

Phase I trial of autologous regulatory T cells for immune aplastic anemia

Nazia Matto,^{1*} Shreyans Gandhi,^{1,2*} Marta Rzepkowska,¹ Sila Gerlevik,¹ Mohammad M. Karimi,³ Austin Kulasekararaj,^{1,2} Abdel Douiri,⁴ Anji Miller,⁵ Lynne Duran,² Haili Cui,² Jen Lewis,² Shahram Kordasti,⁶ Giovanna Lombardi,⁷ Giorgio Napolitani¹ and Ghulam J. Mufti^{1,2}

¹Department of Haematology Medicine, School of Cancer and Pharmaceutical Sciences, SGDP Centre, Denmark Hill, Kings College London; ²King’s College Hospital NHS Foundation Trust; ³School of Cancer and Pharmaceutical Sciences, Comprehensive Cancer Centre, Kings College London; ⁴Division of Population Health Sciences Kings College London; ⁵LifeArc, Lynton House; ⁶System Cancer Immunology, Comprehensive Cancer Centre, King’s College London and ⁷Immunoregulation Laboratory, MRC Centre for Transplantation, School of Immunology & Microbial Sciences, King’s College London, London, UK

**NM and SGa contributed equally as first authors.*

Correspondence: G.J. Mufti
ghulam.mufti@kcl.ac.uk

Received: September 8, 2025.
Accepted: February 13, 2026.
Early view: February 26, 2026.

<https://doi.org/10.3324/haematol.2025.288962>

©2026 Ferrata Storti Foundation
Published under a CC BY-NC license



SUPPLEMENTARY DATA

Contents

SUPPLEMENTARY METHOD	2
CytoF.....	2
Cell Preparation	2
Library Preparation and Sequencing	2
Data Processing and Analysis	2
Cytokine Estimation.....	2
SNP- Array.....	4
Myeloid Gene Panel	4
PNH CLONE ANALYSIS (PNH).....	5
GMP TESTING	5
SUPPLEMENTARY TABLES	6
SUPPLEMENTARY FIGURES.....	10

SUPPLEMENTARY METHOD

CyTOF

A panel of antibodies tagged with a rare metal isotope based on surface markers, transcription factors and cytokines were designed. Cells were first barcoded, pooled together and then stained with Cell-ID Rhodium-103 (Fluidigm) to distinguish live and dead cells. Intracellular staining was performed after fixation and permeabilization using FoxP3/Transcription Factor Staining Buffer Set (eBioscience™). Before data acquisition, stained cells were DNA stained using Cell-ID Intercalator-Ir (Fluidigm).

Single cell sequencing

Cell Preparation

Patient samples were enriched for T cells using the Pan T Cell Isolation Kit (Miltenyi Biotec). The enriched cell populations were co-stained using the following antibodies: CD4, CD8, CD25, CD127 (obtained from, BD® AbSeq Antibody-Oligos, and BD® SampleTag (ST) antibodies BD® Single-Cell Multiplexing Kit, BD Biosciences). The staining protocol followed the manufacturer's guidelines. The AbSeq panel used consisted of the BD® AbSeq Immune Discovery Panel, supplemented with 11 additional single AbSeq oligonucleotides (BD Biosciences).

The cells were sorted into distinct populations: total CD4+ T cells, CD8+ T cells, and regulatory T cells (Tregs). The sorted cell populations were pooled and analysed using the BD Rhapsody™ Express Single-Cell Analysis System. The retrieved beads were washed, and reverse transcription was performed using the BD Rhapsody™ cDNA Kit (BD Biosciences).

Library Preparation and Sequencing

Following reverse transcription, AbSeq, mRNA, SampleTag (ST), and T cell receptor (TCR) libraries were prepared using the BD Rhapsody™. The mRNA libraries were constructed using a custom gene panel (ID: 0002174, BD Biosciences) as well as the BD Rhapsody™ Immune Response Panel HS (BD Biosciences).

The quality of the libraries was evaluated using the Agilent TapeStation 4150 system. The libraries were sequenced on the NovaSeq X Plus (Illumina) or NovaSeq 6000 platforms (Illumina) with paired-end 150 bp reads (PE150).

Data Processing and Analysis

The raw sequencing data was uploaded to the Seven Bridges Genomics platform. Sequencing reads were demultiplexed, aligned to the reference genome, and filtered using the BD Rhapsody™ Sequence Analysis Pipeline v2.2. Generated molecular count matrices per cell, were imported into Seurat v5 for downstream analysis.

Single cells were selected on SampleTag (ST) identifiers, and datasets integrated using canonical correlation analysis (CCA). Uniform Manifold Approximation and Projection (UMAP) embeddings were computed and TCR sequences were incorporated into the Seurat metadata, and clonotype overlap between samples was analyzed (Satija Lab)(17). Clonotypes were defined based on cells sharing identical CDR3 α and CDR3 β sequences.

Cytokine Estimation

Cytokine Analysis was performed by Synnovis using their test code CY-19, CY-20, CY-21 and CY-23. All cytokine levels were measured in pg/ml

1.	IL-1 β , IL-6, IL-8, TNF- α	CY-19
2.	IL-2RA, IL-10, IL-17 α INF- γ	CY-20
3.	IL-15, GM-CSF, MCP-1 VEGF	CY-21
4.	IL-15, IL-13	CY-23

Patient 1

Day	IL-1 β	IL-6	IL-8	TNF- α	IL-2Ra	IL-10	IL-17a	INF- γ	IL-15	GM-CSF	MCP-1	VEGF	IL-5	IL-13
0	<0.80	4.62	119	12.8	2354	7.62	6.15	1.88	4.14	<1.40	480	45.6	2.21	<7.20
14	<0.80	5.96	151	10.7	3145	7.35	8.34	0.91	4.3	<1.40	674	42.8	1.74	141
28	<0.80	10.9	174	10.6	3192	7.06	13.6	1.08	4.24	<1.40	643	56.8	1.1	35.1

Patient 2

Day	IL-1 β	IL-6	IL-8	TNF- α	IL-2Ra	IL-10	IL-17a	INF- γ	IL-15	GM-CSF	MCP-1	VEGF	IL-5	IL-13
0	<0.80	2.86	20.4	14.2	2277	3.76	<2.10	1.63	3.11	<1.40	399	49.2	0.45	<7.2
14	<0.80	2.11	34.4	19	2868	4.45	<2.10	1.31	4.52	<1.40	414	55.8	0.46	<7.2
28	<0.80	2.31	26.9	15.5	2729	4.23	<2.10	0.82	4.72	<1.40	430	48.3	0.28	<7.2

Patient 3

Day	IL-1 β	IL-6	IL-8	TNF- α	IL-2Ra	IL-10	IL-17a	INF- γ	IL-15	GM-CSF	MCP-1	VEGF	IL-5	IL-13
0	<0.80	8	51.8	5.63	2132	2.18	<2.10	5.58	5.34	<1.42	911	67.6	0.95	<7.20
14	<0.80	5.13	46.4	5.16	1959	1.92	<2.10	2.05	4.88	<1.74	1042	57.6	0.4	<7.20
28	<0.80	4.47	41.2	5.08	1829	2.04	<2.10	1.21	4.57	<1.40	874	69.8	0.49	<7.20

Patient 4

Day	IL-1 β	IL-6	IL-8	TNF- α	IL-2Ra	IL-10	IL-17a	INF- γ	IL-15	GM-CSF	MCP-1	VEGF	IL-5	IL-13
0	<0.80	4.66	23.2	10.4	1501	3.45	<2.10	0.92	3.22	<1.40	510	171	0.92	<7.20
14	<0.80	21.7	98.9	11.7	1558	3.56	<2.10	0.83	2.73	<1.40	446	214	0.33	<7.20
28	<0.80	24.3	31	11.1	1792	3.24	<2.10	0.64	2.79	<1.40	430	223	0.56	<7.20

Patient 5

Day	IL-1 β	IL-6	IL-8	TNF- α	IL-2Ra	IL-10	IL-17a	INF- γ	IL-15	GM-CSF	MCP-1	VEGF	IL-5	IL-13
0	<0.80	10.3	100	8.11	3223	3.61	<2.10	1.57	5.74	<1.40	583	54	1.9	<7.20
7	<0.80	7.92	39.4	7.13	3417	3.63	<2.10	1.51	4.96	<1.40	480	40.2	1.8	<7.20
14	<0.80	12	65.4	7.41	3522	3.5	<2.10	2.66	6.88	2.7	556	54.9	1.52	<7.20

Patient 6

Day	IL-1	IL-6	IL-8	TNF- α	IL-2Ra	IL-10	IL-17a	INF- γ	IL-15	GM-CSF	MCP-1	VEGF	IL-5	IL-13
8	<0.80	4.24	31.10	7.88	2570	8.26	<2.10	0.99	2.62	<1.40	326	42.80	0.67	17.30
14	<0.80	4.67	37.40	8.87	2757	13.30	<2.10	1.04	2.93	<1.40	511	55.30	1.07	17.70
29	<0.80	10.10	47.90	12.30	3518	15.10	<2.10	7.64	3.76	<1.40	448	43.60	1.26	14

SNP- Array

The sample was analysed by CytoscanHD microarray which can detect copy number aberrations (CNA) at resolution of 25kb for losses and 50kb for additions and analysed using Chromosome Analysis Suite v4.3 with GRCh37/arr [hg 19] genome build. In the absence of a germline sample.

1.CNA Length>5Mb will be reported. CNA length<5Mb only reported if an overlap with genes forming part of the Sanger Cancer Gene list (www.sanger.ac.uk).

2.Copy-neutral loss of heterozygosity (CN-LOH) reported if extends/includes the telomeres or if >10Mb and interstitial (Schoumans et al, 2016).

3. CN-LOH, balanced translocations and low-level mosaicism (<10% of the cells) maybe undetectable.

A UKAS accredited medical laboratory No.9597

Myeloid Gene Panel

The sequencing method uses QiaSeq targeted amplicon and an Illumina NextSeq 550. Targets covered: ANKRD26 (all coding and 3 UTR); ASXL1(exon 12); BCL2 (all coding); BCOR (all coding); CALR (exon 9); CBL (exons 7+8+9); CEBPA (all coding); CSF3R (exons 13 to 18); CUX1 (all coding); DDX41 (all coding); DNMT3A (all coding); ETV6 (all coding); EZH2 (all coding); FLT3 (exons 14+15+20); GATA1 (all coding); GATA2 (all coding); GNB1 (all coding); HRAS (exons 2+3); IDH1 (exon 4); IDH2 (exon 4); IKZF1 (exons all coding); JAK2 (all coding); KIT (exons 2, 8 to 11, 13+17); KMT2A (all coding); KMT2C (all coding); KRAS (exons 2+3); MPL (all coding); NF1 (all coding); NFE2 (all coding); NPM1 (exon 12); NRAS (exons 2+3); PHF6 (all coding); PPM1 D (all coding); PTPN11 (exons 3+13); RAD21 (all coding); RUNX1 (all coding); SETBP1 (exon 4); SF3B1 (exons 12 to 16); SH2B3 (all coding); SRSF2 (exon 1); STAG2 (all coding); STAT5B (all coding); TET2 (all coding); TP53 (all coding); U2AF1 (exons 2+6); UBA1 (all coding); WT1 (exons 7+9); ZRSR2 (all coding). For regions with read depth >400x, the limit of detection = 5% clone size. For sub-optimal coverage (200-399x), this is 10%.

A UKAS accredited medical laboratory No. 9597

PNH CLONE ANALYSIS (PNH)

PNH clone was detected in 4 patients at a median size of 19.39% (0.39-31.39) in the granulocytes 15.6% (1.85-31.07) in the monocytes, 2.8% (0.25-5.82) in red blood cells. Type II red cell clones were 0.3% (0.22-0.38) and Type III clones 2.6% (0.08-5.44).

GMP TESTING

Suppression assay

Suppression assay is a GMP in-house validated test. To assess the suppressive activity of expanded Tregs, effector T cells (Teff) were stained with a fluorescent proliferation dye (CellTrace, Invitrogen) and co-cultured with allogenic expanded Tregs (1:1, 1:5 and 1:10 ratio) stained with PKH-26 (Sigma) for 5 days in the presence of anti CD3/CD28 beads. After 5 days of culture, cells were harvested and stained with Live/Dead Blue Dead cell stain. The samples were run on MACSQuant Analyser and data were analysed using FlowJo, software.

Mycoplasma detection

Mycoplasma detection using qPCR is an in-house validated test performed at the GMP facility at Guys using validation protocol CRF-PRO-103

Mycoplasma detection is based on the MycoSEQ qPCR method (Life Technologies 4460627). Validation is based upon Ph. Eur. 2.6.7 recommendations. Information provided by the supplier demonstrates that the primers used in the PCR assay provide specific detection of mycoplasma species (*M. argini*, *M. fermentans*, *M. hominis*, *M. hyorhinae*, *M. orale*, *M. pneumoniae*, *M. salivarium* and *S. citri*), but do not lead to amplification of other DNA templates (CHO, Human, Mouse, *B. cereus*, *B. subtilis*, *C. albicans*, *C. perfringens*, *C. sporogenes*, *E. coli*, *E. hirae*, *L. delbrueckii*, *M. luteus*, *S. aureus* and *S. epidermidis*).

Endotoxin

The endotoxin assay by Limulus Amebocyte Lysate (LAL; Ph. Eur. 2.6.14) has been validated by Scottish National Blood Transfusion Service (SNBTS).

Sterility

The sterility assay was performed by rapid test method (Ph. Eur. 2.6.27). The rapid test method (Ph. Eur. 2.6.27) has been validated in collaboration with Scottish National Blood Transfusion service under Protocol VD NAT 837 (Validation of BacT/ALERT Testing for Treg Products (TR005). Method suitability was conducted in collaboration with the SNBTS. The inocula (~100 cfu) were identified using the Vitek 2 automated laboratory System.

Phenotype

This is a qualified in-house GMP quality control assay. A sample of bulk Drug Product was stained with a fluorescent Live/Dead marker, and fluorescent antibodies against cell-surface markers CD4, CD8, CD25, CD127^{low} and then permeabilized and stained using fluorescent anti-Foxp3 antibodies. The samples were run on MACSQuant Analyser and data were analysed using FlowJo, software.

SUPPLEMENTARY TABLES

Table S1: Antibody panel used for CyTOF

Channel	Target	Supplier	Catalogue Number
	FITC anti iNKT cell	BioLegend	342906
153Eu	Va7.2 (3C10)	Standard Biotech	3153024B
159Tb	CD197 (CCR7)	BioLegend	353237
141Pr	CD196 (CCR6)	Standard Biotech	393502
146Nd	CXCR5 (J252D4)	BioLegend	356902
209Bi	CD16 (3G8)	Standard Biotech	3148004B
89Y	CD45 (H130)	Standard Biotech	3089003B
106Cd	CD8a	BioLegend	301002
110Cd	CD3 (UCHT1)	BioLegend	300438
113Cd	CD244 (2-69)	BioLegend	393502
144Nd	CD38 (HIT2)	Standard Biotech	3144014B
145Nd	TCRgd (REA591)	Miltenyi	130-122-291
154Sm	CD161 (HP-3G10)	BioLegend	339902
158Gd	CD194 (CCR4)	Standard Biotech	3158032A
165 Ho	CD127 (IL-7Ra)	Standard Biotech	3165008B
166 Er	CD25 (IL-2R)	BioLegend	356102
167 Er	TIGIT (A15153G)	BioLegend	372702
169 Tm	TIM-3	BioLegend	345019
173 Yb	HLA-DR (L243)	Standard Biotech	3173005B
174 Yb	CXCR3	BioLegend	353733
175 Lu	CD279 (PD-1)	Standard Biotech	3175008B
111Cd	CD4 (RPA-T4)	BioLegend	300570
112Cd	CD56 (HCD56)	BioLegend	318302
147 Sm	CD28	BioLegend	302902
148 Nd	CD45RA	BioLegend	304102
151 Eu	CD103 (Ber-ACT8)	BioLegend	350202
155 Gd	CD27 (L128)	Standard Biotech	3155001B
172 Yb	CCR5	BioLegend	359102
115 In	CD57	BioLegend	359602
150 Nd	CD39	BioLegend	328202
152 Sm	CD31 (WM59)	BioLegend	303102
156 Gd	CD35 (E11)	BioLegend	333402
160 Gd	CD21 (544408)	Bio-Techne	MAB4909
164 Dy	CD95 (Fas)	Standard Biotech	3164008B
176Yb	CLA	BioLegend	321302

Internal Staining

Channel	Target	Supplier	Catalogue Number
139 La	FITC	BioLegend	408305
162 Dy	TCF1	BioLegend	655202
163 Dy	TOX (REA473)	Miltenyi	130-126-455
161 Dy	CD152 (CTLA-4)	Standard Biotools	3161004B
171 Yb	FoxP3 (150D)	eBioscience	14-4776-82
142 Nd	Eomes	eBioscience	14-4877-82
149 Sm	BCL2	BioLegend	658702
170 Er	Tbet	BioLegend	644825
168 Er	Ki67	BioLegend	644825

Table S2: Reagents used for CyTOF staining

	Reagents	Catalogue Number	Supplier
1.	Benzonase	2896216	Biolegend
2.	Heparin	H3149	
3.	Maxpar® Cell staining Buffer	2302177-01	Fluidigm
4.	Rhodium	230ID4D-24	Fluidigm
5.	Iridium	201192B	Fluidigm
6.	Trustain	14-9165-42	Invitrogen
7.	eBioscience™FOXP3/Transcription Factor Staining Buffer kit	2518973	Invitrogen
8.	Paraformaldehyde 16%	YJ380494	Thermo Scientific
9.	Maxpar® Cell Acquisition Solution Plus	201244	Standard Biotools
10.	β2-microglobulin Antibody	316302	Biolegend

Table S3: Reagents used for single cell sequencing

Reagent	Supplier	Catalogue #
BD Horizon™ APC-R700 Mouse Anti-Human CD4	BD Biosciences	564975
BD Pharmingen™ PE Mouse Anti-Human CD8	BD Biosciences	555367
BD Horizon™ BB515 Mouse Anti-Human CD25	BD Biosciences	565096
PE/Cyanine7 anti-human CD127 (IL-7Rα) Antibody	BioLegend	351320
Pan T Cell Isolation Kit, human	Miltenyi Biotec	130-096-535
CD4+ T Cell Isolation Kit, human	Miltenyi Biotec	130-096-533
MACS® BSA Stock Solution	Miltenyi Biotec	130-091-376
autoMACS® Rinsing Solution	Miltenyi Biotec	130-091-222
Hoechst 33258	Invitrogen™	H1398
BD Pharmingen™ Human BD Fc Block™	BD Biosciences	564220
BD Pharmingen™ Stain Buffer (BSA)	BD Biosciences	554657
BD Rhapsody Custom Panel 100– 199 genes (ID:0002174)	BD Biosciences	633778
BD Rhapsody™ Cartridge Kit	BD Biosciences	633733

BD Rhapsody™ cDNA Kit	BD Biosciences	633773
BD Rhapsody™ Enhanced Cartridge Reagent Kit	BD Biosciences	664887
BD Rhapsody™ Immune Response Panel HS	BD Biosciences	633750
BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit	BD Biosciences	633774
BD Rhapsody™ TCR/BCR Amplification Kit.	BD Biosciences	665345
BD® AbSeq Immune Discovery Panel	BD Biosciences	625970
BD® Single-Cell Multiplexing Kit	BD Biosciences	633781
BD™ AbSeq Oligo Mouse Anti-Human CD103	BD Biosciences	940067
BD™ AbSeq Oligo Mouse Anti-Human CD194	BD Biosciences	940047
BD™ AbSeq Oligo Mouse Anti-Human CD195	BD Biosciences	940050
BD™ AbSeq Oligo Mouse Anti-Human CD38	BD Biosciences	940013
BD™ AbSeq Oligo Mouse Anti-Human CD39	BD Biosciences	940073
BD™ AbSeq Oligo Mouse Anti-Human CD45RO	BD Biosciences	940022
BD™ AbSeq Oligo Mouse Anti-Human CD69	BD Biosciences	940019
BD™ AbSeq Oligo Mouse Anti-Human TCR γδ	BD Biosciences	950057
BD™ AbSeq Oligo Mouse Anti-Human Vδ2 TCR	BD Biosciences	940297
BD™ AbSeq Oligo Mouse Anti-Human Vγ9 TCR	BD Biosciences	940295
BD™ AbSeq Oligo Mouse Anti-Human CD95	BD Biosciences	940037
AMPure XP Reagent, 60 mL	Beckman Coulter	A63881
High Sensitivity D5000 ScreenTape	Agilent Technologies	5067-5592
High Sensitivity D5000 Reagents	Agilent Technologies	5067-5593
High Sensitivity D1000 ScreenTape	Agilent Technologies	5067-5584
High Sensitivity D1000 Reagents	Agilent Technologies	5190-6504
Qubit™ 1X dsDNA High Sensitivity (HS) and Broad Range (BR) Assay Kits	Invitrogen™	Q33231

Table S4: Reagents used for expansion of cells under GMP conditions.

	Reagents	Catalogue Number	Supplier
1.	CliniMACS CD8 Reagent, CE	200-070-115	Miltenyi Biotec
2.	CliniMACS CD25 Reagent, CE	200-070-131	Miltenyi Biotec
3.	CliniMACS Tubing Set LS, CE	200-073-204	Miltenyi Biotec
4.	CliniMACS Tubing Set, CE	200-073-104	Miltenyi Biotec
5.	CliniMACS PBS/EDTA Buffer CE 3×1000mL	200-070-025	Miltenyi Biotec
6.	MACS GMP Rapamycin	170-076-308	Miltenyi Biotec
7.	MACS GMP ExpAct Treg kit	170-076-119	Miltenyi Biotec
8.	Aldesleukin/Proleukina	31644/0003	Clinigen
9.	CryoSure-DMSO	WAK-DMSO-70	Wak-Chemie-Medical-GmbH
10.	Plasma-Lyte148	PL 00116/0332	Baxter
11.	Human AB serum	S4190	biowest®
12.	Human serum Albumin (HSA) 20%	PL04500/0012	Biotest
13.	TheraPEAK® X-VIVO® 15	BEBP02-054Q	Lonza

Table S5: Consumables used for expansion of cells within the GMP conditions.

1.	LOVO Med cell processing kit	Part No. R6R4905	Fresenius Kabi
2.	CliniMACS tubing set TS	161-01	Miltenyi Biotec
3.	CliniMACS tubing set LS	162-01	Miltenyi Biotec
4.	Sepax Processing Kit	CT 60.1	Cytiva
5.	G-Rex Bioreactors	P/N 80040S P/N 81100CS	Wolf Wilson

Table S6: Batch Release criteria specifications.

	Identity/Purity	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
1.	ExpAct Treg bead concentration	≤ 100 beads per 3×10^6 cells	≤ 100 beads per 3×10^6 cells	≤ 100 beads per 3×10^6 cells	65 beads per 3×10^6 cells	65 beads per 3×10^6 cells	92.5 beads per 3×10^6 cells
2.	Mycoplasma-PCR	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
3.	Sterility BacT/ALERT	No growth after 5 days	No growth after 5 days	No growth after 5 days	No growth after 5 days	No growth after 5 days	No growth after 5 days
4.	Endotoxin	≤ 7 IU/mL	≤ 7 IU/mL	≤ 7 IU/mL	≤ 7 IU/mL	≤ 7 IU/mL	< 1 IU/mL

Table S7: Collection and Expansion of autologous T-regulatory cells.

	Total Blood Volume (TBV) processed	Isolated Treg cell number from leukapheresis	Treg cell number after expansion	No of cells per infusion on Day 0 and Day 14*	Viability (%)	Phenotype (%) (CD4+, CD25+, CD127 low FoxP3+)	Suppression (1:1, 1:5, 1:10) (%)	CD8* (%)
KCH-001	2.9	181.07×10^6	4.95×10^9	307.5×10^6	97.3	73.91	73.85, 72.65 72.18	3.56
KCH-002	2.4	24.32×10^6	4.16×10^9	248×10^6	97.92	65.11	86.40, 97.60 94.25	4.22
KCH-003	2.5	68.79×10^6	3.16×10^9	420×10^6	98.7	78.69	92.95, 90.70 97.3	3.99
KCH-004	2.7	163.77×10^6	6.81×10^9	317×10^6	98.20	85.48	93.52, 90.75, 14	3.57
KCH-005	2.2	90.06×10^6	4.44×10^9	390×10^6	98.10	46.64	83.57, 90.78 75.55	5.25
KCH-006	2.9	94.8×10^6	2.52×10^9	357×10^6	98.70	47.16	94.08, 85.38, 75.31	4.80

*Patients received same dose on Day 0 and Day 14. T-regs were expanded to the required cell dose/patient kg body weight and met all the release criteria specifications, that included viability $\geq 60\%$, phenotype $\geq 30\%$, suppression $\geq 60\%$, and CD8+ $\leq 10\%$. Expanded Tregs were able to suppress proliferation of effector T cells at all $T_{reg}:T_{eff}$ ratios. TBV processed refers to number of times the patients total blood volume is processed during apheresis.

SUPPLEMENTARY FIGURES

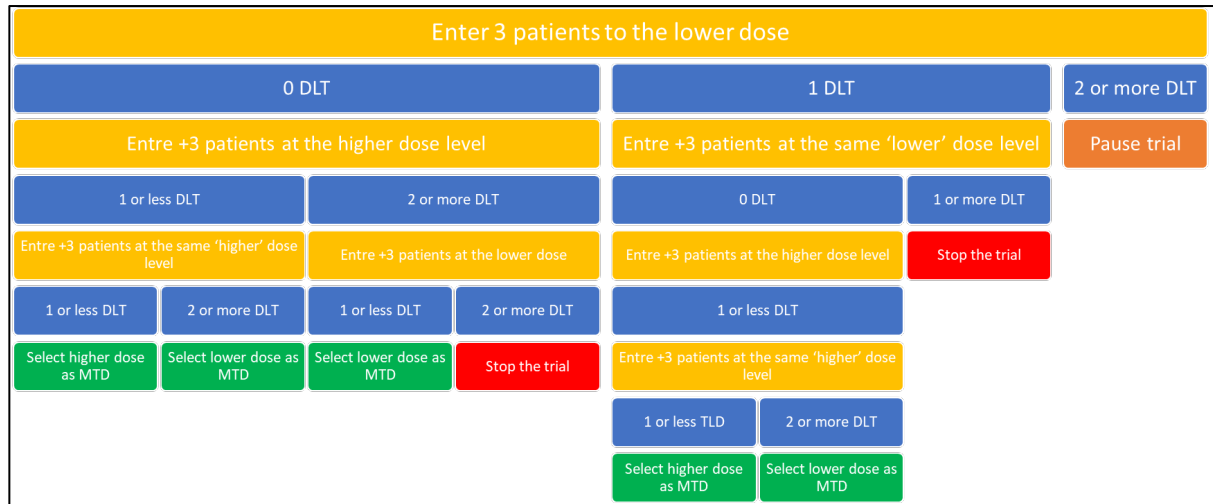


Figure S1: 3+3 Dose escalation design

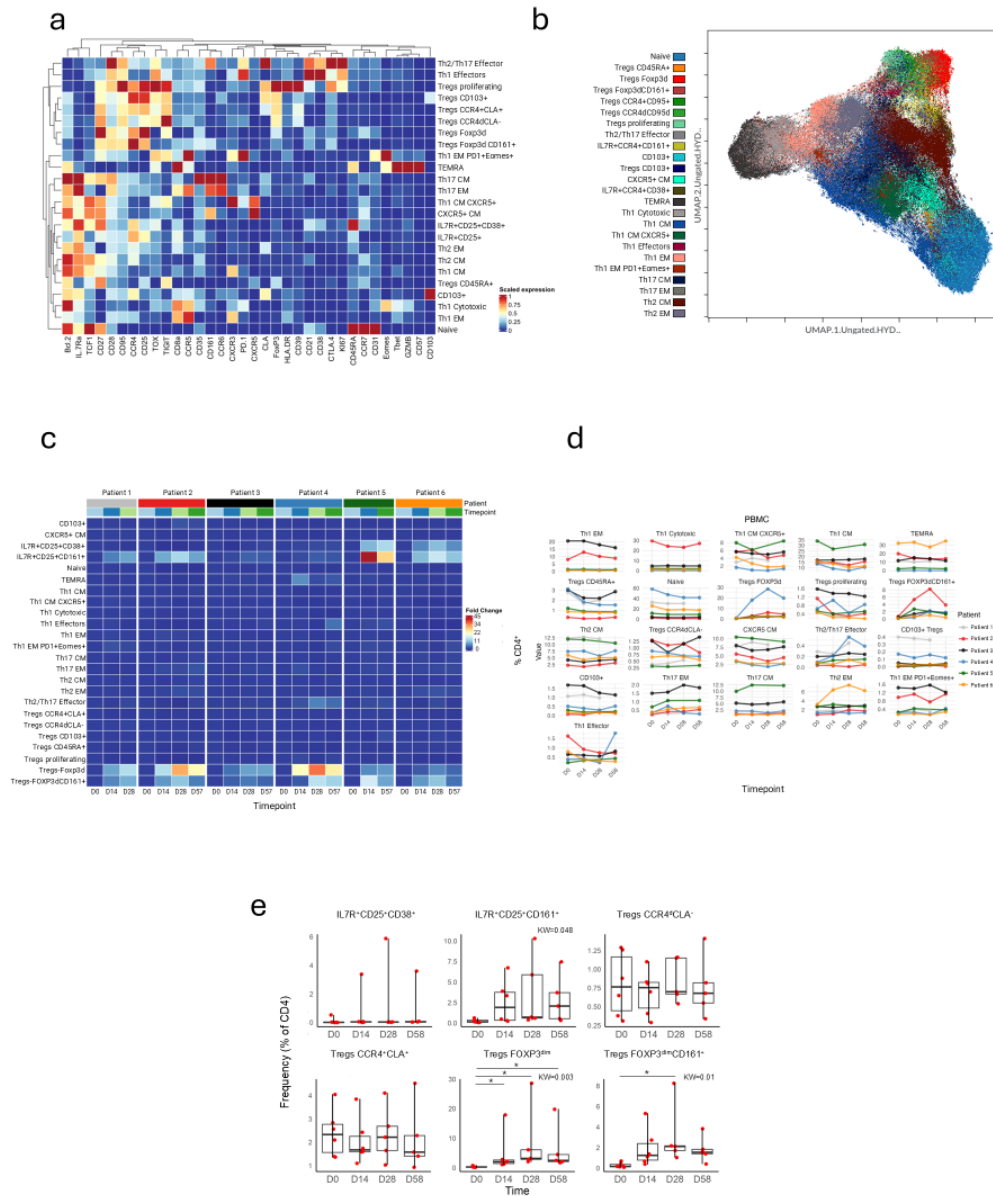
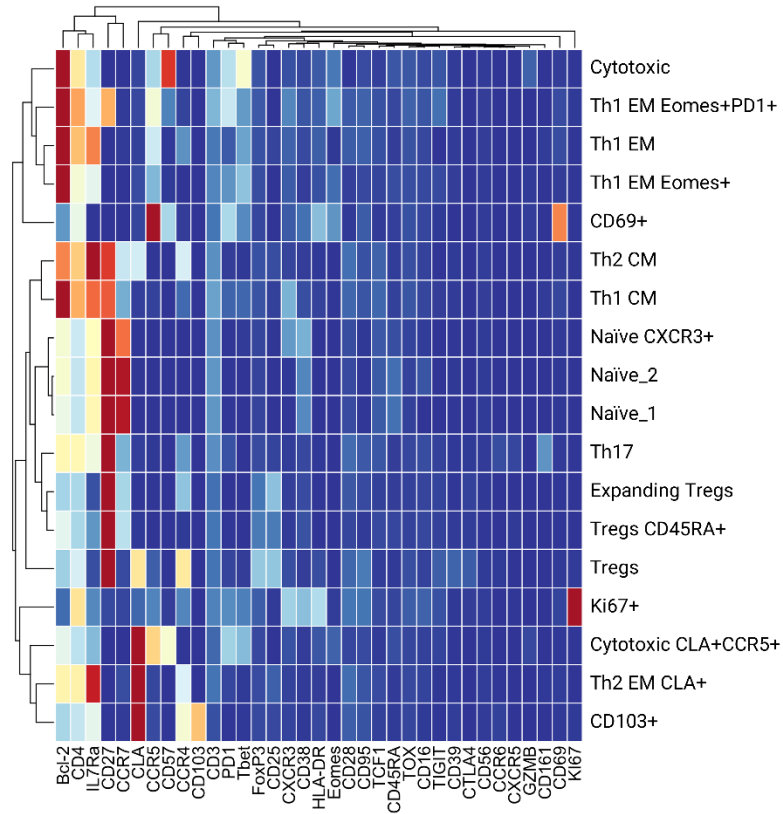


Figure S2: Immune monitoring analysis on peripheral blood samples using mass cytometry (a) Heatmap displaying the median expression levels of CD4⁺ T cell clusters identified in the peripheral blood of treated patients by mass cytometry and phenograph analysis. (b) UMAP visualization of CD4⁺ T cell clusters identified in panel a. (c) Heatmap showing the fold change across different time points of the clusters described in panel a and b in the six treated patients. (d) Plots describing changes in the frequency of the different cluster across different time points in the six patients. (e) Boxplots illustrating the frequency of selected CD4⁺ T cell clusters at different time points. The Kruskal-Wallis test (KW) was used for statistical analysis, and * indicates a p-value < 0.05 after Bonferroni correction for multiple testing. Shown are the only statistically significant comparisons identified

a



b

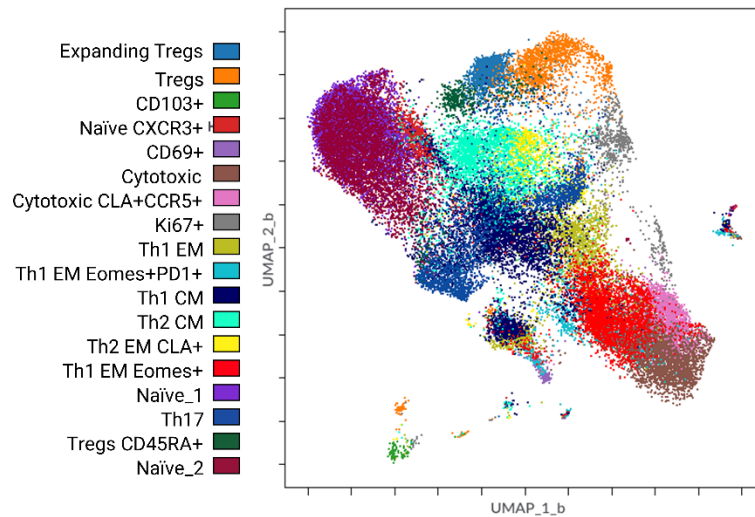


Figure S3: Mass Cytometry Analysis of peripheral blood CD8⁺ T cells in infused patients (a) Heatmap displaying the median expression levels of CD4⁺ T cell clusters identified by Phenograph analysis of mass cytometry samples collected from the bone marrow of Patients 2 and 4 at baseline and six months post infusion. (b) UMAP visualization of CD4⁺ T cell clusters described in panel a.

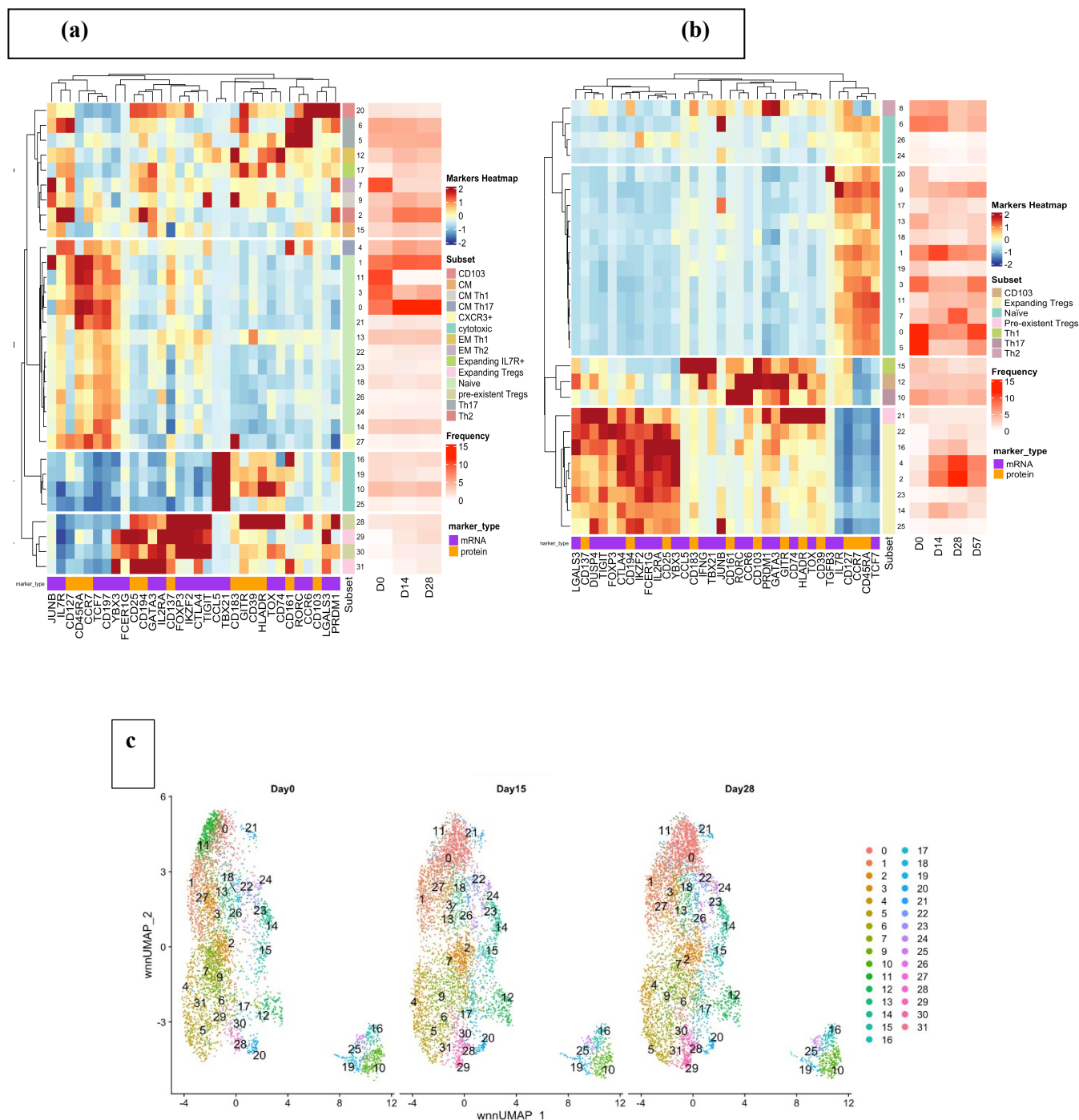


Figure S4: scRNA analysis performed on peripheral blood samples of Patient1 (a) UMAP plot describing the heterogeneity of CD4⁺ T cells from Patient 1 and Patient 4 clusters at different time points as defined by scRNAseq. (c) Combined heatmap showing the expression of selected transcript and protein markers in Patient 1 clusters and their frequency at different time points.

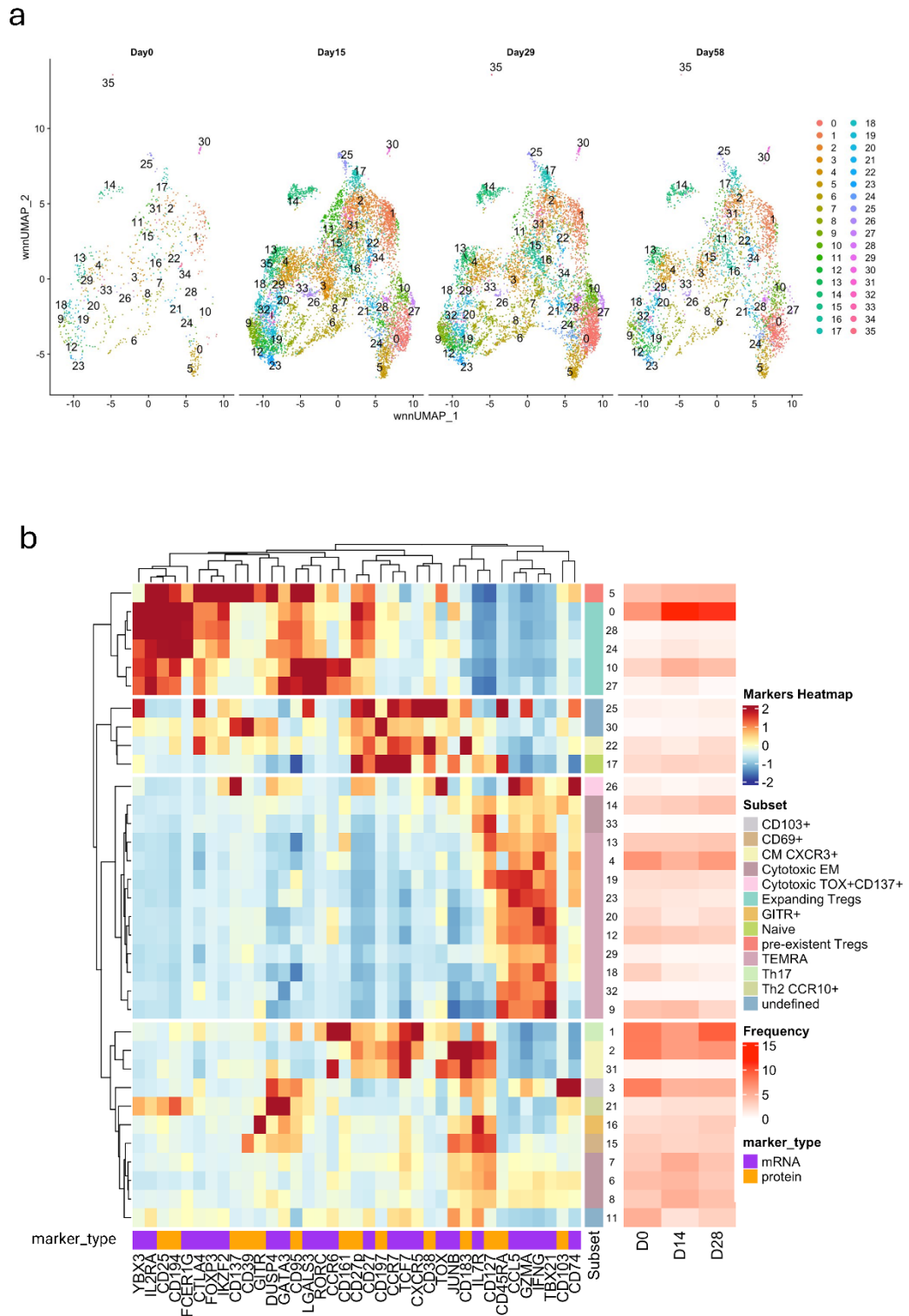


Figure S5: scRNA analysis performed on peripheral blood samples of Patient 2 (a) UMAP plot describing the heterogeneity of CD4⁺ T cells from Patient 2 at different time points as defined by scRNAseq. (b) Combined heatmap showing the expression of selected transcript and protein markers in patient 2 clusters and their frequency at different time points.

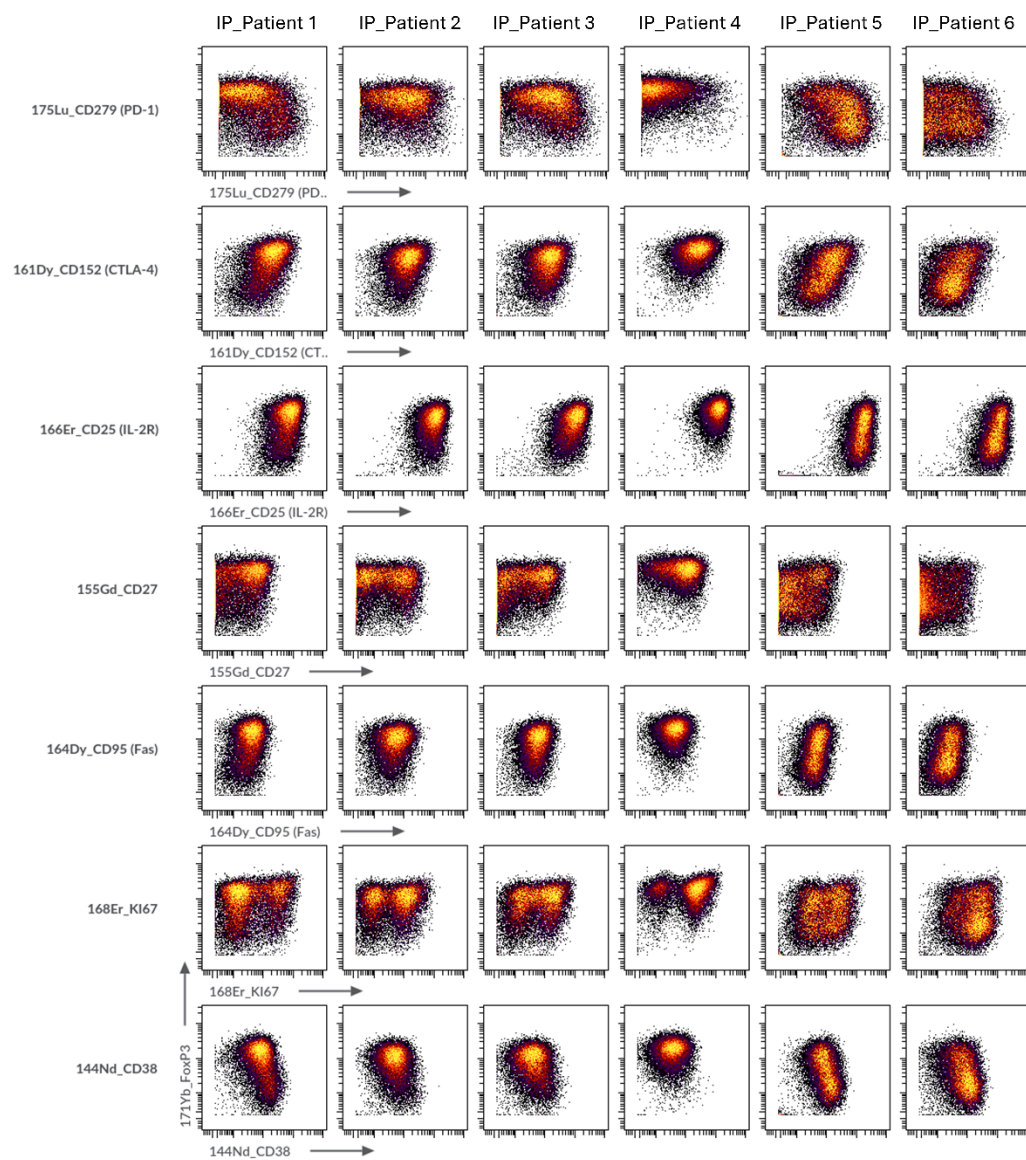


Figure S6: Plots describing the expression of different markers in the infusion products of patients 1 to 6