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POSTER ABSTRACTS

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

Spatial Technologies Reveal the Immune Landscape of Pediatric Acute Myeloid Leukemia

Joost B. Koedijk, MD[1], Inge Van Der Werf[2], Marijn A. Vermeulen, MD PhD[2], Alicia Perzolli, MSc[2], Marta F. Fiocco, Prof. Dr.[3,2,4], Hester A. de Groot-Kruseman, PhD[2], Rubina Moeniralam[2], Kristina B. Christensen[5], Henrik Hasle[6], Stefan Nierkens, PhD[7,8], Mirjam E. Belderbos, MDPhD[2], Michel C. Zwaan, Prof. Dr.[9,1], Olaf Heidenreich, PhD[2,10,11]

[1] Princess Maxima Centre for Pediatric Oncology, Utrecht, Netherlands
[2] Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands
[3] Mathematical Instit, Leiden University, Leiden, Netherlands
[4] Department of Biomedical Data Sciences, Leiden University Medical Center, Leiden, Netherlands
[5] Department of Pathology, Aarhus University Hospital, Aarhus, Denmark
[6] Department of Pediatrics and Adolescent Medicine, Aarhus University Hospital, Aarhus, Denmark
[7] Princess Maxima Center for Pediatric Oncology, Utrecht, Netherlands
[8] Center for Translational Immunology, University Medical Center Utrecht, Utrecht, Netherlands
[9] Department of Pediatric Oncology, Erasmus MC/Sophia Children's Hospital, Rotterdam, Netherlands
[10] Wolfson Childhood Cancer Research Centre, Newcastle University, Newcastle upon Tyne, United Kingdom
[11] Northern Institute for Cancer Research, University of Newcastle, Newcastle upon Tyne, United Kingdom

Pediatric acute myeloid leukemia (AML) is a cancer with a particularly low mutational burden in comparison to other pediatric and adult cancers and therefore, thought to be a poor candidate for T cell-engaging immunotherapies (Gröbner et al., 2018; Pfister et al., 2022). However, little is known about the composition and function of T cells in the bone marrow (BM) of pediatric AML patients. Insight into the frequency and function of BM T cells in children with AML is relevant for naturally occurring AML immune defenses, as well as for T cell-engaging immunotherapies (Koedijk et al., 2021). Here, we performed a multidimensional characterization of the tumor immune microenvironment in children with newly diagnosed AML.

We obtained 82 formalin-fixed and paraffin-embedded (FFPE) diagnostic bone biopsies from a representative cohort of pediatric patients with treatment-naïve *de novo* AML (N=72) and from age- and sex-matched pediatric patients with treatment-naïve early-stage rhabdomyosarcoma without malignant BM infiltration (non-leukemic controls, N=10). We employed immunohistochemistry (IHC) alongside immune-related gene expression profiling (NanoString PanCancer IO 360 ™ Panel) and spatial transcriptomics (NanoString GeoMx ™ Whole Transcriptome Atlas). Moreover, we acquired an additional dataset of pre-treatment immune-related gene expression profiling obtained from the BM of 30 flotetuzumab-treated (CD3 x CD123 bispecific antibody) refractory/relapsed (R/R) adult AML patients (NCT02152956; Vadakekolathu et al., 2020) for TIDE-based immune deconvolution (Jiang et al., 2018).

Using IHC, we identified (a trend towards) a decreased abundance of the overall number of T cells (P=0.09) and CD8 $^{+}$ T cells (P=0.011; Panel A) in the BM of pediatric AML patients in comparison to non-leukemic controls, respectively. Notably, the extent of overall T cell and CD8 $^{+}$ T cell infiltration could differ up to 180-fold between individual AML patients. In general, this difference in T cell infiltration was not associated with the abundance of leukemic blasts or patient's cytogenetic alterations. Nevertheless, children with complex karyotype AML had higher numbers of BM T cells compared to children with normal karyotype AML (P=0.03).Using differential gene expression analysis, we identified genes related to T cell-attracting chemokines, cytotoxicity, and immune checkpoints to be significantly upregulated in T cell-infiltrated- compared to T cell-depleted samples. Moreover, the ratio of anti- to pro-inflammatory macrophages (M2/M1-ratio) was significantly lower in T cell-infiltrated- compared to T cell-depleted pediatric AML samples (P<0.001). Interestingly, this M2/M1-ratio was also significantly lower in R/R adults that responded to flotetuzumab immunotherapy in comparison to non-responders (P<0.001). In fact, this M2/M1-ratio outperformed several other known predictors of response to flotetuzumab immunotherapy (AUROC: 0.852 (95% CI: 0.71-0.99), P=0.001; Jiang et al., 2018; Ayers et al., 2017). In addition, IHC revealed that 9 immune-infiltrated samples, including 6 *KMT2A*-rearranged AML samples, harbored large networks of T- and B cells (representative image of B cell-networks shown in panel B). Using spatial transcriptomics, we dissected the composition of these lymphoid aggregates

and revealed localized anti-tumor immunity in the BM of AML. In comparison to tumor areas, these aggregates showed a higher abundance of activated cytotoxic T cells ($P<0.001$), memory B cells ($P<0.001$), and plasma cells ($P<0.001$), suggesting the presence of tertiary lymphoid structure-like (TLS-like) aggregates in the BM of AML.
In conclusion, we identified a subset of pediatric AML patients with relatively high T cell infiltration and a relatively low abundance of anti-inflammatory macrophages in the BM at diagnosis. Furthermore, we found that the BM M2/M1-ratio may be informative of response to T-cell engaging immunotherapies in adult AML, which needs to be validated in pediatric AML. Lastly, for the first time, we identified TLS-like aggregates in the BM of AML patients, which have been associated with immunotherapy response in many cancers (Schumacher & Thommen, 2022). Additional studies to further characterize the function and relevance of these lymphoid aggregates are ongoing.

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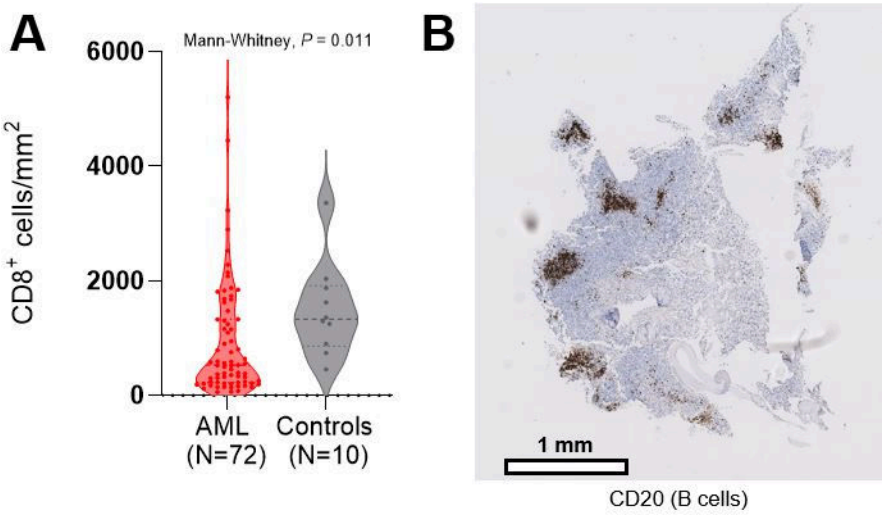

Figure 1

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