Abstract # 2610

TGR-1202 SUPPRESSES ACUTE MYELOID LEUKEMIA (AML) AND ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) CELLS VIA SELECTIVE INHIBITION OF PI3Kδ KINASE

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BACKGROUND

Acute leukemia, characterized by the presence clonal hematopoietic cells in peripheral blood and bone marrow, and notable for an aggressive clinical course, comprises approximately 40% of newly diagnosed leukemias. Treatment for acute leukemias with multi-agent cytotoxic chemotherapy is usually associated with significant toxicity. Advances in therapy have been slow, and nearly all effective therapies lead to marrow suppression and toxicities associated with prolonged cytopenias.

TGR-1202

TGR-1202 is a selective PI3Kδ kinase inhibitor with several fold selectivity over the other PI3K isoforms as well as a 441-kinase panel. Specificity of the molecule was further corroborated in cell-based and Human Whole Blood assays. *In vitro* and *in vivo* xenograft studies demonstrated the therapeutic potential of the molecule in acute leukemia mediated *via* the PI3Kδ pathway.

RESULTS

In Vitro Assays

Enzyme and Cell based Selectivity

	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) with modifications.

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).

CD63 expression in Whole Blood Basophils

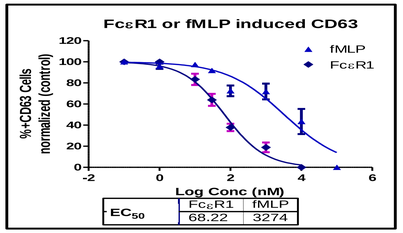


Figure 1: Inhibition of anti-FceR1 (n=7) or fMLP (n=4) induced CD63 expression in Human Whole Blood basophils. Induction of CD63 surface expression on human whole blood basophils was measured using a Flow2CAST kit (Buhlmann Laboratories, Switzerland). Cells were stained with FITC or PE tagged CD63 and CCR3. CD63 positive cells were determined using flow cytometry and normalized to vehicle control.

Anti-B-Cell Proliferative Activity

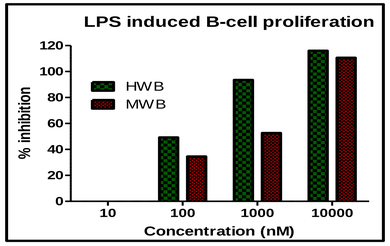


Figure 2: Inhibition of CD19+ or CD45R+ cell proliferation in human (H) or mouse (M) whole blood (WB). CD19 or CD45R are proteins present on B cells. Whole Blood was incubated with test article prior to induction with LPS. 48 h later, positive cells were gated from CD45+ cell and estimated by Flow Cytometry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

Inhibition of AKT Phosphorylation

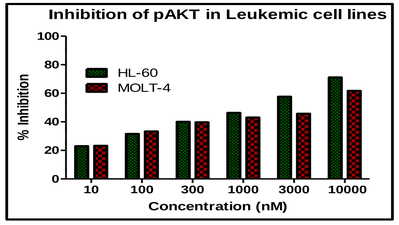


Figure 3: Inhibition of pAKT in AML & ALL derived cell lines with constitutive expression. Cells were treated with compound, lysed, and pAKT determined by Western blotting. Intensity of the bands was determined using ImageJ 1.42q (NIH, USA) and normalized to Actin (loading control).

Induction of Apoptosis

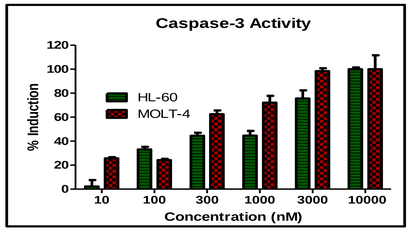


Figure 4: Inhibition of pAKT in AML & ALL derived cell lines with constitutive expression. Cells were treated with compound, lysed, and pAKT determined by Western blotting. Intensity of the bands was determined using ImageJ 1.42q (NIH, USA) and normalized to Actin (loading control).

pAKT in Primary AML Cells

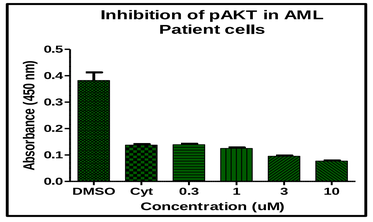
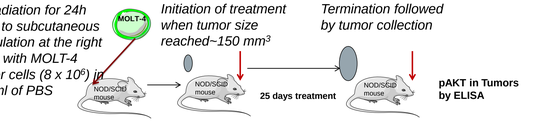


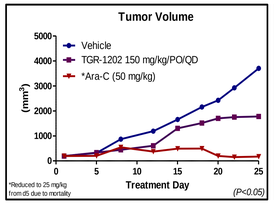
Figure 5: Inhibition of pAKT in primary patient leukemic cells. Cells were isolated from bone marrow of patients (***n=2***) diagnosed with hyper-proliferative acute myeloid leukemia. Leukemic cells were incubated with compound for 48 h and pAKT determined by ELISA. Cytaribine (cyt) was used as a positive control.

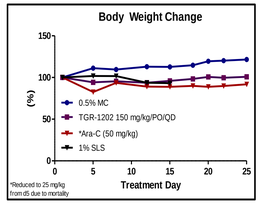
Anti-Tumor Effect of TGR-1202 in a Subcutaneous MOLT-4 Xenograft Model

Experimental Setting



Tumor Burden Inhibition by TGR-1202





Non-Clinical Pharmacokinetics of TGR-1202

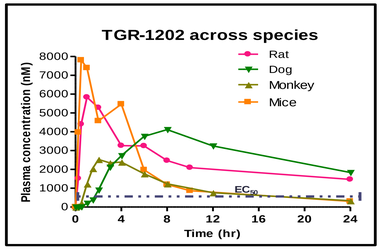
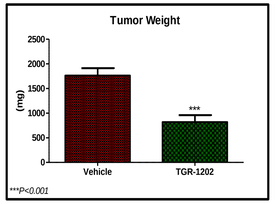
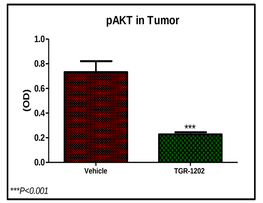


Figure 6: Single Dose oral Pharmacokinetic profile of TGR-1202 across species. EC50 represents the concentration required to inhibit PI3Kδ mediated FcεR1 induced CD63 expression in Human Whole Blood Basophils by 50 %





Ara-c = Cytaribine

CONCLUSIONS

- TGR-1202 is a potent and selective inhibitor of PI3Kδ producing:
  - A translational reduction in proliferation of antigen induced B-cells manifested by a reduction in $CD19^+$ or $CD45R^+$ cells
  - Reduction in pAKT, an effective biomarker in AML and ALL cell lines as well as in cells derived from AML patients
  - Marked anti-tumor activity in a MOLT-4 subcutaneous xenograft mouse model
- TGR-1202 exhibited desirable pharmaceutical/ADME/PK properties along with an excellent safety profile in GLP-TOX studies
- A Phase I trial in patients with select hematologic malignancies is currently planned to open in early 2013