

Single-cell profiling of CD19-directed CAR T-cell phenotypes and immune system dynamics in pediatric B-cell acute lymphoblastic leukemia

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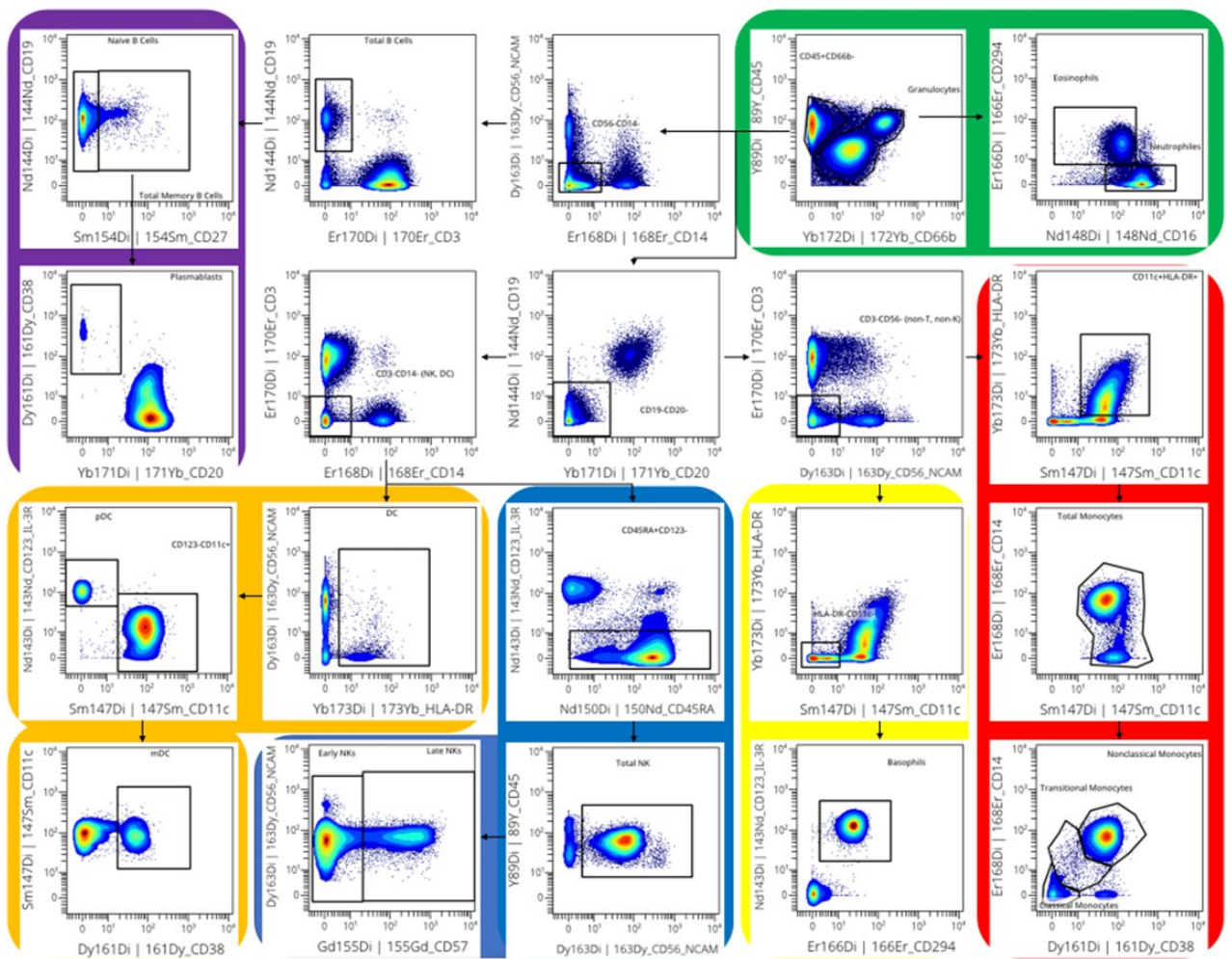
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Supplementary Figure 1. Gating strategy.

(a) Manual gating of CAR+ T-cells as validation of the applied method.

Graph was generated using unfiltered cells. Samples were collected from five representative patients with BCP-ALL in IP, followed by PBMC analysis on days 7 and 28. Color coding: orange – unfiltered cells, blue – CAR+ T-cells.

(b) T cells gating strategy.

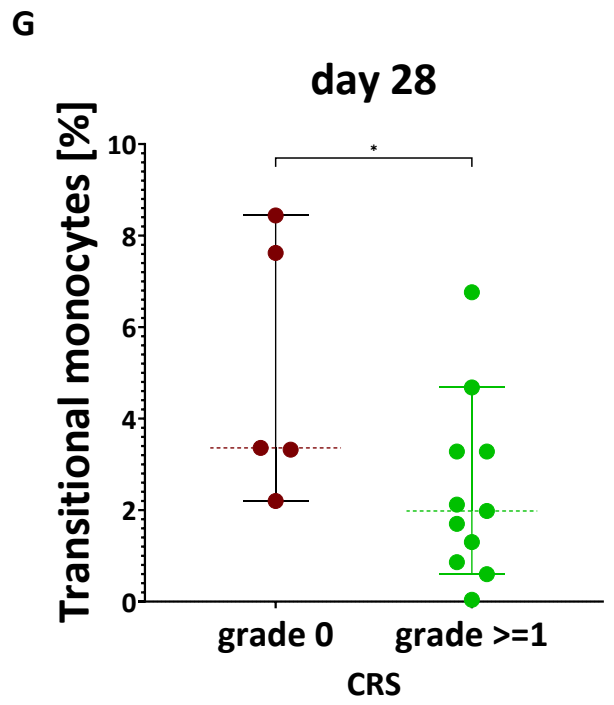
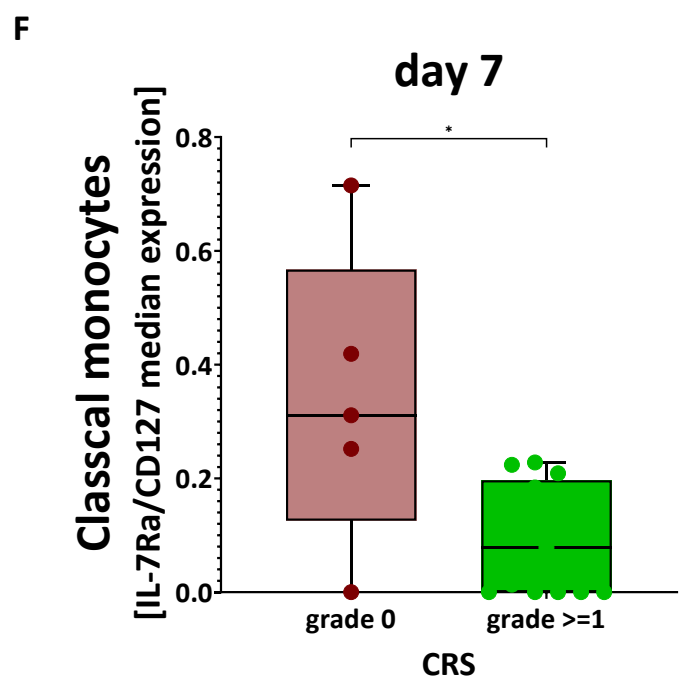
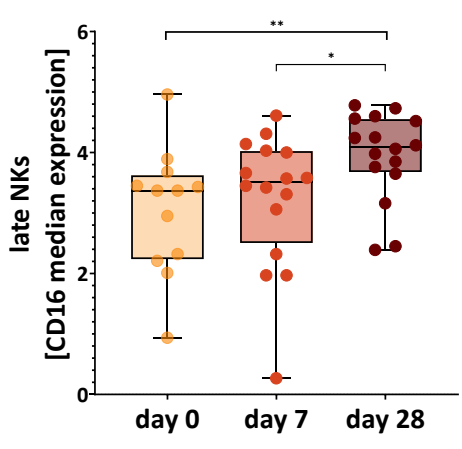
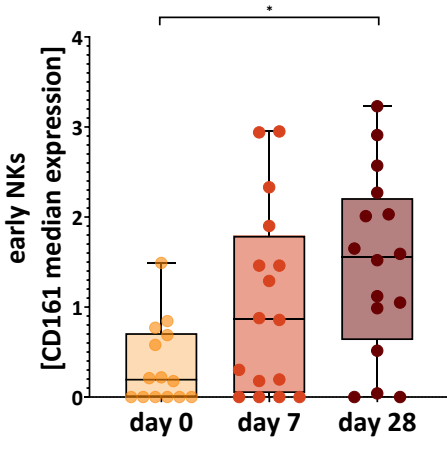
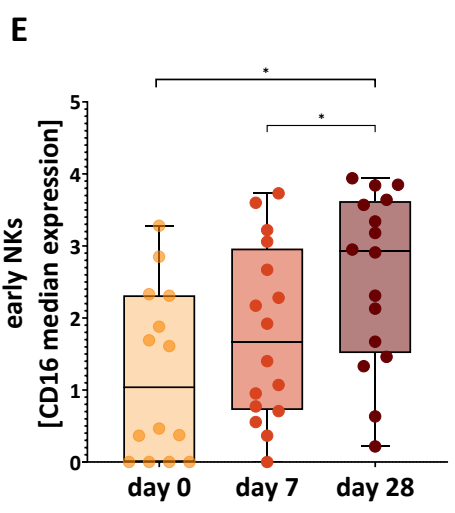
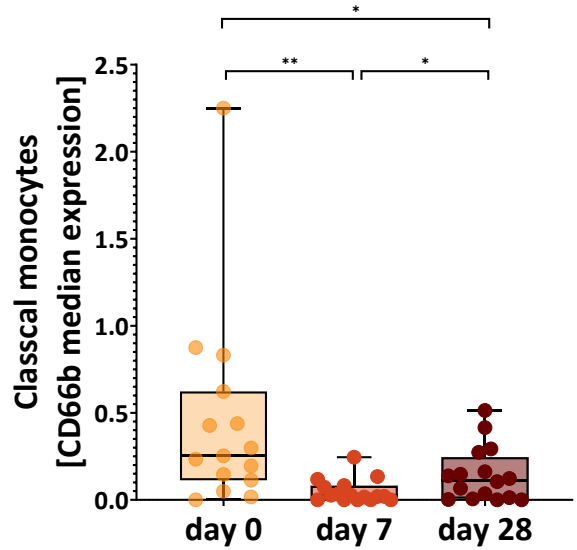
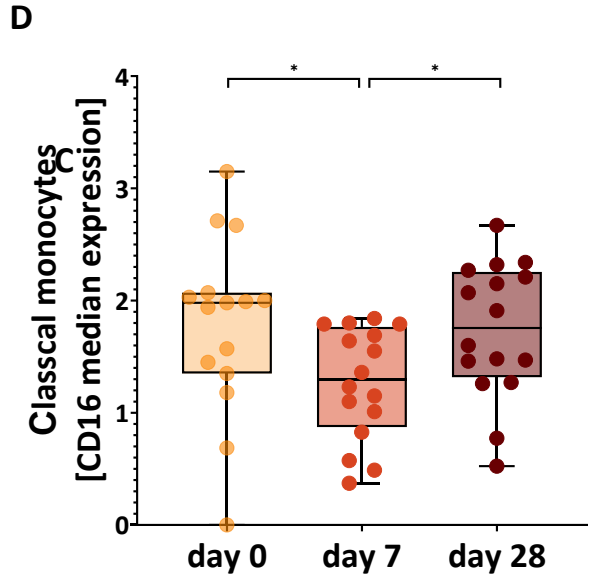
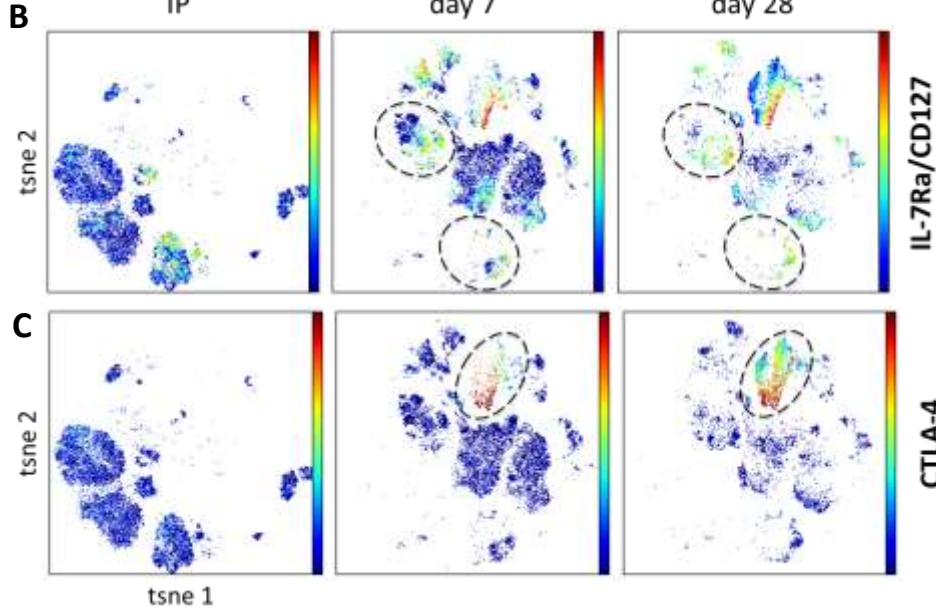
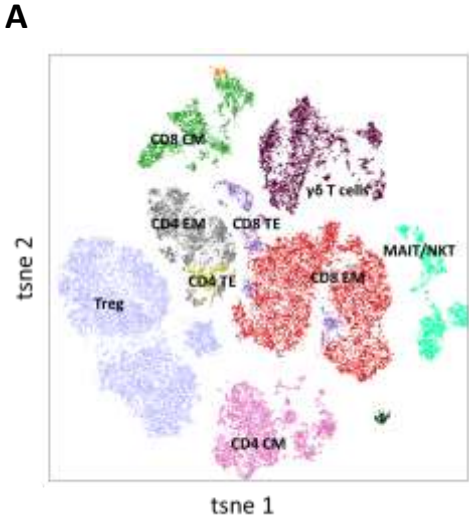
Flow plot showing gating strategy for T cells from Human healthy peripheral blood (4 PBMC from healthy children, ~300,000 cells per sample). Gating and sub sequential analysis was done using OMIQ.

Color green highlights gating for T-reg memory and Th-like 1,2,17; Blue – CD4+; Yellow – CD8+; Red – CD4-CD8- $\gamma\delta$ T cell, Purple – CD4- MAIT/NKT.

(c) Other cells gating strategy.

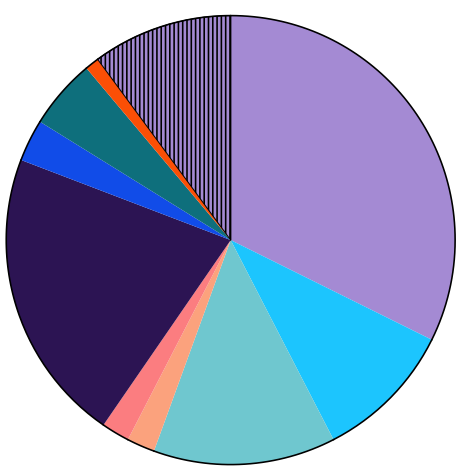
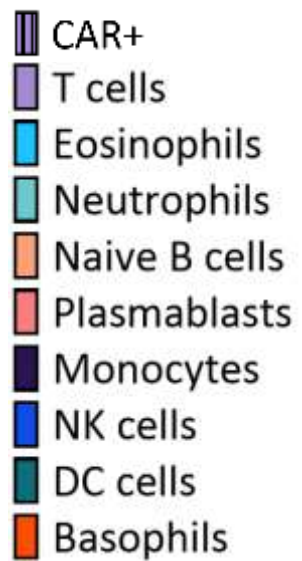
Flow plot showing gating strategy for cells from Human healthy peripheral blood (4 PBMC from healthy children, ~300,000 cells per sample). Gating and sub sequential analysis was done using OMIQ.

Color green highlights gating for neutrophils and eosinophils; Red – monocytes; Yellow – basophils; Blue – NK cells; Orange – DC; Purple – B cells.



Supplementary Figure 2.

- A. CAR+ T-cell subsets at all timepoints classified using Mahalanobis distance.
- B. IL-7R α (CD127) expression on CAR+ T-cells over time (infusion, day 7, and day 28).
- C. CTLA-4 expression on CAR+ T-cells over time (infusion, day 7, and day 28).
- D. CD16 and CD66b expression on classical monocytes decreases at day 7, followed by recovery at day 28 (*p < 0.05; **p < 0.01; ***p < 0.001).
- E. CD16 and CD161 expression on early NK cells increases at day 28, while late NK cells show higher CD16 at day 28 compared to day 0 (*p < 0.05; **p < 0.01; ***p < 0.001).
- F. Expression on classical monocytes of CD127 at day 7 based on CRS incidence (*p < 0.05).
- G. Abundance of transitional monocytes at day 28 based on CRS incidence (*p < 0.05).



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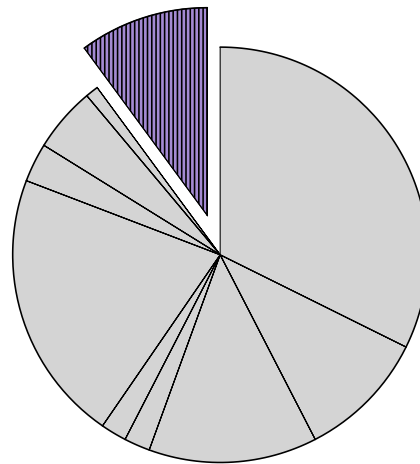
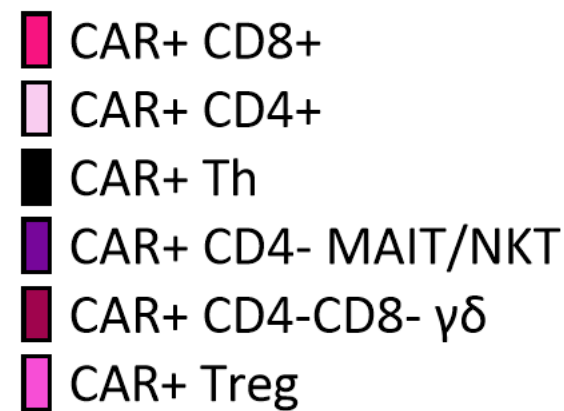
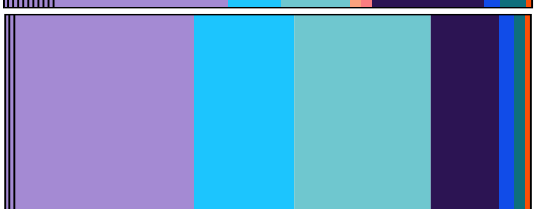
day 0



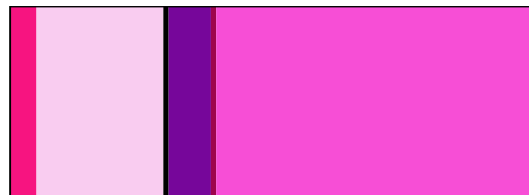
day 7



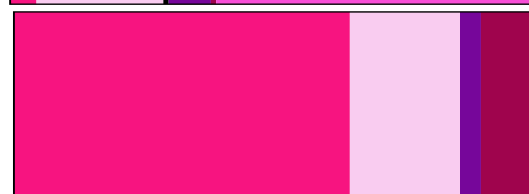
day 28



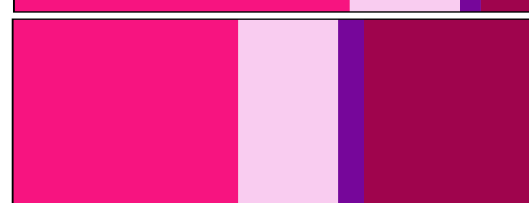
IP



day 7



day 28



Supplementary Figure 3. Visual summary of key findings.

A graphical overview of CAR+ T-cell subtype dynamics and immune responses throughout CD19 CAR-T therapy. The infusion product (IP) primarily consisted of CAR+ Treg memory cells, with smaller proportions of CD4+ central memory and MAIT/NKT subsets. Following infusion, CAR+ Treg memory cells decline, while CD8+ subsets expand over time. Additionally, monocyte and NK cell levels increase post-infusion, highlighting broader immune activation.

Supplementary Table 1. Patient characteristics.

The table portion presents patients' prior treatment history and baseline status.

Patient number	Treatment before CAR-T CD19 infusion			PCR-MRD (1×10^{-4} - negative, >=1x10 ⁻⁴ positive)	normal B cells detected in PBMC at time of infusion (% of lymphocytes)	CD3+ T cell counts at time of aphaeresis (cells/ μ L)	ALC at time of aphaeresis ($10^3/\mu$ L)
19	-	-	-	positive	3.3	1394	13.04
18	-	-	-	negative	1.7	416	0.01
17	-	-	-	negative	0.2	470	0.11
16	-	Blinatumomab	HSCT	negative	no data	no data	no data
15	Inotuzumab	-	HSCT	negative	0	3341	9.99
14	Inotuzumab	-	HSCT	positive	0.3	3596	5.7
13	-	-	HSCT	positive	4.1	2857	3.81
12	-	-	HSCT	negative	0.4	924	0.81
11	-	-	-	negative	37.2	339	0.57
10	-	-	HSCT	negative	no data	no data	no data
9	Inotuzumab	-	HSCT	negative	0.4	519	0.53
8	-	-	-	positive	0	908	1.33
7	-	-	-	negative	2.3	247	0.26
6	Inotuzumab	Blinatumomab	-	positive	0	1077	1.14
5	-	-	HSCT	positive	0	356	0.5
4	Inotuzumab	-	-	positive	1.7	1238	1.33
3	Inotuzumab	-	-	positive	0	536	0.65
2	-	-	-	negative	0.5	842	0.9
1	-	-	HSCT	negative	0.4	160	0.57

Supplementary Table 2. Influence of various factors during therapy on CAR+ T-cell subpopulations within the infusion product.

P-values were determined using the Mann-Whitney U test. Significant p value is highlighted in red in the table.

Variable	CAR+ T-cells in CAR-T CD19 infusion product								
	CD8 Central Memory	CD8 Effector Memory	CD8 Terminal Effector	CD4 Central Memory	CD4 Effector Memory	CD4 Terminal Effector	CD4-MAIT/NKT	CD4-CD8- $\gamma\delta$ T cells	Treg memory
Sex male/female 10/9	p=0.8421	p=0.9673	p=0.7050	p=0.7802	p=0.0172	p=0.1377	p=0.7802	p=0.3555	p=0.9682
Risk group* HR/non-HR 12/7	p=0.8369	p=0.3500	p=0.7892	p=0.7108	p>0,9999	p=0.6340	p=0.9671	p=0.1414	p>0,9999
Blinatumomab** yes/no 2/17	p>0.9999	p=0.5673	p=0.9240	p=0.8421	p>0,9999	p=0.7427	p=0.8421	p=0.2924	p=0.9474
Inotuzumab** yes/no 7/12	p=0.8314	p=0.1997	p=0.7176	p=0.7012	p=0.7012	p=0.5077	p=0.8983	p=0.4662	p=0.7654
H SCT** yes/no 9/10	p=0.7197	p=0.7332	p=0.7950	p=0.1823	p=0.3154	p=0.7377	p=0.7197	p=0.4462	p=0.3154
Burden of the disease*** positive/negative 8/11	p=0.3511	p=0.1221	p= 0.8880	p=0.2723	p=0.8404	p=0.9486	p=0.6574	p=0.2051	p=0.5448
Age at the time of infusion >=10/<10 13/6	p=0.6388	p=0.1997	p=0.1863	p=0.6388	p=0.4670	p=0.0844	p=0.6388	p=0.6384	p=0.7654
CRS grade >=1/0 14/5	p=0.1560	p=0.0913	p=0.0460	p=0.4998	p=0.4998	p=0.9802	p=0.0143	p=0.6213	p=0.2976
ICANS >=1/0 3/16	p=0.9577	p=0.4076	p=0.8947	p=0.7926	p=0.3034	p=0.3509	p=0.1383	p=0.7905	p=0.9577
Outcome**** CR/non-CR 14/5	p=0.2976	p=0.4851	p=0.4847	P=0.8932	p=0.1068	p=0.9486	p=0.4998	p=0.6213	p=0.6868

* at the time of diagnosis

** administration before CAR-T CD19 therapy

*** burden of disease (% of blasts in bone marrow before CAR-T CD19 infusion) – MRD (<1x10⁻⁴ - negative, >=1x10⁻⁴ positive)

**** outcome at 6-months after CAR-T CD19 therapy