TGR-1202: A NOVEL, PI3Kδ SPECIFIC INHIBITOR IN MULTIPLE MYELOMA

TG Therapeutics

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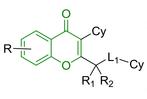
BACKGROUND

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 pharmacokinetic 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.

TGR-1202



TGR-1202 backbone *(full structure not yet disclosed)*

TGR-1202 is a selective PI3Kδ kinase inhibitor with high selectivity over all other PI3K isoforms as well as a 441-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.

RESULTS

Enzyme and Cell based Selectivity of TGR-1202

	IC_{50}/EC_{50} (nM)	Fold-Selectivity		
	PI3Kδ	**PI3Kα**	**PI3Kβ**	**PI3Kγ**
Enzyme	22.23	>10000	>50	>48
Cell-based	24.27	>10000	>34	>17

Table 1. Enzyme assay for inhibition of PI3Kδ and fold-selectivity over other isoforms. Enzyme activity was determined using an PI3K HTRF Assay Kit (Millipore, Billerica, MA).

Cell based specificity against PI3K isoforms for select compounds. Compound specificity towards PI3Kδ was determined in an IgM-induced B cell proliferation assay. For selectivity against PI3K α, β, or γ isoforms, NIH-3T3 or RAW macrophages were seeded in a 6-well tissue culture plate and incubated with compounds at the desired concentrations followed by 20 ng/ml PDGF, 5 μM LPA, or 50 ng/ml c5a. Cells were lysed and AKT phosphorylation was determined by Western Blotting. Intensity of the bands was determined using ImageJ 1.42q (NIH, USA) and normalized to Actin (loading control).

Whole Blood Based Assays

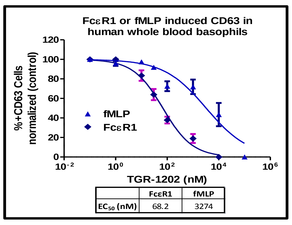


Figure 1: Inhibition of anti-FcεR1 (*n=7*) or fMLP (*n=4*) induced CD63 expression in Human Whole Blood basophils. Induction of CD63 surface expression on human whole blood basophils was measured using a Flow2CAST kit (Buhlmann Laboratories, Switzerland). Cells were stained with FITC or PE tagged CD63 and CCR3. CD63 positive cells were determined using flow cytometry and normalized to vehicle control.

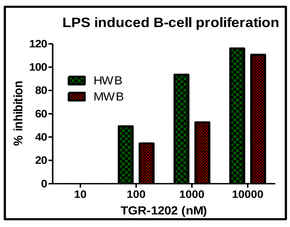


Figure 2: Inhibition of CD19+ or CD45R+ cell proliferation in human (H) or mouse (M) whole blood (WB). CD19 or CD45R are proteins present on B cells Whole Blood was incubated with test article prior to induction with LPS. 48 h later, positive cells were gated from CD45+ cell and estimated by flow cytometry.

In Vitro Combination Studies – Experiment Outline

Cell Line	Cell Type
MM.1S	Multiple myeloma; steroid sensitive
RPMI-8226	Multiple myeloma; steroid resistant

Standards Tested	TGR-1202	Combination Index (CI)
BEN (Bendamustine) - 1 **μM**	**1 μM**	**<1.0 : Synergism** **=1.0 : Additive** **>1.0 : Antagonism**
MEL (Melphalan) - 1 **μM**		
DEX (Dexamethasone) - 1 **μM**		
DOXO (Doxorubicin) – **0.01 μM**		
BORT (Bortezomib) – **0.01 μM**		

In Vitro Combination Studies – Cell Proliferation

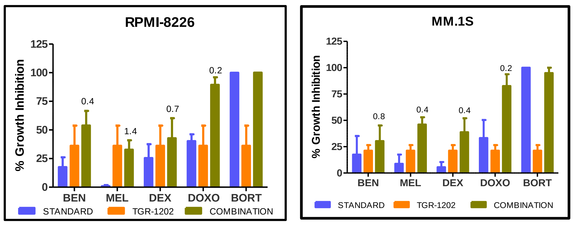
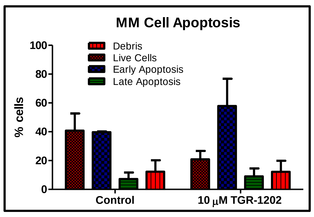


Figure 4: Inhibition of MM Cell Proliferation (*n=3*): Cells were plated in a 96-well plate at a concentration of 10000 cells/well. Wells were treated with TGR-1202, standards or the combinations, and incubated for 72 h at 37°C and 5% CO_2. Proliferation was determined by a MTT assay. Data were expressed as a percent of the DMSO control and plotted accordingly. CI was calculated using CompuSyn V1.0 and the values are depicted above the histogram representing the combination.

Ex vivo MM Patient Cell Assays



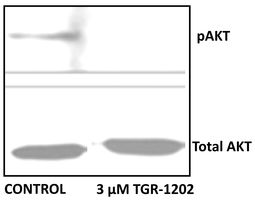


Figure 3. Cell cycle & pAKT in primary Multiple Myeloma patient cells.
Patient 1. 82 y/o male with newly diagnosed stage II IgG Kappa restricted disease; Bone marrow specimen with >70% plasma cells. ***Patient 2.*** 36 y/o male with newly diagnosed stage III IgG Lambda restricted disease; Bone marrow specimen with 70% plasma cells. *Western Blot representative of Patient 1 sample* (***Study by Cleveland Therapeutics Ltd., OH***)

In Vitro Combination Studies – *In Situ* Caspase assay

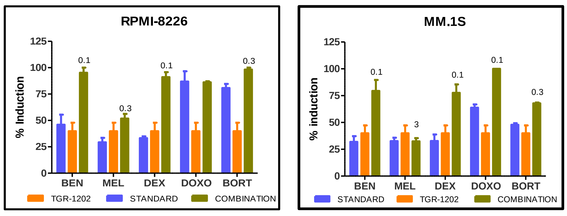


Figure 5: Induction of Caspase-3 activity in MM Cells (*n=2*): Cells were plated in a 6-well plate at a concentration of 0.5 x 10^6 cells/ml. Wells were treated with TGR-1202 , standards, or the combination and incubated for 24 h at 37°C and 5% CO_2. Cells were transferred to microfuge tubes and10 μl of freshly prepared FLICA reagent was added and incubated for 1 h at 37° C and 5% CO_2 away from light. Fluorescence was measured at an excitation wavelength of 490 nm and emission wavelength of 520 nm in a plate reader. Data was expressed as a percent of the maximum response (100%) and plotted accordingly. CI was calculated using CompuSyn version: 1.0 and the values are depicted above the histogram representing the combination.

Non-Clinical Pharmacokinetic Profile of TGR-1202

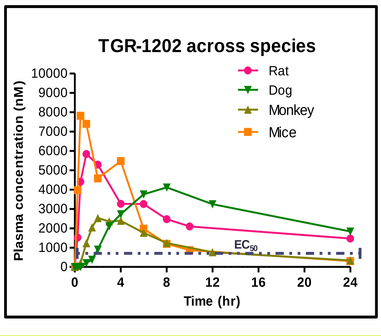


Figure 7: Single Dose oral Pharmacokinetic profile of TGR-1202 across species.

EC50 represents the concentration required to inhibit PI3Kδ mediated FcεR1 induced CD63 expression in Human Whole Blood Basophils by 50 %.

In Vitro Combination Studies – Sub G0 phase of Cell Cycle

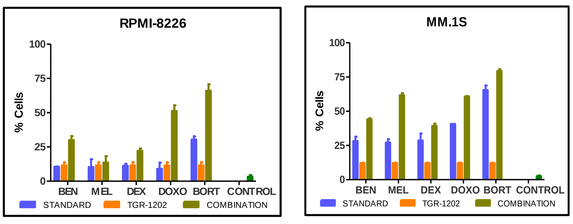


Figure 6: Cell cycle arrest and progression to Sub G0 stage in MM Cells (*n=2*): Cells were plated in a 6-well plate at a concentration of 0.5 x 10^6 cells/ml. Wells were treated with either TGR-1202, standards, or their combinations and incubated for 72 h at 37°C and 5% CO_2. 50 μl of cell cycle reagent was added to the wells and incubated for 30 min at room temperature away from light. Cell suspension was diluted using 300-400 μl PBS and a minimum of 10,000 events were acquired on a Guava easyCyte flow cytometer. Data were analyzed with Express Pro software and % cell population in different cell cycle stages with respect to control.

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 manifested by a reduction in CD19⁺ or CD45R⁺ 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 resulted in both inhibition of cell proliferation and induction of apoptosis.**
- **In an ex-vivo assay with patient MM cells, TGR-1202 inhibited pAKT, an effective biomarker of PI3K suppression, with a corresponding induction of apoptosis.**
- **TGR-1202 exhibits desirable non-clinical pharmaceutical/ADME/PK properties in multiple species along with an excellent safety profile in GLP-TOX studies.**
- **A multi-center Phase I study of single agent TGR-1202 in patients with advanced hematologic malignancies is currently ongoing. Future combination studies with TGR-1202 + novel agents in Multiple Myeloma are warranted.**

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