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**Background** The specific BCL-2 small molecule inhibitor venetoclax has been shown to effectively induce apoptosis in a wide range of malignancies either alone and in combination with other drugs. Based upon these results, there has been considerable growth in its use in clinical studies used alone and in combination with chemotherapy and immune-based therapies. Lymphocytes, and T cells in particular, rely heavily on BCL-2 for survival and function. This has been determined largely by genetic deletion or overexpression of BCL-2 in murine models. However, the adaptive effects of short or long-term small molecule BCL-2 family blockade on surviving immune cell subsets and their function is not fully understood. In the current work, we aimed to better understand the effect of long-term systemic treatment with venetoclax on regulatory T (Treg) cells, which are relatively apoptotically resistant to specific BCL-2 drugging compared to other T cells. We found that venetoclax altered surviving global T cell signaling and induced Treg cells towards a T<sub>H</sub>17-like Treg (Tr17) via PI3K pathway activation and FOXO1 inhibition.

**Methods** Normal and MC38-bearing C57BL/6 FOXP3<sup>ires-GFP</sup> mice were treated systemically with venetoclax for 14 days followed by phenotypic, functional, apoptotic, and genetic (RNA sequencing, ATAC seq, etc.) evaluation of Tregs isolated from immune organs and tumor infiltrating lymphocytes from these animals.

**Results** We show that venetoclax treatment resulted in Treg cell plasticity resulting in the conversion of a subset of canonical Tregs to a Tr17-like cell in normal and tumor-bearing mice. Venetoclax induced Tr17-like cells which were found to express increased IL-17A and IFN $\gamma$ . RNA expression profiling of treated Tregs indicated that the PI3K pathway is activated. PI3K pathway activation was consistently also found in CD8<sup>+</sup> and conventional CD4<sup>+</sup> T cells (Tcons), similar to our recently reported observations in homeostatically expanding naive T cells from stem cell transplanted animals treated with venetoclax.<sup>1</sup> PI3K activation in Tregs resulted in inhibition of FOXO1 through phosphorylation at Ser256, leading to exclusion of FOXO1 from the nucleus. This resulted in transcriptional activation of a Th17-associated genetic program through ROR $\gamma$ t upregulation in Tregs, but not Tcons, and as confirmed by altered genome wide accessibility patterns in treated Tregs.

**Conclusions** Our results indicate that long-term BCL-2 blockade by venetoclax results in Treg plasticity towards a Th17-like functional state through PI3K activation. We believe that this phenomenon may be beneficial for recently reported immune-mediated anti-tumor effects as a result of venetoclax therapy.

## REFERENCE

1. Ludwig LM, Hawley KM, Banks DB, Thomas-Toth AT, Blazar BR, McNerney ME, et al. Venetoclax imparts distinct cell death sensitivity and adaptivity patterns in T cells. *Cell Death & Disease*. 2021;**12**(11):1–13.

**Ethics Approval** All animal experiments were approved by and performed in accordance with the guidelines and regulations set forth by the Institutional Animal Care and Use Committee of the University of Chicago. ACUP # 72295