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ORAL ABSTRACTS

622.LYMPHOMAS: TRANSLATIONAL-NON-GENETIC

Dissecting the Single Cell Landscape of Leukemic Mantle Cell Lymphoma

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Introduction:
Resistance to therapy remains a major challenge for Mantle Cell Lymphoma (MCL) patients, despite a diverse and expanding treatment landscape. There is thus a major need to better understand the mechanistic underpinnings of therapy resistance in order to optimize treatment selection. Given the advent of immunotherapies in recent years, there has been increasing evidence that not only tumor-intrinsic factors affect therapy resistance, but that tumor-immune cell interactions are an additional major contributor. In the context of MCL, such interactions have been poorly characterized, but hold the promise of identifying novel treatment approaches and better understanding resistance mechanisms to commonly applied therapies such as BTK inhibition.

Methods: This study aimed to investigate the single-cell landscape of leukemic MCL using single-cell profiling of 26 patients and 10 healthy donors. In addition, we performed in vitro drug testing on a subset of patient samples (n=12; 6 treatments) to understand the effects of drugs on both tumor and immune cells in MCL . To comprehensively characterize the phenotypic and functional features of immune and tumor cells within a single-cell landscape, we employed high-dimensional full-spectrum cytometry. Therefore, we developed 5 high-dimensional panels covering markers to deeply interrogate T and NK cells, immune evasion and immune checkpoints, cytokines and cytotoxicity, cellular trafficking and intracellular signaling/phosphoproteome (covering >120 proteins). We combined this with single-cell RNA sequencing and a scalable and reproducible bioinformatics pipeline. To assess the disease specificity of the immune landscape, we also obtained single-cell data from other B-NHL including MZL and CLL (n=60 patients).

Results: Our data indicate profound changes in the immune landscape, in particular in the T cell compartment of MCL patients. In this regard, multi-omics factor analysis (MOFA) confirmed a highly distinct T cell landscape in MCL patients, characterized by higher effector marker expression in conventional CD4+ and CD8+ T cells in addition to higher fraction of effector and effector memory T cells compared to healthy donors (Fig. 1A and B). Moreover, our analysis revealed a pronounced expansion of highly suppressive regulatory T cells (~5% of T cells) (Fig.1B). The emergence of this effector Treg (eTreg) subset in MCL patients might limit efficient anti-tumor immunity of CD8+ and conventional CD4+ T cells. Consistent with this, we observed a high fraction of several dysfunctional CD8+ T cells subsets. Notably, we found a remodeling of this eTreg subset in patients undergoing ibrutinib treatment leading to a less pronounced suppressive phenotype and concomitantly observed an increase of proteins crucial for cytotoxic function such as Granzyme B and Interferon-γ in effector T cells.

We further sought to link tumor cell signatures with the healthy immune cell signatures in order to assess potential interactions and mechanisms of immune escape. As such, the tumor cell compartment (MCL cells) displayed high-expression of inhibitory

immune checkpoint ligands such as PVR, HLA-G, CD70 and PD-L1 and antiphagocytic proteins such as CD47 and CD24, which may contribute to the dysfunctional T cell compartment. In line with this, our analysis revealed that high expression of the immune checkpoint ligand CD70 on malignant B cells was inversely associated with an activation module in CD4+ T cells, providing a potential axis contributing to immune escape, which may be leveraged for future therapeutic use. Finally, we identified tumor and immune cell signatures associated with in vitro drug response that may not only be useful for improving treatment selection, but also offer novel biological insights into potential mechanisms of treatment resistance.

Conclusion: This study provides valuable insights into the single-cell landscape of leukemic MCL, offering crucial understanding of how tumor-immune cell interactions may contribute to disease progression and treatment resistance. The findings hold potential for therapeutic exploitation to eventually improve patient outcomes in Mantle Cell Lymphoma.

Disclosures Zenz: *Lilly:* Consultancy, Honoraria; *Beigene:* Consultancy, Honoraria; *AstraZeneca:* Consultancy, Honoraria; *Incyte:* Consultancy, Honoraria; *Abbvie:* Consultancy, Honoraria; *Janssen:* Consultancy, Honoraria; *Gilead:* Consultancy, Honoraria; *Takeda:* Consultancy, Honoraria; *Roche:* Consultancy, Honoraria.
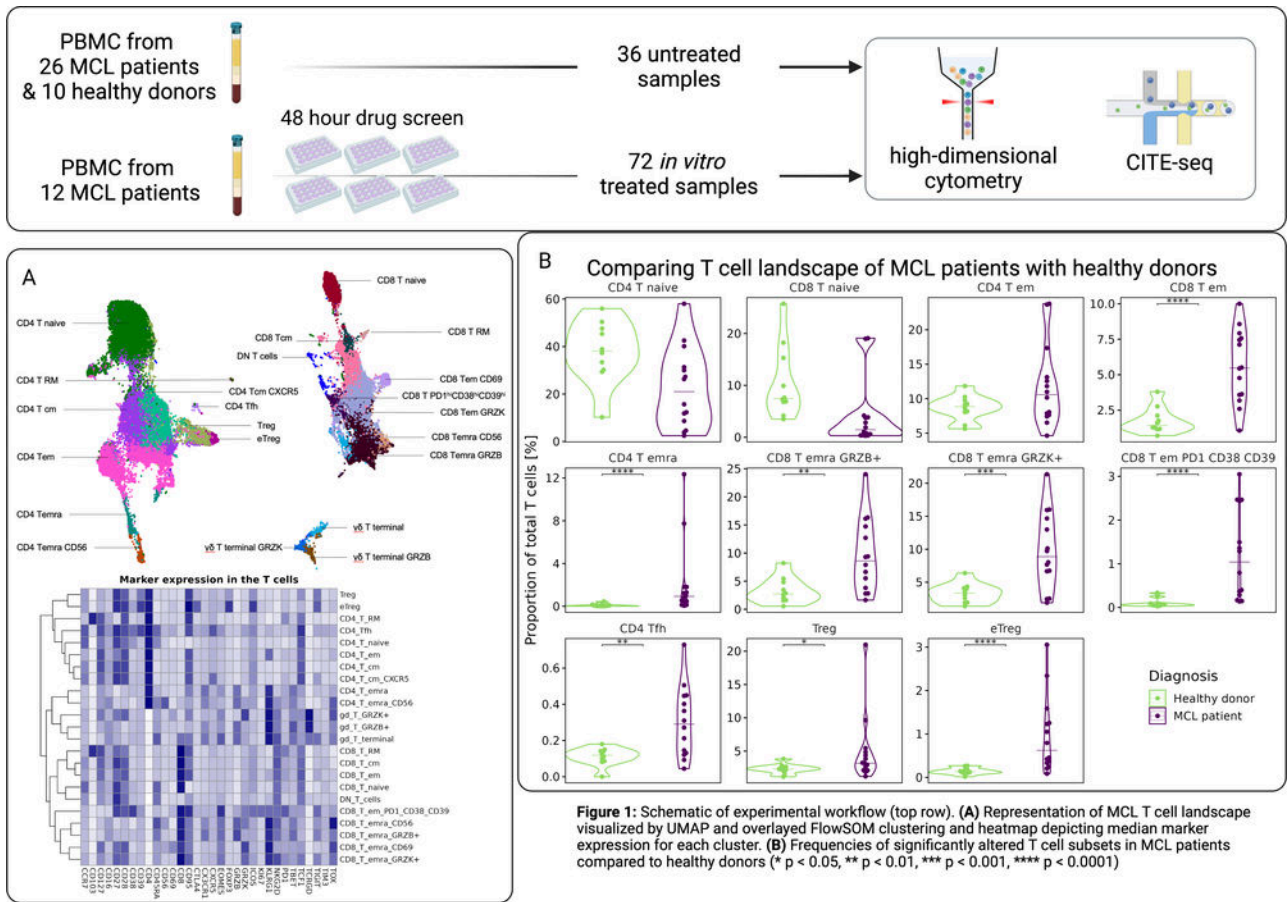

Figure 1: Schematic of experimental workflow (top row). **(A)** Representation of MCL T cell landscape visualized by UMAP and overlayed FlowSOM clustering and heatmap depicting median marker expression for each cluster. **(B)** Frequencies of significantly altered T cell subsets in MCL patients compared to healthy donors (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001)

Figure 1

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