

COMMENTARY TO HABILITATION THESIS¹

Characteristic of the Investigated Matter

The thesis addresses a clinically important challenge in multiple myeloma (MM) therapy: the near-universal development of resistance to proteasome inhibitor (PI) therapy despite the well-established dependence of malignant MM plasma cells on the proteostasis network. Its central premise is that PI resistance arises not primarily from irreversible genetic alterations but rather from adaptive and reversible remodeling of the proteostasis network, with important implications for overcoming therapeutic resistance.

Employed Methodologies

The mechanistic studies were primarily conducted *in vitro* using paired PI-sensitive and PI-resistant MM cell line models. The work employs subunit-selective activity-based proteasome probes to monitor proteasome activity in living cells, complemented by functional reporter assays and standard validation techniques. These approaches are integrated with unbiased quantitative and functional proteomics, LC-MS metabolomics, transcriptomics, and genome-wide CRISPR/Cas9 screening. The findings are further supported by *ex vivo* analyses of patient-derived samples.

Obtained Results

The work demonstrates that selective inhibition of the proteasome $\beta 5$ subunit alone is insufficient to achieve maximal PI cytotoxicity and that effective proteasome blockade requires simultaneous $\beta 5/\beta 2$ co-inhibition, a pharmacological profile clinically achieved only with high-dose carfilzomib [1]. This mechanism was validated in patients, where carfilzomib dose escalation restored clinical responses in more than half of carfilzomib-resistant cases [2]. Furthermore, PI-resistant MM cells tolerate substantial proteasome inhibition through coordinated adaptive remodeling characterized by increased proteasome capacity and metabolic reprogramming, including enhanced redox buffering, oxidative phosphorylation, and sphingolipid remodeling, thereby improving their ability to maintain protein folding and proteostasis [3, 4]. These adaptive vulnerabilities can be therapeutically exploited with nelfinavir, an HIV protease inhibitor [5]. Nelfinavir restores PI sensitivity by inducing proteotoxic stress through membrane lipid bilayer stress independently of direct proteasome inhibition, providing a mechanistic explanation for its clinical synergy with PIs [5].

Applicant's Contribution

Across the five annexed publications, the applicant is first co/author on four manuscripts (Annexes 1, 3, 4, and 5), corresponding author on three (Annexes 1, 4, and 5), and senior co/author on one (Annex 2). Her documented contributions include study conception, development of the key PI-resistant cell models, leadership of the mechanistic work and data interpretation, and manuscript preparation. Collectively, these contributions demonstrate strong and independent scientific direction, based on coherent research topic and international collaborations.

¹ The commentary must correspond to standard expectations in the field and must include a brief characteristic of the investigated matter, objectives of the work, employed methodologies, obtained results and, in case of co-authored works, a passage characterising the applicant's contribution in terms of both quality and content.

- [1] BESSE, Andrej, **Lenka BESSE**, Marianne KRAUS, Max MENDEZ-LOPEZ, Juergen BADER, Bo-Tao XIN, Gerjan DE BRUIN, Elmer MAURITS, Herman S. OVERKLEEF and Christoph DRIESSEN. Proteasome Inhibition in Multiple Myeloma: Head-to-Head Comparison of Currently Available Proteasome Inhibitors. Cell Chemical Biology [online]. 2019, **26**(3), 340+. ISSN 2451-9448. Available at: doi:10.1016/j.chembiol.2018.11.007

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
30	50	40	50

- [2] ZHOU, Xiang, Andrej BESSE, Jessica PETER, Maximilian Johannes STEINHARDT, Cornelia VOGT, Silvia NERRETER, Eva TEUFEL, Emilia STANOJKOVSKA, Xianghui XIAO, Hannah HORNBURGER, Larissa HAERTLE, Max MENDEZ-LOPEZ, Umair MUNAWAR, Angela RIEDEL, Seungbin HAN, Elmer MAURITS, Herman S. OVERKLEEF, Bogdan FLOREA, Hermann EINSELE, K. Martin KORTUEM, Christoph DRIESSEN, **Lenka BESSE** and Leo RASCHE. High-dose carfilzomib achieves superior anti-tumor activity over low-dose and recaptures response in relapsed/refractory multiple myeloma resistant to low-dose carfilzomib by co-inhibiting the 132 and 131 subunits of the proteasome complex. Haematologica [online]. 2023, **108**(6), 1628–1639. ISSN 1592-8721. Available at: doi:10.3324/haematol.2022.282225

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	50	20	40

- [3] SORIANO, G. P., **L. BESSE**, N. LI, M. KRAUS, A. BESSE, N. MEEUWENOORD, J. BADER, B. EVERTS, H. DEN DULK, H. S. OVERKLEEF, B. I. FLOREA and C. DRIESSEN. Proteasome inhibitor-adapted myeloma cells are largely independent from proteasome activity and show complex proteomic changes, in particular in redox and energy metabolism. Leukemia [online]. 2016, **30**(11), 2198–2207. ISSN 1476-5551. Available at: doi:10.1038/leu.2016.102

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
60	10	40	30

- [4] **BESSE, Lenka**, Andrej BESSE, Max MENDEZ-LOPEZ, Katerina VASICKOVA, Miroslava SEDLACKOVA, Petr VANHARA, Marianne KRAUS, Jurgén BADER, Renan B. FERREIRA, Ronald K. CASTELLANO, Brian K. LAW and Christoph DRIESSEN. A metabolic switch in proteasome inhibitor-resistant multiple myeloma ensures higher mitochondrial metabolism, protein folding and sphingomyelin synthesis. Haematologica [online]. 2019, **104**(9, Article e418). ISSN 0390-6078. Available at: doi:10.3324/haematol.2018.207704

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
30	60	40	60

- [5] **BESSE, Lenka**, Andrej BESSE, Sara C. STOLZE, Amin SOBH, Esther A. ZAAL, Alwin J. VAN DER HAM, Mario RUIZ, Santosh PHUYAL, Lorina BUECHLER, Marc SATHIANATHAN, Bogdan I. FLOREA, Jan BOREN, Marcus STAHLMAN, Julia HUBER, Arnold BOLOMSKY, Heinz LUDWIG, J. Thomas HANNICH, Alex LOGUINOV, Bart EVERTS, Celia R. BERKERS, Marc PILON, Hesso FARHAN, Christopher D. VULPE, Herman S. OVERKLEEF and Christoph DRIESSEN. Treatment with HIV-Protease Inhibitor Nelfinavir Identifies Membrane Lipid Composition and Fluidity as a Therapeutic Target in Advanced Multiple Myeloma. *Cancer Research* [online]. 2021, **81**(17), 4581–4593. ISSN 1538-7445. Available at: doi:10.1158/0008-5472.Can-20-3323

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
30	60	30	60