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 murine model of CLL, we characterized the effect of TGR-1202, a selective PI3K delta inhibitor, on T cell compartment dynamics because of immunosuppressive stromal milieu. CLL is an incurable disease that causes high mortality rates due to immune dysfunction because of immunosuppressive stromal milieu. CLL patients with an increased Th17/Th1 ratio have been associated with survival. We investigated the impact of TGR-1202 on the T cell compartment and immune dysfunction in three patient-derived CLL xenografts. We observed a statistically significant reduction in the number of total T cells (p=0.001), Th17 cells (p=0.005), and Th1 cells (p=0.004) in the presence of TGR-1202. Furthermore, we observed a statistically significant increase in the number of Treg cells (p=0.001) and CD4+CD8+ T cells (p=0.001), as well as a significant decrease in the Th17/Th1 ratio, when compared to the control group. Specifically, TGR-1202 caused a significant decrease in the Th17/Treg ratio, indicating a potential therapeutic benefit in CLL patients with high Th17/Treg ratios.

OBJECTIVE

In this series of studies we sought to compare the effects of clinically available PI3K 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

Acute leukemia was induced in male C57BL/6 mice (10-12 weeks old) by intraperitoneal injection of 10^6 freshly isolated CLL cells. After confirmation of disease induction (increased peripheral lymphocytosis and weight loss), mice were randomized to receive TGR-1202 or Duvelisib with daily treatment on days 5-8.

Magnetic Cell Purification

Equal T cell isolation (isolation methods and purity of cells of interest) were performed and flow cytometry was performed on 100,000 events. T cell isolation was achieved with 3x10^6 viable cells/μL, showing a purity of >90%.

Enriched T Cells Assay

Isolated murine T cells were plated in 96-well plates and loaded with 100 nM 3H-thymidine (Perkin Elmer) for 6 hours. The next day, cells were harvested and counted using a liquid scintillation counter. Cytokine expression was measured using FlowJo software (IBM Corp., Armonk, NY).

Flow cytometry analysis was performed using FACScanto or LSRFortessa (BD Biosciences) equipped with Cytometry Data Analysis and FlowJo software (BD Biosciences). Cytokine expression was gated on CD4+ or CD8+ T cells using fluorochrome-conjugated antibodies.

CytoFlow Software Analysis

The murine T cells were stained for CD3, CD4, CD8, and CD127 and analyzed using FlowJo software (Tree Star, Ashland, OR). Data were analyzed using the Mann-Whitney test with 90% confidence intervals.

Statistical analysis was performed using GraphPad Prism (v. 5.0, San Diego, CA) and R (v. 3.5.1) software. Data were analyzed using the non-parametric Mann-Whitney test for survival analysis or the Student’s t-test for comparison of two groups.

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+ and CD8+ T cells were significantly reduced when compared to the control group.
- TGR-1202 relatively maintained the number of Tregs and expression of functional markers comparable to Duvelisib treatment in vivo (55% vs. 65%) and Duvelisib treatment in vivo (60% vs. 40%).
- Greater disruption of Th1/Th2 ratio by oral Duvelisib treatment compared to TGR-1202 treatment may have implications for the future development of TGF-beta-based treatments.

REFERENCES


FUNDING

NIH/NCI (R01-CA177818). The content is solely the responsibility of the authors and does not necessarily represent the official views of NIH/NCI. This work was supported in part by the Blood Cancer, Thermal Cancer and Diabetes Research Program of the National Institutes of Health (NIH) and the American Cancer Society (ACS).