XRK3F2 inhibits p62 signaling and augments myeloma killing by proteasome inhibitors

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Background

Despite advancements in therapy, multiple myeloma (MM) remains an incurable blood cancer. **Our mission is to maximize the efficacy of proteasome inhibitors (PI), the primary treatment for myeloma.**

Proteasomal degradation is essential for MM cells to remove misfolded proteins. Cells can escape PI toxicity, caused by the toxic accumulation of ubiquitin-protein conjugates, by utilizing a secondary degradation pathway.

p62/SQSTM1/Sequestosome-1 is responsible for shifting the degradation of proteotoxic agents towards lysosomal degradation-autophagy, which can decrease PI toxicity. P62 also plays a significant role in multiple signaling pathways, such as p38MAPK and NFκB, which can increase MM cell growth, osteoclast activity, and suppress osteoblasts, leading to bone damage in MM.

The p62-ZZ domain (p62-ZZ) is crucial in these functions but has not been targeted in the clinic.

It has been discovered that XRK3F2 binds to the ZZ domain of p62, which can prevent N-arginylated proteotoxic products from being transported to lysosomes. Our previous studies revealed that XRK3F2 can lead to necroptosis of MM cells.

Hypothesis

We hypothesize that XRK improves MM killing when combined with PIs.

Approach

We tested the cytotoxicity of XRK and PI combinations in vitro, ex vivo co-cultures, and in a human MM xenograft model.

Results

XRK and carfilzomib combination exhibiting synergistic in vitro killing in MM cell lines.

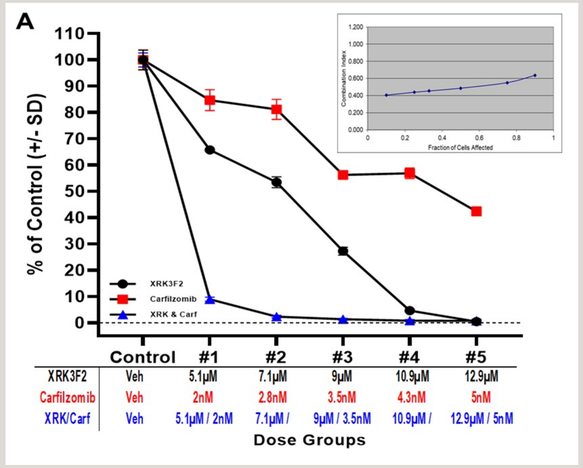
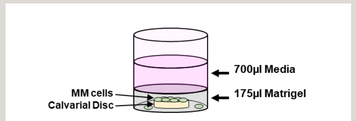


Figure 1: In vitro MTS assay of MM cell lines JJN3 **(A)** and RPMI-8226 **(B)** when treated with Carfilzomib (Carf) and XRK at the indicated doses over 3 days. XRK:Carf ~1282:1 at the physiologically relevant dose range yielded strong synergy based on the combinatorial index of 0.4-0.6 (A insert).

XRK and carfilzomib significantly increased tumor killing in an ex vivo organ co-culture assay (EVOCA) where tumor microenvironment confers PI resistance.

Experimental Schema:



JJN3 MM Cells expressing secreted luciferase (gLUC)
Treatments conditions: 8 DAYS
- Vehicles
- 7μM XRK3F2
- 3.5nM Carfilzomib
- combination

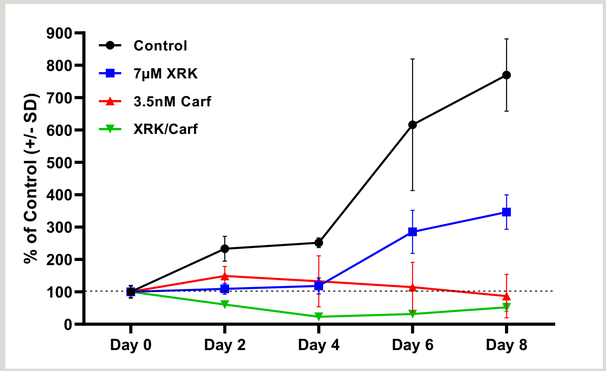


Figure 2: Tumor burden was assessed by the gaussia luciferase levels of the conditioned media at the noted time. XRK and Carfilzomib both suppressed the tumor growth but only the combination eradicate the tumors.

XRK and bortezomib (BTZ) significantly suppressed tumor growth in a plasmacytoma xenograft model, compared to single agent.

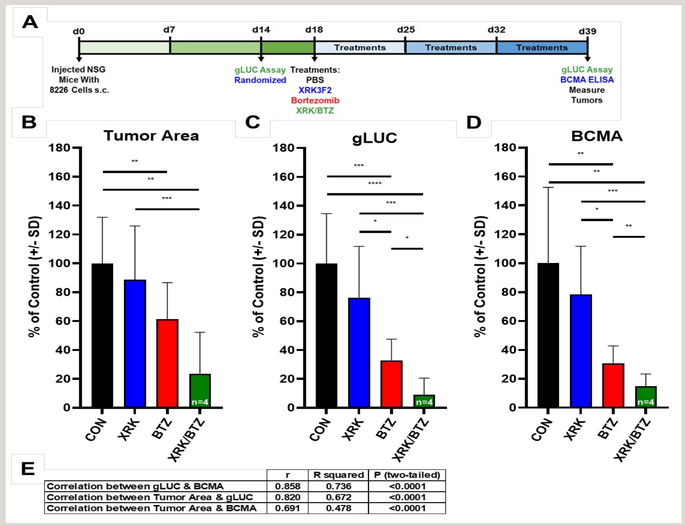


Figure 3: A. Mouse *in vivo* study treatment details. **B**. MM tumor area **C**. plasma gLUC level **D.** plasma BCMA level **E.** Correlation coefficients between gLUC, BCMA, and MM tumor area. The two-tailed p value <0.0001.

Conclusion and Future Direction

Combining the p62-ZZ domain inhibitor XRK with PIs has shown promise in improving the killing of MM. Work is ongoing to validate the combination in xenograft models where tumor cells colonize in bones and in immunocompetent models. sBCMA is a cheap, specific, and sensitive tool for serial tumor measurement, and should be further validated for preclinical and clinical usage.

References

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2. Yun Z, Zhichao J, Hao Y et. al. Leuk Res. 2017 Aug;59:97-104.