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To the editor:

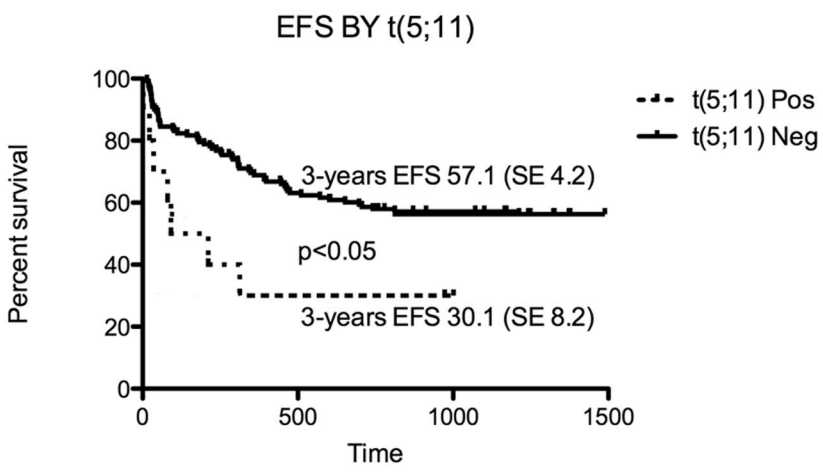
Screening of novel genetic aberrations in pediatric acute myeloid leukemia: a report from the AIEOP AML-2002 study group

Acute myeloid leukemia (AML) is a heterogeneous disease with known specific recurrent genetic aberrations. The continuous and increasing identification of new genetic lesions has permitted the identification of new subgroups of patients with different prognosis.[1] In the present work, we evaluated the incidence of rare genetic abnormalities in pediatric AML such as del(4)(q12)FIP1L1-PDGFRA, t(16;21)(p11;q22)FUS/ERG, t(8;16)(p11;p13)MOZ/CBP, t(11;17)(q23;q12-21)MLL/AF17, t(4;11)(q35;q23)MLL/ArgB2, t(5;11)(q35;p15.5)NUP98/NSD1, t(3;5)(q25;q34)NPM1/MLF1, and MLLPTD in 306 children with newly diagnosed de novo AML other than acute promyelocytic leukemia enrolled in Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) centers from 2000 to 2009,[2] all negative for known recurrent genetic abnormalities involving *MLL*, *CBFB*, and *FLT3* genes (77 males and 77 females, median age at diagnosis 7.2 years, range 17 days to 17 years). RNA was extracted from fresh bone marrow at diagnosis, and multiplex RT-PCR was used. Sequencing by Sanger method was applied to all positive cases to characterize fusion breakpoints. We identified 1 patient each positive for t(16;21)(p11;q22)FUS/ERG, t(11;17)(q23;q12-21)MLL/AF17, and t(4;11)(q35;q23)MLL/ArgB2, respectively, suggesting that these rearrangements are extremely rare in pediatric AML. Two of the 306 patients had del(4)(q12)FIP1L1/PDGFRA, and 4 had t(8;16)(p11;p13)MOZ/CBP. Interestingly, 6 patients (2%) had t(3;5)(q25;q34)NPM1/MLF1, 6 (2%) had MLLPTD, and 6 (2%) were found to carry t(5;11)(q35;p15.5)NUP98/NSD1. In our pediatric cohort, the incidence of this last aberration is lower than that previously reported by Hollink et al.[3] Subsequently, because a strong association of t(5;11) fusion with FLT3ITD has been described (91%),[3] we extended the screening to 42 children with de novo AML harboring the FLT3ITD mutation, enrolled in the AIEOP-LAM 2002 protocol. We found that 6 of 42 (14%) had the NUP98-NSD1 fusion. So, 6 of 12 NUP98/NSD1-positive patients (50%) were FLT3ITD positive, showing a lower association in our pediatric cohort for these 2 aberrancies than that reported by Hollink et al.[3] Then, we looked at the event-free survival (EFS) of patients with t(5;11)NUP98-NDS1 ($n = 12$) and found that it was worse, compared with patients negative for known molecular lesions and enrolled into the LAM 2002-AIEOP protocol (30.1% vs 57.1% at 3 years, $P < .05$).[4] Furthermore, we did not find any difference in either clinical or biologic features between patients with isolated t(5;11) and those with t(5;11) + FLT3ITD (Figure 1). The 8-year EFS of FLT3ITD+ children who did or did not carry t(5,11) was 33.3% and 42.7% ($P = .2$), respectively. This finding suggested that NUP98/NSD1 fusion protein identifies a previously unrecognized subgroup of FLT3ITD patients with an even worse prognosis.

To test whether MLLPTD might also play a role in the occurrence of childhood AML relapse, we analyzed samples from 40 AML patients at relapse, never finding this abnormality. By contrast, 4 patients harbored at relapse the same MLLPTD found at diagnosis, suggesting the stability of this mutation.

In summary, we confirm that t(5,11) is not exceptional in pediatric AML, being frequently associated with FLT3ITD, and identifying patients at high risk of treatment failure. We also suggest a negative role of this translocation in FLT3ITD positive patients to be further considered in the risk stratification of patients. The putative role of the remaining rare abnormalities[5,6] in AML remains to be confirmed in prospective studies with larger cohorts of patients.

A



B

NUP98 partner gene	Age,y	Sex	WBC x $10^{9/L}$	FAB	Cytogentic aberration(s)	Mutations	CR	Relapse (relapse-free survival in mo.)	Dead (OS in mo.)
NDS1	14	M	404	M2	Normal	FLT-3 ITD	+	-	+ (15.8)
NDS1	15	F	327	M5	Normal	FLT-3 ITD	-		+ (52.4)
NDS1	11	M	78	M2	Normal	FLT-3 ITD	+	-	- (33.3)
NDS1	7	M	202	M5	Normal	FLT-3 ITD	-		+ (14.4)
NDS1	3	F	2.8	M4	Normal	FLT-3 ITD	+	-	- (32.4)
NDS1	3	F	21	M5	Normal	-	+	- (44.0)	- (44.0)
NDS1	9	F	17.9	M2	Normal	-	-		- (10.6)
NDS1	16	M	350	M1	Normal	-	+	+(12.4)	+ (20.2)
NDS1	2	M	4.1	M2	Normal	-	-		+(11.4)
NDS1	2	M	83	M2	Normal	-	-		+(16.3)
NDS1	10	F	218.5	M5	Complex Karyotype	FLT-3 ITD	-		+ (3.3)
NDS1	15	F	62.8	M1	Normal	-	-		+ (17.3)

Figure 1. Clinical features of pediatric patients. (A) Probability of event-free survival (EFS) in children with NUP98/NSD1 rearrangement in AML. EFS for patients NUP98/NSD1-positive (n = 12, 30.1%) versus negative patients (n = 142, 57.1%). (B) NUP98/NSD1 rearranged patients' main features.

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To the editor:

P-selectin glycoprotein ligand-1 inhibition blocks increased leukocyte-endothelial interactions associated with sickle cell disease in mice

Sickle cell disease (SCD) is a hemoglobinopathy caused by a single amino acid change in the β globin subunit that predisposes hemoglobin to abnormal polymerization leading to stiff, sickled erythrocytes, and microvascular occlusions.[1] Although the vascular consequences of SCD are triggered by changes in the erythrocyte,[2] a chronic inflammatory state often ensues with many other cell types contributing to SCD pathology. For example, sickling of erythrocytes in the microvasculature is accompanied by increased platelet and leukocyte-endothelial (L-E) interactions that are strongly associated with vascular complications[3] including stroke, priapism, leg ulcers, and nephropathy.[4] Therapeutic targeting of molecules responsible for mediating these adhesive interactions has been shown to be potentially beneficial in preclinical models of SCD.[5] Inhibition of P-selectin in particular has been associated with reduced L-E interactions in mouse models of SCD.[6] The leukocyte ligand for P-selectin is P-selectin glycoprotein ligand-1 (Psgl-1), which also interacts with E- and L-selectin.

Psgl-1 inhibition may be particularly effective in reducing adhesive interactions in SCD since Psgl-1–mediated signaling also affects activation of leukocytes,[7] which in turn affects adhesive properties of the endothelium.[8] To determine the effectiveness of Psgl-1 inhibition on reduction of L-E interactions in the microvasculature, we studied a humanized mouse model of SCD[9] treated with an anti–mouse Psgl-1 antibody. First, C57BL/6J mice underwent a bone marrow transplantation (BMT) procedure as previously described.[8] Recipient mice received bone marrow from wild-type donors ($Hbb^{+/+}$) or donors homozygous for the sickle cell mutation ($Hbb^{h\beta s/h\beta s}$). Eight weeks after BMT, mice receiving $Hbb^{h\beta s/h\beta s}$ marrow (n = 10) were anemic compared with mice receiving $Hbb^{+/+}$ marrow (n = 10; hematocrit - 28.9 ± 1.0 vs 36.7 ± 0.8%, $P < .0001$), (hemoglobin - 10.0 ± 0.5 vs 12.3 ± 0.4 g/dL, $P < .001$) and (RBC counts 7.5 ± 0.5 vs 9.1 ± 0.2 × 10^6/μL, $P < .01$). Sickle-shaped erythrocytes were frequent in the peripheral blood smears of $Hbb^{h\beta s/h\beta s}$ mice and $Hbb^{h\beta s/h\beta s}$ mice exhibited a marked reticulocytosis compared with $Hbb^{+/+}$ mice (reticulocyte - 30.2 ± 4.4 vs 3.4 ± 0.6% of RBCs, $P < .00001$). At the time of sacrifice (20 weeks after BMT), the average spleen size of $Hbb^{h\beta s/h\beta s}$ mice was 370.7 ± 55.4 mg compared with 74.9 ± 2.4 mg in $Hbb^{+/+}$ mice, $P < .000001$. At 16 weeks post BMT, mice were treated with weekly IV injection of anti–Psgl-1 antibody or control IgG antibody for 4 weeks. Intravital microscopy[8] was performed 20 weeks following BMT using intravenous rhodamine to label leukocytes. Leukocyte rolling and firm attachment were increased in $Hbb^{h\beta s/h\beta s}$ mice compared with $Hbb^{+/+}$ mice. However, anti–Psgl-1 treatment completely reversed the increased leukocyte rolling and firm attachment within the $Hbb^{h\beta s/h\beta s}$ mice. (Figure 1A-D). Reduced L-E interactions were associated with reductions in levels of circulating soluble E-selectin (sE-sel), soluble P-selectin (sP-sel), and soluble vascular cell adhesion molecule 1(sVCAM-1; Figure 1G-I). Four weeks of treatment was sufficient to reduce iron deposition and necrotic areas of liver (Figure 1L,O). This was associated with reduced CD68 and TNF-α expression in the liver by RT-PCR (Figure 1E-F). In conclusion, inhibition of Psgl-1 may be an effective treatment for reducing vascular complications of SCD.

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