Combined Carfilzomib and Selective PI3Kδ Inhibition (TGR-1202) Results in Enhanced Myeloma Cell Apoptosis

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Introduction: The PI3K signaling pathway plays a vital role in regulating cell growth, proliferation and survival in multiple myeloma (MM) as well as in many other cancers. TGR-1202, an isoform-specific PI3Kδ inhibitor with efficacy in preclinical models of hematologic malignancies, is currently in Phase 1 clinical development. In multiple myeloma PI3K signaling appears to be very important for many extracellular signals, yet inhibition with pan PI3K inhibitors have not exhibited significant activity. However, literature reports indicate that there are several MM cell lines that express PI3Kδ, and do appear to have differential sensitivity specific isoform inhibition as opposed to pan PI3K inhibition. In this report we sought to evaluate the effects of TGR-1202 alone and in combination with the proteasome inhibitor carfilzomib, with the intent of further understanding the mechanism of action and evaluating the impact of the combination.

Methods: Human myeloma cell lines (MM1s, OCI-MY5, RPMI8226, U266, KMS11, ARH-77, OPM1, OPM2, LP1, JJN3 and L363) were treated with TGR-1202 alone, carfilzomib alone, or with the combination of TGR-1202 and carfilzomib. Annexin V/PI staining and Western blot were used to identify the cellular and molecular sequelae of the combination.

Results: 10 μM TGR-1202 alone did not cause significant cell death in the MM cell lines tested at 48 hours. When cells were treated with the combination of TGR-1202 and carfilzomib, we observed enhanced apoptosis in all of the tested cell lines. In the U266 cell line 3 nM carfilzomib and 10 μM TGR-1202 induced 16% and 14% cell apoptosis respectively. In the combination treatment apoptosis increased to 75%. To explore the molecular mechanisms underlying the combination, we used a Western blot assay to evaluate the impact of the combination on the mTOR signaling pathway, a known reciprocal feedback loop when PI3K is blocked. TGR-1202 alone did not have an obvious effect on the mTOR signaling pathway, yet, combining TGR-1202 with carfilzomib significantly inhibited phospho-mTOR, suggesting total pathway blockade.

Conclusion: The combination of TGR-1202 with carfilzomib induces synergistic apoptosis in MM cell lines. The data presented suggests this occurs through blockade of the entire reciprocal loop of mTOR activation. These findings support the rationale for the future clinical studies of TGR-1202, a selective PI3Kδ inhibitor in combination with the proteasome inhibitor carfilzomib.

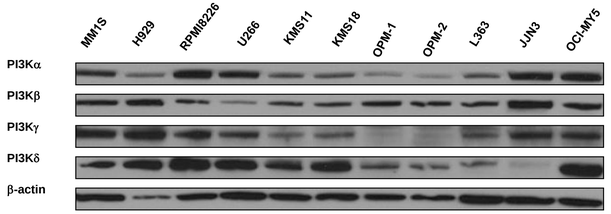

Figure 1. PI3K Isoforms in Myeloma Cell Lines. Differential expression of PI3K110 isoforms in untreated myeloma cell lines.

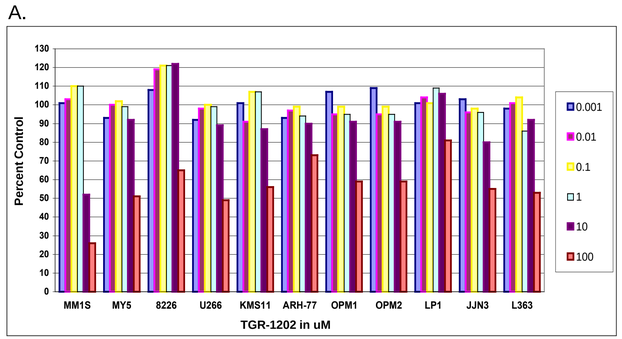

Figure 2. Growth Inhibition By TGR-1202 and carfilzomib. **A.** Myeloma cell lines treated with indicated concentrations of TGR-1202 for 48 hours. **B.** Myeloma cell lines treated for 24 and 48 h with carfilzomib. MTT assays were performed and data are presented as percent of untreated controls.

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PRISM
proteasome research and integrative science for multiple myeloma
PRISM – Onyx Novel Therapies Program (NTP)

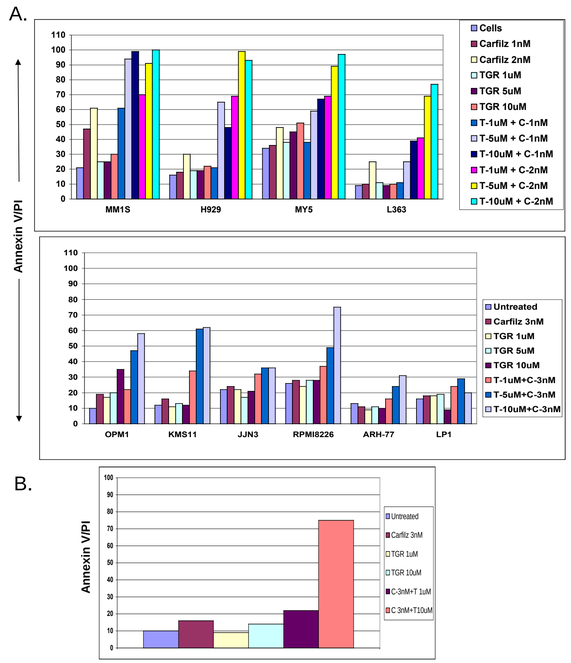

Figure 3. Combination of carfilzomib and TGR-1202 induces Apoptosis in Myeloma Cell Lines. **A**. MM1s, H929, OCI-MY5 and L363 were treated with carfilzomib, 1 or 2 nM, and TGR-1202, 1,5 and 10 μM for 48 hours. In lower panel OPM1, KMS11, JJN3, RPMI8226, ARH-77 and LP1 were treated with carfilzomib 3 nM and the same concentrations of TGR-1202 as above. Enhanced apoptosis was seen in all myeloma cell lines by flow cytometry using annexin V/PI staining. **B**. In U266, treated with a combination of 3 nM carfilzomib and 10 μM TGR1202 induced an observed apoptosis of 16% and 14% respectively.

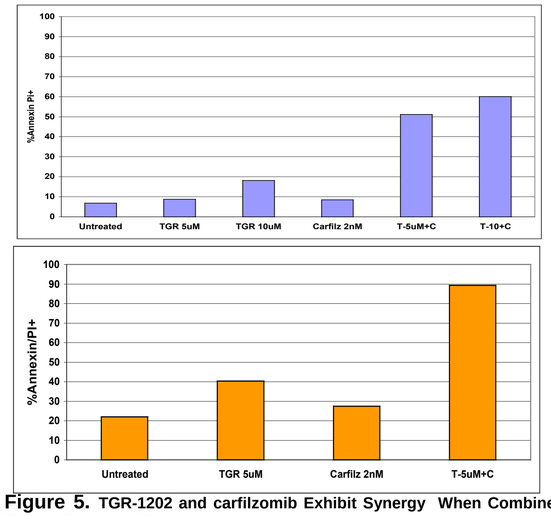

Figure 5. TGR-1202 and carfilzomib Exhibit Synergy When Combined in 2 Patient Samples. Two patient bone marrow aspirates were treated with carfilzomib 2 nM and TGR-1202 5 μM for 24 hours. Flow analysis of the percent apoptosis was obtained from from gating on the CD45+/-, CD38+ and CD138+ plasma cell populations. These were 50% and 90% respectively.

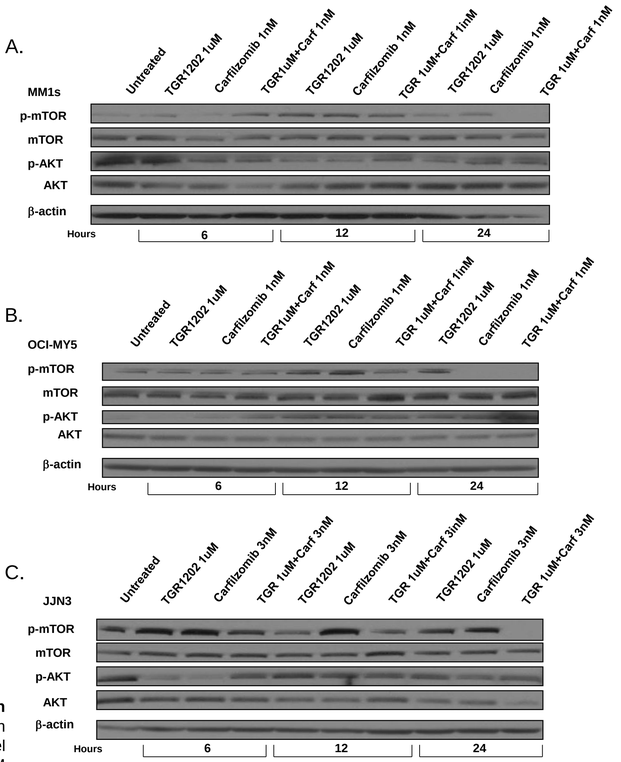

Figure 4. Inhibition of mTOR phosphorylation by the Combination of TGR-1202 and carfilzomib. **A**. MM1s, **B.** OCI-MY5 and **C.** JJN3. Cells were treated with the indicated concentrations of TGR-1202 and carfilzomib for 24 h.

Conclusions

- The combination of PI3Kδ and proteasome inhibition with TGR-1202 and carfilzomib was active in myeloma cell lines and patient samples.
- The combination was active in cell lines that had either high or low expression of PI3Kδ.
- In sensitive cell lines the combination effectively inhibited mTOR phosphorylation suggesting that feedback activation of this pathway was effectively inhibited.
- Further preclinical and clinical evaluation of the combination is warranted.

Disclosures: : SL consultant Onyx, Millennium, Janssen, Celgene, Sanofi, BMS
LB: consultant Onyx