EZH2 mutations and impact on clinical outcome: an analysis in 1,604 patients with newly diagnosed acute myeloid leukemia

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Supplementary Appendix

Supplementary Methods

All molecular studies were performed on DNA from bone marrow aspirates or peripheral blood taken at diagnosis from patients treated in protocols of the Study Alliance Leukemia (SAL). All patient samples were obtained and analyzed with written informed consent of the patients and according to a protocol approved by the local ethical committee. The protocols were in agreement with the Helsinki declaration and registered with NCT numbers 00180115 (AML96), 00180102 (AML2003), 00180167 (AML60+) and 00893373 (SORAML), the analyses were done under the auspices of the SAL-bioregistry (EK98032010). DNA from samples was extracted using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) and quantified with the NanoDrop spectrophotometer. Profiling of *EZH2* mutational status and associated co-mutations was done by targeted resequencing using the TruSight Myeloid assay (Illumina, Chesterford, UK) covering 54 genes frequently mutated in AML: *BCOR*, *BCORL1*, *CDKN2A*, *CEBPA*, *CUX1*, *DNMT3A*, *ETV6*, *EZH2*, *IKZF1*, *KDM6A*, *PHF6*, *RAD21*, *RUNX1*, *STAG2*, *ZRSR2*, *ABL1*, *ASXL1*, *ATRX*, *BRAF*, *CALR*, *CBL*, *CBLB*, *CBLC*, *CDKN2A*, *CSF3R*, *FBXW7*, *FLT3*, *GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, JAK2*, *JAK3*, *KIT*, *KRAS, MLL, MPL, MYD88, NOTCH1, NPM1, NRAS, PDGFRA, PTEN, PTPN11*, *SETBP1*, *SF3B1*, *SMC1A*, *SMC3*, *SRSF2*, *TET2*, *TP53*, *U2AF1* and *WT1*. For each reaction, 50 ng of genomic DNA was used. Library preparation was done as recommended by the manufacturer (TruSight Myeloid Sequencing Panel Reference Guide 15054779 v02, Illumina). Samples were sequenced paired-end (150bp PE) on a NextSeq NGS-instrument (Illumina). Sequence data alignment of demultiplexed FastQ files, variant calling and filtering was done using the Sequence Pilot software package (JSI medical systems GmbH, Ettenheim, Germany) with default settings and a 5% variant allele frequency (VAF) mutation calling cut-off. Human genome build HG19 was used as reference genome for mapping algorithms.

Supplementary Data

Supplementary Table 1 *EZH2* mutations

Pat	Pos (hg19)	Exon	HGVS nom	p.HGVS	VAF [%]	Cov.	Domain	Predicted consequence
1	chr7:148504791	E20	c.2203C>T	p.Gln735Ter	32	1145	SET	inactivating
2	chr7:148512038	E14	c.1640T>A	p.Phe547Tyr	39	8528	CXC	unknown
3	chr7:148526829	E5	c.475G>A	p.Gly159Arg	41	9889	D1	unknown
4	chr7:148543621	E3	c.187C>T	p.Arg63Ter	10	1627		inactivating
5	chr7:148506443	E18	c.2069G>A	p.Arg690His	95	9539	SET	unknown
6	chr7:148526849	E5	c.455delA	p.Asn152Ilefs*15	13	1208	D1	inactivating
7	chr7:148507446	E17	c.2008T>G	p.Phe670Val	85	4364	SET	unknown
8	chr7:148526867	E5	c.437T>C	p.Ile146Thr	51	6570	D1	unknown
9	chr7:148512041	E14	c.1637delA	p.Asn546Ilefs*129	41	2395	CXC	inactivating
9	chr7:148514316	E11	c.1408C>T	p.Gln470Ter	13	1499		inactivating
10	chr7:148513869	E12	c.1412T>G	p.Val471Gly	97	22753		unknown
11	chr7:148511184	E15	c.1718C>T	p.Thr573Ile	82	6075	CXC	unknown
12	chr7:148514412	E11	c.1312A>G	p.Ser438Gly	43	2490		unknown
13	chr7:148543671	E3	c.137G>A	p.Arg46His	48	1848		unknown
14	chr7:148515070	E10	c.1139C>G	p.Ser380Ter	46	4661		inactivating
14	chr7:148506407	E18	c.2105C>T	p.Ala702Val	39	4912	SET	unknown
15	chr7:148507464	E17	c.1990G>A	p.Asp664Asn	46	2917	SET	unknown
16	chr7:148506432	E18	c.2080C>T	p.His694Tyr	49	1501	SET	unknown
16	chr7:148513776	E12	c.1505G>A	p.Arg502Gln	43	1856	CXC	unknown
17	chr7:148507438	E17	c.2016C>G	p.Phe672Leu	45	2328	SET	unknown
18	chr7:148513803	E12	c.1478C>A	p.Pro493His	81	12371		unknown
19	chr7:148513776	E12	c.1505G>A	p.Arg502Gln	91	11811	CXC	unknown
20	chr7:148514989	E10	c.1220delA	p.Asp407Valfs*17	92	8714		inactivating
21	chr7:148523591	E8	c.862C>T	p.Arg288Ter	44	9071	D2	inactivating
22	chr7:148506467	E18	c.2045G>C	p.Ala682Gly	94	30565	SET	activating
23	chr7:148516749	E9	c.938G>A	p.Arg313Gln	44	2889	D2	unknown
24	chr7:148507478	E17	c.1976G>C	p.Arg659Thr	15	941	SET	unknown
24	chr7:148515024	E10	c.1184dupG	p.Glu396Argfs*4	15	1469		inactivating
25	chr7:148511102	E15	c.1800C>A	p.Asp600Glu	49	5001	CXC	unknown
26	chr7:148516749	E9	c.938G>A	p.Arg313Gln	51	2658	D2	unknown
27	chr7:148504785	E20	c.2209G>A	p.Asp737Asn	46	1348	SET	unknown
28	chr7:148506218	E19	c.2140G>C	p.Gly714Arg	97	9524	SET	unknown
29	chr7:148513776	E12	c.1505G>A	p.Arg502Gln	44	9599	CXC	unknown
30	chr7:148504761	E20	c.2233G>A	p.Glu745Lys	23	1575	SET	unknown
31	chr7:148507485	E17	c.1969G>T	p.Asp657Tyr	29	4277	SET	unknown
31	chr7:148544291	E2	c.100C>T	p.Arg34Ter	24	2884		inactivating
32	chr7:148504766	E20	c.2227_2228insATCCTG	p.Val742_Gly743insAspPro	70	1662	SET	unknown
33	chr7:148543643	E3	c.165C>G	p.Ile55Met	6	1242		unknown
34	chr7:148506443	E18	c.2069G>A	p.Arg690His	96	8286	SET	unknown
35	chr7:148504761	E20	c.2233G>A	p.Glu745Lys	51	2314	SET	unknown
36	chr7:148526829	E5	c.475G>A	p.Gly159Arg	25	11952	D1	unknown
37	chr7:148512032	E14	c.1646A>G	p.Glu549Gly	95	11831	CXC	unknown
38	chr7:148523641	E8	c.812C>T	p.Ser271Phe	15	1248	D2	unknown
39	chr7:148526883	E5	c.421C>T	p.Gln141Ter	34	3785	D1	inactivating
40	chr7:148507426	E17	c.2025_2027dupCAA	p.Asn676dup	75	5549	SET	unknown
41	chr7:148526897	E5	c.407A>T	p.Asp136Val	42	1742	D1	unknown
42	chr7:148506429	E18	c.2083T>C	p.Ser695Pro	29	7768	SET	unknown

43	chr7:148512104	E14	c.1574A>G	p.Tyr525Cys	42	11908	CXC	unknown
43	chr7:148525838	E6	c.619C>T	p.Arg207Ter	40	3998		inactivating
44	chr7:148506476	E18	c.2036T>A	p.Val679Glu	29	4129	SET	unknown
45	chr7:148504795	E20	c.2199C>G	p.Tyr733Ter	47	1297	SET	inactivating
45	chr7:148504797	E20	c.2197T>A	p.Tyr733Asn	46	1277	SET	unknown
46	chr7:148508788	E16	c.1876G>A	p.Val626Met	12	824	SET	unknown
46	chr7:148507463	E17	c.1991A>C	p.Asp664Ala	7	1283	SET	unknown
47	chr7:148507436	E17	c.2018A>G	p.Asn673Ser	22	1758	SET	unknown
47	chr7:148544318	E2	c.73C>T	p.Arg25Ter	7	932		inactivating
48	chr7:148523579	E8	c.874T>C	p.Tyr292His	30	5272	D2	unknown
48	chr7:148506476	E18	c.2036T>A	p.Val679Glu	29	4159	SET	unknown
49	chr7:148511182	E15	c.1720A>G	p.Lys574Glu	48	12452	CXC	unknown
50	chr7:148506170	E19	c.2187dupT	p.Asp730Ter	9	1545	SET	inactivating
51	chr7:148506462	E18	c.2050C>T	p.Arg684Cys	37	4666	SET	unknown
52	chr7:148506239	E19	c.2119delinsTAAAT	p.Val707Ter	32	993	SET	inactivating
53	chr7:148523591	E8	c.862C>T	p.Arg288Ter	31	5918	D2	inactivating
54	chr7:148506443	E18	c.2069G>A	p.Arg690His	34	6263	SET	unknown
55	chr7:148506433	E18	c.2079T>A	p.Asn693Lys	45	5742	SET	unknown
56	chr7:148526828	E5	c.476G>A	p.Gly159Glu	48	2545	D1	unknown
57	chr7:148544318	E2	c.73C>T	p.Arg25Ter	80	2865		inactivating
58	chr7:148514983	E10	c.1224_1225dupAA	p.Thr409Lysfs*16	40	1437		inactivating
58	chr7:148544318	E2	c.73C>T	p.Arg25Ter	13	1513		inactivating
59	chr7:148514414	E11	c.1310G>A	p.Trp437Ter	40	5714		inactivating
60	chr7:148507436	E17	c.2018A>G	p.Asn673Ser	45	3177	SET	unknown
60	chr7:148506169	E19	c.2189A>T	p.Asp730Val	35	4068	SET	unknown
61	chr7:148514314	E11	c.1410G>T	p.Gln470His	44	3851		unknown
61	chr7:148529752	E4	c.328_337delinsTG	p.Met110Trpfs*13	37	2976	D1	inactivating
62	chr7:148507497	E17	c.1957C>G	p.Gln653Glu	14	1643	SET	unknown
63	chr7:148507433	E17	c.2021T>C	p.Leu674Ser	93	2800	SET	unknown

VAF: Variant allele frequency

Supplementary Table 2 Co-Mutations

Gene affected	*EZH2*-wt	*EZH2*-mut	p.value
NPM1	507/1541 (33)	13/63 (21)	0.057
FLT3-ITD	339/1541 (22)	8/63 (13)	0.109
FLT3.TKD	117/1541 (8)	6/63 (10)	0.747
ABL1	0/1541 (0)	0/63 (0)	
ASXL1	**129/1541 (8)**	**14/63 (22)**	**0**
ATRX	3/1541 (0)	0/63 (0)	1
BCOR	71/1541 (5)	2/63 (3)	0.821
BCORL1	55/1541 (4)	2/63 (3)	1
BRAF	8/1541 (1)	0/63 (0)	1
CALR	2/1541 (0)	0/63 (0)	1
CBL	32/1541 (2)	0/63 (0)	0.487
CBLB	3/1541 (0)	0/63 (0)	1
CDKN2A	3/1541 (0)	0/63 (0)	1
CEBPA	249/1541 (16)	15/63 (24)	0.152
CEBPA dm	112/1363 (7)	7/54 (11)	0.326
CSF3R	18/1541 (1)	2/63 (3)	0.408
CUX1	34/1541 (2)	4/63 (6)	0.09
DNMT3A	435/1541 (28)	13/63 (21)	0.241
ETV6	13/1541 (1)	1/63 (2)	1
FBXW7	4/1541 (0)	0/63 (0)	1
GATA1	0/1541 (0)	0/63 (0)	
GATA2	92/1541 (6)	6/63 (10)	0.376
GNAS	2/1541 (0)	0/63 (0)	1
HRAS	2/1541 (0)	0/63 (0)	1
IDH1	131/1541 (9)	7/63 (11)	0.621
IDH2	214/1541 (14)	9/63 (14)	1
IKZF	43/1541 (3)	2/63 (3)	1
JAK2	18/1541 (1)	1/63 (2)	1
JAK3	0/1541 (0)	0/63 (0)	
KDM6A	12/1541 (1)	0/63 (0)	1
KIT	75/1541 (5)	2/63 (3)	0.753
KRAS	82/1541 (5)	3/63 (5)	1
MPL	**4/1541 (0)**	**2/63 (3)**	**0.008**
MYD88	3/1541 (0)	0/63 (0)	1
NOTCH1	27/1541 (2)	0/63 (0)	0.576
NRAS	**236/1541 (15)**	**16/63 (25)**	**0.048**
PDGFRA	2/1541 (0)	0/63 (0)	1
PHF6	47/1541 (3)	3/63 (5)	0.692
PTEN	3/1541 (0)	0/63 (0)	1
PTPN11	111/1541 (7)	4/63 (6)	0.993
RAD21	46/1541 (3)	5/63 (8)	0.067
RUNX1	**153/1541 (10)**	**16/63 (25)**	**0**
SETBP1	11/1541 (1)	0/63 (0)	1
SF3B1	43/1541 (3)	2/63 (3)	1
SFRS2	2/1541 (0)	0/63 (0)	1
SMC1A	24/1541 (2)	0/63 (0)	0.639
SMC3	20/1541 (1)	0/63 (0)	0.741
STAG2	80/1541 (5)	6/63 (10)	0.226
TET2	290/1541 (19)	13/63 (21)	0.844
TP53	123/1541 (8)	2/63 (3)	0.248
U2AF1	44/1541 (3)	3/63 (5)	0.618
WT1	112/1541 (7)	4/63 (6)	0.978
ZRSR2	22/1541 (1)	2/63 (3)	0.555

Significant differences ($p<0.05$) are marked in bold

LOH detection in patients with homozygous *EZH2* variants without detectable -7/7q

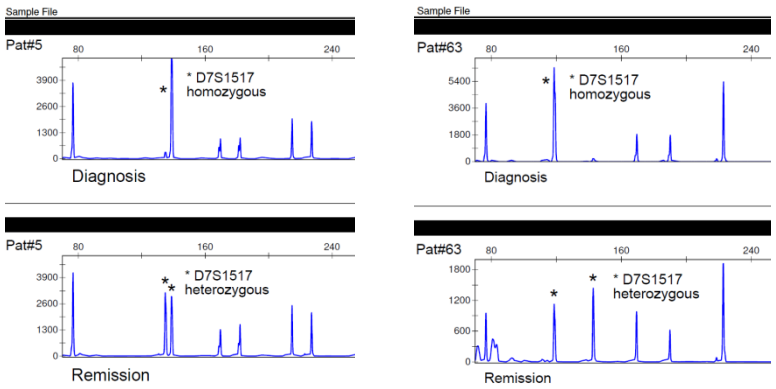
Only 4 patients (out of the 15 *EZH2* homozygous patients) (27%) were affected by monosomy 7. To address the potential impact of 7q36.1 microdeletions/UPD in the remaining patients, we screened NGS data for allelic frequencies of 7q36.1 polymorphisms, pointing at the presence of larger 7q36.1 microdeletions/UPD in patients with homozygous *EZH2* variants without -7/7q:

Supplementary Table 3 Variant allele frequencies (%) of 7q36.1 polymorphisms in patients with homozygous *EZH2* mutations without -7/7q

Ref.	**rs397889839**	**rs55877618**	**rs10268879**	**rs2072407**	**rs183943237**
Type	deletion	insertion	SNP	SNP	SNP
Pos. (hg19)	chr7:148543694	chr7:148504854	chr7:148506363	chr7:148508833	chr7:148506293
Pat#5	73% (homo)	93% (homo)			
Pat#28	70% (homo)		97% (homo)		
Pat#63			96% (homo)		
Pat#57		71% (homo)		90% (homo)	90% (homo)
Pat#37			96% (homo)		
Pat#7				100% (homo)	
Pat#40			100% (homo)		
Control 1	33% (hetero)	59% (hetero)		49% (hetero)	
Control 2	24% (hetero)	54% (hetero)		52% (hetero)	
Control 3		48% (hetero)		48% (hetero)	

Allelic burden [%] of 7q36.1 polymorphisms (rs397889839; rs55877618; rs10268879; rs2072407; rs183943237) in selected patients with homozygous *EZH2* variants. Control DNA was obtained from healthy individuals.

In addition, short tandem repeat (STR) analysis of material from diagnosis and remission showed that cases of patients with homozygous *EZH2* variants were affected by loss of heterozygosity (LOH) at the respective chromosome region (STR loci D7S1517). Since this STR is located about 20 MB centromeric of *EZH2*, this might indicate a larger region of UPD.



Supplementary Figure 1 STR analysis of loci D7S1517 (chr7:123,497,891 - 123,497,966 / GRCh37) from two patients with homozygous *EZH2* variants at the time of diagnosis and complete remission.