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ORAL ABSTRACTS

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

CLEC2A Is a Novel AML-Restricted Immunotherapeutic Target Enriched in KMT2A-Rearranged Acute Myeloid Leukemia

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KMT2A rearrangements (KMT2A-r) are a frequent event in childhood acute myeloid leukemia (AML) and in treatment related AML in adults leading to a highly refractory and deadly subtype of AML regardless of age. Targeted therapies for KMT2A-r AML have had limited success with novel therapies urgently needed. Despite successes in other leukemias, immunotherapy in AML is in its infancy in part due to significant overlap of antigens expressed in AML and normal hematopoietic cells, which would limit efficacy and increase hematopoietic toxicity. We interrogated the AML transcriptome compared to normal hematopoiesis to identify optimal targets that are silent in normal hematopoieisis and are highly expressed in AML. Here we describe a novel immunotherapeutic target, CLEC2A, a member of the C-type lectin family, that is highly enriched in high-risk AML, particularly KMT2A-r AML and absent in normal hematopoeisis. We evaluated the association between KMT2A fusions and CLEC2A expression identifying CLEC2A as a putative oncoprotein and the potential for CLEC2A to serve as an immunotherapeutic target in high-risk AML via antibody-mediated cytotoxicity.

Diagnostic specimens from 1,864 patients with AML (Pediatric 0-29 years from COG Phase III trials N=1486; Adult 18-88 years SWOG N=199 and TCGA-LAML N=179) were interrogated using contemporary NGS to identify genetic aberrations. Conventional karyotyping and mutational analysis were used to determine additional cytogenetic and molecular abnormalities. As CLEC2A was entirely absent in normal bone marrow (TPM 0-0.15), positive CLEC2A expression was defined as mRNA transcripts per million (TPM) >1. Although, CLEC2A was detected in only 18% of AML patients (252/1486; 17% pediatric and 90/378; 24% adult patients), it was highly enriched in those with KMT2A-r AML with 77% (N=193) of CLEC2A positive patients having KMT2A-r, accounting for 48% of the total KMT2A-r cohort, Fig 1A. CLEC2A expression was enriched in specific fusion subgroups: MLLT10 (80%), MLLT4 (71%), and MLLT3 (48%), Fig 1B. CLEC2A expression was low to absent in a majority of other patients, though was seen in other high-risk groups including non-KMT2A MLLT10 fusions, NUP98 fusions, CBFA2T3-GLIS2 fusions, and monosomy 7. CLEC2A is also expressed in a subtype of T-ALL (HOXA activated), which is a fusion driven T-ALL.

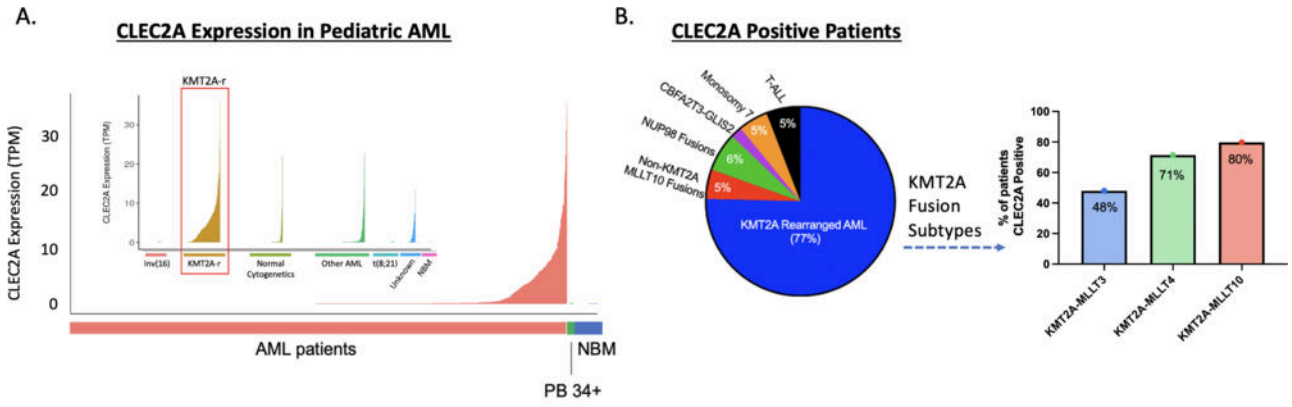
We further evaluated a potential causal link between *KMT2A* fusions and CLEC2A expression and inquired whether KMT2A fusion proteins might directly bind to the CLEC2A promoter and induce CLEC2A expression. We used AutoCUT&RUN profiling of genome-wide occupancy of KMT2A oncoproteins to evaluate direct binding of the KMT2A fusion protein to the CLEC2A promoter in primary AML patient samples with three different *KMT2A* fusions (MLLT10, MLLT4 and MLLT3). We found direct binding of KMT2A fusion proteins to the CLEC2A promoter region, indicating a causal relationship between KMT2A fusions and CLEC2A expression.

We then evaluated the potential of therapeutic targeting of CLEC2A by an antibody-drug conjugate using a HumZap cytotoxicity assay. CLEC2A antibody conjugated to saporin (a toxin with cytolytic activity when internalized) was incubated with CLEC2A+ and CLEC2A- cells with target-specific cytotoxicity evaluated by colorimetric assay. CLEC2A+ OCI-AML2 cells had significantly higher rate of cytotoxicity compared to CLEC2A- control cells at 10nM Ab-toxin conjugate.

Clinical outcomes for patients with and without CLEC2A expression was evaluated. Patients with CLEC2A had significantly worse outcomes (EFS 23% vs. 45.5%, p<0.0001 and OS 38% vs. 63%, p<0.0001 for patients with and without CLEC2A expression, respectively). Given high rates of co-occurring KMT2A-r AML, particularly high-risk fusions (MLLT10 and MLLT4), we evaluated clinical outcomes within the KMT2A-r cohort as these patients have poor EFS and OS. KMT2A-r patients with CLEC2A had significantly worse EFS (24% vs. 39%, p=0.0063) and OS (38% vs. 60%, p=0.0002). Within the KMT2A-r cohort, high rates of relapse were seen for patients with CLEC2A expression compared to those without CLEC2A (72% vs. 55%, p=0.0039).

Here we describe CLEC2A, a novel AML-restricted cell surface target that is an ideal immunotherapeutic target. CLEC2A is highly expressed in KMT2A-r AML, entirely absent in normal hematopoietic cells, directly and causally linked to the KMT2A fusion, and can be used for target-directed cytotoxicity.

Disclosures No relevant conflicts of interest to declare.



A. Waterfall plot of CLEC2A expression (transcripts per million [TPM]) in pediatric AML patients compared to normal bone marrow (NBM) and CD34+ peripheral blood (PB) with expression in specific fusion groups in lay; B. Distribution of cytomolecular alterations in CLEC2A positive patients with the percentage of CLEC2A positive patients in the most enriched KMT2A fusion subgroups.

Figure 1

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