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

Daphne R. Friedman[1,2], Tiffany Simms[1], Sallie D. Allgood[1], Danielle M. Brander[1], Peter Sportelli[3], Hari P. Miskin[3], Swaroop Vakkalanka[4], Srikant Viswanadha[5], J. Brice Weinberg[1,2], Mark C. Lanasa[1]

[1]Duke University, Durham NC, USA; [2]Durham VA Medical Center, Durham NC, USA; [3]TG Therapeutics, Inc., New York NY, USA; [4]Rhizen Pharmaceuticals, La Chaux-de-Fonds, Switzerland; [5]Incozen Therapeutics, Hyderabad, India

Introduction

- The PI3K pathway is a pro-survival mechanism in chronic lymphocytic leukemia (CLL).
- Expression of the δ-isoform 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.

References

- Furman RR et al. (2010). "CAL-101, An Isoform-Selective Inhibitor of Phosphatidylinositol 3-Kinase P110{delta}, Demonstrates Clinical Activity and Pharmacodynamic Effects In Patients with Relpased or Refractory Chronic Lymphocytic Leukemia." ASH Annual Meeting Abstracts 116(21):55.
- Longo, PG et al. (2008). "The Akt/Mcl-1 pathway plays a prominent role in mediating antiapoptotic signals downstream of the B-cell receptor in chronic lymphocytic leukemia B cells. Blood 111(2):846-855.
- Weinberg, JB et al. (2007). "Clinical and molecular predictors of disease severity and survival in chronic lymphocytic leukemia." Am J Hematol 82(12):1063-1070.
- 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: 3914.

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

CLL sample ID	Gender	Race	IGHV	CD38	ZAP70
560	Male	Caucasian	NA	Negative	Negative
583	Male	Caucasian	Unmutated	Negative	Positive
608	Male	Caucasian	NA	Negative	Positive
420	Female	African American	Unmutated	Negative	Positive
322	Male	Caucasian	Unmutated	Negative	Negative
151	Male	Caucasian	Mutated	Negative	Negative
485	Male	Caucasian	Mutated	Negative	Negative
69	Male	Caucasian	Unmutated	Negative	Negative
472	Female	Caucasian	Mutated	Negative	Negative
325	Female	Caucasian	Mutated	Negative	Negative
498	Female	Caucasian	Mutated	Negative	Negative
292	Male	Caucasian	Mutated	Negative	Negative

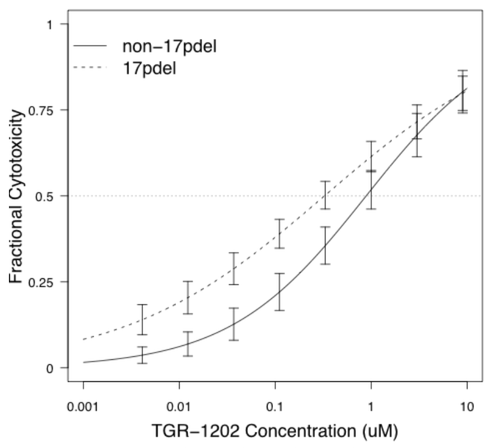


Figure 1. TGR-1202 induces dose-dependent cytotoxicity after three days of *in vitro* incubation with CLL cells, that either have 17p deletion (n = 4) or do not have 17p deletion (n = 3).

CLL sample ID	17p del status	Cytotoxicity ED50 (μM)
560	17p del	0.996
583	17p del	0.148
608	17p del	0.273
420	17p del	0.264
322	Non-17p del	0.477
151	Non-17p del	1.08
485	Non-17p del	1.26
69	Non-17p del	0.973
472	Non-17p del	< 0.1
325	Non-17p del	0.666
498	Non-17p del	< 0.1
292	Non-17p del	1.17

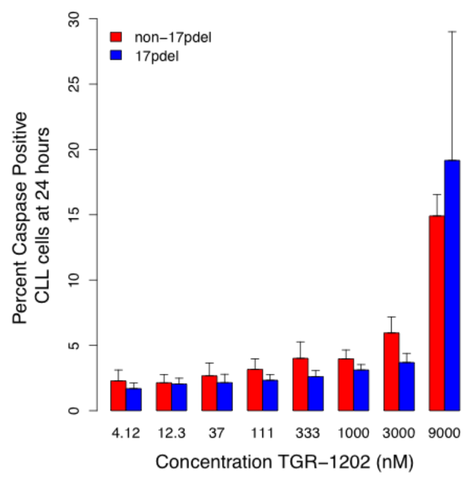


Figure 2. TGR-1202 induces apoptosis in both 17p deletion CLL cells (n = 5) and in non-17p deletion CLL cells (n = 3) at 24 hours, although high concentrations of drug are required.

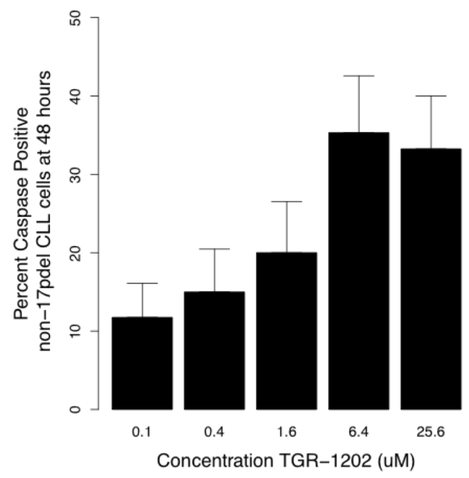


Figure 3. TGR-1202 induces apoptosis in non-17p deletion CLL cells at 48 hours of incubation in a dose-dependent manner.

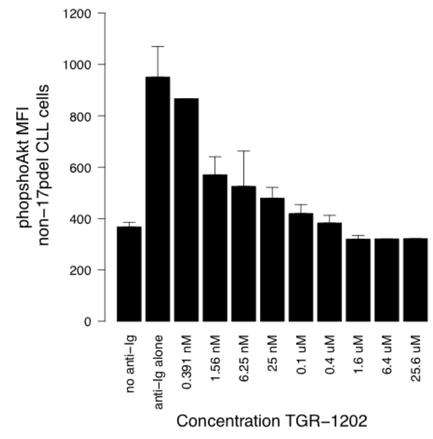


Figure 4. TGR-1202 suppresses the phosphorylation of AKT in non-17p deletion CLL cells in a dose-dependent manner.

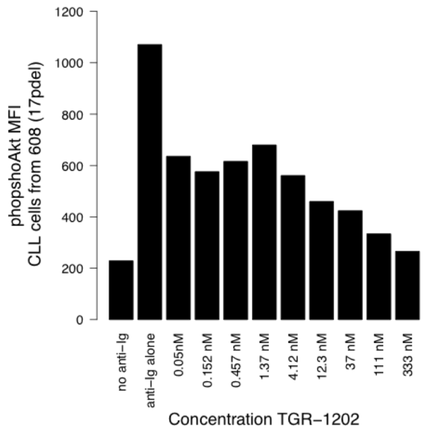
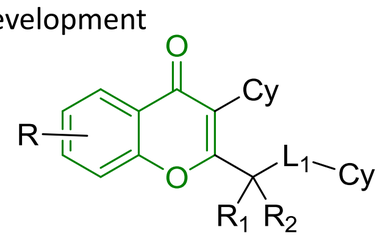


Figure 5. TGR-1202 suppresses the phosphorylation of AKT in 17p deletion CLL cells at low nanomolar concentrations.

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 QD 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

daphne.friedman@duke.edu
mark.lanasa@duke.edu

Presented at the 15th Annual Workshop of the iwCLL, September 9-11, 2013, Cologne Germany