Genome-Scale Crispr-Cas9 Knockout Studies Reveal Mutifactorial and Functionally Overlapping Mechanisms of Myeloma Cell Resistance to Proteasome Inhibition

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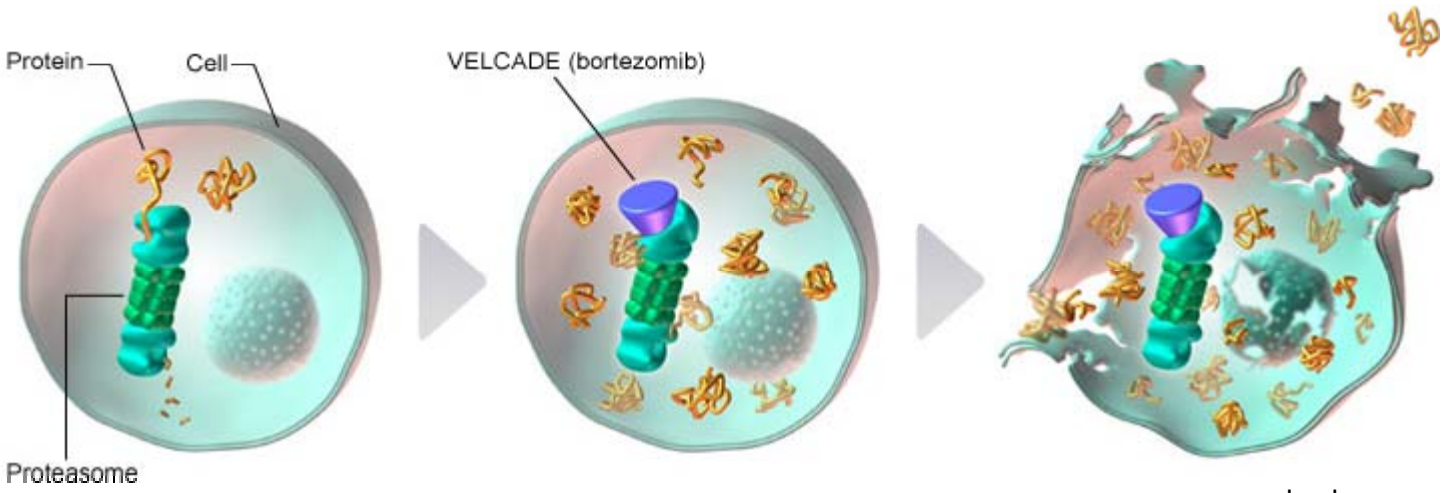
Bortezomib in Multiple Myeloma



www.velcade.com

Bortezomib (trade name *Velcade*) is approved for the treatment of patients with multiple myeloma (MM)

Bortezomib binds to the β5 subunit, leading to full inhibition of ubiquitinated protein hydrolysis.

Extends survival, but does not cure the disease

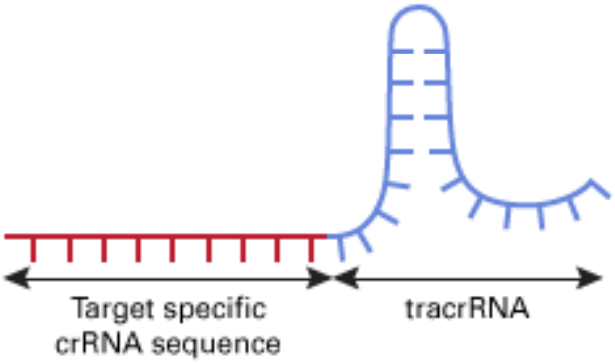
Affects many proteins and hence many pathways

Many possible mechanisms of resistance

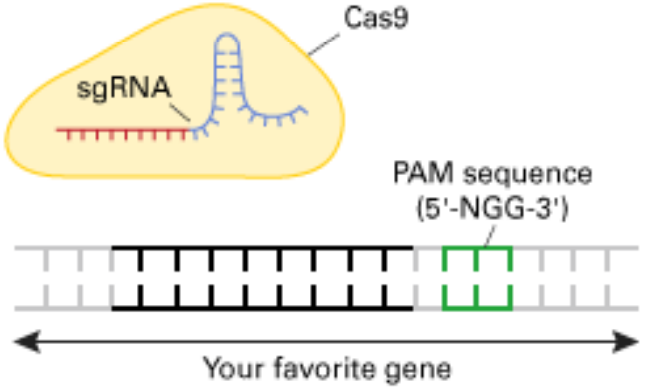
Can we use a genome wide approach to identify genes that regulate bortezomib resistance in MM cells?

CRISPR overview

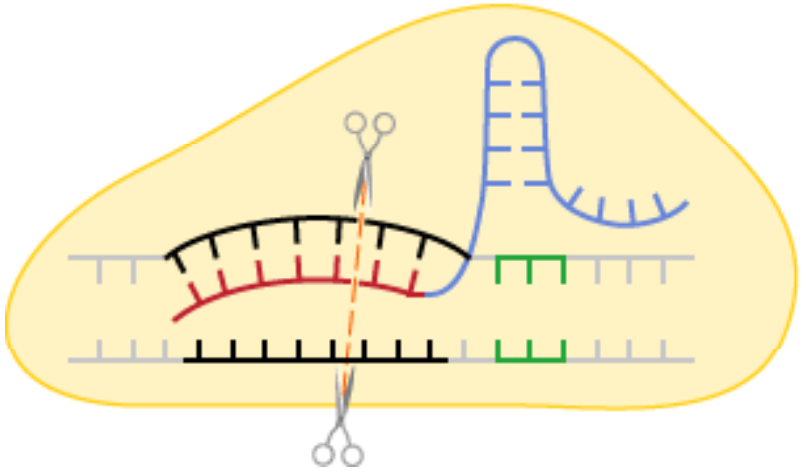
1 sgRNA (single guide RNA)



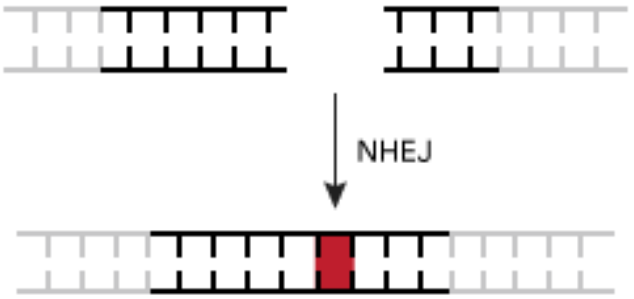
2 sgRNA + CAS9 protein



3 Target specific cleavage



4 Cellular error-prone repair
"knocks out" gene



GeCKO v2 library

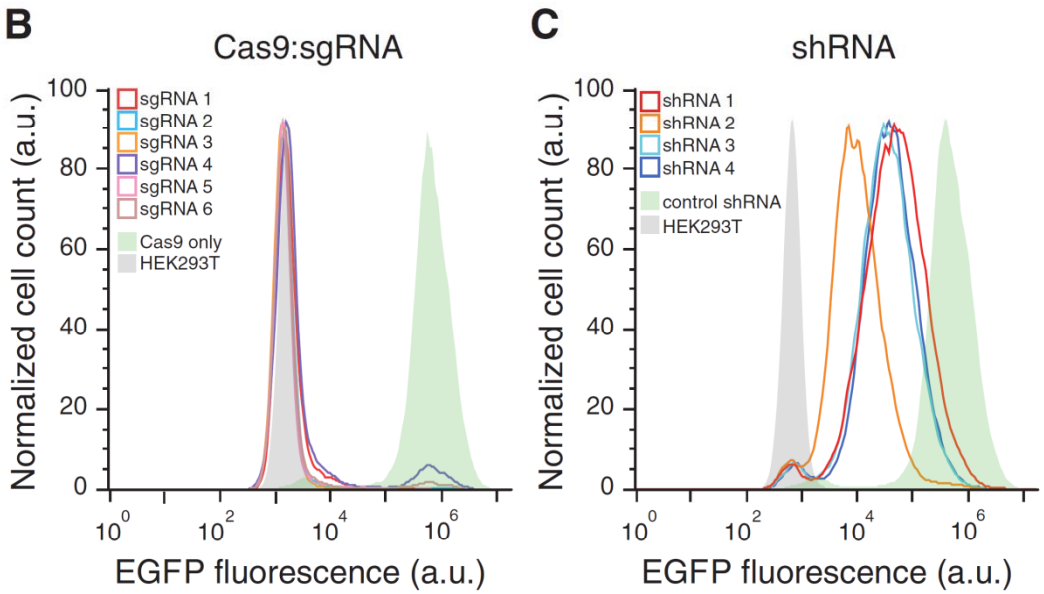
Genome-scale CRISPR Knock-Out

- Human genome-scale CRISPR-Cas9 knockout (GeCKO) v2 library created by the Zhang lab - Broad Institute & MIT
- 2 vector system:
  (1) CAS9
  (2) guide sequences (gdRNAs)
- ~120,000 unique gdRNAs targeting ~20,000 human genes, including control (non-targeting) sgRNAs.
- Divided into 2 sub-libraries: v2.1, v2.2
- 6 gdRNAs per gene - 3 in each 2 sub libraries

Ophir Shalem

Shalem et al Science, 2013

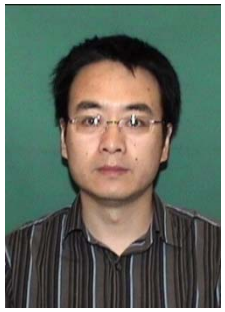
CAS9-sgRNA vs. shRNA



Different sgRNAs designed to KO GFP – complete KO in CAS9

Shalem et al, Science 2013

Experiment workflow
RPMI8226 MM cell line



Yiguo Hu

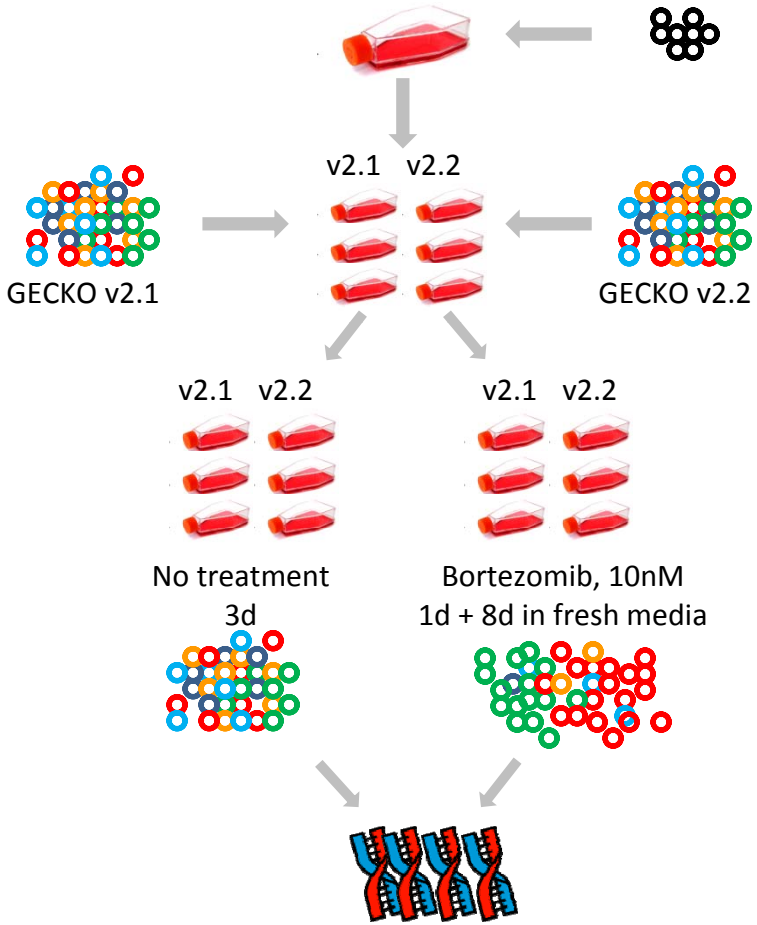
CAS9 transduction

Expansion of the sgRNA library
Lenti-viral transduction to
introducing KO mutations

Bortezomib treatment

Expanding the surviving cells

PCR amplification & Next
generation sequencing

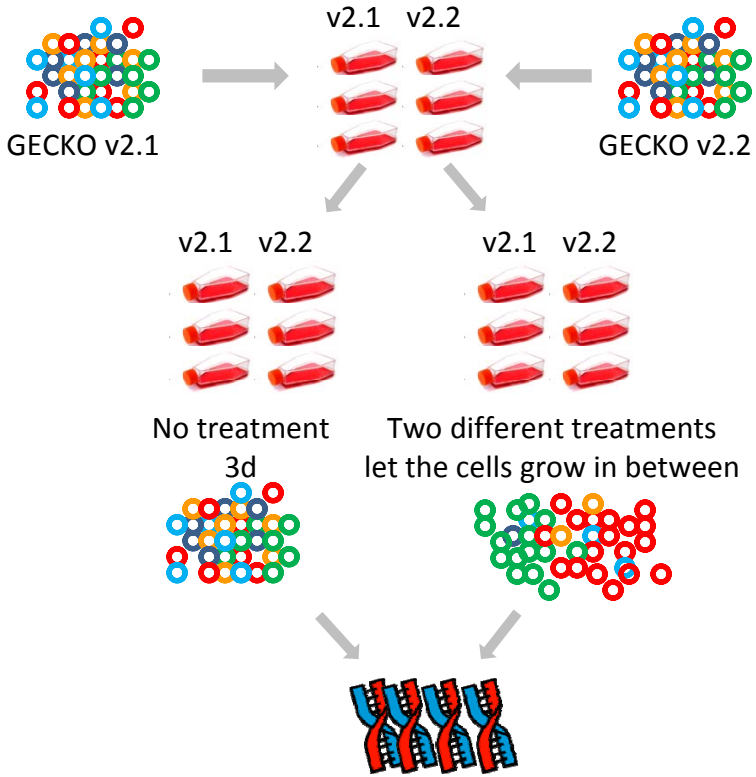


Which sgRNAs are enriched within the surviving of MM cells

Experiment workflow
RPMI8226 MM cell line

Repeat screen with genome-wide sgRNA library

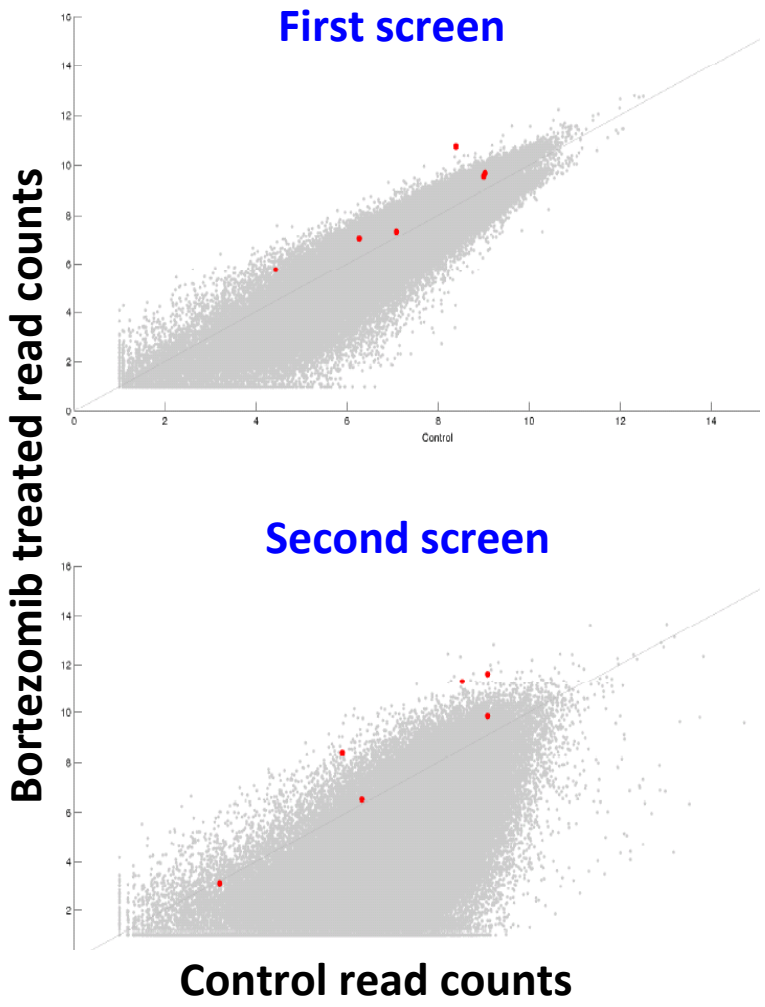
Follow-up of validation studies with smaller library of gdRNA, other cell lines

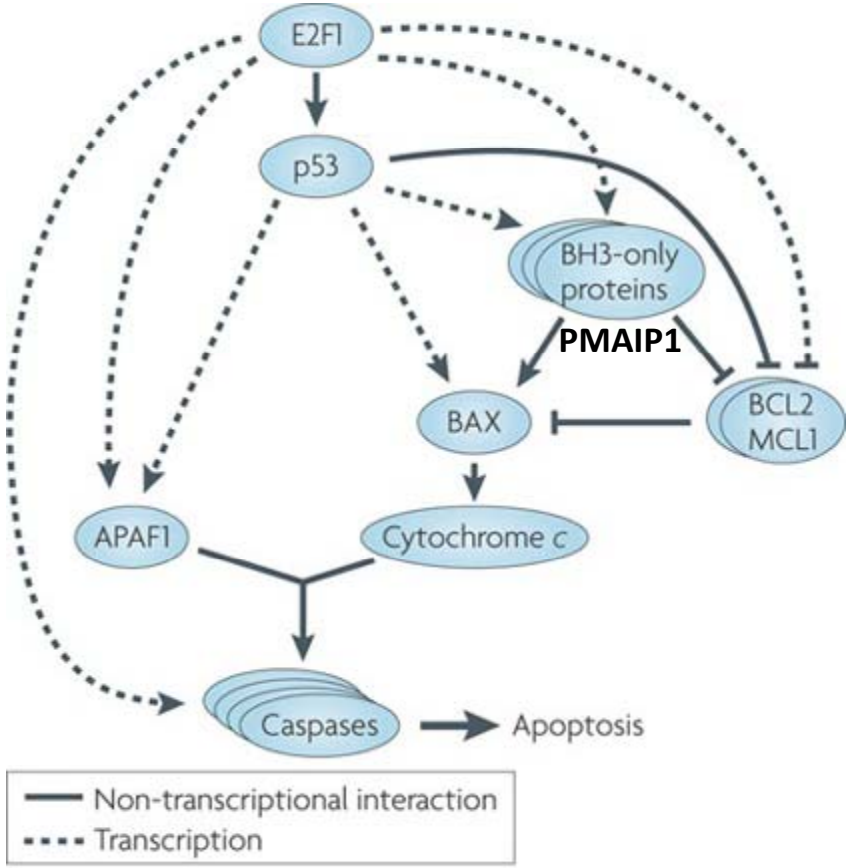


Biological functions of identified candidate genes

- Regulation of apoptosis / Tumor suppressors
  - e.g. *PMAIP1* (Noxa), BAK1
- Proteostatic stress (autophagy, aggressome function, ubiquitination)
  - e.g. *ATG9A, FBXO33, PSMD1, PSMC6*
- Toll like receptors, Regulation of NF-kappaB signaling
  - e.g. *BIRC2, TRAF2*
- Transcriptional regulators:
  - e.g. ZSCAN10, ID1

Example of candidate gene: *PMAIP1* (*NOXA*)





Polager et al, *Nature Reviews Cancer* ,October 2009

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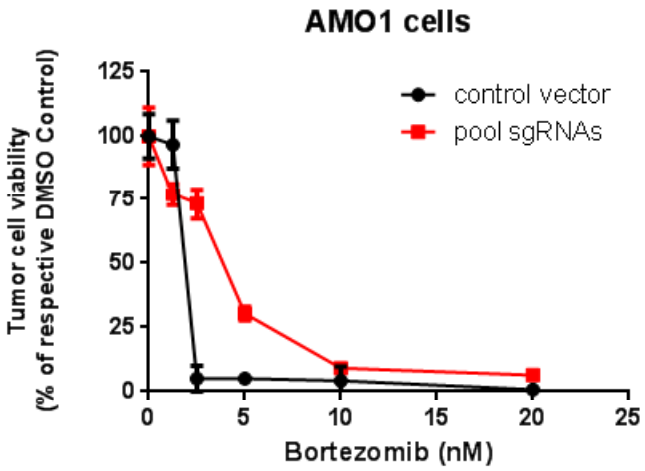
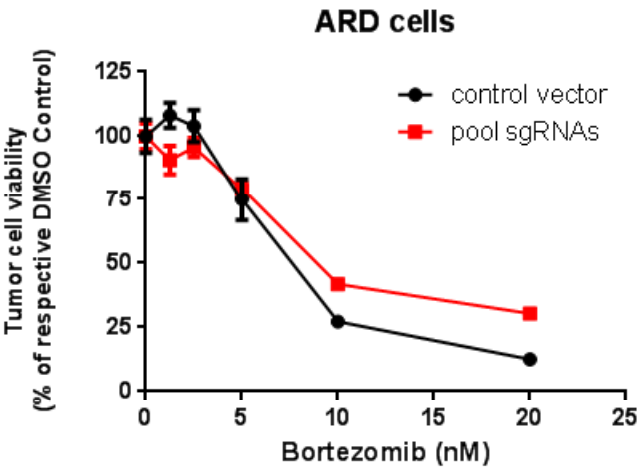
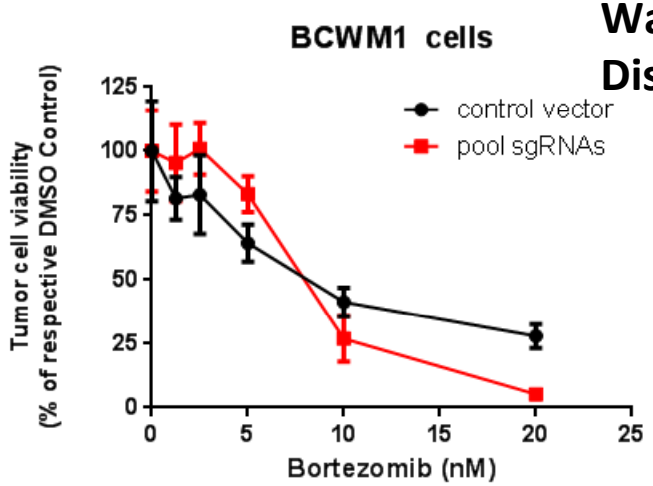
Secondary screens with focused sgRNA – Cell line-specific effects on Bort response

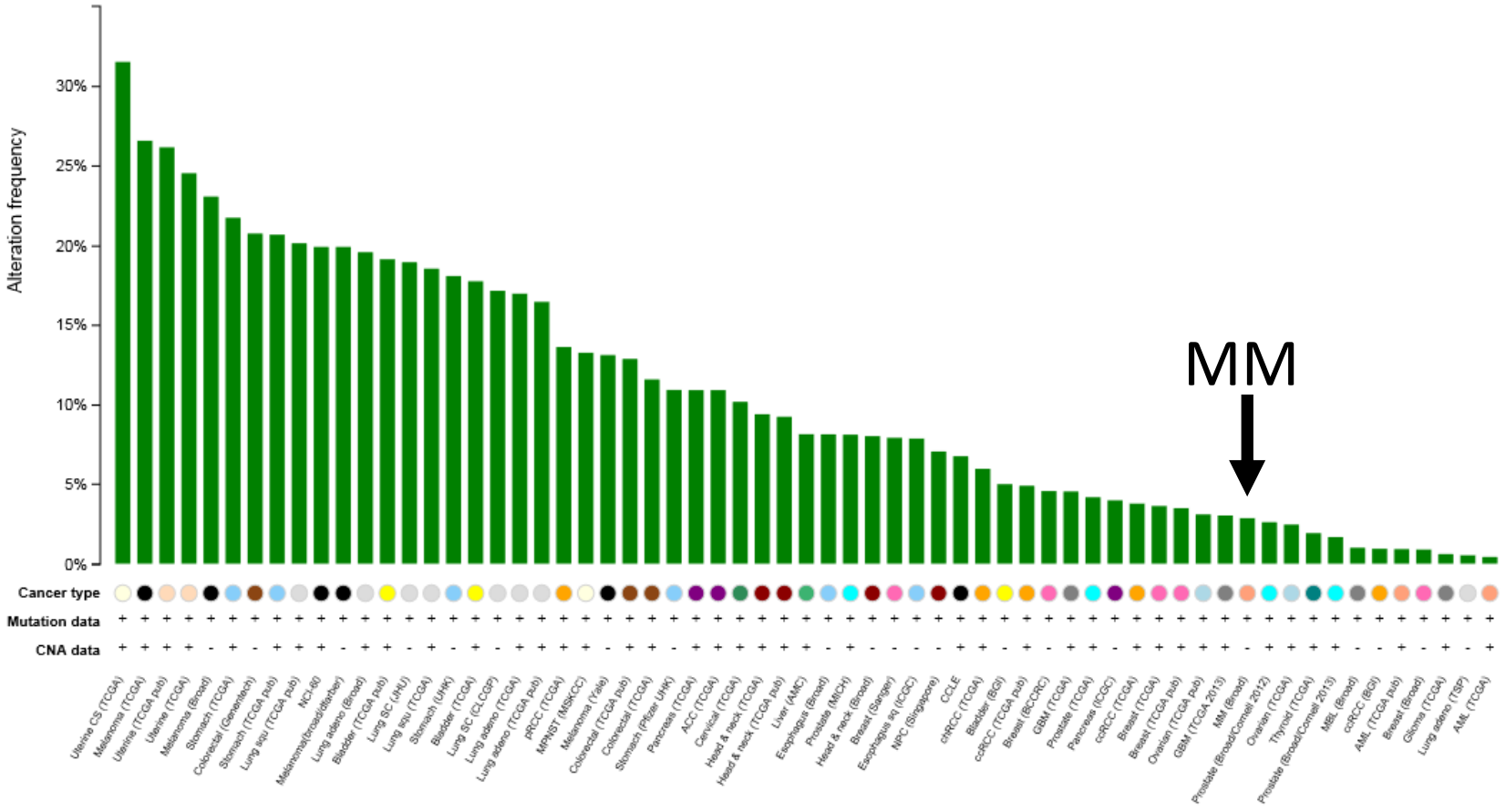






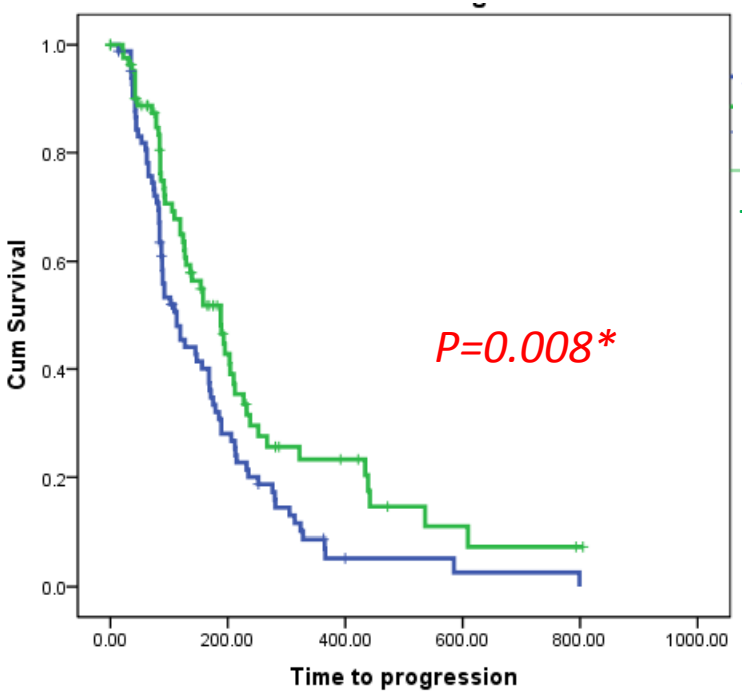
Waldenstrom's macroglobulinemia Disease – bortezomib sensitive

Distribution of mutations in candidate genes - MM vs other tumors



Studies of patients before and after Bortezomib treatment – too few for statistical analysis

Correlation with clinical outcome



Patients with at least on of the candidate genes absent

Other patients

SUMMIT and APEX trials:

Follow up on patients that received Bortezomib

Gene expression levels were measured before treatment

Ongoing - future studies

- Focused screen with smaller sgRNA library against candidate genes for bortezomib resistance
- Importance of whole-genome and focused analyses in diverged genetic models
  - Against panel of MM vs. non-MM cell lines
- Comparative studies with other drugs
  - Ability to multiplex the current setup
- *Invivo* validations

Working Group on Treatment Resistance

- Our results suggest even more complex mechanisms of resistance than previously anticipated
- The complexity of the problems underscores the need to collaborate and address it
- We are inviting colleagues from the MM field and beyond to participate in a Working Group on Treatment Resistance and jointly address the complexity of the problem
  - To participants in the Group, we offer to run CRISPR screens for your agents of choice!

If interested, please contact:

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