Spliceosomal gene mutations are frequent events in the diverse mutational spectrum of chronic myelomonocytic leukemia but largely absent in juvenile myelomonocytic leukemia

Sarah Abu Kar,[1] Anna Jankowska,[1] Hideki Makishima,[1] Valeria Visconte,[1] Andres Jerez,[1] Yuka Sugimoto,[1] Hideki Muramatsu,[2] Fabiola Traina,[3] Manuel Afable,[4] Kathryn Guinta,[1] Ramon V. Tiu,[1,4] Bartlomiej Przychodzen,[1] Hirotoshi Sakaguchi,[2] Seiji Kojima,[2] Mikkael A. Sekeres,[4] Alan F. List,[5] Michael A. McDevitt,[6] and Jaroslaw P. Maciejewski[1,4]

[1]Department of Translational Hematology and Oncology Research, Cleveland Clinic, Cleveland, Ohio, USA; [2]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [3]Hematology and Hemotherapy Center, National Institute of Blood, School of Medical Sciences, University of Campinas, Campinas, Brazil; [4]Department of Hematologic Oncology and Blood Disorders, Taussig Cancer Institute, Cleveland Clinic, Cleveland, Ohio, USA; [5]H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA; and [6]Division of Hematology and Hematological Malignancy, Johns Hopkins University School of Medicine, Baltimore, MD, USA

 doi:10.3324/haematol.2012.064048

Online Supplementary Table S1. List of primers for spliceosomal genes.

SF3B1	
Exon 13-14:	
F: TCCCTTGATTAACAAAAGTCCTG	
R: TGAGTCCAGTCTGGGCAAC	
Exon 15-16:	
F: GTTGATATATTGAGAGAATCTGGATG	
R: TTTAAAATTCTGTTAGAACCATGAAAC	
U2AF1	
Exon2:	
F: GCTGCTGACATATTCCATGTG	
R: AAGTCGATCACCTGCCTCAC	
Exon6-7:	
F: CATTTGGCAAAATCTTGGAC	
R: GGGTTGTGAGGGGAGAAAG	
SRSF2	
Exon1:	
F: AAGGCAACTGCCTGAGAGG	
R: CGGACCTTTGTGAGGTCG	
Exon2:	
F: GTCTGGCGGCGAGATAATG	
R: AAAGACCTACCCCAAATCCC	

Online Supplementary Table S2. Clinical phenotype of cases with spliceosomal mutations.

Diagnosis	MC	SNP-A Lesions						Mutation status		
		Gain	(Start/End)	Loss	(Start/End)	UPD	(Start/End)	*SRSF2*	*SF3B1*	*U2AF1*
CMML-1	46,XY[20]	3q21.2	126881113/127091652	13q14.4q33.1	40874307/101205526	4q12qter	55263953/191273063	c. 284 C>A p. P95H Hetero	WT	WT
CMML-1	46,XY[20]	2p11.2	86916878/87741703	N		N		c. 284 C>A p. P95H Hetero	WT	WT
CMML-1	46,XY[20]	21q22.12; 3q26.1	35006327351603991619809051/162543774	1p35.2	30014720/31140516	N		c. 284 C>G p. P95R Hetero	WT	WT
CMML-1	46,XY[20]	N		N		4q21.21qter	79241320/191290799	c. 284 C>G p. P95R Hetero	WT	WT
CMML-1	46,XY[20]	N		N		9pterp22.2	1/17565098	c. 284 C>A p. P95H Hetero	WT	WT
CMML-1	46,XY,del(12)(p12p13)[16]/46,XY[4]	N		12p12.2p13.31	7855111/20298857	N		c. 284 C>T p. P95L Hetero	WT	WT
CMML-1	46,X,-Y[20]	N		11q23.3	118610902/119245822	N		c. 284 C>A p. P95H Hetero	WT	WT
CMML-1	46,XY[20]	N		12q13.2	51113635/52239176	N		c. 284 C>T p. P95L Hetero	WT	WT
CMML-1	46, XY, +1, der(1;7)(q10;p10)[6]/46,idem,del(1)(q32q42)[13]	N		7q21.2-q36.3	91069834/158672611	N		c. 283 C>G p. P95A Hetero	WT	WT
CMML-1	46,XX[20]	N/A		N/A		N/A		c. 284 C>A p. P95H Hetero	WT	WT
CMML-1	46,XX[20]	N		N		N		c. 284 C>A p. P95H Hetero	WT	WT
CMML-1	46,XY[20]	N		N		N		c. 284 C>T p. P95L Hetero	WT	WT
CMML-1	46,XY[20]	N		N		N		c. 284 C>A p. P95H Hetero	WT	WT
CMML-1	46,XY[20]	N		N		N		c. 284 C>A p. P95H Hetero	WT	WT
CMML-1	46,XY[20]	N		7q35	143527173/143697868	11q12.3qter	62695512/134452384	c. 284 C>G p. P95R Hetero	WT	WT
CMML-1	46,XX[20]	N/A		N/A		N/A		c. 284 C>A p. P95H Hetero	WT	WT
CMML-1	46,XX[20]	N/A		N/A		N/A		c. 284 C>A p. P95H Hetero	WT	WT
CMML-1	46,XX[20]	8p23.2	137747078/137976833	N		N		c. 284 C>A p. P95H Hetero	WT	WT
CMML-1	46,XY,del(20)(q11.2q13.3)[18]/46,XY[2]	N		N		N		c. 284 C>A p. P95H Hetero	WT	WT
CMML-1	46,XX,del(11)(q23)[3]/46,XX[17]	N		N		N		c. 284 C>A p. P95H Hetero	WT	WT
CMML-2	47,XX,+8[20]	8p23.1-q24.3	6200001/146274826	N		N		c. 284 C>A p. P95H Hetero	WT	WT
CMML-2	46,XY,t(7,8)[9]/46,XY[11]	N		N		21q22.11qter	34268175/46885639	c. 283 C>G p. P95A Hetero	WT	WT
CMML-2	46,XY[20]	N		N		4q21.23-qter	85832499/191273063	c. 284 C>A p. P95H Hetero	WT	WT
sAML	46,XX[20]	13q32.1	93932829/94096329	N		1p36.33-p32.3	1/66390902	c. 284 C>G p. P95R Hetero	WT	WT
sAML	46,XX[20]	N		N		N		c. 284 C>A p. P95H Hetero	WT	WT
sAML	46,XY[20]	N		N		N		c. 284 C>G p. P95R Hetero	WT	WT
CMML-1	46,XY,?inv(20)(q11.2q13)[20]	10q24.32, 11p11.2, 14q23.3, 2q11.2, 7q31.1	104239215/104437619;44236665/44352600;96814198/96927739;96327411/96703508;114001355/114148187	N		N		WT	WT	C.470 A>CpQ157P Hetero
CMML-1	46,XY[17]	N		N		7q22.1qter	104330974/158821424	WT	WT	c.470 A>C p.Q157P Hetero
CMML-1	45,XY,-7[18]/46,XY[2]	N			7 whole chromosome 7	N		WT	WT	c.470A>C p.Q157P Hetero
CMML-1	46,XY[20]	N		N		7q11.23qter	76383242/158604512	WT	WT	c.467A>Gp.R156Q Hetero
CMML-2	46,XY[20]	N		7q22.1	99859138/101952271	N		WT	WT	c.470A>Cp.Q157P Hetero
CMML-2	46,XY,del(20)(q11.2q13.3)[19]/46,XY[1].	N		20q11.21q13.2	99859138/101952271	6p25.2pter	1/3830294	WT	WT	c.101C>T p.S34F Hetero
CMML-2	46,XY[20]	N		N		N		WT	WT	c.101C>T p.S34F Hetero
sAML	47,XY,+19[20]	19, 21q22.2	whole chromosome 19, 38637816/38852879	3q28	193343433/193933743	1p12-pter 2p22.1-p22.3 4q28.1-q31.1 5q23.3-q34 6p22.2-p24.1 13q12.12-q12.3 16p12.3-p13.13 21q21.1	825852/119622121,34359719/41118937,126002995/141499529,128889552/160741380,12618912/26319374,23401018/29566989,10873980/20311544,179949299/22474075	WT	WT	C.101 C>T p S34F Hetero
sAML	46,XX,del(17)(q24)[6]/46,XX[24]	21q22.13q22.2	38331484/39721667	2q24.3q32.1	165728844/184500756	1p36.13pter	825852/19244426	WT	WT	c.101C>Tp.S34F Hetero
sAML	46,XX,-7[3]/46,XX,add(12)(p13)[4]/46,XX,-7,add(12)(p13)[2]/46,XX[11]	Yp11.2	3483828/3598704		7 whole chromosome 7	N		WT	WT	c.470A>Cp.Q157P Hetero
sAML	46,X,-X[17]/46,XX[3].	N		N		11q14.1qter	71354680/134437775	WT	WT	c.470A>C p.Q157P Hetero
CMML-1	46,XY,inv(3)(q21q26)[20]	N		N		N		WT	R625L; c.1874 G>T Hetero	WT
CMML-2	46,XY[15]	N		N		N		WT	E622D;c1866 G>T Hetero	WT
sAML	46,XY[20]	N		N		N		WT	K666N; c.1998 G>T Hetero	WT
sAML	46,XY[20]	N		N			13 whole chromosome 13	WT	K666R; c.1997 A>G Hetero	WT
CMML-1	46,XY,inv(1)(p13q21)[cp20]	5q13.2,17q21.31	69110721/70426997,41560151/42107467	Xp11.23	47947275/48050076	N		WT	K666N; c.1998 G>T Hetero	WT

Online Supplementary Table S3. Association between mutational status and cytogenetic lesions (metaphase cytogenetics and single nucleotide polymorphism-array).

Gene	Karyotype		p-value
	Normal	Abnormal	
	WT/M	WT/M	
SF3B1	18/2	52/3	**0,777**
U2AF1	18/1	48/10	**0,195**
SRSF2	8/9	26/17	**0,67**
TET2	7/13	34/29	**0,67**
ASXL1	14/5	36/28	**0,468**
CBL	19/0	52/12	**0,05**
EZH2	20/0	59/5	**0,273**
RAS family	11/3	54/5	**0,163**
IDH family	17/1	61/2	**0,637**
DNMT3A	17/1	56/7	**0,486**
RUNX1	15/4	43/9	**0,736**
TP53	18/0	58/4	**0,57**
UTX	14/0	53/6	**0,588**

* WT: wild type, M: mutant.
[B]*P*=0.05 all others are not significant.

Online Supplementary Table S4. Serial mutational studies for the three spliceosomal genes.

Initial presentation				After progression			
Dx	*U2AF1*	*SF3B1*	*SRSF2*	Dx	*U2AF1*	*SF3B1*	*SRSF2*
CMML-1	WT	WT	M	sAML	WT	WT	M
CMML-1	WT	WT	M	CMML-2	WT	WT	M
CMML-1	WT	WT	M	sAML	WT	WT	M
CMML-1	WT	WT	M	CMML-2	WT	WT	M
CMML-2	M	WT	WT	sAML	M	WT	WT
CMML-1	WT	WT	WT	sAML	WT	WT	WT
CMML-2	M	WT	WT	sAML	M	WT	WT
CMML-1	M	WT	WT	sAML	M	WT	WT

Dx: diagnosis; CMML: chronic myelomonocytic leukemia; WT: wild type; M: mutant;
sAML: secondary acute myelogenous leukemia derived through progression of primary CMML.