Melflufen Is a Highly Effective Anti-neoplastic Agent in Bortezomib-resistant Multiple Myeloma Models

Poster
EP915

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INTRODUCTION

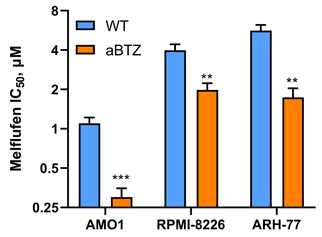
Proteasome inhibitors (PI) are currently at the backbone of treatment regimens of multiple myeloma (MM) therapy (1). Among them, bortezomib (BTZ) is the first-in class boronate-based PI approved for treatment of MM (2). Despite the improved outcomes brought by BTZ-based regimens, patients with MM relapse after BTZ treatment or become BTZ-refractory (2,3), a condition with a very poor prognosis (4). Thus, the development of the BTZ-resistance under continuing selective pressure of BTZ-containing treatments is an important clinical problem. Recent break-through studies unraveled the complexity of molecular mechanisms underlying the acquisition of BTZ-resistance (5,6). Thorough analysis of BTZ-resistant MM cells have revealed complex concerted changes in cell metabolism and protein turnover machinery, associated with myeloma cells becoming independent of proteasome machinery (5). Indeed, resistance to proteasome inhibitors in MM was found to be associated with an undifferentiated phenotype of plasmablasts and even earlier plasma cell progenitors (7), B lymphoid compartments implicated in extramedullary disease (8) and myeloma progeny (9). In fact, proteasome-independent protein turnover machinery relies on elevated activity of proteinases and peptidases (10). Noteworthy, elevated levels of peptidases have been noted in myeloma progression (see EP897), and can be targeted by a lipophilic peptide-conjugate melphalan flufenamide (hereafter referred to as melflufen) (11). Melflufen is a lipophilic peptide-drug conjugate of an alkylating moiety of melphalan (MPH) and a peptidase substrate. Due to its lipophilicity melflufen is easily transported through lipid membranes. Following transport, melflufen can be cleaved in peptidase-enriched compartments (endoplasmatic reticulum, lysosomes, autophagosomes, etc.). This results in a dramatic decrease of lipophilicity and entrapment of alkylating moieties leading to enormous concentration of an alkylating agent within the peptidase-rich compartment. Melflufen has shown great potency in treatment-naïve and melphalan-resistant myeloma models (12, 13), and its efficacy in patients with RRMM has been documented (14). In this study, melflufen is under investigation in a BTZ-resistant MM setting.

OBJECTIVES

1. Evaluate efficacy of melflufen in BTZ-adapted/resistant (aBTZ) MM cell lines
2. Investigate the effect of melflufen on MM progenitor cells by clonal outgrowth
3. Analyze expression of peptidase-encoding genes in aBTZ MM cells
4. Assess the prognostic value of higher aminopeptidase expression in patients with MM

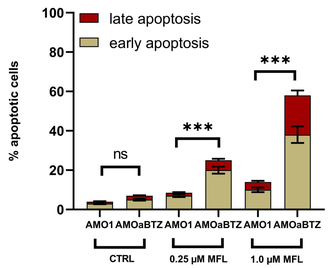
RESULTS

Figure 1. Cytotoxicity of melflufen in BTZ-adapted MM cells.



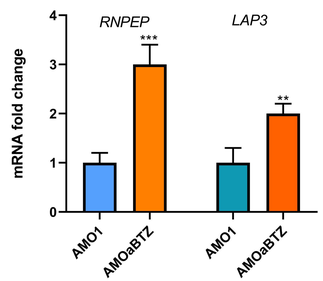
Melflufen's IC50 was measured in MM cell lines AMO1, RPMI-8226, and ARH-77 (in blue) and their BTZ-resistant sublines (aBTZ, in orange). Note: Y scale is log_2-transformed.
N=12. **, p<0.01; ***, p<0.001

Figure 2. Apoptosis.



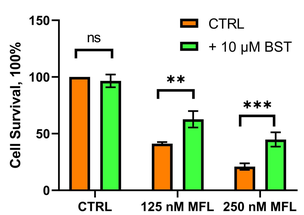
AMO1 and its BTZ-adapted subline were treated with 0.25 and 1.0 µM of MFL for 24 h. Apoptosis was assayed by flow cytometry. ***, p<0.001

Figure 3. Expression of aminopeptidase genes *RNPEP* and *LAP3*.



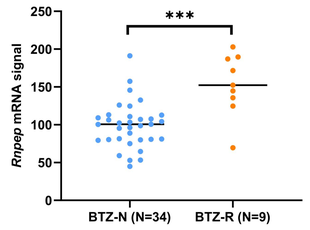
The difference in expression of aminopeptidase-encoding genes *RNPEP* and *LAP3* was determined by RT-qPCR. **, p<0.01; ***, p<0.001. N=6

Figure 4. Effect of peptidase inhibitor bestatin (BST) on melflufen's cytotoxicity.



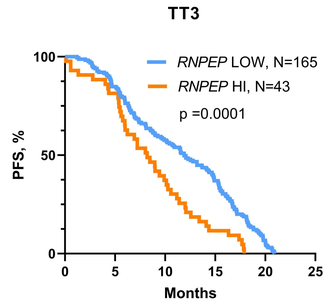
IC_{50} of melflufen (MFL) was measured in BTZ-adapted myeloma cell line AMOaBTZ alone (in orange) or in the presence of peptidase inhibitor bestatin (BST) at 10 µM. N=4. Paired t test **, p<0.01; ***, p<0.001

Figure 5. Aminopeptidase B expression in a murine myeloma model.



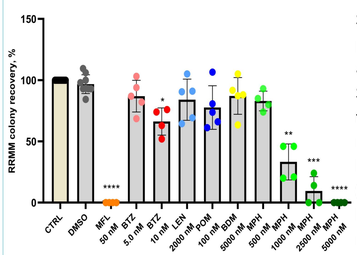
Samples of bortezomib-naïve (BTZ-N, in blue) and -resistant (BTZ-R, in orange) myeloma tumors from a murine Vk*MYC model (GSE111921) analyzed for expression of murine aminopeptidase B (*Rnpep*). Mann Whitney test ***, p<0.001

Figure 6. Progression-free survival (PFS) in MM patients treated by total therapy 3 (TT3).



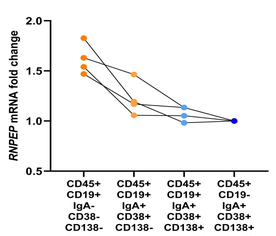
Cox regression analysis of MM patients treated by BTZ-containing TT3 (GSE2658). *RNPEP*-high (HI, in orange) corresponds to the top 20%.

Figure 7. Clonal outgrowth of RRMM patient bone marrow samples.



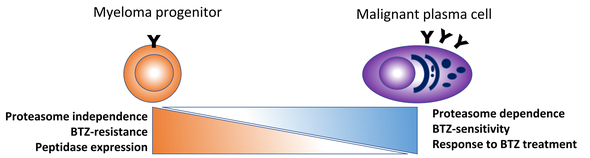
Several bone marrow samples of patients with RRMM were analysed by CFC assay. Suppressive ability (% of colonies from untreated ctrl) of anti-myeloma drugs melflufen (MFL), bortezomib (BTZ), lenalidomide (LEN), pomalidomide (POM), bendamustine (BDM), and melphalan (MPH) was assessed. N=3. ****, p<0.0001

Figure 8. Aminopeptidase B expression in plasma cell (PC) differentiation.



Sorted samples of B cells, immature PC (Im PC), mature PC (Mat PC), and late PC from 4 donors were analyzed for *RNPEP* mRNA expression (GSE141005). Statistics: not enough datapoints for Wilcoxon analysis

CONCLUSIONS



Resistance of multiple myeloma to proteasome inhibitors is a multifaceted complex process involving metabolic adaptation, loss of proteasome dependence, and increased proteasome-independent protein turnover (5,6). Independence of proteasome activity implies increased peptidase activity involved in processing of unfolded proteins (10). In this study we have demonstrated that BTZ-resistant myeloma cells express higher levels of aminopeptidases *LAP3* and *RNPEP* in human and murine models. Higher peptidase expression confirmed higher sensitivity of BTZ-resistant cells to a novel peptidase-enhanced peptide-drug conjugate melflufen as shown by cytotoxicity assays in the presence of a peptidase inhibitor bestatin. As previously shown by gene set enrichment analysis (GSEA), resistance to proteasome inhibitors also coincides with undifferentiated plasma cell phenotype (7). Indeed, we were able to demonstrate that melflufen successfully suppresses clonal outgrowth of RRMM patients bone marrows. It implies potency of melflufen towards myeloma-initiating progenitor cells, a seed of disease relapse (9). This phenomenon is supported by an outstanding long overall survival of RRMM patients treated with melflufen in O-12-M1 study (14). Further investigation of melflufen's activity against myeloma progenitors and BTZ-resistant myeloma cells is currently ongoing.

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DISCLOSURES

KB, FL (Employment, Oncopeptides AB); FL (Equity, Oncopeptides AB); LB, CD (Research grant, Oncopeptides AB)