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Detection of a *GLIS3* fusion in an infant with AML refractory to chemotherapy

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Abstract Infants diagnosed with acute myeloid leukemia (AML) frequently harbor cytogenetically cryptic fusions involving *KMT2A*, *NUP98*, or *GLIS2*. Those with AML driven specifically by *CBFA2T3::GLIS2* fusions have a dismal prognosis and are currently risk-stratified to receive hematopoietic stem cell transplantation (HSCT) in first remission. Here we report an infant with AML who was refractory to multiple lines of chemotherapy but lacked an identifiable fusion despite cytogenetic, fluorescence in situ hybridization (FISH) and targeted next generation sequencing (NGS) testing. Research-grade RNA-seq from a relapse sample revealed in-frame *CBFA2T3::GLIS3* and *GLIS3::CBFA2T3* fusions. A patient-derived xenograft (PDX) generated from this patient has a short latency period and represents a strategy to test novel agents that may be effective in this aggressive subtype of AML. This report describes the first case of AML with a *CBFA2T3::GLIS3* fusion and highlights the need for unbiased NGS testing including RNA-seq at diagnosis, as patients with *CBFA2T3::GLIS3* fusions should be considered for HSCT in first remission.

[Supplemental material is available for this article.]

CASE PRESENTATION

The patient presented at 12 mo of age with diffuse bruising with initial labs notable for white blood cell count of $369 \times 10^9/L$, hemoglobin of 6.6 g/dL, and platelets of $15 \times 10^9/L$. Her exam was notable for several firm nodules consistent with leukemia cutis. Flow cytometry from peripheral blood was diagnostic of AML but not consistent with a RAM phenotype with 81% blasts expressing CD7, CD13, CD33, CD34, CD45, CD56, CD64, CD117, CD123, HLA-DR, and MPO. Cytogenetics and FISH at diagnosis were normal and a next generation sequencing AML panel was notable for two different pathogenic *NRAS* mutations (c.35G > A variant allele frequency 30% and c.34G > A variant allele frequency 5.2%) (Table 1).

Patient was initially treated with daunorubicin, cytarabine, and gemtuzumab. Following the first induction course, her marrow demonstrated minimal residual disease (MRD) of 4.8% with cytogenetic evolution. She had no residual disease following a cycle of mitoxantrone, cytarabine and gemtuzumab. She was next treated with cytarabine and etoposide while being considered for HSCT but was found to have rising MRD to 0.3%. Despite salvage therapy with CPX-351, her MRD remained positive at 0.15%. She was next treated with azacitadine, pevonedistat, fludarabine, and cytarabine but progressed on therapy. Disease was also refractory to several salvage regimens, including topotecan, vinorelbine, thiotepa, and clofarabine (TVTC);

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TABLE 1. Genomic Findings * FFPM represents the normalized RNA-seq fragments supporting the fusion expressed as fusion fragments per million total RNA-seq fragments

Gene/genomic location	Chr	HGVS DNA ref (if genic)	HGVS Protein ref	Variant type	Predicted effect	Allele Frequency	Target coverage
NRAS	1	c.35G>A	p.G12D	Missense	Activating	30	Unknown
NRAS	1	c.34G>A	p.G12S	Missense	Activating	5.2	Unknown
<i>CBFA2T3::GLIS3</i>	16;9	n/a	n/a	<i>CBFA2T3::GLIS3</i> fusion	<i>GLIS3</i> overexpression	n/a	1.4 FFPM

venetoclax and cytarabine (VEN/ARA-C); cladribine, cytarabine, and trametinib (CLAG-trametinib); and finally decitabine, vorinostat, methotrexate, and vincristine (Supplemental Fig. 1). The patient died of progressive leukemia 12 mo after her initial diagnosis.

TECHNICAL ANALYSIS

Variant Interpretation

Exon 1 of the *CBFA2T3* gene (transcript ENST00000268679; NM_005187) was fused with exon 3 of the *GLIS3* gene (transcript ENST00000381971; NM_001042413), leaving *GLIS3* nearly intact. Critical domains, including the amino-terminal conserved region that is shared between *GLIS* genes (NCR), the zinc finger domain (ZFD), and the transactivation domain (TAD), were all present in the fusion transcript. The ZFD domain of *GLIS3* contains the consensus Gli binding site (5'-GACCACCCA) while the TAD domain serves as a potent transactivator (Fig. 1C; Lichti-Kaiser et al. 2012; Jetten 2018). Despite differences in the 3' gene, the gene expression profiling of this patient's *CBFA2T3::GLIS3* fusion appears similar to samples with *CBFA2T3::GLIS2* fusions. First, as with *GLIS2*, the *GLIS3* gene is minimally expressed in non-malignant conditions in whole blood compared with other tissues (Supplemental Fig. 2A,B). Second, in our patient's sample, *GLIS3* is overexpressed when compared with other leukemia as well as normal whole blood samples and leukemia cells lines (Supplemental Fig. 3A–C) by 32.8×, 32.2×, and 32.8-fold, respectively. As with the *GLIS2* fusion, we hypothesize that an increase in expression of the *GLIS3* gene is due to a promoter swap of the *CBFA2T3* gene on the 5' end (Fig. 1A,C). Last, as has been demonstrated in *CBFA2T3::GLIS2* fusion samples, the *FOLR1* gene, which encodes for the cell surface folate receptor, was highly expressed (Supplemental Fig. 3A). *FOLR1* is currently being tested as a therapeutic target for patients with the *CBFA2T3::GLIS2* fusion (Le et al. 2021)

The *CBFA2T3::GLIS3* fusion was validated using RNA from the patient's bone marrow sample. The reverse transcription-polymerase chain reaction (RT-PCR) product was validated by Sanger sequencing (Fig. 1B,D).

A PDX was generated in triple transgenic NOD.scid.II2Rycnull -SGM3 (NSGS) mice, with all mice succumbing to endpoint from leukemia within 3–4 wk from injection (Supplemental Fig. 4). RT-PCR of splenic tissue from the PDX confirmed the presence of the *CBFA2T3::GLIS3* fusion. These PDX models will be utilized to test preclinical therapeutic strategies and will be a valuable resource for the research community (please contact the corresponding author to request material).

SUMMARY

Infants who develop AML frequently have fusions as drivers of their leukemia and are often refractory to conventional chemotherapy (Bolouri et al. 2018). Infants with non-Down

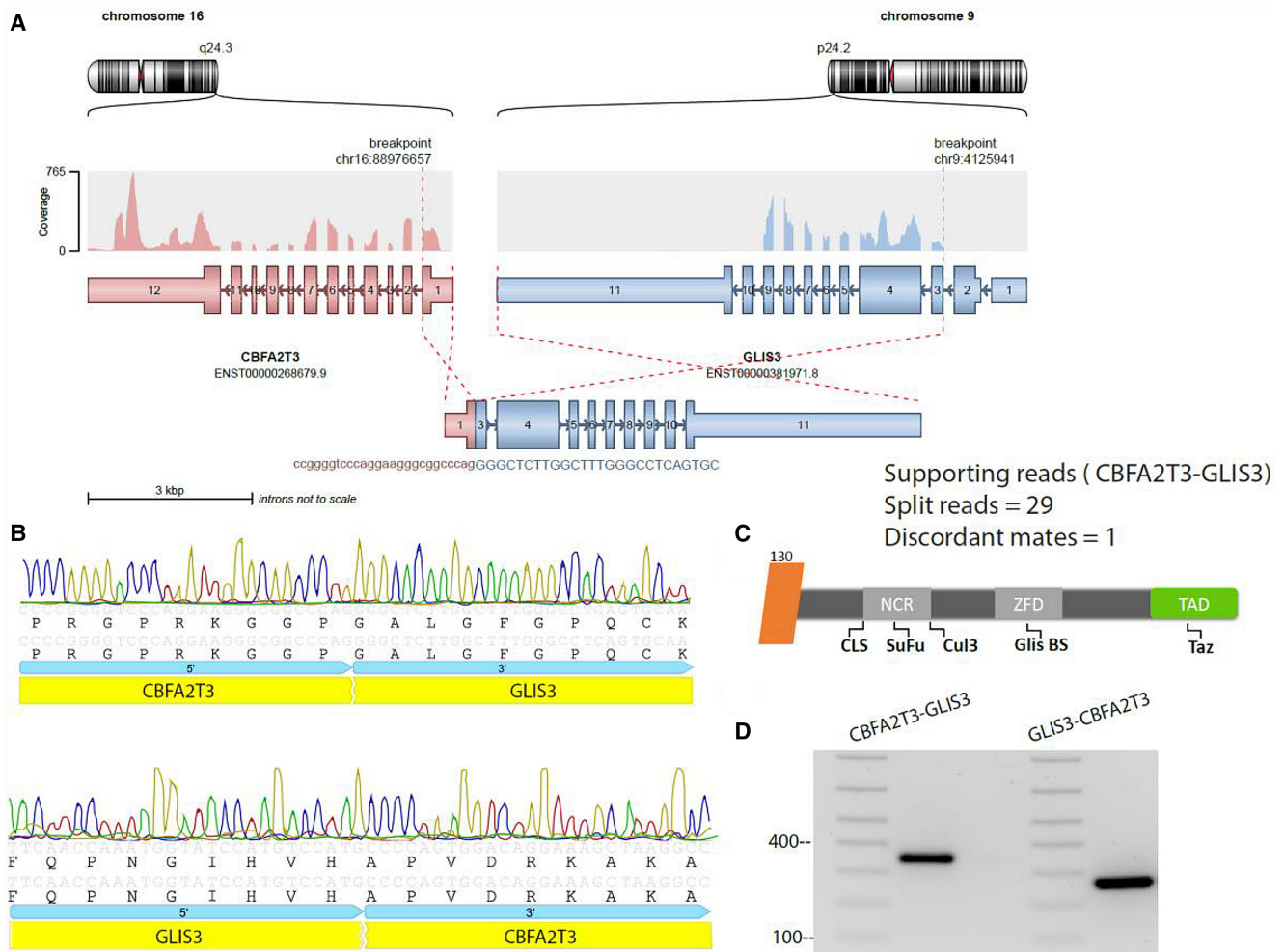


Figure 1. GLIS3 fusions. (A) Illustration of the CBFA2T3::GLIS3 fusion. Coverage indicates aligned read counts on the respective exons. Red dotted lines indicate where the breakpoints are relative to each gene and coordinates are shown above. (B) Chromatograms from Sanger sequencing of the PCR amplicons for CBFA2T3::GLIS3 and GLIS3::CBFA2T3. (C) Illustration of the GLIS3 domains present in the CBFA2T3::GLIS3 fusion protein. Amino-terminal conserved region shared between GLIS genes (NCR), ZFD (zinc finger domain), and trans-activation domain (TAD). The ciliary localization signal Consensus (CLS), Suppressor of Fused (SuFu), Cullin 3 (Cul3), the optimal Glis DNA binding site (Glis BS), and the PDZ binding motif (Taz) subdomain are indicated below. The number above indicates the amino acid position of the fusion breakpoint. (D) One percent agarose gel of PCR product.

syndrome M7 acute myeloid leukemia (AML) were initially found to harbor cytogenetically cryptic *CBFA2T3::GLIS2* fusions on RNA-seq (Masetti et al. 2013). More recently, *CBFA2T3::GLIS2* fusions have been found in other AML subtypes, most commonly in cytogenetically normal (CN)-AML (Smith et al. 2020). The 2017 WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues has designated a new classification of AML with the chromosome 16 [inv (16)(p13.3q24.3)] that results in the fusion between *CBFA2T3* and *GLIS2* (Swerdlow 2017). Across all subtypes, *CBFA2T3::GLIS2* fusions have been associated with poor outcomes and a lack of response to conventional chemotherapy with recent studies demonstrating event-free survival ranging from 8% to 33% (Zangrando et al. 2021). Patients with these fusions are now considered high-risk and are stratified to receive HSCT

on AAML1831 the current Children's Oncology Group clinical trial for patients with newly diagnosed AML.

GLIS3 is known to be involved in development of the pancreas, thyroid, eyes, liver, kidneys, and heart (Pinto and Chetty 2020). To date there is only one prior report of a hematologic malignancy with a *GLIS3* fusion that was diagnosed in an infant with T/megakaryoblastic mixed-phenotype acute leukemia (Klairmont and Choi 2018). A deleterious mutation in *GLIS1* has also been reported in one patient with *ETV6::RUNX1*-positive hyperdiploid acute lymphoblastic leukemia (Chen et al. 2015). This is the first report of a *GLIS3* fusion in a case of pediatric AML. *GLIS3* is one of three identified GLI-similar (GLIS) zinc finger proteins with *GLIS2* acting as a repressor of transcription while *GLIS1* and *GLIS3* serve primarily as transcription factor activators in humans. Recent studies have provided evidence for a regulatory role of GLIS transcription factors in reprogramming, maintenance, and differentiation of several stem and progenitor cell populations (Scoville et al. 2017).

This report highlights the importance of incorporating unbiased NGS into the diagnostic workup of pediatric AML patients. The *CBFA2T3::GLIS3* fusion detected in this patient on RNA-seq would not have been detected on panel-based sequencing currently being used on the AAML1831 protocol. However, identification of this fusion would have changed upfront management for this patient as a workup for HSCT in first remission would have been indicated immediately upon identification. A PDX for this novel fusion is now available and can be used to identify novel therapeutic agents for this difficult to treat subset of patients.

METHODS

RNA was extracted using standard methods. An RNA-seq library from a relapsed bone marrow sample was made using the TruSeq stranded RNA kit (Illumina catalog #20020594), in accordance with the manufacturer's instructions. All manufacturer's controls were used in preparation. The library was analyzed for size, concentration, and presence of primer dimer on the TapeStation 4200 using the HS D1000 assay (Agilent). RNA-seq revealed in-frame *GLIS3-CBFA2T3* and *CBFA2T3-GLIS3* fusions using STAR-fusion and Arriba (Haas et al. 2017; Uhrig et al. 2021). Expression analysis was calculated with the Tukey outlier analysis.

For RT-PCR, cDNA was generated using the Maxima cDNA synthesis kit (Thermo Fisher catalog #K1641) and PCR was performed using Platinum SuperFi Green (Thermo catalog #12369010). Primer sets included

GLIS3-CBFA2T3 fwd/rev: GAGGTTTGCACCTTCTGCTC/CGTCCTCTCGATGTGTGTGT and

CBFA2T3-GLIS3 fwd/rev: GGCTTCAAGACTGAGGGACA/GCTGGAGGTGAAATGAGTCC.

ADDITIONAL INFORMATION

Database Deposition and Access

RNA-seq data have been deposited in the database of Genotypes and Phenotypes (dbGaP) under accession phs002430.

Ethics Statement

Samples were collected as part of an institutional review board approved tissue bank study. Approval for this study was obtained from the University of California, San Francisco,

Committee on Human Research. The guardians provided informed consent in accordance with the Declaration of Helsinki.

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Author Contributions

S.M.S., A.G.L., and S.T. wrote the manuscript. A.G.L. analyzed the data, including identifying the fusion on RNA-seq. S.G.L. generated the RNA-seq library and the RT-PCR data. H.H. and J.M.R. generated the PDX. E.A.S.-C., J.M., and E.S. conceived the study. All authors edited and approved the manuscript.

Competing Interest Statement

The authors have declared no competing interest.

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