Genomic breakpoint-specific monitoring of measurable residual disease in pediatric non-standard-risk acute myeloid leukemia

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Supplemental material

to

Genomic breakpoint specific monitoring of measurable residual disease in pediatric non-standard risk acute myeloid leukemia

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Supplemental Tables

Age	
Median (y)	8,8
Range (y)	[0,1-18,7]

Gender	
Females	15
Males	26

FAB classification	
M0	2
M1	2
M2	3
M3	0
M4	4
M5	24
M6	0
M7	5
unknown	1

Stage of disease	
De novo AML	29
other	12

Primary therapy protocol	
BFM-AML 2004	10
BFM-AML 2012	19
I-BFM Relapsed AML2001/01	12

Table S1. Table summarizing characteristics of patients (n=41) that have been selected for identification of genomics breakpoints. Notably, 8/31 non-SR patients diagnosed with *de novo* AML relapsed during the study and were not counted twice as patients while their MRD samples were analyzed also for relapse follow-up. "Other" thus summarizes secAML (n=10), relAML (n=2; those without MRD-samples analyzed during first line therapy) and MPAL cases.

Therapy Timepoint	Abbreviation	No. of samples
after 1st Induction (d21/d28)	Ind1	35
after 2nd Induction (d56)	Ind2	28
after Consolidation 1 (d84)	Con1	23
after Consolidation 2 (d112)	Con2	13
after Consolidation 3	Con3	3
pre SCT	preSCT	8
post SCT	postSCT	17
day15	d15	5
after relapse	rel	31
other timepoints	other	20

Table S2. Table depicting therapy timepoints of the samples (n=183) used in the study for comparison of gDNA-PCR MRD and FCM-MRD methodology.

Genomic Breakpoint	№ of patients	№ of samples
KMT2A::MLLT3	12	63
KMT2A::MLLT10	8	37
NUP98::NSD1	3	17
KMT2A::MLLT1	2	11
DDX3X::MLLT10	1	11
KMT2A::CREBBP	2	9
NUP98::KDM5A	1	8
KMT2A::ELL	1	7
CBFA2T3::GLIS2	1	6
KMT2A-PTD	2	5
KMT2A::MLLT4	1	4
RUNX1::CBFA2T3	1	3
DEK::NUP214	1	2
Total №	36	183

Table S3. Distribution of identified genomic breakpoint sequences among patients (n=36) as well as among all follow-up (FUP) samples (n=183) with matched gDNA-PCR MRD and FCM-MRD data.

	Antigen	Fluorochrome	Clone	Source
LAIP (DuraClone™)	CD15	FITC	80H5	Beckman Coulter
	CD34	ECD	581	Beckman Coulter
	CD117	PC5.5	104D2D1	Beckman Coulter
	CD33	PC7	D3HL60.251	Beckman Coulter
	CD14	APC-Alexa700	RMO52	Beckman Coulter
	CD11b	APC-Alexa750	Bear1	Beckman Coulter
	HLA-DR	Pacific Blue	IMMU-357.12	Beckman Coulter
CFU (DuraClone™)	CD45	Krome Orange	J33	Beckman Coulter
	CD38	FITC	T16	Beckman Coulter
	CD34	ECD	581	Beckman Coulter
	CD117	PC5.5	104D2D1	Beckman Coulter
	CD33	PC7	D3HL60.251	Beckman Coulter
	CD123	APC-Alexa700	SSDCLY107D2	Beckman Coulter
	CD45RA	APC-Alexa750	2H4LDH11LDB9	Beckman Coulter
	HLA-DR	Pacific Blue	IMMU-357.12	Beckman Coulter
Drop-in markers	CD7	PE	MEM-186	Exbio
	CD11a	PE	MEM25	Exbio
	CD19	PE	LT19	Exbio
	CD56	PE	LT56	Exbio
	CD371	PE	50C1	BioLegend
	NG2	PE	7.1	Beckman Coulter
	CD13	APC	WM15	Exbio
	CD71	APC	MEM-75	Exbio
	CD99	APC	3B2/TA8	Exbio

Table S4. Full details of antibodies used for the study.

genetic subtype	total № of samples	PCR^{pos}/FCM^{pos}	PCR^{neg}/FCM^{neg}	PCR^{pos}/FCM^{neg}	PCR^{neg}/FCM^{pos}	concordance [%]
all	183	78	48	56	1	68,9
KMT2A::MLLT3	63	35	10	17	1	71,4
KMT2A::MLLT10	37	15	4	18	0	51,4
NUP98::NSD1	17	8	1	8	0	52,9
KMT2A::MLLT1	11	6	3	2	0	81,8
DDX3X::MLLT10	11	2	8	1	0	90,9
KMT2A::CREBBP	9	5	3	1	0	88,9
NUP98::KMD5A	8	0	8	0	0	100,0
KMT2A::ELL	7	1	3	3	0	57,1
CBFA2T3::GLIS2	6	4	2	0	0	100,0
KMT2A- PTD	5	1	1	3	0	40,0
KMT2A::MLLT4	4	1	3	0	0	100,0
RUNX1::CBFA2T3	3	0	2	1	0	66,7
DEK::NUP214	2	0	0	2	0	0,0

Table S5. Concordance of gDNA PCR-MRD and FCM-MRD based on genetic subtype. No threshold applied. Any positive=positive.

FAB subtype	total № of samples	PCR^{pos}/FCM^{pos}	PCR^{neg}/FCM^{neg}	PCR^{pos}/FCM^{neg}	PCR^{neg}/FCM^{pos}	concordance [%]
all	183	48	78	56	1	68,9
FAB M0	7	2	2	3	0	57,1
FAB M1	5	2	0	3	0	40,0
FAB M2	16	4	3	9	0	43,8
FAB M3	0	nd	nd	nd	nd	nd
FAB M4	9	2	2	5	0	44,4
FAB M5a/b	114	15	64	34	1	67,9
FAB M6	0	nd	nd	nd	nd	nd
FAB M7	32	23	7	2	0	93,8
w maturation (FAB M2, M4)	25	6	5	14	0	44,0
w/o maturation (all others)	158	42	73	42	1	72,8

Table S6. Concordance of gDNA PCR-MRD and FCM-MRD based on FAB subtype. No threshold applied. Any positive=positive.

Supplemental Figures

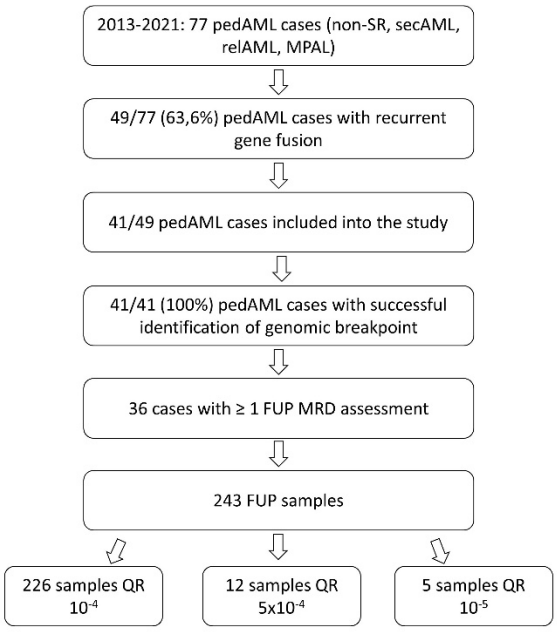
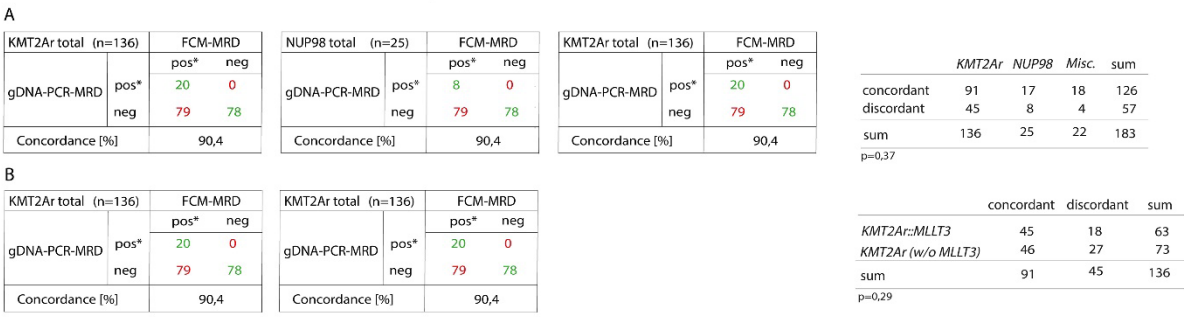


Figure S1. Schematic overview on genomic breakpoint identification in selected pediatric patients with acute myeloid leukemia.



A

KMT2Ar total (n=136)		FCM-MRD	
		pos*	neg
gDNA-PCR-MRD	pos*	20	0
	neg	79	78
Concordance [%]		90,4	

NUP98 total (n=25)		FCM-MRD	
		pos*	neg
gDNA-PCR-MRD	pos*	8	0
	neg	79	78
Concordance [%]		90,4	

KMT2Ar total (n=136)		FCM-MRD	
		pos*	neg
gDNA-PCR-MRD	pos*	20	0
	neg	79	78
Concordance [%]		90,4	

	KMT2Ar	*NUP98*	*Misc.*	sum
concordant	91	17	18	126
discordant	45	8	4	57
sum	136	25	22	183

p=0,37

B

KMT2Ar total (n=136)		FCM-MRD	
		pos*	neg
gDNA-PCR-MRD	pos*	20	0
	neg	79	78
Concordance [%]		90,4	

KMT2Ar total (n=136)		FCM-MRD	
		pos*	neg
gDNA-PCR-MRD	pos*	20	0
	neg	79	78
Concordance [%]		90,4	

	concordant	discordant	sum
KMT2Ar::MLLT3	45	18	63
KMT2Ar (w/o MLLT3)	46	27	73
sum	91	45	136

p=0,29

Figure S2. Comparison of gDNA-PCR MRD and FCM-MRD data based on genetic subtype. No threshold was applied. Any positivity = positive. **(A)** Genetic subtypes were summarized in three major groups. All cases with *KMT2A* rearrangements were summarized in the group "*KMT2Ar*", those with *NUP98* gene fusions in the group "*NUP98*" and all cases with other aberrations in the group "*Miscellaneous*"; p=0,37, ns. **(B)** Concordance of gDNA-PCR MRD and FCM-MRD in the *KMT2Ar* group excluding MLLT3 cases (left) and in the *KMT2A::MLLT3* group only (right); p=0,29, ns. Statistical analysis was done using GraphPadPrism 8.3.0 and Chi-square test.

no maturation (FAB M0, M1, M5, M7)			
(n=158)		FCM-MRD	
		pos*	neg
gDNA-PCR-MRD	pos*	42	42
	neg	1	73
Concordance [%]		72,8	

maturation (FAB M2, M4)			
(n=25)		FCM-MRD	
		pos*	neg
gDNA-PCR-MRD	pos*	6	14
	neg	0	5
Concordance [%]		44,0	

	concordant	discordant	sum
no maturation	115	43	158
maturation	11	14	25
sum	126	57	183

p=0,0039

* no cut-off; any positive=positive

Figure S3. Comparison of gDNA-PCR MRD and FCM-MRD based on FAB classification. No threshold was applied. Any positivity was rated as positive. The presence of maturation leads to reduced concordance of gDNA-PCR MRD and FCM-MRD (p=0,0039). Statistical Analysis was done using GraphPadPrism 8.3.0 and Chi-square test.