Brief report

Long-term disease-free survivors with cytogenetically normal acute myeloid leukemia and *MLL* partial tandem duplication: a Cancer and Leukemia Group B study

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The clinical impact of *MLL* partial tandem duplication (*MLL*-PTD) was evaluated in 238 adults aged 18 to 59 years with cytogenetically normal (CN) de novo acute myeloid leukemia (AML) who were treated intensively on similar Cancer and Leukemia Group B protocols 9621 and 19808. Twenty-four (10.1%) patients harbored an *MLL*-PTD. Of those, 92% achieved complete remission (CR) compared with 83% of patients without *MLL*-PTD ($P = .39$). Neither overall survival nor disease-free survival significantly differed between the 2 groups ($P = .67$ and $P = .55$, respectively). Thirteen *MLL*-PTD$^+$ patients relapsed within 1.4 years of achieving CR. *MLL*-PTD$^+$ patients who relapsed more often had other adverse CN-AML–associated molecular markers. In contrast with previously reported studies, 9 (41%) *MLL*-PTD$^+$ patients continue in long-term first remission (CR1; range, 2.5-7.7 years). Intensive consolidation therapy that included autologous peripheral stem-cell transplantation during CR1 may have contributed to the better outcome of this historically poor-prognosis group of CN-AML patients with *MLL*-PTD. (Blood. 2007;109:5164-5167)

Introduction

The *MLL* partial tandem duplication (*MLL*-PTD) produces an in-frame, elongated protein and occurs in approximately 8% of patients with cytogenetically normal (CN) acute myeloid leukemia (AML).[1] The *MLL*-PTD affects only 1 allele in CN-AML, and the second, wild-type (WT), *MLL* allele is silenced.[2] Unlike MLL chimeric fusion proteins arising from translocations involving 11q23, the MLL-PTD protein retains the C-terminal domains including the histone H3 lysine 4 methyltransferase activity.[3] In a murine knock-in model, the *Mll*-PTD acts as a gain-of-function allele, giving rise to aberrations in skeletal development and conferring proliferation and self-renewal advantages to hematopoietic stem/progenitor cells without causing frank leukemia.[4]

The *MLL*-PTD was the first adverse prognostic molecular marker identified in CN-AML.[5,6] In several subsequent studies, the presence of *MLL*-PTD was associated with a shorter remission duration, with most patients relapsing within 1 year.[7-10] We report herein for the first time that younger adults with *MLL*-PTD (*MLL*-PTD$^+$) treated on 2 recent frontline Cancer and Leukemia Group B (CALGB) protocols had a clinical outcome comparable to that of CN-AML patients without *MLL*-PTD (*MLL*-PTD$^-$) and that a substantial percentage of *MLL*-PTD$^+$ patients are disease-free beyond 2.5 years.

Patients, materials, and methods

A combination of real-time reverse transcriptase–polymerase chain reaction (RT-PCR) and nested RT-PCR/sequencing was used to detect *MLL*-PTD in pretreatment bone marrow (BM) or blood (PB) samples as previously described.[2,7] All patients gave informed consent for the research use of their specimens, in accordance with the Declaration of Helsinki. The clinical trials and companion protocols were approved by the Ohio State University Institutional Review Board and the Cancer and Leukemia Group B. Screening was conducted as described previously for additional molecular markers associated with CN-AML (ie, *FLT3* internal tandem duplication [*FLT3*-ITD], *FLT3* tyrosine kinase domain [*FLT3*-TKD] mutation, *NPM1* mutation, and high *BAALC* and *ERG* expression).[11-14] All screening assays were performed at The Ohio State University Comprehensive Cancer Center.

Patients enrolled on CALGB 9621 and 19808 received induction treatment that included cytarabine, etoposide, and daunorubicin with or without PSC-833, a multi-drug resistance protein inhibitor also called valspodar. Patients who achieved complete remission (CR) underwent autologous peripheral blood stem-cell transplantation (auto-PBSCT) phase.[15,16] Clinical end points were disease-free survival (DFS) and overall survival (OS), as defined previously.[14]

Pretreatment clinical features were compared between the *MLL*-PTD$^+$ and *MLL*-PTD$^-$ groups as well as between *MLL*-PTD$^+$ patients who did and those who did not relapse using Fisher 2-sided exact and Wilcoxon rank

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Table 1. Comparison of CN-AML patients with and without *MLL*-PTD and comparison of *MLL*-PTD+ patients who relapsed with *MLL*-PTD+ patients who did not relapse

Characteristic	*MLL*-PTD			*MLL*-PTD+		
	Negative, n=214	Positive, n=24	*P*	No relapse, n=9	Relapse, n=13	*P*
Age, y			.39			.62
Median	46	49		47	51	
Range	18-59	22-59		22-59	23-59	
Sex, no. of males (%)	100 (47)	13 (54)	.52	5 (56)	7 (54)	1.00
Race, n (%)			1.00			.16
White	186 (88)	22 (92)		7 (78)	13 (100)	
Nonwhite	25 (12)	2 (8)		2 (22)	0 (0)	
Hemoglobin, g/dL			.29			.07
Median	9.3	8.75		9.5	8.3	
Range	4.6-13.6	6.2-11.8		7.8-10.3	6.2-11.2	
Platelet count, $\times 10^9$/L			.78			.15
Median	60.5	64.5		31	72	
Range	7-466	5-395		5-395	23-120	
WBC count, $\times 10^9$/L			<.001			.76
Median	25.15	4.35		4.1	6.7	
Range	0.8-295.0	0.8-64.2		0.8-27.8	0.9-64.2	
Blood blasts, %			.40			.08
Median	56	39.5		26	54	
Range	0-97	5-95		7-75	10-95	
Bone marrow blasts, %			.17			.03
Median	65	55		40.5	66	
Range	12-99	10-94		10-80	38-94	
Centrally reviewed FAB, n (%)			.04			.83
M0	4 (3)	1 (5)		0 (0)	1 (9)	
M1	40 (27)	5 (24)		2 (25)	2 (18)	
M2	46 (32)	10 (48)		4 (50)	5 (45)	
M4	37 (25)	2 (10)		0 (0)	2 (18)	
M5	14 (10)	0 (0)		0 (0)	0 (0)	
M6	1 (1)	2 (10)		1 (13)	1 (9)	
Unclassified	4 (3)	1 (5)		1 (13)	0 (0)	
Extramedullary involvement, n (%)*			.03			1.00
No	146 (69)	21 (91)		8 (89)	11 (92)	
Yes	65 (31)	2 (9)		1 (11)	1 (8)	
Lymphadenopathy, n (%)			.05			NA
No	184 (86)	24 (100)		9 (100)	13 (100)	
Yes	29 (14)	0 (0)		0 (0)	0 (0)	
***FLT3*-ITD, n (%)**			.37			.33
Absent	139 (65)	18 (75)		8 (89)	8 (62)	
Present	75 (35)	6 (25)		1 (11)	5 (38)	
***FLT3*-TKD, n (%)**			1.00			1.00
Absent	180 (91)	18 (95)		6 (100)	10 (91)	
Present	18 (9)	1 (5)		0 (0)	1 (9)	
***NPM1*, n (%)**			<.001			.12
Wild-type	63 (32)	13 (76)		3 (50)	9 (90)	
Mutated	136 (68)	4 (24)		3 (50)	1 (10)	
***BAALC expression*, n (%)†**			.009			.03
Low	83 (54)	3 (19)		3 (60)	0 (0)	
High	72 (46)	13 (81)		2 (40)	9 (100)	
***ERG expression*, n (%)‡**			.27			.23
Low	101 (64)	12 (80)		5 (100)	5 (63)	
High	57 (36)	3 (20)		0 (0)	3 (38)	
Complete remission rate, n (%)	177 (83)	22 (92)	.39	NA	NA	NA
Relapse rate, n (%)	96 (54)	13 (59)	.82	NA	NA	NA
Overall survival			.67	NA	NA	NA
Median, y	3.7	NR		NA	NA	
Alive at 3 y (95% CI), %	52 (45-59)	50 (29-68)		NA	NA	
Alive at 5 y (95% CI), %	45 (38-52)	50 (29-68)		NA	NA	
Disease-free survival			.55	NA	NA	NA
Median, y	2.5	1.0		NA	NA	
Disease-free at 3 y (95% CI), %	48 (40-55)	41 (21-60)		NA	NA	
Disease-free at 5 y (95% CI), %	43 (35-51)	41 (21-60)		NA	NA	

The median follow-up for the 116 of 238 patients alive is 4.7 years, ranging from 1.2 to 8.9 years. For the 199 patients who achieved CR, median disease-free survival duration for the 90 who have not relapsed is 5.4 years, ranging from 1.5 to 8.8 years.

MLL-PTD indicates partial tandem duplication of the *MLL* gene; WBC, white blood cell; FAB, French-American-British; *FLT3*-ITD, internal tandem duplication of the *FLT3* gene; *FLT3*-TKD, tyrosine kinase domain mutation of the *FLT3* gene; CI, confidence interval; NA, not applicable; and NR, not reached.

*Includes involvement of the central nervous system, hepatomegaly, splenomegaly, lymphadenopathy, skin infiltrates, gum hypertrophy, and/or a mediastinal mass.

†For patients on protocol 9621, cut point same as in Baldus et al.[13] For patients on protocol 19808, median *BAALC* expression value used for cut point.

‡For patients on protocol 9621, cut point same as in Marcucci et al.[14] For patients on protocol 19808, median *ERG* expression value used for cut point.

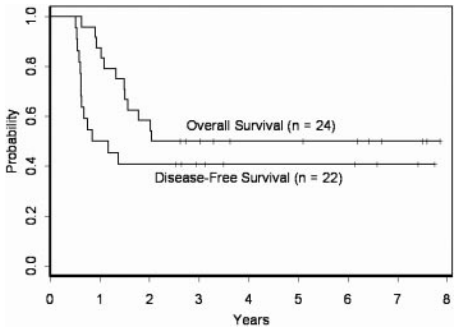

Figure 1. Overall survival and disease-free survival of CN-AML patients with *MLL*-PTD evaluated in this study. Fifty percent of the patients are alive and 41% remain disease-free in CR1 beyond 2.5 years.

sum tests for categoric and continuous variables, respectively. Estimated-probabilities of DFS and OS were calculated using the Kaplan-Meier method, and the log-rank test evaluated differences between survival distributions. All analyses were performed by the CALGB Statistical Center.

Results and discussion

Of the 238 CN-AML patients, 24 (10.1%) harbored an *MLL*-PTD, which represents the largest series of *MLL*-PTD$^+$ CN-AML patients treated similarly and with outcome reported. *MLL*-PTD$^+$ patients differed from *MLL*-PTD$^-$ patients with respect to white blood cell (WBC) counts (median, 4.35 vs 25.15 X10^9/L; $P < .001$), extramedullary involvement (9% vs 31%; $P = .03$), and French-American-British (FAB) subgroups ($P = .04$); a higher percentage of FAB M2 (48% vs 32%) and a lower percentage of FAB M4/M5 (10% vs 35%) was observed in the *MLL*-PTD$^+$ group (Table 1). Among patients with available tissue, *MLL*-PTD$^+$ patients more often presented with wild-type *NPM1* (*NPM1*-WT; 76% vs 32%; $P < .001$) and high *BAALC* expression (81% vs 46%; $P = .009$). In contrast, there was no significant association between the *MLL*-PTD status and the presence of *FLT3*-ITD ($P = .37$); 25% and 35% of *MLL*-PTD$^+$ and *MLL*-PTD$^-$ patients, respectively, had *FLT3*-ITD (Table 1).

CR rates were not significantly different between the *MLL*-PTD$^+$ and *MLL*-PTD$^-$ patients (92% vs 83%; $P = .39$; Table 1). The CR rate for *MLL*-PTD$^+$ patients was similar to that reported in a German study (Döhner et al[9]) that administered idarubicin, cytarabine, and etoposide–based intensive double-induction therapy, and higher than CR rates observed in other studies (Table S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article).[7,8,17] Younger age (< 60 years) and/or inclusion of etoposide may have contributed to the high CR rates in the CALGB and German studies.

With a median follow-up of 4.7 years, no significant differences in DFS ($P = .55$) or OS ($P = .67$) between *MLL*-PTD$^+$ and *MLL*-PTD$^-$ patients were observed (Table 1). The estimated 3-year DFS and OS rates for *MLL*-PTD$^+$ patients were, respectively, 41% and 50% compared with, respectively, 48% and 52% for *MLL*-PTD$^-$ patients (Table 1). Most striking was that 50% of *MLL*-PTD$^+$ patients were alive at last follow-up and 9 (41%) of 22 were still in first CR (CR1), ranging from 2.2 to 7.7 years (Figure 1). This is markedly different from data previously reported, where few adult *MLL*-PTD$^+$ patients maintained a remission beyond 2 years (Table S1). In the Döhner et al[9] study, CN-AML patients aged 16 to 60 years in CR1 were treated with a high-dose cytarabine and mitoxantrone-based (HAM) regimen for the first consolidation therapy and yet 11 of 16 *MLL*-PTD$^+$ patients relapsed within 2 years, and the 5 patients remaining in CR1 were censored with short follow-ups, from only a few months to about a year after achieving remission. Two patients in the Döhner et al[9] study, who achieved a second CR and received allogeneic transplantation, were alive with approximately 3 and a half to 4 years of follow-up. In the current study, 18 of 22 *MLL*-PTD$^+$ patients in CR1 underwent auto-PBSCT, which may have contributed to the reduced number of early relapses in our patients with *MLL*-PTD$^+$ CN-AML. Three of the other 4 received multiple courses of high-dose cytarabine and 1 received 1 course of high-dose cytarabine before refusing further treatment.

Although a considerable fraction of *MLL*-PTD$^+$ patients were alive and relapse-free, the majority relapsed within the first 1.4 years of remission (Figure 1). Thus, we analyzed the *MLL*-PTD$^+$ group for pretreatment and/or molecular characteristics that might explain some of the differences in outcome in this subgroup. Relapsed patients had a higher percentage of BM blasts at diagnosis (median, 66% vs 40.5%; $P = .03$) and more often were high *BAALC* expressers ($P = .03$; Table 1). Nine of 11 *MLL*-PTD$^+$ patients with high *BAALC* relapsed, whereas all 3 patients with low *BAALC* remain in remission (Figure S1A). While not statistically significant, the presence of other adverse molecular prognostic markers was also more frequent among relapsed *MLL*-PTD$^+$ patients compared with *MLL*-PTD$^+$ patients who remain in remission. Thirty-eight percent of the relapsed patients had *FLT3*-ITD compared with only 11% of those still in CR1. Similar trends were observed for *NPM1*-WT and high *ERG* expression. Among relapsed *MLL*-PTD$^+$ patients evaluated for *NPM1* mutations and *ERG* expression, 9 of 10 had only *NPM1*-WT alleles and 3 of 8 were high *ERG* expressers. In contrast, among *MLL*-PTD$^+$ patients remaining in remission, 3 of 6 carried *NPM1* mutations and 0 of 5 were high *ERG* expressers (Table 1; Figure S1B-D).

In conclusion, we have observed for the first time a relatively good outcome for *MLL*-PTD$^+$ patients who achieved CR, with 41% of such patients remaining in first remission beyond 2.5 years. This contrasts with previous studies reporting only a few patients who achieved long-lasting remissions. Besides the multitude of other potential factors, we postulate that treatment advances for younger adults may have contributed to the better outcome observed for the *MLL*-PTD$^+$ patients reported in this study. Although these are encouraging findings, it is clear that the majority of *MLL*-PTD$^+$ patients still relapse early. Our data suggest that the presence of additional, negative prognostic markers may be a contributing factor. Larger studies are necessary to confirm our results and to elucidate the underlying mechanisms of leukemogenesis, which could lead to the development of molecularly targeted curative therapies.

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Authorship

Contribution: S.P.W., A.S.R., G.M., K.M., and C. D. Bloomfield contributed to the design and analysis of this study. S.P.W., A.S.R., G.M., K.M., P.P., and C. D. Bloomfield contributed to the writing of this manuscript and all authors agreed on the final version. S.P.W., P.P., C.L., C. D. Baldus, J.W., and T.V. carried out laboratory-based research. A.S.R. performed statistical analyses. B.L.P., A.J.C., J.E.K., R.A.L., M.A.C., G.M., and C. D. Bloomfield were involved directly or indirectly in care of patients and/or sample procurement.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

A complete list of the members of the CALGB study group can be found in Document S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article.

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