Supplemental Information

GENOMIC LANDSCAPE OF PATIENTS WITH *FLT3*-MUTATED ACUTE MYELOID LEUKEMIA (AML) TREATED WITHIN THE CALGB 10603/RATIFY TRIAL

AUTHORS

Nikolaus Jahn[1,*], Ekaterina Jahn[1,*], Maral Saadati[2], Lars Bullinger[3], Richard A. Larson[4], Tiziana Ottone[5,6], Sergio Amadori[5], Thomas W. Prior[7], Joseph M. Brandwein[8], Frederick R. Appelbaum[9], Bruno C. Medeiros[10], Martin S. Tallman[11], Gerhard Ehninger[12], Michael Heuser[13], Arnold Ganser[13], Celine Pallaud[14], Insa Gathmann[14], Julia Krzykalla[15], Axel Benner[15], Clara D. Bloomfield[16], Christian Thiede[12], Richard M. Stone[17], Hartmut Döhner[1], and Konstanze Döhner[1]

[1]Department of Internal Medicine III, University Hospital of Ulm, Ulm, Germany; [2]Saadati Solutions, Ladenburg, Germany; [3]Department of Hematology, Oncology and Tumor Immunology, Charité University, Berlin, Germany; [4]Department of Medicine and Comprehensive Cancer Center, University of Chicago, Chicago, IL, USA; [5]Department of Biomedicine and Prevention, University Tor Vergata, Rome, Italy; [6]Santa Lucia Foundation, I.R.C.C.S., Neuro-Oncohematology, Rome, Italy; [7]Case Western Reserve University, Cleveland, OH, USA; [8]Department of Medicine, University of Alberta, Edmonton, Canada; [9]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [10]Division of Hematology, Stanford Comprehensive Cancer Center, Stanford University, Stanford, CA, USA; [11]Division of Hematologic Malignancies, Leukemia Service, Memorial Sloan Kettering Cancer Center, New York, NY; [12]Medizinische Klinik und Poliklinik I, Universitätsklinikum Carl Gustav Carus der TU Dresden, Dresden, Germany; [13]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany; [14]Novartis Pharmaceuticals, Basel, Switzerland; [15]Division of Biostatistics, German Cancer Research Center Heidelberg, Heidelberg, Germany; [16]The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA; [17]Department of Medical Oncology, Dana-Farber/Partners CancerCare, Boston, MA, USA

*N.J. and E.J. contributed equally to this work

SUPPLEMENTAL METHODS

Library Enrichment and Sequencing

The library enrichment was performed using the commercially available SureSelectXT in-solution capture technology from Agilent Technologies (Santa Clara, CA, USA). The library comprised the entire coding region of 262 candidate genes involved in hematological malignancies including 20 kinases targeted by midostaurin. For library design Agilent's online tool SureDesign was used. Only exons with a consensus annotation in the RefSeq, Ensembl, CCDS, Gencode, and SNP databases were considered relevant. The UCSC human genome 19 (H. sapiens, hg19, GRCh37, February 2009) served as reference genome for design and determination of genomic coordinates.

Genomic DNA (200ng per sample) extracted from pre-treatment bone marrow (409, 86%) or peripheral blood (66, 14%) specimens was used for molecular screening. SureSelect library preparation and indexing were performed following the manufacturer's instructions for Illumina paired-end sequencing. Samples were then transferred to a cBot (Illumina, San Diego, CA, USA) to create clonal clusters on a flow cell by bridge amplification (Illumina reagent kit: TruSeq PE Cluster Kit v3-cBot-HS). Finally, 2x 100 bp paired-end sequencing by synthesis was carried out on a HiSeq2000 (Illumina, San Diego, CA, USA) using Illumina's TruSeq SBS Kit v3-HS reagents.

Variant calling

The sequencing quality of each sample was assessed using the NGS QC toolkit (2.3.3) and, where necessary, adapter and read end trimming were performed using cutadapt (1.8.3) and in-house scripting respectively.

Paired-end reads were then aligned to the hg19 reference using BWA-MEM (0.7.10). Alignments are sorted and indexed by Picard (1.138) and locally realigned using GATK (3.4.46). For each sample, coverage statistics were calculated using BEDTools (2.24.0) and processed by SAMtools (0.1.19). VarScan2 (2.3.9) was then used for variant calling within the target regions sequenced. All variants were annotated by Annovar (release 22Mar2015) but only non-synonymous mutations affecting exons or splice sites were retained. These were further filtered to remove calls within known regions of segmental duplication, variants annotated in dbSNP (138) but not COSMIC (70) and variants with a minor allele frequency (MAF) above 0.01 in either the 1000 Genomes Project or the Exome Sequencing Project (ESP 6500).

Curation of oncogenic variants

All calls yielded by the computational annotation workflow were subject to further curation. Only variants considered oncogenic were included in the subsequent analyses. The algorithm for mutation reporting is as follows:

a) Removal of all variants that are annotated in SNP databases and occur with a minor allele frequency (MAF) >0.001 in the 1000 Genomes Project, dbSNP150 or the Exome Sequencing Project (ESP 6500).

b) Removal of variants present within regions prone to sequence context specific artifacts, including regions of high depth, enriched for reads of low mapping quality that harbor multiple mismatches

c) Removal of all one bp insertions or deletions present adjacent to regions of more than 5 homopolymer bases (for example insG adjacent to GGGGG) and a variant allele frequency of ≤0.1

d) Removal of all missense variants with a variant allele frequency between ≥0.45 and ≤0.55 or ≥0.9 and 1.0, indicative of polymorphisms, unless they are present with ≥5 counts in COSMIC database (v85) and with ≥1 confirmed somatic.

e) Retention of all frameshift, nonsense or splicing variants with a variant allele frequency ≥0.03

f) Retention of all missense variants with a variant allele frequency between 0.03 and <0.45 or >0.55 and <0.9, indicative of (likely) oncogenic variants.

SUPPLEMENTAL TABLES

Supplemental Table S1: Comparison of clinical characteristics of patients included and excluded into this analysis of entire CALGB 10603/RATIFY trial cohort (N=717).

Characteristic	Excluded, N = 242[1]	Included, N = 475[1]	p-value[2]
Age at registration	48 (40, 53)	48 (39, 54)	1
WBC count at baseline (10E9/L)	34 (13, 89)	35 (12, 72)	0.5
Unknown	7	3	
Sex			0.6
Male	111 (46%)	208 (44%)	
Female	131 (54%)	267 (56%)	
ECOG performance category			0.5
0-1	211 (87%)	422 (89%)	
2	31 (13%)	53 (11%)	
***FLT3* mutation type**			0.073
TKD	47 (20%)	116 (24%)	
ITD allelic ratio <0.5	82 (34%)	126 (27%)	
ITD allelic ratio >=0.5	109 (46%)	232 (49%)	
Unknown	4	1	
Treatment			0.022
Midostaurin	107 (44%)	253 (53%)	
Placebo	135 (56%)	222 (47%)	

[1] Median (IQR); n (%)

[2] Wilcoxon rank sum test; Pearson's Chi-squared test

Supplemental Table S2: List of all genes targeted by custom sequencing panel

ABCA12	*CDKN2B*	*DNMT3B*	*GNB1*	*MAP3K4*	*NXF1*	*RASGRF1*	*STAG1*
ABL1	*CDKN2C*	*DYNC1H1*	*H3F3A*	*MAP3K9*	*OBSCN*	*RB1*	*STAG2*
ACIN1	*CEBPA*	*EED*	*H3F3B*	*MGA*	*OMG*	*RBBP5*	*STAT3*
ACSS3	*CHEK2*	*EEFSEC*	*HAX1*	*MLL3/KMT2C*	*PAX5*	*RBBP6*	*STAT5A*
ADGRV1	*CLTCL1*	*EGFR*	*HCN1*	*MLL5/KMT2E*	*PDGFB*	*RBMX*	*SUZ12*
ALK	*CNNM2*	*ELANE*	*HIPK2*	*MN1*	*PDGFRB*	*RET*	*SYNE1*
ANKRD26	*COPRS*	*EP300*	*HNRNPK*	*MPL*	*PDPK1*	*RHOA*	*TCIRG1*
ARHGEF10	*CREBBP*	*EPHA6*	*HRAS*	*MST1*	*PHF6*	*RMI1*	*TERC*
ARID1A	*CSF1R*	*ETNK1*	*IDH1*	*MYC*	*PHIP*	*ROBO1*	*TERT*
ARID2	*CSF2RB*	*ETV6*	*IDH2*	*MYH9*	*PHKG1*	*ROBO2*	*TET1*
AS3MT	*CSF3R*	*EVI2A*	*IKZF2*	*MYLK2*	*PIK3CA*	*RPS6KA2*	*TET2*
ASXL1	*CSMD1*	*EVI2B*	*INPP5D*	*MYO1F*	*PKN2*	*RPS6KA3*	*TINF2*
ASXL2	*CSMD2*	*EWSR1*	*IRF1*	*NCOA7*	*PLEKHH1*	*RPS6KA6*	*TNK1*
ATRX	*CSNK1A1*	*EZH1*	*IRF4*	*NDE1*	*PLEKHS1*	*RRAS*	*TNK2*
BAP1	*CTC1*	*EZH2*	*IRF8*	*NEK2*	*PPM1D*	*RUNX1*	*TP53*

BCL10	*CTCF*	*FAM175A*	*JAK1*	*NF1*	*PRKAG2*	*RYR2*	*TTC39A*
BCL2	*CTNNB1*	*FAM5C*	*JAK2*	*NF2*	*PRKG2*	*SAMHD1*	*U2AF1*
BCOR	*CUX1*	*FAT4*	*JAK3*	*NFE2*	*PRPF40A*	*SETBP1*	*U2AF2*
BCORL1	*DCC*	*FBXW7*	*JARID2*	*NFE2L1*	*PRPF40B*	*SETD2*	*UBQLN1*
BCR	*DDX23*	*FGFR2*	*KAT6A*	*NFE2L2*	*PRPF8*	*SETDB1*	*UBXN11*
BRAF	*DDX4*	*FLG*	*KDM5C*	*NIPBL*	*PTEN*	*SF1*	*WAC*
BRCC3	*DDX41*	*FLT3*	*KDM6A*	*NOTCH1*	*PTPN11*	*SF3A1*	*WHSC1*
C6	*DDX54*	*FOXP1*	*KDR*	*NOTCH2*	*PTPRF*	*SF3B1*	*WRAP53*
C9orf103	*DHX15*	*FRMD3*	*KIF27*	*NPM1*	*PTPRT*	*SH2B3*	*WT1*
CALR	*DHX33*	*G6PC3*	*KIT*	*NRAS*	*PXDN*	*SMARCB1*	*YLPM1*
CBL	*DICER1*	*GALNT11*	*KMT2A*	*NRXN1*	*RAB11FIP4*	*SMC1A*	*ZBTB33*
CCDC26	*DIS3*	*GALNTL5*	*KMT2D*	*NRXN3*	*RAC1*	*SMC3*	*ZBTB7A*
CCND1	*DKC1*	*GATA1*	*KRAS*	*NSD1*	*RAD21*	*SMG1*	*ZMYM3*
CCND2	*DNAH9*	*GATA2*	*LAMA1*	*NT5C2*	*RAD50*	*SPI1*	*ZNF318*
CDHR1	*DNAJB8*	*GFI1*	*LAMC3*	*NTRK1*	*RAD51*	*SPRED2*	*ZNF687*
CDK4	*DND1*	*GIGYF2*	*LUC7L2*	*NTRK3*	*RASA2*	*SRCAP*	*ZRSR2*
CDKN1B	*DNM2*	*GKAP1*	*MAP3K10*	*NUMA1*	*RASA3*	*SRP72*	
CDKN2A	*DNMT3A*	*GNAS*	*MAP3K11*	*NUP98*	*RASEF*	*SRSF2*	

Supplemental Table S3: Genes targeted by midostaurin (Midostaurin kinome)

JAK3	*KDR*	*KIT*	*MAP3K10*	*MAP3K11*	*MAP3K9*	*MST1*	*NTRK1*	*NTRK3*	*PDGFRB*
PDPK1	*PHKG1*	*PKN2*	*PRKG2*	*RET*	*RPS6KA2*	*RPS6KA3*	*RPS6KA6*	*TNK1*	*TNK2*

Supplemental Table S4: Frequency of gene mutations overall and by *FLT3* mutational subgroups

Characteristic	**Overall, N = 475[1]**	**TKD, N = 116[1]**	**ITD, N = 359[1]**	**p[2]**
NPM1	291 (61%)	71 (61%)	220 (61%)	1.00
DNMT3A	187 (39%)	44 (38%)	143 (40%)	0.74
WT1	100 (21%)	16 (14%)	84 (23%)	0.03
TET2	55 (12%)	10 (8.6%)	45 (13%)	0.32
RUNX1	53 (11%)	10 (8.6%)	43 (12%)	0.40
NRAS	53 (11%)	28 (24%)	25 (7.0%)	<0.001
PTPN11	45 (9.5%)	16 (14%)	29 (8.1%)	0.10
IDH1	39 (8.2%)	9 (7.8%)	30 (8.4%)	1.00
ASXL1	38 (8.0%)	5 (4.3%)	33 (9.2%)	0.12
IDH2 (R140)	34 (7.2%)	7 (6.0%)	27 (7.5%)	0.68
SMC1A	28 (5.9%)	12 (10%)	16 (4.5%)	0.04
CEBPA	26 (5.5%)	2 (1.7%)	24 (6.7%)	0.06
SMC3	24 (5.1%)	9 (7.8%)	15 (4.2%)	0.14
RAD21	23 (4.8%)	8 (6.9%)	15 (4.2%)	0.22
BCOR	20 (4.2%)	3 (2.6%)	17 (4.7%)	0.43
KMT2D	18 (3.8%)	1 (0.9%)	17 (4.7%)	0.09
STAG2	18 (3.8%)	6 (5.2%)	12 (3.3%)	0.40
BCORL1	16 (3.4%)	5 (4.3%)	11 (3.1%)	0.56

ZBTB7A	15 (3.2%)	6 (5.2%)	9 (2.5%)	0.22
MYC	15 (3.2%)	5 (4.3%)	10 (2.8%)	0.38
GATA2	14 (2.9%)	2 (1.7%)	12 (3.3%)	0.53
OBSCN	14 (2.9%)	3 (2.6%)	11 (3.1%)	1.00
KRAS	13 (2.7%)	6 (5.2%)	7 (1.9%)	0.10
NFE2	13 (2.7%)	1 (0.9%)	12 (3.3%)	0.20
CSMD1	11 (2.3%)	3 (2.6%)	8 (2.2%)	0.73
KDM6A	11 (2.3%)	4 (3.4%)	7 (1.9%)	0.48
MGA	11 (2.3%)	3 (2.6%)	8 (2.2%)	0.73
SETD2	10 (2.1%)	2 (1.7%)	8 (2.2%)	1.00
SYNE1	10 (2.1%)	1 (0.9%)	9 (2.5%)	0.46
CREBBP	10 (2.1%)	4 (3.4%)	6 (1.7%)	0.27
ARID2	9 (1.9%)	4 (3.4%)	5 (1.4%)	0.23
MN1	9 (1.9%)	4 (3.4%)	5 (1.4%)	0.23
SF3B1	9 (1.9%)	2 (1.7%)	7 (1.9%)	1.00
SRSF2	8 (1.7%)	3 (2.6%)	5 (1.4%)	0.41
KIT	8 (1.7%)	5 (4.3%)	3 (0.8%)	0.02
ZBTB33	7 (1.5%)	2 (1.7%)	5 (1.4%)	0.68
HNRNPK	7 (1.5%)	3 (2.6%)	4 (1.1%)	0.37
NF1	7 (1.5%)	1 (0.9%)	6 (1.7%)	1.00
PTPRF	6 (1.3%)	0 (0%)	6 (1.7%)	0.34
ZNF318	6 (1.3%)	1 (0.9%)	5 (1.4%)	1.00
NOTCH1	6 (1.3%)	2 (1.7%)	4 (1.1%)	0.64
PHF6	6 (1.3%)	0 (0%)	6 (1.7%)	0.34
CSMD2	6 (1.3%)	4 (3.4%)	2 (0.6%)	0.03
CBL	6 (1.3%)	0 (0%)	6 (1.7%)	0.34
DNAH9	6 (1.3%)	2 (1.7%)	4 (1.1%)	0.64
GNAS	6 (1.3%)	1 (0.9%)	5 (1.4%)	1.00
KMT2A	6 (1.3%)	1 (0.9%)	5 (1.4%)	1.00
FOXP1	6 (1.3%)	4 (3.4%)	2 (0.6%)	0.03
CSF3R	6 (1.3%)	1 (0.9%)	5 (1.4%)	1.00
EP300	6 (1.3%)	1 (0.9%)	5 (1.4%)	1.00
ACIN1	5 (1.1%)	0 (0%)	5 (1.4%)	0.34
RYR2	5 (1.1%)	0 (0%)	5 (1.4%)	0.34
ARID1A	5 (1.1%)	0 (0%)	5 (1.4%)	0.34
ASXL2	5 (1.1%)	1 (0.9%)	4 (1.1%)	1.00
LAMC3	5 (1.1%)	2 (1.7%)	3 (0.8%)	0.60
UBXN11	5 (1.1%)	0 (0%)	5 (1.4%)	0.34
ROBO1	5 (1.1%)	1 (0.9%)	4 (1.1%)	1.00
GPR98	5 (1.1%)	1 (0.9%)	4 (1.1%)	1.00
SRCAP	5 (1.1%)	2 (1.7%)	3 (0.8%)	0.60
EZH2	5 (1.1%)	1 (0.9%)	4 (1.1%)	1.00
FBXW7	5 (1.1%)	2 (1.7%)	3 (0.8%)	0.60
BCR	4 (0.8%)	1 (0.9%)	3 (0.8%)	
MYH9	4 (0.8%)	2 (1.7%)	2 (0.6%)	
ETV6	4 (0.8%)	1 (0.9%)	3 (0.8%)	
DNMT3B	4 (0.8%)	1 (0.9%)	3 (0.8%)	
LAMA1	4 (0.8%)	2 (1.7%)	2 (0.6%)	
U2AF1	4 (0.8%)	1 (0.9%)	3 (0.8%)	
NTRK3	4 (0.8%)	0 (0%)	4 (1.1%)	
CTCF	4 (0.8%)	0 (0%)	4 (1.1%)	
MAP3K11	4 (0.8%)	1 (0.9%)	3 (0.8%)	

CNNM2	4 (0.8%)	0 (0%)	4 (1.1%)
NSD1	4 (0.8%)	2 (1.7%)	2 (0.6%)
SH2B3	4 (0.8%)	0 (0%)	4 (1.1%)
KMT2E	4 (0.8%)	1 (0.9%)	3 (0.8%)
KDM5C	4 (0.8%)	2 (1.7%)	2 (0.6%)
BRCC3	4 (0.8%)	0 (0%)	4 (1.1%)
KMT2C	4 (0.8%)	1 (0.9%)	3 (0.8%)
CUX1	4 (0.8%)	3 (2.6%)	1 (0.3%)
TERT	4 (0.8%)	0 (0%)	4 (1.1%)
EPHA6	4 (0.8%)	1 (0.9%)	3 (0.8%)
FAT4	4 (0.8%)	0 (0%)	4 (1.1%)
INPP5D	4 (0.8%)	1 (0.9%)	3 (0.8%)
TP53	4 (0.8%)	2 (1.7%)	2 (0.6%)
ROBO2	3 (0.6%)	1 (0.9%)	2 (0.6%)
SETBP1	3 (0.6%)	1 (0.9%)	2 (0.6%)
CDHR1	3 (0.6%)	0 (0%)	3 (0.8%)
SF3A1	3 (0.6%)	1 (0.9%)	2 (0.6%)
PLEKHH1	3 (0.6%)	0 (0%)	3 (0.8%)
SETDB1	3 (0.6%)	0 (0%)	3 (0.8%)
EGFR	3 (0.6%)	2 (1.7%)	1 (0.3%)
RBBP6	3 (0.6%)	2 (1.7%)	1 (0.3%)
ATRX	3 (0.6%)	1 (0.9%)	2 (0.6%)
PDGFRB	3 (0.6%)	1 (0.9%)	2 (0.6%)
RPS6KA6	3 (0.6%)	2 (1.7%)	1 (0.3%)
NTRK1	3 (0.6%)	0 (0%)	3 (0.8%)
YLPM1	3 (0.6%)	0 (0%)	3 (0.8%)
RRAS	3 (0.6%)	1 (0.9%)	2 (0.6%)
KAT6A	3 (0.6%)	3 (2.6%)	0 (0%)
PPM1D	3 (0.6%)	2 (1.7%)	1 (0.3%)
HIPK2	3 (0.6%)	0 (0%)	3 (0.8%)
MAP3K10	2 (0.4%)	0 (0%)	2 (0.6%)
TCIRG1	2 (0.4%)	0 (0%)	2 (0.6%)
MPL	2 (0.4%)	1 (0.9%)	1 (0.3%)
DDX41	2 (0.4%)	0 (0%)	2 (0.6%)
RAD50	2 (0.4%)	0 (0%)	2 (0.6%)
WRAP53	2 (0.4%)	0 (0%)	2 (0.6%)
CCND2	2 (0.4%)	1 (0.9%)	1 (0.3%)
CSF1R	2 (0.4%)	0 (0%)	2 (0.6%)
CSF2RB	2 (0.4%)	1 (0.9%)	1 (0.3%)
DHX33	2 (0.4%)	0 (0%)	2 (0.6%)
WHSC1	2 (0.4%)	1 (0.9%)	1 (0.3%)
RPS6KA2	2 (0.4%)	1 (0.9%)	1 (0.3%)
MAP3K9	2 (0.4%)	1 (0.9%)	1 (0.3%)
FLG	2 (0.4%)	2 (1.7%)	0 (0%)
ABCA12	2 (0.4%)	0 (0%)	2 (0.6%)
CTC1	2 (0.4%)	0 (0%)	2 (0.6%)
HCN1	2 (0.4%)	1 (0.9%)	1 (0.3%)
JARID2	2 (0.4%)	0 (0%)	2 (0.6%)
DNM2	2 (0.4%)	0 (0%)	2 (0.6%)
SF1	2 (0.4%)	0 (0%)	2 (0.6%)
EED	2 (0.4%)	1 (0.9%)	1 (0.3%)
PDGFB	2 (0.4%)	2 (1.7%)	0 (0%)

RASA2	2 (0.4%)	0 (0%)	2 (0.6%)
GATA1	2 (0.4%)	0 (0%)	2 (0.6%)
TNK2	2 (0.4%)	0 (0%)	2 (0.6%)
MAP3K4	2 (0.4%)	0 (0%)	2 (0.6%)
JAK3	2 (0.4%)	1 (0.9%)	1 (0.3%)
PRPF40B	2 (0.4%)	1 (0.9%)	1 (0.3%)
DCC	2 (0.4%)	0 (0%)	2 (0.6%)
MYO1F	2 (0.4%)	1 (0.9%)	1 (0.3%)
DIS3	2 (0.4%)	0 (0%)	2 (0.6%)
TINF2	2 (0.4%)	0 (0%)	2 (0.6%)
ZMYM3	2 (0.4%)	0 (0%)	2 (0.6%)
ALK	2 (0.4%)	1 (0.9%)	1 (0.3%)
NUP98	2 (0.4%)	1 (0.9%)	1 (0.3%)
RMI1	2 (0.4%)	0 (0%)	2 (0.6%)
GFI1	2 (0.4%)	0 (0%)	2 (0.6%)
DYNC1H1	1 (0.2%)	1 (0.9%)	0 (0%)
PTPRT	1 (0.2%)	0 (0%)	1 (0.3%)
RET	1 (0.2%)	0 (0%)	1 (0.3%)
DDX54	1 (0.2%)	1 (0.9%)	0 (0%)
ACSS3	1 (0.2%)	0 (0%)	1 (0.3%)
EVI2B	1 (0.2%)	1 (0.9%)	0 (0%)
JAK1	1 (0.2%)	0 (0%)	1 (0.3%)
KDR	1 (0.2%)	0 (0%)	1 (0.3%)
DHX15	1 (0.2%)	0 (0%)	1 (0.3%)
STAT5A	1 (0.2%)	1 (0.9%)	0 (0%)
BRINP3	1 (0.2%)	1 (0.9%)	0 (0%)
BRAF	1 (0.2%)	1 (0.9%)	0 (0%)
IRF4	1 (0.2%)	1 (0.9%)	0 (0%)
COPRS	1 (0.2%)	0 (0%)	1 (0.3%)
PAX5	1 (0.2%)	0 (0%)	1 (0.3%)
PHIP	1 (0.2%)	0 (0%)	1 (0.3%)
RPS6KA3	1 (0.2%)	0 (0%)	1 (0.3%)
SMG1	1 (0.2%)	0 (0%)	1 (0.3%)
EEFSEC	1 (0.2%)	1 (0.9%)	0 (0%)
C6	1 (0.2%)	0 (0%)	1 (0.3%)
NFE2L1	1 (0.2%)	0 (0%)	1 (0.3%)
RASEF	1 (0.2%)	0 (0%)	1 (0.3%)
RASA3	1 (0.2%)	0 (0%)	1 (0.3%)
HRAS	1 (0.2%)	0 (0%)	1 (0.3%)
MYLK2	1 (0.2%)	0 (0%)	1 (0.3%)
RAB11FIP4	1 (0.2%)	0 (0%)	1 (0.3%)
DICER1	1 (0.2%)	1 (0.9%)	0 (0%)
TET1	1 (0.2%)	0 (0%)	1 (0.3%)
SPRED2	1 (0.2%)	0 (0%)	1 (0.3%)
GALNT11	1 (0.2%)	0 (0%)	1 (0.3%)
ZRSR2	1 (0.2%)	0 (0%)	1 (0.3%)
PRKAG2	1 (0.2%)	0 (0%)	1 (0.3%)
JAK2	1 (0.2%)	0 (0%)	1 (0.3%)
BAP1	1 (0.2%)	1 (0.9%)	0 (0%)
NF2	1 (0.2%)	0 (0%)	1 (0.3%)
RASGRF1	1 (0.2%)	0 (0%)	1 (0.3%)
TNK1	1 (0.2%)	1 (0.9%)	0 (0%)

IDNK	1 (0.2%)	0 (0%)	1 (0.3%)
INSRR.NTRK1	1 (0.2%)	1 (0.9%)	0 (0%)
NRXN1	1 (0.2%)	0 (0%)	1 (0.3%)
NXF1	1 (0.2%)	0 (0%)	1 (0.3%)
ANKRD26	1 (0.2%)	0 (0%)	1 (0.3%)
BCL10	1 (0.2%)	0 (0%)	1 (0.3%)
PRPF40A	1 (0.2%)	0 (0%)	1 (0.3%)
SUZ12	1 (0.2%)	0 (0%)	1 (0.3%)
PTEN	1 (0.2%)	0 (0%)	1 (0.3%)
RAD51	1 (0.2%)	0 (0%)	1 (0.3%)
PRPF8	1 (0.2%)	1 (0.9%)	0 (0%)
PLEKHS1	1 (0.2%)	1 (0.9%)	0 (0%)
SPI1	1 (0.2%)	1 (0.9%)	0 (0%)
GALNTL5	1 (0.2%)	1 (0.9%)	0 (0%)
SAMHD1	1 (0.2%)	0 (0%)	1 (0.3%)
NRXN3	1 (0.2%)	1 (0.9%)	0 (0%)
HAX1	1 (0.2%)	0 (0%)	1 (0.3%)
TTC39A	1 (0.2%)	0 (0%)	1 (0.3%)
DKC1	1 (0.2%)	0 (0%)	1 (0.3%)
PHKG1	1 (0.2%)	1 (0.9%)	0 (0%)
WAC	1 (0.2%)	0 (0%)	1 (0.3%)
NUMA1	1 (0.2%)	0 (0%)	1 (0.3%)
FGFR2	1 (0.2%)	0 (0%)	1 (0.3%)
GIGYF2	1 (0.2%)	0 (0%)	1 (0.3%)
RBBP5	1 (0.2%)	0 (0%)	1 (0.3%)
NCOA7	1 (0.2%)	0 (0%)	1 (0.3%)

[1] *n (%)*

[2] *Fisher's exact test (p-value not adjusted for multiple testing)*

Supplemental Table S5: Functional categorization of recurrently mutated genes (>1%).

Methylation	Chromatin	Cohesin	Splicing	Signaling	Transcription	Other	Tumor suppressor
DNMT3A	*ACIN1*	*RAD21*	*HNRNPK*	*CBL*	*ARID1A*	*CSMD2*	*CSMD1*
IDH1	*ARID2*	*SMC1A*	*SF1*	*CSF3R*	*CEBPA*	*DNAH9*	*TP53*
IDH2	*ASXL1*	*SMC3*	*SF3A1*	*FLT3*	*ETV6*	*FBXW7*	
TET2	*ASXL2*	*STAG2*	*SF3B1*	*GNAS*	*FOXP1*	*LAMC3*	
	BCOR		*SRSF2*	*GPR98*	*GATA2*	*NPM1*	
	BCORL1		*U2AF1*	*KIT*	*MGA*	*OBSCN*	
	CREBBP		*ZRSR2*	*KRAS*	*MN1*	*ROBO1*	
	EP300			*NF1*	*MYC*	*RYR2*	
	EZH2			*NOTCH1*	*NFE2*	*SYNE1*	
	KDM6A			*NRAS*	*PHF6*		
	KMT2A			*PTPN11*	*RUNX1*		
	KMT2D			*PTPRF*	*WT1*		
	KMT2E			*UBXN11*	*ZBTB33*		
	KMT2C				*ZBTB7A*		
	SETD2				*ZNF318*		
	SRCAP						

Supplemental Table S6: Genomic classes according to Papaemmanuil E, Gerstung M et al NEJM 2016.

Genomic subgroup (long)	**Genomic subgroup (short)**
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB–MYH11	inv(16)
AML with t(8;21)(q22;q22); RUNX1–RUNX1T1	t(8;21)
AML with MLL fusion genes; t(x;11)(x;q23)	t(11q23;x)
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); GATA2, MECOM(EVI1)	inv(3)
AML with t(6;9)(p23;q34); DEK–NUP214	t(6;9)
AML with t(15;17)(q22;q12); PML–RARA	t(15;17)
AML with NPM1 mutation	NPM1
AML with biallelic CEBPA mutations*	$CEBPA^{biallelic}$
AML with TP53 mutations, chromosomal aneuploidy, or both#	TP53-aneuploidy
AML with mutated chromatin, RNA-splicing genes, or both+	Chromatin-Spliceosome
AML with IDH2R172 mutations and no other class-defining lesions	$IDH2^{R172}$
AML meeting criteria for ≥2 genomic subgroups	2 classes
AML with driver mutations but no detected class-defining lesions	No class
AML with no detected driver mutations	No drivers detected

* Patients with two different mutations in *CEBPA*

Classification in this subgroup requires *TP53* mutation, complex karyotype [3 or more abnormalities, in the absence of 1 of the WHO-designated recurring translocations or inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11),t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with t(9;22)], or in the absence of other class-defining lesions, one or more of the following: −7/7q, −5/5q, −4/4q, −9q, −12/12p, −17/−17p, −18/18q, −20/20q, +11/11q, +13, +21, or +22.

+ Classification in this subgroup requires one or more driver mutations in *RUNX1*, *ASXL1*, *BCOR*, *STAG2*, *EZH2*, *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, or *MLL*-PTD. In the presence of other class-defining lesions — namely, inv(16), t(15;17), t(8;21), t(6;9), inv(3), MLL fusion genes, or complex karyotype or driver mutations in *TP53*, *NPM1*, or $CEBPA^{biallelic}$ — two or more chromatin–spliceosome mutations are required.

Supplemental Table S7: Baseline characteristics of genomic AML classes in the cohort of 451 of 475 patients, in which subcategorization into genomic AML classes was possible.

	NPM1 **(N=287)**	**CBF (N=18)**	***TP53-aneuploidy*** **(N=18)**	**Chromatin-spliceosome (N=68)**	**No Class (N=60)**	**Overall (N=451)**
Age (years)						
Mean (SD)	46.9 (10.1)	44.3 (10.4)	46.3 (9.80)	47.2 (10.5)	39.6 (12.2)	45.9 (10.7)
Median [Min,Max]	48.8 [18.0,59.9]	44.3 [23.4,57.5]	48.4 [22.0,57.4]	50.2 [19.5,59.8]	38.8 [19.4,59.1]	48.1 [18.0,59.9]
Sex						
Male	109 (38.0%)	10 (55.6%)	7 (38.9%)	36 (52.9%)	31 (51.7%)	193 (42.8%)
Female	178 (62.0%)	8 (44.4%)	11 (61.1%)	32 (47.1%)	29 (48.3%)	258 (57.2%)
FLT3 **mutation type**						
TKD	70 (24.4%)	12 (66.7%)	7 (38.9%)	12 (17.6%)	8 (13.3%)	109 (24.2%)
ITD <0.5 allelic ratio	74 (25.8%)	3 (16.7%)	5 (27.8%)	21 (30.9%)	16 (26.7%)	119 (26.4%)
ITD ≥0.5 allelic ratio	143 (49.8%)	3 (16.7%)	6 (33.3%)	35 (51.5%)	35 (58.3%)	222 (49.2%)
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.7%)	1 (0.2%)
WBC count at baseline (10E9/L)						
Mean (SD)	54.8 (55.8)	41.6 (43.7)	40.9 (45.3)	43.8 (47.3)	47.1 (45.7)	51.1 (52.5)
Median [Min,Max]	39.6 [1.40,330]	26.0 [1.40,159]	25.9 [2.70,154]	28.6 [0.600,236]	30.2 [1.20,207]	35.5 [0.600,330]
Missing	1 (0.3%)	0 (0%)	1 (5.6%)	1 (1.5%)	0 (0%)	3 (0.7%)
ECOG performance status						
0-1	255 (88.9%)	17 (94.4%)	17 (94.4%)	64 (94.1%)	50 (83.3%)	403 (89.4%)
2	32 (11.1%)	1 (5.6%)	1 (5.6%)	4 (5.9%)	10 (16.7%)	48 (10.6%)
Treatment						
Placebo	136 (47.4%)	9 (50.0%)	8 (44.4%)	31 (45.6%)	26 (43.3%)	210 (46.6%)
Midostaurin	151 (52.6%)	9 (50.0%)	10 (55.6%)	37 (54.4%)	34 (56.7%)	241 (53.4%)
Allogeneic HCT in CR1						
No	211 (73.5%)	13 (72.2%)	16 (88.9%)	48 (70.6%)	44 (73.3%)	332 (73.6%)
Yes	76 (26.5%)	5 (27.8%)	2 (11.1%)	20 (29.4%)	16 (26.7%)	119 (26.4%)

Supplemental Table S8: Impact of 12 most frequent gene mutations on overall and event-free survival. Log rank test p-values from the univariate tests are indicated without (raw) and with adjustment (adj) for multiple testing via the Bonferroni-Holm procedure (FDR). See corresponding Kaplan Meier estimates in Supplemental Figure 2 and 3). Abbreviations: CI, confidence interval; HR, hazard ratio.

	Overall Surival					Event-free Survival				
	Logrank p-value (raw)	**Logrank p-value (adj)**	**HR**	**Lower CI**	**Upper CI**	**Logrank p-value (raw)**	**Logrank p-value (adj)**	**HR**	**Lower CI**	**Upper CI**
NPM1	<0.001	<0.001	0.60	0.46	0.77	<0.001	<0.001	0.60	0.48	0.74
WT1	<0.001	<0.001	1.83	1.38	2.43	0.018	0.195	1.36	1.05	1.75
ASXL1	0.013	0.125	1.67	1.11	2.52	0.100	0.903	0.72	0.48	1.07
IDH2	0.130	1	0.65	0.37	1.14	0.075	0.748	0.64	0.38	1.05
NRAS	0.097	0.875	0.68	0.43	1.08	0.793	1	1.05	0.71	1.55
DNMT3A	0.984	1	1.00	0.77	1.30	0.275	1	0.88	0.71	1.10
IDH1	0.773	1	1.07	0.68	1.69	0.649	1	0.91	0.61	1.35
PTPN11	0.287	1	0.77	0.48	1.25	0.204	1	0.75	0.48	1.17
RUNX1	0.141	1	1.32	0.91	1.90	0.310	1	0.83	0.58	1.19
SMC1A	0.219	1	0.69	0.37	1.26	0.183	1	1.25	0.90	1.73
SMC3	0.841	1	0.94	0.50	1.77	0.433	1	0.81	0.47	1.38
TET2	0.460	1	0.85	0.56	1.30	0.600	1	1.09	0.79	1.52

Supplemental Table S9: 4-year overall survival rates by genomic AML classes in the cohort of 451 of 475 patients, in which subcategorization into genomic AML classes was possible.

	N	**Number at risk**	**Events**	**Survival**	**Lower 95% CI**	**Higher 95% CI**
NPM1	287	136	120	56.9%	0.51	0.63
CBF	18	11	5	72.2%	0.54	0.96
***TP53*-aneuploidy**	18	5	11	35.3%	0.19	0.67
Chromatin-spliceosome	68	18	45	32.9%	0.23	0.46
No class	60	16	36	36.5%	0.26	0.52

Supplemental Table S10: 4-year event-free survival rates by genomic AML classes in the cohort of 451 of 475 patients, in which subcategorization into genomic AML classes was possible.

	N	Number at risk	Events	Survival	Lower 95% CI	Higher 95% CI
NPM1	287	87	177	37.7%	0.32	0.44
CBF	18	7	9	50.0%	0.32	0.79
***TP53*-aneuploidy**	18	1	17	5.6%	0.01	0.37
Chromatin-spliceosome	68	8	56	16.3%	0.09	0.28
No class	60	3	53	9.5%	0.04	0.22

Supplemental Table S11: Cox proportional hazard model for predictive impact of *FLT3* mutation type on hazard of death or event after treatment with midostaurin in cohort of 451 of 475 patients, in which subcategorization into genomic AML classes was possible. A hazard ratio of >1 indicates a higher and a hazard ratio of <1 a lower risk of death, respectively. Abbreviations: CBF, Core-binding factor AML; CI.95, 95% confidence interval; CR1, first complete remission; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; HCT, hematopoietic cell transplantation; WBC, white blood cell count.

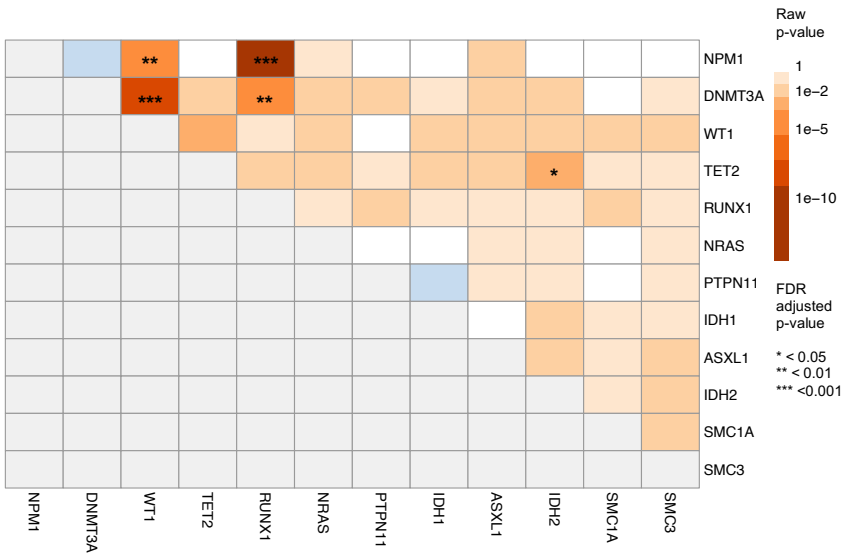
	Overall survival		Event-free survival	
Variable	HR [CI.95]	p-value	HR [CI.95]	p-value
Genomic classes				
CBF vs *NPM1*	0.67 [0.27;1.67]	0.392	0.80 [0.40;1.58]	0.522
TP53-aneuploidy vs *NPM1*	1.94 [1.04;3.61]	0.037	3.67 [2.21;6.09]	<0.001
Chromatin-Spliceosome vs *NPM1*	2.27 [1.61;3.21]	<0.001	1.89 [1.38;2.57]	<0.001
No Class vs *NPM1*	2.13 [1.43;3.17]	<0.001	2.51 [1.79;3.50]	<0.001
Age	1.01 [0.99;1.02]	0.338	1.00 [0.99;1.01]	0.849
Sex				
Female vs male	1.00 [0.76;1.32]	0.995	1.33 [1.06;1.68]	0.015
ECOG (0-1 vs 2)	1.28 [0.85;1.92]	0.236	0.87 [0.60;1.27]	0.475
log2WBC	1.08 [1.00;1.17]	0.045	1.09 [1.02;1.16]	0.014
Allogeneic HCT in CR1	0.58 [0.40;0.83]	0.003	0.72 [0.49;1.06]	0.093
***FLT3* TKD**				
Midostaurin vs Placebo	0.59 [0.32;1.11]	0.100	0.70 [0.43;1.17]	0.174
***FLT3* ITD <0.5 allelic ratio**				
Midostaurin vs Placebo	0.56 [0.32;1.00]	0.049	0.61 [0.39;0.97]	0.035
***FLT3* ITD ≥0.5 allelic ratio**				
Midostaurin vs Placebo	0.66 [0.47;0.94]	0.019	0.67 [0.49;0.91]	0.009
Placebo				
ITD <0.5 allelic ratio vs TKD	1.13 [0.64;2.01]	0.665	1.40 [0.87;2.25]	0.163
ITD ≥0.5 allelic ratio vs TKD	1.49 [0.90;2.47]	0.126	1.52 [1.00;2.32]	0.052

Midostaurin				
ITD <0.5 allelic ratio vs TKD	1.08 [0.58;2.03]	0.811	1.22 [0.74;2.00]	0.430
ITD ≥0.5 allelic ratio vs TKD	1.65 [0.98;2.78]	0.058	1.44 [0.94;2.20]	0.090

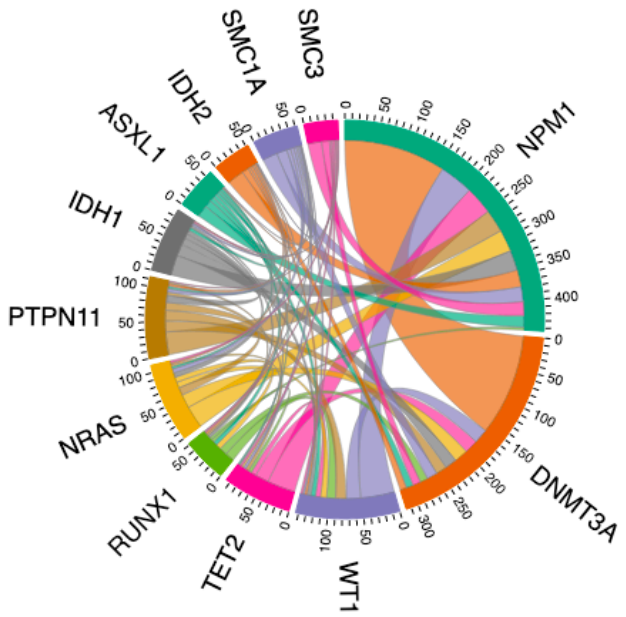
SUPPLEMENTAL FIGURES

Supplemental Figure S1: Mutational exclusivity and co-occurrence of recurrently (>5% of cases) mutated genes. A) Gene pairs that co-occurred more frequently are indicated by blue colors, gene pairs that co-occurred seldom by orange colors. The top 12 genes were tested for mutual exclusivity (66 possible combinations) resulting in 21 significant pairs before adjustment for multiple testing and 5 significant pairs after FDR adjustment: *NPM1-RUNX1* (p<.001), *DNMT3A-WT1* (p<.001), *DNMT3A-RUNX1* (p=.003), *NPM1-WT1* (p=.008) and *IDH2-TET2* (p=.021). Similarly, all combinations were tested for co-occurrence resulting in 2 significant pairs before adjustment for multiple testing: *NPM1-DNMT3A* and *IDH1-PTPN11*, which were not significant after FDR adjustment B) Width of bands reflects the number of cases in which mutations of corresponding genes co-occurred.

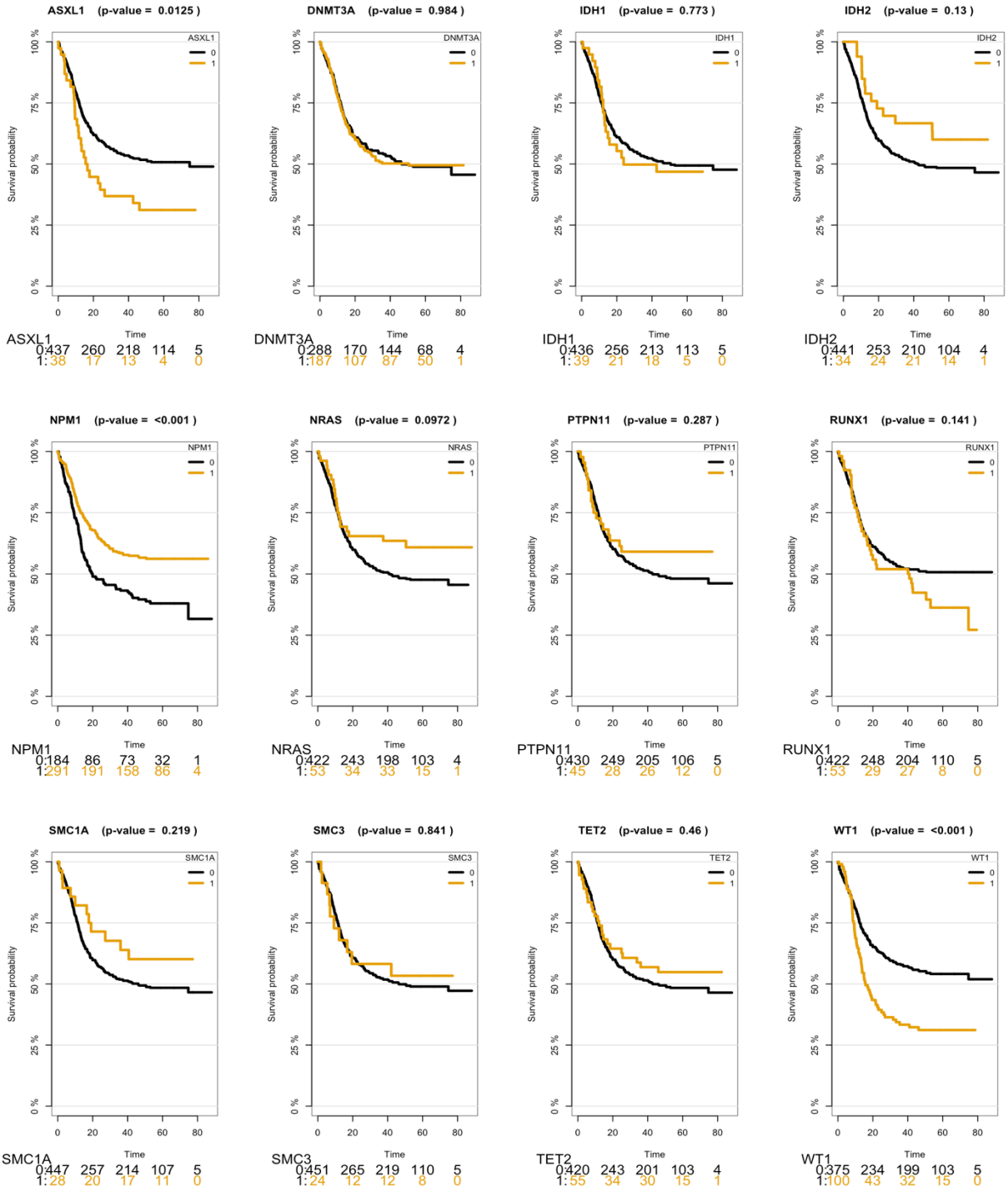
A



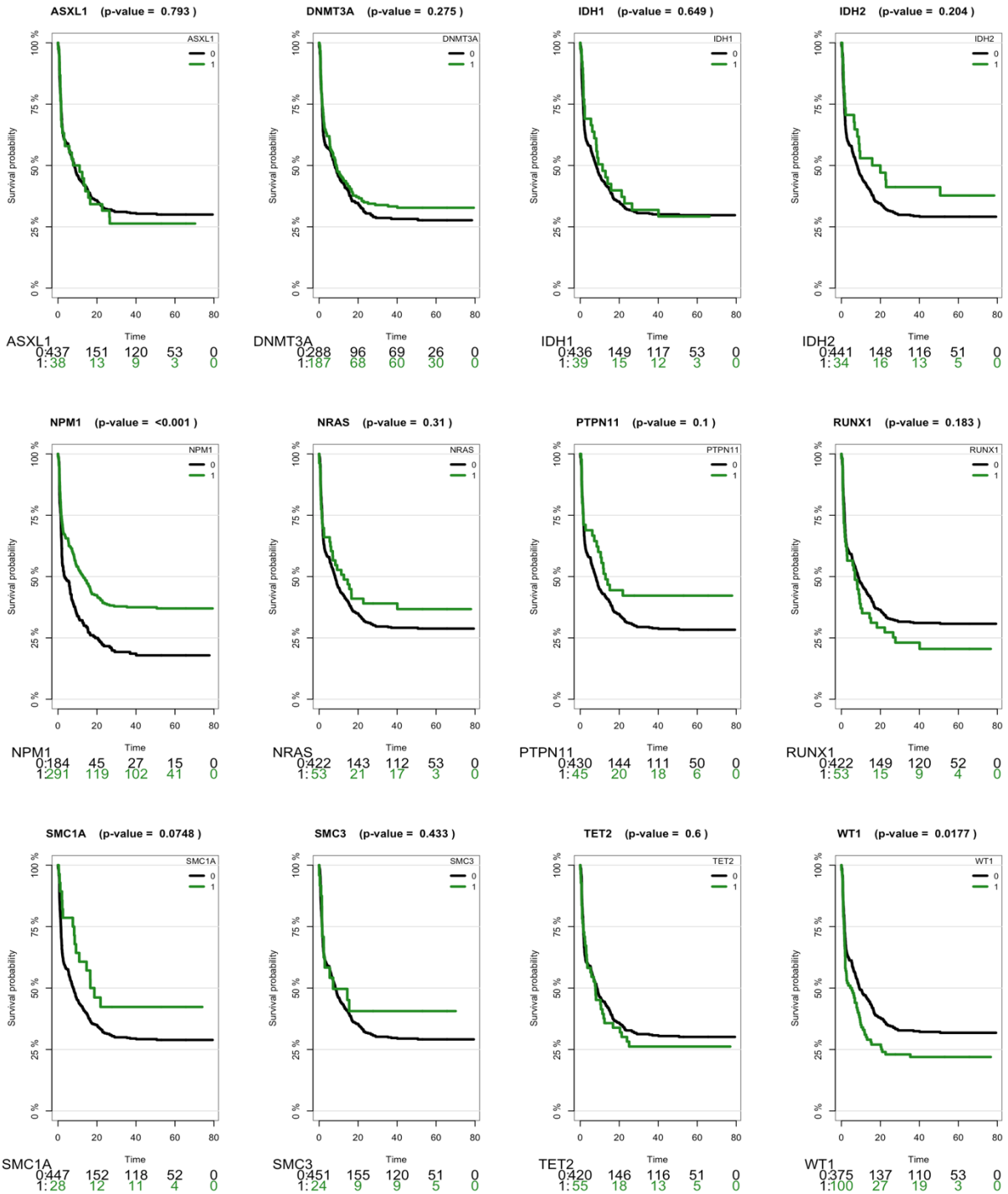
B



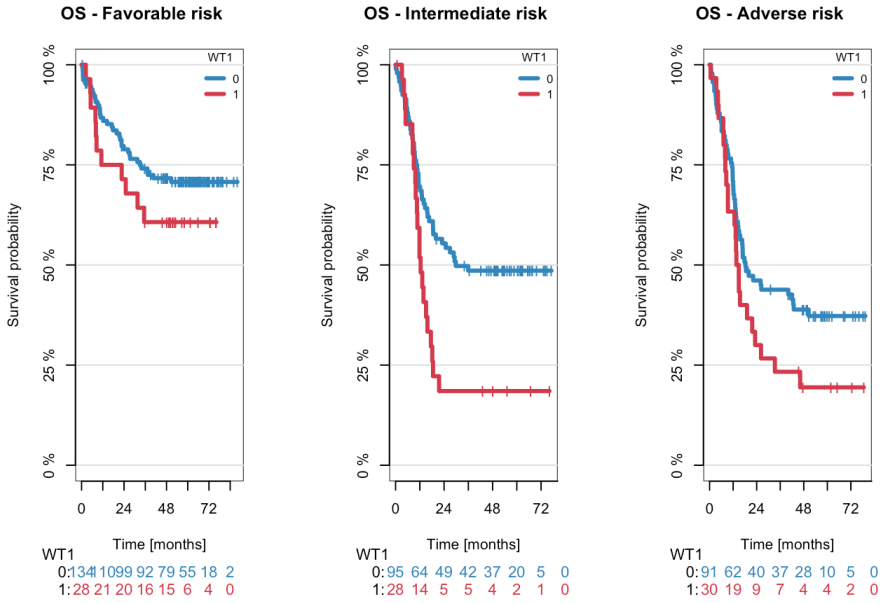
Supplemental Figure S2: Impact of gene mutations on overall survival. Log rank test p-values from the univariate tests are indicated without adjustment for multiple testing. Adjusted p-values are given in Supplemental Table S8.



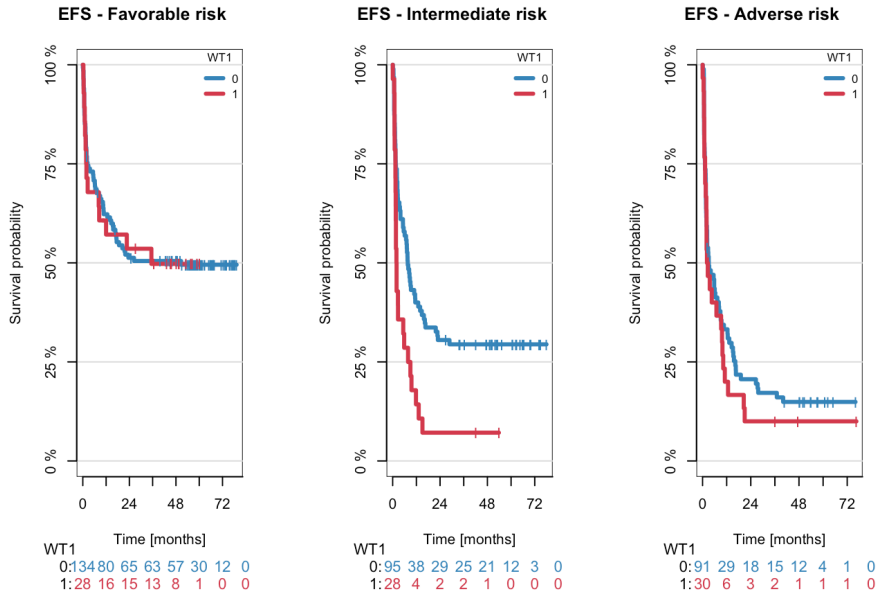
Supplemental Figure S3: Impact of gene mutations on event-free survival. Log rank test p-values from the univariate tests are indicated without adjustment for multiple testing. Adjusted p-values are given in Supplemental Table S8.



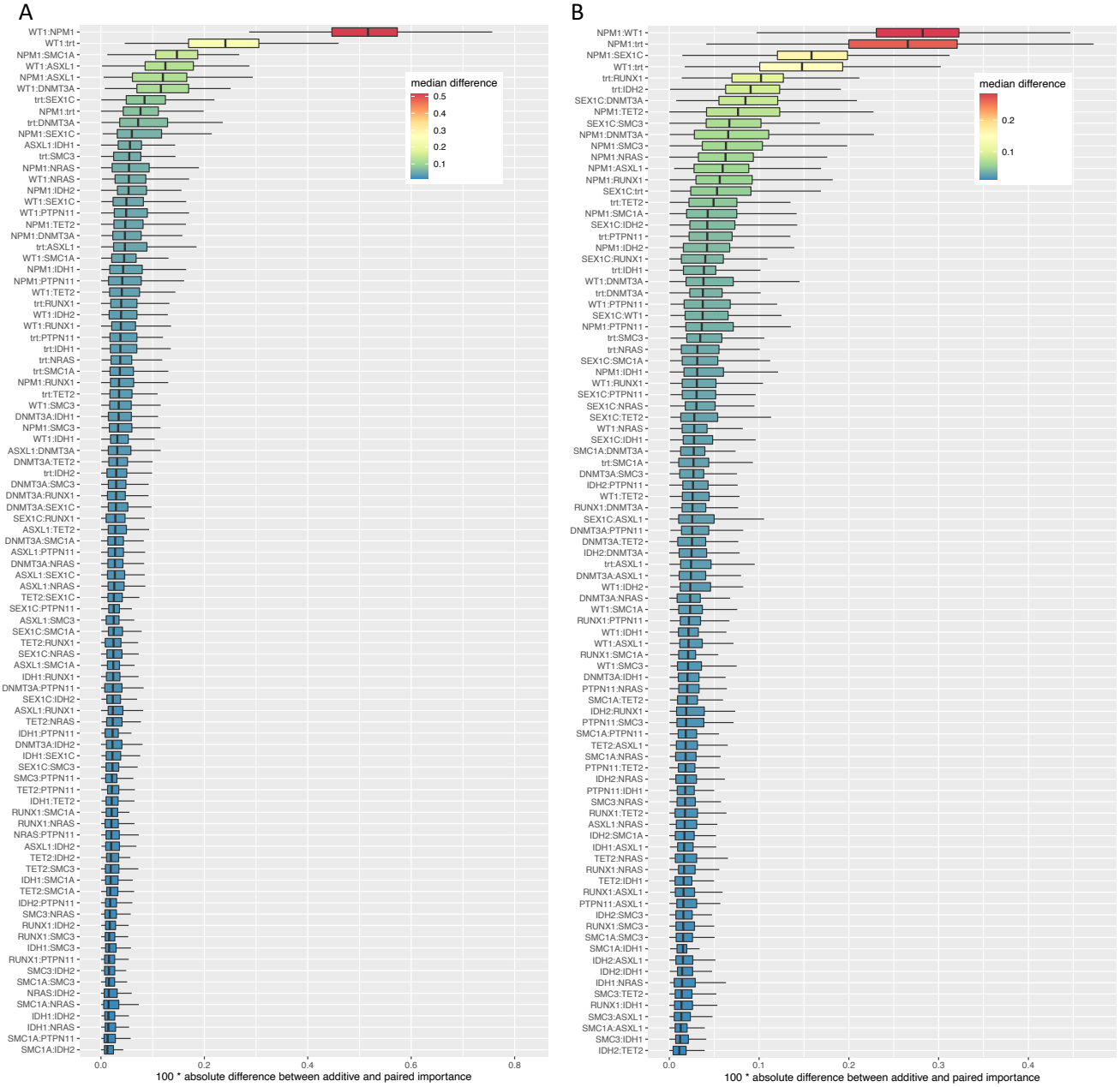
Supplemental Figure S4: Impact of *WT1* mutations on overall survival stratified by ELN2017 risk groups.



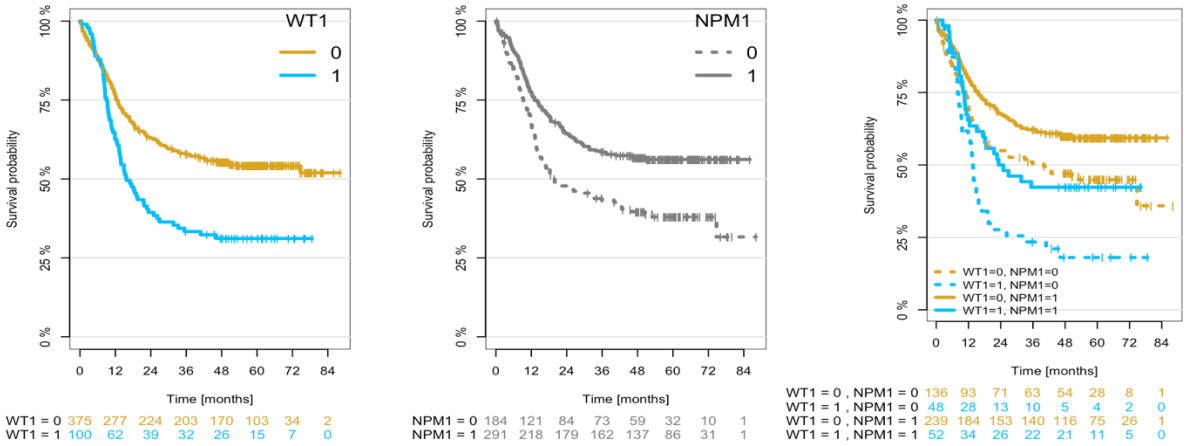
Supplemental Figure S5: Impact of *WT1* mutations on event-free survival stratified by ELN2017 risk groups.

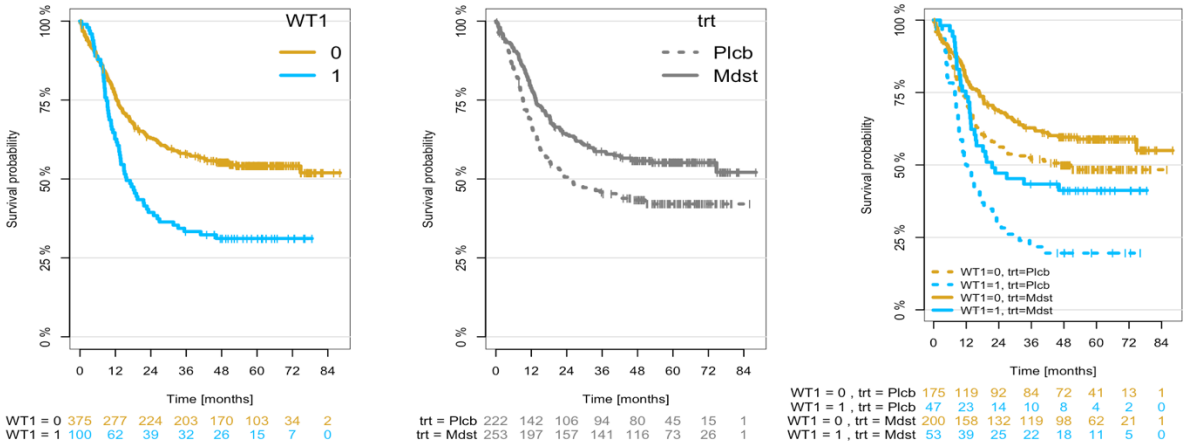


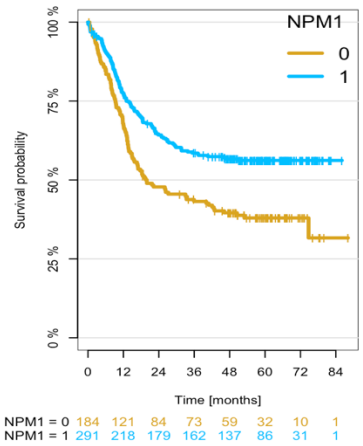
Supplemental Figure S6: Prognostic and possibly predictive impact of pairwise interactions of clinical and/or genetic variables in 475 patients on A) overall and B) event-free survival using random survival forests. The prognostic impact of a variable is measured via "vimp" (variable importance). This measure determines the loss in prediction accuracy using a permuted/noisy version of each variable for model fitting and predicting out-of-bag samples. The assessment of pairwise interactions between variables was based on the comparison of the joint ('paired') VIMP to the sum of their individual VIMPs (called 'additive' importance). Fitting 1000 trees per forest, we constructed 100 forests with different seeds. The following graphic depicts the variable importance for all varibles across these 100 runs in terms of variability (box plots). Higher positive or negative difference between additive and paired vimp values indicate that a variable combination may have prognostic or predictive impact on the survival endpoint.

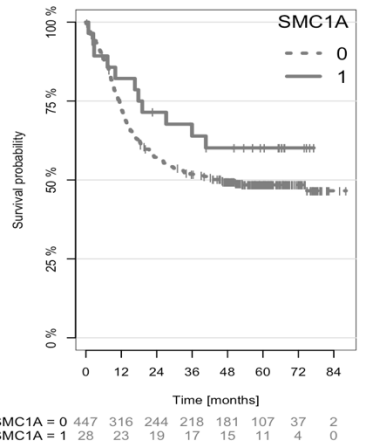


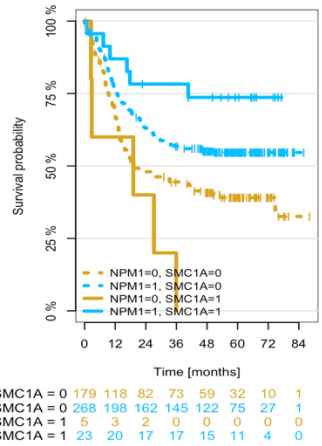
Supplemental Figure S7: The top 10 most interesting interactions regarding overall survival as determined by random survival forests were selected for further inspection. The following Kaplan Meier curves depict the marginal distribution of first variable (left) and the second variable (middle) as well as the combination of the two (right).

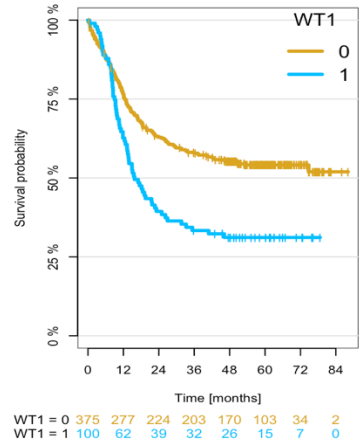


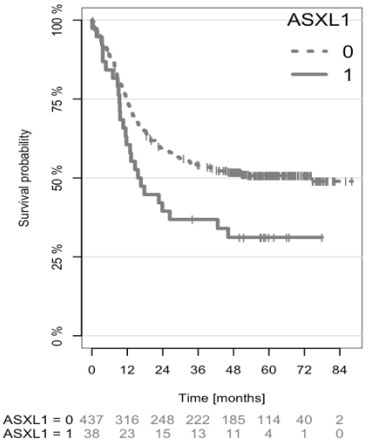


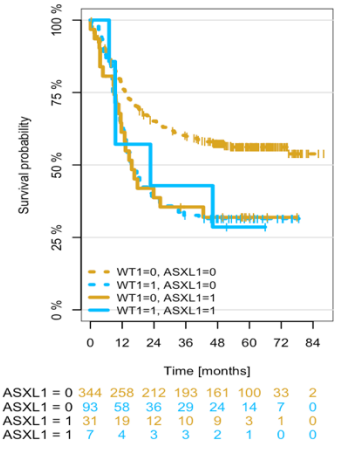

NPM1
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
NPM1 = 0 184 121 84 73 59 32 10 1
NPM1 = 1 291 218 179 162 137 86 31 1

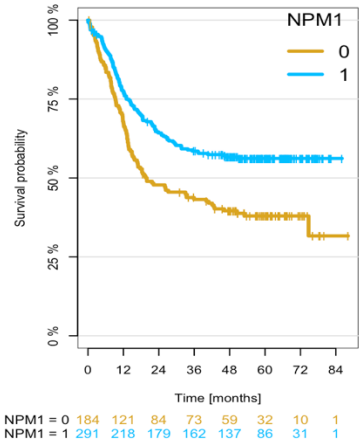

SMC1A
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
SMC1A = 0 447 316 244 218 181 107 37 2
SMC1A = 1 28 23 19 17 15 11 4 0

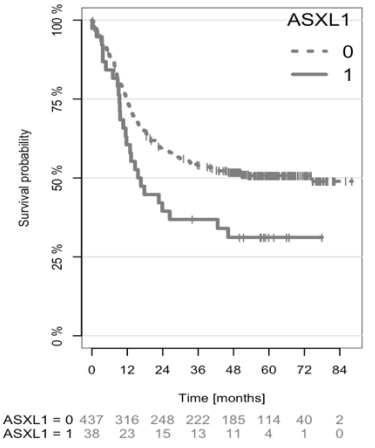

Survival probability
100 %
75 %
50 %
25 %
0 %
NPM1=0, SMC1A=0
NPM1=1, SMC1A=0
NPM1=0, SMC1A=1
NPM1=1, SMC1A=1
0 12 24 36 48 60 72 84
Time [months]
NPM1 = 0 , SMC1A = 0 179 118 82 73 59 32 10 1
NPM1 = 1 , SMC1A = 0 268 198 162 145 122 75 27 1
NPM1 = 0 , SMC1A = 1 5 3 2 0 0 0 0 0
NPM1 = 1 , SMC1A = 1 23 20 17 17 15 11 4 0

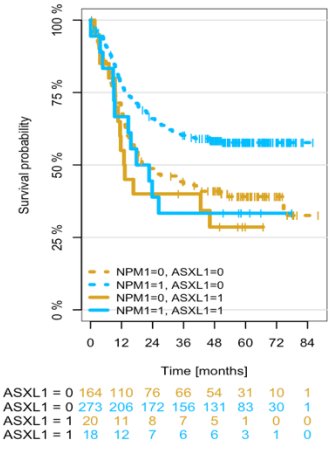

WT1
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
WT1 = 0 375 277 224 203 170 103 34 2
WT1 = 1 100 62 39 32 26 15 7 0

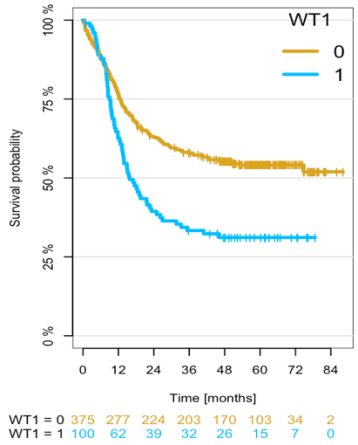

ASXL1
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
ASXL1 = 0 437 316 248 222 185 114 40 2
ASXL1 = 1 38 23 15 13 11 4 1 0

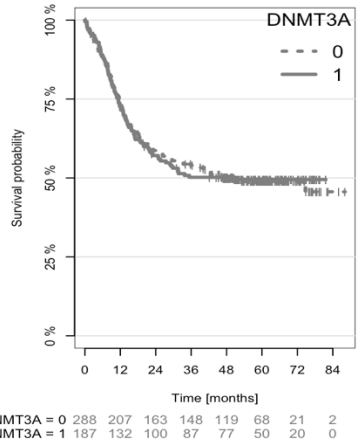

Survival probability
100 %
75 %
50 %
25 %
0 %
WT1=0, ASXL1=0
WT1=1, ASXL1=0
WT1=0, ASXL1=1
WT1=1, ASXL1=1
0 12 24 36 48 60 72 84
Time [months]
WT1 = 0 , ASXL1 = 0 344 258 212 193 161 100 33 2
WT1 = 1 , ASXL1 = 0 93 58 36 29 24 14 7 0
WT1 = 0 , ASXL1 = 1 31 19 12 10 9 3 1 0
WT1 = 1 , ASXL1 = 1 7 4 3 3 2 1 0 0

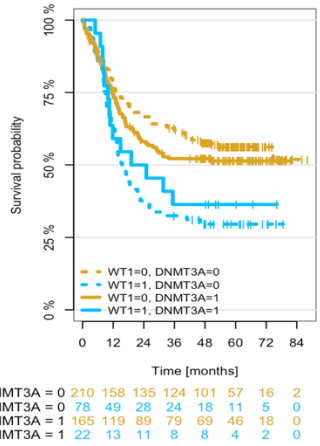

NPM1
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
NPM1 = 0 184 121 84 73 59 32 10 1
NPM1 = 1 291 218 179 162 137 86 31 1

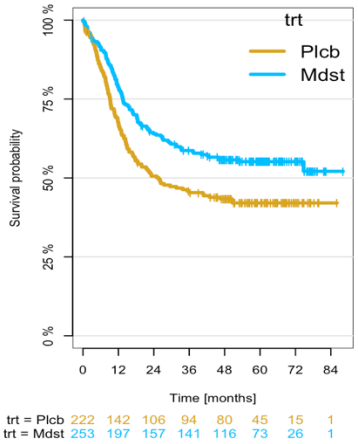

ASXL1
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
ASXL1 = 0 437 316 248 222 185 114 40 2
ASXL1 = 1 38 23 15 13 11 4 1 0

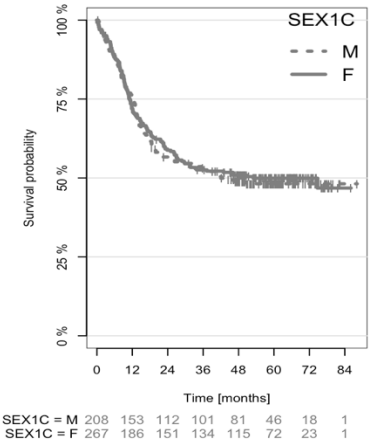

Survival probability
100 %
75 %
50 %
25 %
0 %
NPM1=0, ASXL1=0
NPM1=1, ASXL1=0
NPM1=0, ASXL1=1
NPM1=1, ASXL1=1
0 12 24 36 48 60 72 84
Time [months]
NPM1 = 0 , ASXL1 = 0 164 110 76 66 54 31 10 1
NPM1 = 1 , ASXL1 = 0 273 206 172 156 131 83 30 1
NPM1 = 0 , ASXL1 = 1 20 11 8 7 5 1 0 0
NPM1 = 1 , ASXL1 = 1 18 12 7 6 6 3 1 0

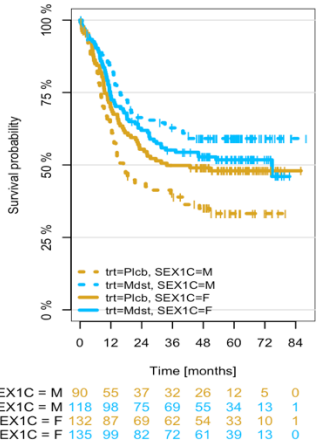

WT1
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
WT1 = 0 375 277 224 203 170 103 34 2
WT1 = 1 100 62 39 32 26 15 7 0

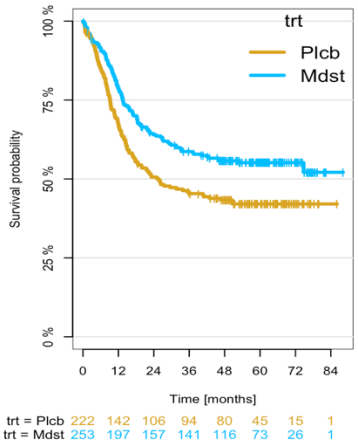

DNMT3A
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
DNMT3A = 0 288 207 163 148 119 68 21 2
DNMT3A = 1 187 132 100 87 77 50 20 0

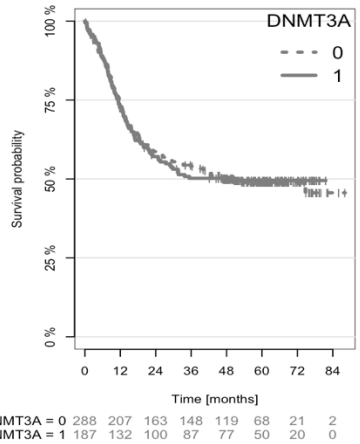

WT1=0, DNMT3A=0
WT1=1, DNMT3A=0
WT1=0, DNMT3A=1
WT1=1, DNMT3A=1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
WT1 = 0 , DNMT3A = 0 210 158 135 124 101 57 16 2
WT1 = 1 , DNMT3A = 0 78 49 28 24 18 11 5 0
WT1 = 0 , DNMT3A = 1 165 119 89 79 69 46 18 0
WT1 = 1 , DNMT3A = 1 22 13 11 8 8 4 2 0

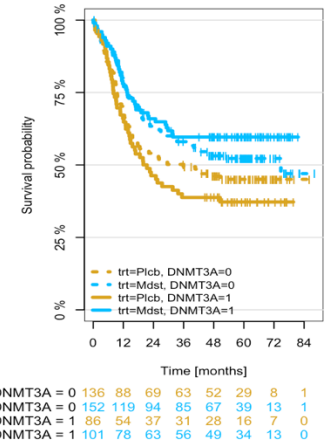

trt
Plcb
Mdst
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
trt = Plcb 222 142 106 94 80 45 15 1
trt = Mdst 253 197 157 141 116 73 26 1

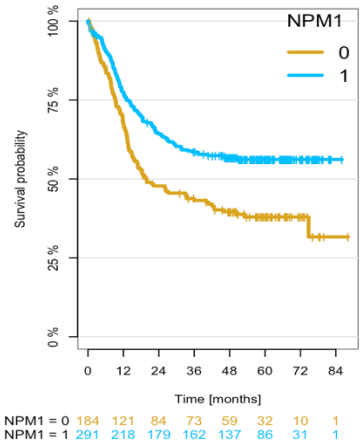

SEX1C
M
F
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
SEX1C = M 208 153 112 101 81 46 18 1
SEX1C = F 267 186 151 134 115 72 23 1

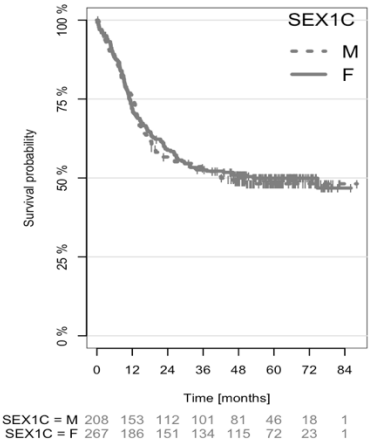

trt=Plcb, SEX1C=M
trt=Mdst, SEX1C=M
trt=Plcb, SEX1C=F
trt=Mdst, SEX1C=F
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
trt = Plcb , SEX1C = M 90 55 37 32 26 12 5 0
trt = Mdst , SEX1C = M 118 98 75 69 55 34 13 1
trt = Plcb , SEX1C = F 132 87 69 62 54 33 10 1
trt = Mdst , SEX1C = F 135 99 82 72 61 39 13 0

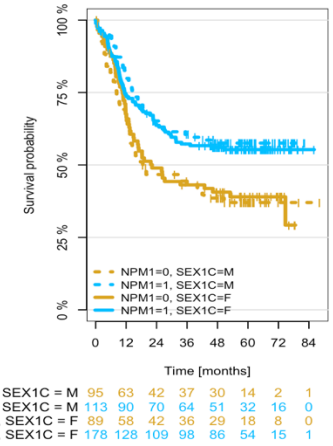

trt
Plcb
Mdst
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
trt = Plcb 222 142 106 94 80 45 15 1
trt = Mdst 253 197 157 141 116 73 26 1

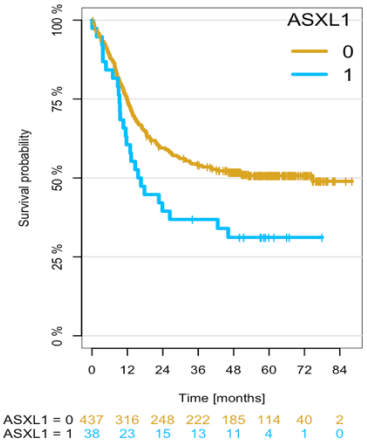

DNMT3A
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
DNMT3A = 0 288 207 163 148 119 68 21 2
DNMT3A = 1 187 132 100 87 77 50 20 0

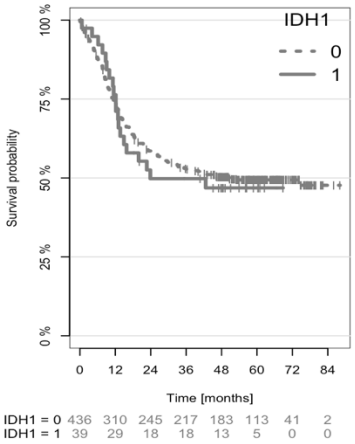

trt=Plcb, DNMT3A=0
trt=Mdst, DNMT3A=0
trt=Plcb, DNMT3A=1
trt=Mdst, DNMT3A=1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
trt = Plcb , DNMT3A = 0 136 88 69 63 52 29 8 1
trt = Mdst , DNMT3A = 0 152 119 94 85 67 39 13 1
trt = Plcb , DNMT3A = 1 86 54 37 31 28 16 7 0
trt = Mdst , DNMT3A = 1 101 78 63 56 49 34 13 0

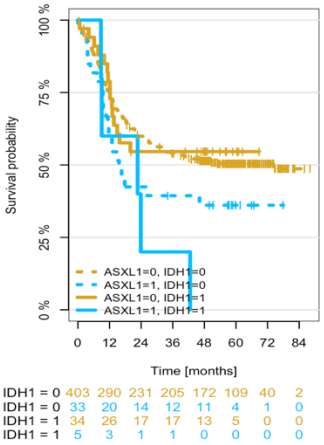

NPM1
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
NPM1 = 0 184 121 84 73 59 32 10 1
NPM1 = 1 291 218 179 162 137 86 31 1


SEX1C
M
F
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
SEX1C = M 208 153 112 101 81 46 18 1
SEX1C = F 267 186 151 134 115 72 23 1

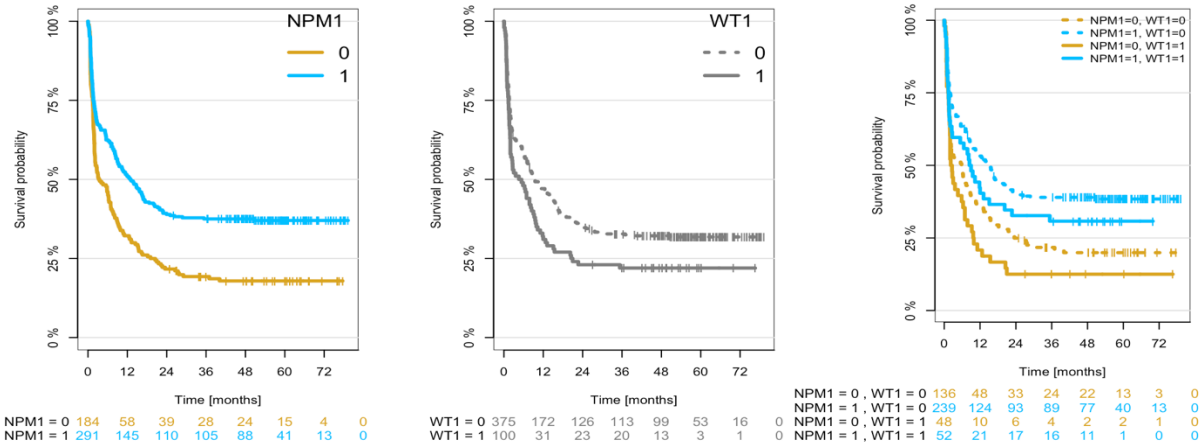

NPM1=0, SEX1C=M
NPM1=1, SEX1C=M
NPM1=0, SEX1C=F
NPM1=1, SEX1C=F
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
NPM1 = 0 , SEX1C = M 95 63 42 37 30 14 2 1
NPM1 = 1 , SEX1C = M 113 90 70 64 51 32 16 0
NPM1 = 0 , SEX1C = F 89 58 42 36 29 18 8 0
NPM1 = 1 , SEX1C = F 178 128 109 98 86 54 15 1

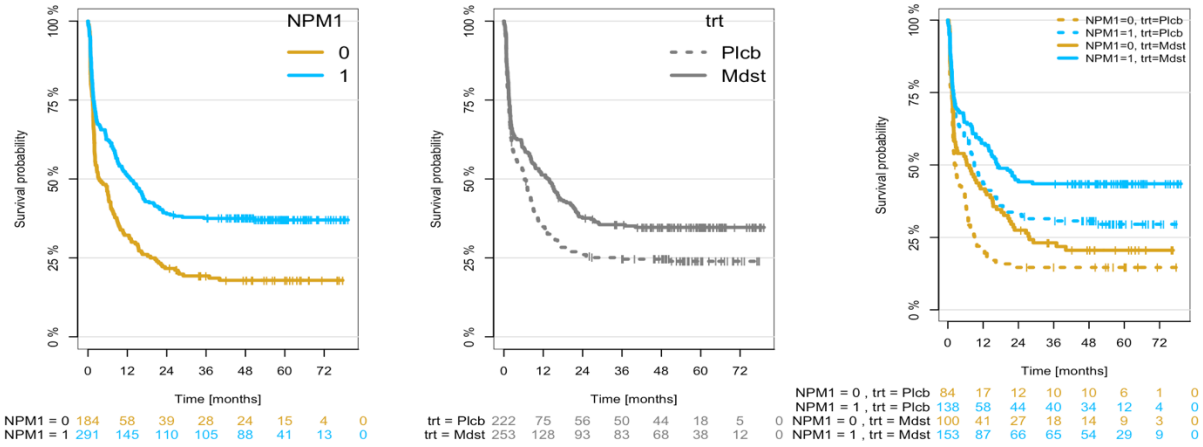

ASXL1
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
ASXL1 = 0 437 316 248 222 185 114 40 2
ASXL1 = 1 38 23 15 13 11 4 1 0

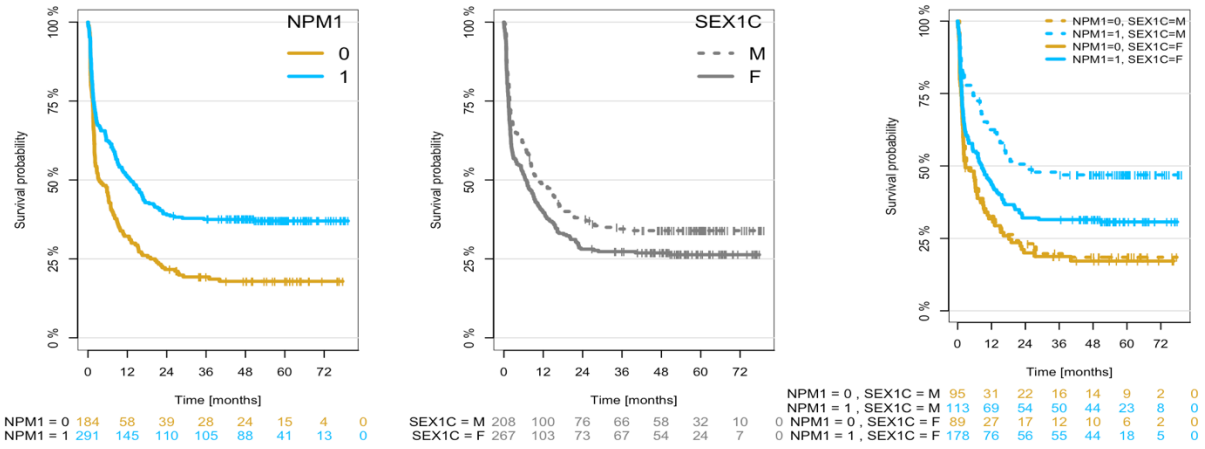

IDH1
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
IDH1 = 0 436 310 245 217 183 113 41 2
IDH1 = 1 39 29 18 18 13 5 0 0

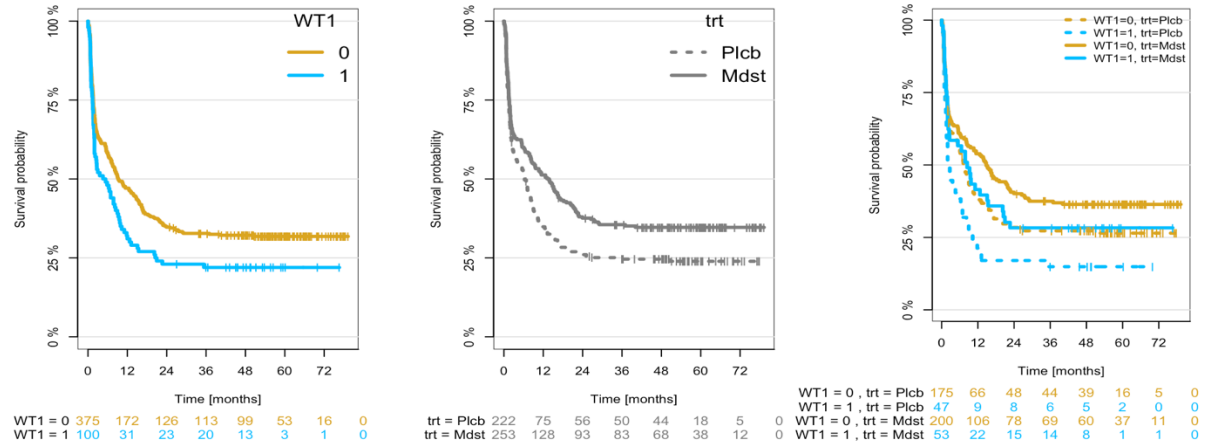

ASXL1=0, IDH1=0
ASXL1=1, IDH1=0
ASXL1=0, IDH1=1
ASXL1=1, IDH1=1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72 84
Time [months]
ASXL1 = 0 , IDH1 = 0 403 290 231 205 172 109 40 2
ASXL1 = 1 , IDH1 = 0 33 20 14 12 11 4 1 0
ASXL1 = 0 , IDH1 = 1 34 26 17 17 13 5 0 0
ASXL1 = 1 , IDH1 = 1 5 3 1 1 0 0 0 0

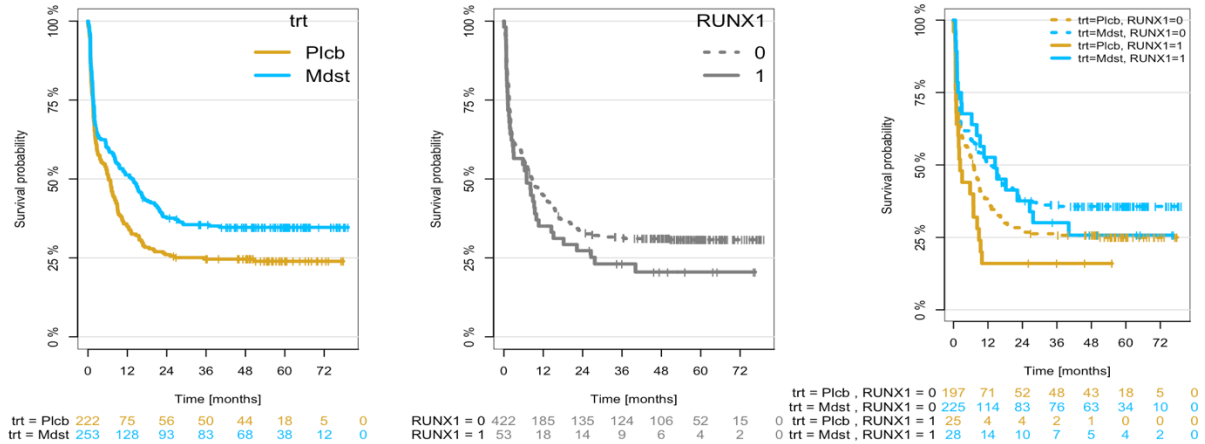
Supplemental Figure S8: The top 10 most interesting interactions regarding event-free survival as determined by random survival forests were selected for further inspection. The following Kaplan Meier curves depict the marginal distribution of first variable (left) and the second variable (middle) as well as the combination of the two (right).

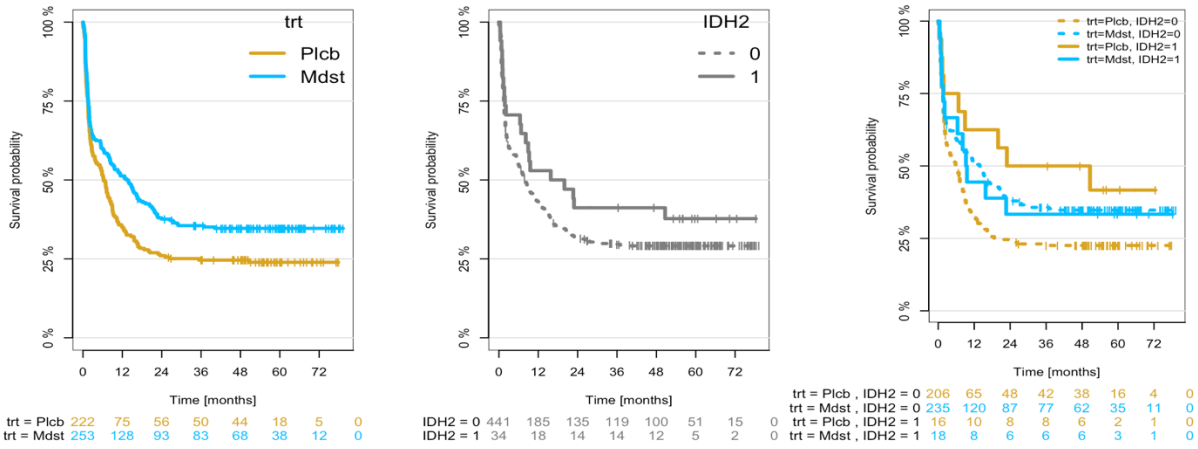


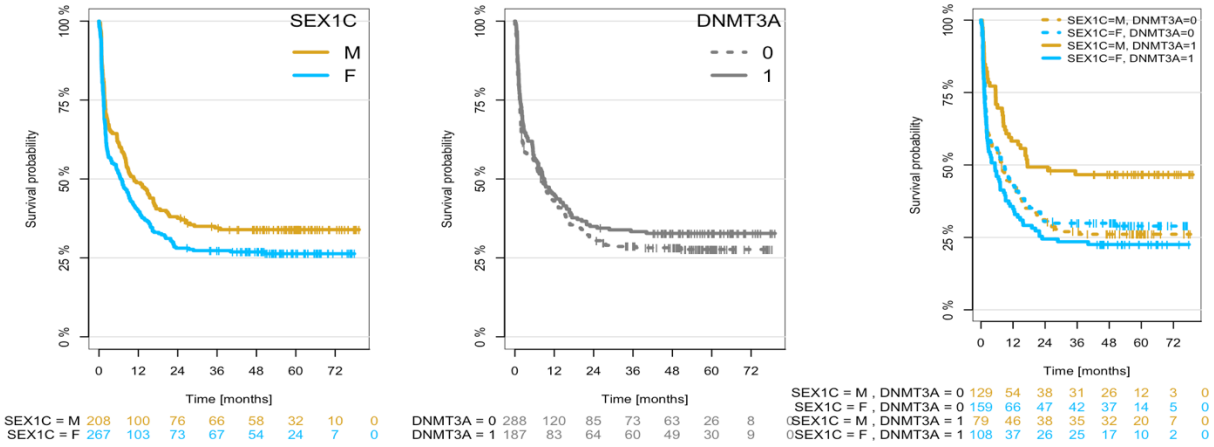


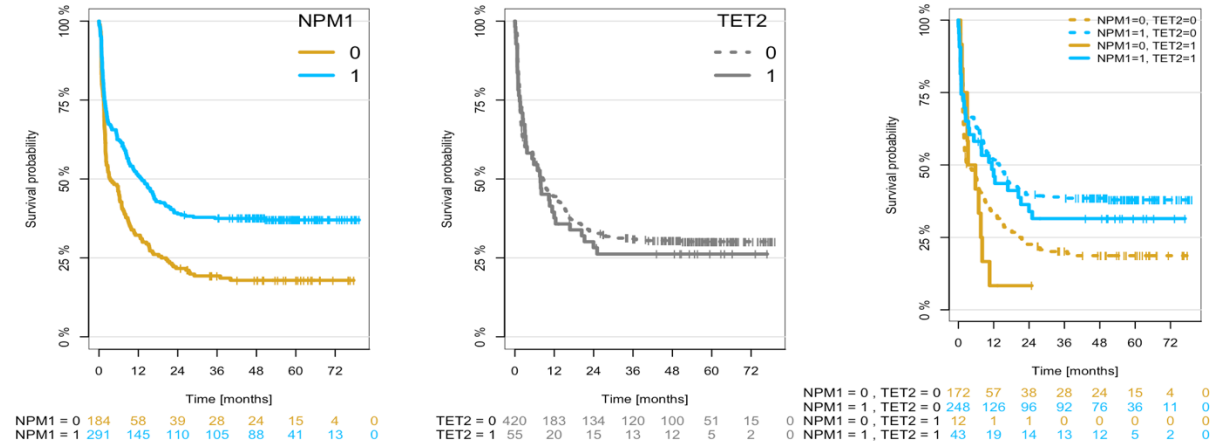

NPM1
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72
Time [months]
NPM1 = 0 184 58 39 28 24 15 4 0
NPM1 = 1 291 145 110 105 88 41 13 0
SEX1C
M
F
Survival probability
0 12 24 36 48 60 72
Time [months]
SEX1C = M 208 100 76 66 58 32 10 0
SEX1C = F 267 103 73 67 54 24 7 0
NPM1=0, SEX1C=M
NPM1=1, SEX1C=M
NPM1=0, SEX1C=F
NPM1=1, SEX1C=F
Survival probability
0 12 24 36 48 60 72
Time [months]
NPM1 = 0 , SEX1C = M 95 31 22 16 14 9 2 0
NPM1 = 1 , SEX1C = M 113 69 54 50 44 23 8 0
NPM1 = 0 , SEX1C = F 89 27 17 12 10 6 2 0
NPM1 = 1 , SEX1C = F 178 76 56 55 44 18 5 0

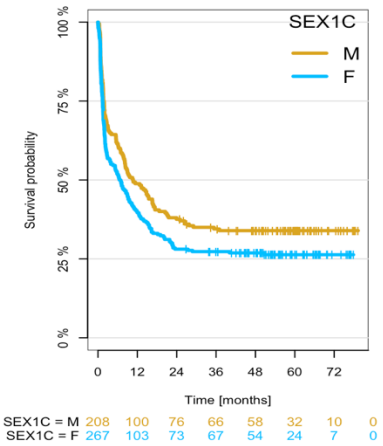

WT1
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72
Time [months]
WT1 = 0 375 172 126 113 99 53 16 0
WT1 = 1 100 31 23 20 13 3 1 0
trt
Plcb
Mdst
Survival probability
0 12 24 36 48 60 72
Time [months]
trt = Plcb 222 75 56 50 44 18 5 0
trt = Mdst 253 128 93 83 68 38 12 0
WT1=0, trt=Plcb
WT1=1, trt=Plcb
WT1=0, trt=Mdst
WT1=1, trt=Mdst
Survival probability
0 12 24 36 48 60 72
Time [months]
WT1 = 0 , trt = Plcb 175 66 48 44 39 16 5 0
WT1 = 1 , trt = Plcb 47 9 8 6 5 2 0 0
WT1 = 0 , trt = Mdst 200 106 78 69 60 37 11 0
WT1 = 1 , trt = Mdst 53 22 15 14 8 1 1 0

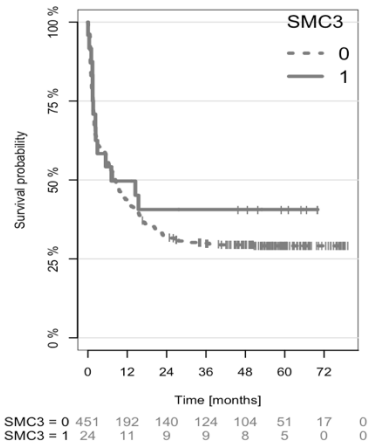

trt
Plcb
Mdst
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72
Time [months]
trt = Plcb 222 75 56 50 44 18 5 0
trt = Mdst 253 128 93 83 68 38 12 0
RUNX1
0
1
Survival probability
0 12 24 36 48 60 72
Time [months]
RUNX1 = 0 422 185 135 124 106 52 15 0
RUNX1 = 1 53 18 14 9 6 4 2 0
trt=Plcb, RUNX1=0
trt=Mdst, RUNX1=0
trt=Plcb, RUNX1=1
trt=Mdst, RUNX1=1
Survival probability
0 12 24 36 48 60 72
Time [months]
trt = Plcb , RUNX1 = 0 197 71 52 48 43 18 5 0
trt = Mdst , RUNX1 = 0 225 114 83 76 63 34 10 0
trt = Plcb , RUNX1 = 1 25 4 4 2 1 0 0 0
trt = Mdst , RUNX1 = 1 28 14 10 7 5 4 2 0

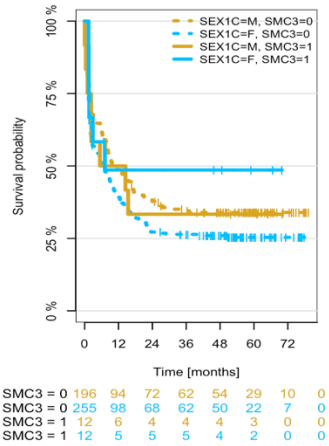

trt
Plcb
Mdst
Survival probability
Time [months]
trt = Plcb 222 75 56 50 44 18 5 0
trt = Mdst 253 128 93 83 68 38 12 0
IDH2
0
1
IDH2 = 0 441 185 135 119 100 51 15 0
IDH2 = 1 34 18 14 14 12 5 2 0
trt=Plcb, IDH2=0
trt=Mdst, IDH2=0
trt=Plcb, IDH2=1
trt=Mdst, IDH2=1
trt = Plcb , IDH2 = 0 206 65 48 42 38 16 4 0
trt = Mdst , IDH2 = 0 235 120 87 77 62 35 11 0
trt = Plcb , IDH2 = 1 16 10 8 8 6 2 1 0
trt = Mdst , IDH2 = 1 18 8 6 6 6 3 1 0

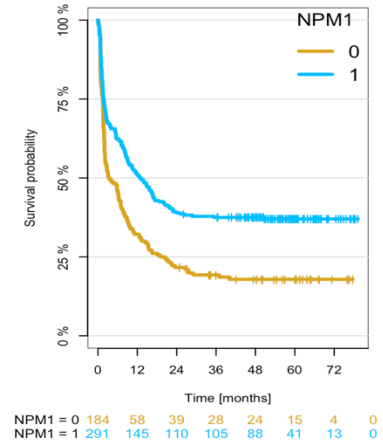

SEX1C
M
F
Survival probability
Time [months]
SEX1C = M 208 100 76 66 58 32 10 0
SEX1C = F 267 103 73 67 54 24 7 0
DNMT3A
0
1
DNMT3A = 0 288 120 85 73 63 26 8 0
DNMT3A = 1 187 83 64 60 49 30 9 0
SEX1C=M, DNMT3A=0
SEX1C=F, DNMT3A=0
SEX1C=M, DNMT3A=1
SEX1C=F, DNMT3A=1
SEX1C = M , DNMT3A = 0 129 54 38 31 26 12 3 0
SEX1C = F , DNMT3A = 0 159 66 47 42 37 14 5 0
SEX1C = M , DNMT3A = 1 79 46 38 35 32 20 7 0
SEX1C = F , DNMT3A = 1 108 37 26 25 17 10 2 0

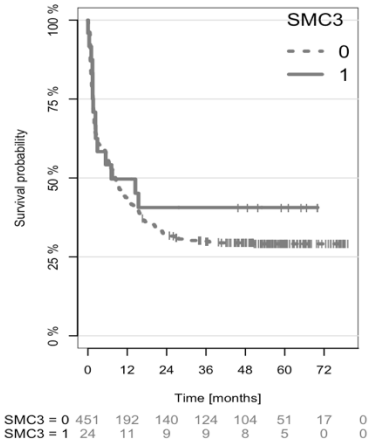

NPM1
0
1
Survival probability
Time [months]
NPM1 = 0 184 58 39 28 24 15 4 0
NPM1 = 1 291 145 110 105 88 41 13 0
TET2
0
1
TET2 = 0 420 183 134 120 100 51 15 0
TET2 = 1 55 20 15 13 12 5 2 0
NPM1=0, TET2=0
NPM1=1, TET2=0
NPM1=0, TET2=1
NPM1=1, TET2=1
NPM1 = 0 , TET2 = 0 172 57 38 28 24 15 4 0
NPM1 = 1 , TET2 = 0 248 126 96 92 76 36 11 0
NPM1 = 0 , TET2 = 1 12 1 1 0 0 0 0 0
NPM1 = 1 , TET2 = 1 43 19 14 13 12 5 2 0

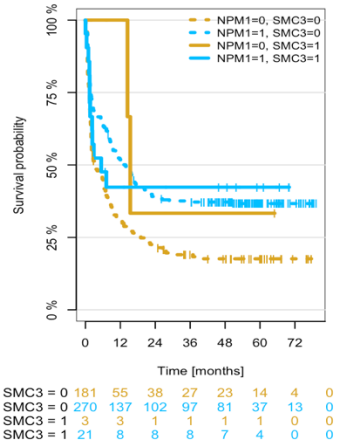

SEX1C
M
F
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72
Time [months]
SEX1C = M 208 100 76 66 58 32 10 0
SEX1C = F 267 103 73 67 54 24 7 0


SMC3
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72
Time [months]
SMC3 = 0 451 192 140 124 104 51 17 0
SMC3 = 1 24 11 9 9 8 5 0 0


SEX1C=M, SMC3=0
SEX1C=F, SMC3=0
SEX1C=M, SMC3=1
SEX1C=F, SMC3=1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72
Time [months]
SEX1C = M , SMC3 = 0 196 94 72 62 54 29 10 0
SEX1C = F , SMC3 = 0 255 98 68 62 50 22 7 0
SEX1C = M , SMC3 = 1 12 6 4 4 4 3 0 0
SEX1C = F , SMC3 = 1 12 5 5 5 4 2 0 0

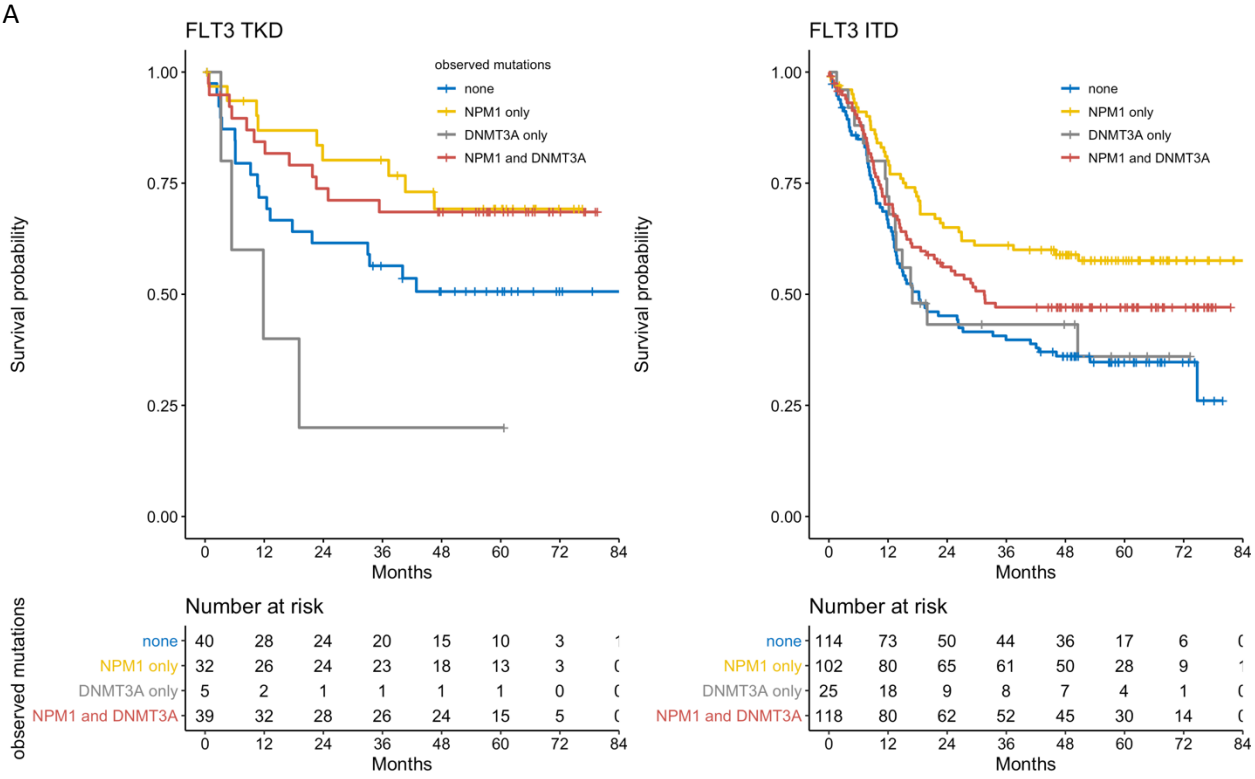

NPM1
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72
Time [months]
NPM1 = 0 184 58 39 28 24 15 4 0
NPM1 = 1 291 145 110 105 88 41 13 0


SMC3
0
1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72
Time [months]
SMC3 = 0 451 192 140 124 104 51 17 0
SMC3 = 1 24 11 9 9 8 5 0 0

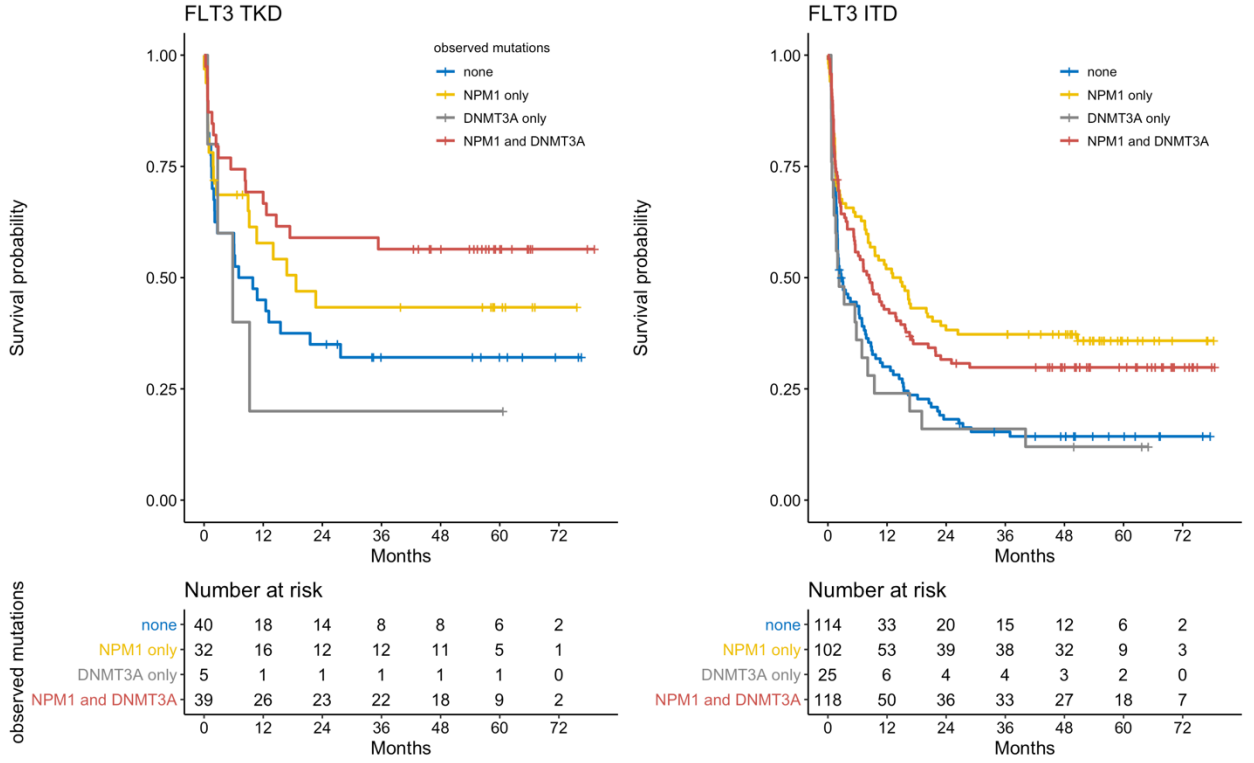

NPM1=0, SMC3=0
NPM1=1, SMC3=0
NPM1=0, SMC3=1
NPM1=1, SMC3=1
Survival probability
100 %
75 %
50 %
25 %
0 %
0 12 24 36 48 60 72
Time [months]
NPM1 = 0 , SMC3 = 0 181 55 38 27 23 14 4 0
NPM1 = 1 , SMC3 = 0 270 137 102 97 81 37 13 0
NPM1 = 0 , SMC3 = 1 3 3 1 1 1 1 0 0
NPM1 = 1 , SMC3 = 1 21 8 8 8 7 4 0 0

Supplemental Figure S9: Kaplan Meier estimates of impact of *NPM1*, *DNMT3A*, and combined genotypes on overall (A) and event-free (B) survival and according to *FLT3* mutation type.

A

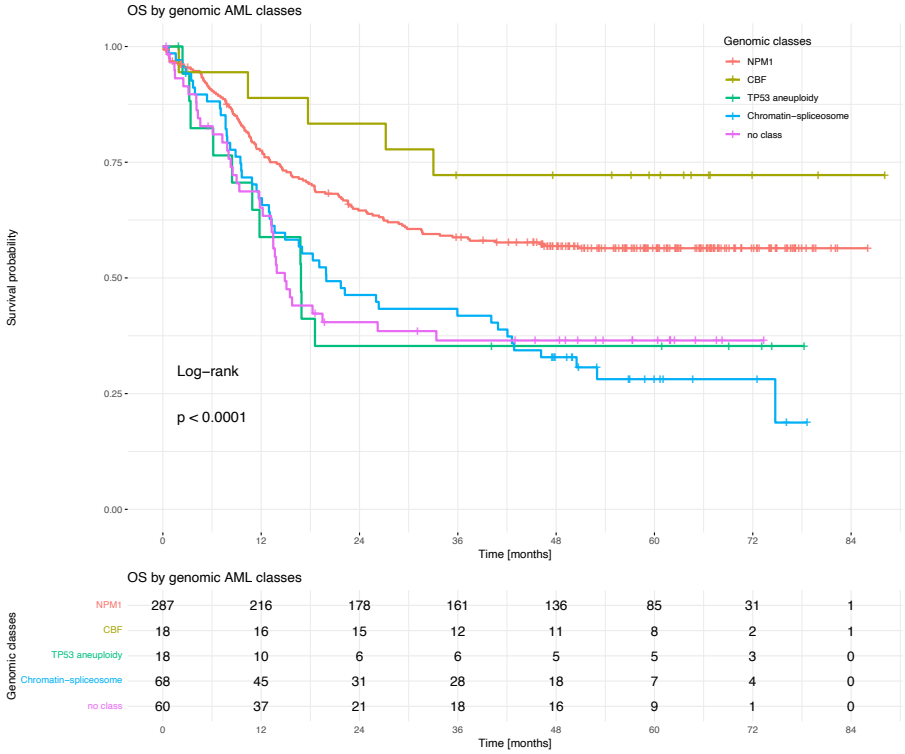


B

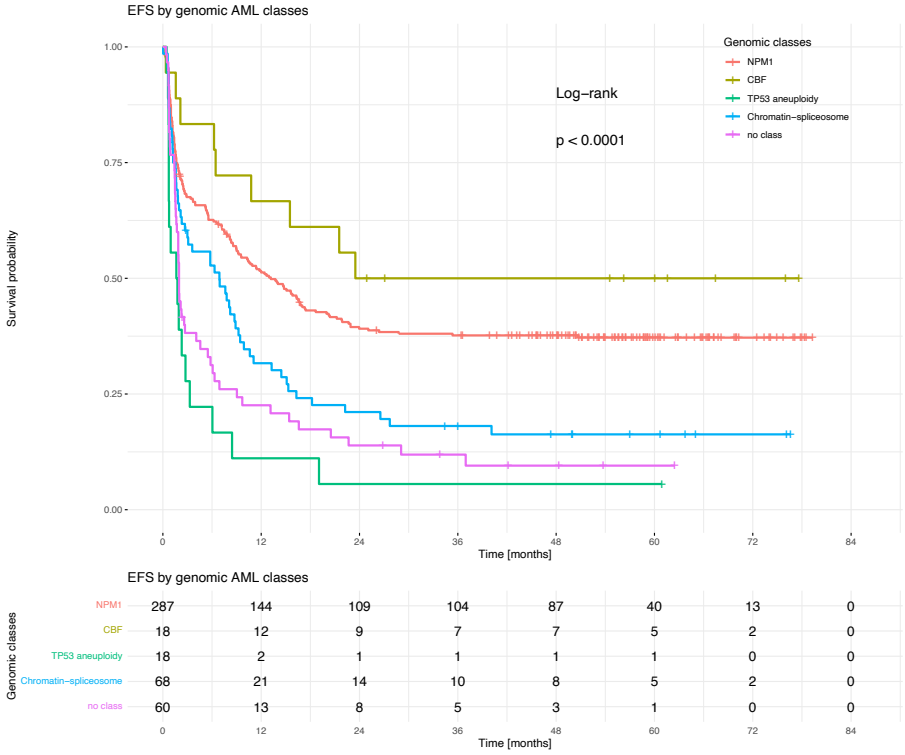


Supplemental Figure S10: Kaplan-Meier estimated A) overall and B) event-free survival curves, and number of events by genomic AML classes and log rank test p-values in cohort of 451 of 475 patients, in which subcategorization into genomic AML classes was possible. Abbreviations: OS, overall survival; EFS, event-free survival.

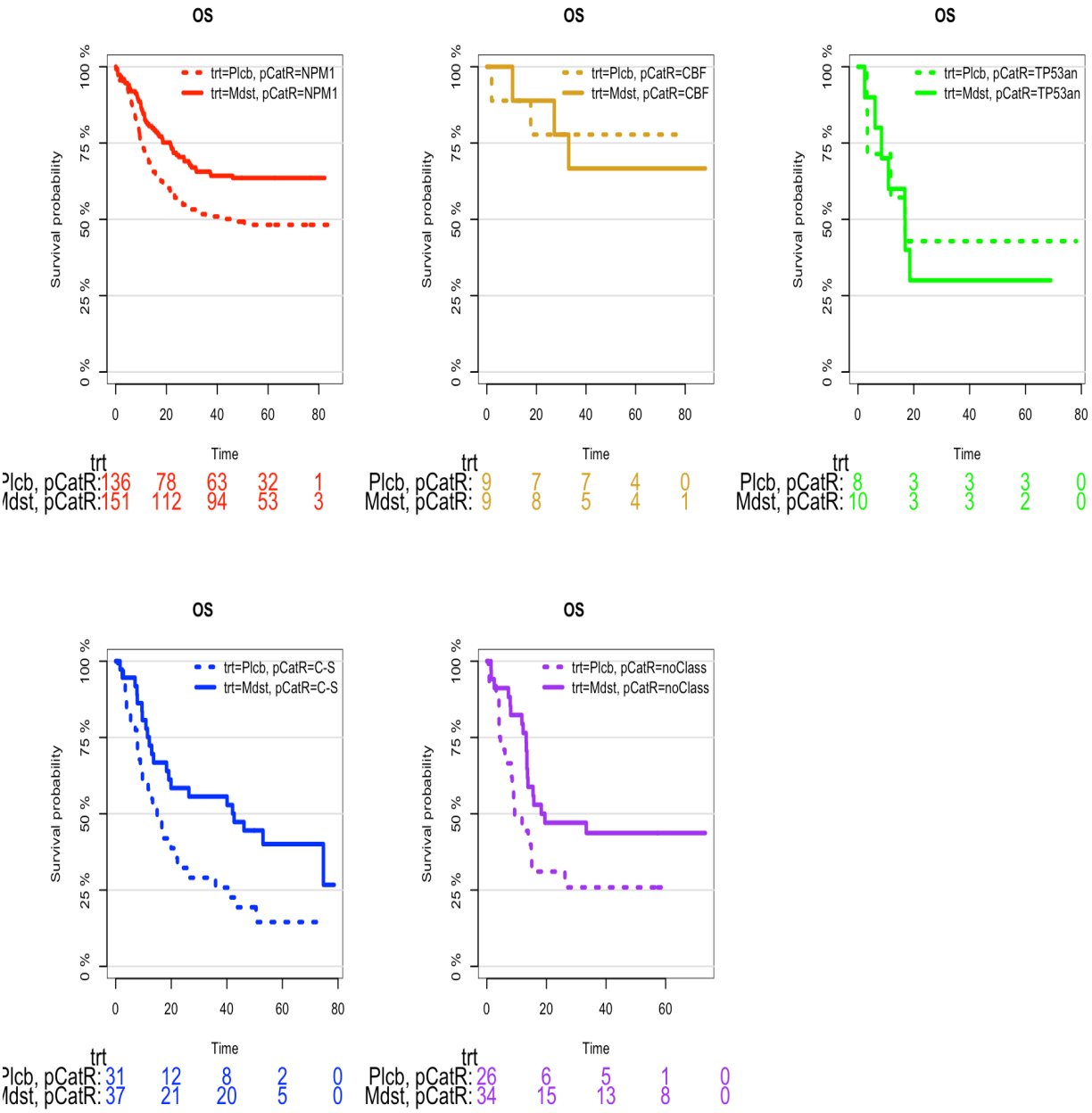
A



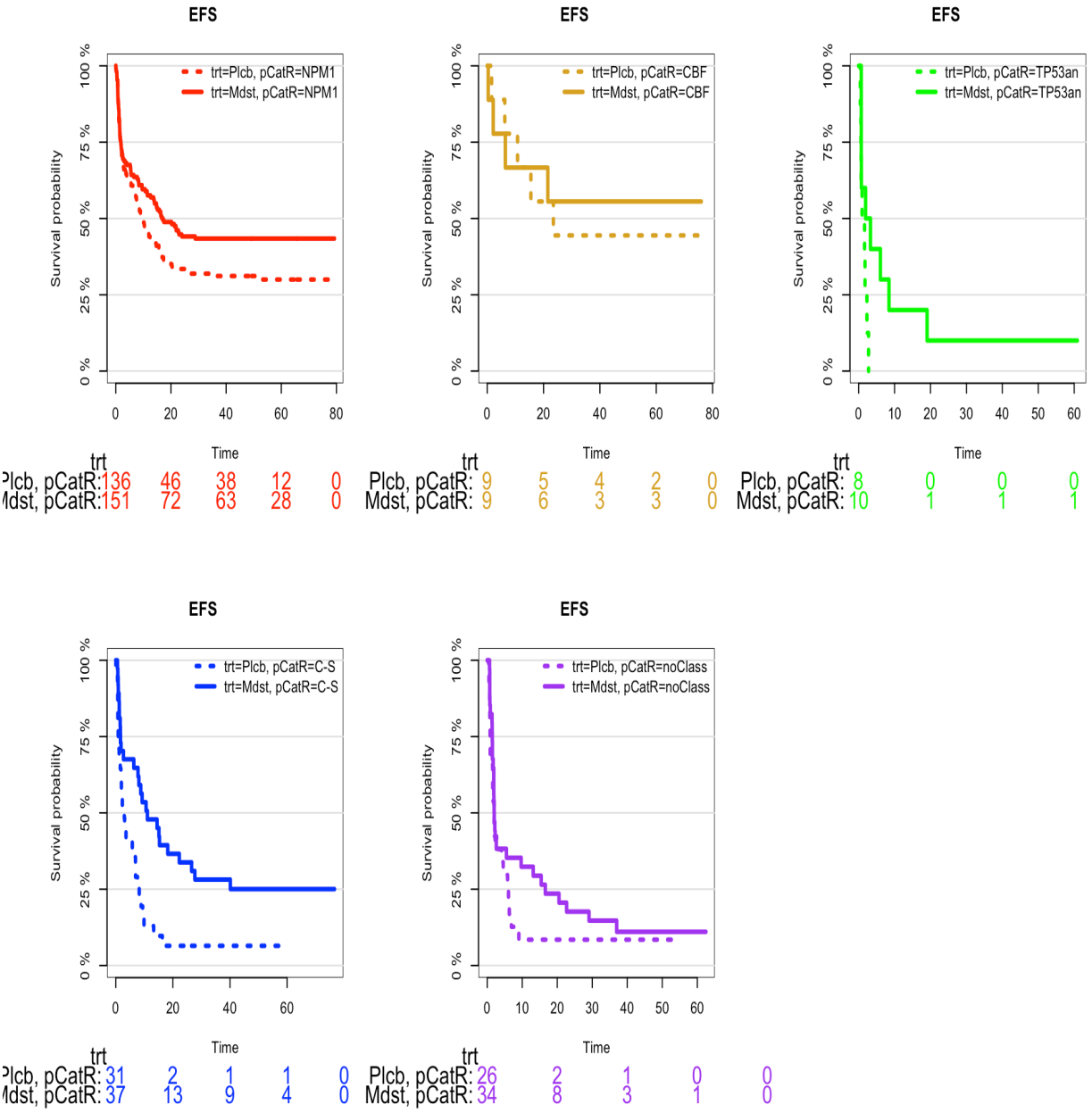
B



Supplemental Figure S11: Kaplan-Meier plots for the marginal overall survival (OS) distribution in the corresponding genomic AML classes (pCat) and treatment (trt) subgroups. Abbreviations: C-S, chromatin-spliceosome; Mdst, midostaurin; Plcb, placebo; TP53an, TP53-aneuploidy.

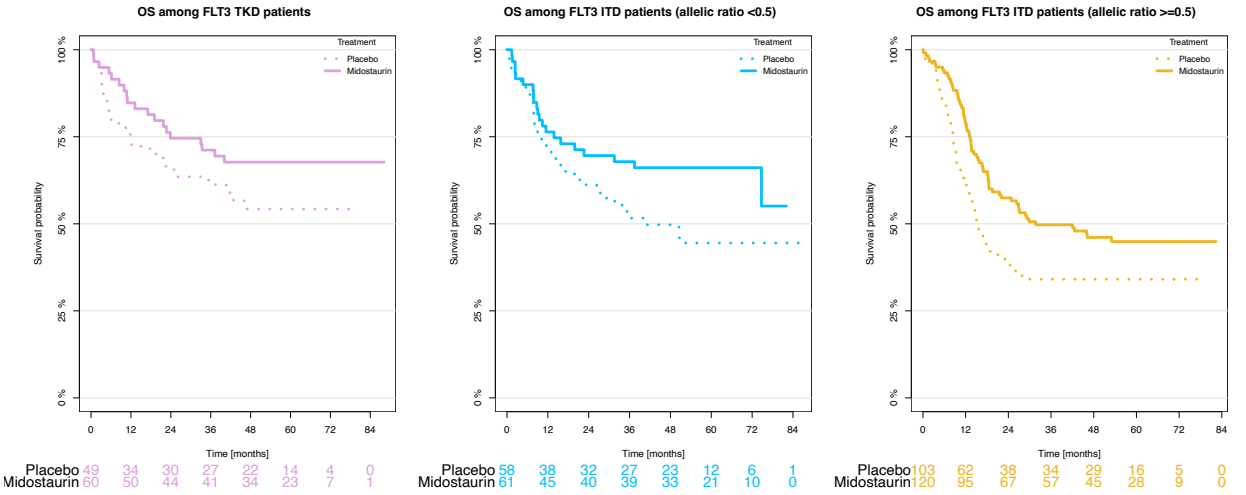


Supplemental Figure S12: Kaplan-Meier plots for the marginal event-free survival (EFS) distribution in the corresponding genomic AML classes (pCat) and treatment (trt) subgroups. Abbreviations: C-S, chromatin-spliceosome; Mdst, midostaurin; Plcb, placebo; TP53an, TP53-aneuploidy.

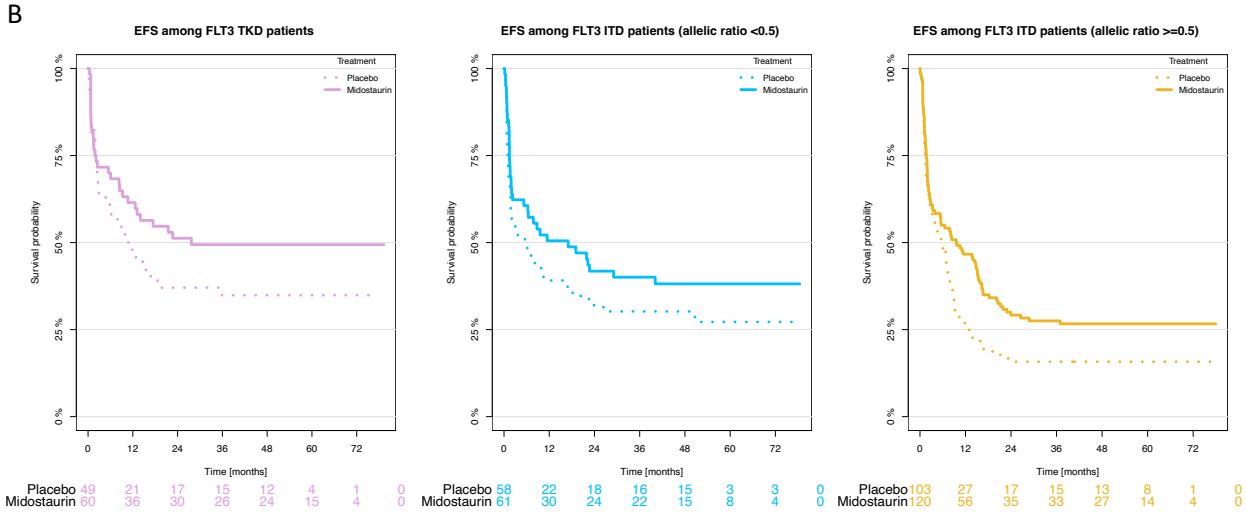


Supplemental Figure S13: Kaplan-Meier estimates for A) overall and B) event-free survival according to *FLT3* mutation type and treatment of 451 patients included into the Cox proportional hazard model.

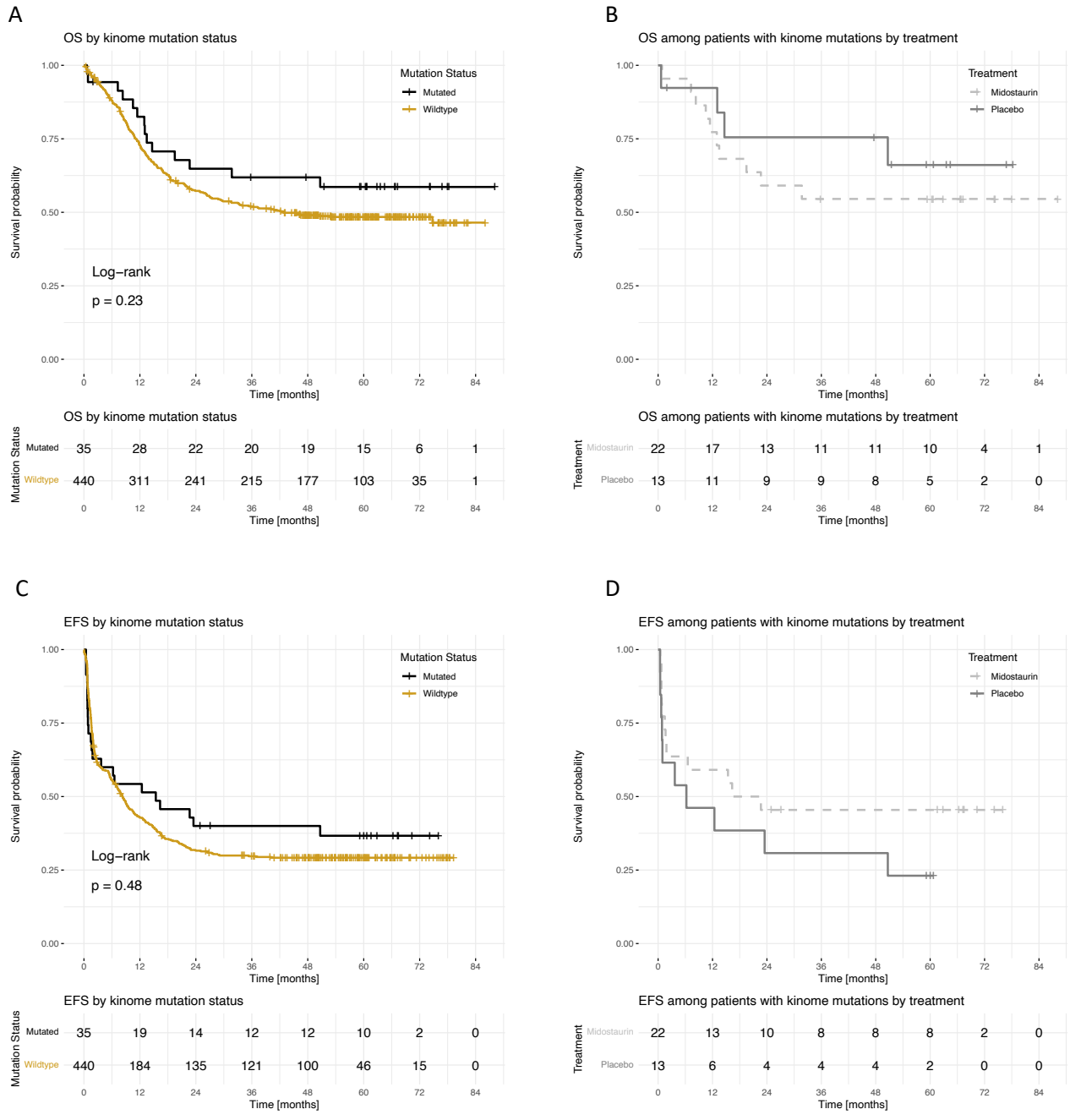
A



B



Supplemental Figure S14: Kaplan Meier estimates for A) overall and C) event-free survival by midostaurin kinome mutation status in entire cohort. Effect of treatment on B) overall and D) event-free survival in subgroup of patients harboring midostaurin kinome mutations.



Supplemental Figure S15: Kaplan Meier estimator for overall (OS) and event-free survival (EFS) according to *FLT3* exon 16 mutation status.