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REGULAR ARTICLE

blood advances

Structural variants involving *MLLT10* fusion are associated with adverse outcomes in pediatric acute myeloid leukemia

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Key Points

- Pediatric *MLLT10*$^+$ AML have poor prognosis and may benefit from targeted therapies.
- Patients with *PICALM::MLLT10*$^+$ and *X::MLLT10*$^+$ exhibit DNA hypermethylation, similarly to *NUP98::NSD1*$^+$.

MLLT10 gene rearrangements with *KMT2A* occur in pediatric acute myeloid leukemia (AML) and confer poor prognosis, but the prognostic impact of *MLLT10* in partnership with other genes is unknown. We conducted a retrospective study with 2080 children and young adults with AML registered on the Children's Oncology Group AAML0531 (NCT00372593) and AAML1031 trials (NCT01371981). Transcriptome profiling and/or karyotyping were performed to identify leukemia-associated fusions associated with prognosis. Collectively, 127 patients (6.1%) were identified with *MLLT10* fusions: 104 (81.9%) with *KMT2A::MLLT10*, 13 (10.2%) with *PICALM::MLLT10*, and 10 (7.9%) *X::MLLT10*: (2 each of *DDX3X* and *TEC*), with 6 partners (*DDX3Y, CEP164, SCN2B, TREH, NAP1L1*, and *XPO1*) observed in single patients. Patients with *MLLT10* (n = 127) demonstrated adverse outcomes, with 5-year event-free survival (EFS) of 18.6% vs 49% in patients without *MLLT10* (n = 1953, $P < .001$), inferior 5-year overall survival (OS) of 38.2% vs 65.7% ($P \leq .001$), and a higher relapse risk of 76% vs 38.6% ($P < .001$). Patients with *KMT2A::MLLT10* had an EFS from study entry of 19.5% vs 12.7% ($P = .628$), and an OS from study entry of 40.4% vs 27.6% ($P = .361$) in those with other *MLLT10* fusion partners. Patients with *PICALM::MLLT10* had an EFS of 9.2% vs 20% in other *MLLT10*$^-$ without *PICALM* (*X::MLLT10*; $P = .788$). Patients with *PICALM::MLLT10* and *X::MLLT10* fusions exhibit a DNA hypermethylation signature resembling *NUP98::NSD1* fusions, whereas patients with *KMT2A::MLLT10* bear aberrations primarily affecting distal regulatory elements. Regardless of the fusion partner, patients with AML harboring *MLLT10* fusions exhibit very high–risk features and should be prioritized for alternative therapeutic interventions.

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The data generated in this study have been deposited in dbGaP under the study ID phs000465.v21.p8 and in the TARGET Data Matrix: (https://target.nci.nih.gov/dataMatrix/TARGET_DataMatrix.html). Genomic sequencing data are available through controlled access as part of the National Institutes of Health genomic data sharing policy to ensure that all approved investigators and institutions abide by the National Institutes of Health Genomic Data User Code of Conduct, the terms of the Data Use Certification, and the Security Best Practices for Controlled Access Data. Data access is restricted for academic use and can be requested here (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000465.v21.p8).

The full-text version of this article contains a data supplement.

Introduction

Current outcomes of pediatric acute myeloid leukemia (AML) remain suboptimal despite treatment intensification, with 5-year overall-survival (OS) rates of 75% to 80% and 5-year event-free survival (EFS) rates of up to 55%.[1-3] Early treatment response and cytogenetic/molecular abnormalities are the most important prognostic factors for survival.[1,4] Because of the significant heterogeneity of pediatric AML, ongoing clinical trials have focused on identifying new prognostic subgroups for whom treatment intensity may be adapted based on novel risk criteria. We, and others, have previously shown that childhood AML is enriched in oncogenic fusions, which can be used to characterize and subclassify patient groups.[5] In the case of rarer fusions, the incidence can be too low to reliably understand its prognostic impact outside of large clinical trials or international collaboration.

The 11q23/*KMT2A*-rearranged (*KMT2A-r*) AML is a heterogeneous genetic subtype, which occurs in 20% to 25% of children with AML. A large International Berlin-Frankfurt-Munster Study Group (I-BFM-SG) collaborative study showed that the prognosis of this subtype is influenced by the fusion partner.[6] The *MLLT10* gene (previously designated *AF10)*, located at 10p12.31, is a known fusion partner of *KMT2A*, and encodes the AF10 protein, which is a transcription factor that exerts its leukemogenic effect by binding unmodified histone H3 and regulating *DOTIL* expression.[7] Van Weelderen et al conducted a more recent I-BFM-SG international study on childhood *KMT2A-r* AML and assessed the outcome of this subgroup depending on the fusion partner. The *KMT2A::MLLT10* fusion was included in the high-risk group that had inferior EFS (30.3% vs 54% in non–high risk) and OS (49.2% vs 70.5%) and a worse cumulative incidence of relapse (59.7% vs 35.2%).[8] Similarly, the current AML phase 3 Children's Oncology Group (COG) trial, AAML1831 (www.clinicaltrials.gov identifier: NCT04293562), includes patients with rearranged *KMT2A::MLLT10* as being at high risk.

However, the role of *MLLT10* and its prognostic implications in partnership with other genes have not been well defined. One of these other partners, *PICALM::MLLT10* (originally called *CALM::AF10*) is generated by the t(10;11)(p12-13;q14-21) translocation, and has been rarely reported in patients with AML, acute lymphoblastic leukemia (ALL, especially T-phenotype), and acute undifferentiated leukemia.[9,10] The *PICALM::MLLT10* fusion gene has been associated with high relapse rates and poor prognosis in adult AML,[9] however, data are lacking in children with AML harboring this translocation.

To better understand the impact of *MLLT10* structural variants in pediatric AML, we conducted a retrospective study in 2080 children and young adults registered on COG AAML0531 and AAML1031 treatment trials. By comprehensive transcriptome and karyotype evaluation, we defined the full spectrum of *MLLT10* fusions, identified new fusion partners, and correlated *MLLT10* structural variants with clinical outcomes in pediatric AML. In addition, transcription and methylation profiles were evaluated to identify dysregulated genes in *MLLT10* fusions with and without *KMT2A*.

Methods

Patients

Patient characteristics and outcome data were collected from 2080 children and young adults aged 0 to 30 years, with AML enrolled on COG AAML0531[11] (August 2006-June 2010) and AAML1031[12] (February 2011-June 2016) treatment trials. Collected data included age, sex, race/ethnicity, date of diagnosis, initial white blood cell count, peripheral blood and bone marrow (BM) blast percentages, World Health Organization classification, presence of extramedullary disease (EM; central nervous system [CNS] or chloromas), karyotype (and RNA sequencing to detect fusions and copy number variants), presence of complex cytogenetics, FLT3-ITD with allelic ratio, other FLT3 mutations, and other molecular alterations (NPM1, CEBPA, WT1, KIT, etc); in addition, we collected data on risk category; treatment protocols; receipt of investigational therapy (gemtuzumab ozogamicin [GO] in AAML0531); allogeneic hematopoietic stem cell transplantation (HSCT); response to therapy, including morphology and measurable (minimal) residual disease (MRD) by flow cytometry after induction courses 1 (end of induction 1 [EOI-1]) and 2 (EOI-2); and treatment outcomes including relapse, refractory disease, occurrence of secondary malignancy, death, and status at last follow-up.

Cytogenetics

Karyotype data for all patients were collected by COG and centrally reviewed using International System for Human Cytogenetic Nomenclature for consistent reporting of lesions. Karyotypes with translocations involving the 10p12.31 locus (*MLLT10*) were compared with results from transcriptome sequencing (as available) to corroborate findings.

All patients provided informed consent, and the study was conducted in accordance with the principles of the Declaration of Helsinki.

RNA sequencing

Transcriptome data were available from 1491 pediatric patients with AML enrolled on COG trials AAML0531 and AAML1031. These data were generated by the British Columbia Genome Sciences Center (Vancouver, BC). Total RNA samples were ribodepleted and prepared for sequencing using a strand-specific messenger RNA library construction protocol. Indexed libraries were pooled and sequenced on an Illumina Hiseq, producing 75–base pair paired-end sequence reads. Sequencing data were aligned to human genome assembly GRCh37. Gene-level counts were quantified using Boston Consulting Group Sequencing Gene's in-house pipeline and annotated using Ensembl version 69 annotations. Gene fusions were identified using 3 calling algorithms: TransAbyss (version 1.4.10), STAR-fusion (version 1.8.1), and Cicero (St. Jude, Memphis, TN). Fusions involving *MLLT10*, identified by at least 2 algorithms or 1 algorithm with corroborating karyotype information, were considered positive for *MLLT10* rearrangement.[13] Differential expression analyses were performed using the R package Limma and gene enrichment analyses were performed using Gene Ontology enrichment (https://geneontology.org/).

Statistical analyses

Data from the AAML0531 and AAML1031 trials were current as of 30 September 2020 and 31 March 2022, respectively. Patient characteristics were compared using the χ^2 test, or Fisher's exact test if data were sparse. The Kruskal-Wallis test was used for comparisons of medians. The Kaplan-Meier method was used to compare OS and EFS. OS was defined as time from study entry until death from any cause. EFS was defined as time from study entry until refractory disease, relapse, or death. Estimates of relapse risk (RR) were obtained by methods that account for competing events. RR was defined as time from EOI-1 for patients in complete remission (CR) until relapse, with deaths without relapse considered competing events. Otherwise, patients were censored at last known contact for OS, EFS, and RR. The statistical significance of predictor variables was tested with the log-rank statistic for OS and EFS, and with the Gray statistic for RR.

Results

Overall, 2080 patients treated on the COG AAML0531 and AAML1031 trials were evaluated by transcriptome profiling and/or karyotyping to identify leukemia-associated fusions and copy number changes linked with prognosis. A total of 127 patients (6.1%) were identified to have primary fusions involving *MLLT10*. Among 127 cases, 104 (81.9%) had *KMT2A::MLLT10*, 13 (10.2%) had *PICALM::MLLT10*, and 10 (7.9%) had *MLLT10* fusions (*X::MLLT10*) with various translocations (Figure 1). Among the latter group, 2 had *DDX3X* and 2 had *TEC* fusions, whereas fusions with other partner genes (*DDX3Y, CEP164, SCN2B, TREH, NAP1L1*, and *XPO1*) were each identified in single patients (supplemental Table 1).

Approximately 2 of 3 patients had confirmatory evidence of an *MLLT10* rearrangement by both RNA sequencing and karyotype data, whereas in another 20% of cases the cytogenetic band reported for the fusion did not map to *MLLT10*. For some cases for which the karyotype was unavailable, RNA sequencing successfully identified a fusion. Conversely, if a sample had low blast count and RNA sequencing did not identify a fusion, the karyotype was able to provide information. Thus, it is important to note, that both karyotype and RNA sequencing are useful and necessary for characterizing structural alterations in patients.

Overall, the median age of our patient cohort was 2.9 years (range, 3 days-21.3 years), 73 were males and 54 females. Patients with *PICALM::MLLT10* were older, with a median age of 14.4 years (range, 7.4-18.9 years) vs 6.6 years (range, 1.4-15.2 years) in those with *X::MLLT10*, and 1.7 years (range, 0-21.3 years) in those with *KMT2A::MLLT10*. Median initial white blood cell count in the whole cohort was 18.5×10^9/L (range, 0.8×10^9/L - 439×10^9/L), and it was higher at 42.9×10^9/L in the *PICALM::MLLT10* group vs 18.4×10^9/L in the *KMT2A::MLLT10* group and 8.6×10^9/L in the *X::MLLT10* group. Overall, 16 and 20 patients had CNS3 and CNS2 disease, respectively. Overall, CNS3 disease was more common in patients with *X::MLLT10*, at 30% (n = 3 of 10) vs 15.4% (n = 2 of 13) and 11.1% (n = 11 of 104) in the *PICALM::MLLT10* and *KMT2A::MLLT10* groups, respectively. Non-CNS EM involvement was overall present in 37 patients (29.1%) and was significantly more common in the *KMT2A::MLLT10* group at 34.6% (n = 36) vs 7.7% (n = 1 of 13) and 0% in the *PICALM::MLLT10* and *X::MLLT10* groups, respectively (Table 1). Sites of EM involvement included skin, gingiva, bone, head/neck, abdomen/pelvis, and spine.

Among patients with *MLLT10* fusions, 76 were treated on COG AAML1031, and 51 on COG-AAML0531; in total, 27 patients received GO as part of their treatment. Allogeneic HSCT in first CR (CR1) was performed in 16 patients (12.6% compared with 16.9% of other AML without an *MLLT10* rearrangement). Nine patients (75%) with *PICALM::MLLT10* fusion underwent HSCT in CR1 vs 5 (50%) and 9 (9.6%) patients in the *X::MLLT10* and *KMT2A::MLLT10* groups, respectively (Table 1). Analysis of the World Health Organization AML categories showed a predominance of acute monoblastic leukemia subtype in 60% and 43.3% of the *X::MLLT10* (n = 6) and *KMT2A::MLLT10* (n = 45) groups, respectively; whereas AML with myelodysplastic syndrome-related changes predominated (30.8%; n = 4) in the *PICALM::MLLT10* cohort (Table 1).

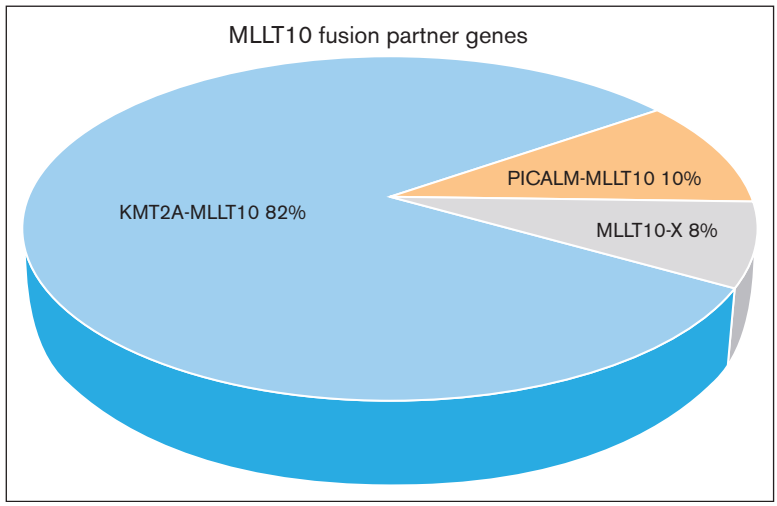

Figure 1. Distribution of the different fusion partners in patients with *MLLT10*$^+$ AML. Among the 127 cases, 104 had *KMT2A::MLLT10*, 13 had *PICALM::MLLT10*, and 10 *X::MLLT10* fusions with various translocations.

Table 1. Comparison of disease characteristics and induction response of patients with AML with and without *MLLT10*

Characteristic	(A) X::MLLT10		(B) PICALM::MLLT10		(C) KMT2A::MLLT10		(D) Other AML		A vs B	B vs C
	n	%	n	%	n	%	n	%	*P* value	*P* value
Total	10		13		104		1953			
Sex										
Male	5	50.0	6	46.2	62	59.6	979	50.1	1.000	.354
Female	5	50.0	7	53.8	42	40.4	974	49.9		
Study										
AAML0531	2	20.0	4	30.8	45	43.3	919	47.1	.660	.389
AAML1031	8	80.0	9	69.2	59	56.7	1034	52.9		
Age group, y										
0-1	2	20.0	0	0.0	58	55.8	344	17.6	.178	<.001
2-10	4	40.0	3	23.1	21	20.2	690	35.3	.650	.728
>11	4	40.0	10	76.9	25	24.0	919	47.1	.102	<.001
Race										
American Indian or Alaska Native	0	0.0	0	0.0	0	0.0	13	0.7	-	-
Asian	1	11.1	0	0.0	3	3.2	92	5.2	.429	1.000
Native Hawaiian or other Pacific Islander	0	0.0	0	0.0	1	1.1	6	0.3	-	1.000
Black or African American	2	22.2	1	8.3	13	13.8	234	13.3	.553	1.000
White	6	66.7	11	91.7	77	81.9	1412	80.3	.272	.686
Multiple races	0	0.0	0	0.0	0	0.0	2	0.1	-	-
Unknown	1		1		10		194			
Ethnicity										
Hispanic or Latino	1	11.1	4	30.8	7	7.2	363	19.2	.360	.024
Not Hispanic or Latino	8	88.9	9	69.2	90	92.8	1528	80.8		
Unknown	1		0		7		62			
***CEBPA* mutation**										
Negative	10	100.0	13	100.0	100	100.0	1783	94.4	-	-
Positive	0	0.0	0	0.0	0	0.0	106	5.6		
Unknown	0		0		4		64			
***NPM1* mutation**										
Negative	10	100.0	13	100.0	101	100.0	1713	90.7	-	-
Positive	0	0.0	0	0.0	0	0.0	177	9.4		
Unknown	0		0		3		61			
***FLT3*-ITD mutation**										
Negative	10	100.0	13	100.0	101	100.0	1570	83.1	-	-
Positive	0	0.0	0	0.0	0	0.0	312	16.5		
Unknown	0		0		3		69			
Response by EOI1										
CR	6	60.0	8	66.7	68	66.7	1434	75.0	1.000	1.000
Not CR	4	40.0	4	33.3	34	33.3	479	25.0		
Not evaluable	0		1		2		40			
Response by EOI2										
CR	8	88.9	7	77.8	82	82.8	1628	88.5	.527	.657
Not CR	1	11.1	2	22.2	17	17.2	212	11.5		
Not evaluable	1		4		5		113			
Age, median (range), y	6.6	(1.4 - 15.2)	14.4	(7.4 - 18.9)	1.7	(0 - 21.3)	10.1	(0.01 - 29.8)	.004	<.001
WBC, median (range), $\times 10^3/\mu L$	8.6	(1.4 - 49.4)	42.9	(2.6 - 439)	18.4	(0.8 - 319.9)	21.7	(0.2 - 918.5)	.032	.095
BM blasts, median (range), %	78.5	(0 - 95)	84.5	(65 - 96)	81	(0 - 100)	67	(0 - 100)	.212	.266

MDS, myelodysplastic syndrome; MLL, mixed-lineage leukemia; MPD, myeloproliferative disorder; WBC, white blood cell count; WHO, World Health Organization.

Table 1 (continued)

Characteristic	(A) X::MLLT10		(B) PICALM::MLLT10		(C) KMT2A::MLLT10		(D) Other AML		A vs B	B vs C
	n	%	n	%	n	%	n	%	*P* value	*P* value
CNS disease										
CNS 1	5	55.6	8	61.5	72	72.7	1329	69.8	1.000	.514
CNS 2	1	11.1	3	23.1	16	16.2	392	20.6	.616	.460
CNS 3	3	33.3	2	15.4	11	11.1	182	9.6	.609	.646
Unknown	1		0		5		50			
Non-CNS EM disease										
No	10	100.0	12	92.3	68	65.4	1694	86.9	1.000	.060
Yes	0	0.0	1	7.7	36	34.6	256	13.1		
Unknown	0		0		0		3			
HSCT in CR1										
Yes	5	55.6	9	75.0	9	9.6	321	16.9	.350	.135
No	4	44.4	3	25.0	85	90.4	1578	83.1		
Unknown	1		1		10		54			
MRD at EOI1										
Negative	7	87.5	4	40.0	81	84.4	1196	70.6	.066	.004
Positive	1	12.5	6	60.0	15	15.6	498	29.4		
Unknown	2		3		8		259			
MRD at EOI2										
Negative	8	100.0	4	80.0	67	94.4	1200	84.4	.188	.295
Positive	0	0.0	1	20.0	4	5.6	221	15.6		
Unknown	2		8		33		532			
WHO classification (pathology, study entry)										
AML with recurrent genetic abnormalities: AML with t(8;21)(q22;q22), AML1/ETO	0	0.0	0	0.0	0	0.0	127	6.5	-	-
AML with recurrent genetic abnormalities: AML with abnormal BM eosinophils and inv(16)(p13q22) or t(16;16)(p13;q22), (CBF/MYH11)	0	0.0	0	0.0	0	0.0	98	5.0	-	-
AML with recurrent genetic abnormalities: AML with 11q23 (MLL) abnormalities	1	10.0	0	0.0	37	35.6	138	7.1	.435	.009
AML with multilineage dysplasia	0	0.0	0	0.0	0	0.0	59	3.0	-	-
AML with multilineage dysplasia: after MDS or MDS/MPD	0	0.0	0	0.0	0	0.0	1	0.1	-	-
AML with multilineage dysplasia: without antecedent MDS or MDS/MPD, but with dysplasia in at least 50% of cells in ≥2 myeloid linea	0	0.0	0	0.0	0	0.0	3	0.2	-	-
AML with myelodysplasia-related changes	0	0.0	4	30.8	4	3.8	81	4.1	.105	.005
AML, not otherwise categorized	1	10.0	1	7.7	3	2.9	77	3.9	1.000	.380
AML, not otherwise categorized: acute erythroid leukemia (erythroleukemia, erythroid/myeloid)	0	0.0	0	0.0	0	0.0	10	0.5	-	-
AML, not otherwise categorized: AML, minimally differentiated	0	0.0	2	15.4	0	0.0	29	1.5	.486	.012
AML, not otherwise categorized: acute erythroid leukemia (pure erythroid leukemia)	0	0.0	0	0.0	0	0.0	1	0.1	-	-
AML, not otherwise categorized: AML without maturation	0	0.0	3	23.1	1	1.0	177	9.1	.229	.004
AML, not otherwise categorized: AML with maturation	0	0.0	1	7.7	0	0.0	166	8.5	1.000	.111
AML, not otherwise categorized: AML, with minimal differentiation	0	0.0	0	0.0	2	1.9	36	1.8	-	1.000

MDS, myelodysplastic syndrome; MLL, mixed-lineage leukemia; MPD, myeloproliferative disorder; WBC, white blood cell count; WHO, World Health Organization.

Table 1 (continued)

Characteristic	(A) X::MLLT10		(B) PICALM::MLLT10		(C) KMT2A::MLLT10		(D) Other AML		A vs B	B vs C
	n	%	n	%	n	%	n	%	*P* value	*P* value
AML, not otherwise categorized: acute myelomonocytic leukemia	0	0.0	1	7.7	6	5.8	176	9.0	1.000	.572
AML, not otherwise categorized: acute monoblastic/acute monocytic leukemia	6	60.0	1	7.7	45	43.3	214	11.0	.019	.015
AML, not otherwise categorized: acute erythroid leukemia (erythroid/myeloid and pure erythroleukemia)	0	0.0	0	0.0	0	0.0	14	0.7	-	-
AML, not otherwise categorized: acute megakaryoblastic leukemia	2	20.0	0	0.0	3	2.9	90	4.6	.178	1.000
AML, not otherwise categorized: acute panmyelosis with myelofibrosis	0	0.0	0	0.0	0	0.0	1	0.1	-	-
AML, not otherwise categorized: myeloid sarcoma	0	0.0	0	0.0	0	0.0	6	0.3	-	-
AML with t(8;21)(q22;q22); RUNX1-RUNX1T1	0	0.0	0	0.0	0	0.0	144	7.4	-	-
AML with inv(16)(p13q22) or t(16;16)(p13;q22); CBFB-MYH11	0	0.0	0	0.0	0	0.0	92	4.7	-	-
AML with t(9;11)(p22;q23); MLLT3-MLL	0	0.0	0	0.0	1	1.0	89	4.6	-	1.000
AML with t(6;9)(p23;q34); DEK-NUP214	0	0.0	0	0.0	0	0.0	17	0.9	-	-
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1	0	0.0	0	0.0	0	0.0	1	0.1	-	-
AML (megakaryoblastic) with t(1;22)(p13;q13); RMB15-MKL1	0	0.0	0	0.0	0	0.0	12	0.6	-	-
Provisional entity: AML with mutated CEBPA	0	0.0	0	0.0	0	0.0	37	1.9	-	-
Provisional entity: AML with mutated NPM1	0	0.0	0	0.0	0	0.0	45	2.3	-	-
Myeloid sarcoma	0	0.0	0	0.0	2	1.9	10	0.5	-	1.000
Other	0	0.0	0	0.0	0	0.0	2	0.1	-	-

MDS, myelodysplastic syndrome; MLL, mixed-lineage leukemia; MPD, myeloproliferative disorder; WBC, white blood cell count; WHO, World Health Organization.

Molecular data showed mutation enrichment in several genes within the *MLLT10* fusion types (Figure 2). *PTPN11* mutations were seen at an overall frequency of 12% across the *MLLT10* cohort, but the majority of those mutations (14 of 15) occurred in the *KMT2A::MLLT10* group.

Additionally, in the *KMT2A* group, *SETD2, FLT3* missense, and *U2AF1* mutations were observed at a frequency of 9.6%, 9.6%, and 4.8%, respectively. Mutations in the epigenetic regulator, plant homedomain finger protein 6 (*PHF6*), and histone methyltransferase gene *EZH2* were observed only in the setting of *PICALM::MLLT10* (69.2% and 23%, respectively) and 2 of 13 patients also had *TP53* mutations. The *EZH2* gene mutations included 2 truncating and 1 missense mutation, all which would lead to loss of function mutations, consistent with the report by Grossmann et al.[14] Within the *X::MLLT10* cases, *SETD2* and *WT1* were the most frequently mutated at 50% and 30%, respectively. Of note, only missense mutations in the tyrosine kinase domain of FLT3 were observed; no patients had internal tandem duplication (*FLT3-ITD*) mutations. Both NRAS and KRAS were frequently observed across all 3 *MLLT10* groups, however, these genes are frequently altered across all subtypes of pediatric AML in general, and do not appear to be causally linked to *MLLT10* fusions.

Secondary cytogenetic abnormalities were present in 22 patients with *KMT2A::MLLT10*; among these, 2 had monosomy 7 and 2 had additional *PICALM::MLLT10* fusions (considered as secondary abnormalities). Furthermore, 15% of the *PICALM::MLLT10* group had an additional *del5q* abnormality.

Structurally, *MLLT10* retains the highly conserved octapeptide motif and leucine zipper domains in the latter half of the gene when fused as the 3′ partner of the rearrangements (Figure 3). Although *MLLT10* reportedly always occupies the 3′ gene position, we observed *MLLT10* in the 5′ gene position with 2 partner genes: *TREH* and *NAP1L1*. For the genes *KMT2A* and *PICALM*, multiple breakpoints occurred when fused to *MLLT10*; representative diagrams of all fusions are shown in supplemental Figure 1.

Patient outcomes

Morphologic CR rates after induction cycles 1 and 2 were similar between *PICALM::MLLT10, X::MLLT10*, and *KMT2A::MLLT10* cases (Table 1; P = not significant), and not significantly different from the non-*MLLT10* AML cohort. However, MRD as assessed by multidimensional flow cytometry after induction cycle 1 was positive in 60% of patients with *PICALM::MLLT10* vs 12.5% in those with *X::MLLT10* vs 15.6% in those with *KMT2A::MLLT10* vs 29.4% in the non-*MLLT10* AML cohort ($P = .01$). Among patients with *MLLT10* fusion and known MRD at EOI-1 (n = 114), there were 22 patients with positive MRD vs 92 patients who were MRD negative. The 5-year disease-free survival (DFS) from EOI-1 for patients who were MRD positive was 9.1% vs 22.4% in those who were MRD negative ($P < .001$). Five-year OS from EOI-1 for

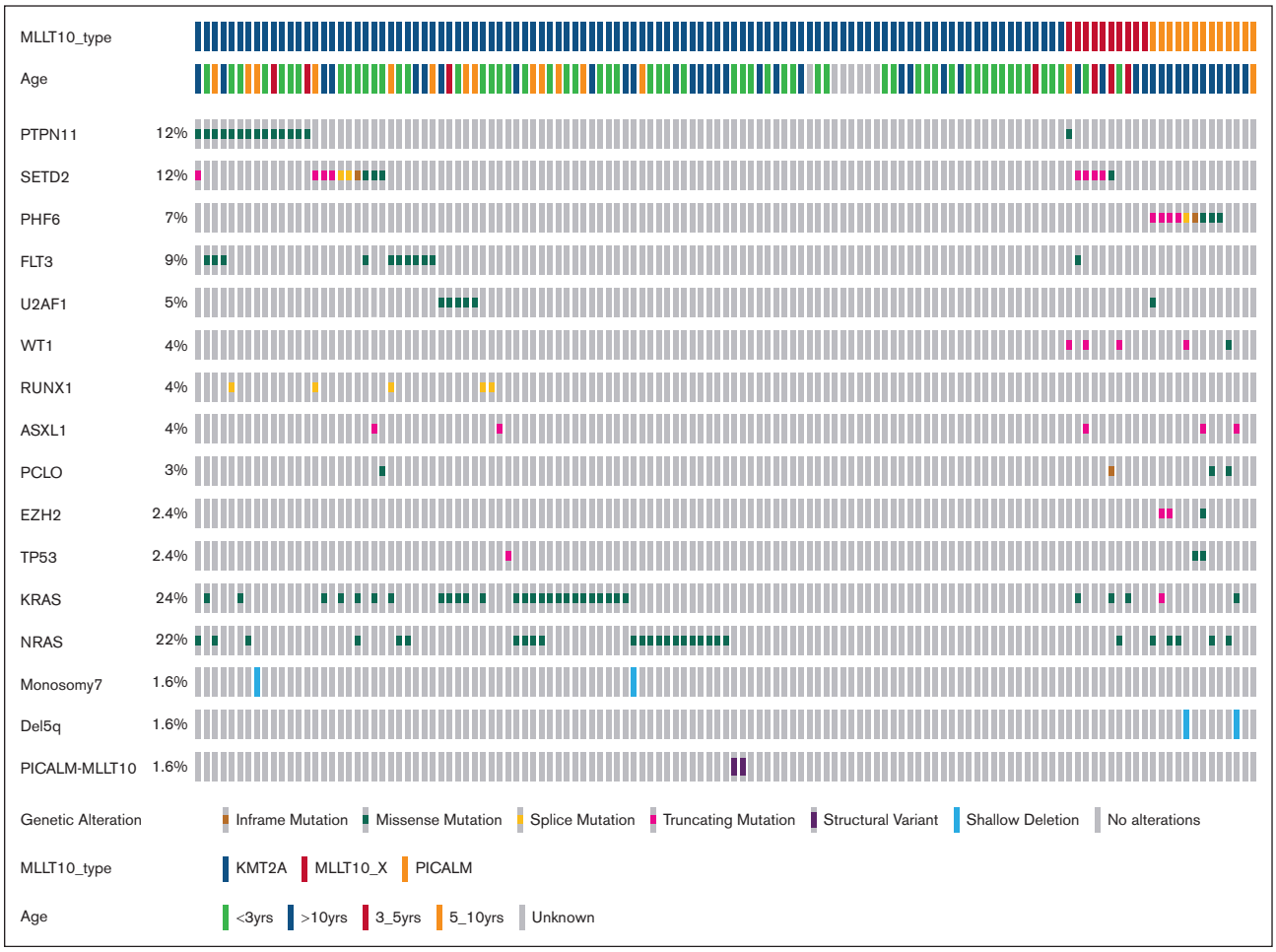

Figure 2. Oncoprint displaying comutational profiles of genes identified at a frequency of >2% within the *MLLT10* cohort, as well as cytogenetic alterations. Each column represents a patient, each row represents a gene or alteration. Columns are ordered by *MLLT10* fusion group. Mutations are color coded according to type.

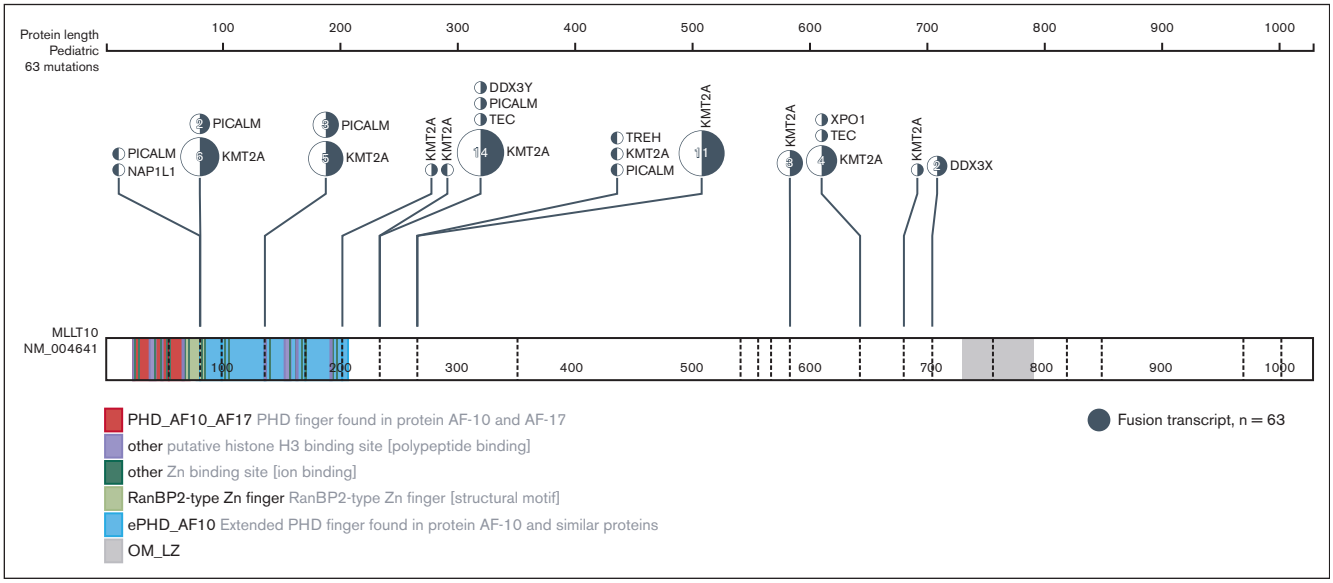

Figure 3. Lollipop plot of *MLLT10* gene. Fusion partners of *MLLT10* are indicated at the *MLLT10* breakpoint. Colored regions of the gene indicate protein domains.

patients who were MRD positive was 13.6% vs 46.4% in those who were MRD negative ($P < .001$).

Collectively, patients with *MLLT10* fusion (n = 127) demonstrated adverse outcomes, with 5-year OS of 38.2% vs 65.7% in patients without *MLLT10* fusion (n = 1953; $P \leq .001$; Figure 4A), poorer 5-year EFS of 18.6% vs 49% ($P < .001$; Figure 4B), and a higher RR of 76% (n = 82) vs 38.6% (n = 1434; $P < .001$; Figure 4C).

Next, we compared the outcomes of patients with other *MLLT10* fusion (*X::* and *PICALM::*) with those of patients with *KMT2A::MLLT10* fusion. Patients with other *MLLT10* fusions had an OS, from study entry, of 27.6% vs 40.4% for those with *KMT2A::MLLT10* ($P = .361$; Figure 4D) and an EFS of 12.7% vs 19.5% for patients with *KMT2A::MLLT10* ($P = .628$; Figure 4E). The 2 cohorts also had similar RR from remission, with 84.7% for *KMT2A::MLLT10* vs 74.6% for other *MLLT10* ($P = .876$). Given the fact that *PICALM::MLLT10* is the most prevalent fusion in the other-*MLLT10* cohort, we further evaluated the outcomes for the other-*MLLT10* cohort with and without PICALM fusion partner. Patients with *PICALM::MLLT10* and *X::MLLT10* had similarly dismal EFS of 9.2% and 20%, respectively ($P = .788$; Figure 4F).

Overall, relapses occurred in 82 patients (64.6%), of whom 59 (72%) were BM relapses; among these, 37 were isolated BM, and 22 were combined BM and EM relapses (18 with CNS relapse, of whom 2 had also chloromas; and 4 with chloromas); 8 patients had isolated CNS relapse, 11 had isolated chloromas, 1 relapse presented as a combined CNS and skin disease, and 3 relapses were from other sites. Most relapses (63.4%) occurred within 1 year from EOI.

Among patients who were *MLLT10*$^{+}$ enrolled on COG AAML0531 trial (n = 51), 27 received GO in Arm B and their 5-year EFS was 22.2% vs 8.3% for Arm A (no GO; $P = .094$), and 5-year OS for Arm B was 43.2% vs 41.7% for Arm A ($P = .566$; supplemental Table 2). In summary, GO appears to benefit EFS in these patients, although not statistically significant, similar to the overall results of the AAML0531 study.[11]

When assessing the impact of HSCT in CR1, time-to-event statistical analyses among those who are *MLLT10*$^{+}$ showed that 5-year OS ± a standard error (SE) of 2 in the HSCT group (n = 16) was 56.3% ± 24.8% vs 43.0% ± 12.9% in the group without HSCT (n = 61; $P = .383$); whereas the 5-year DFS ± 2 SE in the HSCT group was 37.5% ± 24.2% vs 28.7 ± 11.7% without HSCT ($P = .380$). Therefore, survival estimates appeared to be higher for HSCT in CR1, but the large SEs result in nonsignificant statistical differences.

MLLT10 transcriptome and methylome analyses

We interrogated the transcriptome data from 1491 patients treated on COG AAML0531 and AAML1031 to determine whether those with *MLLT10* fusions have a distinct expression profile compared with other AML. Expression profile of 91 patients with *MLLT10* fusions were compared with 1400 non–*MLLT10* rearranged cases.

In this comparison, 1395 genes were differentially expressed, as presented in Figure 5. The list of top 100 upregulated and downregulated differentially expressed genes is provided in supplemental Figure 2. Differentially expressed genes were enriched in homeobox (*HOX*) genes. Seven *HOXA* family genes were among the top 40 upregulated genes, with *HOXA11* identified as 4.7-log fold-change higher on average in patients with rearranged *MLLT10*. Analysis of the top 100 upregulated genes reveals a significant enrichment in the positive regulation of hematopoietic stem cell proliferation for biological processes (22.7-fold enrichment; $P < .0001$; false discovery rate, 0.00281) and activation of HOX genes during differentiation for pathway prediction (14.42-fold enrichment; $P < .0001$; false discovery rate, 0.0043).

To further determine whether patients with *MLLT10* fusions had distinct epigenetic profiles, we performed differential methylation analyses on samples from normal BM and from patients with *MLLT10*-rearranged, *KMT2A*-rearranged, and *NUP98*::*NSD1*-fused AML, on the Infinium HumanMethylation EPIC array. After fitting a multivariate model with all interacting molecular features, the 50 most discriminative regions were extracted (Figure 5). Patients with *PICALM::MLLT10* and the majority of *X::MLLT10* fusions share a distinct hypermethylation signature with patients with high-risk *NUP98::NSD1* fusion; this signature is consistent with gross disruption of HOX clusters, at which *PICALM::MLLT10*, *X::MLLT10*, and *NUP98::NSD1* show severe dysregulation. In contrast, patients with *KMT2A::MLLT10* cluster with other *KMT2A* fusions, and patients with only *FLT3* ITD or *WT1* frameshift variants (commonly observed alongside *NUP98-NSD1* fusions) show only a partial overlap of this hypermethylation signature. Patients with *NUP98-KDM5A* fusion do not show a hypermethylation signature, as noted by Bertrums et al,[15] reflecting a fundamental difference in leukemogenic mechanism between *NUP98* fusions with *NSD1* vs *KDM5A*-like fusion partners.

Gene set enrichment analysis for *HOXA* family was run between subsets of *MLLT10* fusions. No significant differences were observed, but expression trended higher in -*KMT2A* compared with the other fusion subsets (*KMT2A::MLLT10* vs other*::MLLT10*, $P = .11$; *KMT2A::MLLT10* vs *PICALM::MLLT10*, $P = .22$; *KMT2A::MLLT10* vs *X::MLLT10*, $P = .13$). No significant difference was observed between *PICALM::MLLT10* and *X::MLLT10* fusions ($P = .97$) (supplemental Figure 3)

Discussion

Our study identified new *MLLT10* fusion partners not previously described in childhood AML and compared them to other AML subtypes and with known *MLLT10* partners like *KMT2A* and *PICALM*. All patients with aberrant *MLLT10* had a high RR, poor EFS and OS, distinct methylation profiles, and overexpressed *HOXA* genes. Patients with *KMT2A::MLLT10* tend to be younger at diagnosis, have higher rates of EM disease, and exhibit lower EOI MRD levels, whereas patients with *PICALM::MLLT10* and *MLLT10::X* fusions tend to be older with higher MRD levels.

Although 83% of patients with *MLLT10* fusions achieved morphological CR by EOI-2, their RR was still very high at 76%, compared with 39% in other AML subtypes ($P < .0001$), with most relapses (63.4%) occurring within the first year after diagnosis.

Early response to therapy is currently used for risk stratification of therapy in childhood AML, and flow cytometry–based MRD provides a more accurate measure of therapy response[1,16]; despite

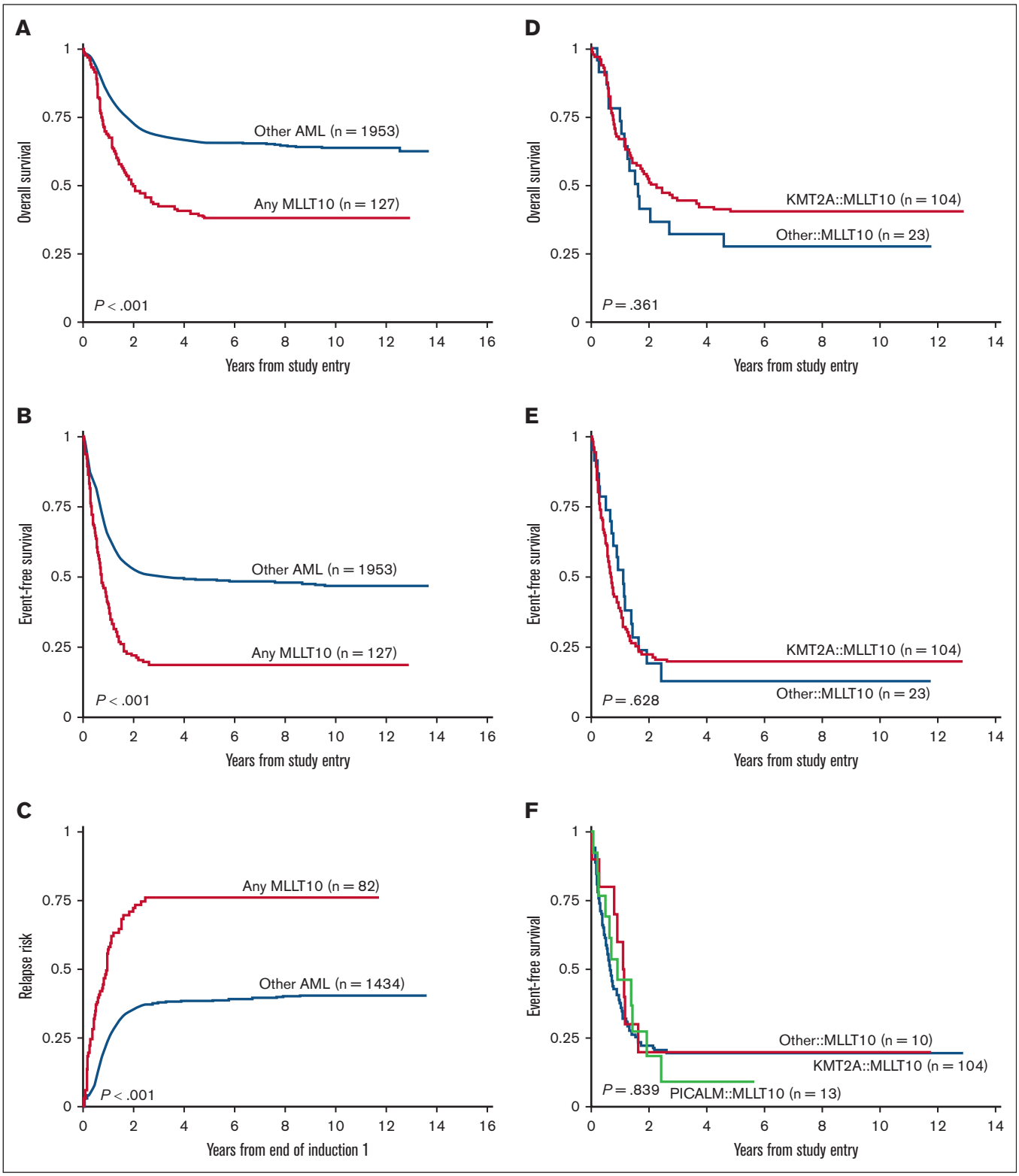

Figure 4. Kaplan-Meier curves. (A) OS, comparing the *MLLT10* cohort with other AML. (B) EFS, comparing the *MLLT10* cohort with other AML. (C) RR, comparing the *MLLT10* cohort with other AML. (D) OS comparing *KMT2A::MLLT10* with other *MLLT10*. (E) EFS, comparing *KMT2A::MLLT10* with other *MLLT10*. (F) EFS, comparing *KMT2A::MLLT10* vs *PICALM::MLLT10* vs *X::MLLT10*.

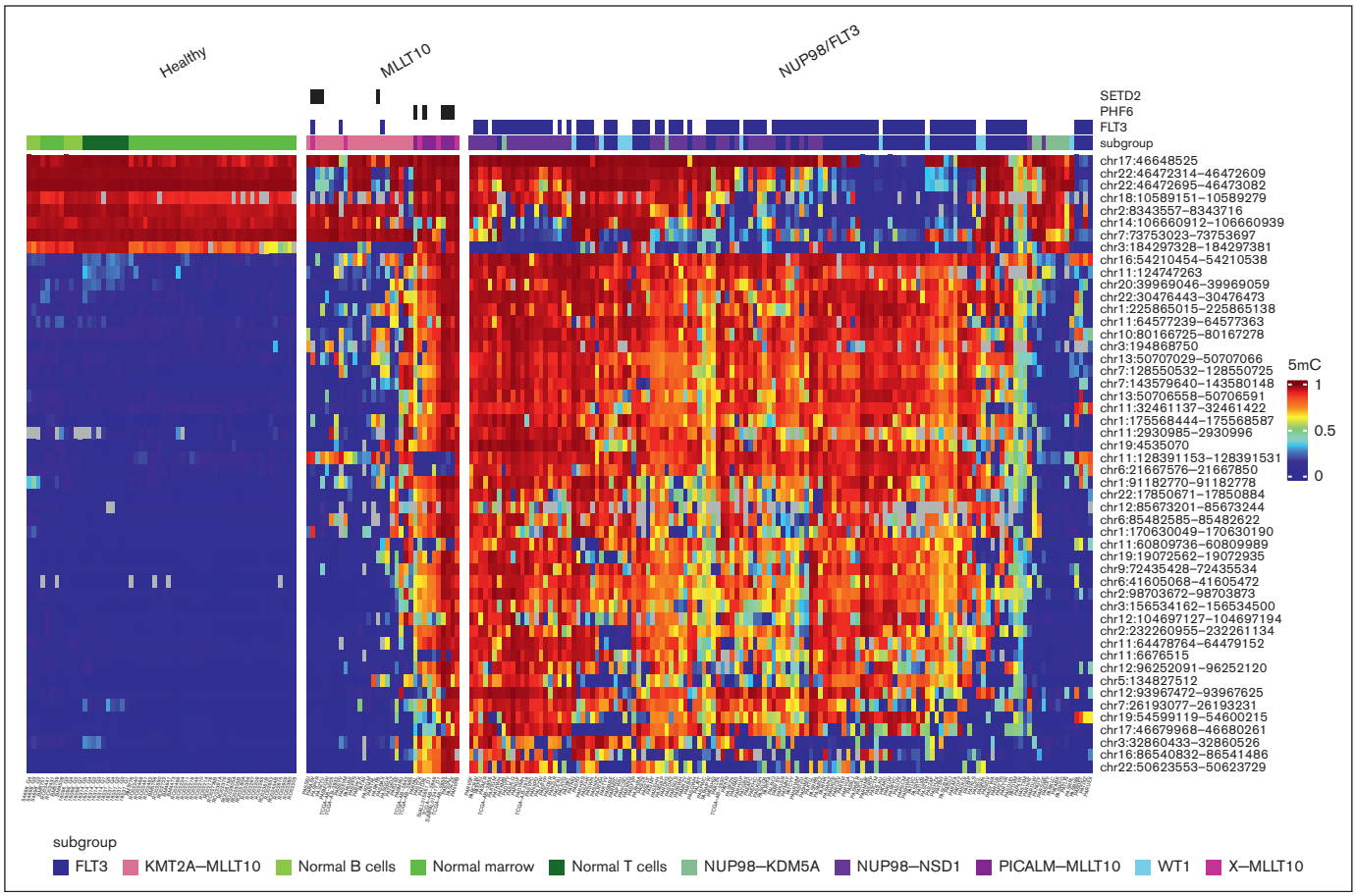

Figure 5. Heat map depicting the top 100 differentially expressed genes, comparing patients with *MLLT10* fusions with other AML subgroups. The right half shows unsupervised methylation analysis on highly variable methylation blocks (ie, perfectly correlated CpG sites adjacent to one another). No testing was performed before clustering, only ranking of features based on variability and clustered heat map of cases and fusion groups at these features. Color bars above heat map correspond to either cytogenetic alterations or *MLLT10* gene partners, as indicated on the right. Heat map depicting the 50 most discriminating DNA methylation regions. Specific AML subtypes are indicated above the heat map, including normal BM (NBM) controls, sorted T cells, sorted B cells, *NUP98* fusion AML cases and fusion-negative cases with *FLT3*-ITD or *WT1* mutations often seen in *NUP98-NSD1* cases.

that, the benefit of MRD measurement is variable among AML subtypes and studies and depends on the sensitivity of the applied method.[17,18] Reverse-transcription polymerase chain reaction analysis of fusion transcripts allows MRD detection with a sensitivity level of up to 0.001%, but it is applicable in only 50% to 60% of pediatric patients with AML with a detectable fusion gene. Next-generation sequencing MRD analysis allows the identification of molecular anomalies, but it is more relevant in AML with normal cytogenetics. Furthermore, the role of next-generation sequencing in MRD detection is still controversial in pediatric AML. Flow cytometry–based MRD has a lower sensitivity than reverse-transcription polymerase chain reaction (up to 0.01%), but it is applicable in >90% of patients with AML, and it is generally the method of choice for MRD detection in clinical AML trials.[19] In our study, flow cytometry–based MRD data were available in most patients (n = 114). Five-year DFS and OS from EOI-1 for patients who were MRD negative (22.4% and 46.4%, respectively) were better than that of patients with EOI-1 MRD positive status (9.1% and 13.6%, respectively).

The study by van Weelderen et al in childhood *KMT2A-r* AML showed that EOI-2 MRD negativity was associated with better EFS (47.6%, MRD negative vs 16.3%, MRD positive; $P < .0001$), and OS (66.0% vs 27.9%; $P < .0001$) and a trend toward lower cumulative incidence of relapse (46.1% vs 65.4%; $P = .016$).[8] In our cohort, although 84% of the patients harboring *KMT2A::MLLT10* fusion had negative MRD at EOI-2, their EFS was very low at 19.5%. Also, the group with other *MLLT10* fusions (*PICALM*- and *X*) had an even lower EFS of 12.7% despite 61% of them being MRD negative by EOI-2. These data suggest that MRD does not adequately predict relapse in these cytogenetic groups.

The small group who had *PICALM::MLLT10* fusion (13 patients) seem to have the worst EFS (9.2% vs 20% in other *MLLT10*; $P = .788$). Our study, to our knowledge, is the first showing outcome data in children with AML harboring this fusion. An adult series of adult patients with AML with *PICALM::MLLT10* fusion gene showed more frequent EM disease, younger age, a high

percentage of therapy-related cases, and a high relapse rate. In our cohort, the *PICALM::MLLT10* group were older, and 2 of 13 (15%) patients had CNS3 disease, 1 (7.7%) had non-CNS EM disease, and 4 (30.8%) had AML with myelodysplastic syndrome-related changes although not therapy related.

A study by Pollard et al in 215 children with *KMT2A-r* AML, showed that adding GO to conventional chemotherapy was associated with a reduced relapse rate, RR (40% GO vs 66% in patients without GO), improved 5-year DFS (GO 57% vs no GO 33%), and a better 5-year EFS (48% with GO vs no GO 29%). In addition, consolidation with HSCT in that study may further have enhanced outcomes (5-year RR with GO and HSCT, 28% vs 73% without GO and HSCT, $P = .006$).[20] In our study, GO appeared to benefit EFS in patients with *MLLT10*$^+$ AML (22.2% GO vs 8.3% no GO) but was not statistically significant ($P = .094$; supplemental Table 2).

Allogeneic HSCT in CR1 is considered by some collaborative groups as the recommended approach in high-risk AML subgroups with an EFS of <30%.[21] Long-term responses were seen in a subset of adult patients with *PICALM::MLLT10*$^+$ AML after HSCT in CR1 but also after high-dose cytarabine.[9] Among our patients with *MLLT10*$^+$ AML who underwent HSCT in CR1 (n = 16), the 5-year DFS was 37.5% (±24.2%) vs 28.7% (±11.7%) in those without HSCT ($P = .380$). Although HSCT in CR1 appeared to benefit survival rates, the difference was not statistically significant. Therefore, novel targeted therapies are urgently needed for this group of patients.

Transcriptome analysis of patients with *MLLT10* fusions showed that *HOXA* genes are overexpressed, especially the *HOXA11* gene. Indeed, *HOXA* gene upregulation (as in *KMT2A* rearrangements) is known to mediate leukemogenesis in *PICALM::MLLT10*–positive acute leukemia.[22] *MLLT10* interacts directly with *DOT1L* in a complex that regulates the methylation of *H3K79*. *MLLT10* fusions are thought to misdirect *DOT1L* to the promoters of *HOXA* genes, leading to hypermethylation of *H3K79*. This hypermethylation causes constitutive activation of *HOXA* activity, preventing cell maturation and differentiation.[22] The *DOT1L-MLLT10* complex has also been implicated in HOXA overexpression in patients with *NUP98-NSD1*–rearranged and *KMT2A*-rearranged fusion positivity. The similarities of hypermethylation seen in *MLLT10* and *NUP98* fusions show severe dysregulation of *HOX* gene clusters and may share mechanistic features leading to poor response to current therapeutic strategies.

Furthermore, variants in the histone methyltransferase *EZH2* were previously identified in patients (27%) with *PICALM::MLLT10*–positive acute leukemias.[14] *EZH2* is the catalytic subunit of the polycomb repressor complex, which decommissions *HOX* clusters, and thus may synergistically deregulate *HOXA* gene expression with *PICALM::MLLT10* in acute leukemia.

Structurally, the octapeptide motif/leucine zipper domain of *MLLT10* (residues 730-795) is required for *DOT1L* association via the *DOT1L* coiled-coiled domain and appears to be preserved in every *MLLT10* fusion, herein. Importantly, *MLLT10* fusions are usually formed by complex reciprocal translocations, such that *X::MLLT10* and *MLLT10::X* are transcribed. The *NAP1L1::MLLT10* fusion occurs via reciprocal translocation, and *TREH::MLLT10* is most likely present in the patient with *MLLT10::TREH.*

As documented by Deshpande et al,[23] and more recently by Gilan et al,[24] *DOT1L* (but not full-length *MLLT10*) is critical to *MLL* fusion–mediated leukemogenesis. It appears that this interaction is also the critical determinant of *MLLT10* fusion–mediated leukemogenesis. With few, if any, exceptions, *X::MLLT10* is thus likely the primary determinant of leukemogenesis. It is unclear whether reciprocal translocations (*MLLT10::X*) disrupt targeting and localization of the DOTCOM/SEC complexes in all, some, or none of the fusions reported herein; development of new cell line models may provide further insight.

Our study highlights the poor outcomes of children with AML and *MLLT10* fusions. There are currently no targeted therapies available for these patients, and intensive chemotherapy is the standard of care for *MLLT10*-rearranged leukemias. Anecdotal reports suggest that allogeneic HSCT could provide DFS benefit in T-cell receptor–negative T-cell ALL (T-ALL), early T-cell precursor ALL, or in adults patients with *MLLT10*-rearranged AML, but larger studies are warranted.[9,25-27] Furthermore, the benefit of HSCT in CR1 for pediatric AML with *MLLT10* fusions remains to be determined.

Early-stage clinical trials (www.clinicaltrials.gov identifiers NCT01684150 and NCT02141828) showed that the *DOT1L* inhibitor, pinometostat, reduces *H3K79* methylation, had acceptable toxicity, and modest efficacy as single agent in adults with *KMT2A*-rearranged acute leukemia.[28] Furthermore, recent work has shown monocytic leukemia zing finger (*KAT6A*) and eleven-nineteen leukemia chromatin reader (*MLLT1*) to be essential for *MLLT10*-driven leukemogenesis.[29,30] Importantly, histone acetyltransferase (*KAT*) inhibitors have shown preclinical efficacy against *PICALM::MLLT10* lines,[29] as has depletion of eleven-nineteen leukemia chromatin reader,[30] but not direct inhibition.[31] In addition, preclinical models demonstrate efficacy of *DOT1L* inhibition in primary *PICALM::MLLT10* AML cells[32] and in vitro AML models.[33]

EZH2 mutations were previously found in 27% of patients with *PICALM::MLLT10*–positive acute leukemias (1 AML and 3 T-ALL),[14] compared with 23% (3 of 13) in our *PICALM::MLLT10* cohort. *EZH1/2* inhibition with valemetostat is currently being evaluated in phase 2 adult trials for relapsed/refractory T-ALL,[34] and could be a promising targeted therapy for patients with *PICALM::MLLT10*–positive AML.

Azacytidine, a DNA demethylating agent, has shown anecdotal efficacy against promoter-hypermethylated infant *KMT2Ar* leukemia.[35] Other targeted therapies such as inhibitors of BET,[36] PARP1 (*Olaparib*),[37] and CDK9 [38] have shown preliminary efficacy in preclinical models of *KMT2A*-rearranged AML.

In our study, expression of *HOXA* genes and *MEIS1* were higher in the *KMT2A::MLLT10* group vs other-*MLLT10* cohorts. Blocking the menin–*KMT2A* interaction disrupts the assembly of oncogenic *KMT2A* wild-type or fusion complexes on chromatin. Preclinical studies demonstrated that menin inhibition downregulates *HOXA* and *MEIS1* transcription and reverses leukemogenesis in *KMT2Ar*- or *NPM1*-mutated leukemia.[39] Revumenib (SNDX-5613) is a potent oral inhibitor of the menin–*KMT2A* interaction. A recently published phase 1 dose-escalation study in children and adults with highly refractory acute leukemia with *KMT2Ar* or *NPM1*

mutation (www.clinicaltrials.gov identifier NCT04065399), menin inhibition with revumenib monotherapy was associated with promising antileukemic activity leading to deep and sustained remissions.[39]

Lastly, *SEDT2* mutations were found in 11% of our patients with *KMT2A::MLLT10* and 50% of those with *X::MLLT10*. Patients with renal cell carcinoma harboring *SEDT2* mutations had a higher response rate and prolonged OS rates when treated with immune checkpoint inhibitors.[40] Therefore, immune checkpoint blockade is a potential alternative therapy for patients with AML with *MLLT10* fusions and *SEDT2* mutations.

In summary, children with AML and *MLLT10* fusions have very dismal prognosis, regardless of the fusion partner, and should be prioritized for novel and alternative therapeutic interventions. It does not appear that these patients benefit from HSCT in CR1, although more studies are warranted. Large prospective trials with targeted therapies are required, although these cases are quite rare; and, it remains to be determined whether patients with rearranged *MLLT10* with different fusion partners would respond differently to specific targeted therapies.

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Authorship

Contribution: S.M., R.E.R., and T.T.Jr conceived and led the study; R.E.R., T.T.Jr, N.B., L.M.H., H.W., A.J.D., R.B.G., and T.A. generated, processed, and analyzed the data; O.A., R.E.R., T.T.Jr, R.B.G., J.E.F., E.A.K., and S.M. drafted the manuscript; and all authors edited and approved the final manuscript.

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