Discovery of Novel Proteasome Activators that Modulate the Immunopeptidome and Enhance Antigen Presentation to Trigger Anti-Myeloma T-cell Immunity

Priyanka S. Rana[1,2], James J. Ignatz-Hoover[1,2], Ehsan Malek[1,2,3], Yuriy Federov[4], Drew Adams[4], and James J. Driscoll[1,2,3].

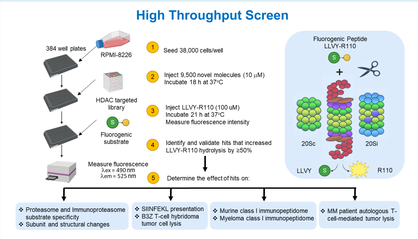
[1]Division of Hematology Oncology, CWRU School of Medicine; [2]Case Comprehensive Cancer Center, CWRU;
[3]Adult Hematologic Malignancies & Stem Cell Transplant Section, Seidman Cancer Center, University Hospitals Cleveland Medical Center;
[4]Small Molecule Drug Discovery Core, CWRU Cleveland, OH

Poster Number: MM-506

ABSTRACT

Transformative advances in immunotherapy have revolutionized cancer treatment and induced durable clinical responses. However, only a fraction of patients respond to immunotherapy, and cancer cells escape immunosurveillance. Proteasomes play a central role in immune surveillance by generating peptides from intracellular proteins; these peptides are presented for recognition by cluster of differentiation (CD)-8+ cytotoxic T lymphocytes (CTLs). Cancer cells employ strategies to downregulate antigen presentation and impair CTL-mediated tumor recognition and lysis. Proteasomes degrade ovalbumin to generate the neo-antigenic peptide “SIINFEKL”, which is presented on the cell surface in complex with the major histocompatibility (MHC) class I H-2Kb molecule and quantitated by flow cytometry. Histone deacetylase (HDAC)-6–specific inhibitors increase proteasome activity and presentation of MHC class I antigens on tumor cells. However, current HDAC6 inhibitors have pharmacologic liabilities that limit efficacy, e.g., low potency and solubility, poor pharmacokinetic properties, and potential genotoxicity. We performed a high-throughput, cell-based screen of 9,600 HDAC-specific compounds to identify novel molecular entities that increased proteasome activity in MM cells. Treatment of tumor cells with the proteasome activators increased levels of the MHC-class I-SIINFEKL complex. Tumor cells were pretreated with the top hits and then co-cultured with CTLs that had been genetically-engineered to express a SIINFEKL-restricted T-cell receptor (TCR). Pre-treatment of lymphoma cells with novel proteasome activators significantly increased tumor lysis by CTLs that expressed the SIINFEKL-restricted TCR. CTL-mediated tumor lysis was dramatically more potent compared to known HDAC6-specific inhibitors. Murine and myeloma immunopeptidome analysis showed that compound A increased overall antigenic peptide abundance and diversity. Moreover, pretreatment with compound A caused a dramatic increase in pan and subtype specific HLA-ABC presentation in MMCLs which in turn enhanced the healthy T-cell mediated lysis of MMCLs. Compound A also enhanced autologous T-cell mediated lysis of MM patient tumor cells. Our results support the development of novel proteasome activators as a paradigm-shifting approach to enhance tumor antigenicity and CTL-mediated antitumor immunity to overcome antigen escape. Our data provide compelling evidence to support the development of drugs that enhance proteasome proteolytic function as a therapeutic strategy to treat cancers and other disease-causing proteinopathies.

STUDY DESIGN



Cell-based HTS identifies HDAC-targeted compounds that increase proteasome ChT-like activity using the cell-permeable substrate LLVY-R110 that is cleaved to release the fluorogenic substrate R110

RESULTS

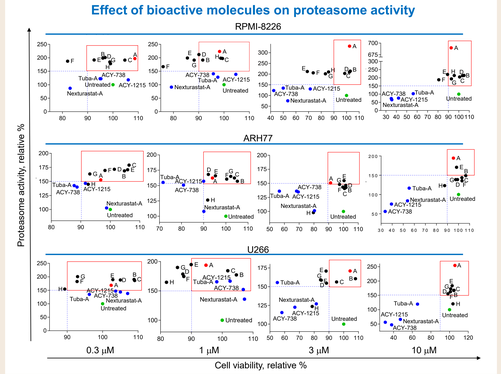


Fig 1. **Novel compounds increase proteasome activity while keeping the cell viability intact:** Three multiple myeloma cell lines RPMI-8226, ARH77 and U266 were treated with compounds at varying concentrations for 48 hrs

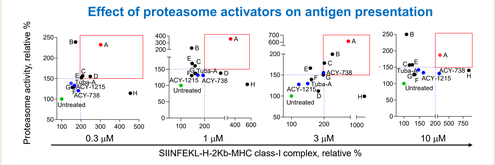


Fig 2. **Proteasome activators increase Antigen presentation:** Effect of the top 10 hits on proteasome ChT-like activity,and presentation of MHC-class I-SIINFEKL complex in E.G7-ova cells.

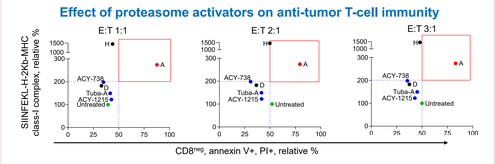


Fig 3. **Proteasome activators increase anti-tumor T-cell immunity:** B3Z mediated killing of E.G7 Ova cells in the presence of proteasome activator. Tumor cells were pretreated with proteasome activators and then co-cultured with CTLs that had been genetically-engineered to express a SIINFEKL-restricted T-cell receptor (TCR). Pre-treatment of lymphoma or multiple myeloma cells with novel HDAC6 inhibitors also increased tumor lysis by CTLs that expressed the SIINFEKL-restricted TCR by 2-fold

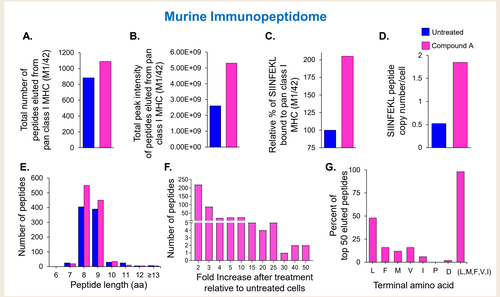


Fig 4. **Compound A increases murine antigenic peptide diversity and abundance:** Pretreatment of E.G7-ova cells with compound A increased the number (A) and total peak intensity of peptides (B) eluted from MHC class I (M1/42). Compound A treatment caused an an increase in SIINFEKL peptide eluted from MHC class I (M1/42) (C) also increased the SIINFEKL copy number analyzed from parallel reaction monitoring of lysates that were spiked with the SIINFEKL peptide (D). The eluted peptides were mostly 8 - 9 amino-acid in length with no significant difference between the treated and untreated (E). Pretreatment of E.G7-Ova cells with compound A increased the presentation of MHC-I specific peptides by up-to 200-fold. (F) More than 90% of the top 50 peptides upregulated by compound A and eluted from the mouse MHC-I antibody bared an aliphatic or aromatic terminal amino-acid (G).

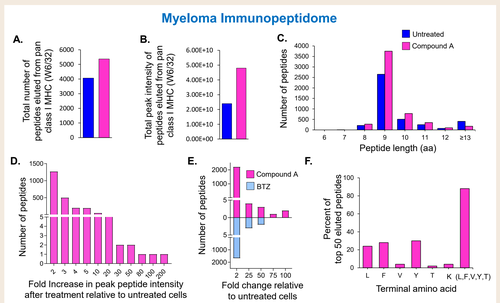


Fig 5. **Compound A increases myeloma antigenic peptide diversity and abundance:** Treatment with compound A increased the number (A) and total peak intensity (B) of peptides eluted from MHC class I (W6/32) in RPMI-8226 cells (A,B) which were mostly 9 amino-acid in length with no significant difference between the treated and untreated (C). Pretreatment with compound A increased the presentation of MHC-I specific peptides by up-to >1000-fold and (D). Whereas pretreatment with BTZ decreased the presentation of MHC-I specific peptides by up-to >1000-fold. More than 80% of the top 50 peptides upregulated by compound A and eluted from the mouse MHC-I antibody bared an aliphatic or aromatic terminal amino-acid (F).

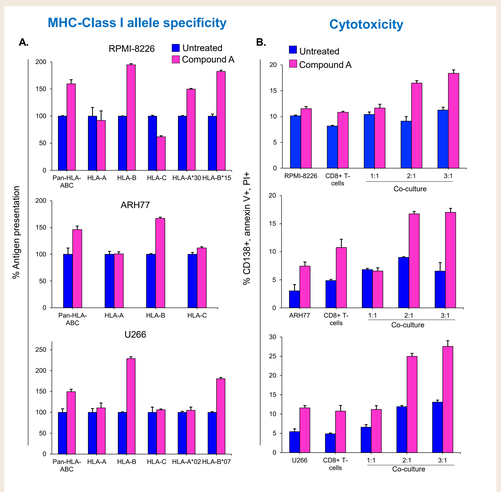


Fig 6. **Compound A enhances allele-specific presentation of MHC class I HLA-ABC and allogenic T-cell mediated cytotoxicity in MMCLs:** Pretreatment with compound A significantly increased pan-HLA-ABC (W6/32) and HLA-B subtype in all three MMCLs. HLA-A subtype was significantly increased in RPMI-8226 cells pretreated with compound A with no change in ARH77 and U266 (A). Compound A treated MMCLs were washed and co-cultured with healthy T-cells which caused an increase in T-cell mediated lysis of MMCLs (B).

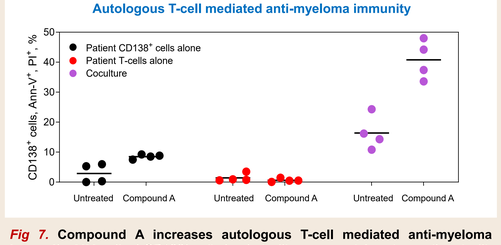
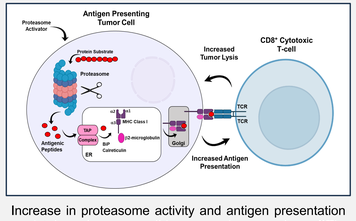


Fig 7. **Compound A increases autologous T-cell mediated anti-myeloma immunity.** MM patient CD138+ cells treated with compound A and co-cultured with autologous T-cells show a significant T-cell mediated MM cytotoxicity as compared to the untreated co-culture. The cytotoxic effects of compound A on MM patient CD138+ cells alone and T-cells alone were not significantly different than the untreated cells

GRAPHICAL MODEL



Increase in proteasome activity and antigen presentation modulates immunopeptidome and promotes CTL-mediated anti-myeloma T-cell immunity.

CONCLUSIONS

- Taken together, we have identified novel bioactive molecules that potently stimulated proteasome activity, increased pan-MHC class I antigen presentation and enhanced the cytotoxic activity of T-cells.
- In murine studies, lead Compound A significantly increased presentation of the immunodominant neoantigen SIINFEKL and modulated antigen abundance and diversity.
- In human studies, mass spectrometry revealed that Compound A treatment of MM cells modulated the antigenic landscape and upregulated specific (neo)antigens
- Compound A enhanced allogenic and autologous T-cell mediated lysis of MMCLs and MM patient BM-derived CD138+ cells respectively.
- Proteasomes govern MHC-class I antigen density to dictate the potency of CTL-mediated antitumor responses.

FUTURE DIRECTIONS

- Pharmacologic modulation of the myeloma immunopeptidome to identify public and private tumor antigwns
- Novel pharmaologics that modulate proteasome activity offer protential to upregulate public and private tumor antigens that can be targeted by TCR-restricted t-cells and cancer vaccines.
- Develop personalized and targeted TCR-directed T cell mediated anti-myeloma

ACKNOWLEDGEMENTS

Research was supported by NIH R01 (5R01AI139141), the UH Seidman Cancer Center, the Vinney Foundation and the Case Comprehensive Cancer Center (to JJD).

Contact Information

Dr. Priyanka S. Rana: p.rana@case.edu
Dr. James J. Driscoll: james.driscoll@uhhospitals.org