The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Precision Lineage Deconvolution in Mixed Phenotype Acute Leukemia Using Cite-Seq Derived Hematopoietic Stages Identifies Lineage Dynamics Associated with Treatment Response

Deepika Dilip, MPH[1], Pallavi Galera, MBBS[2], David Nemirovsky, MS[3], Morgan Lallo, BS[4], Kamal Menghrajani, MD[5], Andriy Derkach, PhD[3], Ross L Levine, MD[6,7], Richard Koche, PhD[4], Wenbin Xiao, MD PhD[8], Jacob Glass, MDPhD[9]

[1] New York Medical College, Valhalla, NY

[2] Department of Pathology and Laboratory Medicine, Hematopathology Service, Memorial Sloan Kettering Cancer Center, New York

[3] Department of Biostatistics and Epidemiology, Memorial Sloan Kettering Cancer Center, New York, NY

[4] Center for Epigenetics Research, Memorial Sloan Kettering Cancer Center, New York, NY

[5] MSKCC Memorial Sloan Kettering Cancer Center, New York, NY

[6] Leukemia Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY

[7] Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY

[8] Department of Pathology and Laboratory Medicine, Hematopathology Service, Memorial Sloan Kettering Cancer Center, New York, NY

[9] Leukemia Service, Department of Medicine, Center for Epigenetics Research, Memorial Sloan Kettering Cancer Center, New York, NY

Introduction:
Mixed phenotype Acute Leukemia (MPAL) is challenging due to ambiguous biology and lack of formal therapeutic guidelines. Although MPAL itself is rare, mixed phenotype lineage expression in secondary AML (sAML-MP) occurs with greater frequency and is associated with poor clinical outcomes. Multimodal single cell sequencing with cellular indexing of transcriptomes and epitopes (CITE-seq) offers more precise lineage characterization and assessment of lineage dynamics. Here we demonstrate a novel, unbiased approach to quantitative multistage hematopoietic lineage assessment in MPAL and sAML-MP using CITE-seq data. In addition, we demonstrate that this approach can be translated from single cell to bulk analysis.

Methods:
Sample preparation: Four MPAL and 6 sAML-MP samples displaying T-cell and myeloid subsets were identified and flow sorted into T and myeloid subgroups. RNA-seq was performed on each subset and analyzed using DESeq2. ComBat was used for batch correction when integrating with disparate data sources.

Single cell lineage deconvolution: CITE-seq samples were processed using Seurat. A single cell deconvolution library was constructed using previously published hematopoietic stage clustering and labeling, and applied to CITE-seq data from five MPAL samples in the same dataset. Deconvolution algorithm parameters were optimized through a bootstrapping approach on *in-silico* sample mixtures.

Bulk and translated lineage deconvolution: Bulk lineage deconvolution was performed using a published library of 13 stages of hematopoiesis derived from healthy donors.This was applied to bulk RNA-seq data from a cohort of published MPAL samples as well as the 6 sAML-MP and 4 MPAL samples described above. A pseudobulk was created for each CITE-seq lineage stage. Bulk lineage deconvolution was then re-run on the MPAL and sAML-MP samples using this library.

Clinical Outcomes: A Wilcoxon signed-rank test was used to analyze differences among deconvolution-derived patient clusters, including response to induction therapy, specific gene mutations, and likelihood of transplant.

Results:
Lineage evolution with treatment: Deconvolution was applied on 17,848 MPAL cells and 35,038 control PBMC/BMMC cells. Antibody Derived Tag (ADT) features were consistent with lineage deconvolution stage assignments. CD38 was negatively correlated with HSC (-0.34, $p < 0.001$) and LMPP (-0.18, $p < 0.001$) while CD34 was positively correlated with both HSC (0.11, $p < 0.001$) and LMPP (0.27, $p < 0.001$) stages. MPAL 5 in particular was assessed at diagnosis and at two later time points. Unsupervised hierarchical clustering of all timepoints resulted in 10 clusters (**Figure A**). The diagnostic time point was largely

contained in clusters 4 (LMPP character, N = 1545 cells, 37.13%) and 9 (HSC character, N = 756 cells, 18.16%). The majority of both relapse timepoints were within cluster 4, with 76.77% (N=357 cells) of T1 and 73.92% (N=1100 cells) of T2 located in it.

Bulk Unsupervised Analysis: Unsupervised analysis of sorted MPAL (N = 8), sorted AML-MP (N = 12), and unsorted MPAL (N = 24) bulk samples resulted in six distinct clusters, each with a distinct lineage signature (**Figure B).** Some were enriched for more differentiated stages such as monocyte or GMP (clusters 6, 5), while others were enriched earlier stages such as LMPP (cluster 2). Secondary AML-MP samples were notably enriched in cluster 4. We found significant associations between lineage clusters and mutations in RUNX1 (p < 0.01), FLT3 (p = 0.004).

Clinical Outcomes: Bulk RNA deconvolution cluster was significantly associated with complete remission (p = 0.0009). On average, individuals with an incomplete/no response to induction chemotherapy had a higher NK signature (p = 0.001), while individuals assigned to transplant had decreased LMPP character (p = 0.0495). Among the single-cell stages, decreased CLP 2 levels were associated with a poor response (p = 0.0224) while CMP / LMPP (p = 0.040) and CD4 N2 (p = 0.022) levels were decreased in patients who were able to undergo HSCT.

Conclusions: Precise identification of lineage signatures in mixed phenotype leukemias shows promise in identifying clinically meaningful biological subsets of these diseases. Prospective analysis of lineage-derived biomarkers should be performed to undertake identification of formal risk stratification and treatment schemas.

Disclosures Menghrajani: *Gilead:* Consultancy. **Levine:** *AstraZeneca:* Consultancy, Honoraria; *Janssen:* Consultancy; *Qiagen:* Membership on an entity's Board of Directors or advisory committees; *Incyte:* Consultancy; *Isoplexis:* Membership on an entity's Board of Directors or advisory committees; *C4 Therapeutics:* Membership on an entity's Board of Directors or advisory committees; *Prelude:* Membership on an entity's Board of Directors or advisory committees; *Auron:* Membership on an entity's Board of Directors or advisory committees; *Zentalis:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *Mission Bio:* Membership on an entity's Board of Directors or advisory committees; *Ajax:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *Novartis:* Consultancy; *Roche:* Honoraria; *Lilly:* Honoraria; *Amgen:* Honoraria.

A MPAL Lineage Analysis at Diagnosis and Two Relapse Timepoints
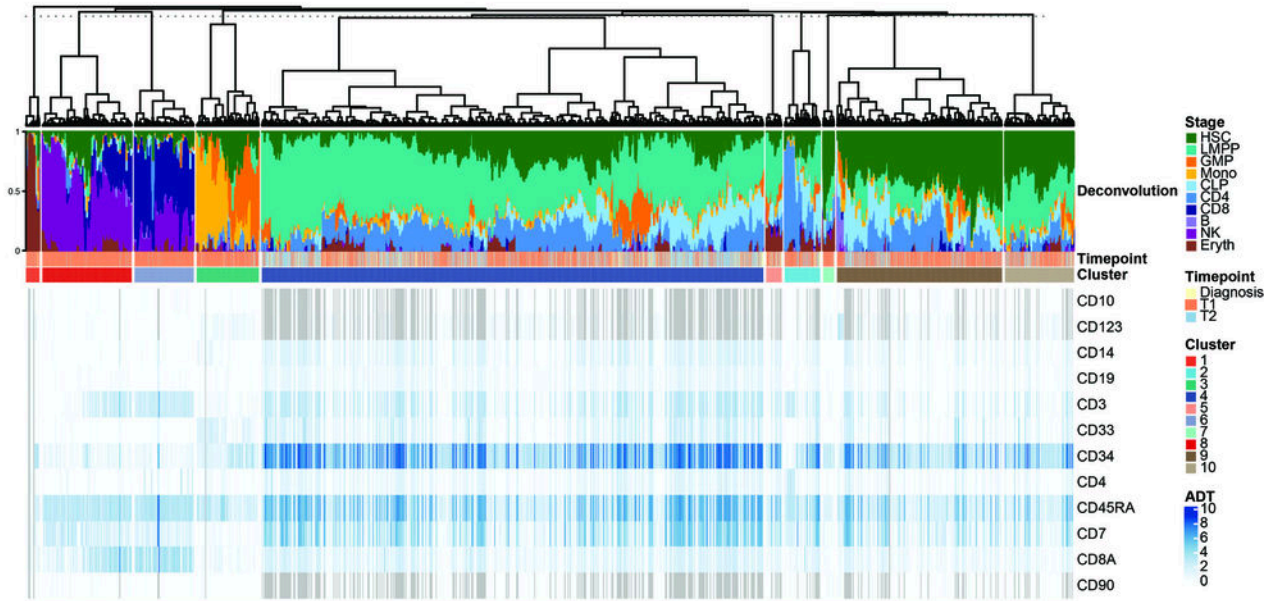
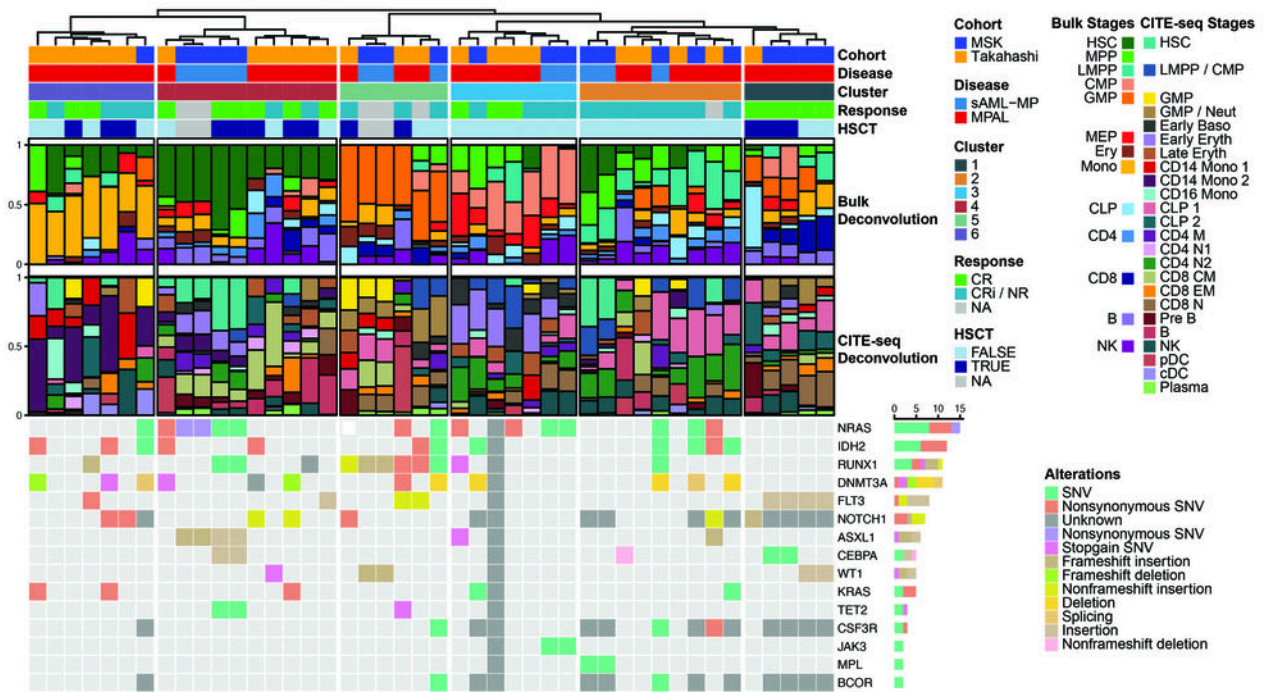## B Bulk and CITE-seq Lineage Deconvolution of MPAL and sAML-MP

Figure 1