SUPPLEMENTAL DATA

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

Contents:

d) Supplemental **Table 4**: Pathogenicity analysis of the 8 TP53 mutations identified among the 476 T-ALL patients

e) Supplemental **Table 5**: Mutational landscape of *TP53*^{Alt} vs *TP53*^{WT} T-ALL

SUPPLEMENTAL METHODS

Patient's protocol and clinical trials

Diagnostic peripheral blood or bone marrow samples from 476 adults and children with T-ALL were analyzed after informed consent was obtained at diagnosis according to the Declaration of Helsinki.

Adult patients aged from 16-59 years, were included in the GRAALL03/05 trials which were registered at clinicaltrials.gov (GRAALL-2003, #NCT00222027; GRAALL-2005, #NCT00327678).

Pediatric patients aged from 1-19 years, were treated in 10 French pediatric hematology departments, members of the FRALLE study group, according to the FRALLE 2000 T guidelines:

Definitions: Good prednisone response (GPR) was defined as < 1000 circulating blasts/μL on day 8, poor prednisone response (PPR) when ≥ 1000 circulating blasts/μL on day 8. Morphologic assessment of a bone marrow aspirate was done at day 21. A chemosensitivity was represented by ≤ 5% blasts, a chemoresistance by > 5%. Complete remission (CR) was defined as: absence of physical signs of leukemia, bone marrow with active hematopoiesis and < 5% leukemic blast cells (identified morphologically), and normal cerebrospinal fluid. Patients were stratified into two groups.

Treatment stratification: Standard risk group (T1) was defined by the presence of all the following criteria: good prednisone response (GPR) at day 8, chemosensitivity (CHs) at day 21, MRD < 10-2 at day 35. High risk group (T2) was defined by the presence of one of the following criteria: poor prednisone response (PPR) at day 8, chemoresistance at day 21 or MRD ≥ 10-2 at day 35. Patients treated according to T2 group were eligible for allogeneic stem cell transplantation (SCT) after late intensification n°1 when a matched sibling or unrelated donor was available.

Immunophenotypic and molecular characterization of T-ALL samples

Peripheral blood (PB) or bone marrow (BM) T-ALL samples were analyzed for immunophenotype, fusion transcripts (SIL-TAL1, CALM-AF10), oncogenic transcripts (HOXA9, TLX1 and TLX3), and T-cell receptor (TCR) recombination and NOTCH1/FBXW7/RAS/PTEN mutations, as previously described.[1–3]

Minimal residual disease assessment

Immunoglobulin/T-cell receptor (Ig/TCR) gene rearrangement based MRD evaluation was centrally assessed for patients who reached complete remission after the first induction cycle, on BM samples after induction (MRD1). MRD was centrally assessed by real-time quantitative allele-specific oligonucleotide PCR and interpreted according to EuroMRD group guidelines.[4–6]

Gene mutation screening

A custom capture Nextera XT gene panel (Illumina, San Diego, CA) targeting all coding exons and their adjacent splice junctions of 63 genes was designed, based on available evidence in hematological neoplasms **(supplemental Table 1).** DNA Libraries were prepared using Nextera Rapid Capture Enrichment protocol and underwent 2x150bp paired-end sequencing on Illumina MiSeq sequencing system with MiSeq Reagent Kit v2 (Illumina). Briefly, sequence reads were filtered and mapped to the human genome (GRCh37/hg19) using in-house software (Polyweb, Institut Imagine, Paris). Annotated variants were selected after filtering out calls according to the following criteria: (i) coverage < 30x, <10 alternative reads or variant allelic fraction (VAF) <7%; (ii) Polymorphisms described in dbSNP, 1000Genomes, EVS, Gnomad and EXAC with a calculated mean population frequency >0.1%. Non-filtered variants were annotated using somatic database COSMIC (version 78) and ProteinPaint (St Jude Children's Research Hospital – Pediatric Cancer data portal), published data and in-silico prediction effect.

SUPPLEMENTAL REFERENCES

1. Asnafi V, Beldjord K, Boulanger E, et al. Analysis of TCR, pT alpha, and RAG-1 in T-acute lymphoblastic leukemias improves understanding of early human T-lymphoid lineage commitment. *Blood*. 2003;101(7):2693–2703.

2. Bond J, Marchand T, Touzart A, et al. An early thymic precursor phenotype predicts outcome exclusively in HOXA-overexpressing adult T-cell acute lymphoblastic leukemia: a Group for Research in Adult Acute Lymphoblastic Leukemia study. *Haematologica*. 2016;101(6):732–740.

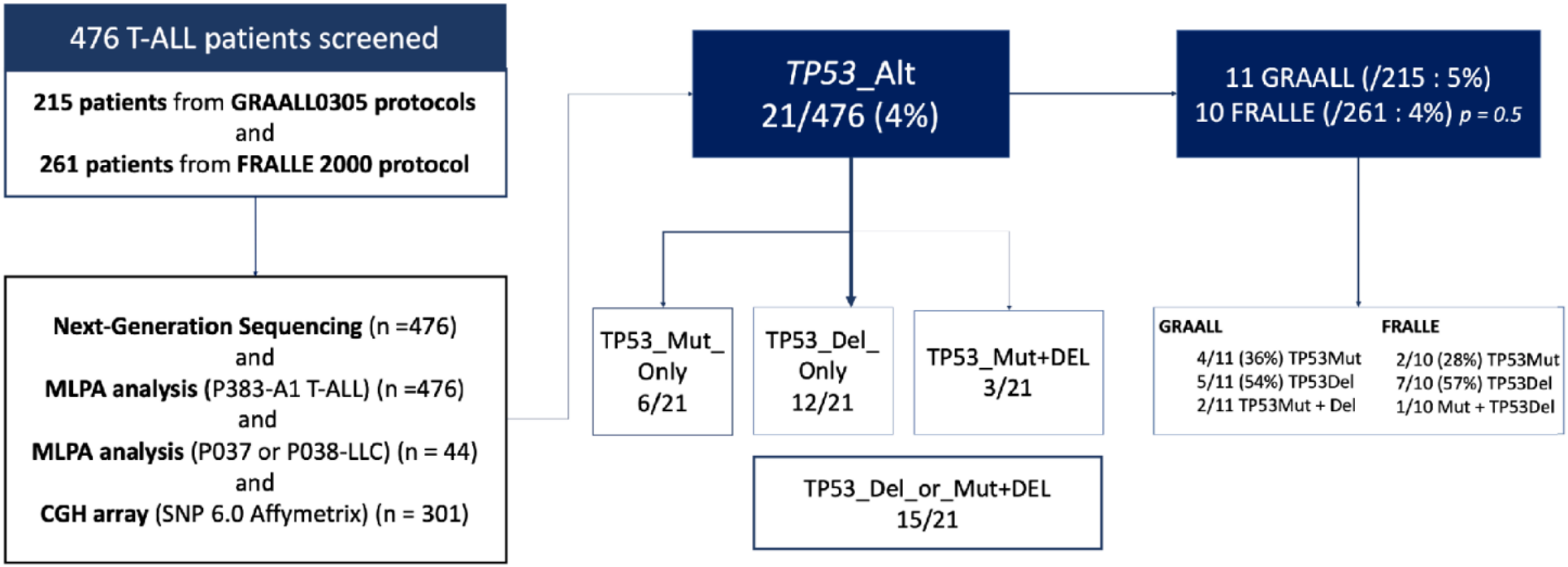
3. Trinquand A, Tanguy-Schmidt A, Ben Abdelali R, et al. Toward a NOTCH1/FBXW7/RAS/PTEN-based oncogenetic risk classification of adult T-cell acute lymphoblastic leukemia: a Group for Research in Adult Acute Lymphoblastic Leukemia study. *J. Clin. Oncol*. 2013;31(34):4333–4342.

4. van Dongen JJM, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia*. 2012;26(9):1908–1975.

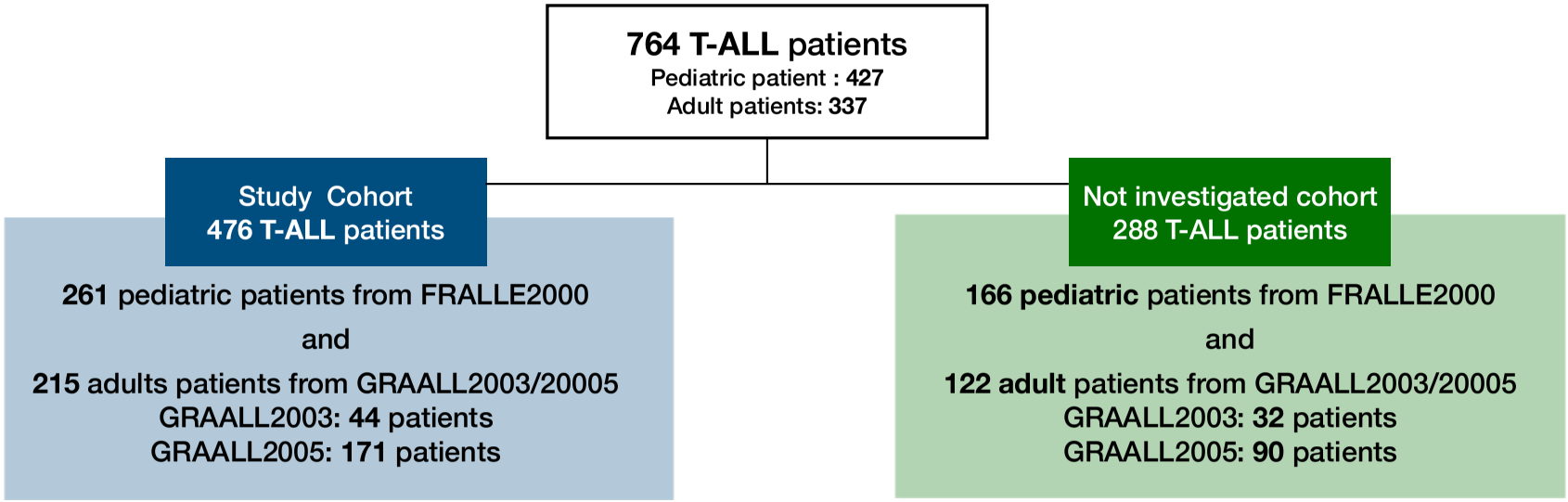
5. Pongers-Willemse MJ, Verhagen OJ, Tibbe GJ, et al. Real-time quantitative PCR for the detection of minimal residual disease in acute lymphoblastic leukemia using junctional region specific TaqMan probes. *Leukemia*. 1998;12(12):2006–2014.

6. van der Velden VHJ, Cazzaniga G, Schrauder A, et al. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia*. 2007;21(4):604–611.

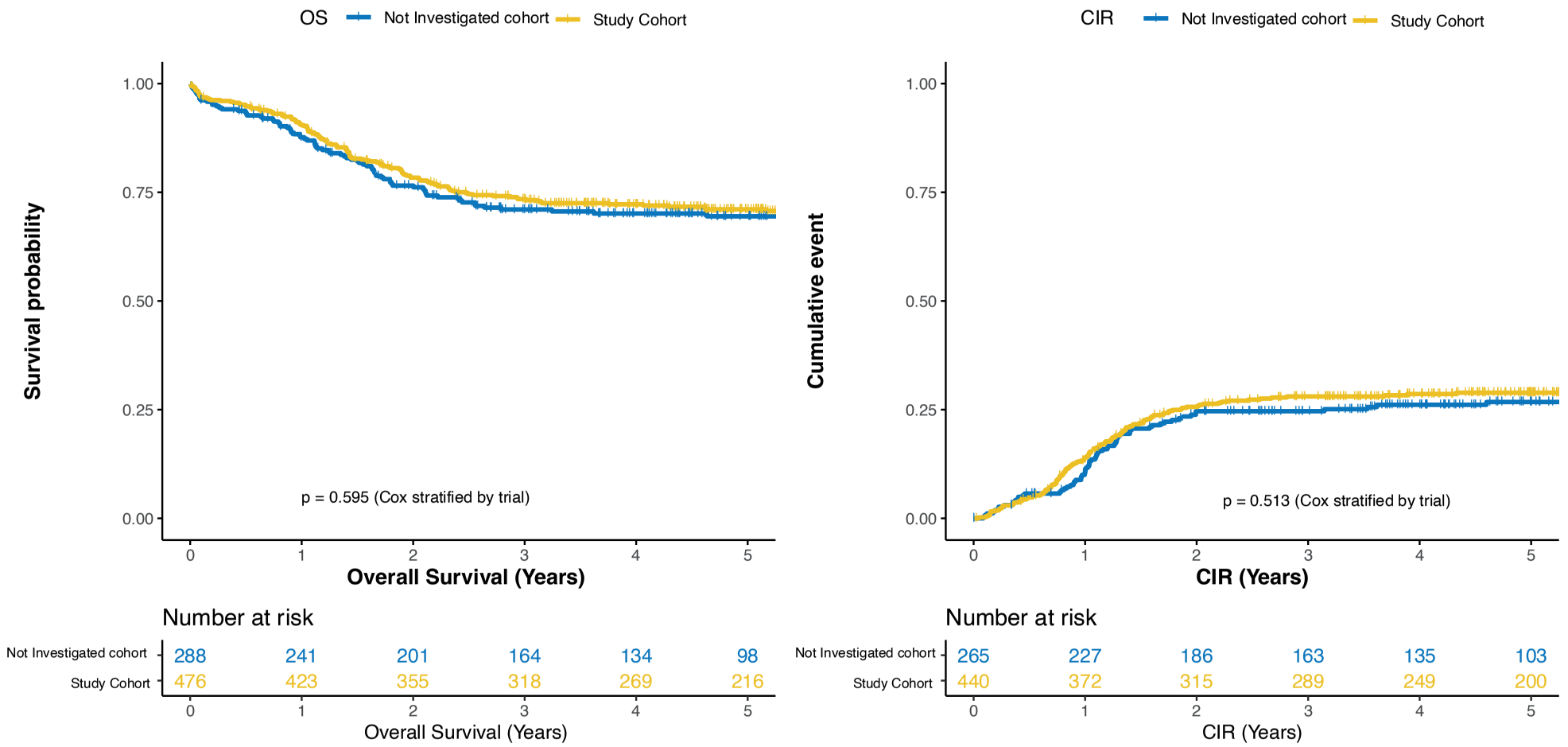
Supplemental Figure 1.
Flow chart of FRALLE2000 and GRAALL0305 patients.



Supplemental Figure 2.
Flow chart of the study cohort patients versus not investigated patients.

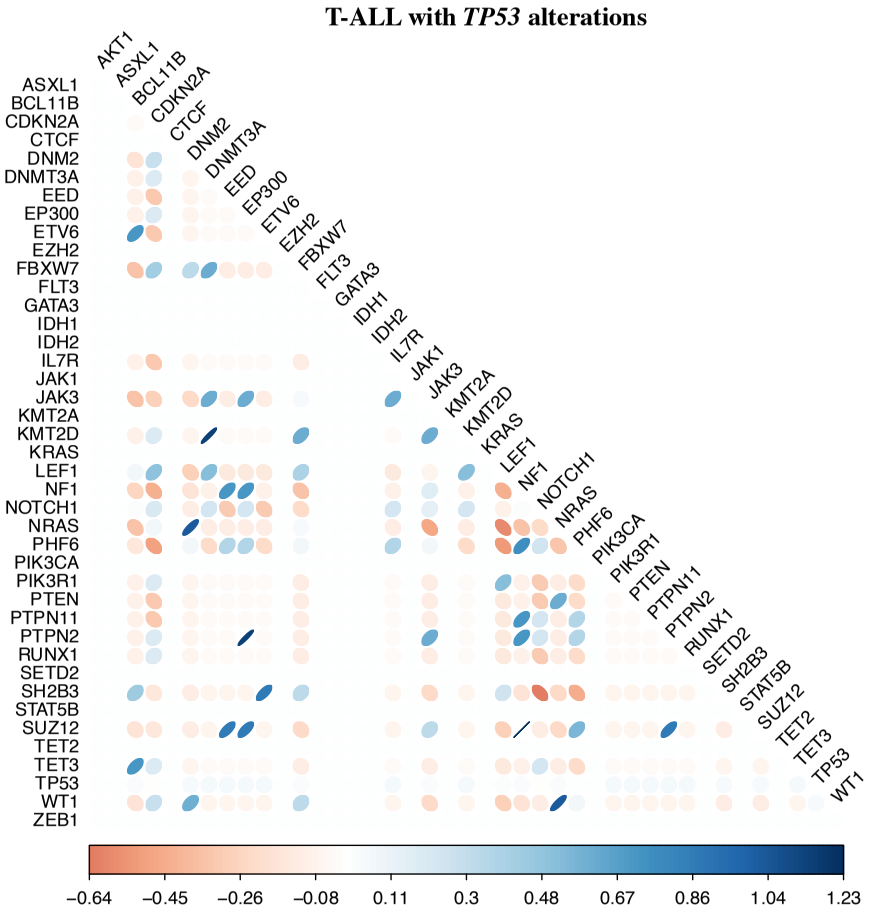


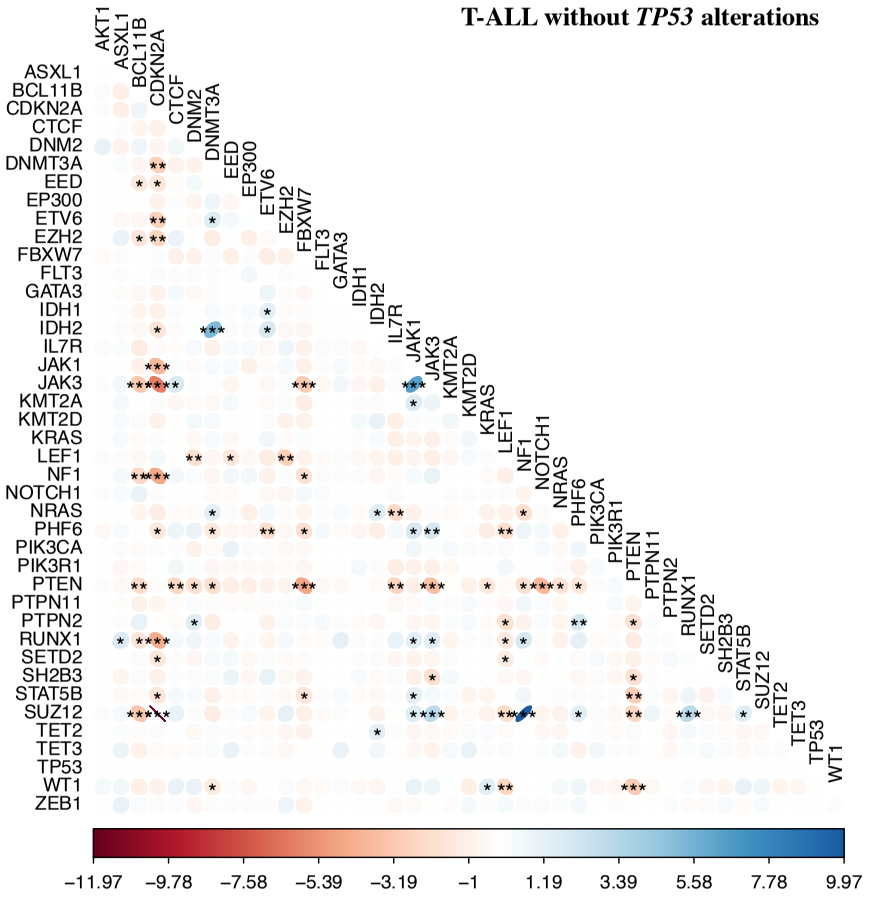
Supplemental Figure 3.
OS and CIR of the study cohort patients versus not investigated patients.



Supplemental Figure 4. Co-occurrences and mutual exclusions of genetic alterations in *TP53*^{Alt} and *TP53*^{WT} T-ALL.

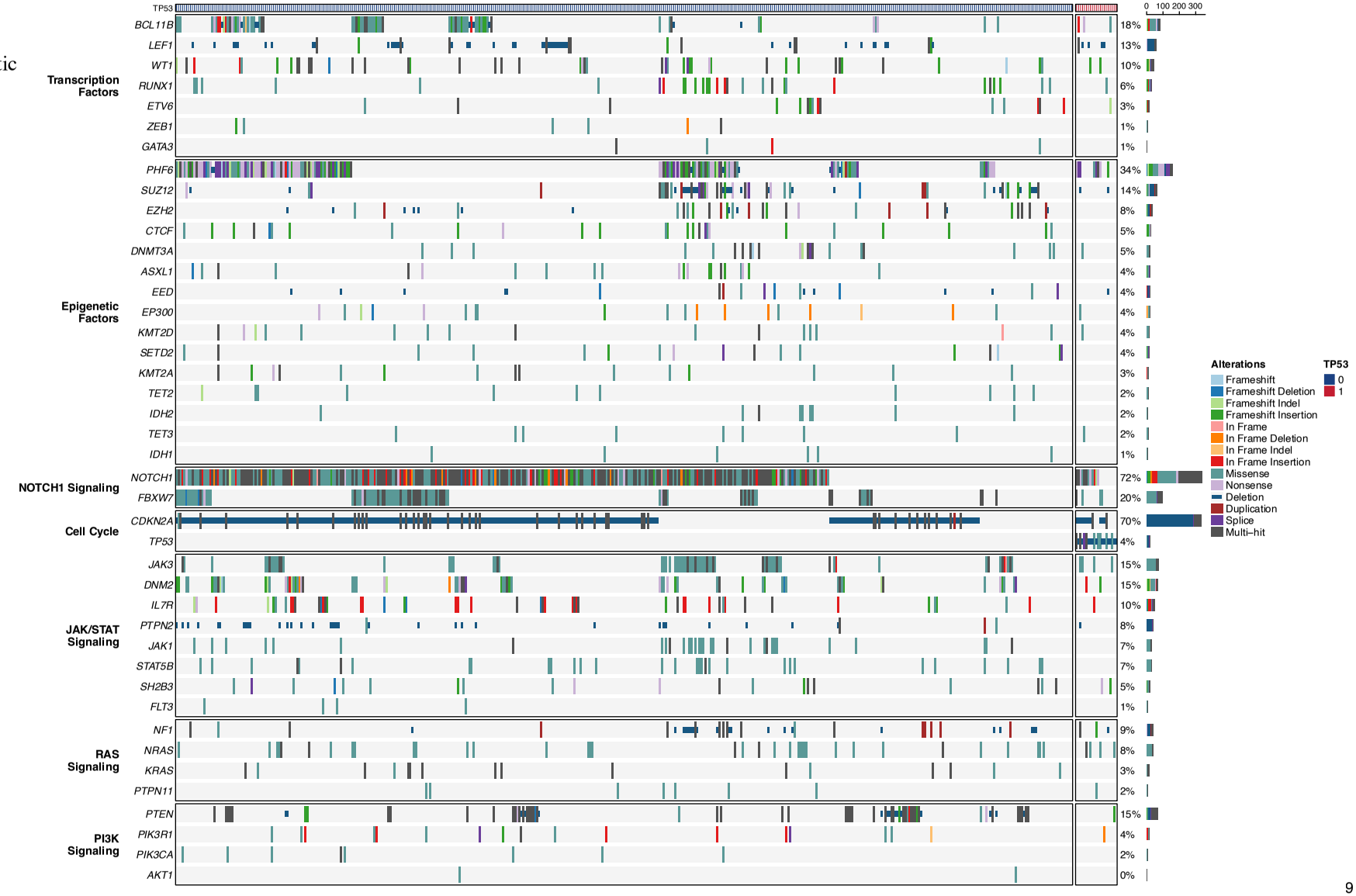
The colors of the scale bar denote the nature of the correlation, with +1 indicating a perfectly positive correlation (blue) and -1 indicating a perfectly negative correlation (orange) between two alterations. The significance level of co-occurrences or mutual exclusions are depicted as followed: * indicating a p-value < 0.05, ** a p-value < 0.01 and *** a p-value < 0.001. The co-occurrences and mutual exclusions were computed with the DISCOVER algorithm (R software)





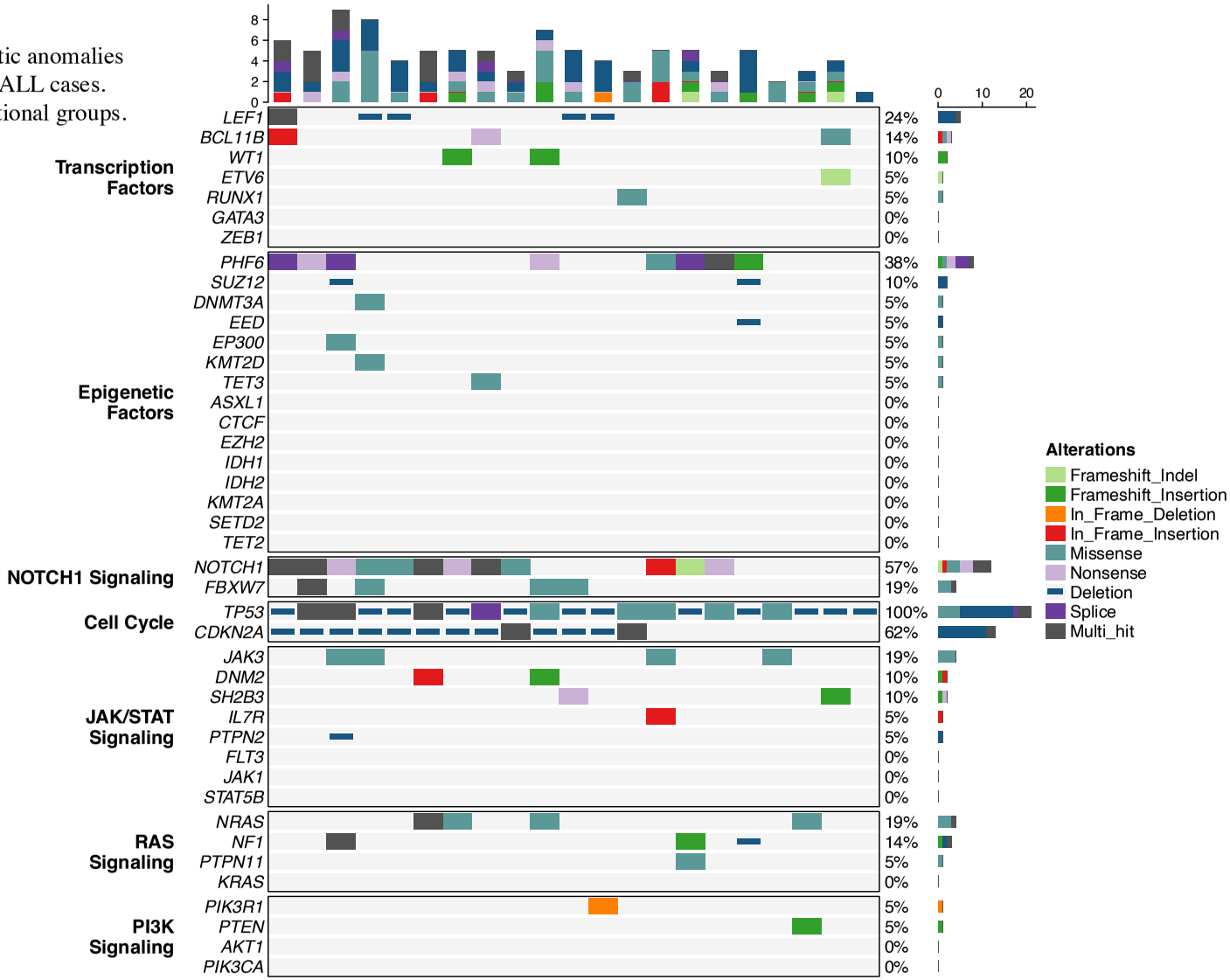
Supplemental Figure 5.

Oncoplot depicting the genetic anomalies observed in *TP53* Altered or Wild-type T-ALL cases of the GRAALL03/05 and FRALLE2000 studies.



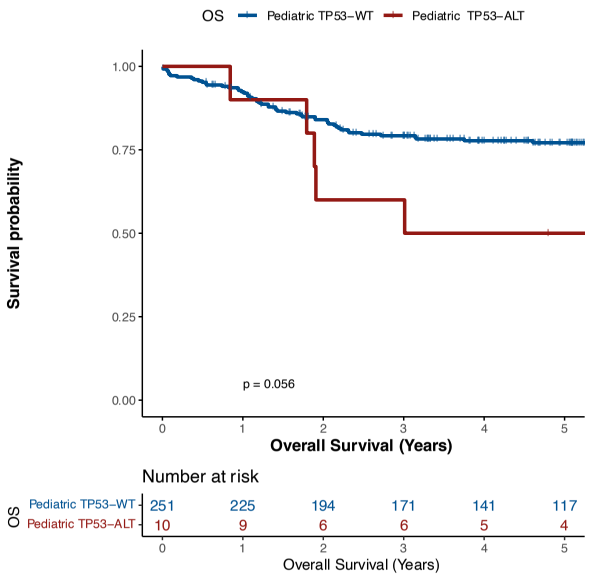
Supplemental Figure 6.

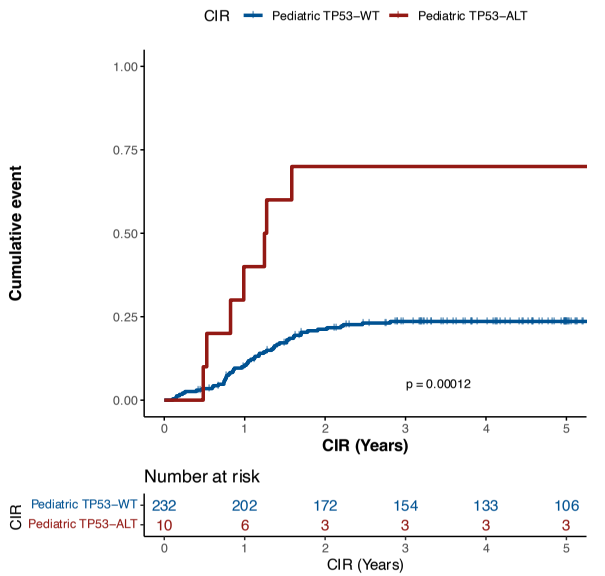
Oncoplot depicting the genetic anomalies observed in *TP53* Altered T-ALL cases. Genes are classified by functional groups.



Supplemental Figure 7.
OS and CIR of FRALLE2000 (pediatric) and GRAALL0305 (adults) patients

FRALLE2000





GRAALL0305