Methods

**Cell culture**

AMO-1 cells, plasma cells from a 64-year old female myeloma patient, were used as a model cell-line for multiple myeloma. Bortezomib and carfilzomib resistant AMO-1 cells were generated and kindly gifted by the Driessen lab (1). Bortezomib, carfilzomib, pomolidimide and bortezomib plus pomolidimide resistant AMO-1 cells were also generated by Dr James Dunford by continual and escalating drug exposure. *(WHERE did we get AMO-1 WT cells from, kindly gifted by MARIA???)*

AMO-1 cells were cultivated in RPMI-1640 medium (*WHERE we get it from*), supplemented with 10% fetal bovine serum (FBS), 100 µg/ml streptomycin and 100 U/ml penicillin (P/S) and 2mM L-glutamine (Invitrogen, UK). Cells were passaged at approximately 1.5-2 million cells per ml (IS THIS THE RIGHT MEASURE??).

Cell viability assay

10% alamarBlue® reagent (ask JIM) was added to cell suspensions. The cells were incubated at 37$^{\circ}$C for 180 minutes. The fluorescence was measured at wavelengths of 360nm for excitiation and 460nm for emission using a FLUOStar OPTIMA microplate reader.

**RNA-Seq**

RNA extraction

RNA was isolated using the Direct-zol RNA miniprep kit (Zymo Research, USA), following the manufacturer’s instructions.

Proteomics

**Phospho**

Cell Lysis

Approximately 20 million cells for each condition in triplicate was pelleted and stored in 500$\mu$l of PBS at -80$^{\circ}$C. 300$\mu$l of fresh lysis buffer (10ml RIPA buffer, 3$\mu$l benzonase, 1 tablet phos stop) was added to each pellet, vortexed and left for 10 minutes on ice and then sonicated. The supernatant was transferred to a fresh (WHAT TYPE) tube.

Protein quant

Protein concentrations were determined by BCA protein assay (Thermofisher, UK). 400$\mu$g of protein at a working concentration of 1$\mu$g/ml was taken from each sample. 100$\mu$g was used for proteomics and 300$\mu$g for phosphoproteomics.

Bibtex:

1)

@article{soriano2016proteasome,

title={Proteasome inhibitor-adapted myeloma cells are largely independent from proteasome activity and show complex proteomic changes, in particular in redox and energy metabolism},

author={Soriano, GP and Besse, L and Li, N and Kraus, M and Besse, A and Meeuwenoord, N and Bader, J and Everts, B and den Dulk, H and Overkleeft, HS and others},

journal={Leukemia},

volume={30},

number={11},

pages={2198--2207},

year={2016},

publisher={Nature Publishing Group}

}