Molecular genetic characterization of myeloid/ lymphoid neoplasms associated with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, *FGFR1* or *PCM1-JAK2*

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Supplemental Material

Molecular genetic characterization of myeloid/lymphoid neoplasms associated with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, *FGFR1* or *PCM1-JAK2*

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Table S1

	PDGFRA	***PDGFRB***	***FGFR1***	***PCM1-JAK2***
total cohort (n=)	35	13	6	7
sex (male/female)	33/2	12/1	5/1	6/1
age in years, median [range]	49 [23-76]	52 [33-70]	60 [48-74]	61 [49-78]
rearrangement type	*FIP1L1-PDGFRA* (n=34) *BCR-PDGFRA* (n=1)	*ETV6-PDGFRA* (n=7) *EBF1-PDGFRB* (n=1) *TNIP1-PDGFRB* (n=1) *CCDC88C-PDGFRB* (n=1) unknown (n=3)	*ZMYM2-FGFR1* (n=4) *BCR-FGFR1* (n=1) unknown (n=1)	*PCM1-JAK2* (n=7)
detection method for rearrangement				
RT-PCR	35	9	5	7
FISH	21	11	6	7
chromosome banding	x	6	6	7
targeted fusion panel		2		

Table S1: Patient characteristics. All known rearrangements were detected by reverse transcriptase PCR (RT-PCR). If possible, FISH and chromosome banding analysis was performed, too.[1-6] The *CCDC88C-PDGFRB* rearrangement was resolved unsing TruSight RNA Fusion Panel (Illumina, San Diego, CA).[7] Four aberrations are listed as unknown. By FISH involvement of *PDGFRB* and *FGFR1* were detected using break-apart probes.

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Table S2

Final diagnosis at time of routine assessment*	Fusion	Gender	Age in years	WBC [x10^9/L]	Platelets [x 10^9/L]	Hemoglobin [g/dL]	PB Eos (%)	Mutation
AL	*PDGFRB*	m	52	N.A.	N.A.	N.A.	N.A.	
ALL	*PDGFRB*	m	33	6.90	23	7.7	N.A.	y
ALL	*FGFR1*	m	68	56.20	20	15.9	4	
AML	*PCM1-JAK2*	m	61	17.80	90	12.7	5	
AML	*PDGFRB*	f	69	5.60	60	9.4	2	y
AML	*PCM1-JAK2*	m	73	6.59	108	8.1	8	
CEL	*PDGFRA*	m	40	N.A.	N.A.	N.A.	N.A.	
HES	*PDGFRA*	m	23	28.30	76	8.8	N.A.	
HES	*PDGFRA*	m	24	55.70	53	14	74	
HES	*PDGFRA*	m	31	14.00	185	13.4	71	
HES	*PDGFRA*	m	33	41.53	135	10.4	62	
HES	*PDGFRA*	m	34	15.40	118	12.2	39	
HES	*PDGFRA*	m	39	8.30	141	11.4	16	y
HES	*PDGFRA*	m	39	9.86	157	8.9	19	
HES	*PDGFRA*	m	39	N.A.	N.A.	N.A.	N.A.	
HES	*PDGFRA*	m	40	7.88	302	13.7	9	
HES	*PDGFRA*	m	41	26.00	94	10.9	70	
HES	*PDGFRA*	m	41	8.80	220	15.1	51	
HES	*PDGFRA*	m	46	6.90	186	14.2	16	
HES	*PDGFRA*	m	47	37.70	250	14.5	47	
HES	*PDGFRA*	m	47	8.00	184	N.A.	N.A.	
HES	*PDGFRA*	m	48	N.A.	N.A.	N.A.	65	
HES	*PDGFRA*	m	49	80.60	133	13.9	24	
HES	*PDGFRA*	m	49	29.00	188	15.2	52	
HES	*PDGFRA*	m	50	25.50	117	9.5	34	
HES	*PDGFRA*	m	50	N.A.	N.A.	N.A.	67	
HES	*PDGFRB*	m	51	N.A.	N.A.	N.A.	63	
HES	*PDGFRA*	m	53	20.00	81	11.8	53	
HES	*PDGFRA*	m	55	10.91	148	12.3	60	
HES	*PDGFRA*	f	56	12.00	135	14.2	48	
HES	*PDGFRA*	m	57	14.66	343	12.5	50	
HES	*PDGFRB*	m	59	N.A.	N.A.	N.A.	N.A.	y
HES	*PDGFRA*	m	61	34.80	175	16.6	75	
HES	*PDGFRB*	m	62	20.60	204	12.5	78	
HES	*PDGFRA*	m	64	19.00	350	12.9	N.A.	
HES	*PDGFRA*	m	65	16.70	226	13.7	N.A.	y
HES	*PDGFRA*	m	66	7.30	181	N.A.	47	
HES	*PDGFRA*	m	68	8.10	197	14.4	N.A.	y
HES	*PDGFRA*	m	70	9.80	201	N.A.	54	
HES	*PDGFRA*	m	73	N.A.	N.A.	N.A.	58	y
HES	*PDGFRA*	f	74	20.00	N.A.	9.01	60	
HES	*PDGFRA*	m	76	10.30	327	13.2	87	
MDS/MPN	*PDGFRB*	m	49	N.A.	N.A.	N.A.	N.A.	
MDS/MPN	*FGFR1*	f	52	N.A.	N.A.	N.A.	14	y
MDS/MPN	*PDGFRA*	m	57	129.40	124	12.1	20	y
MDS/MPN	*PDGFRB*	m	70	6.64	118	12.7	6	
MPN	*PDGFRA*	m	45	4.20	134	13.5	45	
MPN	*PDGFRB*	m	46	58.20	115	10.1	21	
MPN	*FGFR1*	m	48	N.A.	N.A.	N.A.	7	y
MPN	*PCM1-JAK2*	m	49	27.80	96	14	1	
MPN	*PDGFRB*	m	50	7.70	337	9.1	N.A.	
MPN	*PCM1-JAK2*	m	50	13.17	246	10.8	25	
MPN	*PCM1-JAK2*	m	50	9.90	145	12	6	
MPN	*PDGFRB*	m	51	41.90	143	12.8	N.A.	
MPN	*FGFR1*	m	53	45.00	140	16	15	y
MPN	*PDGFRB*	m	53	112.00	52	10.4	25	
MPN	*PDGFRB*	m	58	24.50	213	16.5	4	
MPN	*PCM1-JAK2*	f	68	5.30	213	N.A.	1	
MPN	*FGFR1*	m	70	104.20	217	9.7	N.A.	y
MPN	*FGFR1*	m	74	52.01	N.A.	N.A.	N.A.	y
N.A.	*PCM1-JAK2*	m	78	N.A.	N.A.	N.A.	N.A.	y

Table S2: Patient characteristics. *Samples were collected from 2006-2016. The diagnosis assigned at the respective time is given. Abbreviations: Acute leukemia, AL; acute lymphoblastic leukemia, ALL; acute myeloid leukemia, AML; chronic eosinophilic leukemia, CEL; hypereosinophilic syndrome; HES; myelodysplastic/myeloproliferative neoplasms, MDS/MPN; myeloproliferative neoplasms, MPN; not available; N.A.; male, m; female, f; white blood cells, WBC; eosinophils in peripheral blood, PB Eos; mutation present, y(es).

Table S3

gene	region of interest*	transcript ID
ASXL1	E13	ENST00000375687
BCL2	E01	ENST00000398117
BCOR	coding region	ENST00000378444
BIRC3	coding region	ENST00000263464
BRAF	E15	ENST00000288602
BTK	E15	ENST00000308731
CALR	E09	ENST00000316448
CBL	E08-E09	ENST00000264033
CSF3R	E14, E17	ENST00000373106
CSNK1A1	E03-E04	ENST00000373106
CXCR4	E02	ENST00000241393
DNMT3A	E07-23	ENST00000264709
EGR2	E01-02	ENST00000242480
ETNK1	E03	ENST00000266517
ETV6	coding region	ENST00000396373
EZH2	coding region	ENST00000320356
FLT3-TKD	E20	ENST00000241453
GATA1	coding region	ENST00000376670
IDH1	E04	ENST00000345146
IDH2	E04	ENST00000330062
JAK2	E12+14	ENST00000381652
KIT	E08+E17	ENST00000288135
KRAS	E02-E03	ENST00000256078
MAP2K1	E01-11	ENST00000307102
MPL	E10	ENST00000372470
MYC	E01-03	ENST00000377970
MYD88	coding region	ENST00000396334
NOTCH2	E26, E27, E34	ENST00000256646
NPM1	E11	ENST00000296930
NRAS	E02-E03	ENST00000369535
PHF6	coding region	ENST00000370803
PIGA	E02-06	ENST00000333590
PLCG2	E12, E19, E20, E24, E30	ENST00000359376
PTPN11	E01-E15	ENST00000351677
RUNX1	coding region	ENST00000344691
SAMHD1	E01-16	ENST00000262878
SETBP1	E04	ENST00000282030
SF3B1	E13-E16	ENST00000335508
SRSF2	E01	ENST00000392485
STAT3	E20, E21	ENST00000264657
STAT5B	E02-E19	ENST00000293328
TET2	coding region	ENST00000310581
TP53	E04-10	ENST00000269305
U2AF1	E02+E06	ENST00000291552
UBR5	E58	ENST00000293328
WT1	E07-23	ENST00000332351
XPO1	E14, E15	ENST00000401558
ZRSR2	coding region	ENST00000307771

Table S3: Genes included in panel. *For over 90% of given regions a minimal coverage of 400x was achieved. Abbreviations: tyrosine kinase domain, TKD.

Table S4

rearrangement	gene	mutation	load (%)	gene	mutation	load (%)	gene	mutation	load	gene	VUS	load (%)
FIP1L1-PDGFRA	*RUNX1*	c.521G>A	37	*TET2*	c.3714_3715 del	47						
FIP1L1-PDGFRA	*ASXL1*	c.2458_2459dup	38	*BCOR*	c.2190dup	47	*ETV6*	c.1196_1197 del	24	*ZRSR2*	c.1332_1343dup	89
FIP1L1-PDGFRA	*DNMT3A*	c.1647C>A	3									
FIP1L1-PDGFRA										*SAMHD1*	c.677G>A	57
FIP1L1-PDGFRA	*TET2*	c.5328dup	28									
FIP1L1-PDGFRA										*PTPN11*	c.1658C>T	46
FIP1L1-PDGFRA										*ETV6*	c.1193T>G	56
FIP1L1-PDGFRA	*DNMT3A*	c.2387G>A	24									
ETV6-PDGFRA	*BCOR*	c.4127del	46									
ETV6-PDGFRA	*STAT5B*	c.1882A>T	14									
TNIP1-PDGFRB	*DNMT3A*	c.2192del	41	*NRAS*	c.35G>A	9	*ZRSR2*	c.284C>T	17			
ZMYM2-FGFR1	*RUNX1*	c.239G>A	11									
ZMYM2-FGFR1	*RUNX1*	c.422G>A	23									
BCR-FGFR1	*RUNX1*	c.955dup	33									
FGFR1*	*RUNX1*	c.986_989 dup	11									
ZMYM2-FGFR1	*RUNX1*	c.419G>A	60									
PCM1-JAK2										*PTPN11*	c.1682C>T	50
PCM1-JAK2	*TET2*	c.2717del	37									

Table S4: Variants identified by panel sequencing. * The exact fusion is unknown. The involvement of *FGFR1* was detected by FISH. Mutation load was calculated as mutated/all reads (in %). Abbreviations: variant of uncertain significance, VUS.

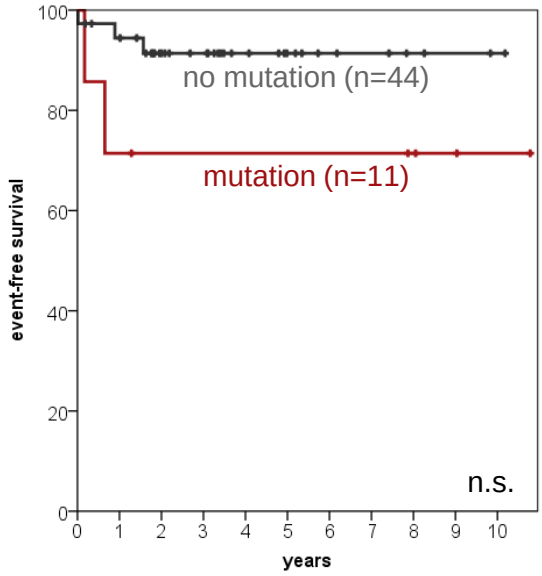
Table S5

rearrangement type	state	gene	mutation		load (%)
FIP1L1-PDGFRA	ID	*ASXL1*	c.2458_2459dup	p.Asp820Glufs*5	38
		BCOR	c.2190dup	p.Pro731Thrfs*9	47
		ETV6	c.1196_1197del	p.Arg399Profs*26	24
type B	CR (imatinib)	*ASXL1*	c.2458_2459dup	p.Asp820Glufs*5	0
		BCOR	c.2190dup	p.Pro731Thrfs*9	0
		ETV6	c.1196_1197del	p.Arg399Profs*26	0
FIP1L1-PDGFRA	ID	*RUNX1*	c.521G>A	p.Arg174Gln	37
		TET2	c.3714_3715del	p.Leu1240Glyfs*2	47
type B	CR (imatinib)	*RUNX1*	c.521G>A	p.Arg174Gln	0
		TET2	c.3714_3715del	p.Leu1240Glyfs*2	0
FIP1L1-PDGFRA	ID	*DNMT3A*	c.1647C>A	p.Cys549*	3
type A	CR (imatinib)	*DNMT3A*	c.1647C>A	p.Cys549*	6
FIP1L1-PDGFRA	ID	*TET2*	c.5328dup	p.Leu1777Serfs*12	28
type A	CR (imatinib)	*TET2*	c.5328dup	p.Leu1777Serfs*12	21
FIP1L1-PDGFRA	ID	*DNMT3A*	c.2387G>A	p.Gly796Asp	24
type A	CR (imatinib)	*DNMT3A*	c.2387G>A	p.Gly796Asp	23
ETV6-PDGFRB	ID	*BCOR*	c.4127del	p.Gly1376Aspfs*4	46
type B	10-fold reduction of *PDGFRB* expression (imatinib + GMALL)	*BCOR*	c.4127del	p.Gly1376Aspfs*4	0
BCR-FGFR1	ID	*RUNX1*	c.955dup	p.Arg319Profs*254	33
type C	all cells positive in chromosome banding analysis	*RUNX1*	c.955dup	p.Arg319Profs*254	45
ZMYM2-FGFR1	treatment naive	*RUNX1*	c.239G>A	p.Arg80His	11
type C	74 cells positive by FISH (ponatinib)	*RUNX1*	c.239G>A	p.Arg80His	51

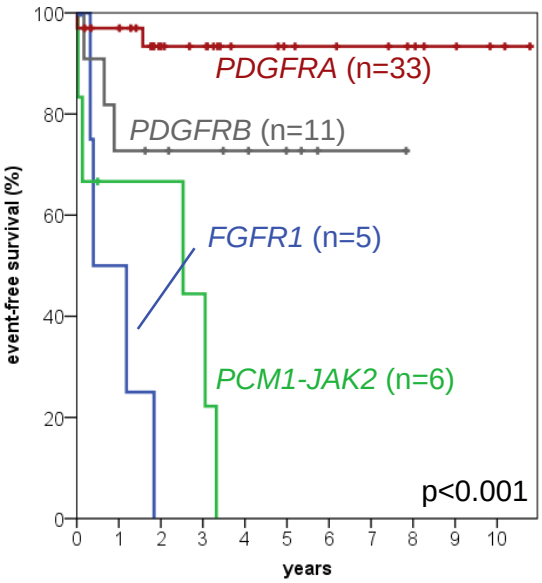
Table S5: Follow-up for patients with mutations. We performed FISH, chromosome banding analysis or *PDGFRB* expression testing for post-treatment samples. Negative RT-PCR was used for complete molecular remission. Treatment information is given, if available, in brackets. Type A are mutations, which developed independently or prior to the rearrangements. Type B and type C are mutations derived from the MLN-Eo clone. Type C indicates subclones, which strongly expanded. Abbreviations: initial diagnosis, ID; complete remission, CR; German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia, GMALL.

Figure S1

A



B



pairwise comparison Log Rank (Mantel Cox)			
significance	*PCM1-JAK2*	*PDGFRA*	*PDGFRB*
FGFR1	0.201	0.000	0.017
PCM1-JAK2		0.000	0.021
PDGFRA			0.063

Figure S1: Event-free survival (EFS). Kaplan–Meier curves show the effect of A) mutations within the *PDGFRA* and *PDGFRB* subgroup and B) fusions types on EFS. Significance was calculated by Log Rank (Mantel Cox) and also compared pairwise in figure B. Indication to change treatment (incl. allogeneic stem cell transplantation) was counted as event. Abbreviations: not significant, n.s..

Figure S2

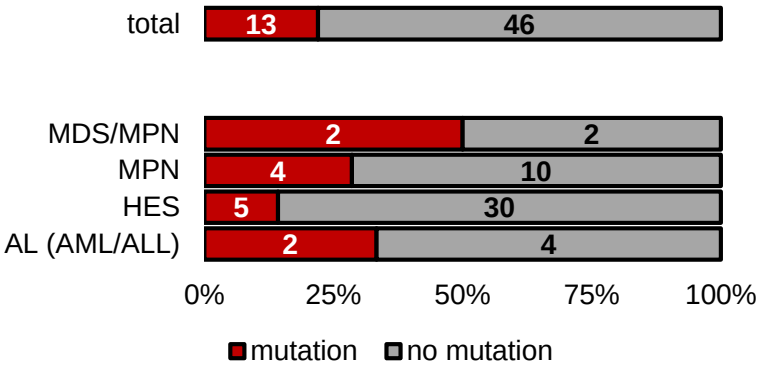


Figure S2: Mutation distribution according to initial final diagnosis. Absolute numbers are indicated in bars. Final diagnosis data was not available for one case and one case is not shown, because chronic eosinophilic leukemia was assigned as diagnosis only to one patient. Abbreviations: Acute leukemia, AL (includes acute lymphoblastic leukemia, ALL; acute myeloid leukemia, AML); hypereosinophilic syndrome, HES; myelodysplastic/myeloproliferative neoplasms, MDS/MPN; myeloproliferative neoplasms, MPN.