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POSTER ABSTRACTS

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

Tracking of Leukemia and Immune Single Cell Phenotypes during Ipilimumab-Based Treatment By Long-Read Sequencing

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Activity of ipilimumab in bone marrow-involved AML has been modest compared to extramedullary AML relapse or solid tumors like melanoma. Single cell transcriptomics (scRNA-seq) could provide deeper understanding of these differences, yet leukemia and immune cell phenotypes defined by somatic mutations or differential isoform expression often cannot be resolved with native short-read scRNA-seq data due to insufficient read coverage. To overcome this limitation, we devised a workflow (*nanoranger*) that leverages improved accuracy of long-read sequencing (Oxford Nanopore, ONT) and enables targeted read-out of molecular features including single-nucleotide variants, gene fusions, isoforms, or T cell receptor (TCR) sequences from intermediate scRNA-seq cDNA libraries.

To determine the performance of *nanoranger*, we undertook a series of assessments. First, we established its genotyping accuracy based on the ability to recover highly polymorphic TCR sequences. TCRs obtained with Illumina versus ONT from the same VD(J) amplicon library were highly concordant (r>0.8) in the number of reads and cells per CDR3 (5,653 [ONT] vs. 5,767 [Illumina]). Second, we tested isoform profiling by targeted sequencing of *PTPRC* which encodes the T cell differentiation markers *CD45RA* (exon 4 present) and *CD45RO* (exon 4 absent). Not only did targeted ONT sequencing increase the single cell coverage of *PTPRC* from 10 to >2,600 cells, but *PTPRC* exon 4 expression also correlated with CD45RA surface marker expression by CITE-seq (r=0.45). Third, we benchmarked somatic mutation detection of a homozygous *TP53* ^{R248G}mutation in the AML cell line Kasumi-1. Our ONT-based approach increased genotyped cells from 0% (via native scRNA-seq) to 22% (*nanoranger*); in >98.8% of these cells, *TP53* ^{R248G} was detectable. Finally, we searched for optimal *nanoranger* targets by assessing whole-transcriptome coverage of 5′ single cell cDNA and found these to be loci at the 5′ end of highly expressed transcripts shorter than 4 kB. Altogether, *nanoranger* reliably recovers molecular features from scRNA-seq libraries with genotyping efficiency dictated by inherent characteristics of the underlying cDNA.

To better understand determinants of response and resistance of CTA-4 blockade in AML, we applied *nanoranger* to bone marrow samples collected in the context of a phase I trial investigating combined decitabine and ipilimumab (NCT02890329). We genotyped 11 genes recurrently mutated in AML across 15,258 single cell profiles from 13 samples (5 responders at screening and best response, 3 non-responders at screening). At screening, somatic mutations were detectable in myeloid progenitor, monocytic and dendritic cells but also in erythroid and megakaryocytic progenitor populations, consistent with detected copy number changes. Further, we clarified AML subclones with integrated analysis of somatic mutations and chromosomal aberrations. In AML1019, we found two separate clones defined by presence or absence of *RUNX1* ^{R320*} and *amp(8p)* or *amp(21p)*. In AML8007, we tracked differential therapeutic responses. At screening, 3 somatic mutations (*DNMT3A*

^{V296M}, *TP53* ^{C176S}, *TP53* ^{R282W}) and 3 chromosomal aberrations (*amp(1p)*, *del(3p)* and *del(5q)*) were detected. At remission, somatic mutations and *del(5q)* persisted, but *amp(1q)* and *del(3p)* became undetectable except in LMPP-like cells. In all 5 responders, somatic mutations remained detectable in all cellular compartments, revealing that the cytoreductive effect of decitabine/ipilimumab was not accompanied by leukemic clone eradication.

The ability of *nanoranger* to perform isoform profiling enabled us to resolve immune phenotypes. Following CTLA-4 blockade, the *PTPRC* isoform *CD45RA* was downregulated in CD4 $^{+}$ and CD8 $^{+}$ T cells, consistent with induction of T cell differentiation by ipilimumab. As *CTLA-4* exists in two isoforms, we tracked alternative splicing of exon 3 and observed lower expression of the membranous isoform in T cells from AML bone marrow compared to circulating or tumor-infiltrating lymphocytes in melanoma, pointing to context-specific isoform expression of *CTLA-4*.

In sum, genotyping with *nanoranger* substantially augmented scRNA-seq analyses of ipilimumab-based therapy in bone marrow AML. This exemplifies the great potential that long-read sequencing-based single cell studies hold to better define response and resistance mechanisms of novel immunotherapies.

Disclosures Neuberg: *Madrigal Pharmaceuticals:* Current equity holder in private company. **Ritz:** *TScan Therapeutics:* Consultancy, Current holder of *stock options* in a privately-held company, 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; *LifeVault Bio:* Consultancy, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees; *Equillium:* 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; *Clade Therapeutics:* Consultancy, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees; *Kite/Gilead:* Research Funding; *Oncternal:* Research Funding; *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:** *Jasper:* Consultancy; *Juno Therapeutics/ BMS/Celgene USA:* Other: Data Safety Monitoring Board; *NMPD - Be the Match, USA:* Membership on an entity's Board of Directors or advisory committees; *Astellas:* Consultancy; *Vor Bipharma:* Consultancy; *Neovii:* Consultancy; *Smart Immune:* Consultancy; *Bluesphere Bio:* Consultancy. **Garcia:** *Prelude:* Research Funding; *Astellas:* Consultancy; *AbbVie:* Consultancy, Research Funding; *Bristol Myers Squibb:* Consultancy; *Pfizer:* Research Funding; *AstraZeneca:* Research Funding; *Gilead:* Consultancy; *New Wave:* Research Funding; *Servier:* Consultancy; *Genentech:* Consultancy, Research Funding. **Livak:** *MBQ Pharma Inc:* Consultancy; *Standard BioTools Inc:* Current equity holder in publicly-traded company. **Wu:** *BioNTech Inc:* Current equity holder in publicly-traded company; *Pharmacyclics:* Research Funding.

OffLabel Disclosure: Combination of ipilimumab and decitabine for immunomodulation of post-transplant or transplant-naive AML/MDS relapse.

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