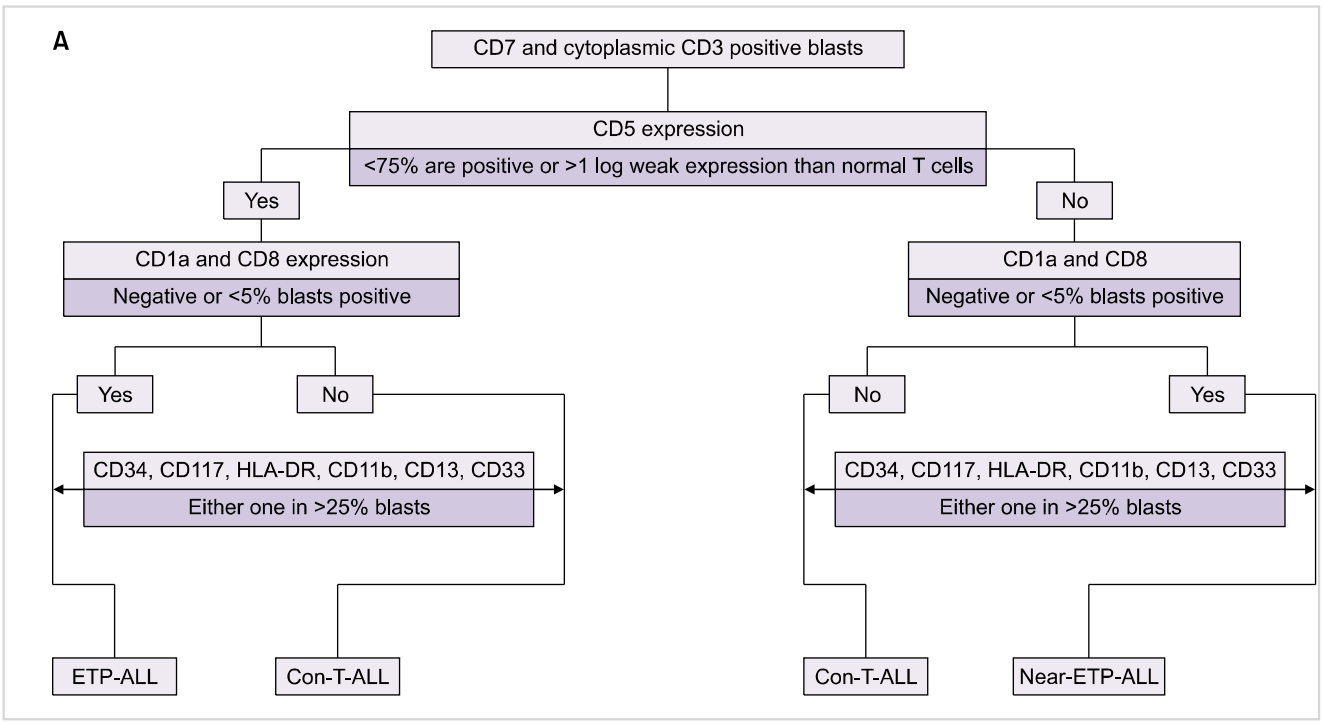
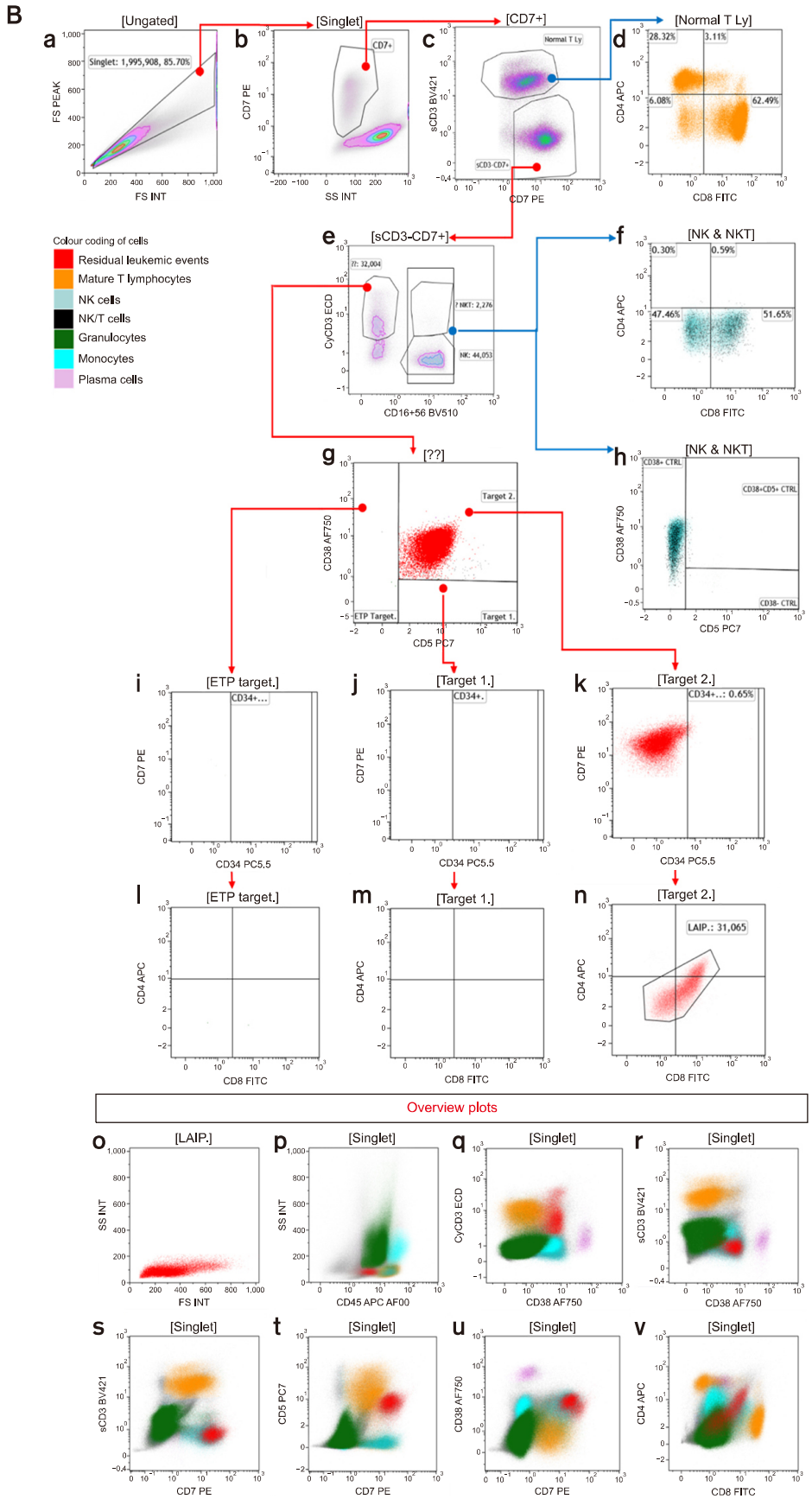
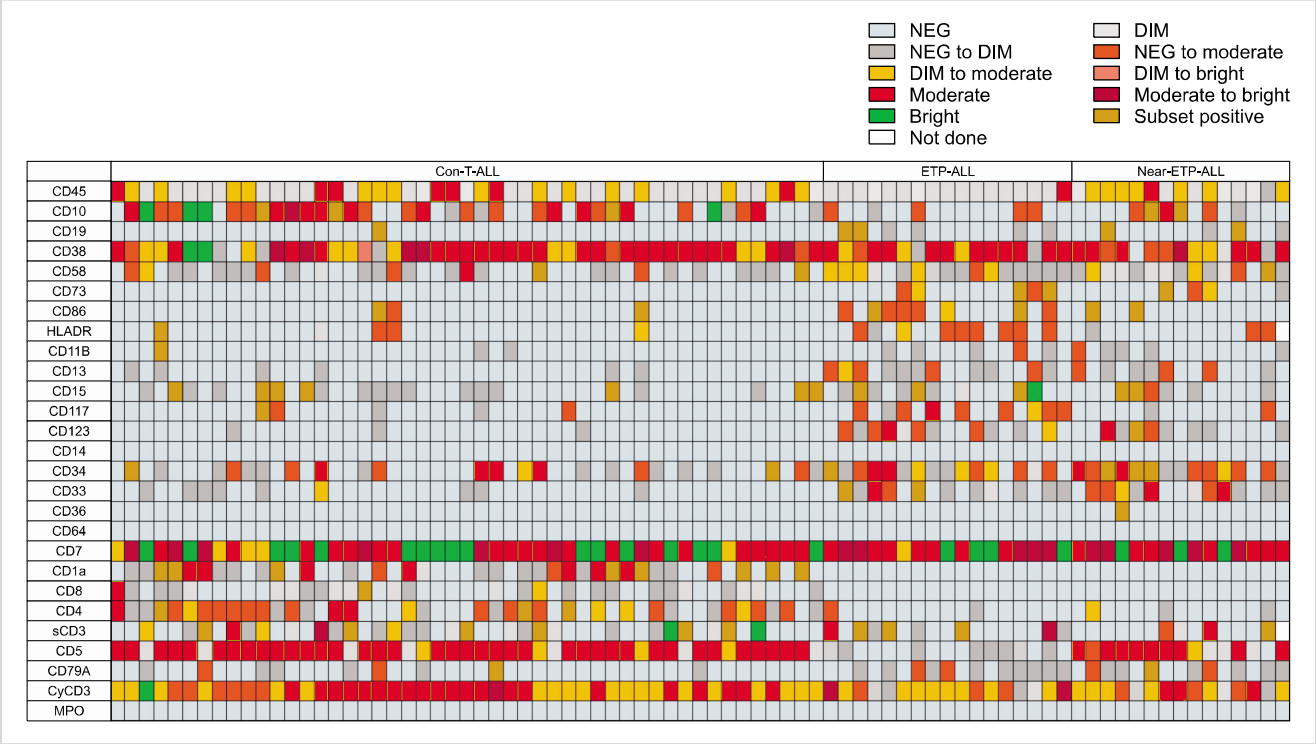


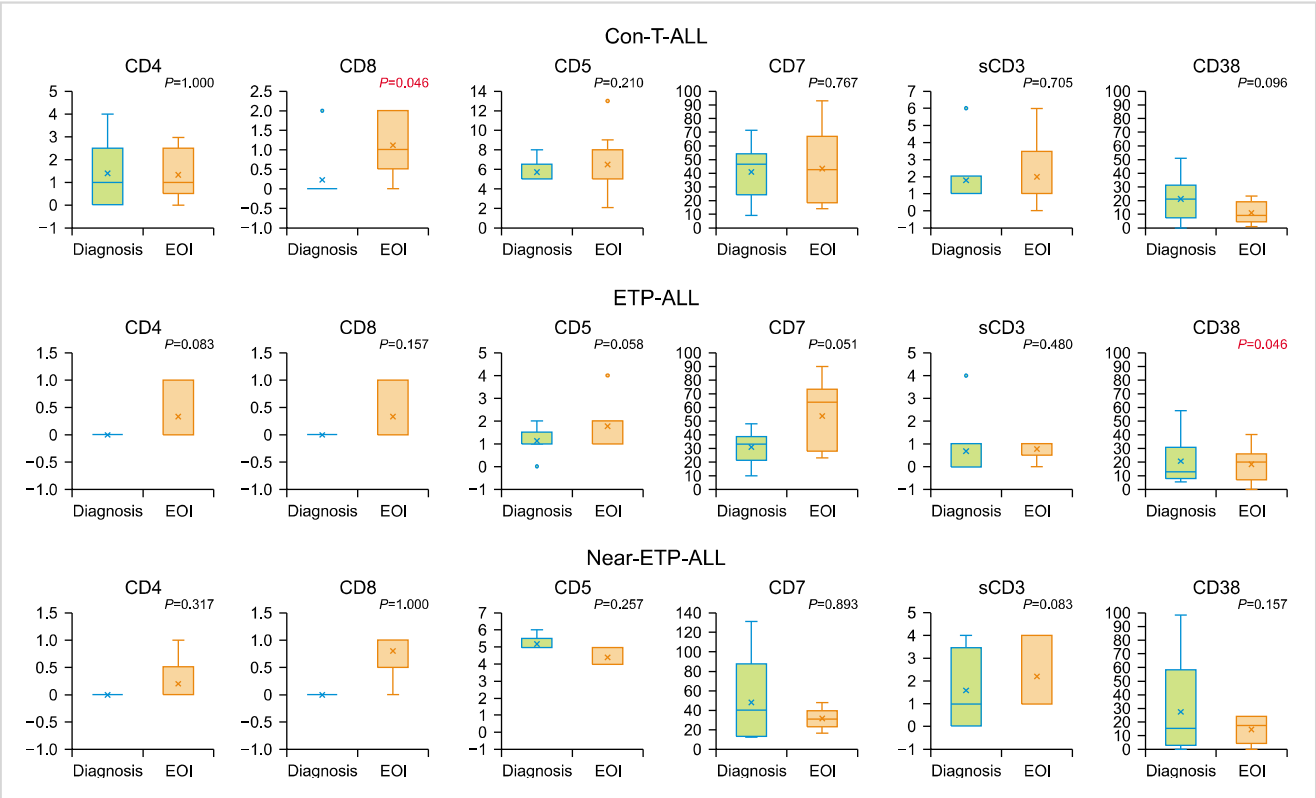
Supplementary Fig. 1. **(A)** Algorithm used in this study to classify T-ALL patients based on flow cytometric immunophenotyping.


B
a
[Ungated]
Singlet: 1,995,908, 85.70%
FS PEAK
FS INT
b
[Singlet]
CD7+
CD7 PE
SS INT
c
[CD7+]
Normal T Ly
sCD3-CD7+
sCD3 BV421
CD7 PE
d
[Normal T Ly]
28.32%
3.11%
6.08%
62.49%
CD4 APC
CD8 FITC
Colour coding of cells
Residual leukemic events
Mature T lymphocytes
NK cells
NK/T cells
Granulocytes
Monocytes
Plasma cells
e
[sCD3-CD7+]
77: 32,004
7 NKT: 2,276
NK: 44,053
CyCD3 ECD
CD16+56 BV510
f
[NK & NKT]
0.30%
0.59%
47.46%
51.65%
CD4 APC
CD8 FITC
g
[??]
Target 2.
ETP Target.
Target 1.
CD38 AF750
CD5 PC7
h
[NK & NKT]
CD38+ CTRL
CD38+CD5+ CTRL
CD38- CTRL
CD38 AF750
CD5 PC7
i
[ETP target.]
CD34+...
CD7 PE
CD34 PC5.5
j
[Target 1.]
CD34+.
CD7 PE
CD34 PC5.5
k
[Target 2.]
CD34+..: 0.65%
CD7 PE
CD34 PC5.5
l
[ETP target.]
CD4 APC
CD8 FITC
m
[Target 1.]
CD4 APC
CD8 FITC
n
[Target 2.]
LAIP.: 31,065
CD4 APC
CD8 FITC
Overview plots
o
[LAIP.]
SS INT
FS INT
p
[Singlet]
SS INT
CD45 APC AF00
q
[Singlet]
CyCD3 ECD
CD38 AF750
r
[Singlet]
sCD3 BV421
CD38 AF750
s
[Singlet]
sCD3 BV421
CD7 PE
t
[Singlet]
CD5 PC7
CD7 PE
u
[Singlet]
CD38 AF750
CD7 PE
v
[Singlet]
CD4 APC
CD8 FITC

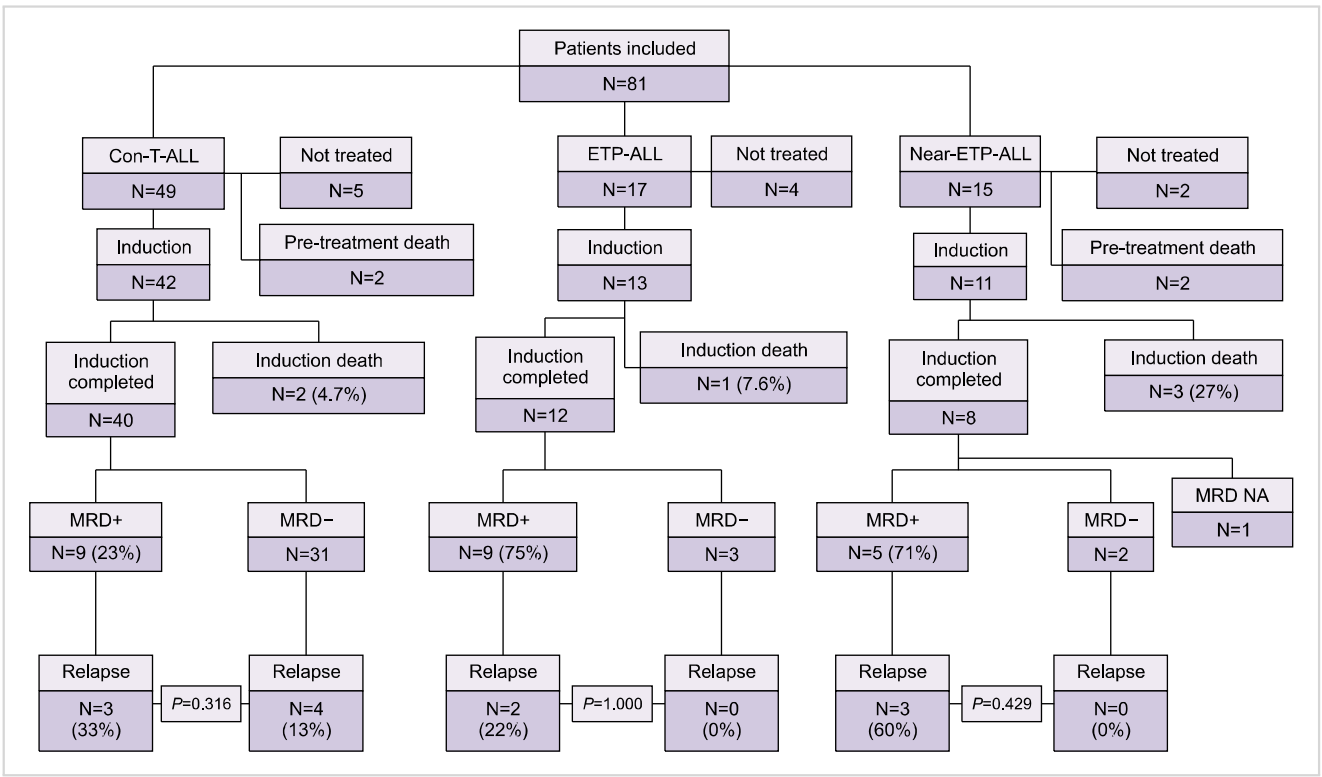
Supplementary Fig. 1. **(B)** Sequential gating for measurable residual disease assessment.
Among all events acquired, singlets were gated on forward scatter (FS) peak vs. area contour plot 'a'; Among these singlets, CD7 positive events (CD7+) were gated on CD7 vs. side scatter (SSC) contour plot 'b'. These CD7+ events comprise mature T-lymphocytes, NK T/NK cells, dim CD7 expressing myeloid blasts, and leukemia associated immunophenotype events (LAIP) events. From these CD7+ events, surface CD3 (sCD3) positive normal T-lymphocytes (normal T Ly, orange events) and sCD3 negative events (sCD3-CD7+) were separated on sCD3 vs. CD7 contour plot 'c'. This sCD3-CD7+ population comprising LAIP and NK and T/NK cells were analyzed in cyCD3 vs. CD16+56 plot 'e' to segregate NK cells and T/NK cells (greyish-green and black dots, respectively). The remaining cyCD3 positive, CD56 & CD16 negative events are our suspected LAIP (labeled as '??'). The distribution of CD4 vs. CD8 and CD5 vs. CD38 expression among NK cells and T/NK cells were analyzed in scatter plots 'f and h'. The suspected LAIP events ('??' gate) were analyzed in CD5 vs. CD38 dot plot 'g' and were divided into target 1 (CD5 positive & CD38 negative), target 2 (both CD5 and CD38 positive), and ETP-ALL target (CD5 negative and CD38 positive or negative). The CD5 and CD38 expression limits in plot 'g' were set based on the CD38 and CD5 expression among NK cells and T/NK cells in plot 'h'. The '??' events falling within each of these target gates in plot 'g' were analyzed in isolation for their CD7 vs. CD34 expression in dot plots i, j, and k, followed by their CD4 vs. CD8 expression in dot plots l, m, and n. The '??' events that were showing normal, i.e., mutually exclusive distribution for CD4 and CD8 (in l, m, and n dot plots) were not considered as LAIP and were considered to be normal mature T-lymphocytes that have deprived their sCD3 during processing. In this example of con-T-ALL-MRD, the '??' events had a moderate expression for both CD5 and CD38 (target 2), was negative for CD34 expression (plot 'k'), and had negative to dim expression for both CD4 and CD8 (plot 'n'). These events were quantified as LAIP events (red dots). The tight clustering of these LAIP events was ensured in FSC vs. SSC plot 'o'. The antigen expression profile of this LAIP cluster was further compared among all the singlet gated events in various immunophenotype combinations across plots 'p' to 'v'. In samples that were MRD negative by this approach, two modifications were made in the gating strategy. First, the sCD3-CD7+ gate in plot 'c' was extended further in the y-axis to include more sCD3 dim to moderate and CD7 bright events. This was to ensure that an sCD3 expressing/upregulated LAIP was not missed. Second, the '??' gate in plot 'e' was extended further in the x-axis to include more cyCD3+ & CD16+ CD56 positive events. This was done to avoid missing any CD56/CD16 expressing/upregulated LAIP.
Abbreviations: Con-T-ALL, conventional T-ALL; ETP-ALL, early-T-cell acute lymphoblastic leukemia.



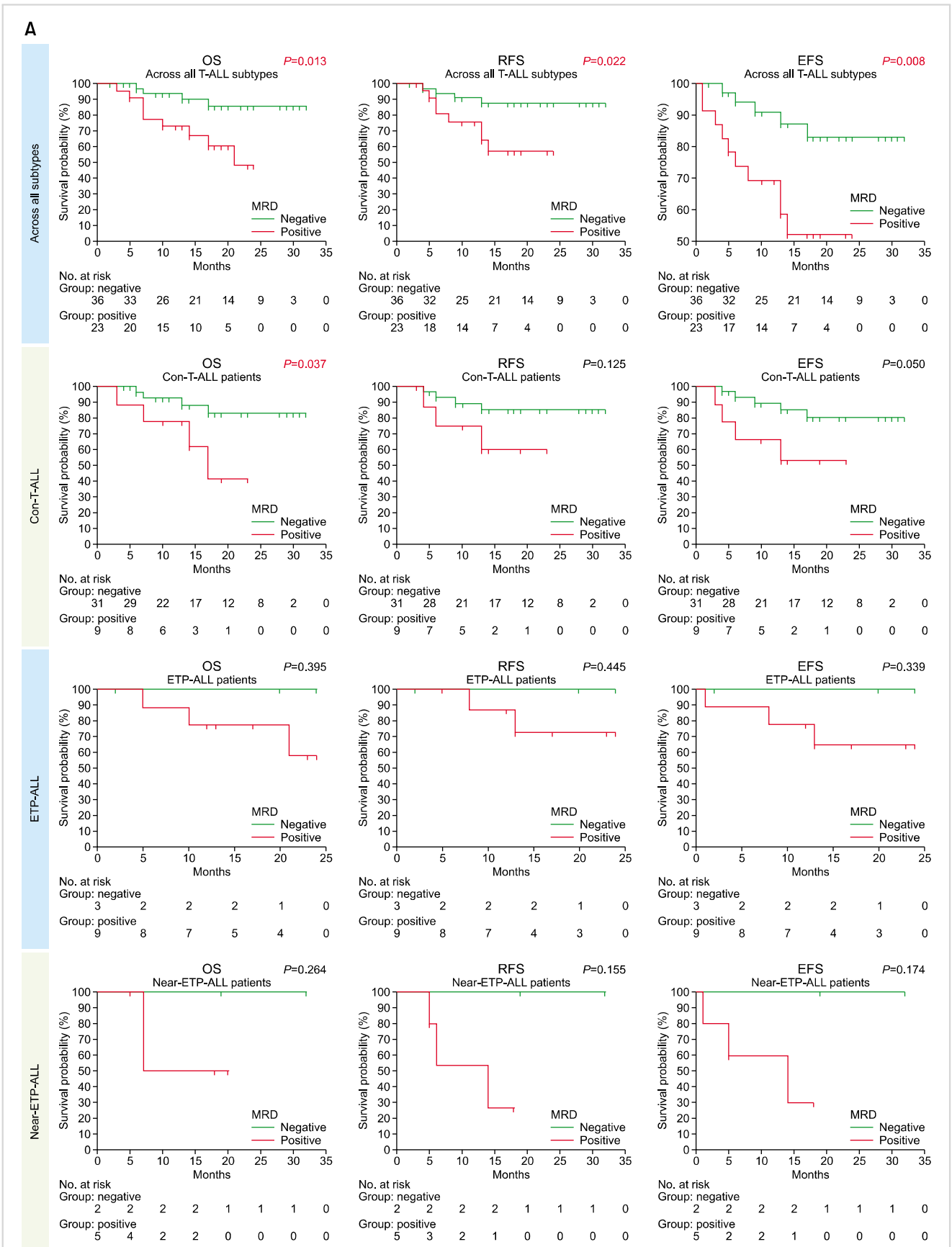
Supplementary Fig. 2. Overview of antigen expression profile among the immunophenotypic subtypes of T-ALL patients analyzed.



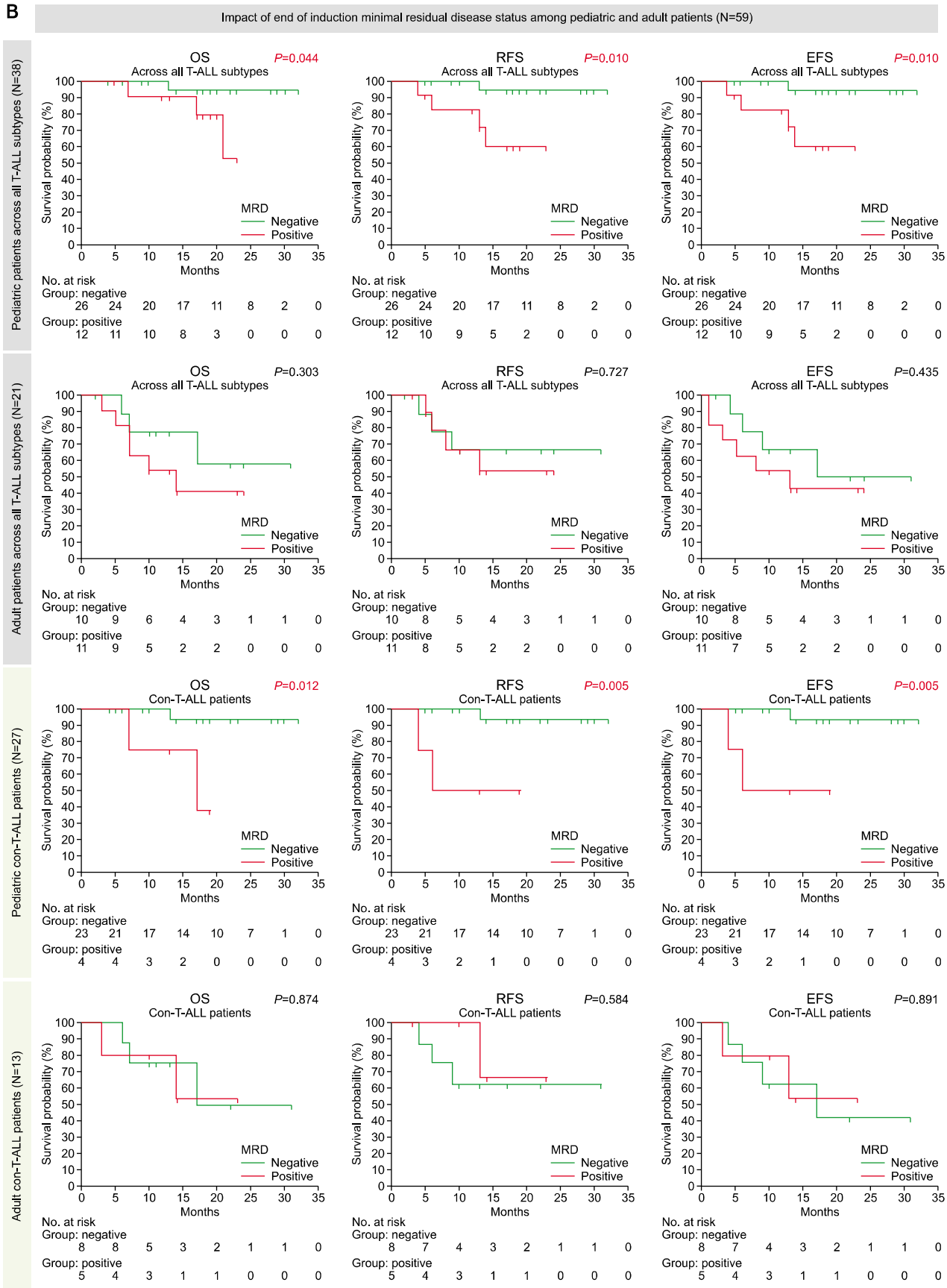
Supplementary Fig. 3. Box and whisker plots comparing changes in the intensity of CD4, CD8, CD7, CD5, CD38, and sCD3 expression between base line and end-of-induction residual blasts.
Abbreviations: First row, con-T-ALL patients; second row, ETP-ALL patients; third row, near-ETP-ALL patients.



Supplementary Fig. 4. Follow-up algorithm of patients included in the study.



Supplementary Fig. 5. **(A)** Kaplan–Meier survival plots depicting the impact of EOI-MRD status on 2-year OS, RFS, and EFS across all T-ALL subtypes. **(B)** Kaplan–Meier survival plots for 2-year OS, RFS, and EFS among pediatric and adult T-ALL patients concerning their EOI-MRD status.



Supplementary Fig. 5. Continued.

Supplementary Table 1A. Flow cytometric immunophenotyping panel for acute leukemia diagnosis.

Tube	BV510	BV421	FITC	PE	ECD	PC5.5	PC7	APC	APC AF700	APC AF750
1	-	-	-	-	-	-	-	-	CD45	
Clone									J.33	
Vendor									BC	
2	CD20	CD123	CD58	CD86	CD73	CD34	CD10	CD19	CD45	CD38
Clone	2H7	9F5	AICD58	FUN-1	AD2	8G12	ALB1	J3-119	J.33	LS198-4-3
Vendor	BD	BD	BC	BC	BD	BD	BC	BC	BC	BC
3	HLA-DR	CD117	CD15	CD13	CD19	CD34	CD56	CD7	CD45	CD11b
Clone	L243	YB5.B8	80H5	SJ1D1	J3-119	8G12	N901(HLDA6)	8H8.1	J.33	BEAR1
Vendor	BD	BD	BC	BC	BC	BD	BC	BC	BC	BC
4	HLA-DR	CD36	CD14	CD123	CD64	CD33	CD117	CD34	CD45	CD38
Clone	L243	FA6.152	RMO52	9F5	22	D3HL60.251	104D2D1	581	J.33	LS198-4-3
Vendor	BD	BC	BC	BC	BC	BC	BC	BC	BC	BC
5	CD3	CD5	CD1a	CD7	CD34	TCR γδ	CD56	CD4	CD45	CD8
Clone	SK7	UCHT2	BL6	8H8.1	581	IMMU510	N901(HLDA6)	13B8.2	J.33	B9.11
Vendor	BD	BD	BC	BC	BC	BC	BC	BC	BC	BC
6		CD117	Cyto MPO	Cyto CD79a	Cyto CD3		CD22	CD34	CD45	CD11b
Clone		YB5.B8	CLB-PO1	HM47	UCHT1		SJ10.1H11	581	J.33	BEAR1
Vendor		BD	BC	BC			BC	BC	BC	BC

Abbreviations: BC, Beckman Coulter; BD, Beckton Dickinson Lifesciences; Cyto, cytoplasmic antigen.

Supplementary Table 1B. Panel for minimal residual disease assessment.

Tube	BV510	BV421	FITC	PE	ECD	PC5.5	PC7	APC	APC AF700	APC AF750
1	CD16 & CD56	Surface CD3	CD8	CD7	Cyto CD3	CD34	CD5	CD4	CD45	CD38
Clone	3G8 & HCD56	UCHT1	B9.11	8H8.1	UCHT1	8G12	BL1a	13B8.2	J.33	LS198-4-3
Vendor	BL	BD	BC	BC	BC	BD	BC	BC	BC	BC
2	-	-	Syto13	-	-	-	-	CD7	CD45	-
Clone	-	-	-	-	-	-	-	8H8.1	J.33	-
Vendor			Invitrogen					BC	BC	

Abbreviations: BC, Beckman Coulter; BD, Beckton Dickinson Lifesciences; BL, BioLegend; Cyto, cytoplasmic antigen.

MRD % calculation

Nucleated cells in the processed bone marrow sample were determined in a separate tube (tube 2 of our MRD panel) using cell-permeant nucleic acid binding Syto13 dye. The formula used for MRD quantification is shown below.

$$\frac{\text{Tube 1 LAIP events}}{\text{Tube 1 Singlet events}} \times \frac{\textit{Syto}13 \text{ positive Singlets\%}}{\text{Tube 1 Singlet\%}} \times \frac{\textit{Syto}13 \text{ positive CD7 events\%}}{\text{Tube 1 CD7 events\%}} \times 100 = \textit{MRD}\%$$

Abbreviation: LAIP, leukemia associated immunophenotype.

MRD assay validation

For lower limit of blank (LLOB) determination, six control samples (non-T-ALL) were processed with our T-MRD panel and one million events were acquired per sample. The mean (±SD) LOB calculated from these four samples was 4.2 (0.95) events. The lowest limit of detection was calculated as 7 events (LLOB+3 times SD of LLOB in control samples), and was rounded off to a cluster of 10 events (0.001%). By spiking assays (treatment naïve T-ALL samples spiked in non-T-ALL control samples) performed in triplicates, we could establish a lowest limit of quantification (LLOQ) as 30 leukemic events in one million acquired events [maximum coefficient of variation (CV) of 11%]. This corresponds to MRD sensitivity of 0.003% with a maximum CV of 14.4%. The table provides dilution experiment results used to calculate LLOQ.

Supplementary Table 1C. Dilution experiments for lower limit of detection calculation.

Dilution experiment			Acquired events	LAIP events	% MRD	LAIP events			% MRD		
						Mean	SD	CV (%)	Mean	SD	CV (%)
For 60 events	Sample 1	Processing 1	11,23,344	65	0.005786	62	4.3	6.9	0.00555	0.00037	6.8
		Processing 2	11,11,446	64	0.005758						
		Processing 3	11,14,246	57	0.005116						
	Sample 2	Processing 1	11,12,026	65	0.005845	63.3	4.7	7.4	0.00584	0.00061	10.5
		Processing 2	11,08,604	58	0.005232						
		Processing 3	10,36,383	67	0.006465						
For 30 events	Sample 1	Processing 1	10,90,239	28	0.002568	31.6	3.5	11.07	0.00305	0.00044	14.4
		Processing 2	10,19,365	32	0.003139						
		Processing 3	10,15,515	35	0.003447						
	Sample 2	Processing 1	10,45,155	32	0.003062	32.6	2.08	6.38	0.00318	0.00022	7.0
		Processing 2	10,17,746	35	0.003439						
		Processing 3	10,18,950	31	0.003042						

Abbreviations: CV, coefficient of variation; LAIP, leukemia associated immunophenotype; MRD, minimal residual disease.

Supplementary Table 2. Comparison of clinical and laboratory characteristics between adult and pediatric patients among each subtype of T-ALL.

Parameters	All categories (N=81)			Con-T-ALL (N=49)			ETP-ALL (N=17)			Near-ETP-ALL (N=15)		
	Adult (N=34)	Pediatric (N=47)	*P*	Adult (N=15)	Pediatric (N=34)	*P*	Adult (N=10)	Pediatric (N=07)	*P*	Adult (N=09)	Pediatric (N=06)	*P*
Sex (male:female)	3.2:1	4.2:1	0.633	6.5:1	3.8:1	0.546	2.3:1	6:1	0.452	6:3	5:1	0.475
Median (range) Hb in g/L	85.6 (61–142)	90 (30–141)	0.867	89 (63–142)	91 (30–141)	0.515	80 (61–128)	97 (30–131)	0.161	88 (69–133)	83 (41–129)	0.456
Median (range) WBC count, $\times 10^9$/L	70.3 (1–480)	148 (1.9–850)	0.002	88 (1.1–349)	110 (1.9–850)	0.056	55 (1–480)	90.4 (3.2–267	0.109	68 (3.6–131)	244 (3–590)	0.272
Median (range) platelet count, $\times 10^9$/L	92.3 (20–290)	119 (22–380)	0.969	52 (20–119)	83 (22–366)	0.308	125 (30–290)	125 (30–245)	0.962	100 (20–218)	149 (32–380)	0.607
Hyperleukocytosis	27%	51%	0.026	27%	53%	0.088	10%	29%	0.323	44%	67%	0.398
Hepatomegaly	36.4%	46.2%	0.401	36%	45%	0.553	20%	40%	0.409	56%	67%	0.735
Splenomegaly	52%	59%	0.526	43%	61%	0.249	50%	40%	0.714	67%	67%	1.000
Lymphadenopathy	75%	81%	0.538	69%	75%	0.692	80%	100%	0.283	78%	100%	0.255
Mediastinal mass	27%	34%	0.523	29%	39%	0.480	40%	20%	0.439	11%	17%	0.756
CNS involvement	4%	3%	0.687	8%	4%	0.569	0%	0%	NA	0%	0%	NA
Induction death	16%	5%	0.127	0%	7%	0.332	14%	0%	0.335	60%	0%	0.026
Induction failure	17.4%	0%	0.007	0%	0%	NA	17%	0%	0.296	75%	0%	0.011
D8BNC	35%	35%	1.000	40%	30%	0.559	57%	50%	0.797	0%	50%	0.053
EOI-MRD positive	52% (N=21)	32% (N=38)	0.117	38.5% (N=13)	15% (N=27)	0.093	67% (N=6)	83% (N=6)	1.000	100% (N=2)	60% (N=5)	0.290
Relapse	33% (N=21)	13% (N=39)	0.120	31% (N=13)	11% (N=27)	0.125	17% (N=6)	17% (N=6)	1.000	100% (N=2)	17% (N=6)	0.035
OS at 24 months	39.8% (N=25)	79.5% (N=41)	<0.001	48.1% (N=13)	79.3% (N=29)	0.123	51.4% (N=7)	66.7% (N=6)	0.222	0% (N=5)	100% (N=6)	0.001
RFS at 24 months	60.4% (N=21)	84.3% (N=39)	0.026	64.3% (N=13)	87.3% (N=27)	0.117	75% (N=6)	80% (N=6)	0.744	0% (N=2)	75% (N=6)	0.006
EFS at 24 months	38% (N=25)	80.3% (N=41)	<0.001	44.5% (N=13)	81.4% (N=29)	0.068	53.6% (N=7)	80% (N=6)	0.234	0% (N=5)	75% (N=6)	0.001

Abbreviations: BM, bone marrow; CNS, central nervous system; D8BNC, day 8 blast not cleared; EFS, event-free survival; EOI-MRD, end-of-induction-measurable residual disease; Hb, hemoglobin; N, number of patients analyzed; NA, not applicable; OS, overall survival; PB, peripheral blood; RFS, relapse-free survival; WBC, white blood cells.

Supplementary Table 3. EOI-MRD status specific clinical and laboratory characteristics across subcategories of T-ALL.

Parameters	All T-ALL subtypes (N=59)			Con-T-ALL (N=40)			ETP-ALL (N=12)			Near ETP-ALL (N=7)		
	MRD- N=36	MRD+ N=23 (39%)	*P*	MRD- N=31	MRD+ N=9 (22.5%)	*P*	MRD- N=3	MRD+ N=9 (75%)	*P*	MRD- N=2	MRD+ N=5 (71%)	*P*
Median (range) age in years	14 (1–31)	23 (9–50)	0.001	13 (1–31)	22 (9- 50)	0.015	19 (15–20)	17 (13–39)	0.727	10 (5–15)	18 (11–34)	0.190
Sex (male:female)	6.2:1	2.8:1	0.241	5.2:1	3.5:1	0.672	All males	3.5:1	0.371	2:0	1.5:1	0.290
Median (range) Hb in g/L	92 (30–142)	88 (41–135)	0.963	93 (30–142)	85 (73–135)	0.799	92 (66–126)	97 (61–131)	0.727	75 (60–90)	75 (41–90)	0.857
Median(range) WBC count, $\times 10^9$/L	131.5 (1.1–736)	40 (1.0–482)	0.003	127 (1.1–736)	49 (9.7–82.5)	0.028	55.7 (10.3–480)	7.2 (1–267)	0.209	470 (350–590)	102 (3–482)	0.190
Median (range) platelet count, $\times 10^9$/L	48 (20–366)	54 (20–380)	0.371	46 (20–366)	45 (20–95)	0.656	145 (45–159)	110 (30–245)	1.000	88 (44–132)	50 (32–380)	0.857
Median (range) BM blast, %	89 (23–99)	86 (22–99)	0.614	87 (23–97)	86 (64–96)	0.935	96 (95–98)	86 (22–94)	0.036	94 (89–99)	92 (74–99)	0.857
Median (range) PB blast, %	85 (2–98)	65 (2–99)	0.199	85 (2–97)	62 (56–88)	0.241	83 (80–86)	45 (2–95)	0.582	94 (90–98)	82 (2–99)	0.571
Hyperleukocytosis (%)	58%	17%	0.002	58%	0%	0.002	33%	11%	0.371	100%	60%	0.290
Hepatomegaly (%)	35.3%	44.4%	0.519	40%	29%	0.576	0%	29%	0.301	50%	100%	0.402
Splenomegaly (%)	56%	50%	0.686	57%	43%	0.509	33%	43%	0.778	100%	75%	0.576
Lymphadenopathy (%)	73%	90%	0.133	72%	88%	0.379	67%	86%	0.490	100%	100%	NA
Mediastinal widening (%)	51.5%	18.2%	0.013	55%	0%	0.003	50%	37.5%	0.747	0%	20%	0.495
D8BNC (%)	39%	39%	0.979	39%	29%	0.629	0%	50%	0.343	100%	33%	0.248
Blasts expressing												
CD19	0%	22%	0.053	0%	0%	NA	0%	33%	0.248	0%	40%	0.290
Surface CD3	40%	32%	0.533	43%	57%	0.519	0%	11%	0.546	50%	25%	0.540
CD79a	14%	39%	0.076	16%	22%	0.672	0%	56%	0.091	0%	40%	0.290
CD56	8%	22%	0.142	10%	0%	0.332	0%	44%	0.157	0%	20%	0.495
Relapse (%)	11%	35%	0.028	4/31 (13%)	3/9 (33.3%)	0.156	0/3 (0%)	2/9 (22%)	0.371	0/2 (0%)	3/5 (60%)	0.147
OS at 24 months	85.7%	48.2%	0.013	83.2%	41.5%	0.037	100%	77.8%	0.395	100%	50%	0.264
RFS at 24 months	87.2%	57%	0.022	85.2%	60.0%	0.125	100%	72.9%	0.445	100%	26.7%	0.155
EFS at 24 months	83%	52%	0.008	80.3%	53.3%	0.050	100%	64.8%	0.339	100%	30%	0.174

Abbreviations: BM, bone marrow; CNS, central nervous system; D8BNC, day 8 blast not cleared; EFS, event-free survival; EOI-MRD, end-of-induction-measurable residual disease; Hb, hemoglobin; NA, not applicable; OS, overall survival; PB, peripheral blood; RFS,relapse-free survival; WBC, white blood cell.