TGR-1202– A novel PI3K-delta inhibitor that differentially regulates T cells in CLL

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ABSTRACT

In the microenvironment of CLL, T cells are characteristically dysfunctional compared to a normal, healthy setting. Idelalisib, an approved PI3Kδ inhibitor has shown successful clinical response in relapsed/refractory CLL, but is associated with a high rate of discontinuation because of immune-related severe adverse events (SAE). More recently, increased toxicity has been reported in treatment-naïve patient trials, potentially as a result of more competent immune response in these patients. The described hepatotoxicity, colitis, and pneumonitis appear to be associated with changes in the T cell compartment and specifically regulatory T cells (Tregs). In the phase I trials of the next generation PI3Kδ inhibitor, TGR-1202, a comparable rate of clinical responses have been reported with apparently less toxicity than prior PI3Kδ inhibitors, even with long term follow-up (Burris et al, ASCO 2016). We previously demonstrated *in vitro* that TGR-1202 relatively preserved the number and function of normal human T cell subsets, including Tregs, when compared to the other clinically available PI3Kδ inhibitors (Maharaj et al, AACR 2016). ***Herein, we aimed to further investigate how TGR-1202 regulates T cell subsets in a preclinical murine model of CLL and determine correlation with incidence of adverse events after oral administration.***

First, we confirmed general immune changes typical of CLL progression. Peripheral white blood cell (WBC) count was significantly higher in leukemic mice compared to wildtype (p=0.02). Following oral PI3K delta inhibitor treatment, WBC count decreased significantly over time (p<0.001 respectively), suggesting comparable efficacy in disease eradication. Both inhibitors also significantly reduced overall circulating CD3+ T cell number, **however TGR-1202 differentially regulated T cell subset ratios previously reported to regulate autoimmunity**. These effects were more pronounced in the CLL setting than wildtype. While Tcon:Teff (CD4:CD8) ratio remained intact, total Treg number was significantly decreased following treatment, leading to increased Tcon:Treg and Teff:Treg ratios. On the Treg population, TGR-1202 maintained expression of functional markers PD-1, CTLA-4 and others closer to control when compared to duvelisib or idelalisib. This indicated greater retention of Treg suppressive capacity *in vivo* in TGR-1202 group. Further, pathology studies are ongoing to assess the toxicity after TGR-1202, duvelisib or idelalisib treatment in these mice.

BACKGROUND

The role of PI3K signaling is widely acknowledged as a key component of cell survival in many hematological malignancies. The PI3K molecule recruits important downstream effector signaling proteins directly following BCR ligation. For example, recruitment of BTK and AKT leads to promotion of cell survival by activating NF-kB and inhibiting apoptotic signals. The p110 delta expressing isoform of PI3K is restricted to hematopoietic cell types; therefore p110 delta represents a viable target for the treatment of B-cell malignancies with little cytotoxicity in other cell types. However, drugs targeting p110 delta may have potential off-target effects in other immune cell types. For example, potential off-target effects in the T-cell compartment may have important implications for immunosuppressive or immunostimulatory mechanisms which may contribute to the progression, or elimination of disease.

Idelalisib (aka "CAL-101") and Duvelisib (aka "IPI-145") are two novel, orally available PI3K inhibitors that show selectivity for p110 delta. In the clinic, rates of objective response for these drugs are 40-60% and nodal responses exceed 70% in R/R CLL. They also show high rates of response in high-risk CLL (e.g. 17p and 11q deletions). In vitro, idelalisib inhibits p110 delta at a concentration 40 to 300-fold lower than the other class 1 PI3K isoforms and exhibits selectivity when profiled against other protein and lipid kinases. In the phase 1 study of single-agent idelalisib in 54 R/R CLL patients who were previously heavily treated, the therapy was well-tolerated generally but 15% of participants discontinued therapy due to adverse effects. **TGR-1202** is a selective inhibitor of p110 delta. Notably, TGR-1202 exhibits a different structure than idelalisib and duvelisib which are very similar compounds chemically. Thus far, TGR-1202 has shown promising activity in B cell lymphomas without significant severe adverse effects. It has been shown to induce cytotoxicity, and inhibit AKT phosphorylation at submicromolar concentrations in both del 17p and non del 17p primary CLL cells in vitro.

OBJECTIVE

In this series of studies we sought to investigate how TGR-1202 regulates T cell subsets in a preclinical murine model of CLL and determine correlation with incidence of immune-mediated adverse events after oral administration.

MATERIALS AND METHODS

CLL Murine Model

25x10^6 splenocytes from leukemic aged euTCL1 mice were injected via tail vein into C57BL/6 mice (Jackson Laboratories). After confirmation of disease induction (increased peripheral lymphocyte count) mice were gavaged once per day with TGR-1202, duvelisib, idelalisib, or vehicle for a total of 21 days.

Magnetic Cell Purification

EasySep T cell isolation kits (StemCell Tech.) were utilized for the enrichment of >95% purity of cells of interest. Company supplied protocols were followed and flow cytometry was performed to elucidate purity. T cell stimulation was achieved with CD3/CD28 soluble cytokines (BD Bioscience, San Jose CA).

Ex Vivo T cell assays

Isolated T cells were plated in a 96 well flat bottom plate at 100,000 cells/well in 200uL complete media with idelalisib (SelleckChem), duvelisib (SelleckChem), or TGR-1202 (supplied by TG Therapeutics), 0-50uM and cultured for 2-5 days.

Flow Cytometry Immunophenotyping

Flow cytometric analysis was performed using fluorochrome-labeled monoclonal antibodies (mAbs; anti-CD3, -CD4, -CD8, -CD25, -CD127, -CD279 (PD-1), -CTLA-4, -FOXP3, BD Bioscience, San Jose CA, eBioscience, San Diego CA, -TGFB-1, -CD39, -CD103 Biolegend, San Diego CA) and the vitality dye Zombie NIR. Data was acquired on an LSRII cytometer (Beckman Coulter), and analyzed with FlowJo software (Tree Star, Ashland, OR). Absolute cell numbers calculated using AccuCheck Counting beads (Invitrogen).

Phospho Flow

Isolated murine CD3+ T cells were stimulated with CD3/28 plate-bound antibody for 30 min and expression of phosphorylation of Serine 473 on AKT was determined using an iQue cytometer and analyzed with accompanying software.

Figure 1. TGR-1202 treatment favors a suppressive human T cell phenotype

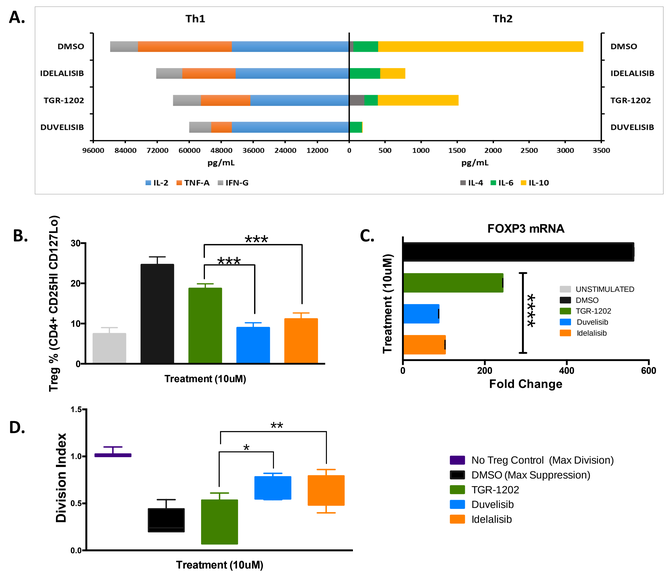

Figure 1. TGR-1202 favors a suppressive human T cell phenotype.
CD3+ T cells were isolated from healthy human peripheral blood and cultured with 10uM of TGR-1202, idelalisib or duvelisib **(A)** Representative cytokine secretion after treatment from supernatant of cultured T cells. TGR-1202 retains production of TH2 cytokines, mainly IL-10. **(B)** Percent Tregs after stimulation and culture with inhibitors obtained by flow cytometry. TGR-1202 relatively preserved the number of Tregs compared to other PI3K delta inhibitors **(C)** FoxP3 mRNA levels were detected by q-RT-PCR in CD3+ T cells isolated from healthy human peripheral blood and stimulated with anti-CD3/CD28 **(D)** Treg suppression assay- Tregs were isolated from healthy human peripheral blood and cultured overnight with each inhibitor. Tregs were then washed and cultured together with responder naive CD4+ autologous T cells. Division index was calculated using FlowJo software as a measure of proliferation of CD4 responder T cells. Data indicates that Tregs treated with TGR-1202 maintained their suppressive capacity compared to Tregs treated with other inhibitors. *p<0.05, **p<0.005, ***p<0.0005.

Figure 2. TGR-1202 demonstrates similar anti-tumor efficacy to other PI3K delta inhibitors in murine CLL

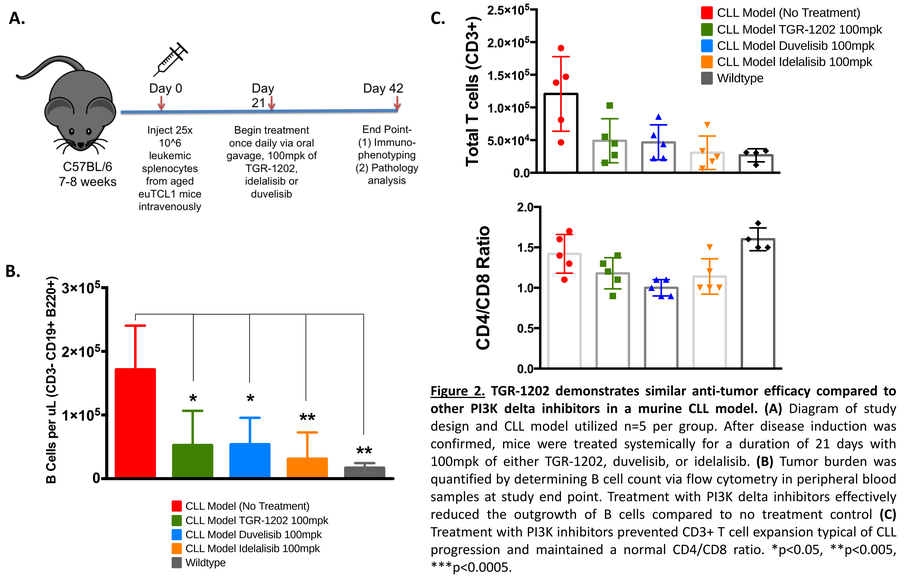

Figure 2. TGR-1202 demonstrates similar anti-tumor efficacy compared to other PI3K delta inhibitors in a murine CLL model. (A) Diagram of study design and CLL model utilized n=5 per group. After disease induction was confirmed, mice were treated systemically for a duration of 21 days with 100mpk of either TGR-1202, duvelisib, or idelalisib. **(B)** Tumor burden was quantified by determining B cell count via flow cytometry in peripheral blood samples at study end point. Treatment with PI3K delta inhibitors effectively reduced the outgrowth of B cells compared to no treatment control **(C)** Treatment with PI3K inhibitors prevented CD3+ T cell expansion typical of CLL progression and maintained a normal CD4/CD8 ratio. *p<0.05, **p<0.005, ***p<0.0005.

Figure 3. *In vivo* administration of TGR-1202 recapitulates the relative preservation of Tregs

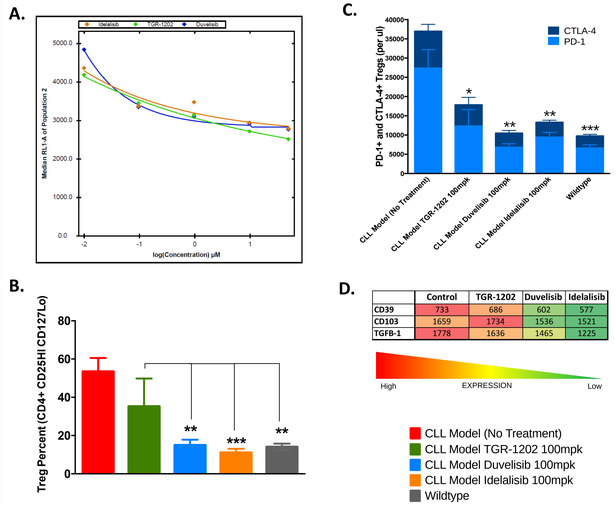

Figure 3. In vivo administration of TGR-1202 recapitulates the relative preservation of Treg function
(A) PI3K delta inhibition reduces phosphorylation of AKT in murine T cells in a dose dependent fashion **(B)** Percent of Tregs in peripheral blood is relatively preserved by TGR-1202 administration but not duvelisib or idelalisib (n=5 mice per group) **(C)** PD-1+ and CTLA-4+ Treg count is higher in TGR-1202 treated mice compared to other PI3K delta inhibitors indicating greater functional capacity. **(D)** Heat map showing basal level expression of markers associated with Treg function on Tregs obtained from spleen at end point. *p<0.05, **p<0.005, ***p<0.0005.

Figure 4. Absolute Treg number is associated with incidence of adverse events

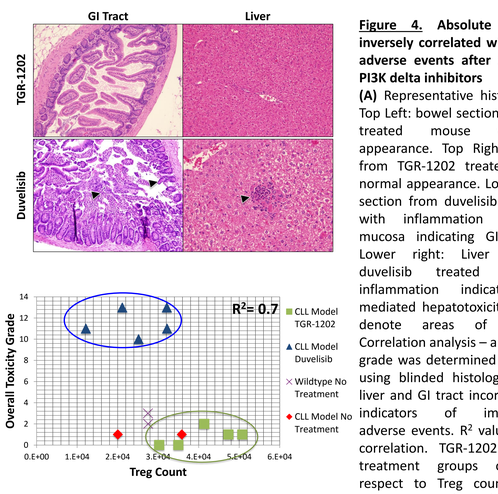

Figure 4. Absolute Treg number inversely correlated with incidence of adverse events after treatment with PI3K delta inhibitors
(A) Representative histologic findings. Top Left: bowel section from TGR-1202 treated mouse with normal appearance. Top Right: liver section from TGR-1202 treated mouse with normal appearance. Lower Left: Bowel section from duvelisib treated mouse with inflammation and denuded mucosa indicating GI tract toxicity. Lower right: Liver section from duvelisib treated mouse with inflammation indicating immune-mediated hepatotoxicity. Black arrows denote areas of interest. **(B)** Correlation analysis – an overall toxicity grade was determined after H&E stain using blinded histological analysis of liver and GI tract incorporating known indicators of immune-mediated adverse events. R^2 value=0.7 indicates correlation. TGR-1202 and duvelisib treatment groups clustered with respect to Treg count and toxicity parameters as shown by ovals.

CONCLUSIONS

- **TGR-1202, duvelisib, or idelalisib oral administration demonstrated comparable efficacy by reducing CLL burden over time in leukemic mice**
- **TGR-1202, duvelisib, and idelalisib treatment comparably reduced PI3K signaling in murine T cells**
- **TGR-1202 maintained the number and functional capacity of Tregs relative to duvelisib or idelalisib treatment in both *ex vivo* normal human T cells and *in vivo* murine CLL model**
- **TGR-1202 administration in CLL mice did not produce significant immune-mediated adverse events in liver or GI tract compared to those observed with duvelisib treatment**
- **Treg count associated with the incidence of immune-mediated adverse events in TGR-1202 and duvelisib treated mice in preliminary histologic data, potentially indicating a role for Tregs in this context**

REFERENCES

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CONFLICT OF INTEREST

Miskin: TG Therapeutics (Employment & Equity Ownership); Maryanski TG Therapeutics (Employment & Equity Ownership)

FUNDING

TG Therapeutics