Abstract 4518

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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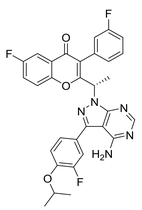
Introduction

- The PI3K pathway is a pro-survival mechanism in chronic lymphocytic leukemia (CLL), 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 PI3K-δ inhibitors, such as idelalisib, has produced responses in relapsed/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), 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 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 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-IgM or anti-IgD. AKT phosphorylation was quantified by median fluorescent intensity (MFI).

About TGR-1202



- TGR-1202 is a novel PI3K-δ 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 PI3K-δ inhibitors in development, and exhibits unique pharmacologic properties including an extended half-life that allows once-daily dosing.
- A Phase I, 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.

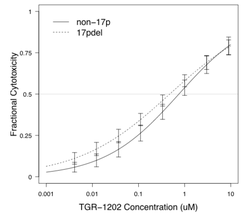
Hypotheses

- 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 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.

Results

Characteristic	17p del	Non-17p del
GENDER		
Male	5	7
Female	5	0
RACE		
Caucasian	9	7
Black	1	0
AGE AT DIAGNOSIS (median)	63	58
ZAP-70		
Positive	4	1
Negative	6	6
CD38		
Positive	1	0
Negative	9	7
IGHV		
Unmutated	3	1
Mutated	4	5
Not Known	3	1

Figure 1. 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).



	Median Cytotoxicity ED50	Number of Samples	p value
17p del	541 nM	9	0.39
Non-17p del	1061 nM	6	

Figure 2. TGR-1202 induces apoptosis in both 17p deletion CLL cells (n = 10) and in non-17p deletion CLL cells (n = 7) at 24 hours.

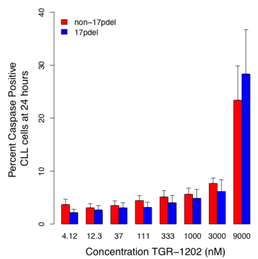


Figure 3. TGR-1202 induces apoptosis in non-17p deletion CLL cells at 48 hours of incubation in a dose-dependent manner.

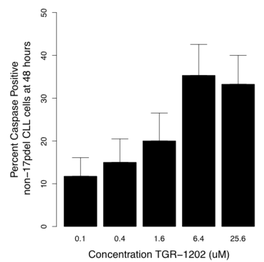


Figure 4. TGR-1202 suppresses the phosphorylation of AKT in non-17p deletion CLL cells in a dose-dependent manner.

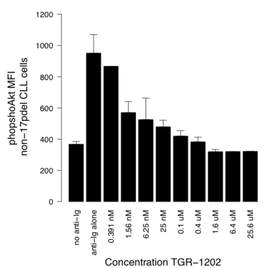
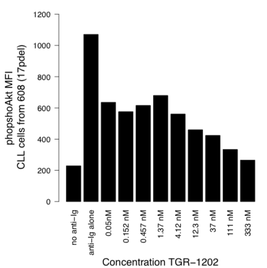


Figure 5. TGR-1202 suppresses the phosphorylation of AKT in 17p deletion CLL cells at low nanomolar concentrations.



Conclusions

- TGR-1202 induces CLL cell cytotoxicity at sub-micromolar concentrations *in vitro*, notably at concentrations several fold lower than those achieved in human PK testing.
- 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.

References

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Conflicts of Interest

Friedman, Lanasa: Research funding
Sportelli, Miskin: Employment & Equity Ownership
Vakkalanka, Viswanadha: Employment & Equity Ownership

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