Quantification of measurable residual disease using duplex sequencing in adults with acute myeloid leukemia

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SUPPLEMENTARY INFORMATION

Quantification of measurable residual disease using duplex-sequencing in adults with acute myeloid leukemia.

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Supplementary Methods

DNA Extraction and Quantification

Genomic DNA (gDNA) from cryopreserved patient bone marrow or peripheral blood mononuclear cells was extracted with the Qiagen PureGene kit. gDNA from a separate young, healthy donor was extracted from a Leukopak purchased from AllCells using an Agilent Genomic DNA extraction kit. For technical spike-in mixtures, gDNA from HCC827, AN3-CA, SW1271, MDA-MB-453, SW48, HCT-15, and SW620 cells was purchased from ATCC and gDNA from OCI-AML3, MOLM-14-L1, and K-562 cells was a gift from Dr. Jerald Radich. All gDNA concentrations were quantified with the Qubit dsDNA High Sensitivity kit and quality assessed on an Agilent TapeStation 2200 using a Genomic DNA Screen Tape.

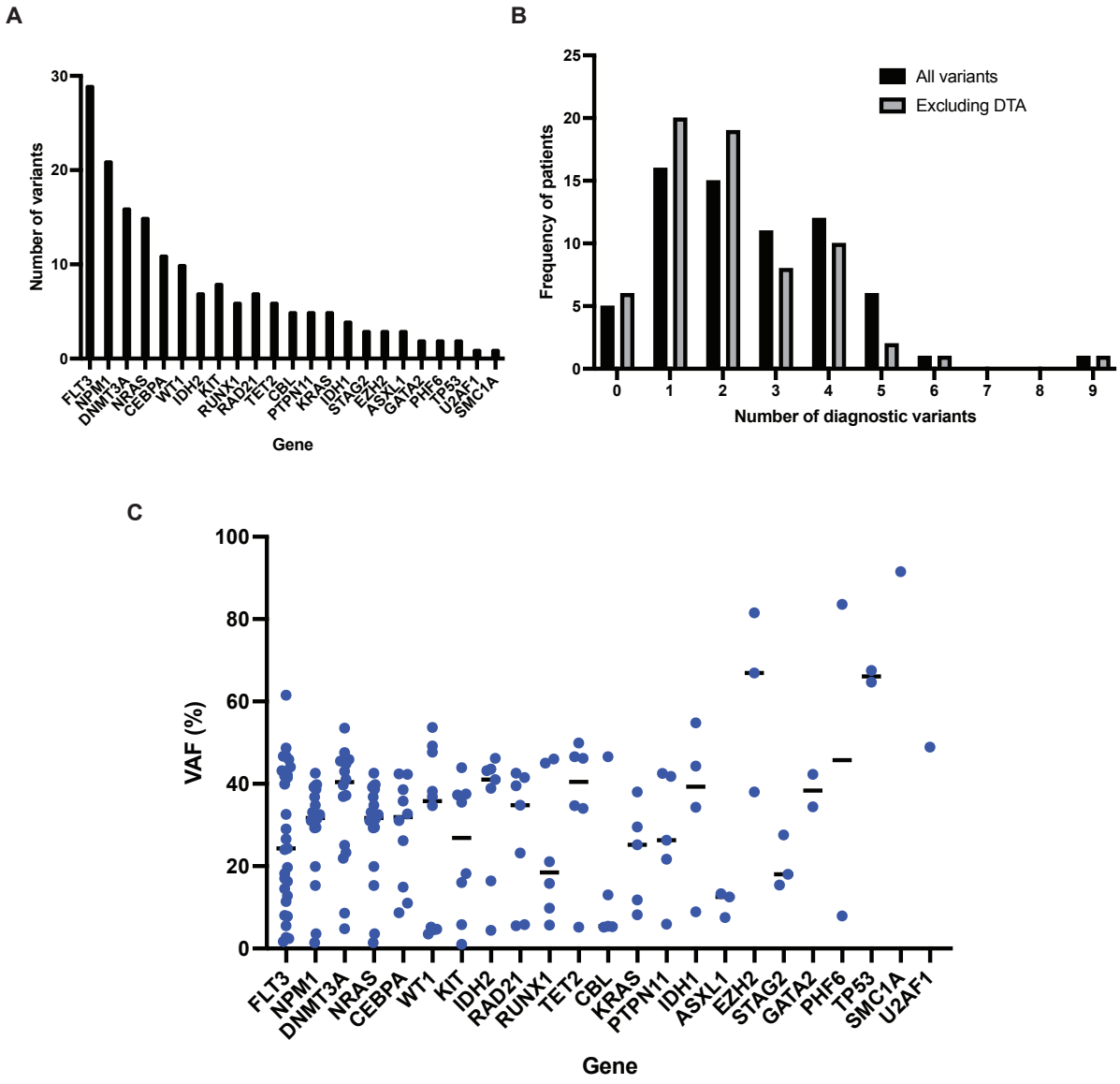
Duplex Sequencing

Retrospective targeted DNA sequencing of 29 genes recurrently mutated in adult AML was performed on paired diagnostic and remission bone marrow or peripheral blood samples utilizing the TwinStrand Duplex SequencingTM AML-29 Panel (**Supplementary Table S1**). Non-error corrected sequencing was performed on diagnostic samples (500ng gDNA) and error-corrected duplex sequencing (DS) was performed on remission samples (1μg gDNA). Briefly, gDNA was sheared to a peak fragment size of 300 bp using a Covaris ultrasonicator. End repair, A-tailing and DuplexSeq^{TM} adapter ligation were performed prior to library conditioning with a cocktail of glycosylases to remove damaged DNA molecules prior to amplification. Following indexing PCR, libraries were hybridized with biotinylated 120-mer DNA probes and purified with streptavidin magnetic beads. Following washes additional PCR was performed, followed by another round of hybridization, capture, washes, and final PCR. Libraries were sequenced using paired-end 150bp sequencing on an Illumina NextSeq 500 (diagnostic samples) or a NovaSeq 6000 (remission samples).

For technical assessment of the 29 gene panel with the DS assay, mutant cell line DNA was spiked into healthy donor DNA at predicted VAFs ranging from $1.0x10^{-2}$ to $3.9x10^{-6}$. Mutant DNA samples harboring a total of 21 unique variants across 13 genes were combined into mutation mixes. Fifteen single nucleotide variants (SNV) were combined into an "SNV mix" and 4 insertions/deletions (indel) were combined into an "indel mix". A serial dilution of *FLT3*-ITD (21 bp) and *NPM1* insertion (4 bp) variants was also generated (1%, 0.1%, 0.01% and 0.003% VAF for each). Four replicate libraries were prepared for each mutation mix, with 1.5µg gDNA input for each library except 50ng for 1% *FLT3/NPM1* and 250ng for 0.1% *FLT3/NPM1*, and pure healthy donor DNA at each DNA input mass. Expected VAFs were based on COSMIC reported zygosities and dilution factors and were adjusted based on DS analysis of SNVs in pure cell line DNA and 1:1 mixes of cell line and healthy donor DNA. Libraries were prepared as above and sequenced on a NovaSeq 6000.

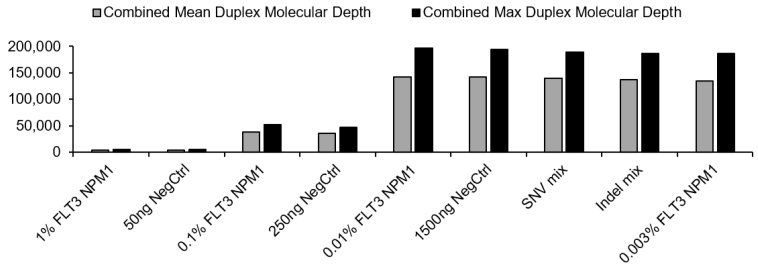
Raw FASTQ files are available in the NCBI Small Reads Archive (SRA) (Accession: PRJNA945188).

Supplementary Figure S1. Detection of mutations at AML diagnosis.
The (A) total number of variants per gene, (B) number of variants per patient (with or without inclusion of *DNMT3A*, *TET2* or *ASXL1* (DTA) genes), and (C) variant allele fraction (VAF) of variants detected in diagnostic samples from 67 acute myeloid leukemia (AML) patients screened for inclusion in this study.



Supplementary Figure S2. Technical assessment of the duplex sequencing assay. (A) Data from 4 technical replicate libraries were merged for each of the mutation mixes or negative controls. Mean and maximum panel-wide duplex molecular depth are plotted for each. (B) The expected vs. observed variant allele fractions (VAFs) are listed for each variant in the cell line DNA spike-in mixtures. *FLT3*-ITD and *NPM1* insTCTG each appear 4 times to reflect the serial dilutions of those mutations. (C) Observed vs. expected VAFs are plotted for each mutation mix, and correlations are calculated and inset. Error bars represent Wilson binomial 95% confidence intervals.

A

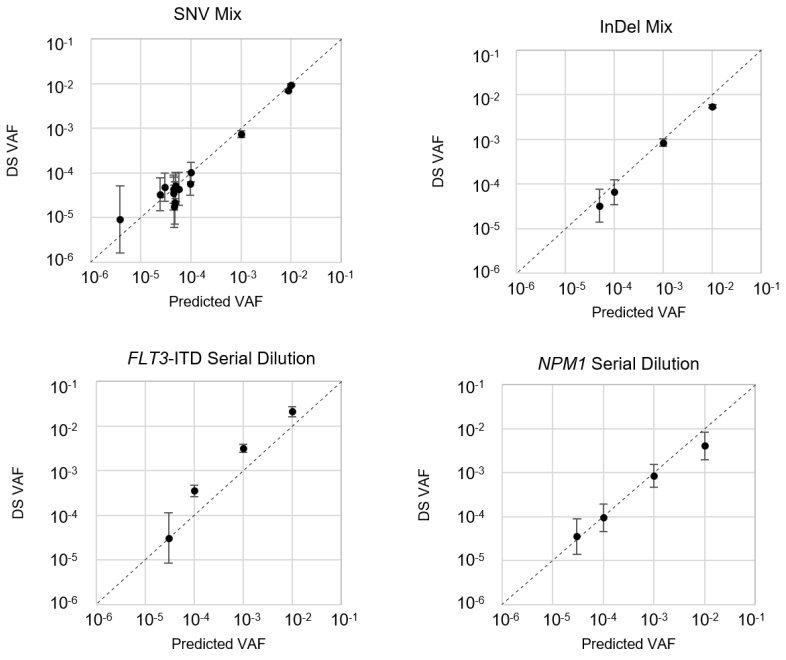


B

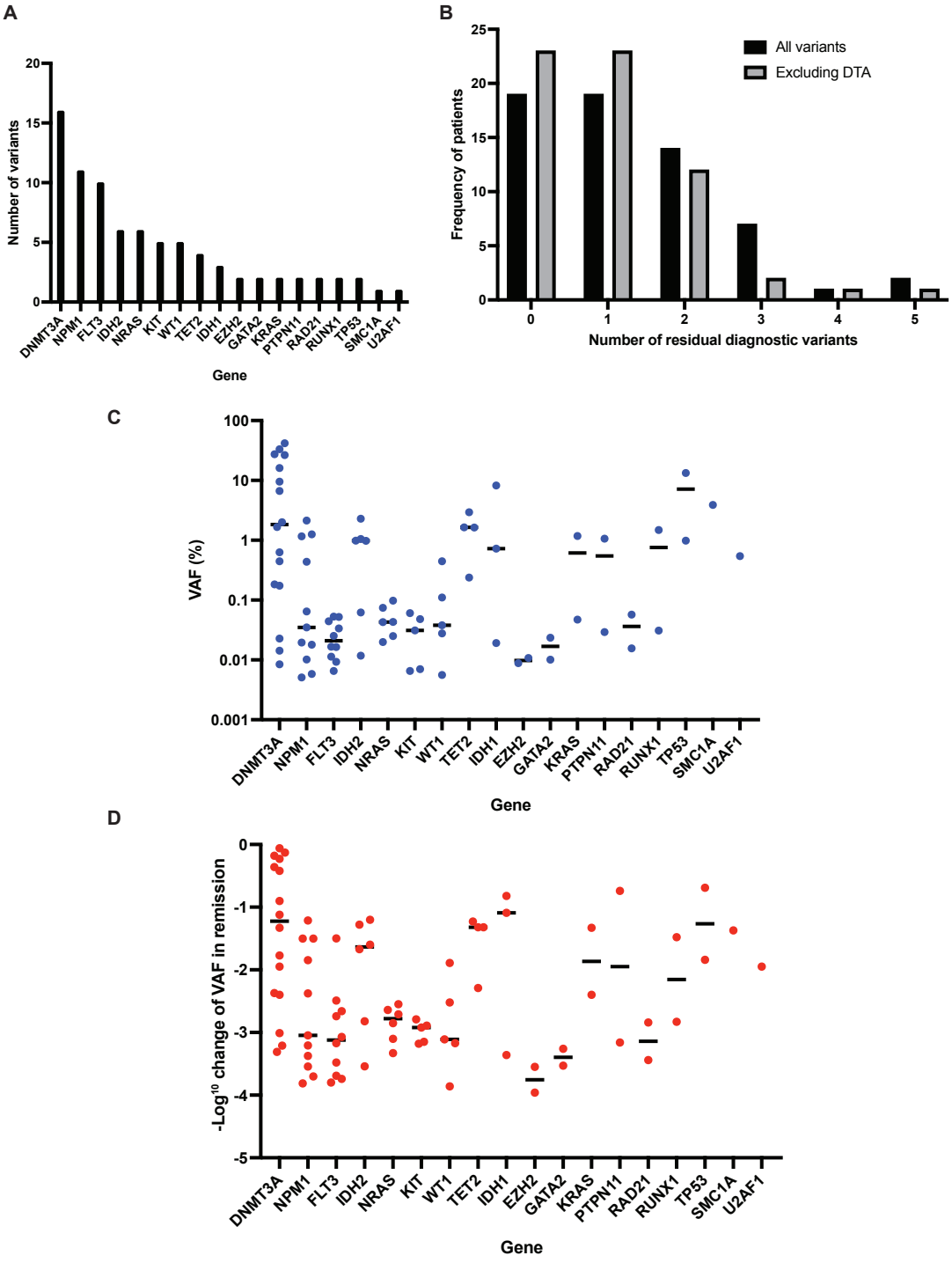
Gene	Variant	Expected	Observed
TP53	C277F	$1.0x10^{-2}$	$9.4x10^{-3}$
FLT3	ITD	$1.0x10^{-2}$	$2.1x10^{-2}$
NPM1	insTCTG	$1.0x10^{-2}$	$4.1x10^{-3}$
TP53	Q136fs	$1.0x10^{-2}$	$5.5x10^{-3}$
NRAS	Q61R	$8.8x10^{-3}$	$7.1x10^{-3}$
TET2	R881G	$1.0x10^{-3}$	$7.4x10^{-4}$
FLT3	ITD	$1.0x10^{-3}$	$3.2x10^{-3}$
NPM1	insTCTG	$1.0x10^{-3}$	$8.4x10^{-4}$
TP53	V218fs	$1.0x10^{-3}$	$8.4x10^{-4}$
TET2	L1329M	$1.0x10^{-4}$	$1.0x10^{-4}$
FLT3	ITD	$1.0x10^{-4}$	$3.5x10^{-4}$
NPM1	insTCTG	$1.0x10^{-4}$	$9.4x10^{-5}$
PTEN	R130fs	$1.0x10^{-4}$	$6.6x10^{-5}$
BRINP3	C726*	$9.6x10^{-5}$	$5.8x10^{-5}$

Gene	Variant	Expected	Observed
EZH2	S734S	$5.7x10^{-5}$	$4.4x10^{-5}$
TP53	S90fs	$5.0x10^{-5}$	$3.2x10^{-5}$
RAD21	D116E	$5.0x10^{-5}$	$4.8x10^{-5}$
HNRNPK	Q182H	$4.9x10^{-5}$	$5.2x10^{-5}$
DNMT3A	M761I	$4.7x10^{-5}$	$2.1x10^{-5}$
ASXL1	W796C	$4.7x10^{-5}$	$1.8x10^{-5}$
KRAS	G13D	$4.6x10^{-5}$	$3.5x10^{-5}$
BRINP3	Q493K	$4.5x10^{-5}$	$4.3x10^{-5}$
FLT3	ITD	$3.0x10^{-5}$	$3.1x10^{-5}$
NPM1	insTCTG	$3.0x10^{-5}$	$3.5x10^{-5}$
KRAS	G12V	$3.0x10^{-5}$	$4.9x10^{-5}$
TP53	R273H	$2.4x10^{-5}$	$3.3x10^{-5}$
RUNX1	Q264R	$3.9x10^{-6}$	$9.1x10^{-6}$

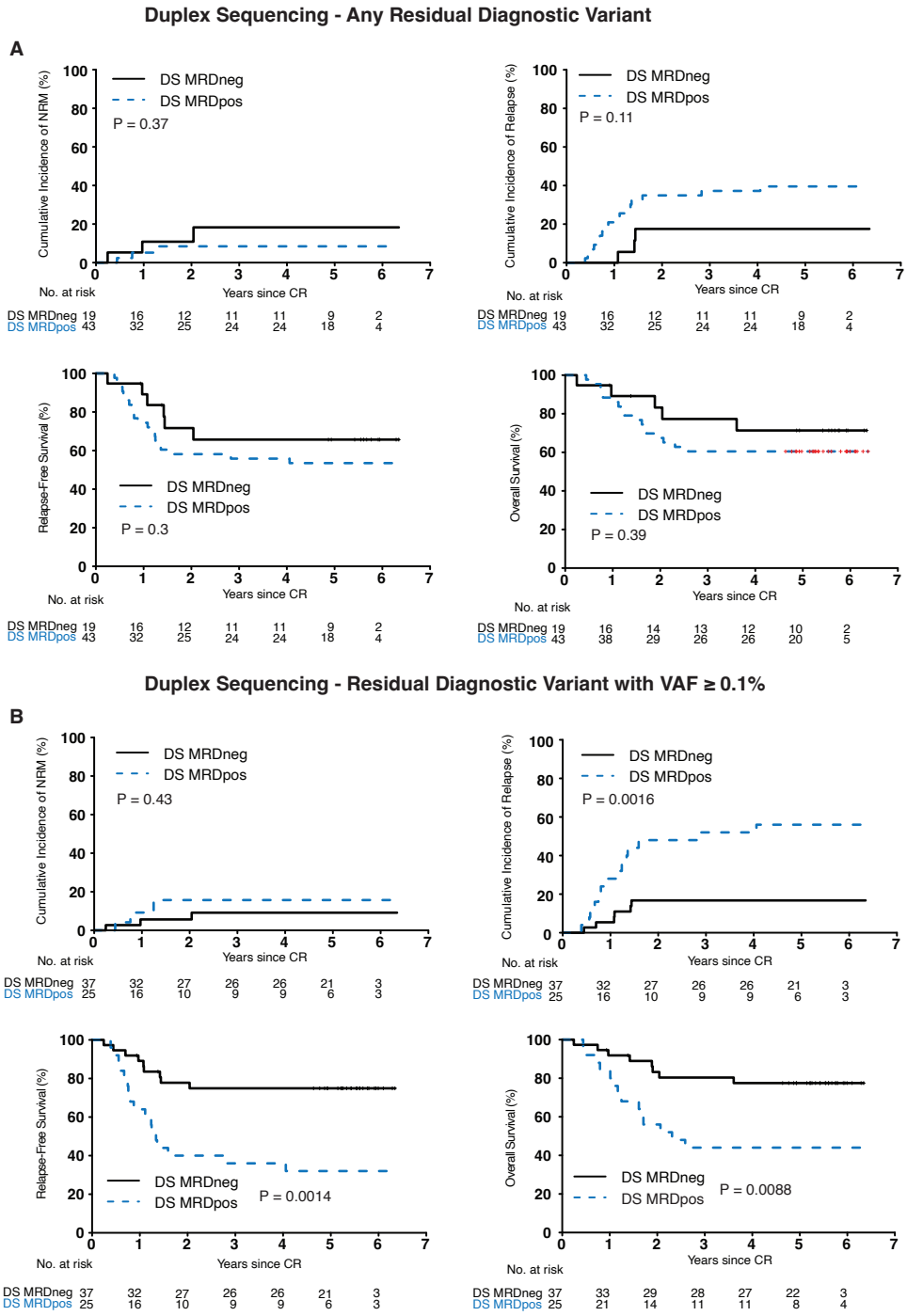
C



Supplementary Figure S3. Detection of residual diagnostic mutations in remission. The (A) total number of variants per gene, (B) number of variants per patient (with or without inclusion of *DNMT3A*, *TET2* or *ASXL1* (DTA) genes), (C) variant allele fraction (VAF), and (D) -log^{10} change in VAF between diagnosis and remission of diagnostic variants detected at the time of remission from 62 acute myeloid leukemia (AML) patients included in this study.

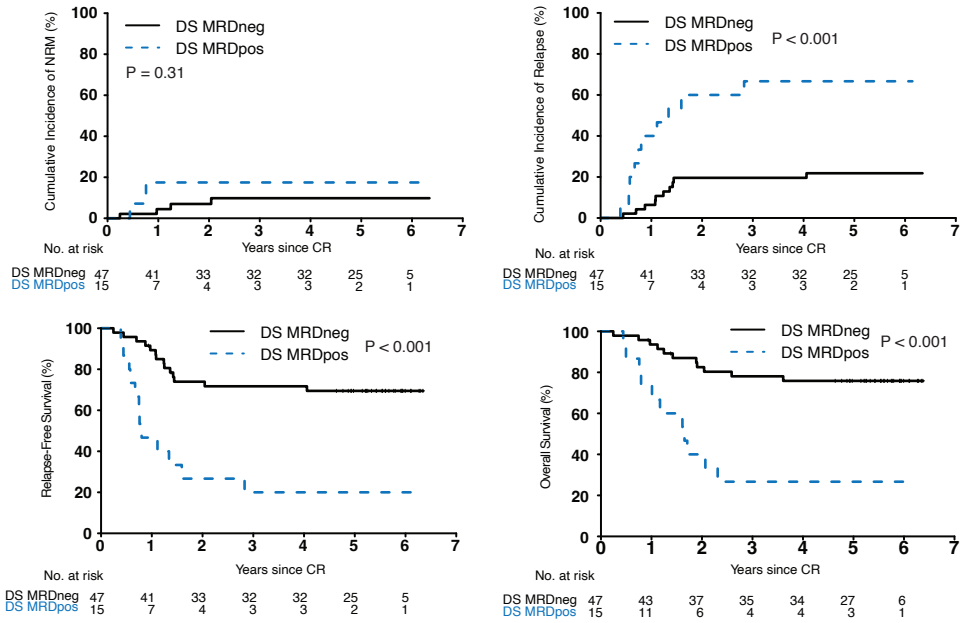


Supplementary Figure S4. Association of different DS MRD status on clinical outcomes. Rates of non-relapse mortality (NRM, top left), relapse (top right), relapse-free survival (bottom left), and overall survival (bottom right) are shown by remission DS MRD status as defined by: (A) any residual diagnostic variant (RDV), (B) RDV with VAF ≥ 0.1%, (C) RDV with VAF ≥ 0.1%, excluding *DNMT3A*, *TET2*, and *ASXL1* (DTA), (D) RDV with no greater than 2 $\log^{10}$ reduction in VAF between diagnosis and remission, (E) RDV with no greater than 2 $\log^{10}$ reduction in VAF between diagnosis and remission, excluding DTA, and (F) deleterious variant with VAF ≥ 0.1%, or ≥ 0.01% for *NPM1/FLT3*-ITD, excluding DTA, agnostic to diagnosis.



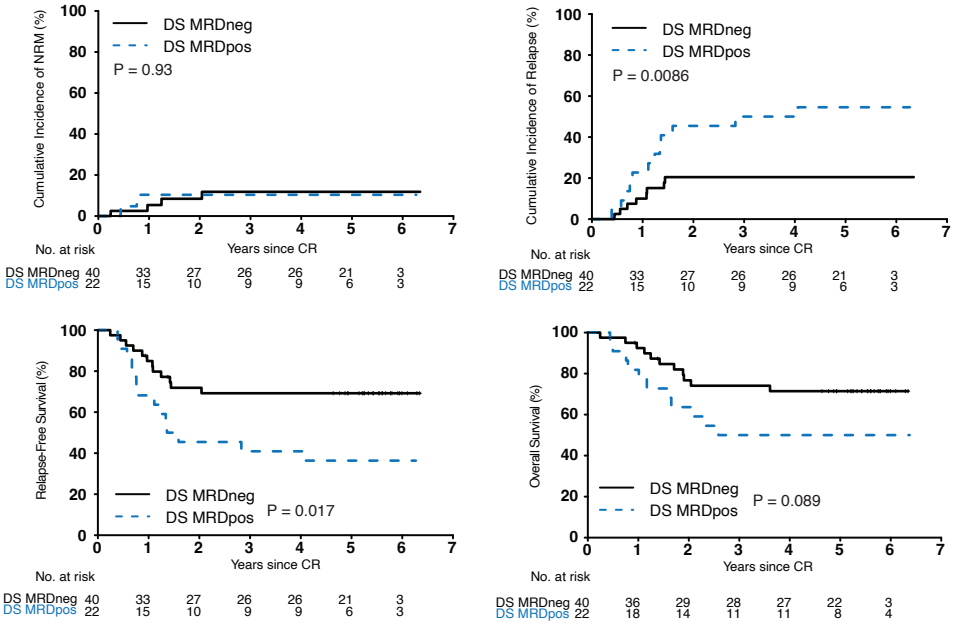
Duplex Sequencing - Residual Diagnostic Variant with VAF ≥ 0.1%, no DTA

C



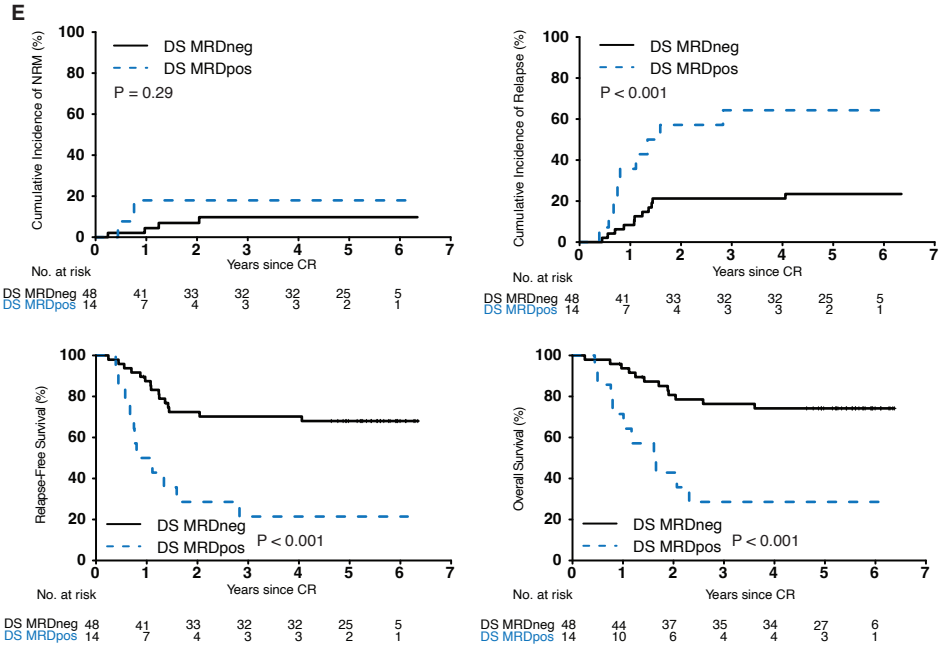
Duplex Sequencing - Residual Diagnostic Variant VAF ≤ 2 Log[10] Decrease

D



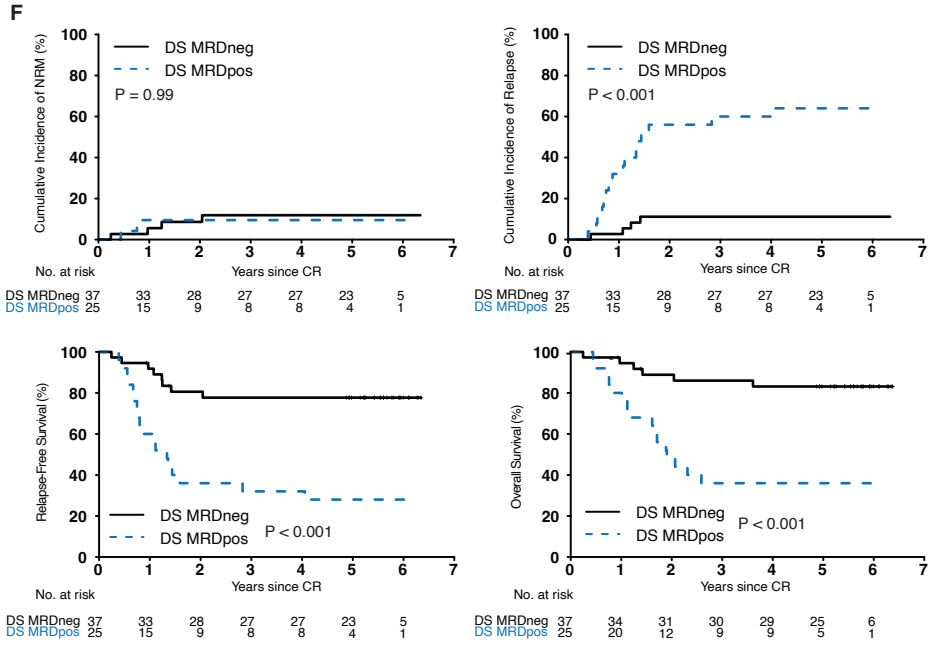
Duplex Sequencing - Residual Diagnostic Variant VAF ≤ 2 Log^{10} Decrease, No DTA

E



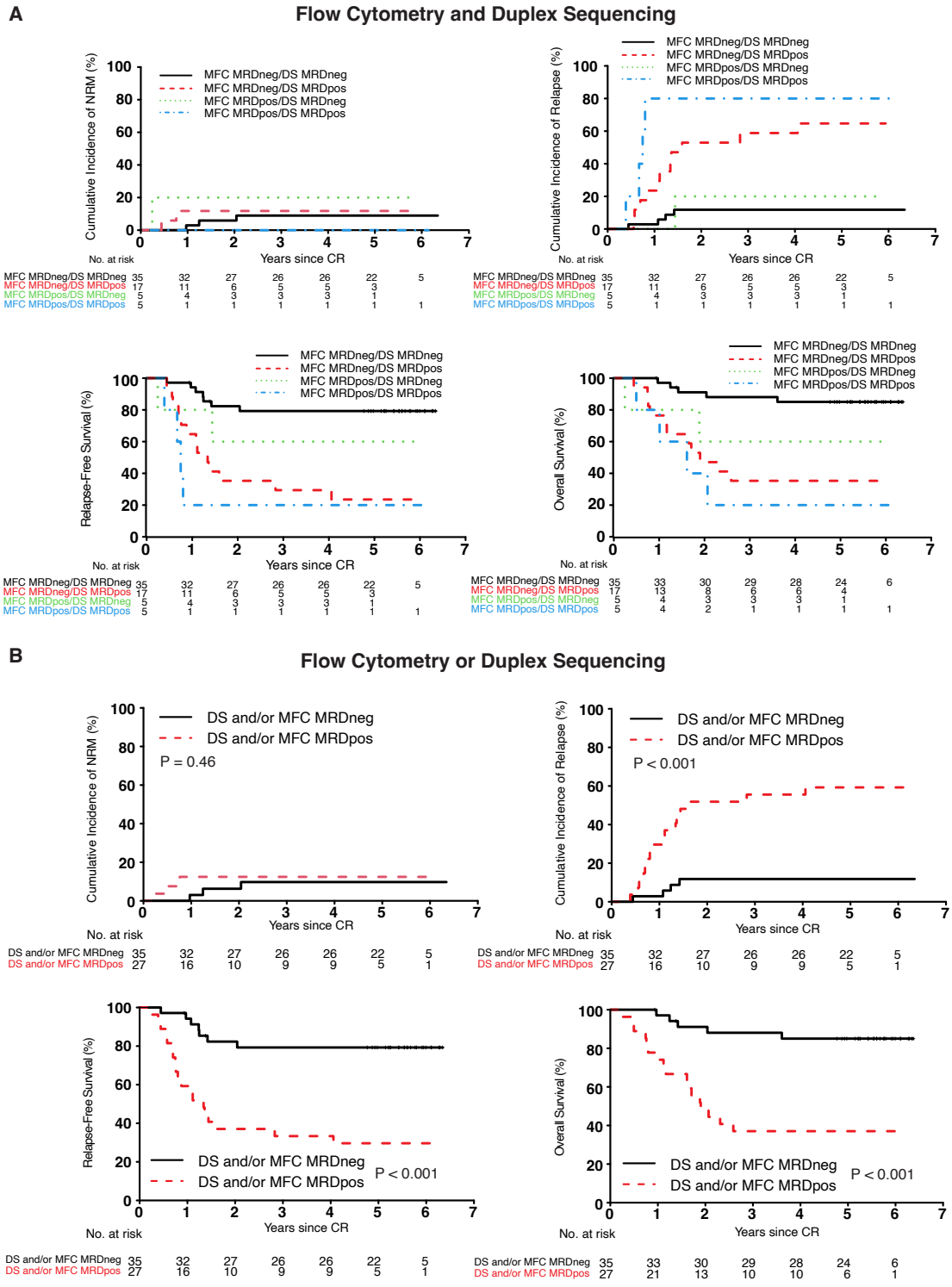
Duplex Sequencing - VAF ≥ 0.1% or ≥ 0.01% for *NPM1*/*FLT3*-ITD, No DTA, agnostic

F



Supplementary Figure S5. Association of DS and/or flow cytometry MRD status on clinical outcomes.

Rates of non-relapse mortality (NRM, top left), relapse (top right), relapse-free survival (bottom left), and overall survival (bottom right) are shown by remission MRD status as determined by (A) DS and multiparametric flow cytometry (MFC) and (B) DS or MFC. Positive, pos; Negative, neg.



Supplementary Table S1. Duplex sequencing panel target regions

Gene	Accession #	Target Region (amino acid)	Exon
ASXL1	NM_015338	363-1542	12-13
CBL	NM_005188	366-477	8-9
CEBPA	NM_001287424	full CDS	1
DNMT3A	NM_022552	286-913	8-23
EZH2	NM_004456	87-208, 244-302, 503-752	4-6, 8, 13-20
FAM5C (BRINP3)	NM_199051	80-142, 396-767	3, 8
FLT3	NM_004119	569-647, 807-847	14-15, 20
GATA2	NM_032638	77-481	3-6
HNRNPK	NM_002140	21-85, 173-215, 319-336, 371-453	4-6, 10, 12, 15-16
IDH1	NM_005896	106-138	4 (partial)
IDH2	NM_002168	126-178	4
KIT	NM_000222	412-448, 788-828	8, 17
KRAS	NM_004985	1-96	2-3
MLL-X (KMT2A-X)	NM_005933 (*KMT2A*)	*MLL* intron 9	intron 9
MYH11-CBFB	NM_022844 (*MYH11*)	*MYH11* intron 30, exon 31	intron 30, exon 31
NPM1	NM_002520	258-282, 283-295	10-11
NRAS	NM_002524	1-96	2-3
PHF6	NM_032458	full CDS	2-10
PTEN	NM_001304717	258-337, 385-440	6, 8
PTPN11	NM_002834	47-110, 484-533	3, 13
RAD21	NM_006265	full CDS	2-14
RUNX1	NM_001754	full CDS	2-9
SMC1A	NM_006306	38-99, 447-515, 578-637, 687-732, 772-902, 1096-1145	2, 9, 11, 13, 15, 16-17, 22
SMC3	NM_005445	184-268, 365-435, 656-705, 882-1035, 1100-1158	9-10, 13, 19, 24-25, 27
STAG2	NM_001282418	42-128, 155-297, 436-472, 513-546, 578-675, 787-844, 892-1155	5-6, 8-10, 15, 17, 19-20, 25, 27-31
TET2	NM_001127208	full CDS	3-11
TP53	NM_000546	full CDS	2-11 + alt. exons
U2AF1	NM_006758	16-44	2
WT1	NM_024426	372-523	7-10

CDS, coding sequence

Supplementary Table S2. Patient clinical characteristics.

Covariate	Trial population	Achieved CR	MRD flow data available	Screened by NGS for molecular targets at diagnosis	Molecular target identified at diagnosis and DS data available
Number of patients	595	416	184	67	62
Randomized arm					
DA	300 (50%)	210 (50%)	89 (48%)	35(52%)	32 (52%)
DA+GO	295 (50%)	206 (50%)	95 (52%)	32 (48%)	30 (48%)
Age					
Median (range)	47 (18-60)	47 (18-60)	47 (18-60)	47 (18-60)	48 (18-60)
Sex					
Female	282 (47%)	193 (46%)	95 (52%)	30 (45%)	28 (45%)
Male	313 (53%)	224 (54%)	89 (48%)	37 (55%)	34 (55%)
Performance status					
0-1	518 (88%)	367 (89%)	155 (85%)	56 (84%)	58 (84%)
2-3	74 (12%)	47 (11%)	28 (15%)	11 (16%)	11 (16%)
Cytogenetic risk					
Favorable	77 (17%)	67 (21%)	34 (21%)	16 (26%)	13 (23%)
Intermediate	263 (58%)	196 (63%)	92 (58%)	31 (51%)	30 (54%)
Adverse	117 (26%)	50 (19%)	34 (21%)	14 (23%)	13 (23%)
Missing	138	93	24	6	6
WBC ($x10^3$)					
Median (range)	11.4 (0.2-545)	11.3 (0.2-545)	13.5 (0.2-214)	18.7 (0.2-214)	18.0 (0.2-214)
Platelets ($x10^3$)					
Median (range)	54 (2-9300)	54 (5-9300)	55 (5-9300)	44 (10-449)	48.5 (10-449)
Hemoglobin (g%)					
Median (range)	9.1 (3.5-29.1)	9.1 (3.5-28.5)	9.2 (3.5-28.5)	9.4 (3.5-13.6)	9.4 (3.5-13.6)
Race					
Asian	26 (4%)	12 (3%)	3 (2%)	1 (2%)	1 (2%)
Black	38 (6%)	24 (6%)	12 (7%)	4 (6%)	4 (6%)
Native American/Alaskan	6 (1%)	3 (1%)	3 (2%)	1 (2%)	1 (2%)
Pacific Islander	4 (1%)	2 (1%)	2 (1%)	0	0
White	497 (83%)	357 (86%)	156 (85%)	49 (88%)	54 (87%)
Unknown	24 (4%)	18 (4%)	8 (4%)	2 (3%)	2 (3%)

WBC, white blood cell count; DA, daunorubicin and cytarabine; GO, gemtuzumab ozogamicin; CR, complete remission; MRD, measurable residual disease; NGS, next generation sequencing; DS, duplex sequencing

Supplementary Table S3. Variants detected at diagnosis and remission.

Patient	Gene	Transcript ID	HGVSc	HGVSp	Chr	Position	Reference	Alternate	Consequence	Diagnostic VAF	Remission VAF	VAF log fold change
SAASZM	CEBPA	NM_001287424.1	c.494_495dupGC	p.Tyr166fs	chr19	33302024	A	AGC	frameshift	0.343	N/A	N/A
SAASZM	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.355	0.00010	-3.54
SAASZM	TET2	NM_001127208.2	c.715dupT	p.Ser239fs	chr4	105234664	G	GT	frameshift	0.340	0.01634	-1.32
SAASZM	TET2	NM_001127208.2	c.5440G>T	p.Gly1814*	chr4	105275950	G	T	stop gained	0.347	0.01643	-1.32
SAATEK	NRAS	NM_002524.4	c.183A>C	p.Gln61His	chr1	114713907	T	G	missense	0.286	N/A	N/A
SAATEK	NRAS	NM_002524.4	c.181C>A	p.Gln61Lys	chr1	114713909	G	T	missense	0.114	N/A	N/A
SAATEK	NRAS	NM_002524.4	c.38G>A	p.Gly13Asp	chr1	114716123	C	T	missense	0.108	N/A	N/A
SAATEK	RUNX1	NM_001754.4	c.1267C>T	p.Arg423Cys	chr21	34792311	G	A	missense	0.098	N/A	N/A
SAATFD	FLT3	NM_004119.2	c.1745_1831dupCCGGCTCCTCAGATAATGAGTACT TCTACGTTGATTTCAGAGAATTATGATCTCA ATGGGAGTTTCCAAGAGAAATTTAG	p.Leu610_Glu611insAlaGlySerSerAspAsnGluTyrPheT yrValAspPheArgGluTyrGluTyrAspLeuLysTrpGluPhePr oArgGluLeuLeu	chr13	28034087	T	TCTTAAATTTTCTCTTGGAAACTCC CATTTGAGATCATATTCATATTCTC TGAAATCAACGTAGAAGTACTCAT TATCTGAGCCGG	ITD	0.467	N/A	N/A
SAATFD	FLT3	NM_004119.2	c.1831_1832insCCGGCTCCTCATAATGAGTACTT CTACGTTGATTTCAGAGAATTATGATCTCAA ATGGGAGTTTCCAAGAGAAATTTAG	p.Leu610_Glu611insAlaGlySerSerTyrAsnGluTyrPheTy rValAspPheArgGluTyrGluTyrAspLeuLysTrpGluPheAsp ArgGluLeuLeu	chr13	28034087	T	TCTTAAATTTTCTCTTGGAAACTCC CATTTGAGATCATATTCATATTCTC TGAAATCAACGTAGAAGTACTCAT TATATGAGCCGG	ITD	0.441	N/A	N/A
SAATFD	IDH2	NM_002168.3	c.419G>A	p.Arg140Gln	chr15	90088702	C	T	missense	0.462	0.00976	-1.67
SAATFT	CBL	NM_005188.3	c.1360_1382delTGA	p.Asp454Glufs	chr11	119278648	TATG	-	deletion	0.054	N/A	N/A
SAATFT	CEBPA	NM_001287424.1	c.560C>G	p.Ala187Gly	chr19	33301990	G	C	missense	0.110	N/A	N/A
SAATGC	DNMT3A	NM_022552.4	c.2645G>A	p.Arg882His	chr2	25234373	C	T	missense	0.476	0.41760	-0.06
SAATGC	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.392	N/A	N/A
SAATGC	NRAS	NM_002524.4	c.38G>A	p.Gly13Asp	chr1	114716123	C	T	missense	0.469	N/A	N/A
SAATGG	CEBPA	NM_001287424.1	p.Asn342_Glu344delinsLys	p.Asn342_Glu344delinsLys	chr19	33301494	CTCCACG	C	deletion	0.424	N/A	N/A
SAATGG	CEBPA	NM_001287424.1	c.172_173dupCC	p.His59fs	chr19	33302346	C	CGG	frameshift	0.386	N/A	N/A
SAATGG	GATA2	NM_032638.4	c.961C>T	p.Leu321Phe	chr3	128483916	G	A	missense	0.423	0.00023	-3.26
SAATGG	NRAS	NM_002524.4	c.38G>A	p.Gly13Asp	chr1	114716123	C	T	missense	0.235	N/A	N/A
SAATHG	GATA2	NM_032638.4	c.1163_1165delTGA	p.Met388del	chr3	129481299	TTCA	-	deletion	N/A	0.15060	N/A
SAATHG	IDH2	NM_002168.3	c.419G>A	p.Arg140Gln	chr15	90088702	C	T	missense	0.389	0.00987	-1.60
SAATHI	DNMT3A	NM_022552.4	c.2644C>T	p.Arg882Cys	chr2	25234374	G	A	missense	0.411	0.27465	-0.18
SAATHI	FLT3	NM_004119.2	c.1751_1780dupCCTCAGATAATGAGTACTTCTACGT TGATT	p.Ser584_Asp593dup	chr13	28034138	A	AAATCAACGTAGAAGTACTCATTAT CTGAGG	ITD	0.017	0.00052	-1.50
SAATHI	FLT3	NM_004119.2	c.1812_1813insAATGAGTACTTCTACGTTGATTTCA GAGAATATGAATATGATCTCG	p.Phe605fs	chr13	28034106	A	ACGAGATCATTATTCATATTCTCTG AAATCAACGTAGAAGTACTCATT	frameshift	N/A	0.00262	N/A
SAATHI	FLT3	NM_004119.2	c.1721_1804dupGCCAGCTACAGATGGTACAGGTGA CCGGCTCCTCAGATAATGAGTACTTCTACGTTGAT TTCAGAGAATAATGAATATGATCTCA	p.Ser574_Leu601dup	chr13	28034114	T	TTGAGATCATTATTCATATTCTCTGA AATCAACGTAGAAGTACTCATTAT CTGAGGAGCCGGTCACCTGTACC ATCTGTAGCTGGC	ITD	N/A	0.00396	N/A
SAATHI	FLT3	NM_004119.2	c.1793_1794insCTACGTTGATTTCAGAGAATATGAA TATGATCTCAAATGGGAGAATGAGTACTTCTACGT TGATTTCAGAGAATATGAA	p.Tyr597_Glu598insAspTyrValAspPheArgGluTyrGluTy rAspLeuLysTrpGluAsnGluTyrPheTyrValAspPheArgGlu Tyr	chr13	28034125	T	TTCATATTCTCTGAAATCAACGTA GAAGTACTCATTCTCGAGGAGCC ATCATTACTATTCTCTGAAATCA ACGTAG	ITD	N/A	0.00083	N/A
SAATHJ	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.326	0.00437	-1.85
SAATHJ	EZH2	NM_004456.4	c.2230dupC	p.Glu744Glyfs	chr7	148807699	C	CG	frameshift	0.669	N/A	N/A
SAATHK	FLT3	NM_004119.2	c.1717_1794dupGAAAGCCAGCTACAGATGGTACAG GTGACCGGCTCCTCAGATAATGAGTACTTCTACGT TGATTTCAGAGAATATGAA	p.Glu598_Tyr599insGluSerGlnLeuGlnMetValGlnValThr GlySerSerAspAsnGluTyrPheTyrValAspPheArgGluTyr Glu	chr13	28034124	A	ATTCATATTCTCTGAAATCAACGTA GAAGTACTCATTCTCGAGGAGCC GGTCACCTGTACCATCTGTAGCT GGCTTTC	ITD	0.266	N/A	N/A
SAATHK	FLT3	NM_004119.2	c.1794_1795insGAAAGCCAGCTACAGATGGTACAG GTTACCGGCTCCTCAGATAATGAGTACTTCTACGT TGATTTCAGAGAATATGAA	p.Glu598_Tyr599insGluSerGlnLeuGlnMetValGlnValThr GlySerSerAspAsnGluTyrPheTyrValAspPheArgGluTyr Glu	chr13	28034124	A	ATTCATATTCTCTGAAATCAACGTA GAAGTACTCATTCTCGAGGAGCC GGTCACCTGTACCATCTGTAGCT GGCTTTC	ITD	0.243	N/A	N/A
SAATHK	FLT3	NM_004119.2	c.1794_1795insGAAAGCCAGCTACAGATGGTACAG GTGACCGGCTCCTCAGATAATGAGTACTTCTACGT TGATTTCAGAGAATAATGAATATGATCTCA ATTTCAGAGAATATGAA	p.Glu598_Tyr599insGluSerGlnLeuGlnMetValGlnValThr GlySerSerAspAsnGluTyrPheTyrValAspPheArgGluTyr AspGluTyrPheTyrValAspPheArgGlnTyrGlu	chr13	28034124	A	ATTCATATTCTCTGAAATCAACGTA GAAGTACTCATTCTCGAGGAGCC ATCAACGTAGAAGTACTCATTATC TCTGTAGCTGGCTTTC TTCATATTCTCTGAAATCAACGTA	ITD	0.170	N/A	N/A
SAATHK	FLT3	NM_004119.2	c.1761_1793dupTGAGTACTTCTACGTTGATTTCAGA GAATATGA	p.Tyr597_Glu598insAspGluTyrPheTyrValAspPheArgGl uTyr	chr13	28034125	T	GAAGTACTCA	ITD	0.114	N/A	N/A
SAATHK	IDH1	NM_005896.3	c.395G>A	p.Arg132His	chr2	209248388	C	T	missense	0.343	N/A	N/A
SAATHK	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.314	N/A	N/A
SAATHK	PTPN11	NM_002834.3	c.181G>T	p.Asp61Tyr	chr12	112450361	G	T	missense	0.217	N/A	N/A
SAATHK	RAD21	NM_006265.2	c.688+1G>T	p.Asp454Asn	chr8	116857266	C	A	splice donor	0.348	N/A	N/A
SAATHK	WT1	NM_024426.4	c.1360G>A	p.Asp454Asn	chr11	32392014	C	T	missense	0.477	N/A	N/A
SAATHT	NRAS	NM_002524.4	c.34G>T	p.Gly12Cys	chr1	114716127	C	A	missense	0.221	N/A	N/A
SAATHW	FLT3	NM_004119.2	c.1796_1810dupATGATCTCAAATCGGAGTT	p.Tyr599_Glu604dup	chr13	28034105	A	AACTCCCATTTGAGATCAT	ITD	0.197	N/A	N/A
SAATHW	FLT3	NM_004119.2	c.1745_1786dupCCGGCTCCTCAGATAATGAGTACT TCTACGTTGATTTCAGAG	p.Arg595_Glu596insAlaGlySerSerAspAsnGluTyrPheTy rValAspPheArg	chr13	28034132	T	TCTCTGAAATCAACGTAGAAGTAC TCATTATCTGAGGAGCCGG	ITD	0.163	N/A	N/A
SAATHW	IDH2	NM_002168.3	c.419G>A	p.Arg140Gln	chr15	90088702	C	T	missense	0.432	N/A	N/A
SAATHW	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.314	N/A	N/A
SAATKY	NPM1	NM_004985.4	c.183A>C	p.Gln61His	chr12	25227341	T	G	missense	0.380	N/A	N/A
SAATKY	NPM1	NM_002520.6	c.863_864insCATG	p.Trp288fs	chr5	171410541	C	CTGCA	frameshift	0.320	N/A	N/A
SAATKY	RAD21	NM_006265.2	c.1193delC	p.Pro398fs	chr8	116852677	TG	T	frameshift	0.058	N/A	N/A
SAATKY	RAD21	NM_006265.2	c.1191_1192delACmsG	p.Pro398fs	chr8	116852679	GT	C	frameshift	0.232	N/A	N/A
SAATLN	CBL	NM_005188.3	c.1380_1382delTGA	p.Asp460del	chr11	119278648	TATG	-	deletion	0.053	N/A	N/A
SAATLN	FLT3	NM_004119.2	c.2503G>T	p.Asp835Tyr	chr13	28018505	C	A	missense	0.128	N/A	N/A
SAATNC	NRAS	NM_002524.4	c.34G>T	p.Gly12Cys	chr1	114716127	C	A	missense	0.313	0.00025	-3.10
SAATNC	WT1	NM_024426.4	c.1142C>A	p.Ser381*	chr11	32396364	G	T	stop gained	0.369	0.00110	-2.52

Patient	Gene	Transcript ID	HGVSc	HGVSp	Chr	Position	Reference	Alternate	Consequence	Diagnostic VAF	Remission VAF	VAF log fold change
SAATND	CBL	NM_005188.3	c.1096-25_1228-103del		chr11	119278405	ATAGTCTTTTAATTTTTTTTAA TCAAAGGAACAATATGAATTA TACTGTGAGATGGGCTCCAC ATTCCAACTATGTAAAATATG TGCTGAAAATGATAAGGATG TAAAGATTGAGCCCTGTGGA CACCTCATGTGCACATCCTG TCTTACATCCTGGCAGGTAC GGATCTAAACAGCGACTTTTT TCAGCTATGTAATAACCTTGG AAAATTCGGTATTATATAGCC TTTACTGATACAAGGGGTGG CCTGGCTTTTGGGGTTAGGT	T	A	exon loss	0.466	N/A	N/A
SAATND	CBL	NM_005188.3	c.1096-8_1228-86del		chr11	119278416	ATTTTTTTTAATCAAAGGAAC AATATGAATTATACTGTGAGA TGGGCTCCACATTCCAACTA TGTAAAATATGTGCTGAAAAT GATAAGGATGTAAGATTGA GCCCTGTGGACACCTCATGT GCACATCCTGTCTTACATCCT GGCAGGTACGGATCTAAACA GCGACTTTTTTCAGCTATGTA ATAACCTTGGAAAATTCGGTA TTATATAGCCTTTACTGATAC AAGGGGTGGCCTGGCTTTT GGGGTTAGGTTTAAACTTTT	AC	A	exon loss	0.130	N/A	N/A
SAATRF	CEBPA	NM_001287424.1	c.1039_1041dupCAG	p.Gln347dup	chr19	33301478	T	TCTG	insertion	0.311	N/A	N/A
SAATRF	CEBPA	NM_001287424.1	c.305_308delACAT	p.Tyr102fs	chr19	33302215	GATGT	G	frameshift	0.327	N/A	N/A
SAATRF	RUNX1	NM_001754.4	c.1268G>A	p.Arg423His	chr21	34792310	C	T	missense	0.057	N/A	N/A
SAATRF	WT1	NM_024426.4	c.1224dupA	p.Gln409fs	chr11	32396281	G	GT	frameshift	0.305	N/A	N/A
SAATTF	FLT3	NM_004119.2	c.1719_1778dupAAGCCAGCTACAGATGGTACAGGT GACCGGCTCCTCAGATAATGAGTACTTCTACGTTG A	p.Glu573_Val592dup	chr13	28034140	A	ATCAACGTAGAAGTACTCATTATC TGAGGAGCCGGTCACCTGTACCA TCTGTAGCTGGCTT	ITD	0.419	N/A	N/A
SAATTF	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.395	N/A	N/A
SAATTF	TET2	NM_001127208.2	c.3629T>C	p.Leu1210Pro	chr4	105243604	T	C	missense	0.499	N/A	N/A
SAATTF	TET2	NM_001127208.2	c.5541G>A	p.Trp1847*	chr4	105276051	G	A	stop gained	0.466	N/A	N/A
SAATTL	FLT3	NM_004119.2	c.2503G>C	p.Asp835His	chr13	28018505	C	G	missense	0.326	N/A	N/A
SAATTL	STAG2	NM_001282418.1	c.819+2_819+4delTAAinsAGAAGGGGC		chrX	124047509	TAA	AGAAGGGGC	splice donor	0.180	N/A	N/A
SAATTL	STAG2	NM_001282418.1	c.819+6_819+8delCTTinsTTG		chrX	124047513	CTT	TTG	splice region	0.276	N/A	N/A
SAATTL	WT1	NM_024426.4	c.1385G>C	p.Arg462Pro	chr11	32392019	C	G	missense	0.382	N/A	N/A
SAATTR	PHF6	NM_032458.2	c.799_800insGA	p.Thr267fs	chrX	134415084	A	AAG	frameshift	0.079	N/A	N/A
SAATTR	WT1	NM_024426.4	c.1385G>A	p.Arg462Gln	chr11	32392019	C	T	missense	0.347	0.00445	-1.89
SAATVB	DNMT3A	NM_022552.4	c.2644C>A	p.Arg882Ser	chr2	25234374	G	T	missense	0.372	0.00630	-1.77
SAATVB	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.314	N/A	N/A
SAATVX	FLT3	NM_004119.2	c.2503G>T	p.Asp835Tyr	chr13	28018505	C	A	missense	0.505	0.00017	-3.48
SAATWX	DNMT3A	NM_022552.4	c.2644C>T	p.Arg882Cys	chr2	25234374	G	A	missense	0.456	0.00183	-2.40
SAATWX	KRAS	NM_004985.4	c.164_181dupTTCTCGACACAGCAGGTC	p.Gly60_Gln61insLeuLeuAspThrAlaGly	chr12	25227342	T	TGACCTGCTGTGTCGAGAA	insertion	0.295	N/A	N/A
SAATWX	KRAS	NM_004985.4	c.175_176insTAGGTCTTCTCGACACAG	p.Thr58_Ala59insValGlyLeuLeuAspThr	chr12	25227348	G	GCTGTGTCGAGAAGACCTA	insertion	0.082	N/A	N/A
SAATWX	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.293	0.00006	-3.70
SAATWX	TET2	NM_001127208.2	c.1659delG	p.Pro555fs	chr4	105235601	TG	T	frameshift	0.462	0.00238	-2.29
SAATYG	EZH2	NM_004456.4	c.2226_2227insAAT	p.Val742_Gly743insAsn	chr7	148807675	C	CATT	insertion	0.815	0.00009	-3.96
SAATYG	FLT3	NM_004119.2	c.2504A>T	p.Asp835Val	chr13	28018504	T	A	missense	0.182	N/A	N/A
SAATYG	FLT3	NM_004119.2	c.1821_1822insTTCCCTAATGAATATGATCTCAAATG GGAGTTTCCAAGA	p.Arg607_Glu608insPheProAsnGluTyrAspLeuLysTrpGluPhePr oArg	chr13	28034097	C	CTCTTGGAAACTCCATTTGAGAT CATATTCATTAGGGAA	ITD	0.207	N/A	N/A
SAATYG	TP53	NM_000546.5	c.711G>C	p.Met237Ile	chr17	7674252	C	G	missense	N/A	0.01087	N/A
SAATYG	U2AF1	NM_006758.2.2	c.101C>A	p.Ser34Tyr	chr21	43104346	G	T	missense	0.489	0.00543	-1.95
SAATYL	CBL	NM_005188.3	c.1259G>A	p.Arg420Gln	chr11	119278541	G	A	missense	N/A	0.00155	N/A
SAATYL	DNMT3A	NM_022552.4	c.2645G>A	p.Arg882His	chr2	25234373	C	T	missense	0.233	0.00014	-3.21
SAATYL	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.199	N/A	N/A
SAATZW	TP53	NM_000546.5	c.844C>T	p.Arg282Trp	chr17	7673776	G	A	missense	0.647	0.13124	-0.69
SAAUBG	EZH2	NM_004456.4	c.2187dupT	p.Asp730fs	chr7	148809078	C	CA	frameshift	0.380	0.00011	-3.55
SAAUBG	KIT	NM_000222.2	c.2447A>T	p.Asp816Val	chr4	54733155	A	T	missense	0.058	0.00007	-2.92
SAAUBG	RAD21	NM_006265.2	c.2T>C	p.Met1?	chr8	116866728	A	G	start lost	0.055	N/A	N/A
SAAUBK	NRAS	NM_002524.4	c.34G>A	p.Gly12Ser	chr1	114716127	C	T	missense	0.195	N/A	N/A
SAAUBW	FLT3	NM_004119.2	c.1770_1829dupCTACGTTGATTTCAGAGAATATGAA TATGATCTCAAATGGGAGTTTCCAAGAGAAAATTT	p.Phe590_Asn609dup	chr13	28034089	T	TAAATTTTCTCTTGGAAACTCCCAT TTGAGATCATATTCATATTCTCTGA AATCAACGTAG	ITD	0.399	0.00034	-3.07
SAAUBW	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.387	0.00035	-3.05
SAAUBW	SMC1A	NM_006306.3	c.1880G>T	p.Arg627Leu	chrX	53405524	C	A	missense	N/A	0.00126	N/A
SAAUBW	TET2	NM_001127208.2	c.4097G>A	p.Arg1366His	chr4	105269662	G	A	missense	0.502	0.02946	-1.23
SAAUBZ	IDH2	NM_002168.3	c.418C>T	p.Arg140Trp	chr15	90088703	G	A	missense	0.404	0.00012	-3.54
SAAUCC	FLT3	NM_004119.2	c.1786_1866dupGAATATGAATATGATCTCAAATGGG AGTTTCCAAGAGAAAATTTAGAGTTTGGTAAGAAT GGAATGTGCCAAATGTTTCTGCAGCATTTCTTTTC CATTGGAAAATCTTTAAAATGCACGTACTCACCATT TGTCTTTGCAGGGAAGGTACTAGGATCAGGTGCT TTTGGA	p.Gly622_Lys623insGluTyrGluTyrAspLeuLysTrpGluP heProArgGluAsnLeuGluPheGlyLysAsnGlyMetCysGln MetPheLeuGlnHisPhePheSerIleGlyLysSerLeuLysCysT hrTyrSerProPheValPheAlaGlyLysValLeuGlySerGlyAla PheGly	chr13	28033962	T	TTCCAAAAGCACCTGATCCTAGTA CCTTCCCTGCAAAGACAAATGGTG AGTACGTGCATTTTAAAGATTTTC CAATGGAAAGAAATGCTGCAGAA ACATTTGGCACATTCCATTCTTAC CAAACTCTAAATTTTCTCTTGGAAA CTCCCATTTGAGATCATATTCATAT TC	ITD	0.204	0.00044	-2.66
SAAUCC	FLT3	NM_004119.2	c.1773_1799dupCGTTGATTTCAGAGAATATGAATAT GA	p.Asp600_Leu601insValAspPheArgGluTyrGluTyrAsp	chr13	28034119	A	ATCATATTCATATTCTCTGAAATCA ACG	ITD	0.078	0.00025	-2.49
SAAUCC	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.348	0.02134	-1.21
SAAUCC	NRAS	NM_002524.4	c.34G>T	p.Gly12Cys	chr1	114716127	C	A	missense	0.300	0.00043	-2.85
SAAUCC	PTPN11	NM_002834.3	c.1530G>C	p.Gln510His	chr12	112489106	G	C	missense	0.059	0.01061	-0.74
SAAUCG	IDH1	NM_005896.3	c.395G>A	p.Arg132His	chr2	208248388	C	T	missense	0.089	0.00723	-1.09
SAAUCI	KIT	NM_000222.2	c.2466T>G	p.Asn822Lys	chr4	54733174	T	G	missense	0.100	0.00007	-3.18
SAAUCI	KRAS	NM_004985.4	c.38_39delGCinsAT	p.Gly13Asp	chr12	25245347	GC	AT	missense	0.118	0.00047	-2.40
SAAUCK	ASXL1	NM_015338.5	c.1774C>T	p.Gln592*	chr20	32434486	C	T	stop gained	0.125	N/A	N/A
SAAUCL	KIT	NM_000222.2	c.2447A>T	p.Asp816Val	chr4	54733155	A	T	missense	0.375	0.00048	-2.89
SAAUCP	DNMT3A	NM_022552.4	c.2645G>A	p.Arg882His	chr2	25234373	C	T	missense	0.251	0.09516	-0.42
SAAUCP	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.153	0.00064	-2.38

Patient	Gene	Transcript ID	HGVSc	HGVSp	Chr	Position	Reference	Alternate	Consequence	Diagnostic VAF	Remission VAF	VAF log fold change
SAAUCP	NRAS	NM_002524.4	c.182A>G	p.Gln61Arg	chr1	114713908	T	C	missense	0.187	0.00043	-2.64
SAAUCP	NRAS	NM_002524.4	c.35G>A	p.Gly12Asp	chr1	114716126	C	T	missense	0.117	N/A	N/A
SAAUCP	SMC1A	NM_006306.3	c.2141A>G	p.Tyr714Cys	chrX	53405067	T	C	missense	N/A	0.00209	N/A
SAAUCW	FLT3	NM_004119.2	c.1837+1_1837+2insAAGGGTTTTCAGAGAATATGAATATGATCTCAAATGGGAGTTTCCAAGAGAAAATTTAGAGTTTGG		chr13	28034080	A	ACCAAACTCTAAATTTTCTCTTGGAAACTCCCATTTGAGATCATATTCATATTCTCTGAAAACCCTT	ITD	0.421	N/A	N/A
SAAUCW	PHF6	NM_032458.2	c.27dupA	p.Gly10fs	chrX	134377638	G	GA	frameshift	0.836	N/A	N/A
SAAUCW	RUNX1	NM_001754.4	c.1268_1276delGCTCGCCGCinsCACCCCG	p.Arg423fs	chr21	34792310	GCGGCGAGC	CGGGGTG	frameshift	0.460	N/A	N/A
SAAUDH	DNMT3A	NM_022552.4	c.2645G>A	p.Arg882His	chr2	25234373	C	T	missense	0.448	0.33074	-0.13
SAAUDH	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.331	0.00005	-3.81
SAAUDH	PTPN11	NM_002834.3	c.1508G>T	p.Gly503Val	chr12	112489084	G	T	missense	0.425	N/A	N/A
SAAUDW	DNMT3A	NM_022552.4	c.1154delC	p.Pro385fs	chr2	25246745	CG	C	frameshift	0.408	0.00174	-2.37
SAAUDW	IDH1	NM_005896.3	c.395G>A	p.Arg132His	chr2	208248388	C	T	missense	0.443	0.00019	-3.36
SAAUDW	NPM1	NM_002520.6	c.863_864insCTTG	p.Trp288fs	chr5	171410541	C	CTGCT	frameshift	0.426	0.00018	-3.37
SAAUDW	PTPN11	NM_002834.3	c.179G>T	p.Gly60Val	chr12	112450359	G	T	missense	0.418	0.00029	-3.16
SAAUDW	RAD21	NM_006265.2	c.1660_1663delAGAA	p.Arg554fs	chr8	116848990	CTTCT	C	frameshift	0.395	0.00057	-2.84
SAAUED	RAD21	NM_006265.2	c.1818_1820delGCA	p.Gln607del	chr8	116847578	TTGC	T	deletion	0.415	N/A	N/A
SAAUEE	FLT3	NM_004119.2	c.1780_1800dupTTCAGAGAATATGAATATGAT	p.Asp600_Leu601insPheArgGluTyrGluTyrAsp	chr13	28034118	G	GATCATATTCATATTCTCTGAA	ITD	0.290	0.00053	-2.74
SAAUEE	FLT3	NM_004119.2	c.1778_1779insCTTCAGAGAATATGAATATGA	p.Asp593_Phe594insPheArgGluTyrGluTyrAsp	chr13	28034140	A	ATCATATTCATATTCTCTGAAG	ITD	0.080	N/A	N/A
SAAUEE	IDH2	NM_002168.3	c.419G>A	p.Arg140Gln	chr15	90088702	C	T	missense	0.436	0.02294	-1.28
SAAUEE	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.398	0.01258	-1.50
SAAUEG	ASXL1	NM_015338.5	c.3031delG	p.Glu1011fs	chr20	32435742	CG	C	frameshift	0.075	N/A	N/A
SAAUEG	PHF6	NM_032458.2	c.319delT	p.Cys107fs	chrX	134393579	T	<DEL>	frameshift	N/A	0.02163	N/A
SAAUEG	WT1	NM_024426.4	c.1369_1405delCAGCGAAAGTTCTCCCGGTCCGACCACCTGAAGACCCinsTATTTTTTTTTTATGTTTCGGAGAAACATGTAGATCT	p.GlnArgLysPheSerArgSerAspHisLeuLysThrHis457*	chr11	32392035	GGGTCTTCAGGTGGTCGGAGCGGGAGAACTTTCGCTG	AGATCTACATGTTCTCCGAAACATAAAAAAAAAATA	stop gained	0.052	N/A	N/A
SAAUEL	CEBPA	NM_001287424.1	c.1049_1050insCAC	p.Leu350_Glu351insThr	chr19	33301470	C	CGTG	insertion	0.423	N/A	N/A
SAAUEL	CEBPA	NM_001287424.1	c.440_441dupCC	p.Ala148fs	chr19	33302078	C	CGG	frameshift	0.358	N/A	N/A
SAAUEL	FLT3	NM_004119.2	c.1730_1801dupAGATGGTACAGGTGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTCAGAGAATATGAATATGATC	p.Gln577_Asp600dup	chr13	28034117	A	AGATCATATTCATATTCTCTGAAATCAACGTAGAAGTACTCATTATCTGAGGAGCCGGTCACCTGTACCATCT	ITD	0.487	N/A	N/A
SAAUEL	NRAS	NM_002524.4	c.35G>A	p.Gly12Asp	chr1	114716126	C	T	missense	0.052	N/A	N/A
SAAUEL	RAD21	NM_006265.2	c.193C>T	p.Arg65*	chr8	116863211	G	A	stop gained	0.426	0.00016	-3.44
SAAUEY	KIT	NM_000222.2	c.1248_1262delGACTTACGACAGGCTinsCCGGTCTTA	p.Thr417_Leu421delinsArgSerTyr	chr4	54723614	GACTTACGACAGGCT	CCGGTCTTA	indel	0.373	0.00061	-2.79
SAAUEY	WT1	NM_024426.4	c.1107delAinsGG	p.Arg370fs	chr11	32396399	T	CC	frameshift	0.407	0.00028	-3.17
SAAUFB	FLT3	NM_004119.2	c.1788_1789insGTTAATGAGTACTTCTACGTTGATTTCAGAGAA	p.Glu596_Tyr597insValAsnGluTyrPheTyrValAspPheArgGlu	chr13	28034130	A	ATTCTCTGAAATCAACGTAGAAGTACTCATTAAC	ITD	0.459	0.00009	-3.69
SAAUFB	WT1	NM_024426.4	c.1141_1144dupTCGG	p.Ala382fs	chr11	32396361	G	GCCGA	frameshift	0.406	0.00006	-3.86
SAAUFE	CBL	NM_005188.3	c.1137_1147delACTATGTAAAA	p.Gln379fs	chr11	119278216	CAACTATGTAAA	C	frameshift	N/A	0.00101	N/A
SAAUFE	DNMT3A	NM_022552.4	c.2645G>A	p.Arg882His	chr2	25234373	C	T	missense	0.455	0.26550	-0.23
SAAUFE	IDH1	NM_005896.3	c.394C>T	p.Arg132Cys	chr2	208248389	G	A	missense	0.548	0.08209	-0.82
SAAUFE	RUNX1	NM_001754.4	c.958C>T	p.Arg320*	chr21	34799310	G	A	stop gained	N/A	0.00156	N/A
SAAUFF	FLT3	NM_004119.2	c.1786_1824dupGAATATGAATATGATCTCAAATGGGAGTTTCCAAGAGAA	p.Glu608_Asn609insGluTyrGluTyrAspLeuLysTrpGluProArgGlu	chr13	28034094	T	TTTCTCTTGGAAACTCCCATTTGAGATCATTCATATTC	ITD	0.240	0.00016	-3.17
SAAUFN	NRAS	NM_002524.4	c.35G>A	p.Gly12Asp	chr1	114716126	C	T	missense	0.421	0.00020	-3.33
SAAUFU	NRAS	NM_002524.4	c.35G>A	p.Gly12Asp	chr1	114716126	C	T	missense	0.350	0.00098	-2.55
SAAUFU	GATA2	NM_032638.4	c.1203_1233delCAACAAGTCCAAGAAGAGCAAGAAAGGGGCGinsGGCA	p.Asn402_Gly410del	chr13	128481259	CGCCCCTTTCTTGCTCTTCTTGGACTTGTTG	TGCC	indel	N/A	0.01450	N/A
SAAUFV	DNMT3A	NM_022552.4	c.2204A>G	p.Tyr735Cys	chr2	25240420	T	C	missense	0.397	0.00444	-1.95
SAAUFV	DNMT3A	NM_022552.4	c.976C>T	p.Arg326Cys	chr2	25247629	G	A	missense	0.369	0.16054	-0.36
SAAUFV	KIT	NM_000222.2	c.2447A>T	p.Asp816Val	chr4	54733155	A	T	missense	0.355	N/A	N/A
SAAUGM	FLT3	NM_004119.2	c.2503G>T	p.Asp835Tyr	chr13	28018505	C	A	missense	0.432	N/A	N/A
SAAUHE	ASXL1	NM_015338.5	c.1900_1922delAGAGAGGCGGCCACCACTGCCAT	p.Glu635fs	chr20	32434622	TCACCACTGCCATAGAGAGGCGGC	T	frameshift	0.133	N/A	N/A
SAAUHE	CBL	NM_005188.3	c.1380_1382delTGA	p.Asp460del	chr11	119278648	TATG	T	deletion	0.052	N/A	N/A
SAAUHE	CEBPA	NM_001287424.1	c.560C>G	p.Ala187Gly	chr19	33301960	G	C	missense	0.087	N/A	N/A
SAAUHE	KIT	NM_000222.2	c.2466T>A	p.Asn822Lys	chr4	54733174	T	A	missense	0.182	N/A	N/A
SAAUHE	KIT	NM_000222.2	c.2466T>G	p.Asn822Lys	chr4	54733174	T	G	missense	0.160	N/A	N/A
SAAUHH	DNMT3A	NM_022552.4	c.2654G>A	p.Arg885Lys	chr2	25234364	C	T	missense	0.459	0.00023	-3.31
SAAUHH	FLT3	NM_004119.2	c.2523C>A	p.Asn841Lys	chr13	28018485	G	T	missense	0.145	N/A	N/A
SAAUHH	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.314	0.00019	-3.21
SAAUHH	PTPN11	NM_002834.3	c.182A>T	p.Asp61Val	chr12	112450362	A	T	missense	0.263	N/A	N/A
SAAUHY	DNMT3A	NM_022552.4	c.2645G>A	p.Arg882His	chr2	25234373	C	T	missense	0.535	0.06667	-0.90
SAAUHY	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.368	0.01162	-1.50
SAAUHY	NRAS	NM_002524.4	c.182A>G	p.Gln61Arg	chr1	114713908	T	C	missense	0.379	0.00074	-2.71
SAAUHY	SMC1A	NM_006306.3	c.1468G>A	p.Asp490Asn	chrX	53409139	C	T	missense	0.915	0.03895	-1.37
SAAUKF	KRAS	NM_004985.4	c.35G>A	p.Gly12Asp	chr12	25245350	C	T	missense	0.025	0.00135	-1.28
SAAUKF	KRAS	NM_004985.4	c.34G>A	p.Gly12Ser	chr12	25245351	C	T	missense	0.252	0.01180	-1.33
SAAUKF	TP53	NM_000546.5	c.499_500delCA	p.Gln167fs	chr17	7675113	CTG	C	frameshift	0.675	0.00986	-1.84
SAAVFE	DNMT3A	NM_022552.4	c.2645G>A	p.Arg882His	chr2	25234373	C	T	missense	0.086	0.00008	-3.01
SAAVFE	FLT3	NM_004119.2	c.1837+3_1837+4insCCCTTCTCAGAGAATATGAATATGATCTCAAATGGGAGTTTCCAAGAGAAAATTTAGAGTTTGGTA		chr13	28034078	T	TTACCAAACTCTAAATTTTCTCTTGGAAACTCCCATTTGAGATCATATTCATATTCTCTGAGAAGGG	ITD	0.615	0.00011	-3.74
SAAVFE	FLT3	NM_004119.2	c.1837+3_1837+4insCCCTTCTCAGAGAATATGCATATGATCTCAAATGGGAGTTTCCAAGAGAAAATTTAGAGTTTGGTA		chr13	28034078	T	TTACCAAACTCTAAATTTTCTCTTGGAAACTCCCATTTGAGATCATATGCATATTCTCTGAGAAGGG	ITD	0.467	N/A	N/A
SAAVFE	RUNX1	NM_001754.4	c.1267C>T	p.Arg423Cys	chr21	34792311	G	A	missense	0.158	N/A	N/A
SAAVFE	STAG2	NM_001282418.1	c.3397C>T	p.Arg1133Trp	chrX	124090694	C	T	missense	0.154	N/A	N/A
SAAVHJ	RUNX1	NM_001754.4	c.501T>G	p.Ser167Arg	chr21	34880564	A	C	missense	0.450	0.01486	-1.48
SAAVID	DNMT3A	NM_022552.4	c.2644C>T	p.Arg882Cys	chr2	25234374	G	A	missense	0.219	0.01658	-1.12
SAAVID	IDH2	NM_002168.3	c.419G>A	p.Arg140Gln	chr15	90088702	C	T	missense	0.164	0.01047	-1.20
SAAVID	RUNX1	NM_001754.4	c.485G>A	p.Arg162Lys	chr21	34880580	C	T	missense	0.211	0.00031	-2.83
SAAVJA	CEBPA	NM_001287424.1	c.1040_1041insCCGCGACAAGGCCAAGCAGCGCAACGTGGAGACGCCGCA	p.Gln346_Gln347insHisArgAspLysAlaLysGlnArgAsnValGluThrPro	chr19	33301479	C	CTGCGGCGTCTCCACGTTGCGCTGCTTGGCCTTGTCGCGG	insertion	0.149	N/A	N/A
SAAVJA	CEBPA	NM_001287424.1	c.222delC	p.Ala75fs	chr19	33302298	CG	C	frameshift	0.262	N/A	N/A
SAAVJA	GATA2	NM_032638.4	c.923G>C	p.Arg308Pro	chr3	128483954	C	G	missense	0.344	0.00010	-3.53
SAAVJP	DNMT3A	NM_022552.4	c.2645G>A	p.Arg882His	chr2	25234373	C	T	missense	0.430	0.02000	-1.33
SAAVJP	FLT3	NM_004119.2	c.1779_1793dupTTTCAGAGAATATGA	p.Asp593_Tyr597dup	chr13	28034125	T	TTCATATTCTCTGAAA	ITD	0.415	0.00007	-3.80
SAAVJP	NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288fs	chr5	171410539	C	CTCTG	frameshift	0.294	N/A	N/A

Supplementary Table S4. Flow cytometry MRD.

Patient	Flow MRD (%)
SAATHG	0.28
SAATLN	0.002
SAATTR	0.6
SAAUCW	0.01
SAAUEE	2.3
SAAUEG	0.21
SAAUFE	6.2
SAAUFU	0.01
SAAUKF	0.2
SAAVJP	0.85

Supplementary Table S5. Univariate cox regression analysis for clinical outcomes based on various duplex sequencing MRD definitions.

	Relapse		Relapse-Free Survival		Overall Survival	
MRD Definition	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
DS - any RDV	2.8 (0.8-9.4)	0.11	1.6 (0.7-4)	0.3	1.5 (0.6-4.2)	0.39
DS - RDV with VAF ≥0.1%	4.7 (1.8-12.3)	0.0016	3.8 (1.7-8.5)	0.0014	3.2 (1.3-7.7)	0.0088
DS - RDV with VAF ≥0.1%, no DTA	5.5 (2.3-13.3)	<0.001	4.6 (2.1-10)	<0.001	4.8 (2-11.1)	<0.001
DS - RDV ≤2 $\log^{10}$ reduction in VAF between diagnosis and remission	3.3 (1.4-8.2)	0.0086	2.6 (1.2-5.6)	0.017	2.1 (0.9-4.8)	0.089
DS - RDV ≤2 $\log^{10}$ reduction in VAF between diagnosis and remission, no DTA	4.5 (1.9-11)	<0.001	4 (1.8-8.7)	<0.001	4.3 (1.8-10.1)	<0.001
DS - VAF ≥0.1% or ≥0.01% for *NPM1/FLT3*-ITD, no DTA, agnostic to diagnosis	8.7 (2.9-26.1)	<0.001	4.8 (2.1-11.1)	<0.001	5.4 (2.1-13.8)	<0.001

MRD, measurable residual disease; HR, hazard ratio; CI, confidence interval; DS, duplex sequencing; RDV, residual diagnostic variant; VAF, variant allele frequency; DTA, *DNMT3A TET2 ASXL1*