MLL Rearrangements Impact Outcome in *HOXA*-deregulated T-lineage Acute Lymphoblastic Leukemia: A Children's Oncology Group Study

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Approximately 15% of newly-diagnosed patients present with T-lineage acute lymphoblastic leukemia (T-ALL).[1] When matched for National Cancer Institute (NCI) risk criteria, patients with T-ALL are at greater risk of relapse than those with B-cell precursor ALL (BCP-ALL), warranting specialized therapies. Unlike BCP-ALL, where molecular abnormalities are commonly utilized for risk-adapted treatment,[2] the recurring molecular lesions found in T-ALL are not. While most patients with T-ALL can be cured, survival is poor for those with refractory disease or relapse.[3,4] With the advent of targeted therapies, efforts are underway to identify lesion-specific treatments for high-risk T-ALL.

In contrast to BCP-ALL, the molecular lesions that deregulate *Homeobox A (HOXA)* genes appear to be widely prevalent in T-ALL and acute myeloid leukemia (AML).[5,6] Deregulated *HOXA* expression commonly occurs in immature, T-cell precursors (ETPs), or can be acquired by leukemic cells harboring lesions involving mixed lineage leukemia gene rearrangements (*MLL*-R) at 11q23, *PICALM-AF10* at t(10;11)(p13;q14), *SET-NUP214* and inv(7)(p15q34).[5] Because there is a paucity of experience regarding the prognostic impact of *HOXA*-deregulating lesions in T-ALL, we utilized a retrospective cohort of 100 T-ALL patients enrolled on COG AALL0434 (NCT00408005) to analyze the cytogenetic and genomic features associated with treatment-related clinical outcomes. We enriched our cohort with 17 cases (17%) for whom induction failed (IF; M3 marrow at Day 29), allowing us to better evaluate the molecular lesions within this subset. The remaining cases were from patients who relapsed (REL; n = 8), or remained in complete continued remission (CCR; n =

75) 4 years. Detailed information regarding our approach is included in the Supplemental Methods, and in Tables S1 and S2.

Deregulated *HOXA* expression is a hallmark of *MLL-R* and *AF10*-R leukemias.[6,7] To identify the prevalence of these and other rearrangements in our 100-member cohort, we performed an iterative evaluation of cytogenetics, FISH, and RNA sequence analyses (Supplemental Methods). In 12 cases, we found *MLL-R*, including *MLL-AF6* (*KMT2A-MLLT4*; n = 4), *del3'MLL* (n = 3), *MLL-ENL* (*KMT2A-MLLT1*; n = 3), *MLL-PICALM* (*KMT2A-PICALM*; n = 1) and *MLL-AF17* (*KMT2A-MLLT6*; n = 1). Eight cases harbored re-arrangements of *AF10*, including *PICALM-AF10* (n = 6), and two with *DDX3X-AF10* lesions[8], one with a novel *CASK* gene fragment in a complex *CASK-DDX3X-AF10* translocation (Table 1, Figure S1). Five cases harbored other previously described lesions, two with inv(7)(p15q34) (#18, #91), two with *NUP98-R* fusions, and one with *HOXA10-TRBC*.[6,9] We identified four novel lesions: the first involving *LINC01250-CCDC91*, where a *trans*-Golgi transport regulator was fused to an intergenic region located 0.1 Mb upstream of *ETV6* (#27); a second involving a fusion between *RPP30-TLX1NB* (#28), which also had a *TRCB-MYB*[5] rearrangement; a third involving *NUP98* rearranged to an intergenic region at 2q32 (#52); and the fourth involved a *STAG2-LMO2* fusion (#96) (Table 1, S3 and S4). Although these newly-identified lesions showed *HOXA* deregulation, they represent distinct genetic subtypes of T-ALL where *HOXA* overexpression reflects stage of maturation arrest (data not shown).

To investigate upregulation of *HOXA* genes in T-ALL cases regardless genetic subtype of T-ALL we performed unsupervised hierarchical clustering using 25 probe sets for genes within the *HOXA* gene family. We identified a cluster of 20 cases that were characterized by increased expression of *HOXA* genes (FDR 0.05) (Figure S2, Table S5). Within this cluster, *HOXA9/10* had > 60-fold increased expression over baseline (*HOXA5* > 28 fold; *HOXA3*, *HOXA7* and *HOXA10* > 3 fold) (Table S5). We validated our profiling approach in independent 90-member patient series, reported by Soulier *et al.*,[6] confirming that *HOXA*-deregulated T-ALL is enriched for *MLL*-R and *AF10*-R (Figure S2, Table S6 and S7).

We hypothesized that subset analyses might identify lesions that were associated with refractory or relapsed disease. We found that *MLL-R*, but not *AF10-R* correlated with IF in T-ALL (P = 0.005) (Table S8). We found that cases with *MLL*-R had an inferior EFS compared to those that did not (P = 0.0035) (Figure 1A). Univariate and multivariate regression analyses indicated that *MLL-R* were significantly associated with IF (P = 0.003, 0.003) and EFS (P = 0.009, 0.008) after adjusting for the effects of age and WBC (Table S9 and S10). Patients bearing the ETP-ALL phenotype have been characterized as having poorly differentiated, stem-cell like immunophenotype. Because *MLL- and AF10*-R leukemias also demonstrate features of undifferentiated leukemias, and the COG immunophenotypic flow analyses for ETP-ALL status were not completely assessed for all patients, we utilized expression profiling to distinguish cases represented by immature, early T-cell precursors (ETPs) using the gene signature developed by Coustan-Smith *et al.* (Table 1, Figure S3). We found an association between ETPs and early treatment response (P = 0.01) (Table S8) and an inferior EFS (P = 0.029) (Figure S4), thus we assessed whether ETPs cases are enriched with translocations harboring *MLL* or *AF10* genes. We found a

marginally significant association between the presence of *MLL*-R and the ETPs status in our cohort (P = 0.07, Table S11). To investigate the effects of ETPs/*MLL*-R on T-ALL patient outcome, we next tested the relationship of *MLL*-R with IF, adjusting for ETPs phenotype, and found that the signature of ETPs/*MLL*-R was associated with IF in T-ALL (P = 0.01) (Table S12 and S13). We extended these observations to assess the impact of ETPs/*MLL*-R on EFS, and found a significant association with refractory disease and relapse (P = 0.005) (Figure 1B). Because MRD has emerged as a prognostic indicator of high-risk disease in T-ALL, we investigated the MRD status in *MLL*-R cases depending on the fusion partner or 3'-deletion. Disease progression was significantly associated with *MLL-AF6*, FISH-identified *del3'MLL*; exceptions occurring only if Day 29 MRD was < 0.1 (Table 1, Figure 1C). Interestingly, no patient with *MLL-ENL* failed therapy, despite Day 29 MRD levels ranging from < 0.01 to 2.8%, supporting reports that they do well with modern therapies. While *AF10*-R have been reported to confer an adverse risk in adult T-ALL[10] in association with ETP features, we observed that only those with Day 29 Induction ≥ 10% failed treatment, in contrast to patients with MRD < 10%, who maintained a durable first remission when treated on AALL0434 and its single delayed intensification phase.

Because the recurring cytogenetic abnormalities that deregulate *HOXA* in T-ALL have not been systematically evaluated, the potential impact of such lesions on outcome has been unclear. Here, we profiled 100 patients for *HOXA*-deregulated T-ALL to determine whether the related molecular lesions might correspond with treatment outcome. The prognostic impact of *MLL-R* and *AF10*-R in T-ALL has been less clear, in part due to their rarity and difficulties in detection, and because of their variable T-cell stage of arrest. In our 100-case series, the molecular repertoire of T-ALL fusions, deletions and inversions was highly heterogeneous, with many lesions occurring with a frequency of 5% or less, and, in ~30% cases cytogenetic analyses were never performed (data not shown). It is therefore not surprising that the prognostic impact of molecular lesions remains an unanswered question in T-ALL.

Since first described by Coustan-Smith *et al.*, patients with the ETP-ALL phenotype have received much attention for their unique biological profile and increased risk for relapse.[11] Recently, ETP-ALL patients have been reported to have similar outcomes as non-ETP patients on the UKALL 2003 and COG AALL0434 studies.[12] When analyzed as a continuous variable on the AALL0434 study, Induction Day 29 MRD ≥ 10% was highly predictive of relapse, if not outright induction failure, but not ETP-ALL.[13] Moreover, *HOXA* deregulation does not confer a worse prognosis in T-ALL,[5] but we identified a subset of *HOXA*-deregulated cases having high end-induction MRD with *MLL-R* and *AF10*-R that failed therapy, suggesting that such patients might benefit from early identification, follow-up MRD monitoring, and/or alternate approaches to therapy. We have also shown that patients with *MLL*-driven immature cells, having ETPs features, were likely to fail therapy, especially when involving *MLL-AF6* or *del3'MLL* rearrangements. While *MLL-AF6* lesions have been reported to confer a worse prognosis in AML,[14] we are the first to show their impact on outcome in T-ALL. In contrast, our results support the findings by Nigro *et al.*[15] showing that in pediatric T-ALL, *AF10*-R tend to be more commonly arrested in more differentiated state, and without an adverse effect on outcome (Figure 1).

There is pressing need to re-evaluate the role of routine cytogenetics/FISH testing in T-ALL. Because IF is a relatively rare event in the current era of modern therapies, the identification of molecular biomarkers relevant to disease resistance and treatment failure has been challenging. Enrichment of the tested cohort in IF cases allowed us to show that *MLL* rearrangements are determinants of high-risk disease in T-ALL. In addition to testing all samples for MRD at the end-Induction and end-Consolidation, we propose that cytogenetic tests be performed on all T-ALL patients at diagnosis specifically including testing for *MLL*-R and *AF10*-R. In cases where Day 29 MRD is 0.1%, follow-up MRD testing might be used to intensify conventional therapy, pursue targeted therapies, or consider transplant in first remission. Further studies are warranted to validate our findings in larger retrospective cohorts or early clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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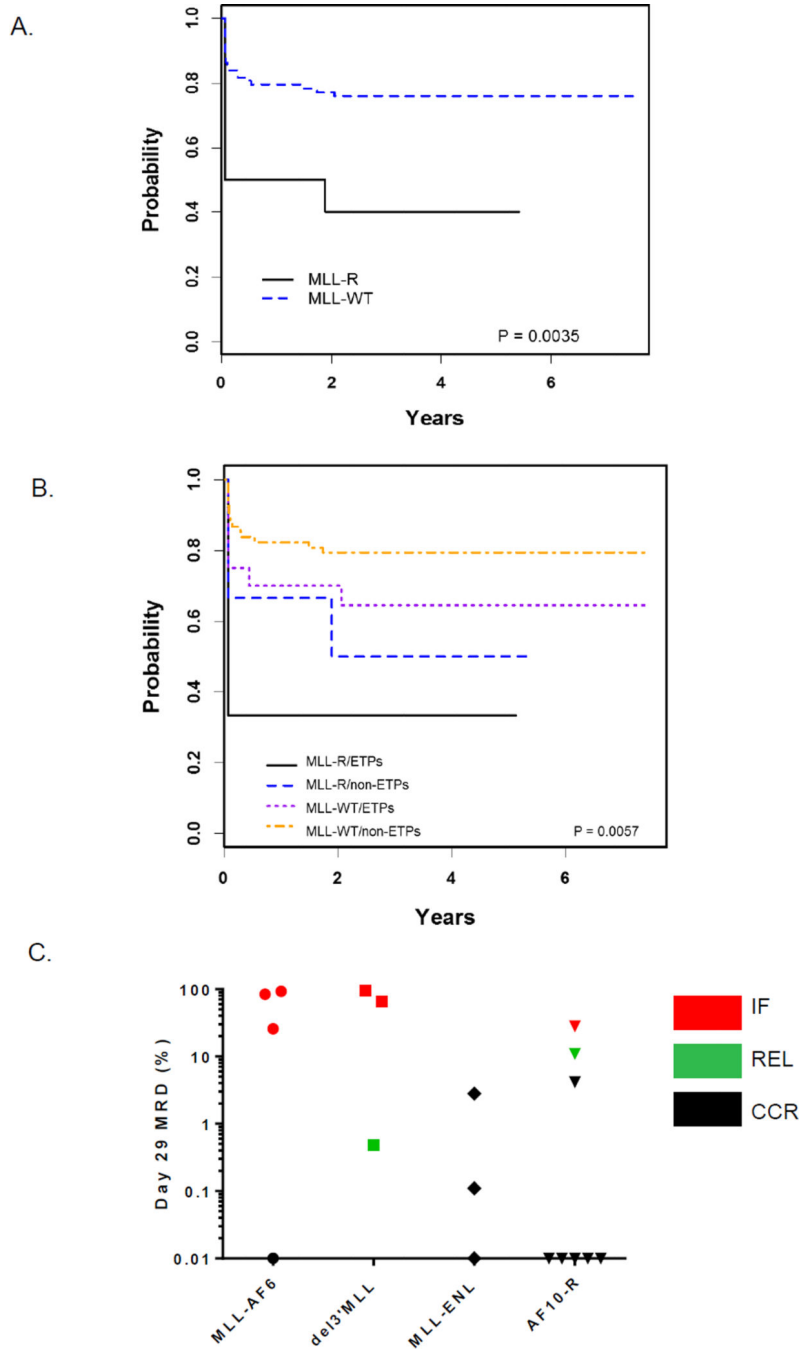
Figure 1.
Event free survival in molecular interrogation cohort of 100 T-ALL (COG AALL0434). **(A)** EFS for *MLL*-R (n = 12; black line) vs those with *MLL* germline (*MLL*-WT, n = 88; blue dash line) (log rank Mantell-Cox, P = 0.0035). **(B)** EFS for *MLL*-R having the ETPs phenotype by GEP (*MLL*-R/ETPs, n = 6; black solid line) vs. *MLL*-R cases without the ETPs signature (MLL-R/non-ETPs, n = 6; blue dash line) vs. non-rearranged MLL with ETPs phenotype by GEP (MLL-WT/ETPs, n = 20; purple, dot line) vs non-rearranged *MLL* without the genomic ETPs signature (*MLL*-WT/non-ETPs, n = 68; yellow dash-dot line)

(log rank Mantell-Cox, P = 0.0057). *MLL*-R/ETPs vs. *MLL*-WT/non-ETPs, P = 0.0004; *MLL*-R/non-ETPs vs. *MLL*-WT/non-ETPs, P = 0.0752; *MLL*-R/ETPs vs. *MLL*-WT/ETPs, P = 0.1364; *MLL*-WT/ETPs vs. *MLL*-WT/non-ETPs, P = 0.1399. **(C)** Post-Induction Day 29 MRD levels in T-ALL patients (COG AALL0434) with specific *MLL*-R (n = 10) and *AF10*-R (n = 8) (Table 1) measured by flow cytometry (circles – *MLL-AF6*, squares – FISH-identified del3' *MLL*, diamond - *MLL-ENL*, triangle - *AF10*-R; red - IF; green - REL; black - CCR).

Table 1

Karyotypic, FISH and molecular classification for 23 patients treated on AALL0434 with features of *HOXA* overexpression and/or *MLL-R/AF10-R*. All FISH analyses were done in CLIA-approved reference centers. No cases of *AF10-R* were initially found at diagnosis.

ID	Age	Gender	WBC	CNS	ETPs by GEP	Karyotype	Day 29 MRD (%)	FISH	RNA seq	Lesion
7	9	Male	6.8	1	ETPs	46,XX,-t(6;11)(q27;q23)[10]/46,XY[10]	25.8	MLL-AF6		MLL-AF6
25	7	Female	196	2		46,XX,t(6;11)(q27;q23),add(12)(p11.2)[6]/46,XX[14]	84	MLL-AF6		MLL-AF6
43	7	Female	144.9	1	ETPs		93	**MLL-AF6**		MLL-AF6
79	16	Female	58.1	1		46,XX,t(7;12)(q34;p13),del(11)(q22q23)[8]/46,XX[2]	<0.01		MLL-AF6	MLL-AF6
67#	8	*Male*	577.5	2		*46,XY,t(4;7)(q12;q36)[6]/43-46,XY,add(20)(p12)[cp5]*	0.48	*del3'MLL*		*del3'MLL*
89	18	Male	6.6	1	ETPs	46,XX,ider(1)(p10)add(1)(p34),del(11)(q23),-21,+mar[3]/46,XY[37]	94.3	del3'MLL		del3'MLL
77#	16	*Male*	379.8	1		*46,XY,der(3)t(3;9)(q12;q34),t(4;7)(q21;q22),add(5)(q13),add(9)(p22),add(9)(q22),del(11)(q23),del(13)(q12q14),der(18)t(1;18)(q21;q23)[19]/46,XY[1]*	65.6	*del3'MLL*		*del3'MLL*
23	12	Male	48.3	1	ETPs	46,XY,t(11;19)(q23;p13.3)[20]	0.11	MLL-ENL		MLL-ENL
31	9	Male	139.1	2		46,XY,t(11;19)(q23;p13.3)[20]/46,XY[3]	<0.01	MLL-ENL		MLL-ENL
85 *	2	Male	440	1			2.8	**MLL-ENL**		MLL-ENL
59	17	Female	260.6	1	ETPs	47,XX,del(1)(q32),del(5)(q22),der(11)t(11;17)(q23;q21),+15,-17,+mar[cp8]/46,XX[12]	<0.01	MLL-AF17		MLL-AF17
21	12	Male	389	1	ETPs	46,XY,der(11)t(11;14)(p13;q11.2)t(11;15)(q21;q22),der(14)t(11;14)(p13;q11.2),der(15)t(11;15)(q21;q22)[4]/46,XY[16]	50.8		MLL-PICALM	MLL-PICALM
1	7	Male	9.6	1		46,XY[20]	<0.01	**PICALM-AF10**	PICALM-AF10	PICALM-AF10
94	5	Female	158.9	1			<0.01	**PICALM-AF10**		PICALM-AF10

ID	Age	Gender	WBC	CNS	ETPs by GEP	Day 29 MRD (%)	Karyotype	FISH	RNA seq	Lesion
46#	7	Male	19.6	1		< 0.01	45,XYY?c;dic(7;12)(p11.2;p11.2),del(9)(p13p24),t(10;11)(p12;q14),-21[3]/47,XYY?c[17]		PICALM-AF10	PICALM-AF10
68	7	Female	91.9	2		4.2	46,X,add(X)(q26),del(5)(q31),t(10;11)(p12;q21)[13]/46,XX[7]	**PICALM-AF10**	PICALM-AF10	PICALM-AF10
72	27	Male	19	1	ETPs	28.4	46,XY,t(10;11)(p13;q21)[8]/45,idem,-9,-9,+mar[4]/46,XY[8]	**AF10-R**	PICALM-AF10	PICALM-AF10
11	14	Male	243.4	1		11	46,XY,add(10)(p13),del(11)(q21)x2,add(12)(p11.2)[5]/46,XY	**PICALM-AF10**		PICALM-AF10
16	5	Male	142.1	1		< 0.01	46,Y,t(X;10)(p10;p10)[18]/46,XY[2]	**AF10-R**	CASK-DDX3X-AF10	CASK-DDX3X-AF10
34	14	Male	72.5	3		< 0.01	46,XY,der(9)(qter->q34::p?24->qter)/46,XY	**AF10-R**	DDX3-AF10	DDX3X-AF10
52	12	Male	116.7	1		48.3	45,XY,add(2)(q21),add(11)(p11.2),der(12;17)(p10;p10)[20]		NUP98-R	NUP98-IGR(2q32.3)
18	11	Male	123	2		< 0.01	46,XY,inv(7)(p15q34),del(12)(p12)[17]/46,XY[10]		TCRB-HOXA10	TCRB-HOXA10
27	7	Female	351.4	1	ETPs	88.3	46,XX,del(2)(q33),del(12)(p12)[20]		LINC01252-CCDC91	IGR(12p13.2)-CCDC91

*MLL-ENL fusion that was missed at diagnosis.

(Italics) cases (#67, #77, #46) that did not fall within 20-member HOXA cluster.

Bold: Supplemental FISH screening at Mayo Clinic; LINC – long intergenic noncoding RNA; IGR - intergenic region.