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

by Aleksandra Oszer, Bartlomiej Pawlik, Natalia Cwilichowska-Puslecka, Pawel Marschollek, Monika Richert-Przygonska, Monika Mielcarek-Siedziuk, Jeremy C. Crawford, Maciej Mazurek, Abhishek Koladiya, Krzysztof Kałwak, Jan Styczynski, Szymon Janczar, Marcin Poreba, M Paulina Velasquez, Kara L. Davis and Wojciech Mlynarski

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

Authors: Aleksandra Oszer[1,2*], Bartlomiej Pawlik[1], Natalia Cwilichowska-Puslecka[3], Pawel Marschollek[4], Monika Richert-Przygonska[5], Monika Mielcarek-Siedziuk[4], Jeremy C Crawford[6], Maciej Mazurek[1], Abhishek Koladiya[2], Krzysztof Kałwak[5], Jan Styczynski[5], Szymon Janczar[1], Marcin Poreba[3], M Paulina Velasquez[7], Kara L Davis[2], Wojciech Mlynarski[1,8]

1 Department of Pediatrics, Oncology and Hematology, Medical University of Lodz, Lodz, Poland

2 Division of Hematology, Oncology, Stem Cell Transplant, and Regenerative Medicine, Department of Pediatrics, Stanford University, Stanford, CA, USA

3 Department of Chemical Biology and Bioimaging, Faculty of Chemistry, Wroclaw University of Science and Technology, Wroclaw, Poland

4 Department of Pediatric Bone Marrow Transplantation, Oncology, and Hematology, Wroclaw Medical University, Wroclaw, Poland

5 Department of Pediatric Hematology and Oncology, Nicolaus Copernicus University Torun, Collegium Medicum, Bydgoszcz, Poland

6 Department of Host-Microbe Interactions, St. Jude Children's Research Hospital, Memphis, TN

7 Department of Bone Marrow Transplantation and Cellular Therapy, St. Jude Children's Research Hospital, Memphis, TN, USA

8 Institute of Medical Expertises, Lodz, Poland

*corresponding author, aleksandra.oszer@umed.lodz.pl

Declarations:

Ethics approval and consent to participate

This study involves human participants and was approved by the Human Research Ethics Committee of the Medical University of Lodz (approval no. RNN/93/22/KE). Participants gave informed consent to participate in the study before taking part, according to the Helsinki declaration and its subsequent amendments. No personally identifiable information was included in the paper.

Data-sharing statement

Data are available upon request to the corresponding author.

Competing interests

BP, NCP, PM, MM, AK, SJ, MP - nothing to disclose. AO – Novartis, ALSAC at St. Jude – support for attending meetings and travel; MRP – Novartis – support for attending meetings and travel; MMS – Novartis - payment and honoraria for lectures, presentations, speakers bureaus, manuscript writing and educational events, support for attending meetings and travel; JCC – patents relating to the assessment and use of CAR T cell for treatment of cancer; KK – Novartis, Medac, Pierre Fabre – speaker's bureau; JS – Novartis, Gilead, AstraZeneca and Chiesi, MSD - payment and honoraria for lectures, presentations, speakers bureaus, manuscript writing and educational events, Novartis, Gilead, AstraZeneca, AbbVie, MSD, Roche - support for attending meetings and travel, AstraZencea, MSD, Chiesi - participation on a Data Safety Monitoring Board and Advisory Board; MPV - V foundation Scholar Award, Assisi Foundation Award, St Baldrick's Research Fund Scholar Award - grants and contracts from any entity, NIH ad hoc grant reviewer, AIRC grant reviewer - payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events, patents in the field of cellular immunotherapy, Rally! Foundation Medical Advisory Board; KLD – advisory board participation at Novartis, research funding from Jazz Pharmaceuticals, BD Biosciences,

Kite-Gilead Pharmaceuticals, Parker Institute for Cancer Immunotherapy; WM – Novartis - honoraria for lectures, President of the Polish Society of Pediatric Oncology and Hematology (unpaid).

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Authors' contributions

AO – conceived the study and secured funding. KLD, WM conceptualized the study and supervised the project. AO, BP, NCP, JCC, SJ, MP, MPV developed methods. AO, BP, NCP and MM performed all experiments. AO and AK developed the software. AO, AK and JCC performed formal data analysis for all of the data generated. PM, MRP, MMS, KK, JS treated patients and/or acquired clinical samples and data. AO, MP, KLD and WM interpreted the results and wrote the first draft of the paper. All authors critically reviewed the manuscript.

Keywords: R/R BCP-ALL, CAR-T CD19 therapy, single-cell analysis, mass cytometry, immunophenotype, CRS

List of Abbreviations: chimeric antigen receptor T-cell (CAR-T), B-cell precursor acute lymphoblastic leukemia (B-ALL), diffuse large B-cell lymphoma (DLBCL), U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA), peripheral blood mononuclear cell (PBMC), cytokine release syndrome (CRS), event-free survival (EFS), overall survival (OS), infusion product (IP), PCR-based

minimal residual disease (PCR-MRD), allogeneic hematopoietic stem cell transplantation (allo-HSCT), relapsed/refractory B-cell precursor acute lymphoblastic leukemia (R/R BCP-ALL), absolute neutrophil count (ANC), regulatory T (Treg)

Main text:

Tisagenlecleucel is the only chimeric antigen T cell (CAR-T) therapy approved by the U.S. Food and Drug Administration and the European Medicines Agency for pediatric B-cell precursor acute lymphoblastic leukemia (B-ALL). Tisagenlecleucel demonstrated remarkable efficacy in relapsed/refractory B-ALL(1) and diffuse large B cell lymphoma (DLBCL)(2).

Significant heterogeneity in CAR+ T-cell infusion products (IP) has been demonstrated in LBCL(2, 3). It remains unclear whether these phenotypic variations impact clinical outcomes (3). While pre-infusion composition of tisagenlecleucel products and their post-infusion behavior in children with B-ALL has not been previously studied in depth, the persistence of CAR+ T-cells in peripheral blood post-infusion may play a crucial role in therapy outcomes (1, 4, 5).

We analyzed relationships between clinical outcomes, pre-treatment patient status, and prior therapies in a cohort of relapsed/refractory B-ALL patients (n = 19, aged 2-16 years) receiving CAR-T therapy with tisagenlecleucel in Poland between September 2022, and August 2024, with follow-up until February 2025. All patients provided informed consent. The study was approved by the Human Research Ethics Committee of the Medical University of Lodz (approval no. RNN/93/22/KE). Treatment response was assessed using PCR-based minimal residual disease (PCR-MRD) in bone marrow biopsies, and toxicity was evaluated using the Penn scale for CRS(6) and ICANS scale for neurotoxicity(7). This study was conducted in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (8). We used mass cytometry to characterize the phenotype of CAR+ T-cells in infusion products and tracked their persistence in peripheral blood on days 7 and 28. Additionally, we profiled T cells and non-CAR T mononuclear cells, specifically NK cells and monocytes, to assess broader immune compartment changes post-infusion (Supplementary Figure 1).

At the time of tisagenleucel infusion, eight patients (42%) had positive measurable residual disease (MRD), as detected by PCR-based assessment in the bone marrow (BM) ($<1\times10^{-4}$: negative; $\geq1\times10^{-4}$:

positive). Among these, only one patient had a high disease burden (88% BM blasts) prior to infusion, whereas the remaining PCR-MRD-positive patients had less than 1% blasts by morphology. Fourteen patients (74%) received CAR-T therapy for first relapse, either after allogeneic hematopoietic stem cell transplantation (allo-HSCT) or without complete remission (CR) after one cycle of re-induction therapy. Four patients were treated for second relapse and one patient for fourth relapse. Prior to CAR-T therapy, nine patients (47%) had received allo-HSCT, two blinatumomab, and six inotuzumab ozogamicin. Cytokine release syndrome occurred in 74% of patients, with a maximum severity of grade 2. Seven patients received tocilizumab, all within seven days post-infusion. ICANS was observed in three patients, with two experiencing grade 2 and one experiencing grade 4 neurotoxicity. Complete remission with B-cell aplasia for longer than 6 months was achieved in 14 patients (73%); however, four subsequently relapsed within one year. One patient experienced disease progression, and two patients died due to leukemia relapse or resistance, with a median follow-up of one year (Figure 1, Supplementary Table 1).

Nineteen patients contributed 77 samples across several timepoints as shown in Figure 2A. All data shown for T-cell subsets are gated on CAR+ T-cells, unless otherwise specified. Tisagenleucel infusion products were primarily composed of MAIT/NKT (7.04%), CD4+ Central Memory (16.6%), and predominantly Treg memory (63.3%) cells, while other subsets comprised less than 2% of CAR+ T-cells (Figure 2B).

CAR+ Treg memory cells, decreased significantly by day 7 (<0.5%, $p<0.0001$) and day 28 ($p<0.001$) compared to infusion products (IP) (Figure 2C). Other CAR+ T-cell subsets also declined, including CD4 Central Memory (day 7: $p<0.001$; day 28: $p<0.05$), CD4- MAIT/NKT (day 7: $p<0.01$).

Conversely, the proportion of CD8 Naïve cells increased post-infusion (day 7: $p<0.0001$; day 28: $p<0.01$), with further expansion between day 7 and 28 ($p<0.01$). Similar trends were observed for CD8 Central Memory (day 7: $p<0.01$; day 28: $p<0.05$), CD8 Effector Memory (day 7: $p<0.0001$; day 28: $p<0.001$), and CD8 Terminal Effector cells (day 7: $p<0.0001$; day 28: $p<0.001$). CD4 Naïve, CD4

Effector Memory, and CD4-CD8- γδ T cells also increased ($p<0.01$), with further expansion between day 7 and 28 ($p<0.05$) (Figure 2D). Further analysis of activation and exhaustion markers in CAR+ T cells revealed an increased expression of IL-7Rα (CD127) on days 7 and 28 post-infusion compared to the infusion product (IP), particularly in CAR+ CD4+ Central Memory and Effector Memory subsets (Supplementary Figure 2A, B). CTLA-4 expression was elevated on days 7 and 28 post-infusion compared to IP, especially in CAR+ CD4−CD8− γδ T cells (Supplementary Figure 2C).

To characterize host immune system changes, we analyzed PBMCs from 16 patients at day 0 (pre-infusion), day 7, and day 28 post-infusion. Monocytes, as well as Early and Late NK cells, significantly increased within 7 days post-infusion ($p<0.01$, $p<0.05$, $p<0.05$, respectively) and remained elevated at day 28 compared to day 0 ($p<0.01$, $p<0.001$, $p<0.01$, respectively) (Figure 3 A-C).

Despite increasing monocyte counts, expression of activation markers (CD16, CD66b) on monocytes decreased significantly by day 7 ($p<0.01$, $p<0.001$) before partially recovering by day 28 ($p<0.05$, $p<0.05$) (Supplementary Figure 2D). In contrast, activation markers on Early and Late NK cells increased over time. CD16 expression on NK cells rose between day 0 and 28 ($p<0.05$) and between day 7 and 28 ($p<0.05$). Additionally, CD161 expression on Early NK cells increased between day 0 and 28 ($p<0.05$) (Supplementary Figure 2E).

No associations were observed between CAR+ T-cell subset composition and patient risk group, disease burden, prior treatment with blinatumomab, inotuzumab ozogamicin, or HSCT. Furthermore, CAR+ T-cell composition was not correlated with treatment outcomes or the development of ICANS (Supplementary Table 2). However, patients who developed CRS (grade ≥1, Penn scale(6)) had a higher frequency of CAR+ CD4- MAIT/NKT and CAR+ CD8 Terminal Effector cells in infusion products compared to those without CRS ($p<0.05$) (Figure 3 D-E). At day 0 pre-infusion, patients who later developed CRS had fewer Early and Late NK cells ($p<0.05$) (Figure 3F-G) compared to those without CRS. At day 7 post-infusion, patients who experienced CRS had fewer Classical Monocytes and lower expression of IL-7RA (CD127) ($p<0.05$) on these cells compared to those without CRS (Figure 3H,

Supplementary Figure 2F). By day 28, CRS patients had lower counts of Transitional Monocytes ($p<0.05$) (Supplementary Figure 2G).

In this study we characterized CAR+ T-cell subsets in infusion products (IP) and tracked their evolution post-infusion, providing insights into treatment response and immune system changes (Supplementary Figure 3).

Infusion products were predominantly composed of CAR+ CD4+ T-cells, with a significant fraction exhibiting a central memory phenotype. Notably, CAR+ regulatory T (Treg) memory cells constituted the largest subset, while naïve CD4+ and CD8+ subsets were largely absent. These findings are consistent with previous reports in DLBCL patients treated with tisagenlecleucel, although those adult studies did not characterize CAR+ Treg memory cells, CD4-CD8- γδ T cells, CD4- MAIT/NKT cells, or T helper cells(3). Post-infusion, the proportion of CAR+ Treg memory cells declined by day 7 post-infusion, while CD8+ naïve and effector subsets expanded, indicating a shift toward a more cytotoxic phenotype. This was accompanied by changes in activation and exhaustion markers in DLBCL, high CAR+ Treg numbers in IP associated with tumor growth due to suppression of cytotoxic CAR+ T-cells, correlating with disease progression and reduced neurotoxicity(1). However, in our pediatric B-ALL cohort, CAR+ Treg memory cells were absent in PBMCs at days 7 and 28 post-infusion, suggesting a transient effect.

Our data also revealed a post-infusion decline in CAR+ CD4+ Central Memory cells and an expansion of CAR+ CD8+ subsets, consistent with previous studies(9).

Beyond CAR+ T-cell dynamics, we document expansion and activation in the NK and myeloid compartments, which may be key determinants of CAR-T efficacy and toxicity. Our findings challenge the assumption that low absolute neutrophil count uniformly enhances CAR-T cell expansion, as previously suggested(10). Instead, we observed that immune recovery, particularly the presence of monocytes and neutrophils, correlates with improved clinical outcomes. This aligns with previous

reports linking neutropenia to B-cell recovery(11), suggesting that hematologic reconstitution may play a role in sustained remission.

The higher frequency of CAR+ CD4- MAIT/NKT and CAR+ CD8+ terminal effector cells in IP correlated with CRS development. This aligns with evidence that highly differentiated effector T cells exhibit impaired in vivo function despite strong in vitro activity and that cytokine signaling can influence T cell efficacy(12).

While tocilizumab has proven effective in managing CRS(13), its impact on the immunological profile of CAR+ T-cells remains insufficiently studied. Our findings suggest that baseline immune profiles and monocyte dynamics may serve as predictors of CRS. Patients who developed CRS exhibited lower pre-infusion levels of NK cell(14). By day 7, these patients displayed reduced classical monocyte numbers and IL-7RA expression, suggesting altered immune recovery, potentially influenced by tocilizumab(15). These findings suggest that immune profiling could aid in identifying high-risk patients and refining CRS management strategies.

This study has several limitations, including a relatively small cohort, interpatient variability, and retrospective data collection. Despite these constraints, our study is the first to characterize the phenotypic composition of tisagenlecleucel CAR+ T-cell subsets in pediatric B-ALL, revealing a significant number of Tregs in IP that do not persist post-infusion. These findings suggest that the same CD19 CAR-T product may exhibit different behaviors in B-ALL compared to lymphoma, as well as in pediatric versus adult patients. Furthermore, our data indicate that early changes in the immune system, particularly monocytes and NK cells, are associated with CAR-T treatment outcomes, underscoring the potential value of comprehensive immune profiling in pediatric B-ALL.

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Figure 1. Patient characteristics and clinical outcomes.

The figure displays a summary of patient characteristics~~, previous treatments,~~ and clinical outcomes. The swimmer plot portion illustrates key events over time, including relapse (R), loss of B-cell aplasia (B), and death (X). Patients who experienced cytokine release syndrome are indicated by a red bar (graded according to the Penn scale), while those who experienced immune effector cell-associated neurotoxicity syndrome are marked with a patterned bar (assessed using the ICE score).

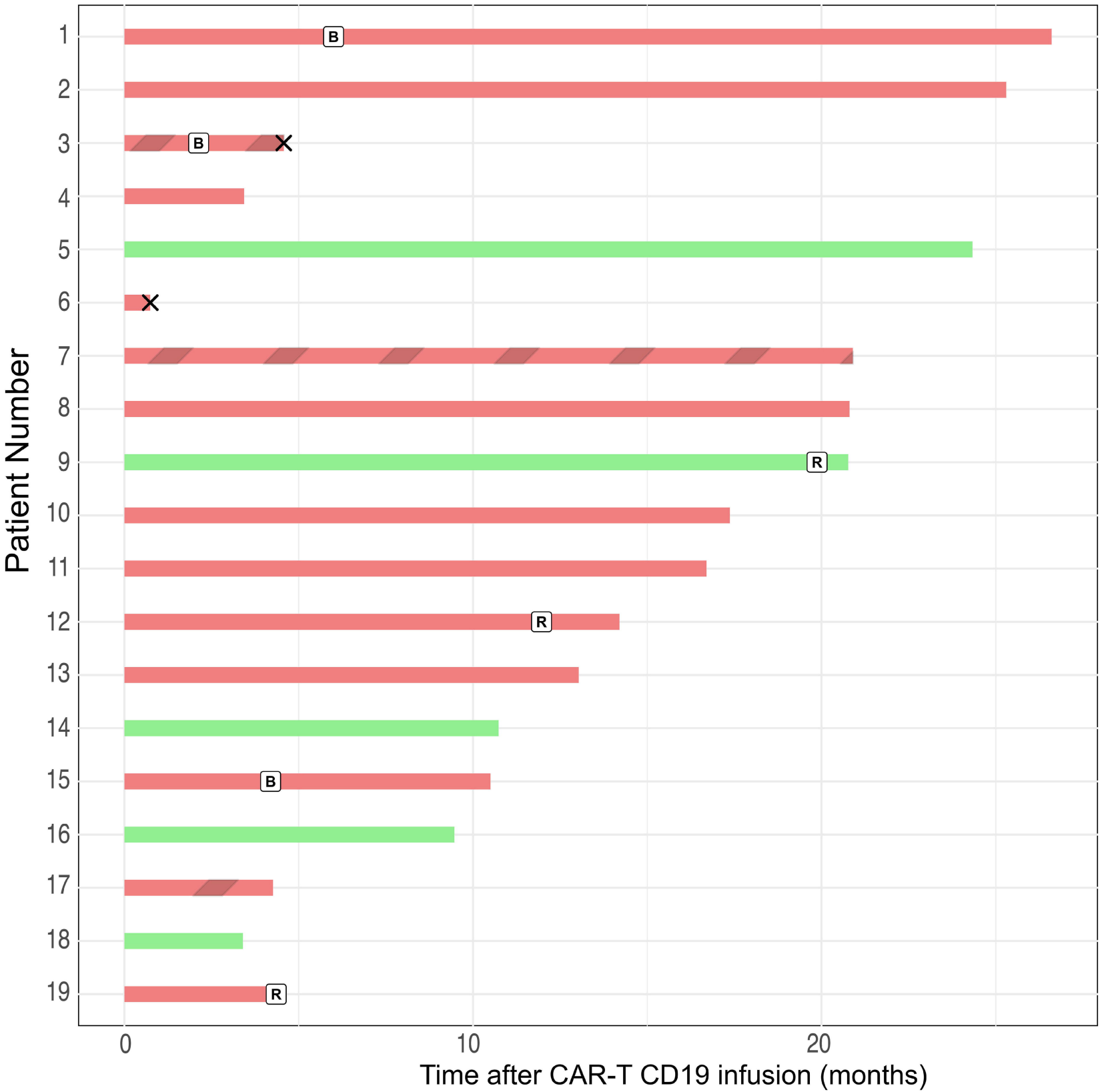
Abbreviations: HSCT, hematopoietic stem cell transplantation; R, relapse; B, loss of B-cell aplasia; X, death.

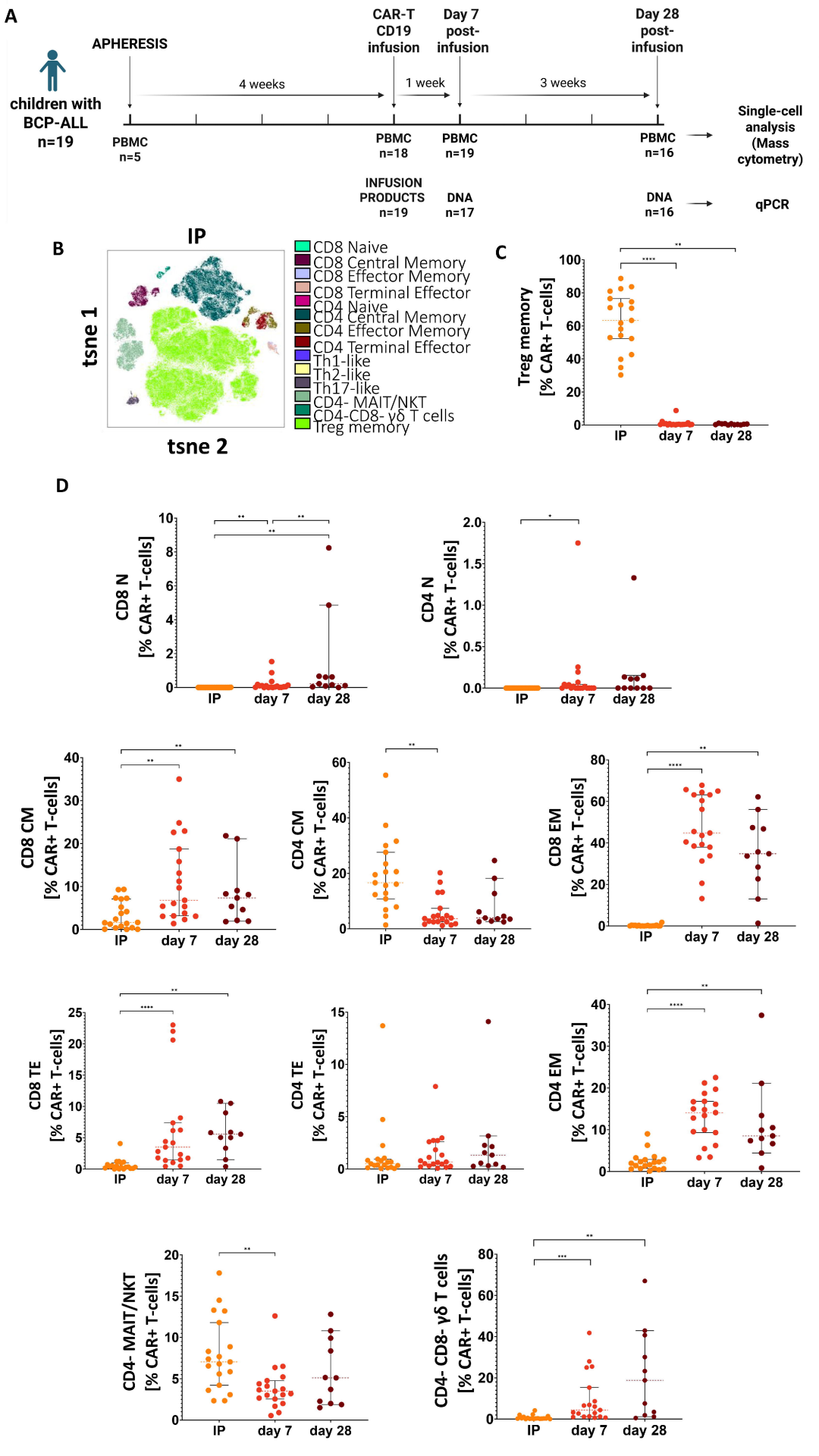
Figure 2. Phenotypic diversity and post-infusion dynamics of CAR$^+$ T-cell subpopulations in CD19 CAR-T therapy.

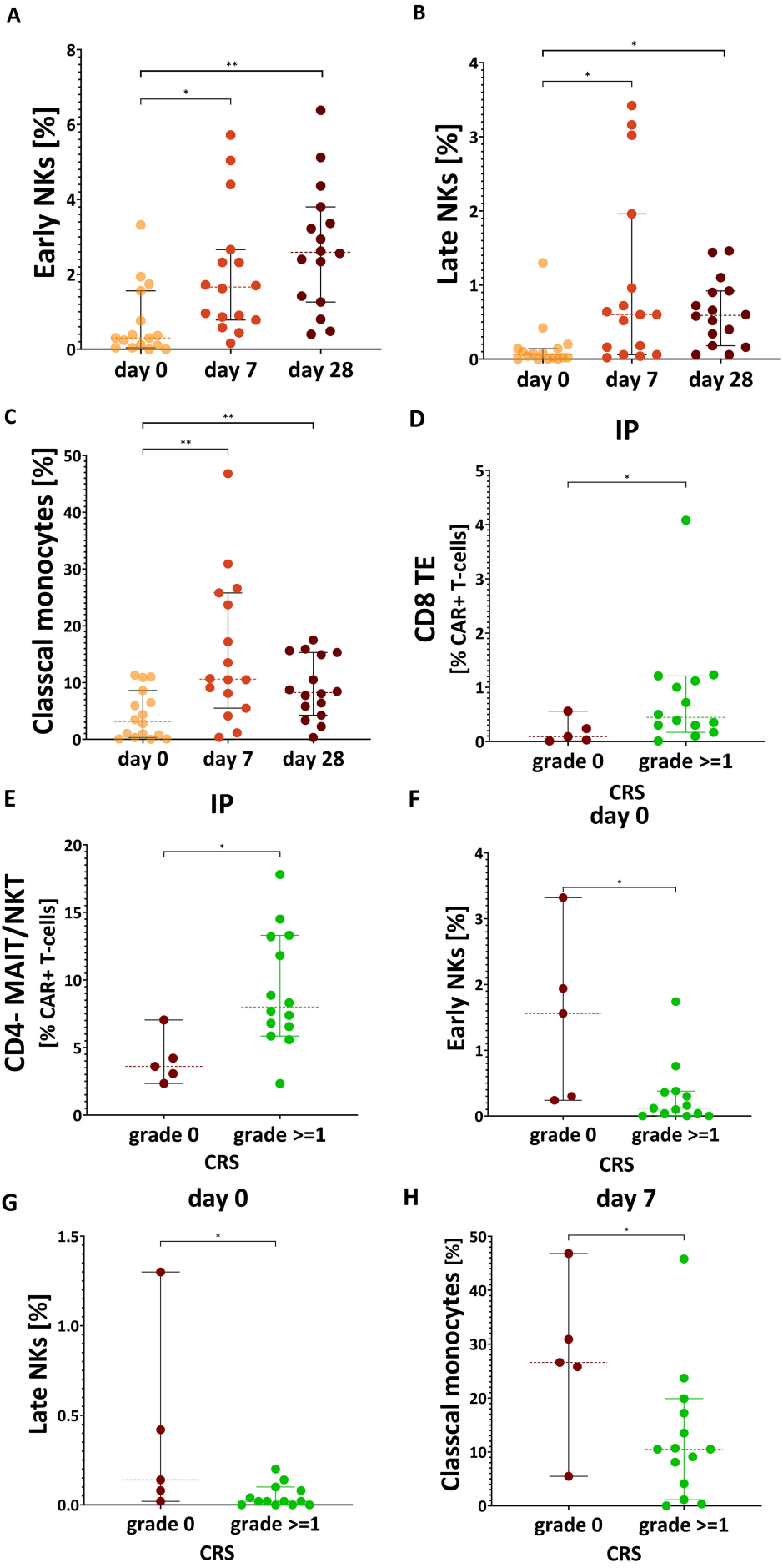
- **A.** Schematic of sample collection and analysis, including mass cytometry and qPCR for validation from pediatric BCP-ALL patients. Correlation between CAR+ cell percentages in PBMCs by mass cytometry and CAR+ transgene expression at day 7 ($R^2 = 0.9043$, $p < 0.0001$) and day 28 ($R^2 = 0.9825$, $p < 0.0001$). Figure created using BioRender.com.
- **B.** T cell subsets within CAR+ T-cells in infusion products using Mahalanobis distance.
- **C.** Longitudinal analysis of CAR+ T-reg memory from infusion to day 7 and day 28 (*p < 0.05; **p < 0.01; ***p < 0.001; ***p < 0.0001, Wilcoxon test, FDR-adjusted).
- **D.** Longitudinal analysis of CAR+ T cell subsets from infusion to day 7 and day 28 (*p < 0.05; **p < 0.01; ***p < 0.001; ***p < 0.0001, Wilcoxon test, FDR-adjusted).

Figure 3. Immune system dynamics and association with cytokine release syndrome.

- A. Longitudinal analysis (day 0, 7, 28) of early NK cells (*p < 0.05; **p < 0.01; ***p < 0.001).
- B. Longitudinal analysis (day 0, 7, 28) of late NK cells (*p < 0.05; **p < 0.01; ***p < 0.001).
- C. Longitudinal analysis (day 0, 7, 28) of classical monocytes (*p < 0.05; **p < 0.01; ***p < 0.001).
- D. Abundance CAR+ CD8 Terminal Effector cells in IP based on development of CRS (*p < 0.05).
- E. Abundance CAR+ CD4$^-$ MAIT/NKT cells in IP based on development of CRS (*p < 0.05).
- F. Pre-infusion PBMC composition at day 0 indicates higher levels of early NK cells in patients who did not develop CRS (*p < 0.05; **p < 0.01).
- G. Pre-infusion PBMC composition at day 0 indicates higher levels of late NK cells in patients who did not develop CRS (*p < 0.05; **p < 0.01).
- H. Abundance of classical monocytes at day 7 based on CRS incidence (*p < 0.05).

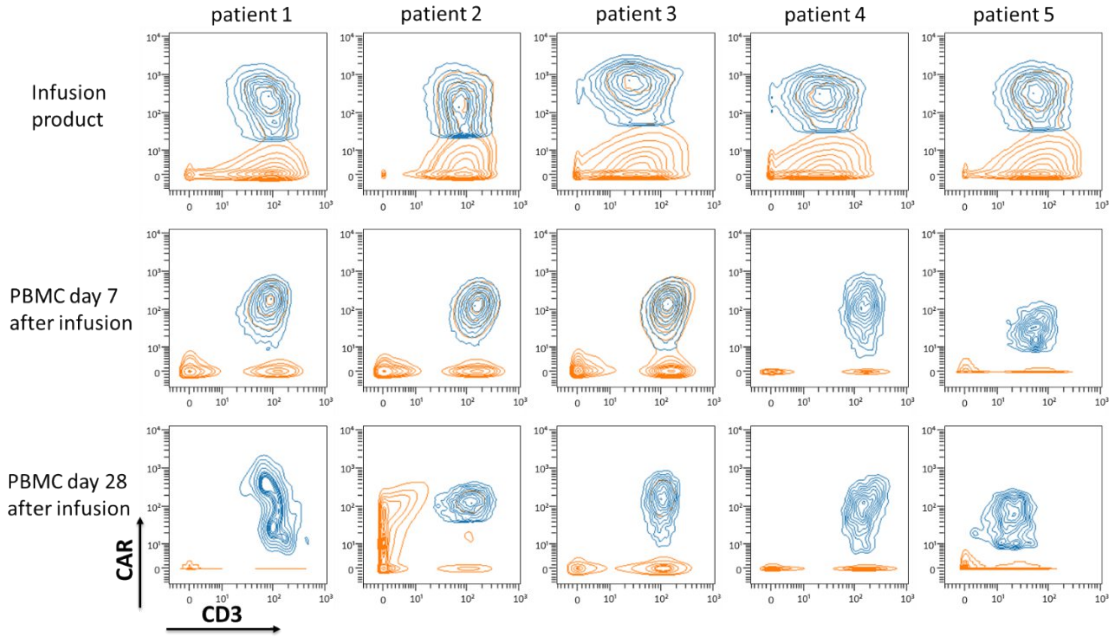

Patient Number
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
B
B
R
R
B
0
10
20
Time after CAR-T CD19 infusion (months)


A
APHERESIS
CAR-T CD19 infusion
Day 7 post-infusion
Day 28 post-infusion
children with BCP-ALL n=19
4 weeks
1 week
3 weeks
PBMC n=5
PBMC n=18
PBMC n=19
PBMC n=16
Single-cell analysis (Mass cytometry)
INFUSION PRODUCTS n=19
DNA n=17
DNA n=16
qPCR
B
IP
tsne 1
tsne 2
CD8 Naive
CD8 Central Memory
CD8 Effector Memory
CD8 Terminal Effector
CD4 Naive
CD4 Central Memory
CD4 Effector Memory
CD4 Terminal Effector
Th1-like
Th2-like
Th17-like
CD4- MAIT/NKT
CD4-CD8- γδ T cells
Treg memory
C
Treg memory [% CAR+ T-cells]
IP
day 7
day 28
D
CD8 N [% CAR+ T-cells]
CD4 N [% CAR+ T-cells]
CD8 CM [% CAR+ T-cells]
CD4 CM [% CAR+ T-cells]
CD8 EM [% CAR+ T-cells]
CD8 TE [% CAR+ T-cells]
CD4 TE [% CAR+ T-cells]
CD4 EM [% CAR+ T-cells]
CD4- MAIT/NKT [% CAR+ T-cells]
CD4- CD8- γδ T cells [% CAR+ T-cells]


A
Early NKs [%]
day 0
day 7
day 28
B
Late NKs [%]
day 0
day 7
day 28
C
Classcal monocytes [%]
day 0
day 7
day 28
D
IP
CD8 TE
[% CAR+ T-cells]
grade 0
grade >=1
CRS
E
IP
CD4- MAIT/NKT
[% CAR+ T-cells]
grade 0
grade >=1
CRS
F
day 0
Early NKs [%]
grade 0
grade >=1
CRS
G
day 0
Late NKs [%]
grade 0
grade >=1
CRS
H
day 7
Classcal monocytes [%]
grade 0
grade >=1
CRS

Supplementary Figure 1.

(a)



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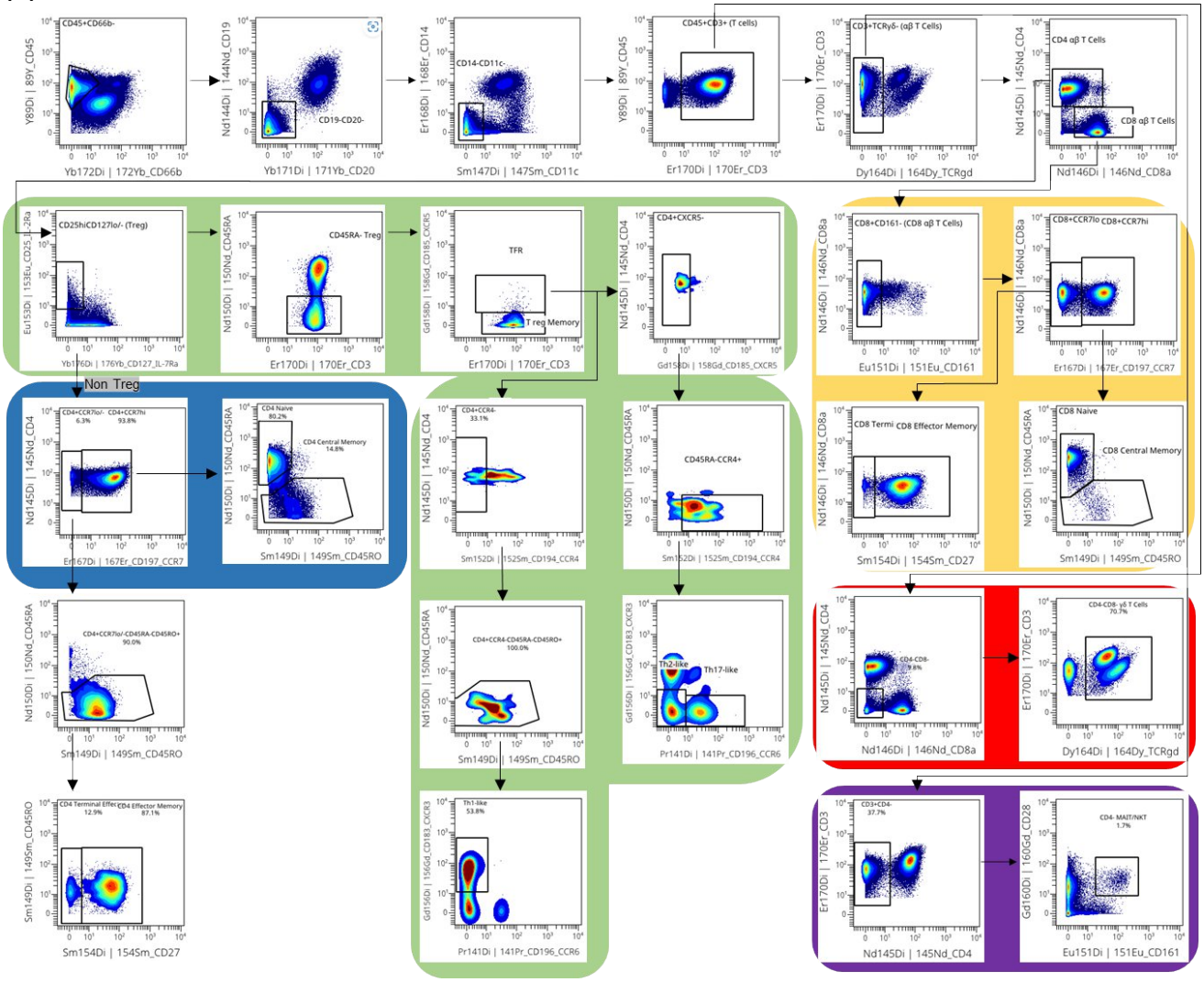
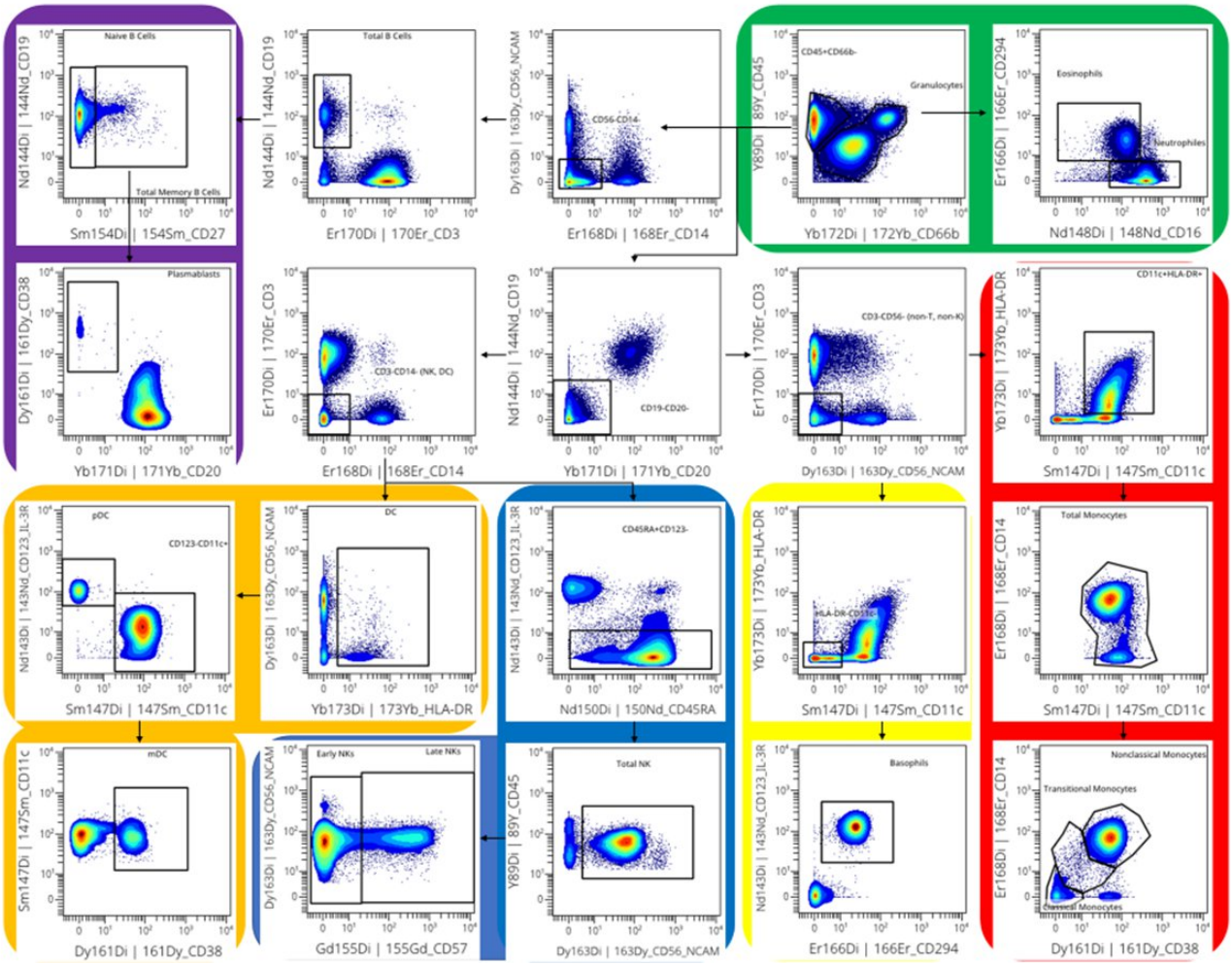


(c)



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.

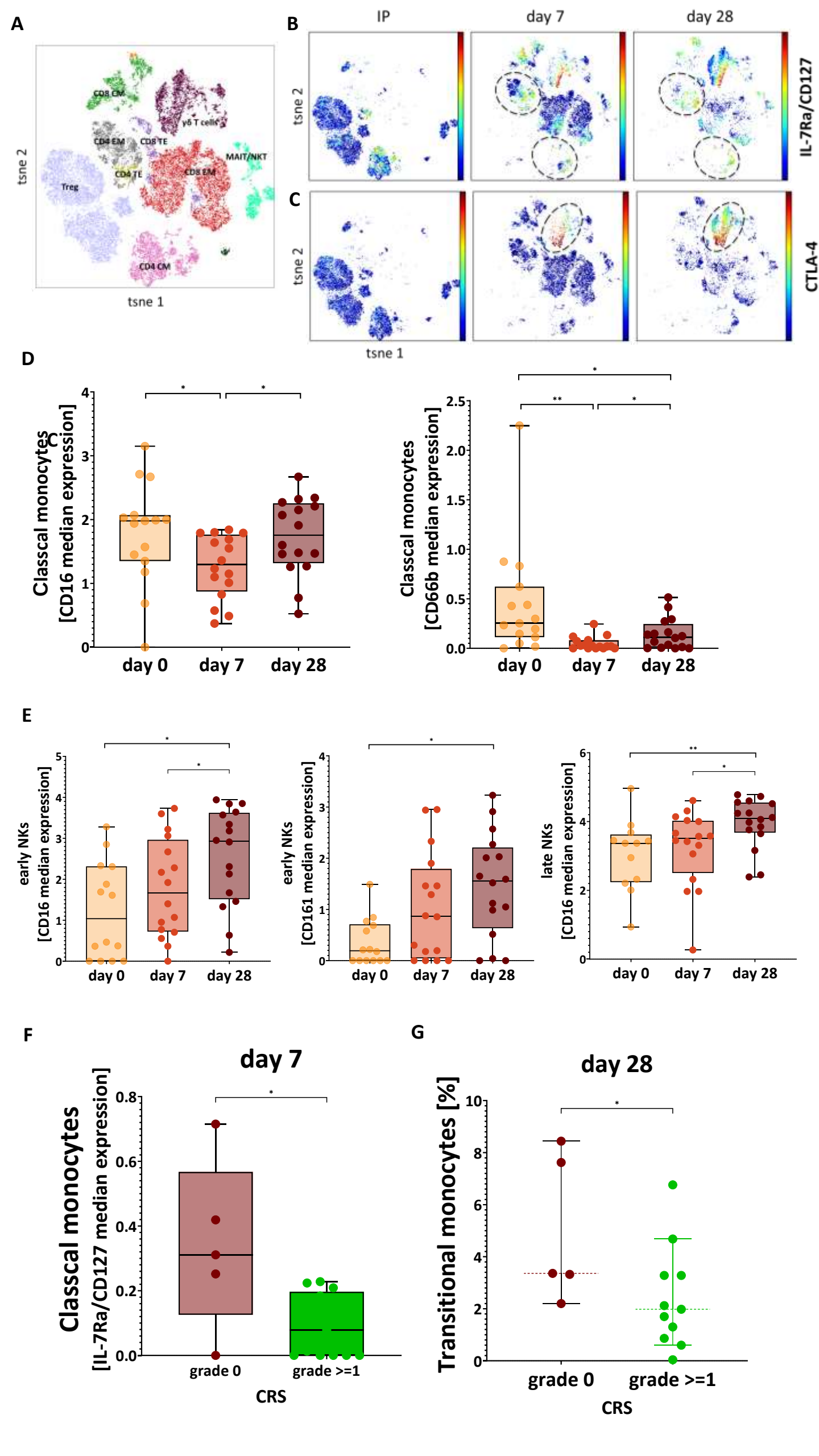
Flow plot showing gating strategy for T cells from Human healthy peripheral blood (4 PBMC from healthy children, ~300,000 cells per sample). Gating and sub sequential analysis was done using OMIQ.

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

(c) Other cells gating strategy.

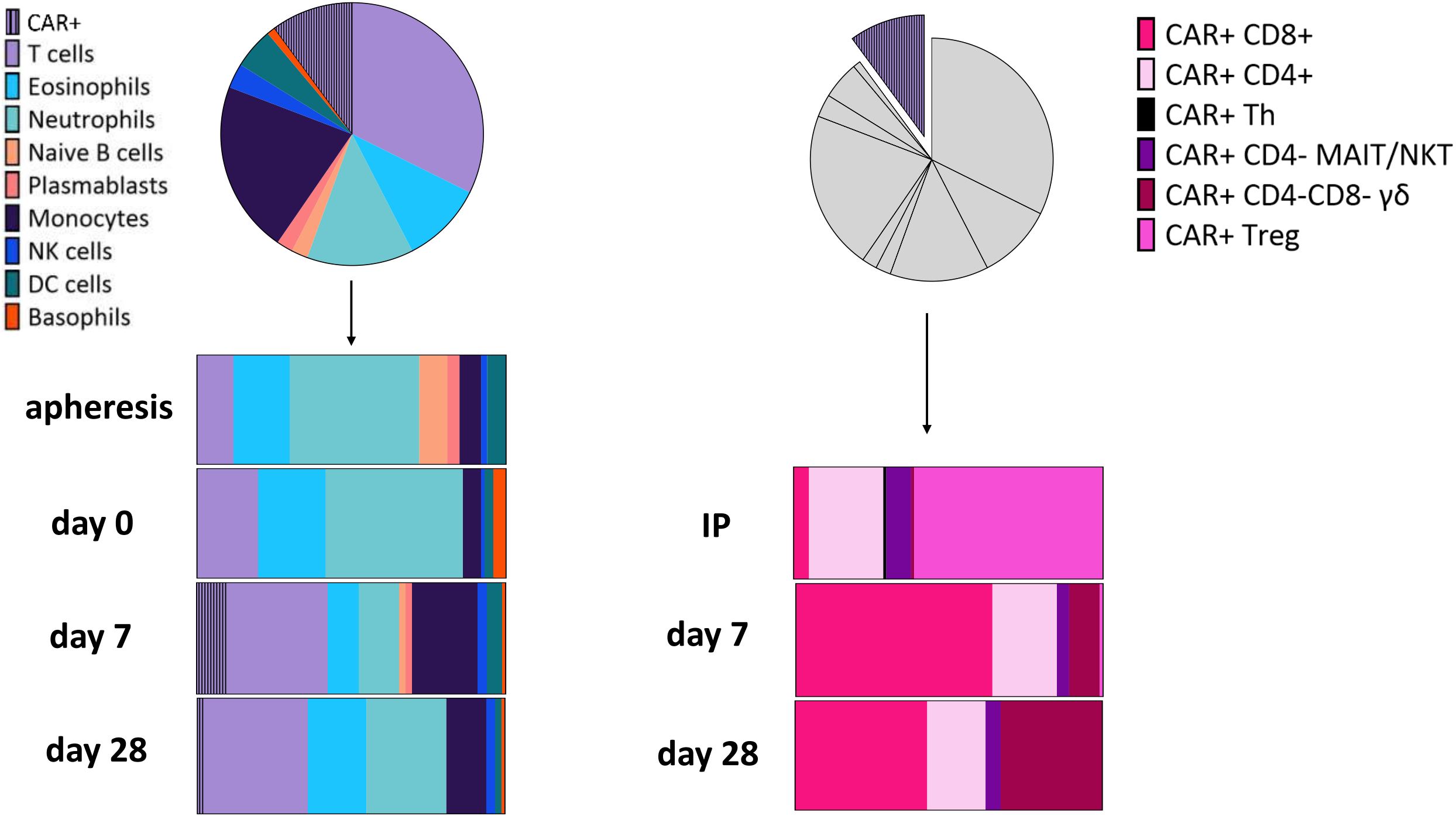
Flow plot showing gating strategy for cells from Human healthy peripheral blood (4 PBMC from healthy children, ~300,000 cells per sample). Gating and sub sequential analysis was done using OMIQ.

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


A
CD8 CM
γδ T cells
CD4 EM
CD8 TE
MAIT/NKT
CD4 TE
CD8 EM
Treg
CD4 CM
tsne 2
tsne 1
B
IP
day 7
day 28
tsne 2
IL-7Ra/CD127
C
tsne 2
tsne 1
CTLA-4
D
Classcal monocytes [CD16 median expression]
day 0
day 7
day 28
Classcal monocytes [CD66b median expression]
day 0
day 7
day 28
E
early NKs [CD16 median expression]
day 0
day 7
day 28
early NKs [CD161 median expression]
day 0
day 7
day 28
late NKs [CD16 median expression]
day 0
day 7
day 28
F
day 7
Classcal monocytes [IL-7Ra/CD127 median expression]
grade 0
grade >=1
CRS
G
day 28
Transitional monocytes [%]
grade 0
grade >=1
CRS

Supplementary Figure 2.

A. CAR+ T-cell subsets at all timepoints classified using Mahalanobis distance.
B. IL-7Rα (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 (*$p < 0.05$).


CAR+
T cells
Eosinophils
Neutrophils
Naive B cells
Plasmablasts
Monocytes
NK cells
DC cells
Basophils
apheresis
day 0
day 7
day 28
CAR+ CD8+
CAR+ CD4+
CAR+ Th
CAR+ CD4- MAIT/NKT
CAR+ CD4-CD8- γδ
CAR+ Treg
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 (<1x10^-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/µL)	ALC at time of aphaeresis (10^3/µL)
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- γδ 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
HSCT** 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