Melflufen Shows Efficacy Against Bortezomib-Resistant Multiple Myeloma Models

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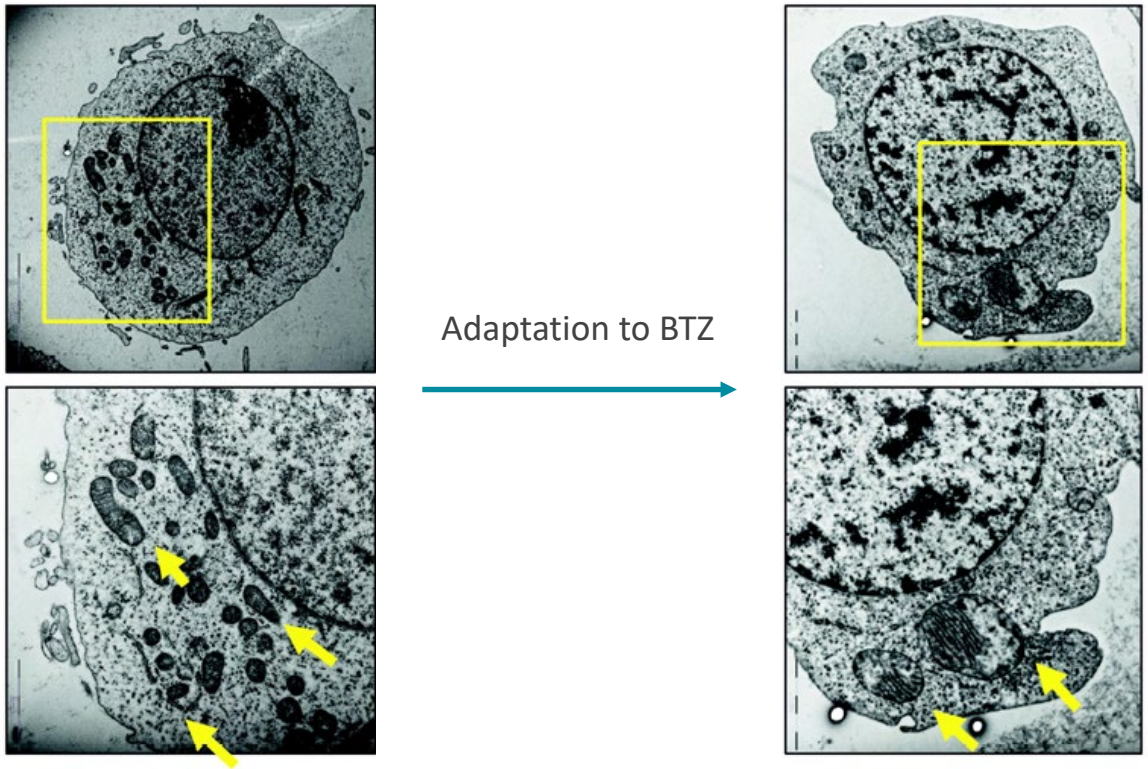
Disclosures

- Konstantin Byrgazov (Oncopeptides AB, employment)
- Ana Slipicevic (Oncopeptides AB, employment)
- Fredrik Lehmann (Oncopeptides AB, employment, equity)
- Christoph Driessen (Oncopeptides AB, research grant)

Resistance to bortezomib

- Bortezomib (BTZ) is approved for multiple myeloma (MM) treatment since 2003, yet MM remains an incurable disease.
- BTZ-resistance is associated with metabolic reprogramming (Soriano, Besse, et al., Leukemia 2016) and mutations in *PSMB5* gene encoding β5 subunit of the proteasome (predominantly observed in cell lines and to some extent in patients (Barrio et al., Leukemia 2019))



Besse et al., Haematologica 2019

Features of BTZ-resistance

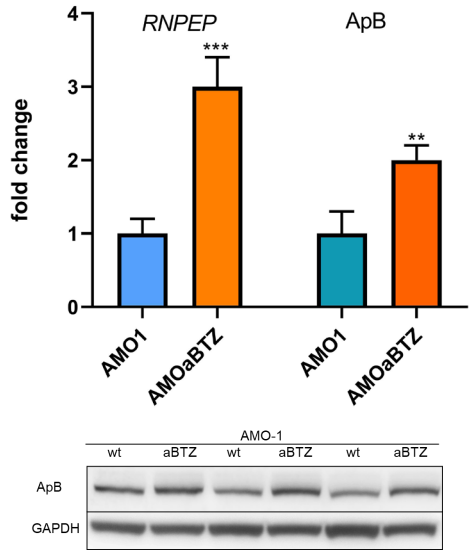
1. Larger Mitochondria
2. Up-regulation of oxidative phosphorylation (OXYPHOS) ↑
3. Larger nucleus/cytosol ratio
4. Partial proteasome independence
5. Aminopeptidase B is up-regulated ↑

Soriano, Besse, et al., Leukemia 2016
Besse et al., Haematologica 2019

Aminopeptidase B

- Aminopeptidase B (ApB) is encoded by *RNPEP* gene and is predominetly located in the nucleus.
- *RNPEP* is up-regulated in BTZ-adapted myeloma cell line AMO1 (AMOaBTZ) (A) and BTZ-resistant MYC-driven MM murine model (B).
- High *RNPEP* expression is associated with shorter PFS in VTD-treated MM patients (8.5 vs 11.9 month, HR 0.71 95% CI 0.49-1.03) (C).

A



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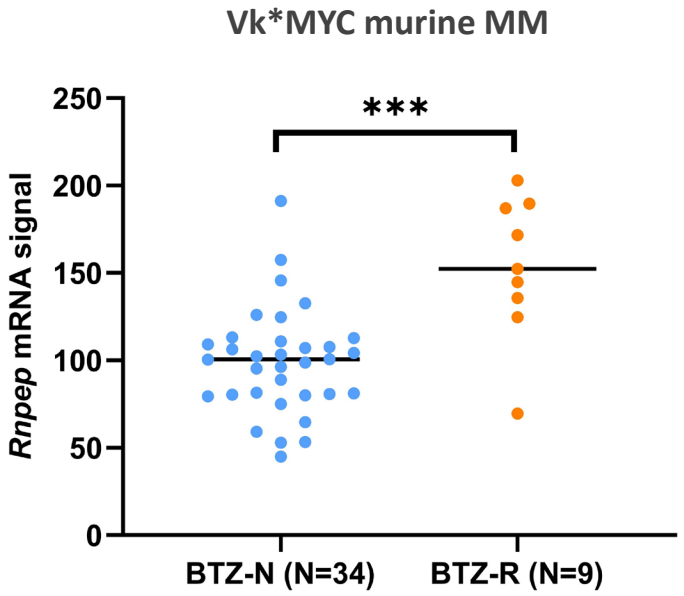
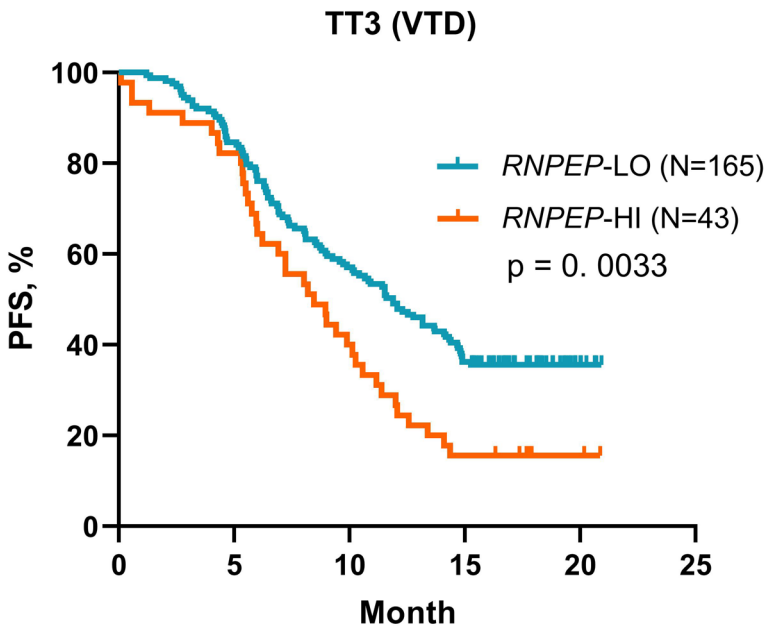


Data source: GSE111921

C



Source: GSE2658

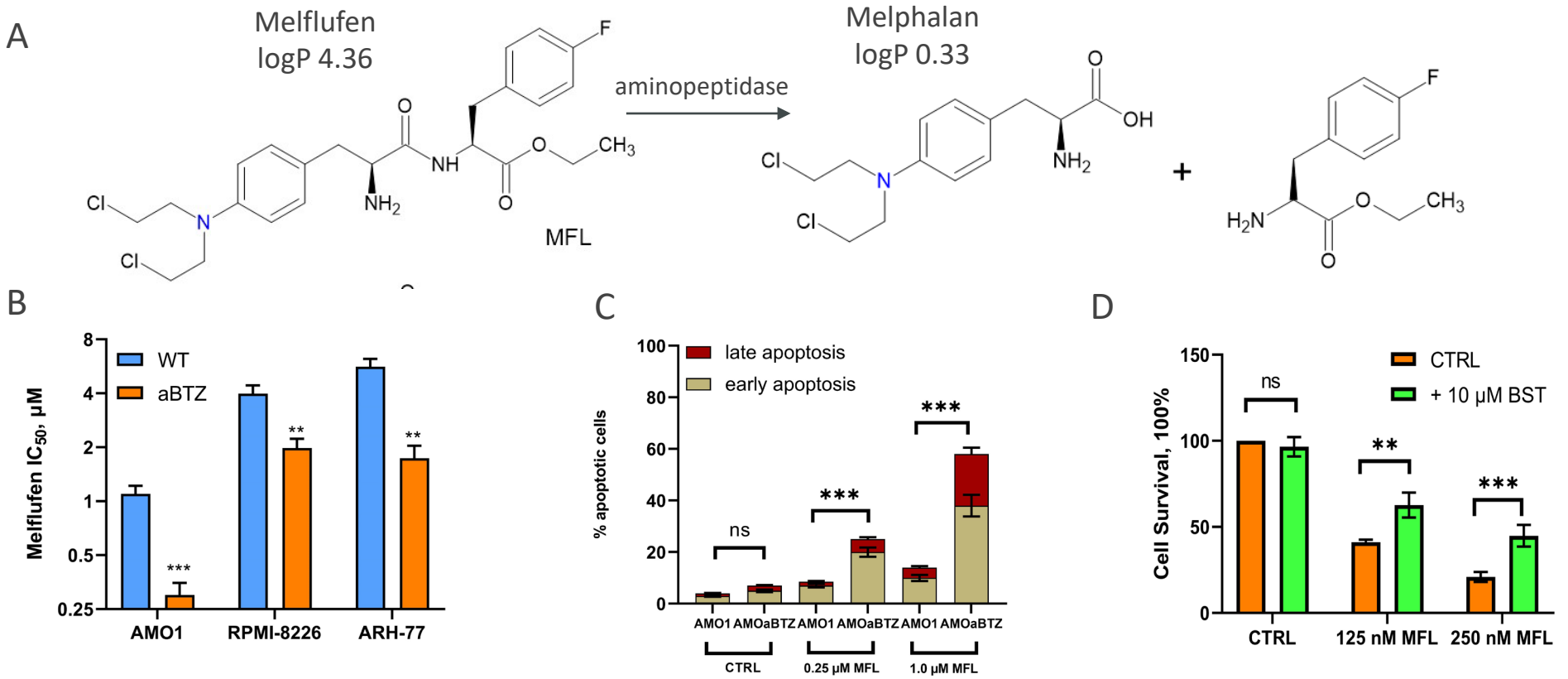
Melflufen is effective against BTZ-resistant MM models *in vitro*

A) Hydrolysis of lipophilic melflufen (logP 4.36) by aminoepptidase resulting in release of hydrophilic melphalan (logP 0.33)

B) Melflufen (MFL) is more effective in BTZ-resistant MM cell lines.

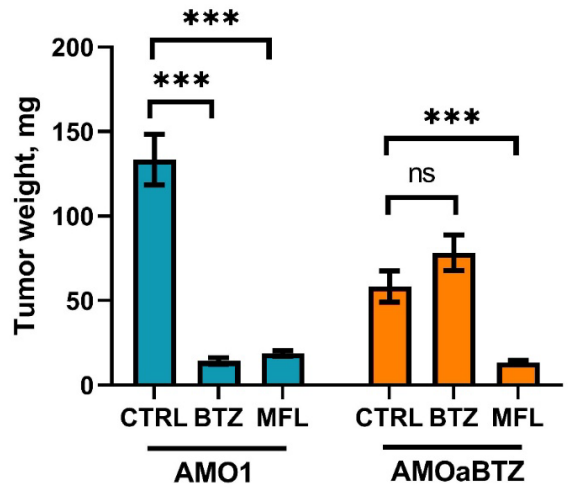
C) MFL induces stronger cell death in BTZ-resistant AMOaBTZ myeloma cells.

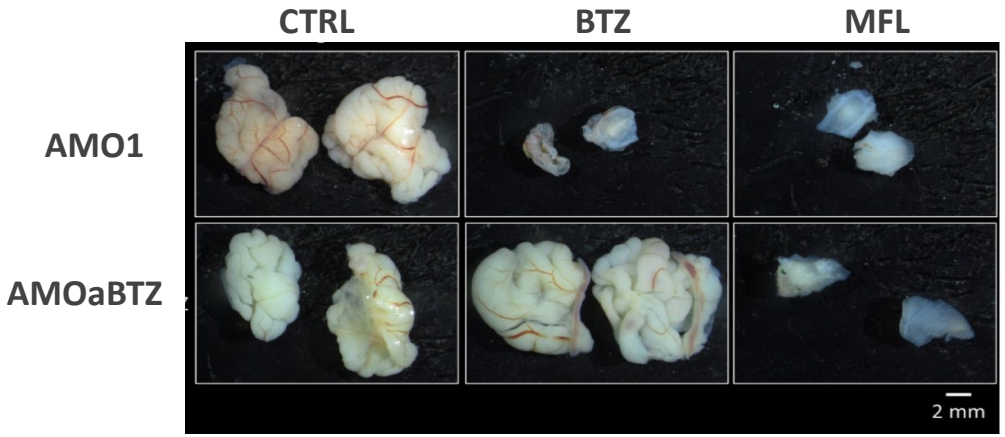
D) Activity of MFL is impaired by aminopeptidase inhibitor bestatin (BST) in AMOaBTZ cells.

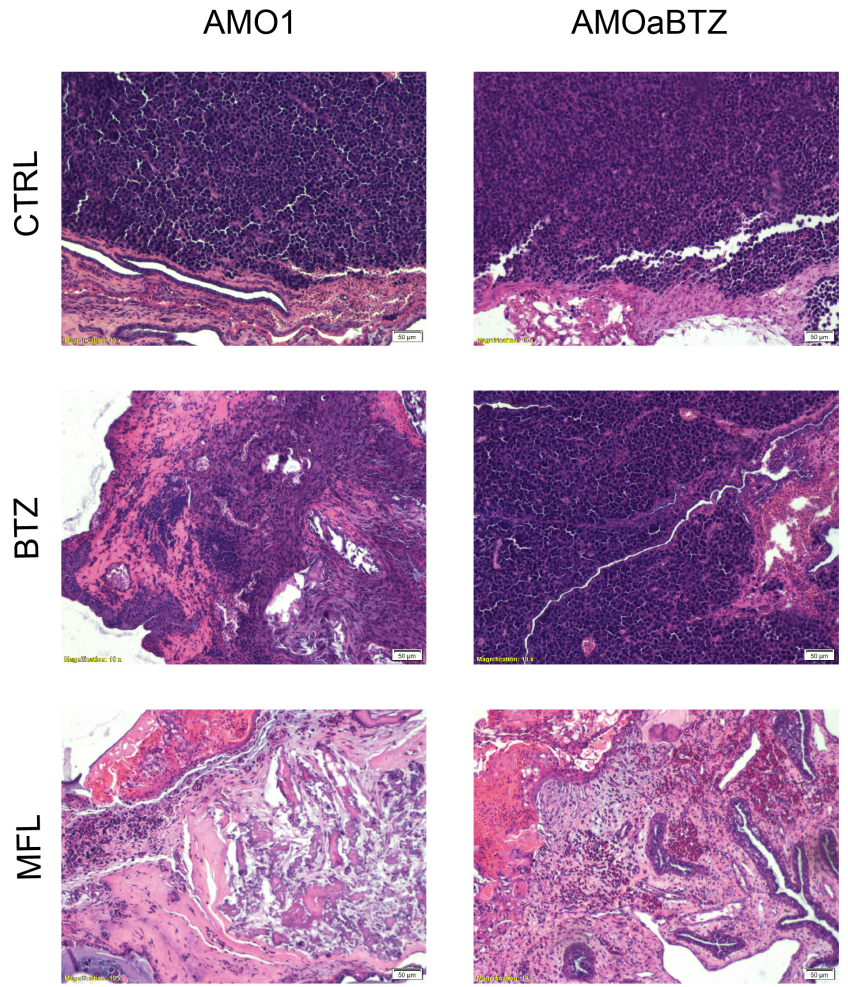


Melflufen shows effect in BTZ-resistant MM model *in vivo*

- Melflufen (MFL) efficiently reduces BTZ-resistant MM tumor growth *in vivo* in the chick chorioallantoic membrane assay model.







***, p<0.001; ns, not significant; ANOVA analysis

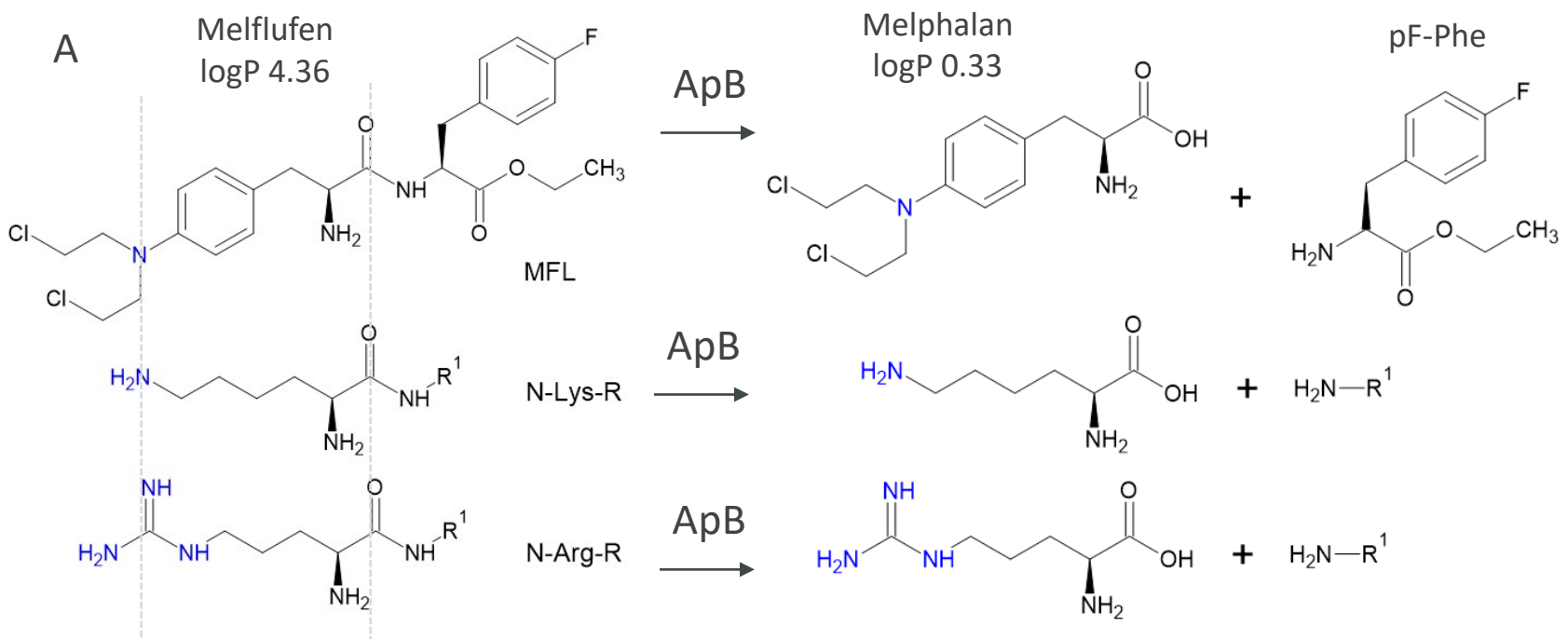
Melflufen is a substrate of aminopeptidase B

- Aminopeptidase B (ApB) hydrolyzes the peptide bond following an N-terminal **basic** amino acid such as arginine (Arg) and lysine (Lys).

  A) Melflufen (MFL) is a peptide-drug conjugate carrying a basic nitrogen mustard on its N-terminus similar to Arg and Lys.

  B) Aminopeptidase B hydrolyzes melflufen *in vitro* leading to release of melphalan and pF-Phe carrier

A



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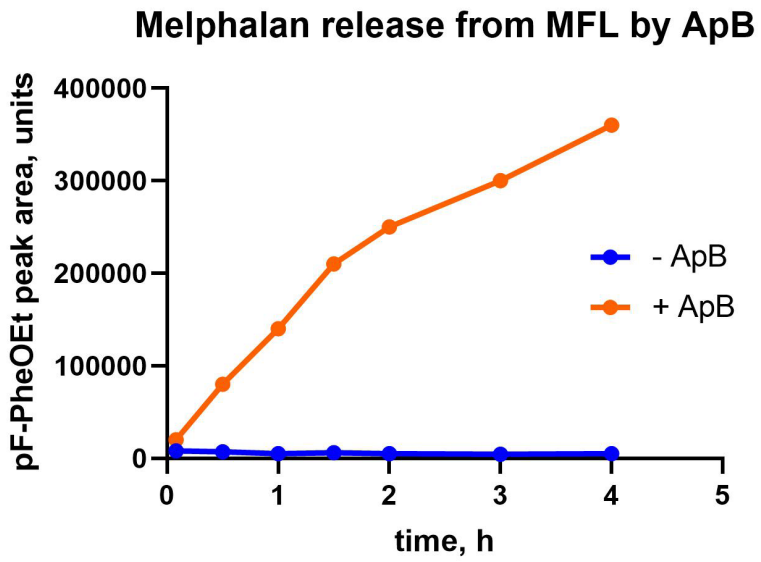


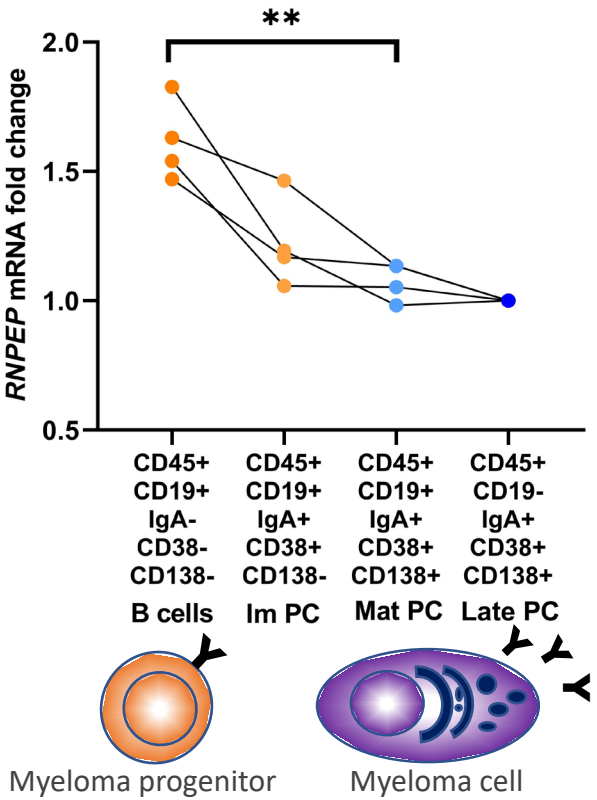
Melflufen suppresses myeloma clonal outgrowth

- Gene expression of aminopeptidase B-encoding *RNPEP* is up-regulated in plasma cell progenitors, memory B cells (Matsui et al., 2008).

  A) Myeloma progenitors are colony forming cells (CFC) giving rise to small myeloma colonies on a methylcellulose semisolid medium.

  B) Already at low concentrations melflufen is superior to other drugs in suppressing MM clonal outgrowth from BM MNC of RRMM patients.

A



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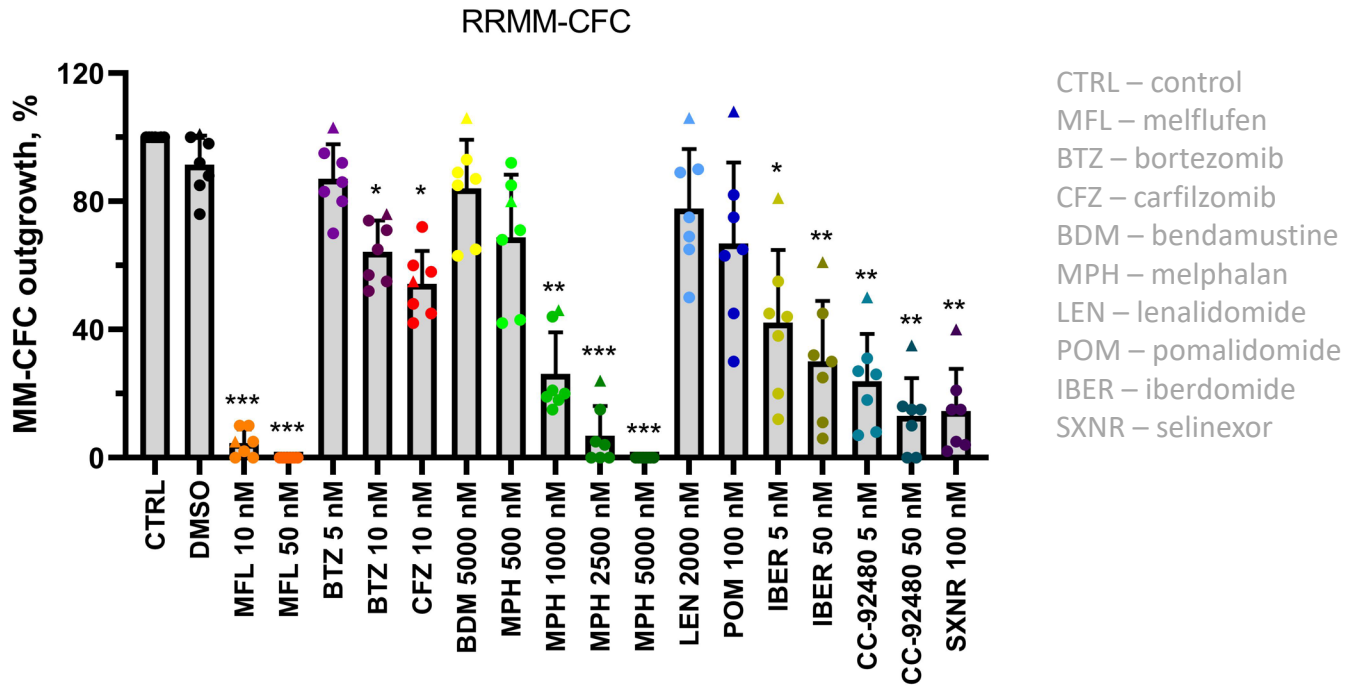

CTRL – control
MFL – melflufen
BTZ – bortezomib
CFZ – carfilzomib
BDM – bendamustine
MPH – melphalan
LEN – lenalidomide
POM – pomalidomide
IBER – iberdomide
SXNR – selinexor

Conclusions

- Resistance to BTZ in myeloma is associated with increased expression of aminopeptidase B.
- High expression of aminopeptidase B is attributed to drug-resistant clones and shorter PFS in VTD-treated MM patients.
- Aminopeptidase B utilizes a novel peptide-drug conjugate melflufen as a substrate. Hydrolysis results in release of melphalan.
- Melflufen is efficacious in killing bortezomib-resistant myeloma cells as well as myeloma progenitors by suppressing clonal outgrowth.