The incidence of acute myeloid leukemia (AML) is 7 cases per million children younger than 15 years and 4.1 per 100,000 men and women per year (1.2% of all new cancer cases).

Heterogeneity in genetics (>700 translocations identified), clinical manifestations and outcome has been described. Currently, prognosis is determined by cytogenetic and molecular abnormalities, as well as by response to induction chemotherapy.

The general therapeutic strategy in patients with AML has not changed substantially in more than 30 years. Overall, de novo AML is cured in ~70% of children, 35-40% of adults (>60 years of age) and in only 5-15% of elderly patients (>60 years of age). Some patients with AML fail to achieve complete remission after induction chemotherapy. Another subset of patients will eventually relapse despite the lack of adverse risk factors.

There is an urgent need to discover better biomarkers to identify high-risk patients before administering chemotherapy and to enable testing of investigational treatments in clinical trials (Tasian SK, et al.).

AML is susceptible to immune system attack, as suggested by the therapeutic efficacy of allogeneic hematopoietic stem cell transplantation. Importantly, AML has a unique ability to promote the generation of an immune-suppressive tumor microenvironment (TME) through the expansion of regulatory T cells, the activation of tryptophan catabolism and the inhibition of immune effector cell functions (Foligoi V, et al.; Munir DH, et al.).

A comprehensive characterization of the immune landscape of AML has not been attempted before (Chen DS, et al.) and has the potential to inform the development of immunotherapeutic clinical trials that are tailored to specific sub-groups of TMEs.

**Objectives**

A. To dissect the immunological ecosystem of the AML bone marrow (BM) microenvironment in a transcriptoric approach.

B. To correlate immune gene signatures with patient outcome.

**Methodology**

**Messenger RNA (mRNA) was purified from bulk mononuclear cells from BM aspirates of AML patients treated with curative intent. 150-200 ng of RNA per sample was incubated with reporter and capture probe mix for Hybridization (Pan-Cancer Immuno Profiling Panel).** This panel has 770 genes from 24 different immune cell types, including genes that are involved in both the adaptive and innate immune responses. The gene expression profiles for all the samples were subsequently analyzed to look at the differences between children and adults, followed by AML samples with regards to disease stage, cytogenetic risk factors, cell types. The cell types were further analyzed to calculate the Tumor Inflammation Signature (TIS)**.

**TIS is a biomarker that measures a suppressed adaptive immune response within the tumor developed for use on the nCounter™ platform.** The signature is a weighted linear algorithm that measures 16 genes associated with presence of antigen presenting cells, TNF, natural killer cell, interferon activity, and T-cell exhaustion markers and normalizes their expression to 16 housekeeper genes. TIS has been developed as a pan-cancer predictor for clinical response to PD-1 checkpoint blockade (Ayash L, et al. Cancer Sci. 2017; 127: 2030-40). Purified target/probe complexes were immobilized on the NanoString cartridge for data collection. Transcript counts were determined using the nCounter® Digital Analyzer at the high-resolution setting (Fig. 1). Digital images were processed with final barcode counts tabulated in reporter code output (RCO) files, which were analyzed using the nSolver® software package (version 5.0) for quality control and data analysis purposes.

The captured transcript counts were normalized to the geometric mean of the reference genes included in the assay and the code set's internal positive controls.

**Figure 1: nCounter® digital profiling of RNA species.**

**Conclusions and Translational Potential**

- **Gene expression profiles are different in children with AML in comparison to adult patients.**

- **Myeloid-derived or 'infamed' gene expression profiles discriminate between shorter and longer RFS and OS.**

- **Defective anti-leukemia immune responses as a result of Adaptive (Compressive) Immune Resistance (AIR).**

- **CD8 T-cell exhaustion, induction of IDO1, PD-L1, and other negative checkpoints by microenvironmental IFNγ.**

- **Inflammation of AML is marked by an immune exclusion phenotype (IDO1 small molecule inhibitors and/or checkpoint blockade strategies).**

**Figure 2: Heat-map (unsupervised hierarchical clustering) summarizing immune gene expression profiles in the TME of patients with AML. High-level overview of immune gene expression (Pan-Cancer Immuno Profiling Panel) in patients with AML. Patient age group (children vs. adults), disease status at time of sampling (complete remission [CR]), disease relapse, new-onset AML and cytogenetic risk group (favorable, intermediate and unfavorable) were selected as covariates.**

**Figure 3: Heat-map (unsupervised hierarchical clustering) summarizing micro-RNA expression profiles in the TME of patients with AML. High-level overview of micro-RNA expression (Human v3 Micro-RNA Expression Panel) in patients with AML. Patient age group (children vs. adults), disease status at time of sampling (complete remission [CR]), disease relapse, new-onset AML and cytogenetic risk group (favorable, intermediate and unfavorable) were selected as covariates.**

**Figure 4: Heat-map (unsupervised hierarchical clustering) summarizing immune cell type-specific scores in the TME of patients with AML. Red denotes up-regulated mRNA levels, blue indicates down-regulated mRNA levels. Patient age group, cytogenetic risk group, relapse-free survival (RFS) and overall survival (OS) were selected as covariates.**

**Figure 5: Heat-map (unsupervised hierarchical clustering) summarizing signature scores in the TME of patients with AML. Housekeeping normalization was performed on the raw gene count data and log transformed. Unsupervised hierarchical clustering was performed on the housekeeping normalized data and the signature scores. Signature scores were calculated as pre-defined linear combinations (weighted averages) of biologically relevant gene sets. Cell type abundance signatures were defined in Dananer P, et al. Other signatures are unpublished. Signatures are interpreted on the log, scale a unit increase in a signature’s score corresponds to a doubling in the biological activity 5 measures. Patient age group (children vs. adults), disease status at time of sampling (complete remission [CR]), disease relapse, new-onset AML and cytogenetic risk group (favorable, intermediate and unfavorable) were selected as covariates.**

**Figure 6: Differential gene expression and analysis.** For each gene, a linear model was fit comparing its log, transformed, normalized expression between the adult and children. This volcano plot displays each gene’s -log (p-value) and log fold change for adults compared to children. Highly statistically significant genes fall at the top of the plot and highly differentially expressed genes fall to either side. Gradient-colored points and horizontal lines indicate various adjusted p-value thresholds. The 40 most statistically significant genes are specifically labeled.

**Figure 7: MetaCore™ was used to capture additional biological systems in our dataset.** Genes that are differentially expressed (FDR-corrected p values<0.05) between adults and children with AML were used for MetaCore process enrichment analysis (GSEA, Thomson Reuter). Top 20 processes which are differentially regulated are shown. A Significant enrichment of MetaCore process generated using up-regulated genes. B. Significant enrichment of MetaCore process generated using down-regulated genes. Left y-axis represents the number of genes detected in each process and right y-axis represents -log p values of MetaCore enrichment scores after multiple testing correction (FDR).

**Figure 8: Comparison of Myeloid and tumor inflammation signature (TIS’s) scores.** TIS, a measure of adaptive anti-tumor activity focused on lymphoid compartment activities, is weakly correlated with a score of myeloid compartment immune activity. High TIS samples had near-unchanged high myeloid score, while low TIS samples saw a wide range of myeloid activity. This pattern is preserved in both adults and children.

**Figure 9: Survival analysis in our cohorts of patients with AML. Kaplan-Meier curves showing relapse-free survival (RFS) and overall survival (OS) in our cohort of children and adults with AML. Relapse was a powerful determinant of unfavorable patient outcome, as opposed to achievement of complete remission.**

**Figure 10: Correlation between immune signature score and patient outcome (RFS).** For each signature, a Cox proportional hazards model was fit estimating the log hazard ratio associated with a unit increase in the signature. Boxes and lines show point estimates and 95% confidence intervals for the log hazard ratio associated with a unit increase in score, or equivalently a doubling of the biological process in question. More precise estimates are given larger boxes. Color denotes results in adults vs. children.

**Figure 11: Immune cell type-specific scores discriminate AML patients with shorter relapse-free survival (RFS) and overall survival (OS).** Kaplan-Meier estimates of RFS and OS in children and adults with AML. Immune cell type-specific scores (see Fig. 2) allowed the stratification of patients with AML into three distinct clusters (A + myeloid-enriched; B myeloid-depleted). The Carman-Browset-Wilcoxon test was used to compare survival curves. Median RFS and OS is indicated. Patients with myeloid-enriched AML experienced shorter RFS and OS compared to patients with myeloid-depleted AMLs. This observation suggests that pre-existing immune resistance may be dysfunctional as a result of immune suppressive circuits in the bone marrow microenvironment. **Hazard Ratio**

GraphPad Prism software was used to analyze and visualize survival data.

**Key References**

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