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Prognostic Features in Acute Megakaryoblastic Leukemia in Children without Down Syndrome: A Report from the AML02 Multicenter Trial and the Children's Oncology Group study POG 9421

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Letter to the Editor

While increased treatment intensity has improved outcomes for children with acute megakaryoblastic leukemia (AMKL), the prognostic and therapeutic implications of megakaryoblastic differentiation remain controversial, with some groups treating such disease as high risk and recommending hematopoietic stem cell transplantation (HSCT) during first remission, while others treat as standard risk in the absence of unfavorable cytogenetics and/or a poor response to induction therapy.(1–5) The t(1;22)(p13;q13) translocation leads to the *RBM15*(*OTT*)-*MKL1*(*MAL*) fusion protein and predominates in infants with AMKL.(6, 7) Retrospective studies have reported conflicting data on long term outcomes for the t(1;22) subgroup.(8, 9) We report the outcomes of children with AMKL treated on the multicenter AML02 protocol (2002–2008) and on Pediatric Oncology Group (POG) protocol 9421 (1995–1999).(5, 10) Details of patient eligibility and treatment have been previously reported; written informed consent was obtained from patients or their guardians in accordance with supervising Institutional Review Boards.

Of the 565 patients enrolled on POG 9421, 49 (8.7%) had AMKL (Table 1). The complete response (CR) rate after 2 cycles of induction therapy was 79.6%, with no difference

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between standard and high-dose cytarabine regimens, which was similar to the non-AMKL cohort (84.5%). After induction, 44 patients with at least partial response (PR) continued on study; 6 underwent protocol-specified HSCT from an HLA-matched sibling donor (4 remain in first CR), while 38 received consolidation chemotherapy (13 remain in first CR). The outcomes for patients with AMKL (5-year rates: EFS, 34.7% ± 7.5%; OS, 36.3% ± 7.5%) were similar to those of patients with other AML subtypes who lacked favorable cytogenetic features (5-year rates: EFS 33.9% ± 2.5%; OS, 45.8% ± 2.6%).

Of the 39 AMKL patients with available cytogenetics, the single patient with the t(1;22) was a long-term survivor after chemotherapy without HSCT. Because specific cytogenetic abnormalities in childhood AML have been associated with poor prognosis, patients were categorized based on the presence or absence of high risk cytogenetic features as defined by analysis of large Medical Research Council (MRC) and Berlin-Frankfurt-Munster (BFM) childhood AML cohorts.(11, 12) The 5-year OS rate was similar between patients with or without high risk cytogenetic abnormalities as defined by BFM (high risk n=11) or MRC (high risk n=3) criteria. With regard to the prognostic impact of HSCT during the first remission, statistical comparison is limited by the small number of patients undergoing transplantation. The 5-year OS rate was 66.7% ± 22% for patients who received a protocol-specified, HLA-matched sibling HSCT in CR or PR, but only 33.5% ± 8.5% for those receiving chemotherapy ($P = 0.2$).

Of the 232 patients enrolled on the AML02 protocol, 26 (11%) had AMKL (Table 1). They lacked favorable cytogenetic features and had a high frequency of miscellaneous cytogenetic abnormalities. Five patients had the t(1;22). The *FLT3* gene was wild-type in the 17 cases analyzed. Twenty-five patients were randomized (12 standard-dose cytarabine, 13 high-dose cytarabine) for induction 1. All patients had morphologic remission after 2 cycles of induction chemotherapy.

MRD was measured in 24 of the 26 AMKL patients after induction 1 and 2: 10 (42%) patients had positive MRD (≥ 0.1%) after induction 1 and 6 (25%) after induction 2. There was no significant difference in the MRD-positive rates between patients with AMKL and those without AMKL (Table 1). Remission induction rates and MRD-negative rates for the AMKL cohort did not differ between the high- and standard-dose cytarabine arms. Of the 6 patients with positive MRD (0.12%–3.92%) after induction 2, 5 underwent HSCT, and 2 are alive in first remission at last follow-up. Of the 18 patients without MRD after the second induction, 10 are alive in first remission, including 4 of the 9 patients who underwent HSCT and 6 of the 9 patients who received chemotherapy only. Notably, of the 12 patients with MRD < 0.1% at both measurements (i.e., post induction 1 and 2), 8 are alive in first remission, including 5 who received only chemotherapy.

The 5 AMKL patients with the t(1;22) had excellent outcomes: all experienced complete remission, the 4 with evaluable MRD samples were negative. All were treated with consolidation chemotherapy without HSCT; 2 participated in the St. Jude NKAML pilot trial of low-dose immunosuppression followed by donor-recipient inhibitory, KIR-HLA–mismatched, NK-cell infusion.(13) All are alive in first remission with a median follow-up of 3.5 years (range, 1.4–6.1 years).

Other than the t(1;22), there were no recurring cytogenetic abnormalities, although complex karyotypes (≥3 independent abnormalities) were common. According to the MRC cytogenetic criteria, no AMKL patient on the AML02 protocol would have been classified as having high risk disease; however, based on karyotypes with 3 or more independent abnormalities, 10 patients would have been classified as having high risk cytogenetics by BFM criteria, including two of the cases with the t(1;22).(11, 12) Outcomes for patients with high risk BFM cytogenetics were similar to those of patients without such characteristics.

The 3-year EFS probability for patients with non-t(1;22) AMKL treated on AML02 (36.3% ± 10.9%) did not significantly differ from that of non-AMKL patients without favorable cytogenetics (56.1% ± 5.3%, $P = 0.19$, Figure 1a). However, OS was significantly inferior for patients with non-t(1;22) AMKL patients compared to those with AML without favorable cytogenetics (3-year estimates, 42.4% ± 11.4% vs. 66.1% ± 5%, $P = 0.02$, Figure 1b). Furthermore, the OS rate for patients with non-t(1;22) AMKL (42.4% ± 11.4%)) was significantly worse than that for patients considered to be standard risk (69.9% ± 6.3%, $P = 0.01$), but not significantly different from that of high risk patients (60.4% ± 8.5%, $P = 0.11$). Of the 21 AMKL patients without the t(1;22), 14 were treated with HSCT in first remission, with 3-year EFS 41.7% ± 13% and 3-year OS 49% ± 13.2%; these outcomes did not differ significantly from the 7 patients treated with consolidation chemotherapy only (3-yr EFS, 28.6% ± 17.1%, $P = 0.78$; 3-yr OS, 25% ± 15.3%, $P = 0.43$).

CD36 expression was documented by flow cytometric immunophenotyping of diagnostic bone marrow samples in 16 of 26 patients. Of the 6 patients with unequivocal positive CD36 expression (i.e., >90% of blasts), all experienced MRD-negative remission after induction 1, and 5 remain alive in first remission, 4 after HSCT and 1 after chemotherapy only. Nine patients had leukemic blasts that did not express CD36; 2 of these patients had the t(1;22) and had good outcomes as noted previously. In contrast, for the 7 patients without the t(1;22) and without CD36 expression, 5 had detectable MRD after induction 1, and only 2 are alive in first remission.

The results of AML02 suggest that the t(1;22) may confer a favorable prognosis compared to other subtypes of AMKL in the context of intensive chemotherapy and adequate supportive care, as the five infants with this karyotype had excellent outcomes. These results support the findings of a retrospective series of 30 pediatric AMKL patients in which 6 of the 11 patients with the t(1;22) were long-term survivors while none of the AMKL patients without the t(1;22) survived.(9) Based on the AML02 results, the t(1;22) is considered a standard-risk feature in the successor AML08 trial (NCT00703820). On the AML08 trial, patients with non-t(1;22) AMKL continue to be regarded as high risk and are recommended to undergo HSCT in first remission. Given the report of detection of the *RBM15*(*OTT*)-*MKL1*(*MAL*) fusion transcript in a patient with normal metaphase cytogenetics,(9) we suggest that infants presenting with AMKL but without the t(1;22) should be evaluated for the fusion transcript by RT-PCR.

Descriptive analyses suggest that MRD of at least 0.1% after induction 2 and lack of CD36 expression on leukemic blasts may be associated with inferior outcome. Although induction was successful among the AMKL patients on AML02, detection of MRD at the end of

induction 2 correlated with a very poor prognosis. While MRD as a measure of early response to therapy has emerged as an independent prognostic factor regardless of megakaryoblastic differentiation (5, 14), CD36 expression may be a prognostic feature specific to AMKL. High CD36 expression was associated with greater *in vitro* sensitivity to cytarabine and daunorubicin as well as favorable outcomes in a small subset of patients treated on POG 9421.(15)

On AML02, patients who did not proceed to HSCT after induction 2 received intensified therapy with cumulative cytarabine exposure of 52–68 g/m^2 over 5 cycles of chemotherapy. Despite this therapy intensification, patients with AMKL without the t(1;22) did not fare better than those who received less intensive regimens such as POG 9421. The lack of improvement despite significant therapy intensification suggests that novel agents are needed, particularly for the subset of patients with non-t(1;22) AMKL. Given the rarity of AMKL in general and of the t(1;22) subgroup in particular, an international effort to combine data from different multicenter trials will be necessary to validate the observation of favorable outcomes for children with the t(1;22) in this study, to explore the favorable prognostic significance of high CD36 expression, and to identify novel therapeutic strategies to improve outcomes for those patients with AMKL lacking these favorable features.

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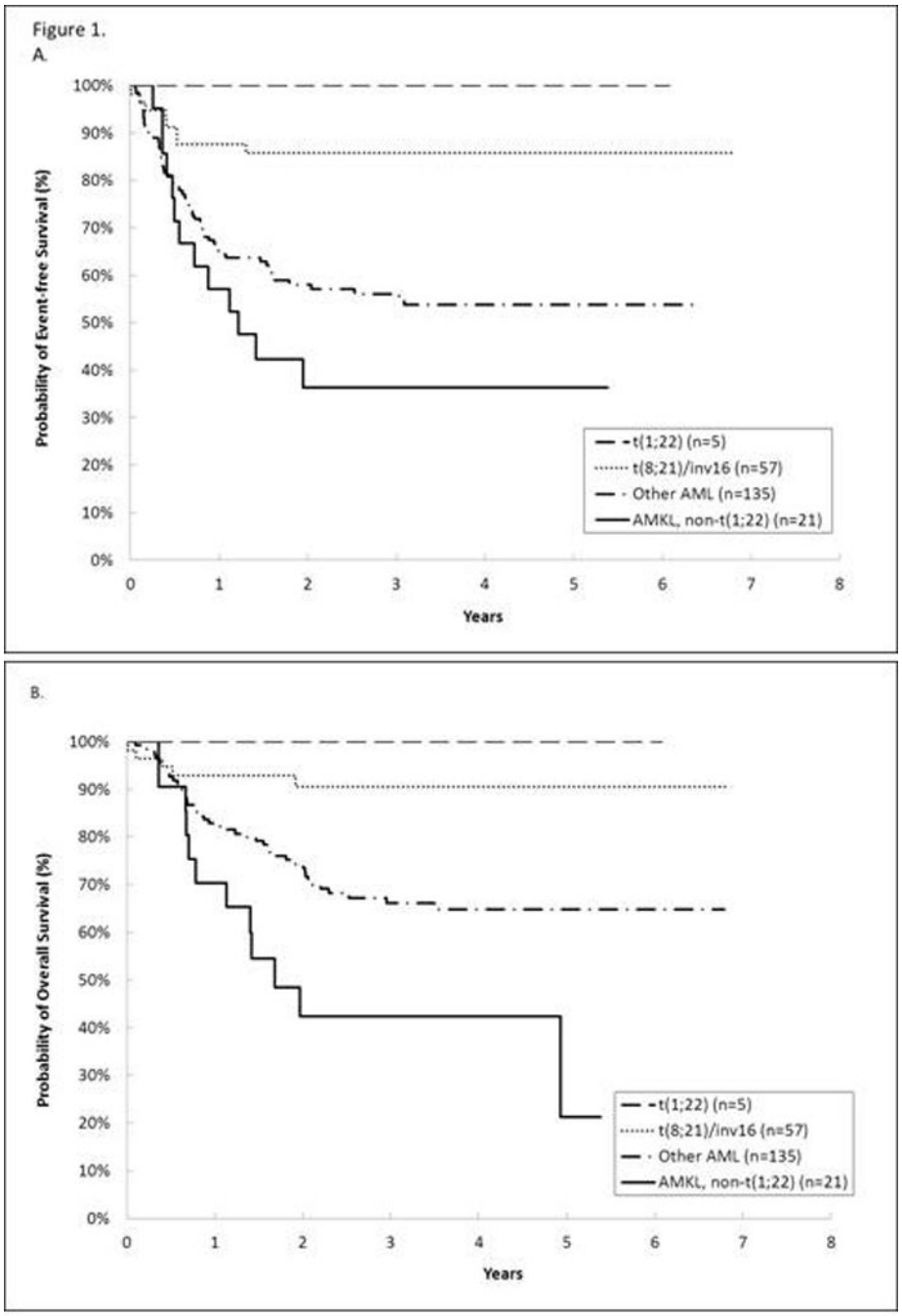

Figure 1.

(a) Event-free survival according to leukemia subtypes in patients treated on AML02. The 3-year rates were 100% for the 5 patients with the t(1;22) AMKL, 85.8% ± 6.1% for the 57 patients with favorable cytogenetics [t(8;21) or inv(16)], 56.1% ± 5.3% for the 135 patients with other AML subtypes, and 36.3% ± 10.9% for the 21 AMKL patients without the t(1;22) (P=0.023)

(b) Overall survival according to leukemia subtypes in patients treated on AML02. The 3-year rates were 100% for the 5 patients with t(1;22) AMKL, 90.6% ± 5.1% for the 57

patients with favorable cytogenetics [t(8;21), inv(16)], 66.1% ± 5% for the 135 patients with other AML subtypes, and 42.4% ± 11.4% for the 21 AMKL patients without t(1;22) ($P<0.001$).

Table 1

AML02 and POG 9421 Patient Characteristics and Response

	AML02			POG 9421		
	AMKL	**Other AML subtypes**	***P* value**	**AMKL**	**Other AML subtypes**	***P* value**
Total Patients, n	26	192		49	516	
Gender, n (%)						
Male	14 (54)	108 (56)	0.84	29 (59)	269 (52)	0.3
Female	12 (46)	84 (44)		20 (41)	247 (48)	
Age at diagnosis (years)						
Median	1.2	10.4	< 0.01	1.8	9.6	< 0.001
Range	0.2–11.3	0.01–21.4		0.1–16.2	0.0–20.4	
Cytogenetics, n (%)						
t(1;22)	5 (19)	0 (0)	< 0.01	1 (2)	0 (0)	< 0.001
normal	3 (11)	50 (26)		13 (27)	131 (25)	
t(8;21)/inv(16)	0 (0)	57 (30)		1 (2)	96 (19)	
11q23	0 (0)	42 (22)		1 (2)	83 (16)	
Miscellaneous	16 (62)	40 (21)		24 (48)	122 (24)	
Not reported	2 (8)	3 (1)		9 (18)	84 (16)	
FLT3 status, n (%)						
ITD	0	28 (15)	< 0.01			NA
PM	0	8 (4)				
Wild type	17 (65)	150 (78)				
Not available	9 (35)	6 (3)		49 (100)	516 (100)	
WBC at diagnosis ($\times 10^3$)						
Median	11.7	22.2	0.06	13.5	24.7	0.004
Range	2.3–72.9	0.3–286.2		0.3–98.4	0.5–667	
Induction Therapy, n (%)						
Standard cytarabine	12 (46)	98 (51)	0.26	26 (53)	258 (50)	0.7
High-dose cytarabine	13 (50)	93 (48)		23 (47)	258 (50)	

	AML02			POG 9421		
	AMKL	**Other AML subtypes**	***P* value**	**AMKL**	**Other AML subtypes**	***P* value**
Not Randomized	1 (4)	1 (1)		0 (0)	0 (0)	
Induction Response, n (%)						
CR (after induction 1)	20 (81)	154 of 190 (81)	> 0.99	NA	NA	
Induction 1 MRD ≥ 0.1%	10 of 24 (42)	65 of 180 (36)	0.65	NA	NA	
CR (after induction 2)	26 (100)	174 of 183 (95)	61	39 (80)	436 (85)	0.2
PR (after induction 2)	0	0		5 (10)	21 (4)	
Induction 2 MRD ≥ 0.1%	6 of 24 (25)	33 of 171 (19)	0.59	NA	NA	
Induction Failure	0	11 (6)	0.37	2 (4)	43 (8)	
Toxic Death	0	2 (1)	> 0.99	2 (4)	12 (2)	
Not evaluable	0	0		1 (2)	4 (1)	
Survival, % (SE)[*]						
EFS[*]	48.7 (10.5)	64.8 (4.4)	0.21	34.7 (7.5)	37.9 (2.3)	0.7
OS[*]	53.9 (10.6)	73.1 (4.0)	0.023	36.3 (7.5)	51.8 (2.4)	0.1

Abbreviations: AMKL, acute megakaryoblastic leukemia; EFS, event-free survival probability; FLT3, fms-related tyrosine kinase 3; ITD, internal tandem duplication; MRD, minimal residual disease; NA, not available; PM, point mutation; PR, partial response; OS, overall survival probability; SE, standard error; CR, complete remission; WBC, white blood cell count.

[*]For AML02, 3-year EFS/OS reported; for POG 9421, 5-year EFS/OS reported