Characterization of Multiple Myeloma cell lines with Acquired-Resistance to Proteasome Inhibitors highlights a link between resistance and metabolic deregulation.

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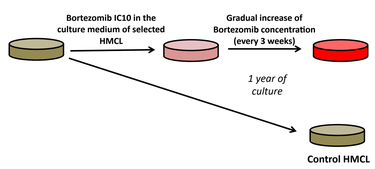
ABSTRACT

Multiple myeloma (MM) is the second most common hematological malignancy characterized by an abnormal clonal proliferation of malignant plasma cells. Despite the introduction of novels agents that have significantly improved clinical outcomes, MM patients invariably relapse. A better understanding of the drug resistance mechanisms and development of biomarkers remain of major interest to improve the treatment of patients.

In order to investigate the mechanisms involved in the resistance to **proteasome inhibitors (PI),** we have derived and characterized **6 Bortezomib-resistant human myeloma cell lines (HMCLs-BR)** from different molecular subgroups including XG1-BR t(11;14), XG2-BR t(12;14), XG7-BR, XG20-BR, XG24-BR t(4;14) and XG19-BR t(14;16).

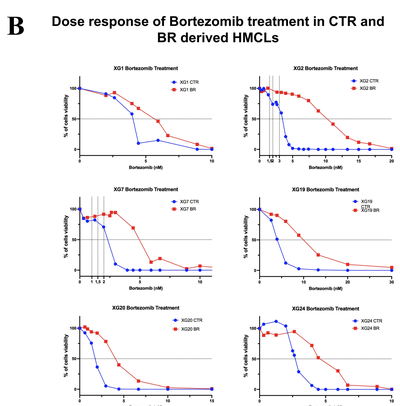
Then, we used a combination of genomic approaches including whole genome sequencing, and comparative transcriptomic analyses on these resistant cell lines and their control counterpart to understand the acquired PIs resistance mechanisms. Finally, we have performed *in vitro* functional characterizations of the HMCL-BR and their parental controls with their metabolic profile. We showed that Btz treatment induces major metabolic modifications suggesting a higher capacity of the BR-HMCL to respond to cell stress.

I. Selection and characterization of BTZ-resistant myeloma cell lines (HMCL-BR):



List of bortezomib resistant MM cell lines (HMCL-BR) developped in the laboratory

Control HMCL	Bortezomib Res HMCL
XG1-CTR	XG1-BR
XG2-CTR	XG2-BR
XG7-CTR	XG7-BR
XG19-CTR	XG19-BR
XG20-CTR	XG20-BR
XG24-CTR	XG24-BR



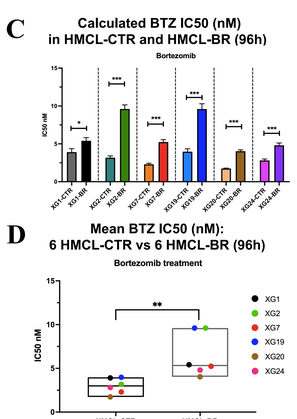
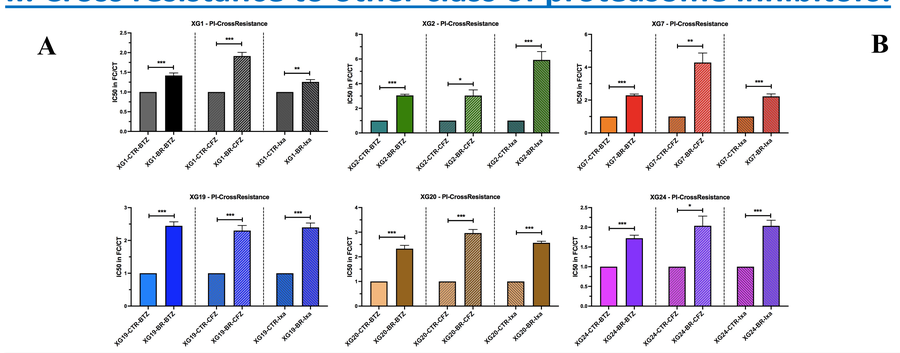


Figure 1: Development and validation of MM cell lines (HMCL) resistant to the proteasome inhibitor Bortezomib (BTZ)

A) XG1, XG2, XG7, XG19, XG20 and XG24 human myeloma cell lines were cultured in presence of increasing concentration of BTZ (HMCL-BR) for 1 year. In parallel the same cell lines were cultured without addition of BTZ (HMCL-CTR). BTZ IC5O was regularly controlled during the culture in the HMCL-CTR vs HMCL-BR to monitor the acquisition of BTZ resistance. **B)** HMCL-CTR or HMCL-BR were treated with different concentrations of BTZ. After 96h, cell viability was monitored using CellTiterGlo (CTG). **C)** HMCL-BR cell lines are more resistant than HMCL-CTR to BTZ treatment with higher calculated IC50 (96h). **D)** The mean BTZ IC50 is significantly different between the two groups. A minimum of 3 independent assays were performed for each cell line.

II. Cross resistance to other class of proteasome inhibitors:



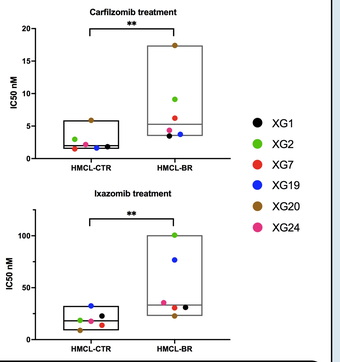
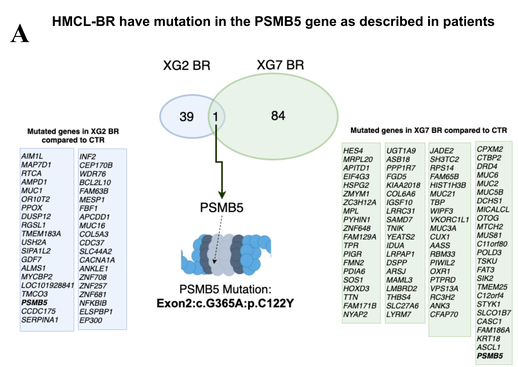


Figure 2: BTZ acquired resistant cell lines are resistant to other Proteasome inhibitors: Carfilzomib and Ixazomib

A) HMCL-CTR or HMCL-BR were treated with different concentrations of BTZ, Carfilzomib (CFZ) or Ixazomib (Ixa). After 96h, cell viability were monitored using CTG, and IC50 were calculated. **B)** HMCL-BR are significantly more resistant to CFZ and Ixa than HMCL-CTR (comparison of the mean IC50 between the two groups). A minimum of 3 independent assays were performed for each cell line.

III. Genomic characterization of HMCL-BR:



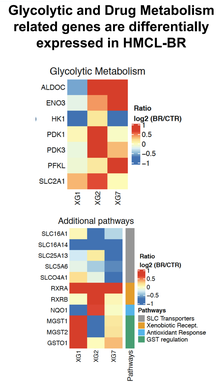


Figure 3: Genomic characterization of HMCL-BR cells.

A) XG2-BR and XG7-BR have a mutation in the PSMB5 subunit that is found in patients as well. **Whole genome sequencing** was performed on HMCL-CTR and -BR cells. Mutated genes were identified in each BR cell line, and PSMB5 was identified as common in XG2-BR and XG7-BR.

Transcriptomic profiles of XG1, XG2 and XG7 -CTR and -BR were analyzed by Affymetrix U133P microarray. Differentialy expressed genes were then identified. **B)** Metabolism and drug clearance related genes are differentially expressed in HMCL-BR

IV. HMCL-BR have a modified metabolism:

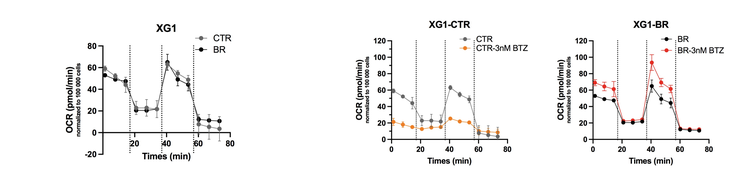


Figure 4: HMCL-BR show higher capacity to respond to cell stress

Using **Seahorse XF96 Extracellular Flux analyzer,** we assessed through real-time and live cell analysis the **mitochondrial oxidative phosphorylation** based on the oxygen consumption rate **(OCR)**, and the **glycolysis** by analyzing the extracellular acidification rate **(ECAR)**. We observed that **A)** XG1-CTR and BR cell lines display similar baseline **glycolytic** rates. **B and C)** XG1-CTR and BR cell lines differentially respond to BTZ treatment, with increased mitochondrial and glycolysis observed in the BR cell line treated with Bortezomib. This is in accordance with the increased gene expression of several glycolytic enzymes found in the resistant myeloma cell lines.

CONCLUSIONS

Altogether, we developed acquired PIs resistant HMCLs that exhibit PSMB5 mutation as observed in patient, and we identified pathways linked to metabolism regulation in these cell lines, that may increase the capacity of the BR-HMCL to respond to cell stress.

These results make our PI-resistant models, an attractive preclinical model to test new therapeutic strategies to overcome PI resistance in MM.

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