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ORAL ABSTRACTS

723.ALLOGENEIC TRANSPLANTATION: LONG-TERM FOLLOW-UP AND DISEASE RECURRENCE

Coordinated Immune Cell Networks in the Bone Marrow Microenvironment Define the Graft Versus Leukemia Response with Adoptive Cellular Therapy

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Donor lymphocyte infusion (DLI) is an established therapy for relapsed acute myeloid leukemia (AML) after hematopoietic stem cell transplant (HSCT), but response rates are poor (˜20%). Interactions between leukemia and immune cells within the leukemia microenvironment may determine responsiveness to adoptive cellular immunotherapies. We hypothesized that systematic characterization of the leukemic marrow microenvironment over treatment course with DLI would define leukemia-immune cell interactions critical to response and hence provide mechanistic understanding of the graft-versus-leukemia (GvL) effect. In particular, we anticipated that use of multi-modal single-cell sequencing across patients and time points before and after therapy accounting for temporal dependencies would facilitate disentangling of the complex microenvironment of leukemic marrow.

To infer cell-cell interactions in a temporally resolved fashion from single cell RNA-sequencing (scRNA-seq) data, we developed DIISCO, a Bayesian model using a Gaussian process regression network. In total, we profiled 30 bone marrow aspirates (14 pre- and 16 post-DLI) from 9 patients (5 responder [R], 4 nonresponder [NR]) with post-HSCT relapsed AML treated with DLI by scRNA-seq, single cell TCR sequencing (scTCR-seq) and surface protein characterization (scCITE-seq). We obtained 51,371 high-quality transcriptomes from the marrow samples along with 43,918 cells from the DLI products of these patients (4 R, 3 NR) for a total of 95,289 cells. Cells were clustered with Phenograph resulting in 57 clusters, including 2 CD4+ T cell, 6 CD8+ T cell (C0, C5, C21, C25, C26, C40), 3 NK, 10 B cell, 3 AML leukemia, and several myelo-erythroid clusters.

DIISCO revealed a cascading response post-DLI that centered around CD8 C0, CD8 C5, AML, and a B cell cluster in R, not observed in NR. Furthermore, C0 appeared to expand after DLI only in R. We observed a strong negative interaction from CD8 C0 to AML after DLI, suggesting an anti-leukemia immune response, supporting the notion that immune cell populations formed a coordinated interactome associated with effective GvL. Evaluation of inferred interacting pairs using DIISCO and with the tool CellPhoneDB pointed toward an activation circuit in R between *CD226* on CD8 C0 and *PVR/NECTIN2* on AML. Conversely, inhibitory interactions between the exhaustion marker *TIGIT* on CD8 C0 and AML were observed in NR. Because DIISCO pinpointed CD8 C0 as a central hub for response, we characterized the identity of this cluster and its post-DLI dynamics. scTCR-seq revealed that this cluster, but not other T cell clusters, underwent marked clonal expansion after DLI (**Figure 1A)**. scRNA-seq revealed high expression of activation/effector genes (*GZMB, IFNG, CX3CR1, PRF1*) compared to other T cells. The clonally expanded cells of C0 also highly expressed *ZNF683*, a transcription factor associated with T cell cytotoxicity. scCITE-seq demonstrated expression of CD45RA and CD57 but not CD28, consistent with an effector memory re-expressing RA (TEMRA) phenotype.

We validated post-DLI expansion of CD8 TEMRA cells (CD3+, CD8+, CD62L-, CD45RA+) in AML R by multi-parameter flow cytometry in an independent cohort of 53 AML patients (29 R, 24 NR) with post-HSCT relapse treated with DLI at our institution (fold change 2.56 pre to post for R, p<0.001; 1.3 pre to post for NR, p=0.474). Moreover, to examine whether CD8+ TEMRA cells were specifically activated after encounter with leukemia, we devised an *in vitro*modified mixed lymphocyte reaction. For 6 AML R, post-DLI donor-derived CD8+ TEMRA cells that were cultured with pre-DLI recipient leukemia cells consistently demonstrated greater CD137 (p=0.004) and IFN-γ secretion compared to co-culture of the leukemia cells with other T cell subsets (p=0.015) (**Figure 1B**). CD8+ TEMRA cells cultured in the absence of leukemia cells did not show elevated expression of either CD137 or IFN-γ, indicating antigen-specific functional activation of CD8+ TEMRAs but not other cell types after encounter with autologous leukemia.

Our results reveal clonal expansion and tumor-specific activation of CD8+ TEMRA cells, supporting an antigen specific functional role of these cells in mediating GvL in AML DLI responders. Further evaluation of this CD8 TEMRA population with the ability to recognize leukemia cells may translate to strategies to optimize adoptive cellular therapy or predict responsiveness to DLI.

Disclosures Livak: *MBQ Pharma Inc:* Consultancy; *Standard BioTools Inc:* Current equity holder in publicly-traded company. **Ritz:** *Equillium:* Research Funding; *Kite/Gilead:* Research Funding; *LifeVault Bio:* Consultancy, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees; *TScan Therapeutics:* Consultancy, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees; *Oncternal:* Research Funding; *Novartis:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Smart Immune:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Garuda Therapeutics:* Consultancy, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees; *Clade Therapeutics:* Consultancy, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees; *Avrobio:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Akron Biotech:* Consultancy, Membership on an entity's Board of Directors or advisory committees. **Soiffer:** *Bluesphere Bio:* Consultancy; *Juno Therapeutics/ BMS/Celgene USA:* Other: Data Safety Monitoring Board; *Neovii:* Consultancy; *Astellas:* Consultancy; *NMPD - Be the Match, USA:* Membership on an entity's Board of Directors or advisory committees; *Smart Immune:* Consultancy; *Vor Bipharma:* Consultancy; *Jasper:* Consultancy. **Wu:** *BioNTech Inc:* Current equity holder in publicly-traded company; *Pharmacyclics:* Research Funding.

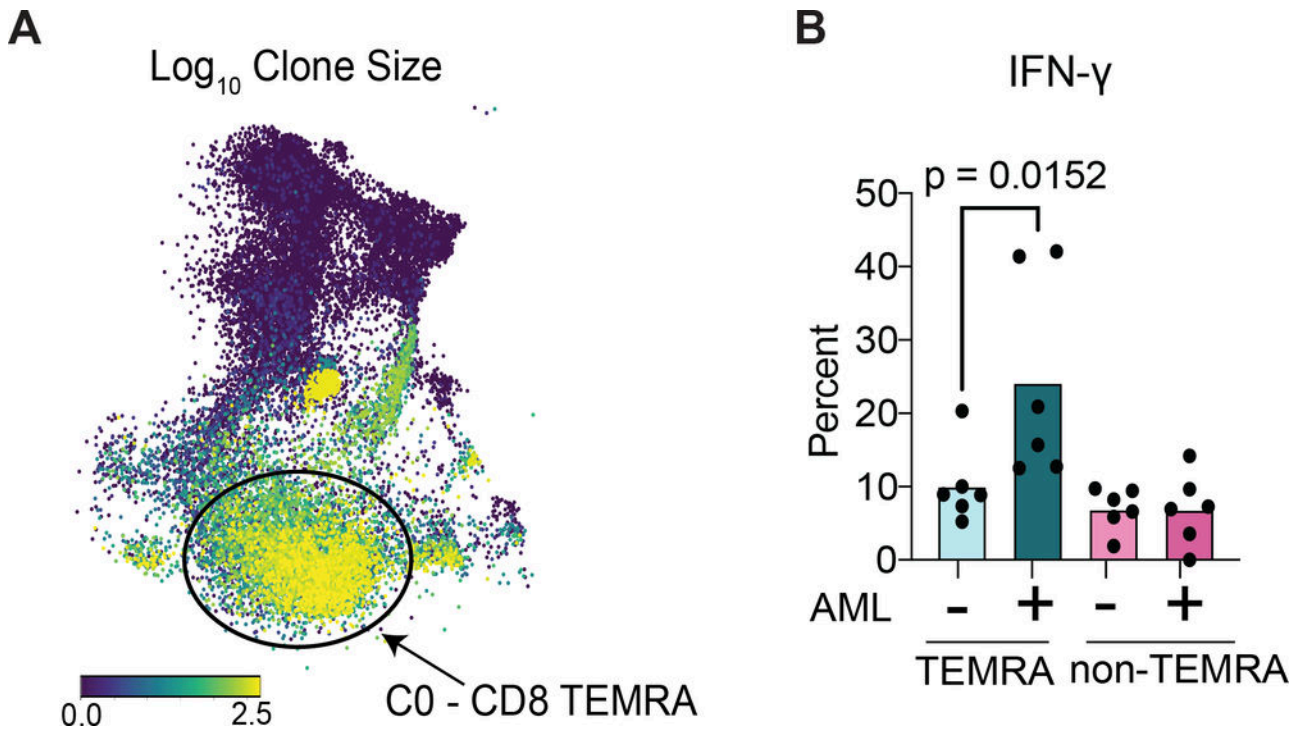

Figure 1

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