583 **EXPLORING THE POTENTIAL OF T CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL) FOR GENERATING LEUKEMIA-SPECIFIC T CELL RESPONSES THAT CAN BE THERAPEUTICALLY HARNESSED WITH IMMUNOTHERAPY**

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**Background** T cell Acute Lymphoblastic Leukemia (T-ALL) is a devastating malignancy found primarily in pediatric populations. Unfortunately, standard of care for T-ALL has not progressed from highly toxic, intensive regimens of chemotherapy, which fails to cure all patients. Immunotherapies designed to activate patients' leukemia-specific T cells may provide a new therapeutic avenue to increase complete response rates, reduce toxicity without the need to engineer (e.g. CAR) cells. However, it is unknown whether T-ALL is capable of being recognized by T cells due given its relatively low mutation-rate. These studies therefore sought to investigate whether signs of leukemia-specific T cell responses are generated by T-ALL. Because T-ALL results in systemic disease and infiltrates multiple lymphoid and non-lymphoid tissues, these studies also determined how the divergent immune contextures of these TMEs impacts T cell responses to T-ALL. From this, we aim to identify immunotherapeutic targets capable of activating T cells across tissues to eradicate leukemia systemically.

**Methods** Primary leukemia cells isolated from a spontaneous murine model (LN3 mice) into immune-competent, congenic (CD45.1) recipient mice. Tissues were harvested at distinct stages of disease for analysis by flow cytometry or utilizing NanoString Technologies' GeoMX Digital Spatial Profiling (DSP) platform.

**Results** Flow cytometric analysis of T cells revealed extensive changes in response to T-ALL that included multiple features of exhaustion typically associated with anti-tumor responses as determined by upregulation of co-inhibitory receptors and TOX. This included a surprisingly high-frequency of PD1+ T cells, which was accompanied by PDL1- and PDL2-expressing myeloid cells that likely are restraining these subsets. Importantly, combination immunotherapy with OX40 agonists while inhibiting PD1 resulted in drastically reduced tumor burden and concomitant expansion of proliferating granzyme-expressing CD8 T cells. To gain better insight into T cell responses within distinct organs, we analyzed tissue sections using DSP. This technique enabled us to evaluate T cells in direct contact with leukemia infiltrates compared to T cells in regions without T-ALL, which further revealed an enrichment of activated subsets. Importantly, these studies have provided critical insight needed to better understand how T cells responding to T-ALL diverge between distinct types of tissues.

**Conclusions** The results from these studies collectively suggest that T cells are activated by T-ALL and that they can be therapeutically harnessed despite relatively low mutation-rates. Future studies will continue analysis of individual organs and use these results to rationally design combinations of immunotherapies by tailoring to activate T cells in all tissue types.

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