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POSTER ABSTRACTS

803.EMERGING TOOLS, TECHNIQUES AND ARTIFICIAL INTELLIGENCE IN HEMATOLOGY

Optical Genome Mapping Allows Detection and Characterization of Cytogenetically Cryptic Oncogenic Fusions in Pediatric Acute Myeloid Leukemia

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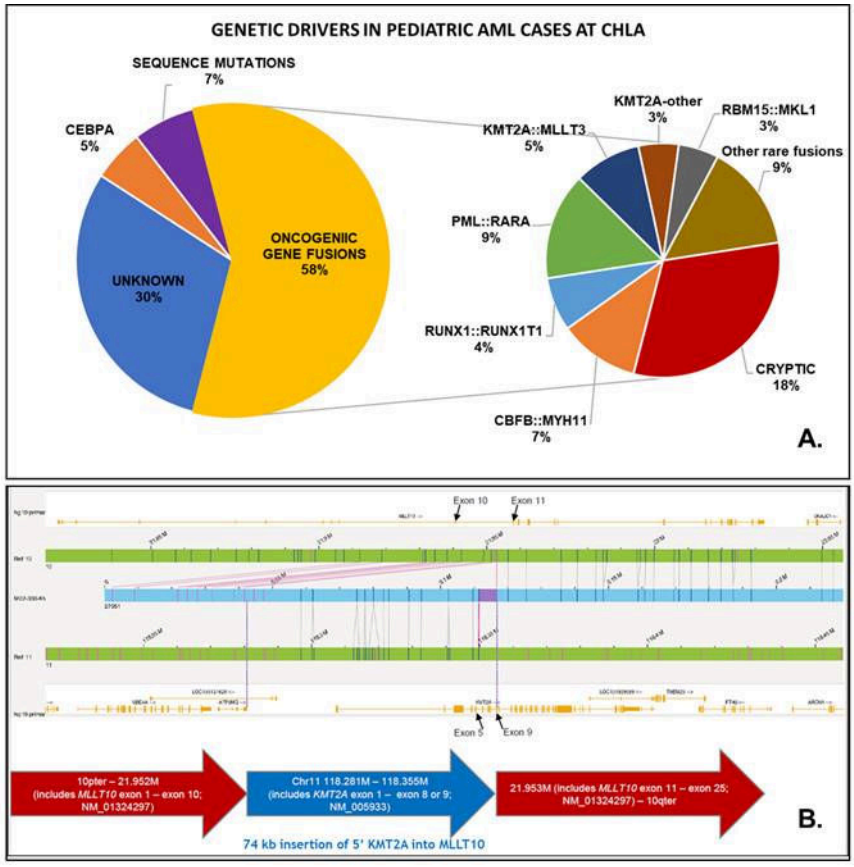
Background: Abnormal gene fusions are predominant oncogenic drivers in pediatric acute myeloid leukemia (AML) and are important diagnostic and prognostic markers. However, several oncogenic fusions specific for or enriched in pediatric AML (like *CBFA2T3::GLIS2, NUP98::NSD1, ETV6::MNX1* and others) are not detectable by conventional cytogenetic analysis (cytogenetically cryptic) and may not be included in fluorescence *in situ* hybridization (FISH) or molecular fusion panels designed for adult patients. Optical genome mapping (OGM) is a powerful new method for genome-wide, high-resolution detection of copy number abnormalities and balanced chromosome rearrangements. This study determined the frequency of cryptic oncogenic fusions in an institutional cohort of pediatric AML cases and evaluated OGM as a method for their detection and characterization.

Methods: Retrospective review of genetic testing results was performed for 93 consecutive *de novo* or relapsed pediatric AML cases tested at the Children's Hospital Los Angeles (CHLA) Center for Personalized Medicine between March 2016 and July 2023. Genetic testing was performed as part of clinical care following standard procedures and consisted of karyotype analysis, FISH testing, chromosomal microarray (CMA) analysis, CHLA custom sequencing panel (OncoKids®) (June 2017 onwards) and RNA-Seq fusion assay (March 2022 onwards). Cases with normal or inconclusive karyotype in which an oncogenic fusion was detected by another clinical assay were selected for OGM testing. High molecular weight DNA from peripheral blood or bone marrow samples was labeled, processed and imaged in a Saphyr System and analyzed in the Bionano Access software following the manufacturer's recommendations (BionanoGenomics, San Diego CA).

Results: Out of 93 pediatric AML cases included in the study, a gene fusion was identified as the primary oncogenic driver in 54 (58%) and a DNA sequence variant in 11 (12%). In 28 cases (30%), the primary genetic abnormality remained undetermined after comprehensive genetic testing. In 17/54 (31%) fusion-driven cases [17/93 (18%) overall cases], the oncogenic fusion remained undetected by karyotype analysis (cryptic). OGM was completed in 13/17 fusion positive cases with non-informative karyotype; it could not be performed in 4 cases due to lack of samples (n=2) or poor DNA quality (n=2), and is still in progress for a subset of 28 unknown cases with available material. OGM analysis successfully revealed all cryptic fusions previously detected by FISH, CMA, OncoKids® and/or RNA-Seq, including *NUP98::NSD1* (n=3), *KMT2A::MLLT10* (n=3), *KMT2A::MLLT3* (n=2)and one case each of *CBFA2T3::GLIS2, NUP98::KDM5A, DEK::NUP214, MYB::GATA1* and *ETV6::MNX1*. Chromosomal mechanism and structure of cryptic fusions were resolved by OGM and included: intrinsically cryptic fusions due to chromosome location of partner genes (*NUP98::NSD1*, n=4; *CBFA2T3::GLIS2*, *NUP98::KDM5A* and *ETV6::MNX1*, n=1 each); complex rearrangements due to incompatible gene orientation (*KMT2A::MLLT10* n=2; *MYB::GATA1*, n=1); three-way rearrangements involving an additional chromosome (*KMT2A::MLLT3* n=2) and small insertions (*KMT2A::MLLT10* and *DEK::NUP214*, n=1 each). OGM also matched the performance of chromosomal microarray (CMA) analysis in detection of copy number variants.

Conclusions: We confirm the significant contribution of cryptic chromosome rearrangements to pathogenesis of pediatric AML, and highlight the importance of tailored testing approaches that effectively detect highly heterogeneous fusion drivers. Our preliminary results show potential of OGM to identify clinically significant cryptic fusions in pediatric AML, and to thus markedly increase diagnostic yield compared to karyotype analysis. As a single assay that detects abnormalities currently interrogated by conventional cytogenetics, FISH and CMA, OGM may allow streamlined and more cost-effective genetic evaluation for pediatric AML.

Disclosures No relevant conflicts of interest to declare.



Figure, top panel (A). Genetic drivers detected by clinical testing in 93 pediatric AML cases at CHLA. Oncogenic fusions accounted for 58% of the cases, and 31% of those were cytogenetically cryptic (18% of all the cases). Bottom panel (B). A cryptic *KMT2A::MLLT10* fusion detected by OGM in an AML with a normal karyotype. The fusion was caused by a submicroscopic 74kb insertion that was also missed by FISH testing with the break-apart *KMT2A* probe.

Figure 1

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