl Sci. 2020;4(2):141-145. doi:10.1017/cts.2019.440.
15. Pozvánka na GCP kurz 2023 Medicus academy. Accessed May 31, 2024. Available from: https://medicus-academy.cz/aktuality/pozvanka-na-gcp-kurz-2023/.
16. 6. celostátní setkání studijních sester a koordinátorů klinických hodnocení 2023. Czecrin. Accessed April 9, 2024. Available from: https://czecrin.cz/akce/6-celostatni-setkani-studijnich-sester-a-koordinatoru-klinickych-hodnoceni-2023/.
17. 6. celostátní setkání studijních sester a koordinátorů klinických hodnocení. Czecrin. Accessed April 29, 2024. Available from: https://czecrin.cz/materialy-ke-stazeni/6-celostatni-setkani-studijnich-sester-a-koordinatoru-klinickych-hodnoceni/.
18. Cinefra M, Cagnazzo C, McMahon L, et al. The Critical Role of the Clinical Research Coordinator for Clinical Trials: A Survey in Oncology. Med Access Point Care. 2017;1:maapoc.0000015. doi:10.5301/maapoc.0000015.
19. Cannito C, Magletta G, Fraul, et al. Presence and activities of clinical research coordinators at Italian Health Care Institutions: A national cross-sectional survey. J Clin Transl Sci. 2022;6(1):e1. doi:10.1017/cts.2021.872.
20. Croghan IT, Viker SD, Limper AH, et al. Developing a clinical trial unit to advance research in an academic institution. Contemp Clin Trials. 2015;45:270-276. doi:10.1016/j.cct.2015.10.001.
21. Knowledge and attitudes regarding clinical trials and willingness to participate among prostate cancer patients - ScienceDirect. Accessed April 9, 2024. Available from: https://www.sciencedirect.com/science/article/abs/pii/S1551714415300987.
22. Shju R, Thankachan S, Akhil A, et al. A Survey Study on Knowledge and Attitude Toward the Ethics Committee and Research Ethical Practices Among Researchers From Kuwait. Sci Eng Ethics. 2023;29(6):37. doi:10.1007/s11948-023-00458-w.



7.16 Annex 16

Salmanton-García J, Wipfler P, Valle-Simón P, Merakou C, Kopsidas I, Bethe U, Steinbach A, Spivak O, Součková L, Mendonça MA, Koniordou M, Hellemans M, Frías-Iniesta J, Davis RJ, Barta I, Azzini AM, Askling HH, Argyropoulos CD, Álvarez-Barco E, Akova M, Bonten MMJ, Cohen-Kandli M, Cox RJ, Flisiak R, Husa P, Jancoriene L, Koscalova A, Launay O, Lundgren J, Mallon P, Marques L, Nauclér P, Ochando J, Pana ZD, Tacconelli E, Tóth K, Trelle S, van Damme P, Zaoutis TE, Zeitlinger M, Albus K, Stewart FA, Hofstraat SHI, Bruijning-Verhagen P, Cornely OA;
VACCELERATE Consortium. Electronic address: trialsites@vaccelerate.eu. VACCELERATE Site Network: Real-time definition of clinical study capacity in Europe. Vaccine. 2023 Jun 13;41(26):3915-3922. doi: 10.1016/j.vaccine.2023.05.006. Epub 2023 May 18. PMID: 37210309; PMCID: PMC10194816.

Summary

Purpose:

The aim of this study was to establish and describe a pan-European infrastructure capable of rapidly identifying and mobilising clinical trial sites for vaccine studies. The study addresses fragmentation in the European clinical trial landscape, which has historically limited Europe’s attractiveness for large-scale vaccine research and delayed trial implementation.

Methodology:

The study was based on the development of the VACCELERATE Site Network, a structured, continuously updated registry of clinical trial sites across Europe. Participating centres provided standardized data on infrastructure, prior trial experience, patient populations, and research capabilities, and the platform enabled rapid feasibility assessments and matchmaking between sponsors and sites through predefined questionnaires and workflows.

Results:

By 2023, the network included 481 sites from 39 European countries, covering a broad spectrum of trial phases and therapeutic expertise, including both adult and paediatric populations. The network has already been actively used for multiple academic and industry trials, demonstrating its functionality as a real-time tool for site identification and trial feasibility assessment across different infectious disease areas.

Conclusion:

The VACCELERATE Site Network represents an effective solution to overcome fragmentation in European clinical research by providing a coordinated, scalable infrastructure for

rapid trial deployment. The study highlights the importance of structured research networks in enhancing the feasibility, efficiency, and global competitiveness of NCCTs in Europe.

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VACCELERATE Site Network: Real-time definition of clinical study capacity in Europe

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ABSTRACT

Background: The inconsistent European vaccine trial landscape rendered the continent of limited interest for vaccine developers. The VACCELERATE consortium created a network of capable clinical trial sites throughout Europe. VACCELERATE identifies and provides access to state-of-the-art vaccine trial sites to accelerate clinical development of vaccines.

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Keywords:
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Methods: Login details for the VACCELERATE Site Network (vaccelerate.eu/site-network/) questionnaire can be obtained after sending an email to. Interested sites provide basic information, such as contact details, affiliation with infectious disease networks, main area of expertise, previous vaccine trial experience, site infrastructure and preferred vaccine trial settings. In addition, sites can recommend other clinical researchers for registration in the network. If directly requested by a sponsor or sponsor representative, the VACCELERATE Site Network pre-selects vaccine trial sites and shares basic study characteristics provided by the sponsor. Interested sites provide feedback with short surveys and feasibility questionnaires developed by VACCELERATE and are connected with the sponsor to initiate the site selection process.

Results: As of April 2023, 481 sites from 39 European countries have registered in the VACCELERATE Site Network. Of these, 137 (28.5 %) sites have previous experience conducting phase I trials, 259 (53.8 %) with phase II, 340 (70.7 %) with phase III, and 205 (42.6 %) with phase IV trials, respectively. Infectious diseases were reported as main area of expertise by 274 sites (57.0 %), followed by any kind of immunosuppression by 141 (29.3 %) sites. Numbers are super additive as sites may report clinical trial experience in several indications. Two hundred and thirty-one (47.0 %) sites have the expertise and capacity to enrol paediatric populations and 391 (79.6 %) adult populations. Since its launch in October 2020, the VACCELERATE Site Network has been used 21 times for academic and industry trials, mostly interventional studies, focusing on different pathogens such as fungi, monkeypox virus, *Orthomyxoviridae*/influenza viruses, SARS-CoV-2, or *Streptococcus pneumoniae*/pneumococcus.

Conclusions: The VACCELERATE Site Network enables a constantly updated Europe-wide mapping of experienced clinical sites interested in executing vaccine trials. The network is already in use as a rapid-turnaround single contact point for the identification of vaccine trials sites in Europe.

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1. Introduction

Before the coronavirus disease 2019 (COVID-19) pandemic was declared in March 2020 [1]. Europe was not the preferred option for global vaccine trial developers, as compared to other regions [2–5], due to its fractured clinical trial infrastructure [6–9]. While regional and international networks were attempting to boost clinical trial and study performance on the continent, none of them was focused on vaccine development [10,11].

In January 2021 the VACCELERATE consortium was set up [12], to harmonize vaccine trial initiative across Europe and create a network in which vaccine trials can be rapidly deployed for early evidence generation and addressing public health needs [13]. Within VACCELERATE, one of its core objectives was to describe the capacity mapping of vaccine trials sites in Europe, in order to facilitate rapid clinical trial site identification, selection and initiation of joint European vaccine trials. Nevertheless, while primarily set up as a vaccine trial network, the network is also ready to participate in epidemiological studies, surveys and other projects and initiatives related to vaccine topics.

Additionally, the VACCELERATE Site Network offers and provides access to the VACCELERATE Academy where investigators can register for educational courses such as good clinical practice (GCP) a training course specific on vaccine trials and a course on conduct of multinational trials. A study nurse course is currently also under development. The VACCELERATE Academy will support capacity building of European sites with limited previous experience in vaccine trials. Altogether, VACCELERATE Site Network will enable to keep sites updated on regulatory developments and state-of-the-art operational procedures.

2. Methods

The VACCELERATE Site Network (vaccelerate.eu/site-network/) [14] collects information on basic contact and site details, main point of contact, prior vaccine trial experience, available infrastructure as well as preferred parameters for study participation (Table 1). Interested sites can initiate their registration through trialsites@vaccelerate.eu. The VACCELERATE Coordination Office (CO) provides the designated point(s) of contact with individual access details to the online platform to complete the site questionnaire. Registration in the VACCELERATE Site Network does not guarantee participation in a vaccine trial sponsored by the VACCELERATE consortium or by third parties. With the aim to enlarge the Site Network by reaching the largest number of vaccine trial sites in Europe, the VACCELERATE National Coordinators (NC) have a key role within the network. They are local liaison partners of the Consortium in their respective countries, being in charge of the guidance for the most appropriate approach to involve new sites and for the local expansion of the network (see Table 2 and Table 3).

The VACCELERATE CO can be approached by vaccine developers or sponsors during clinical trial planning but also while the trial is already active and the need for additional sites arises. The developer/sponsor provides basic information regarding the vaccine trial (i.e., study population, country, study phase…) and defines basic needs and requirements regarding the sites. The registry is then filtered for sites fulfilling these criteria. Registered sites will be identified using information collected in the VACCELERATE Site Management System (ClinicalSite) [15]. Matching registered sites are informed about the clinical trial opportunity per e-mail by VACCELERATE. If desired, sponsors/developers can opt to include further trial information and or (pre-)feasibility questionnaires in the e-mail. VACCELERATE NC can be involved to discuss potential country-specific requirements or challenges and promote the trial in their country-specific VACCELERATE site network. Sites will decide whether they are interested in participating. Refusing to participate in any offered vaccine trial opportunity does not impact registration in the network. Vaccine trial sites keep their decision independence throughout the whole process (Fig. 1). The sponsor then receives the contact details of interested sites and a feasibility and site selection process is to be done by the sponsor or their representative unless VACCELERATE is delegated/contracted to do this for them.

For the cases when VACCELERATE is in charge of the site feasibility, a transparent site selection procedure tool was created to select the best qualified sites for individual studies, both for adult and paediatric trials. First, an online baseline feasibility questionnaire is sent out to a selection of sites that could qualify based on an initial match between the trial site information entered at registration in the Site Network and the specific trial requirements. Feasibility questions address the following categories: general site

Table 1
VACCELERATE Site Network: Survey Captured.

Vaccine Trial Site/Network	
Site name	
Type of institution *(multiple answers possible)*	
Academic research organization	*Pathology Department*
Clinic	*Pharma company/Medical device manufacturer*
Comprehensive cancer centre	*Practice*
Contract research organization	*Society/association*
Ethics committee	*Study office*
Institute/University institution	*Study Group*
Institution	*Trials unit*
Laboratory	*University*
Medical centre	*Working Group*
Network/Consortium	*Pathology Department*
Address	
Street and number	
Postal code	
City/Town	
Country	
Part of national infectious disease network/s?	
Main site for communication	
Name of contact person	
Academic Title | Function	
MD	*Primary care network coordinator*
Microbiologist	*Study nurse*
MSc	*Study physician*
Pharmacist	*Virologist*
PhD	*Other. Please specify*
PI	
Phone number (direct line)	
Email	
Prior clinical trial experience	
Number of trials per centre < 5 years	
Number of subjects per centre < 5 years	
Main focus *(multiple answers possible)*	
Phase I (includes I/II)	
Phase II	
Phase III	
Phase IV	
Observational	
No previous experience	
Previous experience conducting vaccine studies?	
If yes,	
Number of trials per centre < 2 years	
Number of subjects per trial < 2 years	
Main disease area/indication	
Phases	
Phase I	
Phase II	
Phase III	
Phase IV	
Main disease area/indication. Please specify	
Infrastructure	
Dedicated Phase-I-Unit available?	
If yes, number of subjects who can be enrolled in parallel at any given time	
Site pharmacy has prior experience with blinded investigational product?	
Site pharmacy has prior experience with vaccines as investigational product?	
Site pharmacy has prior experience with genetically modified organisms as investigational product?	
Is PCR for SARS-CoV-2 carried out by a lab at your institution?	
No, i.e., by external lab. Please indicate who carries out these PCR for your site	
Yes, i.e., in-house	
Please indicate maximum turn-around time for SARS-CoV-2 PCR at your site? (time from swab to results, regardless of whether done in-house or externally)	
24–48 h	
>48 h	
Preferred parameters for study participation	
Study population	
Healthy subjects	
Adults (≥18)?	
Adolescents and children (<18)?	
Seropositive subjects	
Adults (≥18)?	
Adolescents and children (<18)?	

(continued on next page)

Table 1 (continued)

Vaccine Trial Site/Network
Clinical setting
Phase-I-Unit
Outpatient setting
Inpatient setting
Interest in participating in trials for other than SARS-CoV-2/COVID-19?
I would like to recommend the following site[s]/network(s) for the platform (e.g., site name, PI name, email address…)

COVID-19, coronavirus diseases 2019; **MD**, Medical Doctor; **MSc**, Master of Science; **PCR**, polymerase chain reaction; **PhD**, Doctor of Philosophy; **PI**, principal investigator; **SARS-CoV-2**, severe acute respiratory syndrome coronavirus 2.

Table 2
Registered sites in the VACCELERATE Site Network as of April 2023.

	Paediatric			Adults			Total		
	n	% over total	centres/1M hab	n	% over total	centres/1M hab	n	% over total	centres/1M hab
Total	**231**	100.00 %	0.3	**388**	100.00 %	0.6	**481**	100.00 %	0.7
Albania	1	0.43 %	0.3	1	0.26 %	0.3	1	0.21 %	0.3
Austria	4	1.73 %	0.5	9	2.32 %	1.0	11	2.29 %	1.2
Azerbaijan	1	0.43 %	0.1	2	0.52 %	0.2	2	0.42 %	0.2
Belgium	5	2.16 %	0.4	13	3.35 %	1.1	15	3.13 %	1.3
Bulgaria	5	2.16 %	0.7	16	4.12 %	2.3	16	3.33 %	2.3
Croatia	1	0.43 %	0.2	1	0.26 %	0.2	1	0.21 %	0.2
Cyprus	3	1.30 %	2.6	6	1.55 %	5.1	9	1.88 %	7.7
Czech Republic	1	0.43 %	0.1	8	2.06 %	0.8	8	1.67 %	0.8
Denmark	10	4.33 %	1.7	14	3.61 %	2.4	20	4.17 %	3.5
Estonia	1	0.43 %	0.8	0	0.00 %	0.0	2	0.42 %	0.8
Finland	0	0.00 %	0.0	1	0.26 %	0.2	1	0.21 %	0.2
France	36	15.58 %	0.5	38	9.79 %	0.6	39	8.13 %	0.6
Georgia	1	0.43 %	0.3	1	0.26 %	0.3	1	0.21 %	0.3
Germany	18	7.79 %	0.2	47	12.11 %	0.6	56	11.67 %	0.7
Greece	16	6.93 %	1.5	15	3.87 %	1.4	26	5.42 %	2.4
Hungary	3	1.30 %	0.3	8	2.06 %	0.8	8	1.67 %	0.8
Iceland	1	0.43 %	2.9	0	0.00 %	0.0	1	0.21 %	2.9
Ireland	6	2.60 %	1.3	15	3.87 %	3.2	19	3.96 %	4.0
Israel	3	1.30 %	0.3	7	1.80 %	0.8	7	1.46 %	0.8
Italy	14	6.06 %	0.2	27	6.96 %	0.4	35	7.29 %	0.6
Latvia	1	0.43 %	0.5	0	0.00 %	0.0	1	0.21 %	0.5
Lithuania	4	1.73 %	1.4	6	1.55 %	2.1	8	1.67 %	2.9
Luxembourg	1	0.43 %	1.7	1	0.26 %	1.7	2	0.42 %	3.3
Malta	2	0.87 %	4.5	0	0.00 %	0.0	2	0.42 %	4.5
Netherlands	5	2.16 %	0.3	10	2.58 %	0.6	11	2.29 %	0.6
North Macedonia	1	25.00 %	0.5	0	0.00 %	0.0	1	9.09 %	0.5
Norway	7	3.03 %	1.3	5	1.29 %	0.9	7	1.46 %	1.3
Poland	7	3.03 %	0.2	16	4.12 %	0.4	17	3.54 %	0.4
Portugal	13	5.63 %	1.3	9	2.32 %	0.9	18	3.75 %	1.7
Romania	1	0.43 %	0.1	2	0.52 %	0.1	3	0.63 %	0.2
Serbia	0	0.00 %	0.0	1	0.26 %	0.1	2	0.42 %	0.3
Slovakia	1	0.43 %	0.2	3	0.77 %	0.6	4	0.83 %	0.7
Slovenia	0	0.00 %	0.0	1	0.26 %	0.5	1	0.21 %	0.5
Spain	27	11.69 %	0.6	56	14.43 %	1.2	62	12.92 %	1.3
Sweden	12	5.19 %	1.2	11	2.84 %	1.1	20	4.17 %	2.0
Switzerland	3	1.30 %	0.4	10	2.58 %	1.2	10	2.08 %	1.2
Turkey	7	3.03 %	0.1	8	2.06 %	0.1	11	2.29 %	0.1
Ukraine	2	0.87 %	0.0	8	2.06 %	0.2	8	1.67 %	0.2
United Kingdom	7	3.03 %	0.1	12	3.09 %	0.2	15	3.13 %	0.2

hab, habitants; **M**, million.

information, prior experience in clinical vaccine trials, access to study population, site staff expertise and qualifications, (laboratory) facilities and equipment, and local ethics approval and contracting processes. The information from the baseline feasibility questionnaire is compiled in feasibility reports and this information is used as site selection tool by the Site Selection Board (SSB). The main objectives of the SSB are to agree on site selection criteria for trials, review answers from feasibility questionnaires provided by potential sites against these criteria, and to select the more suitable trial sites across Europe. The information collected by the site selection tool is used as an initial pre-selection filter. Pre-selected sites receive a second feasibility questionnaire detailing trial specific needs and requirements. Combined, the information from baseline and trial-specific feasibility questionnaires is compiled and used for final site selection procedure by the Board.

2.1. Site management system: added value

VACCELERATE Site Network data are stored on the online platform Clinicalsite.org (Healex GmbH, Cologne, Germany) [15]. Clinicalsite.org also permits document uploads, such as curricula vitae of designated clinical trial personnel, reports of previous experience in trials, GCP certificates and other relevant clinical trial training certification.

To further harmonise VACCELERATE standards and processes, minimum good quality standards, key performance indicators and site assessment procedures were also developed by the coordi-

Table 3
Description of the VACCELERATE Site Network.

	Overall (n = 481)		Children (n = 231)		Adults (n = 390)	
	n	%	n	%	n	%
Research topic						
Infectious Diseases	274	57.0	132	57.1	217	55.6
Immunodefficiencies	141	29.3	37	16.0	127	32.6
Diabetes mellitus	10	2.1	7	3.0	7	1.8
Any disease	64	13.3	50	21.6	60	15.4
Prior vaccine trial experience	418	86.9	184	79.7	334	85.6
Number of trials per centre < 5 years	12 (5–30) [0–999]		10 (5–30) [0–960]		15 (7–35) [0–999]	
Number of subjects per centre < 5 years	200 (50–617) [0–100000]		200 (50–1000) [0–100000]		285 (80–825) [0–100000]	
Trial phase						
Phase I	137	28.5	63	27.3	120	30.8
Phase II	259	53.8	119	51.5	222	56.9
Phase III	340	70.7	147	63.6	282	72.3
Phase IV	205	42.6	92	39.8	172	44.1
Observational studies	253	52.6	115	49.8	208	53.3
Previous vaccine trial experience	229	47.6	115	49.8	181	46.4
Number of trials per centre < 2 years	3 (2–5) [0–15]		1 (0–2)[0–2]		4 (2–5) [1–15]	
Number of subjects per trial < 2 years	152 (20–600) [6–1000]		20 (6–203) [6–203]		402 (152–800) [100–1000]	
Trial phase						
Phase I	61	12.7	30	13.0	57	14.6
Phase II	107	22.2	52	22.5	87	22.3
Phase III	164	34.1	88	38.1	128	32.8
Phase IV	69	14.3	38	16.5	56	14.4
Infrastructure						
Pharmacy experience with blinded products	391	81.3	165	71.4	322	82.6
Pharmacy experience with vaccines	280	58.2	125	54.1	236	60.5
Pharmacy experience with GMO	177	36.8	85	36.8	156	40.0
PCR access	443	92.1	196	84.8	353	90.5
Onsite	382	79.4	175	75.8	297	76.2
Outsourced	61	12.7	17	7.4	47	12.1
24–48 h	12	2.5	3	1.3	10	2.6
>48 h	431	89.6	193	83.5	343	87.9
Preferred parameters						
Healthy subjects	418	86.9	193	83.5	110	28.2
Seropositive subjects	344	71.5	144	62.3	93	23.8
Clinical setting						
Phase-I-Unit	129	26.8	49	21.2	119	30.5
Outpatient setting	393	81.7	180	77.9	315	80.8
Inpatient setting	295	61.3	142	61.5	227	58.2

GMO, genetically modified organism; **PCR**, polymerase chain reaction.

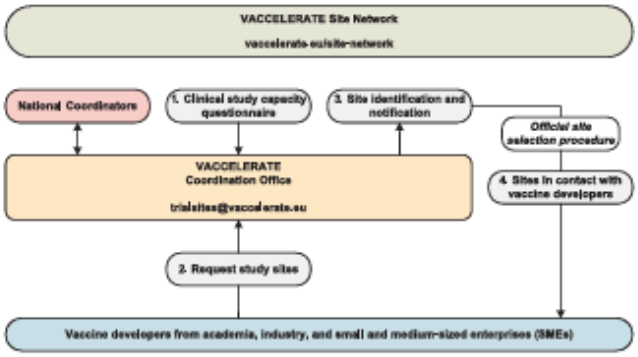


Fig. 1. Management of the VACCELERATE Site Network (1) Potential sites may register after sending an email to trialsites@vaccelerate.eu. Login data to access the online platform where data are stored will be provided. (2) Vaccine developers interested in performing vaccine trials might contact the VACCELERATE Coordination Office with a participant request. (3) Potential sites are identified and filtered according to the vaccine developers, and briefly informed about the vaccine trial, including sometimes a specific feasibility questionnaire, via e-mail. (4) Interested sites can independently decide whether they eventually participate the vaccine trial.

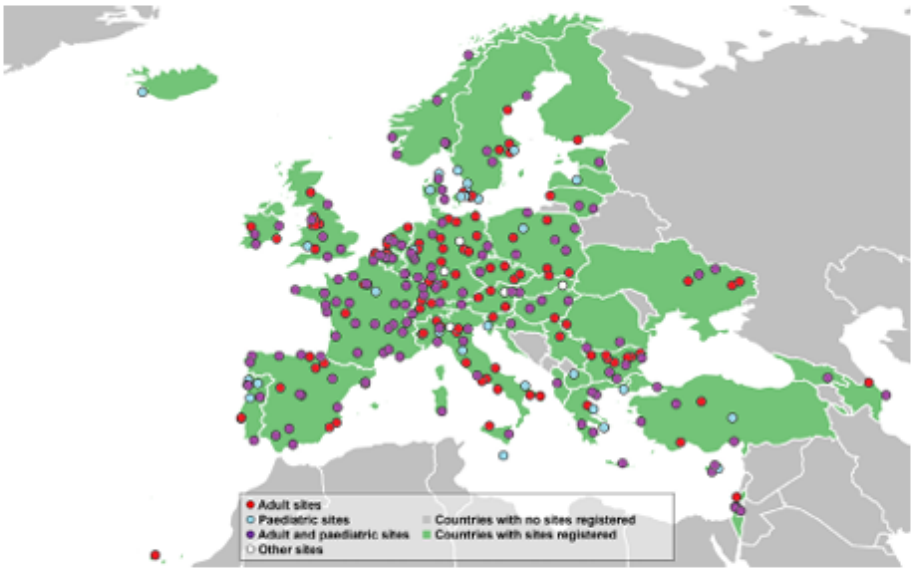


Fig. 2. Current Site Mapping of the VACCELERATE Site Network **Overall registered sites**: Spain (n = 62), Germany (n = 56), France (n = 39), Italy (n = 35), Greece (n = 26), Denmark and Sweden (n = 20, each), Ireland (n = 19), Portugal (n = 18), Poland and United Kingdom (n = 17, each), Bulgaria (n = 16), Belgium (n = 15), Austria, Netherlands, and Turkey (n = 11, each), Cyprus and Switzerland (n = 9, each), Czech Republic, Hungary, Lithuania, and Ukraine (n = 8, each), Israel and Norway (n = 7, each), Slovakia (n = 4), Romania (n = 3), Azerbaijan, Estonia, Luxembourg, and Malta (n = 2, each), and Albania, Croatia, Finland, Georgia, Iceland, Latvia, North Macedonia, Serbia, and Slovenia (n = 1, each).

nation team of the VACCELERATE Site Network. Within ClinicalSite, a certification status was created that can be used to identify experienced and qualified sites. A distinction is made between the following statuses: green, bronze, silver and gold. The obtained status depends on the qualification of the site and staff (sites uploaded GCP and study nurse course certificates, as well as CVs of Principal Investigator/site personnel), as well as their previous experience (participation in at least one phase 2/3 vaccine trial) and if the site implemented VACCELERATE SOPs.

In addition to this certification status, a quality assessment tool was created focussed on criteria related to the previous experience of VACCELERATE sites (i.e., vaccine trial experience, trained PI and staff), indicating capacity building needs. Over the course of the VACCELERATE project, this tool will be able to show the progress of the network and capacity building efforts. For each parameter (experience with vaccine trials, experience PI and experience study coordinator), a site will be classified in one of four categories. The categories all have their own score. The site data for each category can be retrieved from ClinicalSite or the baseline feasibility questionnaire. All information on these parameters can be combined and plotted in a heat map with sites on the y-axis and the different parameters on the x-axis. Sites can move to a new category when they follow the training courses developed in VACCELERATE. Additionally, a total score can be calculated based on the scores of the site for each of the parameters using the assessment tool. Based on the total scores for each site, a gradient scale can be generated and added to the heat map. Besides the assessment tool, performance indicators were also defined in order to measure a VACCELERATE site's performance in trials. The objectives of these performance indicators are to a) aid the selection of sites into studies based on their infrastructure and performance in VACCELERATE studies, b) identify challenges within less well performing organisations requiring extra support/training and plan improvement activities for these sites, and c) monitor and report progress of the VACCELERATE project in improving these indicators.

The following performance indicators were selected because they were considered relevant, objectively measurable, and amenable to improvement by participating sites: a) enrolment/randomisation rates (number of subjects randomised / planned number of subjects randomised), b) subject completion rates (number of subjects completed / enrolled), c) turnaround time for IEC/IRB approval (number of days between submission to ethics committee and approval from ethics committee), d) turnaround time for contract agreement (number of days between first contract sent and all contracts signed), e) completion time of electronic case report forms (eCRFs), and f) response time to electronic case report form (eCRFs) queries.

The above-mentioned performance indicators will be used for both sites that participate in trials with an adult or a paediatric study population. The ClinicalSite data, performance data and assessment tool outcomes can be combined to create a well-rounded quality assessment of sites, distinguishing between sites that performed adult and paediatric trials.

2.2. Data privacy and data protection

The processing of personal data, such as name(s) and surname (s), address, e-mail address, telephone number or curriculum vitae of the person concerned, is always carried out in accordance with the General Data Protection Regulation (EU GDPR) and in accordance with the country-specific data protection regulations applicable to Healex GmbH (Cologne, Germany). Furthermore, participants are informed of the rights to which they are entitled by means of this data protection declaration. Registered points of

Table 4
VACCELERATE Site Network utilisation since set up.

Utilisation date	Pathogen	User	Purpose
October 2020	SARS-CoV-2	Industry trial	Vaccine trial
October 2020	SARS-CoV-2	Industry trial	Vaccine trial
February 2021	SARS-CoV-2	Industry trial	Vaccine trial
February 2021	SARS-CoV-2	Industry trial	Vaccine trial
February 2021	*Orthomyxoviridae*	Industry trial	Vaccine trial
March 2021	SARS-CoV-2	Industry trial	Vaccine trial
March 2021	SARS-CoV-2	Industry trial	Vaccine trial
July 2021	SARS-CoV-2	Industry trial	Vaccine trial
July 2021	SARS-CoV-2	Industry trial	Vaccine trial
August 2021	SARS-CoV-2	Academic trial	Vaccine trial
August 2021	SARS-CoV-2	Academic trial	Vaccine trial
August 2021	SARS-CoV-2	Academic trial	Vaccine trial
September 2021	Fungi	Academic epidemiological study	IFI diagnostic and treatment capacity mapping [18]
March 2022	*Streptococcus pneumoniae*	Industry trial	Vaccine trial
April 2022	*Streptococcus pneumoniae*	Industry trial	Vaccine trial
May 2022	*Orthomyxoviridae*	Industry trial	Vaccine trial
May 2022	Monkeypox virus	Academic epidemiological study	Monkeypox diagnostic and treatment capacity mapping [17]
June 2022	Monkeypox virus	Academic epidemiological study	Status of monkeypox-related clinical trials in Germany
June 2022	Monkeypox virus	Academic epidemiological study	Status of monkeypox-related clinical trials in Europe
July 2022	Monkeypox virus	Academic epidemiological study	Monkeypox epidemiology in children and adult women at outbreak onset [16]
August 2022	NA	Academic epidemiological study	Status of Study Nurse courses in Europe
February 2023	Monkeypox virus	Industrail trial	Feasibility to participate in observational monkeypox paediatric studies
February 2023	Any	Academic epidemiological study	Priority list of pathogens of interest
February 2023	SARS-CoV-2	Academic epidemiological study	Long-COVID treatment and follow up capacity mapping
March 2023	NA	Other	Invitation to study nurse course

IFI, invasive fungal infection; **NA**, not applicable; **SARS-CoV-2**, severe acute respiratory syndrome coronavirus 2.

contact for the sites consent to the transfer of their names and contact details to the sponsor/sponsor representative, in case of a matching and their interest in trial participation as a site with the e-mail expressing their interest.

3. Results

As of April 2023, the VACCELERATE Site Network has collected information from up to 481 institutions from 39 European countries (Fig. 2). Of these, in 274 (57.0 %) the main topic for research was reported to be infectious diseases, in 141 (29.3 %) immunodeficiencies, either acquired or autoimmune, and 64 (13.3 %) sites are open to research in any disease.

More than 400 sites (n = 418, 86.9 %) reported previous experience in vaccine trials, with a median of 12 (IQR 5–30) trials performed per centre in the previous 5 years before the registration, and a median of 200 (IQR 50–617) subjects participating in them. Focusing on the trial phase, most sites (n = 340, 70.7 %) have already participated in phase III trials; with only 137 (28.5 %) sites reporting experience in phase I trials. Specifically for vaccine trials, 229 (47.6 %) claimed having participated in such studies in the past, mainly in phase III (n = 164, 34.1 %) and phase II (n = 107, 22.2 %).

Four in five hospital pharmacies (n = 391, 81.3 %) had experience handling blinded products for research, 280 (58.2 %) vaccines and 177 (36.8 %) genetically modified organisms. Polymerase chain reaction tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were available in 443 (92.1 %) of the participating sites, mainly on-site (n = 382, 79.4 %). Participant-wise, 418 (86.9 %) institutions were open to perform vaccine trials with healthy subjects, while 344 (71.5 %) with infected subjects. Specific data for paediatric and adult populations are depicted in table 2 and 3.

Since its inception in October 2020, the VACCELERATE Site Network has provided access to European sites up to 25 times for different initiatives, such as academic and industry interventional clinical trials or epidemiological studies: two (8.0 %) in 2020, 11 (44.0 %) in 2021, eight (32.0 %) in 2022, and four (16.0 %) until April 2023. Of all the initiatives, 12 (48.0 %) were focused on SARS-CoV-2 initiatives, five (20.0 %) on monkeypox virus and two (8.0 %) on *Orthomyxoviridae*/influenza viruses *Streptococcus pneumoniae*/pneumococcus each (Table 4) [16–18].

3.1. Outlook

The VACCELERATE Site Network serves as a platform where vaccine trial sites, regardless of any previous experience, can register in order to eventually participate in vaccine trials in Europe. In fact, the VACCELERATE consortium itself can ensure capacity building to trial centres that lack the necessary experience, by providing certified courses on different aspects, such as GCP or study nurse courses. Initiated at COVID-19 pandemic onset, the network is open to any vaccine trial that could be accelerated by participating institutions. Additionally, it has also proved to be an excellent initiative to run flash surveys. for timely relevant aspects that could potentially evolve into vaccine trials, and epidemiological studies in Europe [16–18].

Currently, the enrolment in the VACCELERATE Site Network is open to any vaccine trial centre in Europe and reach out to other world regions has begun.

4. Funding statement

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5. Contribution statement

OAC conceived the network idea and is the coordinator of the VACCELERATE Consortium. JSG, AS and UB developed and pro-

grammed the software containing the variables collected for the VACCELERATE Site Network. JSG and PW manage the VACCELERATE Site Network. PVS, CM and IK developed specific strategies and tools to enlarge the VACCELERATE Site Network. JSG performed the analysis, created the figures and wrote the initial manuscript. SHIH and PBV coordinate the work package in which the VACCELERATE Site Network is placed within the VACCELERATE Consortium. All authors worked for the dissemination, enlargement and promotion of the VACCELERATE Site Network and reviewed and approved the final manuscript.

6. Ethics approval

Not applicable.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: VACCELERATE Consortium reports financial support was provided by European Union. VACCELERATE Consortium reports financial support was provided by Federal Ministry of Education and Research Bonn Office.

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Data availability

Data can be made available after a reasonable requestion following the paths described in the manuscript.

References

[1] Mahase E. Covid-19: WHO declares pandemic because of "alarming levels" of spread, severity, and inaction. BMJ 2020;368:m1036.
[2] Lythgoe MP, Middleton P. Comparison of COVID-19 Vaccine Approvals at the US Food and Drug Administration, European Medicines Agency, and Health Canada. JAMA Netw Open 2021;4:e2114531.
[3] Lankinen KS, Pastila S, Klipi T, Nohynek H, Makela PH, Olin P. Vaccinovigilance in Europe–need for timeliness, standardization and resources. Bull World Health Organ 2004;82:828–35.
[4] Centers for Disease Control and Prevention. Vaccine Testing and the Approval Process www.cdc.gov/vaccines/basics/test-approve.html (Last accessed August 3, 2022).
[5] European Vaccination Information Portal. Approval of vaccines in the European Union www.vaccination-info.eu/en/vaccine-facts/approval-vaccines-european-union (Last accessed August 2, 2022).
[6] Leroy O, Geels M, Korejwo J, Dodet B, Imbault N, Jungbluth S. Roadmap for the establishment of a European vaccine R&D infrastructure. Vaccine 2014;32:7021–704.
[7] EU-Response investigators group, Diallo A, Troseid M, Simensen VC, Boston A, Demotes J, Olsen IC, Chung F, Paiva JA, Hites M, Ader F, Arribas JR, Baratt-Due A, Melien O, Tacconelli E, Staub T, Greil R, Tsiodras S, Briel M, Esperou H, Mentre F, Eustace J, Saillard J, Delmas C, LeMestre S, Dumousseaux M, Costagliola D, Rottingen JA, Yazdanpanah Y. Accelerating clinical trial implementation in the context of the COVID-19 pandemic: challenges, lessons learned and recommendations from DisCoVeRy and the EU-SolidAct EU response group. *Clin Microbiol Infect* 2021;28:1-5.
[8] Giannuzzi V, Felisi M, Bonifazi D, Devlieger H, Papanikolaou G, Ragab L, et al. Ethical and procedural issues for applying researcher-driven multi-national paediatric clinical trials in and outside the European Union: the challenging experience of the DEEP project. BMC Med Ethics 2021;22:49.
[9] Magnin A, Iversen VC, Calvo G, Cecetkova B, Dale O, Demlova R, et al. European survey on national harmonization in clinical research. Learn Health Syst 2021;5:e10220.
[10] COMBACTE - Combatting antimicrobial resistance www.combacte.com/ (Last accessed August 7, 2022).
[11] ECRIN - European Clinical Research Infrastructure Network www.ecrin.org (Last accessed July 31, 2022).
[12] VACCELERATE-European Corona Vaccine Trial Accelerator Platform www.vaccelerate.eu (Last accessed August 1, 2022).
[13] Salmanton-García J, Stewart FA, Heringer S, Koniordou M, Alvarez-Barco E, Argyropoulos CD, et al. on behalf of the VACCELERATE Consortium. VACCELERATE Volunteer Registry: A European study participant database to facilitate clinical trial enrolment. Vaccine 2022;40:4090–407.
[14] VACCELERATE Site Network https://vaccelerate.eu/site-network/ (Last accessed August 2, 2022).
[15] clinicalsite.org by Healex GmbH https://healex.systems/ (Last accessed August 13, 2022).
[16] Grothe JH, Cornely OA, Salmanton-García J, on behalf of the VACCELERATE Consortium. Monkeypox in Children and Adult Women in Europe: Results from a Flash VACCELERATE Pilot Survey. *Enferm Infecc Microbiol Clin (Engl Ed)* (Accepted).
[17] Grothe JH, Cornely OA, Salmanton-García J, on behalf of the VACCELERATE Consortium. Monkeypox diagnostic and treatment capacity at epidemic onset A VACCELERATE online survey. *J Infect Public Health* 15:1043-46.
[18] Salmanton-García J, Hoenigl M, Gangneux J-P, Segal E, Alastruey-Izquierdo A, Arikan-Akdagli S, Lagrou K, Özenci V, Vena A, Cornely OA. The current state of laboratory mycology and access to antifungal treatment in Europe: A European Confederation of Medical Mycology survey. *Lancet Microbe* 2022;(Accepted).

7.17 Annex 17

Salmanton-García J, Stewart FA, Heringer S, Koniordou M, Álvarez-Barco E, Argyropoulos CD, Themistocleous SC, Valle-Simón P, Spivak O, Součková L, Merakou C, Amélia Mendonça M, Joanna Davis R, Maria Azzini A, Askling HH, Vene S, Van Damme P, Steinbach A, Shiamakkides G, Seidel D, Olesen OF, Noula E, Macken A, Luís C, Leckler J, Launay O, Isitt C, Hellemans M, Frías-Iniesta J, Di Marzo R, Carcas AJ, Boustras G, Borobia AM, Barta I, Albus K, Akova M, Ochando J, Cohen-Kandli M, Jane Cox R, Husa P, Jancoriene L, Mallon P, Marques L, Mellinghoff SC, Nauclér P, Tacconelli E, Tóth K, Zaoutis TE, Zeitlinger M, Cornely OA, Pana ZD;
VACCELERATE consortium. VACCELERATE Volunteer Registry: A European study participant database to facilitate clinical trial enrolment. Vaccine. 2022 Jul 29;40(31):4090-4097. doi: 10.1016/j.vaccine.2022.05.022. Epub 2022 Jun 2. PMID: 35659449; PMCID: PMC9159788.

Summary

Purpose:

The aim of this study was to develop and implement a pan-European volunteer registry to facilitate rapid and efficient recruitment of participants for clinical trials, particularly in the context of the COVID-19 pandemic. The study addresses a critical bottleneck in clinical research—identification and enrolment of suitable participants—which significantly limits the speed and feasibility of both commercial and NCCTs.

Methodology:

The project was designed as a centralised, digital, multicountry registry platform, integrated within the VACCELERATE infrastructure, allowing individuals across Europe to register their interest in participating in clinical trials. Volunteers provide standardized demographic and clinical data via an online questionnaire, and the system enables matching of participants to ongoing or planned trials based on predefined criteria, while complying with national legal and ethical requirements.

Results:

By mid-2022, the registry was implemented in 12 countries and available in multiple languages, with over 36,000 registered volunteers, demonstrating rapid scalability and high public engagement. Within the first year, more than 15,000 participants were successfully matched to clinical trials, confirming the functionality of the platform as an effective recruitment tool.

Conclusion:

The VACCELERATE Volunteer Registry represents a scalable and sustainable solution to improve recruitment efficiency in European clinical trials by providing a single-entry point for potential participants. The study highlights the importance of digital infrastructure and coordinated networks in overcoming fragmentation and significantly enhancing the feasibility and speed of NCCTs.

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VACCELERATE Volunteer Registry: A European study participant database to facilitate clinical trial enrolment

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ABSTRACT

Introduction: The coronavirus disease 2019 (COVID-19) pandemic has evidenced the key role of vaccine design, obtention, production and administration to successfully fight against infectious diseases and to provide efficient remedies for the citizens. Although clinical trials were rapidly established during this pandemic, identifying suitable study subjects can be challenging. For this reason, the University Hospital Cologne established a volunteer registry for participation in clinical trials first in Germany, which has now been incorporated into the European VACCELERATE clinical trials network and grew to a European Volunteer Registry. As such, VACCELERATE's Volunteer Registry aims to become a common entry point for potential volunteers in future clinical trials in Europe.

Methods: Interested volunteers who would like to register for clinical trials in the VACCELERATE Volunteer Registry can access the registration questionnaire via http://www.vaccelerate.eu/volunteer-registry. Potential volunteers are requested to provide their current country and area of residence, contact information, including first and last name and e-mail address, age, gender, comorbidities, previous SARS-CoV-2 infection and vaccination status, and maximum distance willing to travel to a clinical trial site. The registry is open to both adults and children, complying with national legal consent requirements.

Results: As of May 2022, the questionnaire is available in 12 countries and 14 languages. Up to date, more than 36,000 volunteers have registered, mainly from Germany. Within the first year since its establishment, the VACCELERATE Volunteer Registry has matched more than 15,000 volunteers to clinical trials. The VACCELERATE Volunteer Registry will be launched in further European countries in the coming months.

Conclusions: The VACCELERATE Volunteer Registry is an active single-entry point for European residents interested in COVID-19 clinical trials participation in 12 countries (i.e., Austria, Cyprus, Germany, Greece, Ireland, Lithuania, Norway, Portugal, Spain, Sweden and Turkey). To date, more than 15,000 registered individuals have been connected to clinical trials in Germany alone. The registry is currently in the implementation phase in 5 additional countries (i.e., Belgium, Czech Republic, Hungary, Israel and the Netherlands).

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1. Introduction

The first patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) associated pneumonia were described in Wuhan, central China, in December 2019. [1] After an exponential global expansion, coronavirus disease 2019 (COVID-19) was declared a pandemic 3 months later. [2] The SARS-CoV-2/COVID-19 pandemic has showcased the urgent need for ready-to-use public health tools to adequately face emerging epidemics and pandemics. [3–5] COVID-19 is the perfect example of the enormous impact of emerging global health threats caused by behavioural and climatic changes on our societies. [6].

The European vaccine development landscape is widely scattered, as well as difficult to access and to navigate. [7–10] Therefore, Europe was less attractive for vaccine developers than other regions in the world. [11–14] However, during recent years, advances have been made in order to implement European platforms for therapeutic clinical trials. [15–17] Moreover, the COVID-19 pandemic highlighted the need to bring all European residents closer to phase 2 and phase 3 COVID-19 clinical trials, for example through volunteer registries, [18,19] including healthy volunteers, patients with comorbidities, minorities and/or underrepresented populations. An easily accessible registry of well-characterised potential study volunteers can be a key tool for the early identification of suitable subjects in any phase 2 and phase 3 vaccine clinical trial.

The main goal of the VACCELERATE [20] Volunteer Registry is the implementation of an Europe-wide, dynamic, harmonised and sustainable single-entry volunteer registry for phase 2 and phase 3 clinical trials. While the focus is currently on COVID-19, the registry can be expanded to other indications for vaccine testing and adapted for future health emergencies under the mandate of the EU Health Emergency Preparedness and Response Authority (HERA) Incubator initiative. [21].

2. Overview of the VACCELERATE Volunteer Registry

The VACCELERATE Volunteer Registry (https://www.vaccelerate.eu/volunteer-registry) collects information on basic demographic details (first and last name, e-mail, gender, year of birth, area of residence and country), willingness to travel to a clinical trials site, COVID-19 infection prior to registration, vaccination status (number of doses, time of administration and manufacturer), as well as underlying conditions (adapted for adult and paediatric populations) (Table 1). Volunteers have to consent to data processing, storage and validation prior to submitting their personal data, in accordance with article 13 of the EU General Data Protection Regulation (GDPR). [22] In the case of minors, additional consent by the respective legal guardian(s) is requested, according to the respective national version with regards to local and national regulations. Online registration does not automatically mean participation in a clinical trial. Obtaining informed consent for clinical trial participation falls under the obligation of the respective clinical trial sponsor and/or its representative. Registration in the Volunteer Registry can be withdrawn at any time and without explanation, followed by deletion of the submitted data set. Once volunteers agree to the terms and conditions, their personal data are saved and incorporated into the database.

When a clinical trial becomes ready to enrol, potential study participants are identified and filtered according to the trial's key enrolment criteria. Herewith, potentially eligible candidates are briefly informed about the clinical trial, including contact details of the trial site closest to their area of residence, via e-mail. Interested volunteers will autonomously and independently decide whether they wish to contact the trial site to learn more about the clinical trial and if they wish to participate (Fig. 1).

(1) Potential participants may register via an online questionnaire available at https://www.vaccelerate.eu/volunteer-registry and data are stored. (2) Entities managing or performing clinical

Table 1
VACCELERATE Volunteer Registry - Survey Categories Captured.

ADULTS	CHILDREN
1. Personal data	
	Legal representative's first name
	Legal representative's last name
Volunteer's first name	
Volunteer's last name	
E-Mail	
Year of birth	
Gender (female, male, diverse)	
2. Distance willing to travel to study site (≤10 km, ≤25 km, ≤50 km, ≤100 km, >100 km)	
3. COVID-19 infection (Not infected, infected + month/year of diagnosis)	
4. Vaccination status ([un-]vaccinated [vaccine brand, number of doses and administration month and year])	
5. Pre-existing illnesses	
Cardiovascular diseases	
	Congenital heart defect
High blood pressure	
Coronary heart disease or history of heart attack	
Heart failure	
Lung / Liver / Kidney diseases	
Chronic hepatitis B or C	
	Asthma
	Polycystic kidney disease
	Renal malformation, double kidney
Asthma, COPD, chronic bronchitis or emphysema	
Chronic non-infectious liver disease, including liver cirrhosis	
Chronic kidney disease, including renal insufficiency	
Metabolic diseases	
Diabetes mellitus	
	Overweight
	Congenital metabolic disorder
	Cystic fibrosis
20 kg or more overweight	
Diseases with impairment of the immune system	
HIV	
Cancer currently being treated or having been treated in the last 2 years	
	Congenital immunodeficiencies
	Underlying rheumatological disease
Other pre-existing conditions	
Epilepsy	
Serious chronic illness of the stomach or intestine	
Serious disease of the musculoskeletal system	
Mental illness	
	Hypoxic brain damage
ADULTS	**CHILDREN**
	Failure to thrive
	Chromosomal anomalies (e.g., trisomy 21)
History of stroke	
Pregnancy and breastfeeding	
(Expected date of delivery and end of breastfeeding [month and year])	
Other illness (please specify)	
No pre-existing illness	

COPD, chronic obstructive pulmonary diseases; COVID-19, coronavirus diseases 2019; HIV, human immunodeficiency virus.

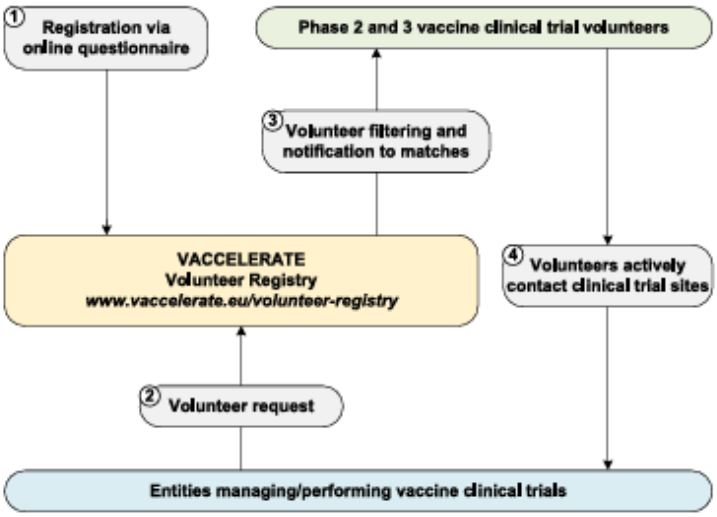


Fig. 1. Volunteer Registry Management.

trials might contact VACCELERATE Volunteer Registry with a participant request. (3) Potential participants are identified and filtered according to the trial's key enrolment criteria, and briefly informed about the clinical trial, including contact details of the trial site closest to their area of residence, via e-mail. (4) Interested volunteers can independently decide whether they contact the trial site to learn more about the clinical trial and if they wish to participate.

In order to determine the need for country-based registries, VACCELERATE National Coordinators (NC) provide information on existing (COVID-19) clinical trial registries in their countries. NCs are the main point of contact for member states reaching out to VACCELERATE, including translations and implementation of consortium activities in their respective countries.

Connecting the VACCELERATE Volunteer Registry with other established national registries is encouraged, for example through linking to these registries on the VACCELERATE website and sharing support requests from clinical trials sponsors. The VACCELERATE Volunteer Registry and established, independent national registries do not share any collected personal data. National versions of the VACCELERATE Volunteer Registry are established as needed upon request of the respective NC, and adjustments are made in terms of required languages (Table 2) and minor/adult cut-offs. While the VACCELERATE Volunteer Registry guarantees compliance with European legislation and requirements, NC may adapt their respective national version with regards to local and national regulations, with a particular focus on data protection and in coordination with local ethics committees.

2.1. Ethics and data protection

The VACCELERATE Volunteer Registry was approved by the Ethics Committee of the Medical Faculty of the University of Cologne (Cologne, Germany) (Study number 20–1536). If required, the corresponding local ethics committee of each participating country may also approve the VACCELERATE Volunteer Registry. Personal data are collected in accordance with article 13 of the EU General Data Protection Regulation (GDPR), [22] with no data transfer either within or outside the EU and no data are shared to any third party.

3. Registry progress and outlook

3.1. Participation

As of May 2022, the VACCELERATE Volunteer Registry is available in 12 countries and 13 languages (Arabic, English, German, Greek, Irish Gaelic, Italian, Lithuanian, Norwegian (Bokmål), Polish, Portuguese, Spanish, Swedish and Turkish). More than 36,000 volunteers from 12 European countries have registered in the VACCELERATE Volunteer Registry (Fig. 2). Among these, 35,443 volunteers (95.81%) have registered from Germany (the first registry, activated at the end of 2020, Fig. 3), 725 (2.0%) from Ireland, 155 (0.4%) from Cyprus, 130 (0.4%) from Austria, 74 (0.2%) from Greece, 50 (0.1%) from Spain, 41 (0.1%) from Sweden, 25 (0.1%) from Portugal, 22 (0.1%) from Norway, 14 (0.04%) from Turkey and 7 each (0.02%) from Italy and Lithuania, respectively. A total of 18,987 (51.3%) registered individuals identified as female, 17,602 (48.0%) as male, and 104 (0.3%) reported other gender identities. Volunteers were born between 1925 and 2022 (overall median age 38 years, adults (n = 32,717) 40 years old, children (n = 3,976) 9 years old). Most of the patients reported no underlying conditions prior to their inclusion in the registry (overall 58.7%, adults 56.7%, children 79.0%). Among the volunteers reporting pre-existing illnesses, cardiovascular diseases (n = 4,293, 11.6%), overweight (n = 3,356, 9.1%, lung diseases (n = 2,913, 7.9%), diabetes mellitus (n = 930, 2.5%), and acquired immunodeficiencies (n = 627, 1.7%) were the most commonly reported ones (Table 3). In less than one year from its launch, the Volunteer Registry was contacted more than 10 times to support identification of participants for clinical trials, with more than 15,000 volunteers matched to clinical trials in Germany alone. The VACCELERATE Volunteer Registry will be launched in further countries and languages during the coming months (Table 2, Fig. 2).

Table 2
VACCELERATE Volunteer Registry Country-Language Correlation.

Country Language	AT	BE*	CY	CZ*	DE	ES	GR	HU*	IE	IL*	IT	LT	NL*	NO	PT	SV	TR
Arabic					x					x							
Czech*				x													
Dutch*		x											x				
English	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
French*		x															
German	x				x						x						
Greek			x				x										
Hebrew*										x							
Hungarian*								x									
Irish Gaelic									x								
Italian					x						x						
Lithuanian												x					
Norwegian, Bokmål														x			
Polish					x												
Portuguese															x		
Russian*										x							
Spanish						x											
Swedish																x	
Turkish			x		x												x

* Country or language to be activated.
AT, Austria; BE, Belgium; CY, Cyprus; CZ, Czechia; DE, Germany; ES, Spain; GR, Greece; HU, Hungary; IE, Ireland; IL, Israel; IT, Italy; LT, Lithuania; NL, Netherlands; NO, Norway; PT, Portugal; SV, Sweden; TR, Turkey.

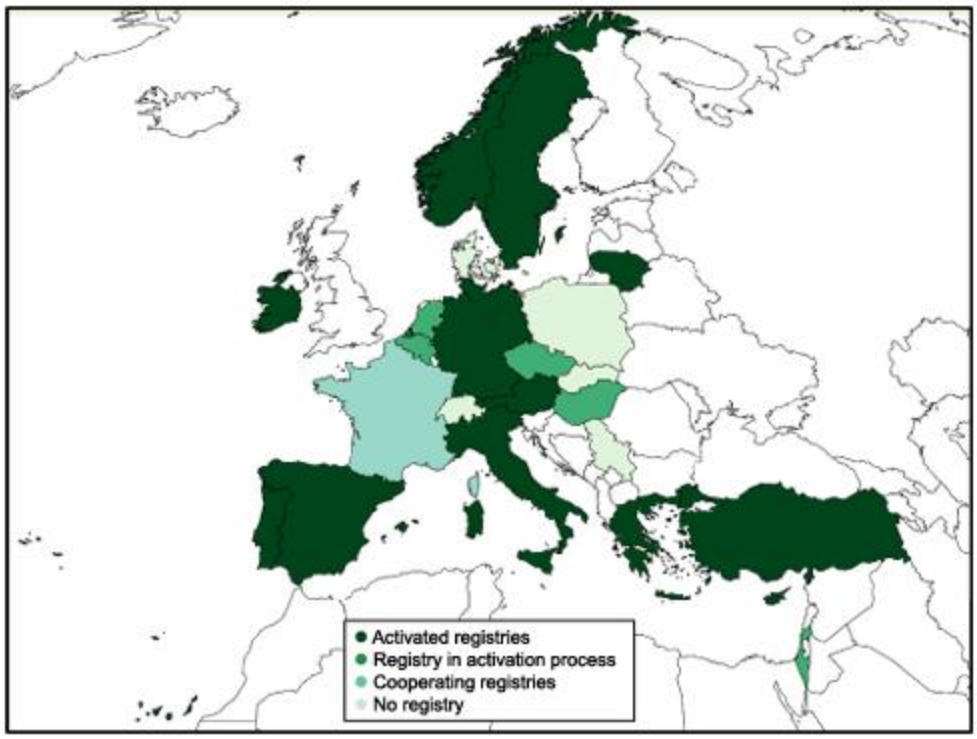


Fig. 2. Current Implementation of the VACCELERATE Volunteer Registry *Active registries: Austria, Cyprus, Germany, Greece, Ireland, Italy, Lithuania, Norway, Portugal, Spain, Sweden, and Turkey; Registries in activation process: Belgium, Czech Republic, Hungary, Israel, and the Netherlands; Cooperating registries: France; No registry: Denmark, Poland, Serbia, Slovakia, and Switzerland.*

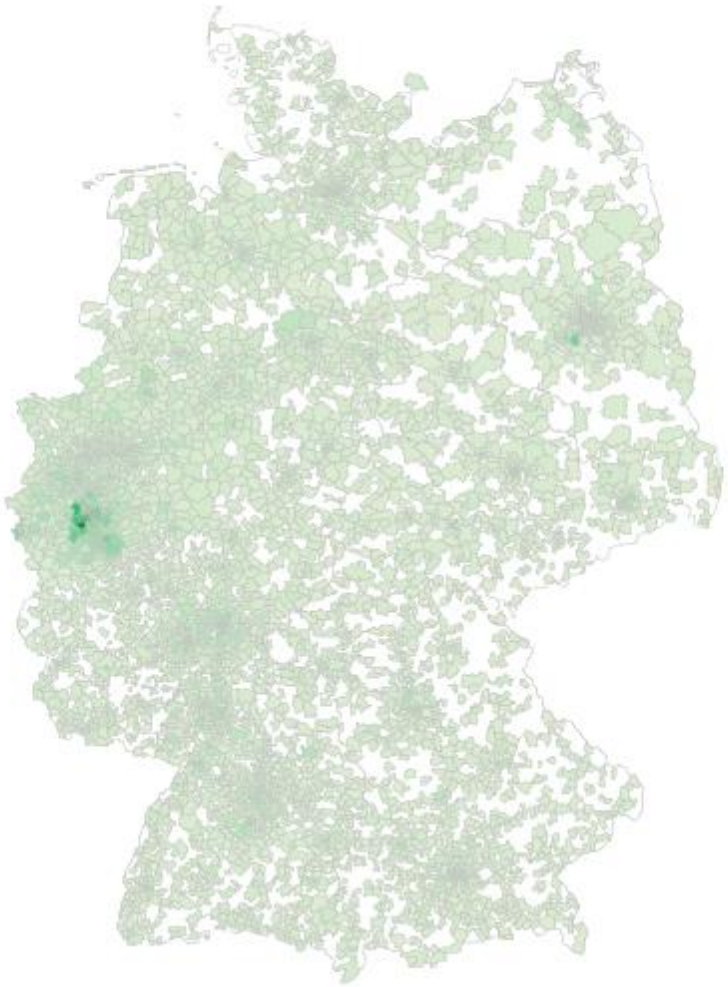


Fig. 3. *Current Geographical Distribution of Volunteers in Germany Heat map of the volunteer distribution in Germany. Coloured areas represent ZIP-code regions with at least one registered volunteer. The darker the area, the more registered volunteers there are. In grey areas, no volunteers are registered.*

3.2. Development of promotional and educational tools for volunteers in clinical trials

VACCELERATE is developing harmonised promotional materials for the Volunteer Registry that can be adapted according to country and language needs. Promotional and educational material targets various populations, such as children and the elderly as well as 'hard-to-reach (HTR)' populations that are largely underrepresented in clinical trials, like migrants or national minorities using languages different from the prevailing national language.

Promotional materials include brochures, content for social media and flyers. All materials will be freely available to the scientific community and industry, as well as to the general public via relevant traditional media (newspapers, radio stations, television channels), public health authorities, patient advocacy groups, scientific associations and societies, VACCELERATE social media channels (LinkedIn® and Twitter®), websites, and additional other stakeholder organisations both at a national (via NC) and pan-European level. NC will take the lead in advertising campaigns promoting national versions of the VACCELERATE Volunteer Registry, and with minor adaptations to match local requirements and needs. Lessons learned and best practice models of successful campaigns will be shared among NC.

Entities outside VACCELERATE were consulted to optimise promotional efforts. The European Patients' Academy on Therapeutic Innovation foundation (EUPATI) [23] was contacted to explore potential synergies, specifically with regards to patient engagement and promotion of the Volunteer Registry. Think Young, [24]

Table 3
Description of the VACCELERATE Volunteer Registry Participants.

	Children (n = 3,976)		Adults (n = 32,717)		Total (n = 36,693)	
	n	%	n	%	n	%
Country						
Germany	3897	98.0	31,546	96.4	35,443	96.6
Ireland	62	1.6	663	2.0	725	2.0
Cyprus	2	0.1	153	0.5	155	0.4
Austria	8	0.2	122	0.4	130	0.4
Greece	1	0.0	73	0.2	74	0.2
Spain	1	0.0	49	0.1	50	0.1
Sweden	0	0.0	41	0.1	41	0.1
Portugal	4	0.1	21	0.1	25	0.1
Norway	0	0.0	22	0.1	22	0.1
Turkey	0	0.0	14	0.0	14	0.0
Italy	1	0.0	6	0.0	7	0.0
Lithuania	0	0.0	7	0.0	7	0.0
Age (years), median (IQR) [range]	9 (5–14), [0–17]		40 (30–53), [18–96]		38 (26–52), [0–96]	
Children						
0–4	896	22.5	0	0.0	896	22.5
5–11	1654	41.6	0	0.0	1654	41.6
12–17	1426	35.9	0	0.0	1426	35.9
Adults						
18–29	0	0.0	7565	23.1	7565	23.1
30–39	0	0.0	8195	25.0	8195	25.0
40–49	0	0.0	6261	19.1	6261	19.1
50–59	0	0.0	6385	19.5	6385	19.5
60–69	0	0.0	3191	9.8	3191	9.8
70–79	0	0.0	970	3.0	970	3.0
80–89	0	0.0	135	0.4	135	0.4
≥ 90	0	0.0	15	0.0	15	0.0
Gender						
Female	1876	47.2	17,111	52.3	18,987	51.7
Male	2090	52.6	15,512	47.4	17,602	48.0
Diverse	10	0.3	94	0.3	104	0.3
Previous COVID-19 infection	69	1.7	1578	4.8	1647	4.5
Number of COVID-19 doses						
None reported	3888	97.8	26,649	81.5	30,537	83.2
At least 1	18	0.5	1253	3.8	1271	3.5
At least 2	60	1.5	3056	9.3	3116	8.5
At least 3	10	0.3	1686	5.2	1696	4.6
At least 4	0	0.0	73	0.2	73	0.2
Underlying conditions						
Cardiovascular diseases	2	0.1	4291	13.1		4293
Overweight	8	0.2	3348	10.2		3356
Lung diseases	165	4.1	2748	8.4		2913
Diabetes mellitus	17	0.4	913	2.8		930
Acquired immunodeficiencies	5	0.1	622	1.9		627
HIV	0	0.0	271	0.8		271
Cancer (active previous last 2 years)	5	0.1	358	1.1		363
Liver diseases	0	0.0	196	0.6		196
Chronic hepatitis B or C	0	0.0	68	0.2		68
Renal diseases	20	0.5	193	0.6		213
Epilepsy	32	0.8	172	0.5		204
Mental illness	61	1.5	1723	5.3		1784
Gastrointestinal illnesses	9	0.2	273	0.8		282
Musculoskeletal system illnesses	9	0.2	318	1.0		327
Other diseases	555	14.0	8024	24.5		8579
Current or expected breastfeeding	0	0.0	1265	3.9		1265
Pregnancy	0	0.0	385	1.2		385
History of stroke	0	0.0	178	0.5		178
Chromosomal anomalies (e.g., trisomy 21)	30	0.8	0	0.0		30
Failure to thrive	14	0.4	0	0.0		14
Underlying rheumatological disease	14	0.4	0	0.0		14
Congenital immunodeficiencies	8	0.2	0	0.0		8
Congenital metabolic disorder	6	0.2	0	0.0		6
Cystic fibrosis	4	0.1	0	0.0		4
Hypoxic brain damage	4	0.1	0	0.0		4
Other diseases	475	11.9	6581	20.1		7056
No pre-existing disease	3141	79.0	18,551	56.7	21,103	59.1

COVID-19, coronavirus disease 2019; HIV, human immunodeficiency virus; IQR, interquartile range.

a not-for-profit organisation (NFPO), was consulted with regards to a) approaches targeting adolescents and young adults, e.g. educational and informational material to minimise information gaps and increase knowledge and b) strategies to improve awareness of, provide access to, and improve quality of information on vaccination processes and participation in clinical trials for the general

public. Local entities were involved as needed to promote the VACCELERATE Volunteer Registry. [25] The European Patients Forum (EPF) was invited to share the perspective of EU patient advocacy groups and to discuss per-country requirements, challenges, and commonalities of participating in the Volunteer Registry. [26].

3.3. Volunteer Registry promotion among underserved/hard-to-reach groups

In order to overcome the traditional underrepresentation of underserved or HTR communities in clinical trials, such as subjects affected by various forms of immunosuppression both on an organic and iatrogenic basis, institutionalized elderly populations, pregnant women, or extreme age groups, understanding country-specific barriers must come first. Identifying the reasons for poor participation will aid to develop suitable methods and to increase access and engagement, while promoting the Volunteer Registry. The VACCELERATE Volunteer Registry group will investigate the access mechanisms of underserved/HTR groups to clinical trials participation, seeking out previous experiences by local authorities, NFPOs, and other relevant organisations.

4. Outlook

We aim for the VACCELERATE Volunteer Registry to become a powerful tool across Europe and act as a central hub for clinical trials, bringing together potential volunteers with entities managing and performing clinical trials, with the ultimate goal of fast-tracking the process of vaccine development and implementation at the pan-European level.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] World Health Organization. Pneumonia of unknown cause www.who.int/csr/don/05-january-2020-pneumonia-of-unkown-cause-china/en/ (Last accessed December 1, 2021).
[2] Mahase E. Covid-19: WHO declares pandemic because of "alarming levels" of spread, severity, and inaction. BMJ 2020;368:m1036.
[3] European Centre for Disease Prevention and Control. Why is pandemic preparedness planning important? www.ecdc.europa.eu/en/seasonal-influenza/preparedness/why-pandemic-preparedness (Last accessed December 3, 2021).
[4] Nature. Has COVID taught us anything about pandemic preparedness? www.nature.com/articles/d41586-021-02217-y (Last accessed November 29, 2021).
[5] Shapiro LI, Kajita GR, Arnsten JH, Tomer Y. Toward better preparedness for the next pandemic. J Clin Invest 2020;130:4543-45.
[6] Baker RE, Mahmud AS, Miller IF, Rajeev M, Rasambainarivo F, Rice BL, et al. Infectious disease in an era of global change. Nat Rev Microbiol 2022;20(4):193–205.
[7] Leroy O, Geels M, Korejwo J, Dodet B, Imbault N, Jungbluth S. Roadmap for the establishment of a European vaccine R&D infrastructure. Vaccine 2014;32:7021–4.
[8] E U-Response investigators group, Diallo A, Troseid M, Simensen VC, Boston A, Demotes J, et al. Accelerating clinical trial implementation in the context of the COVID-19 pandemic: challenges, lessons learned and recommendations from DisCoVeRy and the EU-SolidAct EU response group. Clin Microbiol Infect 2021;
[9] Giannuzzi V, Felisi M, Bonifazi D, Devlieger H, Papanikolaou G, Ragab L, et al. Ethical and procedural issues for applying researcher-driven multi-national paediatric clinical trials in and outside the European Union: the challenging experience of the DEEP project. BMC Med Ethics 2021;22(1). https://doi.org/10.1186/s12910-021-00618-2.
[10] Magnin A, Iversen VC, Calvo G, Čečetková B, Dale O, Demlová R, et al. European survey on national harmonization in clinical research. Learn Health Syst 2021;5(2). https://doi.org/10.1002/lrh2.v5.210.1002/lrh2.10220.
[11] Lythgoe MP, Middleton P. Comparison of COVID-19 Vaccine Approvals at the US Food and Drug Administration, European Medicines Agency, and Health Canada JAMA Netw Open 2021;4(6):e2114531. https://doi.org/10.1001/jamanetworkopen.2021.14531.
[12] Lankinen KS, Pastila S, Kilpi T, Nohynek H, Makela PH, Olin P. Vaccinovigilance in Europe–need for timeliness, standardization and resources. Bull World Health Organ 2004;82:828–35.
[13] Centers for Disease Control and Prevention. Vaccine Testing and the Approval Process www.cdc.gov/vaccines/basics/test-approve.html (Last accessed November 30, 2021).
[14] European Vaccination Information Portal. Approval of vaccines in the European Union www.vaccination-info.eu/en/vaccine-facts/approval-vaccines-european-union (Last accessed December 2, 2021).
[15] ECRIN - European Clinical Research Infrastructure Network www.ecrin.org (Last accessed December 2, 2021).
[16] VACCELERATE-EUVAP European Vaccine Trial Accelerator Platform www.euvap.eu (Last accessed December 1, 2021).
[17] COMBACTE - Combatting antimicrobial resistance www.combacte.com/ (Last accessed December 2, 2021).
[18] COVIREIVAC www.covireivac.fr (Last accessed December 1, 2021).
[19] Chaudhari N, Ravi R, Gogtay NithyaJ, Thatte UrmilaM. Recruitment and retention of the participants in clinical trials: Challenges and solutions. Perspect Clin Res 2020;11(2):64. https://doi.org/10.4103/picr.PICR_206_19.
[20] VACCELERATE-European Corona Vaccine Trial Accelerator Platform www.vaccelerate.eu (Last accessed December 1, 2021).
[21] European Commission. EU Health Emergency Preparedness and Response Authority (HERA) Incubator www.ec.europa.eu/commission/presscorner/detail/en/ip_21_4672 (Last accessed November 24, 2021).
[22] EU General Data Protection Regulation (GDPR) https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02016R0679-20160504&from=EN (Last accessed December 15, 2021).
[23] EUPATI-European Patients' Academy on Therapeutic Innovation www.eupati.eu (Last accessed December 2, 2021).
[24] Think Young www.thinkyoung.eu (Last accessed November 30, 2021).
[25] Vogazianos P, Argyropoulos CD, Haralambous C, Mikellidou CV, Boustras G, Andreou M, et al. Impact assessment of COVID-19 non-pharmaceutical interventions in long term care facilities in Cyprus: Safety improvement strategy. Saf Sci 2021;143:105415. https://doi.org/10.1016/j.ssci.2021.105415.
[26] EPF - European Patients' Forum www.eu-patient.eu (Last accessed November 30, 2021).

7.18 Annex 18

Argyropoulos CD, Leckler J, Salmanton-García J, Constantinou M, Alexandrou A, Themistocleous S, Noula E, Shiamakkides G, Nearchou A, Stewart FA, Albus K, Koniordou M, Kopsidas I, Spivak O, Hellemans M, Hendrickx G, Davis RJ, Azzini AM, Simon PV, Carcas-Sansuan AJ, Askling HH, Vene S, Prellezo JB, Álvarez-Barco E, Macken AJ, Di Marzo R, Luís C, Olesen OF, Frias Iniesta JA, Barta I, Tóth K, Akova M, Bonten MMJ, Cohen-Kandli M, Cox RJ, Součková L, Husa P, Jancoriene L, Launay O, Lundgren J, Mallon P, Armeftis C, Marques L, Naucler P, Ochando J, Tacconelli E, van Damme P, Zaoutis T, Hofstraat S, Bruijning-Verhagen P, Zeitlinger M, Cornely OA, Pana ZD
Enhancing Public Health Communication Regarding Vaccine Trials: Design and Development of the Pan-European VACCELERATE Toolkit
JMIR Public Health Surveill 2023;9:e44491
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Summary

Purpose:

The aim of this study was to design and develop a standardized communication toolkit to improve public understanding of vaccine clinical trials and to enhance recruitment through increased trust and awareness. The study addresses a critical barrier in clinical research—low participation driven by misinformation, vaccine hesitancy, and lack of accessible, reliable information.

Methodology:

The study employed a theory-based, multidisciplinary design approach, grounded in Social Cognitive Theory, to develop a set of harmonised educational and promotional tools tailored to different population groups, including underserved populations. The toolkit was created through a structured process involving content development, selection of trustworthy sources, adaptation to national contexts, and production of diverse materials (e.g., videos, brochures, digital content), ensuring accessibility, inclusiveness, and scalability across Europe.

Results:

The study resulted in the first pan-European, standardised set of communication tools for vaccine clinical trials, including educational cards, videos, brochures, and interactive materials. These tools were translated into multiple languages and designed to address key concerns such as safety, informed consent, and benefits/risks of participation, thereby improving public literacy and engagement with clinical research.

Conclusion:

The VACCELERATE communication toolkit represents an effective strategy to overcome communication barriers and improve recruitment into clinical trials by enhancing trust and understanding among the public. The study highlights the importance of structured, evidence-based communication as a core component of NCCTs infrastructure and demonstrates its role in increasing inclusivity and participation in European clinical research.

Original Paper

Enhancing Public Health Communication Regarding Vaccine Trials: Design and Development of the Pan-European VACCELERATE Toolkit

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Abstract

Background: The pan-European VACCELERATE network aims to implement the first transnational harmonized and sustainable vaccine trial Volunteer Registry, being a single entry point for potential volunteers of large-scale vaccine trials across Europe. This work exhibits a set of harmonized vaccine trial–related educational and promotional tools for the general public, designed and disseminated by the pan-European VACCELERATE network.

Objective: This study primarily aimed to design and develop a standard toolkit to increase positive attitudes and access to trustworthy information for better access and increased recruitment to vaccine trials for the public. More specifically, the produced tools are focused on inclusiveness and equity, and are targeting different population groups, including underserved ones, as potential volunteers for the VACCELERATE Volunteer Registry (older individuals, migrants, children, and adolescents). The promotional and educational material is aligned with the main objectives of the Volunteer Registry to increase public literacy and awareness regarding vaccine-related clinical research or trials and trial participation, including informed consent and legal issues, side effects, and frequently asked questions regarding vaccine trial design.

Methods: Tools were developed per the aims and principles of the VACCELERATE project, focusing on trial inclusiveness and equity, and are adjusted to local country-wise requirements to improve public health communication. The produced tools are selected based on the cognitive theory, inclusiveness, and equity of differently aged and underrepresented groups, and standardized material from several official trustworthy sources (eg, COVID-19 Vaccines Global Access; the European Centre for Disease Prevention and Control; the European Patients' Academy on Therapeutic Innovation; Gavi, the Vaccine Alliance; and the World Health Organization). A team of multidisciplinary specialists (infectious diseases, vaccine research, medicine, and education) edited and reviewed the subtitles and scripts of the educational videos, extended brochures, interactive cards, and puzzles. Graphic designers selected the color palette, audio settings, and dubbing for the video story-tales and implemented QR codes.

Results: This study presents the first set of harmonized promotional and educational materials and tools (ie, educational cards, educational and promotional videos, extended brochures, flyers, posters, and puzzles) for vaccine clinical research (eg, COVID-19 vaccines). These tools inform the public about possible benefits and disadvantages of trial participation and build confidence among participants about the safety and efficacy of COVID-19 vaccines and the health care system. This material has been translated into several languages and is intended to be freely and easily accessible to facilitate dissemination among VACCELERATE network participant countries and the European and global scientific, industrial, and public community.

Conclusions: The produced material could help fill knowledge gaps of health care personnel, providing the appropriate future patient education for vaccine trials, and tackling vaccine hesitancy and parents' concerns for potential participation of children in vaccine trials.

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KEYWORDS

vaccine trials; volunteer registry; educational material; promotional material; patient education; health communication; health promotion; public health; COVID-19; coronavirus; SARS-CoV-2; pandemic; vaccine; vaccination; hesitancy; campaign; misinformation

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Introduction

VACCELERATE [1] is an independent, innovative, and transparent Pan-European academic network with the aim of harmonizing multinational vaccine trial initiatives and conducting capacity mapping of vaccine clinical trials sites and laboratories with standardized methods and protocols across Europe. The network identifies and provides access to state-of-the-art vaccine trial sites to accelerate the development of vaccines and recruit volunteers for vaccine trials through the VACCELERATE Volunteer Registry [2].

The main goal of the VACCELERATE Volunteer Registry [2] is to establish the first transnational harmonized trial participation platform serving as single entry point for the European region. The VACCELERATE Volunteer Registry [2], under the mandate of the European Union's Health Emergency Preparedness and Response Authority (HERA) [3], provides a sustainable platform source for the recruitment of potential volunteers for clinical studies for the current COVID-19 pandemic and future epidemics.

According to the literature, one of the main challenges of a volunteer registry is the registration of a large number of potential trial participants with great diversity [4,5] and dedication in order to be able to recruit and match suitable candidates for the selected clinical trial. Therefore, a great amount of effort is spent looking for strategies to improve communication [6] and convince [7] potential volunteers to take part in vaccine trials.

The enrollment of volunteers in vaccine trials is also influenced by factors such as complacency, conspiracy theories [8] on social media regarding COVID-19, convenience and confidence (the "Three Cs" model of vaccine hesitancy) [7,9], disinformation [10], fake news [11], fear [12], and increasing level of health misinformation [13-15]. The latter leads to an infodemic [16] of misleading information with immediate effects on public health, refusal, and vaccine hesitancy [9] for participation in clinical trials [17,18]. It should be noted that health misinformation is responsible for increased fear and lack of trust, which are major factors for enrollment also in cancer clinical trials [19-21]. Recently, Luís et al [22] reported that there is a lack of public information about COVID-19 vaccine trials among several population groups (eg, minorities and underrepresented populations or individuals), while the available tools for trial participation were also sparse.

The main purpose of this study is the design and development of a standardized toolkit based on the Social Cognitive Theory [23,24] to increase positive attitudes, access to trustworthy information for better access and increased recruitment to vaccine trials. Our secondary objective is to foster engagement of the community through the VACCELERATE Volunteer Registry and smart technology for participating in forthcoming clinical trials [25-28]. The VACCELERATE toolkit has been translated into several languages and is freely and easily accessible to facilitate dissemination among the participating countries of the VACCELERATE Consortium [1].

Methods

The development of the VACCELERATE educational and promotional tools included the following steps: (1) conceptualization of the idea and context based on the selected educational aims and objectives; (2) selection of targeted population groups (eg, older individuals, children, adolescents, and minorities); (3) prioritization of trustworthy sources and websites for seeking information about COVID-19 vaccine trials for the developed materials; (4) performing graphical design (eg, visualization, audio, product, print, and animation design); and (5) acquisition of a digital object identifier (DOI) for each produced material from a DOI registration agency. More details on the developed tools are presented in the *Results* section.

The context of the educational and promotional tools was created and reviewed by a group of experts in the field of infectious diseases and vaccine and pedagogical research. Two graphic designers developed and selected the appropriate color palette and audio (music, speaker voice, and dubbing) for the video story-tales and they adapted the produced material into an efficient pedagogical, attractive, and eye-catching way. The developed tools were (1) icons related to the COVID-19 pandemic (eg, use of a face mask, syringe, microscope, vaccine, handwashing, virus, etc); (2) different versions of logotypes for the VACCELERATE project; (3) typefaces; (4) samples of fonts with different sizes; (5) color palettes (primary and secondary colors); (6) imagery templates and guidelines for social media (eg, Twitter and Instagram); (7) human characters (ie, medical staff, minorities, and older individuals) as prototypes for any future VACCELERATE promotional and or educational material. Special attention was also paid to foster inclusiveness among the characters (different age groups, different ethic and other minority groups, citizens with disabilities, migrants, etc). The provided tools also include QR codes with a URL. The selected URL can be a website (as shown on the VACCELERATE website [29]) or selected animation videos from trustworthy sources of information (eg, COVID-19 Vaccines Global Access [ie, COVAX]; the European Centre for Disease Prevention and Control; the European Patients' Academy on Therapeutic Innovation; the European Vaccine Initiative; Gavi, the Vaccine Alliance; the United Nations International Children's Emergency Fund; and the World Health Organization).

In addition, the produced puzzles test memory in a team-based manner and help develop hand-eye coordination.

Finally, the developed tools have been designed in a such a way that can be adjusted in accordance with local country-wise requirements and requirements to build confidence among participants about the safety and efficacy of COVID-19 vaccines and the health care system, which is a key parameter to improve recruitment among minority communities.

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Results

VACCELERATE Flyers

The flyers (see Multimedia Appendix 1) provide the appropriate amount of information to the public regarding the registration process and the VACCELERATE Volunteer Registry in general. The flyers are A3-sized (29.7×43.18 cm) and include a short description of the VACCELERATE Volunteer Registry objectives and volunteer selection process. In addition, they contain the appropriate contact details (email ID) and link (along with a QR code) for registration in the Volunteer Registry [30]. The registration link directs the interested volunteer to a brief survey (which takes 5-10 minutes to complete) that collects comprehensive information required for clinical trials. The flyer invites all citizens to register, including those with prior COVID-19 vaccination, with specific questions regarding COVID-19 infection, and the invitations to eligible volunteers for future vaccine trials will be sent out by the Coordination Office of VACCELERATE (University Hospital Cologne, Cologne, Germany). Finally, the flyers have been translated into 9 languages (Cypriot Greek, English, French, German, Greek, Hebrew, Italian, Portuguese, and Spanish).

VACCELERATE Posters

The developed posters (see Multimedia Appendix 1) are A0-sized (84.1×118.9 cm) and depict human characters (ie, medical staff, minorities, and older individuals, among others) and a QR code including a URL for the VACCELERATE Volunteer Registry website [30], as well as catchy slogans such as "Be the missing piece" and "Together we can tackle the COVID-19 pandemic." Finally, the posters have been translated into 7 languages, namely English, French, German, Greek, Hebrew, Italian, and Spanish, in collaboration with individual partners and national coordinators of the VACCELERATE Consortium. The promotion of the VACCELERATE Volunteer Registry for participating in COVID-19 vaccine trials could be achieved by affixing these prototype posters to a wall at sites or in buildings with high visibility (eg, hospitals, universities, malls, and public buildings).

VACCELERATE Extended Brochure (Booklet)

The extended VACCELERATE brochure (see Multimedia Appendix 1) comprises valuable information regarding the VACCELERATE Volunteer Registry, such as "What is the VACCELERATE Network," the mission and objectives, the current promotion activities, the importance of registration, and a description of the registration process. The brochure also contains key information for the volunteers, such as the possibility to withdraw their registration at any time. Finally, the extended brochure also includes a QR code that directs the interested reader to the VACCELERATE Volunteer Registry website [30] after scanning it with a smart device.

VACCELERATE Interactive Educational Cards

The educational cards enhance general knowledge and address the misinformation about COVID-19 vaccines, vaccine trials, and COVID-19 vaccination, as well as voluntary trial participation of children and adults. Each set of educational cards represents an educational aim and comprises a specific number of cards and target groups, as shown in Table 1.

The interactive educational cards (Multimedia Appendix 1) have been prepared with the help of 2 graphic designers and under the guidance of a pediatrician and several VACCELERATE participants. The educational cards include a QR code with a URL for promoting educational material on the one side and additional text script and characters on the other. The URL can be a website (eg, the one showed on the VACCELERATE website [29]) or selected videos from trustworthy sources (eg, COVID-19 Vaccines Global Access [ie, COVAX]; the European Centre for Disease Prevention and Control; the European Patients' Academy on Therapeutic Innovation; the European Vaccine Initiative; Gavi, the Vaccine Alliance; the United Nations International Children's Emergency Fund; and the World Health Organization) and produced animation videos from VACCELERATE.

Table 1. Characteristics of interactive educational cards.

Set	Cards, n	Target group	Aim
1	25	Individuals aged >12 years	Increase literacy about vaccine trials, trial registries, participation in vaccine trials, and participant procedures in trials
2	10	Individuals aged ≥12 years	Increase literacy about COVID-19 vaccination and messenger RNA vaccines
3	7	Individuals aged ≥12 years	Increase literacy about vaccine manufacturing, the vaccine approval process, safety monitoring, and awareness against vaccine misinformation
4	4	All individuals irrespective of age	Improve awareness and information about the VACCELERATE Volunteer Registry
5	12	All individuals irrespective of age	Increase literacy about COVID-19 vaccination, expand awareness for COVID-19 vaccine equity, and utilitarianism

VACCELERATE Puzzles

The VACCELERATE puzzles (Multimedia Appendix 1) were developed using graphic techniques under the guidance of the VACCELERATE participants, including a pediatrician, with focus on younger children (aged <12 years) in order to raise their awareness of clinical trials, vaccines, and participation in trials and to promote inclusiveness. The link to the produced puzzles is provided in Multimedia Appendix 1.

One of the developed puzzles comprises 25 pieces and depicts a variety of human characters (ie, medical staff, minorities, older individuals, and children, among others) obtained from the VACCELERATE toolkit, including the appropriate link to the VACCELERATE Volunteer Registry [30]. The concept herein

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is to include as many human characters as possible to highlight the importance of inclusiveness and diversity in vaccine trials, especially for underserved population groups such as racial and ethnic minorities and older individuals.

The other 2 smaller puzzles (8 pieces each) were developed to familiarize children with symbols and icons related to the COVID-19 pandemic, as well as to understand and recognize the effectiveness of vaccines in minimizing the risk of infection and harmful effects of COVID-19.

VACCELERATE Animation Videos

Educational Videos (Adult and Pediatric)

The first produced educational video (see Multimedia Appendix 1) was mainly focused on adults and designed using advanced animation techniques, appropriate subtitles based on a storyboard and educational aims, and audio settings (eg, music and a speaker voice). In addition, the video included the following sections: (1) highlight and increase information regarding clinical trials and their necessity and usefulness for public health (eg, good clinical practices, vaccine trial phases and monitoring, and applied safety protocols); (2) the contribution of volunteers (ie, citizens, patient advocacy groups, and underserved populations) to this effort for capacity mapping and building of registries; and (3) volunteer safety, benefits, and any potential risks during a clinical trial.

The second educational video (see Multimedia Appendix 1) was mainly aimed at children and adolescents. Special attention was given to contain informative animations while delivering the learning aim and keeping each video segment short. The produced video included the following sections: (1) provide information about the significance of vaccine trials; (2) share knowledge and information about the high value of vaccination as the most powerful "weapon" to prevent morbidity and mortality associated with infectious diseases; (3) acknowledge the advancements in technology and safety procedures, which have contributed significantly to the development, evaluation, approval, and monitoring of safe and effective vaccines; and (4) highlight the contribution and importance of pediatric volunteers in vaccine clinical trials and ensure their safety and the benefits and risks of participating in clinical trials.

Promotional Video

The promotional video (see Multimedia Appendix 1) presents short, recorded videos by the VACCELERATE work package leaders and the National Coordinator's pictures to promote, inform, and describe the VACCELERATE Volunteer Registry through specific text scripts. The aim of the video is the inclusiveness and representation of all participating European Union countries and European Union–associated countries of the VACCELERATE Consortium. Currently, we have active representation from 17 countries (Austria, Belgium, Cyprus, Czech Republic, Germany, Greece, Ireland, Israel, Italy, Hungary, Lithuania, the Netherlands, Norway, Portugal, Spain, Sweden, and Turkey) in the VACCELERATE Volunteer Registry.

Discussion

Principal Findings

This paper presents the first set of harmonized promotional and educational tools of the VACCELERATE Volunteer Registry [2]. The produced material, based on the selected population group, includes flyers, posters, interactive educational cards, puzzles, and animation videos. The tools can be downloaded from the official website of VACCELERATE [29] and appropriate references, based on their own DOIs. It should be noted that the provided educational material can be adopted to the user needs and may also be used as base-prototypes for further improvement and production of related tools.

To ensure high rates of vaccination in populations [31] and a large number of participants in vaccine trials, it is necessary to ensure effective, targeted, and trustworthy educational and promotional tools regarding vaccines and vaccine trials, guaranteeing high diversity and inclusiveness in vaccine trials without underrepresentation of minorities.

The developed standardized toolkit is one example of information from trustful sources that can be used against health misinformation and disinformation, lack of trust, fear, and fake news. Furthermore, it can be also used to increase literacy and understanding of the importance of vaccine clinical trials in the general population as part of an emergency preparedness plan.

In addition, the promotional and educational material will be able to bridge the gap in public information (eg, communication approach, target audiences, and type of media produced) regarding COVID-19 vaccine trials, which is covered in only 2.51% of the collected media and only in 4 languages (English, French, German, and Czech) [22]. Another challenge is using visual material and stories in order to inform children about the procedures, importance of, and volunteer participation in vaccine trials and about vaccination.

Limitations

The impact and assessment of the promotional and educational material are beyond the scope of this study. Nevertheless, we are currently in the process of evaluating them for vaccine trials during this year by another expert group (WP4) of the VACCELERATE Consortium.

Conclusions

Communicating with trustworthy information to the public, related to public health issues and vaccine research or trials, constitutes the most important preventive strategy against infectious diseases and threats of future emerging infection diseases.

Future initiatives could involve further improvement of the dissemination process for the developed tools in order to increase the number of volunteer registrations and in particular among population groups, which remain underrepresented in vaccine trials. Another initiative could be the design of an innovative video game [31,32] targeting training and educational material for vaccine clinical trials, addressing vaccine hesitancy and refusal.

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Other prospective initiatives could be the conduct of studies based on citizen science methods (eg, community-based participatory action research [28]) for vaccine research [25-27,33] and future epidemics by engaging community members and service providers as partners in the research process and providing them the appropriate educational tools for tackling current and future public health issues. Such actions will eventually contribute to reduce the knowledge gaps and hesitancy regarding participation in vaccine trials in the general population.

Acknowledgments

This project has received funding from the European Union's Horizon 2020 research and innovation program (grant agreement 101037867; VACCELERATE). The produced educational and promotional tools were developed as part of the VACCELERATE Consortium. For further information, please refer to the official website of the VACCELERATE network [29].

The research leading to these results was conducted as part of the VACCELERATE consortium. For further information please refer to [29].

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information file (Multimedia Appendix 1).

Conflicts of Interest

JS-G has received speaker honoraria from Gilead and Pfizer. The rest of the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Multimedia Appendix 1

Information on the promotional and educational material including DOI number, format, title, and language.
[DOCX File , 28 KB-Multimedia Appendix 1]

References

1. VACCELERATE - European Corona Vaccine Trial Accelerator Platform. European Commission. CORDIS EU Research Results. URL: https://cordis.europa.eu/project/id/101037867 [accessed 2023-03-24]
2. Salmanton-García J, Stewart FA, Heringer S, Koniordou M, Álvarez-Barco E, Argyropoulos CD, VACCELERATE consortium. VACCELERATE Volunteer Registry: a European study participant database to facilitate clinical trial enrolment. Vaccine 2022 Jul 29;40(31):4090-4097 [FREE Full text] [doi: 10.1016/j.vaccine.2022.05.022] [Medline: 35659449]
3. Health Emergency Preparedness and Response (HERA). European Commission. URL: https://health.ec.europa.eu/health-emergency-preparedness-and-response-hera_en [accessed 2023-03-24]
4. McCarthy CR. Historical background of clinical trials involving women and minorities. Acad Med 1994 Sep;69(9):695-698. [doi: 10.1097/00001888-199409000-00002] [Medline: 8074757]
5. Jaklevic MC. Researchers strive to recruit hard-hit minorities into COVID-19 vaccine trials. JAMA 2020 Sep 01;324(9):826-828. [doi: 10.1001/jama.2020.11244] [Medline: 32789501]
6. Langford AT. Health communication and decision making about vaccine clinical trials during a pandemic. J Health Commun 2020 Oct 02;25(10):780-789 [FREE Full text] [doi: 10.1080/10810730.2020.1864520] [Medline: 33719877]
7. Finset A, Bosworth H, Butow P, Gulbrandsen P, Hulsman RL, Pieterse AH, et al. Effective health communication - a key factor in fighting the COVID-19 pandemic. Patient Educ Couns 2020 May;103(5):873-876 [FREE Full text] [doi: 10.1016/j.pec.2020.03.027] [Medline: 32336348]
8. Earnshaw V, Eaton L, Kalichman S, Brousseau N, Hill E, Fox A. COVID-19 conspiracy beliefs, health behaviors, and policy support. Transl Behav Med 2020 Oct 08;10(4):850-856 [FREE Full text] [doi: 10.1093/tbm/ibaa090] [Medline: 32910819]
9. MacDonald N, SAGE Working Group on Vaccine Hesitancy. Vaccine hesitancy: Definition, scope and determinants. Vaccine 2015 Aug 14;33(34):4161-4164 [FREE Full text] [doi: 10.1016/j.vaccine.2015.04.036] [Medline: 25896383]
10. Gottlieb M, Dyer S. Information and disinformation: social media in the COVID-19 crisis. Acad Emerg Med 2020 Jul 24;27(7):640-641 [FREE Full text] [doi: 10.1111/acem.14036] [Medline: 32474977]
11. Naeem SB, Bhatti R, Khan A. An exploration of how fake news is taking over social media and putting public health at risk. Health Info Libr J 2021 Jun 12;38(2):143-149 [FREE Full text] [doi: 10.1111/hir.12320] [Medline: 32657000]
12. Tannenbaum MB, Hepler J, Zimmerman RS, Saul L, Jacobs S, Wilson K, et al. Appealing to fear: A meta-analysis of fear appeal effectiveness and theories. Psychol Bull 2015 Nov;141(6):1178-1204 [FREE Full text] [doi: 10.1037/a0039729] [Medline: 26501228]

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13. Gisondi MA, Barber R, Faust JS, Raja A, Strehlow MC, Westafer LM, et al. A deadly infodemic: social media and the power of COVID-19 misinformation. J Med Internet Res 2022 Feb 01;24(2):e35552 [FREE Full text] [doi: 10.2196/35552] [Medline: 35007204]
14. Suarez-Lledo V, Alvarez-Galvez J. Prevalence of health misinformation on social media: systematic review. J Med Internet Res 2021 Jan 20;23(1):e17187 [FREE Full text] [doi: 10.2196/17187] [Medline: 33470931]
15. Chou WS, Oh A, Klein WMP. Addressing health-related misinformation on social media. JAMA 2018 Dec 18;320(23):2417-2418. [doi: 10.1001/jama.2018.16865] [Medline: 30428002]
16. Infodemic. World Health Organization. URL: https://www.who.int/health-topics/infodemic [accessed 2023-03-24]
17. Lee SK, Sun J, Jang S, Connelly S. Misinformation of COVID-19 vaccines and vaccine hesitancy. Sci Rep 2022 Aug 11;12(1):13681 [FREE Full text] [doi: 10.1038/s41598-022-17430-6] [Medline: 35953500]
18. Surajit B. Vaccine hesitancy and misinformation about clinical trials. Indian J Surg 2021 Jun 24;83(3):615-616 [FREE Full text] [doi: 10.1007/s12262-021-03011-w] [Medline: 34188367]
19. Ginossar T, Diaz Fuentes C, Oetzel J. Understanding willingness to participate in cancer clinical trials among patients and caregivers attending a minority-serving academic cancer center. J Cancer Educ 2022 Feb 14;37(1):179-187. [doi: 10.1007/s13187-020-01802-5] [Medline: 32666504]
20. Habr D, Ferdinand R. Addressing racial/ethnic disparities in cancer clinical trials: everyone has a role to play. Cancer 2021 Sep 15;127(18):3282-3289 [FREE Full text] [doi: 10.1002/cncr.33600] [Medline: 33904590]
21. Jennings W, Stoker G, Bunting H, Valgarðsson VO, Gaskell J, Devine D, et al. Lack of trust, conspiracy beliefs, and social media use predict COVID-19 vaccine hesitancy. Vaccines (Basel) 2021 Jun 03;9(6):593 [FREE Full text] [doi: 10.3390/vaccines9060593] [Medline: 34204971]
22. Luís C, Di Marzo VR, Kaur M, Argyropoulos C, Devane D, Stewart FA, et al. Vaccination rates in Europe are not associated with online media intensity. JCOM 2022;21(05):A05 [FREE Full text] [doi: 10.22323/2.21050205]
23. DiClemente R, Crosby R, Kegler M. Understanding and applying theory in health promotion practice and research. In: Emerging theories in health promotion practice and research strategies for improving public health. San Francisco, CA: Jossey-Bass; 2002.
24. Bandura A. Social cognitive theory: an agentic perspective. Annu Rev Psychol 2001;52:1-26. [doi: 10.1146/annurev.psych.52.1.1] [Medline: 11148297]
25. Beatty AL, Peyser ND, Butcher XE, Carton TW, Olgin JE, Pletcher MJ, et al. The COVID-19 Citizen Science Study: protocol for a longitudinal digital health cohort study. JMIR Res Protoc 2021 Aug 30;10(8):e28169 [FREE Full text] [doi: 10.2196/28169] [Medline: 34310336]
26. Marcus GM, Olgin JE, Peyser ND, Vittinghoff E, Yang V, Joyce S, et al. Predictors of incident viral symptoms ascertained in the era of COVID-19. PLoS One 2021 Jun 17;16(6):e0253120 [FREE Full text] [doi: 10.1371/journal.pone.0253120] [Medline: 34138915]
27. Paul K, Palfinger T. Walking the (argumentative) talk using citizen science: involving young people in a critical policy analysis of vaccination policy in Austria. Evid Policy 2020;16(2):229-247. [doi: 10.1332/174426419X15752578285791]
28. Hills M, Mullett J, Carroll S. Community-based participatory action research: transforming multidisciplinary practice in primary health care. Rev Panam Salud Publica 2007;21(2-3):125-135. [doi: 10.1590/s1020-49892007000200007] [Medline: 17565799]
29. VACCELERATE Official Website. URL: https://vaccelerate.eu/ [accessed 2023-03-24]
30. Volunteer-Registry. Vaccelerate. URL: https://vaccelerate.eu/volunteer-registry-2/ [accessed 2023-03-24]
31. Ohannessian R, Yaghobian S, Verger P, Vanhems P. A systematic review of serious video games used for vaccination. Vaccine 2016 Aug 31;34(38):4478-4483 [FREE Full text] [doi: 10.1016/j.vaccine.2016.07.048] [Medline: 27498211]
32. Ricciardi F, De Paolis LT. A comprehensive review of serious games in health professions. Int J Comput Games Technol 2014;2014:1-11. [doi: 10.1155/2014/787968]
33. Lin A, Vittinghoff E, Olgin J, Peyser N, Aung S, Joyce S, et al. Predictors of incident SARS-CoV-2 infections in an international prospective cohort study. BMJ Open 2021 Sep 21;11(9):e052025 [FREE Full text] [doi: 10.1136/bmjopen-2021-052025] [Medline: 34548363]

Abbreviations

DOI: Digital Object Identifier
ECDC: European Centre for Disease Prevention and Control
HERA: Health Emergency Preparedness and Response Authority

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7.19 Annex 19

Motovska Z, Hlinomaz O, Mrozek J, Kala P, Geisler T, Hromadka M, Akin I, Precek J, Kettner J, Cervinka P, Montalescot G, Jarkovsky J, Belohlavek J, Bis J, Matejka J, Vodzinska A, Muzafarova T, Tomasov P, Schee A, Bartus S, Andrasova A, Olivier CB, Kovarik A, Ostadal P, Demlova R, Souckova L, Vulev I, Coufal Z, Kochman J, Marinov I, Kubica J, Ducrocq G, Karpisek M, Klimsa Z, Hudec M, Widimsky P, Bhatt DL, Group DS. Cangrelor versus crushed ticagrelor in patients with acute myocardial infarction and cardiogenic shock: rationale and design of the randomised, double-blind DAPT-SHOCK-AMI trial. EuroIntervention. 2024 Oct 21;20(20):e1309-e1318. doi: 10.4244/EIJ-D-24-00203. PMID: 39432252; PMCID: PMC11472137.

Summary
Purpose:
The aim of this study is to evaluate the optimal antiplatelet strategy in patients with acute myocardial infarction complicated by cardiogenic shock, a population with extremely high mortality and limited evidence-based therapeutic guidance. The study addresses the uncertainty regarding the efficacy and safety of intravenous versus oral P2Y12 inhibition in this critically ill setting.

Methodology:
The study is designed as a multicentre, randomized, double-blind, placebo-controlled clinical trial comparing intravenous cangrelor with crushed oral ticagrelor in approximately 550 patients across multiple European countries. The trial includes both clinical endpoints (e.g., mortality, myocardial infarction, stroke) and mechanistic laboratory endpoints assessing platelet inhibition and pharmacodynamic response.

Results:
As a study protocol, the publication primarily defines a comprehensive methodological framework, including clearly specified primary and secondary endpoints and detailed trial procedures. It highlights expected challenges such as altered pharmacokinetics in cardiogenic shock and the need for rapid and reliable platelet inhibition in this population.

Conclusion:
The DAPT-SHOCK-AMI trial represents the first randomized effort to directly compare antiplatelet strategies in cardiogenic shock patients and aims to generate evidence to guide clinical practice in this high-risk group. The study exemplifies how investigator-initiated trials can address critical therapeutic gaps in acute cardiovascular care.

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TRIAL DESIGN

Cangrelor versus crushed ticagrelor in patients with acute myocardial infarction and cardiogenic shock: rationale and design of the randomised, double-blind DAPT-SHOCK-AMI trial

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This paper also includes supplementary data published online at: https://eurointervention.pcronline.com/doi/10.4244/EIJ-D-24-00203

ABSTRACT

Cardiogenic shock (CS) is a devastating and fatal complication of acute myocardial infarction (AMI). CS can affect the pharmacokinetics and pharmacodynamics of medications. The unique properties of cangrelor make it the optimal $P2Y_{12}$ inhibitor for CS-AMI, in terms of both efficacy and safety. The DAPT-SHOCK-AMI trial (ClinicalTrials.gov: NCT03551964; EudraCT: 2018-002161-19) will assess the benefits of cangrelor in patients with an initial CS-AMI undergoing primary angioplasty. This randomised, multicentre, placebo-controlled trial of approximately 550 patients (with an allowed 10% increase) in 5 countries using a double-blind design will compare initial $P2Y_{12}$ inhibitor treatment strategies in patients with CS-AMI of (A) intravenous cangrelor and (B) ticagrelor administered as crushed tablets at a loading dose of 180 mg. The primary clinical endpoint is a composite of all-cause death, myocardial infarction (MI), or stroke within 30 days. The main secondary endpoints are (1) the net clinical endpoint, defined as death, MI, urgent revascularisation of the infarct-related artery, stroke, or major bleeding as defined by the Bleeding Academic Research Consortium criteria; (2) cardiovascular-related death, MI, urgent revascularisation, or heart failure; (3) heart failure; and (4) cardiovascular-related death, all (1-4) within 1 year after study enrolment. A platelet reactivity study that tests the laboratory antiplatelet benefits of cangrelor, when given in addition to standard antiplatelet therapy, will be conducted using vasodilator-stimulated phosphoprotein phosphorylation. The primary laboratory endpoints are the periprocedural rate of onset and the proportion of patients who achieve effective $P2Y_{12}$ inhibition. The DAPT-SHOCK-AMI study is the first randomised trial to evaluate the benefits of cangrelor in patients with CS-AMI.

KEYWORDS: adjunctive pharmacotherapy; cardiogenic shock; clinical trials; STEMI

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Introduction

MAGNITUDE OF THE ISSUE

The estimated global annual incidence of acute myocardial infarction (AMI) exceeds 7 million[1]. The average incidence of cardiogenic shock (CS) in patients hospitalised for AMI (CS-AMI) is approximately 7.5%[2]. Most CS-AMI cases have ST-segment elevation MI (STEMI)[3]. An analysis based on extensive US population data (from 1,000 hospitals) documented 44 CS-AMI cases per 100,000 hospitalisations in 2004 and 103 per 100,000 hospitalisations in 2018[3].

The incidence of CS developing during hospitalisation has been decreasing (currently 3.5%), whereas the incidence of initial (primary) shock has been stable or increasing (currently 4.6%)[4,5]. Furthermore, there has been an upward trend in preadmission cardiac arrests in patients with CS-AMI[6].

PHARMACOTHERAPY-RELATED SPECIFICS

CS can affect all aspects of drug pharmacokinetics and pharmacodynamics[7,8]. The splanchnic circulation is hypoperfused because of reflexive vasoconstriction, which is further potentiated during treatment with vasopressor agents – the consequent ischaemia results in liver and kidney dysfunction. The preferred mode of drug administration is parenteral, and drugs that do not undergo metabolism are favoured.

In patients with CS-AMI, metabolism becomes less predictable, which can result in potentially serious adverse events due to overexposure or underexposure to active ingredients[9]. Antithrombotic medications are associated with a risk of bleeding; therefore, drugs with a rapid offset of action and antidotes are particularly beneficial.

EVIDENCE FOR SURVIVAL BENEFIT

Despite advances in medical treatment, reperfusion using primary percutaneous coronary intervention (PCI) remains the only intervention that improves the prognosis of patients with CS-AMI[2,10,11]. An essential component of mechanical reperfusion in AMI is adjuvant antithrombotic pharmacotherapy, which is critical in preventing local thrombus progression and distant embolisation.

ANTIPLATELET THERAPY

Dual antiplatelet therapy using the newer oral $P2Y_{12}$ receptor inhibitors ($iP2Y_{12}$), i.e., ticagrelor or prasugrel, in combination with aspirin, is recommended in patients with AMI undergoing PCI based on the results of the TRITON-TIMI 38 (A Comparison of Prasugrel and Clopidogrel in Acute Coronary Syndrome Subjects Who Are to Undergo Percutaneous Coronary Intervention)[12] and PLATO (A Comparison of Ticagrelor and Clopidogrel in Patients With Acute Coronary Syndrome)[13] randomised trials. However, CS was an exclusion criterion in the TRITON study, and in the ticagrelor arm of the PLATO study, only 25 patients (0.7%) suffered from CS.

STUDY JUSTIFICATION

CS is the most common cause of death in patients with AMI who survive until hospital admission[2]. The in-hospital mortality rate of patients suffering from primary CS at admission is 44.4%[3], and 20% of the deaths occur during the initial PCI procedure[14]. Characteristics including older age, multivessel coronary artery disease, increased time between symptom onset and reperfusion, and postprocedural Thrombolysis in Myocardial Infarction (TIMI) flow <3 were identified as independent predictors of death in patients with CS-AMI[15]. Rapid and effective platelet inhibition can modify two of the prognosis predictors in CS patients with the highest thrombotic risk, i.e., time until reperfusion and angioplasty outcomes **(Figure 1)**.

The efficient inhibition of platelet aggregation is essential for preventing ischaemic events. The short-term risk of reinfarction is 3-4 times higher (9-12%)[3] in patients with CS. Additionally, CS is the strongest independent predictor of stent thrombosis[16]. Ischaemic stroke occurs in 2.4% of patients with CS; this number rises for more invasive circulatory stabilisation methods[17].

Patients with CS-AMI are also at risk of severe bleeding. Typically, bleeding occurs in 1 out of 5 CS-AMI cases during early hospitalisation[18].

Cangrelor is the best studied $iP2Y_{12}$ with a parenteral mode of application **(Table 1)**. The effective inhibition of adenosine diphosphate (ADP)-induced platelet aggregation occurs 2 minutes after initiating treatment, and the antiplatelet effect is maintained throughout the infusion period[19]. Cangrelor metabolism is independent of splanchnic organ function and does not affect liver enzyme-metabolised drugs. Platelet aggregation is restored approximately 60 minutes after stopping the administration of the drug[19].

Cangrelor therapy initiated concomitantly with crushed ticagrelor tablets in patients with STEMI undergoing primary PCI results in prompt and potent platelet inhibition during the intervention; additionally, cangrelor therapy bridges the gap until the full antiplatelet efficacy of ticagrelor is achieved[20].

The unique pharmacokinetic and pharmacodynamic properties of cangrelor make it the optimal $iP2Y_{12}$ for CS-AMI in terms of efficacy and safety. The degree to which $iP2Y_{12}$ suppresses ADP-mediated platelet function depends on the potency of the antiplatelet drug and the baseline (before treatment) prothrombotic condition; this is most pronounced in STEMI patients. A better understanding of the relationship between study medication-related platelet reactivity inhibition (through quantification of the rate of onset and intensity of inhibition during the peri-PCI period) and clinical outcomes in one trial may contribute to developing more effective and safer treatment strategies[21].

Abbreviations

AMI	acute myocardial infarction	**$iP2Y_{12}$**	$P2Y_{12}$ receptor inhibitors
CS	cardiogenic shock	**PCI**	percutaneous coronary intervention
DAPT-SHOCK-AMI	Dual Antiplatelet Therapy For Shock Patients With Acute Myocardial Infarction	**VASP**	vasodilator-stimulated phosphoprotein

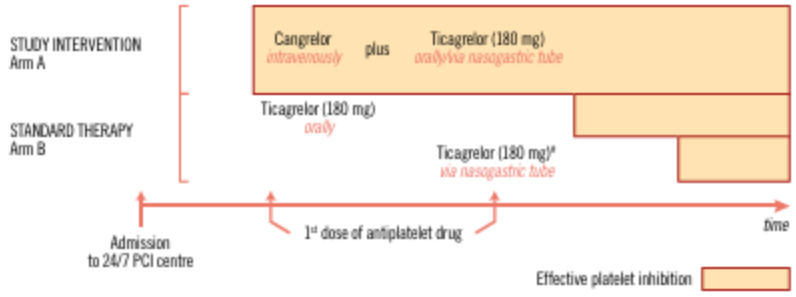


Figure 1. *Benefits of the tested study therapy (study rationale). The first arrow indicates CS-AMI patients' admission to a 24/7 PCI centre, usually directly to the catheterisation laboratory. The upper part of the figure shows patients randomised to cangrelor IV (arm A) achieving effective platelet inhibition immediately after initiation of therapy (large orange bar), irrespective of conscious state and oral intake ability. The lower part shows patients assigned to arm B and treated with ticagrelor. Patients who are able to take ticagrelor orally (second arrow) achieve effective platelet inhibition after hours (medium orange bar). Patients with impaired consciousness cannot receive ticagrelor until after introducing the nasogastric tube (often after arriving at the catheterisation laboratory; third arrow), which represents a further delay in the onset of ticagrelor antiplatelet efficacy (small orange bar). The onset of effective inhibition of platelet reactivity (expressed by colour fill) depends on the antiplatelet drug administered. *Patients with impaired consciousness. CS-AMI: cardiogenic shock complicated acute myocardial infarction; IV: intravenous; PCI: percutaneous coronary intervention*

Table 1. $P2Y_{12}$ receptor inhibitors.

Drug	Structure	Effect	Reversibility	Method of use	Frequency of use
Ticlopidine	Thienopyridine	Indirect	No	Oral	BID
Clopidogrel	Thienopyridine	Indirect	No	Oral	QD
Prasugrel	Thienopyridine	Indirect	No	Oral	QD
Ticagrelor	ATP analogue	Direct	Yes (half-life 6-12 hr)	Oral	BID
Cangrelor	ATP analogue	Direct	Yes (half-life 3 min)	Parenteral	Continuous infusion

ATP: adenosine triphosphate; BID: twice a day; QD: once a day

Study design

STUDY OBJECTIVES

The Dual Antiplatelet Therapy For Shock Patients With Acute Myocardial Infarction trial (DAPT-SHOCK-AMI; ClinicalTrials.gov: NCT03551964, Protocol numbers: 13062017-23-1, EudraCT: 2018-002161-19) is a double-blind, multicentre, international, placebo-controlled trial testing the hypothesis that intravenous cangrelor is (a) more effective in terms of its rate of onset and the proportion of patients achieving effective periprocedural inhibition of ADP-induced platelet aggregation and (b) at least as effective as the recommended treatment of oral (crushed) ticagrelor in reducing major cardiovascular events in patients with initial CS-AMI indicated for primary PCI strategy.

STUDY POPULATION

The study population will be comprised of patients who meet the inclusion criteria, defined as follows: (1) over 18 years of age; (2) AMI according to the ESC/ACC/AHA definition[22] with an indication for emergency PCI (primary PCI strategy); (3) CS due to an AMI present upon admission meeting at least 2 of the following criteria[23]: (a) systolic blood pressure <90 mmHg in the absence of hypovolaemia, (b) need for vasopressor and/or inotropic therapy, and (c) signs of organ hypoperfusion (cyanosis, cold extremities, disorders of consciousness, or heart failure); (4) signed informed consent form as per the applicable legal regulations and regulatory authority requirements; and (5) women with childbearing potential should avoid pregnancy and use a highly effective method of contraception throughout the study period (relevant for long-term use of ticagrelor).

The exclusion criteria are presented in **Supplementary Table 1**.

RANDOMISATION

The patients are randomised in a 1:1 ratio using random permuted blocks, stratified by study centre. Randomisation is performed using an interactive web-response system developed by the Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Czech Republic on the base of the TrialDB system.

STUDY MEDICATION

The patients are randomised into one of two treatment arms **(Figure 2)** according to the study intervention: arm A: cangrelor versus arm B: ticagrelor.

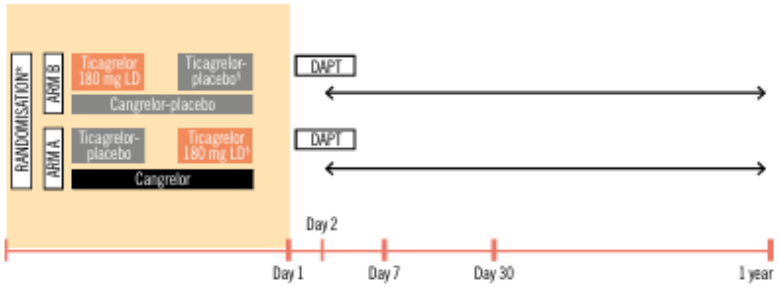


Figure 2. *Study design. Study initiation should be as soon as possible after admission to the hospital. *Study medication administered in addition to initial aspirin IV; †administered 30 minutes before the end of the infusion. Visits: day 7 after randomisation, day 30±5 days, 1 year±14 days. Ticagrelor was in crushed form. DAPT consists of a $P2Y_{12}$ inhibitor plus aspirin. DAPT: dual antiplatelet therapy; IV: intravenous; LD: loading dose*

Patients in arm A will receive the active study medication, cangrelor, as an intravenous (IV) bolus of 30 µg/kg (application <1 min) followed immediately by a continuous infusion at 4 µg/kg. Thirty minutes before the end of the cangrelor infusion, 180 mg of ticagrelor (crushed tablets) will be administered, followed by a maintenance dose of 90 mg every 12 hours (**Supplementary Figure 1**)[24,25].

In arm B of the study, the patients will receive 180 mg of crushed ticagrelor tablets orally as the loading dose, and thereafter a maintenance dose of 90 mg twice daily, as per the guidelines. The placebo dosage, forms, and methods of administration (cangrelor-placebo and ticagrelor-placebo) are identical to those of their respective active substance (cangrelor and ticagrelor). Thus, the cangrelor- and ticagrelor-placebo treatments will be administered in the same way as the IV cangrelor and oral ticagrelor (as crushed tablets), respectively.

Randomisation and initiation of the study medication administration of both compared study arms should be performed immediately (at the earliest possible time) after the patient's admission to the 24/7 PCI centre, which is usually the catheterisation laboratory.

CONCOMITANT THERAPY

The antiplatelet therapy used in this study is $iP2Y_{12}$, which will be administered in addition to an initial aspirin dose of 500 mg IV, followed by 100 mg of aspirin as a daily oral dose. Proton pump inhibitors are recommended to prevent gastrointestinal bleeding. The administration of other standard-care therapies, including additional adjuvant antithrombotic therapy (e.g., a bailout glycoprotein [GP] IIb/IIIa inhibitor and parenteral antithrombin drugs) and mechanical circulatory support, will be left to the discretion of the attending physician.

STUDY FOLLOW-UP

Patients enrolled in the study will be followed for 12 months. A summary of the timing of the visits and examinations that will be performed is presented in **Table 2**. Recommendations regarding treatment during the follow-up period, including dual antiplatelet therapy, will adhere to the appropriate guidelines.

CLINICAL ENDPOINTS

The primary endpoint is defined as a composite of death, myocardial infarction, or stroke 30 days after enrolment into the study. The secondary endpoints are summarised in **Supplementary Table 2**. Other goals include conducting a cost-effectiveness analysis and a magnetic resonance imaging substudy of the predefined endpoints.

PLATELET REACTIVITY STUDY

Patients who meet the enrolment criteria and are randomised at the 5 selected centres will be eligible for the platelet reactivity study. The laboratory antiplatelet effectiveness of the cangrelor and ticagrelor loading dose-based initial $iP2Y_{12}$ strategies will be determined by vasodilator-stimulated phosphoprotein (VASP) phosphorylation using flow cytometry, which is the most specific method for verifying and quantifying the effectiveness of $iP2Y_{12}$ and is associated with clinical outcomes. The determinations will be performed by an accredited facility using standardised sampling kits and protocols specified by the manufacturer. The design of the laboratory study is illustrated in **Figure 3**. The tests will be carried out as follows: before initiating treatment with the antiplatelet study drugs, upon completion of the coronary intervention procedure, 1 hr after PCI, 2 hrs after PCI, at the end of the cangrelor infusion, 1 hr after the end of the infusion, and 2 hrs after the end of the infusion. The primary laboratory endpoint will be assessed at the second and third VASP examinations. Monitoring platelet function dynamics after the intervention will provide important insights into the study – testing a strategy of combined intravenous and oral treatment with $P2Y_{12}$ inhibitors.

SAMPLE SIZE

This study was initially designed to include 304 patients. However, since the beginning of the study, there has been

Table 2. Scheduled visits during the 1-year study follow-up.

	Randomisation Visit 1	Day 7 Visit 2	Day 30±5 days Visit 3	Year 1±14 days Visit 4
Clinical condition	X	X	X	X
ECG	X	X	X	X
Echocardiography	X	X	X	X
*MRI[32]	-	X	X	X
†‡Laboratory sampling	†‡X	†X	†X	†X
Questionnaire on quality of life (EuroQol 5D)[33]	-	-	X	X

*MRI substudy – in selected centres. Laboratory examination involves the following: †examination of the effectiveness of antiplatelet therapy by the determination of VASP phosphorylation via flow cytometry – in selected centres; ‡haematological and biochemical blood tests.
ECG: electrocardiogram; MRI: magnetic resonance imaging; VASP: vasodilator-stimulated phosphoprotein

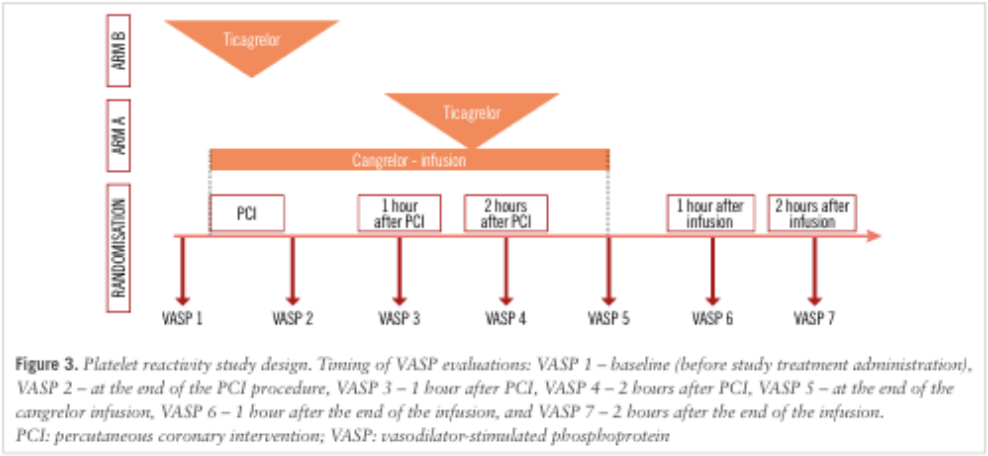


Figure 3. *Platelet reactivity study design. Timing of VASP evaluations: VASP 1 – baseline (before study treatment administration), VASP 2 – at the end of the PCI procedure, VASP 3 – 1 hour after PCI, VASP 4 – 2 hours after PCI, VASP 5 – at the end of the cangrelor infusion, VASP 6 – 1 hour after the end of the infusion, and VASP 7 – 2 hours after the end of the infusion. PCI: percutaneous coronary intervention; VASP: vasodilator-stimulated phosphoprotein*

a substantial amount of new evidence to consider when calculating study population sizes. The power analysis was computed for the superiority and non-inferiority scenarios under the assumption of primary endpoint occurrences in previous studies and registries, as presented in **Supplementary Table 3**.

The null hypothesis for the primary clinical endpoint was the equality of event rates, and the alternative hypothesis was the inequality of event rates between the analysed groups. Based on an expected event rate of 50% in the control group versus 38% in the cangrelor group, a required power of 80%, and a 2-sided statistical significance level of 5%, 536 patients would be needed to detect a 12% difference between groups and reject the null hypothesis. Allowing for a 3% dropout rate, 550 patients should be enrolled in the study (with a permitted 10% increase). The dropout rate is based on several clinical studies.

Based on an expected event rate of 50% in the control group versus 40% in the cangrelor group (difference 10%), a non-inferiority margin of 1%, a required power of 80%, and a 2-sided statistical significance level of 5%, 506 patients would be required to accept the additional non-inferiority hypothesis.

A power analysis for the platelet reactivity study, with the endpoint of effective inhibition of the platelet VASP <50%, was computed for superiority, requiring a power of 80% and a 2-sided statistical significance level of 5%. The null hypothesis for this endpoint was the equality of event rates, whereas, for the alternative hypothesis, it was the inequality of event rates between the analysed groups. Based on an expected event rate of 70% in the control group versus 90% in the cangrelor group, 124 patients will be required to detect a 20% difference between groups and reject the null hypothesis. The required sample size falls within the practical limits of VASP measurements, which is approximately 150.

The power analysis was computed using the PASS 13 software (2014 [NCSS, LLC]).

STATISTICAL ANALYSES

Statistical analyses will be performed using the SPSS Statistics software, version 28.0.1.1 (IBM). The analyses will be performed using an intention-to-treat principle supplemented by a modified intention-to-treat principle, which only includes patients who receive a dose of the study drug. A sensitivity analysis according to the per-protocol population will also be performed.

Standard descriptive statistics will be calculated in the analysis, i.e., absolute and relative frequencies for categorical variables, and medians supplemented with 5th-95th percentiles or means supplemented with standard deviations for continuous variables. The chi-square test will be used to test the statistical significance of differences in the primary endpoint and all other categorical variables, and the Mann-Whitney U test will be used for continuous variables.

Univariate and multivariate logistic regression or Cox proportional hazards models will be used as additional analyses of the influence of patient characteristics on the endpoint occurrence, and the Kaplan-Meier methodology will be adopted to visualise time-to-event data.

The level of statistical significance will be set at $p=0.05$. All the statistical analyses will be performed according to the U.S. Food and Drug Administration Guidance Document "E9 Statistical Principles for Clinical Trials" (FDA-1997-D-0508).

STUDY ORGANISATION

This study is a non-commercial, investigator-initiated study, and it is an international project that will be implemented by teams of investigators in 5 countries (the Czech Republic, France, Germany, Poland, and the Slovak Republic). It adheres to the ethical principles of the Declaration of Helsinki, the International Conference on Harmonization of Good Clinical Practice (R2), and all other applicable legal and regulatory requirements, including the General Data Protection Regulation. The study's organisational structure includes executive, steering, and endpoint adjudication committees, and a data safety monitoring board. External clinical research associate organisations will monitor all entries in the electronic case report forms and the completeness of the source documentation.

The institutions' ethics committees will conduct yearly audits of trial protocols and progress during the study. The auditing process is independent of both trial investigators and trial sponsors.

The records of the procedural findings, coronary angiograms, and PCIs will be submitted to the coordinating centre, where they will be evaluated by an independent panel of experts blinded to how medication was allocated within the study.

The assessment of VASP phosphorylation, as part of the platelet reactivity study, will be performed by an external laboratory and personnel blinded to the allocation of the study medication. The laboratory will enter the results directly into a database that will be inaccessible to the investigators.

Discussion

The increasing average age of the global population and the rising incidence of coronary heart disease indicate that the number of people at risk of CS-AMI is growing. However, conducting randomised studies to assess treatments in patients with CS is challenging. Circulatory instability is usually an exclusion criterion for participation in clinical trials attempting to verify the benefits of antithrombotic pharmacotherapies[12]. The currently available evidence is limited to that from small studies and registry data **(Supplementary Table 3)**.

Minimising thrombotic risk and restoring coronary blood flow at the microcirculatory level is critical for reperfusion and a better prognosis for CS-AMI[26]. The safety and efficacy of adjuvant combination antiplatelet therapy is mainly determined by the selection of the optimal inhibitor for ADP-induced platelet activation to be used alongside aspirin. Adding a third antiplatelet drug, such as a GP IIb/IIIa inhibitor, to the combination with aspirin and especially the highly effective $iP2Y_{12}$, ticagrelor, increases the risk of significant bleeding, thereby nullifying any potential benefits in terms of patient outcomes[27]. Cangrelor, in addition to all the other advantages already mentioned for patients with CS-AMI, reduces the periprocedural need for bailout rescue GP IIb/IIIa inhibitors[28].

The initial $P2Y_{12}$ inhibitor treatment strategy with intravenous cangrelor is compared to the crushed tablet form of ticagrelor. This form of ticagrelor loading dose

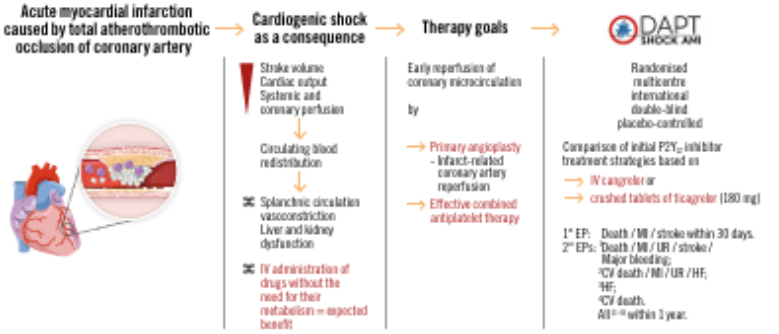


Figure 4. *Study endpoints. The figure summarises the factual basis for conducting the study and outlines the study outcome endpoints.* ✖ *refers to the associated data. CV: cardiovascular; EP: endpoint; HF: heart failure; IV: intravenous; MI: myocardial infarction; UR: urgent revascularisation*

demonstrated a faster onset of effective platelet inhibition than the oral dose (whole tablet)[29]. Therefore, despite the lack of evidence of the benefit on patient prognosis, crushed ticagrelor is recommended as the preferred mode of administration in patients with CS[26,30].

The VASP method was selected to monitor the rate of onset and extent of inhibition of $P2Y_{12}$ platelet receptors by the compared drugs. This choice was based on the unique specificity of the VASP assay for the $P2Y_{12}$ signalling pathway, which makes it the only method for monitoring $P2Y_{12}$ inhibitor efficacy that is not influenced by the $P2Y_1$ receptor functional status[31]. Moreover, unlike other assays, such as the widely used point-of-care VerifyNow (Werfen), VASP phosphorylation measurements are not affected by the co-administration of a GP IIb/IIIa inhibitor[31], which is frequently used during primary angioplasty for CS-AMI.

Conclusions

The DAPT-SHOCK-AMI study aims to provide clinical evidence for selecting the appropriate antiplatelet therapies in patients with AMI complicated by initial CS undergoing primary PCI and, thus, potentially improve the prognosis of this often fatal condition **(Figure 4)**.

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Conflict of interest statement

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References

1. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, Ahmed M, Aksut B, Alam T, Alam K, Alla F, Alvis-Guzman N, Amrock S, Ansari H, Ärnlöv J, Asayesh H, Atey TM, Avila-Burgos L, Awasthi A, Banerjee A, Barac A, Bärnighausen T, Barregard L, Bedi N, Belay Ketema E, Bennett D, Berhe G, Bhutta Z, Bitew S, Carapetis J, Carrero JJ, Malta DC, Castañeda-Orjuela CA, Castillo-Rivas J, Catalá-López F, Choi JY, Christensen H, Cirillo M, Cooper L Jr, Criqui M, Cundiff D, Damasceno A, Dandona L, Dandona R, Davletov K, Dharmaratne S, Dorairaj P, Dubey M, Ehrenkranz R, El Sayed Zaki M, Faraon EJA, Esteghamati A, Farid T, Farvid M, Feigin V, Ding EL, Fowkes G, Gebrehiwot T, Gillum R, Gold A, Gona P, Gupta R, Habtewold TD, Hafezi-Nejad N, Hailu T, Hailu GB, Hankey G, Hassen HY, Abate KH, Havmoeller R, Hay SI, Horino M, Hotez PJ, Jacobsen K, James S, Javanbakht M, Jeemon P, John D, Jonas J, Kalkonde Y, Karimkhani C, Kasaeian A, Khader Y, Khan A, Khang YH, Khera S, Khoja AT, Khubchandani J, Kim D, Kolte D, Kosen S, Krohn KJ, Kumar GA, Kwan GF, Lal DK, Larsson A, Linn S, Lopez A, Lotufo PA, El Razek HMA, Malekzadeh R, Mazidi M, Meier T, Meles KG, Mensah G, Meretoja A, Mezgebe H, Miller T, Mirrakhimov E, Mohammed S, Moran AE, Musa KI, Narula J, Neal B, Ngalesoni F, Nguyen G, Obermeyer CM, Owolabi M, Patton G, Pedro J, Qato D, Qorbani M, Rahimi K, Rai RK, Rawaf S, Ribeiro A, Safiri S, Salomon JA, Santos I, Santric Milicevic M, Sartorius B, Schutte A, Sepanlou S, Shaikh MA, Shin MJ, Shishehbor M, Shore H, Silva DAS, Sobngwi E, Stranges S, Swaminathan S, Tabarés-Seisdedos R, Tadele Atnafu N, Tesfay F, Thakur JS, Thrift A, Topor-Madry R, Truelsen T, Tyrovolas S, Ukwaja KN, Uthman O, Vasankari T, Vlassov V, Vollset SE, Wakayo T, Watkins D, Weintraub R, Werdecker A, Westerman R, Wiysonge CS, Wolfe C, Workicho A, Xu G, Yano Y, Yip P, Yonemoto N, Younis M, Yu C, Vos T, Naghavi M, Murray C. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. *J Am Coll Cardiol.* 2017;70:1-25.

2. Samsky MD, Morrow DA, Proudfoot AG, Hochman JS, Thiele H, Rao SV. Cardiogenic Shock After Acute Myocardial Infarction: A Review. *JAMA.* 2021;326:1840-50.

3. Osman M, Syed M, Patibandla S, Sulaiman S, Kheiri B, Shah MK, Bianco C, Balla S, Patel B. Fifteen-Year Trends in Incidence of Cardiogenic Shock Hospitalization and In-Hospital Mortality in the United States. *J Am Heart Assoc.* 2021;10:e021061.

4. Schrage B, Becher PM, Goßling A, Savarese G, Dabboura S, Yan I, Beer B, Söffker G, Seiffert M, Kluge S, Kirchhof P, Blankenberg S, Westermann D. Temporal trends in incidence, causes, use of mechanical circulatory support and mortality in cardiogenic shock. *ESC Heart Fail.* 2021;8:1295-303.

5. Hunziker L, Radovanovic D, Jeger R, Pedrazzini G, Cuculi F, Urban P, Erne P, Rickli H, Pilgrim T; AMIS Plus Registry Investigators are listed in alphabetic order with the names of the local principal investigators. Twenty-Year Trends in the Incidence and Outcome of Cardiogenic Shock in AMIS Plus Registry. *Circ Cardiovasc Interv.* 2019;12:e007293.

6. Muller A, Maggiorini M, Radovanovic D, Erne P; AMIS PLUS Investigators. Twenty-year trends in the characteristic, management and outcome of patients with ST-elevation myocardial infarction and out-of-hospital resuscitation. Insight from the national AMIS PLUS registry 1997-2017. *Resuscitation.* 2019;134:55-61.

7. Weeks PA, Sieg A, Paruthi C, Rajapreyar I. Antiplatelet Therapy Considerations in Ischemic Cardiogenic Shock: Implications of Metabolic Bioactivation. *J Cardiovasc Pharmacol Ther.* 2015;20:370-7.

8. Osmancik P, Jirmar R, Hulikova K, Peroutka Z, Pompachova A, Motovska Z, Widimsky P. A comparison of the VASP index between patients with hemodynamically complicated and uncomplicated acute myocardial infarction. *Catheter Cardiovasc Interv.* 2010;75:158-66.

9. Boucher BA, Wood GC, Swanson JM. Pharmacokinetic changes in critical illness. *Crit Care Clin.* 2006;22:255-71.

10. Hochman JS, Sleeper LA, Webb JG, Dzavik V, Buller CE, Aylward P, Col J, White HD; SHOCK Investigators. Early revascularization and long-term survival in cardiogenic shock complicating acute myocardial infarction. *JAMA.* 2006;295:2511-5.

11. Thiele H, Akin I, Sandri M, Fuernau G, de Waha S, Meyer-Saraei R, Nordbeck P, Geisler T, Landmesser U, Skurk C, Fach A, Lapp H, Piek JJ, Noc M, Goslar T, Felix SB, Maier LS, Stepinska J, Oldroyd K, Serpytis P, Montalescot G, Barthelemy O, Huber K, Windecker S, Savonitto S, Torremante P, Vrints C, Schneider S, Desch S, Zeymer U; CULPRIT-SHOCK Investigators. PCI Strategies in Patients with Acute Myocardial Infarction and Cardiogenic Shock. *N Engl J Med.* 2017;377:2419-32.

12. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, Neumann FJ, Ardissino D, De Servi S, Murphy SA, Riesmeyer J, Weerakkody G, Gibson CM, Antman EM; TRITON-TIMI 38 Investigators. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med.* 2007;357:2001-15.

13. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, Horrow J, Husted S, James S, Katus H, Mahaffey KW, Scirica BM, Skene A, Steg PG, Storey RF, Harrington RA; PLATO Investigators; Freij A, Thorsén M. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med.* 2009;361:1045-57.

14. Wayangankar SA, Bangalore S, McCoy LA, Jneid H, Latif F, Karrowni W, Charitakis K, Feldman DN, Dakik HA, Mauri L, Peterson ED, Messenger J, Roe M, Mukherjee D, Klein A. Temporal Trends and Outcomes of Patients Undergoing Percutaneous Coronary Interventions for Cardiogenic Shock in the Setting of Acute Myocardial Infarction: A Report From the CathPCI Registry. *JACC Cardiovasc Interv.* 2016;9:341-51.

15. Zeymer U, Vogt A, Zahn R, Weber MA, Tebbe U, Gottwik M, Bonzel T, Senges J, Neuhaus KL; Arbeitsgemeinschaft Leitende Kardiologische Krankenhausärzte (ALKK). Predictors of in-hospital mortality in 1333 patients with acute myocardial infarction complicated by cardiogenic shock treated with primary percutaneous coronary intervention (PCI); Results of the primary PCI registry of the Arbeitsgemeinschaft Leitende Kardiologische Krankenhausärzte (ALKK). *Eur Heart J.* 2004;25:322-8.

16. Iqbal J, Sumaya W, Tatman V, Parviz Y, Morton AC, Grech ED, Campbell S, Storey RF, Gunn J. Incidence and predictors of stent thrombosis: a single-centre study of 5,833 consecutive patients undergoing coronary artery stenting. *EuroIntervention.* 2013;9:62-9.

17. Pahuja M, Chehab O, Ranka S, Mishra T, Ando T, Yassin AS, Thayer KL, Shah P, Kimmelstiel CD, Salehi P, Kapur NK. Incidence and clinical outcomes of stroke in ST-elevation myocardial infarction and cardiogenic shock. *Catheter Cardiovasc Interv.* 2021;97:217-25.

18. Freund A, Jobs A, Lurz P, Feistritzer HJ, de Waha-Thiele S, Meyer-Saraei R, Montalescot G, Huber K, Noc M, Windecker S, Zeymer U, Ouarrak T, Schneider S, Thiele H, Desch S. Frequency and Impact of Bleeding on Outcome in Patients With Cardiogenic Shock. *JACC Cardiovasc Interv.* 2020;13:1182-93.

19. Ferreiro JL, Ueno M, Angiolillo DJ. Cangrelor: a review on its mechanism of action and clinical development. *Expert Rev Cardiovasc Ther.* 2009;7:1195-201.

20. Franchi F, Rollini F, Rivas A, Wali M, Briceno M, Agarwal M, Shaikh Z, Nawaz A, Silva G, Been L, Smairat R, Kaufman M, Pineda AM, Suryadevara S, Soffer D, Zenni MM, Bass TA, Angiolillo DJ. Platelet Inhibition With Cangrelor and Crushed Ticagrelor in Patients With ST-Segment-Elevation Myocardial Infarction Undergoing Primary Percutaneous Coronary Intervention. *Circulation.* 2019;139:1661-70.

21. Alexopoulos D, Xanthopoulou I, Gkizas V, Kassimis G, Theodoropoulos KC, Makris G, Koutsogiannis N, Damelou A, Tsigkas G, Davlouros P, Hahalis G. Randomized Assessment of Ticagrelor Versus Prasugrel Antiplatelet Effects in Patients With ST-Segment-Elevation Myocardial Infarction. *Circ Cardiovasc Interv.* 2017;10:e005383.

22. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD; Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction; Katus HA, Lindahl B, Morrow DA, Clemmensen PM, Johanson P, Hod H, Underwood R, Bax JJ, Bonow RO, Pinto F, Gibbons RJ, Fox KA, Atar D, Newby LK, Galvani M, Hamm CW, Uretsky BF, Steg PG, Wijns W, Bassand JP, Menasché P, Ravkilde J, Ohman EM, Antman EM, Wallentin LC, Armstrong PW, Simoons ML, Januzzi JL, Nieminen MS, Gheorghiade M, Filippatos G, Luepker RV, Fortmann SP, Rosamond WD, Levy D, Wood D, Smith SC, Hu D, Lopez-Sendon JL, Robertson RM, Weaver D, Tendera M, Bove AA, Parkhomenko AN, Vasilieva EJ, Mendis S. Third universal definition of myocardial infarction. *Circulation*. 2012;126:2020-35.

23. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P; ESC Scientific Document Group. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. 2016;37:2129-200.

24. Schneider DJ, Agarwal Z, Seecheran N, Keating FK, Gogo P. Pharmacodynamic effects during the transition between cangrelor and ticagrelor. *JACC Cardiovasc Interv*. 2014;7:435-42.

25. Hochholzer W, Kleiner P, Younas I, Valina CM, Lüffelhardt N, Amann M, Bömicke T, Ferenc M, Hauschke D, Trenk D, Neumann FJ, Stratz C. Randomized Comparison of Oral $P2Y_{12}$-Receptor Inhibitor Loading Strategies for Transitioning From Cangrelor: The ExcelsiorLOAD2 Trial. *JACC Cardiovasc Interv*. 2017;10:121-9.

26. Gorog DA, Price S, Sibbing D, Baumbach A, Capodanno D, Gigante B, Halvorsen S, Huber K, Lettino M, Leonardi S, Morais J, Rubboli A, Siller-Matula JM, Storey RF, Vranckx P, Rocca B. Antithrombotic therapy in patients with acute coronary syndrome complicated by cardiogenic shock or out-of-hospital cardiac arrest: a joint position paper from the European Society of Cardiology (ESC) Working Group on Thrombosis, in association with the Acute Cardiovascular Care Association (ACCA) and European Association of Percutaneous Cardiovascular Interventions (EAPCI). *Eur Heart J Cardiovasc Pharmacother*. 2021;7:125-40.

27. Tavenier AH, Hermanides RS, Fabris E, Lapostolle F, Silvain J, Ten Berg JM, Lassen JF, Bolognese L, Cantor WJ, Cequier A, Chettibi M, Goodman SG, Hammett CJ, Huber K, Janzon M, Merkely B, Storey RF, Zeymer U, Ecollan P, Collet JP, Willems FF, Diallo A, Vicaut E, Hamm CW, Montalescot G, van 't Hof AWJ; ATLANTIC investigators. Efficacy and Safety of Glycoprotein IIb/IIIa Inhibitors on Top of Ticagrelor in STEMI: A Subanalysis of the ATLANTIC Trial. *Thromb Haemost*. 2020;120:65-74.

28. Vaduganathan M, Harrington RA, Stone GW, Deliargyris EN, Steg PG, Gibson CM, Hamm CW, Price MJ, Menozzi A, Prats J, Elkin S, Mahaffey KW, White HD, Bhatt DL; CHAMPION Investigators. Cangrelor With and Without Glycoprotein IIb/IIIa Inhibitors in Patients Undergoing Percutaneous Coronary Intervention. *J Am Coll Cardiol*. 2017;69:176-85.

29. Parodi G, Xanthopoulou I, Bellandi B, Gkizas V, Valenti R, Karanikas S, Migliorini A, Angelidis C, Abbate R, Patsilinakos S, Baldereschi GJ, Marcucci R, Gensini GF, Antoniucci D, Alexopoulos D. Ticagrelor crushed tablets administration in STEMI patients: the MOJITO study. *J Am Coll Cardiol*. 2015;65:511-2.

30. Spagnolo M, Angiolillo DJ, Capodanno D. Evaluating the pharmacokinetic and pharmacodynamic impact of different modes of ticagrelor administration. *Expert Opin Drug Metab Toxicol*. 2023;19:769-84.

31. Siller-Matula JM, Trenk D, Schrör K, Gawaz M, Kristensen SD, Storey RF, Huber K; EPA (European Platelet Academy). Response variability to $P2Y_{12}$ receptor inhibitors: expectations and reality. *JACC Cardiovasc Interv*. 2013;6:1111-28.

32. Alkhalil M, Borlotti A, De Maria GL, Gaughran L, Langrish J, Lucking A, Ferreira V, Kharbanda RK, Banning AP, Channon KM, Dall'Armellina E, Choudhury RP. Dynamic changes in injured myocardium, very early after acute myocardial infarction, quantified using T1 mapping cardiovascular magnetic resonance. *J Cardiovasc Magn Reson*. 2018;20:82.

33. EuroQol Group. EuroQol--a new facility for the measurement of health-related quality of life. *Health Policy*. 1990;16:199-208.

34. Lindholm MG, Kober L, Boesgaard S, Torp-Pedersen C, Aldershvile J; Trandolapril Cardiac Evaluation study group. Cardiogenic shock complicating acute myocardial infarction; prognostic impact of early and late shock development. *Eur Heart J*. 2003;24:258-65.

35. Aissaoui N, Puymirat E, Delmas C, Ortuno S, Durand E, Bataille V, Drouet E, Bonello L, Bonnefoy-Cudraz E, Lesmeles G, Guerot E, Schiele F, Simon T, Danchin N. Trends in cardiogenic shock complicating acute myocardial infarction. *Eur J Heart Fail*. 2020;22:664-72.

36. Helgestad OK, Josiassen J, Hassager C, Jensen LO, Holmvang L, Sørensen A, Frydland M, Lassen AT, Udesen NLJ, Schmidt H, Ravn HB, Møller JE. Temporal trends in incidence and patient characteristics in cardiogenic shock following acute myocardial infarction from 2010 to 2017: a Danish cohort study. *Eur J Heart Fail*. 2019;21:1370-8.

37. Orban M, Limbourg T, Neumann FJ, Ferenc M, Olbrich HG, Richardt G, Hennersdorf M, Empen K, Fuernau G, Desch S, Eitel I, Hambrecht R, Pöss J, Schneider S, Schuler G, Werdan K, Zeymer U, Thiele H, Hausleiter J. ADP receptor antagonists in patients with acute myocardial infarction complicated by cardiogenic shock: a post hoc IABP-SHOCK II trial subgroup analysis. *EuroIntervention*. 2016;12:e1395-403.

38. TRIUMPH Investigators; Alexander JH, Reynolds HR, Stebbins AL, Dzavik V, Harrington RA, Van de Werf F, Hochman JS. Effect of tilarginine acetate in patients with acute myocardial infarction and cardiogenic shock: the TRIUMPH randomized controlled trial. *JAMA*. 2007;297:1657-66.

39. Orban M, Kleeberger J, Ouarrak T, Freund A, Feistritzer HJ, Fuernau G, Geisler T, Huber K, Dudek D, Noc M, Montalescot G, Neumer A, Haller P, Clemmensen P, Zeymer U, Desch S, Massberg S, Schneider S, Thiele H, Hausleiter J. Clopidogrel vs. prasugrel vs. ticagrelor in patients with acute myocardial infarction complicated by cardiogenic shock: a pooled IABP-SHOCK II and CULPRIT-SHOCK trial sub-analysis. *Clin Res Cardiol*. 2021;110:1493-503.

40. Droppa M, Vaduganathan M, Venkateswaran RV, Singh A, Szumita PM, Roberts RJ, Qamar A, Hack L, Rath D, Gawaz M, Fuernau G, de Waha-Thiele S, Desch S, Schneider S, Ouarrak T, Jaffer FA, Zeymer U, Thiele H, Bhatt DL, Geisler T. Cangrelor in cardiogenic shock and after cardiopulmonary resuscitation: A global, multicenter, matched pair analysis with oral $P2Y_{12}$ inhibition from the IABP-SHOCK II trial. *Resuscitation*. 2019;137:205-12.

41. Fiore M, Gerbaud E, Coste P, Cetran L, Marchand H, Seguy B. Optimal platelet inhibition with cangrelor in comatose survivors of out-of-hospital cardiac arrest undergoing primary percutaneous coronary intervention. *Resuscitation*. 2018;130:e1-2.

42. Droppa M, Borst O, Rath D, Müller K, Gawaz M, Bhatt DL, Geisler T. Impact of Intravenous $P2Y_{12}$-Receptor Inhibition with Cangrelor in Patients Presenting with Acute Coronary Syndrome and Cardiogenic Shock - a Case Series. *Cell Physiol Biochem*. 2017;42:1336-41.

43. Kordis P, Bozic Mijovski M, Berden J, Steblovnik K, Blinc A, Noc M. Cangrelor for comatose survivors of out-of-hospital cardiac arrest undergoing percutaneous coronary intervention: the CANGRELOR-OHCA study. *EuroIntervention*. 2023;18:1269-71.

Supplementary data

Supplementary Table 1. Study exclusion criteria.
Supplementary Table 2. Secondary endpoints.
Supplementary Table 3. Outcomes of patients with CS-AMI in registries and clinical trials.
Supplementary Figure 1. Study medication.

The supplementary data are published online at: https://eurointervention.pcronline.com/doi/10.4244/EIJ-D-24-00203

7.20 Annex 20

Součková L, Opatřilová R, Suk P, Čundrle I Jr, Pavlík M, Zvoníček V, Hlinomaz O, Šrámek V. Impaired bioavailability and antiplatelet effect of high-dose clopidogrel in patients after cardiopulmonary resuscitation (CPR). Eur J Clin Pharmacol. 2013 Mar;69(3):309-17. doi: 10.1007/s00228-012-1360-0. Epub 2012 Aug 14. PMID: 22890586.

Summary

Purpose

The aim of this study was to evaluate the bioavailability and antiplatelet effect of high-dose clopidogrel in patients after successful cardiopulmonary resuscitation (CPR). The study addressed the impact of post-cardiac arrest physiological changes on the effectiveness of oral antiplatelet therapy.

Methodology

This prospective observational study assessed platelet inhibition in patients treated with high-dose clopidogrel after CPR using platelet function testing. The pharmacodynamic response was evaluated to determine whether standard oral dosing achieved adequate platelet inhibition in critically ill patients.

Results

The study demonstrated significantly impaired bioavailability and reduced antiplatelet activity despite high-dose clopidogrel administration. A substantial proportion of patients failed to achieve adequate platelet inhibition, indicating that standard dosing strategies may be insufficient following cardiopulmonary resuscitation.

Conclusion

The findings demonstrated that optimisation of antiplatelet therapy in critically ill patients requires evidence generated specifically in this population. The study highlights the important role of investigator-initiated clinical research in improving the safe and effective use of established therapies in complex clinical settings.

Contribution to the habilitation: *This study illustrates the role of investigator-initiated clinical research in optimising existing pharmacotherapy for patient populations that are underrepresented in pre-marketing clinical trials.*

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REVIEW ARTICLE

Impaired bioavailability and antiplatelet effect of high-dose clopidogrel in patients after cardiopulmonary resuscitation (CPR)

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Abstract
Purpose Bioavailability of clopidogrel in the form of crushed tablets administered via nasogastric tube (NGT) has not been established in patients after cardiopulmonary resuscitation. Therefore, we performed a study comparing pharmacokinetic and pharmacodynamic response to high loading dose of clopidogrel in critically ill patients after cardiopulmonary resuscitation (CPR) with patients scheduled for elective coronary angiography with stent implantation.
Methods In the NGT group (nine patients, after cardiopulmonary resuscitation, mechanically ventilated, therapeutic hypothermia), clopidogrel was administered in the form of crushed tablets via NGT. Ten patients undergoing elective coronary artery stenting took clopidogrel per os (po) in the form of intact tablets. Pharmacokinetics of clopidogrel was measured with high-performance liquid chromatography (HPLC) before and at 0.5, 1, 6, 12, 24 h after administration of a loading dose of 600 mg. In five patients in each group, antiplatelet effect was measured with thrombelastography (TEG; Platelet Mapping) before and 24 h after administration.
Results The carboxylic acid metabolite of clopidogrel was detected in all patients in the po group. In eight patients, the maximum concentration was measured in the range of 0.5–1 h after the initial dose. In four patients in the of NGT group, the carboxylic acid metabolite of clopidogrel was undetectable and in the remaining patients was significantly delayed (peak values at 12 h). All patients in the po group reached clinically relevant (>50 %) inhibition of thrombocyte adenosine diphosphate (ADP) receptor after 24 h compared with only two in the NGT group ($p=0.012$). There was a close correlation between peak of inactive clopidogrel metabolite plasmatic concentration and inhibition of the ADP receptor ($r=0.79$; $p<0.001$).
Conclusion The bioavailability of clopidogrel in critically ill patients after cardiopulmonary resuscitation is significantly impaired compared with stable patients. Therefore, other drugs, preferentially administered intravenously, should be considered.

Keywords Clopidogrel · Pharmacokinetics · Thrombelastography · HPLC

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Introduction

Clopidogrel, a thienopyridine derivative, prevents platelet aggregation by selectively blocking the adenosine diphosphate (ADP) receptor and subsequent activation of glycoprotein IIb/IIIa [1, 2]. It proved to be superior to aspirin in reducing coronary and cerebrovascular ischemic events [3], and its addition to aspirin reduced ischemic events compared with aspirin alone in non-ST-elevation (NSTE) acute coronary syndrome—the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) study [4]. Currently, the combination of both drugs is used in patients with ST elevation (STE) [5] and NSTE acute coronary syndromes [6]. Clopidogrel also remains the mainstay of dual antiaggregant therapy after stent implantation [7–9].

Clopidogrel is a prodrug that after absorption from the intestine must undergo hepatic biotransformation to become effective [10]. Only approximately 15 % of the drug is converted to the active form via cytochrome P450 (CYP) (isoenzymes 3A4, 2C19). Most of the drug is metabolized by esterases to an inactive carboxylic acid derivative [11]. In the critically ill, numerous conditions can interfere with these processes [12]: crushing the acid-resistive coating of the tablets and administration with a nasogastric tube (NGT) [13], delayed gastric emptying [12], and compromised splanchnic perfusion with functional and structural changes of intestinal mucosa—just to mention only a few. Acute changes in body compartments, organ dysfunction, and treatment with elimination methods (e.g., renal replacement therapies) are further important factors that interfere with normal drug metabolism and function in the critically ill.

As documented in previous studies, inhibition of platelet aggregation by clopidogrel can vary widely [14–16]. Insufficient platelet inhibition is linked to increased risk of hypercoagulation and stent thrombosis with myocardial reinfarction, which results in increased morbidity and mortality rates [17, 18]. The exact mechanisms of the variable response to clopidogrel therapy are not well understood and are evidently multiple: inadequate drug compliance, underdosing, drug–drug interactions, patient status, polymorphisms of the P2Y12 receptor gene and CYP isoenzymes (e.g., CYP3A4, CYP3A5 and CYP2C19), increased reactivity of resting platelets, and upregulation of other platelet activation pathways (e.g. thrombin, thromboxane A2, and collagen) [19].

After cardiopulmonary resuscitation (CPR) because of acute myocardial infarction (AMI), patients who undergo percutaneous coronary intervention (PCI) with stent implantation are given clopidogrel via an NGT just after admission to the intensive care unit (ICU). Evidently, these patients are at risk of impaired clopidogrel bioavailability, which has not previously been studied. To test this hypothesis, we measured the concentration of a clopidogrel inactive metabolite with high-performance liquid chromatography (HPLC) and platelet reactivity with the modified Thromboelastograph® (TEG) Platelet Mapping. The post-CPR cohort treated with NGT-administered clopidogrel was compared with patients undergoing elective coronary artery stenting and taking intact clopidogrel tablets per os (po).

Methods

Study population

We studied patients older than 18 years in whom clopidogrel in an initial dose of 600 mg was indicated because of coronary artery disease (CAD). Those in whom clopidogrel was taken as chronic medication or treated with thrombolytics or glycoprotein IIb/IIIa inhibitors were excluded [20, 21]. Patients were divided into two groups: (1) the po group, patients who swallowed intact tablets after stenting procedures (patients undergoing planned PCI with stent implantation), and (2) the NGT group, patients who were critically ill after CPR and acute PCI with stent implantation in whom clopidogrel in the form of crushed tablets was administered through an NGT. The study was conducted according to the principles of the Declaration of Helsinki and the law and regulations applicable in the European Union. The local institutional review board approved the protocol. Informed consent was obtained and signed by all patients in the po group beforehand. In the NGT group, informed consent was signed by a doctor unrelated to the study. Patients who regained full consciousness during hospitalization were asked to sign the informed consent subsequently.

Blood sampling

Blood samples to evaluate clopidogrel pharmacokinetics (PK) using HPLC were collected from the artery (NGT group) and peripheral vein (po group) into heparinized test tubes at the following time points: immediately before and 0.5, 1, 6, 12, and 24 h following a high loading dose. Plasma was obtained by blood centrifugation at 14,500 rpm for 5 min, and plasma samples were separated and stored at −70 °C until analysis. Blood samples for evaluating clopidogrel pharmacodynamics (PD) using TEG Platelet Mapping were collected in tubes containing sodium citrate before and 24 h after a high initial dose.

Pharmacokinetic evaluation with HPLC

Qualitative and quantitative analysis of clopidogrel tablets (Plavix®, Sanofi Pharma Bristol-Myers Squibb SNC) was performed in advance [22]. A summary of results from chromatographic analysis also served as a standard for evaluating chromatograms of human plasma. For the study purposes, samples were tempered at room temperature immediately before analysis. The aliquot of 100 µl of trifluoroacetic acid was added to 0.5 ml human plasma, and the solution was vortex mixed thoroughly for 5 min to form plasma protein precipitates. Clear liquid was pipetted over the precipitates and filtered through Sartorius disposable filter Minisart® mesh 0.45 mm into glass vials. Determination of the area under the curve (AUC) and retention time of the inactive carboxyl metabolite of clopidogrel (CLP_{met}) in plasma was performed using the Agilent 1200 HPLC System with a quaternary model pump and an automatic injector coupled to an ultraviolet–visible spectroscopy (UV/VIS) detector operated at 220 nm [23–26]. Data were acquired using ChemStation chromatography software. The column, a particle size of 5 µm, Zorbax XDB-C18 (50 mm×2.1 mm

i.d.), was used for chromatographic separation. Mobile phase was methanol/water (85/15; v/v). Flow rate was 0.5 ml/min, injection volume 10 ml of sample, and analysis time 20 min.

Pharmacodynamic evaluation by TEG Platelet Mapping

The modified TEG Hemostasis Analyzer with Platelet Mapping assay (Haemoscope Corporation, Niles, IL, USA) was used. Blood was drawn exactly according to the manufacturer's recommendations (Table 1) and analyzed according to the manufacturer's instructions [27]. Briefly, the assay relies on evaluation of thrombin-induced clot strength to enable a quantitative analysis of platelet function [28, 29]. Classical measurements (citrated whole blood sample treated with kaolin and sample with heparinase) (channels 1 and 2, Table 1) were extended with ADP receptor activator compound (channel 3, Table 1), which blocks free ADP receptors not inhibited with clopidogrel. This enables indirect measurement of the ADP receptors inhibited with clopidogrel (percent inhibition) [30].

Data analysis

With regard to file size and absence of normal distribution, results are given as median [interquartile range (IQR) if not stated otherwise]. Nonparametric tests were used for inter- and intragroup comparison (Mann–Whitney *U* test and Wilcoxon paired test, as appropriate). For analysis, we used the STATISTICA (StatSoft Inc, Tulsa, OK, USA) software, and $p<0.05$ was considered significant.

Results

Nineteen patients entered the study (nine in the NGT group and ten in the po group). Baseline demographic data, physiological parameters on admission, and CAD risk factors complemented with concurrent medication are shown in Table 2.

All nine patients in the NGT group underwent CPR (median 20 min, range 10–30 min) with subsequent PCI [seven myocardial infarction with ST elevations (STEMI), one non-STEMI (NSTEMI), one malignant arrhythmia) and stenting. From the cath lab, all patients were admitted to the general ICU, where standard therapy followed (mechanical ventilation, invasive hemodynamic monitoring and stabilization, therapeutic hypothermia, etc.). Study blood samples were taken on top of routine tests, an NGT was introduced, and 600 mg clopidogrel given. All patients received clopidogrel within 60 min of stent implantation. All ten po group patients received a coronary artery stent because of advanced CAD and were admitted to the coronary care unit (CCU). Table 3 shows the initial dosage of norepinephrine (NE), lactate values, and liver enzymes in the NGT group on ICU admission. Seven of the nine NGT patients had an increased initial value of lactate. Laboratory liver function tests showed elevated bilirubin in two, elevated alanine aminotransferase (ALT) in seven, and elevated aspartate aminotransferase (AST) in eight patients, respectively.

Table 1 Constituents of thrombelastography (TEG®) Platelet Mapping™ assay channels

	Channel 1	Channel 2	Channel 3
Heparinized blood (μl)	0	360	360
Citrated whole blood sample treated with kaolin (μl)	360	0	0
Activator F™ (A) (μl)	0	10	10
ADP (μl)	0	0	10
$CaCl_2$ 0.1 % (μl)	20	0	0

Channel 1 is a standard TEG® with maximally stimulated platelets. Channel 2 creates a fibrin mesh with no activated platelets. Channel 3 creates a fibrin mesh with platelets stimulated only by the ADP receptors [27]

ADP adenosine diphosphate, *$CaCl_2$* calcium chloride

Table 2 Patient demographic and physiological data, coronary artery disease (CAD) risk factors, and chronic medication

	Per os ($n=10$)	NGT ($n=9$)
Gender (male)	6	6
Age (years)	72 (55–86)	66 (47–81)
Height (cm)	170 (161–180)	173 (160–182)
Weight (kg)	83 (62–105)	83 (68–100)
Blood pressure (mmHg)	137/77 (114/63–175/95)	137/79 (108/60–220/180)
Heart rate (bpm)	77 (55–100)	93 (60–115)
Risk factors		
Smoking	1	2
Hypercholesterolemia	10	5
Hypertension	10	9
Diabetes mellitus	5	4
Family history of CAD	4	1
Length of hospitalization (days)	2 (2–3)	12 (2–26)
Chronic medication		
Lipid-lowering agents	6	3
Aspirin	10	4
Proton pump inhibitors	1	1
ACE Inhibitors	8	5
Calcium-channel blockers	4	2
Diuretics	5	2
Beta-blockers	6	6
Insulin/ADB	4	2

ACE Inhibitors angiotensin-converting enzyme inhibitors, *ADB* antidiabetics

Table 3 Nasogastric-tube (NGT) group: selected hemodynamic data, vasoactive drug dosage, and laboratory tests on intensive care unit admission

Variable	Reference range	Patient no., NTG group								
		11	12	13	14	15	16	17	18	19
Dosage NE (mg/h)	–	20	15	10	15	10	10	5	5	20
Lactate (mmol/l)	0.5–2.20	13.8	11.5	13.7	6	2.2	1.4	6.8	5.7	11.4
Bilirubin (umol/l)	2.0–21	9.1	9.6	4.3	10.9	26.4	10.8	10.9	14	28.3
ALT (ukat/l)	0.00–0.68	2.2	0.3	5.15	2.96	7.29	3.67	0.83	0.54	9.32
AST (ukat/l)	0.00–0.67	3.04	0.34	4.25	2.1	3.78	5.84	1.13	0.83	5.73

NE norepinephrine, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase

Pharmacokinetic evaluation with HPLC

CLP_{met} PK were measured in all patients in both groups (Fig. 1). Maximal CLP_{met} plasmatic concentrations were significantly lower in the NGT group [11,906 (0–17,379) ng/ml] than in the po group [18,455 (18,146–22,198) ng/ml] ($p=0.012$). Also, time to reach maximal concentration of the clopidogrel inactive carboxylic metabolite (T_{max}) tended to be longer in the NGT group (12 h) than in the po group (1 h).

Pharmacodynamic evaluation with TEG Platelet Mapping

Platelet aggregation PD were performed only in the first five patients in each group. Results of the percentage inhibition of thrombocyte ADP receptor before and 24 h after initial clopidogrel dose administration are shown in Fig. 2.

There was no difference between groups prior to clopidogrel administration. Clopidogrel increased inhibition by 45 % (29–53 %) in the po group and by 6 % (6–23 %) in the NGT group ($p=0.032$). As the result, inhibition 24 h after clopidogrel administration was 70 % (67–85 %) in the po group and 26 % (7–51 %) in the NGT group ($p=0.012$). All po group patients reached clinically effective platelet inhibition (i.e., >50 %) [31, 32] compared with only patients in the NGT group ($p=0.037$). Interestingly, one patient in each group showed platelet inhibition >50 % even before taking clopidogrel. A close correlation between peak CLP_{met} plasmatic concentration and increased inhibition of thrombocyte ADP receptor was found ($r=0.79$; $p<0.001$).

Discussion

This study showed impaired bioavailability of clopidogrel in hemodynamically unstable critically ill patients after CPR, as distinct from patients who swallow intact clopidogrel tablets after elective coronary artery stenting. This impairment results in preserved thrombocyte aggregation and increased risk of stent thrombosis. To our knowledge, this is the first such study in critically ill patients after CPR. The study also emphasizes the method of parallel PK (inactive plasma metabolite of clopidogrel measured with HPLC) and PD evaluation (anti-aggregation effect measured with TEG Platelet Mapping). The many reasons for impaired clopidogrel bioavailability are summarized in Table 4 [14, 33, 34].

Evidently, only a few factors can be excluded from the list (e.g., interaction with nutrition; patients not enterally fed during therapeutic hypothermia; omeprazole not given). Determining the impact of other factors is a challenge for further studies (measuring amount lost after crushed tablet passes through the NGT; administration of clopidogrel into duodenal tube, etc.). Nevertheless, detailed identification of the impact of separate factors listed in Table 4 is hardly

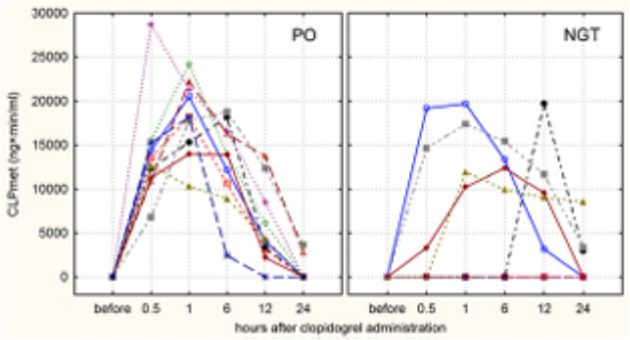


Fig. 1 Comparison of concentrations of the carboxylic acid metabolite of clopidogrel (area under the concentration time curve in ng × min/ml) in the nasogastric tube (NGT) and per os (po) groups

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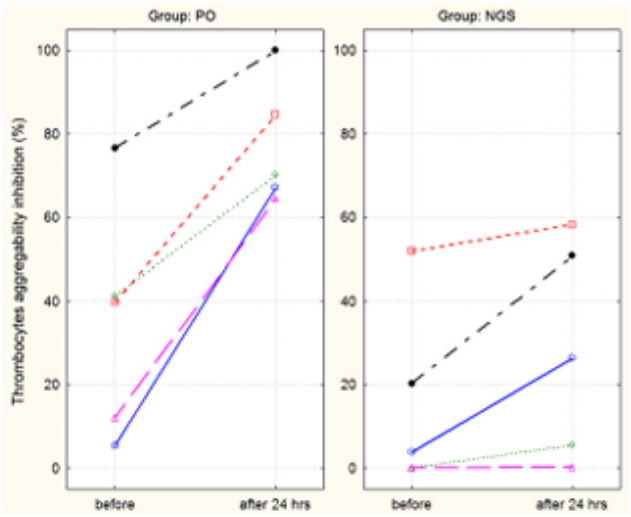


Fig. 2 Inhibition of adenosine diphosphate (ADP) thrombocyte receptors before and 24 h after the initial dose of 600 mg clopidogrel

possible. In fact, previous studies showed impaired bioavailability of clopidogrel in different populations ranging from healthy volunteers to patients with STE acute coronary syndrome (summarized in Table 5). None was performed in patients after CPR, when all potential factors influencing clopidogrel bioavailability are present.

Few factors can influence clopidogrel bioavailability in patients after CPR. To overcome gastroparesis, clopidogrel delivery into the duodenum is achievable, as these tubes can be inserted without endoscopic assistance and with a satisfactory success rate after operator training [40]. Increasing the dose of clopidogrel can be another solution, as an increased initial clopidogrel loading dose from 300 mg to 600 mg led to increased platelet inhibition from 39 % to 57 % within 4 h [41]. Nevertheless, a further increase to 900 mg caused no additional effect [38]. We assume that a dose lower than 600 mg reached the stomach because the tablets had been crushed and administered via NGT. Theoretically, an increased clopidogrel dose could have led to increased clopidogrel bioavailability and function.

Table 4 Reasons for impaired bioavailability of clopidogrel

Clinical factors	Cellular factors	Genetic factors	Drug factors
Poor patient compliance	Accelerated platelet turnover	Polymorphism of CYP	Drug–drug interaction
Underdosing	Reduced CYP metabolite activity	Polymorphism of GPIa	Crushing the tablets
Poor absorption/Intestinal failure/ delayed gastric emptying/ splanchnic hypoperfusion/ malabsorption	Increased ADP exposure	Polymorphism of $P2Y_{12}$	Administration with NGT (but absorption from intestine)
Liver dysfunction	Up-regulation $P2Y_{12}$ pathways	Polymorphism of GPIIIa	Loss of protective tablet coating
Acute coronary syndrome	Up-regulation P2Y-independent pathways (collagen, epinephrine, thromboxane A, thrombin)	Natural inhibition of ADP receptors	
Acute changes in distributive volume (compartments) in patients after CPR (vasoplegia, impaired capillary permeability, etc.)			
Diabetes mellitus/insulin resistance			
Elevated body mass index/smoking			

CPR cardiopulmonary resuscitation, *CYP* cytokine P450, *ADP* adenosine diphosphate

Table 5 Studies monitoring the pharmacokinetics and pharmacodynamics (PK/PD) of clopidogrel in different populations

Study/references	Population	Amount	Methods	PK/PD	Dosage	Year
Preisman et al. [36]	Patients elective or urgent CABG	60	TEG	PD	75 mg	2010
Alstrom et al. [2]	Patients undergoing PCI	30	LTA/TEG/PFA	PD	300 mg	2007
Blinden et al. [37]	Patients chronically receiving clopidogrel before PCI	100	TEG	PD	75 mg	2007
Beckerath [38]	Patients with coronary artery disease	60	Optical aggregometry/ LC-MS	PD/PK	300 mg, 600 mg, 900 mg	2005
Heestermans et al. [35]	Patients STEMI/healthy volunteers	11/10	LTA/LC-MS	PD/PK	600 mg	2008
Zafar et al. [39]	Healthy volunteers	9	LC-MS	PK	300 mg	2009
Our study	Patients STEMI/patients PCI	9/10	TEG/HPLC	PD/PK	600 mg	2010

CABG coronary artery bypass grafting, *TEG* Thromboelastograph Platelet Mapping, *LTA* light transmittance platelet aggregation, *PCI* percutaneous coronary intervention, *PFA* platelet function analyzer, *LC-MS* liquid chromatography–mass spectrometry; *STEMI* myocardial infarction with ST elevation

All patients after CPR were treated with mild hypothermia (32–34 °C) according to current guidelines. Though mild hypothermia does not compromise splanchnic blood flow, as observed in experiments with rats [42] and also in patients on cardiopulmonary bypass [43, 44] and continuous renal replacement therapies [45], it significantly influences liver metabolic activity [46, 47]. This, in theory, could be one of the causes of low levels of CLP_{met} observed in our study. Hypothermia can also influence the classical TEG measurement, but its effect on the method itself is only minor [47–49]. Influence on the advanced TEG measurement (i.e., platelet mapping) has not been tested so far but very probably does not differ from the classical TEG assay.

In our study, natural inhibition of the ADP receptor was relatively high and variable. The median natural ADP receptor inhibition was 35 % in the po group and 15.2 % in the NGT group. Two patients (one in each group) had natural inhibition >50 %, which is considered clinically effective, even before clopidogrel administration. The most probable explanation of this finding seems to be natural inhibition, but platelet hyporeactivity as a reaction to a thrombogenic state—especially in the CPR patient group—cannot be ruled out. All patients in the study had zero levels of CLP_{met} at baseline, which excludes the possibility of a previous clopidogrel medication bias. The high variability of the natural ADP receptor can partially explain the different effect of treatment with clopidogrel. Furthermore, high variability of natural ADP receptor inhibition may account for the acute bleeding episodes during surgery in some patients who are treated with ADP receptor inhibitors [29]. Arterial compared with venous blood was analyzed in CPR patients compared with elective controls. Nevertheless, this should not influence our findings, as platelet function measured by TEG (or comparable methods) does not differ whether arterial or venous blood is analyzed [50, 51].

Study limitations

The bioavailability of clopidogrel after an initial 600 mg loading dose was monitored for 24 h. In theory, prolongation of this interval could have revealed delayed clopidogrel absorption. This was ruled out because of the maintenance dose of 75 mg. Nevertheless, we do not assume that a prolongation of 24 h would bring different results, as the concentrations of CLP_{met} were lower at 24 h compared with those at 1–12 h (see Fig. 1). Besides, clopidogrel level is usually measured 4 h after ingestion [16, 52].

Clinical response was measured (TEG Platelet Mapping) on a small number of patients (five in each group) mainly due to high costs (approximately 150 euros/measurement). Nevertheless the response was uniform (inhibition in the po group, and no effect in the NGT group), raising the suspicion that NGT-administered clopidogrel to patients after CPR is not effective. Larger studies confirming our results are warranted, but the workload and costs must be taken into account. Our study is also limited by the unavailability of a genetic test to prove any genetic influence on clopidogrel absorption and/or metabolism.

Genetics can influence absorption in intestinal mucosa (reduced absorption in patients with P -glycoprotein encoded by the gene ABCB1), hepatic metabolism (different clopidogrel metabolism to the active form by cytochrome P450 variants CYP3A4 and CYP2C19) [53–55]. Indeed, in patients with AMI treated with clopidogrel who had a dysfunctional allele CYP2C19 were more likely to have frequent cardiovascular events than those with a functional allele. This effect was also observed in patients undergoing PCI [54]. Patients who had reduced CYP2C19 allele function had lower levels of the active form of clopidogrel, which was unable to sufficiently inhibit platelet function and thus increased the risk of major adverse cardiovascular events [55].

Consequences for practice

How should we treat patients after CPR in whom clopidogrel is indicated? In our opinion, physicians should be very circumspect and not rely on the drug's effect. Any steps to increase clopidogrel bioavailability are cumbersome (duodenal tube, etc.) and often barely achievable (e.g., improved splanchnic perfusion). Also, TEG Platelet Mapping to monitor the drug's effect is costly and dependent on adequately trained personnel. Other methods of thrombocyte aggregation measurement, such as VerifyNow P2Y12, vasodilator-stimulated phosphoprotein–phosphorylation (VASP-PRI), Multiplate-ADP, and light transmission aggregometry, are not routinely used in these patients [56]. Although no patient in our study developed stent thrombosis during hospitalization, we recommend boosting the effect of aspirin given intravenously with other antiaggregation drugs. Novel and more effective orally administered drugs recently approved in Europe (e.g., prasugrel or ticagrelor) can possibly be of some help, but we recommend relying on their i.v. administration only. Although novel forms of heparin could be considered in the future [57], we believe that i.v.-administered forms of ADP receptor inhibitors (cangrelor, elinogrel) will be more suitable [58]. Until they are authorized for marketing, however, we advise applying glycoprotein IIb/IIIa inhibitors during the periprocedural (PCI) period [59].

Conclusion

In conclusion, our study demonstrates that the bioavailability of clopidogrel and platelet aggregation inhibition in critically ill patients after CPR is significantly impaired compared with in stable patients. Clinicians should consider other methods of antiaggregation in this subgroup of patients.

References

1. Mills DC, Puri R, Hu CJ, Minniti C, Grana GF, Colman RF, Colman RW (1992) Clopidogrel inhibits the binding of ADP analogues to the receptor mediating inhibition of platelet adenylate cyclase. Arteriosc Thromb 12(4):430–436
2. Alstrom U, Tyden H, Oldgren J, Siegbahn A, Stahle E (2007) The platelet inhibiting effect of a clopidogrel bolus dose in patients on long-term acetylsalicylic acid treatment. Thromb Res 120(3): 353–359
3. CAPRIE Steering Committee (1996) A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). Lancet 348(9038):1329–1339
4. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK (2001) Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med 345(7):494–502
5. Antman EM, Hand M, Amstrong PW, Bate ER, Green LA, Halasyamani LK, Hochman JS, Krumholz HM, Lamas GA, Mullany CJ, Pearle DL, Sloan MA, Smith SC (2008) 2007 Focused update of the ACC/AHA 2004 guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association task force on practise guidelines. Circulation 117(2):296–329
6. Mehta SR, Bassand JP, Chrolavicius S, Diaz R, Fox KA, Granger CB, Jolly S, Rupprecht HJ, Widimsky P, Yusuf S (2008) Design and rationale of CURRENT-OASIS: a randomized, 2×2 factorial trial evaluating optimal dosing strategies for clopidogrel and aspirin in patients with ST and non-ST-elevation acute coronary syndromes managed with an early invasive strategy. Am Heart J 156 (6):1080–1088
7. Silber S, Albertsson P, Aviles FF, Camici PG, Colombo A, Hamm C, Jorgensen E, Marco J, Nordrehaug JE, Ruzyllo W, Urban P, Stone GW, Wijns W (2005) Guidelines for percutaneous coronary interventions. The Task Force for Percutaneous Coronary Interventions of the European Society of Cardiology. Eur Heart J 26 (8):804–847
8. Sabatine MS, Cannon CP, Gibson CM, López-Sendón JL, Montalescot G, Theroux P, Lewis BS, Murphy SA, McCabe CH, Braunwald E (2005) Effect of clopidogrel pretreatment before percutaneous coronary intervention in patients with ST-elevation myocardial infarction treated with fibrinolytics: the PCI-CLARITY Study. JAMA 294(10):1224–1232
9. Berger PP, Steinhubl S (2002) Clinical implications of Percutaneous Coronary Intervention-Clopidogrel in Unstable angina to prevent Recurrent Events (PCI-CURE) Study: a US perspective. Circulation 106(17):2284–2287
10. PLAVIXTM, RxMed: Pharmaceutical Information, Sanofi/Bristol-Mayers Squibb www.RXlist.com
11. Pereillo JM, Maftouh M, Andrieu A, Uzabiaga MF, Fedeli O, Savi P, Pascal M, Herbert JM, Maffrand JP, Picard C (2001) Structure and stereochemistry of the active metabolite of clopidogrel. Drug Metab Dispos 30(11):1288–1295
12. Chapman MJ, Nguyen NQ, Fraser RJ (2007) Gastrointestinal motility nad prokinetics in the critically ill. Curr Opin Crit Care 13(2):187–194
13. Beckwith MC, Feddema SS, Barton RG, Graves C (2004) A guide to drug therapy in patients with enteral feeding tubes: dosage form selection and administration methods. Hosp Pharm 39(3):225–237
14. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA (2007) Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. J Am Coll Cardiol 49(14):1505–1516
15. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM (2003) Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 107(23): 2908–2929
16. O'Donoghue M, Wiviott SD (2006) Clopidogrel response variability and future therapies: clopidogrel: does one size fit all? Circulation 114(22):600–606
17. Taubert D, Kastrati A, Harlfinger S, Gorchakova O, Lazar A, von Beckerath N, Schömig A, Schömig E (2004) Pharmacokinetics of clopidogrel after administration of a high loading dose. Thromb Haemost 92(2):311–316
18. Gurbel PA, Bliden KP, Hayes KM, Yoho JA, Herzog WR, Tantry US (2005) The relation of dosing to clopidogrel responsiveness and the incidence of high posttreatment platelet aggregation in patients undergoing coronary stenting. J Am Coll Cardiol 45 (9):1392–1396
19. Ernesto O, Martin H, Ronald D (2007) Clopidogrel resistance. Heart Lung Circ 16(3):17–28
20. Michelson AD (2009) Methods for the measurement of platelet function. Am J Cardiol 103(3):20–26
21. Coulter SA, Cannon CP, Ault KA, Antman EM, Van de Werf F, Adgey AA, Gibson CM, Giugliano RP, Mascelli MA, Scherer J,

Barnathan ES, Braunwald E, Kleiman NS (2000) High levels of platelet inhibition with abciximab despite heightened platelet activation and aggregation during thrombolysis for acute myocardial infarction: results from TIMI (thrombolysis in myocardial infarction). Circulation 101(23):2690–2695
22. Vocilkova L, Opatrilova R, Sramek V (2009) Determination of clopidogrel by chromatography. Curr Pharm Anal 5(4):424–431
23. Caplain H (1999) Pharmacokinetics od clopidogrel. Semin Thromb Hemost 25(2):25–28
24. Nirogi RVS (2006) Quantification of clopidogrel in human plasma by sensitive liquid chromatography/tandem mass spectrometry. Mass Spectrom 20(11):1695–1700
25. Harlfinger S (2004) Pharmacokinetics of clopidogrel and its active matabolite in human plasma. Annual Scientific Session of the American College of Cardiology. New Orleans, LA. March 7–10
26. Kastrati A (2004) Are we making efficient use of clopidogrel? Eur Hear J 25(6):454–456
27. Collyer TC, Gray DJ, Sandhu R, Berridge J, Lyons G (2009) Assesment of platelet inhibition secondary to clopidogrel and aspirin therapy in preoperative acute surgical patients measured by Thrombelastography Platelet Mapping. Br J Anaesth 102 (4):492–498
28. Craft RM, Chavez JJ, Bresee SJ, Wortham DC, Cohen E, Carroll RC (2004) A novel modification of the Thrombelastograph assay, isolating platelet function, correlates with optical platelet aggregation. J Lab Clin Med 143(5):301–309
29. Bochsen L, Wiinberg B, Kjelgaard-Hansen M, Steinbruchel DA, Johanson PI (2007) Evaluation of TEG platelet mapping assay in blood donors. Thromb J 5(3):509–518
30. Hobson AR, Agarwala RA, Swallow RA, Dawkins KD, Curzen NP (2006) Thrombelastography: current clinical applications and its potential role in interventional cardiology. Platelets 17 (8):509–518
31. Gurbel PA, Tantry US (2007) Clopidogrel resistance? Thromb Res 120(3):311–321
32. Uchiyama S (2011) Clopidogrel resistance: identifying and overcoming a barrier to effective antiplatelet treatment. Cardiovasc Ther 29(6):100–111
33. Michelson AD (2004) Platelet function testing in cardiovascular diseases. Circulation 110(19):489–493
34. Nguyen TA, Diodati JG, Pharand CH (2005) Resistance to clopidogrel: a review of the evidence. J Am Coll Cardiol 45(8): 1157–1164
35. Heestermans CM, van Werkum JW, Seesing TH, von Beckerath CM, Hackeng CM, Schömig E, Verheugt FW, ten Berg JM (2008) Impaired bioavalability of clopidogrel in patients with ST-segment elevation myocardial infarction. Thromb Response 122(6):776–777
36. Preisman S, Kogan A, Itzkovsky K, Leikin G, Raanani E (2010) Modified Thromboelastography evaluation of platelet dysfunction in patients undergoing coronary artery surgery. Eur J Cardiothorac Surg 37(6):1367–1374
37. Blinden KP, DiCiara J, Tantry US, Bassi AK, Chaganti SK, Gurbel PA (2007) Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention. J Am Coll Cardiol 49(6):657–666
38. von Beckerath N, Taubert D, Pogatsa-Murray G, Schömig E, Kastrati A, Schömig A (2005) Absorption, metabolization, and antiplatelet effects of 300-, 600-, and 900-mg loading doses of clopidogrel: results of the ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect) Trial. Circulation 112 (19):2946–2950
39. Zafar MU, Farkouh ME, Fuster V, Chesebro JH (2009) Crushed clopidogrel administred via nasogastric tube has faster and greater absorption than oral whole tablets. J Interv Cardiol 22(4):385–389
40. Rafferty G, Tham TCK (2010) Endoscopically placed enteral feeding tubes. World J Gastrointest Endosc 2(5):155–164
41. Müller I, Seyfarth M, Rüdiger S, Wolf B, Pogatsa-Murray G, Schömig A (2001) Effect of a high loading dose of clopidogrel on platelet function in patients undergoing coronary stent placement. Heart 85(1):92–93
42. Deniz T, Agalar C, Agalar F, Comu FM, Caglayan O, Alpay Y, Saygun O (2010) The effect of hypothermia on splanchnic flows and lung in a two-hit hemorrhagic shock model. J Surg Res 158 (1):121–126
43. Croughwell ND, Newman MF, Lowry E et al (1997) Effect of temperature during cardiopulmonary bypass on gastric mucosal perfusion. Br J Anaesth 78:34–38
44. Thoren A, Elam M, Ricksten SE (2001) Jejunal mucosal perfusion is well maintained during mild hypothermic cardiopulmonary bypass in humans. Anesth Analg 92:5–11
45. Rokyta R Jr, Matejovic M, Krouzecky A, Opatrny K Jr, Ruzicka J, Novak I (2004) Effects of continuous venovenous haemofiltration-induced cooling on global haemodynamics, splanchnic oxygen and energy balance in critically ill patients. Nephrol Dial Transplant 19 (3):623–630
46. Tortorici MA, Kochanek PM, Poloyac SM (2007) Effects of hypothermia on drug disposition, metabolism, and response: a focus of hypothermia-mediated alterations on the cytochrome P450 enzyme system. Crit Care Med 35(9):2196–2204
47. Polderman KH (2009) Mechanisms of action, physiological effects, and complications of hypothermia. Crit Care Med 37(7): S186–S202
48. Cundrle I Jr, Sramek V, Zvonicek V (2010) Influence of temperature adjustment on thrombelasthography results. Intensive Care Med 36(2):152
49. Ruzicka J, Stengl M, Bolek L, Benes J, Matejovic M, Krouzecky A (2012) Hypothermic anticoagulation: testing individual responses to graded severe hypothermia with thromboelastography. Blood Coagul Fibrinolysis. Mar 3. [Epub ahead of print]
50. Kafian S, Mobarrez F, Kalani M, Wallén H, Samad BA (2011) Comparison of venous and arterial blood sampling for the assessment of platelet aggregation with whole blood impedance aggregometry. Scand J Clin Lab Invest 71(8):637–640
51. Durila M, Kalinčik T, Jurčenko S, Pelichovská M, Hadačová I, Cvachovec K (2010) Arteriovenous differences of hematological and coagulation parameters in patients with sepsis. Blood Coagul Fibrinolysis 21(8):770–774
52. Price MJ, Coleman JL, Steinhubl SR, Wong GB, Cannon CP, Teirstein PS (2006) Onset and offset of platelet inhibition after high-dose clopidogrel loading and standard daily therapy measured by a point-of-care assay in healthy volunteers. Am J Cardiol 98(5):681–684
53. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W, Braunwald E, Sabatine MS (2009) Cytochrome P-450 polymorphisms and response to clopidogrel. N Engl J Med 360(4):354–362
54. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Méneveau N, Steg PG, Ferrières J, Danchin N, Becquemont L, French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Investigators (2009) Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med 360(4):363–375
55. Brand JT, Close SL, Iturria SJ, Payne CD, Farid NA, Ernest CS 2nd, Lachno DR, Salazar D, Winters KJ (2007) Common

polymorhism of CYP2C19 and CYP2C9 affect the pharmacokinetc and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost 5(12):2429–2436

56. Steinhubl SR, Talley JD, Braden GA (2001) Point of care measured platelet inhibition correlates with a reduced risk of an adverse cardiac event after percutaneous coronary intervention: results of the GOLD(AU-Assessing Ultegra) multicenter study. Circulation 103(21):2528–2530
57. Rao SV, Melloni C, Myles-Dimauro S, Broderick S, Kosinski AS, Kleiman NS, Dzavik V, Tanguay JF, Chandna H, Gammon R, Rivera E, Alexander JH, Fier I, Roach J, Becker RC, EMINENCE Investigators (2010) Evaluation of a new heparin agent in percutaneous coronary intervention: results of the phase 2 evaluation of M118 IN pErcutaNeous Coronary intErvention (EMINENCE) Trial. Circulation 121(15):1713–1721
58. Mahan CE, Fanikos J (2011) New antithrombotics: the impact on global health care. Thromb Res 127:518–524
59. Christian W, Hamm CW, Bassand JP, Agewall S, Bax J, Boersma E, Bueno H, Caso P, Dudek D, Gielen S, Huber K, Ohman M, Petrie MC, Sonntag F, Uva MS, Storey RF, Wijns W, Zahger D (2011) ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J 1–56

7.21 Annex 21

Ruzsíková A, Součková L, Suk P, Opatřilová R, Kejdušová M, Šrámek V. Quantitative analysis of drug losses administered via nasogastric tube – In vitro study. International Journal of Pharmaceutics, 2015;478 (1), 368-371. Doi: 10.1016/j.ijpharm.2014.11.065

Summary

Purpose

The aim of this study was to quantify drug losses during administration through a nasogastric tube (NGT) and to evaluate the influence of different dosage forms and administration techniques on the amount of drug delivered. The study addressed a common but poorly investigated problem in intensive care, where inaccurate drug delivery may compromise treatment efficacy.

Methodology

An in vitro model was used to compare five administration methods and six oral dosage forms commonly administered via NGT. Drug losses were determined gravimetrically, and the suitability of individual administration techniques was systematically evaluated.

Results

Drug losses ranged from 4% to 38%, depending on the dosage form and administration method. Administration techniques involving crushing the medication and preparing the suspension directly in the syringe resulted in the lowest losses, whereas intact pellets frequently caused tube obstruction and were unsuitable for clinical practice. The greatest losses occurred during flushing through the nasogastric tube.

Conclusion

The study demonstrated that the administration technique significantly influences the amount of drug delivered via a nasogastric tube. These findings provide practical recommendations for optimising drug administration in critically ill patients and highlight the role of investigator-initiated research in improving the safe and effective use of established therapies in routine clinical practice.

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Quantitative analysis of drug losses administered via nasogastric tube – In vitro study

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ABSTRACT

Purpose: Drug administration through nasogastric tube (NGT) is a standard practice but the real amount of the delivered drug is unknown. Therefore, we designed a study to determine the losses of various dosage forms administered by different methods through NGT.

Methods: In vitro model was used. Five different administration methods (A–E) and six dosage forms (simple compressed tablets – T/S; film coated tablets – T/FC; enteric coated tablets – T/EC; capsules with powder filling – C/P; capsules containing extended release pellets – C/ER; capsules containing gastro-resistant pellets – C/GR) were investigated. Measurement was repeated six times for each drug-method combination. The overall losses were determined by gravimetry. In method A partial losses associated with each step of drug administration were also determined.

Results: Significant drug losses were measured (4–38%). Only methods A (crushing–beaker–syringe–water–NGT) and B (crushing–water–syringe–NGT) were suitable for administration of all tested dosage forms. Method B proved the most effective for all kinds of tablets and C/GR ($p < 0.05$) and tended to be more effective also for C/ER ($p = 0.052$) compared to method A. C/P showed minimal losses for both tested methods (B and E). Flushing of the drug through NGT causes major losses during drug administration compared to crushing and transfer ($p < 0.05$). All methods for intact pellets (C–E) were found inappropriate for clinical practice due to NGT clogging.

Conclusions: Choosing a suitable administration method can significantly affect the amount of drugs delivered through NGT.

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1. Introduction

Enteral feeding is the preferred route of nutrition in ICU patients (Kreymann et al., 2006). The frequent inability to swallow necessitates insertion of the orogastric or more frequently nasogastric tube (NGT) into the stomach (National Collaborating Centre for Acute Care (UK), 2006). However, many obstacles can hamper adequate delivery of medications to the gastrointestinal tract and their absorption when NGT is used – e.g., crushing and dissolving of the tablets can lead to significant losses. Furthermore, not all oral dosage forms are suitable for administering via NGT (e.g., slow release tablets, enteric coated tablets and irritating drugs) (Gilbar and Pract, 1999; Zhu and Zhou, 2013). Impaired stomach emptying, gut mucosa damage, intestinal and splanchnic hypoperfusion are further factors which can reduce drug bioavailability (Clarke, 2008; Gilbar and Pract, 1999; Součková et al., 2013). In practice, all these aspects result in a limited drug administration into gastrointestinal tract (do Nascimento et al., 2012).

From the vast spectrum of drugs administered via NGT in ICU patients, only the bioavailability of proton pump inhibitors (PPI) was extensively studied comparing intravenous versus gastric routes (Olsen and Devlin, 2008; Täubel et al., 2001), administration via NGT versus *per os* (Sostek et al., 2003) and evaluating modifications of NGT administration (Freston et al., 2004; Tsai et al., 2000).

Ponrouch et al. investigated in vitro the impact of various technical parameters on availability of four PPIs in pediatric

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practice. They found that diameter and type of PPI but not the length of the NGT influence the recovery of the active drug at the end of NGT (Ponrouch et al., 2010).

Devlin et al. also found a big difference between various PPIs and their forms in drug effectivity. The results were not influenced by nurse experience (Devlin et al., 2006).

Data on other drugs except of PPIs are limited. In an elegant combined in vitro and clinical study, Kotake et al. found that up to 30% of amiodarone is lost during drug administration via NGT (Kotake et al., 2006).

As adequate data on drug losses during preparation for NGT administration are lacking, we designed an in vitro study assessing the impact of dosage form and way of preparation on drug losses when administered via NGT.

2. Materials and methods

The whole experiment was performed by one investigator (1st author of the manuscript) using an in vitro model. All medications passed through 14F (i.e., 4.7 mm outer and 3.0 mm inner diameters, respectively) feeding tube (Medicoplast, Germany) mounted to a ring stand in 30° angle to mimic the most often ICU patient position. Measurement was repeated six times for each combination of drug and method. During drug administration to the tube, the mixture of water and the drug in the syringe was constantly shaken to prevent sticking of the drug particles to the inner walls of the syringe as much as possible. The flushed mixture of the drug and water was collected in an evaporating basin at the end of NGT and evaporated to dryness. The losses were determined by gravimetry (difference between the initial drug weight and the weight of drug following passing the NGT and evaporation). Ohaus Explorer E12145 analytical scales with accuracy of four decimal positions were used for gravimetric measurements.

Six different dosage forms were tested:

- T/S – simple compressed tablet (sotalol, Sotahexal®, Hexal AG, Germany).
- T/EC – tablet with enteric coating (pantoprazole, Controloc®, Takeda, Germany).
- T/FC – simple film-coated tablet (clopidogrel, Trombex®, Zentiva, Czech Republic).
- C/P – capsule with powder filling (lactobacilles, Lacidofil®, Institut Rosell, France).
- C/ER – capsule containing extended-release pellets (theophylline, Euphyllin CR N 300®, Nycomed, Germany).
- C/GR – capsule with gastro-resistant pellets (omeprazole, Helicid®, Zentiva, Czech Republic).

Following administration techniques were used: methods A and B were used for tablets (T/S, T/EC, T/ES), methods B and E were used for capsules with powder filling (C/P), and all methods for capsules with pellets (C/ER and C/GR).

2.1. Method A (crushing–beaker–syringe–water–NGT)

Technique consisted of further steps: crushing the tablets or pellets in a mortar, transferring the crushed powder into a beaker, transferring crushed powder from the beaker to a syringe, adding water (15 ml) into the syringe, mixing the solution in the syringe, injecting the mixture into NGT and flushing the tube with water (5 ml). This method was not used for capsules with powder filling (C/P). For this method, drugs were weighted after each step of administration to define its specific contribution to the overall loss.

2.2. Method B (crushing–water–syringe–NGT)

Tablets and pellets were crushed in a mortar, in case of the capsules containing powder, the powder was poured out into the mortar. Water (10 ml) was added to the mortar and the crushed powder was mixed with water, the mixture was drawn into a syringe, then it was injected into NGT. After injection, additional 10 ml of water were poured into the mortar, the solution was drawn in the syringe and the tube was flushed with the solution.

2.3. Method C (pellets–beaker–NGT–water)

Pellets were poured from the capsule into a small beaker with a spout. The pellets were then slowly poured from the beaker to NGT. After that, the tube was flushed with 20 ml of water.

2.4. Method D (pellets–NGT–water)

Pellets were poured from the capsule right into NGT. The tube was flushed with 20 ml of water.

2.5. Method E (pellets–syringe–water–NGT)

Pellets were poured into a syringe, water added, mixture was shaken and injected into NGT.

2.6. Statistical analysis

The statistical analysis was carried out using Statistica 11 software (StatSoft Inc., USA). Data are presented as mean and 95% confidence interval. Unpaired *t*-test was used to compare differences between groups, $p < 0.05$ was considered significant.

3. Results

Eighteen combinations of drug and method have been studied. Average losses for methods A and B are shown in Fig. 1. Method B has proven as the most effective for all kinds of tablets (T/S, T/EC, T/FC) and capsules with gastro-resistant pellets (C/GR). Similarly, capsules containing extended-release pellets (C/ER) tended to be more

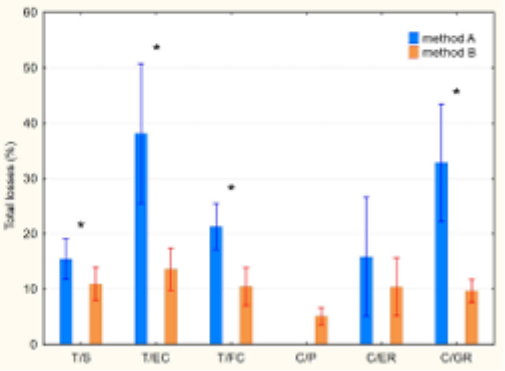


Fig. 1. Total losses (mean and 95% confidence interval) according to dosage form and administration technique by method A (crushing–beaker–syringe–water–NGT) and method B (crushing–water–syringe–NGT). Types of dosage forms: T/S – simple compressed tablet, T/EC – enteric coated tablet, T/FC – simple film coated tablet, C/P – capsule with powder filling (method B only), C/ER – capsule containing extended release pellets, C/GR – capsule containing gastro-resistant pellets. * means $p < 0001$ between methods A and B.

effectively delivered by method B than A ($p = 0.052$) and capsules with powder filling (C/P) have also showed minimal losses when administered by method B. All methods preserving intact pellets (C–E) were found inappropriate for clinical practice as NGT clogged during drug administration (methods C and D in 100% for both kinds of pellets, method E in 100% for C/ER and 33% for C/GR).

Proportion of particular steps of method A on total loss is presented in Fig. 2. Drug delivery through NGT (flushing) contributes more to the total losses than crushing and the transfer of drugs to the syringe ($p < 0.01$).

4. Discussion

We have demonstrated that significant drug losses can be detected when drugs are administered via NGT. Different dosage forms differ in the losses, which can be further influenced by administration method. Moreover pellets clog the NGT and therefore, are not suitable for NGT administration. All the steps during NGT drug administration are associated with losses. According to our subanalysis of method A, flushing of the dissolved drug suspension through the NGT plays the major role.

We showed that dissolving the crushed drug directly in the mortar diminishes losses that occur when the drug is transferred into the beaker and then dissolved. Evidently, some amount of powder remains in the mortar. Furthermore, repeated rinsing of the mortar with 10 ml of water, which was afterwards used for NGT flushing, probably increased the amount of delivered drug in method B. Interestingly, the custom to use either method A or B differs between various ICU/HDUs in our hospital.

Evidently, different dosage forms differ in their potential to be administered via NGT – e.g., a simple film coated tablet (T/FC) is more easily crushed in a mortar compared to a tablet with enteral coating (T/EC), where crushing requires high pressure and some pieces of the drug frequently flew off the mortar. Losses by crushing the drugs measured in our study (<10% of the drug) are in agreement with the losses associated with crushing in the study provided by Kotake (cca 7.4%) (Kotake et al., 2006).

Some drugs cannot be evidently administered through NGT because of clogging. This is true for pellets and even elaborated manipulations (method E – pellets poured into the syringe, mixing with water and then injecting) only diminish the risk of clogging which in clinical practice is unacceptably high (33.3%). Measurements were provided using a 14F feeding tube as this size of NGT is used most often in our ICU. The usage of large bore NGT (which is not risk free) could at least partially circumvent this drawback. Diameter of the tube also played an important role in the study of Ponrouch, where all the 6F tubes clogged (Ponrouch et al., 2010).

Our study has several limitations. Losses were measured by gravimetry, which does not measure only active substances of the drug. Therefore, we cannot separate losses of the active drug from additives (coating etc.). More sophisticated methods (mass spectrophotometry or high power liquid chromatography) can give more accurate answers (Devlin et al., 2006; Ponrouch et al., 2010).

The usage of a new NGT does not totally mimic clinical setting, because sticking of enteral nutrition on the inner surface of NGT can modify absorption of the drug.

Administration of the drug via NGT represents only the first step of the drug route to the site of action. Some other obstacles exist in ICU patients, primarily impaired gastrointestinal motility and absorption can further decrease bioavailability. Therefore, even positive results of this study cannot be directly transferred into clinical practice. Despite quite low losses during NGT administration, Trombex (clopidogrel) administration in ICU patients was associated with insufficient blood levels (Součková et al., 2013).

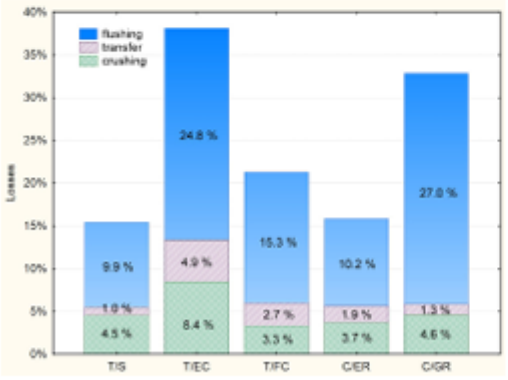


Fig. 2. Mean losses associated with particular steps (crushing, transfer, flushing) of drug administration via NGT by method A (crushing–beaker–syringe–water–NGT). Types of dosage forms: T/S – simple compressed tablet, T/EC – enteric coated tablet, T/FC – simple film coated tablet, C/ER – capsule containing extended release pellets, C/GR – capsule containing gastro-resistant pellets.

5. Conclusion

We have proved that the administration technique can significantly affect the amount of drug delivered through NGT. Regardless of dosage form, dissolving of crushed tablet or capsule directly in mortar seems the most promising method. Out of various dosage forms, pellets seem to be most tricky to administer through NGT.

Declaration of personal interests

None.

Declaration of funding interests

None.

References

Clarke, S., 2008. Drug administration via nasogastric tube. Paediatr. Nurs. 20, 32.
Devlin, J.W., Bakshi, A., Bungay, K., Olsen, K.M., 2006. An in vitro comparison of different providers to deliver four proton pump inhibitor products through a feeding tube. Aliment. Pharmacol. Ther. 24, 1603–1611. doi:http://dx.doi.org/10.1111/j.1365-2036.2006.03169.x.
do Nascimento, M.M.G., Reis, A.M.M., Wick, J.Y., Ribeiro, A.Q., 2012. Drug administration through feeding tubes; an integrated qualification program. Nutr. Hosp. 27, 1309–1313.
Freston, J.W., Kukulka, M.J., Lloyd, E., Lee, C., 2004. A novel option in proton pump inhibitor dosing: lansoprazole orally disintegrating tablet dispersed in water and administered via nasogastric tube. Aliment. Pharmacol. Ther. 20, 407–411. doi:http://dx.doi.org/10.1111/j.1365-2036.2004.02086.x.
Gilbar, P.J., Pract, D.H.C.P., 1999. A guide to enteral drug administration in palliative care. J. Pain Symptom Manag. 17, 197–207. doi:http://dx.doi.org/10.1016/S0885-3924(98)00124-9.
Kotake, T., Takada, M., Goto, T., Komamura, K., Kamakura, S., Morishita, H., 2006. Serum amiodarone and desethylamiodarone concentrations following nasogastric versus oral administration. J. Clin. Pharm. Ther. 31, 237–243. doi:http://dx.doi.org/10.1111/j.1365-2710.2006.00730.x.
Kreymann, K.G., Berger, M.M., Deutz, N.E.P., Hiesmayr, M., Jolliet, P., Kazandjiev, G., Nitenberg, G., van den Berghe, G., Wernerman, J., Ebner, C., Hartl, W., Heymann, C., Spies, C., 2006. ESPEN Guidelines on enteral nutrition: intensive care. Clin. Nutr. 25, 210–223. doi:http://dx.doi.org/10.1016/j.clnu.2006.01.021.
National Collaborating Centre for Acute Care (UK), 2006. Nutrition support for adults. Oral Nutrition Support, Enteral Tube Feeding and Parenteral Nutrition, National Institute for Health and Clinical Excellence: Guidance. National Collaborating Centre for Acute Care (UK), London.

Olsen, K.M., Devlin, J.W., 2008. Comparison of the enteral and intravenous lansoprazole pharmacodynamic responses in critically ill patients. Aliment. Pharmacol. Ther. 28, 326–333. doi:http://dx.doi.org/10.1111/j.1365-2036.2008.03728.x.

Ponrouch, M.P., Sautou-Miranda, V., Boyer, A., Bourdeaux, D., Montagner, A., Chopineau, J., 2010. Proton pump inhibitor administration via nasogastric tube in pediatric practice: comparative analysis with protocol optimization. Int. J. Pharm. 390, 160–164. doi:http://dx.doi.org/10.1016/j.ijpharm.2010.01.040.

Sostek, M.B., Chen, Y., Skammer, W., Winter, H., Zhao, J., Andersson, T., 2003. Esomeprazole administered through a nasogastric tube provides bioavailability similar to oral dosing. Aliment. Pharmacol. Ther. 18, 581–586. doi:http://dx.doi.org/10.1046/j.1365-2036.2003.01667.x.

Součková, L., Opatřilová, R., Suk, P., Čundrle Jr, I., Pavlík, M., Zvoníček, V., Hlinomaz, O., Šrámek, V., 2013. Impaired bioavailability and antiplatelet effect of high-dose clopidogrel in patients after cardiopulmonary resuscitation (CPR). Eur. J. Clin. Pharmacol. 69, 309–317. doi:http://dx.doi.org/10.1007/s00228-012-1360-0.

Täubel, J.J., Sharma, V.K., Chiu, Y.L., Lukasik, N.L., Pilmer, B.L., Pan, W.J., 2001. A comparison of simplified lansoprazole suspension administered nasogastrically and pantoprazole administered intravenously: effects on 24 h intragastric pH. Aliment. Pharmacol. Ther. 15, 1807–1817. doi:http://dx.doi.org/10.1046/j.1365-2036.2001.01098.x.

Tsai, W.L., Poon, S.K., Yu, H.K., Chang, C.S., Yeh, H.Z., Ko, C.W., Chen, G.H., 2000. Nasogastric lansoprazole is effective in suppressing gastric acid secretion in critically ill patients. Aliment. Pharmacol. Ther. 14, 123–127. doi:http://dx.doi.org/10.1046/j.1365-2036.2000.00680.x.

Zhu, L.-L., Zhou, Q., 2013. Therapeutic concerns when oral medications are administered nasogastrically. J. Clin. Pharm. Ther. 38, 272–276. doi:http://dx.doi.org/10.1111/jcpt.12041edit.

7.22 Annex 22

Blahynková H, Součková L, Papiež A. Patient and Public Involvement v klinickém výzkumu: od regulatorních požadavků k partnerské spolupráci s pacienty. Klin Farmakol Farm. 2026;40(2):112-115 | DOI: 10.36290/far.2026.025

Summary

Purpose:

The article aimed to present the concept of Patient and Public Involvement (PPI) in clinical research and to demonstrate its evolution from a regulatory expectation to a genuine partnership between patients and researchers. It discusses the role of patient involvement in improving the relevance, quality, feasibility, and ethical conduct of clinical research while aligning with modern regulatory frameworks, including ICH GCP R3, Quality by Design, and the revised Declaration of Helsinki.

Methodology:

This publication is a narrative review that synthesises current international regulatory guidance, ethical principles, and published evidence on Patient and Public Involvement. It describes the integration of patients throughout the clinical research lifecycle, including research prioritisation, protocol development, endpoint selection, study documentation, patient-reported outcomes, and dissemination of research results.

Results:

The review demonstrates that meaningful patient involvement contributes to more patient-centred clinical trial design, improves recruitment and participant retention, enhances the relevance of study endpoints, and supports the development of understandable study materials. It also discusses the role of patient advocates, identifies barriers to effective implementation such as tokenism and insufficient institutional expertise, and highlights the need for education and structured frameworks to enable meaningful partnerships between researchers and patients.

Conclusion:

The publication highlights Patient and Public Involvement as a fundamental component of contemporary clinical research that extends beyond regulatory compliance to become an essential element of high-quality, ethical, and patient-centred research. It demonstrates that meaningful partnerships with patients improve study design, facilitate the translation of research into clinical practice, and strengthen evidence generation, reinforcing the central role of patient engagement within the modern clinical research ecosystem.

Patient and Public Involvement v klinickém výzkumu: od regulatorních požadavků k partnerské spolupráci s pacienty

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Klinický výzkum se v posledních letech stále více orientuje na systematické zapojení pacientů a veřejnosti (Patient and Public Involvement, PPI), přičemž pacient již není vnímán pouze jako účastník studie, ale také jako aktivní partner podílející se na plánování výzkumu. Tento přístup zvyšuje relevanci výzkumných otázek, zlepšuje proveditelnost studií i využitelnost jejich výsledků v klinické praxi. Principy zapojení pacientů jsou reflektovány v moderních metodologických a regulatorních rámcích, včetně zásad International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice (ICH GCP R3), Quality by Design či nejnovější revizi Declaration of Helsinki.

Zapojení pacientů je dnes zároveň často vyžadováno nebo výrazně preferováno ve veřejných grantových programech, například v rámci Horizon Europe, National Institute for Health and Care Research nebo Patient-Centered Outcomes Research Institute. Pacienti mohou přispět k formulaci výzkumných otázek, výběru relevantních cílových ukazatelů, zlepšení srozumitelnosti studijních dokumentů i ke snížení zátěže účastníků studií.

Důležitým prostředníkem mezi pacienty a výzkumníky může být pacientský advokát, který podporuje smysluplné zapojení pacientů v jednotlivých fázích výzkumu. Systematické PPI tak představuje důležitý posun směrem ke kvalitnějšímu a etičtějšímu klinickému výzkumu.

Klíčová slova: Patient and Public Involvement, klinický výzkum, design klinických hodnocení, pacientský advokát, patient-reported outcomes.

Patient and Public Involvement in clinical research: from regulatory requirements to partnerships with patients

In recent years, clinical research has increasingly focused on the systematic involvement of patients and the public (Patient and Public Involvement, PPI), with patients no longer viewed merely as study participants but also as active partners contributing to research planning. This approach enhances the relevance of research questions, improves the feasibility of studies, and increases the applicability of their results in clinical practice. The principles of patient involvement are reflected in modern methodological and regulatory frameworks, including the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice (ICH GCP R3), Quality by Design, and the latest revision of the Declaration of Helsinki.

Patient involvement is also frequently required or strongly preferred in public grant programs today, such as Horizon Europe, the National Institute for Health and Care Research, or the Patient-Centered Outcomes Research Institute. Patients can contribute to formulating research questions, selecting relevant endpoints, improving the clarity

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The manuscript is original and has not been published or submitted elsewhere.

Ethical principles compliance:
The authors attest that their study was approved by the local Ethical Committee and is in compliance with human studies and animal welfare regulations of the authors' institutions as well as with the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects adopted by the 18th WMA General Assembly in Helsinki, Finland, in June 1964, with subsequent amendments, as well as with the ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, updated in December 2018, including patient consent where appropriate.

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of study documents, and reducing the burden on study participants.
A patient advocate can serve as an important intermediary between patients and researchers, supporting meaningful patient involvement at each stage of research. Systematic PPI thus represents an important step toward higher-quality and more ethical clinical research.

Key words: Patient and Public Involvement, clinical research, clinical trial design, patient advocate, patient reported outcomes.

Proměna role pacientů v klinickém výzkumu

Moderní klinická farmakologie a vývoj léčiv postupně opouštějí tradiční paternalistický model klinického výzkumu, v němž byl pacient vnímán především jako účastník studie a zdroj dat. Rostoucí komplexita studií, důraz na klinickou relevanci a výzvy spojené s náborem a retencí účastníků vedly k přehodnocení této role. Do popředí se proto dostává systematické zapojení pacientů a veřejnosti do výzkumu (Patient and Public Involvement, PPI), které redefinuje roli pacienta z pasivního účastníka klinického hodnocení na aktivního partnera a spolutvůrce výzkumného procesu (1–4). Význam PPI je dnes zohledněn také v moderních metodologických a regulatorních rámcích klinického výzkumu (1, 5), současně je zapojení pacientů stále častěji požadováno nebo výrazně podporováno v rámci veřejných výzkumných grantů a mezinárodních výzkumných programů.

Cílem tohoto článku je představit koncept zapojení pacientů do klinického výzkumu, popsat jeho přínosy pro design a realizaci klinických studií a diskutovat roli pacientského advokáta jako důležitého prostředníka mezi pacientskou komunitou a výzkumníky.

Regulatorní a etické ukotvení zapojení pacientů

Zapojení pacientů do klinického výzkumu není pouze konceptuální změnou v přístupu k výzkumu, ale je stále více zakotveno také v regulatorních, etických a metodologických rámcích moderního klinického hodnocení (2, 6). Oblast PPI prochází v posledních letech dynamickým evolučním vývojem jak na mezinárodní úrovni, tak v prostředí České republiky. Aktualizovaná verze ICH GCP R3 představuje výrazný milník v tomto procesu (1, 7, 8) a zdůrazňuje orientaci na pacienta, integraci moderních technologií a princip kvality budované již ve fázi návrhu studie. Koncept Quality by Design, který je v této směrnici opakovaně uváděn, představuje zásadní změnu v chápání kvality klinických hodnocení. Kvalita již nemá být zajišťována primárně prostřednictvím kontrol a auditů, ale musí být systematicky integrována do návrhu studie již v raných fázích její přípravy. Zapojení pacientů v této fázi umožňuje včas identifikovat prvky protokolu, které mohou být nadměrně zatěžující, logisticky obtížně proveditelné nebo klinicky málo relevantní. Spolupráce s pacienty může vést k optimalizaci studijních návštěv, odběrů biologického materiálu i využití decentralizovaných prvků studie (9).

Vedle klinických cílů pacienti často upozorňují na praktické aspekty účasti ve studii, jako je cestování, administrativní zátěž, invazivita vyšetření či dopad na kvalitu života (10). Integrace těchto perspektiv může přispět k realističtějšímu designu klinických studií a zlepšení jejich proveditelnosti.

Směrnice ICH GCP R3 reflektuje technologický pokrok jako klíčový prostředek k posílení patient-centric přístupu v klinickém výzkumu. Využití digitálních technologií, včetně umělé inteligence, nositelné elektroniky, elektronických informovaných souhlasů nebo vzdáleného monitorování umožňuje realizaci decentralizovaných a hybridních klinických studií, které mohou významně snížit zátěž pacientů a zvýšit dostupnost výzkumu pro širší populaci. Elektronické informované souhlasy představují důležitý nástroj pacientsky orientovaného výzkumu. Jejich úspěšné využití však vyžaduje jednoduché a bezpečné ověření identity.

Význam zapojení pacientů je současně zdůrazněn i v etických dokumentech upravujících klinický výzkum. Nejnovější revize Declaration of Helsinki, přijatá World Medical Association v roce 2024, zdůrazňuje význam respektu k účastníkům výzkumu a jejich komunitám a podporuje jejich zapojení do různých fází výzkumného procesu, včetně návrhu studie a šíření výsledků.

Rostoucí význam zapojení pacientů je patrný také v oblasti financování výzkumu. Mnohé veřejné grantové agentury dnes požadují nebo významně podporují smysluplné zapojení pacientů či pacientských advokátů do výzkumných projektů. Tento požadavek je explicitně formulován například v rámci programů Horizon Europe (11), britského National Institute for Health and Care Research nebo amerického Patient-Centered Outcomes Research Institute.

Navzdory rostoucímu významu PPI však oblast jeho praktické implementace zůstává v mnoha zemích, včetně České republiky, ve fázi postupného budování zkušeností a institucionální expertizy. Zatímco některé zahraniční systémy již disponují etablovanými modely participace pacientů, česká nemocniční a akademická centra teprve vytvářejí praktické mechanismy pro systematické zapojování pacientů do výzkumu. V tomto kontextu nabývá na významu sdílení zkušeností mezi institucemi, inspirace mezinárodními frameworky a adaptace ověřených přístupů na podmínky českého zdravotnického a výzkumného prostředí. Postupné budování praktické expertizy, vzdělávání pacientských zástupců i výzkumných týmů a rozvoj partnerské spolupráce tak představují klíčové předpoklady pro dlouhodobě udržitelnou implementaci PPI do klinického výzkumu.

Přínosy zapojení pacientů pro klinický výzkum

1) Zapojení pacientů do přípravy a realizace klinických studií zvyšuje jejich relevanci a proveditelnost. Pacienti přinášejí perspektivu reálné zkušenosti s onemocněním, která doplňuje odborný pohled výzkumníků a pomáhá formulovat výzkumné otázky.

Jedním z klíčových přínosů zapojení pacientů je možnost lépe definovat výzkumné otázky tak, aby odrážely skutečné potřeby pacientské populace. Zatímco výzkumníci se často zaměřují na biologické mechanismy onemocnění nebo regulatorní požadavky, pacienti mohou upozornit na aspekty onemocnění, které mají zásadní dopad na jejich každodenní život (12, 13), funkční schopnosti nebo kvalitu života. Zapojení pacientů tak může přispět k identifikaci dosud nenaplněných potřeb (unmet needs) a pomoci zaměřit výzkum na klinicky relevantní problémy.

2) Design klinických studií

Pacienti mohou rovněž významně přispět k optimalizaci designu klinických studií. Jejich perspektiva může být cenná při výběru klinicky významných endpointů (4, 12), zejména v případě ukazatelů zaměřených na kvalitu života nebo funkční stav pacientů (11). Současně mohou pomoci identifikovat prvky protokolu, které představují nadměrnou zátěž pro účastníky, například časté studijní návštěvy, invazivní vyšetření nebo logisticky náročné postupy.

Zohlednění těchto faktorů může vést k realističtějšímu nastavení studijních procedur a přispět ke zlepšení náboru a retence účastníků (1). Praktické zkušenosti ukazují, že studie, jejichž design reflektuje potřeby a možnosti pacientů, dosahují často vyšší míry náboru i lepší adherence k protokolu.

3) Studijní dokumenty a komunikace s účastníky

Materiály určené účastníkům klinických hodnocení a náborové dokumenty představují klíčové rozhraní mezi výzkumným týmem a pacientem. Jejich kvalita, srozumitelnost a praktická použitelnost mají přímý dopad na úspěšnost náboru, informovanost účastníků i jejich dlouhodobou spolupráci ve studii. Zapojení pacientů do jejich přípravy umožňuje identifikovat nejasné formulace, nadměrnou administrativní zátěž a postupy, které jsou v reálných podmínkách obtížně proveditelné. Pacienti mohou například pomoci upravit jazyk informovaných souhlasů tak, aby byl srozumitelný pro širší populaci, nebo upozornit na informace, které jsou pro potenciální účastníky studie zvláště důležité.

Zapojení pacientů při vývoji nástrojů hodnocení výsledků

Jedním z klíčových aspektů patient-centric přístupu v klinickém vývoji je validace nástrojů pro hodnocení výsledků (Clinical Outcome Assessments, COA), zejména pacientem hlášených výstupů (Patient-Reported Outcomes, PRO). Regulační autority považují rané fáze klinického vývoje (fáze I–II) za optimální období pro ověření jejich „vhodnosti pro daný účel" (fit-for-purpose) (7, 13, 14). Hodnocení srozumitelnosti dotazníku až v registrační studii může představovat významné vědecké i regulatorní riziko, protože případné nedostatky mohou negativně ovlivnit interpretaci klíčových cílových ukazatelů.

V raných fázích vývoje se proto využívají kognitivní rozhovory (3, 14) (cognitive interviews, cognitive debriefing), jejichž cílem je ověřit, že pacienti správně rozumějí instrukcím, jednotlivým položkám i odpovědním škálám a že hodnocené parametry odpovídají jejich zkušenosti s onemocněním. Tento postup umožňuje včas identifikovat nejasné formulace, nevhodně nastavené škály či nepřiměřené období pro vybavení si příznaků (recall period), odhalit rozdíly v interpretaci mezi skupinami pacientů a snížit zátěž respondentů (13). Včasné testování tak významně snižuje regulatorní riziko a posiluje vědeckou robustnost klinického programu.

V pediatrické populaci vyžaduje testování srozumitelnosti vývojově přiměřený přístup. U mladších dětí se používají jednodušší jazykové formulace, obrázkové škály nebo alternativní techniky, například formou hry či kresby. Současně se ověřuje vhodnost hodnoticích škál i nastavení recall period. Pokud to věk a kognitivní schopnosti umožňují, dítě by mělo odpovídat samostatně. Není-li spolehlivý self-report možný, přechází se k hodnocení pečovatelem (Observer-Reported Outcome, ObsRO), které se zaměřuje pouze na objektivně pozorovatelné projevy.

Role pacientského advokáta v klinickém výzkumu

Klíčovou postavou PPI je pacientský advokát, jehož roli je nutné přesně vymezit. V kontextu klinického výzkumu nejde o právního zástupce, ale o školeného experta s přímou životní zkušeností s konkrétním onemocněním a jeho léčbou anebo znalostí procesů klinického výzkumu. Na rozdíl od běžného účastníka klinické studie, který zůstává zdrojem dat, vystupuje pacientský advokát jako aktivní člen výzkumného týmu, často v roli Patient Research Partner (5, 11).

Pacientský advokát přináší do výzkumu perspektivu pacientské komunity a pomáhá identifikovat aspekty, které jsou pro nemocné skutečně významné. Vedle tradičních klinických ukazatelů se tak do popředí dostávají parametry kvality života, funkční zdatnosti, psychosociálních dopadů léčby a zvládnutelnosti nežádoucích účinků. Tyto faktory mohou zásadně ovlivňovat nejen ochotu pacientů účastnit se klinických studií, ale i jejich důvěru ve smysl výzkumu a praktickou využitelnost výsledků.

Pacientské organizace představují důležitý zdroj zpětné vazby a zkušeností pacientské komunity. Jejich zapojení podporuje komunikaci mezi pacienty a zdravotnickými pracovníky a přispívá k rozvoji péče orientované na potřeby pacientů.

Výzvy a limity zapojení pacientů

Navzdory rostoucí podpoře PPI přetrvávají významné překážky jeho efektivní implementace, včetně dominance vědeckého žargonu, nerovné dynamiky moci a omezené diverzity zapojených pacientů. V některých případech může zapojení pacientů zůstávat spíše formální než skutečně partnerské.

V literatuře je tento fenomén popisován jako riziko tokenismu, kdy jsou pacienti do výzkumu zapojováni primárně za účelem naplnění formálních požadavků grantových schémat či institucionálních standardů, aniž by měli reálný vliv na rozhodovací procesy (7, 14), definování výzkumných priorit nebo interpretaci výsledků. Takový přístup může oslabovat důvěru pacientské komunity a snižovat skutečný přínos participace pro kvalitu výzkumu.

Další významnou výzvu představuje riziko kooptace pacientských zástupců, kdy pacient postupně přebírá perspektivu výzkumného týmu a ztrácí schopnost reprezentovat zkušenosti širší pacientské komunity (1). Další výzvou je opakované zapojování stejného okruhu zkušených pacientských zástupců, což může vést k omezené reprezentativnosti

pacientské populace. K odlišení smysluplného zapojení od symbolického přístupu přispívají standardizační nástroje, jako jsou směrnice GRIPP2, a vzdělávací iniciativy zaměřené na rozvoj kompetencí pacientských zástupců, jako je EUPATI. Přístup k PPI do výzkumu vykazuje v evropském prostoru značnou variabilitu. Severské země patří dlouhodobě mezi lídry, zatímco ve střední Evropě, včetně České republiky a Německa, se PPI postupně institucionalizuje (3, 8). Významným krokem bylo založení národní platformy EUPATI v roce 2022, která vytvořila rámec pro vzdělávání pacientů v oblasti klinického výzkumu, vývoje léčiv a regulace. Společně s dalšími iniciativami, například CAATS při Masarykově univerzitě, které se ve spolupráci s Centrem precizní medicíny ve Fakultní nemocnici Brno zaměřuje na identifikaci dostupných terapeutických možností pro dětské pacienty s velmi vzácnými onemocněními a současně podporuje komunikaci mezi pacienty, klinickými pracovišti a výzkumnými týmy. Podobné iniciativy mohou přispět nejen k lepší orientaci pacientů v možnostech léčby a klinického výzkumu, ale také k posílení jejich role jako informovaných partnerů v procesu vývoje a hodnocení nových terapií.

Zkušenosti z praxe ukazují, že včasné zapojení pacientů vede k hmatatelným změnám v designu studií, snížení zátěže účastníků a zvýšení etické přijatelnosti i efektivity klinického výzkumu (15). Zohlednění perspektivy pacientů již ve fázi návrhu studií zároveň umožňuje generovat data, která jsou lépe využitelná pro hodnocení zdravotnických technologií a rozhodování o úhradách. Parametry kvality života a funkční přínosy léčby se tak stávají důležitým mostem mezi klinickým výzkumem, regulací a reálnou dostupností inovativních terapií pro pacienty. Současně roste zájem o hodnocení skutečného dopadu participace pacientů na kvalitu a efektivitu výzkumu. Jedním z přístupů je systematické dokumentování změn ve studijních protokolech iniciovaných pacienty, například úprav studijních návštěv, administrativní zátěže nebo informovaných souhlasů, které umožňuje lépe posoudit přínos PPI pro realizaci klinických studií.

Závěr

Zapojení pacientů podle principů ICH GCP R3 představuje významnou změnu v klinickém výzkumu. Moderní technologie, náborové strategie i materiály pro účastníky by měly vznikat ve spolupráci s pacienty, aby odpovídaly jejich potřebám a zkušenostem.

Smysluplná participace pacientů vyžaduje transparentní komunikaci, respekt k jejich zkušenostem a reprezentativní zastoupení různých skupin pacientů. Pouze za těchto podmínek může být PPI skutečným partnerstvím, které přispívá ke kvalitě, etické integritě a dlouhodobé udržitelnosti klinického výzkumu.

LITERATURA

1. Egher C, Zvonareva O. Knowledge-based Representation: Patient Engagement in Drug Development. Health Expect. 2024;27(1):e13912.
2. Geißler J, Isham E, Hickey G, et al. Patient Involvement in Clinical Trials. Commun Med. 2022;2(1):94.
3. U.S. Food and Drug Administration (FDA). Patient-Focused Drug Development: Collecting Comprehensive and Representative Input [Internet]. FDA; 2020 [cited 11-02-2026]. Available from: http://resource.nlm.nih.gov/9918230998606676.
4. U.S. Food and Drug Administration (FDA). Patient-Focused Drug Development: Incorporating Clinical Outcome Assessments Into Endpoints for Regulatory Decision-Making [Internet]. FDA; 2023 [cited 11-02-2026]. Available from: https://www.hhs.gov/guidance/document/patient-focused-drug-development-incorporating-clinical-outcome-assessments-endpoints.
5. Amjad A, Ratcliffe N, Smith AL, et al. Patient and Public Involvement in Laboratory Based Research: Reflections on Six Studies.
6. Arumugam A, Phillips LR, Moore A, et al. Patient and Public Involvement in Research: A Review of Practical Resources for Young Investigators. BMC Rheumatol. 2023;7(1):2.
7. The Lancet Healthy Longevity. Increasing Patient and Public Involvement in Clinical Research. Lancet Healthy Longev. 2024;5(2):e83.
8. Tong A, Scholes-Robertson N, Hawley C, et al. Patient-Centred Clinical Trial Design. Nat Rev Nephrol. 2022;18(8):514-523.
9. Jackson-Perry D, Cart-Richter E, Haerry D, et al. Patient and Public Involvement in HIV Research: A Mapping Review and Development of an Online Evidence Map. J Int AIDS Soc. 2024;27(11):e26385.
10. U.S. Food and Drug Administration (FDA). Patient-Focused Drug Development: Methods to Identify What Is Important to Patients [Internet]. FDA; 2022 [cited 11-02-2026]. Available from: https://www.hhs.gov/guidance/document/patient-focused-drug-development-methods-identify-what-important-patients-guidance.
11. U.S. Food and Drug Administration (FDA). Patient-Focused Drug Development: Selecting, Developing, or Modifying Fit-for-Purpose Clinical Outcome Assessments [Internet]. FDA; 2022 [cited 11-02-2026]. Available from: https://www.hhs.gov/guidance/document/patient-focused-drug-development-methods-identify-what-important-patients-guidance.
12. Daws SA, Bassi A, Buckland S, et al. Characterising Meaningful Patient and Public Involvement in the Pharmaceutical Industry Research Setting: A Retrospective Quality Assessment. BMJ Open. 2023;13(8):e071339.
13. Sand AS, Grimsgaard S, Pettersen I. Patient and Public Involvement in Health Research: A Nordic Perspective. Scand J Public Health. 2020;48(1):119-121.
14. Schilling I, Herbon C, Jilani H, et al. Aktive Beteiligung von Patientinnen an klinischer Forschung – Eine Einführung. Z Evid Fortbild Qual Gesundhwes. 2020;155:56-63.
15. Zeissler ML, Bakshi N, Bartlett M, et al. Patient and Public Involvement and Engagement in the Development of a Platform Clinical Trial for Parkinson's Disease: An Evaluation Protocol. J Parkinsons Dis. 2024;14(4):809-821.

8 Conclusion

This habilitation thesis examined IICTs from a system perspective, extending beyond the traditional focus on individual studies, methodological approaches, or regulatory requirements. Across the different areas addressed, it shows that the value and translational potential of NCCTs depend not solely on scientific excellence, but on the interaction of rigorous methodology, proportionate regulation, sustainable research infrastructures, multidisciplinary expertise, meaningful patient involvement, transparent evidence generation and dissemination, and effective pathways for implementation into healthcare.

The publications included in this thesis illustrate the broad spectrum of contributions made by NCCTs. They demonstrate how NCCTs and related concrete IICTs can contribute to the optimisation of existing therapies, comparative evaluation of therapeutic strategies, development of validation of approaches support precision medicine, investigation of ATMPs, and generation of evidence addressing clinically relevant questions arising from routine healthcare. Beyond individual clinical studies, the work presented also addresses the conditions necessary for such research to be conducted effectively, including research capacity, regulatory support, collaborative infrastructures, and patient involvement. Together, these contributions illustrate the complementary role of IICTs within the wider clinical research landscape, particularly in addressing questions driven by clinical need and public-health relevance.

A central perspective emerging from this thesis is an integrated view of IICTs within the broader European clinical research ecosystem. Rather than viewing methodology, regulation, research infrastructures, professional competencies, evidence transparency, patient engagement, and implementation as independent components, the work presented demonstrates their interdependence across the research continuum – from the formulation of clinically relevant questions and generation of robust evidence to its interpretation, dissemination, and potential implementation in healthcare. This systems perspective shifts the focus from success of an individual clinical trial towards the conditions required for clinically relevant research to generate sustainable value beyond the study itself.

An important implication of this perspective is that the generation of scientifically robust evidence represents a necessary, but not sufficient, condition for research impact. Evidence must also be accessible and transparent, sufficiently relevant to patients and clinical practice, and capable of being interpreted and integrated within healthcare decision-making. Strengthening NCCTs therefore requires investment not only in individual studies, but in the ecosystem that enables them: methodological and regulatory expertise, multidisciplinary research teams, national and European research infrastructures, professional education and capacity building, meaningful patient involvement, and mechanisms supporting evidence translation and implementation. As clinical research continues to evolve through precision medicine, advanced therapies, real-world data and evidence, artificial intelligence, and increasing integration between research and healthcare delivery, investigator-initiated clinical research

will continue to have an important complementary role within the evolving research landscape. Its close connection to clinical practice enables clinically driven questions to be investigated across the therapeutic lifecycle, including questions of comparative effectiveness, optimisation of established interventions, treatment individualisation, and areas in which evidence gaps persist despite limited commercial incentives. At the same time, emerging methodological and technological approaches create opportunities to connect clinical trials more closely with data generated in routine care and to support iterative cycles of evidence generation, evaluation, and refinement.

Ultimately, this thesis supports the recognition of IICTs not as a collection of isolated research activities, but as an integral component of a continuously learning clinical research and healthcare ecosystem.

Its contribution begins with clinically relevant uncertainty: transforming unanswered questions from clinical practice into methodologically rigorous research, generating transparent and clinically meaningful evidence, and creating the conditions for that evidence to inform subsequent healthcare decisions. Realising this potential requires the interaction of investigators, patients, healthcare professionals, research infrastructures, regulators, funders, and healthcare systems across national and European levels.

Within such an ecosystem, the value of IICTs extends beyond the individual trial. It lies in the capacity to connect clinical need with evidence generation and evidence with practice, while continuously returning new uncertainties from healthcare to research. Strengthening this cycle is essential for a sustainable, responsive, and patient-centred clinical research environment capable of addressing questions that matter to patients, healthcare professionals, and society.

Bibliography

1. Kandi V, Vadakedath S. Clinical Trials and Clinical Research: A Comprehensive Review. *Cureus*. Published online February 16, 2023. doi:10.7759/cureus.35077

2. Joshi G, Bhandari TK, Joshi P, et al. The Current Landscape of Clinical Trials. *J Clin Med*. 2025;14(7):2519. doi:10.3390/jcm14072519

3. Markey N, Howitt B, El-Mansouri I, Schwartzenberg C, Kotova O, Meier C. Clinical trials are becoming more complex: a machine learning analysis of data from over 16,000 trials. *Sci Rep*. 2024;14(1):3514. doi:10.1038/s41598-024-53211-z

4. Konwar M, Bose D, Gogtay N, Thatte U. Investigator-initiated studies: Challenges and solutions. *Perspect Clin Res*. 2018;9(4):179. doi:10.4103/picr.PICR_106_18

5. Fuentes Camps I, Rodríguez A, Agustí A. Non-commercial *vs* . commercial clinical trials: a retrospective study of the applications submitted to a research ethics committee. *Br J Clin Pharmacol*. 2018;84(6):1384-1388. doi:10.1111/bcp.13555

6. Hoffmann JM, Bauer A, Grossmann R. Academic vs. industry-sponsored trials: A global survey on differences, similarities, and future improvements. *J Glob Health*. 2024;14:04204. doi:10.7189/jogh.14.04204

7. Diallo A, Trøseid M, Simensen VC, et al. Accelerating clinical trial implementation in the context of the COVID-19 pandemic: challenges, lessons learned and recommendations from DisCoVeRy and the EU-SolidAct EU response group. *Clin Microbiol Infect*. 2022;28(1):1-5. doi:10.1016/j.cmi.2021.10.011

8. Ecrin | Facilitating European Clinical Research. Accessed July 20, 2026. https://ecrin.org/

9. Salmanton-García J, Wipfler P, Valle-Simón P, et al. VACCELERATE Site Network: Real-time definition of clinical study capacity in Europe. *Vaccine*. 2023;41(26):3915-3922. doi:10.1016/j.vaccine.2023.05.006

10. Djurisic S, Rath A, Gaber S, et al. Barriers to the conduct of randomised clinical trials within all disease areas. *Trials*. 2017;18(1):360. doi:10.1186/s13063-017-2099-9

11. World Health Organization. Global action plan for clinical trial ecosystem strengthening. Published online 2025.

12. Blümle A, Wollmann K, Bischoff K, et al. Investigator initiated trials versus industry sponsored trials - translation of randomized controlled trials into clinical practice (IMPACT). *BMC Med Res Methodol*. 2021;21(1):182. doi:10.1186/s12874-021-01359-x

13. Del Álamo M, Lémeret S, Nieto C, et al. Funding multinational investigator-initiated clinical studies in Europe: why and how? *Trials*. 2024;25(1):689. doi:10.1186/s13063-024-08548-1

14. Capili B, Anastasi JK. Pragmatic Clinical Trials: A Study Design for Real-World Evidence. *AJN Am J Nurs*. 2025;125(2):56-58. doi:10.1097/AJN.0000000000000014

15. Merali Z, Wilson JR. Explanatory Versus Pragmatic Trials: An Essential Concept in Study Design and Interpretation. *Clin Spine Surg Spine Publ*. 2017;30(9):404-406. doi:10.1097/BSD.0000000000000588

16. Uittenboogaard A, Mageto S, Mohamed SKE, et al. Investigator-Initiated Clinical Pharmacokinetic Studies in Resource-Limited Settings: Minimal Requirements and Practical Guidance. *J Clin Pharmacol*. 2025;65(6):675-687. doi:10.1002/jcph.6184

17. Wichman C, Smith LM, Yu F. A framework for clinical and translational research in the era of rigor and reproducibility. *J Clin Transl Sci*. 2021;5(1):e31. doi:10.1017/cts.2020.523

18. Mrudula Dhengale*1 HB. Challenges and Innovations in Conducting Clinical Trials in Rare and Orphan Diseases. Published online May 18, 2025. doi:10.5281/ZENODO.15459246

19. Rare Diseases Clinical Trials Toolbox | Ecrin. Accessed July 20, 2026. https://ecrin.org/rare-diseases-clinical-trials-toolbox

20. European Medicine Agency. ICH E6(R3) Guideline for Good Clinical Practice. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e6r3-guideline-good-clinical-practice-step-5_en.pdf

21. Wang F, Wang X, Liu E, Song F, Chen Y. Impacts of clinical research units on clinical research — a systematic review of empirical studies. *Syst Rev*. 2025;14(1):94. doi:10.1186/s13643-025-02813-3

22. Klatte K, Subramaniam S, Benkert P, et al. Development of a risk-tailored approach and dashboard for efficient management and monitoring of investigator-initiated trials. *BMC Med Res Methodol*. 2023;23(1):84. doi:10.1186/s12874-023-01902-y

23. Competencies for Australian Academic Clinical Trialists.

24. Madeira C, Pais A, Kubiak C, Demotes J, Monteiro EC. Investigator-initiated clinical trials conducted by the Portuguese Clinical Research Infrastructure Network (PtCRIN). *Contemp Clin Trials Commun*. 2016;4:141-148. doi:10.1016/j.conctc.2016.08.002

25. European Commission. REGULATION (EU) 2016/679 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation) (Text with EEA relevance). https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R0679

26. Smith J. FAST-EU pilot delivers first results: 70-Day approval timelines prove feasible ahead of Biotech Act. https://www.regulatoryrapporteur.org/industry-news/fast-eu-pilot-delivers-first-results-70-day-approval-timelines-prove-feasible-ahead-of-biotech-act/1142.article

27. Madeira C, Hořavová L, Dos Santos F, et al. Investigator Initiated Clinical Trials (IICTs): A Systematic Search in Registries to Compare the Czech Republic and Portugal in Terms of Funding Policies and Scientific Outcomes. *Ther Innov Regul Sci*. 2021;55(5):966-978. doi:10.1007/s43441-021-00293-w

28. De Jong AJ, Gardarsdottir H, Santa-Ana-Tellez Y, De Boer A, Zuidgeest MG. Experiences with low-intervention clinical trials—the new category under the European Union Clinical Trials Regulation. *Clin Trials*. 2025;22(4):494-500. doi:10.1177/17407745241309293

29. Magnin A, Iversen VC, Calvo G, et al. European survey on national harmonization in clinical research. *Learn Health Syst*. 2021;5(2):e10220. doi:10.1002/lrh2.10220

30. Demlova Regina. *EU Support for Non-Commercial Clinical Trial Sponsors (SUPPORT-NCS)*. https://www.muni.cz/en/research/projects/76627

31. European Health and Digital Executive Agency. Unlocking the impact: discover how four EU-funded Joint Actions are contributing to a healthier Europe. https://hadea.ec.europa.eu/news/unlocking-impact-discover-how-four-eu-funded-joint-actions-are-contributing-healthier-europe-2023-12-04_en

32. Cimino J, Braun C. Building a competitive infrastructure to support clinical research in healthcare institution. *Eur J Clin Invest*. 2021;51(9):e13641. doi:10.1111/eci.13641

33. Strategy Report on Research Infrastructures ROADMAP2021. https://roadmap2021.esfri.eu/

34. European Commission. CONSOLIDATED STATUTES OF THE EUROPEAN CLINICAL RESEARCH INFRASTRUCTURE NETWORK (ECRIN-ERIC). https://ecrin.org/sites/default/files/inline-files/Amended%203%20ECRIN%20Statutes_22112023.pdf

35. CZECRIN. https://czecrin.cz/

36. Williams CM, Skinner EH, James AM, Cook JL, McPhail SM, Haines TP. Comparative effectiveness research for the clinician researcher: a framework for making a methodological design choice. *Trials*. 2016;17(1):406, s13063-016-1535-1536. doi:10.1186/s13063-016-1535-6

37. Ford I, Norrie J. Pragmatic Trials. Drazen JM, Harrington DP, McMurray JJV, Ware JH, Woodcock J, eds. *N Engl J Med*. 2016;375(5):454-463. doi:10.1056/NEJMra1510059

38. World Medical Association. Declaration of Helsinki Medical Research Involving Human Participants. https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/

39. ICH Expert Working Group. ICH HARMONISED TRIPARTITE GUIDELINE CHOICE OF CONTROL GROUP AND RELATED ISSUES IN CLINICAL TRIALS E10. https://database.ich.org/sites/default/files/E10_Guideline.pdf

40. HTA CG. Practical Guideline for Quantitative Evidence Synthesis: Direct and Indirect Comparisons. https://health.ec.europa.eu/document/download/1f6b8a70-5ce0-404e-9066-120dc9a8df75_en?filename=hta_practical-guideline_direct-and-indirect-comparisons_en.pdf

41. Landewé RBM, Smolen JS, Weinblatt ME, et al. Can we improve the performance and reporting of investigator-initiated clinical trials? Rheumatoid arthritis as an example. *Ann Rheum Dis*. 2014;73(10):1755-1760. doi:10.1136/annrheumdis-2014-205821

42. European Medicine Agency. Reflection paper on the use of Artificial Intelligence (AI) in the medicinal product lifecycle. https://www.ema.europa.eu/system/files/documents/scientific-guideline/reflection-paper-use-artificial-intelligence-ai-medicinal-product-lifecycle-en.pdf

43. Burr CD, Qian S, Winter P, et al. Realising the digital twin: a thematic review and analysis of the ethical, legal, and social issues for digital twins in healthcare. *AI Soc*. 2026;41(5):5243-5267. doi:10.1007/s00146-025-02833-6

44. Weirauch U, Kreuz M, Birkenbihl C, et al. Design specifications for biomedical virtual twins in engineered adoptive cellular immunotherapies. *Npj Digit Med*. 2025;8(1):493. doi:10.1038/s41746-025-01809-6

45. World Health Organization. Repurposing of medicines – the underrated champion of sustainable innovation: policy brief. https://www.who.int/europe/publications/i/item/WHO-EURO-2021-2807-42565-59178

46. Jacobson RM, Pignolo RJ, Lazaridis KN. Clinical Trials for Special Populations: Children, Older Adults, and Rare Diseases. *Mayo Clin Proc*. 2024;99(2):318-335. doi:10.1016/j.mayocp.2023.03.003

47. European Medicine Agency. Scientific guidance on post-authorisation efficacy studies. https://www.ema.europa.eu/system/files/documents/scientific-guideline/wc500219040_en.pdf

48. De Maria Marchiano R, Di Sante G, Piro G, et al. Translational Research in the Era of Precision Medicine: Where We Are and Where We Will Go. *J Pers Med*. 2021;11(3):216. doi:10.3390/jpm11030216

49. Ashley EA. Towards precision medicine. *Nat Rev Genet*. 2016;17(9):507-522. doi:10.1038/nrg.2016.86

50. Dwan K, Gamble C, Williamson PR, Kirkham JJ, the Reporting Bias Group. Systematic Review of the Empirical Evidence of Study Publication Bias and Outcome Reporting Bias — An Updated Review. Boutron I, ed. *PLoS ONE*. 2013;8(7):e66844. doi:10.1371/journal.pone.0066844

51. Brett J, Staniszewska S, Mockford C, et al. Mapping the impact of patient and public involvement on health and social care research: a systematic review. *Health Expect*. 2014;17(5):637-650. doi:10.1111/j.1369-7625.2012.00795.x

52. Warner K, See W, Haerry D, Klingmann I, Hunter A, May M. EUPATI Guidance for Patient Involvement in Medicines Research and Development (R&D); Guidance for Pharmaceutical Industry-Led Medicines R&D. *Front Med*. 2018;5:270. doi:10.3389/fmed.2018.00270

53. European Commission. Policy, strategy, how to apply and work programmes Cluster 1.Health. https://research-and-innovation.ec.europa.eu/funding/funding-opportunities/funding-programmes-and-open-calls/horizon-europe/cluster-1-health_en?utm_source=chatgpt.com

54. Staniszewska S, Brett J, Simera I, et al. GRIPP2 reporting checklists: tools to improve reporting of patient and public involvement in research. *BMJ*. Published online August 2, 2017:j3453. doi:10.1136/bmj.j3453

55. HRA. Public involvement guidance for researchers. https://www.hra.nhs.uk/planning-and-improving-research/best-practice/public-involvement/guidance-for-researchers/

56. Crocker JC, Ricci-Cabello I, Parker A, et al. Impact of patient and public involvement on enrolment and retention in clinical trials: systematic review and meta-analysis. *BMJ*. Published online November 28, 2018:k4738. doi:10.1136/bmj.k4738

57. Van Spall HGC, Desveaux L, Finch T, et al. A Guide to Implementation Science for Phase 3 Clinical Trialists. *J Am Coll Cardiol*. 2024;84(20):2063-2072. doi:10.1016/j.jacc.2024.08.068

58. Peters DH, Adam T, Alonge O, Agyepong IA, Tran N. Republished research: Implementation research: what it is and how to do it: Implementation research is a growing but not well understood field of health research that can contribute to more effective public health and clinical policies and programmes. This article provides a broad definition of implementation research and outlines key principles for how to do it. *Br J Sports Med*. 2014;48(8):731-736. doi:10.1136/bmj.f6753

59. Committee on the Learning Health Care System in America; Institute of Medicine; Smith M, Saunders R, Stuckhardt L, et al., editors. *Best Care at Lower Cost: The Path to Continuously Learning Health Care in America.* 2013. https://www.ncbi.nlm.nih.gov/books/NBK207218/

60. Kraus VB. Biomarkers as drug development tools: discovery, validation, qualification and use. *Nat Rev Rheumatol*. 2018;14(6):354-362. doi:10.1038/s41584-018-0005-9

61. Hendrikse NM, Llinares Garcia J, Vetter T, Humphreys AJ, Ehmann F. Biomarkers in Medicines Development—From Discovery to Regulatory Qualification and Beyond. *Front Med*. 2022;9:878942. doi:10.3389/fmed.2022.878942

62. Collins FS, Varmus H. A New Initiative on Precision Medicine. *N Engl J Med*. 2015;372(9):793-795. doi:10.1056/NEJMp1500523

63. Sherman RE, Anderson SA, Dal Pan GJ, et al. Real-World Evidence — What Is It and What Can It Tell Us? *N Engl J Med*. 2016;375(23):2293-2297. doi:10.1056/NEJMsb1609216