Disclosures

Susan Lee
Sr. Research Associate

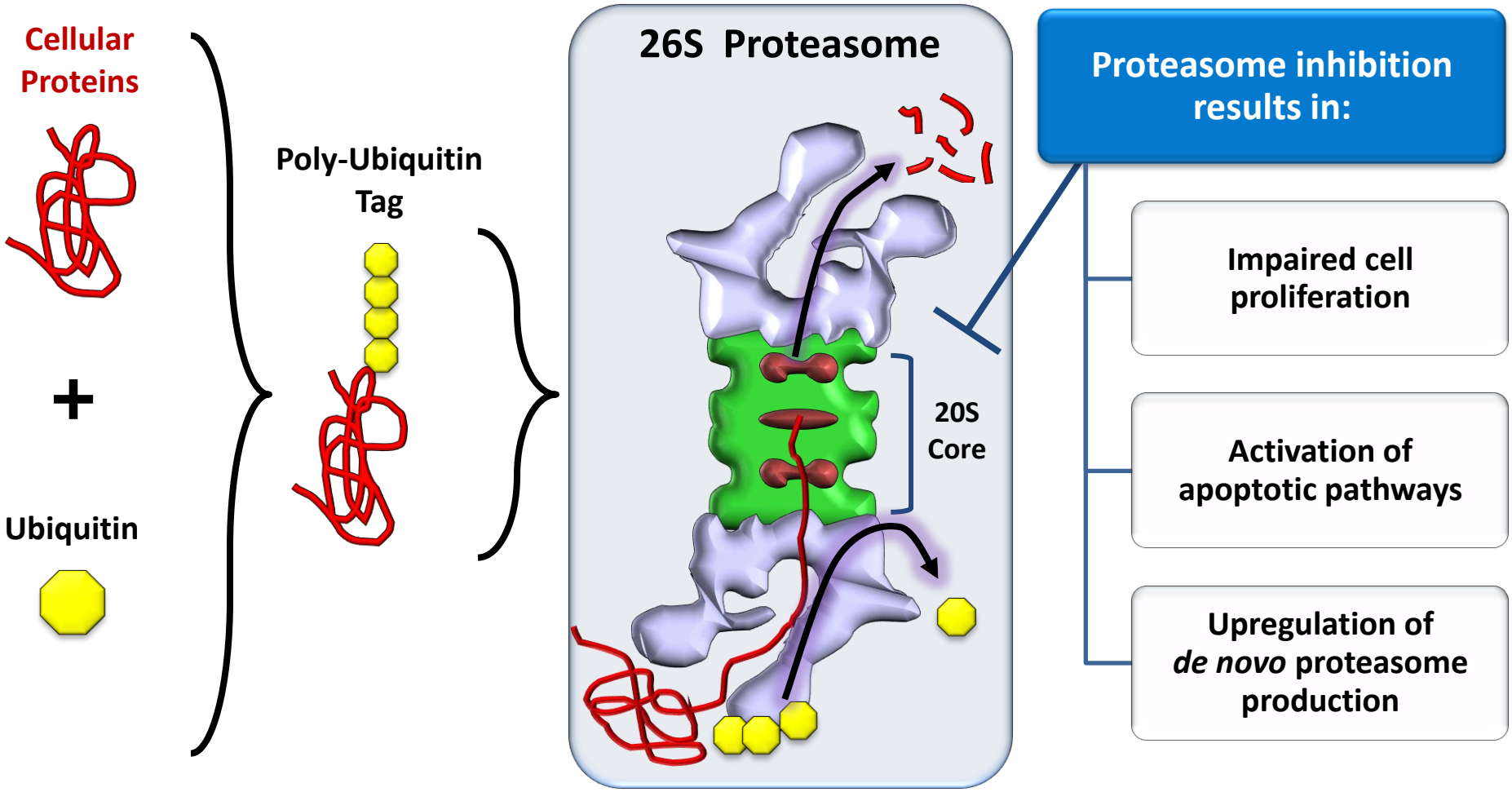
Employee of Onyx Pharmaceuticals

Potent Inhibition of Multiple Proteasome Subunits by Carfilzomib in Solid Tumor and Multiple Myeloma Patients

Lee, S.J., Woo, T.M., Vallone, M., Arastu-Kapur, S., Chan, E.T., Wong, A.F., Le, M.H., Hannah, A.L., Kirk, C.J.

Onyx Pharmaceuticals, South San Francisco, CA

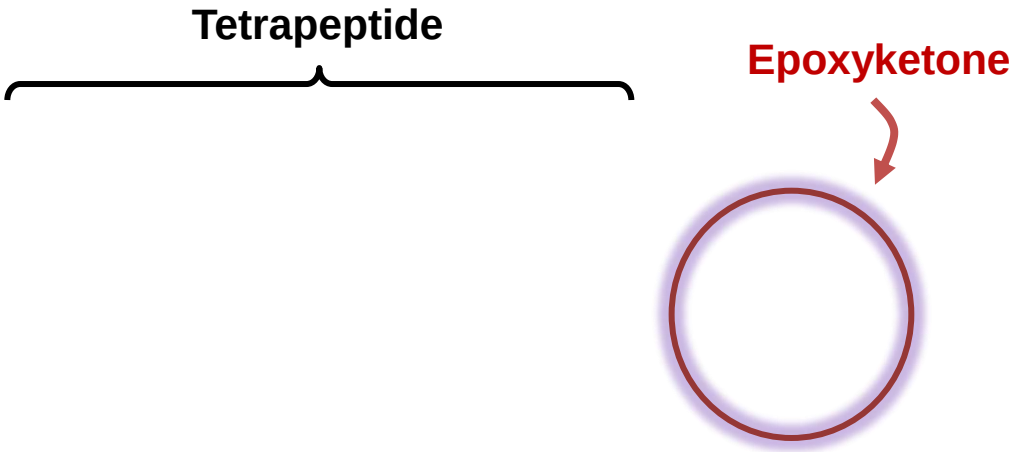
The Ubiquitin Proteasome Pathway



Adapted from Orlowski RZ, et al. *Clin Cancer Res.* 2008;14:1649-1657.
Meiners S, et al. *J Biol Chem*. 2003;21517-21525.

Carfilzomib: A Novel Agent Designed to Promote Selective and Sustained Proteasome Inhibition

- Carfilzomib is a next generation, highly selective, irreversible proteasome inhibitor
  - Potent and sustained target suppression
  - Improved antitumor activity
  - Minimal off-target activity with low neurotoxicity

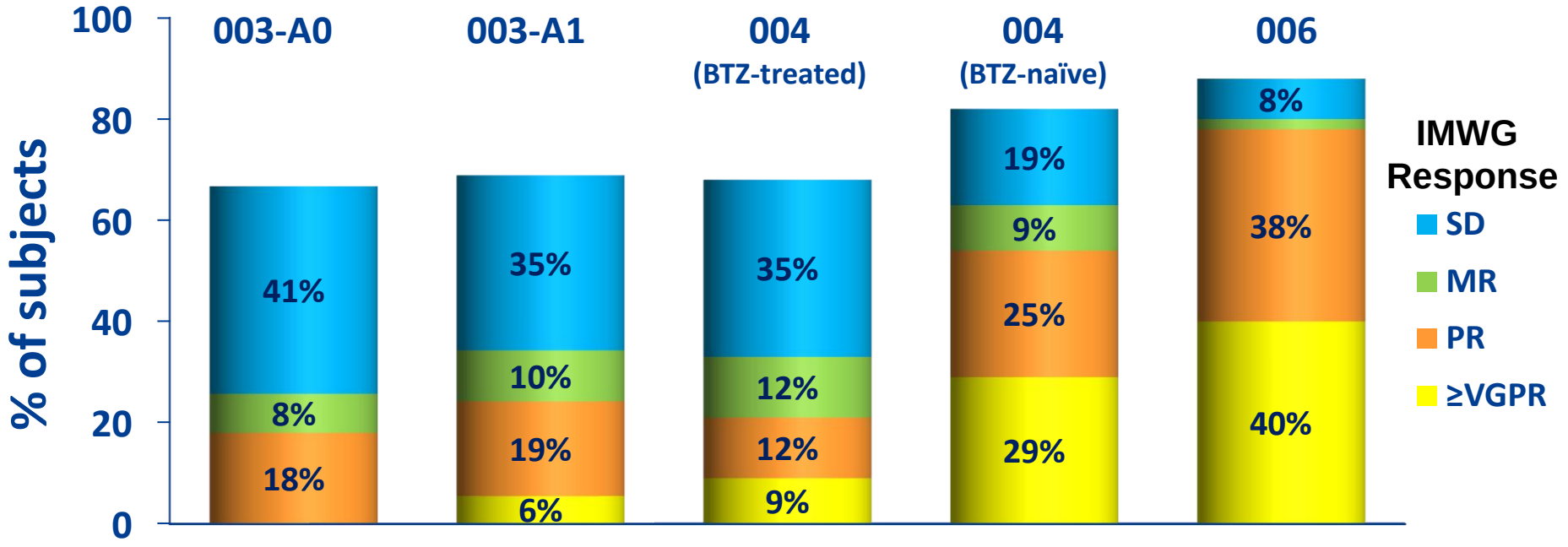


Adapted from Kuhn DJ, et al. *Blood*. 2007;110:3281-3290.

Clinical Program Overview

Trial	N	Design/Population	Status
003-A0	46	Ph 2 Single-Agent (RR / MM)	Completed
003-A1	266	Ph 2b Single-Agent (RR / MM)	Completed
004	165	Ph 2 Single-Agent (R / MM)	Enrolled
005	50	Ph 2 Single-Agent (RR MM with Renal Impairment)	Enrolled
011	84	**FOCUS:** Single Agent vs. Best Supportive Care (R&R MM)	Ongoing
006	84	Ph1/2 Combination with Len/Dex (Relapsed MM)	Enrolled
009	700	**ASPIRE:** Combination CRd vs. Rd (Relapsed MM)	Ongoing
007	145	Ph 1b/2 Single-Agent Dose-Escalation (Relapsed Solid Tumors &MM)	Ongoing

Carfilzomib Active in MM



N	39	257	34	64	50
# Prior Therapies	5 (median)	5 (median)	1-3	2 (median)	1-3
ORR (≥PR)	18%	25%	21%	54%	78%
Dosing	20 mg/m^2	20/27 mg/m^2	20 mg/m^2	20/27 mg/m^2	15-27 mg/m^2 + Len (10-25 mg) + Dex (40 mg qw)

Jagannath S, et al. ASH 2009. ASH/ASCO joint session oral presentation;Stewart AK, et al. EHA 2010. Abstract 1099 (oral presentation).
Siegel D, et al. ASH 2010. Abstract 985 (oral presentation).Vij R, et al. ASH 2010. Abstract 1938 (poster presentation).
Wang M, et al. Lymphoma and Myeloma 2010. Poster presentation; Martin T, et al. Lymphoma and Myeloma 2010. Poster presentation.

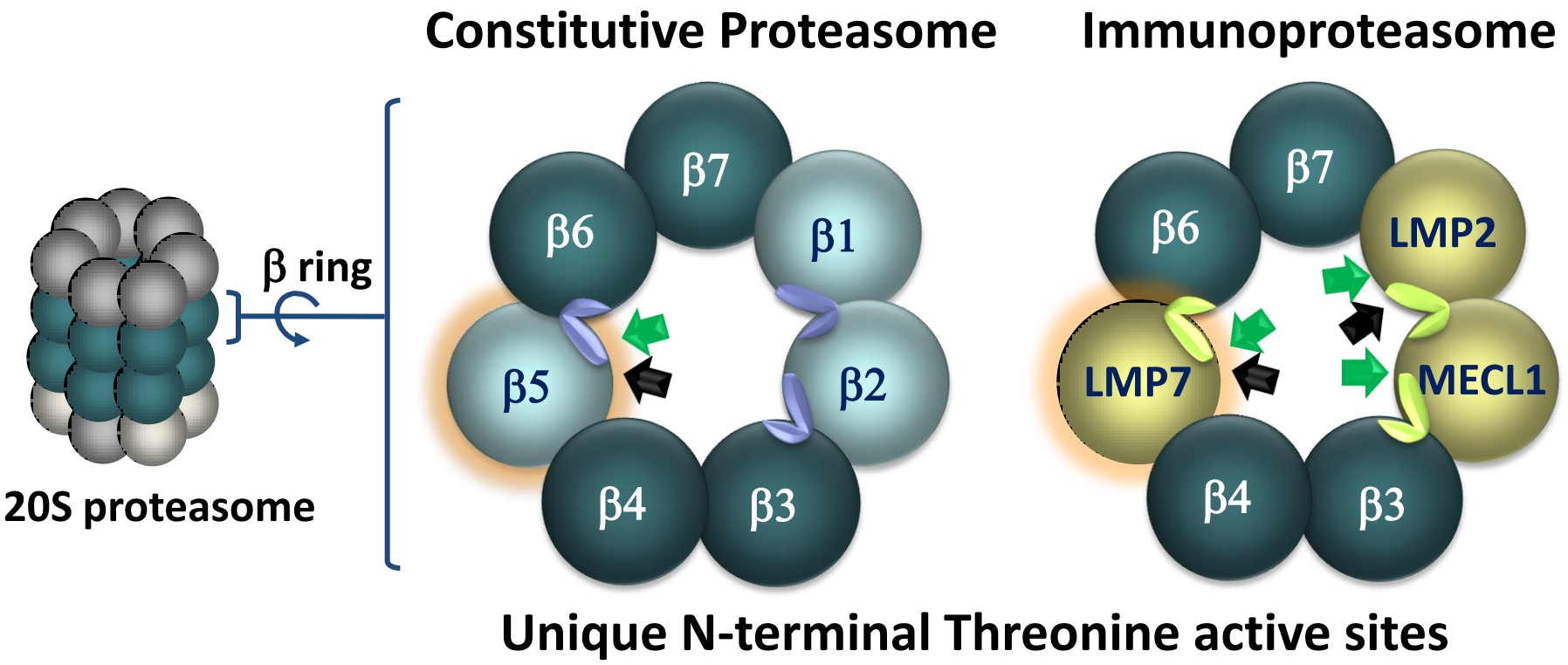
Carfilzomib is Well Tolerated as a Single Agent

All treatment-emergent adverse events of Grade ≥ 3 (≥10%)

	003 (A0) (N=46)	003 (A1) (N=266)	004 (N=143)	005 (N=50)	All Studies (N=505)
Hematologic					
Thrombocytopenia	26%	27%	15%	20%	23%
Anemia	37%	22%	12%	28%	21%
Lymphopenia	28%	19%	12%	18%	18%
Neutropenia	4.3%	10%	13%	6%	10%
Non-hematologic					
Pneumonia	11%	8.3%	13%	12%	10%
Fatigue	8.7%	7.1%	5.6%	14%	7.5%
Hyponatremia	13%	7.5%	2.1%	4%	6.1%

Singhal SB, et al. ASH 2010. Abstract 1954 (poster presentation).

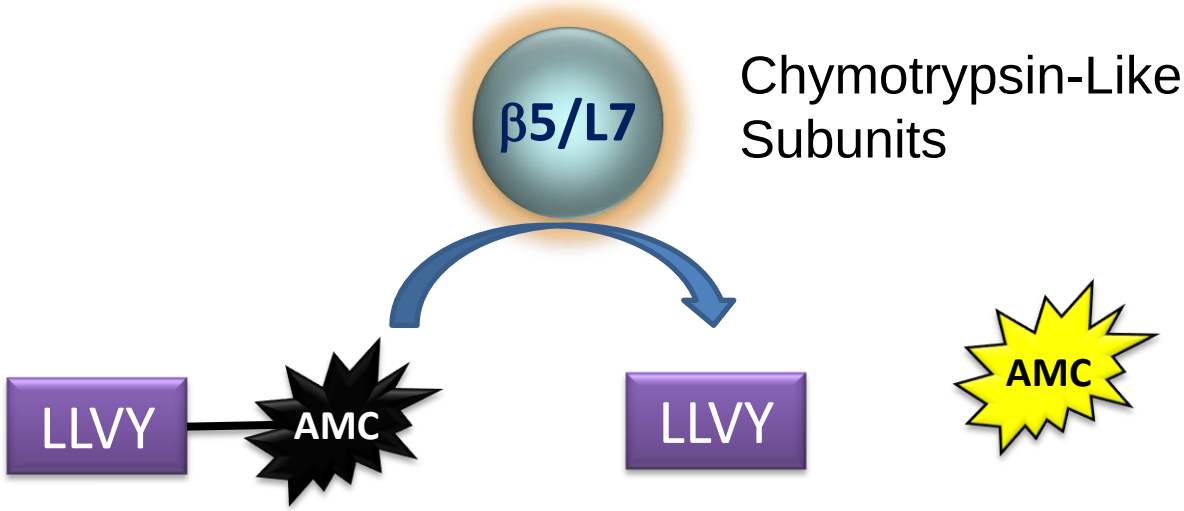
Two Classes of Proteasomes



Carfilzomib	• 1º Target: chymotrypsin-like active sites (ß5 / LMP7); 2º Target: LMP2 & MECL1
Bortezomib	• 1º Target: chymotrypsin-like active sites (ß5 / LMP7); 2º Target: LMP2

Adapted from Groettrup M, Kirk C, and Basler M. *Nat Rev Immunol*. 2010;10(1):73-78.

Standard Assays for Measuring Proteasome Activity Involve Substrate Cleavage



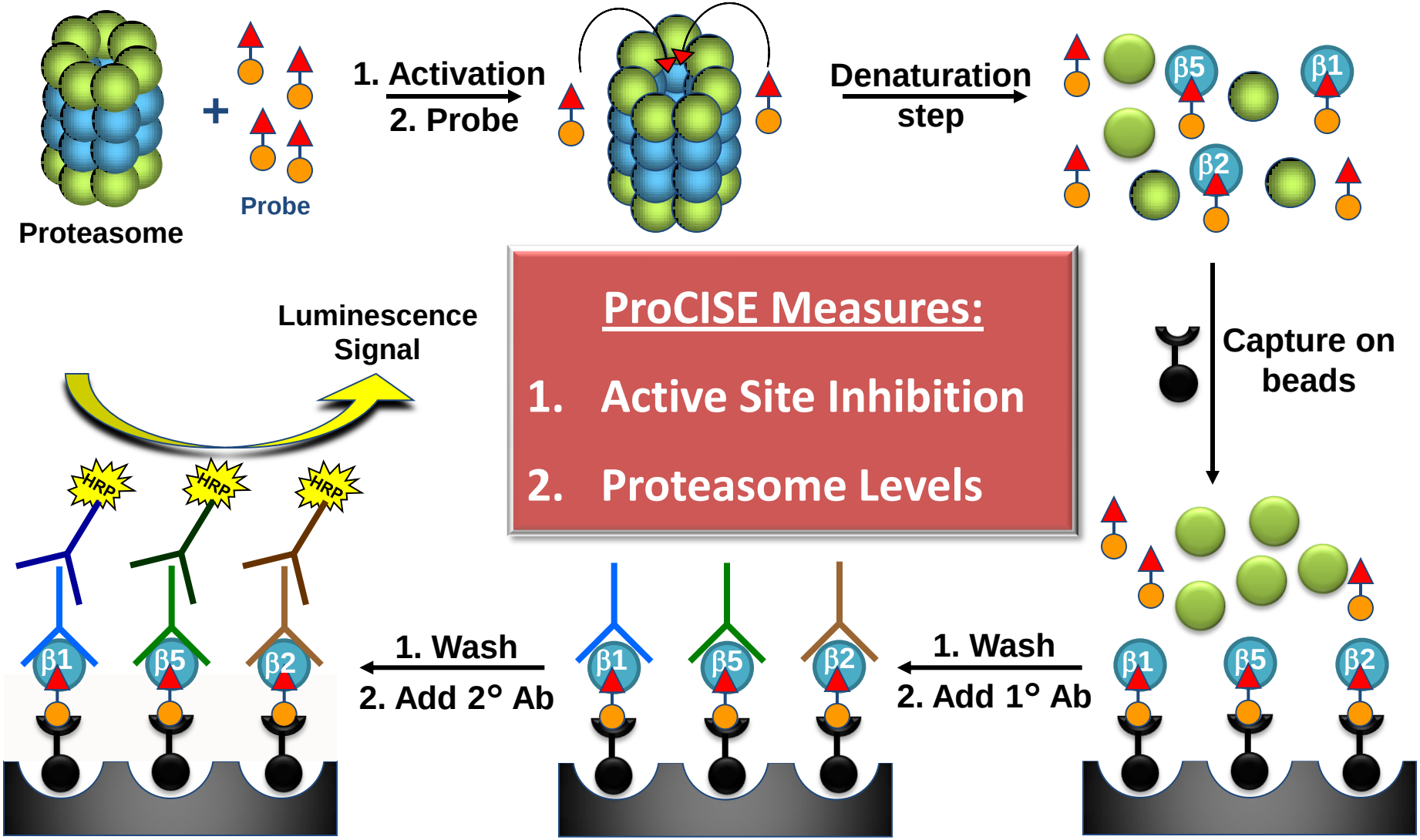
- Inhibition of **both** Beta5 & LMP7 necessary to induce Myeloma tumor cell death[1]
  - MM cells express both classes of proteasome
- Substrates for other active sites are poorly defined
  - Role of individual active sites in drug response is currently unknown

[1]Parlati, F., et al. Carfilzomib can induce tumor cell death through selective inhibition of the chymotrypsin-like activity of the proteasome. Blood, Vol. 114, Issue 16, 3439-3447, October 15, 2009

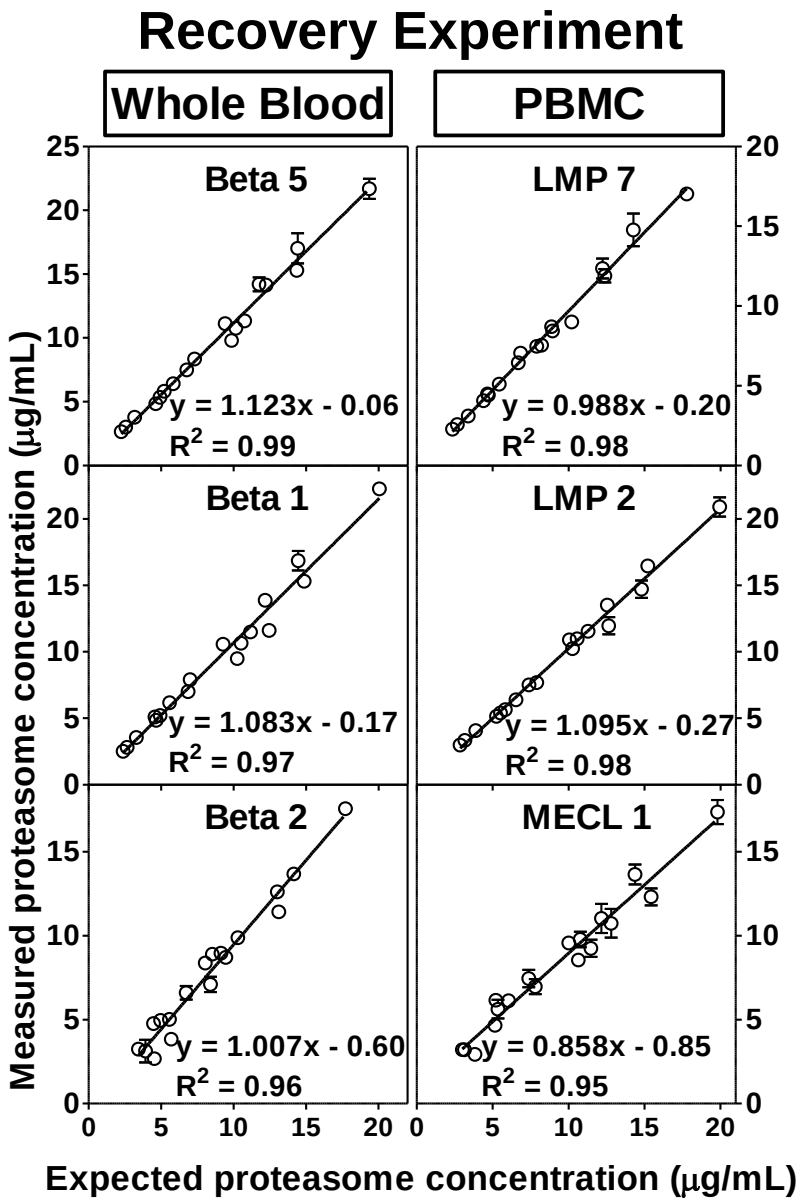
ProCISE

(**Pro**teasome **C**onstitutive-**I**mmuno **S**ubunit **E**LISA)

Measures All 6 Proteasome Subunits

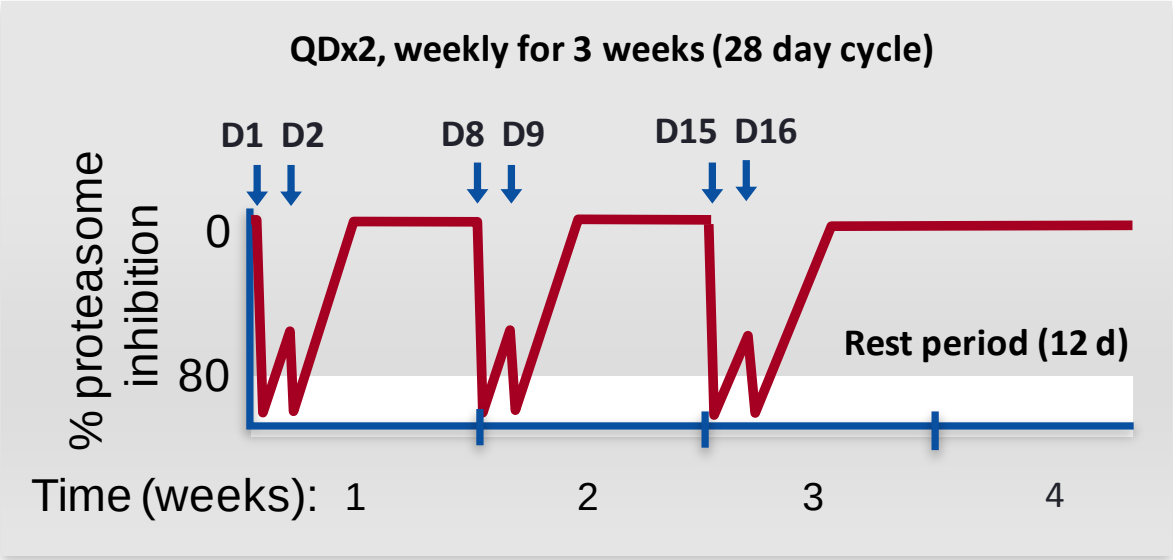


ProCISE Validation



- Low day-to-day variability
- Beta5 & LMP7 activity is equivalent to LLVY activity
- Dynamic range allows for detection of up to 90% inhibition

Carfilzomib Dosing Schedule & PD Analysis

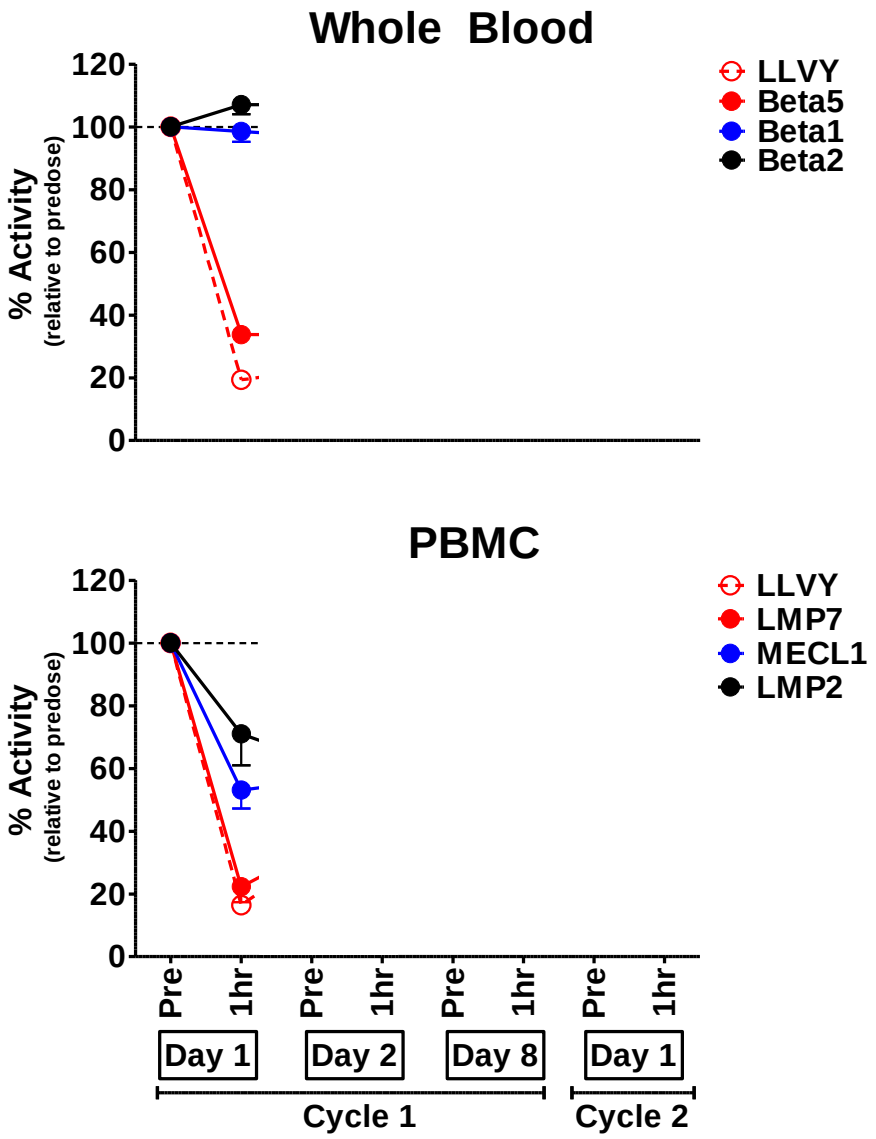


	CD138+ (Bone Marrow)	Blood	PBMC
LLVY	CT-L	CT-L	CT-L
ProCISE	Beta5 LMP7 MECL1	Beta5 Beta2 Beta1	LMP7 LMP2 MECL1
# Patients Analyzed	40	74	71

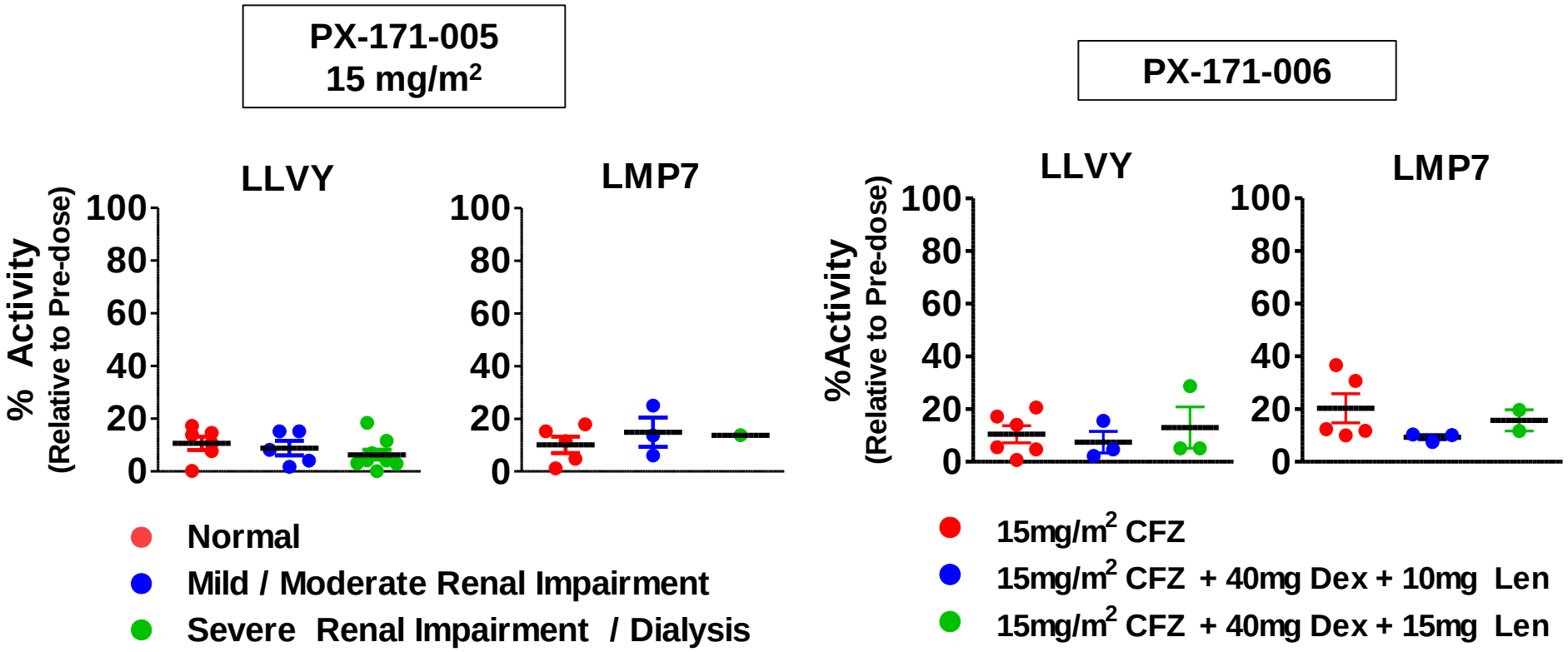
Kinetics of Proteasome Inhibition in Carfilzomib Treated Patients

- **Potent Inhibition After 1st Dose**
  - **>80% for CT-L**
- **Cumulative Inhibition in Blood due to irreversible nature of CFZ**
- **Prolonged Inhibition in PBMCs for 48 hr**
- **Recovery of activity in PBMC following rest period**

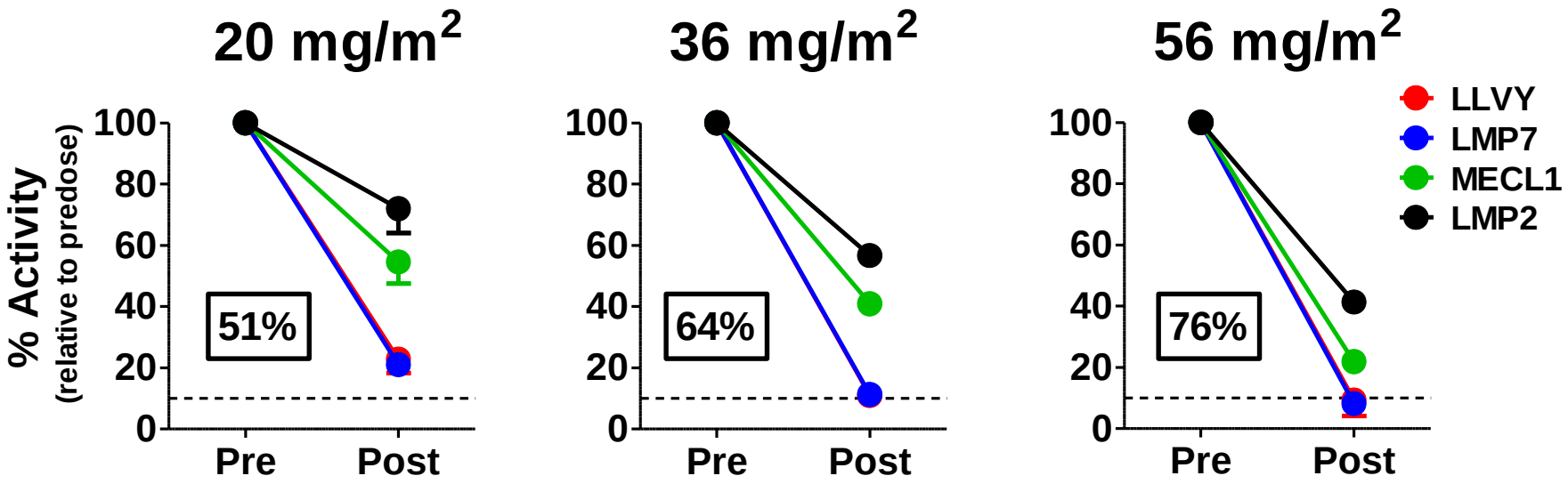
Dose: 15 mg/m^2



Renal Status or Co-Administration of other Agents Does Not Alter Carfilzomib Pharmacodynamics

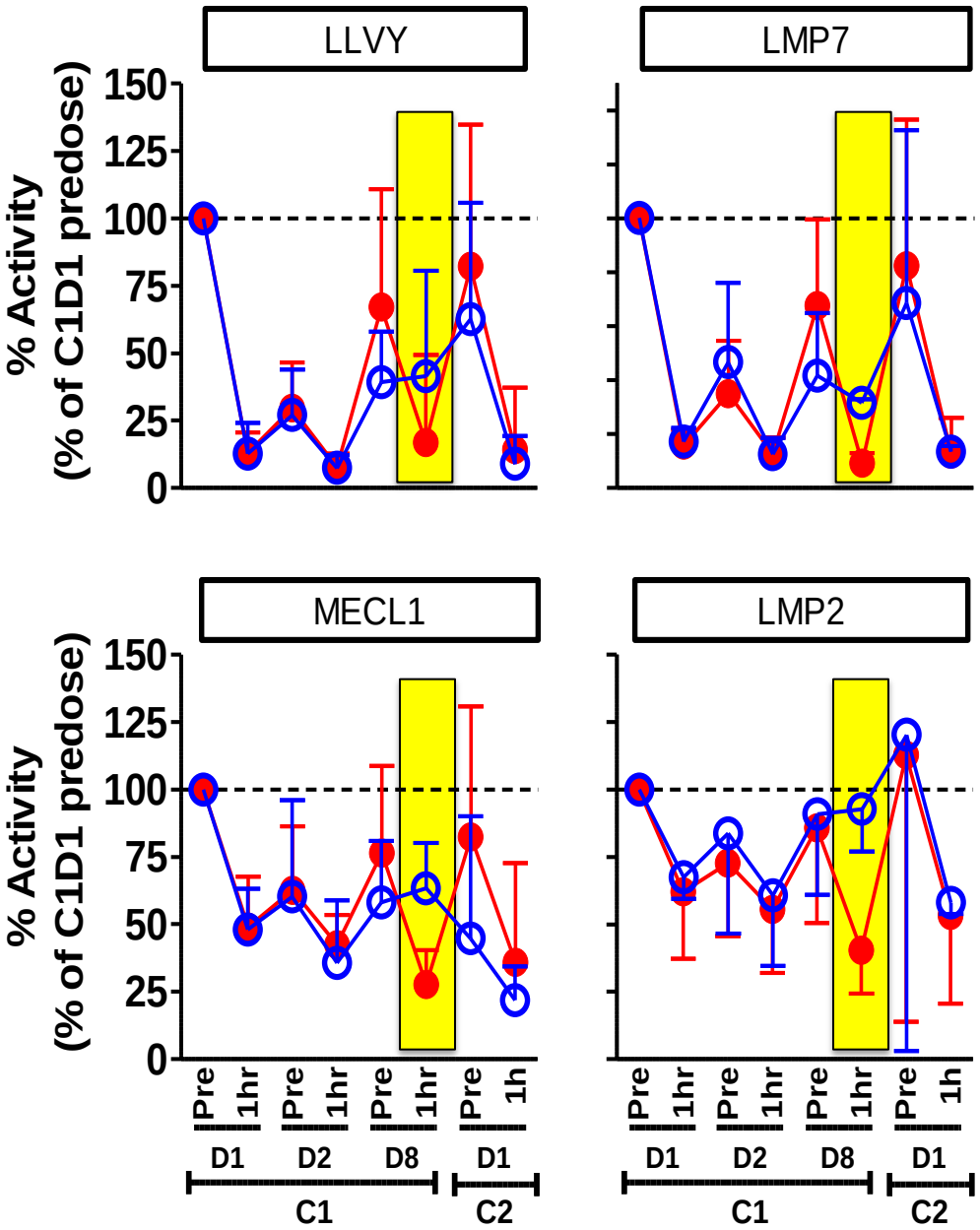


High Dose Carfilzomib Results in Near Complete Inhibition of Immunoproteasome Subunits



- Immunoproteasome subunits show dose-dependant inhibition by CFZ
- At 56mg/m^2, CT-L activity is below the limit of detection (≤10% activity) & total immunoproteasome inhibition reaches 76%
- Only inhibition of Beta5 is seen in Whole Blood

Patient Response May Correlate to Proteasome Subunit Inhibition



≥ PR N = 10-20 (15 – 27mg/m²)

PD N = 3-5 (15 – 56mg/m²)

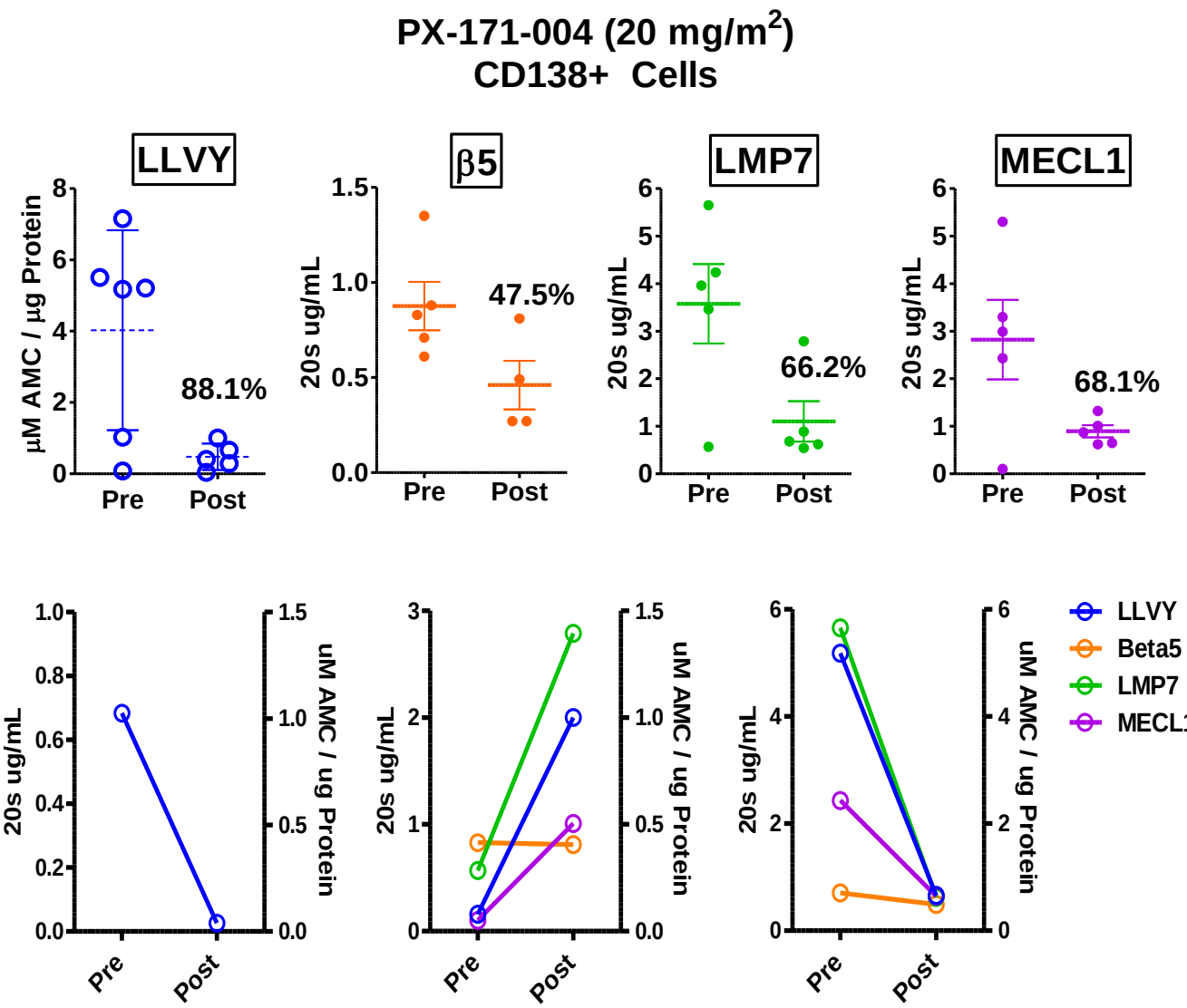
Differences in proteasome inhibition are observed between responding & non-responding patients

Proteasome Content in Patient-Derived Tumor Cells

Baseline CD138+ Bone Marrow Proteasome Activity					
Trial	**Ave # Prior Regimens** (range)	**LLVY (μM AMC / μg Protein) (N = 25)**	**β5 (ng/μg Protein (N = 17)**	**LMP7 (ng/μg Protein) (N = 19)**	**% LMP7**
003, 004, 005	**5** (1 -13)	**10.2 ± 3**	**0.9 ± 0.1**	**2.5 ± 0.4**	**73.7%**
Proteasome Activity in Cell Lines[1]					
MM1.S			**3.9 ± 0.5**	**2.9 ± 0.2**	**45%**
8226			**4.0 ± 0.5**	**2.4 ± 0.2**	**37%**
Arh77			**4.0 ± 0.2**	**3.4 ± 0.2**	**46%**

[1]Parlati, F., et al. Carfilzomib can induce tumor cell death through selective inhibition of the chymotrypsin-like activity of the proteasome. Blood, Vol. 114, Issue 16, 3439-3447, October 15, 2009

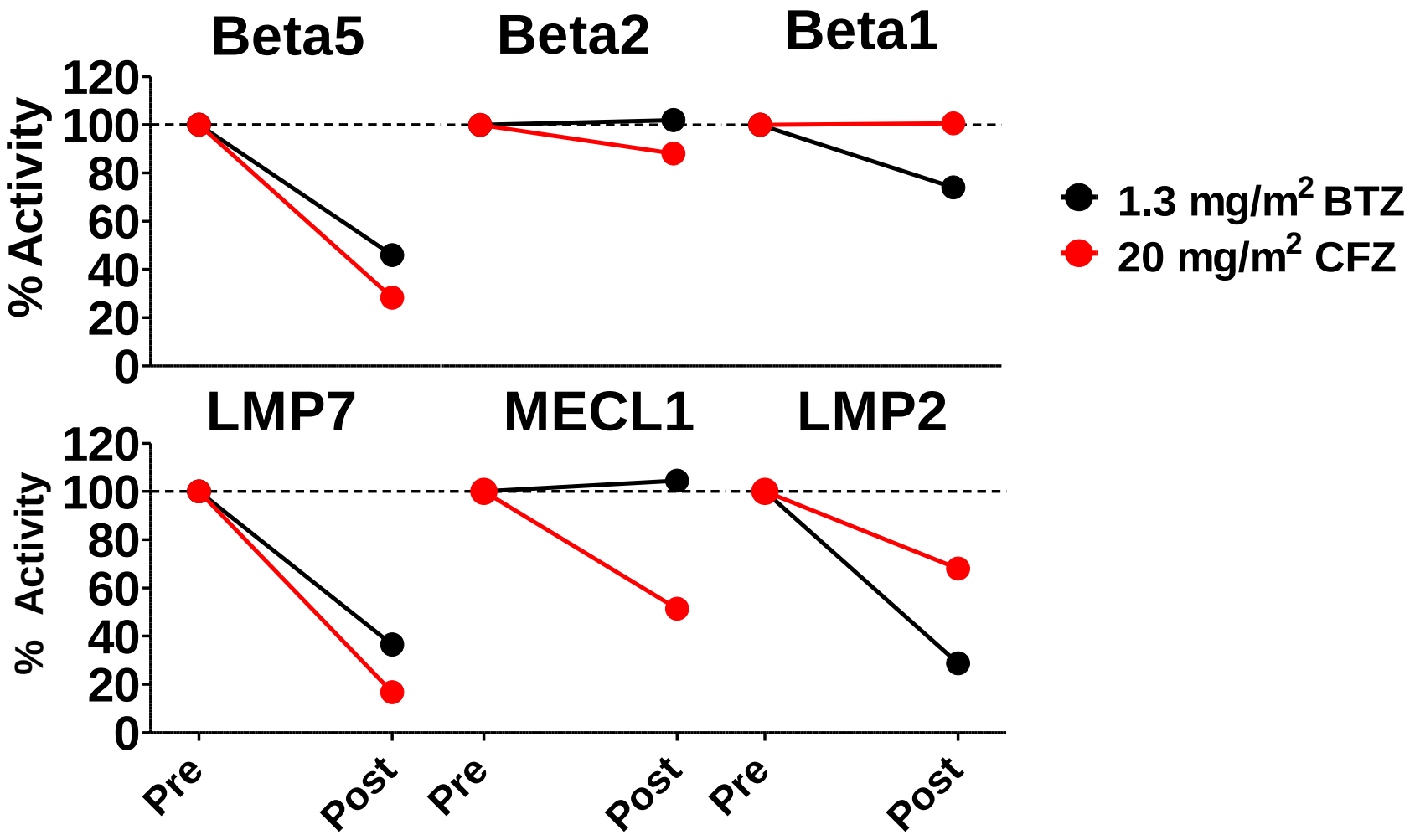
Carfilzomib Administration Results in Proteasome Inhibition in Tumor Cells



Levels of inhibition similar to Whole Blood & PBMC

2 of 3 patients show inhibition in Bone Marrow Tumor Cells

Comparison of the Proteasome Inhibition Profile[19] of Carfilzomib and Bortezomib



Conclusions

- ProCISE is the first assay to measure all 6 proteasome subunits in patient samples
- Carfilzomib administration results in ≥ 85% inhibition of CT-L subunits (Beta5 / LMP7)
- Inhibition in MM tumor cells similar to Blood & PBMC
- Levels of inhibition with carfilzomib compare favorably to that of other classes of proteasome inhibitors
- Near complete inhibition of the immunoproteasome is achieved at well-tolerated high-dose CFZ and is currently under further clinical investigation
- Potent immunoproteasome inhibition may correlate with response in MM patients

Acknowledgements

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