Table 1. Enzyme and Cell based Selectivity Assays

<table>
<thead>
<tr>
<th>IC50/EC50 (nM)</th>
<th>Fold-Selectivity</th>
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<tbody>
<tr>
<td>P3Kδ</td>
<td>P3Kμ</td>
</tr>
<tr>
<td>TGR-1202 Enzyme</td>
<td>22.23</td>
</tr>
<tr>
<td>Cell-based</td>
<td>24.27</td>
</tr>
<tr>
<td>TGR-2537 Enzyme</td>
<td>13.83</td>
</tr>
<tr>
<td>Cell-based</td>
<td>31.52</td>
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Enzyme activity was determined using an P3Kδ HTRF Assay Kit (MRIPore, Billerica, MA) with modifications. Cell based specificity towards P3Kδ was determined in an IgM-induced B cell proliferation assay. For selectivity against P3K α, β, or γ isoforms, pAKT was measured in NIH-3T3 or RAW macrophages upon induction with specific antigens.

Cell cycle analysis was performed by flow cytometry. Cells were treated with either 1 µM TGR-1202, 20 ng/ml UTX or a combination (10 ng/ml UTX + 1 µM TGR-1202) and incubated for 72 h.

RESULTS

Figure 1. TGR-2537 induced lymphoma cell death as a single agent

Enzyme activity was determined by flow cytometry using the Annex-V kit from Immunotech. 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

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

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

Activation of caspase 3 was measured using the Green FLICA assay kit in B-lymphoma cell lines treated with either 1 µM TRC-3200, 100 µg/ml UTX, or combination for 24 h. Note that TGR-1202 alone did not activate caspase-3 in the test condition.

Cytotoxicity of B- and T-cell lymphoma cells was determined using the Cell Titer-Blue assay. Cells were exposed to TGR-2537 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-2537 are potent and selective inhibitors of P3Kδ, and have single agent activity against B-lymphoma cell lines, and highly potent in 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 P3Kδ 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.

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