

Supplemental information

Clinical Outcomes in Patients with Multi-Hit TP53 Chronic Lymphocytic Leukemia Treated with Ibrutinib

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Christian Brieghel¹, Kathrine Aarup¹, Mathias H. Torp², Michael A. Andersen¹, Christina W. Yde², Xin Tian³, Adrian Wiestner⁴, Inhye E. Ahn^{1*} and Carsten U. Niemann^{1*}

1. Department of Hematology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark
2. Center for Genomic Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark
3. Office of Biostatistics Research, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA
4. Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA

*These authors contributed equally to this work

Supplementary Table S1.

Annotated *TP53* variants

Patient ID	c description	p description	VAF, %	Effect	N
A20	c.733G>T	p.Gly245Cys	96.85	missense	1
B01	c.712T>A	p.Cys238Ser	28.43	missense	1
B01	c.818G>A	p.Arg273His	2.00	missense	2
B01	c.743G>A	p.Arg248Gln	1.81	missense	3
B01	c.524G>A	p.Arg175His	1.52	missense	4
B01	c.961A>T	p.Lys321*	1.45	nonsense	5
B01	c.733G>A	p.Gly245Ser	1.37	missense	6
B01	c.837_839delGAG	p.Arg280del	1.18	in-frame	7
B01	c.569C>T	p.Pro190Leu	1.11	missense	8
B01	c.438G>A	p.Trp146*	0.86	nonsense	9
B01	c.746G>C	p.Arg249Thr	0.71	missense	10
B01	c.814G>A	p.Val272Met	0.66	missense	11
B01	c.673-1G>T	Splice	0.56	splice	12
B01	c.586C>T	p.Arg196*	0.45	nonsense	13
B01	c.747G>T	p.Arg249Ser	0.44	missense	14
B01	c.734G>T	p.Gly245Val	0.37	missense	15
B01	c.375+1G>C	Splice	0.37	splice	16
B01	c.581T>A	p.Leu194His	0.33	missense	17
B01	c.722C>T	p.Ser241Phe	0.32	missense	18
B01	c.782+1G>T	Splice	0.30	splice	19
B01	c.469G>T	p.Val157Phe	0.29	missense	20
B01	c.742C>T	p.Arg248Trp	0.28	missense	21
B01	c.694A>T	p.Ile232Phe	0.26	missense	22
B01	c.994-1G>C	Splice	0.23	splice	23
B01	c.749C>T	p.Pro250Leu	0.23	missense	24
B01	c.586_587insT	p.Arg196fs	0.23	frame shift	25
B01	c.585delCinsTG	p.Arg196fs	0.23	frame shift	26
B01	c.814G>T	p.Val272Leu	0.20	missense	27
B02	c.613T>C	p.Tyr205His	82.87	missense	1
B02	c.536A>G	p.His179Arg	0.68	missense	2
B02	c.535C>A	p.His179Asn	0.59	missense	3
B02	c.638G>A	p.Arg213Gln	0.47	missense	4
B02	c.638G>A	p.Arg213Gln	0.47	missense	5
B02	c.487T>C	p.Tyr163His	0.42	missense	6
B02	c.452C>A	p.Pro151His	0.32	missense	7
B02	c.380C>T	p.Ser127Phe	0.30	missense	8
B02	c.711G>A	p.Met237Ile	0.22	missense	9
B03	c.623A>T	p.Asp208Val	18.67	missense	1
B03	c.715A>G	p.Asn239Asp	1.33	missense	2
B03	c.493C>T	p.Gln165*	0.58	nonsense	3
B03	c.637C>T	p.Arg213*	0.45	nonsense	4
B03	c.637C>T	p.Arg213*	0.45	missense	5
B03	c.742_743insACC	p.Asn247_Arg248insHis	0.25	in-frame	6
B04	c.528C>G	p.Cys176Trp	82.56	missense	1
B05	c.524G>A	p.Arg175His	32.00	missense	1
B05	c.574C>T	p.Gln192*	1.26	nonsense	2
B05	c.497C>A	p.Ser166*	0.97	nonsense	3
B05	c.736A>G	p.Met246Val	0.76	missense	4
B05	c.314delG	p.Gly105fs	0.51	frame shift	5
B05	c.838A>G	p.Arg280Gly	0.28	missense	6
B05	c.515T>A	p.Val172Asp	0.22	missense	7
B07	c.742C>T	p.Arg248Trp	94.59	missense	1
B07	c.559+15C>T	Splice	0.26	in-frame	2
B07	c.559+11_559+12insT	Splice	0.26	in-frame	3
B08	c.707A>G	p.Tyr236Cys	49.78	missense	1
B08	c.841G>T	p.Asp281Tyr	1.98	missense	2
B08	c.743G>A	p.Arg248Gln	1.10	missense	3
B08	c.701A>C	p.Tyr234Ser	0.55	missense	4
B08	c.818G>A	p.Arg273His	0.51	missense	5
B08	c.408A>C	p.Gln136His	0.42	missense	6
B08	c.733G>A	p.Gly245Ser	0.40	missense	7
B08	c.673-2A>T	Splice	0.38	splice	8
B08	c.659A>G	p.Tyr220Cys	0.38	missense	9
B08	c.782+1G>C	Splice	0.34	splice	10
B08	c.818G>T	p.Arg273Leu	0.32	missense	11
B08	c.359A>T	p.Lys120Met	0.28	missense	12

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Patient ID	c description	p description	VAF, %	Effect	N
B08	c.673-1G>C	Splice	0.23	splice	13
B08	c.673-2A>C	Splice	0.23	splice	14
B08	c.517G>T	p.Val173Leu	0.21	missense	15
B09	c.524G>A	p.Arg175His	94.71	missense	1
B11	c.1009C>T	p.Arg337Cys	87.18	missense	1
B11	c.701A>G	p.Tyr234Cys	1.02	missense	2
B11	c.730G>A	p.Gly244Ser	0.27	missense	3
B12	c.536A>G	p.His179Arg	96.14	missense	1
B12	c.596G>A	p.Gly199Glu	0.29	missense	2
B14	c.596G>A	p.Gly199Glu	54.77	missense	1
B14	c.832C>T	p.Pro278Ser	0.83	missense	2
B16	c.844_845insCCA	p.Asp281_Arg282insPro	1.04	in-frame	1
B18	c.413C>T	p.Ala138Val	12.25	missense	1
B18	c.638_639delGAinsAG	p.Arg213Gln	11.92	in-frame	2
B18	c.527G>A	p.Cys176Tyr	1.97	missense	3
B18	c.701A>G	p.Tyr234Cys	1.78	missense	4
B18	c.743G>A	p.Arg248Gln	0.90	missense	5
B18	c.832C>T	p.Pro278Ser	0.71	missense	6
B18	c.722C>T	p.Ser241Phe	0.67	missense	7
B18	c.560-2A>T	Splice	0.62	splice	8
B18	c.391A>T	p.Asn131Tyr	0.60	missense	9
B18	c.673-2A>G	Splice	0.53	splice	10
B18	c.415A>T	p.Lys139*	0.52	nonsense	11
B18	c.794T>C	p.Leu265Pro	0.40	missense	12
B18	c.818G>A	p.Arg273His	0.38	missense	13
B18	c.770T>C	p.Leu257Pro	0.36	missense	14
B18	c.395A>G	p.Lys132Arg	0.35	missense	15
B18	c.392A>T	p.Asn131Ile	0.33	missense	16
B18	c.944_945delCTinsAA	p.Ser315*	0.30	nonsense	17
B18	c.375+1G>T	Splice	0.28	splice	18
B18	c.830G>A	p.Cys277Tyr	0.27	missense	19
B18	c.742C>T	p.Arg248Trp	0.26	missense	20
B18	c.488A>G	p.Tyr163Cys	0.25	missense	21
B18	c.716A>G	p.Asn239Ser	0.24	missense	22
B18	c.395A>T	p.Lys132Met	0.24	missense	23
B18	c.584T>C	p.Ile195Thr	0.23	missense	24
B18	c.527G>C	p.Cys176Ser	0.23	missense	25
B18	c.830G>T	p.Cys277Phe	0.22	missense	26
B18	c.672+1G>T	Splice	0.22	splice	27
B18	c.747G>T	p.Arg249Ser	0.21	missense	28
B18	c.527G>T	p.Cys176Phe	0.21	missense	29
B18	c.574C>T	p.Gln192*	0.20	nonsense	30
B19	c.746_751delGGCCCA	p.Arg249_Pro250del	91.85	in-frame	1
B19	c.481G>A	p.Ala161Thr	0.91	missense	2
B19	c.1004_1008delGTGAG	p.Arg335fs	0.76	frame shift	3
B20	c.782+1G>T	Splice	1.90	splice	1
B20	c.733G>A	p.Gly245Ser	1.87	missense	2
B20	c.527G>T	p.Cys176Phe	1.39	missense	3
B20	c.535C>T	p.His179Tyr	1.29	missense	4
B20	c.842A>G	p.Asp281Gly	1.13	missense	5
B20	c.746G>C	p.Arg249Thr	0.94	missense	6
B20	c.854A>T	p.Glu285Val	0.74	missense	7
B20	c.527G>A	p.Cys176Tyr	0.73	missense	8
B20	c.404G>A	p.Cys135Tyr	0.73	missense	9
B20	c.417G>T	p.Lys139Asn	0.67	missense	10
B20	c.469G>T	p.Val157Phe	0.66	missense	11
B20	c.97-1G>T	Splice	0.66	splice	12
B20	c.782+1delG	Splice	0.65	in-frame	13
B20	c.745A>G	p.Arg249Gly	0.65	missense	14
B20	c.281C>A	p.Ser94*	0.63	nonsense	15
B20	c.583_584insA	p.Ile195fs	0.60	frame shift	16
B20	c.731G>A	p.Gly244Asp	0.58	missense	17
B20	c.559+1G>A	Splice	0.58	splice	18
B20	c.623A>T	p.Asp208Val	0.54	missense	19
B20	c.695T>C	p.Ile232Thr	0.52	missense	20
B20	c.524G>A	p.Arg175His	0.51	missense	21
B20	c.713G>T	p.Cys238Phe	0.43	missense	22
B20	c.329G>C	p.Arg110Pro	0.41	missense	23
B20	c.743G>A	p.Arg248Gln	0.39	missense	24

(continued)

Patient ID	c description	p description	VAF, %	Effect	N
B20	c.407A>C	p.Gln136Pro	0.38	missense	25
B20	c.1036G>T	p.Glu346*	0.36	nonsense	26
B20	c.559+1G>C	Splice	0.36	splice	27
B20	c.313G>C	p.Gly105Arg	0.36	missense	28
B20	c.775G>T	p.Asp259Tyr	0.35	missense	29
B20	c.481G>A	p.Ala161Thr	0.32	missense	30
B20	c.1001G>T	p.Gly334Val	0.30	missense	31
B20	c.841G>T	p.Asp281Tyr	0.30	missense	32
B20	c.746G>T	p.Arg249Met	0.28	missense	33
B20	c.455_456insC	p.Pro153fs	0.28	frame shift	34
B20	c.97-1G>C	Splice	0.28	splice	35
B20	c.158_159insAATG	p.Trp53fs	0.26	frame shift	36
B20	c.329G>T	p.Arg110Leu	0.23	missense	37
B21	c.673-2A>G	Splice	16.19	splice	1
B21	c.469G>T	p.Val157Phe	0.23	missense	2
B21	c.332T>A	p.Leu111Gln	0.22	missense	3
B21	c.713G>A	p.Cys238Tyr	0.20	missense	4
B22	c.590T>G	p.Val197Gly	89.22	missense	1
B23	c.476_477delCCinsAG	p.Ala159Glu	96.72	in-frame	1
B24	c.733G>T	p.Gly245Cys	91.38	missense	1
B24	c.712T>G	p.Cys238Gly	0.27	missense	2
B25	c.638G>A	p.Arg213Gln	35.63	missense	1
B25	c.638G>A	p.Arg213Gln	35.63	missense	2
B25	c.686_687delGT	p.Cys229fs	18.68	frame shift	3
B25	c.782+1G>T	Splice	1.58	splice	4
B25	c.993+1G>A	Splice	1.38	splice	5
B25	c.404G>A	p.Cys135Tyr	1.14	missense	6
B25	c.93delT	p.Leu32fs	1.08	frame shift	7
B25	c.718A>G	p.Ser240Gly	0.78	missense	8
B25	c.818G>A	p.Arg273His	0.73	missense	9
B25	c.335delG	p.Gly112fs	0.64	frame shift	10
B25	c.643A>G	p.Ser215Gly	0.59	missense	11
B25	c.844C>G	p.Arg282Gly	0.58	missense	12
B25	c.422G>A	p.Cys141Tyr	0.45	missense	13
B28	c.626_627delGA	p.Arg209fs	97.80	frame shift	1
B28	c.848G>A	p.Arg283His	0.31	missense	2
B29	c.919+1G>A	Splice	96.27	splice	1
B30	c.993+1G>T	Splice	77.51	splice	1
B32	c.990_991insT	p.Gln331fs	38.11	frame shift	1
B33	c.709A>G	p.Met237Val	99.18	missense	1
B33	c.848G>A	p.Arg283His	0.34	missense	2
B34	c.243_245delACC	p.Pro82del	3.84	in-frame	1
B34	c.241delA	p.Thr81fs	2.05	frame shift	2
B34	c.239delC	p.Pro80fs	1.19	frame shift	3
B34	c.234delA	p.Ala79fs	0.55	frame shift	4
B34	c.314_315insG	p.Ser106fs	0.52	frame shift	5
B34	c.237delT	p.Pro80fs	0.51	frame shift	6
B34	c.320A>C	p.Tyr107Ser	0.49	missense	7
B34	c.810delT	p.Phe270fs	0.47	frame shift	8
B34	c.848G>A	p.Arg283His	0.45	missense	9
B34	c.804delC	p.Asn268fs	0.37	frame shift	10
B34	c.806_807insG	p.Ser269fs	0.23	frame shift	11
B35	c.439delG	p.Val147fs	86.21	frame shift	1
B36	c.403T>G	p.Cys135Gly	88.24	missense	1
B37	c.460_461delGG	p.Gly154fs	90.58	frame shift	1
B37	c.455_457delCGCinsACA	p.Pro152_Pro153delinsHisThr	89.93	in-frame	2
B37	c.466delCinsATG	p.Arg156fs	0.23	frame shift	3
B38	c.151G>T	p.Glu51*	92.27	nonsense	1
B39	c.652_654delGTG	p.Val218del	42.37	in-frame	1
B40	c.844C>T	p.Arg282Trp	84.79	missense	1
B41	c.652delG	p.Val218fs	45.21	frame shift	1
B43	c.818G>T	p.Arg273Leu	75.75	missense	1
B45	c.817C>T	p.Arg273Cys	7.88	missense	1
B45	c.626_627delGA	p.Arg209fs	0.68	frame shift	2
B45	c.358A>G	p.Lys120Glu	0.20	missense	3
B46	c.659A>G	p.Tyr220Cys	86.08	missense	1
B46	c.817C>T	p.Arg273Cys	2.42	missense	2
B47	c.797G>A	p.Gly266Glu	51.66	missense	1
B47	c.535C>T	p.His179Tyr	1.04	missense	2

(continued)

Patient ID	c description	p description	VAF, %	Effect	N
B47	c.743G>A	p.Arg248Gln	0.40	missense	3
B47	c.469G>T	p.Val157Phe	0.24	missense	4
B48	c.530C>G	p.Pro177Arg	94.07	missense	1
B49	c.469G>T	p.Val157Phe	13.51	missense	1
B49	c.641A>G	p.His214Arg	1.80	missense	2
B49	c.358A>G	p.Lys120Glu	0.85	missense	3
B49	c.856G>C	p.Glu286Gln	0.70	missense	4
B49	c.560-1G>A	Splice	0.63	splice	5
B49	c.772G>A	p.Glu258Lys	0.51	missense	6
B49	c.742C>T	p.Arg248Trp	0.44	missense	7
B49	c.635_636delTT	p.Phe212fs	0.40	frame shift	8
B49	c.841G>A	p.Asp281Asn	0.36	missense	9
B49	c.743G>A	p.Arg248Gln	0.27	missense	10
B50	c.847C>T	p.Arg283Cys	49.97	missense	1
B51	c.713G>A	p.Cys238Tyr	81.25	missense	1
B52	c.659A>G	p.Tyr220Cys	98.55	missense	1

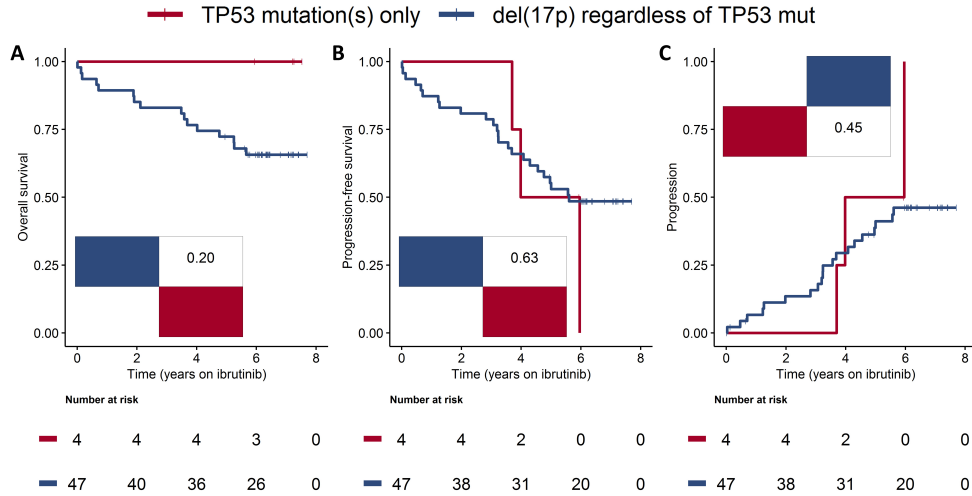
Supplementary Table S2.

Characteristic		Total (n=112)	Single-hit TP53 (n=94)	Multi-hit TP53 (n=18)	P
		No. (%)	No. (%)	No. (%)	
Age, years	Median (IQR)	72.7 (66.2-77.0)	73.2 [65.9-77.4]	71.9 (68.5-73.8)	0.44
Sex	Female	41 (36.6)	37 (39.4)	4 (22.2)	0.19
	Male	71 (63.4)	57 (60.6)	14 (77.8)	
Binet stage	A	49 (65.3)	43 (68.3)	6 (50.0)	0.17
	B	18 (24.0)	15 (23.8)	3 (25.0)	
	C	8 (10.7)	5 (7.9)	3 (25.0)	
	missing	37	31	6	
B2M, mg/L	<4	43 (86.0)	37 (86.0)	6 (85.7)	>0.99
	>4	7 (14.0)	6 (14.0)	1 (14.3)	
	missing	62	51	11	
IGHV status	M-CLL	59 (73.8)	49 (72.1)	10 (83.3)	0.50
	U-CLL	21 (26.2)	19 (27.9)	2 (16.7)	
	missing	32	26	6	
TP53mut	wild type	10 (16.4)	10 (23.3)	0 (0.0)	0.026
	mutated	51 (83.6)	33 (76.7)	18 (100.0)	
	missing	51	51	0	
del(17p)	absent	33 (29.5)	33 (35.1)	0 (0.0)	0.0014
	present	79 (70.5)	61 (64.9)	18 (100.0)	
Treatment status	TN	35 (31.2)	30 (31.9)	5 (27.8)	>0.99
	RR	77 (68.8)	64 (68.1)	13 (72.2)	

Abbreviations: B2M, beta-2-microglobulin; IGHV, immunoglobulin heavy-chain variable region; M-CLL, mutated CLL; U-CLL, unmutated CLL; *TP53* mut, *TP53* mutational status; del(17p), deletion of chromosome 17p; TN, treatment-naïve; RR, relapsed-refractory; IQR, interquartile range.

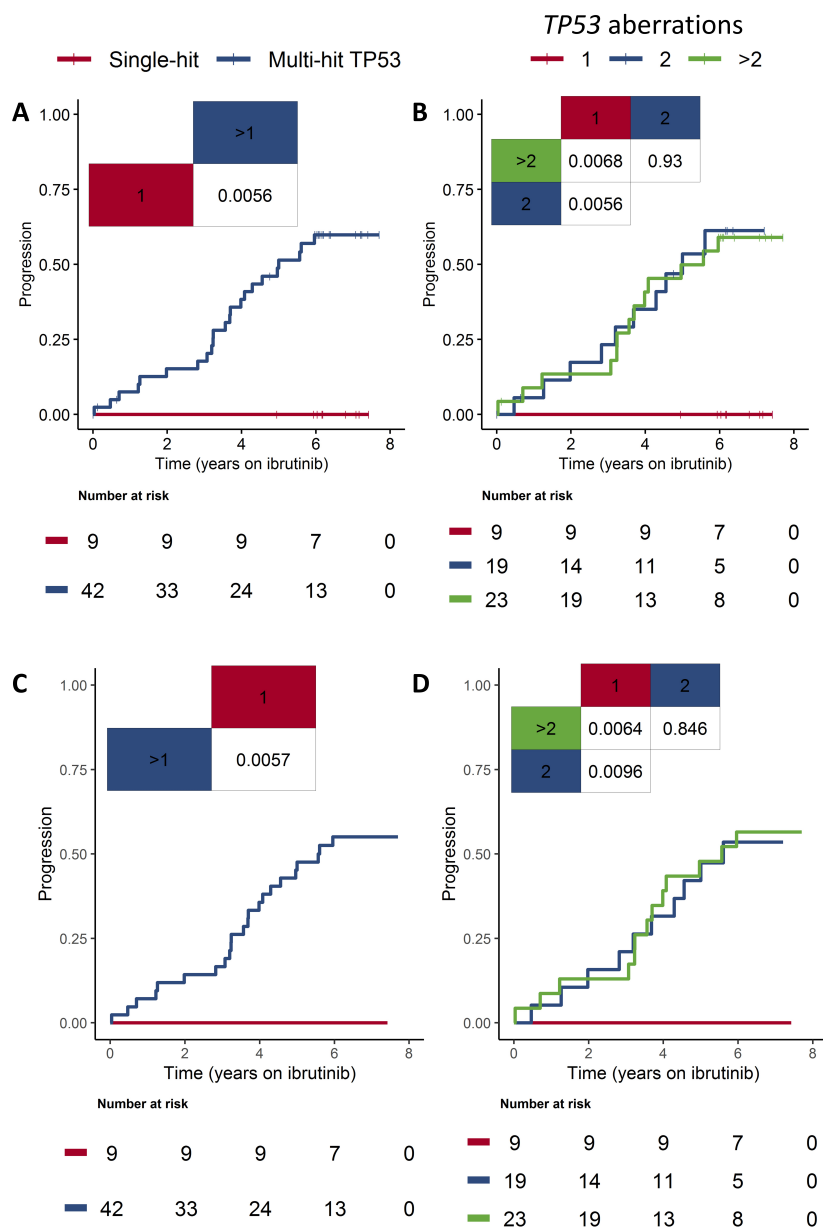
NOTE: Baseline characteristics in 112 Danish *TP53* aberrant patients treated with single-agent ibrutinib. *P* indicates intergroup difference between single- and multi-hit *TP53*.

Supplementary Figure S1.



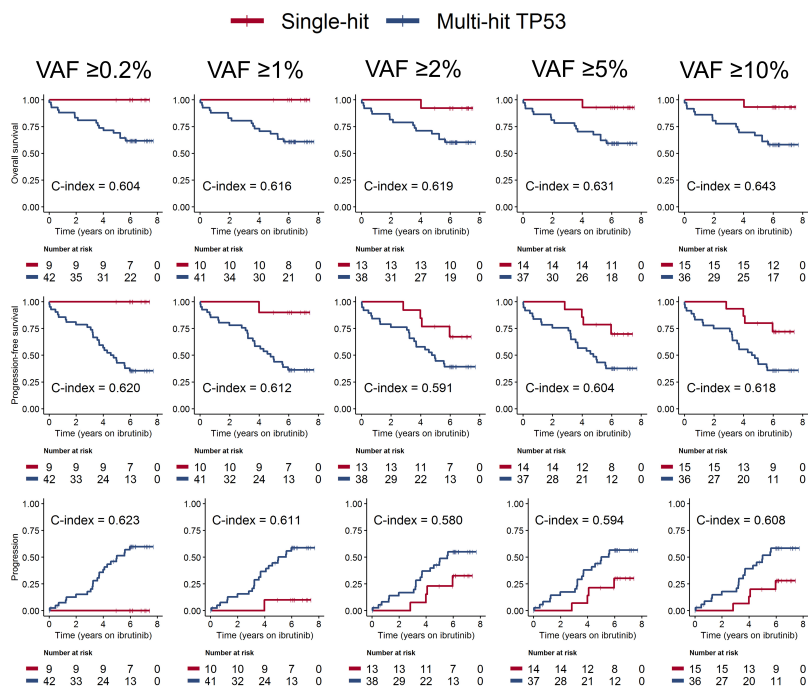
(A) Overall survival, (B) progression-free survival, and (C) time to progression stratified for patients with *TP53* mutations only and patients with del(17p) regardless of *TP53* mutational status.

Supplementary Figure S2.



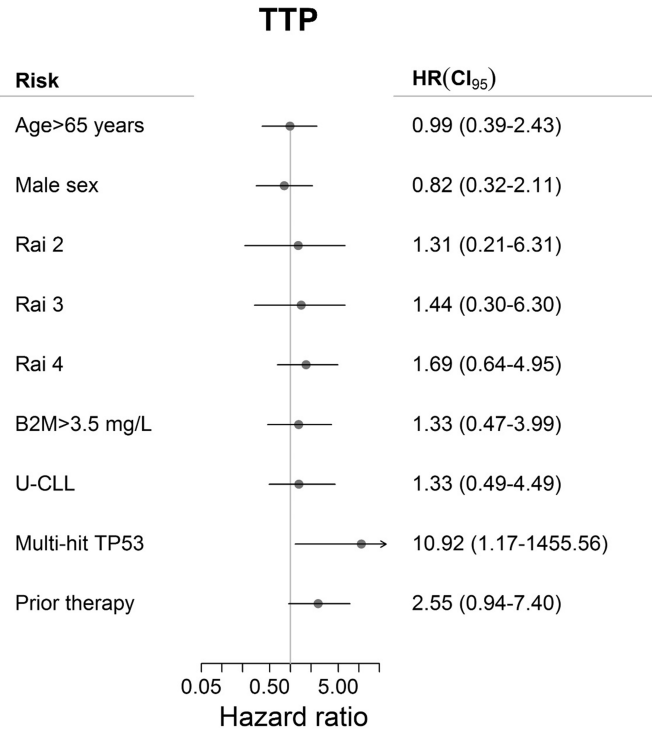
(A-B) Time to progression (TTP) and (C-D) cumulative incidence of relapse (CIR) considering death as competing risk. (A, C) Significant difference was demonstrated for patients with single-hit (red) compared to multi-hit *TP53* (blue), whereas (B, D) similar outcome was demonstrated for multi-hit *TP53* patients carrying 2 (blue) and >2 *TP53* aberrations (green).

Supplementary Figure S3.



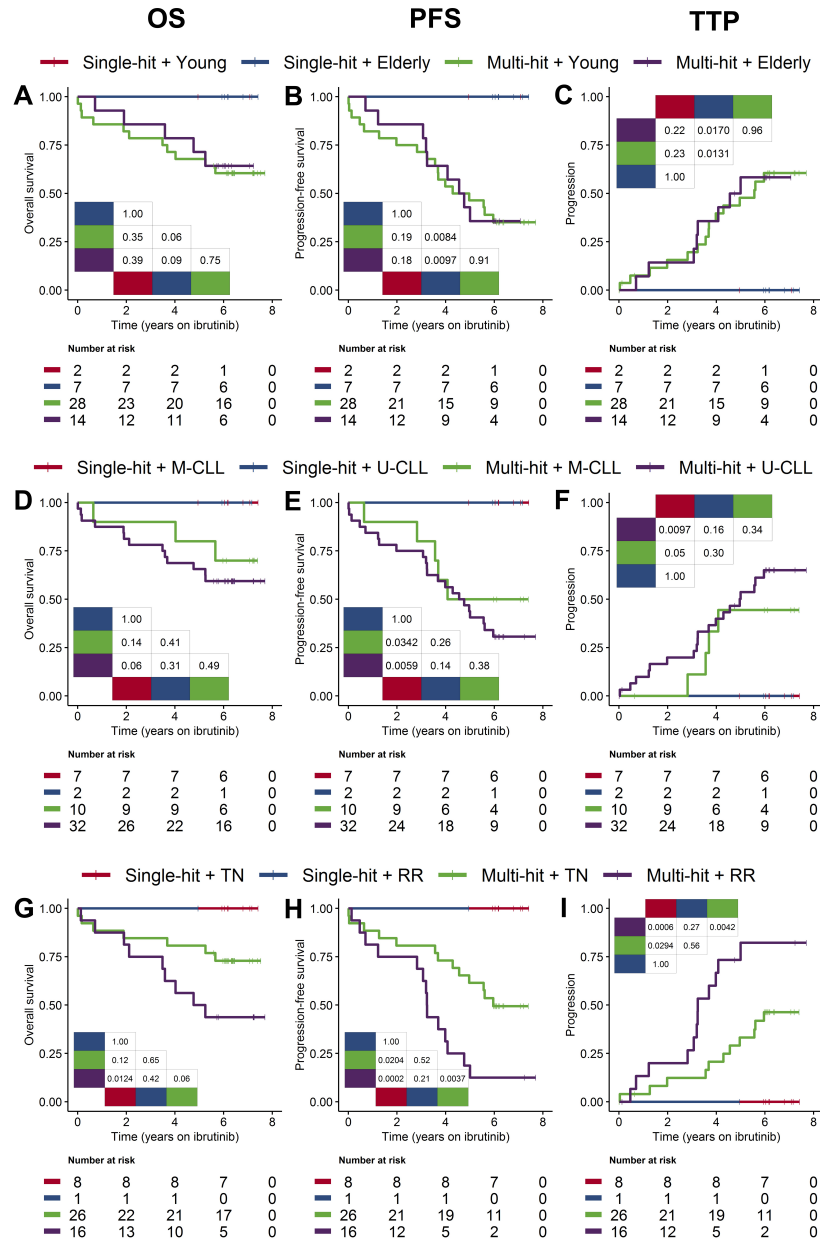
Gradually excluding low burden *TP53* mutations below 1%, 2%, 5% and 10% variant allele frequency (VAF; left to right) for overall survival (OS; top), progression-free survival (PFS; middle), and time to progression (TTP; bottom) also resulted in an increased number of patients with single-hit *TP53*. OS was similar when using a low VAF cutoff compared to a high VAF cutoff and showed better discrimination capabilities with a high VAF cutoff. By contrast, the highest discrimination capabilities for PFS and TTP were seen with the lowest VAF cutoff of 0.2%, and both PFS as well as TTP for the group of patients single-hit *TP53* became shorter when excluding *TP53* mutations, especially when excluding *TP53* mutations below 2% VAF.

Supplementary Figure S4.



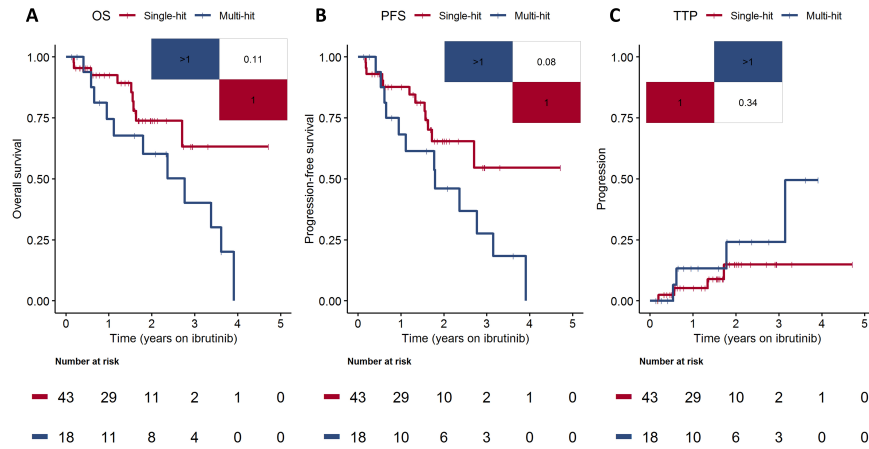
Multivariable analysis with Firth's penalized likelihood for time to progression (TTP). Multi-hit *TP53* was the only independent risk factor associated with shorter TTP. Abbreviations: B2M, beta-2-microglobulin; U-CLL, unmutated immunoglobulin heavy-chain variable region gene (IGHV) status.

Supplementary Figure S5.



(A, D, G) Overall survival (OS), (B, E, H) progression-free survival (PFS), and (C, F, I) time to progression (TTP) based on single and multi-hit *TP53* status, and further stratified on (A-C) age, (D-F) IGHV mutational status, and (G-I) treatment status.

Supplementary Figure S6.



Validation of multi-hit *TP53* in 61 of 112 patients with *TP53* aberrant chronic lymphocytic leukemia with known *TP53* mutational status. By including only patients with known *TP53* mutational status, no difference in (A) OS, (B) PFS or (C) TTP was demonstrated in patients with single-hit *TP53* (red) compared to patients with multi-hit *TP53* (red).