**BACKGROUND**

The bone marrow microenvironment contributes to the pathogenesis of Multiple Myeloma (MM) by promoting the oncogenic process, including drug resistance. High expression levels of the PI3Kδ isoform in patient MM cells implicate this target as a novel and attractive interventional strategy aimed at attenuating the progression of the disease. Herein, we describe the biological and pharmacological properties of TGR-1202, a novel small molecule PI3Kδ inhibitor with potential to be developed as a clinical candidate for the treatment of MM either as a single agent or in combination with currently available cytotoxic / targeted agents.

**RESULTS**

**In Vitro Combination Studies – Experiment Outline**

**Cell Line**

- MM.1S
- Multiple myeloma; steroid sensitive

**Cell Type**

- RPMI-8226
- Multiple myeloma; steroid resistant

**Standards Tested**

- BEN (Bendamustine) - 1 µM
- MEL (Melphalan) - 1 µM
- DEX (Dexamethasone) - 1 µM
- DOXO (Doxorubicin) - 0.01 µM
- BORT (Bortezomib) - 0.01 µM

**TGR-1202**

TGR-1202 is a selective PI3Kδ kinase inhibitor with high selectivity over all other PI3K isoforms as well as a 443-kinase panel, designed with a unique backbone compared to other PI3K inhibitors in development. In vitro studies (ASH 2012) demonstrated the therapeutic potential of the molecule in leukemias mediated via the PI3Kδ pathway. TGR-1202 is currently under clinical development for patients with relapsed and refractory hematological malignancies.

**In Vitro Combination Studies – Experiment Outline**

**Inhibition**

- **Cell Cycle**
  - MM.1S
  - RPMI-8226
  - Ex vivo MM Patient Cell Assays

**In Vitro Combination Studies – In Situ Caspase assay**

**In Vitro Combination Studies – Sub G0 phase of Cell Cycle**

**CONCLUSIONS**

- TGR-1202 is a potent and selective inhibitor of PI3Kδ resulting in a concentration dependent in-vitro reduction in proliferation of antigen induced B-cells
- TGR-1202 demonstrated synergism in in-vitro combination studies with standard MM agents in both steroid sensitive and steroid resistant MM cell lines, and in MM cells from newly diagnosed patients with myeloma restricted disease
- In an ex-vivo assay with patient MM cells, TGR-1202 inhibited PI3Kα, an effective biomarker of PI3K suppression, with a corresponding induction of apoptosis.
- TGR-1202 exhibits desirable non-clinical pharmacological/ADME/PK properties in multiple species along with an excellent safety profile in GLP-Tox studies.

**Abstract P53**

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**Enzyme and Cell based Selectivity of TGR-1202**

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**Whole Blood Based Assays**

**Ex vivo MM Patient Cell Assays**

**TGR-1202 across species**

- Rat
- Dog
- Mouse

**Cell Cycle arrest and progression to sub-G0 stage in MM Cells**

- Cells were plated in a 6-well plate at a concentration of 0.5 x 10^5 cells/well. Cells were treated with TGR-1202, standards, or the combination and incubated for 24 h at 37°C and 5% CO2. Cells were transferred to lacZ/cyclin D1 or RFP/light chain of freshly prepared PI3Kδ reagent was added and incubated for 1 h at 37°C and 5% CO2, away from light. Fluorescence was measured at an excitation wavelength of 640 nm and emission wavelength of 520 nm, in a plate reader. Data was expressed as a percent of the maximum response (%Rmax) and plotted accordingly. O was calculated using CompuSyn version 2.1. The x’s and 50%’s are depicted above the histogram representing the combination.

**Figure 1:** Inhibition of anti-Fas/anti-CD19 (IgM) and anti-CD19 (IgG) induced CD86 expression in Human Whole Blood following incubation with PI3Kδ selective inhibitors. Example Western Blot (loading control: β-actin).

**Figure 2:** Inhibition of CD69 or CD69+/CD86+ cell proliferation in human (H) or murine (M) MM cell lines (MM.1S, RPMI-8226, MM-1S). Cells were stimulated with Fcγ receptors and PI3Kδ-selective inhibitors. Western Blot shows representative results for CD86 expression and PI3Kδ inhibition.

**Figure 3:** Cell cycle arrest in Primary Multiple Myeloma patient cells. 

**Figure 4:** Inhibition of MM Cell Proliferation (µM) Cells were plated in a 96-well plate at a concentration of 10000 cells/well. Cells were treated with TGR-1202, standards, or the combination and incubated for 24 h at 37°C and 5% CO2. Cells were treated to radioactive end-point of 2 µCi of [3H]thymidine. The percentage of [3H] thymidine incorporation was recorded.

**Figure 5:** Inhibition of Caspase-3 activity in MM Cells (µM). Cells were plated in a 6-well plate at a concentration of 0.5 x 10^5 cells/well. Cells were treated with TGR-1202, standards, or the combination and incubated for 24 h at 37°C and 5% CO2. Cells were stained with Annexin V and Fluoromount for staining/IgM, and quantitated using FlowJo software. The fold change represents the percentage of Annexin V binding and was calculated using CompuSyn version 2.1. The x’s and 50%’s are depicted above the histogram representing the combination.

**Figure 6:** Cell cycle arrest and progression to sub-G0 stage in MM Cells (µM). Cells were plated in a 6-well plate at a concentration of 0.5 x 10^5 cells/well. Cells were treated with either TGR-1202, standards, or their combinations and incubated for 24 h at 37°C and 5% CO2. Staining with propidium iodide was added to the wells and incubated for 30 min at room temperature away from light. Cell suspension was diluted into 100-400 µl PBS and a minimum of 16000 events were acquired on a FACSCalibur flow cytometer. Data were analyzed with FlowJo Pro software and cell population in different cell cycle phases is depicted on the histogram.