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POSTER ABSTRACTS

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

Deconvoluting Clonal and Cellular Architecture in *IDH*-Mutant Acute Myeloid Leukemia

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Introduction
Mutations in Isocitrate dehydrogenase 1/2 (*IDH*) occur in 20% of acute myeloid leukemias (AML) and frequently co-occur with *DNMT3A*, *NPM1*, *SRSF2*, and *NRAS*. In clonal hematopoiesis (CH), *IDH* mutations confer high risk of AML transformation. Recently developed methods linking single cell genotype to single cell transcriptomic profiles and cell lineage offer the opportunity to define the transcriptomic consequences of each mutation across leukemic cell lineages and clonal phylogenies. Here, we develop an integrative single cell laboratory and analytical framework to study the genetic, clonal and cellular heterogeneity of *IDH* mutated CH and AML, across disease stages (diagnosis and relapse) and deliver a high-resolution map of *IDH*-mutant AML.

Methods
Bone marrow or peripheral blood mononuclear samples from 15 AML patients, 2 CH individuals, and 12 unaffected age-matched donors were profiled by single cell RNA-sequencing. For the AML cases, a median of 3 samples per patient were profiled under treatment with IDH inhibitor (IDHi) monotherapy (n=5 patients), IDHi in combination with intensive chemotherapy (NCT02632708, n=6) or in combination with hypomethylating agent (n=2). This dataset comprised 259,610 cells across AML (n=190,133), CH (n=16,705) and normal controls (n=52,772). Genotyping of Transcriptomes (Nam et al., 2019) (for *IDH1/2*, *NPM1*, *SRSF2*, and *NRAS* mutations) across 6 AML patients enabled genotyping 23% of cells at diagnosis for at least one clone-defining mutation.

Results
Through meta-analysis of 3,653 diagnostic AML samples profiled by a targeted DNA panel (Papaemmanuil et al., 2016; Tazi et al., 2022), we identified *DNMT3A*, *SRSF2* and *NPM1* as critical co-operative mutations in *IDH*-mutant AML. Amongst the 15 AML patients, 6 had *IDH1* mutation, 8 had *IDH2* mutations, and 1 had both *IDH1* and *IDH2*. The co-occurring mutations in *DNMT3A* (n=11 patients), *NPM1* (n=5), *SRSF2* (n=5) reflected the genomic landscape of *IDH*-mutant AML.
At diagnosis, *IDH*-mutant AML was enriched in immature hematopoietic stem and progenitor-like blasts. Compared to normal hematopoiesis, *IDH*-mutant AML blasts upregulated inflammatory response markers including TNFα/NFκB-signaling and downregulated proliferation-associated pathways including G2M Checkpoint, and MYC and E2F Targets, and DNA damage response.
To investigate how co-mutations modify the *IDH*-mutant phenotype, we established an integrative single-cell profiling approach to assign single cells to distinct phylogenetic subclones and extract clone-specific transcriptional programs. *SRSF2* and *NPM1* co-mutations modulated the AML blast phenotype, towards erythroid (*SRSF2*) or granulomonocytic lineage (*NPM1*). *NRAS*- or *SRSF2*-mutant subclones defined distinct gene expression-derived clusters and upregulated proliferation and metabolic pathways. Furthermore, *NPM1* co-mutated blasts upregulated KMT2A-target genes.
Next, we tracked clonal responses to combination IDHi and chemotherapy using GoT and targeted DNA sequencing. In 4 cases, the ancestral clone, defined by *IDH* and *DNMT3A* mutations, retained clonal dominance in both mature and immature myeloid compartments at complete response (median 4 weeks). Conversely, subclones defined by mutations in *NPM1*, *NRAS*, or *SRSF2*, were either reduced or not detectable (limit of detection 2%). In two cases, mutations were not detected after therapy.
In patients who relapsed after IDHi monotherapy treatment, blasts upregulated stemness, fatty acid metabolism, OxPHOS, and inflammation at relapse compared to diagnosis. Furthermore, MHC Class II was downregulated at relapse, pointing to immune evasion of AML as a potential relapse mechanism.
Finally, we investigated the role of *IDH2* mutations at CH. *IDH*-mutant CH-derived monocytes upregulated inflammation-associated pathways, mirroring the phenotypes observed in *IDH*-mutant AML at diagnosis.

Conclusion
We defined how *IDH* mutations and their frequent co-mutations dysregulated hematopoiesis. We leveraged state-of-the-art single cell techniques to assign leukemic cells to phylogenetic subclones and delineated the role of serially acquired co-mutations in shaping lineage bias and transcriptional programs. Furthermore, we defined genetic, clonal and transcriptional changes at three stages of the AML disease process: diagnosis, remission, and relapse under IDH inhibitor therapy.

Disclosures Cai: *Imago Biosciences:* Consultancy, Current equity holder in private company. **Levine:** *Isabl Inc:* Current Employment. **Medina Martinez:** *Isabl Inc.:* Current Employment, Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees. **Menghrajani:** *Gilead:* Consultancy. **Mims:** *Jazz Pharmaceuticals:* Membership on an entity's Board of Directors or advisory committees. **Patel:** *Isabl Inc:* Current Employment. **Loghavi:** *Gerson Lehrman Group:* Consultancy; *Abbvie:* Consultancy; *Blueprint Medicine:* Consultancy; *Caris Diagnostics:* Consultancy; *Amgen:* Research Funding; *Astellas:* Research Funding; *QualWorld:* Consultancy; *Guidepoint:* Consultancy; *Daiichi Sankyo:* Consultancy; *Recordati/ EUSA Pharma:* Consultancy; *Abbvie:* Current equity holder in publicly-traded company. **Bolton:** *Servier:* Research Funding; *GoodCell:* Membership on an entity's Board of Directors or advisory committees. **Viny:** *Arima Genomics:* Membership on an entity's Board of Directors or advisory committees. **Roshal:** *Celgene:* Other: Provision of services; *Auron Therapeutics:* Other: Ownership/Equity interests; Provision of services; *Physicians' Education Resource:* Other: Provision of services; *Roche:* Other: Funding; *Beat AML:* Other: Funding; *NGM:* Other: Funding. **Levine:** *Prelude:* Membership on an en-

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