

Molecular response patterns in relapsed/refractory AML patients treated with selinexor and chemotherapy

Supplementary information

Supplementary Tables

Supplementary Table S1. Genes included in our custom myeloid panel.

Gene	Exons	Gene	Exons	Gene	Exons
ASXL1	12	GATA2	2-6	RUNX1	complete
ASXL2	11+12	IDH1	4	SETBP1	4
BCOR	complete	IDH2	4	SF3B1	13-16
BCORL1	complete	JAK2	12, 14	SMC1A	2, 11, 16, 17
BRAF	Exon15	KDM6A	complete	SMC3	10, 13, 19, 23, 25, 28
CALR	9	KIT	2, 8-11, 13, 17	SRSF2	1
CBL	8, 9	KRAS	2-5	STAG1	complete
CEBPA	complete	MPL	10	STAG2	complete
CSF3R	14-17	MYC	2	TET2	3-11
CSNK1A1	3, 4	NF1	complete	TP53	2-11
DDX41	complete	NPM1	12	U2AF1	2, 6
DNMT3A	complete	NRAS	2-5	WT1	7, 9
ETNK1	3	PHF6	complete	ZBTB7A	2, 3
ETV6	complete	PPM1D	1-6	ZRSR2	complete
EZH2	complete	PTPN11	3, 13		
FLT3	14-16, 20	RAD21	complete		

Supplementary Table S2. Primer sequences used for NGS MRD analysis.

Primer name	Primer sequence
SF3B1_NGS_64 8F	GGTAAACACAAGGGACTGGNNNNNNNNNNNNNGCA TTCCTTCTTATTGCCCTTC
SF3B1_NGS_64 8R	CGGACTACAGCTCCATCATNNNNNNNNNNNNNACTT CTAAGATGTGGCAAGATGG
SRSF2_NGS_6 9_120LF	GGTAAACACAAGGGACTGGNNNNNNNTGAGGACGCTA TGGATGCC
SRSF2_NGS_6 9_120LR	CGGACTACAGCTCCATCATNNNNNNNNcctcagccccgttacC TG

Supplementary Table S3. Comparison of patient demographics and baseline characteristics of included and excluded patients.

Characteristic	Included patients (n=15)	Excluded patients (n=27)	P
Age			0.019
Median (years)	49.1	59.6	
Range (years)	29-72	35-78	
Sex			0.78
Male - no. (%)	9 (60)	15 (56)	
Female - no. (%)	6 (40)	12 (44)	
ECOG performance status at diagnosis			0.48
0 - no. (%)	10 (66)	4 (15)	
1 - no. (%)	3 (20)	0 (0)	
2 - no. (%)	1 (7)	0 (0)	
Missing data - no. (%)	1 (7)	23 (85)	
ECOG performance status			0.49
0 - no. (%)	6 (40)	8 (30)	
1 - no. (%)	5 (33)	12 (44)	
2 - no. (%)	1 (7)	1 (4)	
3 - no. (%)	1 (7)	0 (0)	
Missing data - no. (%)	2 (13)	6 (22)	
Type of AML			0.37
De novo - no. (%)	11 (73)	19 (70)	

Secondary* - no. (%)	4 (27)	5 (19)	
Therapy-related -no. (%)	0 (0)	3 (11)	
Cytogenetic risk group			0.874
Favorable/intermediate – no. (%)	9 (60)	16 (59)	
Adverse – no. (%)	4 (27)	8 (30)	
missing data - no. (%)	2 (13)	3 (11)	
ELN at SAIL screening (9)			0.571
Favorable/intermediate – no. (%)	5 (33)	3 (11)	
Unfavorable – no. (%)	7 (47)	7 (26)	
Missing data – no. (%)	3 (20)	17 (63)	
WBC count			0.38
Median - ($\times 10^9/l$)	4.17	8.53	
Range - ($\times 10^9/l$)	0.6-12.9	0.4-85.0	
Hemoglobin			0.075
Median – g/dL	9.21	10.22	
Range – g/dL	7.1-12.5	8.3-13.4	
Platelet count			0.027
Median - ($\times 10^9/l$)	46.64	101.38	
Range - ($\times 10^9/l$)	10-98	3-346	
Prior HCT			0.307
Yes – no. (%)	8 (53)	10 (37)	
No – no. (%)	7 (47)	17 (63)	
aGVHD in patients receiving an allograft or DLIs (n=12; n=7)			0.4
Yes – no. (%)	8 (73)	2 (7)	
No – no. (%)	3 (27)	0 (0)	
Missing data - no. (%)	0 (0)	25 (93)	
cGVHD in transplanted receiving an allograft or DLIs (n=12; n=7)			0.4
Yes – no. (%)	3 (20)	0 (0)	
No – no. (%)	8 (53)	2 (7)	
Missing data - no. (%)	4 (27)	25 (93)	
Treatment after SAIL			0.011

Yes – no. (%)	15 (100)	9 (33)	
No – no. (%)	0 (0)	5 (19)	
Missing data - no. (%)	0 (0)	13 (48)	
Type of treatment after SAIL			0.077
alloHCT- no. (%)**	9 (60)	7 (26)	
DLI - no. (%)	3 (20)	2 (7)	
Intensive Chemotherapy - no. (%)	2 (13)	0 (0)	
Non-intensive chemotherapy - no. (%)	1 (7)	0 (0)	
No treatment - no. (%)	0 (0)	5 (19)	
Missing data - no. (%)	0 (0)	13 (48)	

Abbreviations: ECOG, performance status of the Eastern Cooperative Oncology Group; ELN, European LeukemiaNet; WBC, white blood cell count; HCT, hematopoietic stem cell transplantation; alloHCT, allogeneic hematopoietic stem cell transplantation; aGvHD, acute graft-versus-host disease; cGvHD, chronic graft-versus-host disease; DLI, donor lymphocyte infusion; P, P-value from two-sided chi-squared tests for categorical variables and from two-sided Student's T or Kolmogorov-Smirnov tests for continuous variables.

* secondary AML developing after an antecedent hematologic disorder;

** three patients received a second alloHCT.

Supplementary Table S4. Patient demographics and baseline characteristics (n=15).

Characteristic	Included patients (n=15)
Age	
Median (years)	49.1
Range (years)	29-72
Sex	
Male - no. (%)	9 (60)
Female - no. (%)	6 (40)
ECOG performance status at diagnosis	
0 - no. (%)	10 (66)
1 - no. (%)	3 (20)
2 - no. (%)	1 (7)
Missing data - no. (%)	1 (7)
ECOG performance status	
0 - no. (%)	6 (40)
1 - no. (%)	5 (33)
2 - no. (%)	1 (7)
3 - no. (%)	1 (7)
Missing data - no. (%)	2 (13)
Type of AML	
De novo - no. (%)	11 (73)
Secondary* - no. (%)	4 (27)
Therapy-related -no. (%)	0 (0)
Cytogenetic risk group	
Favorable/intermediate – no. (%)	9 (60)
Adverse – no. (%)	4 (27)
missing data - no. (%)	2 (13)
ELN at SAIL screening (9)	
Favorable/intermediate – no. (%)	5 (33)
Unfavorable – no. (%)	7 (47)
Missing data – no. (%)	3 (20)
WBC count	
Median - ($\times 10^9/l$)	4.17
Range - ($\times 10^9/l$)	0.6-12.9
Hemoglobin	
Median – g/dL	9.21
Range – g/dL	7.1-12.5

Platelet count	
Median - ($\times 10^9/l$)	46.64
Range - ($\times 10^9/l$)	10-98
Prior HCT	
Yes – no. (%)	8 (53)
No – no. (%)	7 (47)
aGvHD in patients receiving an allograft or DLIs (n=12)	
Yes – no. (%)	8 (73)
No – no. (%)	3 (27)
cGvHD in transplanted receiving an allograft or DLIs (n=12)	
Yes – no. (%)	3 (27)
No – no. (%)	8 (73)
Treatment after SAIL	
Yes – no. (%)	15 (100)
No – no. (%)	0 (0)
Type of treatment after SAIL	
alloHCT- no. (%)	9 (60)
DLI - no. (%)	3 (20)
Intensive chemotherapy - no. (%)	2 (13)
Non-intensive chemotherapy - no. (%)	1 (7)
No treatment - no. (%)	0 (0)
Missing data - no. (%)	0 (0)

Abbreviations: ECOG, performance status of the Eastern Cooperative Oncology Group; ELN, European LeukemiaNet; WBC, white blood cell count; HCT, hematopoietic stem cell transplantation; alloHCT, allogeneic hematopoietic stem cell transplantation; aGvHD, acute graft-versus-host disease; cGvHD, chronic graft-versus-host disease; DLI, donor lymphocyte infusion; P, P-value from two-sided chi-squared tests for categorical variables and from two-sided Student's T or Kolmogorov-Smirnov tests for continuous variables.

* secondary AML developing after an antecedent hematologic disorder;

** three patients received a second alloHCT.

Supplementary Table S5. Comparison of clinical characteristics between CR/CRI (n=7) and all other patients (n=8).

Characteristic	All (n=15)	CR/CRI after induction chemotherapy n=7	All others (MLFS, SD, PR, PD, after induction) n=8	P
Age				0.586
Median (years)	49.1	46.7	51.3	
Range (years)	29-72	34-72	29-69	
Sex				0.398
Male - no. (%)	9 (60)	5 (71)	4 (50)	
Female - no. (%)	6 (40)	2 (29)	4 (50)	
ECOG performance status at diagnosis				0.061
0 - no. (%)	10 (66)	7 (100)	3 (38)	
1 - no. (%)	3 (20)	0 (0)	3 (38)	
2 - no. (%)	1 (7)	0 (0)	1 (13)	
Missing data - no. (%)	1 (7)	0 (0)	1 (13)	
ECOG performance status				0.545
0 - no. (%)	6 (40)	3 (43)	3 (38)	
1 - no. (%)	5 (33)	2 (29)	3 (38)	
2 - no. (%)	1 (7)	0 (0)	1 (13)	
3 - no. (%)	1 (7)	1 (14)	0 (0)	
Missing data - no. (%)	2 (13)	1 (14)	1 (13)	
Type of AML				0.31
De novo - no. (%)	11 (73)	6 (86)	5 (62)	
Secondary* - no. (%)	4 (27)	1 (14)	3 (38)	
Therapy-induced -no. (%)	0 (0)	0 (0)	0 (0)	
Cytogenetic risk group				0.853
Favorable/intermediate – no. (%)	9 (60)	5 (71)	4 (50)	
Adverse – no. (%)	4 (27)	2 (29)	2 (25)	
Missing data - no. (%)	2 (13)	0 (0)	2 (25)	
ELN at SAIL screening (9)				0.079
Favorable/intermediate – no. (%)	5 (33)	4 (50)	1 (12)	

Unfavorable – no. (%)	7 (47)	2 (25)	5 (63)	
Missing data – no. (%)	3 (20)	2 (25)	2 (25)	
WBC count				0.54
Median - ($\times 10^9/l$)	4.17	3.43	4.91	
Range - ($\times 10^9/l$)	0.6-12.9	0.6-12.6	0.7-12.9	
Hemoglobin				0.664
Median – g/dL	9.21	9.41	9.01	
Range – g/dL	7.1-12.5	7.1-12.5	7.3-11.0	
Platelet count				0.443
Median - ($\times 10^9/l$)	46.64	52.71	40.57	
Range - ($\times 10^9/l$)	10-98	15-98	10-86	
Prior HCT				0.782
Yes – no. (%)	8 (53)	4 (57)	4 (50)	
No – no. (%)	7 (47)	3 (43)	4 (50)	
AlloHCT after SAIL				0.833
Yes – no. (%)	9 (60)	4 (57)	5 (63)	
No – no. (%)	6 (40)	3 (43)	3 (37)	
aGVHD in patients receiving an allograft or DLIs (n=12)				0.387
Yes – no. (%)	8 (73)	5 (83)	3 (60)	
No – no. (%)	3 (27)	1 (17)	2 (40)	
cGVHD in transplanted receiving an allograft or DLIs (n=12)				0.621
Yes – no. (%)	3 (27)	2 (33)	1 (20)	
No – no. (%)	8 (73)	4 (67)	4 (80)	
Treatment after SAIL				n.a.
Yes – no. (%)	15 (100)	7 (100)	8 (100)	
No – no. (%)	0 (0)	0 (0)	0 (0)	
Type of treatment after SAIL				0.335
AlloHCT- no. (%)	9 (60)	4 (57)	5 (62)	
DLI - no. (%)	3 (20)	2 (29)	1 (12)	
Intensive chemotherapy - no. (%)	2 (13)	0 (0)	2 (26)	
Non-intensive chemotherapy - no. (%)	1 (7)	1 (14)	0 (0)	
No treatment - no. (%)	0 (0)	0 (0)	0 (0)	

Abbreviations: CR, complete remission; CRi, complete remission with incomplete hematologic recovery; MLFS, morphologic leukemia-free state; SD, stable disease; PR, partial remission; PD, progressive disease; ECOG, performance status of the Eastern Cooperative Oncology Group; ELN, European LeukemiaNet; WBC, white blood cell count; HCT, hematopoietic stem cell transplantation; alloHCT, allogeneic hematopoietic stem cell transplantation; aGvHD, acute graft-versus-host disease; cGvHD, chronic graft-versus-host disease; DLI, donor lymphocyte infusion; P, P-value from two-sided chi-squared tests for categorical variables and from two-sided Student's T or Kolmogorov-Smirnov tests for continuous variables.

* secondary AML developing after an antecedent hematologic disorder;

Supplementary Table S6. Comparison of molecular characteristics between CR/CRI (n=8) and all other patients (n=10).

Muation class	Mutated gene	All (n=15)	CR/Cri after induction n=7	All others (SD, PR, PD, MLFS, after induction) n=8	P
Epigenetic modifiers	<i>ASXL1</i>				0.605
	Mutated – no. (%)	3 (20)	1 (33)	2 (67)	
	Wildtype – no. (%)	12 (80)	6 (50)	6 (50)	
	<i>ASXL2</i>				0.038
	Mutated – no. (%)	3 (20)	3 (100)	0 (0)	
	Wildtype – no. (%)	12 (80)	4 (33)	8 (67)	
	<i>DNMT3A</i>				0.605
	Mutated – no. (%)	3 (20)	1 (33)	2 (67)	
	Wildtype – no. (%)	12 (80)	6 (50)	6 (50)	
	<i>IDH2</i>				0.333
	Mutated – no. (%)	1 (7)	0 (0)	1 (100)	
	Wildtype – no. (%)	14 (93)	7 (50)	7 (50)	
	<i>KDM6A</i>				0.333
	Mutated – no. (%)	1 (7)	0 (0)	1 (100)	
	Wildtype – no. (%)	14 (93)	7 (50)	7 (50)	
	<i>TET2</i>				0.605

	Mutated – no. (%)	3 (20)	1 (33)	2 (67)	
	Wildtype – no. (%)	12 (80)	6 (50)	6 (50)	
Nucleo-phosmin	<i>NPM1</i> – no. (%)				0.919
	Mutated – no. (%)	2 (13)	1 (50)	1 (50)	
	Wildtype – no. (%)	13 (87)	6 (46)	7 (54)	
Cohesin complex	<i>RAD21</i>				n.a.
	Mutated – no. (%)	0 (0)	0 (0)	0 (0)	
	Wildtype – no. (%)	15 (100)	7 (100)	8 (100)	
	<i>SMC1A</i>				0.333
	Mutated – no. (%)	1 (7)	0 (0)	1 (100)	
	Wildtype – no. (%)	14 (93)	7 (50)	7 (50)	
Signal transduction	<i>CBL</i>				0.333
	Mutated – no. (%)	1 (7)	0 (0)	1 (100)	
	Wildtype – no. (%)	14 (93)	7 (50)	7 (50)	
	<i>FLT3</i> – no. (%)				0.104
	Mutated – no. (%)	2 (13)	2 (100)	0 (0)	
	Wildtype – no. (%)	13 (87)	5 (38)	8 (62)	
	<i>KRAS</i>				0.333
	Mutated – no. (%)	1 (7)	0 (0)	1 (100)	
	Wildtype – no. (%)	14 (93)	7 (50)	7 (50)	
	<i>NF1</i>				0.333
	Mutated – no. (%)	1 (7)	0 (0)	1 (100)	
	Wildtype – no. (%)	14 (93)	7 (50)	7 (50)	
Spliceosome	<i>PTPN11</i>				0.919
	Mutated – no. (%)	2 (13)	1 (50)	1 (50)	
	Wildtype – no. (%)	13 (87)	6 (46)	7 (54)	
	<i>SF3B1</i>				0.919
	Mutated – no. (%)	2 (13)	1 (50)	1 (50)	
	Wildtype – no. (%)	13 (87)	6 (46)	7 (54)	
	<i>SRSF2</i>				0.605
	Mutated – no. (%)	3 (20)	1 (33)	2 (67)	
	Wildtype – no. (%)	12 (80)	6 (50)	6 (50)	
	<i>U2AF1</i>				0.333
Myeloid TF's	Mutated – no. (%)	1 (7)	0 ()	1 (100)	
	Wildtype – no. (%)	14 (93)	7 (50)	7 (50)	
	<i>ZBTB7A</i>				0.268
	Mutated – no. (%)	1 (7)	1 (100)	0 (0)	
	Wildtype – no. (%)	14 (93)	6 (43)	8 (57)	
	<i>BCOR</i>				0.333
	Mutated – no. (%)	1 (7)	0 (0)	1 (100)	
	Wildtype – no. (%)	14 (93)	7 (50)	7 (50)	

Tumor suppressors	<i>CEBPA</i>				0.333
	Mutated – no. (%)	1 (7)	0 (0)	1 (100)	
	Wildtype – no. (%)	14 (93)	7 (50)	7 (50)	
	<i>CUX1</i>				0.070
	Mutated – no. (%)	3 (20)	0 (0)	3 (100)	
	Wildtype – no. (%)	12 (80)	7 (58)	5 (42)	
	<i>GATA2</i>				0.919
	Mutated – no. (%)	2 (13)	1 (50)	1 (50)	
	Wildtype – no. (%)	13 (87)	6 (46)	7 (54)	
	<i>RUNX1</i>				0.31
	Mutated – no. (%)	4 (27)	1 (25)	3 (75)	
	Wildtype – no. (%)	11 (73)	6 (55)	5 (45)	
	<i>SETBP1</i>				0.333
	Mutated – no. (%)	1 (7)	0 (0)	1 (100)	
	Wildtype – no. (%)	14 (93)	7 (50)	7 (50)	
	<i>PPM1D</i>				0.333
	Mutated – no. (%)	1 (7)	0 (0)	1 (100)	
	Wildtype – no. (%)	14 (93)	7 (50)	7 (50)	
	<i>TP53</i>				0.104
	Mutated – no. (%)	2 (13)	2 (100)	0 (0)	
	Wildtype – no. (%)	13 (87)	5 (38)	8 (62)	
	<i>WT1</i>				0.155
	Mutated – no. (%)	2 (13)	0 (0)	2 (100)	
	Wildtype – no. (%)	13 (87)	7 (54)	6 (46)	

Abbreviations: CR, complete remission; CRI, complete remission with incomplete hematologic recovery; MLFS, morphologic leukemia-free state; SD, stable disease; PR, partial remission; PD, progressive disease; P, P-value from two-sided chi-squared tests for categorical variables; TF, transcription factor.

Supplementary Table S7. Response patterns of gene mutations under SAIL treatment.

Gene	Molecular Response association	Based on number of patients (n)
<i>RUNX1</i>	Molecular non-response	4
<i>GATA2</i>	Molecular non-response	2
<i>TET2</i>	Molecular non-response	3
<i>ASXL1</i>	Molecular non-response	3
<i>ASXL2</i>	Molecular non-response	3
<i>BCOR</i>	Molecular non-response	1
<i>CUX1</i>	Molecular non-response	3
<i>DNMT3A</i>	Molecular non-response	3
<i>FLT3</i>	Molecular response	2
<i>NPM1</i>	Molecular non-response	2
<i>PTPN11</i>	Molecular non-response	2
<i>SRSF2</i>	Molecular non-response	3
<i>TP53</i>	Molecular response	2
<i>SF3B1</i>	Molecular response	2
<i>WT1</i>	Molecular non-response	2

Note: Molecular response was defined as VAF negativity in the follow-up sample after SAIL treatment in comparison to the relapse sample.

Supplementary Table S8. Univariate Analysis for OS and EFS in 15 SAIL patients.

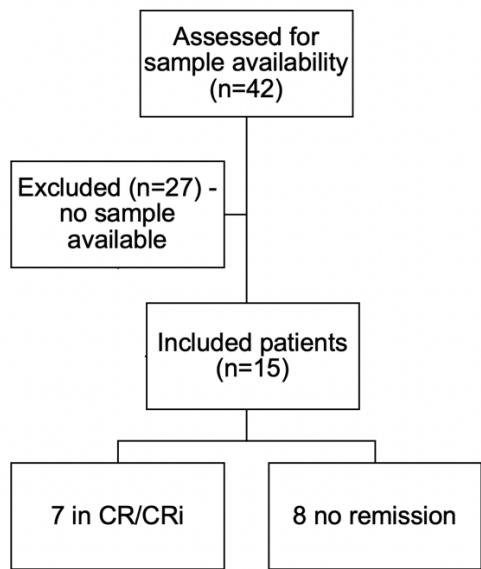
Endpoint	Variables in the model	Univariate analysis		
		HR	95% CI	P
OS	Age <median vs >median	0.129	0.025-0.666	0.014
	Male vs. female	0.513	0.144-1.832	0.304
	ECOG at SAIL screening 0 or 1 vs. 2	0.500	0.100-2.496	0.398

	alloHCT after SAIL yes vs. no	0.331	0.094-1.172	0.087
	Type of AML primary vs. secondary/therapy-induced	0.222	0.055-0.906	0.036
	Hemoglobin (g/dL) <median vs. >median	2.096	0.580-7.571	0.259
	WBC count ($\times 10^9/l$) <median vs. >median	0.713	0.201-2.529	0.601
	Platelet count ($\times 10^9/l$) <median vs. >median	1.828	0.504-6.635	0.359
	<i>FLT3</i> mut vs wt	0.770	0.096-6.184	0.805
	<i>ASXL1</i> mut vs wt	0.770	0.162-3.661	0.743
	<i>ASXL2</i> mut vs wt	0.727	0.150-3.528	0.693
	<i>CUX1</i> mut vs wt	1.137	0.235-5.502	0.873
	<i>DNMT3A</i> mut vs wt	1.354	0.279-6.585	0.707
	<i>GATA2</i> mut vs wt	0.519	0.064-4.189	0.538
	<i>SRSF2</i> mut vs wt	0.964	0.204-4.564	0.963
	<i>TET2</i> mut vs wt	3.881	0.914-16.470	0.066
	<i>TP53</i> mut vs wt	4.351	0.787-24.066	0.092
EFS	Age <median vs >median	0.485	0.079-2.966	0.433
	Male vs. female	0.704	0.116-4.265	0.703
	ECOG at SAIL 0 and 1 vs. 2 and 3	0.289	0.018-4.648	0.381
	alloHCT after SAIL yes vs. no	0.134	0.012-1.507	0.104
	Type of AML primary vs. secondary/therapy-induced	1.122	0.122-10.328	0.919
	Hemoglobin (g/dL) <median vs. >median	3.934	0.422-36.690	0.229
	WBC count ($\times 10^9/l$) <median vs. >median	0.696	0.113-4.270	0.695
	Platelet count ($\times 10^9/l$) <median vs. >median	1.605	0.261-9.872	0.610
	<i>FLT3</i> mut vs wt	2.179	0.302-15.721	0.440

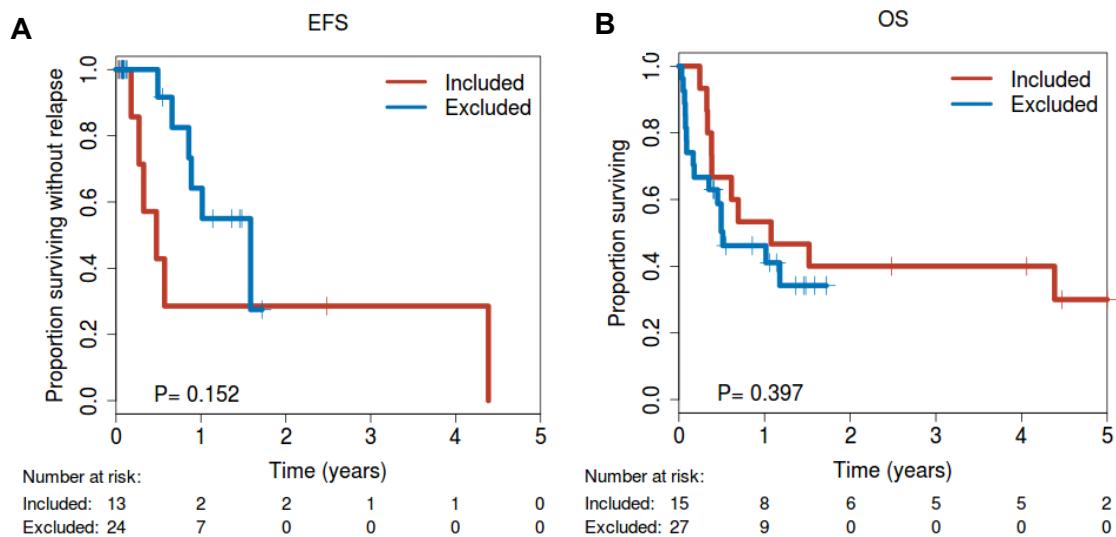
	<i>DNMT3A</i> mut vs wt	0.296	0.031- 2.827	0.290
	<i>GATA2</i> mut vs wt	0.638	0.071- 5.772	0.689
	<i>TP53</i> mut vs wt	1.420	0.234- 8.597	0.703

Note: Genes were considered for univariate analysis if they were detectable in at least 3 patients. Hazard ratios greater than or less than 1 indicate an increased or decreased risk, respectively, of an event for the first category listed.

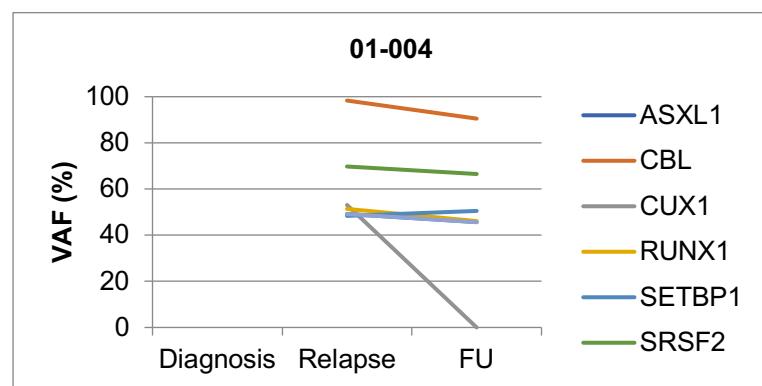
Patient number was too small for a useful calculation for the following variables: *BCOR*, *CBL*, *CEBPA*, *IHD2*, *KDM6A*, *KRAS*, *NF1*, *PPM1D*, *RAD21*, *RUNX1*, *SETBP1*, *SMC1A*, *TET2*, *U2AF1*, *ZBTB7A*.

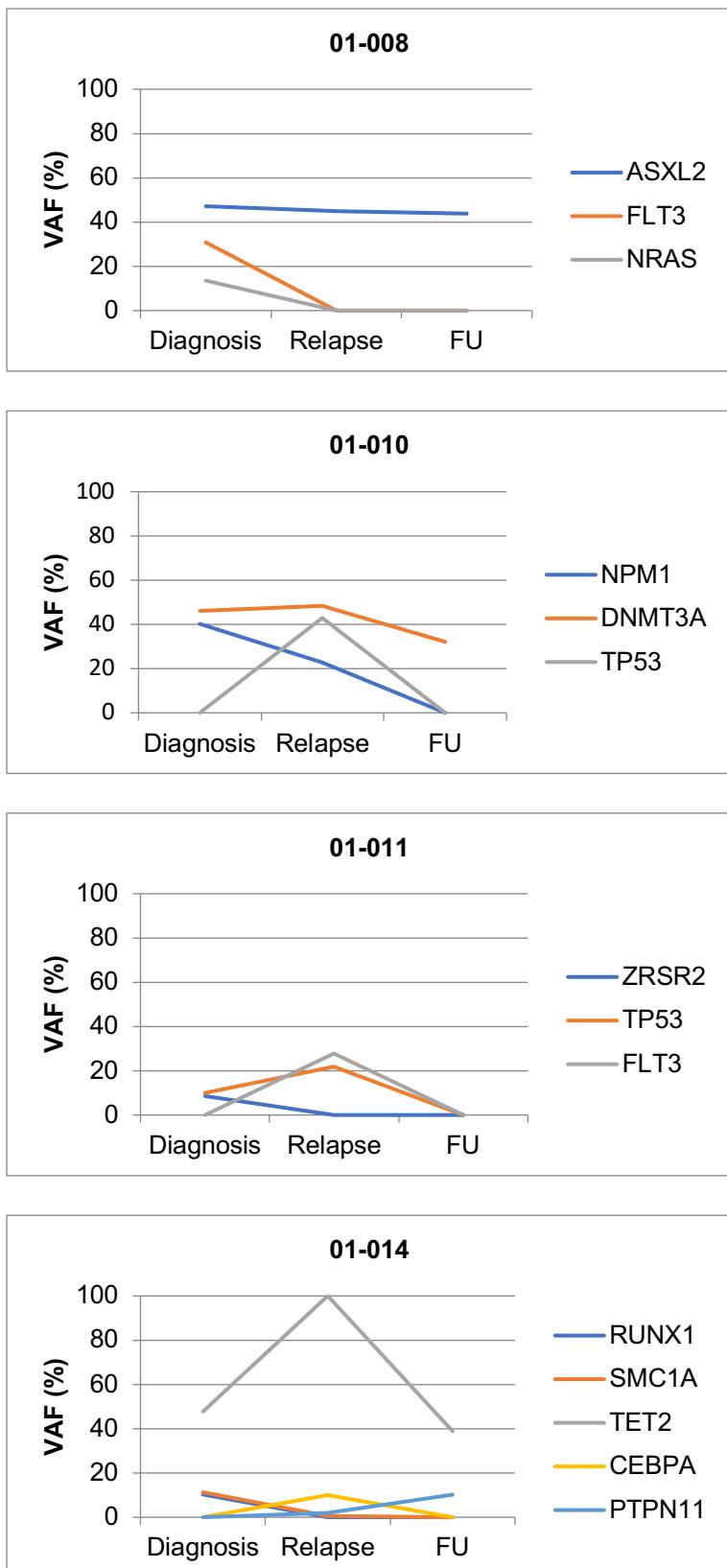
Supplementary Figures**Supplementary Figure S1. Consort diagram of studied patients.**

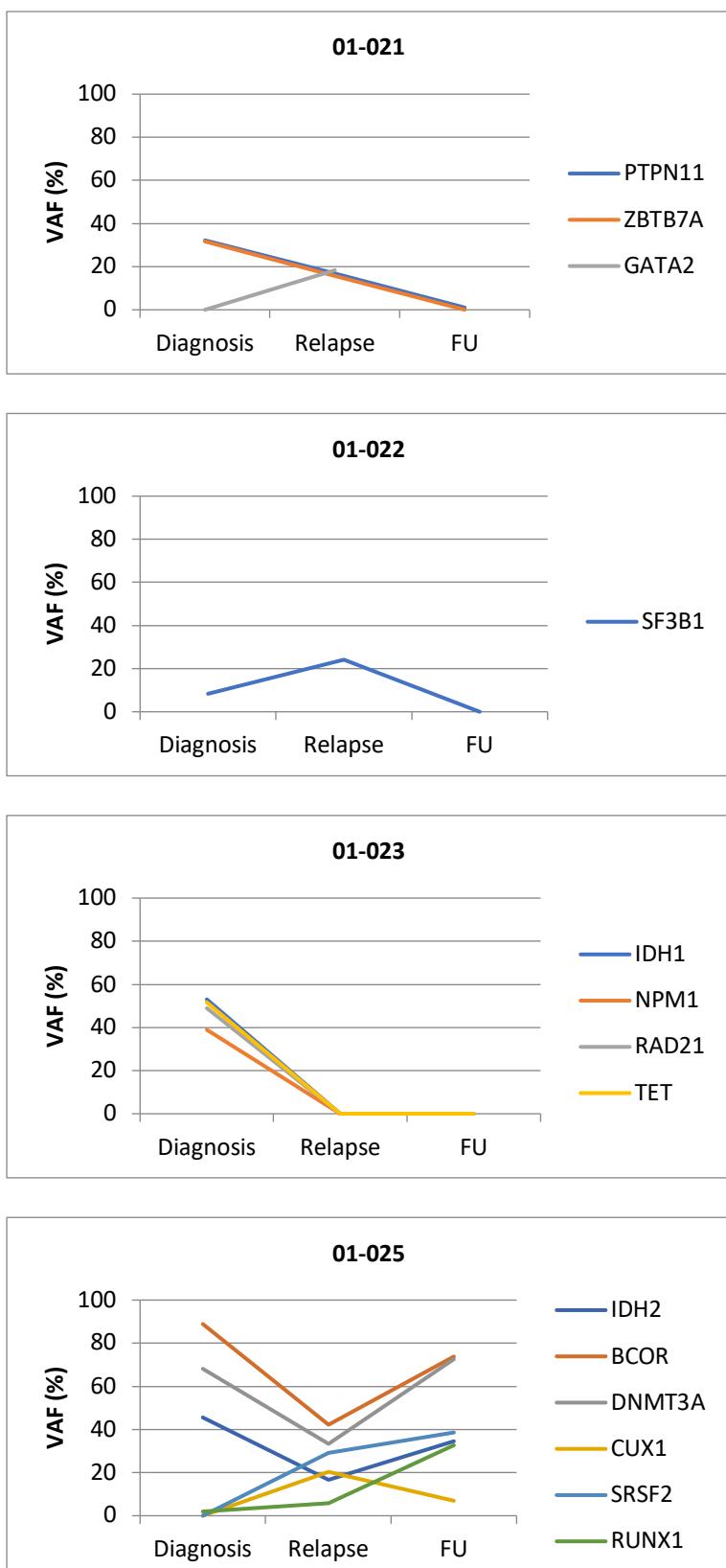
Supplementary Figure S2. Comparison of (A) EFS and (B) OS between included and excluded patients.

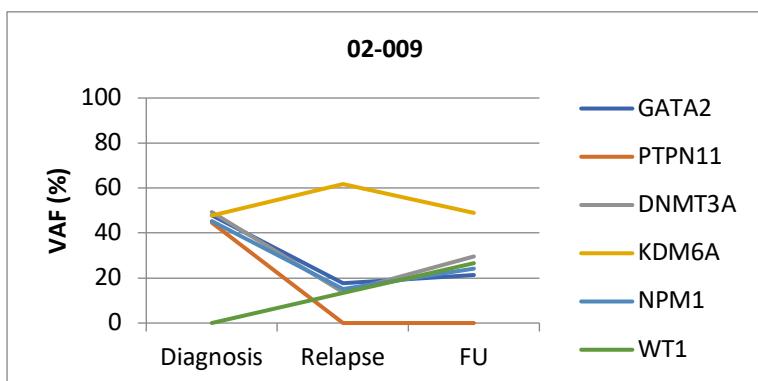
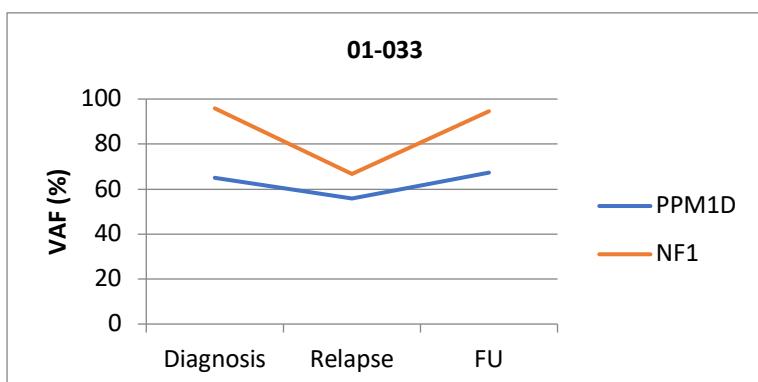
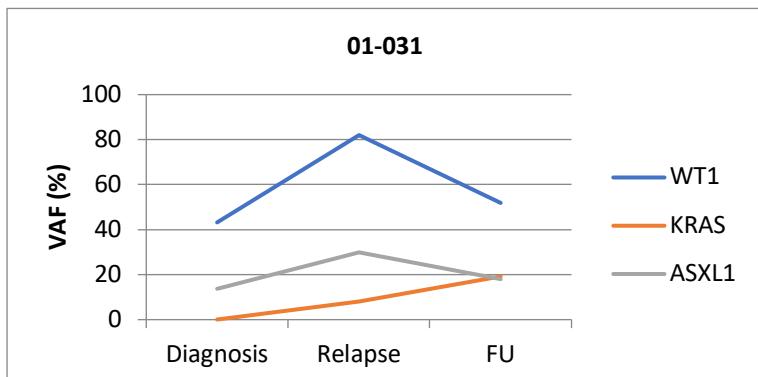
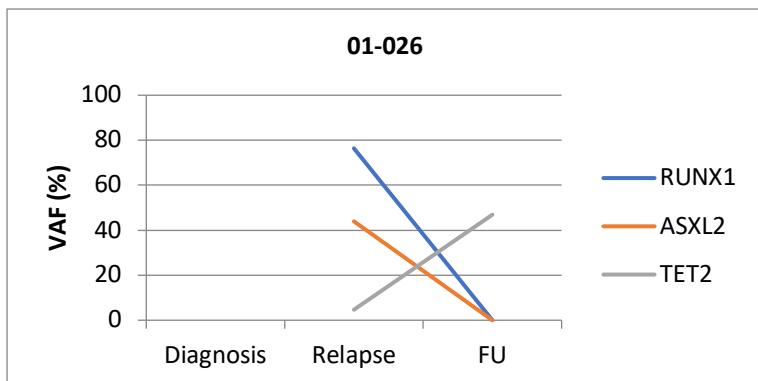


Supplementary Figure S3. Patient-specific clonal evolution from diagnosis to relapse/refractoriness and follow-up after cycle one of SAIL treatment.

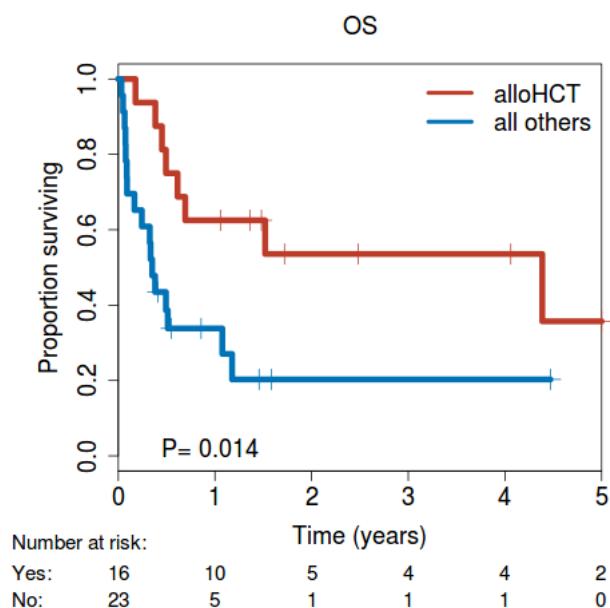




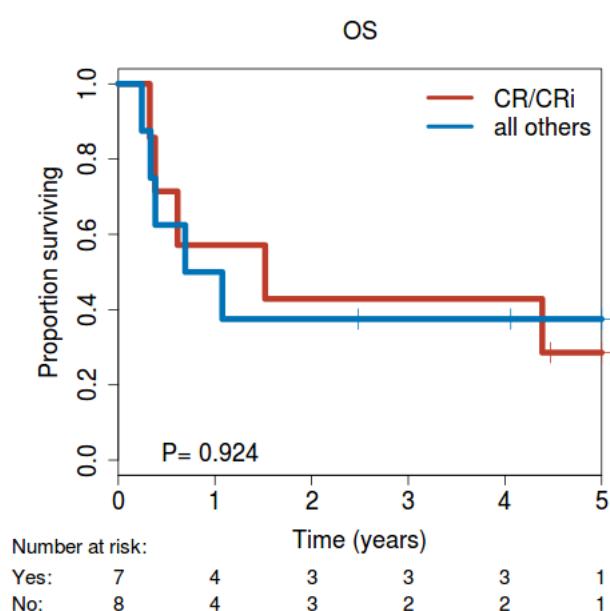




Supplementary Figure S4. OS of patients undergoing alloHCT after SAIL induction vs all other patients.



Supplementary Figure S5. OS in patients achieving CR/CRI vs. patients with other response to SAIL induction therapy.



Supplementary Figure S6. OS in patients with declining and persisting clones after one cycle of SAIL treatment.