Abstract # 3725

NOVEL PI3K INHIBITORS DEMONSTRATE MARKED CYTOTOXICITY IN MODELS OF T-CELL LYMPHOMA, CAUSED APOPTOSIS AND WERE SYNERGISTIC WITH THE NOVEL ANTI-CD20 MONOCLONAL ANTIBODY UBLITUXIMAB IN MODELS OF B-CELL LYMPHOMA

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BACKGROUND

Activation of PI3K has been shown to be required for the proliferation and survival of cancer cells. A selective PI3K-delta inhibitor, GS-1101/CAL-101, has produced promising results in the treatment of lymphoid malignancies. More recently, new chemical entities targeting PI3K have been developed, with pharmacologic and pharmacodynamic features distinct from CAL-101. TGR-1202 and TGR-5237 are two novel and structurally related PI3K inhibitors that possess marked selectivity of the PI3Kδ isoform over other PI3K isoforms and a panel of 442 kinases. Here we present data that confirmed the specificity of these PI3Kδ inhibitors using cell-based and Human Whole Blood assays, and demonstrated the activity of TGR-1202 and TGR-5237 in B- and T- cell lymphoma models.

While CAL-101 as a single agent has demonstrated promising clinical activity, we hypothesize that combining PI3Kδ inhibitors and immunotherapy will become a highly effective strategy for the treatment of B-cell lymphoma. To investigate the hypothesis, we studied the pharmacologic interaction of TGR-1202 with a novel anti-CD20 monoclonal antibody, ublituximab. Ublituximab is a next generation anti-CD20 antibody currently in clinical development, and is characterized by a specific glycosylation profile, with a low fucose content, that enhances its antibody-dependent cell-mediated cytotoxicity (ADCC) response against malignant B cells. Here we present data that demonstrated marked synergy between TGR-1202 and ublituximab.

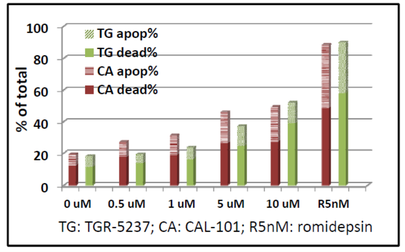
RESULTS

Table 1. Enzyme and Cell based Selectivity Assays

	IC_{50}/EC_{50} (nM)	Fold-Selectivity		
	PI3Kδ	PI3Kα	PI3Kβ	PI3Kγ
TGR-1202				
Enzyme	22.23	>10000	>50	>48
Cell-based	24.27	>10000	>34	>17
TGR-5237				
Enzyme	13.83	>1000	>54	>9
Cell-based	31.52	>1000	>30	>12

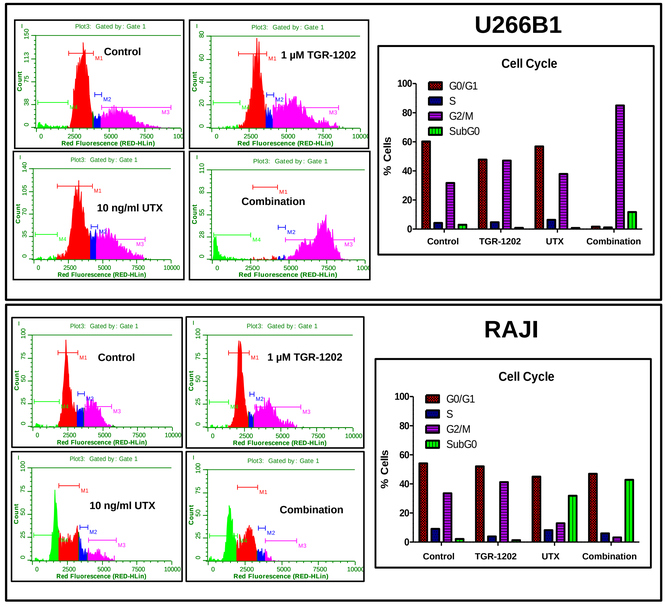
Enzyme activity was determined using an PI3K HTRF Assay Kit (Millipore, Billerica, MA) with modifications. **Cell based** specificity towards PI3Kδ was determined in an IgM-induced B cell proliferation assay. For selectivity against PI3K α, β, or γ isoforms, pAKT was measured in NIH-3T3 or RAW macrophages upon induction with specific antigens.

Figure 1. TGR-5237 induced lymphoma cell death as a single agent



Enzyme activity was determined by flow cytometry using the Annex-V kit from Invitrogen. WSU-NHL cells were exposed to the indicated drugs for 48 hours.

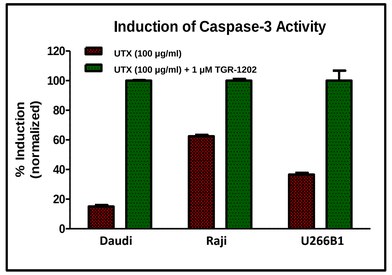
Figure 2. TGR-1202 and ublituximab (UTX) synergistically induced apoptosis (sub G0) and G2/M arrest in B-lymphoma cells



Cell cycle analysis was performed by flow cytometry. Cells were treated with either 1 µM TGR-1202, 10 ng/ml UTX or a combination (10 ng/ml UTX + 1 µM TGR-1202) and incubated for 72 h.

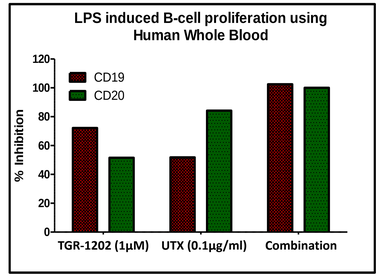
RESULTS

Figure 3. Ublituximab markedly enhanced caspase-3 activation induced by TGR-1202 in lymphoma cells



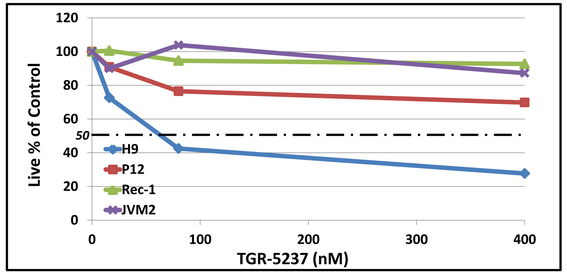
Activation of caspase 3 was measured using the Green FLICA assay kit in B-cell lymphoma cell lines treated with either 1 µM TRG-1202, 100 µg/ml UTX, or combination for 24 h. **Note that TGR-1202 alone did not activate caspase-3 in the test condition.**

Figure 4. TGR-1202 and ublituximab synergistically inhibited proliferation of B cells from human whole blood



LPS induced proliferation of CD19+ or CD20+ cells was determined by flow cytometry. Human Whole Blood was incubated with test agents prior to induction with LPS. 72 h later, CD19+ or CD20+ cells were gated from CD45+ cells and estimated by Flow Cytometry.

Figure 5. Select T-lymphoma cell line was hypersensitive to TGR-5237



Cytotoxicity of B- and T-cell lymphoma cells was determined using the Cell Titer-Glo assay. Cells were exposed to TGR-5237 at indicated concentrations for 6 days. Survival of treated cells was expressed as a percentage of the untreated control cells. Note the concentration to achieve 50% inhibition for the cutaneous T-cell lymphoma line H9 was less than 80nM.

CONCLUSIONS

- **TGR-1202 and TGR-5237 are potent and selective inhibitors of PI3Kδ, and have single agent activity against B-lymphoma cell lines, and may be highly potent against select T-lymphoma cell lines.**
- **Combination of TGR-1202 and the novel anti-CD20 antibody, ublituximab, is highly effective in the induction of G2/M arrest and apoptosis in B-lymphoma cell lines.**
- **Combining selective PI3Kδ inhibitors and anti-CD20 immunotherapy may represent a promising strategy to treat B-cell lymphoma. Clinical studies evaluating the combination of TGR-1202 and ublituximab are warranted.**