The PI3Kδ 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), with expression of the δ isoform largely restricted to lymphocytes. Inhibition of PI3K activity in vitro induces CLL cell apoptosis and death.
• Clinical evaluation of PI3Kδ inhibitors, such as idelalisib, has produced responses in relapsed/refractory CLL patients.
• TGR-1202 is a novel PI3Kδ specific inhibitor that inhibits AKT phosphorylation and induces apoptosis in B-cell lymphoma cell lines (Friedman et al, ASH 2012), and has also demonstrated clinical activity in patients with relapsed/refractory CLL (Savona et al, ASH 2013).
• We previously evaluated the in vitro effects of TGR-1202 and idelalisib 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.

Methods

• Blood was collected from 40 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 or 48 hours and tested for apoptosis by activated caspase-3 and 7AAD staining by flow cytometry.
• After 72 hours of incubation with TGR-1202, cytotoxicity was evaluated 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-IGκB or anti-IκB. AKT phosphorylation was quantified by median fluorescent intensity (MFI).

About TGR-1202

• TGR-1202 is a novel PI3Kδ 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 and structure differentiated from other PI3Kδ inhibitors in development, and exhibits unique pharmacologic properties including an extended half-life that allows once-daily dosing.
• A Phase 1, first-in-human, clinical trial of TGR-1202 is ongoing, evaluating QD oral administration of TGR-1202 in patients with relapsed or refractory non-Hodgkin’s lymphoma, CLL (including 17p del), Hodgkin’s lymphoma, and select other lymphoproliferative disorders.
• TGR-1202 has been well tolerated to date with notably, no drug-related hepatotoxicity observed.
• Patients with relapsed/refractory CLL have demonstrated marked nodal reductions (nPRs) accompanied by significant lymphocytosis—a pharmacodynamic effect commonly associated with BCR-targeted agents.
• Dose escalation continues in the Phase I study as expansion cohorts have been opened at select dose levels.

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 PI3Kδ inhibitor, with a mechanism of action that does not depend on p53, we hypothesize that 17p and non-17p deleted CLL samples will have similar in vitro responses to TGR-1202.

Results

• TGR-1202 induces dose-dependent cytotoxicity after three days of in vitro incubation with CLL cells, that either have 17p deletion (n = 9) or do not have 17p deletion (n = 6).

Conclusions

• TGR-1202 induces CLL cell cytotoxicity at sub-micronolar concentrations in vitro, notably at concentrations several fold lower than those achieved in human PI3Kδ testing.
• TGR-1202 induces CLL cell apoptosis, however, the relatively high concentrations required for TGR-1202 and other PI3Kδ 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.

References


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