

Development of a Whole Cell Assay to Monitor Proteasome Activity in Multiple Myeloma

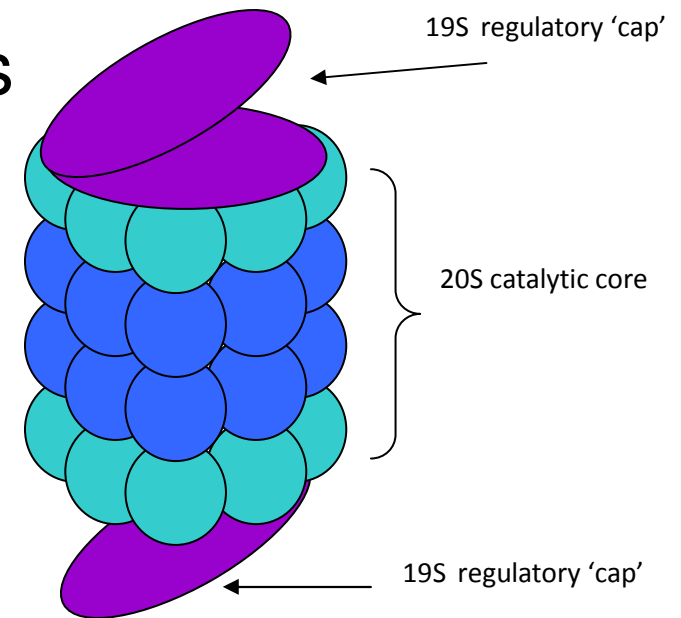
Andrea Liggett

UKMF Scientific Day

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The Proteasome

- Protein degradation via ATP-ubiquitin proteasome pathway
- Control of vital cellular processes
- 3 elements of catalytic activity:
 - β_5 , chymotrypsin-like (CT-L)
 - β_2 , trypsin-like (T-L)
 - β_1 , peptidylglutamate peptide hydrolysing (PGPH)



Proteasome Inhibitors

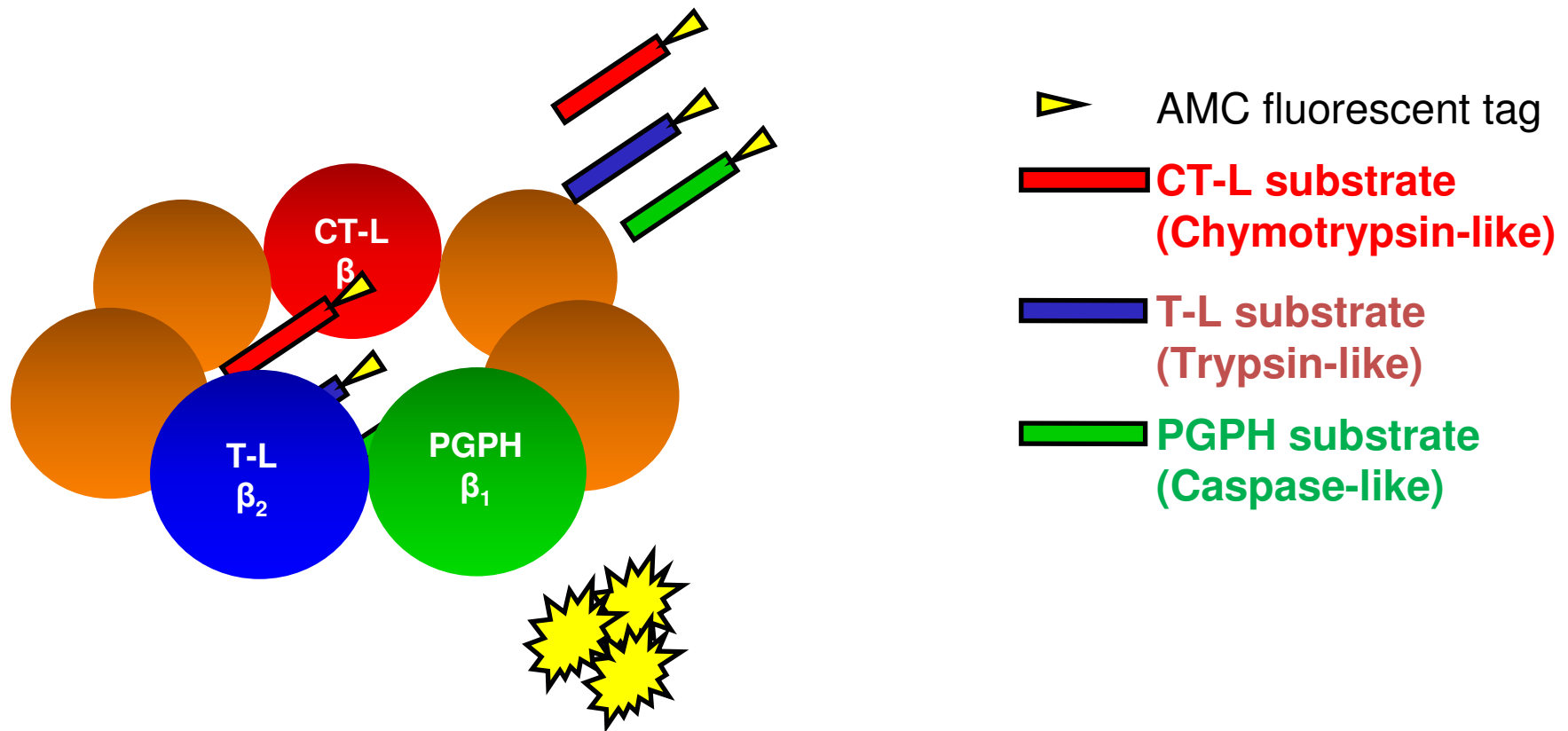
- Proteasome inhibitors are a novel class of anti-cancer agents
 - **Bortezomib (Velcade, PS-341)**
- Enhance effectiveness of chemotherapeutic agents and overcome chemoresistance in patients with Multiple Myeloma.
- Better understanding of molecular pathogenesis and novel therapies may improve survival.

AIMS

- Develop a clinically – relevant whole cell proteasome assay
- Apply this to the investigation of three different proteasome inhibitors
- Profile proteasome activity in Multiple Myeloma patient samples

MATERIALS & METHODS

Conventional Fluorogenic Assay



Monitoring Proteasome Activity in whole cells

- conventional fluorogenic assays use extracts (> 5×10^6 cells), thus limiting their use with clinical samples

- fluorescent substrates:

- ❖ **Succ-LLVY-AMC** for **CT-L, β_5**

- ❖ **Z-LLE-AMC** for **PGPH, β_1**

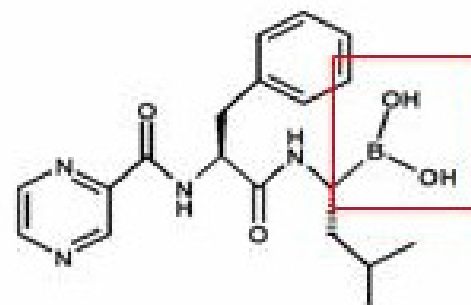
- modified by adapting a side chain as a methyl ester to aid cell entry

Monitoring Proteasome Activity in whole cells

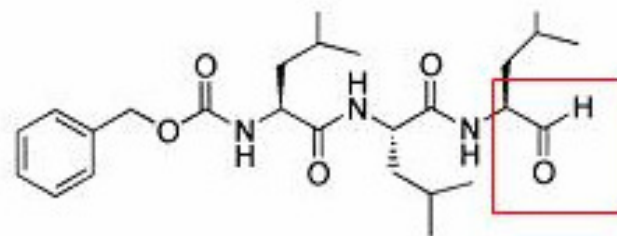
- U266 & OPM2 Multiple Myeloma cell lines
- CD138⁺ cells from bone marrow samples of patients with Multiple Myeloma
- Rate of increase in fluorescence = turnover of substrate by β_5 and β_1 subunits of the proteasome respectively
- Measured using the ABI Cytofluor system

Proteasome Inhibitors

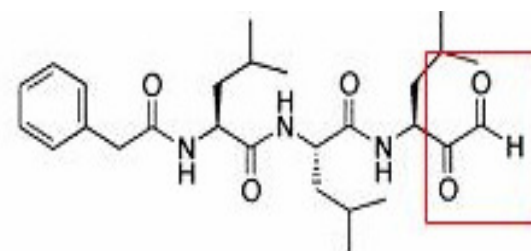
- Dipeptide boronic acid inhibitor
 - **Bortezomib (Velcade, PS-341)**
- 1st generation proteasome inhibitor



- Peptide aldehyde inhibitor
 - **MG132**



- Novel tripeptide α – ketone aldehyde inhibitor
 - **BZLLL**



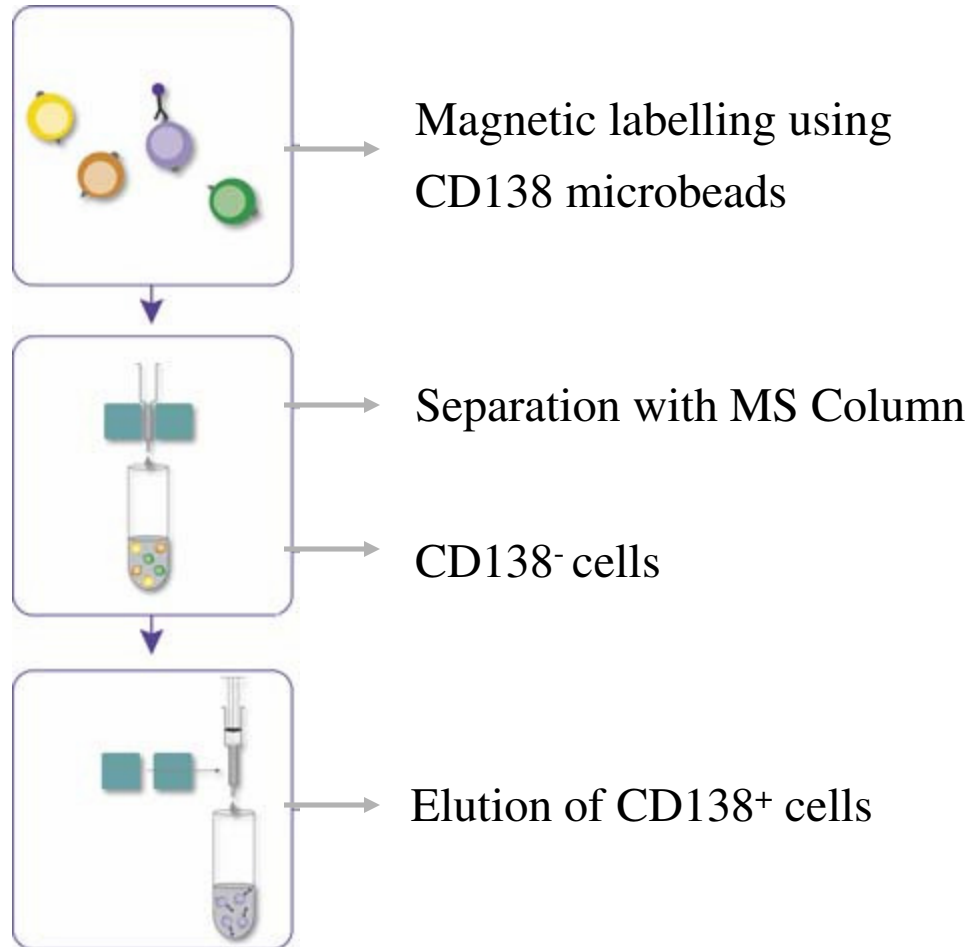
MACS Beads Separation



MS apparatus

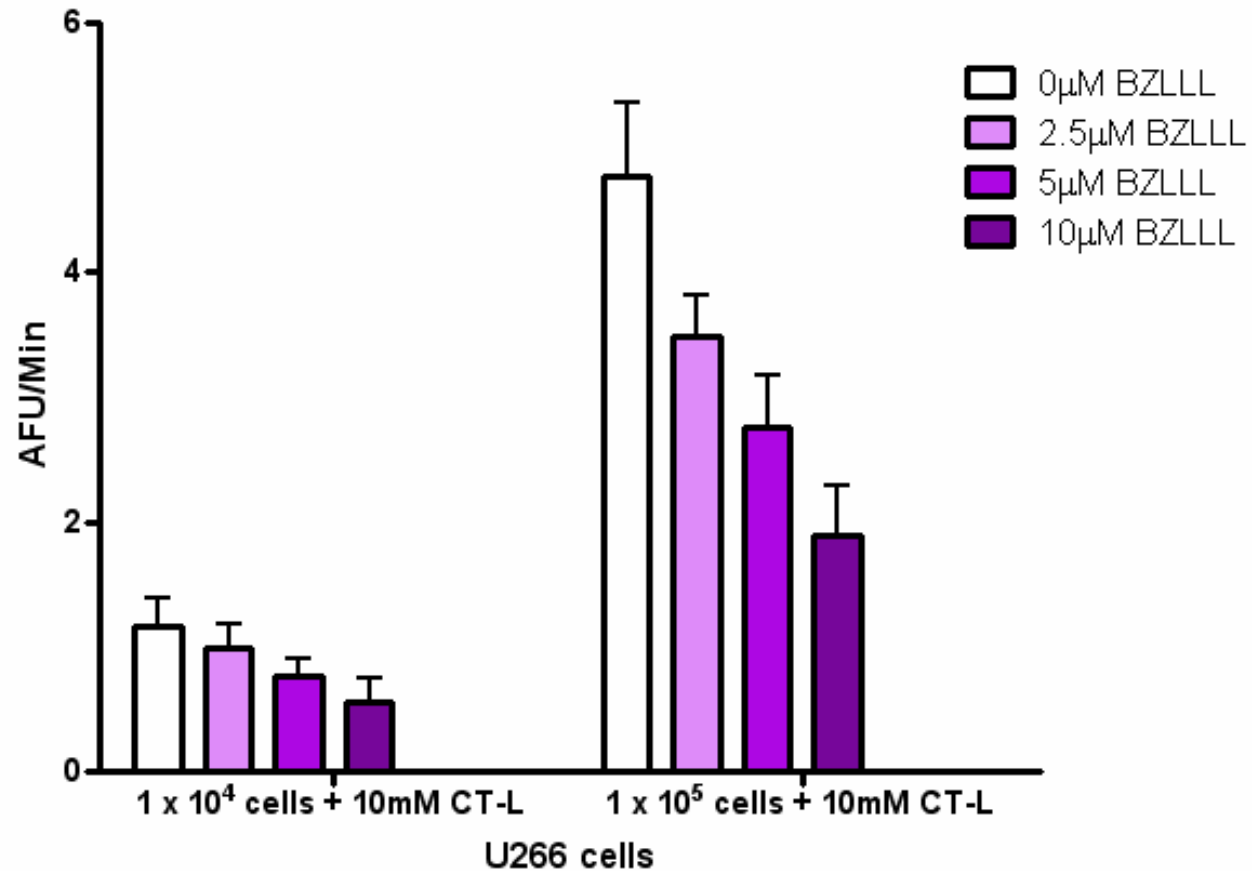


AutoMACS System



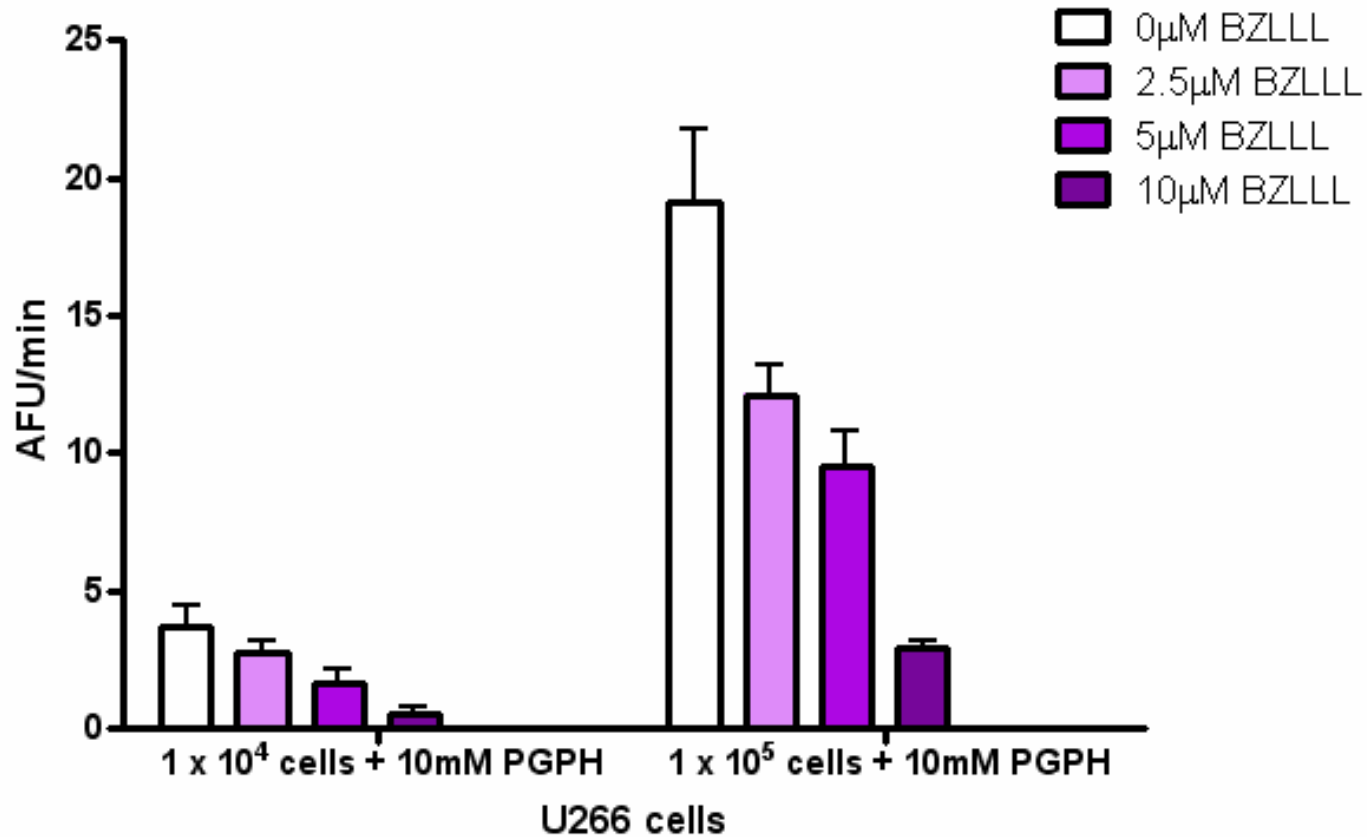
RESULTS

Turnover of CT-L substrate in U266 cells at increasing concentrations of BZLLL



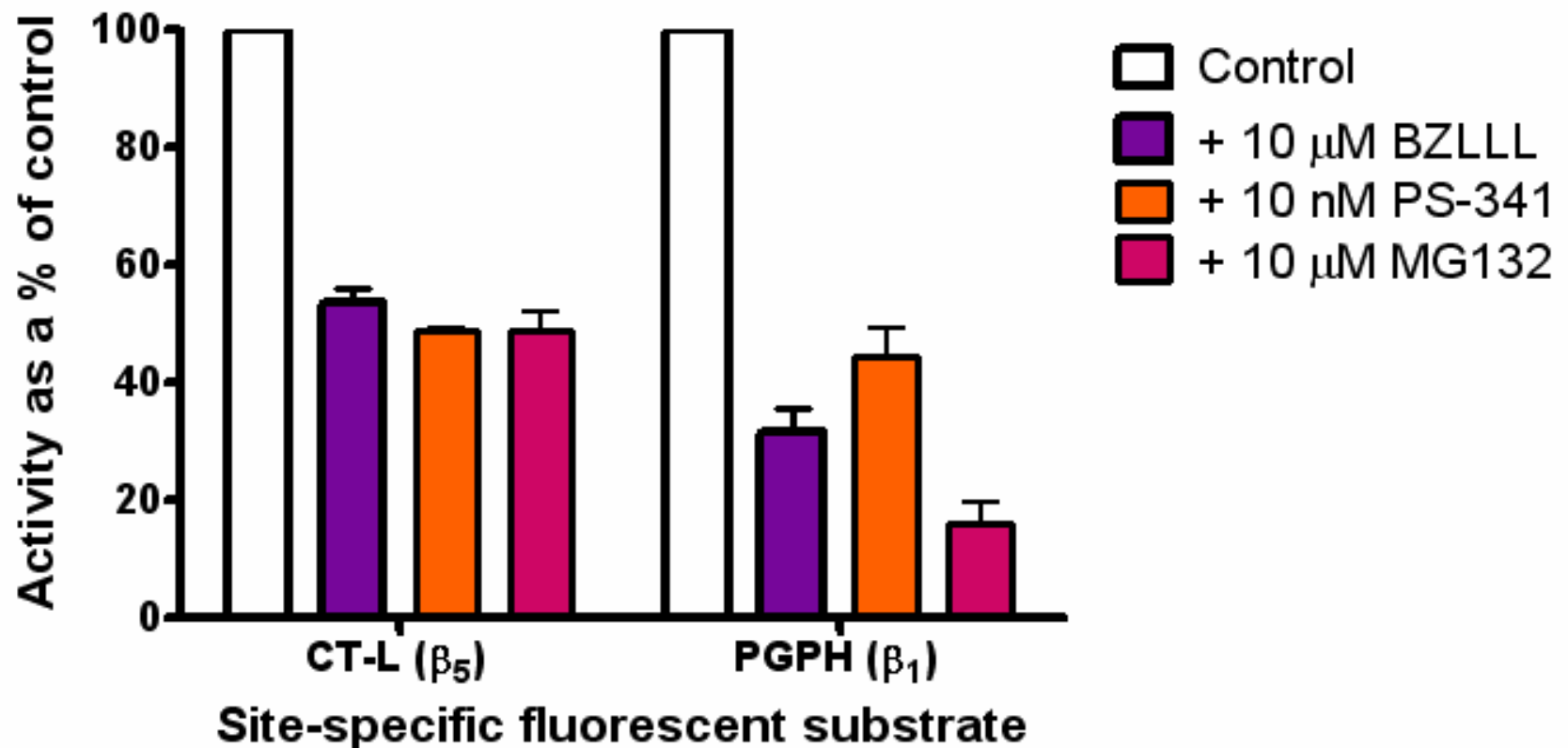
Number of cells	0 μM BZLLL (%)	2.5 μM BZLLL (%)	5 μM BZLLL (%)	10 μM BZLLL (%)
1 x 10 ⁴	100	85 ± 15	66 ± 12	48 ± 17
1 x 10 ⁵	100	73 ± 7	58 ± 9	40 ± 8

Turnover of PGPH substrate in U266 cells at increasing concentrations of BZLLL



Number of cells	0 μM BZLLL (%)	2.5 μM BZLLL (%)	5 μM BZLLL (%)	10 μM BZLLL (%)
1 x 10 ⁴	100	75 ± 12	44 ± 14	15 ± 7
1 x 10 ⁵	100	63 ± 6	50 ± 7	15 ± 2

Peptidyl Glutamate Peptide Hydrolysing (PGPH) and Chymotrypsin-like (CT-L) proteasome activities in the presence of three proteasome inhibitors.



Monitoring Proteasome Activity in primary CD138+ Multiple Myeloma plasma cells (1×10^4).

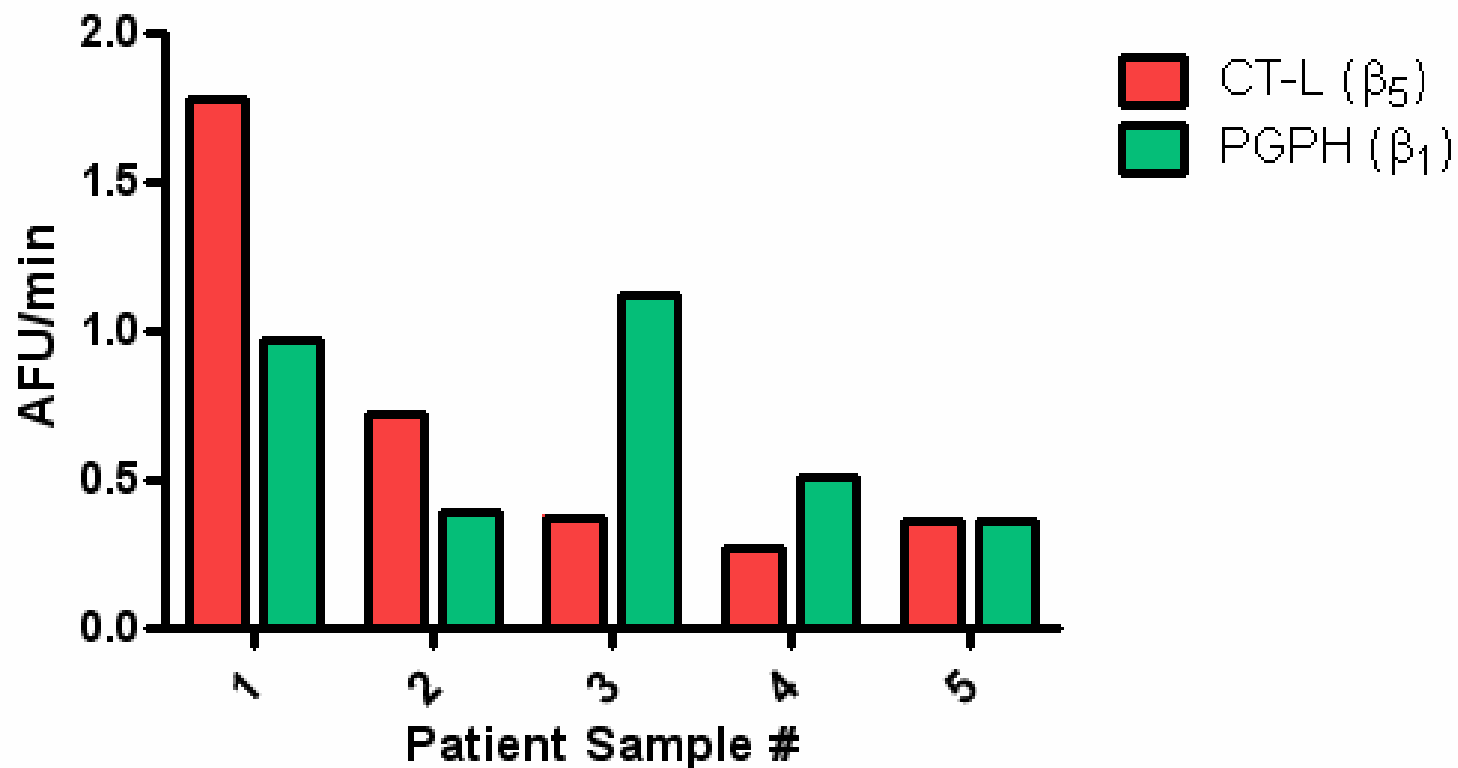
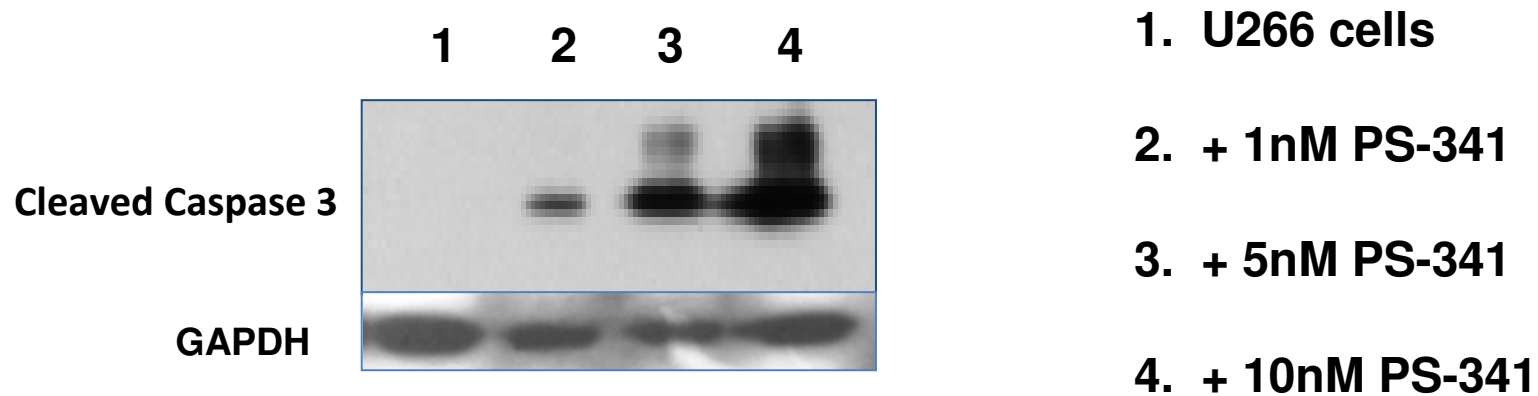


Table 1: Proteasome Inhibition & caspase – 3 activation

Drug Treatment	Time to MAX CT-L inhibition (hrs)	Time to MAX PGPH inhibition (hrs)	50% Apoptosis (hrs)*
10µM BZLLL	9.9 ± 0.8	9.4 ± 2.6	12.9 ± 2.7
10nM PS341	7.7 ± 0.6	7.8 ± 0.6	10.4 ± 0.3
10µM MG132	8.5 ± 0.9	6.7 ± 1.1	12.9 ± 1.4

* Apoptosis was measured using the Enzchek® Caspase – 3 Assay # 2

Effect of Proteasome Inhibition on Caspase 3 Activation



Conclusion

- **CT-L** and **PGPH** activities have been profiled in 1×10^4 whole cells.
- Both substrates and inhibitors are respectively permeabilising and killing the cells.
- Modification of the **T-L** substrate **Z-Ala-Arg-Arg-AMC** will provide a comprehensive method to profile total proteasome activity in **clinically relevant** numbers of **whole cells**.

Acknowledgements

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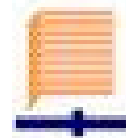
Clinical Haematologists



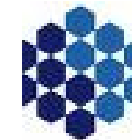
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