Poster: MM-501

Overexpression of Proteasome PSMC ATPases Mitigates ER Stress and Correlates With Reduced Overall Survival in Multiple Myeloma

JJ IGNATZ-HOOVER [1,3], ER CHAN [2], J D'CRUZ[4], PS RANA [1,3], E MALEK[1], JJ DRISCOLL[1,3]

[1] Division of Malignant Hematology, University Hospitals, Seidman Cancer Center, Cleveland OH 44106, USA

[2] Department of Bioinformatics, Case Western Reserve University, Cleveland OH 44106, USA

[3] Hematopoetic and Immune Cancer Biology Program, Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland OH 44106 USA

[4] University School Chagrin Falls OH 44022

University Hospitals Seidman Cancer Center

Case Western Reserve University | Case Comprehensive Cancer Center

INTRODUCTION

- Multiple myeloma (MM) is a plasma cell malignancy that produces large amounts of protein, making it exquisitely sensitive agents that disrupt proteostasis.
- The 26S proteasome is a massive 2.5 MDa multi-subunit proteolytic machine that is composed of a 20S catalytic core and capped at either of both ends by 19S regulatory particles.
- Proteasome inhibitors, e.g., bortezomib (BTZ), primarily bind the PSMB5 subunit that has chymotrypsin-like peptidase activity. Bortezomib yields clinical response alone and in multidrug regimens, but drug resistance and relapse eventually occur.
- We hypothesized that by correlating the expression of proteasome subunits in MM patient tumor cells with clinical outcomes following treatment with proteasome inhibitors, we could gain novel insight into myeloma biology and identify actionable drug targets.

AIMS

- To define novel MM prognostic biomarkers in the ubiquitin proteasome system (UPS).
- To determine the effect of the above hits on PI resistance *in vitro.*
- To prevent or overcome drug resistance by identifying actionable drug targets

METHODS

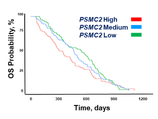
- We correlated genomic expression profiles to clinical outcomes from two publicly available datasets.

1. **A**ssessment of **P**roteasome Inhibition for **Ex**tending Remissions (**APEX** trial).[1] Phase 3 RCT- Compared single agent bortezomib to high dose DEX in MM patients who previously received chemotherapy.
2. Multiple Myeloma Research Foundation (**MMRF**)'s **C**linical **O**utcomes in MM to **P**ersonal **A**ssessment of Genetic Profiles **S**tudy (**CoMMpass** study)[2]

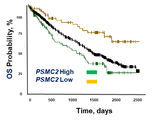
- The most commonly altered hit, PSMC2- a member of the heterohexameric ring of the 19S proteasome was overexpressed in MM cell lines using a C terminal FLAG tagged third generation lentiviral constructs.
- We assessed the effects of exogenous PSMC2 on key proteasome subunits by IB, proteasome structure by IP and native gel, and proteasome activity by a LLVY-SUC proteasome assay.
- We tested the effects of PSMC2 overexpression on resistance to the PI bortezomib by Annexin/PI staining.
- We tested the effects of PSMC2 overexpression on endoplasmic reticulum stress by treating the cells with bortezomib or tunicamycin, an antibiotic that inhibits N glycosylation of ER proteins, and assessing the real time viability of MMCLs treated with using Incucyte fluorescent microscopy, IB analysis of ER stress protein expression, and activation of a viral ER stress sensor by flow cytometry

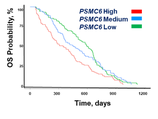
RESULTS

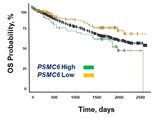
1A.



1B.





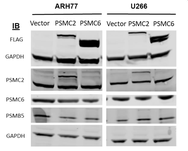


1C.

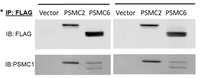
	Response Duration	p-value	PFS	p-value	OS	p-value
PSMC1	0.53	0.0032	1.64	0.01	1.8136	0.034
PSMC2	1.12	0.57	1.18	0.39	1.9337	0.014
PSMC3	0.93	0.74	1.21	0.32	1.67	0.053
PSMC4	1	0.98	0.927	0.69	0.85	0.57
PSMC5	0.75	0.14	1.49	0.03	1.73	0.046
PSMC6	1.35	0.15	1.63	0.01	2.30	0.0014

Figure 1 Kaplan Meier survival analysis. A. KM analysis of the APEX trial reveals poorer OS) with increasing PSMC6 and PSMC2 expression comparing upper (red), middle (blue), and lower (green) tertiles of RNA expression (PSMC6 p= $1x10^{-4}$ PSMC2 p= $7x10^{-6}$ n=380). **B.** KM analysis of PSMC6 reveals poorer OS with higher expression (Green=Upper 50%, Orange=Lower 50%, Black=Total cohort, Hazard Ratio (HR)=2.29 p=0.0014 n=221). Higher PSMC2 expression also correlated with poorer OS (Green= Upper 33%, Orange=Lower 66%, Black=Total cohort, HR=1.993 p=0.0136 n=216) **C.** Cox univariate analysis of PSMC1-6 RNAseq with respect to response duration, PFS, and OS. Significant HRs and related p-values are highlighted.

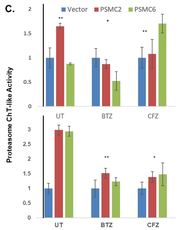
3A.



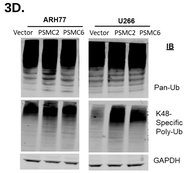
3B.



3C.



3D.



3E.

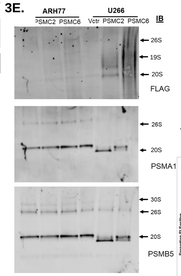
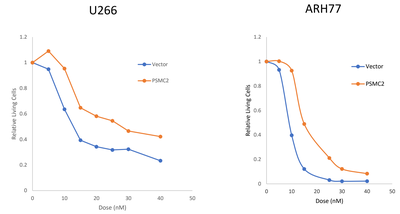


Figure 3 Overexpressed PSMC subunits incorporate into higher order proteasome structures and increase CHTL activity. 2A. IB of PSMC2/PSMC6 overexpression does not change basal PSMC levels. 2B. Co-IP of PSMC2/6 yields PSMC1, suggesting PSMC2-FLAG is incorporated into the 19S proteasome. **C.** PSMC2 overexpression increases CTL proteasome activity. **D.** PSMC2 overexpression modulates processing of K48 UB chains. PSMC2 overexpression. changes the distribution of native proteasome.

4A.



4B.

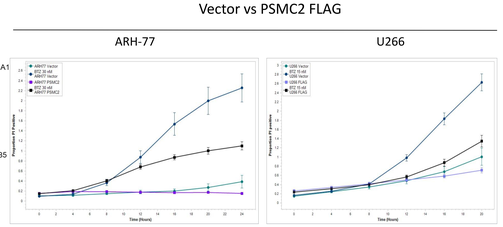


Figure 4 PSMC2 overexpression increases drug resistance. A. ARH77 and U266 FLAG and vector cells were challenged with BTZ for 18 hrs and then assayed by annexin PI staining. LD50 increased from 10 nm to 15 nm in ARH77 cells and from 12 to 25 in U266 Cells. **B.** ARH77 and U266 cells were treated with BTZ and analyzed for PI positivity every 4 hours using an Incucyte SX5.

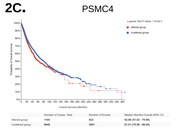
2A.



2B.

	OS	PFS	Disease-specific survival
PSMC1	0.764	0.939	0.162
PSMC2	0.284	**1.2E-03**	0.126
PSMC3	0.428	0.252	0.798
PSMC4	**2.00E-03**	**0.03**	**0.0248**
PSMC5	0.336	0.713	0.713
PSMC6	0.349	0.142	0.657

2C.



2D.

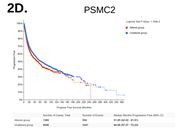
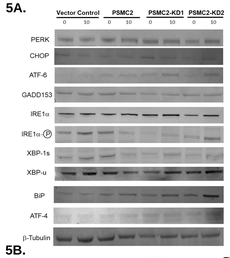
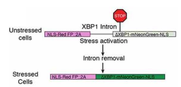


Figure 2 PSMC2 is the most common heterohexameric ATPase mutation in human cancer. A. Oncoprint of PSMC 1-6 alterations in TCGA Pancancer Atlas. PSMC2 is the most common and most discrete change. (n=10965) **B.** Cox univariate hazard ratios correlating PSMC subunits to clinical outcomes. **C.** Kaplan-Meier survival curves for pan-cancer PSMC4 expression reveals a median overall survival of 62 months compared to 81 months. **D.** Kaplan-Meier survival curves for pan-cancer PSMC2 expression reveals a median progression free survival of 51 months in the PSMC2 altered cohort compared to 64 months in the unaltered cohort ($p=1.2x10^{-3}$).

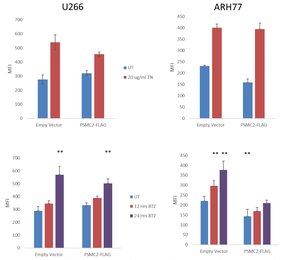
5A.



5B.



5C.



5D.

Figure 5 PSMC2 modulates ER stress in Multiple Myeloma Cell lines. A. ARH77 FLAG Vector control, PSMC2-FLAG, or PSMC2 knockdown cells were treated with 10 nM BTZ for 18 hour and analyzed by WB ER stress sensors activation. **B.** ER Stress Sensor (ERSS) described by others.[3] Briefly, the sensor shares an intron with XBP1U, a precursor of the XBP1S. Upon cell stress, intron is spliced, and GFP is expressed. **C.** ARH77 and U266 cells were transduced with ERSS and= challenged with 20 ug/ml tunicamycin for 24 hours and ER stress was quantitated by Flow cytometry. **D.** ARH77 and U266 cells were transduced with ERSS, treated with 10 nM BTZ and assayed by flow at 12 and 24 hours. In both cell lines, PSMC2 overexpression blunts the level of GFP signal on BTZ challenge.

CONCLUSIONS

- Transcriptomic analysis of UPS genes reveals that *PSMC2* expression correlates with OS in both the APEX and CoMMpass datasets. Additional *PSMC* subunits correlate to OS and PFS in the CoMMpass database, but PSMC2 is the most often altered.
- Overexpression increases MMCL resistance to bortezomib and the ER stress inducer tunicamycin. This contrasts what others have shown utilizing a CRISPR based KO model [4,5,6]
- Our overexpression data better models the clinical cohort where PSMC2 is largely overexpressed. This overexpressed PSMC2 increases proteasome activity and improves MMCL responses to ER stress.
- Targeting the ER stress response may re-sensitive PSMC2 high cell lines to PIs.

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ACKNOWLEDGEMENTS

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CONTACT INFORMATION

Primary Author:
James Ignatz-Hoover, MD PhD
James.Ignatz-Hoover2@uhhospitals .org

Corresponding Author
James Driscoll, MD PhD
James.Driscoll@uhhospitals.org