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

Sarah M. Choi,[1] Rajan Dewar,[1] Patrick W. Burke[2] and Lina Shao[1]

[1]Department of Pathology and [2]Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

Correspondence: choism@med.umich.edu

doi:10.3324/haematol.2017.185249

Supplemental Methods:

Patients: This study was approved by the Institutional Review Board at the University of Michigan. Clinical and pathology records were obtained through retrospective review of the electronic medical chart. Prior history of chemotherapy or radiation was noted, along with laboratory values at diagnosis [hemoglobin, absolute neutrophil count (ANC), platelet count, bone marrow blast percentage] (Table S1-S2). The clinical and treatment course were also recorded (transformation to AML, transplant status, chemotherapeutic regimen used). Subtype of MDS (WHO 2016 criteria)[1] at the time of diagnosis as well as flow cytometric immunophenotyping results were also catalogued. Conventional cytogenetics/karyotype results were also recorded. A prognostic score was calculated using the revised International Prognostic Scoring System without and with age adjustment (IPSS-R and IPSS-RA, respectively).[2] Overall Survival (OS) was calculated from the diagnosis date to date of death, censoring for patients alive at the time of study completion.

The control cohort (Table S2) was comprised of patients with low (n=3), intermediate (n=5), high (n= 13), very high (n=17) IPSS-RA scores. There were 12 patients with complex karyotype of $\geq$ 3 abnormalities, 11 patients with other karyotype abnormalities, and 15 patients with normal karyotype. In terms of pathologic diagnosis, 27 patients had MDS with excess blasts and 11 had MDS with multilineage dysplasia.

Cytogenetics and cytogenomic array: *MLL*-PTD was detected by whole genome Affymetrix CytoScan® array analysis in all the cases as described previously.[3] All cases with available karyotype were stratified according to the Comprehensive Cytogenetic Scoring System of the Revised International Prognostic Scoring System.

Molecular analysis: Mutational analysis of *NPM1*, *FLT3*, *JAK2* V617F and *CEPBA* were performed as previously described.[4-8] *KIT* and *IDH1/2* mutation analysis was done by Sanger sequencing.

Statistical analysis: Statistical analysis was performed with survival curve analysis using log rank (Mantle-Cox) test and unpaired t test with Welch's correction.

Supplemental Tables:

Table S1. Laboratory values at diagnosis for *MLL*-PTD patients

	Hgb , g/dL	ANC, x10^9/L	Plts, x10^9/L	BM blasts, %
Patient 1	8.2	1.3	115	0.8
Patient 2	7	0.2	22	9
Patient 3	8.1	0.1	80	7
Patient 4	7.5	0.1	16	15.4
Patient 5	10.5	0.2	111	15
Patient 6	8.1	0.4	107	11
Patient 7	8.9	2.5	28	5.2
Patient 8	7.7	2.7	203	4

Table S2. Control cohort characteristics of non-*MLL*-PTD MDS patients

													Indicates alive at conclusion of study
Age at dx, years	Sex	Disease category at diagnosis	Hgb, g/dL	ANC, $x10^9$/L	Plts, $x10^9$/L	Bone marrow blasts, %	Cytogenetic Karyotype	IPSS-R		IPSS-RA		Overall survival, months
64.8	M	MDS-MLD	9.6	2	37	0.5	44,XY,t(1;12)(q21;q24.1),-3,add(3)(p10),der(5)t(3;5)(p13;q23),-6,-7,+11, der(11)t(3;11)(q21;q13)[15]/46,XY[5]	6	High	5.9	High	13.5
67.0	M	MDS-EB-2	7.7	3.4	69	10.5	42-44,XY,add(3)(p12),der(4)t(4;12)(q27;q12),add(5)(q13),add(7)(p11.2),add(11)(p11.2),-12,del(13)(q12q22),-14,-17,del(20)(q11.2q13.1),add(22)(q13)[cp20]	9	Very high	8.99	Very High	3.8
71.4	F	MDS-MLD	7.5	0.5	61	2.8	45,XX,-7[3]/46,idem,+mar[5]/46,XX[14]	6.5	Very high	6.52	Very High	28.2
67.5	M	MDS-EB-2	10.6	0.6	132	14	47,XY,+8[18]/46,XY[2]	5.5	High	5.44	High	29.6
76.9	M	MDS-MLD	12.1	1.6	104	2	45,X,-Y,-7,+mar[15]/45,X,-Y[5].	3	Low	3.24	Intermediate	35.4
85.3	M	MDS-EB-1	6.6	0.48	16	9	43,XY,der(3)t(3;16)(p12;q13),der(5)t(3;5)(p12;q13),-12,der(13)t(12;13)(q12;q34), der(17)t(17;20)(p13;p11.2),-20,idic(22)(p11.2)[15]/ 44,sl,+del(12)(q11.2)[1]/ 43,sl,idic(7)(q36)[1]/ 44,sl,+idic(22)[1]/ 46,XY[2]	9	Very high	9.08	Very High	3.5
75.5	F	MDS-EB-1	9.7	1.5	81	6.6	46,X,idic(X)(q13)[2]/47,sl,+idic(X)[9]/47,sl,+mar[2]/46,X,del(X)(q13q28)[1]/46,XX[6]	5.5	High	5.62	High	17.6
77.4	F	MDS-EB-2	8	1.7	145	11.6	46,XX,del(5)(q13q33)[12]/46,XX[8]	5	High	5.19	High	23.4
94.5	M	MDS-EB-1	9.1	0.2	45	5.75	45,XY,del(5)(q22q33),der(17;20)(q10;p10)[2]/46,sl,del(7)(q11.2q36),+8[5]/44,sl,-7[5]/85-94,slx2,add(11)(q12)x2,+1-2mar,4-11dmin[cp4]/46,XY[2]/46,sl,del(2)(p21p25),add(11)(q12),-21,+2mar[1]/45,sdl2,+X[1]	7.5	Very high	7.81	Very High	1.8
64.8	F	MDS-EB-1	8.8	2.5	62	9.4	45,XX,del(3)(q26q27),-5,add(7)(q11.2*),add(8)(q24),add(12)(p13)[2]/43,sl,-12,add(17)(p13),add(20)(q11.2),-21[16]/43,sdl,-add(7)(q11.2*),+add(7)(q11.2**)[2]		Very high	7.44	Very High	18.2
63.0	M	MDS-EB-1	13.4	3	20	5	46,XY,t(1;21)(p36.3;q22)[17]/46,XY[3]	5	High	4.83	High	8.4
65.4	F	MDS-EB-1	8.4	1.9	69	6	45,XX,del(2)(q31q37),idic(5)(q11.2),del(6)(p21p25),del(7)(q11.2q36),del(12)(p11.2p13),dic(20;21)(p13;p11.2)[2] /45,sl,-del(7)(q11.2q36),-7,+r[4] /46,XX[14]	7.5	Very High	7.44	Very High	42.8
67.0	M	MDS-EB-2	7.7	3.4	69	10	42-44,XY,add(3)(p12),der(4)t(4;12)(q27;q12),add(5)(q13),add(7)(p11.2),add(11)(p11.2),-12,del(13)(q12q22),-14,-17,del(20)(q11.2q13.1),add(22)(q13)[cp20]	8	Very high	7.97	Very High	3.8

67.5	M	MDS-EB-2	10.6	0.55	132	14	47,XY,+8[18]/46,XY[2]	5.5	High	5.44	High	29.6
55.1	M	MDS-EB-2	11.2	0.7	126	10	42-45,XY,del(3)(p21),del(5)(q31q35),-7,add(9)(q34),inv(12)(p13q13),add(17)(p13),-18,-20,-21,-22,+1-4mar[cp10]/46,XY[10]	6.5	Very high	6.24	Very High	14.8
62.5	M	MDS-EB-1	6.7	5	471	8.6	46,XY,del(3)(q12q24)[20]	6.5	Very high	6.37	Very High	17.3
67.7	M	MDS-EB-2	8.4	0.4	165	18.2	47,XY,+11[5]/46,XY[15]	6.5	Very high	6.46	Very High	21.3
76.9	M	MDS-EB-1	12.1	1.6	104	2.2	45,X,-Y,-7,+mar[18]/46,XY[2]	4	Intermediate	4.21	Intermediate	35.4
51.8	M	MDS-MLD	9.5	0.56	34	2.2	47,XY,t(1;3)(p13;q21),del(4)(q21q25),del(11)(q14q24),+19,del(20)(q11.2q13.3)[4]	7.5	Very high	7.27	Very High	15.1
69.8	M	MDS-RS-MLD	7.2	2.3	18	1.5	44-46,XY,der(2)ins(2;6)(q23;p24p12)add(2)(q23),der(3)t(3;12)(p24;p13),add(4)(q12),der(5;22)(p10;q10),-6,der(12)t(3;12)t(?6;12)(q12;q24),der(19)dup(19)(q13.1q13.4)add(19)(q13.4),+mar[cp17]/43-44,sl,add(X)(p11.2),-der(2)ins(2;6)add(2),+add(2)(q32),-der(3)t(3;12),+3,+6,-7,-der(12)t(3;12)t(6;12),+add(12)(p13),add(19)(p13),add(?21)(p11.2),+del(?22)(q11.2q13),-mar[cp3]	6.5	Very high	6.5	Very High	9.7
80.6	M	MDS-EB-2	8.5	0.2	41	10	46,XY[19]/47,XY,+19[1]	5.5	High	5.74	High	10.4
36.3	M	MDS-EB-1	9.3	1.2	38	5	45,XY,-7,del(20)(q11.2q13.3)[7]	7	Very high	6.49	Very High	9.4
74.0	F	MDS-EB-2	9.4	0.7	40	12.5	46-48,XX,del(1)(p22p36.1),del(2)(p24),del(3)(p21),-5,der(6)t(1;6)(p13;q25),der(7)add(7)(p21)del(7)(q32q36),+8,+8,+8,del(11)(q22q23),-12,+13,del(13)(q12q14)x2,add(16)(q11.2),add(17)(p13),add(19)(p13),-21,+0-2mar[cp20]	9.5	Very high	9.51	Very High	9.5
61.9	M	MDS-MLD	9	1.7	234	1.8	46,XY[20]	2	Low	1.68	Low	64.5
66.9	M	MDS-MLD	8	0.8	81	4.6	46,XY[20]	3.5	Intermediate	3.4	Intermediate	62.2
56.1	M	MDS-MLD	8.3	1.1	78	0.8	46,XY[20]	2.5	Low	1.98	Low	35.5
74.6	F	MDS-MLD	9.8	0.8	59	15	46,XX[20]	5.5	High	5.6	High	21.2
79.2	M	MDS-EB-1	10	2.2	68	9	46,XY[20]	3.5	Intermediate	3.8	Intermediate	18.9
73.2	F	MDS-EB-2	8.2	1	365	12	46,XX[20]	5	High	5.08	High	31.5
59.1	M	MDS-EB-2	7.5	0.5	27	2.6 (with Auer rod)	46,XY[20]	5	High	4.73	High	16.7
74.0	F	MDS-MLD	6	1.1	190	1.8	46,XX[20]	2.5	Low	2.65	Low	19.4
59.7	F	MDS-EB-2	7.4	3.9	61	18	46,XX[20]	6	High	5.79	High	7.5
60.9	F	MDS-EB-1	10.1	1.1	46	6.2	46,XX[20]	4	Intermediate	3.73	Intermediate	12.4
86.5	M	MDS-EB-2	7.2	0.4	17	12.5	46,XY[20]	7	Very High	7.25	Very High	4.6
60.3	M	MDS-EB-2	8.8	1.2	77	17.5	46,XY[20]	5.5	High	5.28	High	2.9
59.5	M	MDS-EB-2	8.9	0.5	47	9	46,XY[20]	5.5	High	5.26	High	31.1
76.8	M	MDS-MLD	7.7	1.3	14	10.6	46,XY[20]	6.5	Very High	6.62	Very High	17.6
82.5	M	MDS-EB-2	9.1	2.4	30	12.7	46,XY[20]	6	High	6.25	Very High	10.8

References

1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405.
2. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12):2454-2465.
3. Wang Y, Miller S, Roulston D, Bixby D, Shao L. Genome-Wide Single-Nucleotide Polymorphism Array Analysis Improves Prognostication of Acute Lymphoblastic Leukemia/Lymphoma. J Mol Diagn. 2016;18(4):595-603.
4. Behdad A, Weigelin HC, Elenitoba-Johnson KS, Betz BL. A clinical grade sequencing-based assay for CEBPA mutation testing: report of a large series of myeloid neoplasms. J Mol Diagn. 2015;17(1):76-84.
5. Gale RE, Green C, Allen C, et al. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood. 2008;111(5):2776-2784.
6. Murphy KM, Levis M, Hafez MJ, et al. Detection of FLT3 internal tandem duplication and D835 mutations by a multiplex polymerase chain reaction and capillary electrophoresis assay. J Mol Diagn. 2003;5(2):96-102.
7. Szankasi P, Jama M, Bahler DW. A new DNA-based test for detection of nucleophosmin exon 12 mutations by capillary electrophoresis. J Mol Diagn. 2008;10(3):236-241.
8. Reading NS, Lim MS, Elenitoba-Johnson KS. Detection of acquired Janus kinase 2 V617F mutation in myeloproliferative disorders by fluorescence melting curve analysis. Mol Diagn Ther. 2006;10(5):311-317.