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Prognostic value and oncogenic landscape of TP53 alterations in adult and pediatric T-ALL

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1 **Prognostic value and oncogenic landscape of *TP53* alterations in adult and**
 2 **pediatric T-ALL**

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54 Among hematological malignancies, T-cell acute lymphoblastic leukemia (T-ALL)
55 represents a class of aggressive tumors with dismal clinical outcome in case of relapse or
56 refractory diseases. While the intensification of multi-agent chemotherapy protocols has
57 dramatically improved prognosis, refractory and relapsed cases are clinically challenging due
58 to limited therapeutic options.^{1,2} p53 is a transcription factor and a master tumor suppressor
59 gene frequently altered in cancer.³ In contrast to carcinomas and other hematological
60 malignancies, *TP53* alterations (*TP53*^{Alt}), encompassing mutations (*TP53*^{Mut}) and/or pan-exon
61 deletions (*TP53*^{Del}), are remarkably rare at diagnosis in T-ALL, and their clinical implication
62 remains elusive.⁴⁻⁶ Critically, *TP53*^{Alt} have been reported to be acquired in up to 20% of the
63 relapsed T-ALL cases, where they convey a deleterious prognosis.⁷⁻⁹ Here, we produce the
64 first comprehensive analysis of *TP53*^{Alt} and the associated oncogenetic landscape in an
65 extensive cohort of 476 patients newly diagnosed with T-ALL.

66

67 We investigated the clinical characteristics linked to *TP53*^{Alt} in 476 patients, including 215
68 adults, and 261 children. Adult patients were enrolled in the GRAALL-2003-2005 trials
69 (GRAALL-2003, NCT00222027; GRAALL-2005, NCT00327678) and pediatric patients
70 were enrolled in the FRALLE 2000 trial (Supplemental Figure 1). Based on DNA availability
71 for molecular analysis, 215 adult patients out of 337 and 261 pediatric patients out of 427
72 were included in this study. No difference in clinical outcomes was observed between the
73 included patients and the entire cohort (Supplemental Figure 2-3, Supplemental Table 1).
74 Diagnostic peripheral blood or bone marrow samples were collected after informed consent
75 was obtained, according to the Declaration of Helsinki. All samples contained $\geq 80\%$ blasts,
76 immunophenotypic of T-ALL samples, minimal residual disease (MRD) assessment and
77 Multiplex ligation-dependent probe amplification analysis (MLPA) (P383 T-ALL from MRC
78 Holland) were performed as previously described.¹⁰⁻¹²

79 Genomic analysis was performed by pan-exon targeted next-generation sequencing (NGS) of
80 DNA extracted from diagnostic samples; DNA libraries were prepared using Nextera XT kit
81 (Illumina) and sequenced on an Illumina MiSeq. The NGS panel included 63 genes known to
82 be mutated in T-ALL (Supplemental Table 2.). Genetic lesion co-occurrences and mutual
83 exclusions were computed using the DISCOVER R package. We performed a computational
84 approach previously described for the detection of copy number variants (CNVs) from next-
85 generation sequence data,^{13,14} including systematic analysis of the depth of *TP53* gene
86 coverage. This is based on variations in depth of coverage of aligned sequence reads, using a
87 locally developed algorithm. The CNVs detected were confirmed by high-resolution CGH
88 and/or MLPA analysis (kit P037-CLL-1 and P038-CLL-2 from MRC-Holland). Diagnostic
89 DNA was hybridized on Affymetrix (Santa Clara, CA) Cytogenetics whole Genome 2.7M
90 Arrays (CGH-array), according to the manufacturer's recommendations. Data analysis was
91 performed with the Chromosome Analysis Suite (ChAS) software (Affymetrix®). Gene copy
92 number aberrations were compared with the Database of Genomic variants (DGV)
93 <http://projects.tcag.ca/variation> to study only non-variant GCV aberrations.

94 Comparisons for categorical and continuous variables between subgroups were performed
95 with Fisher's exact test and Mann-Whitney test respectively. Overall survival (OS) was
96 calculated from the date of diagnosis to the last follow-up date censoring patients alive. The
97 cumulative incidence of relapse (CIR) was calculated from the complete remission (CR) date
98 to the date of relapse censoring patients alive without relapse at the last follow-up date.
99 Relapse and death in CR were considered competitive events. Univariate and multivariate
100 analyses assessing the impact of categorical and continuous variables were performed with a
101 Cox model. Proportional-hazards assumption was checked before conducting multivariate
102 analyses. In univariate and multivariate analyses, age and log₁₀(WBC) were considered
103 continuous variables. All analyses were stratified on the trial. Variables with a p-value less

104 than 0.1 in univariate analysis were included in the multivariable models. Statistical analyses
105 were performed with STATA software (STATA 12.0 Corporation, College Station, TX). All
106 p-values were two-sided, with $p < 0.05$ denoting statistical significance. Additional details are
107 included in the Supplemental Methods.

108

109 The incidence of $TP53^{Alt}$ at diagnosis in these cohorts was 4% (21/476). $TP53^{Mut}$ were
110 detected in 9 patients (6 adults and 3 pediatric cases, Figure 1A, Supplemental Tables 3 and
111 4) and $TP53^{Del}$ were identified in 15 patients (7 adult and 8 pediatric cases), 3 patients
112 harbored both $TP53^{Mut}$ and $TP53^{Del}$. Patients with $TP53^{Alt}$ did not significantly differ from
113 $TP53$ wild-type ($TP53^{Wt}$) patients regarding sex, age and central nervous system (CNS)
114 involvement (Table 1), but were associated with an immature phenotype (combining IM0 T-
115 ALL; TCRd and TCRg germline, IMd T-ALL; TCRd rearranged but not TCRg and IMg T-ALL;
116 both TCRd and TCRg rearranged)¹² (8/20, 40% vs 81/399, 20%, $p = 0.048$). The oncogenetic
117 landscape of $TP53^{Alt}$ was comparable to $TP53^{Wt}$ T-ALLs (Figure 1B and 1C, Supplemental
118 Figures 4, 5 and 6 and Supplemental Table 5). To investigate the prognostic value of $TP53^{Alt}$,
119 survival analyses were performed on the series of 476 patients. $TP53^{Alt}$ did not confer
120 increased poor prednisone response, defined by a peripheral blood blast count $> 1.0 \times 10^9/L$
121 at the end of the induction phase) (38% vs 45%, $p = 0.7$) (Table 1). Although $TP53^{Alt}$ did not
122 significantly influence the morphological complete response rate at the end of the induction
123 course (81% vs 93%, $p = 0.07$), patients harboring $TP53^{Alt}$ were associated with delayed early
124 medullary blast clearance, as confirmed by end of induction MRD1 assessment, with more
125 positivity ($\geq 10^{-4}$) in $TP53^{Alt}$ as compared with $TP53^{Wt}$ cases (75% vs 35%, $p = 0.01$). Patients
126 (both adult and pediatric) with $TP53^{Alt}$ had an inferior outcome compared to $TP53^{Wt}$ (Table 1,
127 Figures 1D-E, Supplemental Figure 7) with an increased CIR (5y-CIR: 65% vs. 27%; specific
128 hazard ratio (SHR) 3.1, 95%CI (1.67 - 5.78); $p < 0.001$) and a shorter OS (5y-OS: 48% vs.

129 72%; hazard ratio: 2.34, 95%CI (1.30 - 4.24); $p = 0.005$). In multivariate analysis $TP53^{Alt}$
130 predicted a statistically lower OS (HR: 2.87, 95%CI (1.56 - 5.26); $p = 0.001$) and higher CIR
131 (SHR, 2.90, 95%CI 1.55 - 5.44), $p = 0.001$) even after adjustment on the 4-genes
132 *NOTCH1/FBXW7/RAS/PTEN* (*NFRP*) classifier, which identified poor prognosis patients in
133 both GRAALL and FRALLE trials.^{10,15}

134 The limited number of MRD1 assessments available among the $TP53^{Alt}$ T-ALL patients
135 (12/21, 57%), did not allow us to include this endpoint in the multivariate analysis, justifying
136 further studies to establish whether this alteration remains an independent prognostic factor
137 when combined with MRD1 status in T-ALL.

138 This study provides the largest comprehensive analysis of $TP53^{Alt}$ in T-ALL, describing for
139 the first time both their clinical profile and, most importantly, the extremely poor prognosis
140 impact associated with $TP53^{Alt}$ at diagnosis in T-ALL, urging the need to develop innovative
141 targeted therapies for patients harboring $TP53^{Alt}$.

142 About 25% pediatric and 50% adult T-ALL patients relapse, with a 5-year survival inferior
143 to 20% in both age groups.^{1,2} The sole therapeutic approaches with a curative potential for T-
144 ALL relapsed cases are limited to conventional chemotherapy or hematopoietic stem cell
145 transplantation (HSCT). Molecular genetic analyses and sequencing studies have recently led
146 to the identification of recurrent T-ALL alterations associated with the prognostic, allowing
147 to refine the stratification of the relapse risk.^{10,14,16,17} Yet, a significant proportion of T-ALL
148 relapses remain unpredicted, underlining the urge for new predictive markers.

149 Negative outcomes observed in $TP53^{Alt}$ T-ALL are likely to be related to p53-induced
150 therapeutic resistance previously described for other malignancies combining loss of p53
151 tumor suppressor activity and acquisition of novel functions that disrupt the DNA-damage
152 response pathway.^{18,19} APR-246 (Eprentapopt), a small molecule interacting with mutated
153 p53 protein to restore its WT conformation^{20,21}, has recently shown promising results in

154 myeloid malignancies and in B-ALL²²⁻²⁵ justifying further studies to explore the potential
155 efficacy of this new drug in T-ALL *TP53*^{Mut}.

156

157

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173 **Authorship Contributions**

174 V.A, O.H, M.S and G.P.A conceived and designed the research and oversaw the project; M.B, C.G,
175 N.G, J-M.C, F.H, Y.C, Y.B, E.M, V.G, A.P, P.R, A.B, D.B, O.H, N.B, V.A provided study materials
176 or patients; M.S, G.P.A and V.A performed molecular analyses; M.S and G.P.A collected and
177 assembled data; N.B and M.S performed statistical analysis; M.S, G.P.A, R.B, O.H V.A, N.B
178 analyzed and interpreted data; M.S, G.P.A, N.B and V.A wrote the manuscript. M.S and G.P.A
179 contributed equally to this work.

180

181 **Disclosure of Conflicts of Interest**

182 The authors declare no competing financial interests

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243 Hematopoietic Stem-Cell Transplantation for TP53-Mutant Acute Myeloid Leukemia and
244 Myelodysplastic Syndromes. *J Clin Oncol*. 2022;JCO2200181.
- 245

246 **Table Legends**

247 **Table 1: Clinico-biological and outcome characteristics of adult and pediatric T-ALL**
 248 **(GRAALL and FRALLE protocols) according to TP53 status.**

249 Abbreviations: T-ALL: T-cell acute lymphoblastic leukemia; WBC, white blood count; CNS,
 250 central nervous system; ETP, early thymic precursor; *High Risk* classifier, *NOTCH1/FBXW7-*
 251 *RAS/PTEN* classifier as previously described^{10,15}; CR, complete remission; MRD, minimal
 252 residual disease; Allo-HSCT, allogenic hematopoietic stem cell transplantation; CIR,
 253 cumulative incidence of relapse; OS, overall survival; HR: hazard ratio, SHR: specific hazard
 254 ratio, CI: confidence interval.

255 MRD1 correspond to MRD evaluation after induction and was performed by allele-specific
 256 oligonucleotides polymerase chain reaction. T-cell receptor status and oncogenic were
 257 performed as described in supplemental methods.

258 * CNS involvement; CNS3 in FRALLE2000 trial, CNS2 and/or CNS3 in GRAALL2003 and
 259 GRAALL2005 trial

260 ** T-ALL are divided into three subclasses as following **(i)** immature (no detectable TCRb
 261 VDJ): IM0 (TCRd and TCRg germline), IMd (TCRd rearranged but not TCRg) and IMg
 262 (both TCRd and TCRg rearranged); **(ii)** T-ALL with TCRab lineage (including both early-
 263 cortical IMb/Pre-ab and mature sTCRab+) and **(iii)** mature sTCRgd.¹²

264 *** Low Risk Classifier: *NOTCH1* and/or *FBXW7* (*N/F*) mutation without *N/K-RAS* and
 265 *PTEN* (*R/P*) alteration. High-Risk Classifier: *N/F* mutation with *R/P* alteration, *N/F* Wild-
 266 type with or without *R/P* alteration.^{10,15}

267
 268 ¹Statistics presented: Median (Minimum-Maximum)

269 ²Statistical tests performed: Fisher's exact test; Wilcoxon rank-sum test.

270 ³Univariate and multivariate Cox analyses stratified on protocol

271 p-values < 0.05 are indicated in bold

272

273 **Figure Legends**

274 Figure 1: ***TP53* Alterations in the GRAALL03/05 and FRALLE2000 studies**

275 **A.** Lollipop plots indicating the observed *TP53* mutations in 476 T-ALL patients. More
276 details reporting the pathogenicity of the *TP53* mutations are provided in Supplemental Table
277 4.

278 **B.** Circos plots illustrating pairwise relationships across the relatively common mutated
279 genes in *TP53^{Alt}* T-ALL. The width of the ribbon corresponds to the number of cases who
280 have simultaneous presence of a first and a second gene mutation.

281 **C.** Frequency of alterations per gene among the *TP53^{Alt}* vs *TP53^{WT}* T-ALL. The width of the
282 circles is proportional to the frequency of alterations observed in the two T-ALL subgroups
283 (*TP53^{Alt}* in red, vs *TP53^{WT}* T-ALL in blue).

284 **D, E** Clinical impact of *TP53* Alterations in the GRAALL0305 and FRALLE2000 studies.
285 Overall survival (**D**) and cumulative incidence of relapse (**E**). The red curve represents the
286 *TP53^{Alt}* T-ALL, and the black curve the *TP53^{WT}* patients

287

288 Table

Table 1.

Variable	<i>TP53^{Alt}</i> n = 21 (4%)	<i>TP53^{WT}</i> n = 455 (96%)	Total n = 476	p-value ²
Male	14 / 21 (67%)	343 / 455 (75%)	357 / 476 (75%)	0.4
Age (y) ¹	23.4 (4.0-51.8)	15.3 (1.1-59.1)	15.3 (1.1-59.1)	0.5
WBC (G/l) ¹	25 (5-674)	66 (0-980)	64 (0-980)	0.01
CNS Involvement*	3 / 21 (14%)	48 / 453 (11%)	51 / 474 (11%)	0.5
Immunophenotype				
ETP phenotype	6 / 16 (38%)	50 / 291 (17%)	56 / 307 (18%)	0.09
Immature (IM0/d/g)**	8 / 20 (40%)	81 / 399 (20%)	89 / 419 (21%)	0.048
ab lineage	6 / 20 (30%)	205 / 399 (51%)	211 / 419 (50%)	0.07
Mature TCRgd	3 / 20 (15%)	63 / 399 (16%)	66 / 419 (16%)	>0.9
Oncogenetic classification				
<i>TLX1</i>	1 / 18 (6%)	53 / 397 (13%)	54 / 415 (13%)	0.5
<i>TLX3</i>	2 / 18 (11%)	70 / 397 (18%)	72 / 415 (17%)	0.8
<i>SIL-TAL1</i>	1 / 18 (6%)	56 / 397 (14%)	57 / 415 (14%)	0.5
<i>CALM-AF10</i>	0 / 18 (0%)	13 / 397 (3%)	13 / 415 (3%)	>0.9
High-Risk Classifier***	13 / 21 (62%)	196 / 455 (43%)	209 / 476 (44%)	0.12
Treatment Response				
Prednisone response	13 / 21 (62%)	246 / 446 (55%)	259 / 467 (55%)	0.7
Chemosensitivity	12 / 21 (57%)	325 / 446 (73%)	337 / 467 (72%)	0.1
MRD1 > 10 ⁻⁴	9 / 12 (75%)	114 / 328 (35%)	123 / 340 (36%)	0.01
Complete Remission	17 / 21 (81%)	423 / 455 (93%)	440 / 476 (92%)	0.07
Allo-HSCT	4/20 (20%)	97 / 436 (22%)	101 / 456 (22%)	0.7
Outcome				
5-year CIR [95% CI]	65% [11;43]	27% [23;32]	29% [25;33]	<0.001
5-year OS [95% CI]	48% [26;67]	72% [68;76]	71% [67;75]	0.005

Univariate and Multivariate Analysis ³						
CIR	Univariate			Multivariate		
	SHR	95%CI	p	SHR	95%CI	p
Age	1.01	(0.98 ; 1.03)	0.57	-	-	-
CNS	1.57	(0.85 ; 2.59)	0.08	1.28	(0.77 ; 2.13)	0.34
Log(WBC)	1.62	(1.2 ; 2.18)	0.002	1.62	(1.19 ; 2.19)	0.002
Prednisone response	0.67	(0.47 ; 0.95)	0.03	0.93	(0.64 ; 1.35)	0.70
High-risk Classifier**	2.78	(1.94 ; 3.99)	< 0.001	2.58	(1.78 ; 3.74)	< 0.001
<i>TP53^{Alt}</i>	3.11	(1.67 ; 5.78)	< 0.001	2.90	(1.55 ; 5.44)	0.001
OS	HR	95%CI	p	HR	95%CI	p
Age	1.03	(1.01 ; 1.05)	0.001	1.05	(1.03 ; 1.07)	< 0.001
CNS	2.00	(1.28 ; 3.14)	0.002	1.64	(1.02 ; 2.64)	0.04
Log(WBC)	1.99	(1.48 ; 2.67)	< 0.001	2.01	(1.51 ; 2.86)	< 0.001
Prednisone response	0.54	(0.38 ; 0.76)	< 0.001	0.83	(0.57 ; 1.20)	0.31
High-risk Classifier	2.93	(2.06 ; 4.17)	< 0.001	2.90	(2.01 ; 4.18)	< 0.001
<i>TP53^{Alt}</i>	2.34	(1.30 ; 4.24)	0.005	2.87	(1.56 ; 5.26)	0.001

Table 1.

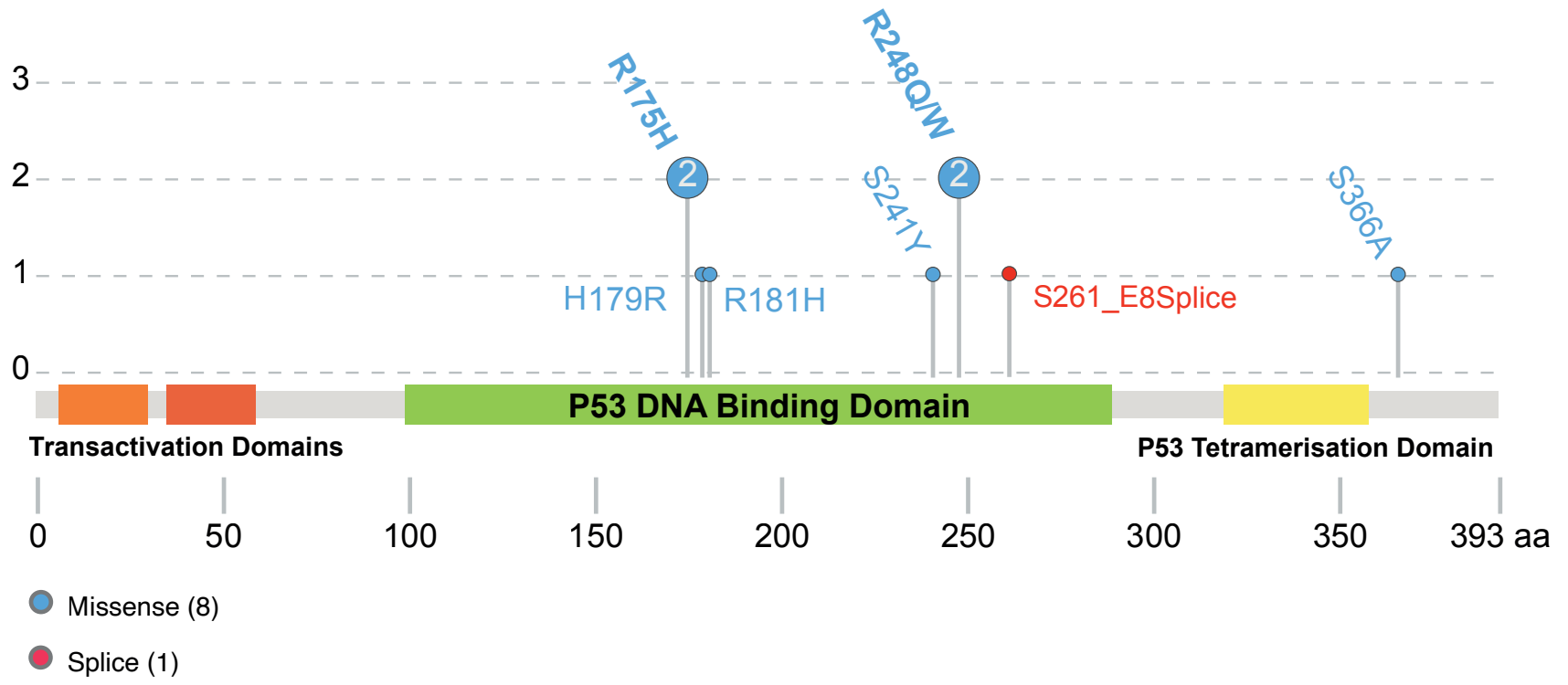
Table 1.

Variable	<i>TP53^{Alt}</i> n = 21 (4%)	<i>TP53^{WT}</i> n = 455 (96%)	Total n = 476	p-value ²
Male	14 / 21 (67%)	343 / 455 (75%)	357 / 476 (75%)	0.4
Age (y) ¹	23.4 (4.0-51.8)	15.3 (1.1-59.1)	15.3 (1.1-59.1)	0.5
WBC (G/l) ¹	25 (5-674)	66 (0-980)	64 (0-980)	0.01
CNS Involvement*	3 / 21 (14%)	48 / 453 (11%)	51 / 474 (11%)	0.5
Immunophenotype				
ETP phenotype	6 / 16 (38%)	50 / 291 (17%)	56 / 307 (18%)	0.09
Immature (IM0/d/g)**	8 / 20 (40%)	81 / 399 (20%)	89 / 419 (21%)	0.048
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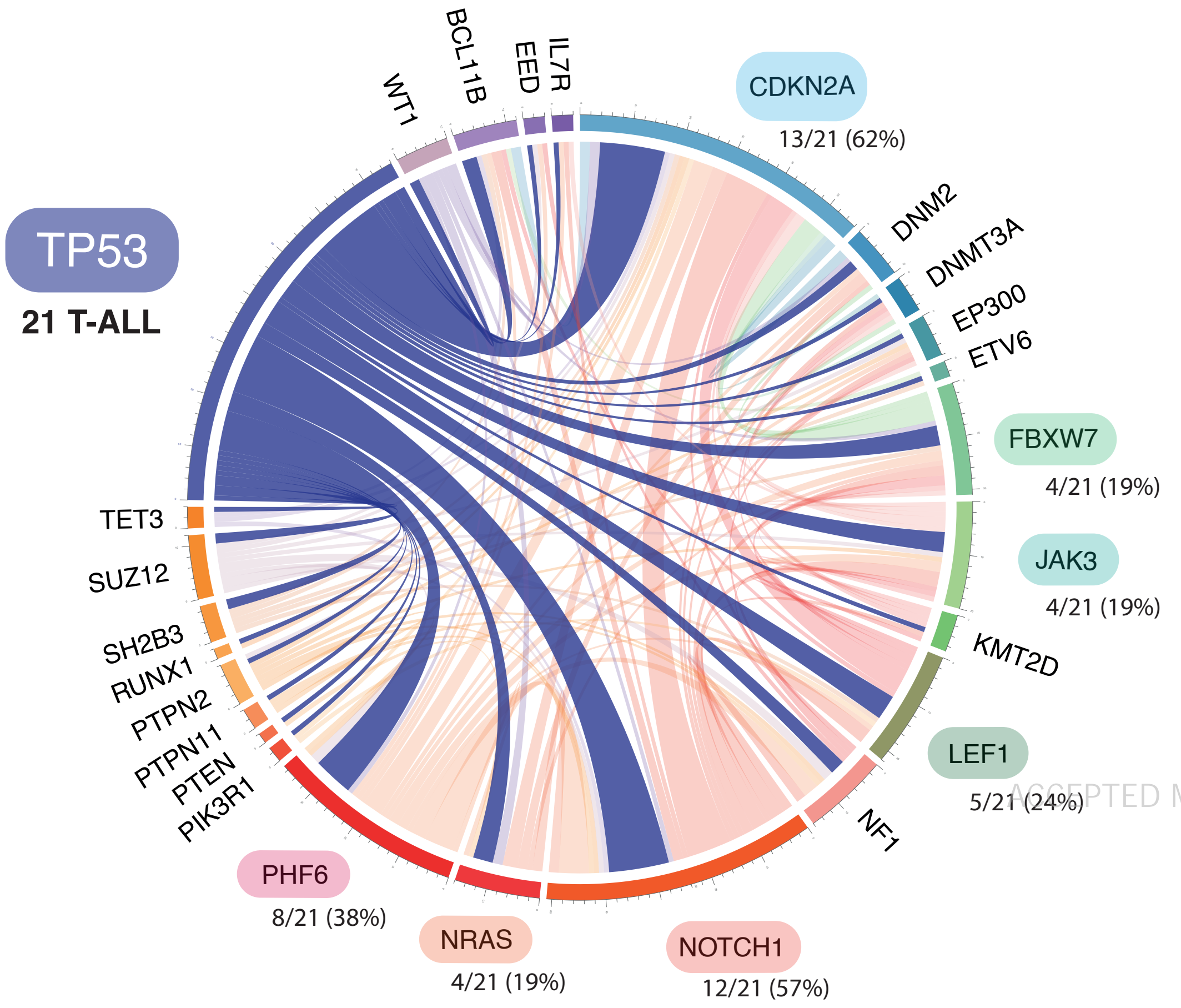
Univariate and Multivariate Analysis³						
CIR	Univariate			Multivariate		
	SHR	95%CI	p	SHR	95%CI	p
Age	1.01	(0.98 ; 1.03)	0.57	-	-	-
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High-risk Classifier**	2.78	(1.94 ; 3.99)	< 0.001	2.58	(1.78 ; 3.74)	< 0.001
<i>TP53^{Alt}</i>	3.11	(1.67 ; 5.78)	< 0.001	2.90	(1.55 ; 5.44)	0.001
OS	HR	95%CI	p	HR	95%CI	p
Age	1.03	(1.01 ; 1.05)	0.001	1.05	(1.03 ; 1.07)	< 0.001
CNS	2.00	(1.28 ; 3.14)	0.002	1.64	(1.02 ; 2.64)	0.04
Log(WBC)	1.99	(1.48 ; 2.67)	< 0.001	2.01	(1.51 ; 2.86)	< 0.001
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<i>TP53^{Alt}</i>	2.34	(1.30 ; 4.24)	0.005	2.87	(1.56 ; 5.26)	0.001

Figure 1.

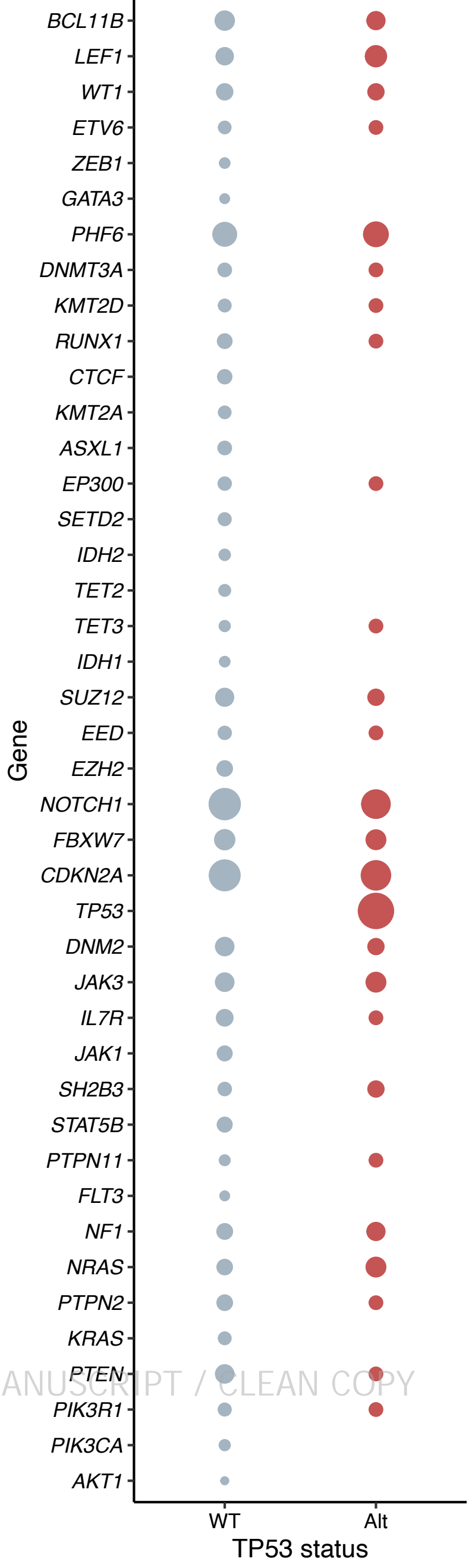
A



B

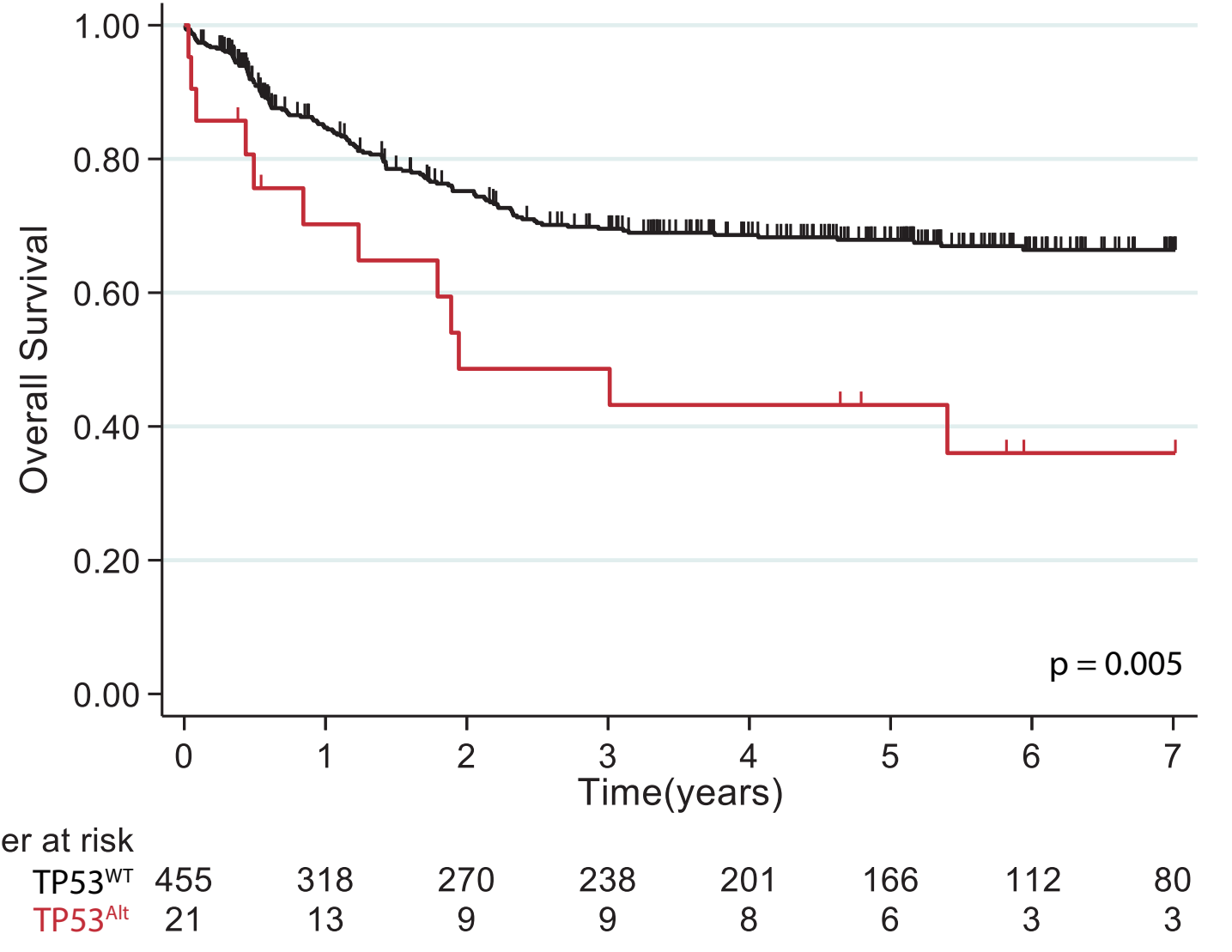


C

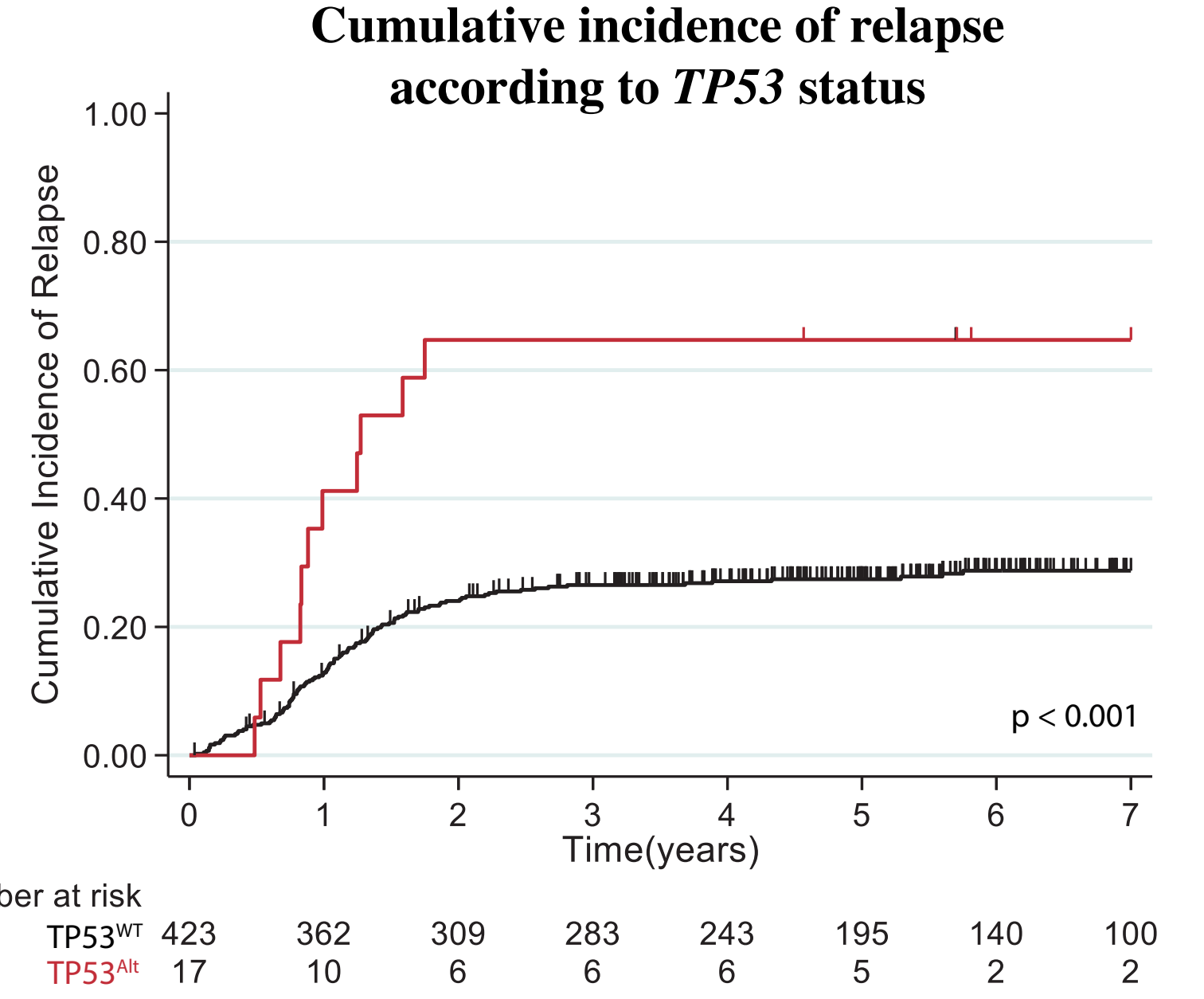


Overall Survival according to TP53 status

D



E



Frequency (%) ● 25 ● 50 ● 75 ● 100