

Partial tandem duplication of *KMT2A* (*MLL*) may predict a subset of myelodysplastic syndrome with unique characteristics and poor outcome

Partial tandem duplication (PTD) of the *KMT2A* (*MLL*) gene is detected in approximately 5-10% of cases of acute myeloid leukemia (AML)¹⁻⁵ and within cases showing normal karyotype, confers a worse prognosis.^{2,6,7} In these patients, the duplications are variable in size and most commonly involve exons 2 or 3, spanning through exon 6 or exons 8-11.¹ Unlike many other translocations involving the *KMT2A* gene, *MLL*-PTD cannot be detected using conventional cytogenetics.⁸ Other methods, such as the cytogenomic array, can be used to identify patients with this genetic alteration who may be poten-

tial candidates for therapy (e.g., demethylating agents, histone deacetylase inhibitors).^{9,9}

As in AML, *MLL*-PTD can also be seen in a subset of patients with myelodysplastic syndrome (MDS) and its acquisition has been observed during leukemic transformation;^{4,10} however, the clinical significance of *MLL*-PTD at diagnosis, particularly with regard to survival, has not been well characterized in these patients. In the study herein, we describe unique pathologic and clinical characteristics in a series of MDS patients with *MLL*-PTD, and analyze the impact of *MLL*-PTD on therapy response and clinical outcomes.

We reviewed the records of consecutive patients with a diagnosis of MDS who had cytogenomic microarray studies performed between March 2014 and June 2017 (n=83). Cases with *MLL*-PTD were identified and compared to a control cohort of patients (n=38) without

Table 1A. Pathologic characteristics of *MLL*-PTD cases.

	Disease category at diagnosis	<i>MLL</i> -PTD	Cytogenetics	Molecular analysis results
<i>MLL</i>-PTD MDS				
Patient 1	MDS-MLD	exons 2-10; 24 kb	Normal karyotype	Negative for <i>JAK2</i> V617F mutation.
Patient 2	MDS-EB-1	exons 2-8 on one allele; 347 kb including entire gene on other allele	Normal karyotype	Negative for <i>FLT3</i> ITD and D835, <i>IDH1/2</i> mutations.
Patient 3	MDS-EB-2	exons 2-5; 11 kb	Normal karyotype	Negative for <i>FLT3</i> ITD and D835 mutation.
Patient 4	MDS-EB-2	exons 2-10; 17 kb	Normal karyotype	Negative for <i>FLT3</i> ITD and D835, and <i>CEPBA</i> mutations.
<i>MLL</i>-PTD MDS with subsequent AML transformation				
Patient 5	MDS-EB-2	exons 2-10; 21 kb	Normal karyotype	Negative for <i>FLT3</i> ITD and D835, <i>CEPBA</i> , <i>IDH1/2</i> , <i>NPM1</i> mutations.
Patient 6	MDS-EB-2	exons 2-10; 33 kb	Gain of 4	Positive for <i>IDH2</i> R172K. Negative for <i>FLT3</i> ITD and D835, <i>CEPBA</i> , <i>IDH1</i> , <i>NPM1</i> , and <i>KIT</i> mutations.
<i>MLL</i>-PTD therapy-related MDS (t-MDS)				
Patient 7 (Later transformed to AML)	t-MDS-EB-1	exons 2-10; 17 kb	Normal karyotype	Negative for <i>FLT3</i> ITD and D835, <i>CEPBA</i> , <i>IDH1/2</i> , <i>NPM1</i> mutations.
Patient 8 (Later transformed to AML)	t-MDS-MLD	exons 2-8; 16 kb	Normal karyotype	Positive for <i>FLT3</i> D835 and <i>IDH1</i> R132F. Negative for <i>FLT3</i> ITD and <i>IDH2</i> mutations.
<i>MLL</i>-PTD <i>de novo</i> AML				
Patient 9	AML	exons 2-8; 16 kb	Inadequate	Negative for <i>FLT3</i> ITD and D835, <i>CEPBA</i> , <i>IDH1/2</i> , <i>NPM1</i> mutations.
Patient 10	AML	exons 2-10; 21 kb	Normal karyotype	Negative for <i>FLT3</i> ITD and D835, <i>CEPBA</i> , <i>IDH1/2</i> , <i>NPM1</i> mutations.
Patient 11	AML	exons 2-8; 17 kb	Normal karyotype	Positive for <i>FLT3</i> ITD and D835. Negative for <i>CEPBA</i> , <i>IDH 1/2</i> , <i>NPM1</i> , <i>KIT</i> , <i>BCR-ABL</i> , <i>PML-RARα</i> .
Patient 12	AML	exons 2-10; size unspecified	Trisomy 11	Positive for <i>FLT3</i> ITD. Negative for <i>CEPBA</i> , <i>IDH 1/2</i> , <i>NPM1</i> mutations.
<i>MLL</i>-PTD detected at AML transformation from MPN or MDS/MPN				
Patient 13 (History of PMF)	PMF	exons 3-5; 9 kb	Normal karyotype	Positive for <i>FLT3</i> ITD. Negative for <i>FLT3</i> D835, <i>IDH 1/2</i> mutation.
Patient 14 (History of CMML)	CMML	exons 2-8; 16 kb	Normal karyotype	Negative for <i>FLT3</i> ITD and D835. Negative for <i>IDH 1/2</i> and <i>JAK2</i> V617F mutation.

PTD: partial tandem duplication; MDS: myelodysplastic syndrome; ITD: internal tandem duplication; AML: acute myeloid leukemia; PMF: primary myelofibrosis; CMML: chronic myelomonocytic leukemia; t-MPS: therapy-related myelodysplastic syndrome; MPN: myeloproliferative neoplasms; EB: excessive blasts.

Table 1B. Clinical characteristics of *MLL*-PTD cases.

	Age at diagnosis years	Sex	Prognostic score and category				Therapy				OS months	Alive at end of study	
			IPSS-R		IPSS-RA		Supportive	Hypomethylating agent	Induction 7+3	Transplant FLAG			
<i>MLL</i> -PTD MDS													
Patient 1	39.9	M	2	low	0.8	very low	X					16.73	
Patient 2	68.7	F	6	high	5.97	high		X				6.61	
Patient 3	67.6	M	5	high	4.94	high		X			X	12.56	X
Patient 4	45.8	M	7	very high	6.64	very high	X					3.45	
<i>MLL</i> -PTD MDS with subsequent AML transformation													
Patient 5	66.3	M	4.5 intermediate		4.4 intermediate				X	X		13.12	
								(failed)					
Patient 6	67.6	M	6.5	very high	6.46	very high	X		X			1.81	
<i>MLL</i> -PTD therapy-related MDS (t-MDS)													
Patient 7	70.2	F	5	high	5.01	high	X			X		10.19	
(Later transformed to AML)													
Patient 8	64.9	F	3.5 intermediate		3.33 intermediate					X (failed)		2.30	
(Later transformed to AML)													
<i>MLL</i> -PTD <i>de novo</i> AML													
Patient 9	66.8	F						X (failed)	X	X		33.90	X
Patient 10	60.5	M						X (failed)	X	X		18.81	X
Patient 11	52.3	F						X		X		15.12	X
Patient 12	68.2	F						X		X		11.18	X
<i>MLL</i> -PTD detected at AML transformation from MPN or MDS/MPN													
Patient 13	67.6	M								X		0.66	
(History of PMF)													
Patient 14	60.3	M								X	X	20.75	X
(History of CMML)										(residual CMML)			

PTD: partial tandem duplication; OS: overall survival; MDS: myelodysplastic syndrome; AML: acute myeloid leukemia; PMF: primary myelofibrosis; CMML: chronic myelomonocytic leukemia; t-MDS: therapy-related myelodysplastic syndrome; MPN: myeloproliferative neoplasms; OS: overall survival; IPSS-R: revised international prognostic scoring system; IPSS-RA: age-adjusted IPSS-R.

MLL-PTD who were derived from the same original cohort (Online Supplementary Tables S1-S2). Cases of AML with *MLL*-PTD were also reviewed. All patients had been evaluated and treated at the same institution during the same time period. Overall survival (OS) was calculated from the diagnosis date to date of death, censoring for patients alive at the time of study completion. Additional methods are described in Online Supplementary Methods.

A total of 14 cases with *MLL*-PTD were identified by the cytogenomic microarray (Table 1A,B). Excluding patients with therapy-related myeloid neoplasms, *MLL*-PTD was detected in 6-7% of patients with MDS that were screened by the array during the same time period. These patients were predominantly male, ranging in age from 39.9-68.7 years of age at diagnosis (mean 59.3 years, median 66.9 years).

The majority of *MLL*-PTD MDS cases were classified as MDS with excess blasts (MDS-EB; by WHO 2016 criteria),¹¹ showed normal karyotype by conventional cyto-

genetics, and had variable revised international prognostic scoring system (IPSS-R) scores ranging from low to very high (Table 1A,B). Cases of *MLL*-PTD presented with lower absolute neutrophil count ($P=0.012$) and platelet count ($P=0.046$) compared to non-*MLL*-PTD MDS cases with high to very high age-adjusted IPSS-R (IPSS-RA), but comparable hemoglobin and bone marrow blast percentage (Figure 1A). Flow cytometric immunophenotyping did not reveal specific immunophenotypic aberrations distinguishing *MLL*-PTD cases from non-*MLL*-PTD cases (*data not shown*).

MLL-PTD MDS patients showed worse OS compared to MDS patients without *MLL*-PTD, even when compared to those with high to very high IPSS-RA (Figure 1B, $P<0.0001$; Figure 1C, $P=0.002$). The median OS for *MLL*-PTD MDS was 9.85 months from the time of diagnosis compared to 31.5 months for the high to very high IPSS-RA group. OS was also worse when comparing cases of MDS with poor to very poor cytogenetic features, which

had a median OS of 18.2 months (Figure 1D, $P=0.027$). *MLL*-PTD MDS patients, with and without acute leukemic transformation, also showed worse OS compared to patients with *MLL*-PTD who presented with *de novo* AML (Figure 1E, $P=0.043$). All four patients with *de novo* AML were alive at the conclusion of this study.

Patients with *MLL*-PTD MDS who transformed to AML as well as *de novo MLL*-PTD AML received either 7+3¹² or FLAG¹³ induction therapy (Table 1B).

Three of the five *MLL*-PTD patients who received 7+3

induction therapy showed residual disease in day 14 marrows, whereas two *de novo* AML cases, both of which also had a *FLT3*-ITD mutation, achieved CR and were subsequently transplanted. All cases that failed initial 7+3 induction therapy showed complete response to FLAG re-induction therapy. One patient with therapy-related myelodysplastic syndrome (t-MDS) failed initial FLAG induction therapy. Two additional MDS patients received initial FLAG induction therapy but passed away due to infection before disease response evaluation could be completed.

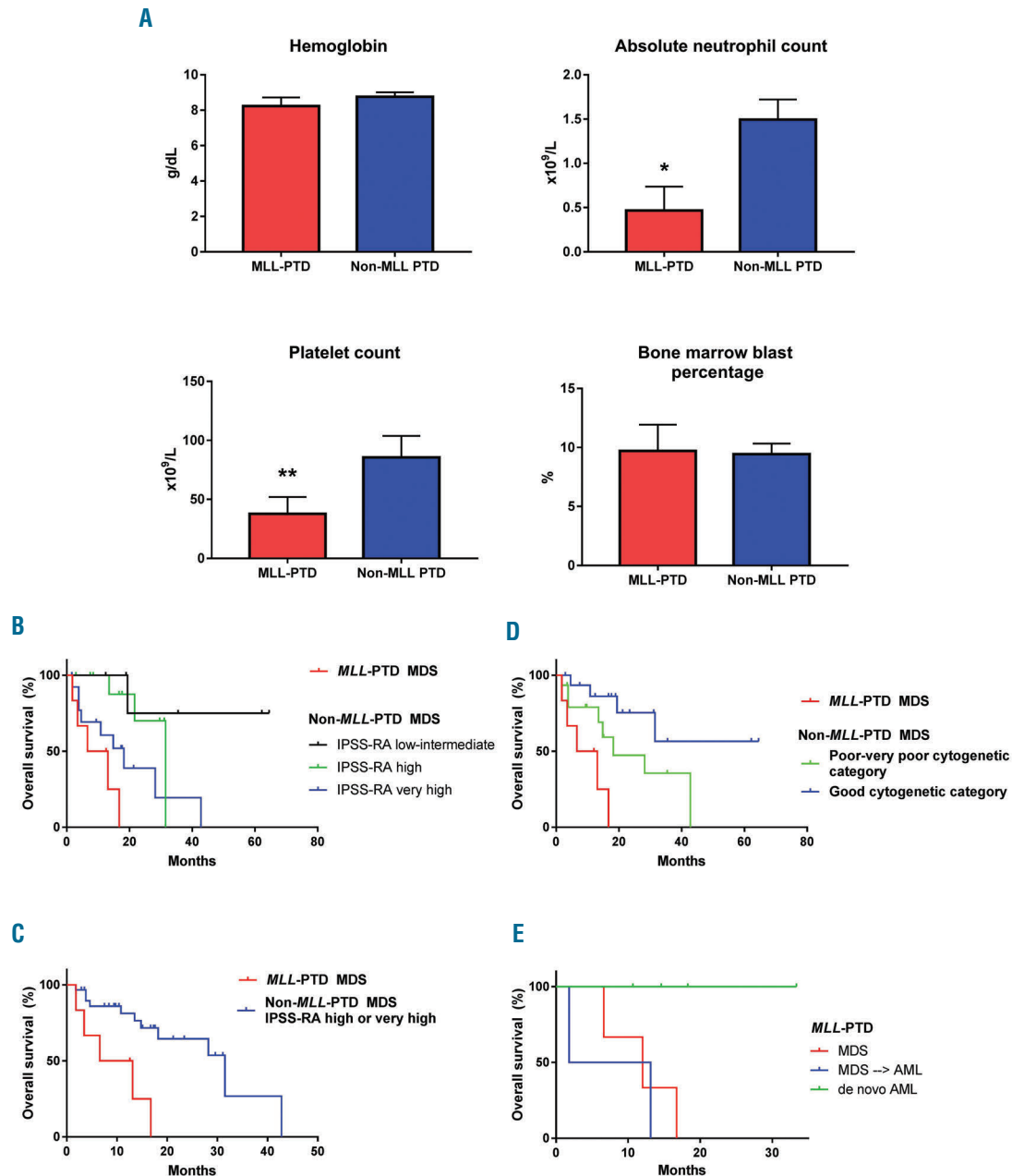


Figure 1. *MLL*-PTD MDS presents with lower absolute neutrophil and platelet counts and displays worse prognosis than high risk non-*MLL*-PTD MDS. (A) Hemoglobin, absolute neutrophil count, platelet count, and bone marrow blast percentage were compared between *MLL*-PTD MDS and non-*MLL*-PTD MDS with high to very high IPSS-RA score [mean value and standard error of the mean are depicted; * ($P=0.012$) ** ($P=0.046$)]. (B) Survival analysis comparing *MLL*-PTD MDS to non-*MLL*-PTD MDS based on IPSS-RA prognostic group ($P<0.0001$). (C) Survival analysis comparing *MLL*-PTD MDS to high or very high risk non-*MLL*-PTD MDS based on IPSS-RA score ($P=0.002$). (D) Survival analysis comparing *MLL*-PTD MDS to non-*MLL*-PTD MDS with poor to very poor cytogenetic and good cytogenetic groups ($P=0.027$). (E) Survival analysis comparing *MLL*-PTD MDS, transformed *MLL*-PTD MDS, and *de novo MLL*-PTD AML cases ($P=0.043$). PTD: partial tandem duplication; MDS: myelodysplastic syndrome; IPSS-RA: age-adjusted IPSS-R.

All *MLL*-PTD patients who proceeded to transplant were still alive at the conclusion of this study, except for one patient with t-MDS who relapsed with AML (Table 1B). All patients who did not receive a transplant passed away, with the longest survival time being 16.7 months. The single *MLL*-PTD MDS patient who received a transplant remains alive at the time of writing.

The study herein identifies a subset of MDS defined by the presence of *MLL*-PTD that is associated with more advanced disease with excess blasts and worse outcome, compared to *MLL*-PTD MDS, even those with high risk IPSS scores and complex karyotype. Though the sample size is small, the effect size is pronounced and statistically significant in all comparisons. The overall prevalence of *MLL*-PTD in MDS (6-7% of cases) is similar to that observed in AML and slightly higher than reported in a previous study of mutational analysis in MDS.¹⁰ It is of note that the control cohort of high and very high risk IPSS-RA in our study shows a better median OS of 31.5 months than has been reported in the larger multinational IPSS-R study, which showed a median survival of 19 months for high risk and 10.8 months for very high risk groups.¹⁴ This finding could potentially be reflective of differences in therapy and clinical practice or of the smaller numbers of patients in this single institution review. It is also significant because despite improved survival of high and very high risk MDS patients being treated at the same institution during the same time interval, patients with *MLL*-PTD still fared very poorly. Furthermore, their median survival of 9.85 months is even lower than very high risk MDS patients as predicted by the IPSS-R study.¹⁴ This finding suggests that the finding of *MLL*-PTD in an MDS patient is indeed a very poor prognostic factor and taken a step further, these patients may potentially benefit from expeditious transplantation if feasible. Supporting this is the observation that all patients with *MLL*-PTD who received a transplant (other than those with therapy-related myeloid neoplasms) were still alive at the conclusion of this study.

For those patients who developed *MLL*-PTD AML, in cases where 7+3 induction chemotherapy was not successful, complete response was achievable in most cases treated with FLAG re-induction therapy. The presence of additional *FLT3*-ITD mutation may possibly predict a better response to 7+3 induction therapy; however, the sample population is too small to draw a definitive conclusion. Co-occurrence of *FLT3*-ITD and *MLL*-PTD has been reported previously in AML, though the significance of this finding on outcome or response is currently unknown.¹⁵ The potential use of demethylating agents and histone deacetylase inhibitors, as has been proposed for cases of *MLL*-PTD AML,^{3,9} is one area of further exploration. Of note, *de novo* *MLL*-PTD AML patients, which exclude patients with underlying MDS, showed the best outcomes of patients in this study, possibly due to early transplantation, and may have better prognosis than has been previously reported.

In summary, our findings suggest that *MLL*-PTD MDS presents as high grade disease with excess blasts and typically normal karyotype. Testing for *MLL*-PTD might be considered in all patients with MDS, and its discovery could potentially warrant consideration for early transplantation. Because the size of our sample is limited,

additional studies on a larger cohort of patients to further define the features of this subgroup and delineate appropriate therapy are needed.

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