The PI3K-δ inhibitor TGR-1202 induces cytotoxicity and inhibits phosphorylation of AKT in 17p deleted and non-17p deleted CLL cells in vitro

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Introduction

- The PI3K pathway is a pro-survival mechanism in chronic lymphocytic leukemia (CLL).
- Expression of the δ-isofom of PI3K is largely restricted to lymphocytes.
- Inhibition of PI3K activity in vitro induces CLL cell apoptosis and death.
- Clinical evaluation of PI3K-δ inhibitors, such as GS-1101, has produced responses in relapsed and/or refractory CLL patients.
- TGR-1202 is a novel PI3K-δ specific inhibitor that inhibits AKT phosphorylation and induces apoptosis in B-cell lymphoma cell lines (Friedman et al., ASH 2012).
- We previously evaluated the in vitro effects of TGR-1202 and GS-1101 on cytotoxicity, apoptosis, and AKT phosphorylation in a small series of primary CLL samples, and found equal efficacy.
- Herein, we evaluate the effect of TGR-1202 on CLL lymphocytes, specifically evaluating differences between 17p deleted CLL samples and non-17p deleted CLL samples.

Hypotheses

- We hypothesize that TGR-1202 induces cytotoxicity and apoptosis, and inhibits AKT phosphorylation in CLL cells obtained from a larger cohort of patients.
- 17p deletion confers inferior outcomes after conventional chemotherapy due to inactivation and/or deletion of the p53 pathway.
- Since TGR-1202 is a PI3K-δ inhibitor, with a mechanism of action that does not rely on p53, we hypothesize that 17p and non-17p deleted CLL samples will have similar in vitro responses to TGR-1202.

Methods

- Blood was collected from CLL patients seen at the Duke Center for CLL and enrolled in IRB approved protocols at the Duke University and Durham VA Medical Centers.
- CLL lymphocytes were isolated using negative selection yielding greater than 95% purity of CLL lymphocytes.
- Primary CLL cells were incubated with serial dilutions of TGR-1202 for 24 hours or 48 hours and tested for apoptosis by activated caspase-3 and 7AAD staining measured by flow cytometry.
- After 72 hours of incubation with TGR-1202, CLL cells were evaluated for cytotoxicity using the colorimetric MTS reagent.
- Phosphorylated AKT (S473) was measured by flow cytometry after one hour of incubation with either compound and ten minutes of incubation with anti-IGM or anti-IGD. AKT phosphorylation was quantified by median fluorescent intensity (MFI).

Results

![Image](image1.png)

**Figure 1.** TGR-1202 induces dose-dependent cytotoxicity after three days of in vitro incubation with CLL cells, that either have 17p deletion (n = 4) or do not have 17p deletion (n = 3).

![Image](image2.png)

**Figure 2.** TGR-1202 induces apoptosis in both 17p deletion CLL cells (n = 5) and in non-17p deletion CLL cells (n = 3) at 24 hours, although high concentrations of drug are required.

![Image](image3.png)

**Figure 3.** TGR-1202 induces apoptosis in non-17p deletion CLL cells at 48 hours of incubation in a dose-dependent manner.

![Image](image4.png)

**Figure 4.** TGR-1202 suppresses the phosphorylation of AKT in non-17p deletion CLL cells in a dose-dependent manner.

![Image](image5.png)

**Figure 5.** TGR-1202 suppresses the phosphorylation of AKT in 17p deletion CLL cells at low nanomolar concentrations.

![Table](table1.png)

**Table 1.** CLL sample ID, Gender, Race, IGHV, CD38, ZAP70

<table>
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<th>CLL sample ID</th>
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</table>

![Image](image6.png)

**Figure 6.** TGR-1202 suppresses the phosphorylation of AKT in non-17p deleted CLL cells in a dose-dependent manner.

![Image](image7.png)

**Figure 7.** TGR-1202 suppresses the phosphorylation of AKT in 17p deletion CLL cells at low nanomolar concentrations.

![Image](image8.png)

**Figure 8.** TGR-1202 suppresses the phosphorylation of AKT in non-17p deletion CLL cells in a dose-dependent manner.

References

- Friedman, Dr et al. (2012). Comparison of the PI3K-δ inhibitors TGR-1202 and GS-1101 in Inducing Cytotoxicity and Inhibiting Phosphorylation of Akt in CLL Cells in vitro.” ASH Annual Meeting Abstracts 120: 391A.

About TGR-1202

- TGR-1202 is a novel PI3K-δ specific inhibitor with high selectivity over other Class I PI3K isoforms as well as a panel of 441-kinases.
- TGR-1202 was designed with a unique backbone compared to other PI3K inhibitors in development.

TGR-1202 backbone (full structure not yet disclosed)

- A Phase I, first-in-human, clinical trial of TGR-1202 is ongoing, evaluating GD oral administration of TGR-1202 and is enrolling patients with relapsed and/or refractory:
  - non-Hodgkin’s lymphoma
  - CLL (including 17p-del)
  - peripheral T-cell lymphoma; and
  - select other lymphoproliferative disorders.

- The dose escalation portion of this study will determine the maximum tolerated dose of TGR-1202 using a standard 3+3 design
- TGR-1202 has been well tolerated to date with no DLTs observed. Dose escalation continues in this Phase I study with higher dose cohorts.

Conclusions

- TGR-1202 induces CLL cell cytotoxicity at sub-micromolar concentrations in vitro.
- TGR-1202 induces CLL cell apoptosis, however, the relatively high concentrations required for TGR-1202 and other PI3K-δ inhibitors (Friedman, ASH 2012) compared to the cytotoxicity results may indicate alternate mechanisms of cell death for this class of agents.
- TGR-1202 inhibits AKT phosphorylation in CLL cells at low nanomolar concentrations in vitro.
- These effects appear to be independent of 17p deletion status, suggesting that p53 is not necessary for efficacy of TGR-1202 therapy in CLL.

Conflicts of Interest

Friedman, Lanasa: Research funding Sportelli, Miskin: Employment Vakkalanka, Viswanadha: Employment

Contact

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