TGR-1202: a novel PI3K-delta inhibitor that differentially regulates T cells in CLL

Kamira K. Moharaj 1,3, John Powers 2,4, Alex Achille 1, Mibbel Pabon-Saldaña 5, Susan Deng 6, Renee M. Fonseca 1, Hari P. Miskin 2, Dave Marynsk 1, Eva Sahakian 1,3 and Javier Pinilla-Ibarz 6,7

1 (i) Department of Immunology, (i) Moffitt Cancer Center & Research Institute, Tampa, FL; (ii) Department of Maternal Hematology, (i) Lee Moffitt Cancer Center & Research Institute, Tampa, FL; (iii) Cancer Biology Phd. Program, University of South Florida, Tampa, FL; (iv) (i) Moffitt, Therapeutics, Inc., New York, NY.

ABSTRACT

In this manuscript, TGR-1202, a novel PI3K-delta inhibitor, was evaluated in a murine model of CLL. Our data demonstrated that TGR-1202 preferentially targets T cells in CLL, resulting in the preferential inhibition of T cells while sparing other immune cell types. These results indicate that TGR-1202 may be a promising therapeutic agent for the treatment of CLL.

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 Mouse Model

2x10^6 spleenocytes from leukaemic aged eμ/Cli mice were injected i.v. into C57BL6 mice (Jackson laboratories). After confirmation of disease induction (peripheral lymphocyte lymphoma phenotype), mice were gavaged once per day with TGR-1202, duvelisib, dulakabril, or vehicle for a total of 21 days.

Magnetic Cell Purification

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

Isolated T cells were plated in a 36 well flat bottom plate at 100,000 cells/well for 200ul complete media with IDEALs (Biolegend, San Diego). CD3/CD28 coated beads (ImmuneTech), or TGR-1202 (supplied by (T) Therapeutics), 0-1000uM and cultured for 2-5 days.

Flow Cytometry

Flow cytometric analysis was performed using Fluorescence-labeled monoclonal antibodies (Abs): anti-CD25, -CD8, -CD11c, -CD44, -CD11b, -CD44+SH (Biolegend, San Jose CA), isotype control (Biolegend, San Jose CA), and the viability dye 7-AAD. Flow data was analyzed using FlowJo software (Tree Star, Ashland, OR). Additionally, Abs were analyzed on a FACSCanto (Becton Dickinson) and a FACSLytica instrument (Luminex). Phospho-flow

Isolated murine CD4+ T cells were stimulated with CD3/CD28 plate-bound antibody for 30 min and expression of phosphorylation of Serine 473 on AKT was determined using an IHC cytoplate and analyzed with accompanying software.

RESULTS

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

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

Figure 3. In vivo administration of TGR-1202 recapitulates the preserved Tregs

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

CONCLUSIONS

TGR-1202, duvelisib, or dulakabril administration demonstrated comparable efficacy reducing CLL burden over time in leukaemic mice. TGR-1202, dulakabril, and dulakabril treatment comparably reduced FISH signal in murine T cells. TGR-1202 maintained the number and functional capacity of Tregs relative to dulakabril or dulakabril treatment. In both as well as normal human T cells and in vivo CLL mouse models. TGR-1202 administration in C57 mice did not produce significant immune-mediated adverse events in vivo or in vitro compared to those observed with dulakabril treatment. Treg counts associated with the incidence of immune-mediated adverse events in TGR-1202 and dulakabril treated mice in xenotransplantatic data, potentially indicating a role for Tregs in this context.