SUPPLEMENTAL METHODS

Clinical lentiviral manufacture

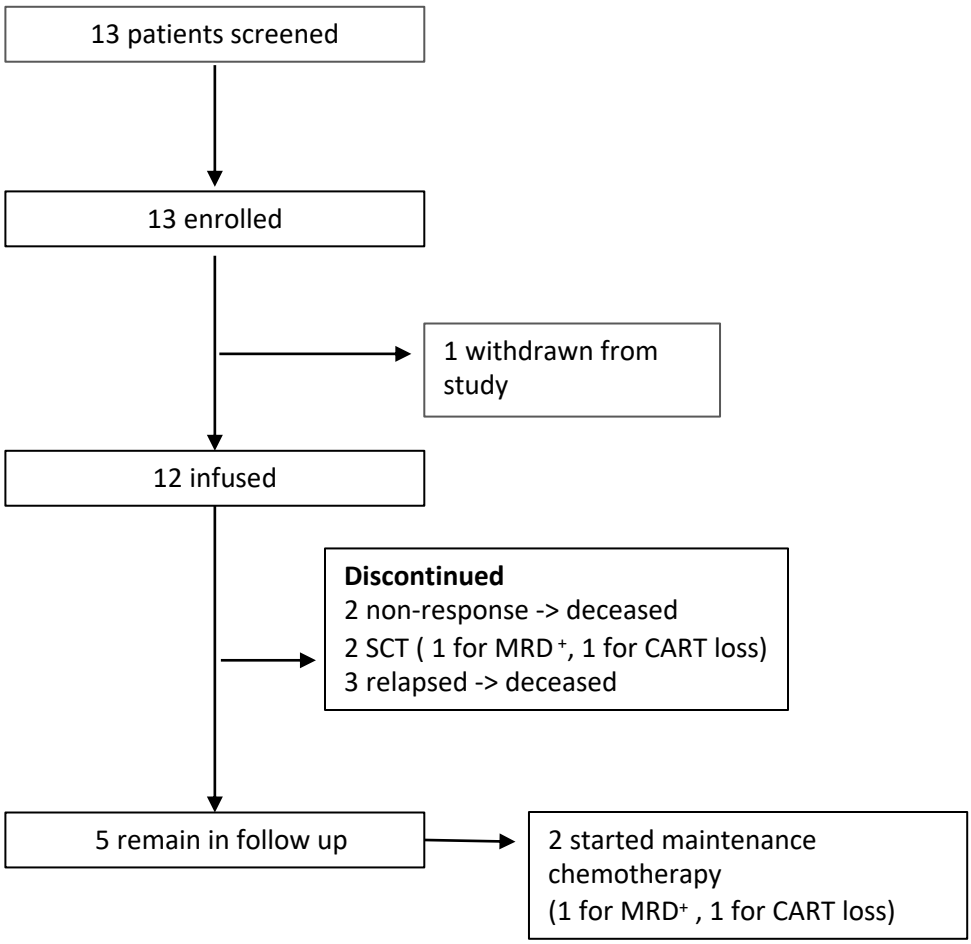
A third generation self-inactivating (SIN) lentiviral vector [12] encoding the αCD19CAT-41BBz or the αCD22-9A8-41BBz cassette under control of a human PGK or EF1α promoter respectively and incorporating, HIV CPPT, RRE, and mutated WPRE were manufactured in accordance with EMEA–Guidelines on Development and Manufacture of Lentiviral Vectors (CHMP/BWP/2458/03) at Rayne Cell Therapy Suite (RCTS) at King's College London.

Manufacture of Advanced Therapeutic Investigational Medicinal Product

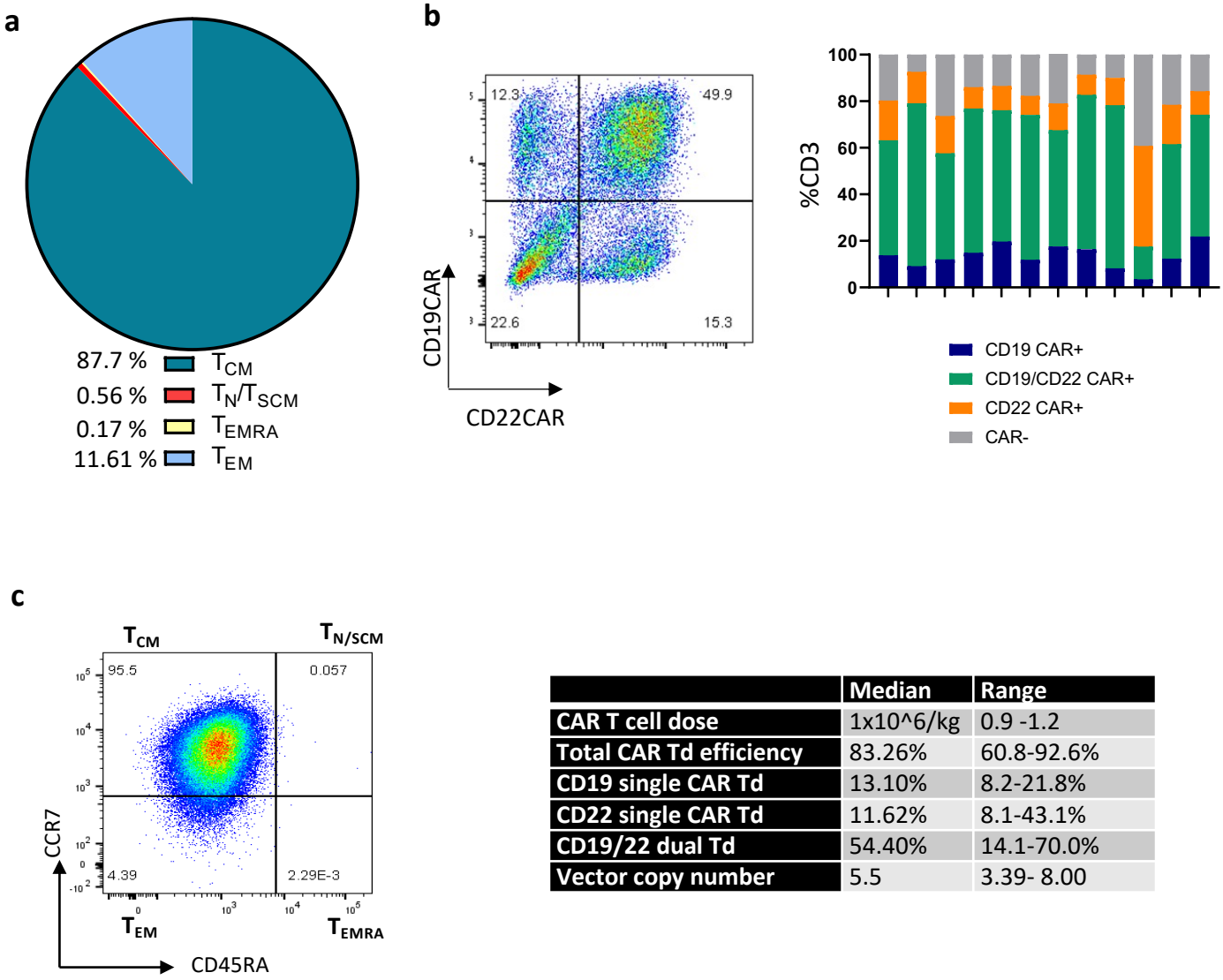
Products were generated from fresh or frozen autologous PBMCs following leucapheresis of the patient. PBMCs were washed and activated with CTS Dynabeads CD3/CD28 at a 3:1 bead: lymphocyte ratio. Lentiviral transduction was performed in Retronectin-coated cell culture bags at an MOI of 3.5 for the CD19CAR vector and 1.5-2.5 for the CD22CAR vector, selected to give balanced populations of CD19 and CD22CAR populations. On day 4 cells were washed and expanded for up to 3 days in a WAVE bioreactor (Cytiva). Dynabeads were magnetically removed on day 7 and cells cryopreserved either the same or next day. Cells were cultured in X-VIVO15 media (Lonza) supplemented with 5% Human AB serum (LSP). No exogenous cytokines were added to the culture media. Release assays performed prior to infusion included assessments of sterility (Bacterial culture, Mycoplasma PCR), endotoxin levels (LAL), residual bead count, viability and transduction efficiency by flow cytometry. Cellular material was separately tested for viral copy number using qPCR for the lentiviral packaging signal Ψ.

Flow Cytometry of CAR T-cell Products

Flow cytometry was performed with a BD LSR II, Aria, Canto II, Lyric (BD Biosciences) and data analysed using FlowJo vX (Tree Star, Inc., Ashland OR), FACs DIVA 8.0.1. or BD FACSuite. CATCAR and 9A8CAR expression was detected with recombinant CD19 and CD22 anti-idiotype antibodies (Evitria) via fluorochrome-conjugated anti-Rat or anti-Rabbit IgG secondary antibodies (Biolegend). Antibodies for phenotypic analysis included: CD3 APC-Cy7 (Biolegend), CD4 PE-Cy7 (BD), CD8 V500 (BD), CD45 FITC (BD), CD45RA FITC (BD), CCR7 APC (BD), CD95 PE-Cy7 (BD), Anti-Rat IgG PE (Biolegend), Anti-Rabbit IgG BV421 (Biolegend), Cell viability solution 7-AAD (BD), Human Fc Block (BD). Fluorescence minus one (FMO) controls determined expression thresholds.



Supplementary Figure 1. Screening, Enrollment, Treatment, and Follow-up. SCT= allogeneic stem cell transplantation; MRD= minimal residual disease



	Median	Range
CAR T cell dose	1x10^6/kg	0.9 -1.2
Total CAR Td efficiency	83.26%	60.8-92.6%
CD19 single CAR Td	13.10%	8.2-21.8%
CD22 single CAR Td	11.62%	8.1-43.1%
CD19/22 dual Td	54.40%	14.1-70.0%
Vector copy number	5.5	3.39- 8.00

Supplementary Figure 2. **Characteristics of AUTO 1/22 infused product.**
CAR T-cell populations were identified by pre-gating on live, CD45+/CD3+ cells. CAR-expressing cells were then identified by binding of anti-idiotype antibodies specific for the CD19 and CD22 CARs. **a.** AUTO1/22 memory phenotype composition, TCM= T central memory (CD45RA-CD62L+), TN/TSCM = T naïve/stem cell like memory T cells (CD45RA+CD62L+), TEMRA = terminally differentiated memory (CD45RA+CD62L-), TEM= effector memory (CD45RA-CD62L-). Below is a representative example of product memory phenotype. **b.** Proportion of single CD19 , single CD22 and double CD19/22 transduced CART cells in each infused product. Each bar represents the product infused in a single patient. The plot to the left exemplifies CAR expression in patient product with single CD19 transduced T cells in the left upper quadrant, double CD19/22 transduced T cells in the right upper quadrant and single CD22 transduced T cells in the lower right quadrant. **c.** CART cell dose, transduction (Td) efficiency (expressed as percentage of T cells expressing CD19 single, CD22 single or CD19/22 dual CAR), composition between single CD19, single CD22 and double CD19/22 transduced product in the whole cohort of pts as well as vector copy number. Median, ranges and interquartile ranges (IQR) are reported.

Criteria	N=12 N (%)
CRS maximum grade (ASTCT)	
Grade 1	5 (41%)
Grade 2	6 (50%)
Grade 3-4	0
ICANS maximum grade (ASTCT)	
Grade 1	4 (36%)
Grade 2	1 (8%)
Grade 3	0
Grade 4 (MRI leukoencephalopathy)	1 (8%)
Cytopenia not resolving by /recurring after day 28 maximum grade (CTCAE)	
Grade 1	0
Grade 2	1 (8%)
Grade 3	2(17%)
Grade 4	8 (67%)
B cell aplasia	
At day 30	11 (92%)
At last follow up (median 8.7 months)	7 (58%)

Criteria:	N=12 N (%)
Cytopenia not resolving by / recurring after day 28 Maximum Grade (CTCAE)	
Grade 1	0 (0%)
Grade 2	1 (8%)
Grade 3	2(17%)
Grade 4	8 (67%)
B cell aplasia	
At day 30	11 (92%)
At last follow up (median 8.7 months)	7 (58%)

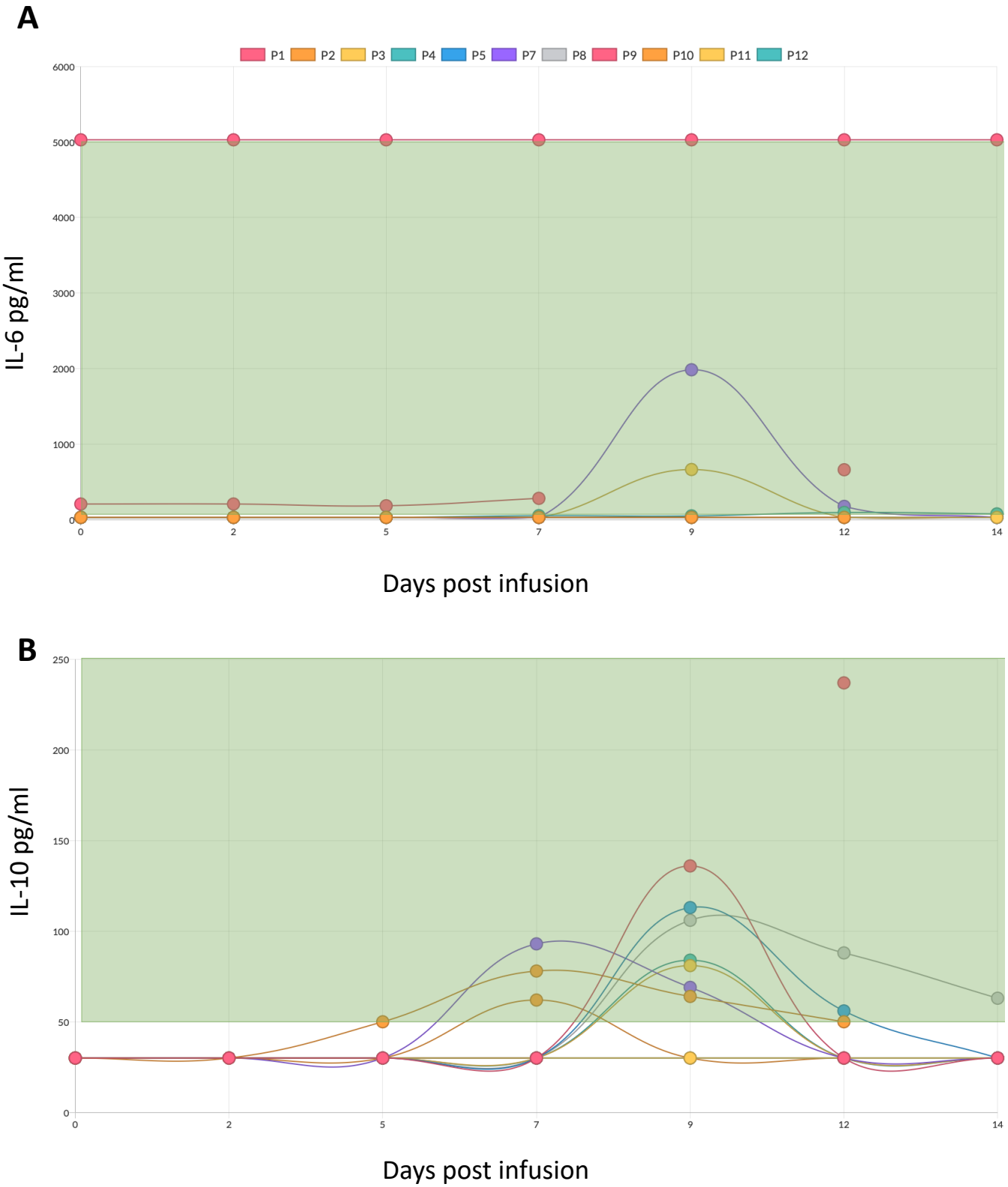
Supplementary Table 1. Adverse events of special interest

Frequency of adverse events noted post CAR T cell infusion, by grade and type of toxicity. CRS - cytokine release syndrome, ICANS- immune effector cell associated neurological syndrome and both were graded according to ASTCT consensus guidelines (Lee et al, 2018) Cytopenias were defined as reduced neutrophil or platelet count since B lymphopenia is expected following CAR targeting. Cytopenias were graded according to CTCAE v5.0. B cell aplasia was defined as <5 B cells per µl blood post CAR T cell infusion

Criteria: Other, non-laboratory Grade 3-4	N=12 N (%)
Infection	
Grade 3 (catheter-related, lung infection, device related)	3 (25%)
Grade 4 (sepsis, lung infection)	2 (17%)
Febrile neutropenia	
Grade 3	4 (33%)
Typhilitis	
Grade 4	1 (8%)
Diarrhoea	
Grade 3	1 (8%)
Respiratory	
Grade 3 (pleural effusion)	1 (8%)
Grade 4 (hypoxia)	1 (8%)

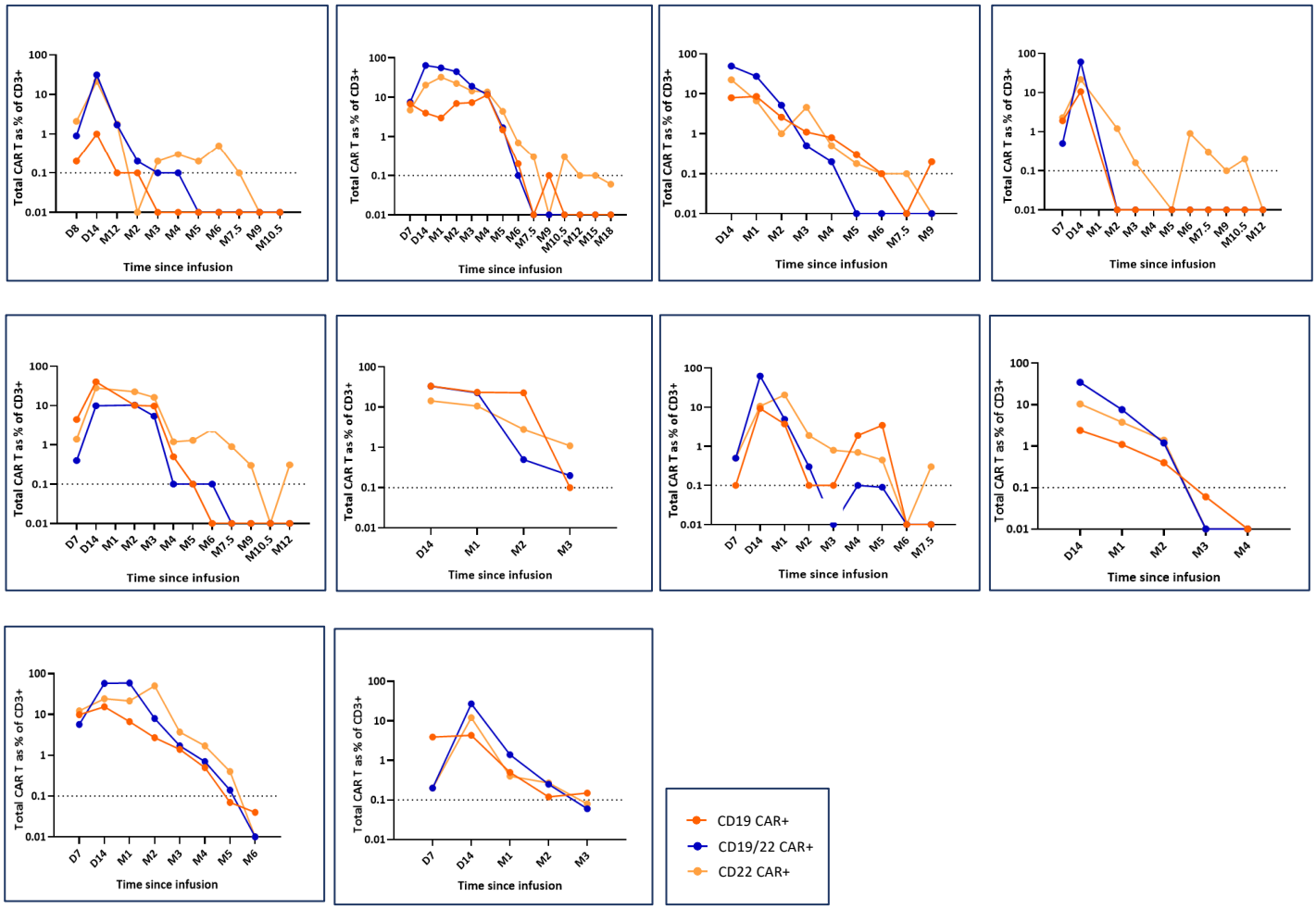
Criteria: Other, non-laboratory Grade 3-4	N=12 N (%)
Hypotension/ Hypertension	
Grade 3	3 (25%)
Tumour Lysis syndrome	
Grade 3	1 (8%)
Pain	
Grade 3 (abdominal, bone)	2 (17%)
Depression	
Grade 3	1 (8%)

Supplementary Table 2. Summary of post-infusion non-laboratory CTCAE grade 3-4 adverse events in all patients

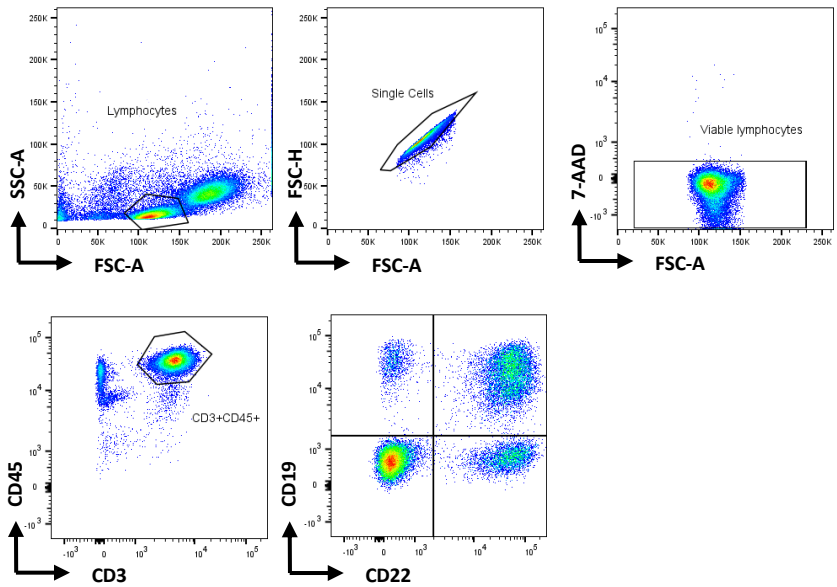


Supplementary Figure 3. Serum cytokine measurements in the first 2 weeks post infusion

Cytokine profile of IL-6 (A), IL-10 (B). As measured per patient by an ISO-accredited method using cytometric bead array analysis (BD Biosciences). IL-2, IL-4, TNF, IFNγ were measured at the same timepoints but are not graphically shown as they were below the lower limit of detection in all patients at all timepoints. The validated lower limit of this assay is 50pg/ml and upper limit 5000pg/ml (depicted with pale green area). Readings for patient P6 were not available. *Patients P4, P5, P9, P10, P12 had CRS grade 1, all other patients had CRS grade 2*

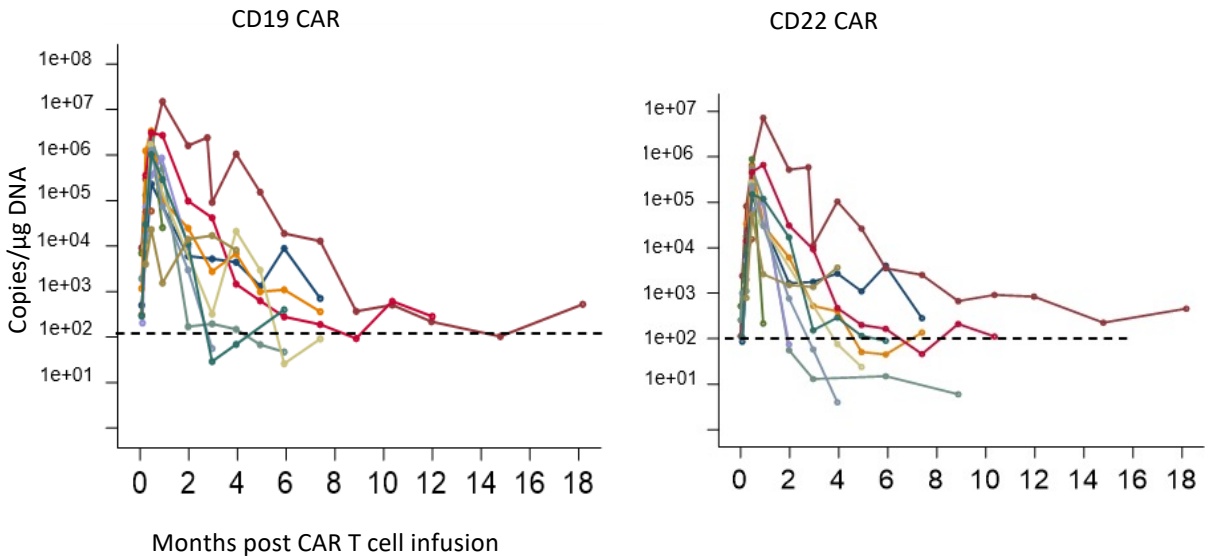


Supplementary Figure 4. Expansion and persistence of CAR T-cell populations as measured by flow cytometry of peripheral blood samples post infusion.



Supplementary Figure 5.Example gating strategy

CAR T-cell populations were identified by pre-gating on live, CD45+/CD3+ cells. CAR-expressing cells were then identified by binding of anti-idiotype antibodies specific for the CD19 and CD22 CARs.



Supplementary Figure 6. Expansion and persistence of CAR T-cell populations as measured by qPCR for transgene-specific sequences in peripheral blood samples of all patients post infusion. Transgene-specific sequences for the CD19 and CD22 CAR were detected by qPCR in peripheral blood samples taken on days 0, 2, 7, 14 and 28, monthly up to 6 months, 6 weekly to 1 year then 3 monthly up to 18 months post infusion. The validated threshold for detection was 100 copies/ug DNA.

PK analysis	Peripheral blood CD19CAR (cohort 3, N=12)	Peripheral blood CD22CAR (cohort 3, N=12)
Time of last measurement (days)		
Median	202.5	202.5
Range	14 to 553	14 to 553
Cmax concentration (copies/ug DNA)		
Geometric mean	937,947.80	270,171.20
CV%	506.88	321.16
Time to Cmax (days)		
Median	14	14
Range	13 to 28	13 to 28
AUC (0 to 28), (copies/ug DNA)		
Geometric mean	9,492,498.00	2,586,767.00
CV%	528.08	356.59
Half life (days)		
Median	15.41	17.69
Range	2.15 to 34.44	1.17 to 40.20
Duration of CART persistence (days)		
Median	135	105
Range	14 to 553	14 to 553
N patients with CAR T≥100 copies/ug at last follow-up	7	5
Median follow-up in months	8.7	8.7

Supplementary Table 3. Summary of CAR T cell pharmacokinetic parameters as measured in peripheral blood by digital droplet PCR detecting CAR-specific transgene sequences.

Cmax, maximum concentration; AUC, area 1200 under the curve; AUC (0 to 28) AUC from time zero to day 28; AUC (0 to t) AUC from time zero until last measurement; Time to Cmax is the time to reach peak CAR T cell concentration. CAR T cell persistence was defined as the median interval in days from infusion to first value <100 copies per μg DNA, or the last follow-up if this threshold level was not reached.