Modulation of T cell Compartment in a Preclinical CLL Murine Model by a Selective PI3K delta Inhibitor, TGR-1202

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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.***

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 TGR-1202 or duvelisib 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, Th1:Th2 ratio was increased, 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 and CTLA-4 closer to control when compared to duvelisib (TGR-1202 vs. control PD-1: p=ns, CTLA-4: p=ns ; duvelisib vs. control PD-1: p=0.02* CTLA-4: p=0.03*). This indicated greater retention of Treg suppressive capacity *in vivo* in TGR-1202 group. Interestingly, overall Th17 number was reduced in both treatments over time but there was greater disruption of Treg:Th17 ratio in duvelisib group (duvelisib vs. control Treg/Th17 p=0.04* ; TGR-1202 vs. control Treg/Th17 p=ns). In perspective, maintenance of this balance has been extensively reported to mediate autoimmunity. In order to determine susceptibility to apoptosis, we quantified CD95 (FASL) expression. Lower CD95 mean fluorescence intensity (MFI) was observed on CD4, CD8 and Treg populations in both periphery and spleen of TGR-1202 groups compared to those treated with duvelisib. Pathology studies are ongoing to assess the toxicity after TGR-1202 or duvelisib 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 p110d 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 p110d. Notably, TGR-1202 exhibits a vastly 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 compare the effects of clinically available PI3Kδ inhibitors TGR-1202 and Duvelisib on T cells with an emphasis on regulatory T cells in a preclinical CLL murine model

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 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 murine T cells were plated in 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, -CXCR3, -CCR4, -CCR6 -CD279 (PD-1), -CTLA-4, -FOXP3, -CD95, BD Bioscience, San Jose CA, eBioscience, San Diego CA, -TGFB-1, -GITR, -CD39, 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 was determined using an iQue cytometer and analyzed with accompanying software

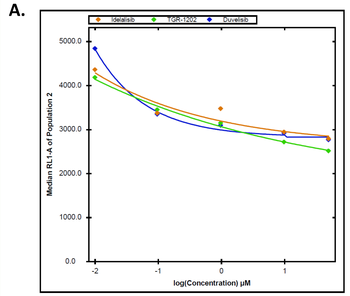
Cytometric Bead Array

Supernatant was collected from CD3+ T cells that were isolated from mice treated with drugs and incubated for 48 hours. Th1/Th2/Th17 cytokines were measured with CBA (BD Biosciences, San Diego, CA) according to manufacturer's protocol.

Plasma Concentration Assay

Peripheral blood was collected from sub-mandibular bleeds 1 hour post drug administration and plasma was isolated by centrifuging. TGR-1202 plasma concentrations were determined using a validated LC-MS/MS method (NorthEast Bioanalytical Laboratories, Hamden, CT).

Figure 1. PI3K delta inhibitors target murine T cells



pAKT (S473) Fold Change of MFI

Conc [uM]	Idelalisib	TGR-1202	Duvelisib
DMSO	1	1	1
0.01	1.133	1.085	1.260
0.1	0.878	0.900	0.870
1	0.903	0.817	0.802
10	0.764	0.708	0.757
50	0.723	0.654	0.720

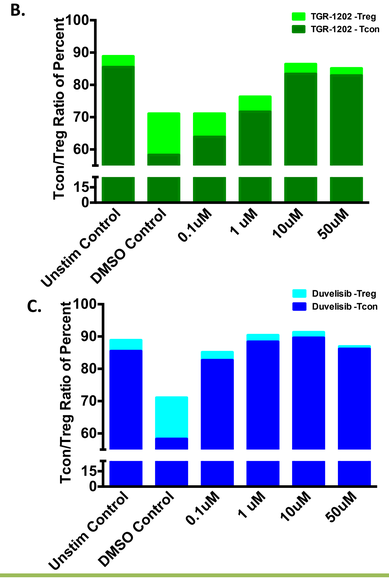


Figure 1. PI3K delta inhibitors target murine T cells. CD3+ T cells were isolated from murine wildtype splenocytes and stimulated with CD3/28 plate bound. **(A)** Phosphorylation of AKT (Serine 473) was inhibited within 30min in a dose-dependent manner after treatment with Idelalisib, TGR-1202, or Duvelisib. 48h of treatment with **(B)** TGR-1202 or **(C)** Duvelisib increased the T conventional (CD4+ CD25-) to regulatory T cells (CD4+ CD25Hi CD127Lo) ratio in murine CD3+ T cells.

Figure 2. TGR-1202 maintains Treg number & expression of suppressive Treg markers

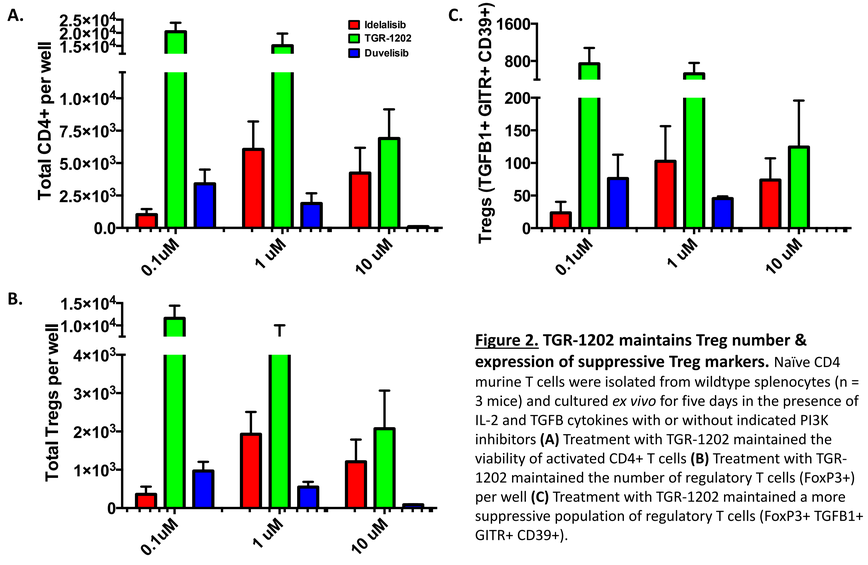


Figure 2. TGR-1202 maintains Treg number & expression of suppressive Treg markers. Naïve CD4 murine T cells were isolated from wildtype splenocytes (n = 3 mice) and cultured *ex vivo* for five days in the presence of IL-2 and TGFB cytokines with or without indicated PI3K inhibitors **(A)** Treatment with TGR-1202 maintained the viability of activated CD4+ T cells **(B)** Treatment with TGR-1202 maintained the number of regulatory T cells (FoxP3+) per well **(C)** Treatment with TGR-1202 maintained a more suppressive population of regulatory T cells (FoxP3+ TGFB1+ GITR+ CD39+).

Figure 3. TGR-1202 demonstrates efficacy in a murine CLL model

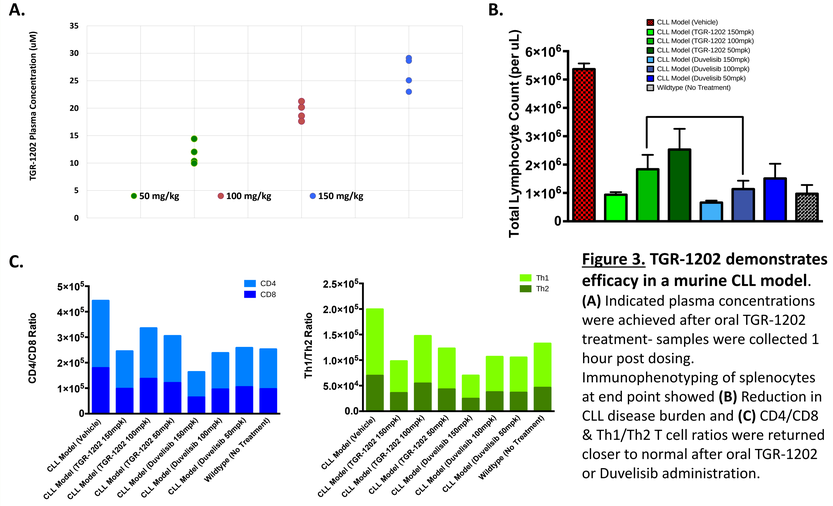


Figure 3. TGR-1202 demonstrates efficacy in a murine CLL model. **(A)** Indicated plasma concentrations were achieved after oral TGR-1202 treatment- samples were collected 1 hour post dosing. Immunophenotyping of splenocytes at end point showed **(B)** Reduction in CLL disease burden and **(C)** CD4/CD8 & Th1/Th2 T cell ratios were returned closer to normal after oral TGR-1202 or Duvelisib administration.

Figure 4. TGR-1202 administration favors suppressive T cell phenotype

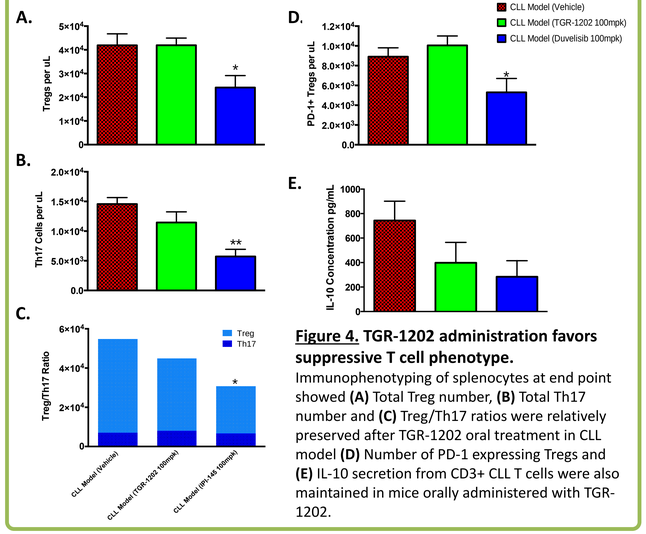


Figure 4. TGR-1202 administration favors suppressive T cell phenotype. Immunophenotyping of splenocytes at end point showed **(A)** Total Treg number, **(B)** Total Th17 number and **(C)** Treg/Th17 ratios were relatively preserved after TGR-1202 oral treatment in CLL model **(D)** Number of PD-1 expressing Tregs and **(E)** IL-10 secretion from CD3+ CLL T cells were also maintained in mice orally administered with TGR-1202.

CONCLUSIONS

- **TGR-1202 or Duvelisib oral administration demonstrated comparable efficacy by reducing CLL burden over time in leukemic mice**
- **TGR-1202 & Duvelisib targeted the T cell population *in vivo***
- **CD4/CD8 and TH1/TH2 ratio was not different between mice treated with TGR-1202 or Duvelisib**
- **TGR-1202 relatively maintained the number of Tregs and expression of functional markers compared to Duvelisib treatment in vivo and ex vivo**
- **Greater disruption of Treg/Th17 ratio by oral Duvelisib treatment compared to TGR-1202 treatment which may have implications for occurrence of autoimmune-like organ toxicity**

REFERENCES

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3) Winkler DG, Faia KL, Dinitto JP, et al. PI3K-δ and PI3K-γ inhibition by IPI-145 abrogates immune responses and suppresses activity in autoimmune and inflammatory disease models. Chem Biol. 2013;20(11):1364-74.

CONFLICT OF INTEREST

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

FUNDING

TG Therapeutics