

## Supplementary Appendix

### Measurable Residual *FLT3-ITD* before Allogeneic Transplant for Acute Myeloid Leukemia

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## **eMethods**

### **Clinical cohort**

This cohort study included patients aged  $\geq 18$  from the Pre-MEASURE study<sup>1</sup> who received alloHCT for *FLT3*-ITD mutated AML during CR1 at Center for International Blood and Bone Marrow Transplant Research (CIBMTR) reporting sites in the US between 2013-2019. Patients provided written informed consent for participation in the National Marrow Donor Program institutional review board-approved CIBMTR database (NCT01166009) and repository (NCT04920474) protocols. Research was performed in compliance with all applicable federal regulations pertaining to the protection of human research participants and with approval of the CIBMTR observational research group. STROBE reporting guidelines were followed. Race and ethnicity data were collected by CIBMTR as pre-transplant essential data and were based on patient self-report extracted from the medical record by transplant center data managers in categories based on the US Office of Management and Budget's Revisions to the Standards for the Classification of Federal Data on Race and Ethnicity.

### **Samples and DNA Isolation**

Post-transplant, pre-conditioning baseline whole blood samples from *FLT3*-ITD mutated AML patients from the Pre-MEASURE study were collected within 100 days prior to transplant and high-quality genomic DNA (gDNA) extracted as previously described.<sup>1</sup> Patients were eligible for re-analysis in this study if they had at least 700ng of gDNA remaining and DNA concentration  $\geq 20$ ng/uL.

### ***FLT3*-ITD targeted next-generation sequencing (NGS)**

*FLT3*-ITD mutations were detected using the Invivoscribe *FLT3*-ITD MRD assay (Invivoscribe Inc., San Diego, CA) following manufacturer's instructions. In short, 700ng of gDNA was amplified

by polymerase chain reaction on a Mastercycler X50a (Eppendorf AG, Hamburg, Germany) using oligonucleotides designed with Illumina adapters containing unique molecular indices targeting the exon 14/15 region of the *FLT3* gene. Following PCR amplification, the PCR products were subjected to two rounds of cleanup using Ampure XP reagent (Beckman Coulter Inc., Brea, CA) on a Zephyr G3 NGS Workstation (PerkinElmer Health Sciences, Inc., Shelton, CT). Samples were processed in batches of 24, including 21 patient samples, a positive control, a negative control, and a no-template control (NTC). Resulting libraries were subjected to quality control evaluation using the D1000 screentape assay on the TapeStation 4200 instrument (Agilent Technologies). A run passed if patient samples and positive/negative controls had peaks between 200-1000bp with a concentration  $>1.0$  ng/uL and  $<1.0$ ng/uL for NTC.

Equal molar quantities of each library were pooled and subjected to paired-end 300-bp sequencing on the MiSeq instrument (Illumina). A sequencing run passed if the MiSeq™ Cluster Density was  $\geq 500$  K/mm<sup>2</sup>, the total MiSeq™ Run Q30 Score was  $\geq 60\%$ , and at least 10 million MiSeq™ reads passed filter.

Data analysis was performed on demultiplexed FASTQ files using the *FLT3* ITD MRD v1.2 Software (Invivoscribe, Inc.) using the NIH HPC Biowulf cluster (<http://hpc.nih.gov>). If the positive control, negative control, or NTC samples did not pass, the protocol was repeated from the beginning for all samples. If any sample was not evaluable, the protocol was repeated from the beginning for that sample.

For assay validation, 21 competency standards were created by Invivoscribe, Inc. consisting of serial dilutions of *FLT3*-ITD cell lines and patient sample positive controls with a size range of ITDs ranging from 30bp to 171bp and targeted variant allele fractions (VAFs) ranging from 5% to 0.0001%. Competency standards were analyzed in singlet at both the National Institutes of Health

laboratory and the Invivoscribe, Inc. laboratory. All samples above the validated LOD of 0.005% VAF were confirmed by both laboratories, with highly consistent results (eFigure 1).

### **Duplex Sequencing**

For the 48 patients that tested negative using the Invivoscribe *FLT3*-ITD MRD assay but experienced relapse within the first year after transplant and did not have a detectable *NPM1* mutation using the previously published anchored multiplex PCR (AMP) NGS assay<sup>1</sup>, targeted NGS was performed on pre-transplant blood DNA utilizing a custom error-corrected duplex sequencing assay (TwinStrand Biosciences, Inc., Seattle, WA) targeting 15 AML-associated genes (eTable 4). Briefly, 500ng gDNA was enzymatically fragmented. End repair, A-tailing and DuplexSeq™ 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 generated using pre- and post-PCR separation on the Sciclone G3 NGS workstation and Zephyr G3 NGS workstation (PerkinElmer Health Sciences, Inc., Shelton, CT).

The resulting libraries were subjected to paired-end 150-bp sequencing on a NovaSeq 6000 (Illumina, San Diego, CA), according to manufacturer's instructions. Libraries were pooled for sequencing such that each sample had a unique dual index.

Raw sequencing FASTQ files were analyzed using the TwinStrand DuplexSeq™ FASTQ to VCF Parallel App version 4.1.0 per manufacturer instructions. Resulting variant calls with a VAF  $\geq$  0.01% were filtered to identify residual variants predicted to have a deleterious variant consequence. Remaining variants underwent manual curation to confirm pathogenicity.

## **Statistical Analysis**

Overall survival and cumulative incidence of relapse with non-relapse mortality as a competing risk were considered as the primary endpoints. The follow-up time was collected with day of transplant as time 0. The day from sample collection to transplant date was also collected when available for an alternative starting time. Median follow-up time was calculated for censored patients. Kaplan-Meier estimation and log rank tests were used to calculate overall survival and relapse-free survival endpoints. Cox proportional hazards models were fitted, with forward selection for variable selection, and the proportional hazards assumptions were validated. Fine-Gray regression models were used to examine the cumulative incidence of relapse with transplant related mortality as a competing risk, and Bayesian information criterion (BIC) was used for model selection. The interactions between NGS MRD and clinical characteristics including age and conditioning intensity were included in the stepwise selection process and significant results will be reported if any. Statistical significance was defined as p-value < 0.05. Receiver operator curve was used to identify the best *FLT3*-ITD VAF on relapse prediction by month post-transplant, and the threshold was defined using Youden's statistic.

## **Data Availability**

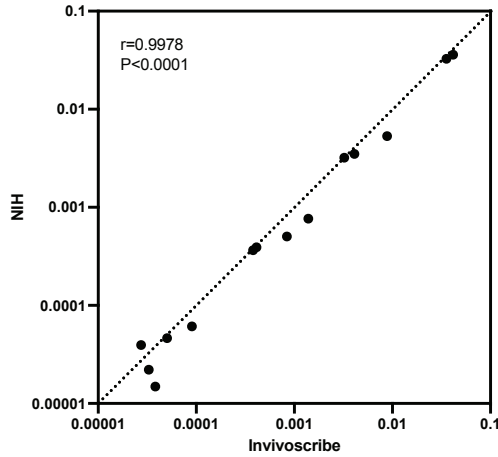
FASTQ files are available in the NCBI Sequence Read Archive (SRA) (Accession: PRJNA1021545).

**eFigure 1. Validation of the Invivoscribe *FLT3*-ITD MRD assay.** (A) Samples, *FLT3*-ITD size (bp), expected variant allele fraction (VAF), observed variant read frequency (VRF), and *FLT3*-ITD MRD result for competency standards as evaluated at the Invivoscribe and National Institutes of Health (NIH) laboratories. (B) Graphical comparison of VRF of *FLT3*-ITD variants as detected the Invivoscribe and NIH laboratories for competency standards. The Pearson correlation is shown in the graph inset and a value of equivalence line displayed as a dashed line.

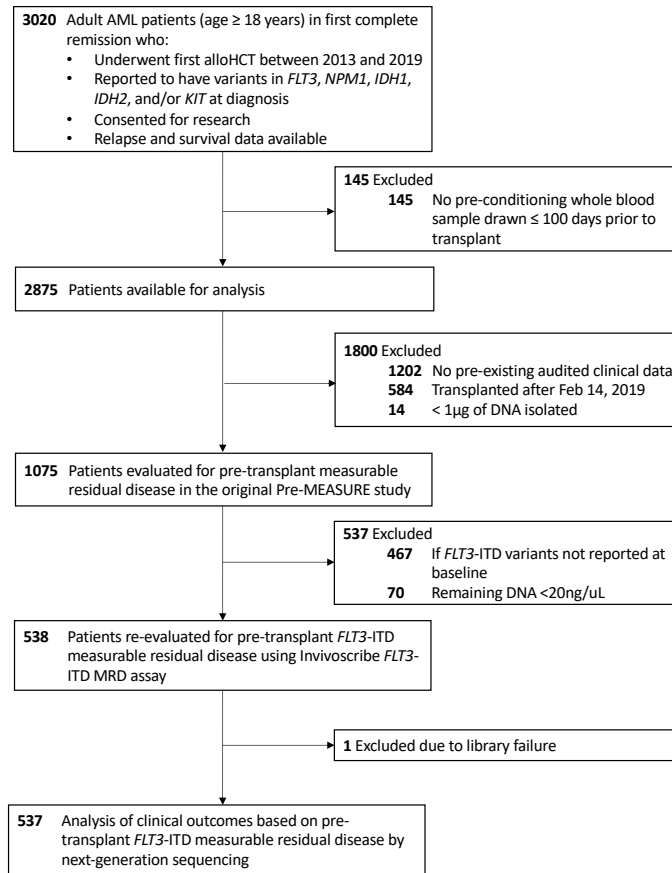
**A**

Sample	Targeted VAF	Invivoscribe			NIH		
		VRF	ITD_Size	ITD Result	VRF	ITD_Size	ITD Result
Cell Line 30bp Insert	5.00E-02	4.16E-02	30	Positive	3.59E-02	30	Positive
Cell Line 30bp Insert	5.00E-03	4.10E-03	30	Positive	3.50E-03	30	Positive
Cell Line 30bp Insert	5.00E-04	3.80E-04	30	Positive	3.65E-04	30	Positive
Cell Line 30bp Insert	5.00E-05	2.74E-05	30	Positive	3.95E-05	30	Positive
Cell Line 30bp Insert	1.00E-06	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Cell Line 126bp Insert	5.00E-02	8.82E-03	126	Positive	5.32E-03	126	Positive
Cell Line 126bp Insert	5.00E-03	8.40E-04	126	Positive	5.05E-04	126	Positive
Cell Line 126bp Insert	5.00E-04	5.05E-05	126	Positive	4.63E-05	126	Positive
Cell Line 126bp Insert	5.00E-05	1.38E-05	126	Positive	Not Detected	Not Detected	Not Detected
Cell Line 126bp Insert	1.00E-06	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Clinical Positive 42bp Insert	5.00E-02	3.56E-02	42	Positive	3.27E-02	42	Positive
Clinical Positive 42bp Insert	5.00E-03	3.23E-03	42	Positive	3.21E-03	42	Positive
Clinical Positive 42bp Insert	5.00E-04	4.11E-04	42	Positive	3.92E-04	42	Positive
Clinical Positive 42bp Insert	5.00E-05	3.28E-05	42	Positive	2.21E-05	42	Positive
Clinical Positive 42bp Insert	1.00E-06	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Clinical Positive 171bp Insert	5.00E-02	1.39E-03	171	Positive	7.67E-04	171	Positive
Clinical Positive 171bp Insert	5.00E-02	3.83E-05	93	Positive	1.49E-05	93	Positive
Clinical Positive 171bp Insert	5.00E-03	9.04E-05	171	Positive	6.10E-05	171	Positive
Clinical Positive 171bp Insert	5.00E-04	1.93E-05	171	Positive	Not Detected	Not Detected	Not Detected
Clinical Positive 171bp Insert	5.00E-05	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Clinical Positive 171bp Insert	1.00E-06	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Clinical Negative Pool	n/a	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Positive Control	5.00E-05	5.07E-05	30	Positive	6.47E-05	30	Positive
Negative Control	n/a	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
No Template Control	n/a	Undetermined	Not Detected	Undetermined	Undetermined	Undetermined	Undetermined

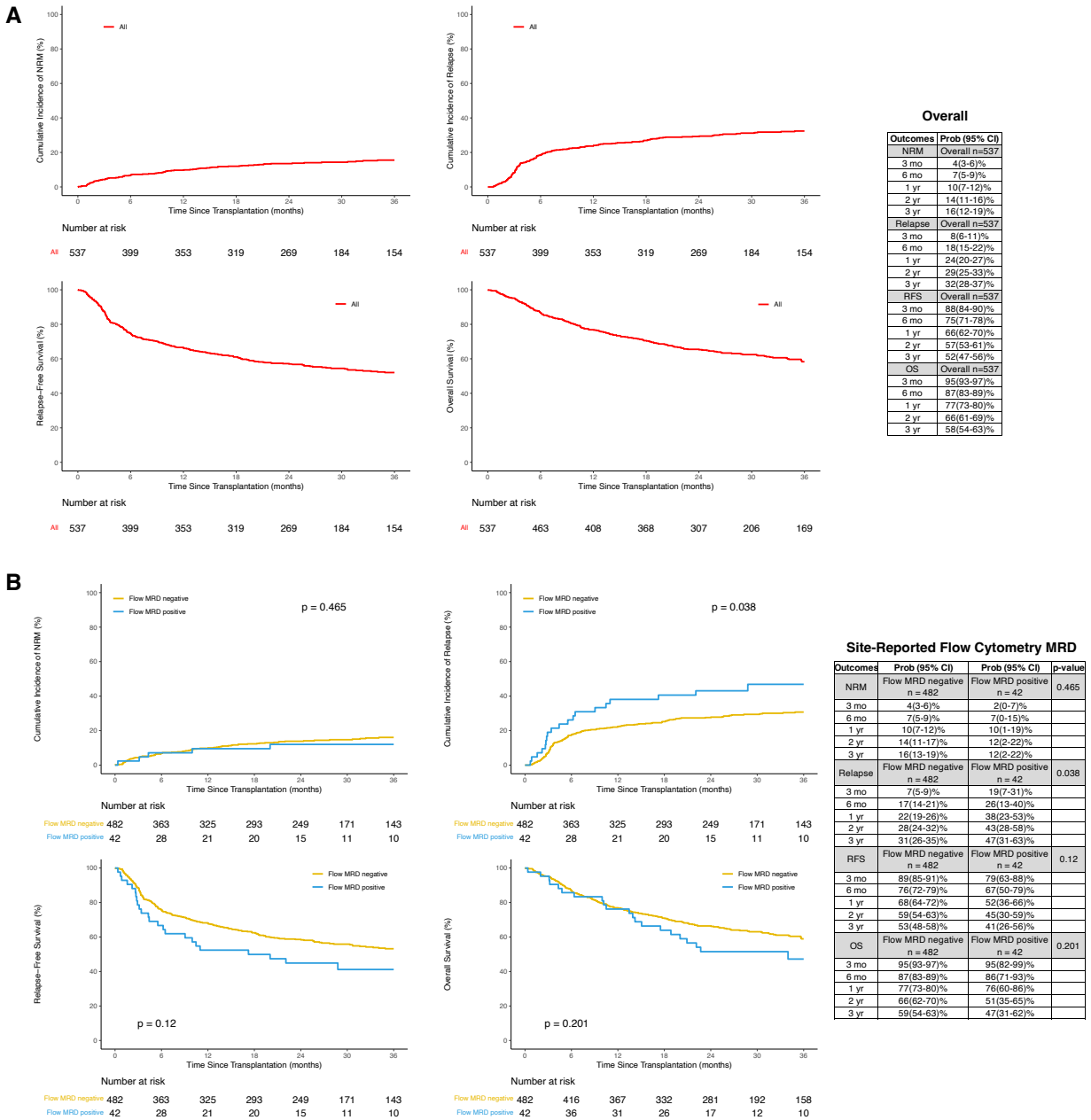
**B**



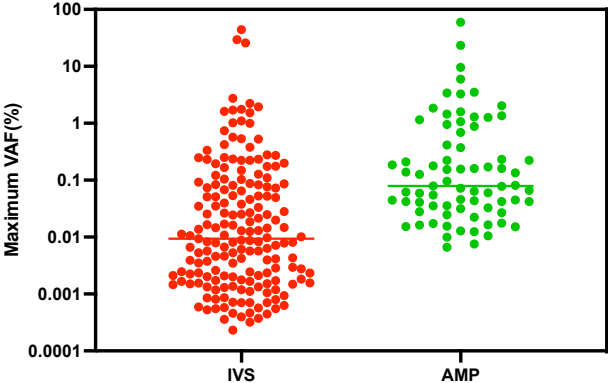
**eFigure 2. Patient selection flow chart.**



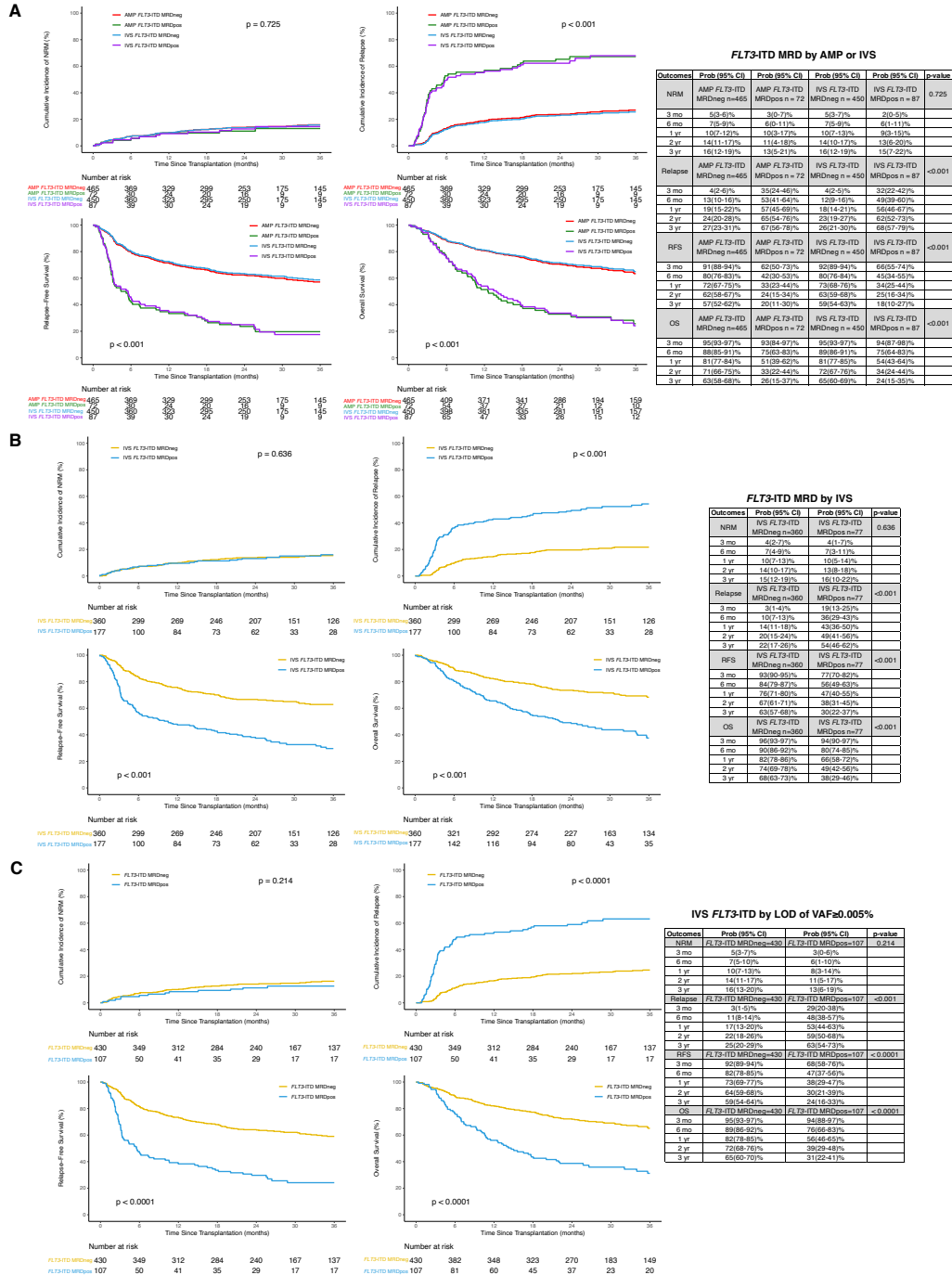
**eFigure 3. Baseline characteristics for *FLT3*-ITD mutated AML patients and the association with clinical outcomes after allogeneic hematopoietic cell transplant.** Cumulative incidence of non-relapse mortality (NRM, top left) and relapse (top right), relapse-free survival (RFS, bottom left) and overall survival (OS, bottom right) shown at 36 months based on baseline patient characteristics (A) overall and (B) site-reported MRD status by flow cytometry. Point estimates at different time points are shown in the table (far right). Overall P values: Gray's test for non-relapse mortality (NRM) and relapse; log-rank test for relapse-free survival (RFS) and overall survival (OS). Confidence interval, CI; Probability, prob; Month, mo; Year, yr.



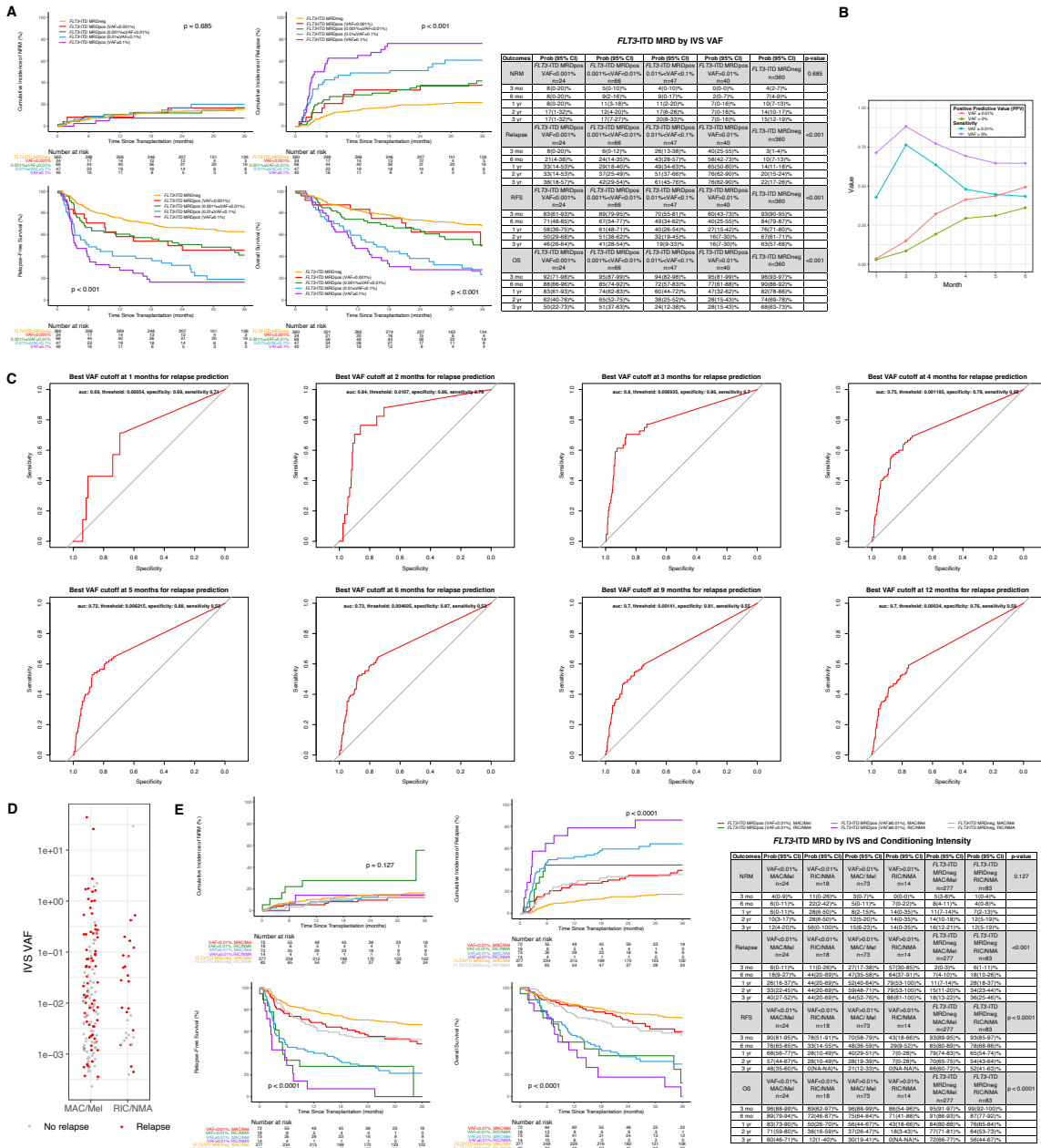
**eFigure 4. *FLT3*-ITD residual disease burden levels as detected by multiple next generation sequencing assays.** Maximum variant allele fraction (VAF) of *FLT3*-ITD variants per AML patient in pre-transplant blood as detected by Invivoscribe (IVS) or anchored multiplex PCR (AMP) targeted next generation sequencing (NGS) measurable residual disease (MRD) assays.



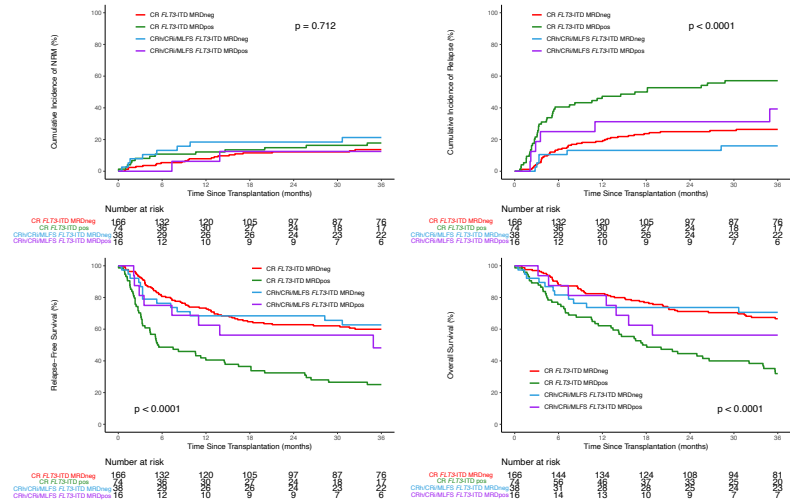
**eFigure 5. FLT3-ITD MRD status for FLT3-ITD mutated AML patients and the association with clinical outcomes after allogeneic hematopoietic cell transplant.** Cumulative incidence of non-relapse mortality (NRM, top left) and relapse (top right), relapse-free survival (RFS, bottom left) and overall survival (OS, bottom right) shown at 36 months (A) comparison of Invivoscribe (IVS) and anchored multiplex PCR (AMP) assays with VAF $\geq$ 0.01% as MRD positive; (B) all IVS detected variants as MRD positive; (C) IVS with VAF $\geq$ 0.005% (limit of detection of the assay) as MRD positive. Point estimates at different time points are shown in the table (far right). Overall P values: Gray's test for non-relapse mortality (NRM) and relapse; log-rank test for relapse-free survival (RFS) and overall survival (OS). Confidence interval, CI; Probability, prob; Month, mo; Year, yr.



**eFigure 6. *FLT3*-ITD MRD status by different variant allele fraction (VAF) groups for *FLT3*-ITD mutated AML patients and the association with clinical outcomes after allogeneic hematopoietic cell transplant.** For clinical outcomes in (A) Invivoscribe (IVS) detected *FLT3*-ITD variants by VAF group, and (E) IVS detected *FLT3*-ITD variants by VAF group and conditioning intensity, cumulative incidence of non-relapse mortality (NRM, top left) and relapse (top right), relapse-free survival (RFS, bottom left) and overall survival (OS, bottom right) shown at 36 months. Point estimates at different time points are shown in the table (far right). Overall P values: Gray's test for non-relapse mortality (NRM) and relapse; log-rank test for relapse-free survival (RFS) and overall survival (OS). (B) Positive predictive value (PPV) and sensitivity of *FLT3*-ITD variant positivity pre-transplant (divided by VAF group) for predicting relapse by month post-alloHCT. (C) Receiver operator curve analysis of *FLT3*-ITD VAF on relapse prediction by month post-alloHCT. At the clinically-relevant 3 month post-alloHCT timepoint, a VAF threshold of 0.0069% was able to best classify relapsing patients with an accuracy of 85%. (D) IVS *FLT3*-ITD VAF subdivided by conditioning regimen (with relapse outcomes depicted (relapse, red; no relapse, grey)). Confidence interval, CI; Probability, prob; Month, mo; Year, yr; Reduced intensity conditioning without melphalan, RIC; Reduced intensity conditioning with melphalan, Mel; non-myeloablative conditioning, NMA; myeloablative conditioning, MAC.



**eFigure 7. NGS MRD status for *FLT3*-ITD mutated AML patients and the association with clinical outcomes after allogeneic hematopoietic cell transplant stratified by complete remission (CR) status.** Cumulative incidence of non-relapse mortality (NRM, top left) and relapse (top right), relapse-free survival (RFS, bottom left) and overall survival (OS, bottom right) shown at 36 months based on category of CR status and *FLT3*-ITD MRD status. Point estimates at different time points are shown in the table (far right). Overall P values: Gray's test for non-relapse mortality (NRM) and relapse; log-rank test for relapse-free survival (RFS) and overall survival (OS). Confidence interval, CI; Probability, prob; Month, mo; Year, yr. Complete remission, CR; CR with incomplete hematologic recovery, CRh/Cri/MFLS.



**FLT3-ITD MRD by IVS and CR status**

Outcome	CR FLT3-ITD MRDneg, n=166	CR FLT3-ITD MRDpos, n=74	CRh/Cri/MFLS FLT3-ITD MRDneg, n=38	CRh/Cri/MFLS FLT3-ITD MRDpos, n=16	p-value
<b>NRM</b>					0.712
3 mo	3(0-6%)	8(2-14%)	8(0-17%)	0(0-0%)	
6 mo	5(2-9%)	11(4-18%)	13(2-24%)	0(0-0%)	
1 yr	8(4-12%)	12(5-20%)	18(6-31%)	6(0-19%)	
2 yr	12(7-17%)	15(7-23%)	18(6-31%)	12(0-30%)	
3 yr	14(8-19%)	18(9-27%)	21(8-35%)	12(0-30%)	
<b>Relapse</b>					<0.001
3 mo	4(1-7%)	22(12-31%)	3(0-6%)	19(0-39%)	
6 mo	14(8-19%)	41(23-52%)	11(1-20%)	25(3-47%)	
1 yr	19(12-25%)	47(26-59%)	13(2-24%)	31(8-55%)	
2 yr	25(15-32%)	53(31-64%)	13(2-24%)	31(8-55%)	
3 yr	28(16-33%)	57(32-69%)	16(4-28%)	39(13-66%)	
<b>RFS</b>					<0.0001
3 mo	93(88-96%)	70(58-79%)	89(74-96%)	81(52-94%)	
6 mo	81(74-86%)	49(37-59%)	78(63-87%)	75(46-87%)	
1 yr	73(66-79%)	41(29-51%)	68(51-81%)	62(31-81%)	
2 yr	63(55-70%)	32(22-43%)	68(51-81%)	56(30-76%)	
3 yr	60(52-67%)	25(16-35%)	63(45-76%)	48(22-70%)	
<b>OS</b>					<0.0001
3 mo	97(93-99%)	69(56-84%)	82(71-91%)	100%	
6 mo	89(82-92%)	76(64-84%)	82(65-91%)	88(59-97%)	
1 yr	82(76-87%)	62(50-72%)	74(67-85%)	81(52-94%)	
2 yr	71(64-77%)	45(33-55%)	74(67-85%)	56(30-76%)	
3 yr	67(60-73%)	32(21-43%)	71(63-83%)	56(30-76%)	

**eTable 1. Patient baseline clinical characteristics.**

Variables	Excluded*	In Study	Total	Variables	Excluded*	In Study	Total
<b>Age Group</b>				<b>Donor Type</b>			
Median (range)	53.5 (19.8-75.0)	55.6 (18.0-76.1)	55.4 (18.0-76.1)	Cord Blood	12 (17%)	61 (11%)	73 (12%)
<60	48 (68%)	339 (63%)	387 (64%)	Haploidentical Related	7 (10%)	52 (10%)	59 (10%)
>=60	23 (32%)	198 (37%)	221 (36%)	HLA-identical Sibling	3 (4%)	70 (13%)	73 (12%)
<b>Sex</b>				<b>Matched Unrelated</b>			
Female	37 (52%)	296 (55%)	333 (55%)	Mismatched	6 (8%)	44 (8%)	50 (8%)
Male	34 (48%)	241 (45%)	275 (45%)	<b>Multiple Donors</b>			
<b>Conditioning Intensity</b>				<b>ATG Usage</b>			
MAC	42 (59%)	321 (60%)	363 (60%)	No	50 (70%)	392 (73%)	442 (73%)
RIC/NMA	29 (41%)	216 (40%)	245 (40%)	Yes	21 (30%)	144 (27%)	165 (27%)
<b>Site-reported Flow Cytometry</b>				<b>Race and Ethnicity</b>			
Negative	65 (96%)	464 (92%)	529 (92%)	American Indian or Alaska Native	0 (0%)	2 (0%)	2 (0%)
Positive	3 (4%)	41 (8%)	44 (8%)	Asian	7 (10%)	30 (6%)	37 (6%)
<b>Graft Type</b>				<b>Black or African American</b>			
Bone Marrow	9 (13%)	70 (13%)	79 (13%)	Multiracial	0 (0%)	1 (0%)	1 (0%)
Cord Blood	13 (18%)	73 (14%)	86 (14%)	Native Hawaiian or Pacific Islander	0 (0%)	1 (0%)	1 (0%)
Peripheral Blood	49 (69%)	394 (73%)	443 (73%)	White (Hispanic or Latino)	5 (7%)	27 (5%)	32 (5%)
<b>Karnofsky Score</b>				<b>White (Other)</b>			
<90	29 (41%)	222 (42%)	251 (42%)	54 (78%)	428 (82%)	482 (82%)	
>=90	41 (59%)	312 (58%)	353 (58%)	<b>ELN Risk Group</b>			
<b>HCT-Comorbidity Index</b>				<b>Favorable</b>			
0	9 (13%)	112 (21%)	121 (20%)	6 (8%)	25 (5%)	31 (5%)	
1-2	27 (38%)	170 (32%)	197 (33%)	<b>Intermediate</b>			
3+	35 (49%)	247 (47%)	282 (47%)	42 (59%)	301 (56%)	343 (57%)	
<b>Baseline NPM1 Mutation</b>				<b>Adverse</b>			
Positive	37 (52%)	280 (52%)	317 (52%)	23 (32%)	210 (39%)	233 (38%)	
				<b>AML Group</b>			
				<b>De novo</b>			
				66 (93%)			
				492 (92%)			
				558 (92%)			
				<b>Therapy-related</b>			
				3 (4%)			
				18 (3%)			
				21 (3%)			
				<b>Transformed MDS/MPS</b>			
				2 (3%)			
				27 (5%)			
				29 (5%)			

\*FLT3-ITD mutated AML patients from the Pre-MEASURE study were excluded due to sample availability or analysis failure as outlined in eFigure 2

**eTable 2. Residual *FLT3*-ITD variants detected in the blood of AML patients prior to transplant using the Invivoscribe *FLT3*-ITD MRD assay.**

Patient ID	Read Depth	ITD Result	ITD Size (bp)	ITD READS	VAF (%)	ITD sequence
CH 012215	490850	Positive	105	2794	0.5690	TACAGATGGTACAGGTGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTTCAGAGAATATGAATATGATC TCAAAATGGAGTTTCCAAAGAAAGCCAGC
CH 012215	490850	Positive	24	1983	0.4040	CTAGTTGATTTTCAGAGAATATGA
CH 012215	490850	Positive	27	5	0.0010	ATGAATATGATCTCAAAATGGGAGTTTC
CH 012215	490850	Positive	48	38	0.0077	GGTACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTTCAGAGG
CH 017056	854116	Positive	185	7	0.0008	TGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC CAAGAGAAAATTTAGAGTTTGGTAAGAAATGCGAAATGTCGAAATGTTTTCGAGCAATTTCTTTCCATTGGAAAAT CTTTAAATGCACGTACTCACCATTGTCCTTTGCA
CH 017056	854116	Positive	75	33	0.0039	TCAGATAATGAGTACTTCTACGTTGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012565	409307	Positive	15	352	0.0860	TGATTTTCAGAGAATA
CH 012565	409307	Positive	90	115	0.0281	TACAGATAATGAGTACTTCTACGTTGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012493	603371	Positive	42	1346	0.223	TATGAAAGCCAGC
CH 012478	783451	Positive	27	208	0.0265	TTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012365	726640	Positive	81	1208	0.166	AGAGAA
CH 012273	688466	Positive	24	86	0.0125	CTACGTTGATTTTCAGAGAATATGA
CH 012231	990128	Positive	39	266	0.0269	TGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012235	1306473	Positive	63	3250	0.249	ATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012235	1306473	Positive	15	462	0.0354	GAGCCCTCAATATGG
CH 012232	744754	Positive	33	188	0.0252	ATAATGAGTACTTCTACGTTGATTTTCAGAGAAT
CH 012310	993617	Positive	99	2699	0.272	CGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012301	659843	Positive	60	250	0.0379	TTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012209	915628	Positive	5	939	0.103	AGAAAATTTAGAGTTTGAAGATG
CH 012190	668324	Positive	51	356	0.0533	CCCTC
CH 012189	643785	Positive	18	160	0.0249	GACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012159	921967	Positive	48	624	0.0677	TGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012449	833952	Positive	165	3	0.00036	ATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012449	833952	Positive	30	1456	0.175	CAGGTGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC GTTTCCAAAGAAAATTTAGAGTTTGTCCAGGACTTCTACGTTGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012449	833952	Positive	78	215983	25.9	GAGAAATGAATATGATCTCAAAATGGGAGTTTC
CH 012449	833952	Positive	78	9	0.00492	GG GAGCTAAAATGGGAGTTTCCAAAGAGAAAATTTAGAGTTTGTCCAGGACTTCTACGTTGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012449	833952	Positive	84	19	0.00276	TAT GATAATGAGTACTTCTACGTTGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012449	833952	Positive	87	32139	3.85	AGTTGAG CAGGTGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012449	833952	Positive	78	42	0.00887	CAAAATGGGAGTTTCCAAAGAGAAAATTTAGAGTTTGTCC GTTTCCAAAGAGTTT
CH 012428	795071	Positive	87	148	0.0186	ACTTCTAAGTTGAGGAAAGAGAGGAAAGAGGAGCTAAATGGGAGTTTC
CH 014416	758145	Positive	36	347	0.0458	CAAAATGGGAGTTTCCAAAGAGAAAATTTAGAGTTTGTCC GAGTTTGGAAA
CH 013785	494597	Positive	30	39	0.00789	GGCTCCTCAGATAATGAGTACTTCTACGTTGATTTTC
CH 014322	536054	Positive	51	115	0.0215	TACTTCTACGTTGATTTTCAGAGAATATGAATATGATCTCAAAATGGGAGTTTC
CH 012070	592420	Positive	57	49	0.00827	TATGAATATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTAGAGTTTGTCC AGAGAATATGAATATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTAGAGTTTGTCC





Patient ID	Read Depth	ITD Result	ITD Size (bp)	ITD READS	VAF (%)	ITD sequence
CH 016883	615728	Positive	60	33	0.00536	GATTTCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTAGAGTTG GGTACAGGTGACCGGCTCCTCAGATAATGATGATCTACGTTACGTTGATTTCCAGAGAAATGAAATGATCTCAAAATG GGAGCA
CH 016883	615728	Positive	81	511	0.083	
CH 016981	675750	Positive	24	6721	0.995	CTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA GATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016414	607306	Positive	54	15	0.00247	
CH 016385	492968	Positive	21	71	0.0144	TCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016411	509072	Positive	48	411	0.0807	CGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016545	686643	Positive	69	3608	0.525	CAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016406	971392	Positive	63	1067	0.11	ACGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016697	476611	Positive	63	5	0.00105	ATGGTACAGGTGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016455	724367	Positive	36	4	0.000552	GGTGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016475	849657	Positive	18	3	0.000353	AATATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016475	849657	Positive	30	6	0.000706	TTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016415	741778	Positive	24	178	0.024	CTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016415	741778	Positive	27	80	0.0108	GATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016415	741778	Positive	33	17	0.00229	TGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016415	741778	Positive	36	3	0.000404	TGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016415	741778	Positive	36	7	0.000944	CAAATGGGAGTTTCCAAAGAGAAATATCCAAAGAGAAAATTTA
CH 016415	741778	Positive	39	1856	0.25	TAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016415	741778	Positive	39	22	0.00297	GCTCCTCAGATAATGAGTACTTCTACGTTGATTTAGGGA
CH 016415	741778	Positive	42	18	0.00243	TCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016415	741778	Positive	54	16	0.00216	TACAGATGGTACAGGTGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTCCAAAGAGAAAATTTA
CH 016415	741778	Positive	54	5	0.000674	AGGTGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTCCAAAGAGAAAATTTA
CH 016415	741778	Positive	63	7	0.000944	AGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTAGAGTTTGGTAAAGAG
CH 016415	741778	Positive	63	899	0.121	TACAGATGGTACAGGTGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTCCAAAGAGAAAATTTA
CH 016415	741778	Positive	75	10	0.00135	TCAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016415	741778	Positive	87	88	0.0119	CCTCAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA TTTAGAGTTTG
CH 016415	741778	Positive	90	299	0.0403	GGCTCCTCAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA GAAAATTTAGAGTTT
CH 016441	882075	Positive	51	188	0.0213	AGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016441	882075	Positive	63	215	0.0244	GGTACAGGTGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016458	1142063	Positive	57	123	0.0108	CTCCTCAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016559	1072267	Positive	39	4	0.000373	TTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016519	647231	Positive	60	227	0.0351	AATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016501	500122	Positive	27	8543	1.71	TATGAAATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016501	500122	Positive	69	1329	0.286	ATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016520	499749	Positive	63	4	0.0008	CGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016546	582978	Positive	21	511	0.0877	TCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016525	615561	Positive	39	9	0.00146	ATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016521	755028	Positive	33	2883	0.362	AAATGGGAGTTTCCAAAGAGAAAATTTAGAGCCG
CH 016521	755028	Positive	27	7675	1.02	TGATTTCCAGAGAGTCCCAATGGGCTTCA
CH 016504	895209	Positive	63	14	0.00156	TGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016535	756021	Positive	36	3	0.000397	ATCAATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016509	1540859	Positive	21	804	0.0522	TGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA
CH 016610	704760	Positive	24	310201	44	CTACGTTGATTTCCAGAGAAATGAAATGATGATCTCAAAATGGGAGTTTCCAAAGAGAAAATTTA

Patient ID	Read Depth	ITD Result	ITD Size (bp)	ITD READS	VAF (%)	ITD sequence
CH 016585	696296	Positive	90	1614	0.232	GGTGACCGGCTCCTCAGATAAGTACGTTGATTTACAGAGAAATGAAATATGATCTCAAAATGGGAGTT TCCAAGAGAAAATTT
CH 016801	1166447	Positive	18	154	0.0132	TGATTTCCAGAGAAATGA
CH 016801	1166447	Positive	24	1753	0.15	ATTTCCAGAGAAATGAAATGATC
CH 016801	1166447	Positive	30	11	0.000943	GAAATATGATCTCAAAATGGGAGTTTCCAAGA
CH 016801	1166447	Positive	33	4912	0.421	GATAATGAGTACTTCTACGTTGATTTCCAGAGAT
CH 016801	1166447	Positive	48	21	0.0018	CAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATC
CH 016801	1166447	Positive	60	331	0.0284	TTGATTTCCAGAGAAATGAAATGATCTCAAAATGGGAGTTTCCAAGAGAAATTTAGAGT
CH 016801	1166447	Positive	75	5	0.000429	CTCAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATCTCAAAATGGGAGTTTCCAAGAGAAAA
CH 016672	547370	Positive	147	4	0.000731	TGAAATGATCTCAAAATGGGAGTTTCCAAGAGAAAAATTTAGAGTTTGGTAAAGTGGAAATGTGCCAAATGTTTCT GCAGCAATTTTCCATTTGAAAATCTTTAAAATGCACGTACTCACCATTGCTCTTCCAGGGAAGAGGGG
CH 016672	547370	Positive	39	10615	1.94	CCGGCTCCTCAGATAAGTACTTCTACGTTGATTTGC
CH 016593	400325	Positive	75	15	0.00375	AGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATCTCAAAATGGGAGTTTCCAAGAGAAAAATTT
CH 016614	603001	Positive	54	169	0.028	GATTTCCAGAGAAATGAAATGATCTCAAAATGGGAGTTTCCAAGAGAAAAATTTA
CH 016563	898529	Positive	51	19	0.00211	GCTCCTCAGATAAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATC
CH 016563	898529	Positive	75	2997	0.334	ATGGTACAGGTGACCGGCTCCTCAGATAAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATGATCTGGG
CH 016568	759671	Positive	69	35	0.00461	GAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATCTCAAAATGGGAGTTTCCAAGAGAAAAATTTA
CH 016579	676426	Positive	18	4	0.000591	TGATTTCCAGAGAAATGGGG
CH 016579	676426	Positive	21	12	0.00177	TCAGAGAAATGAAATGATC
CH 016604	576104	Positive	60	3082	0.535	ACGTTGATTTCCAGAGAAATGAAATGATCTCAAAATGGGAGTTTCCAAGAGAAAAATTTAA
CH 016635	554972	Positive	39	46	0.00829	ATTTCCAGAGAAATGAAATGATCTCAAAATGGGAGTTTCCAAGAGAAAAATTTAGAT
CH 016605	748232	Positive	81	15	0.002	TAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATCTCAAAATGGGAGTTTCCAAGAGAAAAATTTAGAT CCGTC
CH 016695	901991	Positive	60	1633	0.181	AATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATCTCAAAATGGGAGTTTCCAAGAGAAAAATTTAGAT
CH 016695	901991	Positive	60	2106	0.233	TTGATTTCCAGAGAAATGAAATGATCTCAAAATGGGAGTTTCCAAGAGAAAAATTTAGAGT
CH 016641	664879	Positive	15	98	0.0147	AATGAAATGATC
CH 016641	664879	Positive	18	9	0.00135	TCTCAAAATGGGAGTTTCC
CH 016646	889316	Positive	24	177	0.0199	GATTTCCAGAGAAATGAAATGAA
CH 016704	652523	Positive	60	3	0.00046	TACAGATGTTACAGGTGACCGGCTCCTCAGATAAGTACTTCTACGTTGATTTCCAGAGAAAAATTTAGAGT
CH 016656	750824	Positive	48	10	0.00133	ACAGGTGACCGGCTCCTCAGATAAGTACTTCTACGTTGATTTCCAGAGAAAAATTTAGAGT
CH 016700	1089339	Positive	102	5	0.000459	ACCGGCTCCTCAGATAAGTACTTCTACGTTGATTTCCAGAGAAAAATTTAGAGTCTCAAAATGGGAGTTTCCA CAGGTGACCGGCTCCTCAGATAAGTACTTCTACGTTGATTTCCAGAGAAAAATTTAGAGTCTCAAAATGGGAGTTTCCA
CH 016700	1089339	Positive	105	47	0.00431	GTTTCCAAGAGAAAAATTTAGAGTTTGGTCCC
CH 016655	872046	Positive	39	8	0.000707	CAGAGAAATGAAATGATCTCAAAATGGGAGTTTGGGG
CH 016785	1116876	Positive	21	6424	0.737	GTTGATTTCCAGAGAAATGAA
CH 016684	1094416	Positive	45	184	0.0165	CTACGTTGATTTCCAGAGAAATGA
CH 016684	1094416	Positive	48	95	0.00868	TGATTTCCAGAGAAATGAAATGATCTCAAAATGGGAGTTTCCAAGA
CH 016693	895764	Positive	45	87	0.00971	CAGATAATGAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATC
CH 016670	799283	Positive	60	18	0.00225	GGTGACCGGCTCCTCAGATAAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATC
CH 016717	582837	Positive	39	5	0.000658	ACAGGTGACCGGCTCCTCAGATAAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATC
CH 016696	913661	Positive	90	695	0.0761	CCGGCTCCTCAGATAAGTACTTCTACGTTGATTTCCAGAGAAATGAAATGATCTCAAAATGGGAGTTTCCA AGAGAAAAATTTAGAA
CH 016741	951706	Positive	21	1159	0.122	TGATTTCCAGAGAAATGAAATG





**eTable 3. Residual variants other than FLT3-ITD detected in the blood of FLT3-ITD MRD false-negative AML patients prior to transplant.**

Patient ID	Chromosome	Position	Reference	Alternate	Gene	Transcript	HGVSc	HGVSp	Alt depth	Depth	VAF (%)	Consequence
CH 014489	17	7673739	C	A	TP53	NM_000546.5	c.880G>T	p.Glu294*	16	13813	0.116	nonsense
CH 014489	17	7674229	C	T	TP53	NM_000546.5	c.733G>A	p.Arg175Ser	13	11734	0.111	nonsense
CH 012021	17	7675087	C	T	TP53	NM_000546.5	c.524G>A	p.Arg175His	5	9019	0.055	nonsense
CH 012046	11	11927775	C	T	CBL	NM_005188.4	c.1027C>T	p.Arg343*	2	9363	0.021	nonsense
CH 012046	11	119278220	G	A	CBL	NM_005188.4	c.1151G>A	p.Cys384Tyr	3	10261	0.029	nonsense
CH 012046	11	119278261	C	T	CBL	NM_005188.4	c.1192C>T	p.His398Tyr	5	10216	0.049	nonsense
CH 012046	11	119278280	G	A	CBL	NM_005188.4	c.1211G>A	p.Cys404Tyr	13	10013	0.130	nonsense
CH 012046	15	90088701	C	T	IDH2	NM_002168.3	c.419G>A	p.Arg140Gln	268	6439	4.162	nonsense
CH 012046	4	54733154	A	T	KIT	NM_002222.2	c.2447A>T	p.Asp816Val	3	8338	0.036	nonsense
CH 012082	17	7673801	C	T	TP53	NM_000546.5	c.819G>A	p.Arg273His	5	10024	0.050	nonsense
CH 012082	17	7674846	A	G	TP53	NM_000546.5	c.584T>C	p.Ile195Thr	2	8706	0.023	nonsense
CH 012522	17	7674889	T	C	TP53	NM_000546.5	c.647A>G	p.His214Arg	2	12128	0.016	nonsense
CH 012256	17	7674220	G	A	TP53	NM_000546.5	c.742C>T	p.Arg248Tyr	2	12021	0.017	nonsense
CH 012256	17	7675076	G	A	TP53	NM_000546.5	c.635C>T	p.His179Tyr	3	11177	0.027	nonsense
CH 012256	11	119278528	G	A	CBL	NM_005188.4	c.1247G>A	p.Cys416Tyr	26	13986	0.186	nonsense
CH 012256	11	119278665	C	T	CBL	NM_005188.4	c.1384C>T	p.Arg462*	2	13923	0.014	nonsense
CH 012185	11	119278280	G	A	CBL	NM_005188.4	c.1211G>A	p.Cys404Tyr	2	14235	0.014	nonsense
CH 012185	2	208248388	G	A	IDH1	NM_001282386.1	c.394C>T	p.Arg132Cys	90	11518	0.781	nonsense
CH 012662	21	34799294	GCTTA	G	RUNX1	NM_001754.4	c.987+2_987+5delTAAG		4	7963	0.050	splicing
CH 012562	17	7674220	G	A	TP53	NM_000546.5	c.742C>T	p.Arg248Tyr	3	7125	0.042	nonsense
CH 012562	17	7674892	C	T	TP53	NM_000546.5	c.638G>A	p.Arg213Gln	5	6375	0.078	nonsense
CH 012562	17	7675133	T	C	TP53	NM_001276697.2	c.1A>G	p.Met17	2	6664	0.030	start loss
CH 012562	17	7675231	G	C	TP53	NM_000546.5	c.380C>T	p.Ser127Phe	2	6265	0.032	nonsense
CH 012362	15	90088701	C	T	IDH2	NM_002168.3	c.419G>A	p.Arg140Gln	101	14075	0.778	nonsense
CH 012334	13	28018486	CATG	C	FLT3	NM_004119.3	c.2508_2510delCAT		8	14000	0.057	indel
CH 012126	17	119278280	G	C	CBL	NM_005188.4	c.1211G>A	p.Cys404Tyr	6	17467	0.034	nonsense
CH 012126	17	11927775	G	C	CBL	NM_005188.4	c.1027C>G	p.His178Asp	3	12509	0.024	nonsense
CH 012135	17	7673578	G	A	TP53	NM_000546.5	c.1027C>T	p.Arg343*	2	15454	0.013	nonsense
CH 012135	17	7674946	A	G	TP53	NM_000546.5	c.949C>T	p.Gln317*	26	16036	0.162	nonsense
CH 012135	17	7674946	A	G	TP53	NM_000546.5	c.584T>C	p.Ile195Thr	8	13579	0.059	nonsense
CH 012135	11	119278280	G	A	CBL	NM_005188.4	c.1211G>A	p.Cys404Tyr	2	19437	0.010	nonsense
CH 012298	17	7674251	CA	C	TP53	NM_000546.5	c.710delT		2	8692	0.023	frameshift
CH 012298	11	119278540	G	A	CBL	NM_005188.4	c.1259G>A	p.Arg20Gln	2	12922	0.015	nonsense
CH 012353	13	28034203	T	C	FLT3	NM_004119.3	c.1715A>G	p.Tyr572Cys	2	10360	0.019	nonsense
CH 012353	17	7673805	C	T	TP53	NM_000546.5	c.814G>A	p.Val72Met	2	17201	0.012	nonsense
CH 012353	11	119278280	G	A	CBL	NM_005188.4	c.1211G>A	p.Cys404Tyr	2	19581	0.010	nonsense
CH 012353	15	90088701	C	T	IDH2	NM_002168.3	c.419G>A	p.Arg140Gln	21	12843	0.166	nonsense
CH 012385	17	7674219	C	T	TP53	NM_000546.5	c.419G>A	p.Arg248Gln	6	14833	0.040	nonsense
CH 012385	17	7674872	A	G	TP53	NM_000546.5	c.656T>C	p.Tyr220His	3	12415	0.024	nonsense
CH 012416	17	7673775	G	A	TP53	NM_000546.5	c.844C>G	p.Arg282Gly	3	18885	0.016	nonsense
CH 012416	17	7674918	C	A	TP53	NM_000546.5	c.612G>T	p.Glu204Asp	2	12558	0.016	nonsense
CH 012416	11	119278508	G	A	CBL	NM_005188.4	c.1228-1G>A		35	17608	0.199	splicing
CH 012416	15	90088701	C	T	IDH2	NM_002168.3	c.419G>A	p.Arg140Gln	4176	11925	35.019	nonsense
CH 012416			TCTCAGCTAGCCCTCATGGTTAC				c.1375_1376insCGCCGCCACCTGGGCCCTC					
CH 012394	11	32392042	T	T	WT1	NM_024426.6	CCACAGTGCACAGGATACACGGAGGAG					
CH 012394	17	7675051	C	T	TP53	NM_000546.5	CCACTGCACCCAGCCCTGTAATAGGATTT					
CH 012394	2	208248387	C	T	IDH1	NM_001282386.1	TTTTAAGTAAACAATGAGGGTCTAGCTGA					
CH 012394	11	32396361	GT	CCCA	WT1	NM_024426.6	G					
CH 012394	11	32396385	GT	CCCA	WT1	NM_024426.6	G					
CH 012394	11	32396385	GT	CCCA	WT1	NM_024426.6	G					
CH 012394	11	32392019	G	A	WT1	NM_024426.6	c.1399C>T	p.Arg467Tyr	149	20361	0.732	nonsense
CH 012464	17	7675070	G	A	TP53	NM_000546.5	c.541C>T	p.Arg181Cys	3	25136	0.012	nonsense
CH 012092	11	119278208	G	A	CBL	NM_005188.4	c.1139T>C	p.Leu380Pro	22	30845	0.071	nonsense
CH 017016	17	7673802	G	A	TP53	NM_000546.5	c.817C>T	p.Arg273Cys	65	25553	0.254	nonsense
CH 016723	17	7673786	G	A	TP53	NM_000546.5	c.833C>G	p.Proz73Arg	90	29250	0.308	nonsense
CH 016723	17	7675189	C	T	TP53	NM_000546.5	c.422G>A	p.Cys141Tyr	3	22407	0.013	nonsense
CH 012416	11	32392042	T	T	WT1	NM_024426.6	c.559+1G>A	p.Lys459fs	2	14925	0.013	frameshift
CH 012394	17	7675051	C	T	TP53	NM_000546.5	c.395G>A	p.Arg132His	4	22486	0.018	splicing
CH 012394	11	32396361	GT	CCCA	WT1	NM_024426.6	c.1158_1159insTGGGTGG	p.Ala387fs	155	26048	0.595	frameshift
CH 012394	11	32396387	GT	CCCA	WT1	NM_024426.6	c.1152_1153delClnsTGGG	p.Arg385fs	136	26261	0.518	frameshift
CH 012394	11	32396385	GT	CCCA	WT1	NM_024426.6	c.1124_1125insGGCCCT	p.Val376fs	152	26468	0.574	frameshift
CH 012394	11	32392019	G	A	WT1	NM_024426.6	c.1399C>T	p.Arg467Tyr	149	20361	0.732	nonsense
CH 012464	17	7675070	G	A	TP53	NM_000546.5	c.541C>T	p.Arg181Cys	3	25136	0.012	nonsense
CH 012092	11	119278208	G	A	CBL	NM_005188.4	c.1139T>C	p.Leu380Pro	22	30845	0.071	nonsense
CH 017016	17	7673802	G	A	TP53	NM_000546.5	c.817C>T	p.Arg273Cys	65	25553	0.254	nonsense
CH 016723	17	7673786	G	A	TP53	NM_000546.5	c.833C>G	p.Proz73Arg	90	29250	0.308	nonsense
CH 016723	17	7675189	C	T	TP53	NM_000546.5	c.422G>A	p.Cys141Tyr	3	22407	0.013	nonsense

Patent ID	Chromosome	Position	Reference	Alternate	Gene	Transcript	HGVSc	HGVSp	Alt depth	Depth	VAF (%)	Consequence
CH 016723	11	119278280	G	A	CBL	NM_005188.4	c.1211C>A		9	31440	0.029	missense
CH 016480	17	7675413	G	GAAAT	TP53	NM_000546.5	c.980_983dupATT	p.Phe328fs	2	14263	0.014	frameshift
CH 016480	17	7673783	C	T	TP53	NM_000546.5	c.836G>A	p.Gly279Glu	4	14167	0.028	missense
CH 016480	17	7673802	G	A	TP53	NM_000546.5	c.817C>T	p.Arg273Cys	2	14079	0.014	missense
CH 016480	17	7674220	G	C	TP53	NM_000546.5	c.742C>G	p.Arg248Gly	3	13768	0.022	missense
CH 016480	17	7674249	C	T	TP53	NM_000546.5	c.713C>A	p.Cys238Tyr	2	11308	0.018	missense
CH 016619	17	7673608	C	T	TP53	NM_000546.5	c.920-1G>A		2	11936	0.017	splicing
CH 016619	17	7673801	C	T	TP53	NM_000546.5	c.818G>A	p.Arg273His	2	13139	0.015	missense
CH 016619	17	7674195	GTGA	A	TP53	NM_000546.5	c.764_766delTCA	p.Ile255del	2	11037	0.018	indel
CH 016619	17	7674198	A	T	TP53	NM_000546.5	c.764T>A	p.Ile255Asn	6	11123	0.054	missense
CH 016619	17	7675087	GC	G	TP53	NM_000546.5	c.668delG	p.Pro223fs	2	10134	0.020	frameshift
CH 016619	17	7675087	C	T	TP53	NM_000546.5	c.524G>A	p.Arg175His	2	10264	0.019	missense
CH 016619	17	7675094	C	T	TP53	NM_000546.5	c.517G>A	p.Val173Met	3	10292	0.029	missense
CH 016619	17	7675118	G	A	TP53	NM_000546.5	c.493C>T	p.Gln165*	3	9503	0.032	nonsense
CH 016619	17	7675160	G	A	TP53	NM_000546.5	c.451C>T	p.Pro151Ser	2	10147	0.020	missense
CH 016619	11	119278262	A	G	CBL	NM_005188.4	c.1193A>G	p.His398Arg	7	14534	0.048	missense
CH 016492	17	7673795	C	T	TP53	NM_000546.5	c.824G>A	p.Cys275Tyr	2	12554	0.016	missense
CH 016492	17	7673801	C	T	TP53	NM_000546.5	c.818G>A	p.Arg273His	7	12394	0.056	missense
CH 016492	17	7675087	C	T	TP53	NM_000546.5	c.524G>A	p.Arg175His	8	10881	0.074	missense
CH 016849	11	119278181	A	C	CBL	NM_005188.4	c.1112A>T	p.Tyr371Phe	16	18941	0.084	missense
CH 016849	11	119278235	A	C	CBL	NM_005188.4	c.1166A>C	p.Lys389Thr	36	21798	0.165	missense
CH 016891	17	7673795	C	T	TP53	NM_000546.5	c.824G>A	p.Cys275Tyr	2	11843	0.017	missense
CH 016891	17	119278280	G	A	CBL	NM_005188.4	c.1211G>A	p.Cys404Tyr	2	16639	0.012	missense
CH 016891	17	7675184	G	T	TP53	NM_000546.5	c.427G>A	p.Val143Met	2	7866	0.025	missense
CH 016891	17	7675558	G	GGGCTCGAGCGTAGGATCTGACT	TP53	NM_000546.5	c.35_36insTGAGCGAGAGGGGAAAGCA		2	7323	0.027	frameshift
CH 016754	17	7675097	C	A	TP53	NM_000546.5	GGAGGAGCCGAGTCAGATCCTAGCC	p.Pro13fs	4	9710	0.041	missense
CH 016703	11	119278220	G	A	CBL	NM_005188.4	c.514G>T	p.Val172Phe	2	12234	0.016	missense
CH 016819	17	7669678	G	A	TP53	NM_000546.5	c.1151G>A	p.Cys384Tyr	4	17088	0.023	missense
CH 016819	17	7673825	A	C	TP53	NM_000546.5	c.1112C>T	p.Ser371Phe	4	18231	0.022	missense
CH 016819	17	7674228	C	A	TP53	NM_000546.5	c.794T>G	p.Leu265Val	4	16736	0.054	missense
CH 016819	17	7674249	C	T	TP53	NM_000546.5	c.734G>T	p.Gly245Val	9	18216	0.015	missense
CH 016898	17	7676380	C	T	TP53	NM_000546.5	c.713G>A	p.Cys238Tyr	2	13476	0.015	missense
CH 016889	17	7673820	G	A	TP53	NM_000546.5	c.96T>G>A		2	11221	0.018	splicing
CH 016885	17	7673771	C	G	TP53	NM_000546.5	c.799C>T	p.Arg267Tyr	3	26279	0.011	missense
CH 016885	17	7673776	G	C	TP53	NM_000546.5	c.848G>C	p.Arg283Pro	105	37570	0.277	missense
CH 016885	17	7673795	C	A	TP53	NM_000546.5	c.843C>G	p.Asp281Glu	5	37989	0.013	missense
CH 016889	17	7674262	A	A	TP53	NM_000546.5	c.824G>T	p.Cys275Phe	4	36946	0.011	missense
CH 016891	17	7674871	T	C	TP53	NM_000546.5	c.700T>A	p.Tyr234Asn	6	22691	0.026	missense
CH 016891	17	7675236	C	C	TP53	NM_000546.5	c.659A>G	p.Tyr220Cys	19	31548	0.060	missense
CH 016891	4	54733172	A	T	KIT	NM_000222.2	c.376-1G>A		5	23628	0.021	splicing
CH 016905	17	7674192	A	T	TP53	NM_000546.5	c.2465A>T	p.Asn822Ile	16	31033	0.052	missense
CH 016905	17	7675087	C	T	TP53	NM_000546.5	c.770T>G	p.Leu257Arg	2	15468	0.013	missense
CH 016905	17	7675177	A	T	TP53	NM_000546.5	c.524G>A	p.Arg175His	3	13795	0.022	missense
CH 016905	11	119278208	T	C	CBL	NM_005188.4	c.434T>A	p.Leu145Gln	93	13066	0.710	missense
CH 016905	11	119278214	A	C	CBL	NM_005188.4	c.1139T>C	p.Leu380Pro	20	19127	0.105	missense
CH 016905	11	32392716	G	A	WT1	NM_024426.6	c.1145A>C	p.Lys382Thr	2	19498	0.010	missense
CH 016963	17	7673801	C	A	TP53	NM_000546.5	c.1303C>T	p.Arg435*	8	17390	0.046	nonsense
CH 016963	17	7673810	A	G	TP53	NM_000546.5	c.818G>T	p.Arg273Leu	13	7460	0.174	missense
CH 016963	17	7673820	G	A	TP53	NM_000546.5	c.809T>C	p.Phe270Ser	302	7460	4.048	missense
CH 016963	17	7675081	G	A	TP53	NM_000546.5	c.799C>T	p.Arg267Tyr	2	7451	0.027	missense
CH 016963	17	7675087	C	T	TP53	NM_000546.5	c.530C>T	p.Pro177Leu	9	6654	0.135	missense
CH 016963	17	7676039	C	T	TP53	NM_000546.5	c.524G>A	p.Arg175His	3	6763	0.044	missense
CH 017103	17	7673804	A	T	TP53	NM_000546.5	c.329G>T	p.Arg170Leu	2	6556	0.031	missense
CH 017103	17	7673808	C	A	TP53	NM_000546.5	c.815T>A	p.Val272Ile	4	25462	0.016	missense
CH 017103	17	7674237	C	T	TP53	NM_000546.5	c.811G>T	p.Gln271*	4	25497	0.016	nonsense
CH 017103	17	7674937	TC	T	TP53	NM_000546.5	c.726G>A	p.Cys242Tyr	11	20584	0.052	missense
CH 017103	17	7675085	A	C	TP53	NM_000546.5	c.592delG	p.Gln198fs	3	21212	0.014	frameshift
CH 017103	17	7676085	A	C	TP53	NM_000546.5	c.526T>G	p.Cys176Gly	12	22165	0.054	missense
CH 017103	17	7676000	G	A	TP53	NM_000546.5	c.368C>T	p.Thr123Ile	57	22643	0.252	missense

Patient ID	Chromosome	Position	Reference	Alternate	Gene	Transcript	HGVSc	HGVSp	Alt depth	Depth	VAF (%)	Consequence
CH.017103	11	119278513	C	G	CBL	NM_005188.4	c.1232C>G	p.Ser411*	4	26935	0.015	nonsense
CH.017103	15	90088701	C	T	IDH2	NM_002168.3	c.419G>A	p.Arg140Gln	5	17647	0.028	missense
CH.017042	17	7674291	C	A	TP53	NM_000546.5	c.711G>T	p.Met237Ile	4	13729	0.029	missense
CH.017042	17	7675156	G	A	TP53	NM_000546.5	c.455C>T	p.Pro152Leu	3	17168	0.017	missense
CH.017042	17	7675184	C	T	TP53	NM_000546.5	c.427G>A	p.Val143Met	2	16227	0.012	missense
CH.017040	17	7674226	T	G	TP53	NM_000546.5	c.736A>C	p.Met248Leu	2	17601	0.011	missense
CH.017040	17	7674946	A	G	TP53	NM_000546.5	c.594T>C	p.Ile195Thr	3	16967	0.018	missense
CH.017040	17	7675051	C	A	TP53	NM_000546.5	c.559+1G>T		2	18440	0.011	splicing
CH.017040	11	119278164	G	A	CBL	NM_005188.4	c.1096-1G>A		2	19887	0.010	splicing
CH.017040	11	119278530	C	T	CBL	NM_005188.4	c.1249C>T	p.Pro417Ser	22	22198	0.099	missense

**eTable 4. Duplex sequencing panel target regions.**

<b>Gene</b>	<b>Accession ID</b>	<b>Exons</b>
<i>CBL</i>	NM_005188.4	7,8,9
<i>FLT3</i>	NM_004119.3	4,7,10-17,29
<i>IDH1</i>	NM_005896.4	7,8
<i>IDH2</i>	NM_002168.4	4,7
<i>JAK2</i>	NM_004972.4	12,14
<i>KIT</i>	NM_000222.3	8,9,10,11,17
<i>KRAS</i>	NM_004985.5	2,3,4
<i>NPM1</i>	NM_002520.7	10,11
<i>NRAS</i>	NM_002524.5	2,3,4
<i>PTPN11</i>	NM_002834.5	3,7,8,12,13
<i>RUNX1</i>	NM_001754.5	full gene
<i>SF3B1</i>	NM_012433.4	13,14,15,16
<i>TP53</i>	NM_000546.6 NM_001276698.3	full gene
<i>WT1</i>	NM_024426.6	8,9,10
<i>BRAF</i>	NM_004333.6	15

## eReferences

1. Dillon LW, Gui G, Page KM, et al. DNA Sequencing to Detect Residual Disease in Adults With Acute Myeloid Leukemia Prior to Hematopoietic Cell Transplant. *JAMA*. 2023;329(9):745-755.