



# COMPARATIVE ANALYSIS OF *in vitro* CHEMOSENSITIVITY TO FOUR PROTEASOME INHIBITORS IN HUMAN MYELOMA CELL LINES



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## BRIEF OVERVIEW

Proteasome inhibitors (PI) are effective chemotherapeutic agents in the treatment of multiple myeloma (MM). Wide inter-individual variation in PI response is a major limitation in achieving consistent therapeutic effect in MM. Yet few studies have compared efficacy of the PIs bortezomib/Bz, carfilzomib/Cz, ixazomib/Ix and oprozomib/Opz in a range of myeloma subtypes. In our current study, we performed comprehensive *in vitro* chemosensitivity profiling of response to four (4) PIs (Bz, Cz, Ix and Opz) in a panel of fifty (50) human myeloma cell lines (HMCLs) representing the biological and genetic heterogeneity of MM.

Comparison of the cellular responses to PI treatment among HMCLs showed wide range of variability in IC<sub>50</sub> values identifying some lines as highly sensitive and some lines relatively refractory to PI treatment. We observed statistically significant pair-wise correlation between IC<sub>50</sub> values of the proteasome inhibitors. However, it was interesting to note that although all 4 drugs belong to the same drug class (PI), not all cell lines responded the same across all PI treatments.

This demonstrates tumor heterogeneity even in response to inhibitors of the same class, and further demonstrates tumors refractory to one PI may still respond to another.

## OBJECTIVE

**To generate an *in vitro* chemosensitivity profile of proteasome inhibitor (PI) response in human myeloma cell lines (HMCLs)**

- To maintain a repository of human myeloma cell lines (HMCLs).
- To determine *in vitro* chemosensitivity of HMCLs to 4 different PIs (Bortezomib, Carfilzomib, Ixazomib, Oprozomib) using CellTiter Glo and Caspase 3/7 assays.

## METHODS

- Cell lines:** Fifty (50) Human myeloma cell lines (HMCLs) were procured and maintained in HMCL media with IL-6.
- In vitro* chemosensitivity assays:** HMCLs were treated with increasing concentrations of the four (4) proteasome inhibitors Bz, Cz, Ix, Opz used as single agents. *In vitro* cytotoxicity assays were performed using CellTiter-Glo® Luminescent kit (Promega), while Caspase3/7 activity were evaluated using Caspase-Glo®3/7 Assay kit (Promega). Values were normalized to untreated controls and the half maximal inhibitory concentration (IC<sub>50</sub>) interpolated by calculating the nonlinear regression using sigmoidal dose-response equation (variable slope). The IC<sub>50</sub> values were used to compare response to PI treatment.
- Gene expression profiling data analysis:** mRNA sequencing data was downloaded from the Keats lab data repository at Translational Genomics Research Institute (TGen) (<http://www.keatslab.org/data-repository>). Gene expression profiling (GEP) data were normalized and analyzed using Partek Genomics suite to identify gene expression signatures associated with cytotoxicity.

## RESULTS

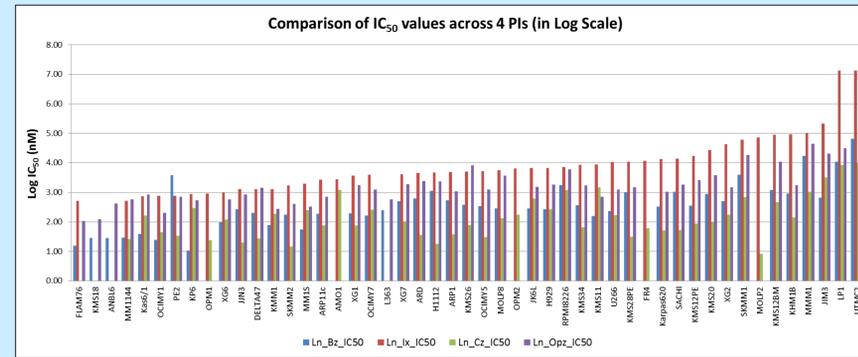


Figure: Wide range of chemosensitivity to four proteasome inhibitors in HMCLs.

	Mean IC <sub>50</sub> (nM)	IC <sub>50</sub> Range (nM)
<b>Bortezomib (Bz)</b>	17.84	2.78-124.31
<b>Carfilzomib (Cz)</b>	11.13	0.68-55.34
<b>Ixazomib (Ix)</b>	106.75	15.11-1250
<b>Oprozomib (Opz)</b>	45.82	7.6-776.03

Table: Numerical summaries of IC<sub>50</sub> values in HMCLs.

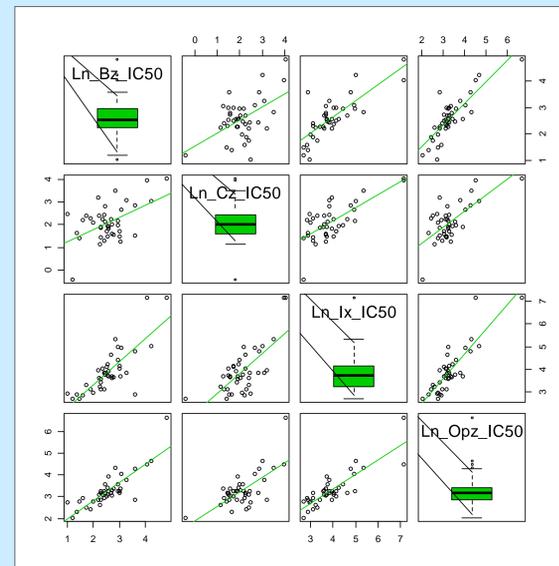


Figure: Scatterplot matrix demonstrating pair-wise correlation of IC<sub>50</sub> values between PIs across the HMCL panel.

	Ln_Bz_IC50	Ln_Cz_IC50	Ln_Ix_IC50	Ln_Opz_IC50
<b>Ln_Bz_IC50</b>	1			
<b>Ln_Cz_IC50</b>	0.515 (0.0006)	1		
<b>Ln_Ix_IC50</b>	0.791 (<.0001)	0.629 (<.0001)	1	
<b>Ln_Opz_IC50</b>	0.840 (<.0001)	0.686 (<.0001)	0.852 (<.0001)	1

Table: Pearson product-moment correlation (PPMC) test with adjusted p-values (Holm's method).

## GENE EXPRESSION ANALYSIS

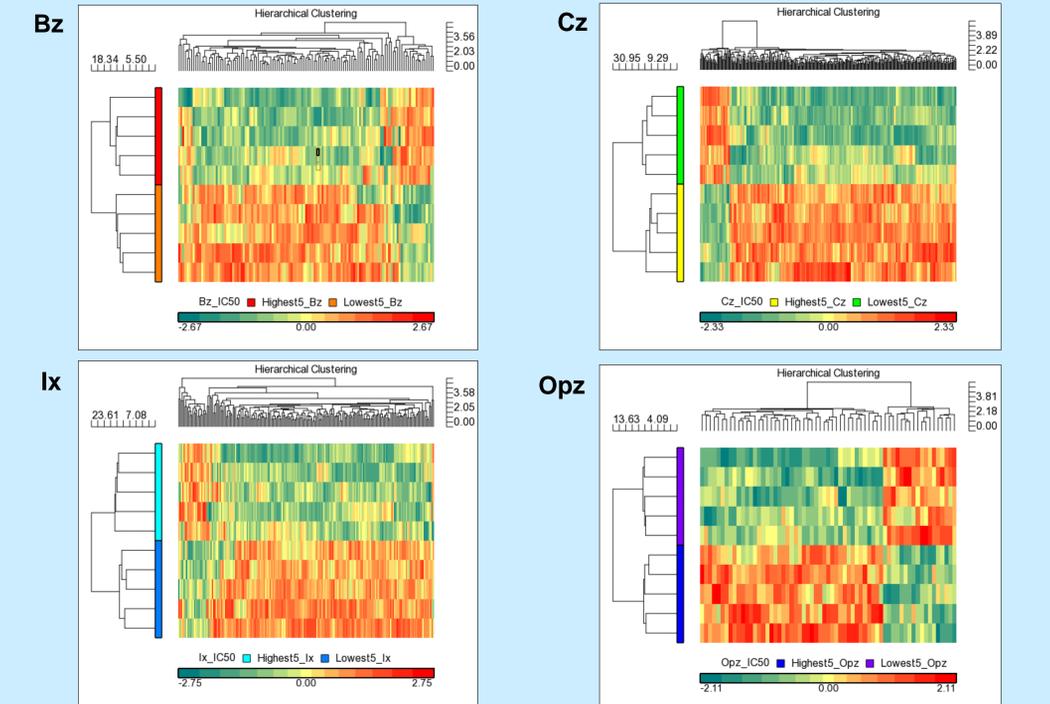


Figure: Heatmaps representing top genes that discriminate between the cell lines with Highest 5 vs Lowest 5 IC<sub>50</sub> values (p<0.01) for each PI.

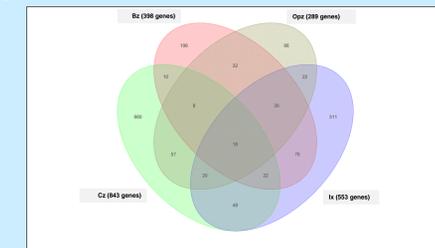


Figure: Venn Diagram showing relationship between GEP signatures across 4 PIs (p<0.05).

## CONCLUSIONS

- Wide range of variability in *in vitro* chemosensitivity to PIs across HMCLs..
- Considerable pair-wise correlation between PI response.
- Presence of outliers: HMCLs sensitive to one PI, comparably less-sensitive to other PIs.
- Subgroup analysis revealed significant correlation between carfilzomib IC<sub>50</sub> and chromosome number (p =0.0095).
- Gene clusters were identified that correlated with PI response: potential for developing predictive scoring algorithms for clinical evaluation of drug of choice.

## REFERENCES

- Rajkumar SV. Multiple myeloma: 2013 update on diagnosis, risk-stratification, and management. Am J Hematol. 2013;88(3):226-235.
- Munshi NC and Anderson KC. New strategies in the treatment of multiple myeloma. Clin Cancer Res. 2013;19(13):3337-3344.

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