
Genome wide molecular analysis of minimally differentiated acute myeloid leukemia

Fernando P.G. Silva,¹ Inês Almeida,¹ Bruno Morolli,¹ Geeske Brouwer-Mandema,² Hans Wessels,³ Rolf Vossen,⁴ Harry Vrieling,¹ Erik W.A. Marijt,² Peter J.M. Valk,⁵ Hanneke C. Kluin-Nelemans,^{2,6} Wolfgang R. Sperr,⁷ Wolf-Dieter Ludwig,⁸ and Micheline Giphart-Gassler¹

¹Department of Toxicogenetics; ²Department of Hematology; ³Department of Clinical Cytogenetics and ⁴Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands; ⁵Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands; ⁶Department of Hematology, University Medical Center Groningen, Groningen, The Netherlands; ⁷Department of Internal Medicine I, Medical University of Vienna, Vienna, Austria and ⁸Robert-Rössle-Clinic at the HELIOS Clinic Berlin-Buch, Department of Hematology, Oncology, and Tumor Immunology, Berlin, Germany

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Supplementary Information

Primers used for PCR and sequencing were the same unless otherwise specified. All PCR reactions consisted of an activation step at 95°C for 10 min, followed by incubation for 30 to 45 cycles for 20 sec at 95°C, 30 sec at a specific annealing temperature and extension at 72°C for an appropriated time (*Online Supplementary Table S2*).

The final steps consisted of an extension step at 72 °C for 5 min, which was followed by denaturation at 95 °C for 1 min in the samples analyzed by melting curve analysis.

Reactions mixes varying from 10 to 15 mL contained 10 ng of DNA, 6 pmol of each primer, 200 mM of dNTPs, 1.5 to 3.0

mM of MgCl₂ (*Online Supplementary Table S1*), 1 U of DNA polymerase, 1 x reaction buffer and 15 mL mineral oil. Reactions mixes for melting curve analysis further contained 1 x LCGreen PLUS (Idaho Technology, Salt Lake City, UT, USA).

In some reactions 1 M of betaine (Sigma-Aldrich, Saint-Louis, MO, USA) was added as a specificity enhancer. Reaction and condition variables as well as primers are presented in *Online Supplementary Table S2*.

For the immunophenotyping results at diagnosis: percentage of cells positive for a surface marker, please refer to the corresponding PDF [PDF_haematol.2009.009324.immunophenotyping.pdf](#)

Online Supplementary Table S1. Detailed description of mutations in *RUNX1*, *RAS*, *PTPN11*, *JAK2*, *NPM1* and *CEBPA*.

Patient	Gene	Allele 1	Protein 1	Allele 2	Protein 2
1	<i>PTPN11</i>	226 G>A	E76K		
1	<i>JAK2</i>	1849 G>T	V617F		
2	<i>NRAS</i>	183 A>C	Q61H		
2	<i>PTPN11</i>	181 G>C	D61H		
5	<i>PTPN11</i>	181G>T	D61Y		
7	<i>RUNX1</i>	237 G>T	W79C	237 G>T	W79C
9	<i>KRAS</i>	35 G>A	G12D		
23	<i>JAK2</i>	1849 G>T	V617F	1849 G>T	V617F
24	<i>RUNX1</i>	416G>A	R139G	416G>A	R139G
24	<i>NRAS</i>	38 G>A	G13D		
26	<i>RUNX1</i>	887-10C>A	A297fs	887-10C>A	A297fs
27	<i>RUNX1</i>	239G>A	R80H	239G>A	R80H
28	<i>RUNX1</i>	142G>T	D48Y		
28	<i>JAK2</i>	1849 G>T	V617F		
30	<i>RUNX1</i>	237G>C	W79C	237G>C	W79C
30	<i>PTPN11</i>	179 G>T	G60V		
31	<i>RUNX1</i>	342_343insAAGAAGTCTGTTC	A115fs		
32	<i>RUNX1</i>	512A>T	D171V	512A>T	D171V
35	<i>NRAS</i>	38 G>A	G13D		
35	<i>CEBPA</i>	937_939dupAAG	K313dup	206_209delACCC	D69Gfs
36	<i>RUNX1</i>	425_426insCGTCCCCGAAG	R142fs		
37	<i>RUNX1</i>	314_315insT	M106fs	314_315insT	M106fs
38	<i>RUNX1</i>	164_165insTGCTT	L55fs	164_165insTGCTT	L55fs
38	<i>RUNX1</i>	383_384insAGAAT_329_383dup	V128_A129insEY_E111_V128dup		
41	<i>RUNX1</i>	955_956insC	R319fs		
44	<i>PTPN11</i>	227 A>T	E76V		
47	<i>RUNX1</i>	249 G>T	K83N	249 G>T	K83N
55	<i>RUNX1</i>	314_315delTG	V105fs	314_315delTG	V105fs
56	<i>NRAS</i>	182 A>G	Q61R		
59	<i>KRAS</i>	35 G>A	G12D		
59	<i>NPM1</i>	860_863insTCTG	W288fs		
60	<i>PTPN11</i>	226 G>C	E76Q		

Online Supplementary Table S2. Sequence and annealing temperature of primer pairs used for mutation screening and $MgCl_2$ concentrations used for mutation screening.

Primer	Screened Patients	Forward (5'-3')	Reverse (5'-3')	Annealing		MgCl ₂ Conc. (mM)	LCGreen PLUS	Betaine
				T _m (°C)	time (seconds)			
<i>RUNX1</i> exon 6	2, 3, 22, 26, 29, 41, 43, 52	TGATCTCTCCCTCCCTCCT	CAGTTGGTCTGGGAAGGTGT	59	40	1.5	No	Yes
<i>RUNX1</i> exon 7	2, 3, 22, 26, 29, 41, 43, 52	AAATCCCACCCCACTTTACA	GCTCAGCTGCAAAGAATGTG	59	40	1.5	No	Yes
<i>RUNX1</i> exon 8	2, 3, 22, 26, 29, 41, 43, 52	CTCCGCAACCTCCTACTCAC	CCTGACCTACAGCGAGATCC	59	40	1.5	No	Yes
<i>KRAS</i> codon 12/13	All	AGGCCTGCTGAAAATGACTG	TGGATCATATTCGTCCACAAAA	65	40	1.5	Yes	No
<i>KRAS</i> codon 61	All	AGGATTCCTACAGGAAGCAAG	TACACAAAGAAAGCCCTCCC	58.3	40	2.5	Yes	No
<i>NRAS</i> codons 12/13	All	GGTTTCCAACAGTTCTTGC	CACTGGGCCTCACCTCTATG	63.7	40	1.5	Yes	No
<i>NRAS</i> codon 61	All	GCAATAGCATTGCATTCCCT	TCGCCTGTCCTCATGTATTG	57.8	40	1.5	No	No
<i>PTPN6</i> exon 3	All	CCTCTACTCCTGCACCGACT	CTCATCCCTGCTTGGGAAT	64	40	2.0	Yes	No
<i>PTPN6</i> exon 13	All	TATGCCTGGACCTGAGGTTT	ACAGTGGGAAGGTGGTGGT	62.7	40	1.5	Yes	No
<i>PTPN11</i> exon 3	All	TGTAACCTTTATTGTCCCTTG	AGCAGCAGACTTTGTGGTCA	65.7	40	2.0	Yes	No
<i>PTPN11</i> exon 13	All	TTCTTCATGATGTTTCCCTCG	CCAAGAGGCCTAGCAAGAGA	58.9	40	2.0	Yes	No
<i>JAK2</i> codon 617	All	AAGCAGCAAGTATGATGAGCA	CAGATGCTCTGAGAAAGGCA	62.9	40	1.5	Yes	No
<i>SPI1</i> exon 1	All	TCTGCATTGGCCCCA	GCAGGCGTCCGAGGG	63	40	1.5	Yes	No
<i>SPI1</i> exon 2	All	CATGGTGCAGGCCCTGA	GCCCAGGCTGGGCTG	65.5	40	1.5	Yes	No
<i>SPI1</i> exon 3	All	CCTGCCAACCCCTGAGCT	GCCCCACCACAGGCCT	67	40	1.5	Yes	No
<i>SPI1</i> exon 4	All	TCCTGACTCTGATCTCCTTCCC	GCAGGGCACAGACACGG	67	40	2.0	Yes	No
<i>SPI1</i> exon 5	All	CCGGGCCCTGTGCGTACGCAAGG	CCGGGAGCGTCTCCTGTGTCCG	59	90	1.5	No	Yes
<i>PURA</i>	5, 8, 9, 25, 33, 37, 39, 44, 60	GAGAGTGGCTGACTGGCTGT	GGGGGTTTCATTGAGTTTGA	59	45	2.0	No	No
<i>CEBPA</i>	All	GCCGGGAGAACTCTAATCC	AGGCACCGGAATCTCCTAGT	59	120	2.0	No	Yes
<i>PILRB</i> exon 1	18, 24, 26, 29, 44, 45	GGAGCAATTAGGCAAGGGATA	CCCAGATGTTCTGCCTTTGT	59	40	2.0	No	No
<i>PILRB</i> exon 2	18, 24, 26, 29, 44, 45	AGGTCTCTCCCCACTCACT	GTGGTCACAAAACCTCAC	60	40	2.0	No	No
<i>PILRB</i> exon 3	18, 24, 26, 29, 44, 45	ACCATGCCTTTCATCCAGAC	CTTGAGGAGACAGTGCCTTCT	59	40	2.0	No	No
<i>PILRB</i> exon 4	18, 24, 26, 29, 44, 45	CTGGCCCTGCATCTAAAAAC	GGTACTGCAGAGCAGGAAGG	59	40	2.0	No	No
<i>CEBPB</i> set1	All	GTGTCTTCCGGGCACCCGCGTT	[FAM]AGAGGTCGGAGAGGAAGTCG	59	30	1.5	No	Yes
<i>CEBPB</i> set2	All	GCCTCCTCCGGGCAGCAC	[TET]GTGTCTTGTCTGGACGAGGAGACGTG	59	30	2.0	No	Yes
<i>CEBPB</i> set3	All	AGCAGCGGGAGCCTCT	[HEX]GTGTCTTGGCCGCTAGCAGT	59	30	2.5	No	Yes
<i>PILRA</i> exon 1	18, 24, 26, 29, 44, 45	GACTTGGCACTGACCACAAG	GAGGGAGGGAGTGTAGTAGGG	65.5	45	2.0	No	No
<i>PILRA</i> exon 2	18, 24, 26, 29, 44, 45	CGAGACAAAGGTGGGACATC	TGTTAGGGCCAAAGAGAGA	58	45	2.0	No	No
<i>PILRA</i> exon 3	18, 24, 26, 29, 44, 45	CTTTCACCCAGACACCCTGT	GAGCCTCACAATGCAGACA	58	45	2.0	No	No
<i>PILRA</i> exon 4	18, 24, 26, 29, 44, 45	CAGAGAAGGTGGGATGGAGA	GGTTTGTGGGTTCTTAGCA	59.3	45	2.0	No	No
<i>PILRA</i> exon 5	18, 24, 26, 29, 44, 45	TCCTGTCCTTGTGCCATA	GTACTTGAATGGATGCAGATTTT	57.3	45	2.0	No	No
<i>TET2</i> exonw 1/2	2, 4, 19	GTTGCTATGAAGACAAGAATGTTTATTTCCGTACATTTGTGACTTGTGCTCCA		57	120	2.0	No	No

Immunophenotyping results at diagnosis: percentage of cells positive for a surface marker.

Patient number	1	2	3	4	5	6	7	8	9	18	19	20	21	22	23	24	25
CD10	0	0	0	0.5	0.5	2	3	6	1	0.5	0.5			0		1	
CD114												75	11		10		1
CD116												1	2		2		5
CD117												67	67	36	60	51	81
CD11b		0.5						2				18	1		4		37
CD11c	23	1	6			15	1			1	1						
CD13	10	94	50	56	77	13	32	51	77	75	18	95	96	33	31	88	68
CD133												2	92		20	38	56
CD135												16	1		13		3
CD14	15	0.5	6	42	4	10	0.5	4	1	0.5	2	0	2	0	5	26	1
CD15	10	0.5	6		8	10	4	6	20	12	36	2	2	0	0	60	1
CD16																	
CD19	2	0.5	4		2	3	0.5	2	1	2	1	20	1	5	3	32	2
CD1a														0		0	
CD2	8		7		9	17	1	9		5		2	1	3	3	9	13
CD20	1				2		4								0		
CD22			6		2		0.5			3	1			6			
CD24														1			
CD253a												1	0	1	2	0	1
CD3	5	0.5	8	10	10	18	0.5		2	5	14			2		9	
CD33	71	5	50	44	67	70	3	84	74	35	10	86	5	42	77	70	69
CD34	71	95	86	50	58	56	74	0	77	91	75	97	95	93	60	89	91
CD36												2	2	1	19	38	7
CD38				79								96	76	96	39		68
CD4											4	1	2	3	1	86	11
CD41												7	1	0	3		2
CD45				96	89									99		99	
CD5					8		2							3			
CD54														24			
CD56	1		1			3	0.5			2		1	1	1	2	0	3
CD57														1			
CD61	2		0		1	0	0.5	0.5	3	0.5	0.5	5	1	0	2	10	2
CD64												19	2	0	2	44	3
CD65												5	2	0	1	85	4
CD7	44	34	6	62	33	72	28	6	28	6	19	86	1	3	1	7	22
CD79a																	7
CD8														2			
CD90												2	0		1		2
cyCD22	1											6	2	13	9		4
cyCD3	5											3	1	0	2	10	14
cyIgM																	
HLA-DR	77	91	85	48	78	40	15	4	54		68	92	43	51	54	46	23
LF												6	0	0	1	0	1
MPO7		0.5				10			1			6	1	2	2	74	1
slg																	
T-cell receptor gamma/delta														2			
T-cell receptor alpha/beta														0			
TdT	0.5	0.4	79	0	0	3	83	12	1	87	81	10	2	95	6	58	7

Patient number	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	
CD10		0			1	0	3	1	0	2	0	1	1	0	0	1	0	
CD114	11		51	44														
CD116	29		0	25														
CD117	22	31	84	56	89	3	59	78	12	76	1	69	23	2	49		70	
CD11b	35		4	30														
CD11c																3	1	20
CD13	76	86	91	90	68	89	88	25	84	62	75	78	51	94	68	81	45	
CD133	40		85	46	83	79	82											66
CD135	8		0	11														
CD14	17	4	1	10	2	0	1	1	0	0	0	1	13	31	1	2		
CD15	9	39	0	10	5	6	8	22	3	4	0	8	16	82	1	2		
CD16																		
CD19	2	1	3	6	3	0	3	2	0	22	44	2	5	6	3	2	1	
CD1a					0	0	0							0			0	
CD2	7	11	4	8	4	3	9	8	0	3	6	12	36	3	7	10	1	
CD20		1									1			2	4	3	1	
CD22		4									5			3	13	4	31	
CD24		1									34			36				
CD253a	1	0	1	1	1	0	1	4	0	0	0	2		0				
CD3		8			3	4	6	6	0	1	2	7	2	2	6	9	5	
CD33	39	18	35	72	21	56	5	71	98	95	11	33	68	54	8	67	18	
CD34	76	86	88	76	89	92	91	71	96	94	94	82	45	89	56	78	70	
CD36	30	7	51	50	6	4	27	6	0	9	2	4	77	20				
CD38	92		72	94														
CD4	16	36	2	7	70	30	62	3	2	2	5	31	32	16	3	4		
CD41	1	4	33	25				7	0	0	0	3	12	2				
CD45		97			86	99	58	94	99	99	93	98	99	72	49	90		
CD5		8									4			2	6	9	1	
CD54		48												34				
CD56	19	2	5	5	0	0	19	1	74	0	0	4	3	1	8		14	
CD57																		
CD61	3	4	39	26	1	2	9	4	0	6	2	2	15	1	2	0	6	
CD64	25	22	1	24	7	2	12	22	65	17	7	15	27	35				
CD65	30	7	1	23	8	5	12	5	34	62	0	16	18	39				
CD7	7	7	10	57	4	3	14	17	0	73	3	10	4	4	11	9	26	
CD79a		9			25	27	7				8			25				
CD8									0					2	4	4		
CD90	28		42	9														
cyCD22	4	6	3	10							16			2				
cyCD3	10		4	4	2	6	6		2					3				
cylgM														4				
HLA-DR	85	92	58	86	49	82	75	69	0	87	91	79	88	85	41	54	5	
LF	6	17	1	4	1	5	1	17	0	6	2	4	2	10				
MPO7	5	3	1	12	4	4	3	8	6	96	0	12	8	4	3		3	
slg					3							3		6				
T-cell receptor gamma/delta														1				
T-cell receptor alpha/beta														2				
TdT	58	76	13	22	27	45	68	1	0	16	83	77	0	41	0	44	1	

Patient number	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
CD10	2	0	0	0	0	0	0	0	0	5	0	0		0.5	0.5	0.5		1
CD114																		
CD116																		
CD117				0	65	15		70	5	80	60	0	66			29	47	61
CD11b																		
CD11c																19	77	
CD13	85	80	70	0	0	70	0	70	80	80	51	72	82	6	76	82		83
CD133																		
CD135																		
CD14	11	0	0	0	0	0	0	10	0	0	4	0	0	0.5	0.5	1	4	11
CD15	27	0	10	50	0	0	0	0	0	0	5	0	35	2	0.5	0.5	30	7
CD16														1	1	1		
CD19	1	0	0	0	0	0	0	7	0	5	3	0	0	1	1	4	3	1
CD1a																		
CD2		0	0	0	0	0	0	10	0	0	7	2	0	3	4	3	10	4
CD20	1													1	1	3	3	1
CD22																	2	1
CD24																		
CD253a																		
CD3	2													3	5	2	9	3
CD33	51	20	55	0	0	2	0	15	60	1	32	84	46	94	80	83	87	94
CD34	71	60	90	90	85	95	95	75	95	95	70	97	88	93	2	91	1	80
CD36																	20	18
CD38																		
CD4														1	2	1	82	62
CD41																	6	3
CD45																	98	98
CD5														3	5	3	8	27
CD54																		
CD56			80	0	0	80		10	0	1			0				1	4
CD57																		
CD61	1	0	0	0	0	0		0	0	0		0		1	0.5	0.5		
CD64																		
CD65		15	0	5	0	0	0	0	5	1	7	7			0.5	5		
CD7	80	0	25	0	0	0	10	30	0	95	7	10	0	4	4	4	11	35
CD79a														0.5	1	27		
CD8														1	2	0.8	4	1
CD90																		
cyCD22																		
cyCD3														0	0	0		
cyIgM																		
HLA-DR	80												84	94	17	71	64	96
LF																		
MPO7	8	0		2	0	6	1	2	1	0	8	0	0	1		1	2	4
slg																		
T-cell receptor gamma/delta																		
T-cell receptor alpha/beta																		
TdT	81												49	0.1	0.1		1	1