Prognostic relevance of clonal hematopoiesis in myeloid neoplastic transformation in patients with follicular lymphoma treated with radioimmunotherapy

Zhuoer Xie,[1,2] Terra Lasho,[1] Arushi Khurana,[1] Alejandro Ferrer,[1] Christy Finke,[1] Abhishek A. Mangaonkar,[1] Stephen Ansell,[1] Jenna Fernandez,[1] Mithun Vinod Shah,[1] Aref Al-Kali,[1] Naseema Gangat,[1] Jithma Abeykoon,[1] Thomas E. Witzig[1] and Mrinal M. Patnaik[1]

[1]Mayo Clinic, Department of Internal Medicine, Hematology Division, Rochester, MN and [2]Malignant Hematology Department, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL, USA

Correspondence: M. Patnaik
Patnaik.Mrinal@mayo.edu

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Supplemental information.

DNA sequencing method: briefly 200 ng of target DNA was fragmented using the Covaris LE220 plus sonicator. The ends were repaired using the Sureselect End-Repair-A-Tailing enzyme mix. Adapter ligated DNA fragments were size selected to enrich for 200 bp inserts (~320 bp total library size) using AMPURE XP bead purification. The size selected adapter-modified fragments were enriched, and specific indexes were added by 12 cycles of PCR using universal Index Primers.

The Custom Capture hybrid-target enrichment probes were designed using Agilent SureSelect design software (Agilent Technologies, Santa Clara, CA). The targeted gene panel was comprised of 62962 single probes with size 1.805Mbp, and covered the coding regions, UTRs, and overlapping intron/exon regions for 205 genes described and/or enriched for CHIP mutations. The custom capture was carried out using the Agilent Bravo liquid handler following Agilent's SureSelect XT Low. Purified capture products were then amplified using the SureSelect Post- Capture primer mix for 14 cycles. Libraries were validated and quantified on the Agilent Bioanalyzer. Samples were sequenced by 150 paired end reads, 21 samples to a Flow Cell, on an Illumina NovaSeqSP with an expected expected depth of $\geqslant$ 1,000X coverage (Illumina, SanDiego, CA).

Secondary bioinformatics analysis included quality assessment and alignment to the hg19 build reference genome using Novoalign (Novo- craft Technologies, Malaysia), followed by GATK based single nucleotide and small insertion/deletion variant calling, structural variation discovery, and annotation. The quality of sequencing chemistry was evaluated using FastQC.('FASTQC'). After alignment, PCR duplication rates and percent reads mapped on target were used to assess the quality of the sample preps. Realignment and recalibration steps were implemented in the GATK. Somatic single nucleotide variations (SNVs) were then genotyped using SomaticSniper, whereas insertions and deletions were called by GATK Somatic Indel Detector. Each variant in coding regions was Strand-Aware Variant Annotation Tool '), as well as ClinVar, dbNSFP, OMIM, and the Human Gene Annotation Database to predict biological effects. Interpretation for relevant alterations included absence in international normal variant allele databases (GnomAD, ExAC), deleterious effect on protein function by multiple phenotype prediction models, somatic and functional annotation in literature, consequence of variant (nonsense, truncating, etc.) and location proximal to important domains.

Supplementary Table 1. The CH panel used in the study covered 289 genes.

ANKRD26
ARID1A
ASXL1
ASXL2
ATM
BCL10
BCL11B
BCL6
BCOR
BCORL1
BIRC3
BRAF
BRCC3
BTG1
BTG2
CALR
CARD11
CBL
CBLB
CCND3
CD58
CD70
CD79A
CD79B
CDKN2A
CDKN2B
CEBPA
CHD2
CNOT3
CREBBP
CRLF2
CSF1R
CHEK2
CSF3R
CTCF
CUX1
DDX3X
DDX41
DIS3
DNMT3A
DNMT3B
EBF1
EED
EP300
ETNK1
ETV6
EZH2
ELANE
ERCC6L2
EZR
FAM46C
FAS
FBXO11
FBXW7
FLT3
FOXP1
FYN
GATA1
GATA2
GATA3
GNA13
GNAS
GNB1
HIST1H1B
HIST1H1C
HIST1H1D
HIST1H1E
HIST1H3B
HRAS
ID3
IDH1
IDH2
IDH3A
IKBKB
IKZF1
IKZF2
IKZF3
IL7R
INTS12
IRF4
IRF8

ITK
JAK1
JAK2
JAK3
JARID2
KDM6A
KDM5A
KDM5C
KIT
KLHL6
KMT2A
KMT2B
KMT2C
KMT2D
KRAS
LEF1
LRBA
LRRK2
LTB
LUC7L2
MALT1
MAP2K1
MAP3K14
MAPK1
MED12
MEF2B
MPL
MXRA5
MYD88
MECOM
NF1
NOTCH1
NOTCH2
NPM1
NRAS
P2RY8
PAPD5
PAX5
PDS5B
PDSS2
PHF6
PHIP
PIK3CA
PML
POT1
POU2AF1
POU2F2
PPM1D
PRDM1
PRPF40B
PRPF8
PTEN
PTPN1
PTPN11
RAD21
RBBP4
RHOA
RIT1
RPL10
RPL5
RPS15
RPS2
RUNX1
SETBP1
SETD2
SETDB1
SF1
SF3A1
SF3B1
SGK1
SMC1A
SMC3
SOCS1
SPRY4
SRSF2
STAG1
STAG2
STAT3
STAT5A
STAT5B
STAT6
STK11
STK35
SUZ12

SWAP70
TBL1XR1
TCF3
TET1
TET2
TET3
TMEM30A
TNF
TNFAIP3
TNFRSF14
TP53
TRAF3
TYW1
U2AF1
U2AF2
UBR5
VPS13B
VPS13C
VWF
WT1
XBP1
XPO1
ZNF471
ZRSR2

Supplementary table 2. The pathogenic variants identified in the entire cohort.

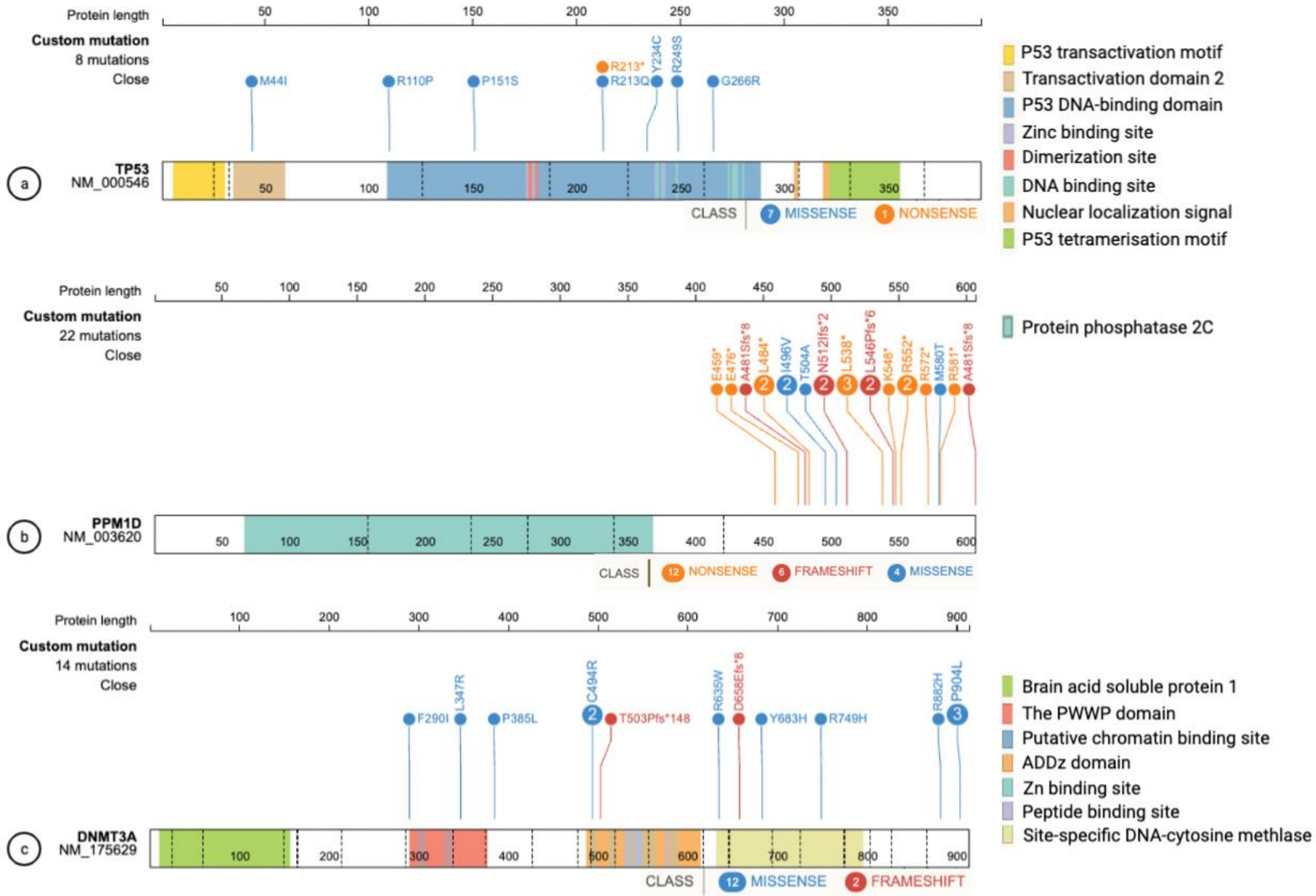
	Gene	Chromosome	Nucleotide change	Amino acid change	Mutation type	CH type
PT_17	***JAK2***	9	c.1711G>A	G571S	Missense	M-CH
	PPM1D	17	c.1654C>T	R552*	Nonsense	
	TP53	17	c.329G>C	R110P	Missense	
PT_18	***ASXL1***	20	c.4342C>T	Q1448*	Nonsense	LM-CH
	NOTCH1	9	c.1295C>T	T432M	Missense	
	PPM1D	17	c.1375G>T	E459*	Nonsense	
	PPM1D	17	c.1613T>G	L538*	Nonsense	
	SMC3	10	c.2000G>T	G667V	Missense	
PT_19	***PPM1D***	17	c.1535delA	N512Ifs*2	Frameshift	M-CH
	TP53	17	c.701A>G	Y234C	Missense	
PT_20	***CBL***	11	c.1247G>T	C416F	Missense	LM-CH
	CREBBP	16	c.1909G>T	E637*	Missense	
	KMT2D	12	c.11584C>T	Q3862*	Nonsense	
	LTB	6	c.199C>A	Q67K	Missense	
	STAT6	12	c.1256A>G	D419G	Missense	
PT_21	***PPM1D***	17	c.1642A>T	K548*	Nonsense	M-CH
PT_22	***EZH2***	7	c.338G>A	W113*	Nonsense	LM-CH
	IDH2	15	c.515G>A	R172K	Missense	
	PPM1D	17	c.1440dupA	A481Sfs*8	Frameshift	
	STAG2	X	c.1664_1667dupCACA	Q556Hfs*5	Frameshift	
PT_23	***KMT2D***	12	c.8704C>T	Q2902*	Nonsense	L-CH
PT_24	***DNMT3A***	2	c.1974_1978del	D658Efs*8	Frameshift	M-CH
PT_25	***DNMT3A***	2	c.1480T>C	C494R	Missense	LM-CH
	KMT2D	12	c.11905C>T	Q3969*	Nonsense	
	KMT2D	12	c.8488C>T	R2830*	Nonsense	
PT_26	***CDKN2A***	9	c.238C>T	R80*	Nonsense	LM-CH
	CREBBP	16	c.4336C>T	R1446C	Missense	
	DNMT3A	2	c.2711C>T	P904L	Missense	
	PPM1D	17	c.1741C>T	R581*	Nonsense	
	PPM1D	17	c.1654C>T	R552*	Nonsense	
	PPM1D	17	c.1714C>T	R572*	Nonsense	
PT_27	***HIST1H1C***	6	c.280G>A	V94M	Missense	LM-CH
	PPM1D	17	c.1486A>G	I496V	Missense	
	PPM1D	17	c.1636dupC	L546Pfs*6	Frameshift	
PT_28	***PPM1D***	17	L538*	L538*	Nonsense	M-CH
	PPM1D	17	A481Sfs*8	A481Sfs*8	Frameshift	
	TET2	4	c.3804-2A>G	splice effect	Splice Effect	
PT_29	***ASXL1***	20	c.1782C>A	C594*	Nonsense	M-CH
	TP53	17	c.747G>T	R249S	Missense	
PT_30	***ARID1B***	6	c.662del	N221Tfs*42	Frameshift	LM-CH
	DNMT3A	2	c.1506del	T503Pfs*148	Frameshift	
	MED12	X	c.6348_6359dupCCAGCAGCAACA	H2116_Q2119dup	Duplication	
	STAT3	17	c.1973A>G	K658R	Missense	
PT_31	***DNMT3A***	2	c.1040T>G	L347R	Missense	M-CH
	DNMT3A	2	c.2173+1G>A	splice effect	Splice Effect	
PT_32	***BRCC3***	X	c.721C>T	Q241*	Nonsense	M-CH
	PPM1D	17	c.1613T>G	L538*	Nonsense	
PT_33	***ASXL2***	2	c.3715G>T	E1239*	Nonsense	LM-CH
	CARD11	7	c.583G>C	V195L	Missense	
	DNMT3A	2	c.2173+1G>A	splice effect	Splice Effect	
	DNMT3A	2	c.2645G>A	R882H	Missense	
	DNMT3A	2	c.2083-2A>G	splice effect	Splice Effect	
	EP300	22	c.784G>T	G262*	Nonsense	
	PHIP	6	c.2785G>T	E929*	Nonsense	
	PPM1D	17	c.1510A>G	T504A	Missense	
	PPM1D	17	c.1451T>A	L484*	Nonsense	
	TET1	10	c.2077G>T	E693*	Nonsense	
PT_34	***JAK2***	9	c.365G>A	R122H	Missense	LM-CH
	STAT6	12	c.3G>A	p.Met1?	Nonsense	
PT_35	***DNMT3A***	2	c.2711C>T	P904L	Missense	M-CH
	TP53	17	c.451C>T	P151S	Missense	
PT_36	***DNMT3A***	2	c.1903C>T	R635W	Missense	M-CH
PT_37	***DNMT3A***	2	c.2711C>T	P904L	Missense	M-CH
	DNMT3A	2	c.868T>A	F290I	Missense	
PT_38	***DNMT3A***	2	c.1A>C	p.Met1?	Nonsense	M-CH
	DNMT3A	2	c.2246G>A	R749H	Missense	
	PPM1D	17	c.1636dupC	L546Pfs*6	Frameshift	

	PPM1D	17	c.1535delA	N512Ifs*2	Frameshift	
PT_39	***PPM1D***	17	c.1451T>G	L484*	Nonsense	M-CH
PT_40	***PPM1D***	17	c.1739T>C	M580T	Missense	M-CH
	SBDS	7	c.127G>T	V43L	Missense	
	TP53	17	c.638G>A	R213Q	Missense	
	TP53	17	c.796G>A	G266R	Missense	
PT_41	***DNMT3A***	2	c.1154C>T	P385L	Missense	M-CH
PT_42	***DNMT3A***	2	c.2047T>C	Y683H	Missense	M-CH
PT_43	***ARID1A***	1	c.3036T>G	Y1012*	Nonsense	LM-CH
	CREBBP	16	c.4297_4305del	Y1433_D1435del	Deletion	
	KMT2D	12	c.5104C>T	R1702*	Nonsense	
	NF1	17	c.3826C>T	R1276*	Nonsense	
PT_44	***DNMT3A***	2	c.2173+1G>A	splice effect	Splice Effect	M-CH
PT_45	***MED12***	X	c.6348_6359dupCCAGCAGCAACA	H2116_Q2119dup	Duplication	M-CH
PT_53	***CD79B***	17	c.586T>G	Y197D	Missense	LM-CH
	CREBBP	16	c.4336C>T	R1446C	Missense	
	HIST1H1C	6	c.305C>T	S102F	Missense	
	KMT2D	12	c.15289C>T	R5097*	Nonsense	
	TP53	17	c.637C>T	R213*	Nonsense	
PT_54	***PHIP***	6	c.607G>T	D203Y	Missense	M-CH
	PPM1D	17	c.1426G>T	E476*	Nonsense	
	TP53	17	c.132G>T	M44I	Missense	
	DNMT3A	2	c.1480T>C	C494R	Missense	
PT_55	***PTEN***	10	c.253+42C>T	splice effect	Splice Effect	M-CH
PT_56	***EZH2***	7	c.1852-6C>T	splice effect	Splice Effect	L-CH
PT_57	***PPM1D***	17	c.1486A>G	I496V	Missense	M-CH
PT_58	***CREBBP***	16	c.4424C>T	P1475L	Missense	LM-CH
	EZH2	7	c.1937A>C	Y646S	Missense	
	KMT2D	12	c.13745_13764del	G4582Efs*17	Frameshift	
	KMT2D	12	c.14515+2T>G	splice effect	Splice Effect	
	POU2AF1	11	c.16+3A>T	splice effect	Splice Effect	
	TNFRSF14	1	c.169T>C	C57R	Missense	

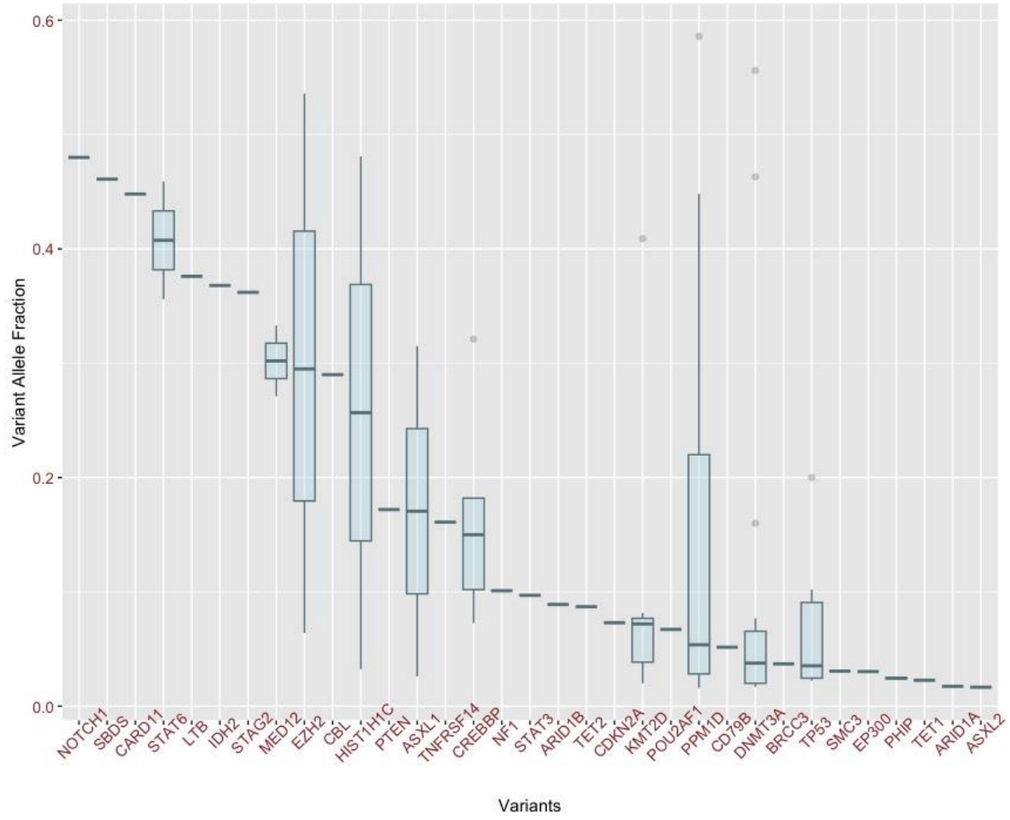
Supplementary Table 3. Detailed information for group 1, including the time differences between the paired samples.

	Age	Gender	Total number of mutations	Type of Mutation	Time to Zevalin (y)	Timing diff for the paired sample (y)	TMN	Zevalin To TMN	Zevalin to Last FU	Death	OS
PT_9	79	M	0	-	1.86	1.01	Y	9.60	10.00	Y	11.86
PT_10	38	F	0	-	0.83	0.24	N		6.91	N	7.74
PT_11	36	M	0	-	0.43	1.03	N		15.55	N	15.98
PT_12	43	M	0	-	2.27	0.72	N		14.45	N	16.73
PT_13	60	F	0	-	2.81	4.93	N		13.10	N	15.91
PT_14	42	F	0	-	7.30	1.14	N		10.82	Y	18.12
PT_15	55	M	0	-	3.38	1.05	N		15.65	N	19.03
PT_16	63	F	0	-	9.13	1.05	N		15.27	N	24.40
PT_30	40	F	4	LM-CH	12.73	0.27	Y	6.60	14.32	N	27.05
PT_31	50	M	2	M-CH	8.41	1.05	Y	7.88	7.90	N	16.30
PT_32	67	F	2	M-CH	4.11	0.93	Y	9.81	12.01	Y	16.12
PT_33	45	M	10	LM-CH	1.30	5.20	N		16.98	N	18.28
PT_34	43	F	1	L-CH	7.12	1.13	N		16.80	N	23.92
PT_35	36	F	2	M-CH	32.42	1.02	N		5.39	N	37.82
PT_36	46	M	1	M-CH	19.64	1.01	N		16.21	N	35.85
PT_37	48	F	2	M-CH	10.16	1.17	N		16.63	N	26.79
PT_38	69	M	4	M-CH	4.99	0.52	N		16.93	N	21.92
PT_39	67	M	1	M-CH	5.02	1.07	N		14.85	Y	19.87
PT_40	70	F	4	M-CH	3.61	0.52	N		12.21	Y	15.82
PT_41	65	M	1	M-CH	3.40	0.99	N		16.50	N	19.90
PT_42	66	F	1	M-CH	10.35	1.01	N		7.18	Y	17.53
PT_43	36	F	4	LM-CH	1.04	1.48	N		18.37	N	19.41
PT_44	49	F	1	M-CH	2.46	0.80	N		14.65	N	17.11
PT_45	28	F	1	M-CH	14.03	0.50	N		14.73	N	28.76

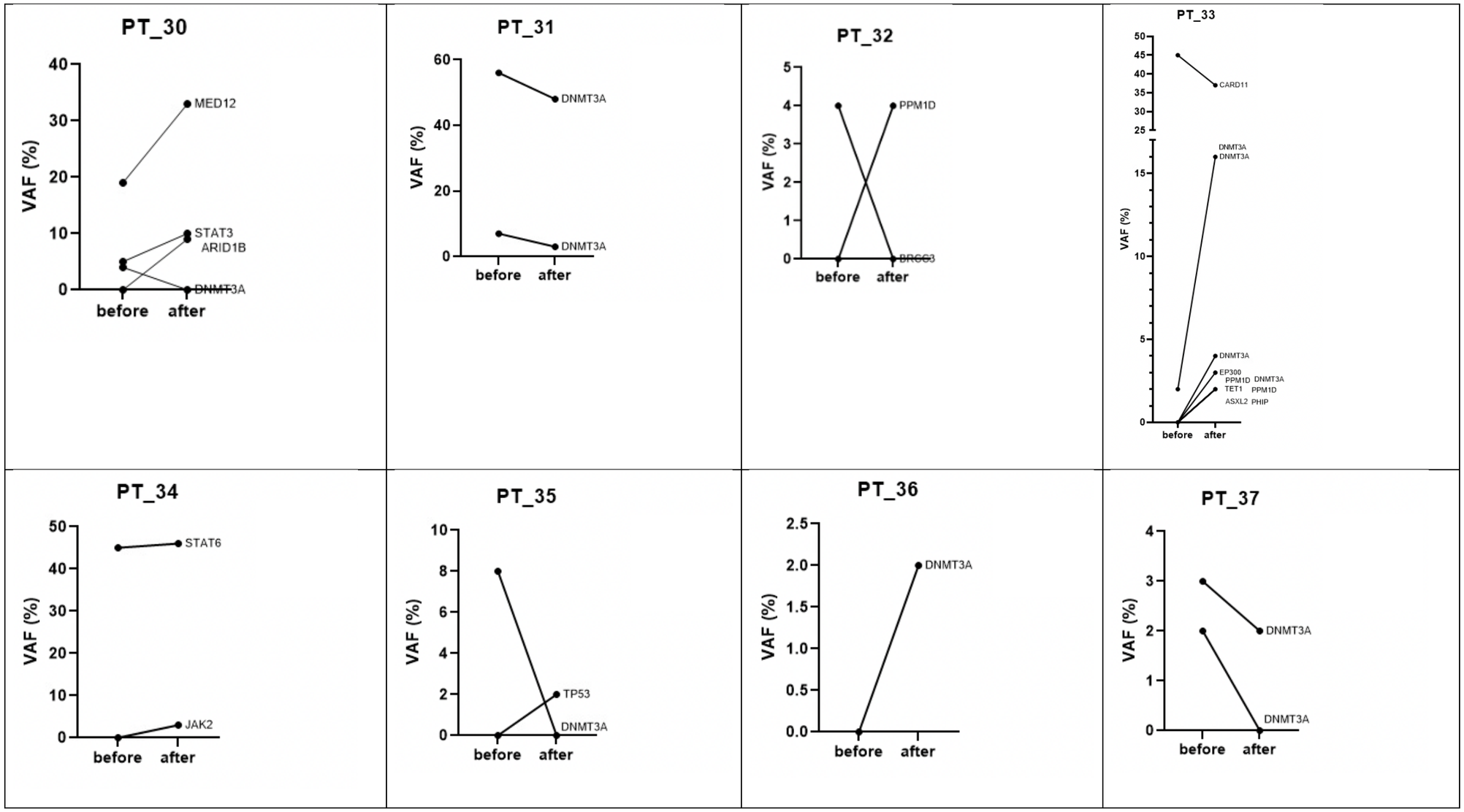
Supplementary Figure 1. Lollipop plot showing type and location along the protein sequence of *TP53* mutation, *PPM1D* mutation, and *DNMT3A* mutation. The number of recurrently detected alterations is indicated by the text within each disc, as well as by disc size. Colors indicate the type of mutation: blue, missense; orange, nonsense; red, frameshift. Most of the *TP53* mutations were missense mutations and occurred in the DNA binding domain, while 82% of the *PPM1D* mutations were truncating mutations that occurred in the terminal exon. *DNMT3A* mutations were largely missense mutations and occurred throughout the genome, with 6 involving the catalytic methyltransferase domain, including 1 dominant negative R882 mutation that has been implicated commonly in MDS/AML

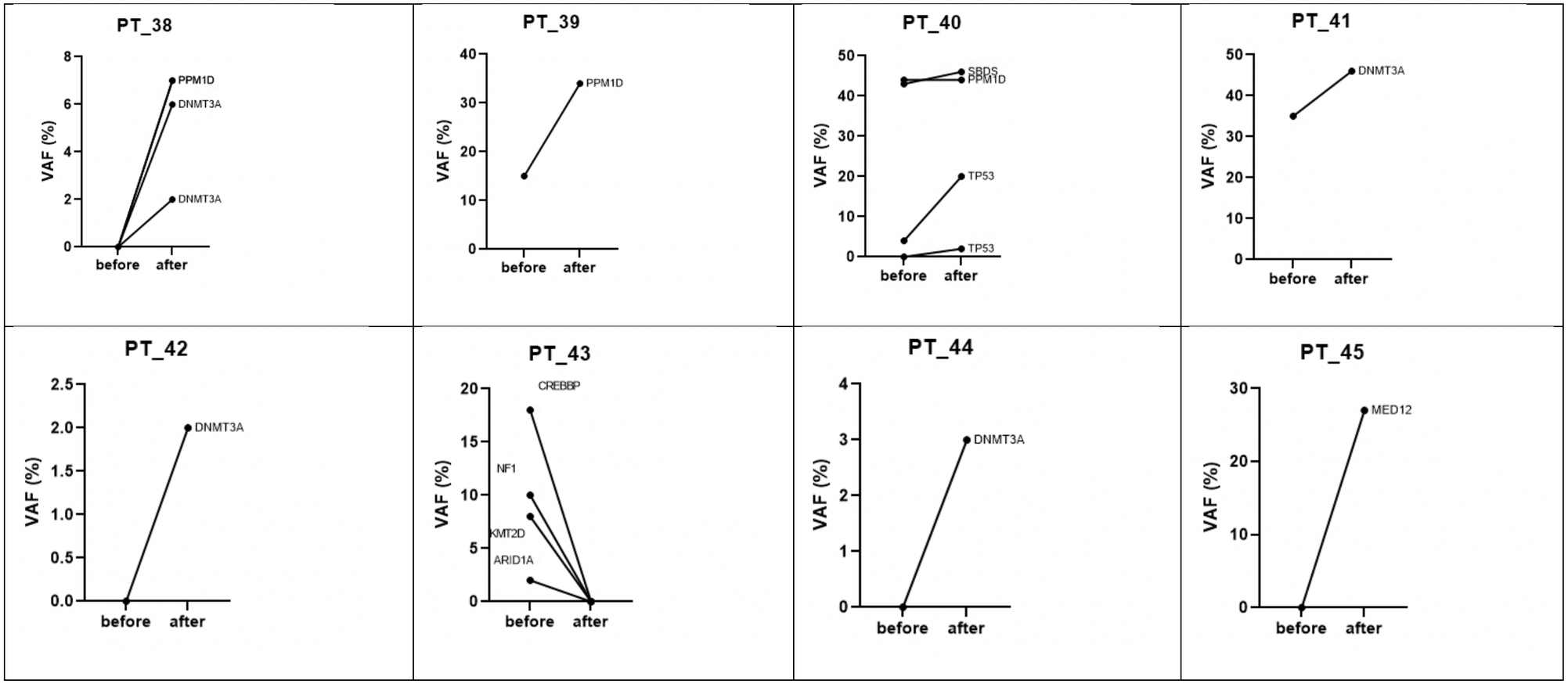


Supplemental figure 2. Variant allele fraction is sorted from the highest to the lowest.

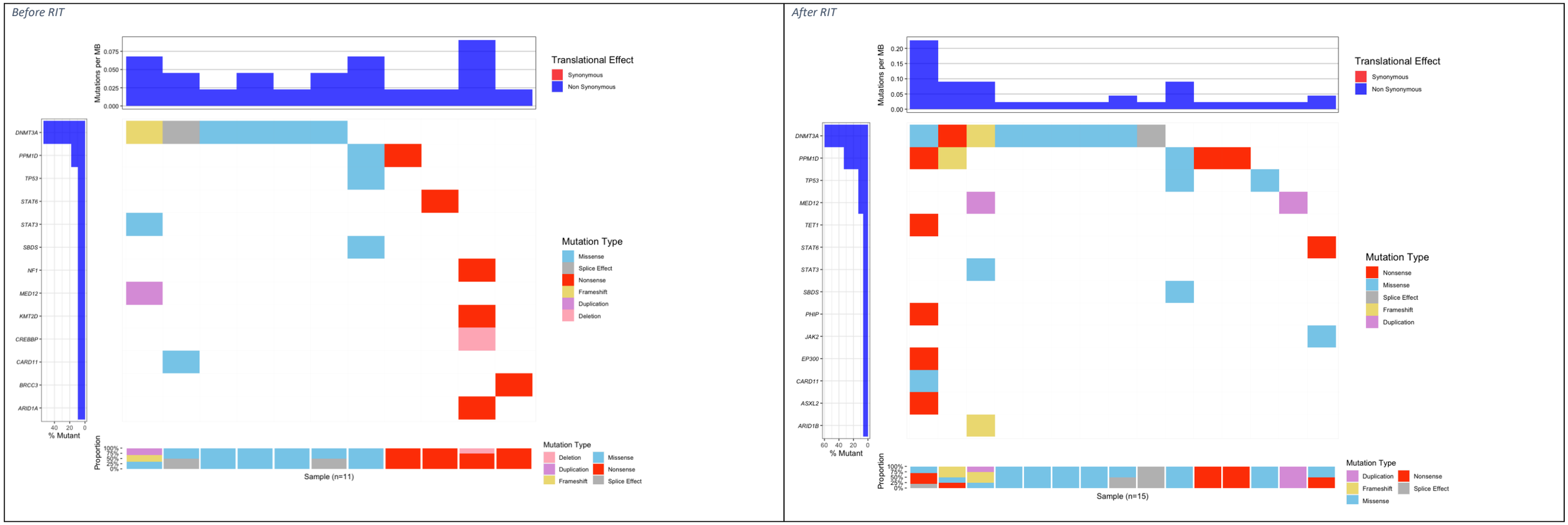


Supplemental figure 3. Clone evolution before and after radioisotope therapy in group 1.

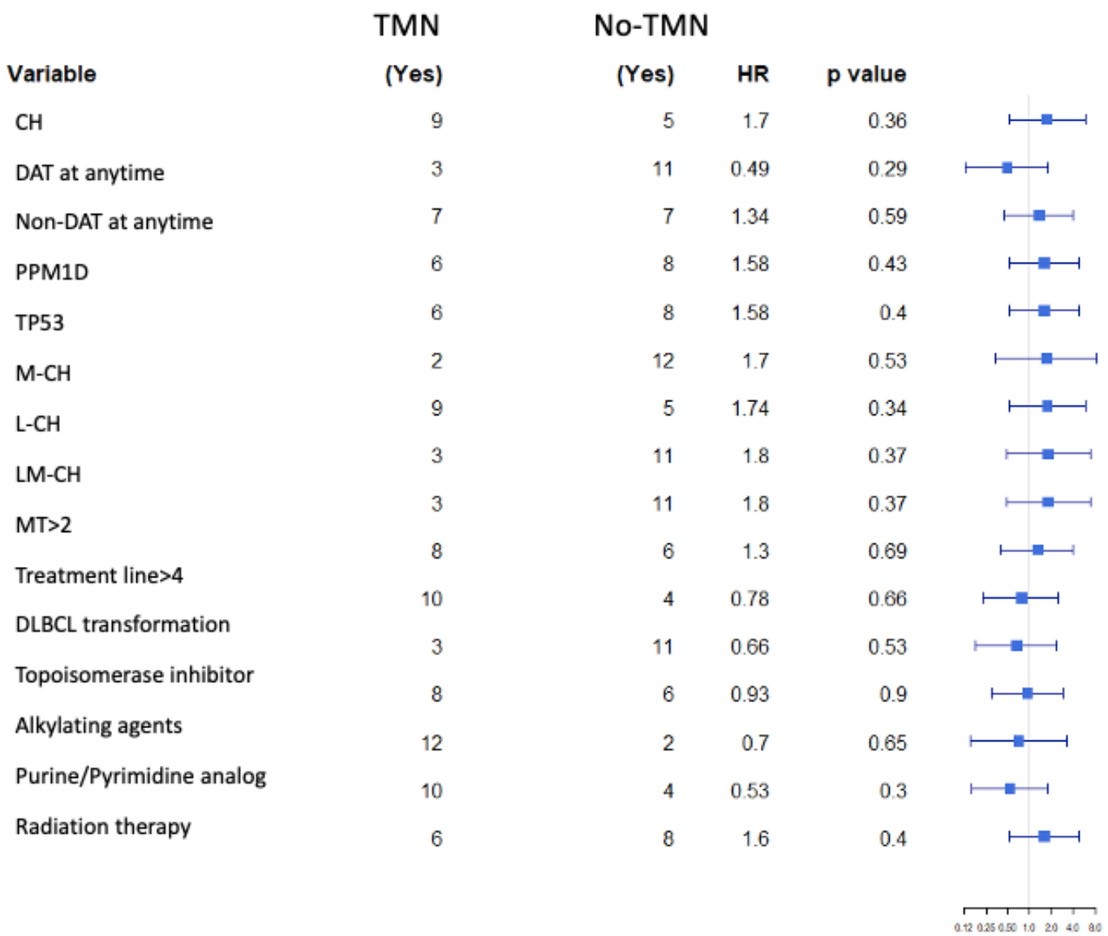



PT_38
VAF (%)
PPM1D
DNMT3A
DNMT3A
before
after
PT_39
VAF (%)
PPM1D
before
after
PT_40
VAF (%)
SBDS
PPM1D
TP53
TP53
before
after
PT_41
VAF (%)
DNMT3A
before
after
PT_42
VAF (%)
DNMT3A
before
after
PT_43
VAF (%)
CREBBP
NF1
KMT2D
ARID1A
before
after
PT_44
VAF (%)
DNMT3A
before
after
PT_45
VAF (%)
MED12
before
after

Supplementary Figure 4. Oncoplot before and after radioisotope therapy in group 1.

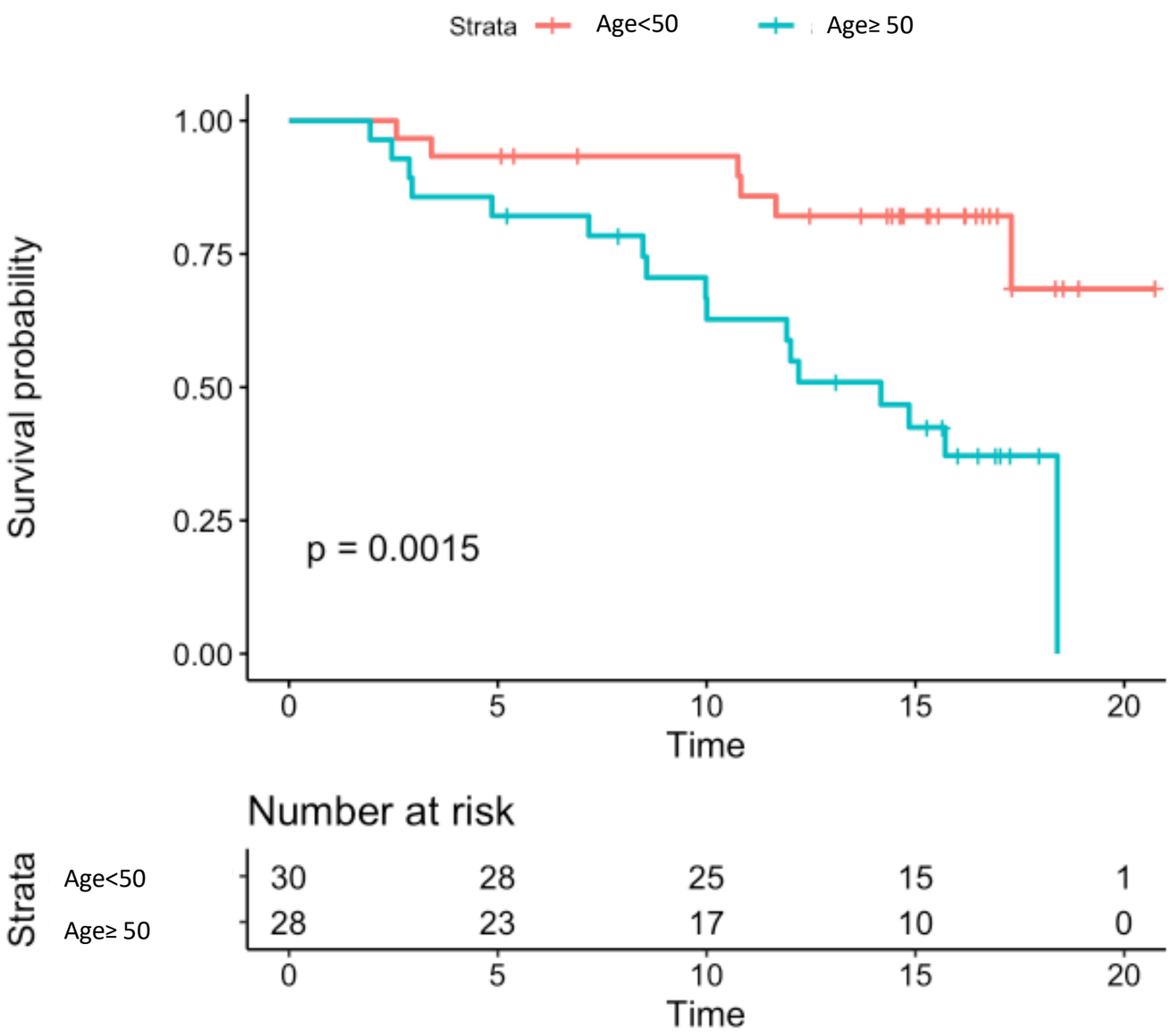


Supplementary Figure 5. Forest plot for the variables on TMN development (univariable analysis);

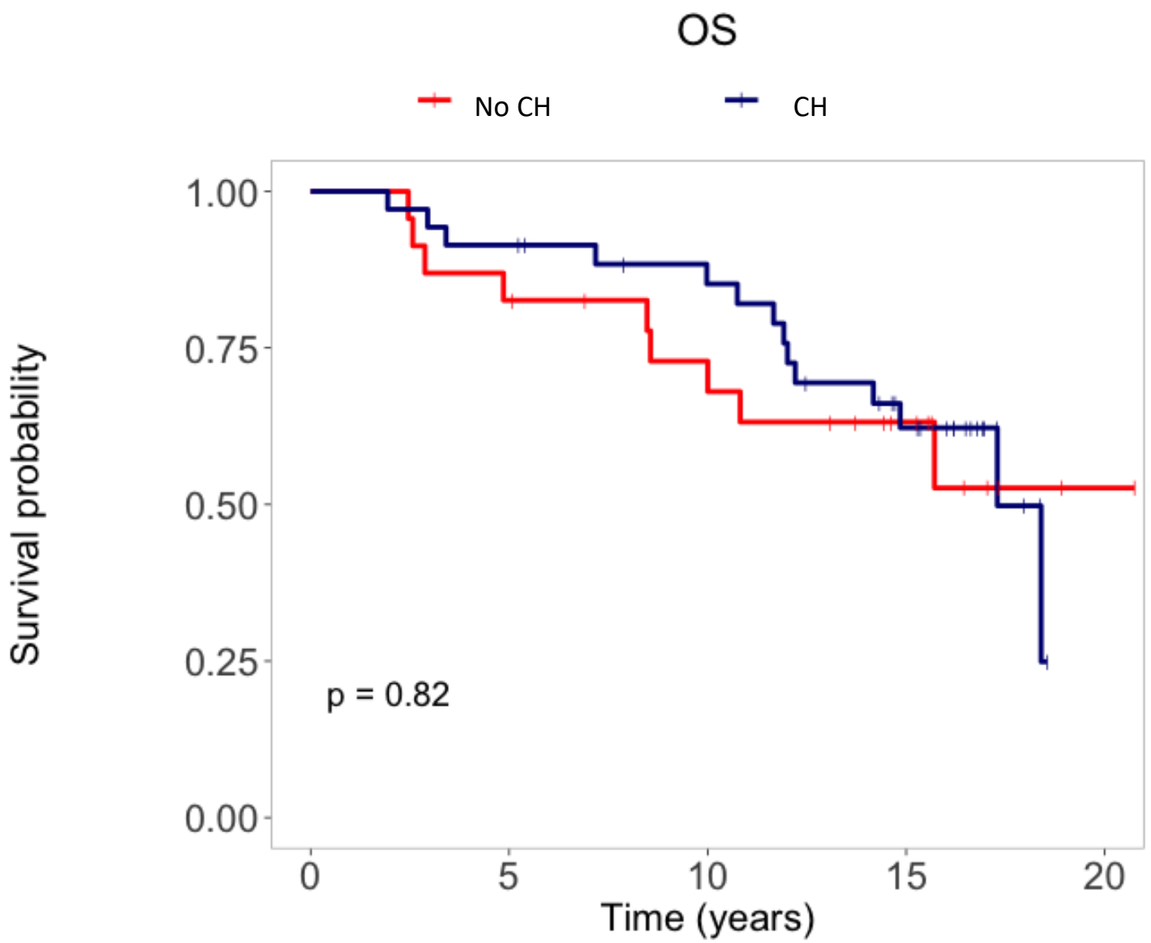


Abbreviation: DAT: *DNMT3A*, *ASXL1,* and *TET2*; AnytimeDAT: DAT mutations at any time points. AnytimeNonDAT: mutations other than DAT mutations at any time points. DDR: DNA damage response and repair; M-CH: myeloid CH; L-CH: lymphoid CH; LM-CH: lymphoid and myeloid CHIP; MT>2: more than 2 mutations.

Supplementary Figure 6. Age>50 is a risk factor for shorter OS. Group 0 indicates age<50, group 1 indicates age ≥ 50



Supplementary Figure 7. Overall survival stratified by CH status.



Number at risk

No CH	23	19	15	9	1
CH	35	32	27	16	0