Genome-wide genotyping of acute myeloid leukemia with translocation t(9;11)(p22;q23) reveals novel recurrent genomic alterations

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SUPPLEMENTAL METHODS, TABLES, AND FIGURES

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Supplemental Methods

Patient samples and treatment

Adult patients were entered on 1 of 6 treatment trials of the German-Austrian AMLSG [AML HD93 (n=6), AML HD98A (clinicaltrials.gov identifier NCT00146120; n=15), AML HD98B (n=3), AMLSG 06-04 (NCT00151255; n=2), AMLSG 07-04 (NCT00151242; n=9), AMLSG 12-09 (NCT01180322, n=2)]. 3 patients were treated off protocol. Written informed consent and local ethics committee approval was available for all patients. The t(9;11) was identified by conventional cytogenetics and/or reverse-transcriptase PCR as previously described (Table S1)(1).

DNA extraction

Peripheral blood or bone marrow samples obtained at diagnosis and during remission were enriched for leukemic blasts using Ficoll gradient centrifugation as described previously(2, 3). Cells were stored at -80°C or -196°C, respectively. DNA extraction was performed using the Allprep DNA/RNA mini kit (Qiagen, Leiden). DNA quality and integrity was assessed using a Nanodrop ND-1000 spectrophotometer (Wilmington, DE).

Single-nucleotide polymorphism (SNP)-array-based genotyping

DNA from each sample was processed using *Nsp* I and *Sty* I restriction enzymes according to manufacturer's instruction protocol for Affymetrix SNP 6.0 arrays. SNP genotype calls were generated by applying the birdseed algorithms in Genotyping console v2.0, v3.0, v4.0 (Affymetrix) using at least 50 arrays in each analysis. CHP- and CEL-file raw data were processed according to previously described algorithms using reference alignment(4), dChipSNP(5), and circular binary segmentation (CBS)(6). CBS results were filtered to eliminate all lesions with less than 5 consecutive markers (SNP and/or copy number variants) and with mean segment deviation above 0.2 for gains and below -0.2 for genomic losses. Pair-wise segmentation was performed for each diagnostic sample with matched remission sample. For patient samples lacking any matched material we computed the

segmentation against a reference pool of 300 samples from our local database as previously described(2). From the unpaired cohort only those lesions were counted as truly somatically acquired that were found to be altered in at least one sample from the paired sample cohort. All other lesions were eliminated from the data set. All calls were also visually inspected using dChipSNP and crosschecked against public databases of copy number polymorphisms (http://projects.tcag.ca/variation/ and http://www.genome.ucsc.edu/) to exclude inherited copy number variants and false calls due to experimental artefacts (inter-batch effects or noise). For the detection of true copy-neutral loss-of-heterozygosity a threshold of >20 Mb in size and/or with >20 consecutive markers containing less than 10% intervening or conflicting calls was defined according to prior experiences(2, 7).

Gene mutation and gene expression analysis

Gene mutation/expression status was determined for a set of genes known to be frequently altered in AML [*FLT3* (internal tandem duplication, ITD; point mutations in the tyrosine kinase domain, TKD), *NPM1*, *IDH1*/2, *DNMT3A*, *TET2*, *NRAS*, and deregulated expression of *EVI1*]. Mutation/expression assays were performed as previously described(8, 9).

***MLL3* and *KLF5* expression using Affymetrix Human *GeneChip* U133 plus 2.0 microarrays**

Cases were selected for gene expression analysis based on tissue availability for RNA extraction or availability of former microarray-based gene expression analysis. RNA was extracted from cell pellets using Allprep DNA/RNA mini kit (Quiagen, Leiden), processed, and hybridized to Affymetrix HG *GeneChip* U133 plus 2.0 microarrays according to manufacturer's protocol as previously described(10). Data were normalized using the RMA algorithm and log2 transformed. To measure *KLF5* and *MLL3* expression levels t(9;11)-rearranged AML cases were dichotomized into high (n=10) vs. low (n=10) expression cases of *KLF5* or *MLL3* based on median expression measured by Affymetrix HG U133 plus 2.0

microarrays (*KLF5*, probe-set: 209212_s_at; *MLL3*, probe-set 222414_at). No material was available for the cases with gain of 13q (MLL#14 and MLL#17).

Statistical analysis

The Kaplan-Meier method, the Wilcoxon rank order-, and the Fisher's exact test were used for calculation of distribution estimation and survival distributions of overall survival (OS), event-free survival (EFS), and relapse-free survival (RFS). Definition of complete remission (CR) and survival endpoints such as OS, EFS, and RFS followed the recommended consensus criteria(11).

Table S1: Patient characteristics

Sample ID	AML-Type	Primary Cancer (Therapy Regimen)	Gender	Blast %*	Karyotype
MLL#1	AML		1	80	46,XY,t(9;11)(p22;q23)[20]
MLL#2	t-AML	Breast Cancer (4x EC/Rx)	2	90	46,XX,t(9;11)(p21~22;q23))[15]
MLL#3	t-AML	Breast Cancer (3x FEC; 3x Docetaxel/Rx)	2	n.d.	46,XX,t(9;11)(p22;q23))[30]
MLL#5	t-AML	Melanoma	2	85	46,XX,t(9;11)(p22;q23))[24]
MLL#6	t-AML	Cervical Cancer (6xCEV/Rx)	2	80	46,XX,t(9;11)(p22;q23)[17]/46,XX[5]
MLL#7	AML		1	90	46,XY,t(9;11)(p22;q23)[14]/46,XY[1]
MLL#8	t-AML	Breast Cancer (regimen unknown)	2	86	46,XX,t(9;11)(p21;q23))[18]
MLL#9	AML		1	59	46,XY,t(9;11)(p22;q23))[16]
MLL#10	AML		2	88	46,XX,t(9;11)(p22;q23))[24]
MLL#11	AML		1	96	46,XY,t(9;11)(p22;q23))[22]
MLL#12	AML		1	90	46,XY,t(9;11)(p22;q23))[23]
MLL#13	AML		1	90	46,XY,t(4;11;9)(q27;q23;p22))[20]
MLL#14	t-AML	Germ Cell Tumor (3xPEB)	1	62	46,XY,t(9;11)(p22;q23)[10]/ 47,XY,+8,t(9;11)(p22;q23)[5]
MLL#15	AML		1	96	46,XY,t(9;11)(p22;q23))[11]
MLL#16	t-AML	Breast Cancer (regimen unkown)	2	97	46,XX,t(9;11)(p22;q23)[10]/ 47,XX,t(9;11)(p22;q23),+8[4]
MLL#17	AML		2	n.d.	46,XX,t(9;11)(p22;q23)[3]/ 47,XX,t(9;11)(p22;q23),+13[8]
MLL#18	AML		1	80	46,XY,t(9;11)(p22;q23))[14]
MLL#19	t-AML	Hodgkin Lymphoma (regimen unkown)	1	85	46,XY,t(9;11)(p22;q23))[15]
MLL#20	AML		2	85	46,XX,t(9;11)(p22;q23))[14]
MLL#21	AML		2	75	47,XX,+8,t(9;11)(p22;q23)[9]/46,XX[2]
MLL#22	t-AML	Breast Cancer (4x high-dose EC)	2	74	46,XX, t(9;11)(p22;q23))[14]
MLL#23	AML		2	90	47,XX,+8,t(9;11)(p22;q23))[10]
MLL#24	AML		2	100	47,XX,t(9;11)(p22;q23),+21[12]/ 47,XX,del(7)(p11),t(9;11)(p22;q23),add(12)(q24),+21[3]
MLL#25	AML		1	90	46,XY,t(9;11)(p22;q23))[12]
MLL#26	t-AML	Hodgkin Lymphoma (2xCOPP/ABVD/Rx)	1	90	47,XY,t(9;11)(p22;q23),+der(9)t(9;11)(p22;q23))[17]
MLL#27	AML		2	90	47,XX,+8,t(9;11)(p11;q23))[7]
MLL#28	t-AML	Breast Cancer (CMF)	2	96	46,XX,t(9;11)(p21;q23))[10]
MLL#29	AML		2	96	46,XX,t(9;11)(p22;q23),dup(11)(q13q25))[15]
MLL#30	AML		2	90	46,XX,t(9;11)(p22;q23))[21]
MLL#31	t-AML	Hodgkin Lymphoma (8xBEACOPPesc.)	2	90	46,XX,t(9;11)(p22;q23)[13] 47,XX,t(9;11)(p22;q23),+19[2]
MLL#32	AML		2	50	47,XX,t(9;11)(p22;q23),+21[18]/46,XX,t(9;11)(p22;q23)[2]
MLL#33	AML		1	100	45,X,-Y,t(9;11)(p21;q23)[9] 46,XY,-Y,+8,t(9;11)(p21;q23)[2]
MLL#34	AML		1	95	47,XY,+8,t(9;11)(p13;q23)[8]/46,XY,t(9;11)(p13;q23)[5]
ML#L36	AML		2	85	49,XX,+der(8)(q22),add(9)(p13),t(9;11)(p22;q23),+add(11) (p13),add(12)(p11),+14)[17]
MLL#38	t-AML	Breast Cancer (regimen un known)	2	90	46,XX,t(9;11)(p22;q23))[12]
MLL#40	t-AML	Primary tumor unknown	1	n.d.	46,XY,t(9;11;9;11)(p22;q23;p22;q13)[20]
MLL#41	t-AML	Hodgkin Lymphoma (8xBEACOPP)	1	80	46,XY,t(9;11)(p22;q23)[18] 47,XY,t(9;11)(p22;q23),+der(9)t(9;11)(p22;q23)[2]
MLL#43	n.d.		2	n.d.	48,XX,+8,t(9;11)(p22;q23),+der(9)t(9;11)(p22;q23)[25]

Sample ID	AML-Type	Primary Cancer (Therapy Regimen)	Gender	Blast %*	Karyotype
MLL#44	n.d.		1	n.d.	46,XY,t(9;11)(p22;q23)[5] 46,XY,t(9;11)(p22;q23),der(12)t(1;12)(q21;p12)[15]
MLL#45	t-AML	DLBCL (6xR-CHOP+2xR)	2	84	46,XX,t(9;11)(p22;q23)[11] 52,XX,+3,+6,+8,t(9;11)(p22;q23),+12,+13,+18 [9]

The t(9;11) was identified by conventional cytogenetics and/or reverse-transcriptase PCR.
*Blast percentage is referring to BM blast count; gender: 1=male, 2=female; n.d.=no data; DLBCL=diffuse large B-cell lymphoma; therapy-regimen: EC=epirubicin and cyclophosphamide; FEC=5-fluorouracil, epirubicin, cyclophosphamide; CEV=carboplatin, etoposide, vincristine; PEB=cisplatin, etoposide, bleomycin; COPP=cyclophosphamide, vincristine, procarbacine, prednisone; ABVD=adriamycin, bleomycin, vinblastine, dacarbazine; CMF=cyclophosphamide, methotrexate, 5-fluorouracil; BEACOPP(esc)=bleomycin, etoposide, adriamyicin, cyclophosphamide, vincristine, procarbazine, prednisone; CHOP=cyclophosphamide, doxorubicin, vincristin, prednisone, RrRituximab; Rx=radiotherapy

Table S2: Observed copy number alterations (CNAs) for each AML case with t(9;11) (paired analysis). CNAs are listed for each case with start and end positions as indicated.

Case-ID	AML-Type	Chromosome	Cytoband	Start*	End*	CNA-type	CNA-size (kb)	Gene mutation /*EVI1* expression
MLL#1	AML	1	q21.1	143,744,483	143,945,015	loss	200.532	**- / *EVI1* low**
MLL#2	t-AML	-	-	-	-	-	-	**- / *EVI1* low**
MLL#3	t-AML	2	p11.2	88398741	88607713	loss	208.972	**- / *EVI1*high**
		2	p24.3	15744487	16253263	loss	508.776	
		3	q26.33	182932061	183120944	loss	188.883	
		3	q13.13	110433975	110620383	loss	186.408	
		4	q35.2	189117413	191261892	loss	2144.479	
		7	q11.22	66429845	68686152	loss	2256.307	
		7	q21.3-q22.1	97677076	98303945	loss	626.869	
		7	p11.2-q11.21	55942408	62803669	loss	6861.261	
		7	q11.22-q11.23	69500954	76146102	loss	6645.148	
		7	q36.1-q36.3	151675439	158096843	loss	6421.404	
		10	p11.21	35092677	35319381	loss	226.704	
		12	q24.13-q24.33	111,930,392	130,623,782	loss	18693.39	
		13	q34	110843036	111823964	loss	980.928	
MLL#5	t-AML	9	p24.3-p21.3	30,910	20,362,786	gain	20331.876	**- /*EVI1*high**
		11	q23.3-q25	117,859,771	134,449,982	loss	16590.211	
MLL#6	t-AML	-	-	-	-	-	-	**- / *EVI1*high**
MLL#7	AML	-	-	-	-	-	-	**- / *EVI1*: n.d.**
MLL#8	t-AML	1	q31.1-q31.3	185,407,441	194,143,602	loss	8736.161	**FLT3-TKD (D835)/*EVI1* low**
		2	p12	76,531,878	78,808,834	loss	2276.956	
MLL#9	AML	-	-	-	-	-	-	**- / *EVI1*high**
MLL#10	AML	9	p21.3	20,376,290	20,721,909	loss	345.619	**IDH1-SNP 105 / EVI1high**
		11	q23.3	117,861,238	117,946,853	loss	85.615	
MLL#12	AML	-	-	-	-	-	-	**- / *EVI1* low**
MLL#15	AML	-	-	-	-	-	-	**- / *EVI1* low**
MLL#17	AML	6	q13	70308225	70323531	loss	15.306	**FLT3-TKD (D835) / *EVI1* high**

Case-ID	AML-Type	Chromosome	Cytoband	Start*	End*	CNA-type	CNA-size (kb)	Gene mutation /*EVI1* expression
		13	q11-q34	17,994,924	112,391,207	gain	94396.283	
		16	p13.11	14956257	15023746	loss	67.489	
		23	q26.2	130643148	130801050	loss	157.902	
MLL#21	AML	8	p23.3-q24.3	2,269	146,268,947	gain	146266.678	**- / *EVI1* low**
		18	q12.2	33,146,609	33,471,499	loss	324.89	
MLL#40	t-AML	-	-	-	-	-	-	**n.d. / n.d**
MLL#45	t-AML	-	-	-	-	-	-	**- / n.d.**

*hg18; (-) no CNA/LOH/mutation detectable; n.d.=no mutation/ expression data available.

Table S3: Recurrent copy number alterations (CNAs) in unpaired AML cases with t(9;11) (unpaired analysis). CNAs are listed for each case with start and end positions as indicated.

Case-ID	AML-Type	Chromosome	Cytoband	Start*	End*	CNA-type	CNA-size (kb)	Gene mutation /*EVI1* expression
MLL#13	AML	11	q23	117,861,238	117,873,353	loss	12.115	**- / *EVI1* high**
MLL#14	t-AML	8	p23.3-q24.3	21,242	146,268,972	gain	146248.730	**- / *EVI1* low**
		13	q21.33	72,097,687	73,119,202	gain	1022.515	
MLL#20	AML	7	q35-q36.2	142,841,489	152,604,848	loss	9763.359	**- / *EVI1* low**
MLL#22	t-AML	13	q12.12	22,537,900	22,791,657	gain	253.757	**- / *EVI1*high**
MLL#23	AML	8	p23.1-q24.3	21,242	146,268,972	gain	146247.730	***IDH1*-SNP 105 / *EVI1* low**
MLL#26	t-AML	11	q23.3-q25	117,859,771	134,449,982	gain	16590.211	**n.d. / n.d.**
MLL#27	AML	8	p23.1-q24.3	21,242	146,268,972	gain	146247.730	**n.d. / n.d.**
MLL#29	AML	9	p22.1	19,198,991	19,545,619	gain	46.628	***IDH1*-SNP 105 / *EVI1* low**
MLL#32	AML	11	q23	117,863,133	117,873,755	loss	10.622	**- / *EVI1* high**
		13	q12.12-q12.13	24,083,858	24,478,645	gain	394.787	
MLL#33	AML	8	p23.3-q24.3	21,242	146,268,972	gain	146247.730	**- / *EVI1* low**
MLL#34	AML	8	P23.3-q24.3	151,222	146,268,972	gain	146117.750	**- / n.d.**
MLL#36	AML	8	p23.1-q24.3	21,242	146,268,972	gain	146247.730	**- / n.d.**
MLL#43	n.n.	8	p23.3-q24.3	21,242	146,268,972	gain	146247.730	**n.d. / n.d.**

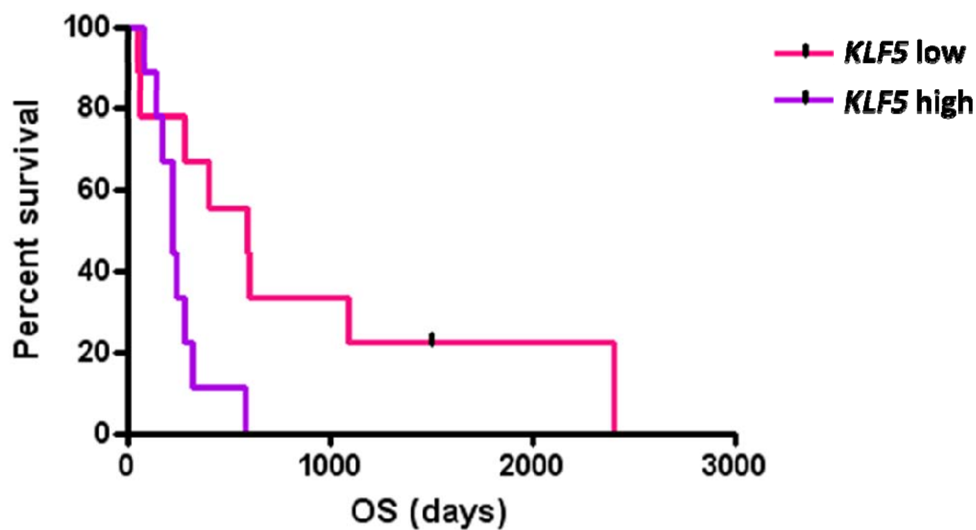
*hg18; (-) no CNA/LOH/mutation detectable; n.d.=no mutation/ expression data available; n.n.=not known;

Table S4: Median *KLF5*-expression levels based on Affymetrix HG in *MLL*-rearranged leukemia samples

Sample Name*	Treatment Protocol	*EVI1*-expression status	Median Expression Level**	*KLF5* - Group
MLL#5	07-04	high	-0.903568792	Low expression
1[§]	93	n.d.	-0.857772398	
2[§]	07-04	low	-0.809994268	
MLL#28	93	high	-0.78643899	
3[§]	98B	n.d.	-0.785838652	
MLL#16	98A	low	-0.745515871	
MLL#12	98A	low	-0.587627459	
4[§]	98A	high	-0.507879782	
5[§]	05-04	n.d.	-0.400304842	
MLL#11	07-04	low	-0.345617819	
MLL#25	93	low	-0.22851615	High expression
MLL#24	93	high	-0.222498465	
6[§]	93	n.d.	-0.10512166	
MLL#22	98A	high	-0.102599192	
7[§]	06-04	low	0.255415869	
MLL#9	98A	high	0.386407328	
8[§]	98A	low	0.950099897	
MLL#18	98A	high	1.629785967	
9[§]	98A	high	1.655306768	
10[§]	98A	n.d.	2.512278509	

*Samples marked with "§" were not available for SNP-array analysis; **Median expression level of Affymetrix *KLF5* probe set: 209212_s_at; n.d.=no data

Figure S1



Higher *KLF5* expression was associated with lower overall survival as shown (p=0.02, logrank test).

Supplementary References

1. Scholl C, Breitinger H, Schlenk RF, Döhner H, Frohling S, Döhner K. Development of a real-time RT-PCR assay for the quantification of the most frequent MLL/AF9 fusion types resulting from translocation t(9;11)(p22;q23) in acute myeloid leukemia. Genes Chromosomes Cancer. 2003 Nov;38(3):274-80.

2. Kühn MWM, Radtke I, Bullinger L, Goorha S, Cheng J, Edelmann J, et al. High-resolution genomic profiling of adult and pediatric core-binding factor acute myeloid leukemia reveals new recurrent genomic alterations. Blood. 2012 Mar 8;119(10):e67-75.

3. Bullinger L, Krönke J, Schon C, Radtke I, Urlbauer K, Botzenhardt U, et al. Identification of acquired copy number alterations and uniparental disomies in cytogenetically normal acute myeloid leukemia using high-resolution single-nucleotide polymorphism analysis. Leukemia. 2010 Feb;24(2):438-49.

4. Pounds S, Cheng C, Mullighan C, Raimondi SC, Shurtleff S, Downing JR. Reference alignment of SNP microarray signals for copy number analysis of tumors. Bioinformatics. 2009 Feb 1;25(3):315-21.

5. Lin M, Wei LJ, Sellers WR, Lieberfarb M, Wong WH, Li C. dChipSNP: significance curve and clustering of SNP-array-based loss-of-heterozygosity data. Bioinformatics. 2004 May 22;20(8):1233-40.
6. Olshen AB, Venkatraman ES, Lucito R, Wigler M. Circular binary segmentation for the analysis of array-based DNA copy number data. Biostatistics. 2004 Oct;5(4):557-72.

7. Radtke I, Mullighan CG, Ishii M, Su X, Cheng J, Ma J, et al. Genomic analysis reveals few genetic alterations in pediatric acute myeloid leukemia. Proc Natl Acad Sci U S A. 2009 Aug 4;106(31):12944-9.

8. Schlenk RF, Döhner K, Krauter J, Fröhling S, Corbacioglu A, Bullinger L, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med. 2008 May 1;358(18):1909-18.

9. Gröschel S, Lugthart S, Schlenk RF, Valk PJ, Eiwen K, Goudswaard C, et al. High expression predicts outcome in younger adult patients with acute myeloid leukemia and is associated with distinct cytogenetic abnormalities. J Clin Oncol. 2010 Apr 20;28(12):2101-7.

10. Rucker FG, Russ AC, Cocciardi S, Kett H, Schlenk RF, Botzenhardt U, et al. Altered miRNA and gene expression in acute myeloid leukemia with complex karyotype identify networks of prognostic relevance. Leukemia. 2013 Feb;27(2):353-61.

11. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010 Jan 21;115(3):453-74.