Genomics improves risk stratifi cation of adults with T-cell acute lymphoblastic leukemia enrolled in measurable residual disease-oriented trials

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Supplemental Information

Methods

Targeted deep sequencing (TDS)

DNA or cryopreserved cells from T-ALL patients (n=145) were collected from the Carlos III Spanish National DNA Bank (PT13/0001/0037 and PT13/0010/0067), La Fe Biobank (PT13/0010/0026) and the IGTP Biobank (PT17/0015/0045). Cell sorting was applied in samples with less than 70% infiltration except for 18 cases that cryopreserved cells were not available (blast range [30-69%]). Positivity for CD45+ dim (identified in the two-dimension representation CD45-APC *vs* SSC) and CD7+ criteria was used to purify T-ALL population. DNA was mainly isolated from bone marrow (BM) and occasionally from peripheral blood (PB). Sequence was performed in a MiSeq instrument (Illumina, San Diego, CA, USA), employing a paired-end read length of 2 x 75 bp protocol at a mean depth of coverage of >280X. FASTQ files from TDS were aligned to the hg19 reference genome using Burrows-Wheeler Aligner (BWA), version 0.7.15[1]. Mapped reads were recalibrated using Genome Analysis Toolkit (GATK), version 3.4.46[2], regions with indels were realigned using the GATK tool. PCR duplicates were marked using Picard tools, version 1.138[3]. Variants were called using a combination of SamTools version 1.10[4] and VarScan2 version 2.4.0[5]. Variants were annotated using ANNOVAR version 2018-04-16[6]. Variants described in population databases such as 1000Genomes, ExAC, gnomAD, and Exome Variant Server, with a minimum population frequency >1% were excluded from further analyses. Candidate variants were selected after filtering out calls according to the following criteria: coverage <30X and <8 reads on the alternative allele. Mapping errors were removed by visual inspection using the Integrative Genomics Viewer (IGV) browser[7].

Patients and treatment protocols

For clinical and outcome correlations, 29 patients from our initial cohort were excluded (3 pediatric cases, treated according to the SEOP-PETHEMA 2015 trial; 3 cases treated with the intermediate risk trial [RI-08, NCT02036489]; 6 patients treated with the OLD-07 [NCT01366898)] or FRAGILE07 trial [NCT01358201)], according their advanced age and

comorbidities, and 17 patients treated with the LAL2019 [on going trial, NCT04179929]). For all included patients (n=116), full clinical information was available, including MRD data. The cytogenetic classification was based on the Genesca et al.[8] study, considering a complex karyotype (CK) with ≥3 cytogenetic alterations instead of the classical cut-off of 5 genetic alteration[9]. The reason is that our cytogenic analysis showed that patients with yet ≥3 alterations presented a very worse outcome and dismal prognosis[8]. Patients were treated with two consecutive MRD-oriented high-risk adult ALL protocols (ALL-AR [Ph$^-$]-03[NCT00853008], ALL-HR [Ph$^-$]-11 [NCT01540812]). Briefly, in the ALL-AR (Ph$^-$)-03 trial (n=32) response to induction chemotherapy was evaluated by cytomorphology and flow cytometry. Good responders (<5% blasts; cytological complete remission [CR]) proceeded to consolidation chemotherapy, and whenever a good MRD response was maintained (MRD≤0.05%) they followed maintenance chemotherapy treatment. Poor responders (>5% blasts) received intensification of induction treatment, followed by allo-SCT. Poor MRD responders after consolidation treatment (MRD≥0.05%), were also allocated to allo-SCT. In the ALL-HR (Ph$^-$)-11 (n=84) treatment allocation was exclusively based on fully centralized flow cytometry. An MRD level of ≤0.1% after induction treatment allocated patients to consolidation chemotherapy, and values of MRD≤0.01%, after consolidation treatment, to pursuit with maintenance chemotherapy. The remaining patients were assigned to allo-SCT. MRD assessment was made following the EuroFlow guidelines[10].

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Table S1. Response to treatment of T-ALL patients included in the aging cluster and in the resistance cluster.

	Patients resistance cluster (n=17)	**Patients no-resistance cluster (n=99)**	**P**	**Patients aging cluster (n= 15)**	**Patients no-aging cluster (n=101)**	**P**
Slow response at day +14	13/16 (81%)	35/83 (42%)	0.006	12/14 (86%)	36/85 (42%)	0.003
N. of induction cycles to CR						
1	9 (53%)	84 (85%)	0.006	9 (60%)	84 (83%)	0.075
2	8 (47%)	15 (15%)		6 (40%)	17 (17%)	
CR (Ind-1 + Ind-2)	13 (77%)	89 (90%)	0.124	9 (60%)	93 (92%)	0.003
Exitus Ind-1 or Ind-2	4 (24%)	7 (7%)	0.055	5 (33%)	6 (6%)	0.005

Results expressed as number of cases/total cases (percentage). CR: complete remission; +14: fourteen days after induction treatment; d+35: thirty-five days after induction treatment; P: p value.

Table S2. Clinical-biological characteristics and response to treatment of T-ALL patients grouped according to the GOG mutational profile.

	Genetic group		
	GOG$^-$ (n = 97)	**GOG$^+$ (n =19)**	**P**
Patient-related features			
Median age, y (range)	37 (16-61)	38 (16-59)	0.618
Gender, M/F	70/27	16/3	0.393
Disease-related features			
Median WBC, 10^9/L (range)	52.8 (0.5-525.4)	95.1 (6.5-414.6)	0.271
ECOG			
0	36/95 (38%)	7/17 (41%)	0.939
1	45/95 (47%)	8/17 (47%)	
2	12/95 (13%)	2/17 (12%)	
≥3	2/95 (2%)	0	
Adenopathies	48/82 (59%)	7/16 (44%)	0.276
Splenomegaly	31/93 (33%)	8/18 (44%)	0.366
Hepatomegaly	21/92 (23%)	4/18 (22%)	1.000
Mediastinal mass	38/94 (40%)	11/19 (58%)	0.161
CNS involvement	13/91 (14%)	1/19 (5%)	0.457
Immunophenotype			
ETP-ALL	22/92 (24%)	0	0.112
Pre-T	14/92 (15%)	4/18 (28%)	
Cortical	38/92 (41%)	9/18 (50%)	
Mature	18/92 (20%)	4/18 (22%)	
Cytogenetics			
0-2 abn.	56/97 (58%)	10/19 (53%)	0.431
CK≥3	9/97 (9%)	1/19 (5%)	
NE	32/97 (33%)	8/19 (42%)	
Response-related features			
Slow response at day +14	44/86 (51%)	4/13 (31%)	0.170
N. of induction cycles to CR			
1	76/97 (78%)	17/19 (90%)	0.357
2	21/97 (22%)	2/19 (10%)	
CR post Ind-1	77/97 (79%)	18/19 (95%)	0.190
CR (Ind-1 + Ind-2)	84/97 (87%)	18/19 (95%)	0.461
MRD <0.1% at day +35	60/72 (83%)	14/17 (82%)	1.000
Treatment			
Chemotherapy	56/67 (69%)	13/15 (87%)	0.213
Allo-SCT	21/67 (31%)	2/15 (13%)	

Results expressed as number of cases/total cases (percentage). MRD values were considered for those patients that reach CR. Y: years; CNS: central nervous system; ETP-ALL: early T-cell precursor acute lymphoblastic leukaemia; CR: complete remission; abn: abnormalities; CK: complex karyotype; MRD: measurable residual disease; d+14: fourteen days after induction treatment; d+35: thirty-five days after induction treatment; Allo-SCT: allogeneic stem cell transplantation; P: p value.

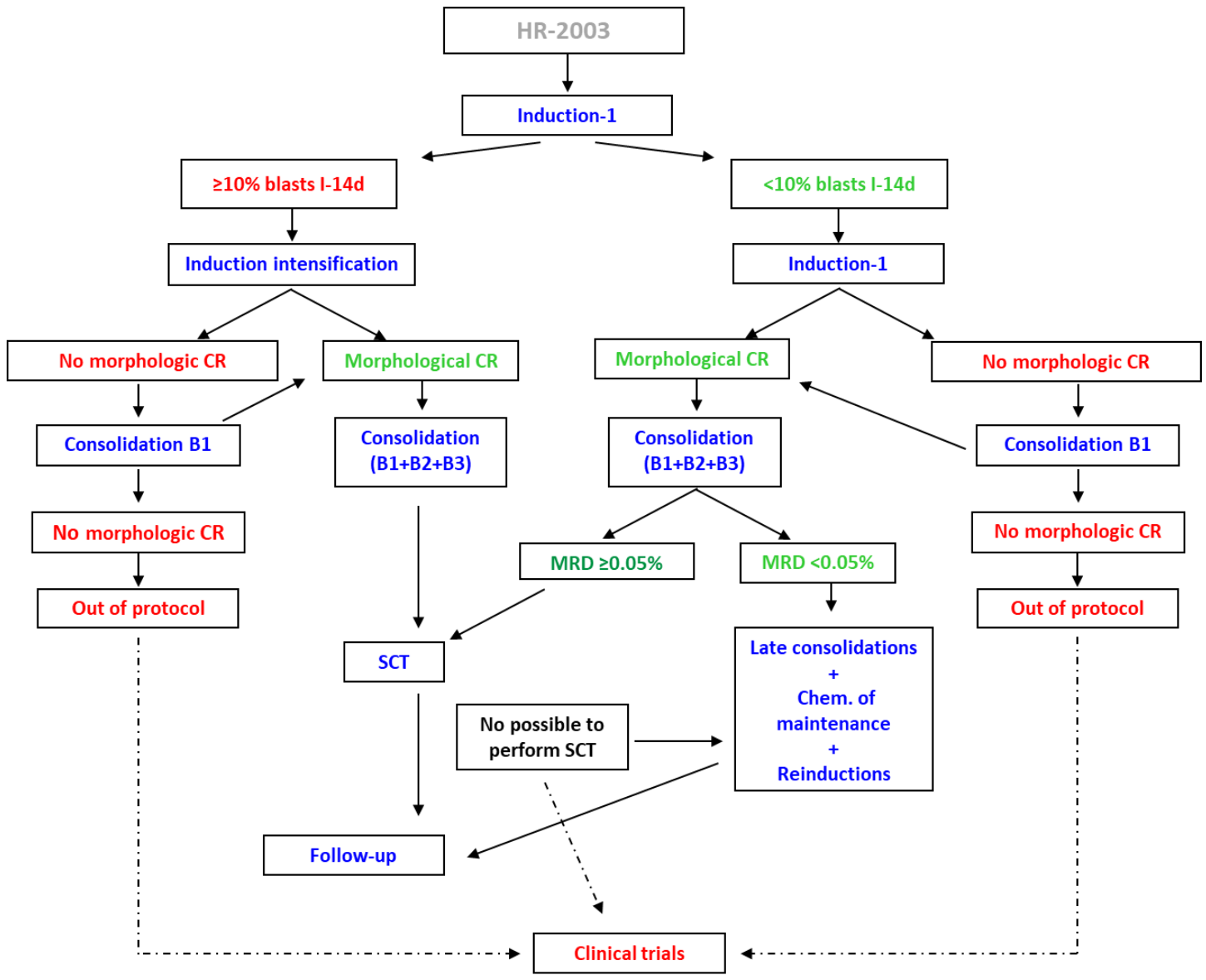
Table S3. Prognostic factors for cumulative incidence of relapse identified in the univariable analyses in the PETHEMA adult T-ALL cohort.

		Univariable analyses	
Disease/patient feature	**N**	**HR (95% CI)**	**P**
Age*	102	0.986 (0.965 – 1.009)	0.230
WBC count ($x10^9$/L)*	100	1.001 (0.998 – 1.003)	0.510
CNS involvement			
No	87	Reference	0.380
Yes	10	0.644 (0.239 – 1.736)	
ETP-ALL			
No ETP-ALL	78	Reference	0.340
ETP-ALL	17	1.365 (0.725 – 2.571)	
Karyotype			
0-2 abn.	62	Reference	0.150
CK≥3	7	1.929 (0.794 – 4.688)	
PETHEMA treatment protocol			
ALL-AR-03	27	Reference	0.580
ALL-HR-11	75	1.196 (0.632 – 2.262)	
N/KRAS			
Non-mutated	92	Reference	0.026
Mutated	10	2.550 (1.121 – 5.800)	
MRD at day +35			
<0.1%	74	Reference	0.110
≥0.1%	15	1.772 (0.871 – 3.608)	

*Age and WBC were considered as continuous variables. N: number of cases; HR: hazard ratio; CI: confidence interval; OS: overall survival; WBC: white blood cell; ETP-ALL: early T-cell precursor acute lymphoblastic leukaemia; Abn: abnormalities; CK: Complex Karyotype; CNS: central nervous system; MRD: measurable residual disease P: p value.

Supplemental Figure 1

A



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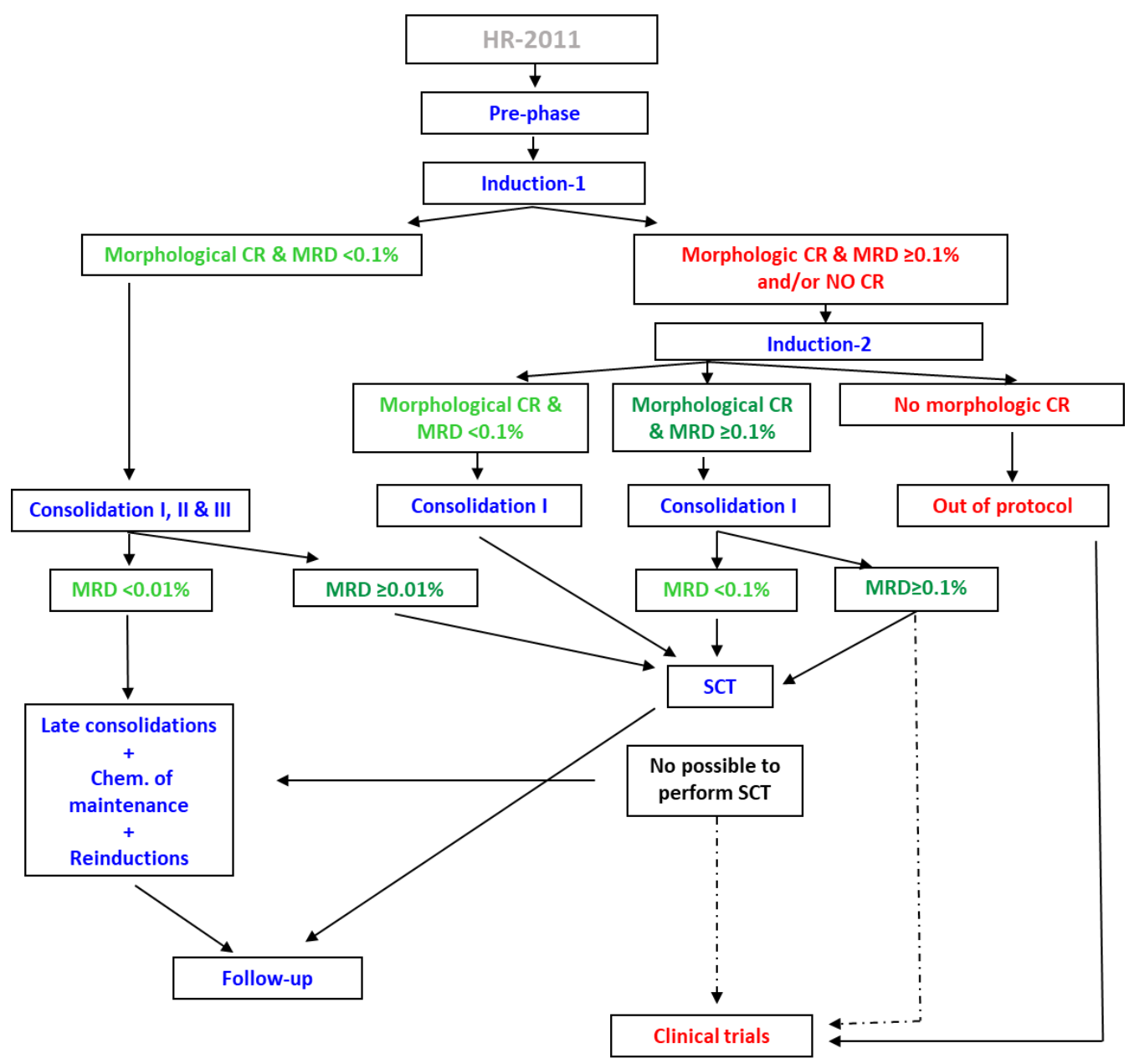
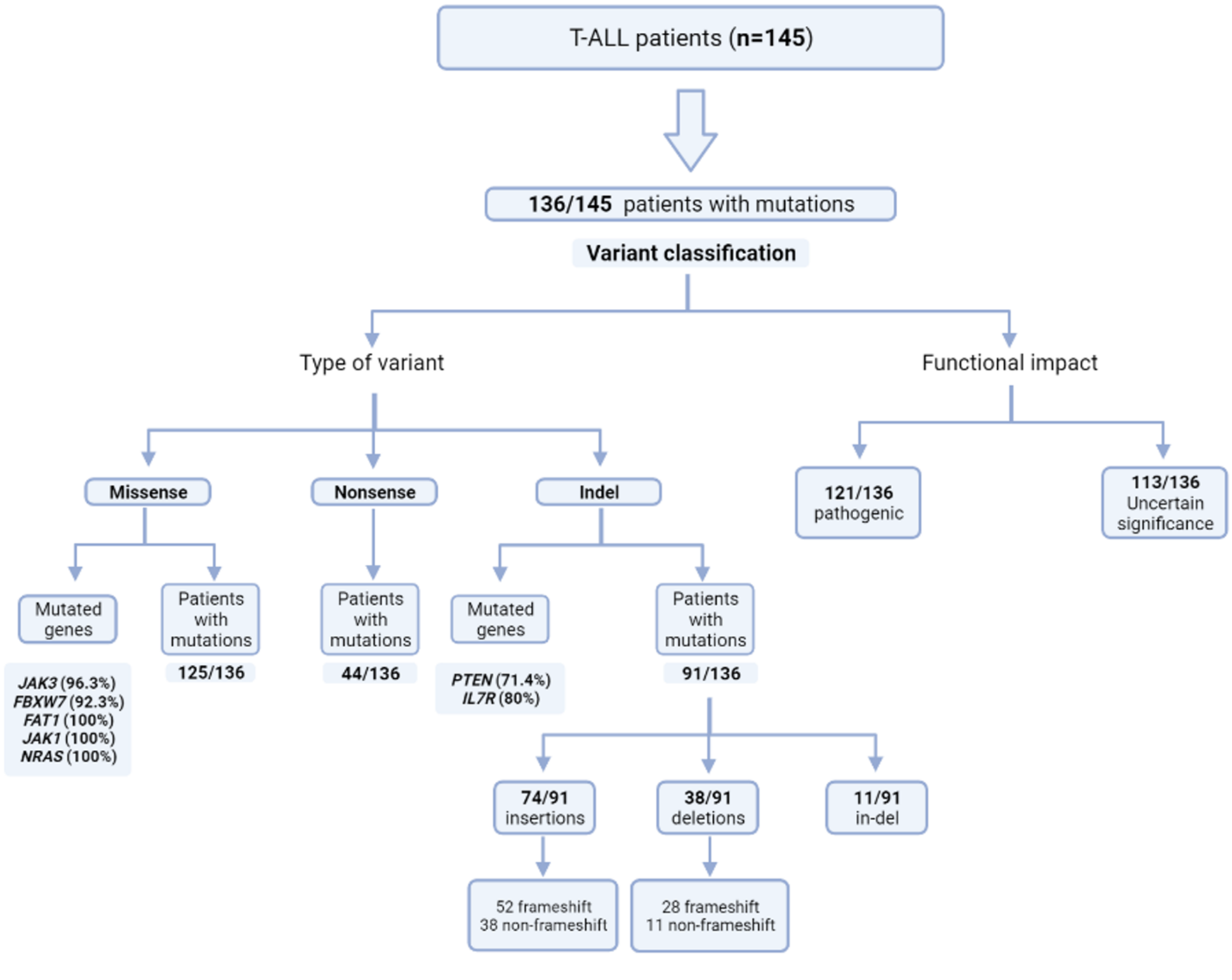


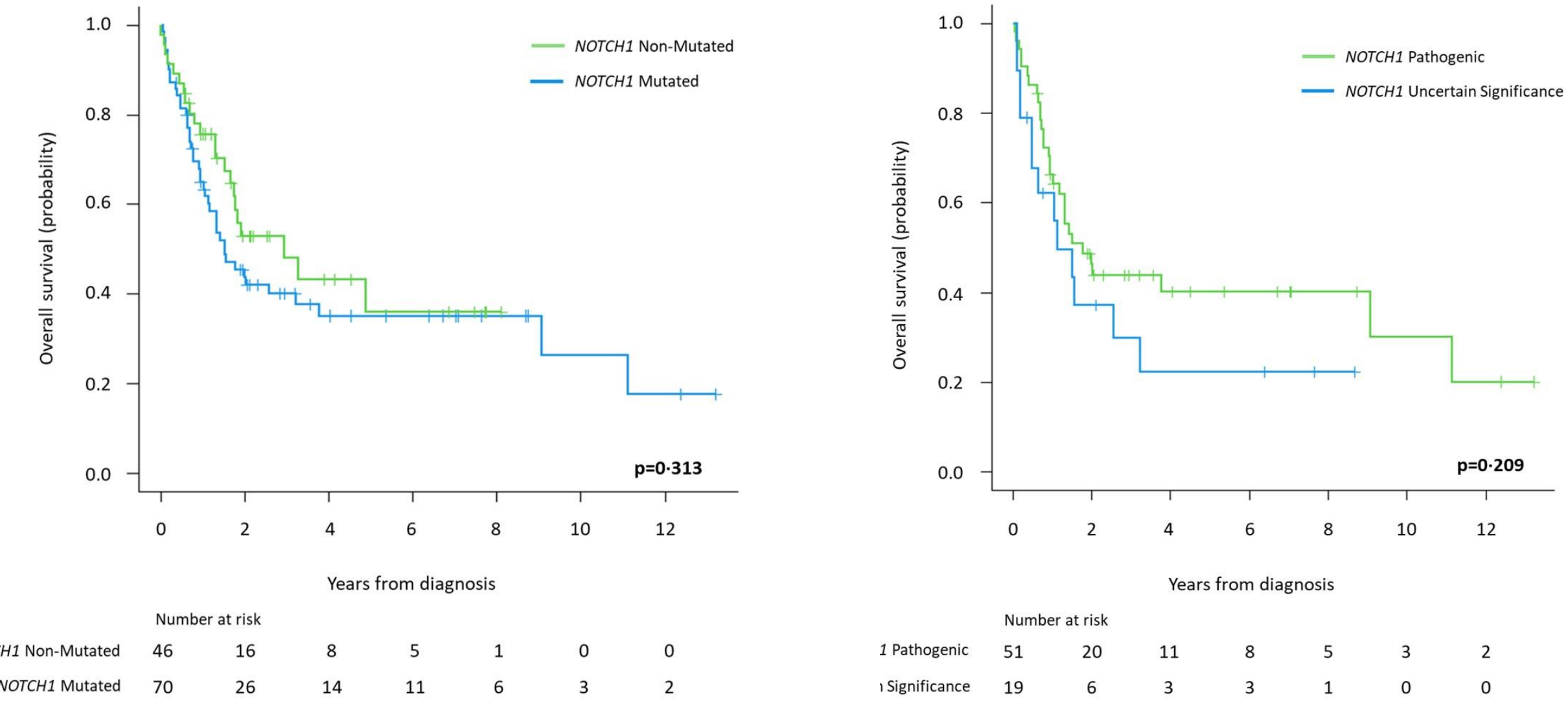
Supplemental Figure 1. (A) Flowchart of the HR2003 trial. Percentage of blasts cells at day +14 were evaluated by cytometry and according to this value patients continued with standard induction chemotherapy (<10%) or induction intensification (>10%). After induction treatment, CR patients followed 3 standard consolidation blocks or one consolidation block (No CR). No MRD stratification criteria was employed at the end of induction treatment. Good responders after consolidation treatment (MRD <0.05) continued with standard maintenance and reinduction chemotherapy. Patients with >0.05 MRD proceeded to SCT together with those with >10% blast cells at day +14. In blue treatment indication. In read poor responders and clinical trial treatment option, and in green good responders. In dark green patients with positive MRD. (B) Flowchart of the HR2011 trial. Patients proceeded to pre-phase and standard induction treatment. MRD levels were measured by cytometry at the end of induction treatment (I +35d) and patients were stratified according: MRD<0.1 standard consolidation treatment; MRD >0.1 induction intensification followed by SCT. Good responders after consolidation treatment (MRD <0.01) continued with standard maintenance and reinduction chemotherapy. In blue treatment indication. In read poor responders and clinical trial treatment option, and in green good responders. In dark green patients with positive MRD.

Supplemental Figure 2



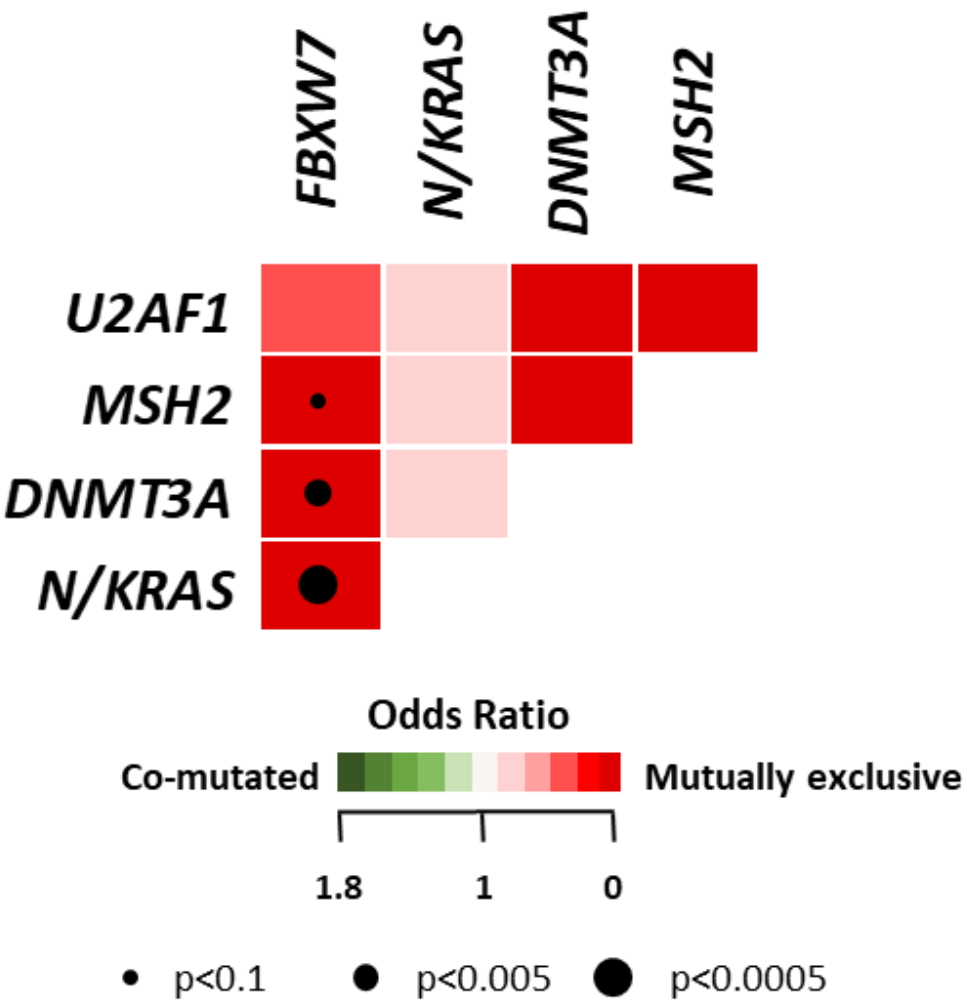
Supplemental Figure 2. Variant classification according to the type of variant detected (missense, nonsense, and indel) and their functional impact (pathogenic or uncertain significance). In-del: insertion and deletion.

Supplemental Figure 4



Supplemental Figure 3. Impact of *NOTCH1/FBXW7* mutations in OS. (A) OS from diagnosis according to *NOTCH1* global mutational status. OS (5y) was estimated of 35% (95% CI, 23%-47%) in *NOTCH1* mutated patients and 36% (95% CI, 16%-56%) in non-mutated patients (p=0.313). (B) OS from diagnosis according to the functional impact of the *NOTCH1* variants. OS (5y) was estimated of 40% (95% CI, 25% -55%) in patients with pathogenic *NOTCH1* variants and 22% (95% CI, 1%-43%) in patients with uncertain significance *NOTCH1* variants (p=0.243). (C) OS from diagnosis according to the *NOTCH1* variant clonality status. OS (5y) was estimated of 38% (95% CI, 24%-52%) in patients with clonal *NOTCH1* variants (VAF > 25%), compared with 25% (95% CI, 0%-50%) in patients with subclonal *NOTCH1* variants (VAF < 30%) (p=0.380). (D) OS from diagnosis according to *FBXW7* and *NOTCH1* mutational status. OS (5y) was estimated of 62% (95% CI, 9%-73%) in patients with *FBXW7* variants (with and without *NOTCH1* variants) compared with 27% (95% CI, 15%-41%) in patients with only *NOTCH1* variants (p=0.036). The OS of patients without *NOTCH1* and *FBXW7* mutations was 37% (95% CI 18%).

Supplemental Figure 5



Supplemental Figure 4. Co-ocurrence between genes with prognostic impact in the T-ALL cohort. Positive (odds ratio >1) and negative (odds ratio <1) correlations are depicted as green and red, respectively. Black circle diameters indicate the degree of significance.