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POSTER ABSTRACTS

641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

T Cell Dysfunction and Exhaustion in Patients with CLL: The Impact of Long Term Ibrutinib Treatment

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Introduction:
Chronic lymphocytic leukemia (CLL) is a B-cell malignancy associated with defects in the immune system and is dependent on the microenvironmental niche for survival. Ibrutinib, the first irreversible inhibitor of Bruton's tyrosine kinase targets B-cell receptor signaling to kill tumor cells. In addition, ibrutinib also appears to regulate the tumor microenvironment and T-cell immunity, but this mechanism unknown. Increased expression of inhibitory receptors, and functional defects such as reduced proliferative capacity and cytotoxicity are common in T cells from patients with CLL. The effect of ibrutinib on T-cell immunity has not been extensively studied in the setting of long term ibrutinib treatment. Therefore, we investigated the phenotype and function of CD4+ and CD8+ T-cells from patients with CLL treated with ibrutinib, treatment naïve CLL and age-matched healthy controls.
Methods:
CLL patient samples were obtained from treatment naïve patients at diagnosis, and at the 3- and 5-year timepoints. Samples from patients on continuous ibrutinib who had not relapsed at the 3- and 5-year timepoints; and pre-ibrutinib were obtained as well. Age matched healthy donor-derived peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Ficoll. PBMCs from CLL samples and healthy donors were labeled with CellTrace Violet and treated with anti-CD3 and anti-CD28 (ThermoFisher) for 7 days. T-cells were stimulated to identify proliferation capacity and to identify immune profiles.
Results:
Efficient T-cell activation and proliferation requires multiple signals such as T-cell receptor (TCR) stimulation, a costimulatory signal, and cytokine production. We analyzed the proliferation of T cells in-vitro upon stimulation with anti-CD3/CD28 beads using CellTrace Violet on PBMCs and found that this stimulation resulted in increased expansion of CD4+ T cells and CD8+ T cells in all the samples. We also found significant difference in the proliferation between untreated and treated CLL samples. Interestingly, proliferating T cells had different expression pattern of the exhaustion markers and inhibitory molecules such as TIM3, TIGIT, LAG3, CD160, KLRG1, CD244 and PD-1 at different phases of the disease. Increased expression of CD160, CD244, and PD1 are the key features of T-cell exhaustion. We found significant increase in the percentage of T cells from CLL patients expressing CD244, CD160 and PD1 compared to healthy donor controls but not between naïve and ibrutinib treated samples. Long term ibrutinib treatment in 3yr and 5yr samples does not change the exhaustion state in patients. We also found T-cells from ibrutinib treated samples express higher levels of CD57 which is known as a marker of senescence (Figure 1A).
The phenotypes of untreated CLL samples are CCR7+CD45RA- central memory T-cells (35%) and CCR7+CD45RA+ naïve T cells (25%) ($p<0.05$) after stimulation in-vitro on day 3. In the same setting, ibrutinib treated samples had higher levels of effector memory T-cells ($p<0.05$) (80%) compared to untreated CLL samples (Figure 1B). These results suggest that the phenotype has changed to central memory phenotype in untreated samples. Functional T-cell exhaustion in CLL also leads to failure in cytokine production. Therefore, we measured different cytokine levels after stimulation in all the patient samples and

in healthy controls. We found significant increase in the levels of inflammatory cytokines such as IFN-γ ($p<0.05$) and TNF-α ($p<0.05$) in long term ibrutinib treated samples compared to naïve CLL samples and healthy controls.

Conclusion:

Several studies have showed that T-cell counts are increased after treatment with ibrutinib in patients with CLL but not in extended therapy. Our findings suggest that long term ibrutinib treatment does increase T-cell number and proliferative capacity in CLL. The proliferating T cells express higher levels of exhaustion markers and inhibitory molecules in-vitro making them dysfunctional. However, these T-cells still maintained good proliferative potential, on par to healthy donors and better than untreated patients which can be applied in expanding improved T cell grafts for various types of adoptive T cell therapy, such as TCR gene therapy, and CAR therapy. Also better understanding of these subtypes may lead to new strategies and to improve antitumor function of these cells.

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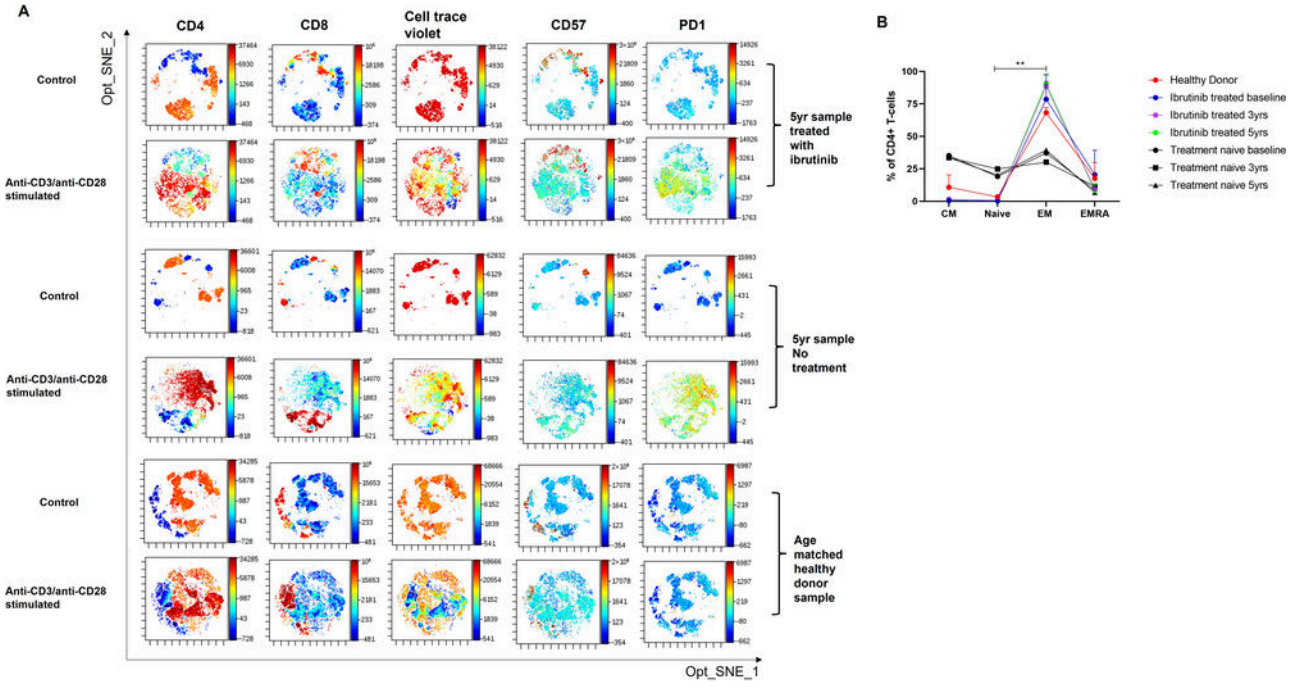

Figure 1: Profiling of CD4 and CD8 T cells in patients with CLL compared to healthy donors. (A) Data analyzed using Cytobank shows the different expression pattern on CD4+ and CD8+ T cells. (B) Treatment naïve CLL samples has higher number of central memory and naïve T cells compared to treated CLL samples and healthy donor.

Figure 1

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