

Autologous T cell therapy for PRAME⁺ advanced solid tumors in HLA-A*02⁺ patients: a phase 1 trial

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Supplementary Table 1: PRAME is a highly prevalent target across many solid tumors

Tumor Indication / Sub-Indication	PRAME Prevalence (%)
Sarcoma	
• Synovial Sarcoma	100
Endometrial Carcinoma	97
• Uterine Carcinosarcoma	100
Melanoma	
• Cutaneous Melanoma	95
• Uveal Melanoma	91
Ovarian Serous Carcinoma	84
Lung Cancer	
• Non-small Cell Squamous Lung Carcinoma	68
• Non-small Cell Lung Adenocarcinoma	25
Kidney Cancer	
• Renal Papillary Cell Carcinoma	40
Cholangiocarcinoma	33
Esophageal Carcinoma	27
Head And Neck Squamous Cell Carcinoma	27
Breast Carcinoma	26
• Triple-negative Breast Cancer	63
Bladder Urothelial Carcinoma	18
Liver Hepatocellular Carcinoma	18

PRAME prevalence is based on applying a target-specific, MS-based expression threshold to TCGA RNAseq data⁵⁵. Data for uveal melanoma is based on qPCR testing of screening biopsies from clinical patients.

Supplementary Table 2: Patient characteristics

ID	Sex	Age (Years)	Cancer Diagnosis	Prior Lines of Systemic Therapies	Tumor Burden (Sum of Target Lesion Diameter at Baseline) [mm]	No. of Target and Non-Target Lesions	Liver Lesions at Base-line	Brain Lesions at Base-line	LDH at Baseline [x ULN]	Number of Infused CD3 ⁺ CD8 ⁺ Dex ⁺ T cells ¹ [x10 ⁹]	Dose Level
1	Female	61	Ovarian Cancer	6	219.7	5	Yes	No	1.84	0.078	DL1
2	Female	18	Cut. Melanoma	4	95.0	9	No	Yes	2.15	0.099	EC1
3	Male	63	Head & Neck Cancer	4	90.6	3	Yes	No	0.89	0.105	DL1
4	Female	40	Head & Neck Cancer	6	206.0	5	No	No	0.87	0.118	DL1
5	Male	65	Cut. Melanoma	7	128.0	5	No	No	0.55	0.204	EC1
6	Male	72	Head & Neck Cancer	7	147.0	6	No	No	0.95	0.247	DL2
7	Male	42	Uveal Melanoma	2	198.0	11	Yes	Yes	1.30	0.248	EC1
8	Female	55	NSCLC	8	155.1	6	No	Yes	1.28	0.271	DL2
9	Female	50	Head & Neck Cancer	3	29.0	4	Yes	No	2.83	0.302	DL2
10	Male	57	Synovial Sarcoma	2	108.7	12	No	No	1.54	0.350	DL2
11	Male	65	Thymus Cancer	4	125.0	5	Yes	No	2.67	0.358	EC2
12	Male	40	Synovial Sarcoma	3	133.0	9	No	No	1.55	0.366	DL2
13	Male	20	Synovial Sarcoma	2	147.9	2	No	No	0.82	0.385	DL2
14	Male	49	Synovial Sarcoma	3	84.0	3	No	No	0.76	0.409	DL2
15	Male	59	Mucosal Melanoma	4	209.0	11	Yes	No	4.94	0.499	DL3
16	Female	61	Uterine Cancer	2	133.0	14	Yes	Yes	0.71	0.532	DL3
17	Male	50	Cut. Melanoma	4	211.0	9	Yes	No	6.61	0.548	DL3
18	Female	62	Uveal Melanoma	4	109.0	13	Yes	No	4.65	0.586	DL3
19	Male	63	Synovial Sarcoma	4	37.6	4	No	No	0.71	0.627	DL3

20	Female	59	Synovial Sarcoma	4	141.1	2	No	No	1.42	0.808	DL3
21	Female	52	Cut. Melanoma	5	37.0	5	Yes	Yes	1.56	1.070	DL4
22	Male	30	Cut. Melanoma	7	41.0	3	No	No	0.78	1.160	DL4
23	Female	51	Cut. Melanoma	4	178.7	11	Yes	No	1.47	1.210	DL4
24	Male	51	Cut. Melanoma	7	23.0	3	No	No	0.82	1.300	DL4
25	Female	70	Cholangiocarcinoma	3	187.1	6	Yes	No	1.15	1.570	DL4
26	Male	46	MPNST	1	197.6	9	No	No	1.38	1.570	DL4
27	Male	79	Cut. Melanoma	5	56.8	3	No	No	0.91	1.630	DL4
28	Male	24	Cut. Melanoma	7	117.5	3	No	No	1.52	1.720	DL4
29	Female	40	Melanoma (Unk. Primary)	2	21.0	5	No	Yes	0.76	1.730	DL4
30	Male	53	Head & Neck Cancer	1	30.0	6	Yes	Yes	0.56	1.920	DL4
31	Female	66	Ovarian Cancer	10	102.9	11	Yes	No	1.52	1.970	DL4
32	Female	72	Cut. Melanoma	6	52.0	6	No	No	1.24	2.090	DL4
33	Female	40	Synovial Sarcoma	1	123.0	7	No	No	1.49	3.940	DL5
34	Male	71	Uveal Melanoma	1	99.2	6	Yes	No	1.16	4.160	DL5
35	Female	57	Ovarian Cancer	5	207.3	15	Yes	No	4.74	4.680	DL5
36	Female	52	Lung Cancer (Small Cell Neuroendocrine)	4	55.0	6	No	Yes	0.88	5.090	DL5
37	Male	54	Pancreatic NET	3	55.0	5	Yes	No	0.70	5.120	DL5
38	Female	31	Cut. Melanoma	3	43.0	6	No	No	1.01	5.120	DL5
39	Female	49	Synovial Sarcoma	2	61.0	3	No	No	0.86	6.010	DL5
40	Female	67	Ovarian Cancer	3	50.6	13	No	No	1.36	8.840	DL5
Median (min, max) or Percentage	50 % (Male)	53.5 (18, 79)		4 (1, 10)	108.9 (21.0, 219.7)	6 (2, 15)	42.5 % (Yes)	20.0 % (Yes)	1.26 (0.55, 6.61)	0.940 (0.078, 8.840)	
	50 % (Female)						57.5 % (No)	80.0 % (No)			

Patients 1 to 27 were treated during phase 1a dose escalation and patients 28 to 40 in phase 1b dose extension. Overall, a heavily pre-treated patient population with various solid tumor types and poor prognostic features were included.

¹Transduced viable CD8 T cells

LDH: lactate dehydrogenase; MPNST: malignant peripheral nerve sheath tumor; NET: neuroendocrine tumor; NSCLC: non-small cell lung cancer; SCN: small cell neuroendocrine; ULN: upper limit of normal.

Supplementary Table 3: Product characteristics

ID	%CD3	%CD8 in %CD3 ⁺	%Dex ⁺ in %CD3 ⁺ CD8 ⁺	Viability	VCN per transduced cell	Response
1	99%	19%	17%	86	5.88	SD
2	96%	24%	11%	96	9.09	PR
3	100%	28%	20%	90	5.00	SD
4	100%	68%	46%	86	4.35	SD
5	99%	26%	13%	97	7.69	PR
6	97%	61%	17%	91	5.88	SD
7	100%	26%	69%	89	5.80	SD
8	99%	44%	53%	92	5.66	SD
9	99%	25%	43%	91	6.98	PR
10	99%	59%	20%	87	5.00	PR
11	99%	50%	45%	88	4.44	PD
12	99%	40%	25%	91	8.00	SD
13	99%	40%	48%	95	8.33	cPR
14	98%	36%	39%	95	5.13	cPR
15	98%	29%	61%	88	4.92	PR
16	99%	70%	45%	88	4.44	SD
17	99%	25%	43%	89	6.98	PR
18	99%	42%	55%	91	3.64	cPR
19	97%	21%	29%	86	3.45	PD
20	100%	56%	46%	90	4.35	SD
21	99%	46%	32%	93	6.25	cPR
22	99%	39%	20%	88	5.00	PR
23	99%	25%	52%	89	3.85	cPR
24	99%	46%	65%	89	7.69	cPR
25	100%	50%	42%	87	4.76	PD
26	98%	19%	54%	94	3.70	PD
27	99%	47%	69%	90	7.25	SD
28	98%	35%	56%	88	3.57	PR
29	100%	67%	60%	93	6.67	SD
30	99%	77%	49%	78	6.12	cPR
31	99%	50%	71%	91	7.04	cPR
32	99%	41%	46%	88	4.35	PD
33	100%	51%	80%	94	5.00	PR
34	99%	61%	58%	91	5.17	cPR
35	99%	60%	64%	88	4.69	PD
36	100%	44%	67%	86	5.97	SD
37	99%	64%	75%	86	4.00	SD
38	100%	45%	80%	90	5.00	cPR
39	100%	58%	76%	83	5.26	PR
40	99%	63%	81%	93	6.17	cPR
Median (min, max) or Percentage	99 (96, 100)	45 (19-77)	49 (11-81)	89.5 (78-97)	5.15 (3.44, 9.09)	

cPR: confirmed partial response; PD: progressive disease; PR: partial response; SD: stable disease; VCN: vector copy number.

Supplementary Table 4: Treatment-emergent adverse events after IMA203 treatment

MedDRA system organ class Preferred term	All Grades		≥Grade 3	
	No.	%	No.	%
Patient with any adverse event	41	100.0	41	100.0
Adverse events of special interest	38	92.7	2	4.9
Cytokine release syndrome ¹	38	92.7	2	4.9
Immune effector cell-associated neurotoxicity syndrome	5	12.2	0	0.0
Blood and lymphatic system disorders	41	100.0	41	100.0
Neutropenia ¹	35	85.4	34	82.9
Anaemia	31	75.6	20	48.8
Thrombocytopenia ¹	30	73.2	15	36.6
Lymphopenia	25	61.0	25	61.0
Leukopenia	24	58.5	23	56.1
Lymphocytosis	2	4.9	1	2.4
Cytopenia	1	2.4	1	2.4
Leukocytosis	1	2.4	1	2.4
Gastrointestinal disorders	35	85.4	2	4.9
Nausea	25	61.0	0	0.0
Diarrhoea ¹	18	43.9	1	2.4
Vomiting	14	34.1	1	2.4
Constipation	12	29.3	0	0.0
Abdominal pain ¹	4	9.8	1	2.4
General disorders and administration site conditions	24	58.5	4	9.8
Fatigue	16	39.0	1	2.4
Pyrexia	13	31.7	1	2.4
Swelling face	2	4.9	1	2.4
Condition aggravated ²	1	2.4	1	2.4
Metabolism and nutrition disorders	20	48.8	3	7.3
Hypokalaemia ¹	14	34.1	2	4.9
Hyponatraemia	12	29.3	0	0.0
Failure to thrive	1	2.4	1	2.4
Investigations	18	43.9	4	9.8
Alanine aminotransferase increased	11	26.8	1	2.4
Aspartate aminotransferase increased	10	24.4	1	2.4
Blood creatinine increased	8	19.5	1	2.4
Blood alkaline phosphatase increased	6	14.6	1	2.4
Blood fibrinogen decreased	1	2.4	1	2.4
Respiratory, thoracic and mediastinal disorders	10	24.4	5	12.2
Pleural effusion	4	9.8	1	2.4
Hypoxia	3	7.3	2	4.9
Respiratory failure	2	4.9	1	2.4
Bronchial obstruction	1	2.4	1	2.4
Laryngeal inflammation	1	2.4	1	2.4
Skin and subcutaneous tissue disorders	9	22.0	2	4.9
Rash maculo-papular	9	22.0	2	4.9
Vascular disorders	7	17.1	4	9.8
Hypotension	5	12.2	1	2.4
Hypertension	4	9.8	3	7.3

MedDRA system organ class Preferred term	All Grades		≥Grade 3	
	No.	%	No.	%
Infections and infestations	6	14.6	2	4.9
Appendicitis	1	2.4	1	2.4
COVID-19	1	2.4	1	2.4
Enterococcal infection	1	2.4	1	2.4
Infection	1	2.4	1	2.4
Orchitis	1	2.4	1	2.4
Sepsis ^{2,3}	1	2.4	1	2.4
Septic shock ²	1	2.4	1	2.4
Renal and urinary disorders	6	14.6	2	4.9
Proteinuria ¹	5	12.2	1	2.4
Acute kidney injury	1	2.4	1	2.4
Nervous system disorders	4	9.8	1	2.4
Headache	4	9.8	1	2.4
Cardiac disorders	3	7.3	1	2.4
Atrial fibrillation ⁴	3	7.3	1	2.4
Endocrine disorders	2	4.9	1	2.4
Inappropriate antidiuretic hormone secretion	2	4.9	1	2.4
Injury, poisoning and procedural complications	2	4.9	2	4.9
Humerus fracture ¹	1	2.4	1	2.4
Infusion related reaction	1	2.4	1	2.4
Eye disorders	1	2.4	1	2.4
Ulcerative keratitis	1	2.4	1	2.4
Hepatobiliary disorders	1	2.4	1	2.4
Cholangitis	1	2.4	1	2.4
Immune system disorders	1	2.4	1	2.4
Contrast media allergy	1	2.4	1	2.4
Musculoskeletal and connective tissue disorders	1	2.4	1	2.4
Muscle spasms ¹	1	2.4	1	2.4
Reproductive system and breast disorders	1	2.4	1	2.4
Vaginal haemorrhage	1	2.4	1	2.4

Treatment-emergent AEs reported in at least 25% of patients and all TEAEs with ≥Grade 3 regardless of relatedness to study treatment. ¹Two patients received an experimental second infusion of IMA203. TEAEs after second infusion are included in this table. They had these ≥Grade 3 TEAEs only after second infusion: First patient: abdominal pain, cytokine release syndrome, diarrhoea, hypokalaemia, proteinuria; Second patient: humerus fracture, muscle spasms, neutropenia, thrombocytopenia; ²Fatal Adverse events were not considered related to any study drug; ³Patient died from sepsis of unknown origin and did not receive IMA203 TCR T cells; ⁴DLT: Dose limiting toxicity in dose escalation at DL2 reported on March 17, 2021

Supplementary Table 5: IMA203 pharmacokinetic parameters

PK parameters	Non-responder (n=19)	Responder (n=21)
C _{max} (Copies/μg gDNA) Median (Min, Max)	2.71 x10 ⁵ (3.95 x10 ⁴ , 1.34 x10 ⁶)	3.29 x10 ⁵ (1.24 x10 ⁵ , 1.87 x10 ⁶)
T _{max} (days) Median (Min, Max)	3 (1, 13)	4 (1, 15)
AUC (days*copies/μg) Median (Min, Max)	3.37 x10 ⁶ (4.84 x10 ⁵ , 2.11 x10 ⁷)	4.54 x10 ⁶ (1.85E+06, 2.35 x10 ⁷)

AUC: area under the curve; gDNA: genomic DNA; PK: pharmacokinetic.

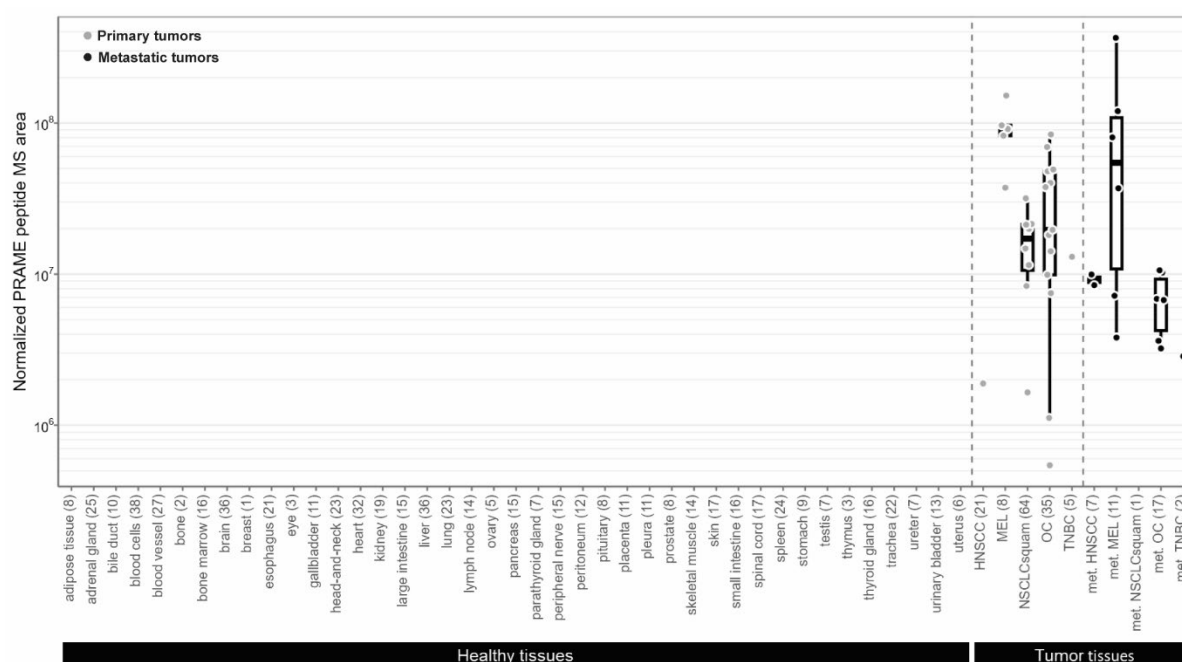
Supplementary Table 6: List of reagents used for phenotypical T-cell analyses

Reagent	Manufacturer	Clone	Order Number	Lot	Dilution	Application/ Product References
CD19 BV510	Biolegend	HIB19	302242	B221987/ B239285/ B339100	1:40	1. Wang X, et al. 2010. <i>Haematologica</i> . 95:884 2. Yoshino N, et al. 2000. <i>Exp. Anim. (Tokyo)</i> 49:97.
CD3 BV570	Biolegend	UCHT1	300436	B344592/ B355927	1:20	1. Thakral D, et al. 2008. <i>J. Immunol.</i> 180:7431. 2. Yoshino N, et al. 2000. <i>Exp. Anim. (Tokyo)</i> 49:97.
CD3 PerCP-Cy5.5	Biolegend	OKT3	300328	B208442/ B338476	1:80	1. Smeltz RB. 2007. <i>J. Immunol.</i> 178:4786. (Activ) 2. Rivollier A, et al. 2004. <i>Blood</i> 104:4029. (Activ)
CD3 AF700	Biolegend	HIT3a	300324	B218705/ B279655	1:100 / 1:200	1. Smeltz RB. 2007. <i>J. Immunol.</i> 178:4786. (Activ) 2. Rivollier A, et al. 2004. <i>Blood</i> 104:4029. (Activ)
CD4 PerCP	Biolegend	RPTA-4	300528	B342971	1:80	1. Zenaro E, et al. 2009. <i>J. Leukoc. Biol.</i> 86:1393 2. Yoshino N, et al. 2000. <i>Exp. Anim. (Tokyo)</i> 49:97.
CD8 BUV805	BD	RPA-T8	749366	1347807/ K11122	1:600 / 1:1200	1. Kourtis N, Wang Q, Wang B, et al. <i>Nat Cancer</i> . 2022 Jul;3(7):885-898.
CD8 AF488	Biolegend	RPA-T8	301021	B201763/ B284960/ B230874/ B284960	1:100	1. Awasthi, S., et al. 2011. <i>J. Virol</i> 85:10472
CD8 BV421	Biolegend	RPA-T8	301036	B242747/ B314490	1:200	1. Yoshino N, et al. 2000. <i>Exp. Anim. (Tokyo)</i> 49:97. (FC) 2. Rout N, et al. 2010. <i>PLoS One</i> 5:e9787. (FC)
Vbeta8 PE-Cy7	Biolegend	JR2 (JR.2)	348108	B258443	1:100	1. Lavoie PM, et al. 2005. <i>Int. Immunol.</i> 17:931
CD45RA BUV395	BD	HI100	740298	1327771/ K11117	1:300 / 1:600	1. Koristka S, Cartellieri M, Theil A, et al. <i>J Immunol.</i> 2012; 188(3):1551-8. (FC)
CD45RA APC Fire 750	Biolegend	HI100	304152	B218733	1:150	1. Alanio C, et al. 2010. <i>Blood</i> 115:3718. (FC) 2. Iannello A, et al. 2010. <i>J. Immunol.</i> 184:114. (FC)

CCR7 BV421	Biolegend	G043H7	353208	B229001	1:40	1. Barcelo H, <i>et al.</i> 2018. <i>Curr Protoc Cytom.</i> 84:e35. 2. Karlsson H, <i>et al.</i> 2015. <i>PLoS One.</i> 10: 0144787.
CD197 (CCR7) BV785	Biolegend	G043H7	353230	B227401/ B321505	1:40	1. Ogishi M, <i>et al.</i> <i>J Exp Med.</i> 2023 Jan 2;220(1):e20220484.
CD62L BUV496	BD	DREG-56	741155	1347785/ K11118/ K11936	1:300	1. Kishimoto TK, Warnock RA, Jutila MA, <i>et al.</i> <i>Blood.</i> 1991; 78(3):805-811. 2. Kishimoto TK, Jutila MA, Butcher EC. <i>Proc Natl Acad Sci U S A.</i> 1990; 87(6):2244-2248.
CD62L BV785	Biolegend	DREG-56	304830	B235783/ B277488	1:300	1. Weist B, Wehler P, El Ahmad L, Schmueck Henneresse M, Millward J, Nienen M, <i>et al.</i> <i>Kidney Int.</i> 2015;88:1293-1303 2. Boardman D, Philippeos C, Fruhwirth G, Ibrahim M, Hannan R, Cooper D, <i>et al.</i> <i>Am J Transplant.</i> 2017;17:931-943
CD28 BV650	Biolegend	CD28.2	302946	B229718/ B329064	1:80	1. Yoshino N, <i>et al.</i> 2008. <i>Exp. Anim. (Tokyo)</i> 49:97. (FC) 2. Rout N, <i>et al.</i> 2010. <i>PLoS One</i> 5:e9787. (FC)
CD45RO PE-Fire640	Biolegend	UCHL1	304264	B317498	1:80	1. Yoshino N, <i>et al.</i> 2000. <i>Exp. Anim. (Tokyo)</i> 49:97. (FC) 2. Thakral D, <i>et al.</i> 2008. <i>J. Immunol.</i> 180:7431. (FC)
CD45RO BV 605	Biolegend	UCHL1	304238	B246176/ B250227	1:80	1. Yoshino N, <i>et al.</i> 2000. <i>Exp. Anim. (Tokyo)</i> 49:97. (FC) 2. Thakral D, <i>et al.</i> 2008. <i>J. Immunol.</i> 180:7431. (FC)
CD27 BV750	BD	0323	751671	2136515/ 219972 - 980/ K11123/ K11713	1:50 / 1:50 / 1:25 / 1:25	1. Björkström NK, Béziat V, Cichocki F, <i>et al.</i> <i>Blood.</i> 2012; 120(17):3455-65. (FC)
CD27 PE Dazzle 594	Biolegend	M-T271	356422	B220331	1:200	1. Shuwa HA, <i>et al.</i> <i>Med.</i> 2021 Jun 11;2(6):720-735.e4.
CD57 APC	Biolegend	HNK-1	359610	B210684/ B340445	1:80 / 1:160	1. Yang C, <i>et al.</i> <i>Nat Commun.</i> 2019 Sep 2;10(1):3931.
PD1 BV785	Biolegend	EH12.2 H7	329930	B339372/ B351259	1:25	1. Vojnov L, <i>et al.</i> 2010. <i>J. Virol.</i> 84:753. (FC) 2. Radziewicz H, <i>et al.</i> 2010. <i>J. Immunol.</i> 184:2410. (FC)
PD-1 BV650	Biolegend	EH12.2 H7	329950	B239981	1:100	1. Vojnov L, <i>et al.</i> 2010. <i>J. Virol.</i> 84:753. (FC) 2. Radziewicz H, <i>et al.</i> 2010. <i>J. Immunol.</i> 184:2410. (FC)
TIM-3 AF488	R&D Systems	344823	FAB2365 G	ABVG0415 021/ 0517031/ 0621091	1:40	1. Gleason, M.K. <i>et al.</i> (2012) <i>Blood</i> 119 :3064. 2. Ndhlovu, L.C. <i>et al.</i> (2012) <i>Blood</i> 119:3734.
LAG-3 BUV737	eBioscience	3DS223 H	367- 2239-42	2400295/ 2480223/ 2516571	1:40	1. Yu T, Nie FQ, <i>et al.</i> <i>Cell Death Dis.</i> 2024 Jan 5;15(1):12.

LAG-3 APC	eBioscience	3DS223 H	17-2239- 42	4307377/ 2305268	1:40	1. Yu T, Nie FQ, <i>et al.</i> Cell Death Dis. 2024 Jan 5;15(1):12.
TIGIT PerCP - eFluor710	eBioscience	MBSA43	46-9500- 42	4318928/ 2284173	1:40	1. Ueda T, <i>et al.</i> Nat Biomed Eng. 2023 Jan;7(1):24-37.
Tetramer, 50 µg	Immatics	-	-	-	-	1. Tsimberidou AM, <i>et al.</i> Cancer Immunol Res. 2023 Jul 5;11(7):925-945.
Streptavidin- PE	Life Technologies	-	S866	-	-	-
LD FVS eFluor450	ThermoFisher	-	65-0863- 14	2296706	1:4000	1. Ulges, <i>et al.</i> Proc. Natl Acad. Sci. USA 113, 10145–10150 (2016)
LD FVS510	BD	-	564406	7138874/ 345532	1:1000	1. Siegel M, <i>et al.</i> Front Immunol. 2024 Aug 20;15:1406804.

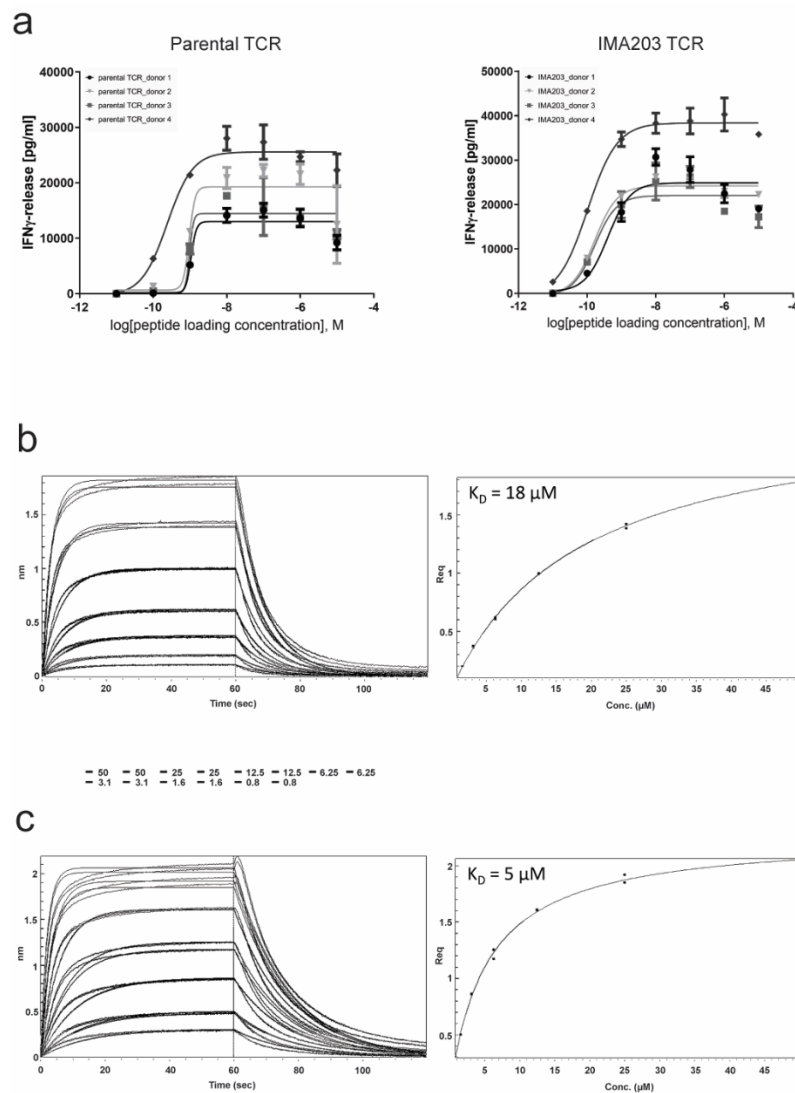
All antibodies listed in the table are monoclonal, host-species mouse, have been verified for reactivity in human samples and quality tested for application in flow cytometry.



Supplementary Figure 1: PRAME peptide is abundant on tumors while not quantifiable on normal tissue

PRAME-004 peptide presentation detected using liquid chromatography-MS in healthy normal tissue, primary tumor, and metastatic tumor tissues. Box-and-whisker plots represent normalized signal intensities over multiple samples (if available) and have been defined in the logarithmic space. Boxes display median, 25th and 75th percentile. Whiskers extend to the lowest data point still within 1.5 interquartile range of the lower quartile, and the highest data point still within 1.5 interquartile range of the upper quartile.

adipose: adipose tissue; adrenal gl: adrenal gland; bladder: urinary bladder; bloodvess: blood vessel; esoph: esophagus; gall bl: gallbladder; intest. la: large intestine; intest. sm: small intestine; nerve cent: central nerve; nerve periph: peripheral nerve; parathyr: parathyroid gland; perit: peritoneum; pituit: pituitary; skel. mus: skeletal muscle; HNSCC: head and neck cancer; MEL: melanoma; met: metastatic; NSCLCsquam: squamous non-small cell lung carcinoma; OC: ovarian cancer; TNBC: triple-negative breast cancer.

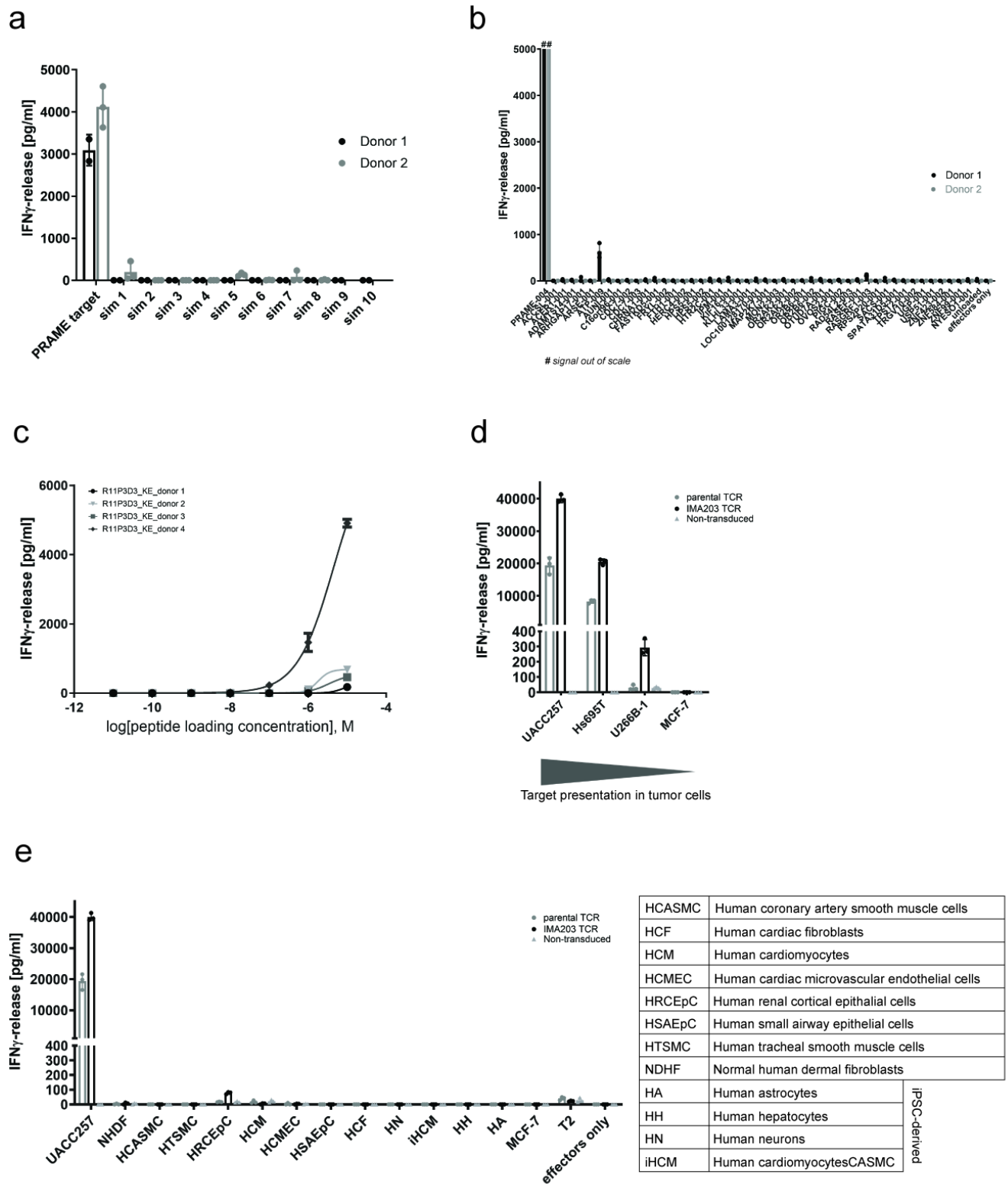


Supplementary Figure 2: PRAME-004 target recognition by the IMA203 TCR

The pairing optimized IMA203 TCR recognizes its target peptide with high affinity and functionality. a) Functional avidity of T cells lentiviral transduced with parental TCR (left panel) or IMA203 TCR (right panel) and co-cultured with T2 cells pulsed with increasing amounts of PRAME target peptide. IFN- γ secretion measured after 24 h of co-culture in four different donors. Error bars indicate standard deviation of technical triplicates. b) and c) Pairing-optimized IMA203 TCR exhibits increased affinity towards PRAME-pHLA. Binding affinity of b) non-modified parental TCR variant and c) engineered IMA203 TCR was determined by biolayer interferometry (Octet). Biotinylated PRAME-pHLA was immobilized on streptavidin sensors and the TCRs were applied in duplicates at 0.8 μ M, 1.6 μ M, 3.1 μ M, 6.25 μ M,

12.5 μM , 25 μM , and 50 μM concentration, respectively, for association/dissociation analysis.

Binding affinity (equilibrium dissociation constant K_D) was determined at steady state.



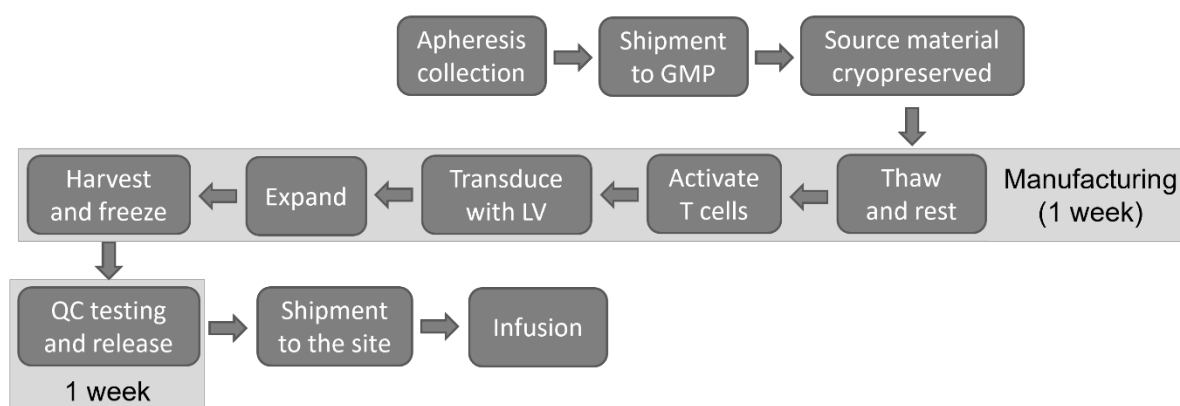
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Supplementary Figure 3: Specificity and efficacy of the IMA203 TCR

a) The IMA203 TCR recognizes the target peptide PRAME-004 but not peptides with high similarity to the target sequence (sim 1 to sim 10). TCR-expressing CD8⁺ T cells from two donors were co-cultured with peptide-loaded T2 cells at 4:1 ratio in duplicates for donor 1 and triplicates for donor 2. IFN- γ release was measured after 20 h. Error bars indicate standard deviation. No values for similar peptides sim 9 and sim 10 available for donor 2. b) IMA203 TCR testing for potentially cross-reactive peptides. TCR-transduced CD8⁺ T cells from two donors were tested for activation upon co-culture with peptide-loaded T2 cells using PRAME-004 (positive control, target peptide) and 45 out of 57 TCR motif-based similar peptides, E:T ratio 1:1. The remaining twelve peptides were tested in different assays, no reactivity was observed. Signals of positive controls were out of scale. No reactivity was observed besides weak signals towards ATP-009 and RASEF-001 in one assay. IFN- γ release after 20 h co-culture was measured by ELISA.

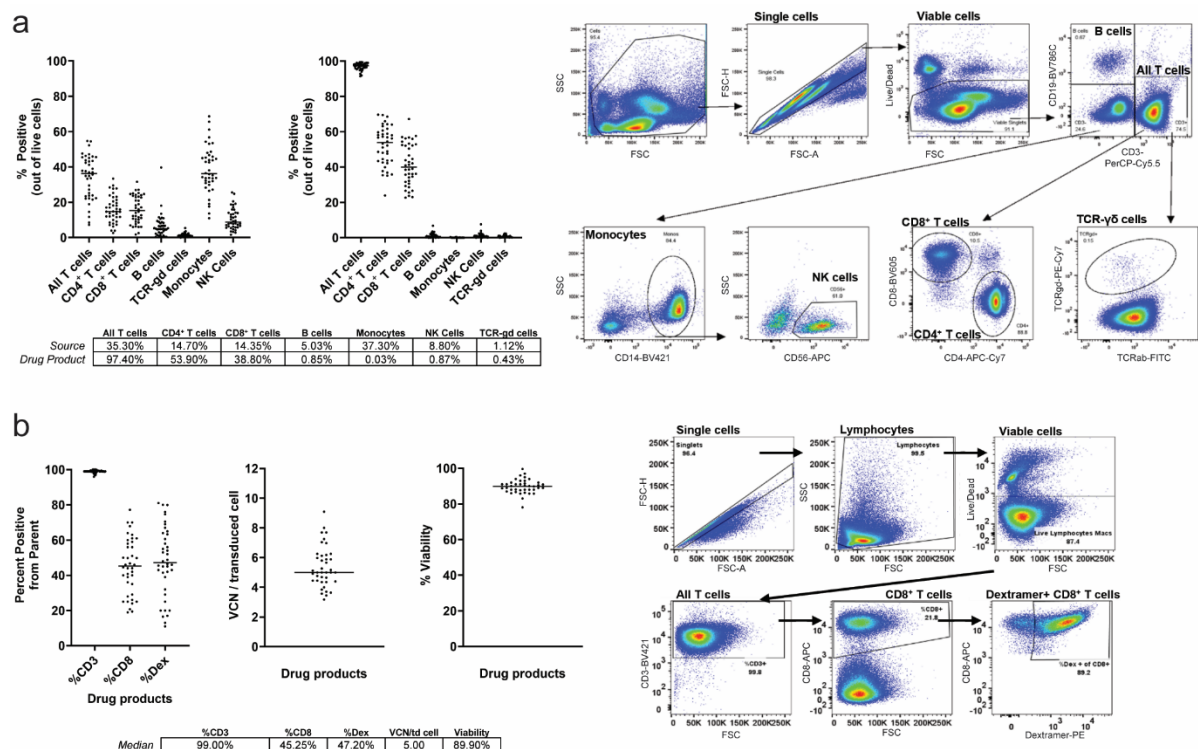
c) The weak recognition of similar peptide ATP-009 from b) was further investigated. IMA203 TCR lentiviral transduced T cells were co-cultured with T2 cells pulsed with increasing amounts of ATP-009 peptide. IFN- γ secretion was measured after 24 h of co-culture in four different donors. Error bars indicate standard deviation of technical triplicates. d) IMA203 TCR and parental TCR recognize endogenously presented target peptide. TCR transduced T cells or non-transduced controls were co-cultured with HLA-A*02:01-positive tumor cells lines presenting different levels of PRAME-HLA copies per cell. IFN- γ secretion was assessed after 24 h by ELISA (UACC257: 1100 PRAME copies/cell; Hs695T: 450 PRAME copies/cell; U266B-1: 30 PRAME copies/per cell; MCF-7: PRAME-004 negative). Only IMA203 TCR transduced cells react to U266B1. Error bars indicate standard deviation of technical triplicates. e) IMA203 TCR-transduced T cells do not recognize HLA-A*02:01-positive primary normal cells or iPSC-derived cells as measured after 24 h of co-culture by IFN- γ ELISA. Low level of cytokine secretion only observed for human renal cortical epithelial cells in one of two donors (reactive donor shown in graph, same donor as in panel d)). Error bars indicate standard deviation of technical ELISA triplicates.

Version independent



Supplementary Figure 4: Manufacturing process

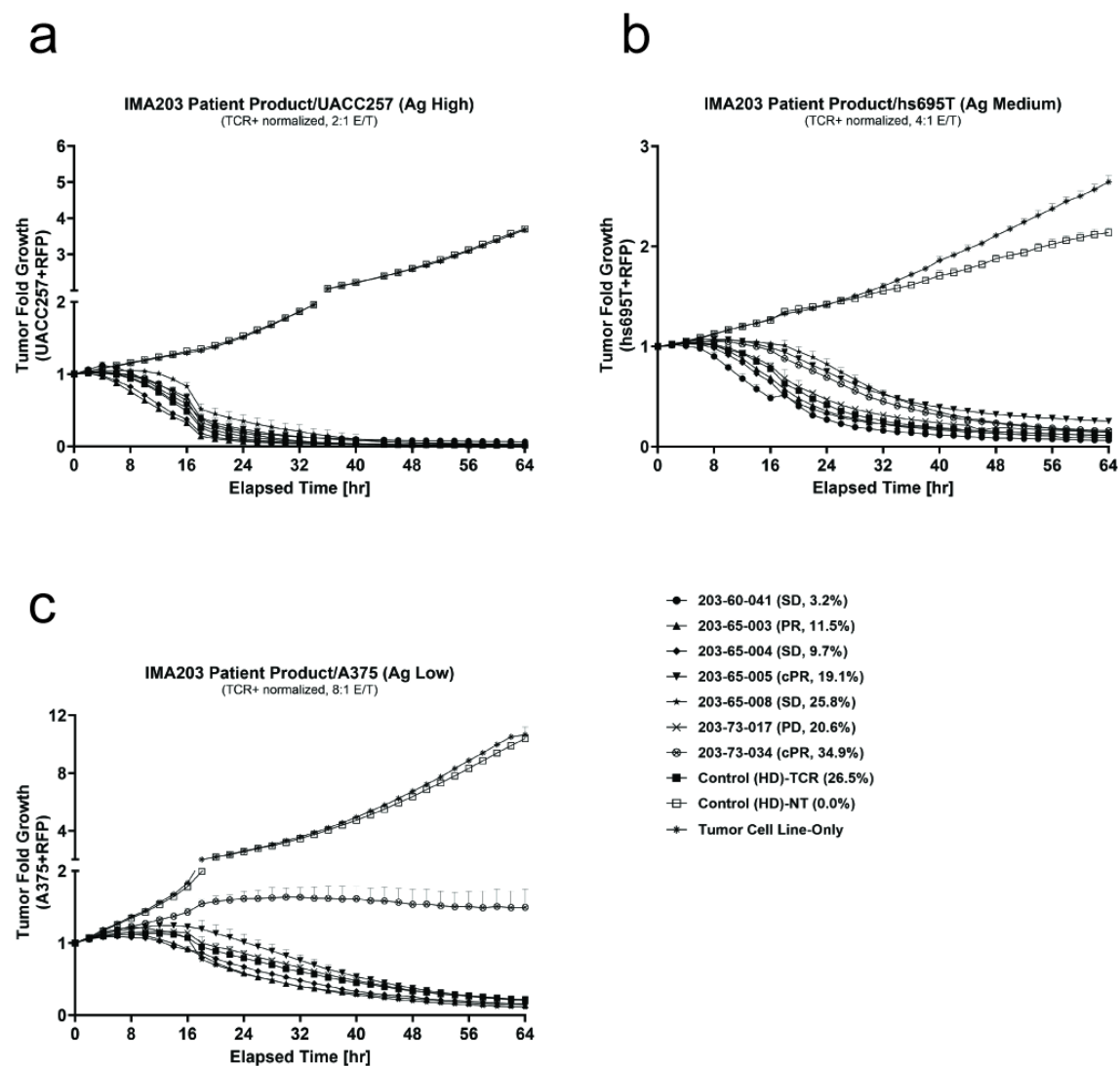
The short IMA203 manufacturing process has 14 days turnaround from start of manufacturing to product release.



Supplementary Figure 5: Composition and critical quality attributes of IMA203 drug product

a) Frequency of measured lymphoid and myeloid cells among drug product starting material (left panel) for 40 infused patients and the corresponding drug product (right) and sequential gating strategy for flow analyses. b) Key critical quality attribute data from IMA203 infused patients (n=40). Solid line in plots indicates median with tabular report for all values above.

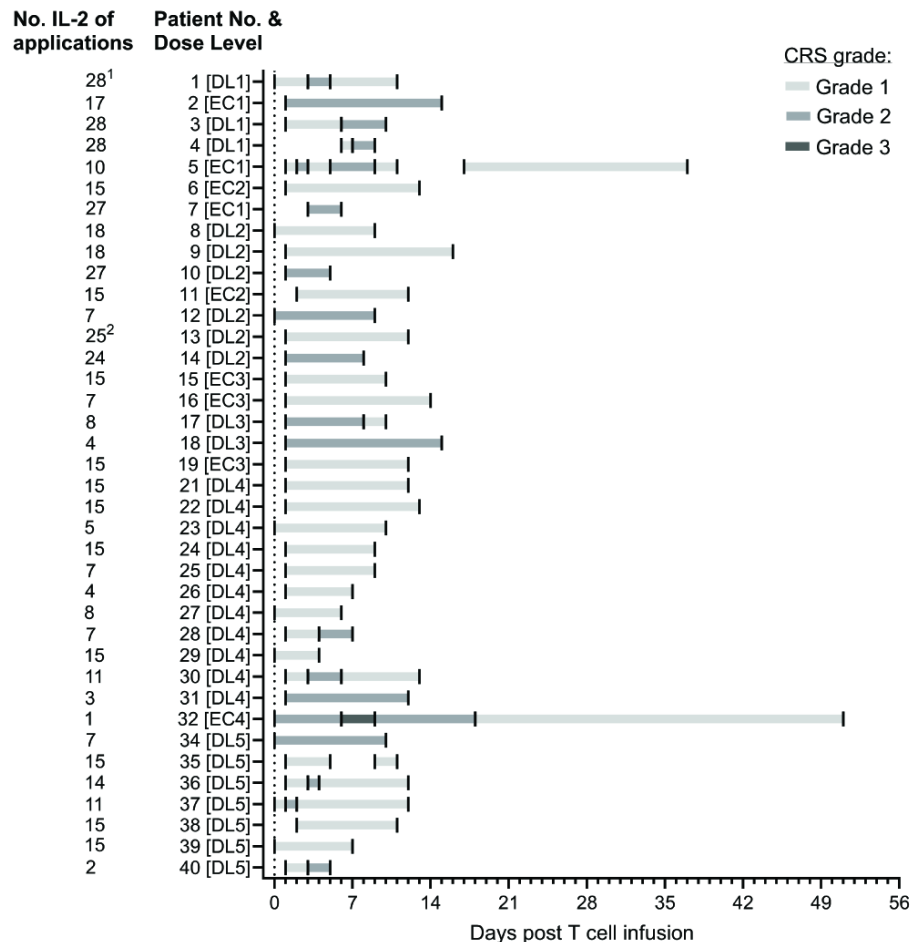
%CD3: CD3⁺ T cells out of viable cells; %CD8: CD8⁺ T cells out of CD3⁺ T cells; %Dex: dextramer-positive out of CD3⁺CD8⁺ T cells, as shown in adjacent gating strategy; VCN/td: vector copy number per transduced cell.



Supplementary Figure 6: Drug products manufactured from patients demonstrated *in vitro* cytotoxicity against high, medium, or low PRAME-expressing tumor cell lines

Cytotoxic potential of patient products against antigen-positive tumor cell lines. IMA203 T cells were co-cultured with PRAME-positive a) UACC257-RFP tumor cells at a 2:1 E:T ratio b) hs695T-RFP tumor cells at a 4:1 E:T ratio, and c) A375-RFP tumor cells at an 8:1 E:T ratio. Effectors were normalized to IMA203 TCR expression. Tumor fold growth was measured over a 64 h period using the IncuCyte live-cell analysis system; n=7 for UACC267 and hs695T; n=6 for A375. Graphs depict mean values and standard deviations.

Ag: antigen; cPR: confirmed partial response; E:T: effector-to-target ratio; PR: partial response; RFP: red fluorescent protein; SD: stable disease.

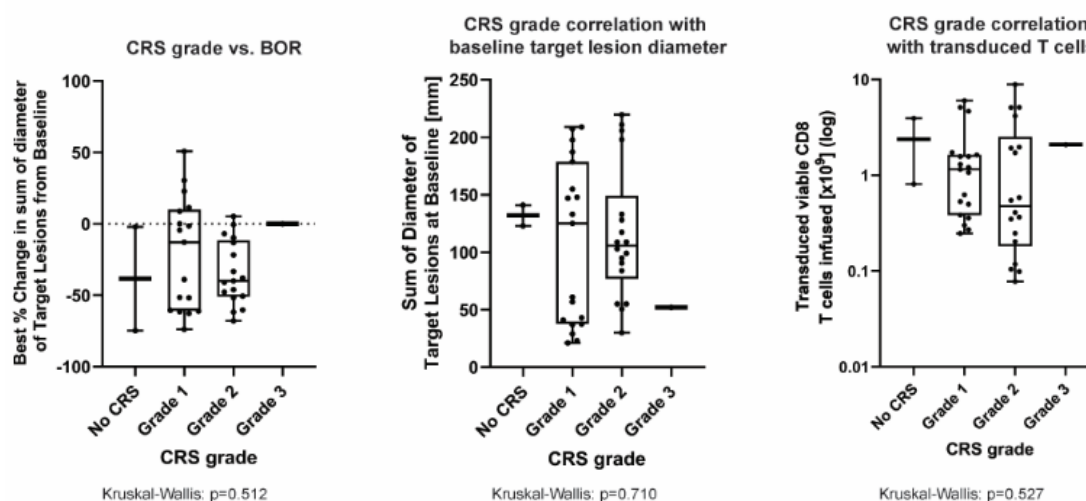


Supplementary Figure 7: Median duration of cytokine release syndrome in patients treated with IMA203 is 10 days

Swimmer plot showing CRS occurrence and severity in each patient over time. CRS was reported for 38 of 40 patients. The number of IL-2 applications for each patient are stated on the left. Two patients received a second infusion of IMA203, only CRS events after the first infusion are shown in the plot. The following CRS events were observed after the second infusion: Patient 18 (DL3): Grade 1 CRS from day 3 to 12, Grade 3 CRS from day 43 to 47 and Grade 1 CRS from grade 48 to 49 after T-cell infusion. Patient 23 (DL4): Grade 1 CRS from day 1 to 4 after T-cell infusion.

¹Patient received a dose of only 26.5×10^6 IU instead of 28×10^6 IU. ²Patient received a dose of only 22.5×10^6 IU instead of 28×10^6 IU.

a



b

CRS grade correlation with response

CRS grade	Responder	Non-Responder	Total
No CRS/Grade 1	9	11	20
Grade 2/Grade 3	11	7	18

Fisher's exact test: $p=0.352$

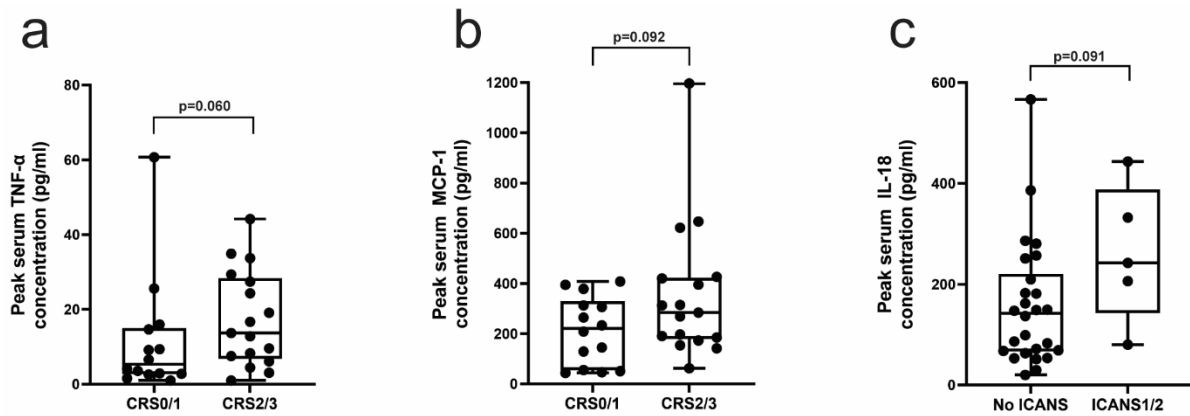
Supplementary Figure 8: Cytokine release syndrome is independent of response and T-cell product dose

Patients are only counted once with the highest CRS grade that was determined during the trial period. In case of exploratory second TCR T-cell infusion, the baseline characteristics, response information, and highest CRS grade of first infusion were used for analysis. a) Left: Best change in sum of target lesion diameter from baseline of patients with no CRS ($n=2$) or maximum severity of CRS Grade 1 ($n=17$), 2 ($n=17$), or 3 ($n=1$). No significant correlation was observed (Kruskal-Wallis, $p=0.512$). Middle: Sum of diameter of target lesions at baseline [mm] of patients with no CRS ($n=2$) or maximum severity of CRS Grade 1 ($n=19$), 2 ($n=18$), or 3 ($n=1$). No significant correlation was observed (Kruskal-Wallis, $p=0.710$). Right: T-cell dose (as number of total infused viable CD8⁺ T cells) of patients with no CRS ($n=2$) or maximum severity of CRS Grade 1 ($n=19$), 2 ($n=18$), or 3 ($n=1$). No significant correlation was observed (Kruskal-Wallis, $p=0.527$). Box plots depict box bounds as the first and third quartiles, with horizontal lines inside the boxes as the median. Whiskers reach the minimum and maximum

value of the data. Each dot in the plot represents one patient. b) Number of patients with no CRS/Grade 1 CRS (mild cases) or Grade 2/Grade 3 CRS grouped by response or no response. The association of CRS grade and response was assessed using Fisher's exact test, no significant difference was observed between responder and non-responder.

BOR: best overall response; cPR: confirmed partial response; CRS: cytokine release syndrome; PD: progressive disease; PR: partial response; SD: stable disease.

[#]Patients 36 (DL5) and 39 (DL5) were not included in the analysis since BOR could not yet be determined at data cut-off. *Patient 19 (DL3) was not included since change in sum of target lesion diameter was not evaluable.

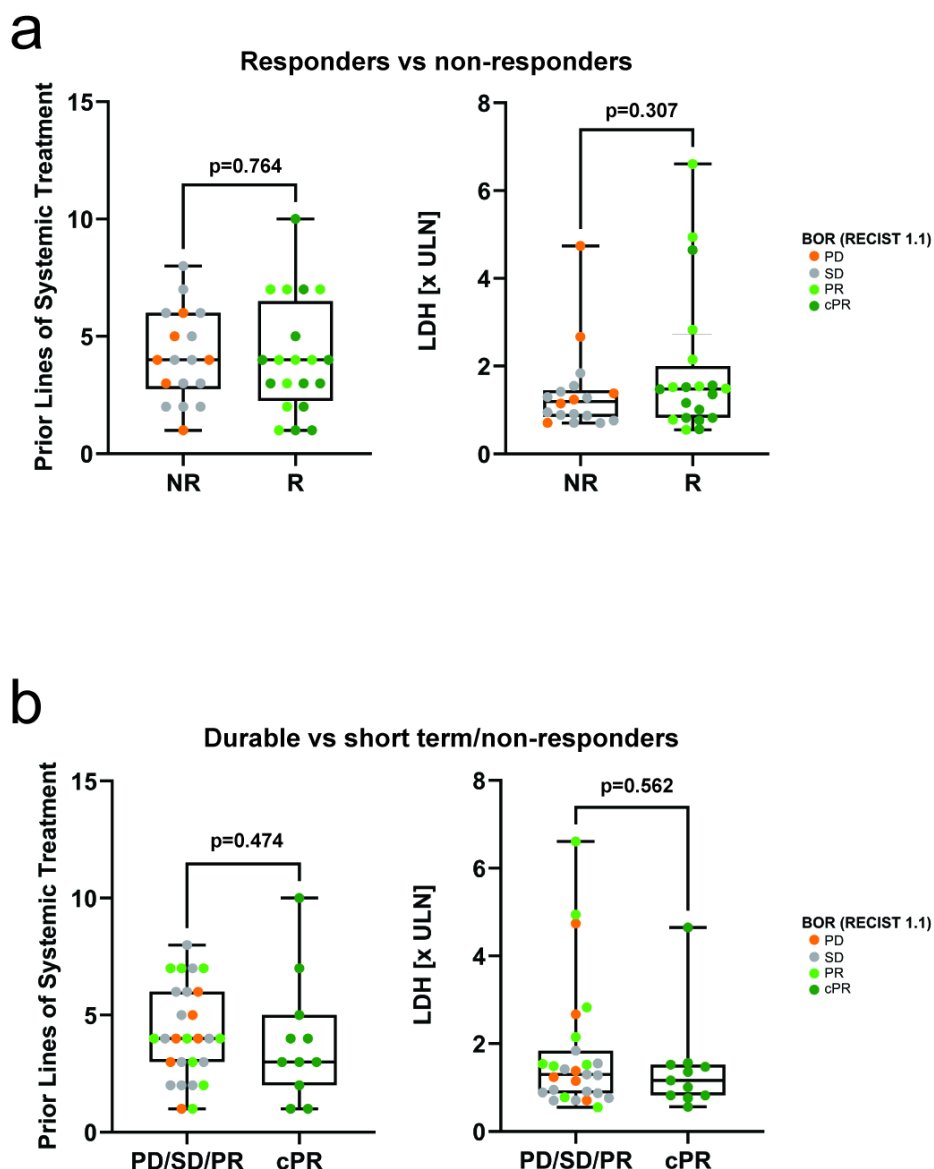


Supplementary Figure 9: Higher TNF- α and MCP-1 serum peak levels were associated with severity of cytokine release syndrome while higher IL-18 serum peak levels were associated with ICANS events

Serum cytokine profiles associated with CRS or ICANS. a) Peak serum TNF- α concentration [pg/ml]. b) Peak serum MCP-1 concentration [pg/ml] was analyzed for patients grouped with no CRS/Grade 1 CRS (mild cases) and Grade 2/Grade 3 CRS. c) Peak serum IL-18 concentration [pg/ml] was analyzed for patients with no ICANS and grouped with ICANS Grade 1/Grade 2.

For the association of serum cytokines with CRS grade or with ICANS two-tailed Mann Whitney statistical test was used (n=31). The p-values are depicted in the respective graphs. Box plots depict median as horizontal line within box, with box bounds as the first and third quartiles. The upper and lower whisker represents the maximum and minimum value of the given data. Dots represent individual data points.

CRS: cytokine release syndrome; ICANS: immune effector cell-associated neurotoxicity.



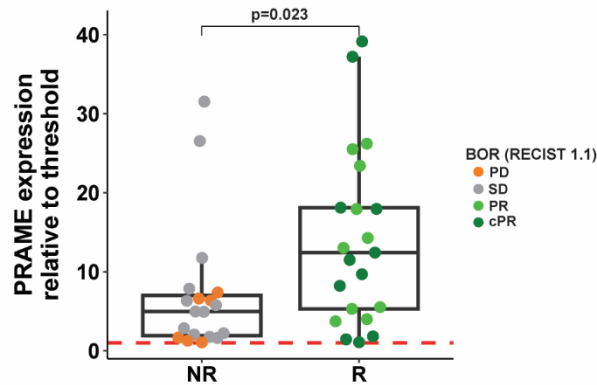
Supplementary Figure 10: Prior lines and lactate dehydrogenase at baseline do not impact response or durable response

No correlation between objective response rate and prior lines of treatment or LDH at baseline.

a) Patients were split into responder (R, includes PR and cPR, n=20) and non-responder (NR, includes PD and SD, n=18) based on BOR according to RECIST 1.1. No significant correlation was observed for the number of systemic treatment lines prior to IMA203 therapy (p=0.764) or LDH [xULN] at start of lymphodepletion prior to IMA203 therapy (p=0.307). b) Patients were split into long-term responder (cPR, n=11) and non-/short term responder (PD/SD/PR, n=27)

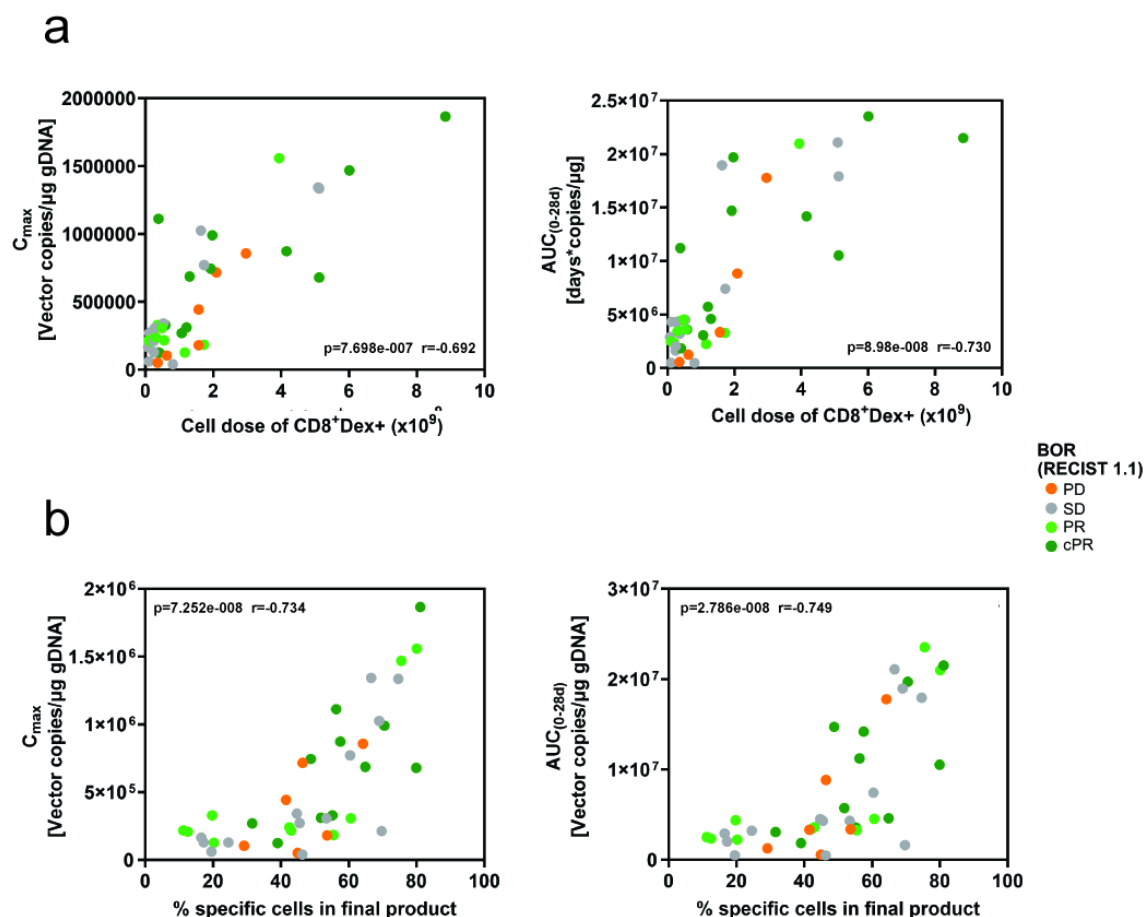
based on BOR according to RECIST 1.1. No significant correlation was observed for the number of systemic treatment lines prior to IMA203 therapy ($p=0.474$) or LDH [$\times$ ULN] at start of lymphodepletion prior to IMA203 therapy ($p=0.562$). Each point represents one patient and the respective BOR (color-coded) according to RECIST1.1. Patients 36 (DL5) and 39 (DL5) were not included in the analysis since BOR could not yet be determined at data cut-off. Group comparisons were performed using two-tailed Mann Whitney statistical test. Box plots depict median as horizontal line within box, with box bounds as the first and third quartiles. The upper and lower whisker represents the maximum and minimum value of the given data.

BOR: best overall response; cPR: confirmed partial response; DL: dose level; LDH: lactate dehydrogenase; NR: non-responder; PD: progressive disease; PR: partial response; R:responder; RECIST: Response Evaluation Criteria in Solid Tumors; SD: stable disease; ULN: upper limit of normal.



Supplementary Figure 11: PRAME expression is associated with clinical responses

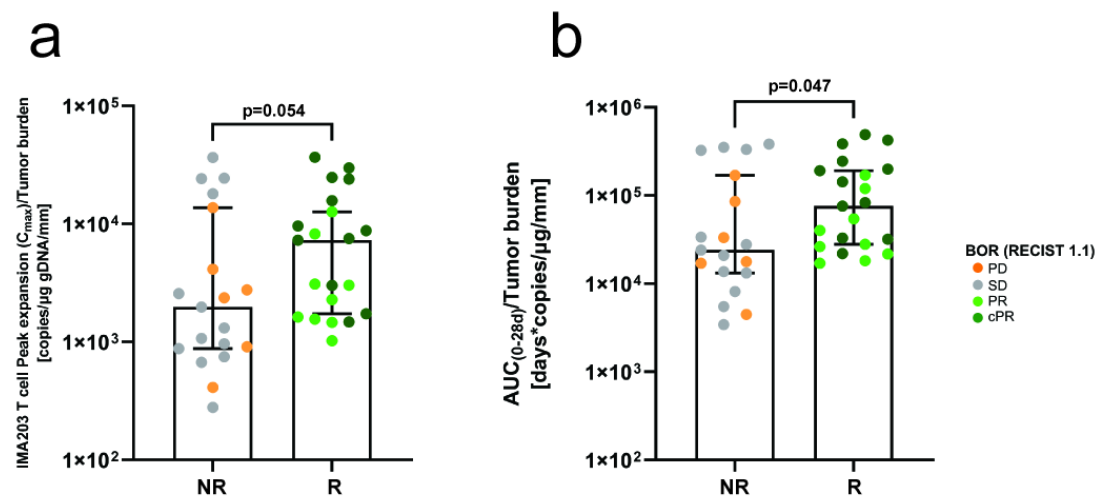
PRAME mRNA expression from patient tumor biopsies ($n = 40$) was measured by qPCR (IMADetect® assay). ΔCt values meeting the predefined threshold criteria (ΔCt threshold) were used as predictive for target peptide presentation. Measured ΔCt is normalized to the ΔCt threshold ($2^{-(\Delta\text{Ct}-\text{Threshold})}$). Two-sided Mann-Whitney U statistical test was used. Box plots depict median as horizontal lines within boxes, with box bounds as the first and third quartiles. Lower whisker is the minimum value of the data within 1.5 times the interquartile range below the 25th percentile. Upper whisker is the maximum value of the data within 1.5 times the interquartile range above the 75th percentile. BOR: best overall response; cPR: confirmed partial response; NR: non-responder; PD: progressive disease; PR: partial response; R: responder; SD: stable disease.



Supplementary Figure 12: IMA203 T-cell expansion post-infusion correlates with dose and transduction efficiency

Association of peak cell expansion (C_{max}) and $AUC_{(0-28d)}$ a) infused T-cell dose (viable CD8⁺ dextramer-positive) and b) %dextramer-positive in the viable CD3⁺CD8⁺ T-cell population of final product was assessed. Spearman correlation was used for correlation coefficient (r) and two-sided p -value calculation. Dots represent individual data points ($n=40$).

AUC: area under the curve; BOR: best overall response; cPR: confirmed partial response; CRS: cytokine release syndrome; PD: progressive disease; PR: partial response; SD: stable disease.



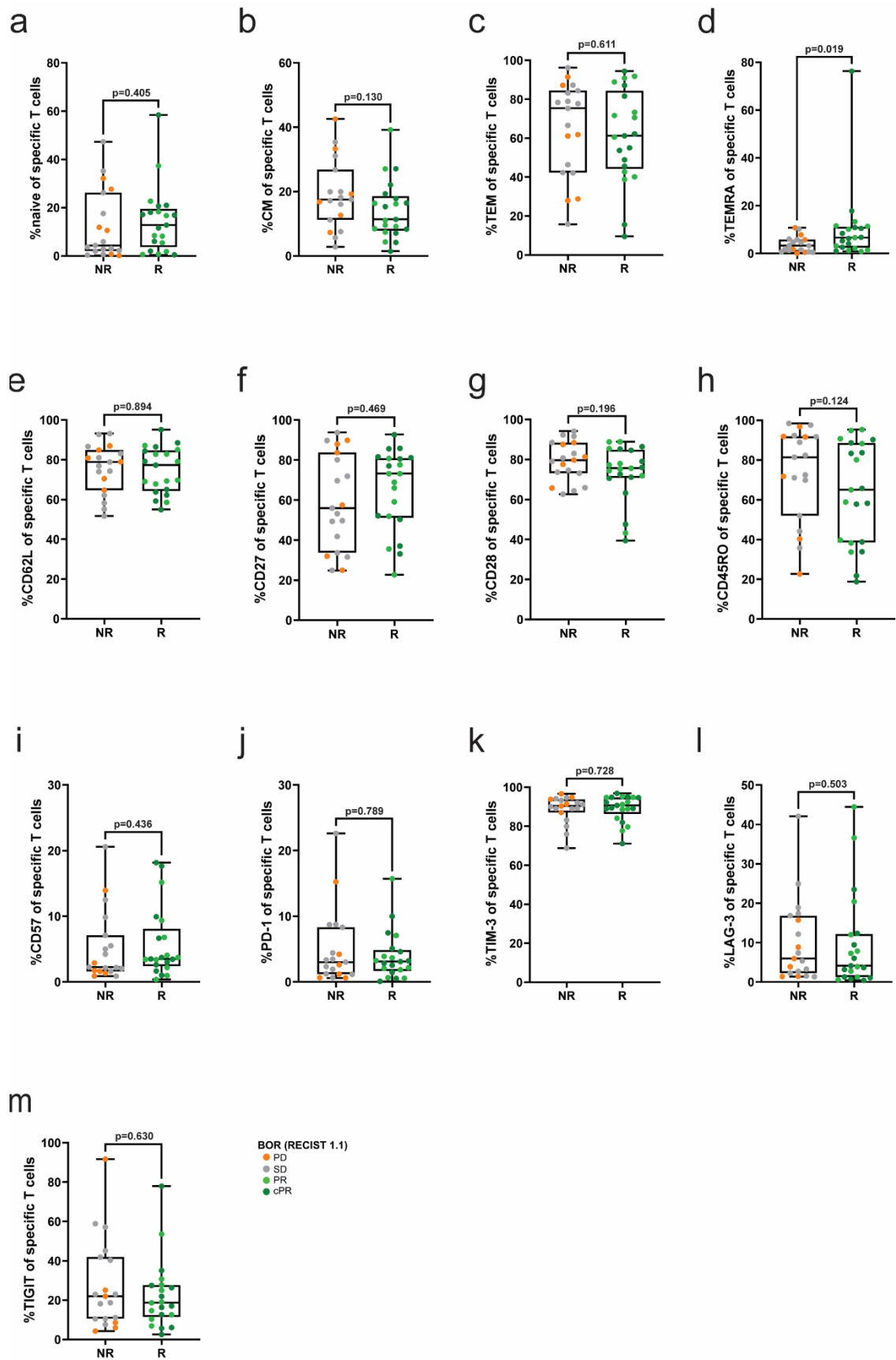
Supplementary Figure 13: Greater T-cell exposure normalized to tumor burden correlates with response

a) T-cell peak expansion (C_{max}) normalized to tumor burden is associated with clinical outcome.

b) Cumulative T-cell persistence over the first 28 days, expressed as $AUC_{(0-28d)}$, is associated with positive clinical outcome when normalized with tumor burden. Samples are grouped and each point represents one patient and the respective BOR (color-coded) according to RECIST 1.1. Statistical analysis was performed using Mann-Whitney U test, with 95% CI median. PD/SD: $n=19$; PR/cPR: $n=21$.

AUC: area under the curve; BOR: best overall response; cPR: confirmed partial response;

PD: progressive disease; PR: partial response; SD: stable disease.

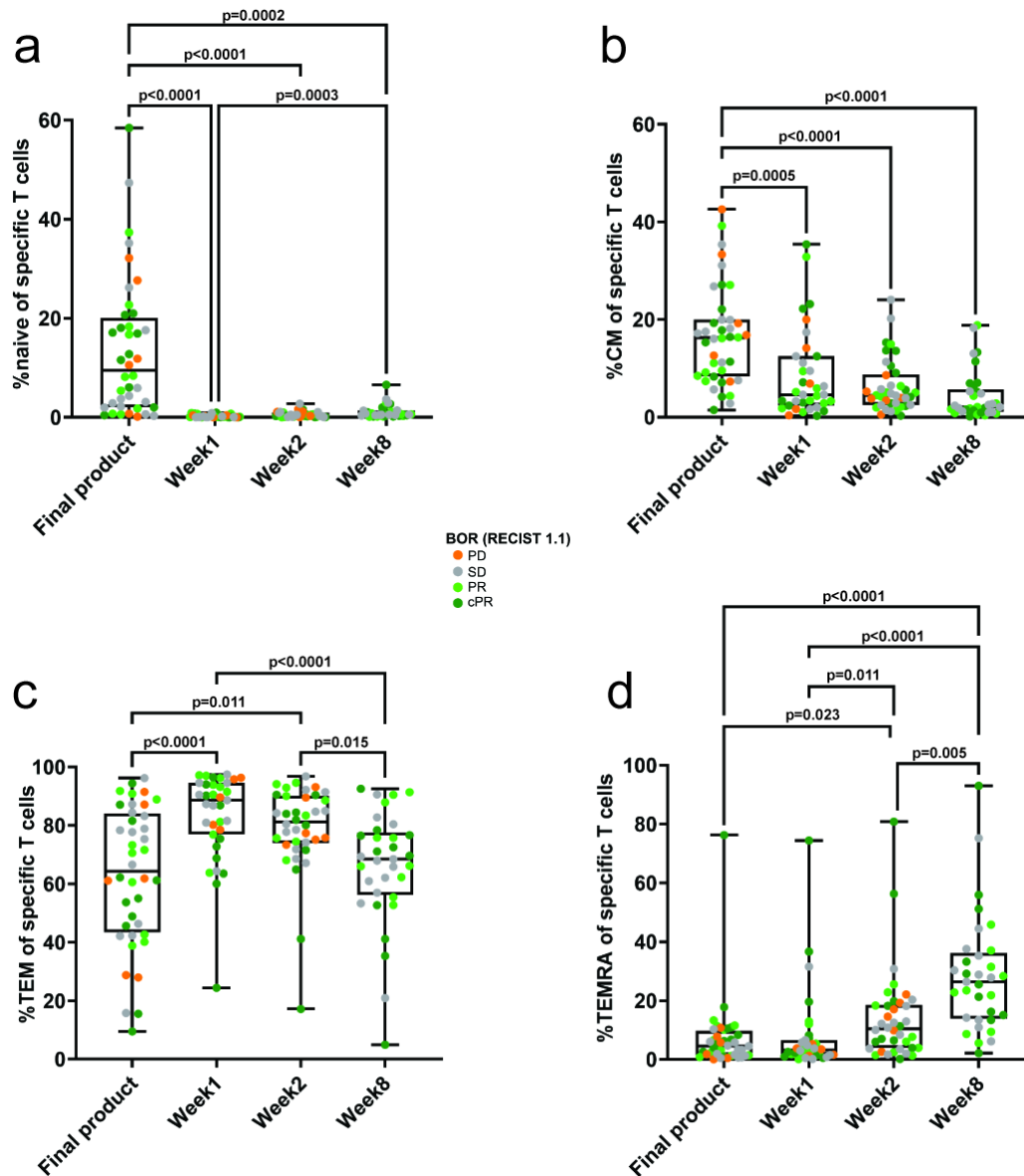


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Supplementary Figure 14: With the exception of TEMRA, presence of memory phenotype in drug product did not correlate with responses

Analysis of T-cell phenotypes according to clinical responses. Flow cytometric quantification of a) TN, b) CM, c) TEM, d) TEMRA and e) CD62L, f) CD27, g) CD28, h) CD45RO, i) CD57, j) PD-1, k) TIM-3, l) LAG-3, and m) TIGIT expression on CD3⁺CD8⁺ tetramer-positive-gated lymphocytes. Statistical analysis was performed using two-tailed Mann Whitney statistical test. Box plots depict median as horizontal line within box, with box bounds as the first and third quartiles. The upper and lower whisker represents the maximum and minimum value of the given data.. Each point represents one patient and the respective BOR (color-coded) according to RECIST1.1. Gating strategy is depicted in Supplementary Fig. 20.

CM: central memory T cells; TEM: effector memory T cells; TEMRA: effector memory T cells expressing CD45RA; TN: naïve T cells.

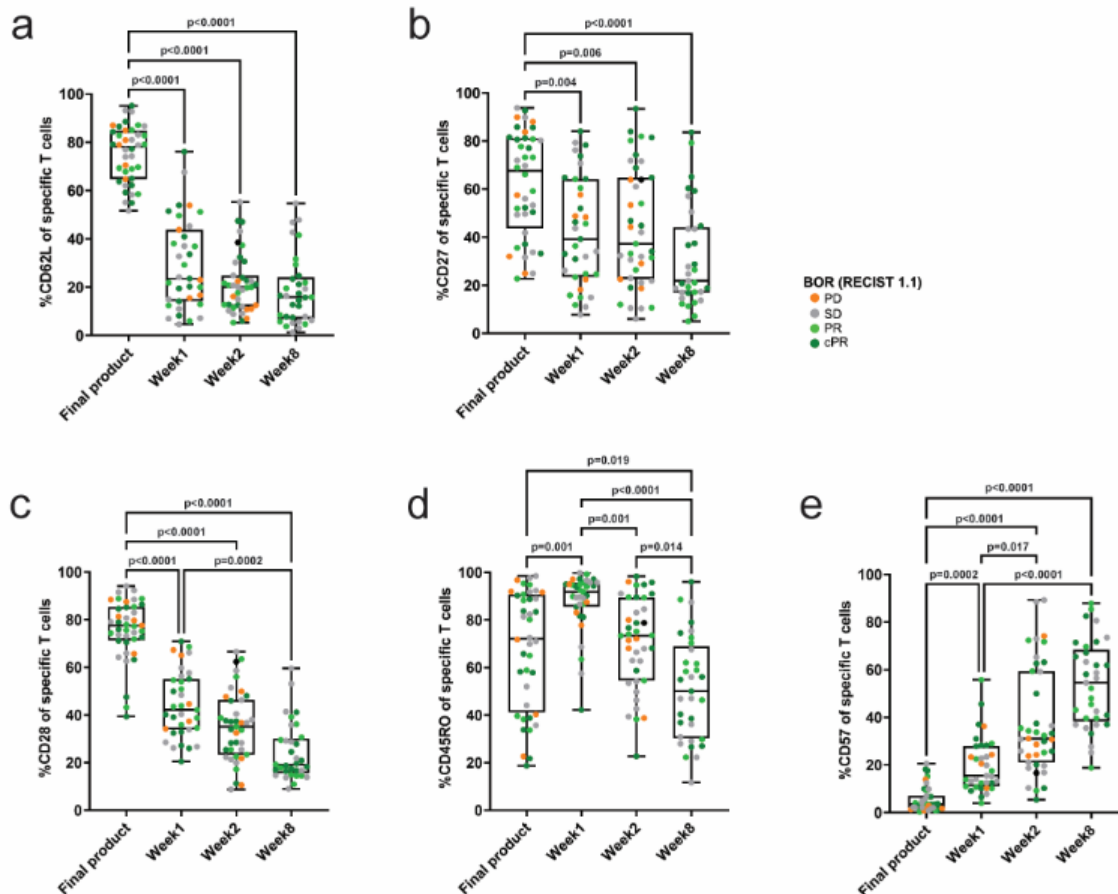


Supplementary Figure 15: Kinetics of naïve, central, effector and differentiated memory phenotype in peripheral blood

Longitudinal memory phenotyping of peripheral blood T cells. Frequency of memory T cell subsets of PRAME-specific T cells (tetramer-positive CD8⁺ T cells) in the final product (n=40) and post-infusion samples at week 1 (n=35), week 2 (n=38), and week 8 (n=33) for a) TN and memory phenotypes b) CM, c) TEM, and d) TEMRA. Statistical significance was assessed by Kruskal-Wallis one-way ANOVA and p-values were calculated by Dunn's multiple comparison tests. Boxes represent the interquartile range including the median value (line within boxes). Whiskers depict minimum and maximum values and each point represents one patient and

the respective BOR (color-coded) according to RECIST1.1. Gating strategy is depicted in Supplementary Fig. 20.

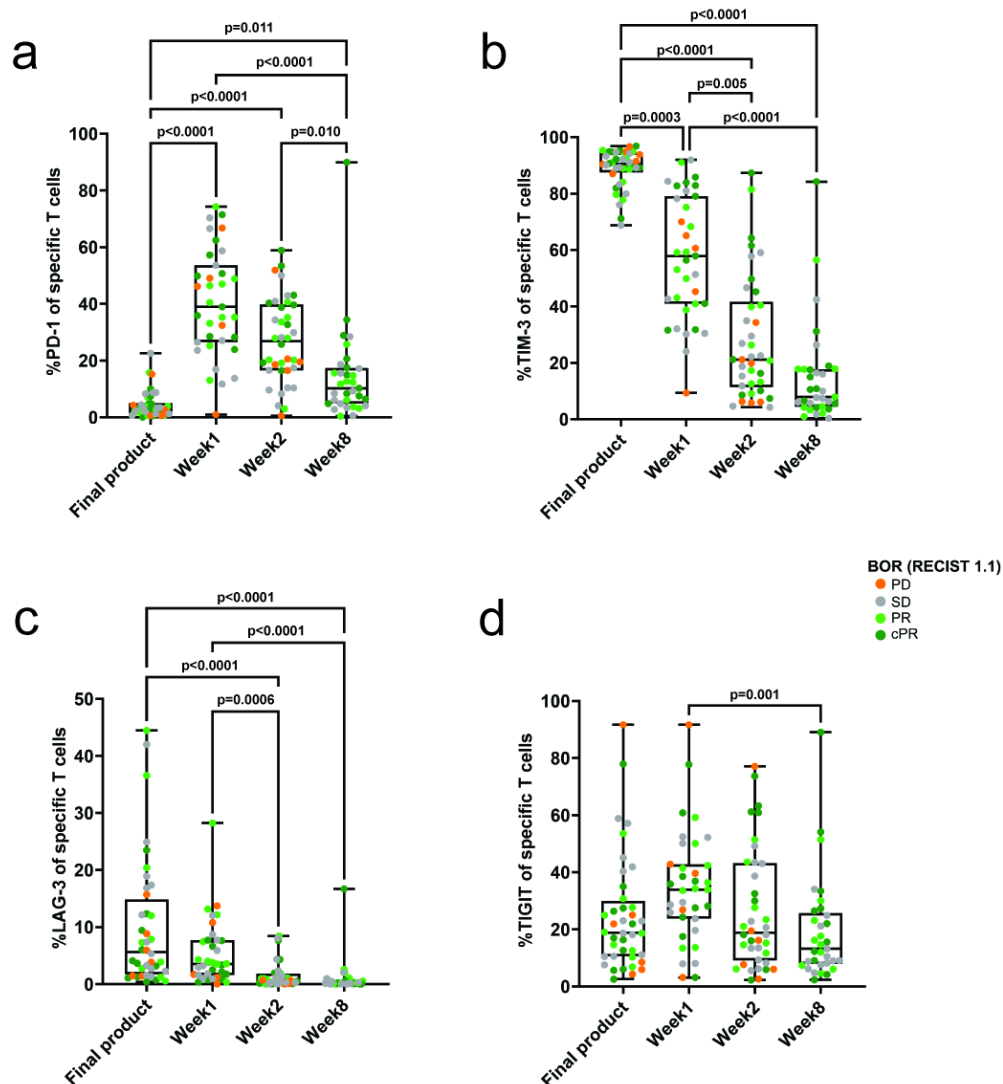
BOR: best overall response; CM: central memory T cells; cPR: confirmed partial response; PD: progressive disease; PR: partial response; RECIST: Response Evaluation Criteria in Solid Tumors; SD: stable disease; TEM: effector memory T cells; TEMRA: effector memory T cells expressing CD45RA; TN: naïve T cells.



Supplementary Figure 16: Kinetics of homing, activation, and exhaustion markers in peripheral blood

Longitudinal phenotyping of peripheral blood T cells. Frequency of memory/co-stimulation-related markers in T cell subsets of PRAME-specific T cells (tetramer-positive CD8⁺ T cells) in the final product (n=40) and post-infusion samples at week 1 (n=35), week 2 (n=38), and week 8 (n=33). a) CD62L, b) CD27, c) CD28, d) CD45RO, and e) CD57. Statistical significance was assessed by Kruskal-Wallis one-way ANOVA and p-values were calculated by Dunn's multiple comparison tests. Boxes represent the interquartile range including the median value (line within boxes). Whiskers depict minimum and maximum values and each point represents one patient and the respective BOR (color-coded) according to RECIST1.1. Gating strategy is depicted in Supplementary Fig. 20.

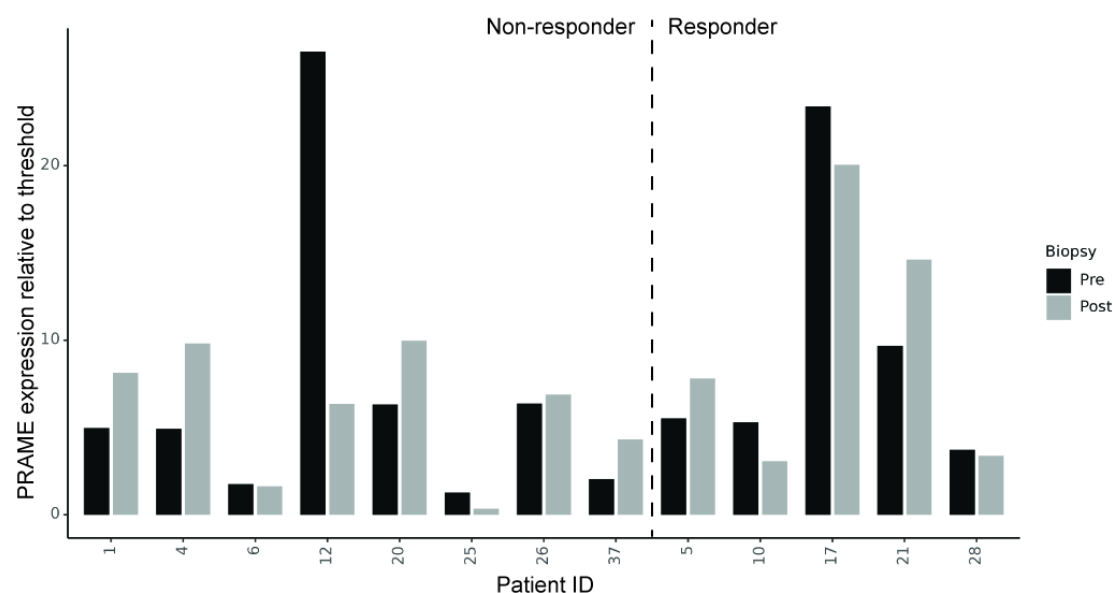
BOR: best overall response; cPR: confirmed partial response; PD: progressive disease; PR: partial response; RECIST: Response Evaluation Criteria in Solid Tumors; SD: stable disease.



Supplementary Figure 17: Kinetics of exhaustion phenotype in peripheral blood

Longitudinal analysis of activation/exhaustion profile of peripheral blood T cells. Frequency of activation/exhaustion-related markers in T cell subsets of PRAME-specific T cells (tetramer-positive CD8⁺ T cells) in the final product (n=40) and post-infusion samples at week 1 (n=35), week 2 (n=38), and week 8 (n=33). a) PD-1, b) TIM-3, c) LAG-3, and d) TIGIT. Statistical significance was assessed by Kruskal-Wallis one-way ANOVA and p-values were calculated by Dunn's multiple comparison tests. Boxes represent the interquartile range including the median value (line within boxes). Whiskers depict minimum and maximum values and each point represents one patient and the respective BOR (color-coded) according to RECIST1.1. Gating strategy is depicted in Supplementary Fig. 20.

BOR: best overall response; cPR: confirmed partial response; PD: progressive disease; PR: partial response; RECIST: Response Evaluation Criteria in Solid Tumors; SD: stable disease.



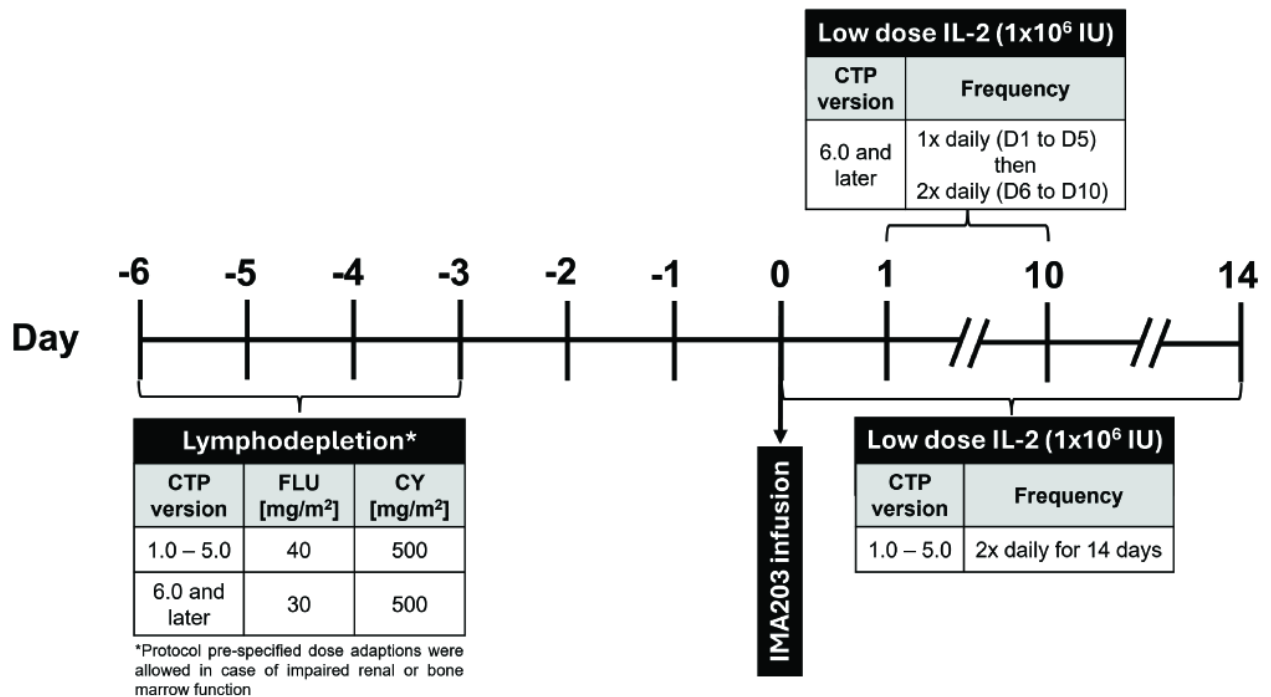
Supplementary Figure 18: PRAME is not downregulated in post-treatment biopsies

PRAME mRNA expression from patient pre- and post-treatment biopsies was measured by qPCR (validated in-house assay). Measured DCt is normalized to the DCt threshold.

No downregulation can be observed in post-treatment biopsies, besides in two non-responder (12, 25) and two responder (10, 17).

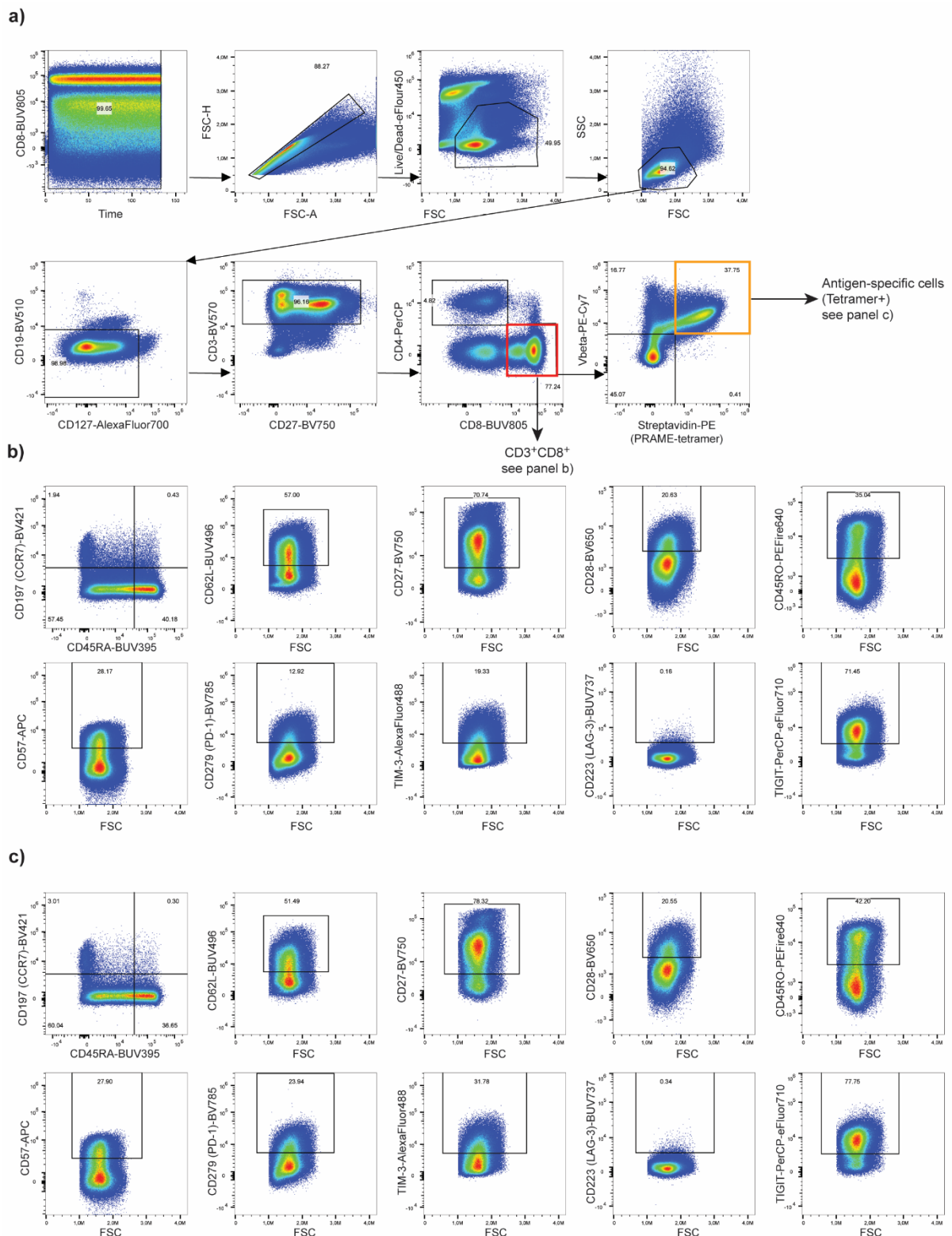
DCt: delta cycle threshold; qPCR: quantitative polymerase chain reaction.

Supplementary Figure 19: Treatment schedule



The figure depicts the treatment schedule according to the respective Clinical Trial Protocol versions consisting of a lymphodepletion regimen on four consecutive days, followed by the IMA203 infusion at day 0 and IL-2 treatment thereafter.

CTP: clinical trial protocol; CY: cyclophosphamide; D: day; FLU: fludarabine; IL: interleukin; IU: international units.



Supplementary Figure 20: Sequential gating strategy for flow cytometry analyses

a) Pre-gating strategy was applied to patient samples. b) Gating strategy to evaluate frequencies of memory, homing, activation and exhaustion markers within CD3⁺CD8⁺ T cells (pre-gated in panel a). c) To analyze antigen-specific cells, the same gating strategy as shown

in b) was applied. Sequential gating strategy in a) and b) was applied to Extended Fig. 3. Sequential gating strategy in a) and c) was applied to Extended Fig. 7, as well as Supplementary Fig. 14, 15, 16, and 17.