

T cell receptor gene therapy targeting KRAS G12V for advanced pancreatic cancer in a single-arm phase 1/2 clinical trial

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Adoptive T cell transfer targeting KRAS G12D can induce tumor regression in advanced epithelial cancers. The safety, efficacy of T cell receptor (TCR)-T cells targeting KRAS G12V, and potential benefit of repeat infusions remain unknown. In a single-arm phase 1/2 clinical trial (NCT04146298), we treated five patients with recurrent pancreatic cancer using adoptive transfer of autologous T cells engineered to express an HLA-A*11:01-restricted KRAS G12V₈₋₁₆-specific TCR. Three patients received multiple infusions. Primary endpoints were safety and objective response rate. The most frequent adverse event of grade 3 or higher was hematologic toxicities due to lymphodepletion. One patient with liver metastases experienced a complete response lasting 5.5 months, and two patients experienced short-term stable disease. TCR-T cells were detected in peripheral blood of four patients at 1 month after their first infusion. No clinical responses were observed with TCR-T retreatments. In two patients, there was evidence of hyper-acute rejection of TCR-T cells after retreatment, which was likely mediated by persistent antibodies targeting the engineered KRAS G12V-reactive TCR induced from the prior TCR-T cell infusion. This study provides early results demonstrating safety, therapeutic potential, and considerations for repeat infusion of HLA-A*11:01-restricted TCR-T cells targeting KRAS G12V in patients with recurrent pancreatic cancer.

INTRODUCTION

Adoptive cell transfer with tumor-infiltrating lymphocytes (TILs) reactive against cancer mutations, known as neoantigens,¹ has been shown to mediate objective tumor regression in individual patients with metastatic melanoma,² cholangiocarcinoma,³ colorectal

cancer,⁴ ovarian cancer,⁵ and breast cancer.^{5,6} Adoptive cell transfer with T cell receptor (TCR)-engineered T cells targeting neoantigens also has induced objective tumor regression in individual patients with metastatic breast cancer,⁵ pancreatic cancer,⁷ and colorectal cancer.⁸ Previous case reports demonstrated that adoptive cell transfer of TILs or TCR-T cells targeting a common oncogenic KRAS G12D mutation could induce durable tumor regression in one patient with metastatic colorectal cancer and one patient with metastatic pancreatic cancer, respectively.^{4,7} However, it is unknown whether adoptive cell transfer with TCR-T cells targeting other KRAS mutations such as KRAS G12V can mediate tumor regression in patients with metastatic epithelial cancer. Notably, more than 90% of patients with pancreatic ductal adenocarcinoma (PDAC) have

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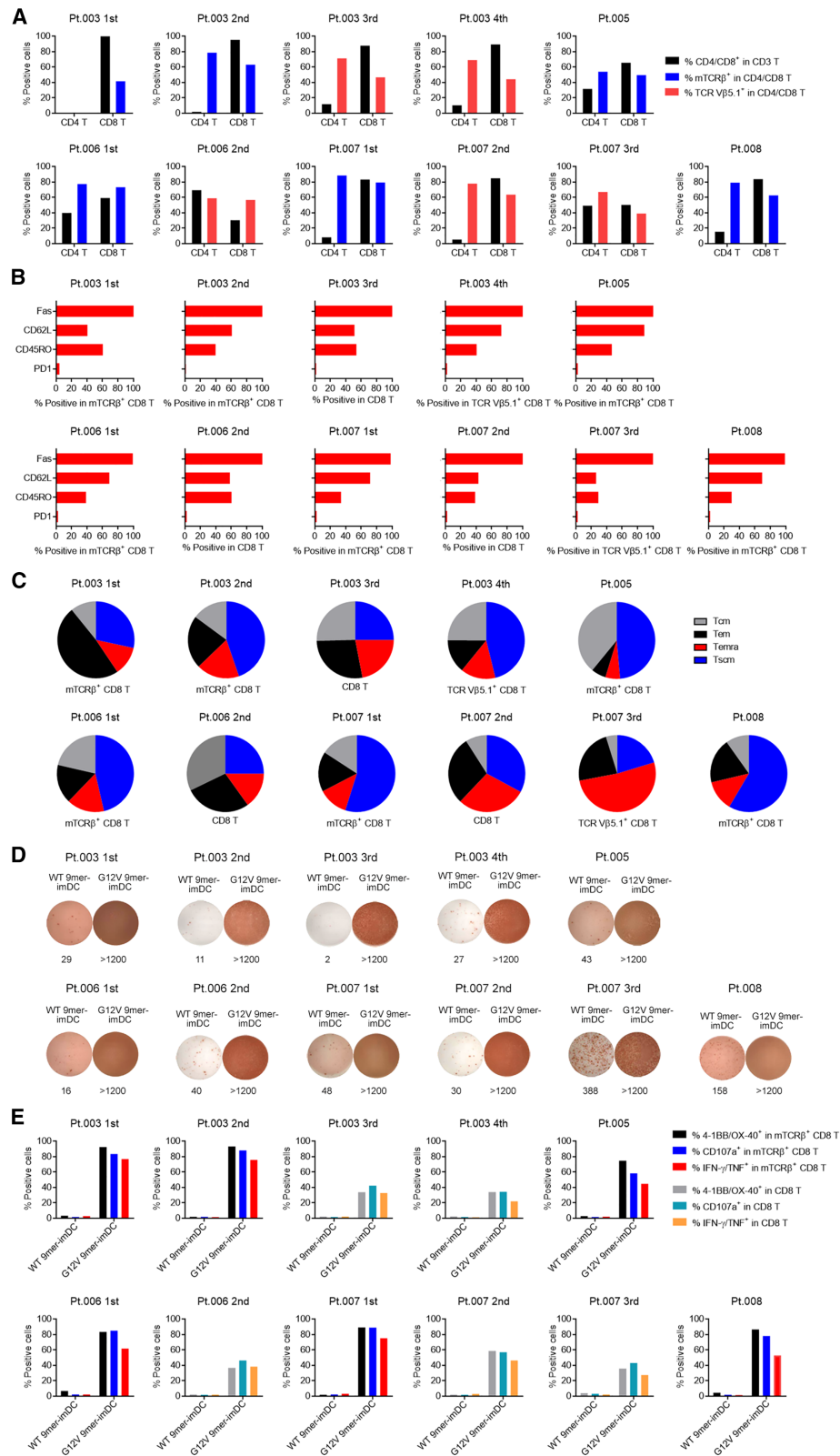
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KRAS mutations, the most prevalent being KRAS G12D (41%), G12V (34%), and G12R (16%).⁹ Recent preclinical studies have identified TCRs targeting these KRAS mutations that enable the testing of this hypothesis.^{10–17}

For adoptive cell transfer with autologous T cells including TILs, chimeric antigen receptor (CAR)-T cells, and TCR-T cells, typically only a single infusion is given to patients in most studies.^{8,18–24} In one early study, upon disease progression about 13 months after adoptive transfer of TILs containing about 25% ERBB2IP mutation-specific T_H1 cells, a patient with metastatic cholangiocarcinoma was retreated with TILs containing a >95% pure population of ERBB2IP mutation-reactive T_H1 cells and experienced a deeper and longer lasting tumor regression.³ Repeat TIL infusions in patients with metastatic melanoma was feasible but mediated no objective responses, although it appeared that no lymphodepletion was used in this study.²⁵ More recently, repetitive intraventricular dosing of B7-H3 CAR-T cells in pediatric and young adult patients with diffuse intrinsic pontine glioma was found to be tolerable with clinical activity worthy of further investigation.^{26,27} For TCR-T cell therapy, it is unknown whether repeat infusions in patients with metastatic epithelial cancer will be tolerable and effective.

Previously, we identified an HLA-A*11:01-restricted TCR targeting the KRAS G12V_{8–16} (VVGAVGVGK) neoepitope in a patient with pancreatic cancer,²⁸ which recognized the identical 9-amino-acid-long neoepitope as another reported TCR.¹⁰ Furthermore, T cells engineered to express this TCR specifically recognized and killed pancreatic cancer organoids and slowed the growth of an established organoid-derived xenograft in immunodeficient mice,²⁸ demonstrating that this 9-mer KRAS G12V neoepitope is naturally processed and presented by cancer cells at sufficient levels to be recognized by the TCR, consistent with mass spectrometry-based detection of this neoepitope in cancer cells by others.¹⁷ Given that virtually all patients with advanced PDAC will die of their disease despite the best standard of care treatments such as gemcitabine with Nab-paclitaxel, FOLFIRINOX, and nanoliposomal irinotecan with fluorouracil,^{29,30} we initiated an open-label, single-arm phase 1/2 clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04146298) NCT04146298) to evaluate the safety and activity of adoptive transfer of autologous T cells genetically engineered to express an HLA-A*11:01-restricted mutant KRAS G12V_{8–16}-specific TCR in HLA-A*11:01⁺ patients with advanced pancreatic cancer that expressed mutant KRAS G12V. We also investigated the feasibility of administering repeat infusions

to patients upon disease progression. Here, we report the results of the non-prespecified interim analysis of this ongoing trial, including treatment-related adverse events (AEs), clinical responses, T cell persistence, plasma cytokine levels, and plasma anti-TCR antibodies.

RESULTS

Manufacture and characterization of HLA-A*11:01-restricted KRAS G12V-reactive TCR-T cell products

To generate TCR-T cell products, cryopreserved peripheral blood mononuclear cells (PBMCs) were thawed, stimulated with polymeric nanomatrix conjugated to humanized CD3 and CD28 agonist (GMP-grade T cell TransAct) and IL-2, and transduced with lentiviral particles encoding the TCR. Given the CD8 coreceptor dependency of our TCR,²⁸ if TCR-T cells contained less than 30% CD8 T cells, we depleted CD4 T cells using CD4 microbeads. Then, about 9–14 days after the initial stimulation, transduced T cells underwent a rapid expansion protocol using irradiated feeder cells, IL-2, and anti-CD3 to further expand the cells. Subsequently, about 10–17 days after the second stimulation, the cells were harvested and adoptively transferred into the autologous patient ([Figures S1–S11](#)). All T cell products given to patients passed quality control testing and release testing criteria in accordance with the certificate of analysis including sterility, endotoxin, TCR expression, replication competent lentivirus, cell viability, and viable cell number ([Table S1](#)).

For the first infusion of all five patients, our TCR (TCR-001) contained the murine TCR- α and TCR- β constant regions, which promote the pairing of the introduced TCR, facilitate detection of the engineered T cells, and have been used in clinical studies.^{5,7,8} This autologous product containing human-mouse hybrid TCR-001-transduced T cells is referred to as LV-TCR-001-m Td. In three patients (Pt.003, Pt.006, and Pt.007) who received repeat infusions, a different and fully human TCR (LV-4148 TCR-h Td) that recognizes the same HLA-A*11:01-restricted KRAS neoepitope¹⁰ was used to avoid the risk of rejection in the event that antibodies formed against the murine TCR sequences present in the initial infusion products.

Furthermore, we characterized the phenotype and *in vitro* function of the TCR-T cell products. As shown in [Figures 1A–1C](#), there was considerable variability of transduction efficiency between patients and TCRs (CD8 T, 38.0%–78.3%; CD4 T, 52.8%–87.5%; [Table S2](#)), with very few PD1⁺ T cells, >97% Fas⁺ T cells and a variable frequency of T cell subsets including stem cell memory T (Tscm; CD62L⁺

Figure 1. Characterization of the infusion product for five patients undergoing a total of 11 cell infusions

(A) Flow cytometric analysis of the frequency of CD4 and CD8 T cells and transduction efficiency of CD4 and CD8 T cells as measured by detection of mouse TCR β constant chain, which is engineered into TCR-001, or detection of human TCR V β 5.1, which is expressed by 4148 TCR. (B and C) Flow cytometric analysis of select cell surface proteins (B) and T cell subsets (C) on the infusion product is shown. Data are gated on mTCR β ⁺ CD8 T cells, CD8 T cells, or TCR V β 5.1⁺ CD8 T cells as indicated. Tscm: CD62L⁺ and CD45RO[−]; Tcm: CD62L⁺ and CD45RO⁺; Tem: CD62L[−] and CD45RO⁺; Temra: CD62L[−] and CD45RO[−]. (D and E) IFN- γ ELISPOT images (D) and 4-1BB/OX-40 expression on mTCR β ⁺ CD8 T cells or CD8 T cells (E) are shown after an overnight coculture of the infusion product with HLA-A*11:01⁺ healthy donor-derived imDC pulsed with KRAS wild-type (WT) or KRAS G12V 9-mer peptide. Flow cytometric analysis of CD107a expression and IFN- γ /TNF intracellular production gated on mTCR β ⁺ CD8 T cells or CD8 T cells (E) is shown after 5-h stimulation of the infusion product with HLA-A*11:01⁺ healthy donor-derived imDC pulsed with KRAS WT or KRAS G12V 9-mer peptide.

Table 1. Clinical characteristics and responses of PDAC patients receiving KRAS G12V A11 TCR-T cell therapy

Patient	Pt.003	Pt.005	Pt.006	Pt.007	Pt.008
Gender	male	male	male	male	male
Age at screening	63	58	59	59	59
Primary Tumor type	PDAC	PDAC	PDAC	PDAC	PDAC
Primary Tumor position	body of pancreas	head of pancreas	head of pancreas	body of pancreas	body of pancreas
Prior treatments	1. Gemcitabine+Nab-paclitaxel, 6 cycles; 2. surgery; 3. S-1, 9 months	1. mFOLFIRINOX+a-PD1 (Keytruda), 6 cycles; 2. surgery; 3. mFOLFIRINOX+a-PD1 (Keytruda), 10 cycles; 4. S-1+a-PD1(Keytruda), 5 cycles; mFOLFIRINOX, 2 cycles	1. surgery; 2. Gemcitabine+Nab-paclitaxel, 8 cycles; 3. mFOLFIRINOX, 5 cycles	1. surgery; 2. Gemcitabine+Nab-paclitaxel, 6 cycles	1. Gemcitabine+Nab-paclitaxel, 3 cycles; 2. surgery; 3. Gemcitabine+oxaliplatin, 3 cycles; mFOLFIRINOX+Trimetinib+a-PD1 (Sintilimab), 5 cycles
Metastatic sites (sum of longest diameters)	liver (40.8 mm)	liver (15.0 mm)	around celiac trunk, retroperitoneum (26.3 mm)	peri-pancreas (16.5 mm)	liver, stomach, adrenal gland and retroperitoneal lymph node (71.0 mm)
CA19-9 baseline level (U/mL)	5716.1	191.6	995.9	1341.9	1548.2
ECOG performance status	1	1	0	0	1
KRAS mutation (frequency)	KRAS G12V (7.1%)	KRAS G12V (17.0%)	KRAS G12V (15.0%)	KRAS G12V (19.0%)	KRAS G12V (5.0%)
HLA-I	A*11:01/A*02:01; B*13:01/B*51:01; C*03:04/C*14:02	A*33:03/A*11:01; B*58:01/B*40:01; C*03:02/C*04:01	A*24:02/A*11:01; B*54:01/B*40:01; C*01:02/C*07:02	A*02:01/A*11:01; B*39:01/B*15:01; C*01:02/C*07:02	A*02:01/A*11:01; B*35:01/B*15:11; C*03:03/C*04:01
Best overall response	1st, CR (23.4 weeks); 2ND, PD; 3rd & 4th, SD (7.9 weeks)	PD	1st, SD (9.9 weeks); 2ND, PD	1st, PD; 2ND, PD; 3rd, not evaluated	SD (7.3 weeks)
PFS	29.4 weeks	6 weeks	15.9 weeks	4.7 weeks	14.3 weeks
OS	16.0 months	5.8 months	12.6 months	18.0 months	3.9 months

CD45RO⁺), central memory T (Tcm; CD62L⁺ CD45RO⁺), effector memory T (Tem; CD62L⁺ CD45RO⁺), and terminally differentiated CD45RA⁺ effector memory T (Temra; CD62L⁺ CD45RO⁺) in the TCR-T cell products.³¹ Our T cell expansion process appeared to generate a notable frequency of Tscm subsets (ranging from 20.3% to 58.5% with a median of 44.7%) despite a relatively long cell manufacture time (ranging from 20 days to 30 days with a median of 26 days). Both TCR-001-engineered and 4148 TCR-engineered T cell products were highly specific for the KRAS G12V₈₋₁₆ 9-mer, and the transduced CD8 T cells upregulated high levels of 4-1BB/OX-40 and CD107a and produced effector cytokines including IFN- γ and TNF when stimulated *in vitro* with HLA-A*11:01-expressing healthy donor-derived imDC pulsed with 1 μ g/mL KRAS G12V₈₋₁₆ 9-mer peptide (Figures 1D and 1E).

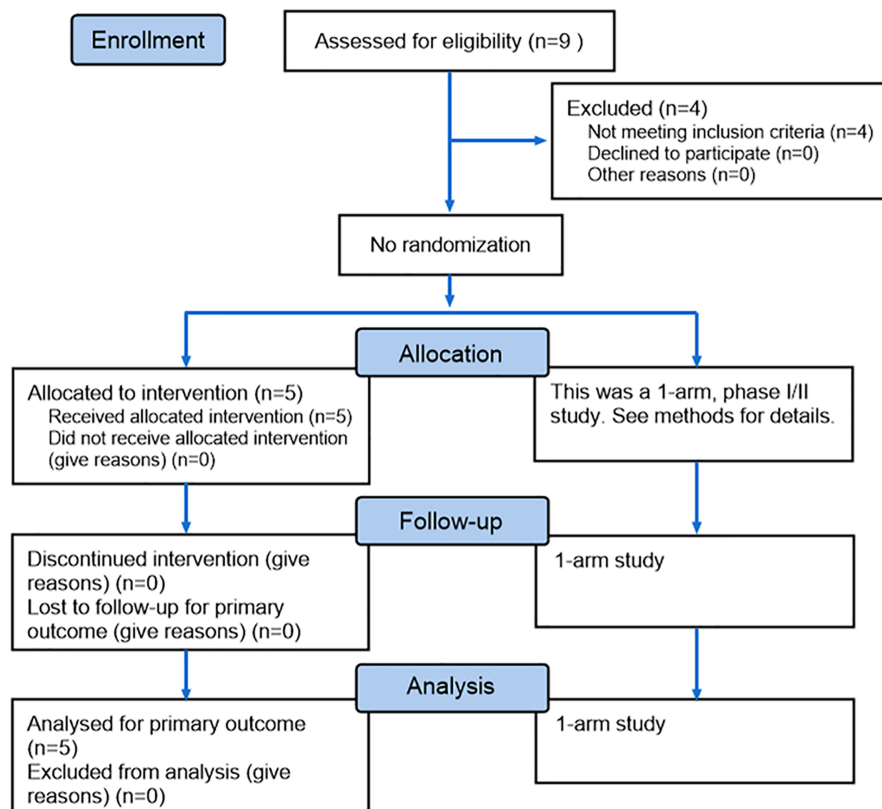
Patient and treatment characteristics

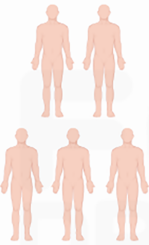
Between September 2021 and July 2023, five patients with recurrent pancreatic cancer were enrolled for TCR-T treatment on our ongoing study (Table 1; Figure 2A; Table S3). These five patients previously received standard therapies including neoadjuvant chemotherapy (for Pt.003, Pt.005, and Pt.008), radical resection surgery, and adjuvant chemotherapy and had disease progression with primary tumor recurrence or metastasis before the first TCR-T treatment (Table 1). Whole-

exome sequencing of their primary tumors demonstrated that they were HLA-A*11:01⁺ patients with KRAS G12V mutation in tumor (Table 1). Patients received a non-myeloablative lymphodepletion regimen consisting of 500 mg/m²/day cyclophosphamide and 30 mg/m²/day fludarabine for 3 days (Table S2), which significantly depleted lymphocytes and monocytes but not neutrophils, platelets, and hemoglobin (Figure S12), prior to infusion with autologous HLA-A*11:01-restricted KRAS G12V₈₋₁₆ reactive TCR-T cell products including human-mouse hybrid TCR-001-transduced T cells (LV-TCR-001-m Td) or the fully human 4148 TCR-transduced T cells (LV-4148 TCR-h Td) (Figures 2B, S1–S11C, and 3A). Three patients (Pt.003, Pt.006, and Pt.007) received TCR-T cell retreatment upon disease progression (Figure 2B). During some of the cell infusions, the anti-human PD-1 drug Tislelizumab was administered (Table S2; Figure 3A) at the discretion of investigators. No IL-2 was given to patients.

Treatment-related adverse events

One of the primary objectives was to evaluate the frequency and severity of treatment-related AEs after TCR-T cell infusion. As shown in Table S4, there was a total of 11 cell infusions for the 5 patients, and the reported AEs of all grades included hematological toxicity in all five patients (100%), cytokine release syndrome (CRS) in all five patients (100%) and 8 of 11 treatments (73%),

A**B**

	# infusion	Lymphodepletion	TCR-T cells	a-PD1	Follow-up
PDAC patients enrollment 	Pt.003	1st	✓	LV-TCR-001-m Td	✗
		2nd	✓	LV-TCR-001-m Td	✗
		3rd	✗	LV-4148 TCR-h Td	✓
		4th	✓	LV-4148 TCR-h Td	✓
	Pt.005	1st	✓	LV-TCR-001-m Td	✗
	Pt.006	1st	✓	LV-TCR-001-m Td	✓
		2nd	✓	LV-4148 TCR-h Td	✓
	Pt.007	1st	✓	LV-TCR-001-m Td	✓
		2nd	✓	LV-4148 TCR-h Td	✓
		3rd	✓	LV-4148 TCR-h Td	✗
	Pt.008	1st	✓	LV-TCR-001-m Td	✓

Primary objectives
 —Safety assessment
 —Objective response rate (ORR)

Secondary objectives
 —TCR-T persistence
 —Overall survival (OS)

Figure 2. CONSORT diagram and flowchart of the clinical study

Description of CONSORT diagram (A) and flowchart (B) of the clinical trial.

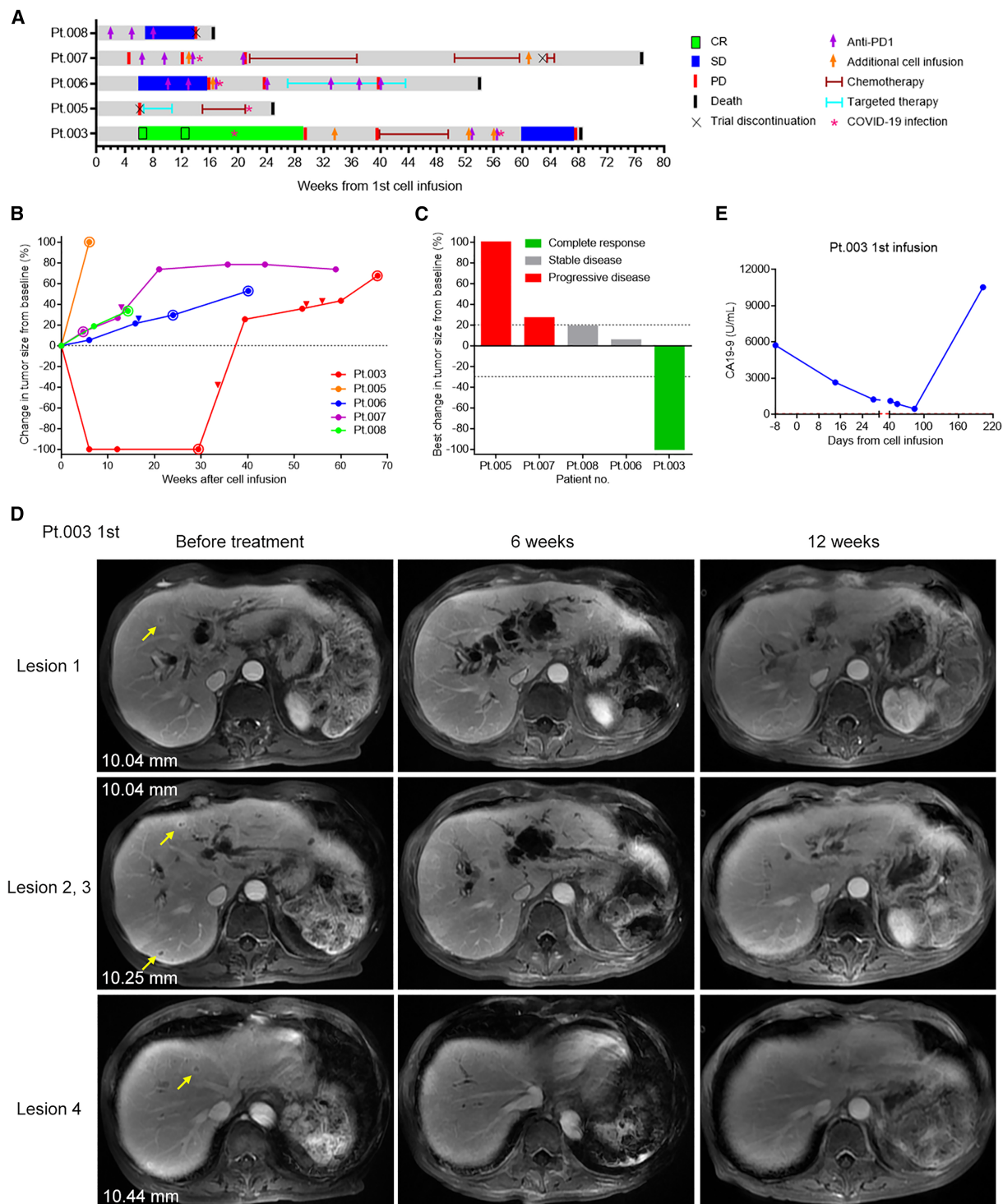


Figure 3. Clinical responses to therapy with autologous T cells genetically engineered to express an HLA-A*11:01-restricted mutant KRAS G12V-specific TCR
 (A) Clinical event timeline for five patients with recurrent pancreatic cancer from first infusion of TCR-T cells. (B) Spider plot showing percent change in target tumor measurements. Hollow circles indicate progressive disease with new lesions. Solid triangles indicate additional cell infusion. (C) Waterfall plot of the best clinical response for each evaluable patient. (D) Contrast-enhanced magnetic resonance (MR) images before and 6 weeks and 12 weeks after the first cell infusion of TCR-001-transduced T cells in Pt.003. Yellow arrows highlight site of liver lesions before therapy. (E) Serum concentrations of CA19-9 before and after the first cell infusions in Pt.003.

Table 2. Summary of hematological toxicity

Adverse events	Pt.003				Pt.005	Pt.006		Pt.007			Pt.008
	1st	2nd	3rd*	4th		1st	2nd	1st	2nd	3rd	
Lymphopenia	Gr4 (D1)	Gr4 (D5)	—	Gr4 (D7)	Gr4 (D7)	Gr4 (D28)	Gr4 (D3)	Gr4 (D3)	Gr4 (D7)	Gr4 (D7)	Gr4 (D7)
Anemia	Gr2	Gr2	Gr3 (No)	Gr3 (No)	Gr2	Gr1	Gr2	—	Gr1	Gr2	Gr1
Neutropenia	—	Gr1	—	—	Gr1	Gr3 (D14)	Gr2	—	—	Gr1	Gr1
Leukopenia	—	Gr3 (D1)	—	—	Gr2	Gr3 (D28)	Gr3 (D1)	—	—	Gr3 (D1)	Gr1
Thrombocytopenia	—	—	—	Gr2	Gr3 (D7)	—	—	Gr1	—	Gr2	—

The time in brackets indicates the time when recovered to $\leq$ grade 2 and “No” in parentheses indicates no recovery to $\leq$ grade 2. “*” indicates that the patient didn’t receive lymphodepletion. “—” = no adverse events reported.

chills/pyrexia in all five patients (100%) and 8 of 11 treatments (73%), hypotension in 1 of 5 patients (20%) and 1 of 11 treatments (9%), fatigue in 1 of 5 patients (20%) and 1 of 11 treatments (9%), and erythema in 1 of 5 patients (20%) and 1 of 11 treatments (9%). The most frequently reported AEs of grade 3 or higher were lymphodepletion-related hematologic toxicities in all five patients (100%) and 10 of 11 treatments (91%), including leukopenia in 3 of 5 patients (60%) and 4 of 11 treatments (36%), neutropenia in 1 of 5 patients (20%) and 1 of 11 treatments (9%), lymphopenia in 5 of 5 patients (100%) and 10 of 11 treatments (91%), thrombocytopenia in 1 of 5 patients (20%) and 1 of 11 treatments (9%), and anemia in 1 of 5 patients (20%) and 2 of 11 treatments (18%), which occurred within 28 days after cell infusion and generally recovered within 28 days with a median of 7 days (Table 2). Persistent grade 3 anemia was observed in Pt.003 prior to the third cell infusion. No CRS of grade 3 or higher occurred, and grade 1 and grade 2 CRS mostly was observed within 1 day of cell infusion in all reported patients and was self-limiting within 3 days without tocilizumab treatment. Notably, body temperature rose to nearly 40°C about 1–3 h after cell infusion for Pt.003’s second TCR-T treatment and Pt.007’s third TCR-T treatment, potentially due to hyper-acute rejection against the TCR-T cell products (Figure S13). Temperatures returned to normal after receiving indomethacin and physical cooling. About 12 h after the second cell infusion, Pt.003 also experienced hypotension, was treated with norepinephrine, and recovered within 2 days. Pt.008 had erythema on body trunk and limbs 1 day after cell infusion and recovered after about 1 week. No neurotoxicity was observed, and there were no treatment-related deaths.

Clinical responses and hyper-acute rejection of TCR-T cells

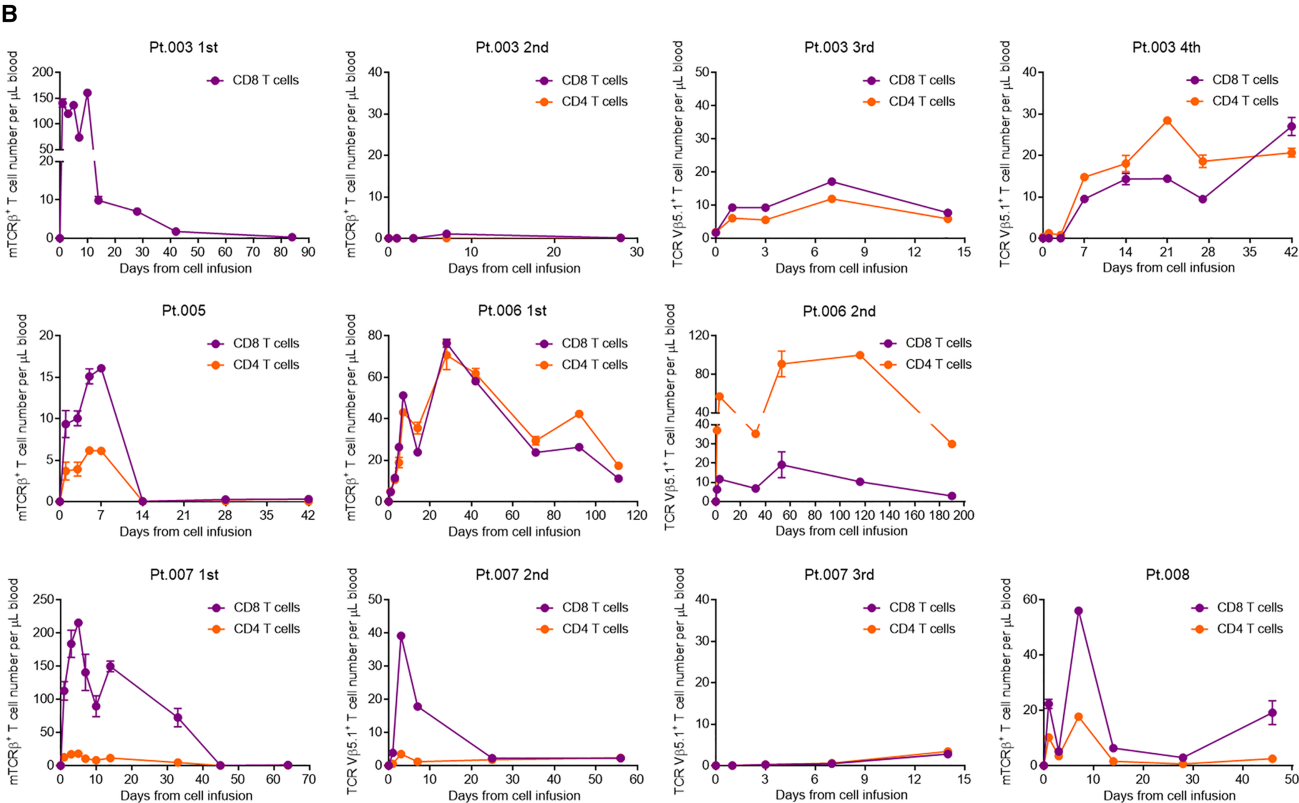
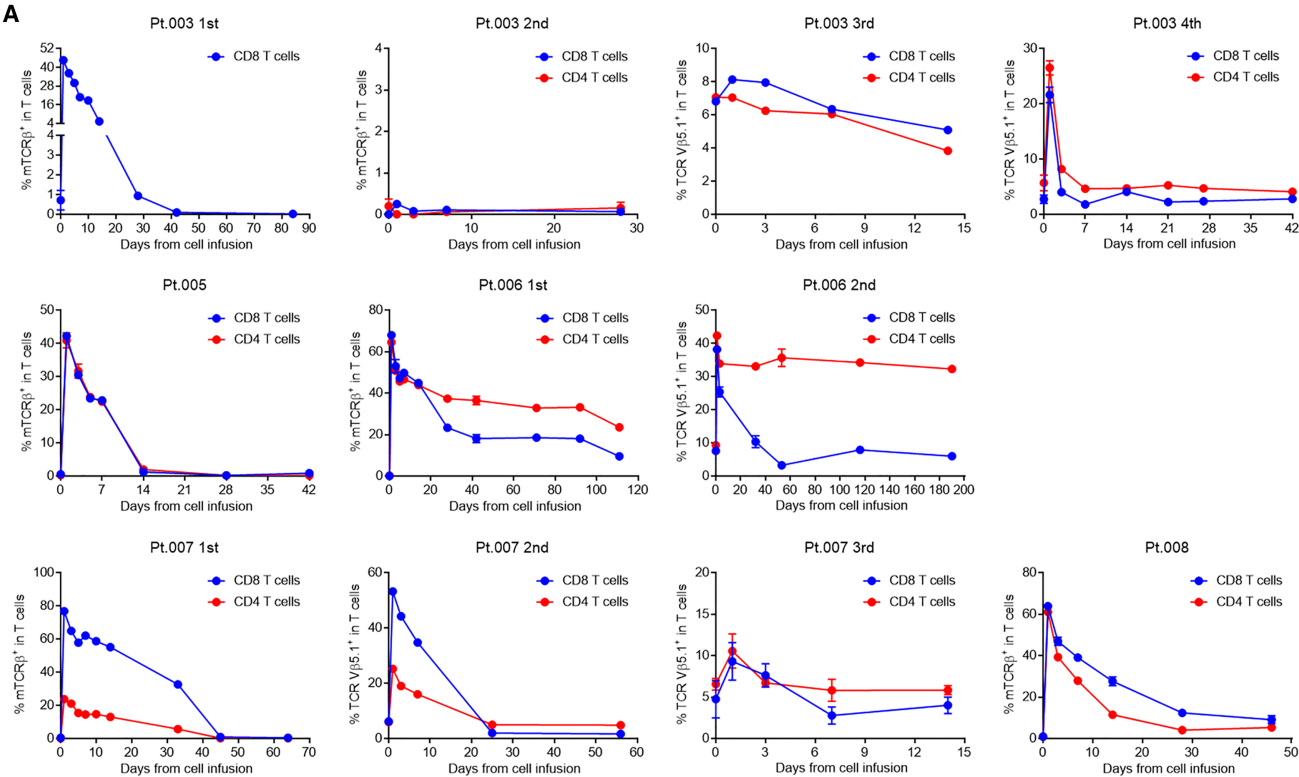
We treated five patients with recurrent PDAC with autologous HLA-A*11:01-restricted KRAS G12V₈₋₁₆ reactive TCR-T cells. These five patients all received human-mouse hybrid TCR-001-engineered T cell products during the first cell infusion, and three patients among them additionally received human-mouse hybrid TCR-001-engineered T cell products or fully human 4148 TCR-engineered T cell products. One patient (Pt.003) experienced a complete response (CR), and two patients (Pt.006 and Pt.008) experienced short-duration stable disease without tumor regression as best response according to Response Evaluation Criteria in Solid Tumor (RECIST) 1.1 criteria (Table 1; Figures 3B and 3C). Median progression-free sur-

vival and overall survival were 14.3 weeks and 12.6 months, respectively (Table 1). A clinical course description for each patient is provided in supplemental information and Figure 3A.

Pt.003 (63-year-old male) exhibited a CR of four liver metastatic lesions 6 weeks after the first cell infusion, a response that lasted 23.4 weeks (Table 1; Figure 3D). When Pt.003 experienced progressive disease 29.4 weeks after the first cell infusion, it was at the primary tumor site and not in the liver. After 1 month, a second cell infusion of TCR-001-engineered T cells was given to determine whether retreatment would be effective. Unexpectedly, within 1–3 h after repeat infusion, Pt.003 experienced a high fever and chills (Figure S13) with signs consistent with rejection of the engineered T cells. Hence, when Pt.003, Pt.006, and Pt.007 had progressive disease after the prior cell infusion of human-mouse hybrid TCR-001-engineered T cell product, the fully human 4148 TCR-engineered T cell products were given to avoid the potential hyper-acute rejection due to the induction of anti-mouse TCR constant regions antibody during the prior cell infusion of human-mouse hybrid TCR-001-engineered T cell product (as described later). Notably, Pt.005 and Pt.008 did not receive retreatment with the fully human 4148 TCR-engineered T cell products due to tumor progression-related thrombocytopenia or coagulation disorders. However, the third and fourth infusions of 4148 TCR-engineered T cell products in Pt.003 mediated stable disease for only 7.9 weeks (Table 1; Figure 3B), and Pt.006 and Pt.007 did not respond to the second infusion of 4148 TCR-engineered T cell products (Table 1; Figure 3B). Surprisingly, Pt.007 experienced signs of hyper-acute rejection against the fully human 4148 TCR-engineered T cell product with a high fever and chills within 1–3 h after the third cell infusion (Figure S13). Notably, serum levels of CA19-9 in Pt.003 after the first and fourth TCR-T treatment significantly and gradually decreased, while CA19-9 levels in Pt.003 after the two other treatments and in other patients during TCR-T treatment only transiently decreased or gradually increased (Figures 3E and S14), which generally was consistent with the evaluation by RECIST 1.1 criteria.

Attenuated *in vivo* persistence of transferred TCR-T cells after repeat infusions

We evaluated the persistence and peak T cell expansion of TCR-T cells in post-treatment PBMCs using flow cytometry in the five



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patients who underwent a total of 11 cell infusions (Figure 4; Table S2). For TCR-001-engineered T cell infusion, in Pt.006 (first infusion) and Pt.008, we could detect TCR-T cells in PBMCs at every time point for which PBMC samples were available (Figure 4A), while in Pt.003 (first infusion), Pt.005, and Pt.007 (first infusion), TCR-T cells persisted for 28, 7, and 33 days, respectively (Figure 4A), showing no clear correlation between the length of cell persistence and response to therapy. Importantly, in Pt.003 (second infusion), autologous TCR-001-transduced T cells were rapidly depleted *in vivo* within 1 day post treatment (Figures 4A and S15), indicating the possible existence of anti-mouse TCR constant region antibodies that may exert antibody-dependent cell-mediated cytotoxicity. For the fully human 4148 TCR-engineered T cell infusion, in Pt.006 (second infusion), TCR-T cells could be detected in PBMCs at every time point for which PBMC samples were available and persisted for more than 190 days (Figure 4A), while in Pt.003 (third infusion), Pt.003 (fourth infusion), and Pt.007 (second infusion), TCR-T cells persisted only for 7, 1, and 7 days respectively (Figure 4A). More importantly, we found that in Pt.007 (third cell infusion, i.e., second infusion with 4148 TCR-transduced T cells), the fully human 4148 TCR-transduced T cells were rapidly depleted from the peripheral blood within 3 days post treatment and were detected only in 1 day and 3 days post-treatment PBMCs at low level (Figures 4A and S20). The persistence profile of TCR-T cells in post-treatment PBMCs was further confirmed by KRAS G12V₈₋₁₆-HLA-A*11:01-tetramer staining (Figures S21 and S22).

Across all cell infusions, excluding the two cases with hyper-acute rejection, the median maximum percentage of TCR-engineered CD8 T cells in PBMCs occurred on day 1 and was 44.6% (range, 8.13%–76.75%, Figure 4A), and the median peak concentration (C_{max}) of the TCR-T cells was 39.1 cells per μ L blood (range, 0.0085–215.4, Figure 4B). The median T_{max} (time to C_{max}) and median T_{last} (time of last measurable TCR-T) were 7 days (range, 1–53) and 28 days (range, 1–190), respectively (Figure 4B; Table S2). Notably, in Pt.003 (fourth infusion), the absolute number of endogenous TCR V β 5.1⁺ T cells in the blood significantly increased from 7 days post treatment and likely represented COVID-19 infection-induced lymphocytosis (Figure 4B) rather than the 4148 TCR-transduced T cells because we did not detect KRAS G12V reactivity in the day 7 post-treatment PBMC samples (Figure S17). Peak T cell expansion was significantly lower in patients who had shorter-term T cell persistence (Pt.003 third and fourth cell infusions) (Figure 4). The persistence and expansion pattern of CD4 TCR-T cells were similar to that of CD8 TCR-T cells (Figure 4). Furthermore, the TCR-T cells within post-treatment PBMCs appeared to retain effector functions since they specifically upregulated 4-1BB/OX-40

and CD107a and produced effector cytokines including IFN- γ and TNF upon *in vitro* stimulation with KRAS G12V₈₋₁₆ 9-mer, although it was not to the extent of their respective infusion products (Figures 5 and S15–S20).

Finally, we characterized the phenotype of persistent TCR-T cells in the about-1-month-post-treatment PBMCs for cell infusions with relatively long-term T cell persistence and found that among persistent TCR-transduced CD8 T cells there were very few PD1⁺ T cells, and most cells displayed a T_{scm} phenotype (CD62L⁺ CD45RO[–] and Fas⁺), except for Pt.003 after the first cell infusion where the engineered T cells were predominantly effector memory phenotype (Figure S23).

Changes in C-reactive protein and cytokines after TCR-T cell infusion

For all five patients, we measured serum C-reactive protein (CRP) and plasma cytokines in the clinical lab and collected plasma at various time points during cell infusion for research studies. Transient increase of serum CRP levels after cell infusion was found in Pt.003 (first and second infusion), Pt.005, and Pt.007 (third infusion) (Figure S24), which correlated with hyper-acute rejection in Pt.003 (second infusion) and Pt.007 (third infusion), as well as short-term persistence (potential rejection) in Pt.005. The level of plasma IL-6 in Pt.003 (second infusion), Pt.005, and Pt.007 (third infusion) rapidly reached the peak with high level within 1 day, 3 days, and 1 day post treatment, respectively (Figures S25–S35). However, there was little change in plasma concentration of IFN- γ , TNF, and other cytokines during treatment in all five patients (Figures S25–S35), suggesting a relatively weak effector cytokine response *in vivo* by TCR-T cells.

Induction of anti-TCR antibody in plasma after TCR-T cell infusion

As TCR-001 was constructed by fusing human TCR variable regions with mouse TCR constant regions and Pt.003 experienced hyper-acute rejection against autologous TCR-001-engineered T cell products within 1–3 h after the second cell infusion, we investigated whether human anti-mouse TCR constant region antibodies post treatment were induced using indirect assays. As shown in Figures 6A and S36A, incubation of healthy donor-derived TCR-001-transduced T cells and Pt.003 autologous TCR-001-transduced T cells with Pt.003 first D203 plasma could significantly suppress the staining of fluorescence-conjugated anti-mouse TCR β constant region antibody, indicating the existence of human anti-mouse TCR β constant region antibody in plasma. In Pt.003 and Pt.007, human anti-mouse TCR β constant region antibodies were induced from

Figure 4. *In vivo* persistence of the TCR-T cells in five patients undergoing a total of 11 cell infusions

(A and B) The *in vivo* persistence of the transferred TCR-T cells in the peripheral blood was determined by flow cytometric analysis of mouse TCR β or human TCR V β 5.1 in Pt.003, Pt.005, Pt.006, Pt.007, and Pt.008. Data are gated on CD3⁺ CD8 T cells or CD4 T cells. Mouse TCR β ⁺ T cells and human TCR V β 5.1⁺ T cells represent the TCR-001-transduced T cells and the 4148 TCR-transduced T cells, respectively. The percentage of mouse TCR β ⁺ T cells or human TCR V β 5.1⁺ T cells in CD8 or CD4 T cells (A) and the absolute number of mouse TCR β ⁺ T cells or human TCR V β 5.1⁺ T cells in blood (B) are shown. Dots indicate the individual value or the mean of 2–4 biological replicates. Error bars represent SD of 2–4 biological replicates (some small bars may not be visible).

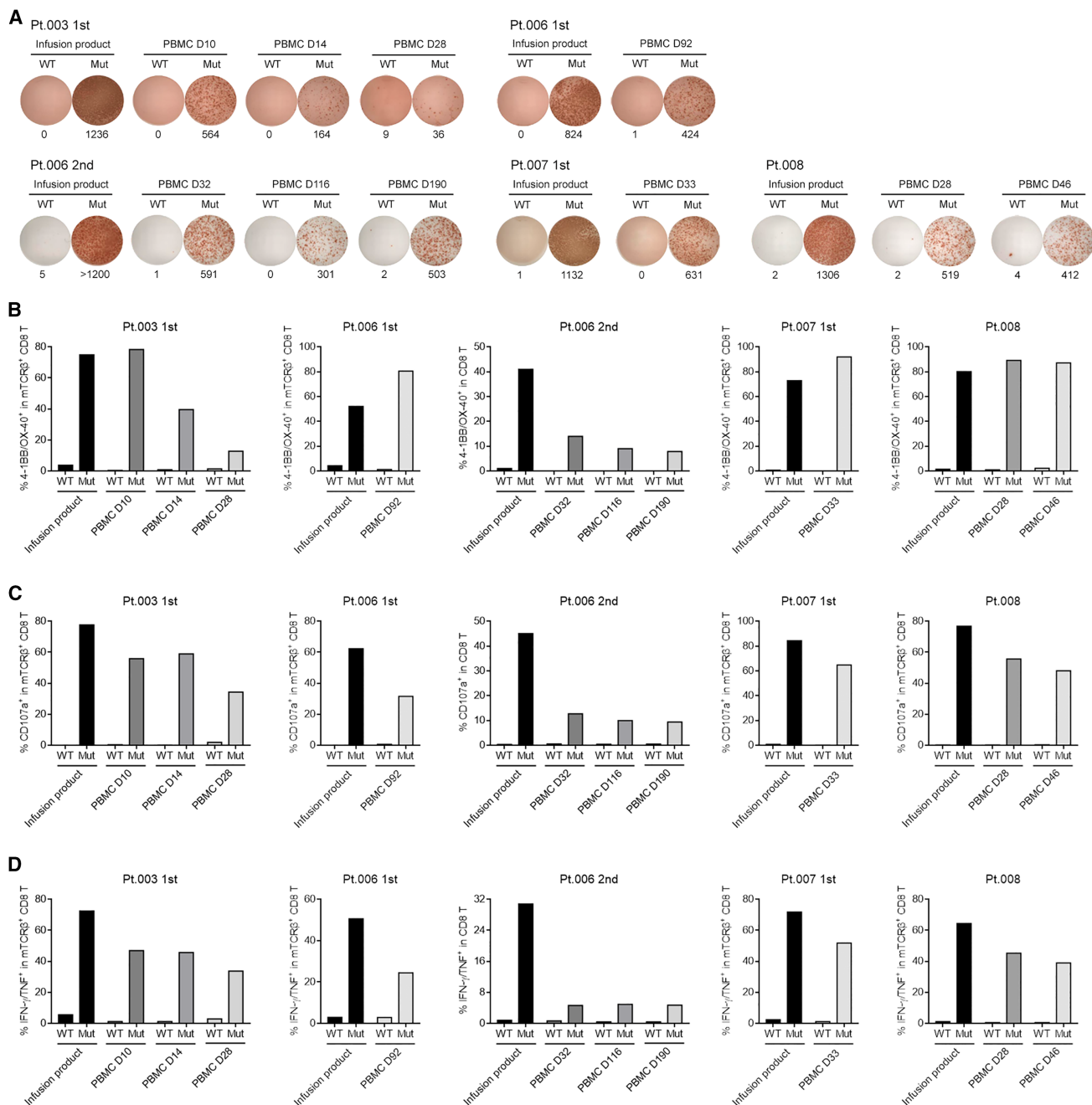
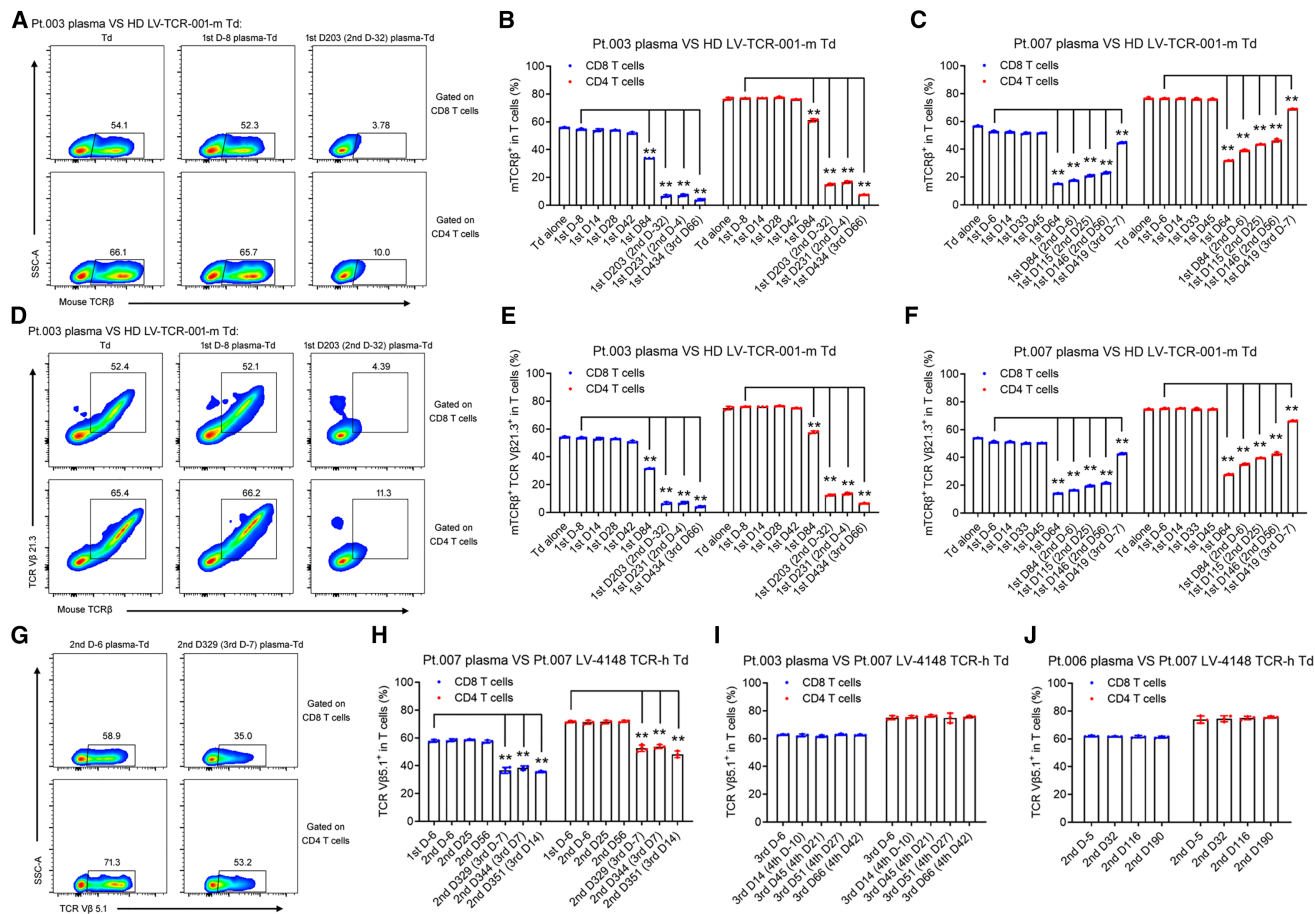


Figure 5. Evaluation of *in vitro* effector functions of TCR-001-engineered T cells persisting in the PBMC at various times after treatment

(A and B) IFN- γ ELISPOT (A) and 4-1BB/OX-40 expression on mTCR β ⁺ CD8 T cells or CD8 T cells (B) in the indicated PBMC sample or the infusion product after pulsing overnight with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9-mer peptide. (C and D) Flow cytometric analysis of CD107a expression (C) and intracellular IFN- γ /TNF production (D) on mTCR β ⁺ CD8 T cells or CD8 T cells is shown after 5-h stimulation of the indicated PBMC sample or the infusion product with 1 μ g/mL KRAS WT or KRAS G12V (Mut) 9-mer peptide.

84 days to 64 days after the first infusion, respectively (Figures 6B and 6C), a time when the corresponding TCR-001-transduced T cells could not be detected in PBMC (Figure 4). The induction of human anti-mouse TCR β constant region antibodies likely contributed to

hyper-acute rejection against the TCR-001-engineered T cell product during the second cell infusion for Pt.003. As shown in Figures 6D and S36B, incubation of healthy donor-derived TCR-001-transduced T cells and Pt.003 autologous TCR-001-transduced T cells with



hyper-acute rejection against 4148 TCR-engineered T cells during the third infusion in Pt.007. No human anti-human TCR V β 5.1 antibodies were detected in the plasma of Pt.003 and Pt.006 after 4148 TCR-engineered T cell infusion (Figures 6I and 6J). The short-term T cell persistence of TCR-001-engineered T cells in Pt.005 and 4148 TCR-engineered T cells in Pt.003 could in theory be due to endogenous T cell immunity against the TCR-T cells. However, we did not detect T cell responses against the TCR-T cells in the post-treatment PBMCs of Pt.005 and in the post-treatment PBMCs of the fourth cell infusion of Pt.003 (Figure S39). We further validated the presence of human anti-mouse TCR β constant region antibodies in the plasmas from Pt.003 and Pt.007 by detecting human IgG on plasma-incubated TCR-T cells using a previously described assay.⁸ We found that TCR-001-engineered T cells bearing mouse TCR constant regions incubated with plasmas from Pt.003 and Pt.007 stained positively for human IgG (Figure S40A), and this correlated with decreased staining of fluorescence-conjugated anti-mouse TCR β antibodies (Figure S40B). Intriguingly, the same plasmas from Pt.003 and Pt.007 (prior to LV-4148 TCR-T infusion) did not suppress staining of fluorescence-conjugated anti-mouse TCR β antibodies on 4148 TCR-engineered T cells that we engineered to express the same mouse TCR constant region as TCR-001 TCR-T cells (Figure S40C), and this was correlated with a lack of human IgG antibodies detected on these cells (Figure S40D), indicating that human anti-mouse TCR β constant region antibodies in these plasmas also bind to epitopes that are outside of the mouse TCR constant regions (e.g., the antibodies may bind to an epitope containing both the mouse constant and human variable regions).⁸ We also detected human IgG antibodies on 4148 TCR-engineered T cells bearing the same mouse constant regions as TCR-001 after incubation with plasma of Pt.007 after infusion of LV-4148-TCR-T cells (second infusion) (Figure S40D). This was correlated with greatly reduced staining of fluorescence-conjugated anti-human TCR V β 5.1 antibody in mTCR β^+ 4148 TCR-engineered T cells (Figure S40E) but not in mTCR β^- non-4148 TCR-engineered T cells (Figure S40F), suggesting that the anti-human TCR V β 5.1 antibodies in the plasma were directed toward a unique sequence in the engineered V β 5.1 TCR (e.g., its idiotype) and not solely toward a conserved V β 5.1 sequence. These results demonstrated that both human anti-mouse TCR constant region antibodies and human anti-human TCR variable region antibodies could be induced in some patients after TCR-T cell infusion.

Because anti-TCR antibodies can induce the activation of T cells,^{32,33} we next tested whether the plasma containing human anti-mouse TCR β constant region antibodies and human anti-human TCR V β 21.3 antibodies could activate the engineered T cells. We found that the plasma from Pt.003 and Pt.007 could induce activation of TCR-001-engineered T cells (Figures S41A–S41C). The plasma from Pt.007 after infusion of LV-4148 TCR-T cells (second infusion) containing human anti-human TCR V β 5.1 antibodies also could induce the activation of fully human 4148 TCR-engineered T cells (Figures S41D–S41F). Notably, the plasma containing human anti-mouse TCR β constant region antibodies and human anti-human

TCR V β 21.3 antibodies from Pt.003 and Pt.007 did not induce the activation of non-TCR-001-engineered T cells (Figure S42), indicating the antigen specificity of the anti-TCR antibodies in plasma. Furthermore, the plasma containing human anti-mouse TCR β constant region antibodies from Pt.003 and Pt.007 could induce the activation of TCR-001-engineered T cells but not 4148 TCR-engineered T cells bearing mouse TCR constant region (Figures S43A–S43F), whereas the plasma containing human anti-human TCR V β 5.1 antibodies after infusion of LV-4148 TCR-T cells (second infusion) from Pt.007 could induce the activation of 4148 TCR-engineered T cells bearing mouse TCR constant region (Figures S43D–S43F). Non-TCR-001-engineered T cells and non-4148 TCR-engineered T cells could not be activated by the plasma from Pt.003 and Pt.007 (Figures S43G–S43L), further validating the antigen specificity of the anti-TCR antibodies in plasma.

DISCUSSION

Our study reveals that adoptive cell transfer with TCR-T cells targeting a KRAS G12V mutation was tolerable in all five patients with advanced pancreatic cancer. All patients experienced the expected transient hematologic toxicities mainly due to lymphodepletion. One of the five patients experienced a CR lasting 5.5 months. Clinical responses were not observed in three patients after receiving one or more repeat cell infusions, which could have been due in part to acute rejection of the TCR-T cells. Our results therefore demonstrate the potential of extending TCR-T cells to target a KRAS mutation other than KRAS G12D in patients with pancreatic cancer.⁷ More importantly, we report the novel finding that anti-human TCR variable region antibodies can be induced after cell infusion of TCR-T cells, which likely will limit the persistence and efficacy of repeat cell infusions in some patients.

The moderate AEs observed in our clinical study may correlate with the modified lymphodepletion regimen and absence of IL-2 administration after cell infusion. Compared to lymphodepletion regimens in previous TCR-T cell therapies,^{8,21} patients were given reduced doses of cyclophosphamide and fludarabine, which efficiently depleted lymphocytes and monocytes but not neutrophils, platelets, and hemoglobin, thus reducing the risk of infection during the treatment. Furthermore, no grade 3 or 4 CRS and neurotoxicity were observed in our study. Grade 2 CRS in one patient (Pt.003) likely was due to hyper-acute rejection of TCR-T cells after the second cell infusion; transient hypotension was resolved by administration of norepinephrine. While IL-2 is commonly administered after TCR-based adoptive immunotherapies, its requirement and impact on the therapeutic efficacy of cell therapy in humans is not known. Although high-dose IL-2^{7,8} or low-dose IL-2²⁰ may contribute to TCR-T cell persistence *in vivo*, some studies^{21,34} did not give IL-2 to patients and observed objective tumor regression and long-term T cell persistence *in vivo*. We did not administer IL-2 in this study to minimize toxicities and test the therapeutic potential of our TCR-T in the absence of exogenous IL-2 support. Considering that TCR-T cells persisted for more than 1 month or more during the first cell infusion in some of our patients, IL-2 does not appear essential

for persistence, though it may improve function and efficacy of TCR-T therapy.

In a previous report,⁷ two patients with metastatic pancreatic cancer were treated with autologous T cells engineered with two HLA-C*08:02-restricted KRAS G12D-reactive TCRs. One patient experienced an objective partial response, while the other patient only had short-term stable disease, and both patients had a relatively high level of T cell persistence in the blood for months after cell infusion. In a recent clinical study,⁸ 3 of 7 patients with metastatic colorectal cancer had objective partial responses by RECIST including regressions of metastases to the liver, lungs, and lymph nodes lasting 4 to 7 months after receiving autologous personalized neoantigen-reactive TCR-T cells. In another clinical study,³⁵ no objective clinical responses were observed in 16 patients with different types of refractory solid cancers treated with autologous personalized neoantigen-reactive TCR-T cells using a non-viral precision genome-editing method, likely due in part to a relatively low number of TCR-T cells infused, ranging from 2.0×10^8 to 5.4×10^9 with a median of 1.3×10^9 . In our current study, one of five patients with recurrent pancreatic cancer experienced an objective CR with complete regression of four liver metastatic lesions lasting 5.5 months after the first infusion of TCR-001-engineered T cells. The numbers of TCR-001-engineered T cells infused in our five patients ranged from 15.2×10^9 to 17.2×10^9 with a median of 17.1×10^9 . Three patients (Pt.006, Pt.007, and Pt.008) had T cell persistence for a month or more without evidence of an exhausted T cell phenotype *in vivo*. Despite a favorable T cell phenotype, patients still did not respond to TCR-T cells during the first cell infusion even after receiving several doses of anti-PD1 antibody, suggesting that lack of efficacy was not due to insufficient engraftment or suboptimal T cell phenotypes but potentially other factors such as defects in T cell infiltration into tumor, an immunosuppressive tumor microenvironment, or poor recognition of tumor cells by TCR-T cells. While our previous preclinical study²⁸ found that TCR-001-engineered T cells could recognize and kill pancreatic cancer organoid cells (PAC-229) established from Pt.007's primary tumor, it is possible that the tumor recognition *in vivo* by TCR-001 was insufficient for antitumor activity, leading to no clinical response. In future, the potency of TCR-T cells may be increased through armoring with molecules such as IL-7/CCL19,^{36–38} dominant-negative-TGFβRII,^{39,40} or other molecules to potentially overcome defects in T cell infiltration into tumor or the immunosuppressive tumor microenvironment. Repeat cell infusions of TCR-001-engineered T cells or 4148 TCR-engineered T cells in Pt.003, Pt.006, and Pt.007 did not lead to objective clinical responses. In these cases, short-term T cell persistence was observed, with evidence of hyper-acute rejection of the TCR-T cells. For 4148 TCR-engineered T cell treatment during the second infusion, Pt.006 patient had no objective clinical response although 4148 TCR-engineered T cells demonstrated persistence *in vivo*. Notably, both TCR-001 and 4148 TCR specifically recognized the 9-amino-acid-long KRAS G12V_{8–16} neoepitope presented on HLA-A*11:01 at concentrations as low as 10 ng/mL and 1 ng/mL respectively.^{10,28} The functional avidity of 4148 TCR appears to be slightly stronger than that of

TCR-001; we found that 4148 TCR-transduced T cells recognized and killed pancreatic cancer organoids naturally expressing KRAS G12V mutation and HLA-A*11:01 more strongly than that of TCR-001-transduced T cells (Figures S44 and S45). Given that we only evaluated the 4148 TCR in the setting of retreatment, it would be interesting to evaluate the efficacy of 4148 TCR-engineered T cells when used as a first cell treatment. Moreover, the 10-mer KRAS G12V 7–16 neoepitope that binds HLA-A*11:01 appears to be expressed at higher levels on cancer cells than the 9-mer peptide,^{14,15,17} making the 10-mer potentially a more attractive target. While we have not yet isolated a TCR that targets the KRAS G12V 10-mer neoepitope, such TCRs have been reported,^{17,41} and we eagerly await clinical results of its targeting.

In one study, T cell persistence correlated with clinical responses in TCR-T cell treatment.²¹ TCR-T cell persistence may be impacted by many factors such as lymphodepletion, IL-2 infusion, T cell expansion, T cell differentiation status, and TCR affinity.^{8,20,21,42} During the third infusion of Pt.003, no lymphodepletion was given, which correlated to a much lower maximum percentage of TCR-engineered CD8 T cells in the blood as well as short-term T cell persistence, supporting the notion that lymphodepletion is important for TCR-T cell persistence. In our study, persistent TCR-engineered CD8 T cells *in vivo* comprised mainly Tscm subsets, potentially due to the relatively high frequencies of Tscm subsets (about 20%–50%) in the T cell infusion products. The relatively high frequency of Tscm cells in the infusion product was unexpected given that the cells underwent two *in vitro* expansions and were in culture for 20 to 30 days (median 26 days). Most cell products were generated using TheraPEAK X-Vivo 15 media containing Cell-Vive serum substitute, which may contribute to an increased percentage of these less differentiated T cell subsets, but this remains to be formally proven. Despite this, we noted that TCR-T cells after the primary stimulation, transduction, and expansion phase contained a higher frequency of Tscm cells than the infusion product, which indicates that the rapid expansion step leads to increased T cell differentiation (data not shown), which was also observed in a recent clinical study.⁸ In the future, we can generate T cell infusion products containing a higher frequency of less differentiated T cells by stimulating and transducing a larger number of initial PBMCs and omitting the second stimulation (rapid expansion) step. Importantly, we found that in some patients, poor T cell persistence after multiple cell infusions was likely caused by antibodies that bound to the anti-KRAS G12V TCRs and mediated hyper-acute rejections of the TCR-T cells. Antibody responses to the allogeneic TCRs did not occur in all patients, and thus, if repeat TCR-T treatments are planned, one should evaluate whether there are deleterious anti-mouse TCRβ constant region antibodies and anti-human TCR Vβ antibodies in the plasma of these patients prior to TCR-T retreatment. Strategies that may further mitigate hyper-acute rejection of TCR-T include the use of B-cell-depleting agents such as rituximab or CD19-CAR-T cells in conjunction with TCR-T, though this has not yet been tested. Moreover, a different TCR could be used, and if a human-mouse hybrid TCR was initially used, then a different, fully human TCR could be used

on the repeat infusion, as was done in this study. While the introduction of a transgenic TCR into T cells expressing endogenous TCRs can result in the formation of mixed TCR dimers that lead to harmful and sometimes lethal autoimmunity in mice,^{43,44} this was not observed in our patients, consistent with the clinical TCR-gene therapy experience in humans.⁴⁵ We also minimized the formation of TCR-mixed dimers by introducing two point mutations in the constant regions of the human 4148 TCR alpha and beta chains to promote their pairing via formation of a disulfide bridge. In the future, we could further reduce the potential risk of autoimmunity due to mixed TCR dimers by knocking out the endogenous TCRs using gene editing technologies such as CRISPR-Cas9.^{46,47}

In an early study,⁴⁸ in six of 26 (23%) patients, post-treatment sera contained human anti-mouse TCR variable region antibodies to lymphocytes transduced with a mouse TCR. Antibodies were found in both responding and non-responding patients. In contrast, 17 patients treated with the fully human anti-MART-1 TCR-T cells similarly were screened, and none had evidence of post-treatment serum IgG binding to the human MART-1 TCR-transduced lymphocytes. In a recent clinical study of seven patients,⁸ personalized and autologous neoantigen-reactive TCRs containing mouse TCR constant regions were used, and one patient (4293), who did not respond to therapy, developed anti-TCR IgG antibodies in the day 141 post-treatment serum sample. While the exact epitope(s) recognized by the anti-TCR IgG antibodies in this patient were not determined, it appears likely that the variable regions of the TCR were recognized, at least in part, because the serum only weakly bound to two of four other TCR-transduced T cells that contained the murine constant regions. In our study, two of five patients (Pt.003 and Pt.007) receiving human-mouse hybrid TCR-001-engineered T cells developed anti-mouse TCR β constant region antibodies and anti-human TCR V β antibodies, whereas one of three patients (Pt.007) receiving the fully human 4148 TCR-engineered T cells developed anti-human TCR V β antibodies. The seemingly higher rate of anti-TCR antibodies observed in our study could be due to differences in the intensity of lymphodepletion regimens used (less intense in our study), the inherent immunogenicity of the transgenic TCRs (e.g., in relation to the HLA genotype of the patients), the methods and timepoints of the serum/plasma samples used to detect anti-TCR antibody responses, and/or other patient-related factors that could impact immunity such as age, general health, and previous treatments. To our knowledge, our results are the first to demonstrate the presence of a detrimental antibody response against both the murine TCR constant regions as well as the human TCR V β variable regions contained within the TCR-T cell products.

In conclusion, the data from this clinical trial showed that adoptive transfer of autologous HLA-A*11:01-restricted KRAS G12V-reactive TCR-T cells was safe and was associated with an objective response in one of five HLA-A*11:01⁺ patients with advanced pancreatic cancer that expressed mutant KRAS G12V. The presence of antibodies directed toward the therapeutic TCR after the first cell infusion should be evaluated in patients who are being considered for retreatment with the same cell product since these cells may be rejected.

The limitations of the study

Our study has several limitations. First, a major limitation is the small sample size, which limits the generalizability and statistical power of our findings. Thus, treatment of additional patients is required to determine the efficacy of our KRAS G12V-reactive TCR-T cell product. Second, there was some variability in the treatment between patients, such as the number of cells and number of cell infusions, the dose of anti-PD1, and the use of lymphodepletion regimen, which need to be better controlled in the future. Third, the mechanisms underlying the response and lack of response to the TCR-T cell treatment were not comprehensively investigated and require further study.

MATERIALS AND METHODS

Sex as a biological variable

This study involved only male patients with pancreatic cancer. However, sex was not considered as a biological variable, as the incidence and outcome of human pancreatic cancer is similar for both sexes.

Study design

This study was an open-label, single-arm phase 1/2 study designed to evaluate the safety and activity of adoptive transfer of autologous T cells genetically engineered to express an HLA-A*11:01-restricted mutant KRAS G12V-specific TCR after non-myeloablative lymphodepletion in HLA-A*11:01⁺ patients with advanced pancreatic cancer that expressed mutant KRAS G12V.

Trial oversight

The study was approved by Shanghai Changhai Hospital Ethics Committee (research protocol #2019-034), conducted according to the principles of the Declaration of Helsinki, and was registered on [ClinicalTrials.gov](https://www.clinicaltrials.gov) (NCT0414298). All patients provided written informed consent before enrollment.

Patient eligibility

Patients had to be 18 to 75 years old and have the HLA-A*11:01 allele and KRAS G12V mutation in tumor tissue determined by whole-exome sequencing as described previously.⁴⁹ Tumors had to be histologically confirmed to be pancreatic cancer, and patients had to have metastatic disease and to have received standard chemotherapy, or they had to have recurrent pancreatic cancer and had to have received surgery and adjuvant chemotherapy. At least 1 measurable lesion had to be evaluable by computed tomography (CT) or magnetic resonance (MR) scans (per RECIST v1.1). Eastern Cooperative Oncology Group performance status had to be 0 or 1 with a patient life expectancy of at least 12 weeks. Patients had to be seronegative for HIV antibody, hepatitis B surface antigen and core antibody, and hepatitis C antibody. Adequate liver, kidney, and bone marrow function was a requirement. Neither sex nor gender was considered in the study design.

Key exclusion criteria included active systemic infections, coagulation disorders, or any other major medical illnesses; concurrent opportunistic infections; concurrent systemic steroid therapy;

history of severe immediate hypersensitivity reaction to any of the medicines used in this study (e.g., cyclophosphamide or fludarabine); active coronary ischemic symptoms; and being on any other investigational agents.

Treatment

After TCR-T cells were successfully manufactured and met the release criteria, patients received a modified lymphodepletion,⁵⁰ which consisted of intravenous cyclophosphamide (500 mg/m² once daily) and fludarabine (30 mg/m² once daily) administered on days -4 to -2 before cell infusion. On day 0, TCR-T cells were infused to the patient intravenously in the patient care unit over approximately 30–50 min. During the treatment, anti-human PD1 antibody (tislelizumab, Beigene) was administered with at least 3 weeks interval at different times after cell infusion in some patients. If the patient experienced stable disease or better after the first infusion, repeat infusions would be considered after meeting patient eligibility upon disease progression.

Clinical assessments

Peripheral blood samples were taken for safety and *in vivo* persistence analysis of TCR-T cells during the study period. Safety was assessed by means of AE monitoring, graded according to the NCI Common Terminology Criteria for Adverse Events v5.0. CRS and immune-effector-cell-associated neurotoxicity syndrome (ICANS) were graded according to the ASTCT 2018 guideline.⁵¹

Tumors were assessed by CT or MR scans performed at baseline before treatment, at about 6 weeks after treatment, and at regular intervals thereafter until disease progression. Tumor response was evaluated by RECIST v1.1.

The primary objectives were to measure the frequency and severity of treatment-related AEs and the objective response rate by RECIST v1.1. The secondary objectives were to measure the persistence of TCR-T cells in peripheral blood by flow cytometry and overall survival of the patient.

Vector manufacture

The two TCRs (“TCR-001” and “4148 TCR”) used here are HLA-A*11:01-restricted TCRs that specifically target the 9-amino-acid-long KRAS G12V neopeptide VVGAVGVGK. “TCR-001” was originally isolated from a patient (Pt.001) in Changhai Hospital as described in our previous study.²⁸ TCR-001 contains the human TCR β chain TRBV11-2 (also known as TCR V β 21.3). The full-length TCR-001 was constructed by fusing TRB V-D-J-encoding sequences and TRA V-J-encoding sequences to the modified murine TRB and TRA constant regions as previously described^{52,53} in order to promote pairing of the introduced TCR and facilitate identification of the transduced T cells by flow cytometry using anti-mouse-TCR- β -constant regions antibodies (BioLegend). The 4148 TCR was originally isolated from a patient (Pt. 4148) in Surgery Branch, NCI, NIH, as described in Cafri et al.¹⁰ 4148 TCR contains the human TCR β chain TRBV5-1 (also known as TCR V β 5.1). The fully

human 4148 TCR was constructed by ligating TRB V-D-J-encoding sequences and TRA V-J-encoding sequences with human TRBC1 (UniProtKB-P01850, aa 176) and TRAC (UniProtKB-P01848, aa 140), respectively, with two site-directed mutations, TRBC1 serine-57-cysteine and TRAC threonine-48-cysteine, which allowed the formation of an additional disulfide bridge between two TCR chains.⁵⁴ Each TCR was codon optimized, synthesized, and cloned into the lentiviral plasmid by OBIO Technology Corp. (Shanghai). See Figure S5 in our previous study for TCR-001-expressing lentiviral plasmid schematic,²⁸ and see Figure S46 in this manuscript for 4148 TCR-expressing lentiviral plasmid schematic.

Lentiviral particles for each TCR were produced under GMP by OBIO Technology Corp. (Shanghai) by transient transfection of 293T cells with plasmids encoding gag/pol, Rev, envelope VSV-G, and the TCRs. Supernatants were harvested, and TCR-expressing lentiviral particles were purified and titrated. The lentiviral particles were tested for host cell DNA (HCD) and host cell protein with acceptable levels.

Preparation of the infusion product

All patient-derived materials were collected under an IRB approved protocol. Cell manufacture was performed in our clean room facility (Cell Production Facility, Human Applications Lab). Venous blood from the patients before the first cell infusion was used as the starting material for TCR-T cell preparation. PBMCs were isolated using Lymphoprep (Alere Technologies) density gradient centrifugation and frozen in CryoStor CS10 (Stemcell Technologies). Thawed PBMCs (2×10^6 /mL, 2 mL per well of 24-well plate) were stimulated with GMP-grade T cell TransAct (Miltenyi Biotec) in AIM-V CTS media (Gibco, only used for the preparation of the infusion product for Pt.003's first treatment) or TheraPEAK X-Vivo 15 media (LONZA) containing 5% heat-inactivated human AB serum (Gemini, only used for the preparation of the infusion product for Pt.003's first treatment) or GMP-grade Cell-Vive T-NK Xeno-Free Serum Substitute (BioLegend) and 300 IU/mL IL-2 (SL Pharm). 2 days later, the activated T cells (5×10^5 /mL, 2 mL per well of 24-well plate) were transduced with the lentiviral particles encoding the TCRs. The following day, the cells were transferred to a G-Rex 6-well plate (Wilson Wolf) and further cultured in the above culture media. At about 9–14 days after the initial stimulation, aliquots of the cells were further expanded or cryopreserved. See Figures S1–S11 A for a schematic. If TCR-T cells contained less than 30% CD8 T cells, CD4 T cells would be depleted by CliniMACS CD4 GMP MicroBeads (Miltenyi Biotec) before cryopreservation.

To prepare the infusion product, fresh or thawed TCR-T cells underwent a 10- to 17-day rapid expansion protocol similar to a previous study⁷ using AIM-V CTS media (Gibco, only used for the preparation of the infusion product for Pt.003's first treatment) or TheraPEAK X-Vivo 15 media (LONZA) containing 5% heat-inactivated human AB serum (Gemini, only used for the preparation of the infusion product for Pt.003's first treatment) or GMP-grade Cell-Vive T-NK Xeno-Free Serum Substitute (BioLegend), lethally

irradiated healthy donor PBMC feeder cells, 30 ng/mL Anti-CD3 mAb GMP grade (clone OKT3, Takara), and 3,000 IU/mL IL-2 (SL Pharm) in G-Rex 500 flask (Wilson Wolf). After the rapid expansion, the TCR-T cell cultures were harvested for patient treatment. See [Figures S1–S11B](#) for a schematic.

Characterization of the infusion product

Flow cytometry was used to evaluate transduction efficiency and expression of select cell surface markers. “TCR-001” construct contains the mouse TCR- α and β chain constant regions, and thus, transduction efficiency can be evaluated by measuring expression of the mouse TCR- β constant regions using the anti-mouse TCR- β constant region antibody. The fully human “4148 TCR” construct contains human TCR β chain TRBV5-1, and thus, transduction efficiency can be evaluated by measuring expression of the human TRBV5-1 using the anti-human TCR V β 5.1 antibody. The following, anti-human flow cytometry antibodies were used in this report: CD3-Pacific blue (OKT3), CD4-APC-Fire750 (SK3), CD8-PE-Cy7 (SK1), mouse TCR- β constant region-FITC/PE (H57-597), PD1-FITC (EH12.2H7), CD62L-APC (DREG-56), CD45RO-FITC (UCHL1), Fas-PerCP Cy5.5 (DX2), TCR V β 5.1-PE (IM2285), and TCR V β Repertoire Kit. All antibodies were from BioLegend except for TCR V β 5.1-PE (IMMU 157) and TCR V β Repertoire Kit (Beckman Coulter).

For preparation of cells for flow cytometry, cells were harvested and then re-suspended in FACS buffer (1 $\times$ PBS containing 1% FBS and 2 mM EDTA) and incubated with titrated fluorochrome-conjugated antibodies for about 30 min in the fridge and the dark. Cells were then washed with FACS buffer and re-suspended with FACS buffer followed by data acquisition with the FACSria Fusion flow cytometer. Flow cytometry data were analyzed using FlowJo software.

We performed coculture assays to evaluate the *in vitro* function of the infusion product. Immature dendritic cells (DCs) were used as antigen-presenting cells in these cultures. DCs were generated from PBMCs using the plastic adherence method as described previously with minor modification.⁵³ Briefly, HLA-A*11: 01⁺ healthy donor PBMCs were thawed, washed, re-suspended in AIM-V media (Gibco), and then transferred to a tissue culture-treated 6-well plate and incubated at approximately 1 $\times$ 10⁶ cells/cm² in an incubator at 37°C, 5% CO₂. After 90 min, the non-adherent cells were collected, and the adherent cells in the plates were vigorously washed with RPMI and then further incubated with AIM-V media for 60 min. The media and non-adherent cells were removed, and the adherent cells in the plates were then vigorously washed again with RPMI and then incubated with DC media, which is composed of RPMI containing 5% heat-inactivated human AB serum (Gemini), 100 U/mL penicillin and 100 μ g/mL streptomycin (Gibco), 1:100 GlutaMax (Gibco), 0.25 μ g/mL Amphotericin B (Sangon), 25 mM HEPES (Sangon), 800 IU/mL GM-CSF (Novoprotein), and 40 ng/mL IL-4 (Novoprotein). DCs were used in experiments after day 5 of initiation of the cultures.

The cocultures comprised infusion product T cells (1 $\times$ 10⁵/well of 96-well plate) incubated with DCs (3–5 $\times$ 10⁴/well) that were sepa-

ately pulsed for 2 h with 1 μ g/mL of the HPLC-purified KRAS peptides (Sangon) wild-type (WT) 9-mer (VVGAGGVGK) and mutated G12V (Mut) 9-mer (VVGAVGVGK). The cells were cocultured overnight in a 96-well ELISPOT plate (Dakewe) for IFN- γ ELISPOT and flow cytometric analysis of T cell activation (4-1BB and OX-40 expression) in 50/50 media that consisted of a 1 to 1 mixture of AIM-V media (GIBCO) or X-VIVO15 media (LONZA) with complete media (CM). CM is composed of RPMI supplemented with 10% human AB serum, 1:100 GlutaMax, 25 mM HEPES, 0.25 μ g/mL Amphotericin B, 100 U/mL penicillin, and 100 μ g/mL streptomycin. No exogenous cytokines were added to the coculture. After an overnight incubation (18–24 h), cells were collected for flow cytometric analysis of T cell activation (4-1BB and OX-40 expression), and the plate was processed and developed to visualize IFN- γ spots according to manufacturer's instructions. For intracellular cytokine staining (ICS), 2 $\times$ 10⁵ infusion product cells and 0.6–1 $\times$ 10⁵ KRAS peptides-pulsed DCs were seeded per well of a 96-well round-bottom plate. FITC or PE-conjugated anti-CD107a antibody (H4A3, BioLegend), Brefeldin A (BioLegend), and Monensin (BioLegend) were added at the onset of stimulation. After 5 h of stimulation in an incubator at 37°C and 5% CO₂, the cells were transferred to a 96-well round-bottom plate. Cells were washed once with 200 μ L of FACS buffer and then stained with PE Cy7-conjugated anti-CD8 antibody (SK1), Pacific blue-conjugated anti-CD3 antibody (UCHT1), APC/Fire 750-conjugated anti-CD4 antibody (SK3), and PE or FITC-conjugated anti-mTCR β antibody (H57-597) (all from BioLegend). The cells were incubated in the dark at 4°C for 30 min and then washed once with 200 μ L of FACS buffer. The PBMCs were then incubated in fixation buffer (BioLegend) in the dark at room temperature for 20 min. The PBMCs were washed once with 200 μ L of FACS buffer and then twice with 200 μ L of permeabilization buffer (BioLegend). The cells were stained with APC-conjugated anti-IFN- γ antibody (B27) and PerCP/Cy5.5-conjugated anti-TNF antibody (MAB11) (all from BioLegend). After a 30-min incubation in the dark at room temperature, the PBMCs were washed once with 200 μ L of permeabilization buffer. The cells were re-suspended in FACS buffer and analyzed on a BD FACSria Fusion flow cytometer.

Plasma cytokine assay

Blood was collected in BD Vacutainer K2 EDTA blood collection tubes before and after cell infusion, and plasma was harvested for cytokines and proteins assays by the Department of Laboratory in Changhai Hospital. An IMULITE 1000 immunoassay system inflammation panel including TNF, IL-1 β , IL2R, IL-6, IL-8, and IL-10 (Siemens Healthcare, only used for the assays of plasmas from Pt.003's first treatment) or multi-cytokine detection kit (immunofluorescence method) including IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, IFN- γ , TNF, and IFN- α (Hangzhou Cellgene Biotech Co.) was used to evaluate concentrations of various cytokines and proteins in the plasma according to manufacturer's instructions. The human IFN- γ ELISA kit (Dakewe) was only used for the assays of plasmas from Pt.003 during the first infusion.

***In vivo* persistence of engineered T cells**

Whole blood was collected in BD Vacutainer K2 EDTA blood collection tubes, and PBMCs were isolated using Lymphoprep (Alere Technologies) density gradient centrifugation. The “TCR-001” transduced T cells were tracked by flow cytometric analysis of mouse TCR β expression, while the “4148 TCR” transduced T cells were tracked by flow cytometric analysis of human TRBV5-1 (also known as TCR V β 5.1). However, it should be noted that not all V β 5.1⁺ T cells in the PBMC were the engineered 4148-TCR-T cells because there was a low basal percentage of endogenous non-engineered V β 5.1⁺ T cells in the peripheral blood (about 2%–10%) in our patients (Figure 4A), especially after recovering from lymphodepletion. Thus, in order to better estimate the peak concentration (C_{max}) of the V β 5.1⁺ 4148-TCR-T cells, we used the time point with the peak absolute number before Tlast (the time of last measurable TCR-T) and for which we had evidence for specific KRAS G12V reactivity of the PBMC *in vitro* (Figures 5, S16, S17, S19, and S20). The absolute number of mouse TCR β ⁺ T cells or human TCR V β 5.1⁺ T cells in blood was calculated as follows: the percentage of mouse TCR β ⁺ T cells or human TCR V β 5.1⁺ T cells in lymphocytes of PBMCs $\times$ the absolute number of lymphocytes in routine blood test. In some experiments, APC-conjugated KRAS G12V₈₋₁₆-HLA-A*11:01 tetramer (prepared by Shanghai Polaris Biology Co.) was used.

***In vitro* function of engineered T cells persisting in peripheral blood**

IFN- γ ELISPOT and T cell activation (4-1BB and OX-40 expression) and ICS with flow cytometric analysis were used to evaluate function of the transduced T cells circulating in the PBMCs at the indicated timepoints after cell infusion. For both assays, PBMC and infusion product were thawed and rested overnight in 50/50 media in the absence of exogenous cytokines. PBMCs were rested in a 24-well plate, and the infusion product was rested in a G-Rex 24-well plate (Wilson Wolf). For IFN- γ ELISPOT and T cell activation (4-1BB and OX-40 expression) by flow cytometric analysis, in brief, about 2×10^5 PBMCs or 1×10^5 infusion product per well of a 96-well ELISPOT plate was stimulated overnight with 1 μ g/mL of the HPLC-purified KRAS WT or mutated G12V 9-mer peptides. After overnight culture (18–24 h), cells were collected for flow cytometric analysis of T cell activation (4-1BB and OX-40 expression), and the plate was processed and developed to visualize IFN- γ spots according to manufacturer's instructions. For ICS, about 3×10^5 PBMCs or 2×10^5 infusion product cells were seeded per well of a 96-well round-bottom plate. The cells were stimulated with 1 μ g/mL of the HPLC-purified KRAS peptides. PE or FITC-conjugated anti-CD107a antibody (H4A3, BioLegend), Brefeldin A (BioLegend), and Monensin (BioLegend) were added at the onset of stimulation. After 5 h of culture, the cells were stained with anti-cell surface antibodies, fixed, permeabilized, stained with anti-cytokine antibodies, and assayed by flow cytometry.

Indirect assay of anti-TCR antibody in plasma by flow cytometry

To detect the presence of anti-TCR antibody in the pre- and post-infusion plasma of patients, human-mouse hybrid “TCR-001” or

fully human “4148 TCR” transduced T cells from patients or healthy donor were incubated with the indicated plasma sample and then assayed for the expression of mouse TCR β , human TCR V β 21.3, or human TCR V β 5.1 by flow cytometry. Pre-infusion or post-infusion plasma samples (100 μ L/well) were added to TCR-T cells ($1-2 \times 10^5$ /well in 100 μ L 50/50 media) in a 96-well round-bottom plate and incubated at 37°C, 5% CO₂ for 2 h. T cells then were washed twice with 200 μ L of FACS buffer, stained with PE Cy7-conjugated anti-CD8 antibody (SK1), Pacific blue-conjugated anti-CD3 antibody (UCHT1), APC/Fire 750-conjugated anti-CD4 antibody (SK3), FITC or PE-conjugated anti-mTCR β antibody (H57-597), FITC-conjugated anti-human TCR V β 21.3 antibody (IG125), or PE-conjugated anti-human TCR V β 5.1 antibody (IMMU 157) for about 30 min in the fridge and the dark and then assayed by flow cytometry. In some experiments, PE-conjugated anti-human IgG Fc antibody (M1310G05, BioLegend) was used.

Assay to measure the potential of plasma to activate TCR-T cells

Pre-infusion or post-infusion plasmas were added to TCR-T cells from patients (2×10^6 /mL in 50/50 media) at a volume ratio of 1:1 and incubated in an incubator at 37°C, 5% CO₂ for 2 h. Then T cells were washed twice with PBS and re-suspended by 50/50 media. For flow cytometric analysis of T cell activation (4-1BB and OX-40 expression), T cells (1×10^5 /well) were cultured overnight in a 96-well round-bottom plate in 50/50 media. After an overnight incubation (18–24 h), cells were washed once with 200 μ L of FACS buffer and then stained with PE Cy7-conjugated anti-CD8 antibody (SK1), Pacific blue-conjugated anti-CD3 antibody (UCHT1), APC/Fire 750-conjugated anti-CD4 antibody (SK3), PE-conjugated anti-mTCR β antibody (H57-597), APC-conjugated anti-4-1BB antibody (4B4-1), and FITC-conjugated anti-OX-40 antibody (ACT35) (all from BioLegend). The cells were incubated in the dark at 4°C for 30 min, washed once with 200 μ L of FACS buffer, and then assayed by flow cytometry. For flow cytometric analysis of intracellular cytokines in T cells, T cells (1×10^5 /well) were cultured in a 96-well round-bottom plate in 50/50 media in the presence of FITC-conjugated anti-CD107a antibody (H4A3, BioLegend), Brefeldin A (BioLegend), and Monensin (BioLegend). After 5 h of culture, the cells were stained with anti-cell surface antibodies, fixed, permeabilized, stained with anti-cytokine antibodies, and assayed by flow cytometry.

T cell-mediated immune response against TCR-T cells

We tested for the potential development of a T cell-mediated immune response against TCR-T cells using a modified method.⁴⁸ Briefly, patients' pre-infusion or post-infusion PBMCs, infusion product (TCR-T cells), and mock T cells were thawed and rested overnight in 50/50 media in the absence of exogenous cytokines. Autologous TCR-T cells or mock T cells were stained with 5 μ M of Cell-Trace Yellow (#C34573, Invitrogen) according to the manufacturer's protocol. Then, PBMCs (1×10^5 per well) were cocultured with Cell-Trace Yellow-stained TCR-T cells or mock T cells at a ratio of 1:1 in a 96-well round-bottom plate in 50/50 media. After overnight culture (18–24 h), cells were washed once with 200 μ L of

FACS buffer, stained with PE Cy7-conjugated anti-CD8 antibody (SK1), Pacific blue-conjugated anti-CD3 antibody (UCHT1), APC/Fire 750-conjugated anti-CD4 antibody (SK3), APC-conjugated anti-4-1BB antibody (4B4-1), and FITC-conjugated anti-OX-40 antibody (ACT35) for about 30 min in the fridge and the dark, and then T cell activation (4-1BB and OX-40 expression) in Cell-Trace Yellow-negative CD3⁺ CD8⁺ T cells or CD3⁺ CD4⁺ T cells was assayed by flow cytometry.

Cytotoxicity assay

To determine the TCR-T cells mediated cytotoxicity, luciferase-based cytotoxicity assays were performed. In brief, 1×10^4 organoid target cells stably transduced with firefly luciferase were incubated with the indicated TCR-T cells at various effector to target ratios overnight (18–24 h) in white 96-well plates in RPMI 1640 medium containing 10% FBS. Steady-Lumi II reagent (RG059M, Beyotime, China) (100 μ L per well) was added to cells containing 100 μ L medium for each well, and the resulting luminescence was analyzed on a microplate reader (TECAN, SPARK) in the model of luminometric measurement as counts per second (CPS) for each well detection. Target-only cells as maximum CPS and Target-only cells plus 1% Tween 20 (ST825-100mL, Beyotime, China) as minimum CPS were used to determine the assay range. Percent specific lysis was calculated as $(1 - [\text{sample} - \text{CPS min CPS}]/[\text{max CPS} - \text{min CPS}]) \times 100\%$. Three technical replicates were performed for each condition.

Statistical analysis

Related quantitative data were analyzed using GraphPad Prism version 8.0.2 statistic software (GraphPad Software, San Diego, CA, USA). Means $\pm$ SD were shown in related graphs. Statistical comparisons between two groups were analyzed with two-tailed unpaired *t* test, and $p < 0.05$ was considered statistically significant. For statistical comparisons of more than two groups, one-way ANOVA analysis with Tukey's multiple-comparison test was used, and $p < 0.05$ was considered statistically significant.

DATA AND CODE AVAILABILITY

All data related to the findings in this study are available in the main text and the supplementary materials. Any additional information required to reanalyze the data reported in this paper is available from the corresponding author contact upon request.

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AUTHOR CONTRIBUTIONS

G.J., E.T., S. Guo, J.Y., and G.T. conceived of and supervised the study; X.X., S. Guo, and Z.C. designed the study; X.X., G.T., H.G., B.Z., S.Y., and X.D. developed the methodology; X.X., B.Z., S.Y., X.D., P.L., Y.L., X.S., X.Y., and H.W. performed the experiments; S.

Gao, X.K., B.L., M.S., W.J., and K.Z. acquired the clinical data (acquired and managed patients, provided facilities, and so on); X.X., S. Guo, H.G., B.Z., and Y.S. analyzed and interpreted the data (for example, statistical analysis, computational analysis); S. Guo, J.Y., L. Gao, S. Gao, X.W., L. Gu, J. Huang, and J. Hao were responsible for the clinical care of patients; C.Z., Y.S., C.W., and C.Z. helped the study and provided technical support; X.X., G.J., E.T., B.Q., and J.Y. wrote, reviewed, and revised the manuscript. All authors reviewed the results and approved the final version of the manuscript.

DECLARATION OF INTERESTS

E.T. is a consultant for AstraZeneca and Pathfinder Oncology.

SUPPLEMENTAL INFORMATION

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Supplemental Information

T cell receptor gene therapy targeting

KRAS G12V for advanced pancreatic cancer

in a single-arm phase 1/2 clinical trial

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Supplemental Materials

Supplemental information

Description of patient clinical courses

Pt.003 was a patient with pancreatic adenoductal carcinoma in the body of the pancreas. At initial diagnosis, the patient received neoadjuvant chemotherapy with Gemcitabine + Nab-paclitaxel for 6 cycles for about 4 months. After radical surgical resections of primary tumor and left adrenal gland, the patient received adjuvant chemotherapy with oral S1 (Tegafur Gimeracil Oteracil Potassium Capsule) for 9 months. The patient had progressive disease with four liver metastatic lesions about 1 year after the surgery and experimental TCR-T cell therapy was offered. After a modified lymphodepletion, the patient received autologous TCR-001-engineered T cells. In subsequent follow-up, four liver metastatic lesions disappeared reaching a complete response six weeks after the cell infusion, maintaining 23.4 weeks. When the patient had progressive disease 29.4 weeks after the first cell infusion, it was at the primary tumor site and not in the liver. After one month, the second cell infusion of TCR-001-engineered T-cell product was given considering the objective response after the first cell infusion. Unexpectedly, the patient experienced hyper-acute rejection against autologous TCR-001-engineered T-cell product with high fever and chills within 1-3 hours after the second cell infusion. The patient had progressive disease in recurrent primary tumor 6 weeks after the second cell infusion and received chemotherapy with oral S1 for about 2 months. 4.5 months after the second cell infusion, the patient received the fully-human 4148 TCR-engineered T-cell product (the third infusion) without lymphodepletion but 4148 TCR-engineered T cells persisted for only 7 days. After 3 weeks, the patient received 4148 TCR-engineered T cells (the fourth infusion) after lymphodepletion again. In subsequent follow-up, the patient had stable disease four weeks after the fourth cell infusion, maintained until 7.9 weeks. Then the patient had progressive disease with new metastatic lesions in the omentum and peritoneum 12 weeks after the fourth cell infusion, and received supportive care treatment. The patient died with disease 16.0 months after the first cell infusion.

Pt.005 was a patient with pancreatic adenoductal carcinoma in the head of the pancreas. At initial diagnosis, the patient received neoadjuvant chemotherapy with modified Folfirinox + anti-PD1 (Keytruda) for 6 cycles for about 4 months. After radical surgical resections of primary tumor, the patient received adjuvant chemotherapy with modified Folfirinox + anti-PD1 (Keytruda) for 10 cycles. The patient had progressive disease with multiple liver metastatic lesions about 8 months after the surgery and continued to receive chemotherapy with oral S1 + anti-PD1 (Keytruda) for 5 cycles and modified Folfirinox + anti-PD1 (Keytruda) for 2 cycles. Then experimental TCR-T cell therapy was offered 15.7 months after the surgery. After a modified lymphodepletion, the patient received autologous TCR-001-engineered T cells. In subsequent follow-up, multiple liver metastatic lesions progressed six weeks after cell infusion. Then, the patient received target therapy with Trimetinib for one month, chemotherapy with liposomal irinotecan + 5-FU for 3 cycles, and supportive care treatment. The patient died with disease 5.8 months after the cell infusion.

Pt.006 was a patient with pancreatic adenoductal carcinoma in the head of the pancreas. At initial diagnosis, the patient received radical surgical resections of primary tumor and adjuvant chemotherapy with Gemcitabine + Nab-paclitaxel for 8 cycles. The patient had progressive disease with metastatic lesions around the celiac trunk and retroperitoneum 14.5 months after the surgery and continued to received chemotherapy with modified Folfirinox for 5 cycles. Then experimental TCR-T cell therapy was offered 21.5 months after the surgery. After a modified lymphodepletion, the patient received autologous TCR-001-engineered T cells. In subsequent follow-up, the patient had stable disease six weeks after the cell infusion, maintained for 9.9 weeks, and received anti-PD1 (Tislelizumab) for 2 cycles. When the patient had progressive disease with metastatic tumor 3.6 months after the first cell infusion, the second cell infusion using the fully-human 4148 TCR-engineered T-cell product was given. The patient had progressive disease with new lesions in lumbar vertebra 7.4 weeks after the second cell infusion, and received targeted therapy with Lenvatinib + anti-PD1 (Tislelizumab) and then supportive care treatment. The patient died with disease 12.6 months after the first cell infusion.

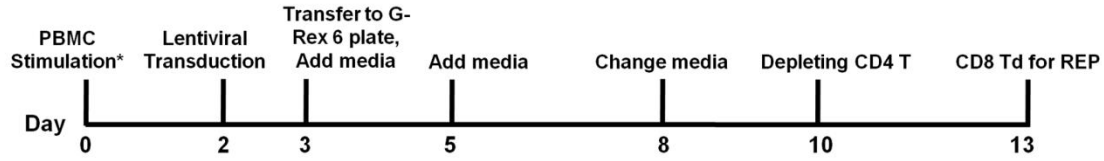
Pt.007 was a patient with pancreatic adenoductal carcinoma in the body of the pancreas. At initial diagnosis, the patient received radical surgical resections of primary tumor and adjuvant chemotherapy with Gemcitabine + Nab-paclitaxel for 6 cycles. The patient had progressive disease with recurrent lesions in the peri-pancreas about 13 months after the surgery and experimental TCR-T cell therapy was offered. After a modified lymphodepletion, the patient received autologous TCR-001-engineered T cells. In subsequent follow-up, the patient had progressive disease with new multiple liver metastatic lesions 4.7 weeks after the cell infusion and received anti-PD1 (Tislelizumab) for 2 cycles. The second cell infusion of the fully-human 4148 TCR-engineered T-cell product was given to the patient 3 months after the first cell infusion. However, the patient had progressive disease with recurrent lesions in the peri-pancreas 8.1 weeks after the second cell infusion, and received chemotherapy with Gemcitabine + Nab-paclitaxel for 5 cycles and 3 cycles with 3.5 months interval. After chemotherapies, the patient felt pain relief in the back and recurrent lesions in the peri-pancreas were regressing but multiple liver metastatic lesions still existed. Then, the third cell infusion of 4148 TCR-engineered T cells was given to the patient. Unexpectedly, the patient experienced hyper-acute rejection against autologous 4148 TCR-engineered T cells with high fever and chills within 1-3 hours after the third cell infusion. The disease was rapidly progressing without tumor measurement 2 weeks after the third cell infusion and the patient received Gemcitabine + Nab-paclitaxel for 1 cycle and supportive care treatment. The patient died with disease 18.0 months after the first cell infusion.

Pt.008 was a patient with pancreatic adenoductal carcinoma in the body of the pancreas. At initial diagnosis, the patient received neoadjuvant chemotherapy with Gemcitabine + Nab-paclitaxel for 3 cycles. After radical surgical resections of primary tumor and splenectomy, the patient received adjuvant chemotherapy with Gemcitabine + Oxaliplatin for 3 cycles and modified Folfirinox + Trimetinib + anti-PD1 (Sintilimab) for 5 cycles. The patient had progressive disease with metastases in multiple organs including liver, stomach, adrenal gland and retroperitoneal lymph node before experimental TCR-T cell therapy was offered 9.6 months after the surgery. After a modified

lymphodepletion, the patient received autologous TCR-001-engineered T cells and also received anti-PD1 (Tislelizumab) for 3 cycles. In subsequent follow-up, the patient had stable disease 7 weeks after the cell infusion, maintained for 7.3 weeks. The patient had progressive disease with hematuria and new metastatic lesions in bladder 14.3 weeks after the cell infusion, and received supportive care treatment. The patient died with disease 3.9 months after the cell infusion.

Supplemental Figures.

A. Generating the Infusion Product, Transduction Phase



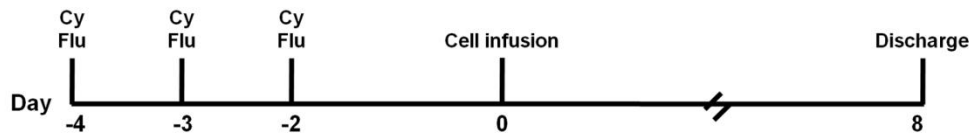
* PBMC stimulation: T cell TransAct (1: 100 volume), IL-2 (300 IU/mL).

B. Generating the Infusion Product, Rapid Expansion Phase



* Rapid expansion: irradiated PBMC, OKT3 (30 ng/mL), IL-2 (3000 IU/mL).

C. Treatment scheme



Cy: Cyclophosphamide 500 mg/m²/day IV

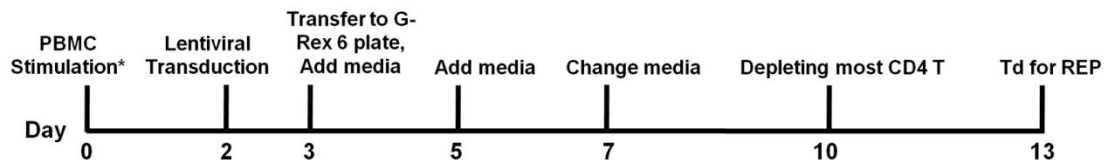
Flu: Fludarabine 30 mg/m²/day IV

Cell infusion: 17.2×10^9 IV (40.7% TCR-transduced in CD8 T cells)

Figure S1. Cell therapy manufacture and treatment scheme during 1st infusion for patient Pt.003.

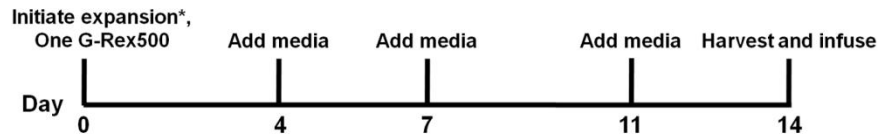
(A-C) Autologous TCR-001-engineered T cells (LV-TCR-001-m Td) for the infusion product were generated and harvested for patient Pt.003 treatment during 1st infusion. T-cell transduction phase (A) and T-cell rapid expansion phase (B) are shown. Additional details are described in the METHODS DETAILS section. The treatment scheme is also shown (C).

A. Generating the Infusion Product, Transduction Phase



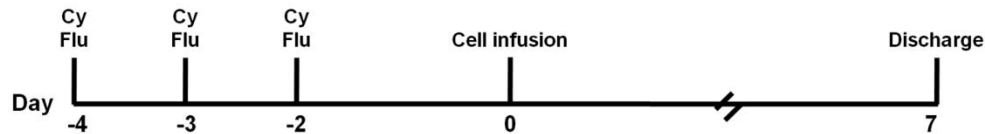
* PBMC stimulation: T cell TransAct (1: 100 volume), IL-2 (300 IU/mL).

B. Generating the Infusion Product, Rapid Expansion Phase



* Rapid expansion: irradiated PBMC, OKT3 (30 ng/mL), IL-2 (3000 IU/mL).

C. Treatment scheme



Cy: Cyclophosphamide 500 mg/m²/day IV

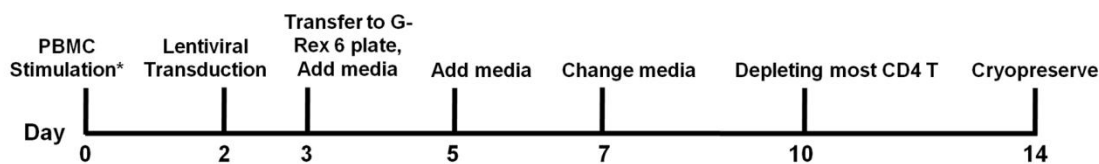
Flu: Fludarabine 30 mg/m²/day IV

Cell infusion: 17.2×10⁹ IV (62.0% TCR-transduced in CD8 T cells)

Figure S2. Cell therapy manufacture and treatment scheme during 2nd infusion for patient Pt.003.

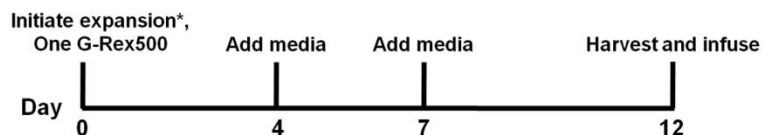
(A-C) Autologous TCR-001-engineered T cells (LV-TCR-001-m Td) for the infusion product were generated and harvested for patient Pt.003 treatment during 2nd infusion. T-cell transduction phase (A) and T-cell rapid expansion phase (B) are shown. Additional details are described in the METHODS DETAILS section. The treatment scheme is also shown (C).

A. Generating the Infusion Product, Transduction Phase



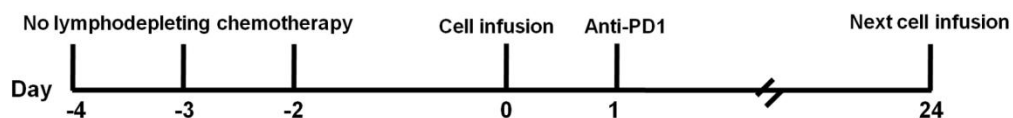
* PBMC stimulation: T cell TransAct (1: 100 volume), IL-2 (300 IU/mL).

B. Generating the Infusion Product, Rapid Expansion Phase



* Rapid expansion: irradiated PBMC, OKT3 (30 ng/mL), IL-2 (3000 IU/mL).

C. Treatment scheme



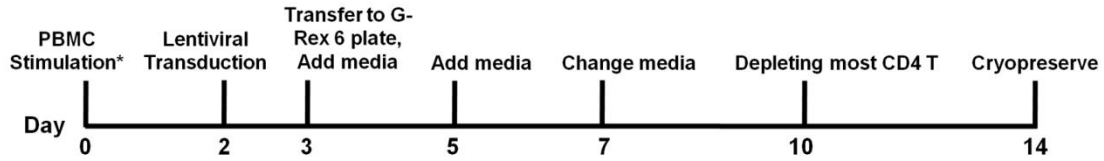
Anti-PD1: Tislelizumab (Beigene, Ltd), 200 mg IV

Cell infusion: 12.7×10^9 IV (45.7% TCR-transduced in CD8 T cells)

Figure S3. Cell therapy manufacture and treatment scheme during 3rd infusion for patient Pt.003.

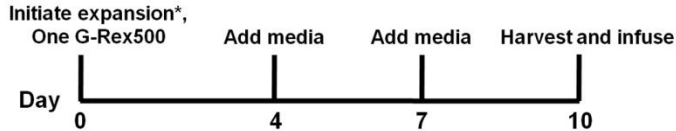
(A-C) Autologous 4148 TCR-engineered T cells (LV-4148 TCR-h Td) for the infusion product were generated and harvested for patient Pt.003 treatment during 3rd infusion. T-cell transduction phase (A) and T-cell rapid expansion phase (B) are shown. Additional details are described in the METHODS DETAILS section. The treatment scheme is also shown (C).

A. Generating the Infusion Product, Transduction Phase



* PBMC stimulation: T cell TransAct (1: 100 volume), IL-2 (300 IU/mL).

B. Generating the Infusion Product, Rapid Expansion Phase



* Rapid expansion: irradiated PBMC, OKT3 (30 ng/mL), IL-2 (3000 IU/mL).

C. Treatment scheme

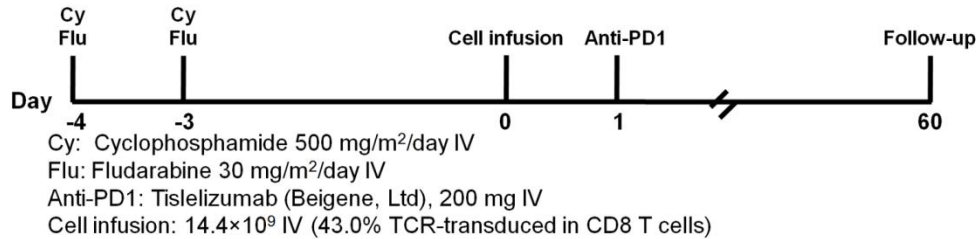
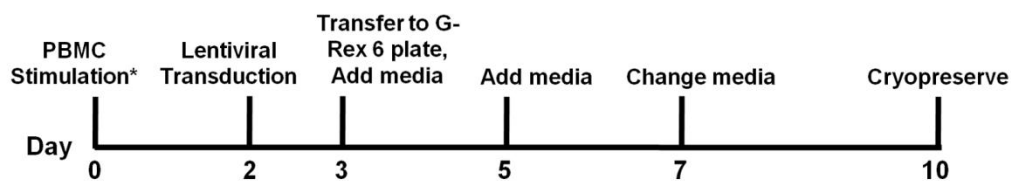


Figure S4. Cell therapy manufacture and treatment scheme during 4th infusion for patient Pt.003.

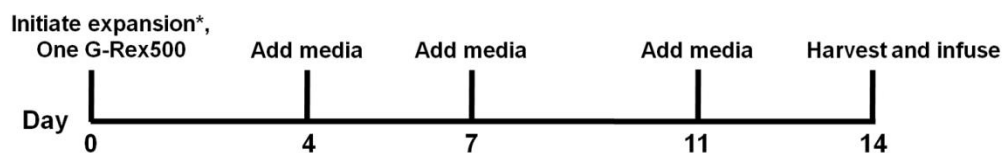
(A-C) Autologous 4148 TCR-engineered T cells (LV-4148 TCR-h Td) for the infusion product were generated and harvested for patient Pt.003 treatment during 4th infusion. T-cell transduction phase (A) and T-cell rapid expansion phase (B) are shown. Additional details are described in the METHODS DETAILS section. The treatment scheme is also shown (C).

A. Generating the Infusion Product, Transduction Phase



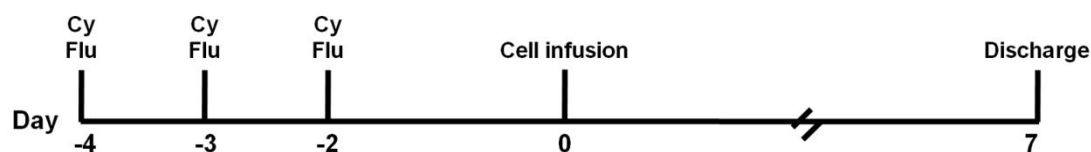
* PBMC stimulation: T cell TransAct (1: 100 volume), IL-2 (300 IU/mL).

B. Generating the Infusion Product, Rapid Expansion Phase



* Rapid expansion: irradiated PBMC, OKT3 (30 ng/mL), IL-2 (3000 IU/mL).

C. Treatment scheme



Cy: Cyclophosphamide 500 mg/m²/day IV

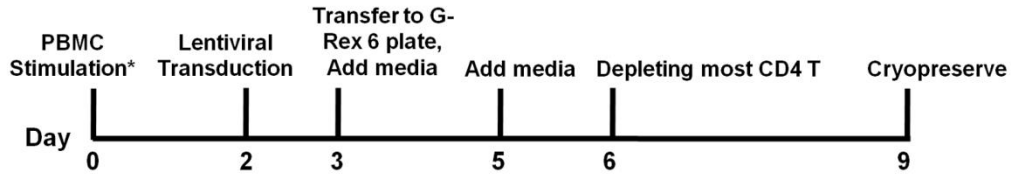
Flu: Fludarabine 30 mg/m²/day IV

Cell infusion: 15.2×10⁹ IV (48.6% TCR-transduced in CD8 T cells)

Figure S5. Cell therapy manufacture and treatment scheme during 1st infusion for patient Pt.005.

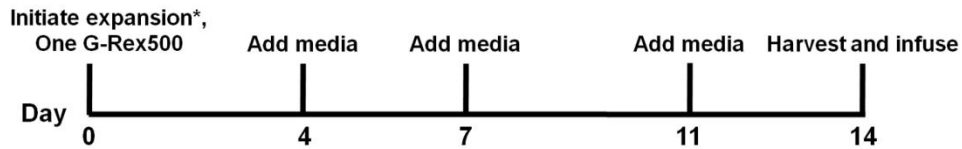
(A-C) Autologous TCR-001-engineered T cells (LV-TCR-001-m Td) for the infusion product were generated and harvested for patient Pt.005 treatment during 1st infusion. T-cell transduction phase (A) and T-cell rapid expansion phase (B) are shown. Additional details are described in the METHODS DETAILS section. The treatment scheme is also shown (C).

A. Generating the Infusion Product, Transduction Phase



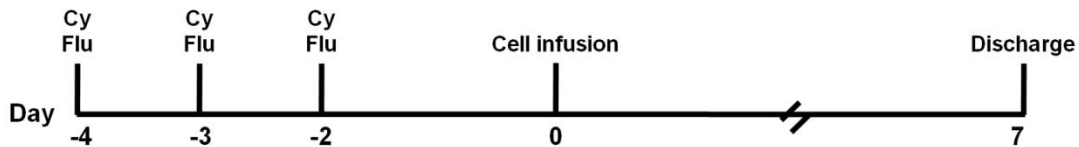
* PBMC stimulation: T cell TransAct (1: 100 volume), IL-2 (300 IU/mL).

B. Generating the Infusion Product, Rapid Expansion Phase



* Rapid expansion: irradiated PBMC, OKT3 (30 ng/mL), IL-2 (3000 IU/mL).

C. Treatment scheme



Cy: Cyclophosphamide 500 mg/m²/day IV

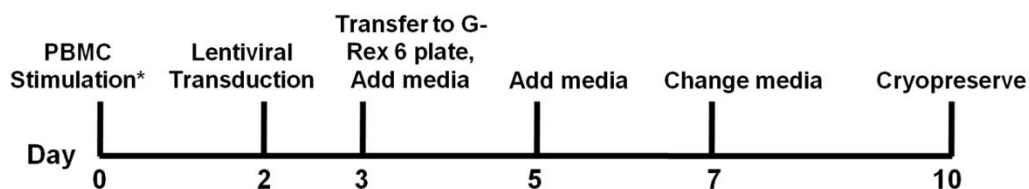
Flu: Fludarabine 30 mg/m²/day IV

Cell infusion: 17.2×10⁹ IV (72.1% TCR-transduced in CD8 T cells)

Figure S6. Cell therapy manufacture and treatment scheme during 1st infusion for patient Pt.006.

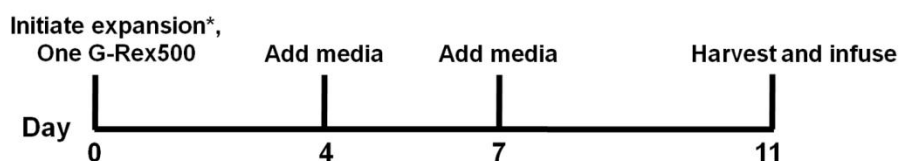
(A-C) Autologous TCR-001-engineered T cells (LV-TCR-001-m Td) for the infusion product were generated and harvested for patient Pt.006 treatment during 1st infusion. T-cell transduction phase (A) and T-cell rapid expansion phase (B) are shown. Additional details are described in the METHODS DETAILS section. The treatment scheme is also shown (C).

A. Generating the Infusion Product, Transduction Phase



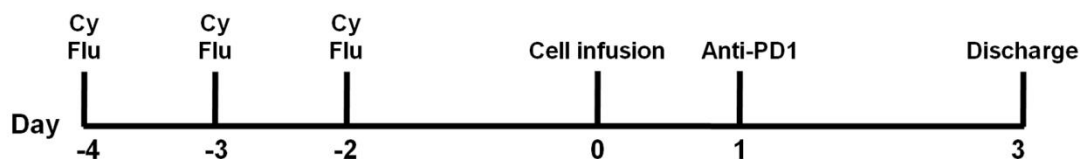
* PBMC stimulation: T cell TransAct (1: 100 volume), IL-2 (300 IU/mL).

B. Generating the Infusion Product, Rapid Expansion Phase



* Rapid expansion: irradiated PBMC, OKT3 (30 ng/mL), IL-2 (3000 IU/mL).

C. Treatment scheme



Cy: Cyclophosphamide 500 mg/m²/day IV

Flu: Fludarabine 30 mg/m²/day IV

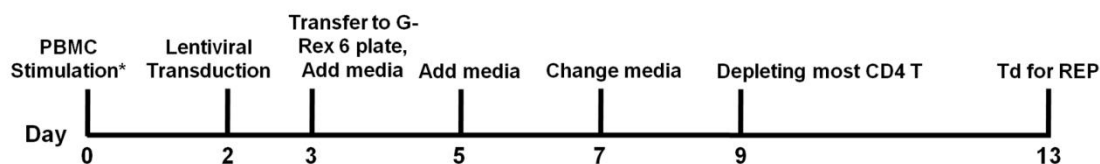
Anti-PD1: Tislelizumab (Beigene, Ltd), 200 mg IV

Cell infusion: 16.5×10⁹ IV (55.6% TCR-transduced in CD8 T cells)

Figure S7. Cell therapy manufacture and treatment scheme during 2nd infusion for patient Pt.006.

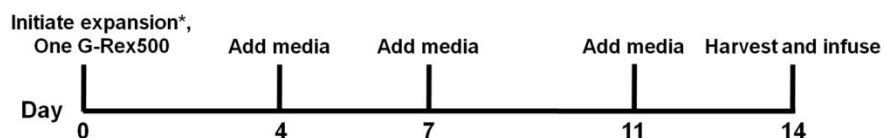
(A-C) Autologous 4148 TCR-engineered T cells (LV-4148 TCR-h Td) for the infusion product were generated and harvested for patient Pt.006 treatment during 2nd infusion. T-cell transduction phase (A) and T-cell rapid expansion phase (B) are shown. Additional details are described in the METHODS DETAILS section. The treatment scheme is also shown (C).

A. Generating the Infusion Product, Transduction Phase



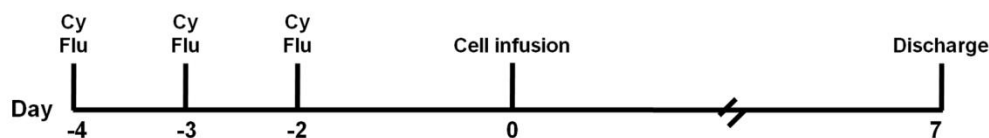
* PBMC stimulation: T cell TransAct (1: 100 volume), IL-2 (300 IU/mL).

B. Generating the Infusion Product, Rapid Expansion Phase



* Rapid expansion: irradiated PBMC, OKT3 (30 ng/mL), IL-2 (3000 IU/mL).

C. Treatment scheme



Cy: Cyclophosphamide 500 mg/m²/day IV

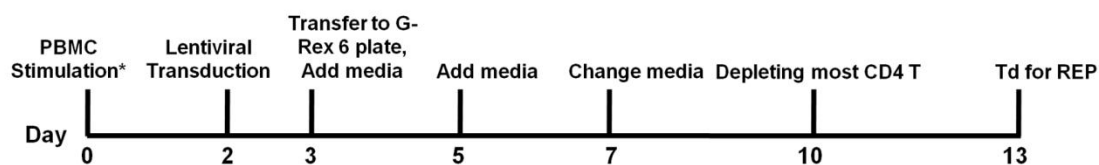
Flu: Fludarabine 30 mg/m²/day IV

Cell infusion: 16.5×10⁹ IV (78.3% TCR-transduced in CD8 T cells)

Figure S8. Cell therapy manufacture and treatment scheme during 1st infusion for patient Pt.007.

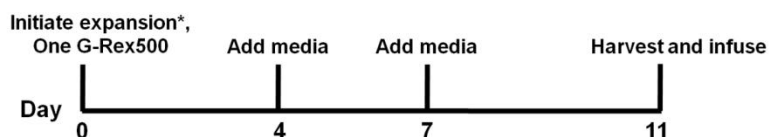
(A-C) Autologous TCR-001-engineered T cells (LV-TCR-001-m Td) for the infusion product were generated and harvested for patient Pt.007 treatment during 1st infusion. T-cell transduction phase (A) and T-cell rapid expansion phase (B) are shown. Additional details are described in the METHODS DETAILS section. The treatment scheme is also shown (C).

A. Generating the Infusion Product, Transduction Phase



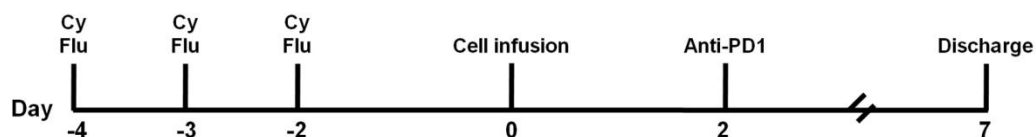
* PBMC stimulation: T cell TransAct (1: 100 volume), IL-2 (300 IU/mL).

B. Generating the Infusion Product, Rapid Expansion Phase



* Rapid expansion: irradiated PBMC, OKT3 (30 ng/mL), IL-2 (3000 IU/mL).

C. Treatment scheme



Cy: Cyclophosphamide 500 mg/m²/day IV

Flu: Fludarabine 30 mg/m²/day IV

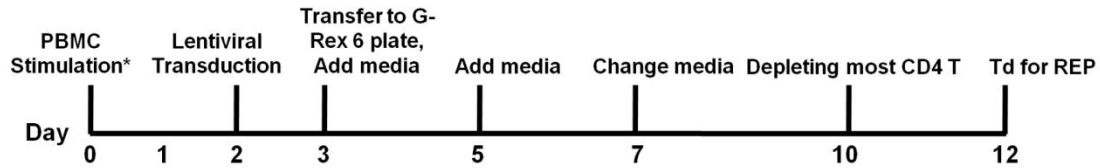
Anti-PD1: Tislelizumab (Beigene, Ltd), 200 mg IV

Cell infusion: 16.8×10⁹ IV (62.5% TCR-transduced in CD8 T cells)

Figure S9. Cell therapy manufacture and treatment scheme during 2nd infusion for patient Pt.007.

(A-C) Autologous 4148 TCR-engineered T cells (LV-4148 TCR-h Td) for the infusion product were generated and harvested for patient Pt.007 treatment during 2nd infusion. T-cell transduction phase (A) and T-cell rapid expansion phase (B) are shown. Additional details are described in the METHODS DETAILS section. The treatment scheme is also shown (C).

A. Generating the Infusion Product, Transduction Phase



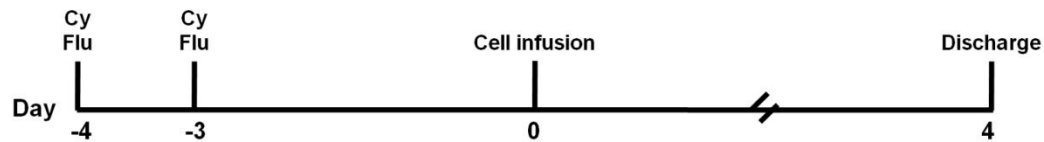
* PBMC stimulation: T cell TransAct (1: 100 volume), IL-2 (300 IU/mL).

B. Generating the Infusion Product, Rapid Expansion Phase



* Rapid expansion: irradiated PBMC, OKT3 (30 ng/mL), IL-2 (3000 IU/mL).

C. Treatment scheme



Cy: Cyclophosphamide 500 mg/m²/day IV

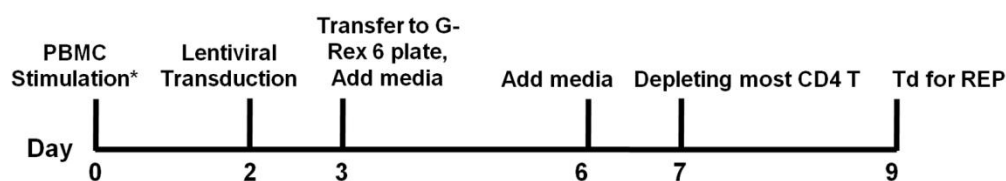
Flu: Fludarabine 30 mg/m²/day IV

Cell infusion: 17.1×10^9 IV (38.0% TCR-transduced in CD8 T cells)

Figure S10. Cell therapy manufacture and treatment scheme during 3rd infusion for patient Pt.007.

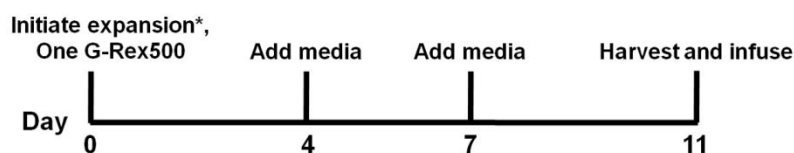
(A-C) Autologous 4148 TCR-engineered T cells (LV-4148 TCR-h Td) for the infusion product were generated and harvested for patient Pt.007 treatment during 3rd infusion. T-cell transduction phase (A) and T-cell rapid expansion phase (B) are shown. Additional details are described in the METHODS DETAILS section. The treatment scheme is also shown (C).

A. Generating the Infusion Product, Transduction Phase



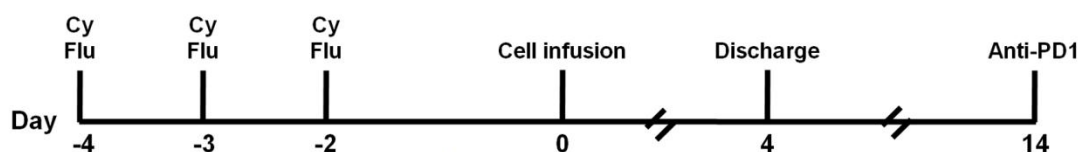
* PBMC stimulation: T cell TransAct (1: 100 volume), IL-2 (300 IU/mL).

B. Generating the Infusion Product, Rapid Expansion Phase



* Rapid expansion: irradiated PBMC, OKT3 (30 ng/mL), IL-2 (3000 IU/mL).

C. Treatment scheme



Cy: Cyclophosphamide 500 mg/m²/day IV

Flu: Fludarabine 30 mg/m²/day IV

Anti-PD1: Tislelizumab (Beigene, Ltd), 200 mg IV

Cell infusion: 17.1×10^9 IV (61.6% TCR-transduced in CD8 T cells)

Figure S11. Cell therapy manufacture and treatment scheme during 1st infusion for patient Pt.008.

(A-C) Autologous TCR-001-engineered T cells (LV-TCR-001-m Td) for the infusion product were generated and harvested for patient Pt.008 treatment during 1st infusion. T-cell transduction phase (A) and T-cell rapid expansion phase (B) are shown. Additional details are described in the METHODS DETAILS section. The treatment scheme is also shown (C).

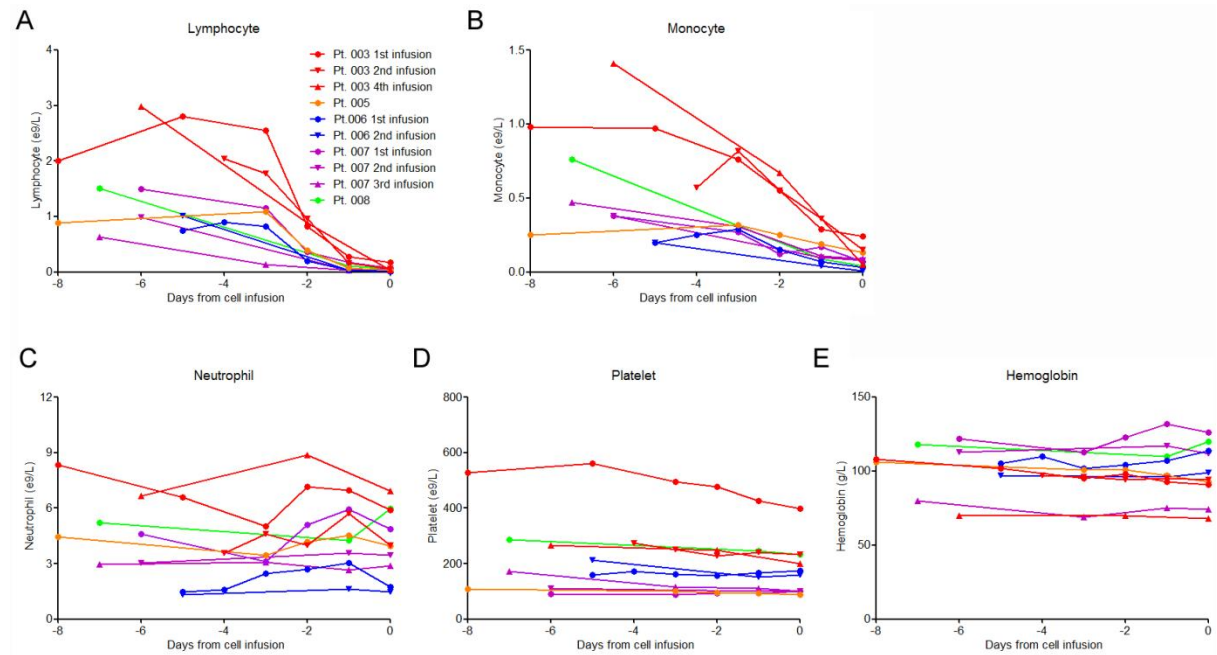


Figure S12. Changes in various routine clinical blood tests in five patients after a total of 10 lymphodepletion regimens.

(A-E) Routine clinical blood tests were performed during non-myeloablative lymphodepletion.

Changes in values of lymphocyte (A), monocyte (B), neutrophil (C), platelet (D) and hemoglobin (E) are shown.

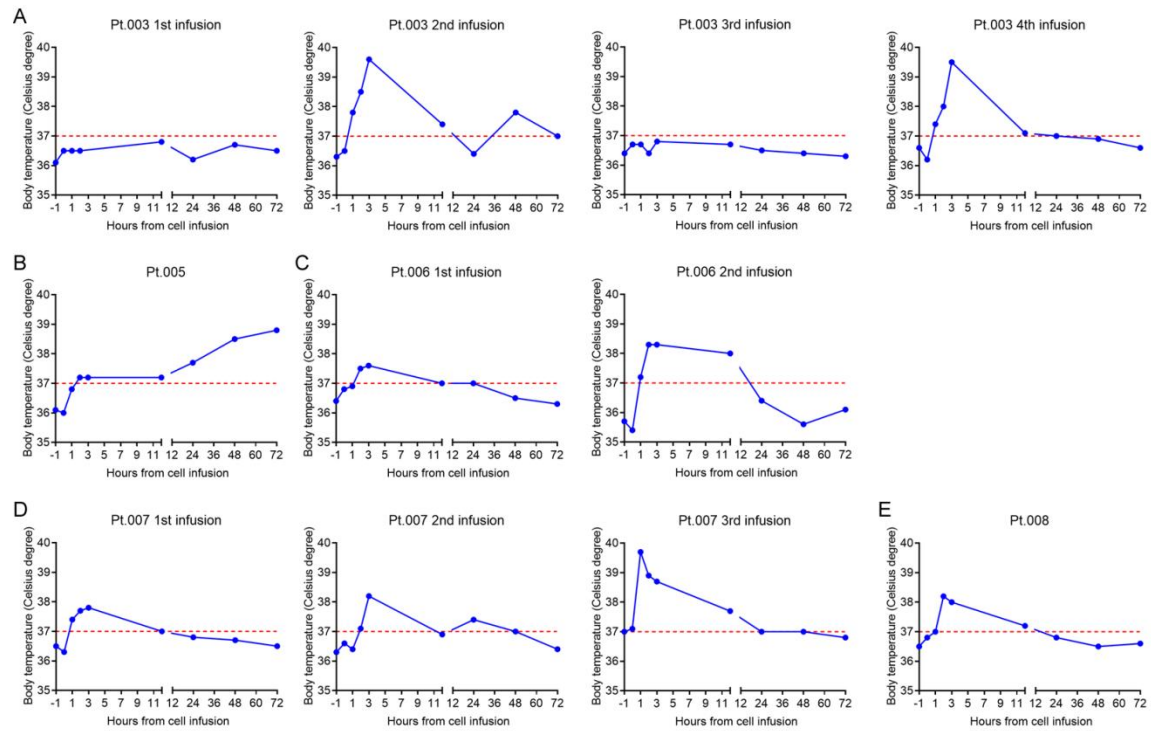


Figure S13. Body temperature within 72 hours of five patients undergoing a total of 11 cell infusions.

(A-E) Changes in body temperature before and 72 hours after cell infusion in Pt.003 (A), Pt.005 (B), Pt.006 (C), Pt.007 (D), and Pt.008 (E). Red dashed line indicates the upper limit of normal value.

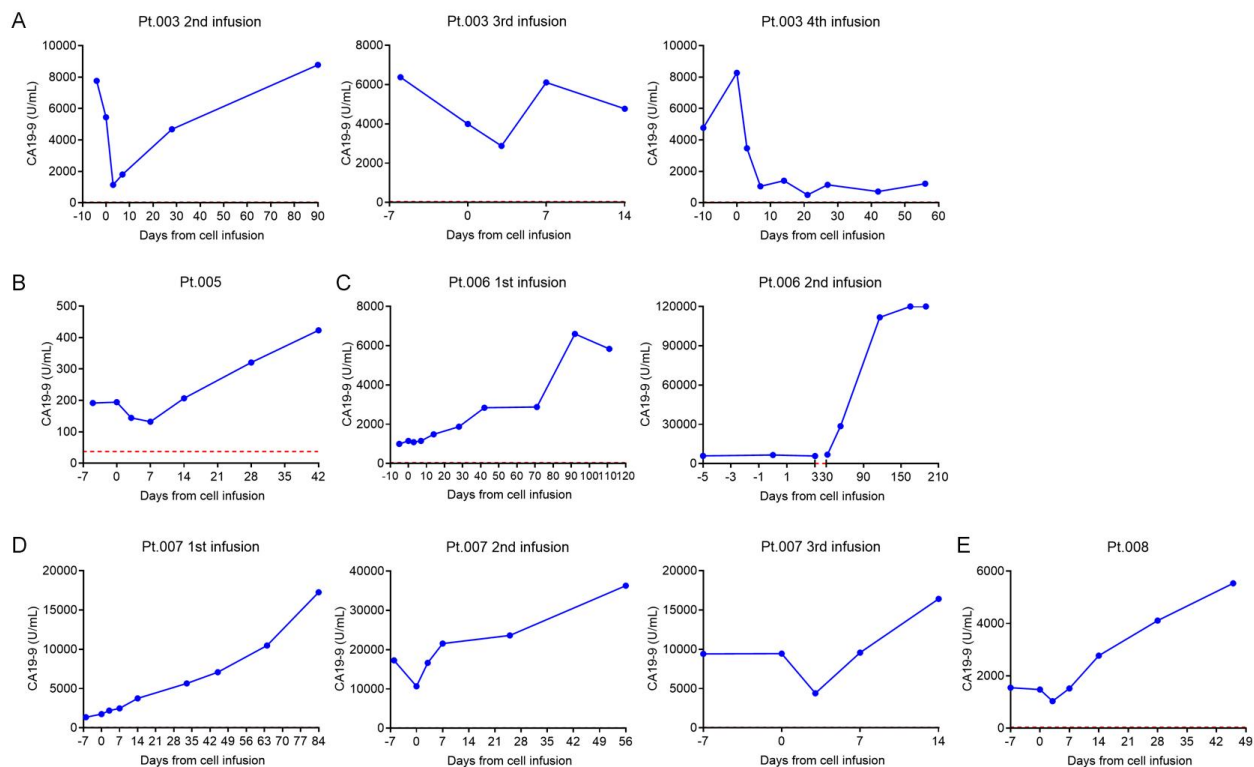


Figure S14. CA19-9 levels in five patients receiving a total of 11 cell infusions.

(A-E) Serum concentrations of CA19-9 before and after cell infusions in Pt.003 (A), Pt.005 (B), Pt.006 (C), Pt.007 (D), and Pt.008 (E). Red dashed line indicates the upper limit of normal value.

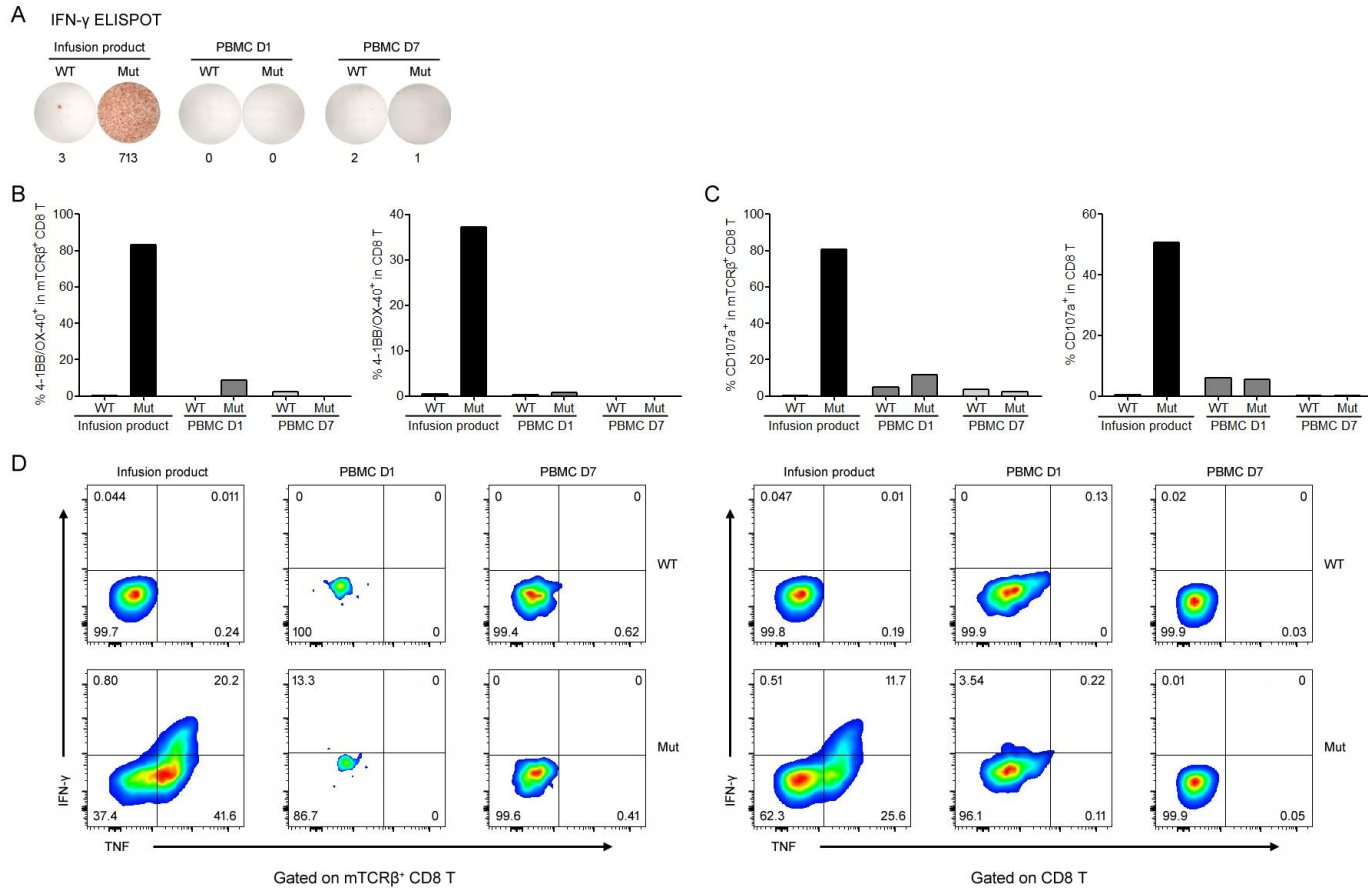


Figure S15. Evaluation of *in vitro* effector functions of TCR-001-engineered T cells persisting in the PBMC after the second infusion of Pt.003.

(A-B) IFN- γ ELISPOT (A) and 4-1BB/OX-40 expression on mTCR β^+ CD8 T cells or CD8 T cells (B) in the indicated PBMC sample or the infusion product after an overnight co-culture of with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9mer peptide. (C-D) Flow cytometric analysis of CD107a expression (C) and intracellular IFN- γ /TNF production (D) on mTCR β^+ CD8 T cells or CD8 T cells is shown after 5h stimulation of the indicated PBMC sample or the infusion product with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9mer peptide. Left panels are gated on mTCR β^+ CD8 T cells and right panels are gated on CD8 T cells (B-D).

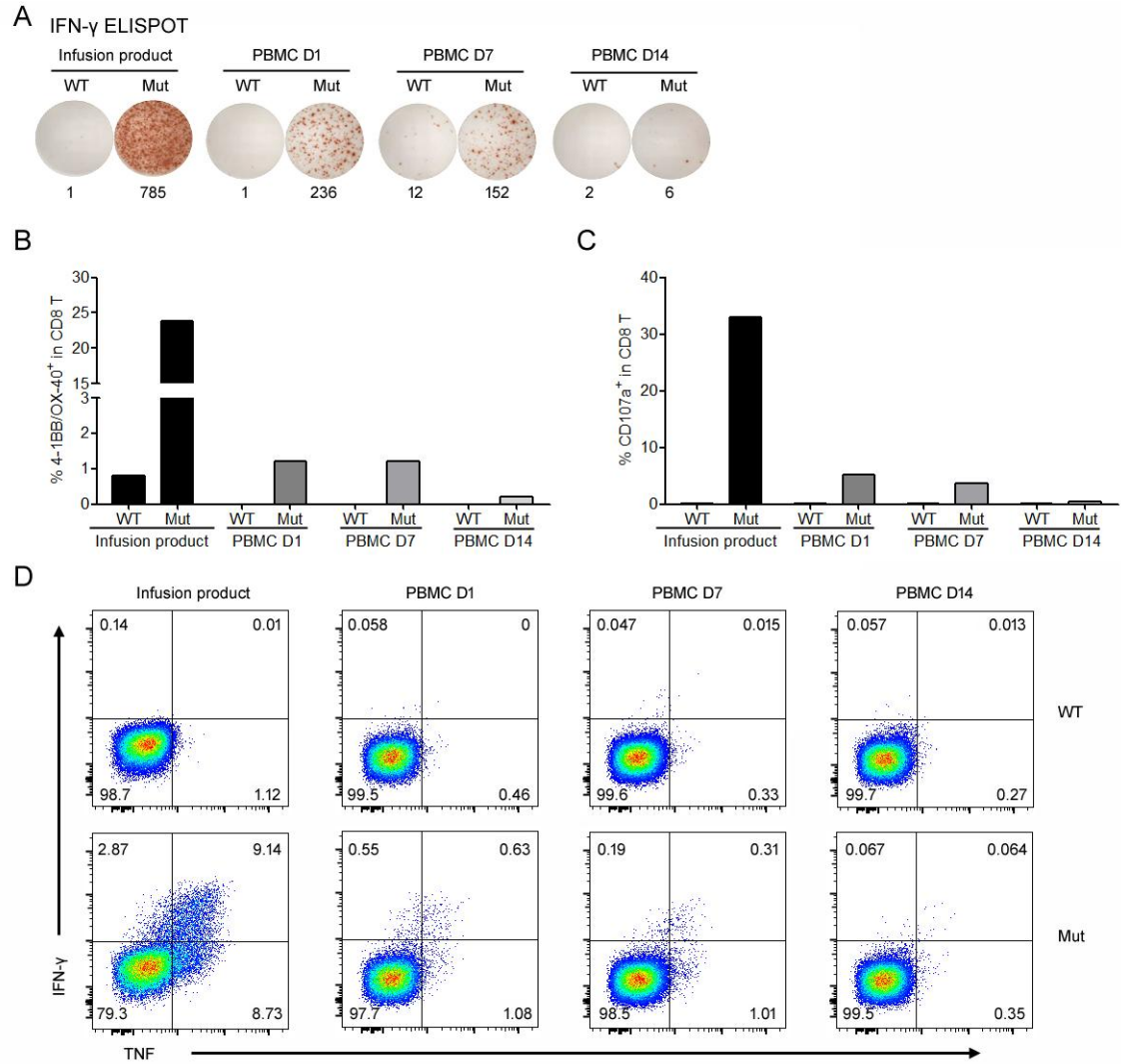


Figure S16. Evaluation of *in vitro* effector functions of 4148 TCR-engineered T cells persisting in the PBMC after the third infusion of Pt.003.

(A-B) IFN- γ ELISPOT (A) and 4-1BB/OX-40 expression on CD8 T cells (B) in the indicated PBMC sample or the infusion product after an overnight co-culture of with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9mer peptide. (C-D) Flow cytometric analysis of CD107a expression (C) and intracellular IFN- γ /TNF production (D) on CD8 T cells is shown after 5h stimulation of the indicated PBMC sample or the infusion product with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9mer peptide.

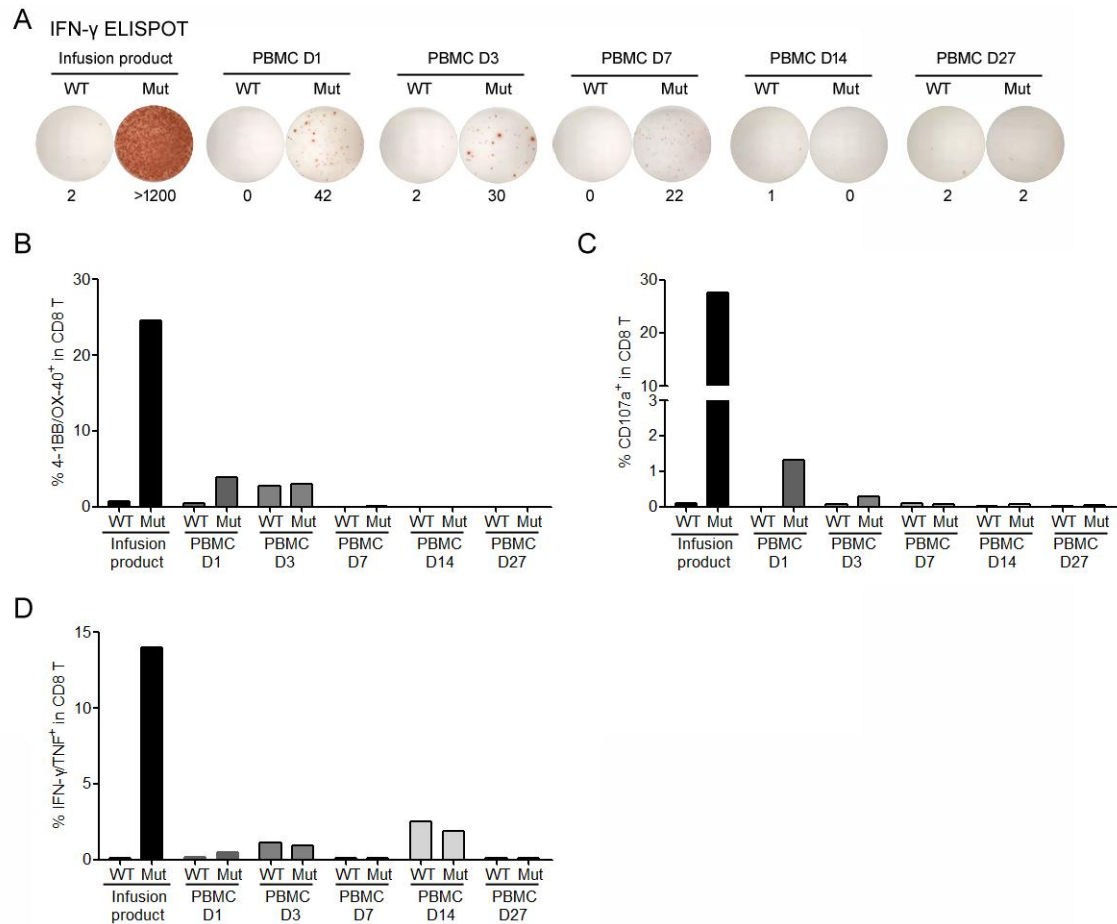


Figure S17. Evaluation of *in vitro* effector functions of 4148 TCR-engineered T cells persisting in the PBMC after the fourth infusion of Pt.003.

(A-B) IFN- γ ELISPOT (A) and 4-1BB/OX-40 expression on CD8 T cells (B) in the indicated PBMC sample or the infusion product after an overnight co-culture of with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9mer peptide. (C-D) Flow cytometric analysis of CD107a expression (C) and intracellular IFN- γ /TNF production (D) on CD8 T cells is shown after 5h stimulation of the indicated PBMC sample or the infusion product with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9mer peptide.

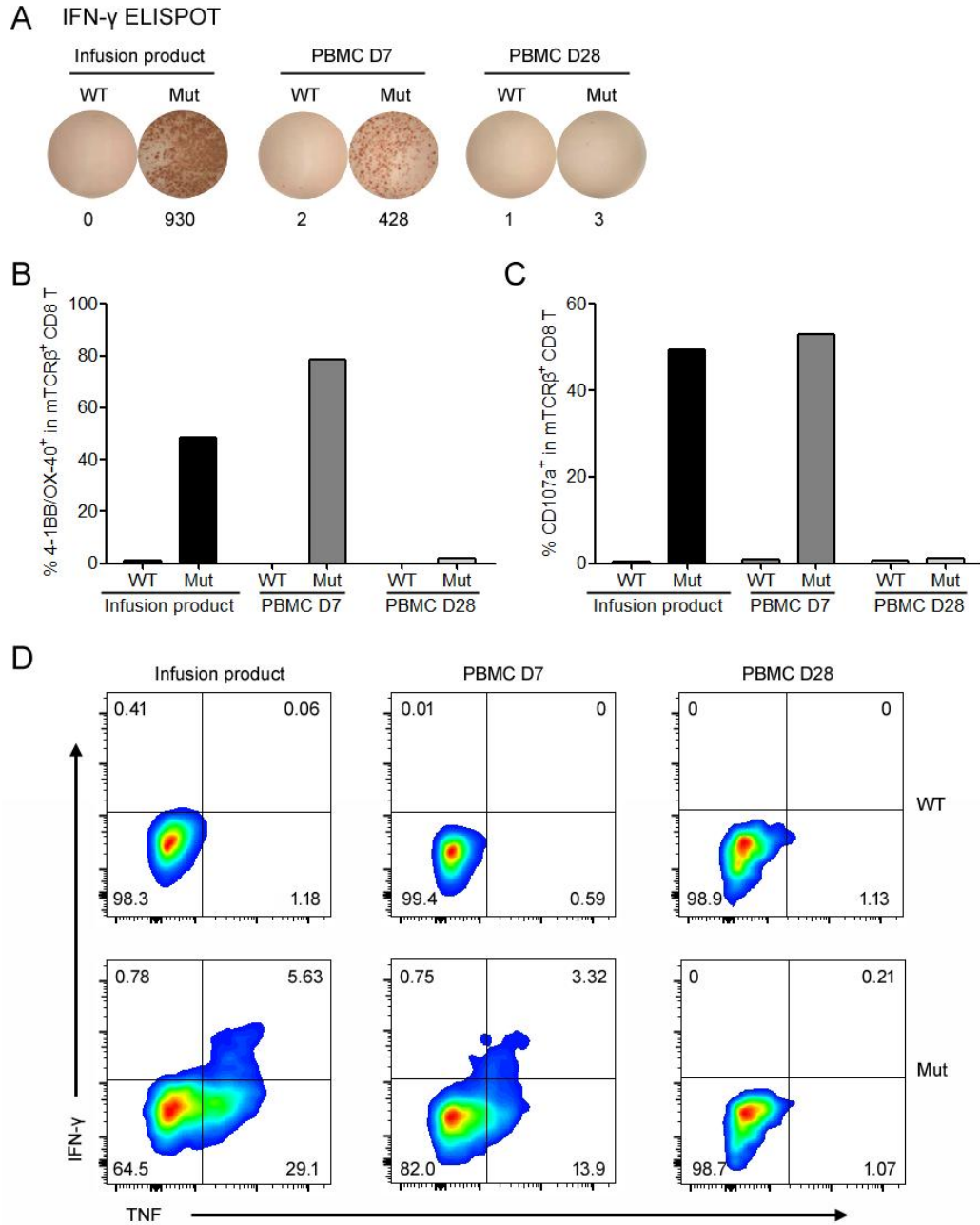


Figure S18. Evaluation of *in vitro* effector functions of TCR-001-engineered T cells persisting in the PBMC after the first infusion of Pt.005.

(A-B) IFN- γ ELISPOT (A) and 4-1BB/OX-40 expression on mTCR β ⁺ CD8 T cells (B) in the indicated PBMC sample or the infusion product after an overnight co-culture of with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9mer peptide. (C-D) Flow cytometric analysis of CD107a expression (C) and intracellular IFN- γ /TNF production (D) on mTCR β ⁺ CD8 T cells is shown after 5h stimulation of the indicated PBMC sample or the infusion product with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9mer peptide.

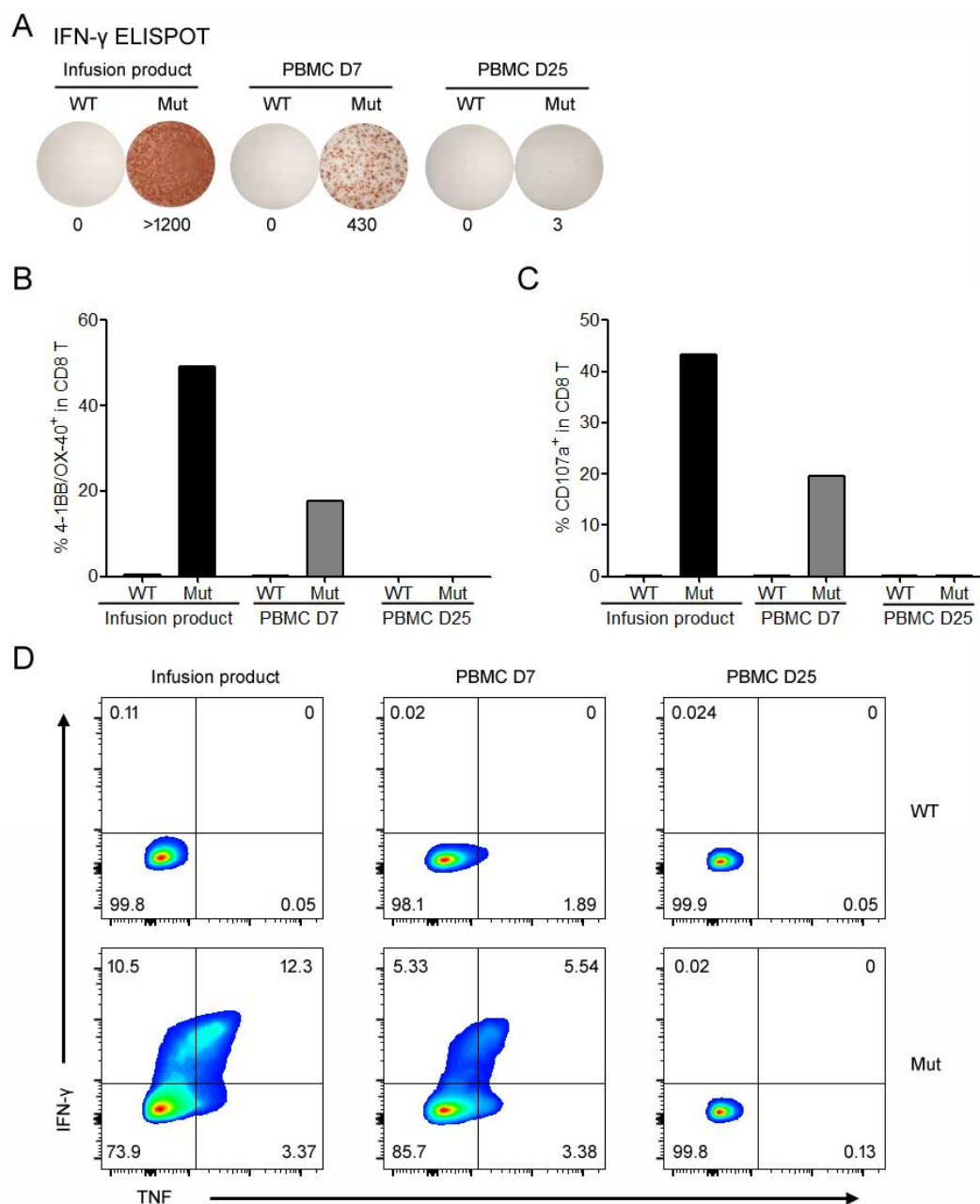


Figure S19. Evaluation of *in vitro* effector functions of 4148 TCR-engineered T cells persisting in the PBMC after the second infusion of Pt.007.

(A-B) IFN- γ ELISPOT (A) and 4-1BB/OX-40 expression on CD8 T cells (B) in the indicated PBMC sample or the infusion product after an overnight co-culture of with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9mer peptide. (C-D) Flow cytometric analysis of CD107a expression (C) and intracellular IFN- γ /TNF production (D) on CD8 T cells is shown after 5h stimulation of the indicated PBMC sample or the infusion product with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9mer peptide.

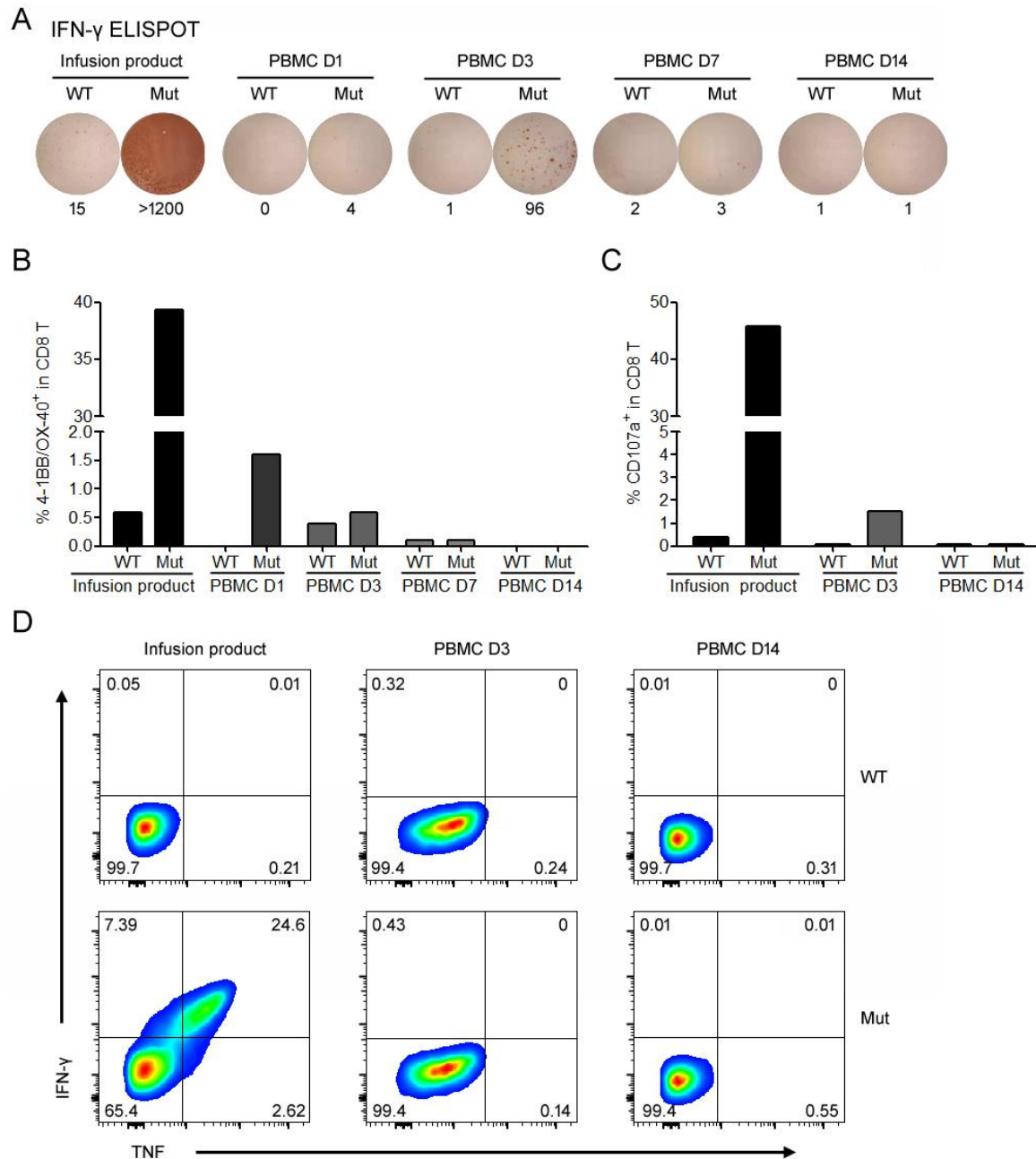


Figure S20. Evaluation of *in vitro* effector functions of 4148 TCR-engineered T cells persisting in the PBMC after the third infusion of Pt.007.

(A-B) IFN- γ ELISPOT (A) and 4-1BB/OX-40 expression on CD8 T cells (B) in the indicated PBMC sample or the infusion product after an overnight co-culture of with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9mer peptide. (C-D) Flow cytometric analysis of CD107a expression (C) and intracellular IFN- γ /TNF production (D) on CD8 T cells is shown after 5h stimulation of the indicated PBMC sample or the infusion product with 1 μ g/mL KRAS wild-type (WT) or KRAS G12V (Mut) 9mer peptide.

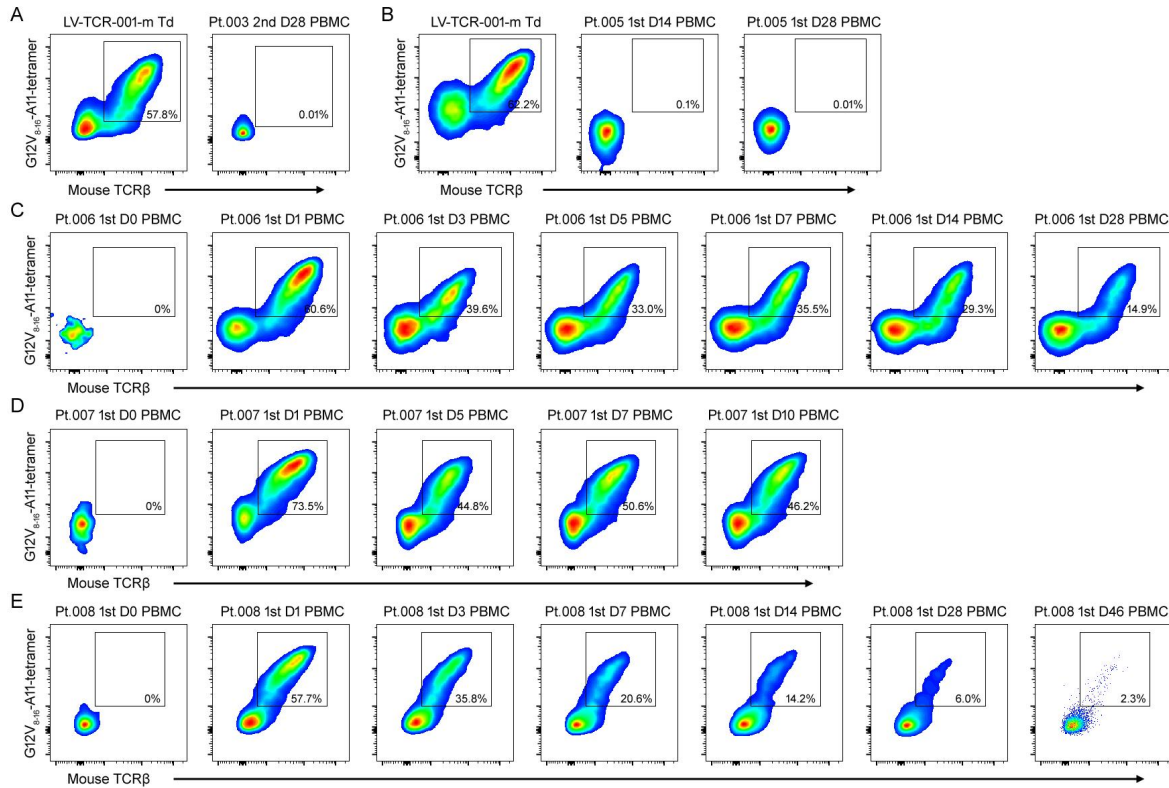


Figure S21. Evaluation of TCR-001-engineered T cells persisting in the PBMC after cell infusion by tetramer staining.

(A-E) The *in vivo* persistence of the transferred TCR-001-engineered T cells in the peripheral blood was determined by flow cytometric analysis of KRAS G12V₈₋₁₆-HLA-A*11:01-tetramer and mouse TCR β in Pt.003 (A), Pt.005 (B), Pt.006 (C), Pt.007 (D), and Pt.008 (E). Data are gated on CD3⁺ CD8 T cells. LV-TCR-001-m Td cells were used as positive control.

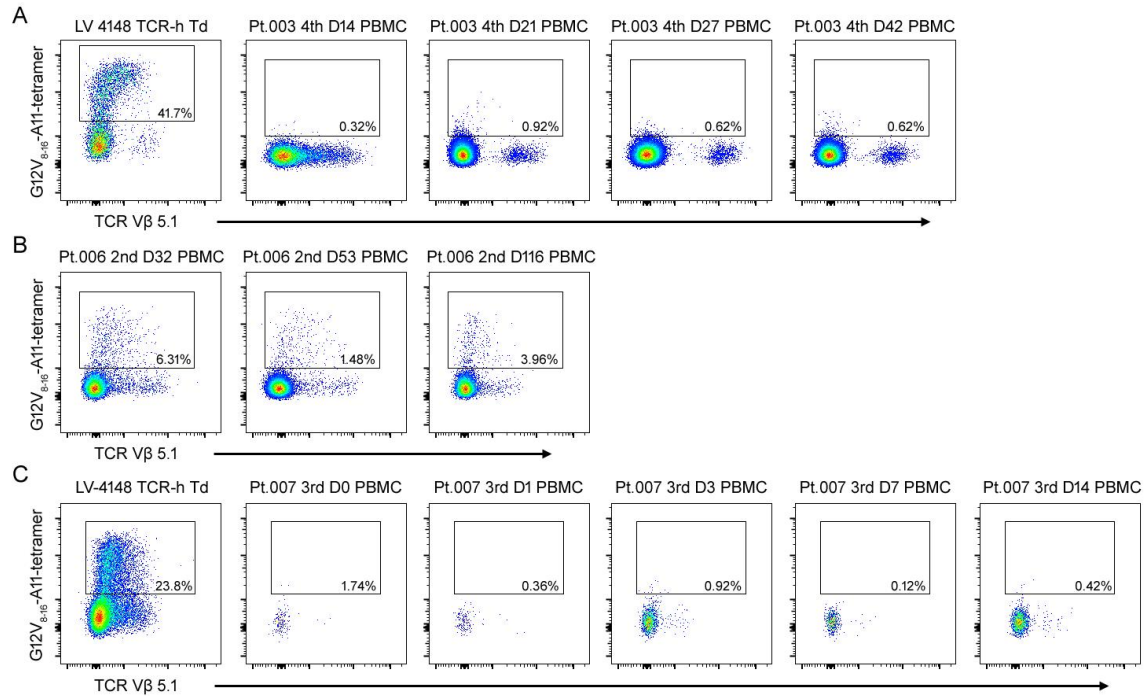


Figure S22. Evaluation of 4148 TCR-engineered T cells persisting in the PBMC after cell infusion by tetramer staining.

(A-C) The *in vivo* persistence of the transferred 4148 TCR-engineered T cells in the peripheral blood was determined by flow cytometric analysis of KRAS G12V₈₋₁₆-HLA-A*11:01-tetramer and TCR Vβ 5.1 in Pt.003 (A), Pt.006 (B), and Pt.008 (C). Data are gated on CD3⁺ CD8 T cells. LV-4148 TCR-h Td cells were used as positive control. Notably, the staining of PE-conjugated TCR Vβ 5.1 antibody was inhibited by APC-conjugated KRAS G12V₈₋₁₆-HLA-A*11:01-tetramer likely due to epitope competition (e.g., peptide/MHC tetramers bind to the CDR1 and CDR2 loops of the TCR variable region which likely are also targeted by the TCR Vβ 5.1 antibody), steric hindrance, or peptide/MHC tetramer mediated downregulation of the TCR.

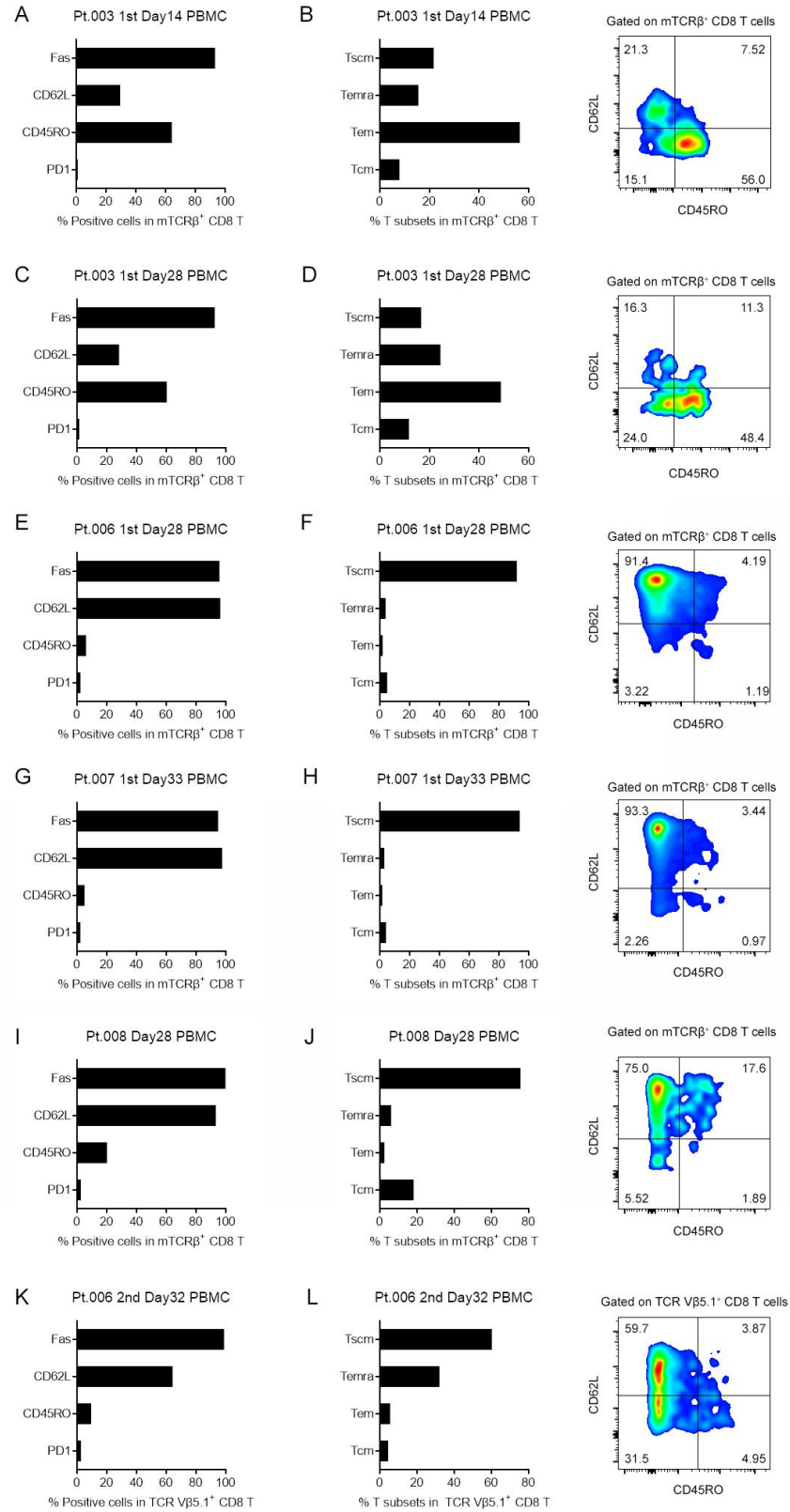


Figure S23. Phenotypes of persistent TCR-engineered CD8 T cells in post-treatment PBMC.

(A-F) After autologous TCR-001-engineered T-cell products were infused to Pt.003 (A-D), Pt.006 (E, F), Pt.007 (G, H) and Pt.008 (I, J) patients, flow cytometric analysis of select cell surface proteins expressed on persistent TCR-001-engineered T cells is shown in about one month post-treatment PBMC. Data are gated on mTCR β^+ CD8 T cells. The percentages of various cell surface proteins positive cells on mTCR β^+ CD8 T cells are shown (A, C, E, G, I). The percentages of T cell subsets (Left) on mTCR β^+ CD8 T cells based on CD62L and CD45RO expression are shown (B, D, F, H, J) with corresponding flow cytometric plot (Right). (K-L) After autologous 4148 TCR-engineered T-cell products were infused to Pt.006 patient, flow cytometric analysis of select cell surface proteins expressed on persistent 4148 TCR-engineered T cells is shown in about one month post-treatment PBMC. Data are gated on TCR V β 5.1 $^+$ CD8 T cells. The percentages of various cell surface proteins positive cells on TCR V β 5.1 $^+$ CD8 T cells are shown (K). The percentages of T cell subsets (Left) on TCR V β 5.1 $^+$ CD8 T cells based on CD62L and CD45RO expression are shown (L) with corresponding flow cytometric plot (Right).

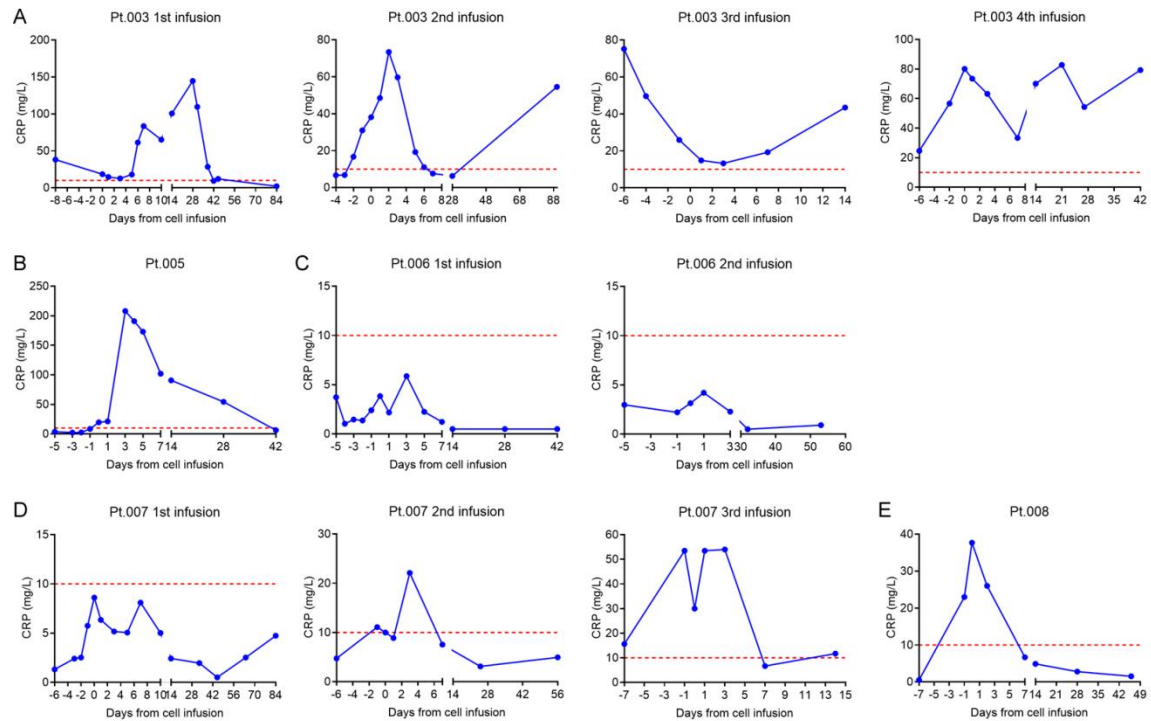


Figure S24. C-reactive protein levels in the serum of 5 patients undergoing a total of 11 cell infusions.

(A-E) Changes in C-reactive protein (CRP) during cell infusion in Pt.003 (A), Pt.005 (B), Pt.006 (C), Pt.007 (D), and Pt.008 (E) are shown. Red dashed line indicates the upper limit of normal value.

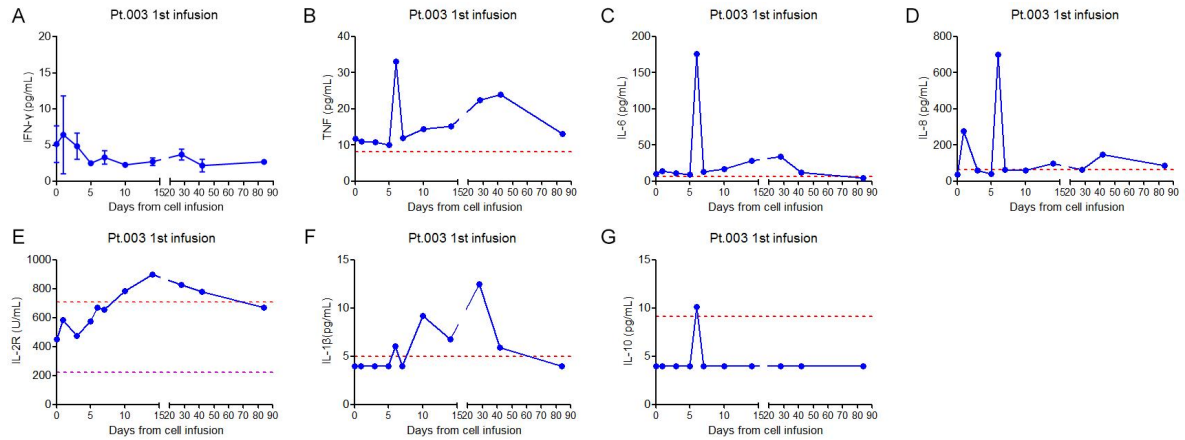


Figure S25. Changes in plasma cytokines during the first cell infusion in Pt.003 patient.

(A) Plasma IFN- γ was assayed by human IFN- γ ELISA kit (Dakewe). Error bars represent SD of 3 technical replicates. (B-G) Plasma TNF (B), IL-6 (C), IL-8 (D), IL-2R (E), IL-1 β (F) and IL-10 (G) were assayed by IMULITE 1000 immunoassay system inflammation panel (Siemens Healthcare). Red dashed line indicates the upper limit of normal value.

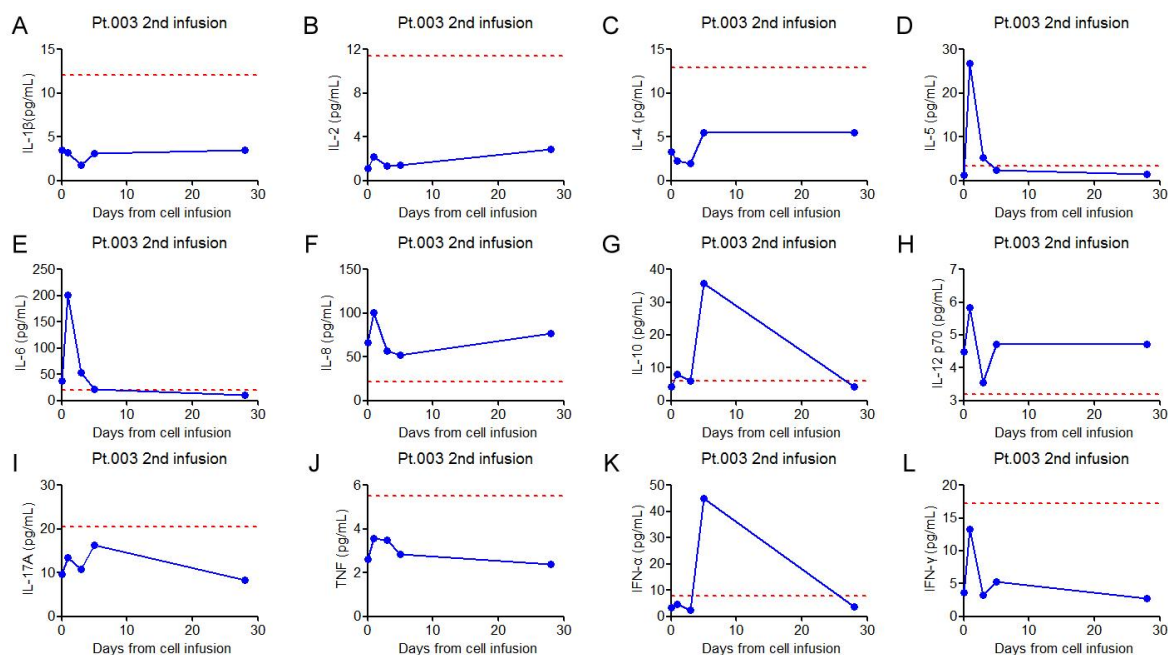


Figure S26. Changes in plasma cytokines during the second cell infusion in Pt.003 patient.

(A-L) Plasma IL-1 β (A), IL-2 (B), IL-4 (C), IL-5 (D), IL-6 (E), IL-8 (F), IL-10 (G), IL-12 p70 (H), IL-17A (I), TNF (J), IFN- α (K), and IFN- γ (L) were assayed by multi-cytokine detection kit (Hangzhou Cellgene Biotech Co., Ltd). Red dashed line indicates the upper limit of normal value.

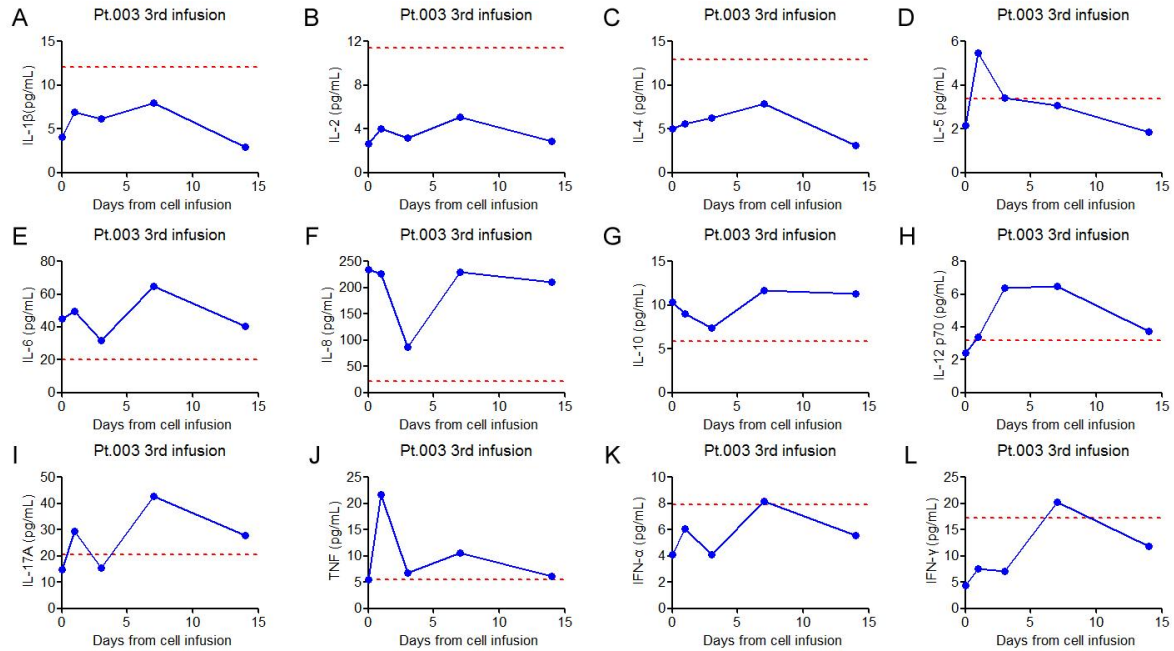


Figure S27. Changes in plasma cytokines during the third cell infusion in Pt.003 patient. (A-L) Plasma IL-1 β (A), IL-2 (B), IL-4 (C), IL-5 (D), IL-6 (E), IL-8 (F), IL-10 (G), IL-12 p70 (H), IL-17A (I), TNF (J), IFN- α (K), and IFN- γ (L) were assayed by multi-cytokine detection kit (Hangzhou Cellgene Biotech Co., Ltd). Red dashed line indicates the upper limit of normal value.

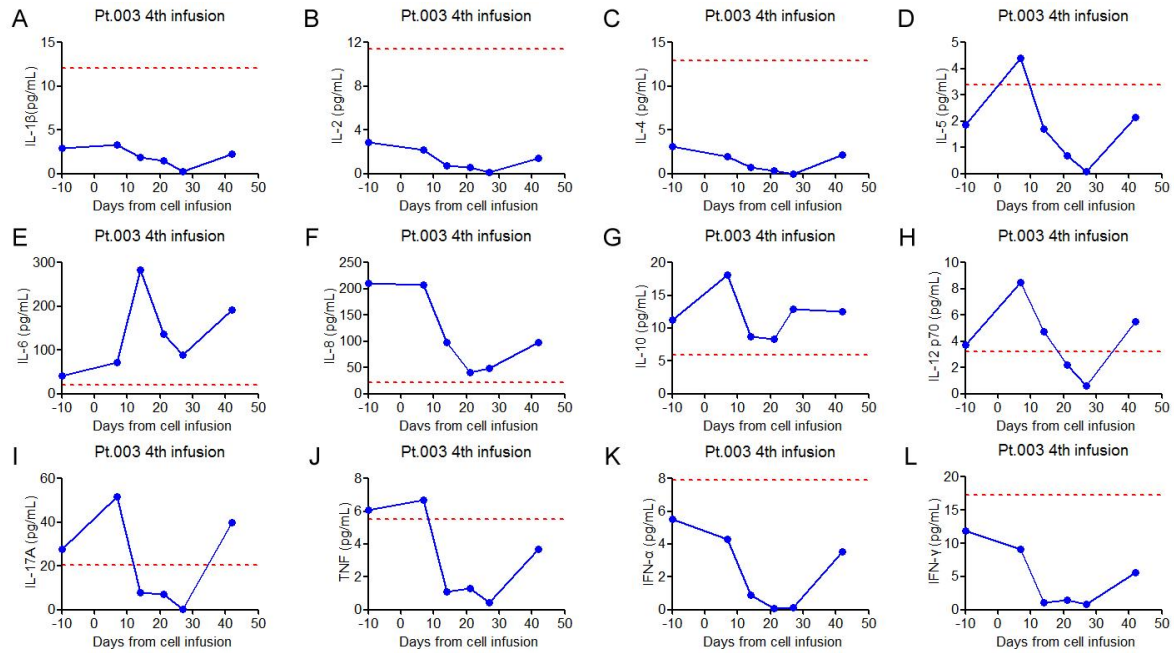


Figure S28. Changes in plasma cytokines during the fourth cell infusion in Pt.003 patient.

(A-L) Plasma IL-1 β (A), IL-2 (B), IL-4 (C), IL-5 (D), IL-6 (E), IL-8 (F), IL-10 (G), IL-12 p70 (H), IL-17A (I), TNF (J), IFN- α (K), and IFN- γ (L) were assayed by multi-cytokine detection kit (Hangzhou Cellgene Biotech Co., Ltd). Red dashed line indicates the upper limit of normal value.

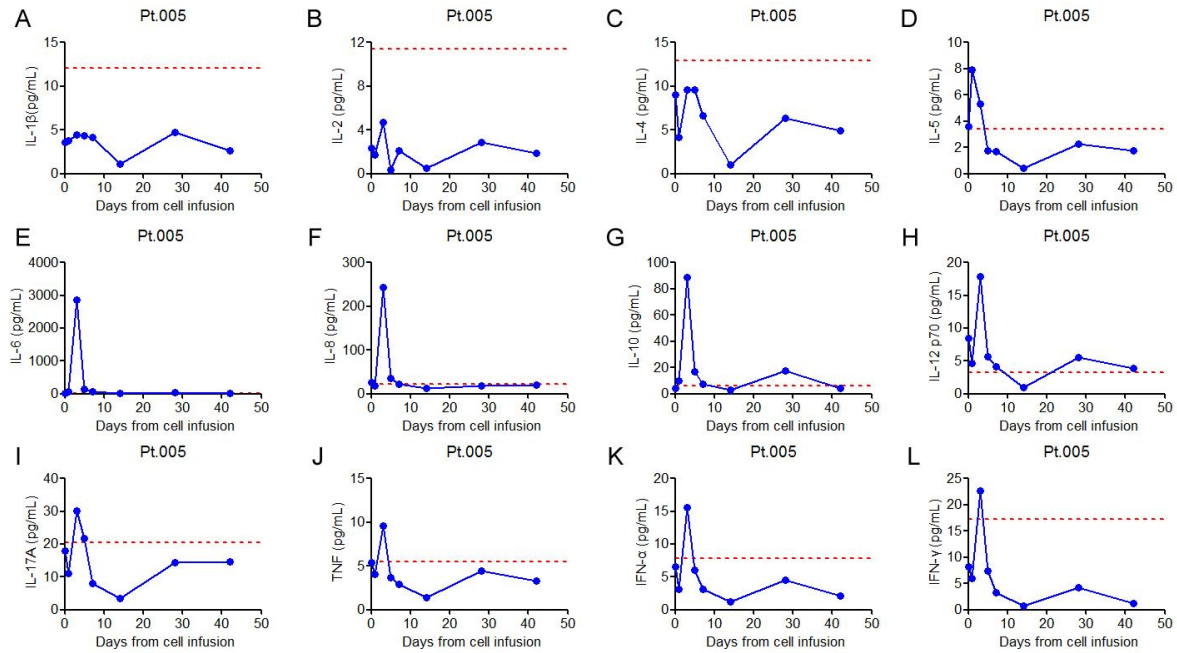


Figure S29. Changes in plasma cytokines during the first cell infusion in Pt.005 patient.

(A-L) Plasma IL-1 β (A), IL-2 (B), IL-4 (C), IL-5 (D), IL-6 (E), IL-8 (F), IL-10 (G), IL-12 p70 (H), IL-17A (I), TNF (J), IFN- α (K), and IFN- γ (L) were assayed by multi-cytokine detection kit (Hangzhou Cellgene Biotech Co., Ltd). Red dashed line indicates the upper limit of normal value.

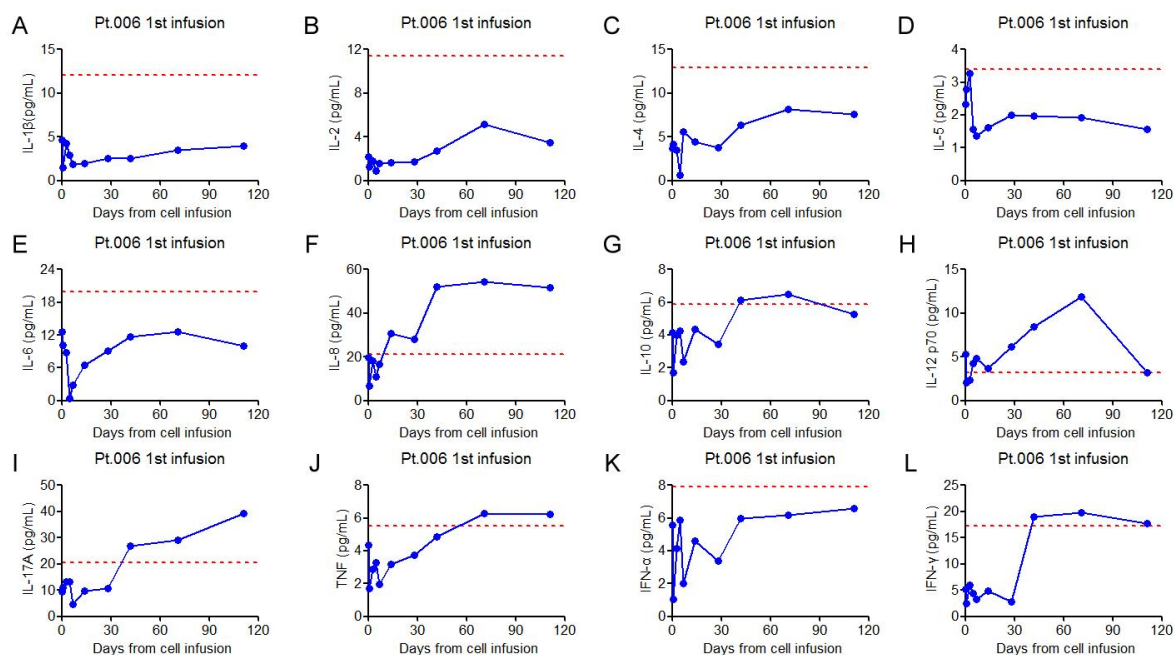


Figure S30. Changes in plasma cytokines during the first cell infusion in Pt.006 patient.

(A-L) Plasma IL-1 β (A), IL-2 (B), IL-4 (C), IL-5 (D), IL-6 (E), IL-8 (F), IL-10 (G), IL-12 p70 (H), IL-17A (I), TNF (J), IFN- α (K), and IFN- γ (L) were assayed by multi-cytokine detection kit (Hangzhou Cellgene Biotech Co., Ltd). Red dashed line indicates the upper limit of normal value.

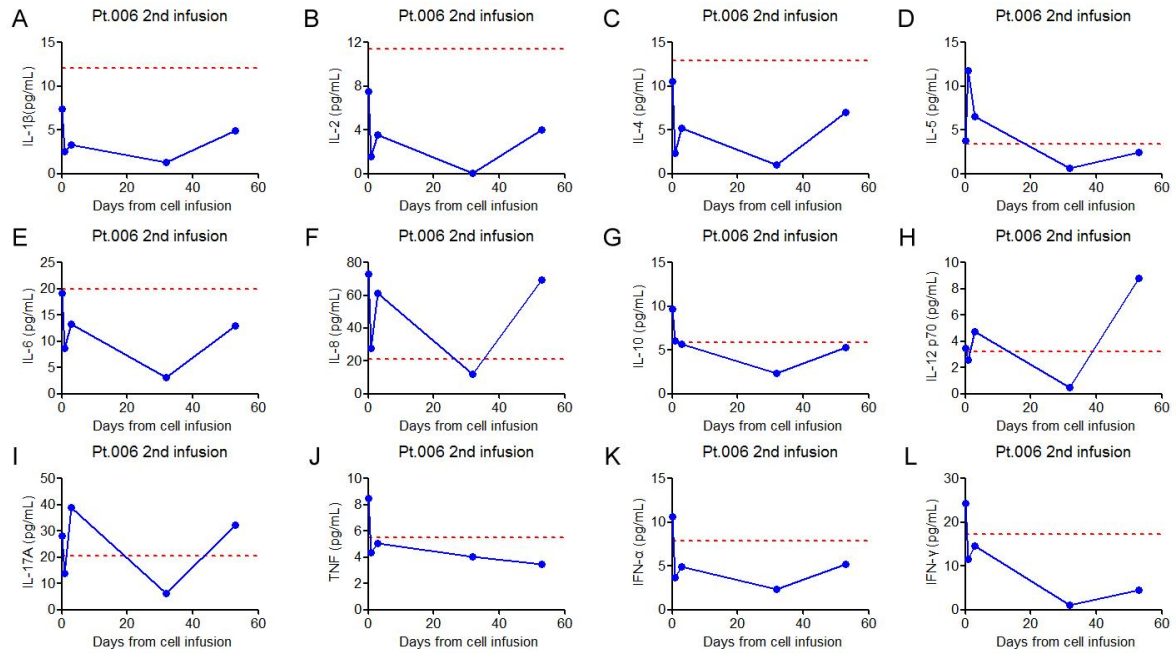


Figure S31. Changes in plasma cytokines during the second cell infusion in Pt.006 patient.

(A-L) Plasma IL-1 β (A), IL-2 (B), IL-4 (C), IL-5 (D), IL-6 (E), IL-8 (F), IL-10 (G), IL-12 p70 (H), IL-17A (I), TNF (J), IFN- α (K), and IFN- γ (L) were assayed by multi-cytokine detection kit (Hangzhou Cellgene Biotech Co., Ltd). Red dashed line indicates the upper limit of normal value.

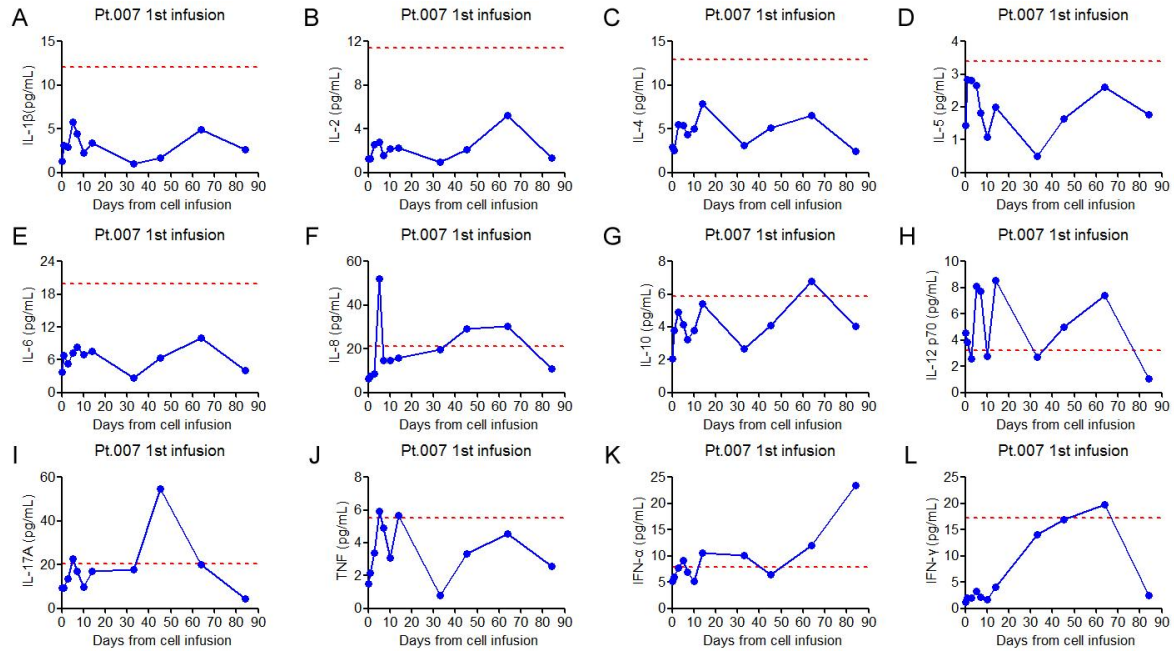


Figure S32. Changes in plasma cytokines during the first cell infusion in Pt.007 patient.

(A-L) Plasma IL-1 β (A), IL-2 (B), IL-4 (C), IL-5 (D), IL-6 (E), IL-8 (F), IL-10 (G), IL-12 p70 (H), IL-17A (I), TNF (J), IFN- α (K), and IFN- γ (L) were assayed by multi-cytokine detection kit (Hangzhou Cellgene Biotech Co., Ltd). Red dashed line indicates the upper limit of normal value.

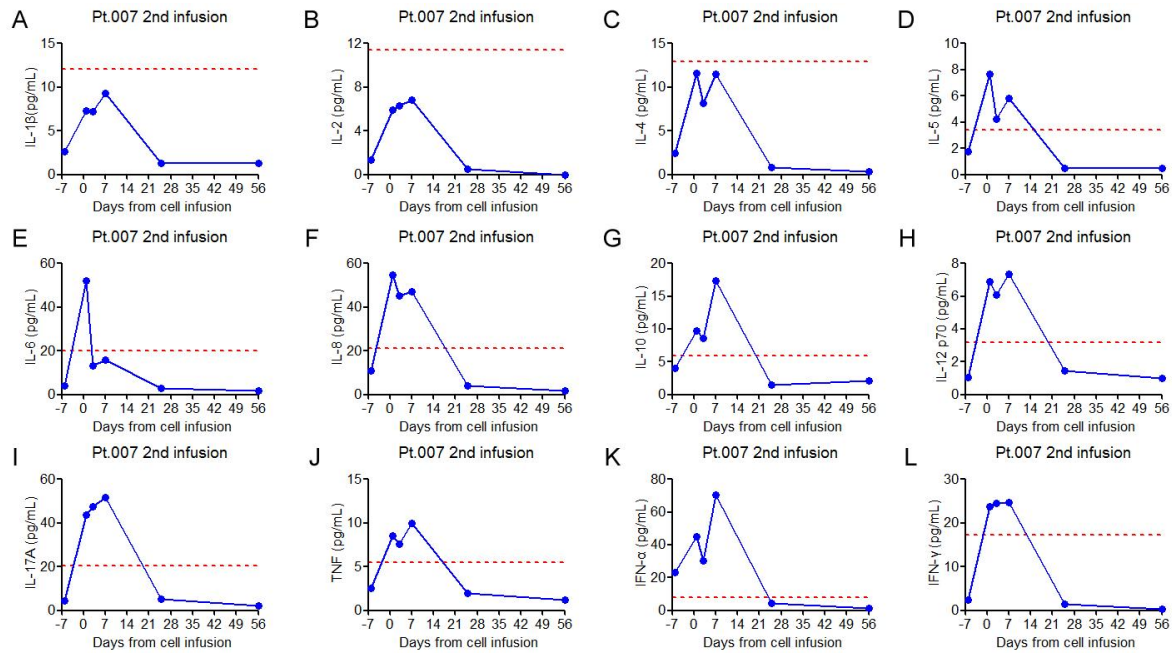


Figure S33. Changes in plasma cytokines during the second cell infusion in Pt.007 patient.

(A-L) Plasma IL-1 β (A), IL-2 (B), IL-4 (C), IL-5 (D), IL-6 (E), IL-8 (F), IL-10 (G), IL-12 p70 (H), IL-17A (I), TNF (J), IFN- α (K), and IFN- γ (L) were assayed by multi-cytokine detection kit (Hangzhou Cellgene Biotech Co., Ltd). Red dashed line indicates the upper limit of normal value.

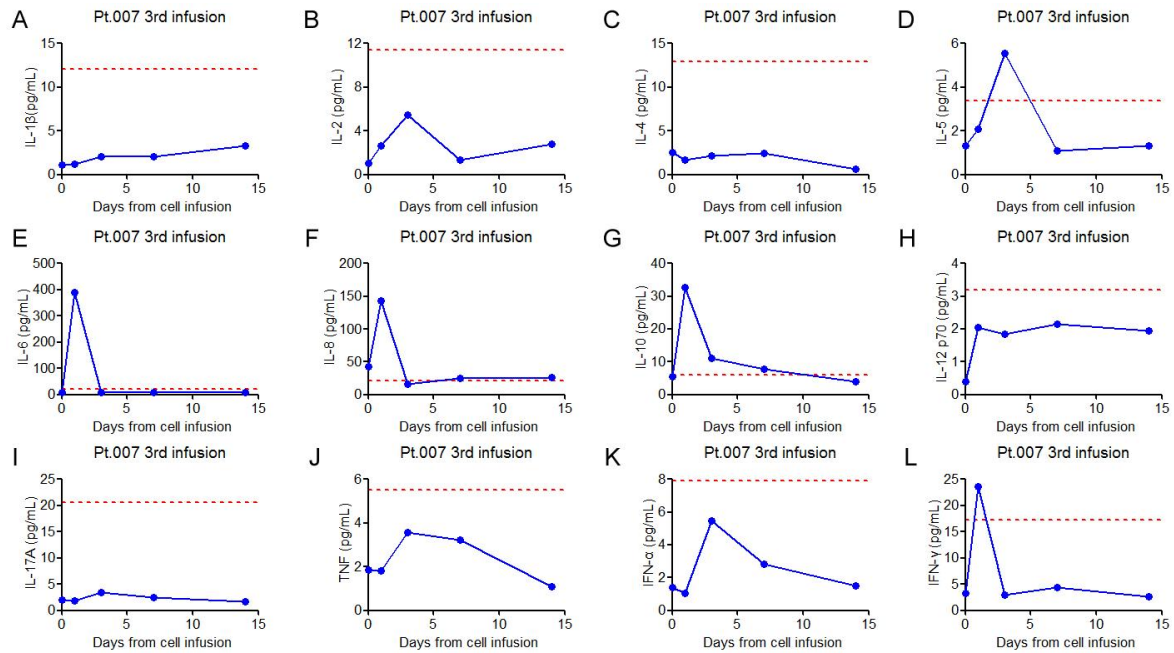


Figure S34. Changes in plasma cytokines during the third cell infusion in Pt.007 patient.

(A-L) Plasma IL-1 β (A), IL-2 (B), IL-4 (C), IL-5 (D), IL-6 (E), IL-8 (F), IL-10 (G), IL-12 p70 (H), IL-17A (I), TNF (J), IFN- α (K), and IFN- γ (L) were assayed by multi-cytokine detection kit (Hangzhou Cellgene Biotech Co., Ltd). Red dashed line indicates the upper limit of normal value.

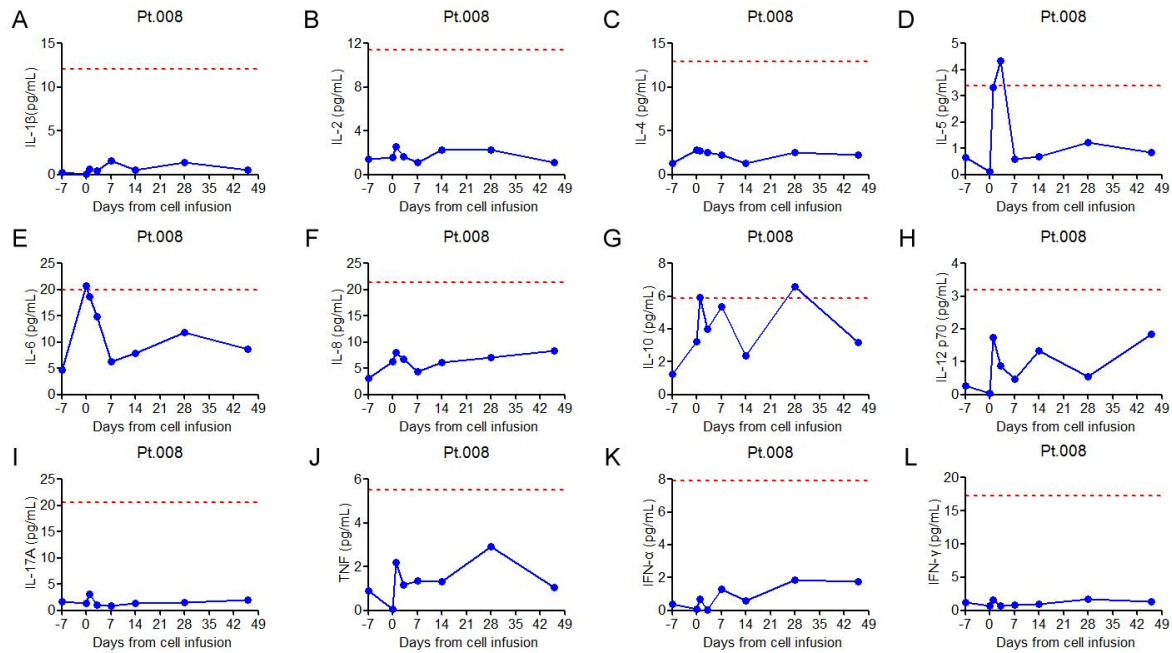


Figure S35. Changes in plasma cytokines during the first cell infusion in Pt.008 patient.

(A-L) Plasma IL-1 β (A), IL-2 (B), IL-4 (C), IL-5 (D), IL-6 (E), IL-8 (F), IL-10 (G), IL-12 p70 (H), IL-17A (I), TNF (J), IFN- α (K), and IFN- γ (L) were assayed by multi-cytokine detection kit (Hangzhou Cellgene Biotech Co., Ltd). Red dashed line indicates the upper limit of normal value.

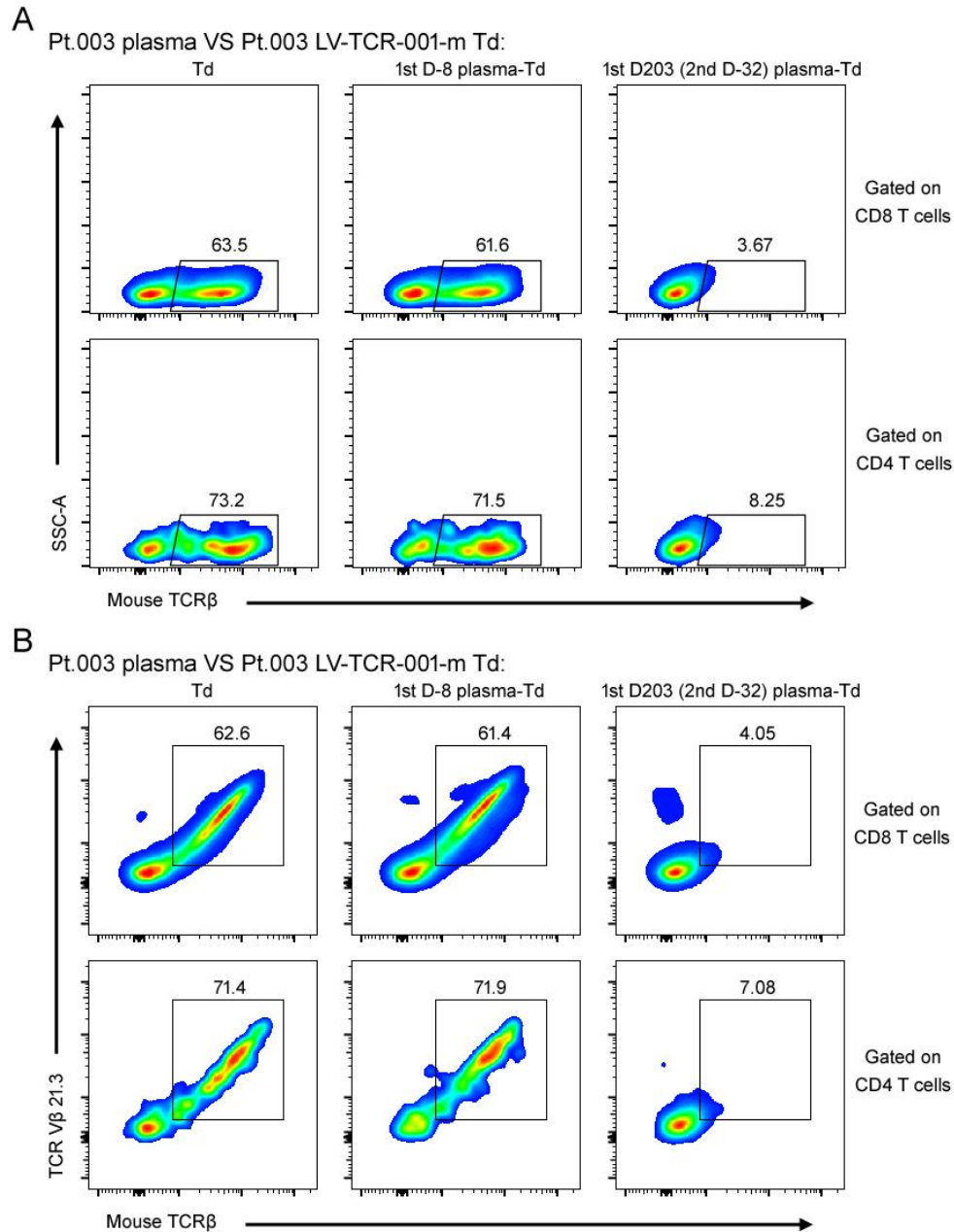


Figure S36. Evaluation of anti-TCR antibody in plasma during cell infusion of TCR-001-engineered T cells in Pt.003 patient.

(A-B) After Pt.003 derived TCR-001-transduced T cells (Pt.003 LV-TCR-001-m Td) were incubated with pre-infusion or post-infusion plasmas for 2 hours, the expression of mouse TCR β or human TCR V β 21.3 in CD8 T cells or CD4 T cells was determined by flow cytometric analysis. Representative flow cytometric plots of mouse TCR β (A) or human TCR V β 21.3 (B) expression are shown for Pt.003 plasma-incubated Pt.003 LV-TCR-001-m Td cells. Numbers in plots indicate the percentage of mouse TCR β^+ (A) or human TCR V β 21.3 $^+$ (B) cells among CD8 T cells or CD4 T cells.

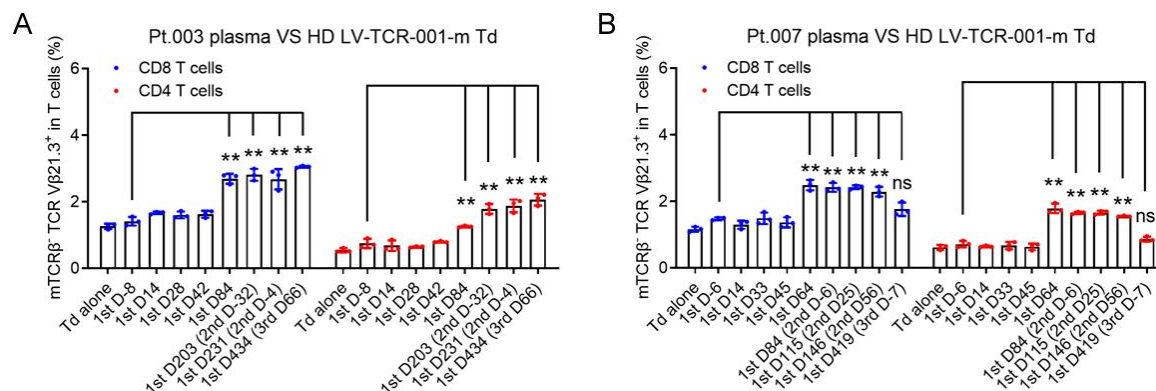


Figure S37. Anti-human TCR Vβ21.3 antibody in plasma did not bind mouse TCRβ-negative human TCR Vβ21.3⁺ endogenous T cells.

(A-B) Healthy donor-derived TCR-001-transduced T cells (HD LV-TCR-001-m Td) were incubated with pre-infusion or post-infusion plasmas for 2 hours and the expression of mouse TCRβ or human TCR Vβ21.3 on CD8 T cells or CD4 T cells was determined by flow cytometric analysis. Summarized data of the percentage of mouse TCRβ⁺ human TCR Vβ21.3⁺ cells in T cells in Pt.003 plasma-incubated (A) or Pt.007 plasma-incubated (B) HD LV-TCR-001-m Td cells are shown. Error bars represent SD of 3 biological replicates. Dots indicate biological replicates. **P < 0.01 by 1-way ANOVA test followed by Tukey's multiple-comparison test.

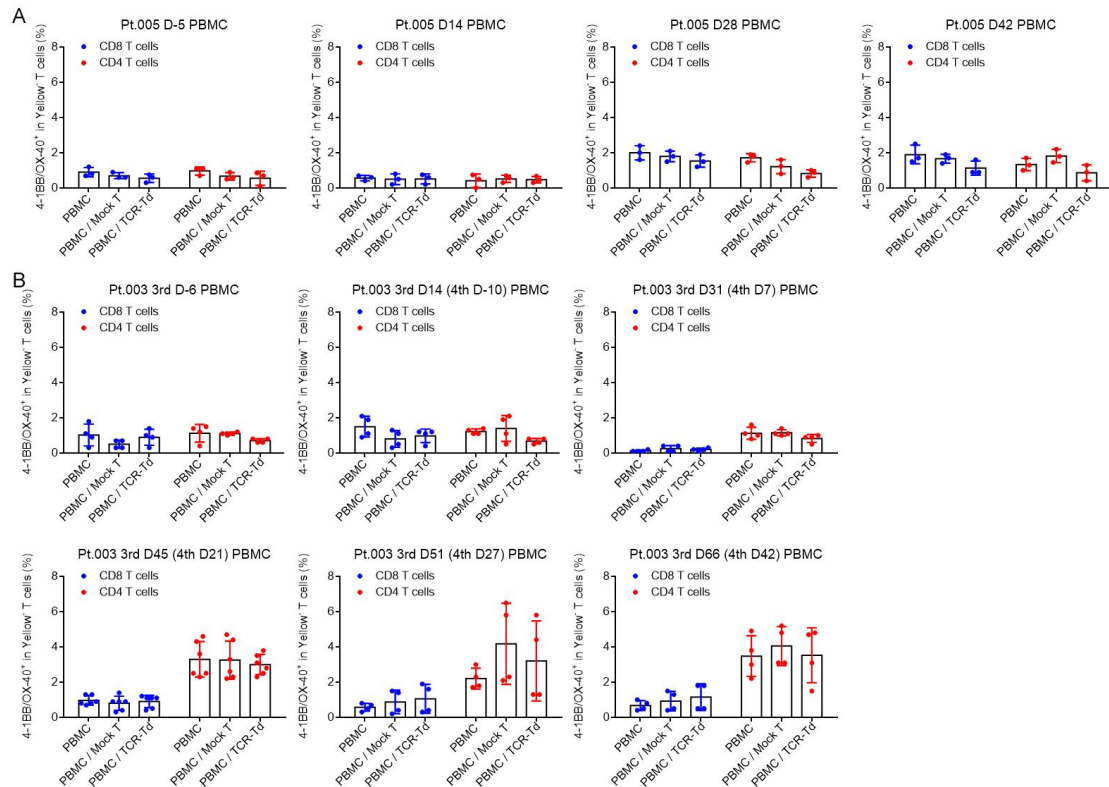


Figure S39. Evaluation of T-cell reactivity against TCR-Td cells in the PBMC after cell infusion of TCR-T cells in Pt.005 and Pt.003.

(A) Pt.005 pre-infusion or post-infusion PBMC cells were thawed. Overnight coculture of PBMCs (1×10^5 per well) with Cell-Trace Yellow-stained autologous TCR-001-transduced T cells (Pt.005 LV-TCR-001-m Td) or Mock T cells was analyzed by flow cytometry for expression of 4-1BB/OX-40 on Yellow⁺ T cells as the markers for T-cell reactivity. Error bars represent SD of 3 biological replicates. Dots indicate biological replicates. (B) Pt.003 pre-infusion or post-infusion PBMCs after the third and fourth infusion were thawed. Overnight coculture of PBMC cells (1×10^5 per well) with Cell-Trace Yellow-stained autologous 4148 TCR-transduced T cells (Pt.003 LV-4148 TCR-h Td) or Mock T cells was analyzed by flow cytometry for expression of 4-1BB/OX-40 on Yellow⁺ T cells as the markers for T-cell reactivity. Error bars represent SD of 4 or 6 biological replicates. Dots indicate biological replicates.

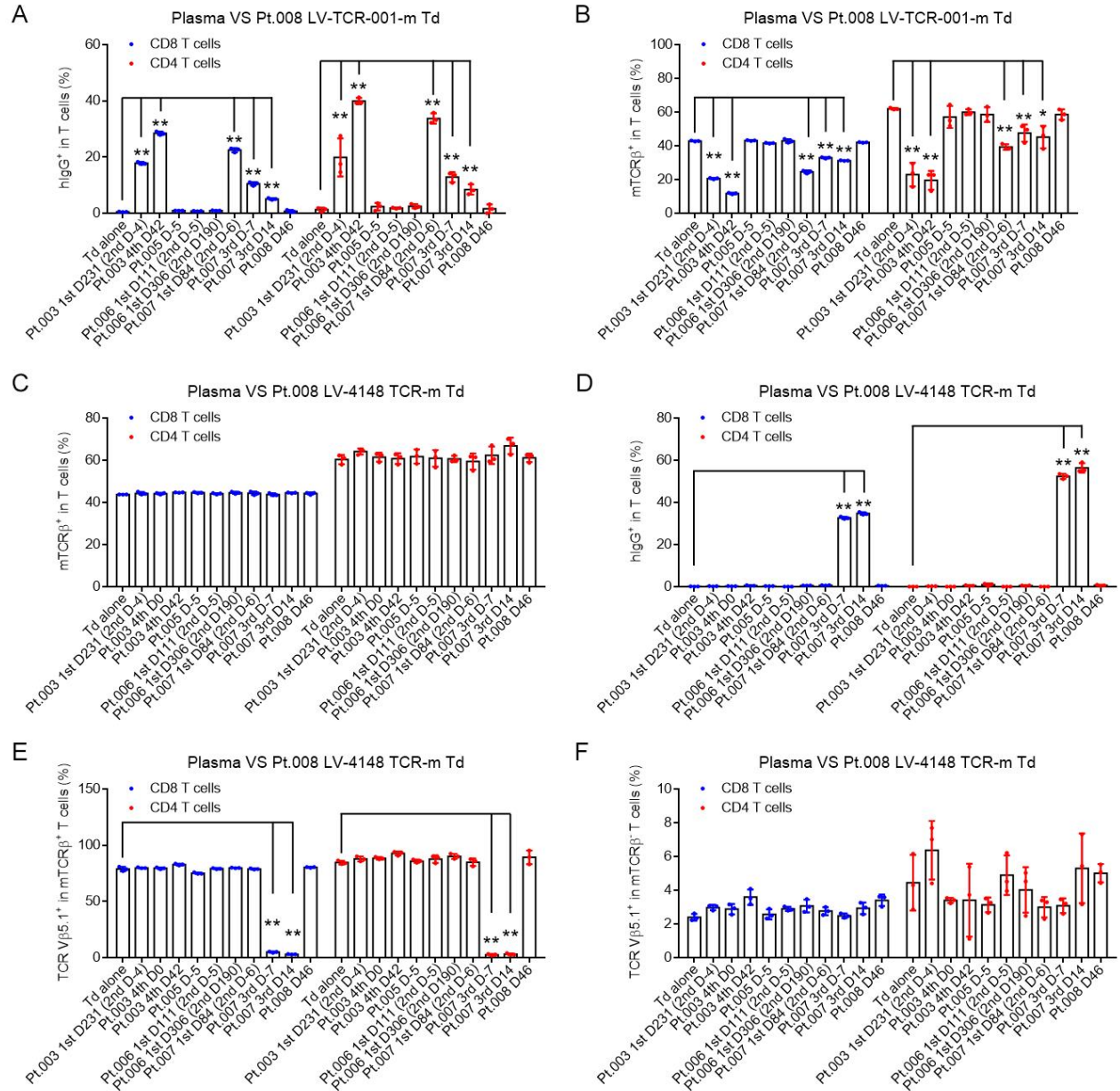


Figure S40. Validation of anti-TCR antibody in plasma after cell infusion of TCR-001 or 4148 TCR-engineered T cells in patients.

(A-B) Pt.008-derived TCR-001-transduced T cells (Pt.008 LV-TCR-001-m Td) were incubated with pre-infusion or post-infusion plasmas for 2 hours and expression of human IgG or mouse TCR β on CD8 T cells or CD4 T cells was determined by flow cytometric analysis. Summarized data of human IgG (A) or mouse TCR β (B) expression on various patient plasma-incubated Pt.008 LV-TCR-001-m Td cells are shown. Error bars represent SD of 3 biological replicates. Dots indicate biological replicates. *P < 0.05 or **P < 0.01 by 1-way ANOVA test followed by Tukey's multiple-comparison test. (C-F) Pt.008 derived human-mouse hybrid 4148 TCR-transduced T cells (Pt.008 LV-4148 TCR-m Td) were incubated with pre-infusion or post-infusion plasmas for 2 hours and expression of mouse TCR β (C), human IgG (D), or human TCR V β 5.1 (E-F) on CD8 T cells or CD4 T cells was

determined by flow cytometric analysis. Summarized data of mouse TCR β (C), human IgG (D) or human TCR V β 5.1 (E-F) expression on various patient plasma-incubated Pt.008 LV-4148 TCR-m Td cells are shown. Error bars represent SD of 3 biological replicates. Dots indicate biological replicates. **P < 0.01 by 1-way ANOVA test followed by Tukey's multiple-comparison test.

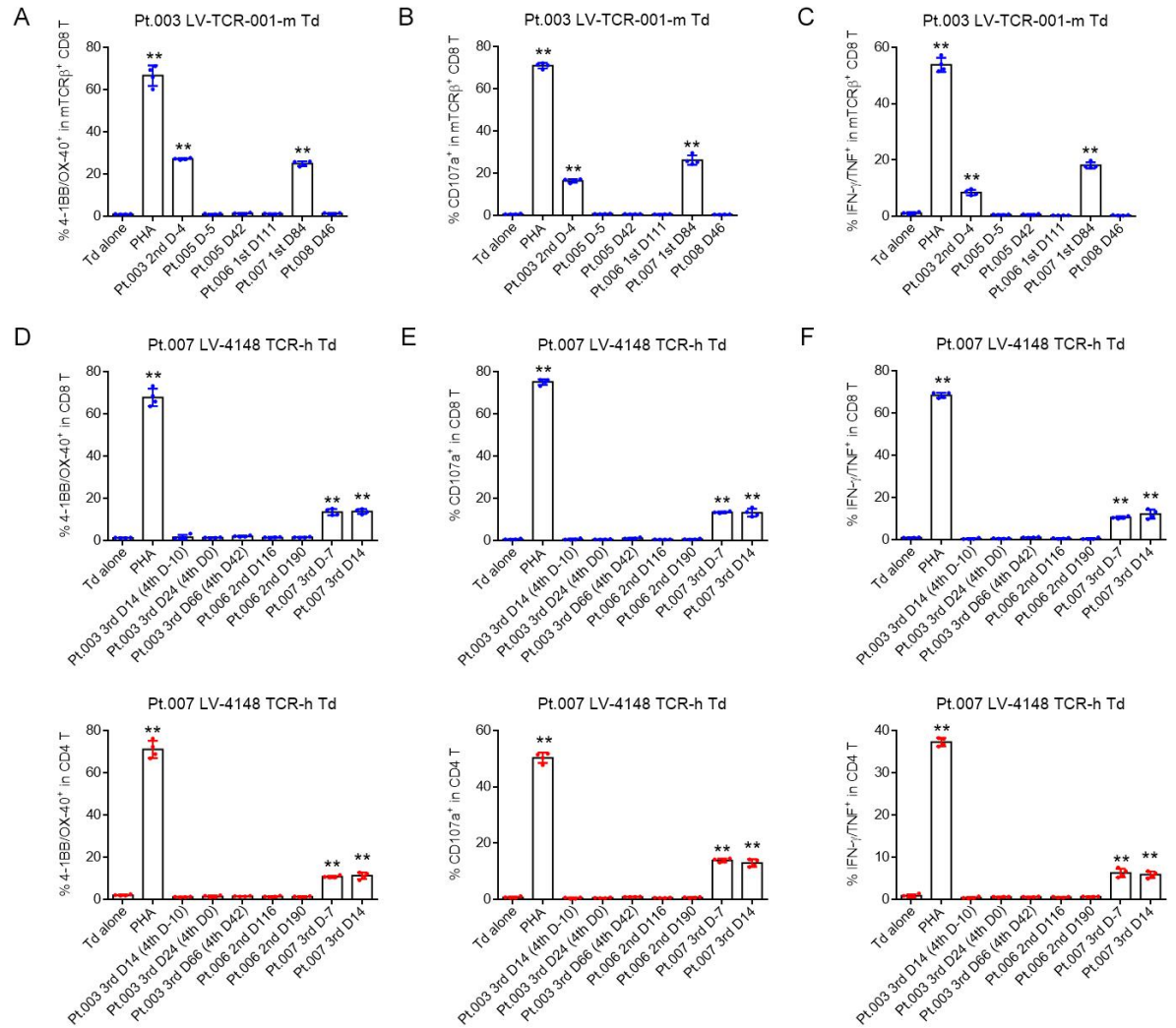


Figure S41. Select plasma from post cell infusion patients induce activation of TCR-T cells.

(A-F) Pt.003 derived TCR-001-transduced T cells (Pt.003 LV-TCR-001-m Td) (A-C) or Pt.007 derived 4148 TCR-transduced T cells (Pt.007 LV-4148 TCR-h Td) (D-F) were incubated with pre-infusion or post-infusion plasmas for 2 hours and then cultured overnight for detection of 4-1BB/OX-40 expression (A, D) or for 5 hours for detecting CD107a expression (B, E) and intracellular IFN-γ/TNF production (C, F). Summarized data are shown. Error bars represent SD of 4 biological replicates. Dots indicate biological replicates. **P < 0.01 vs. “Td alone” group by 1-way ANOVA test followed by Tukey’s multiple-comparison test.

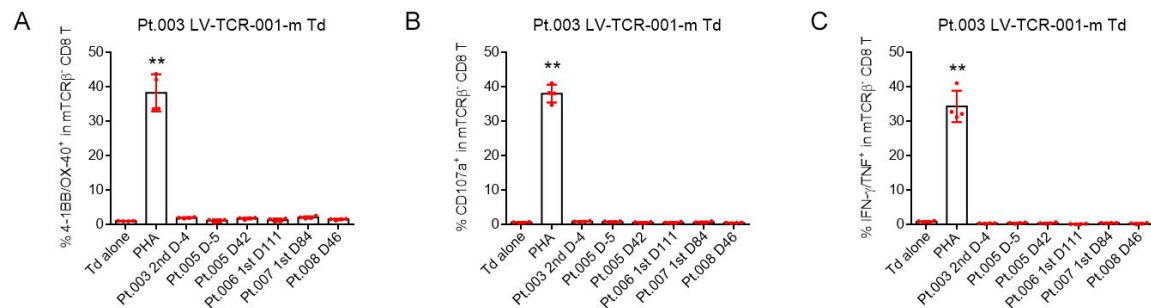


Figure S42. Anti-TCR antibody in plasma after cell infusion did not induce activation of non-TCR-001-engineered (mTCRβ-negative) T cells.

(A-C) Pt.003 derived TCR-001-transduced T cells (Pt.003 LV-TCR-001-m Td) (A-C) were incubated with pre-infusion or post-infusion plasmas for 2 hours and plasma-incubated Td cells were cultured overnight for detecting 4-1BB/OX-40 expression (A) or for 5 hours for detecting CD107a expression (B) and intracellular IFN-γ/TNF production (C). Summarized data in mTCR⁻ CD8 T cells are shown. Error bars represent SD of 4 biological replicates. Dots indicate biological replicates. **P < 0.01 vs. “Td alone” group by 1-way ANOVA test followed by Tukey’s multiple-comparison test.

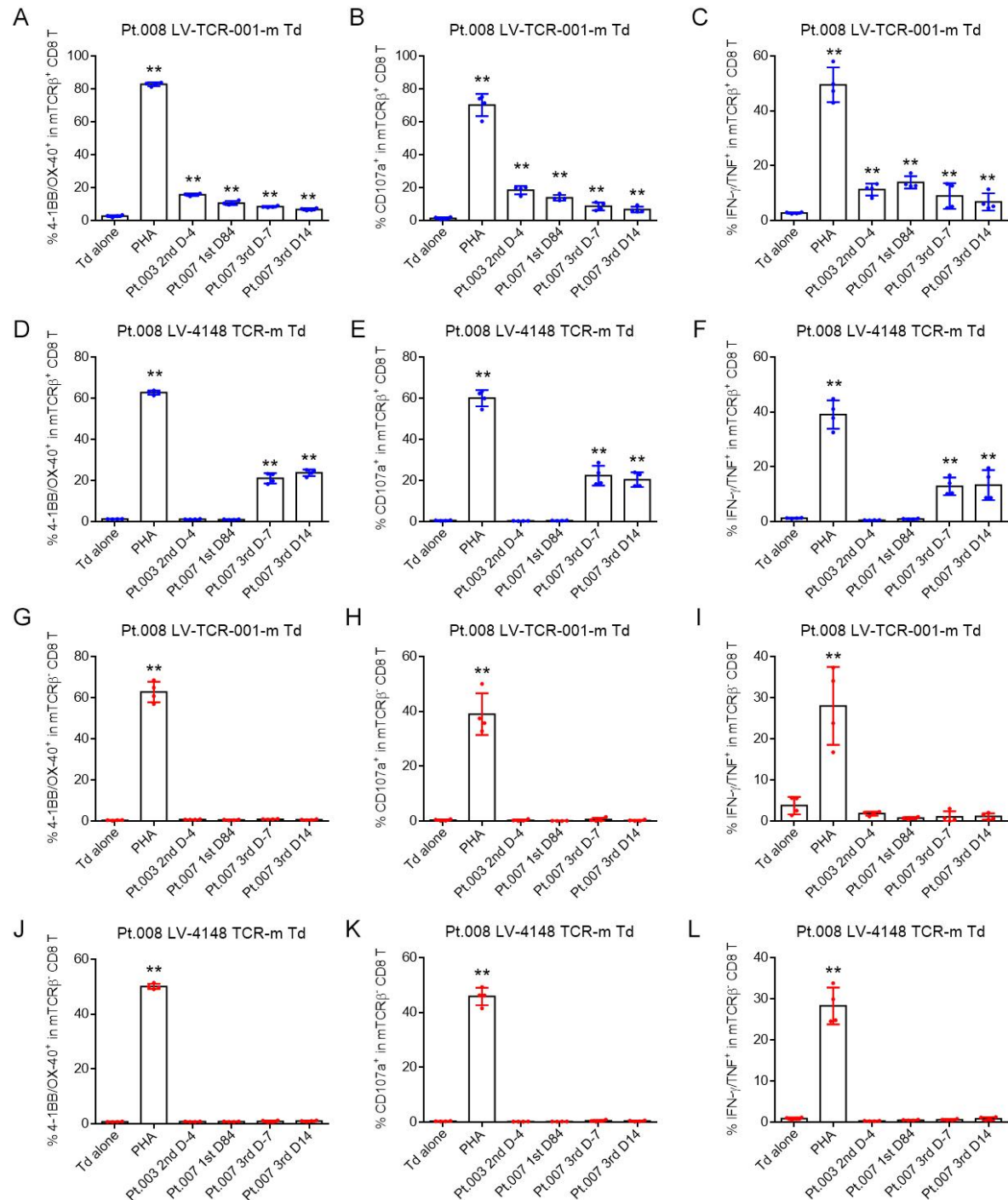


Figure S43. The plasmas from Pt.003 and Pt.007 induce activation of TCR-T cells.

(A-L) Pt.008 derived TCR-001-transduced T cells (Pt.008 LV-TCR-001-m Td) (A-C and G-I) or Pt.008 derived human-mouse hybrid 4148 TCR-transduced T cells (Pt.008 LV-4148 TCR-m Td) (D-F and J-L) were incubated with pre-infusion or post-infusion plasmas from Pt.003 and Pt.007 for 2 hours and then cultured overnight for detection of 4-1BB/OX-40 expression (A, D, G, J) or for 5 hours for detecting CD107a expression (B, E, H, K) and intracellular IFN-γ/TNF production (C, F, I, L).

Summarized data in mTCR⁺ CD8 T cells (A-F) or mTCR⁻ CD8 T cells (G-L) are shown. Error bars represent SD of 4 biological replicates. Dots indicate biological replicates. **P < 0.01 vs. “Td alone” group by 1-way ANOVA test followed by Tukey’s multiple-comparison test.

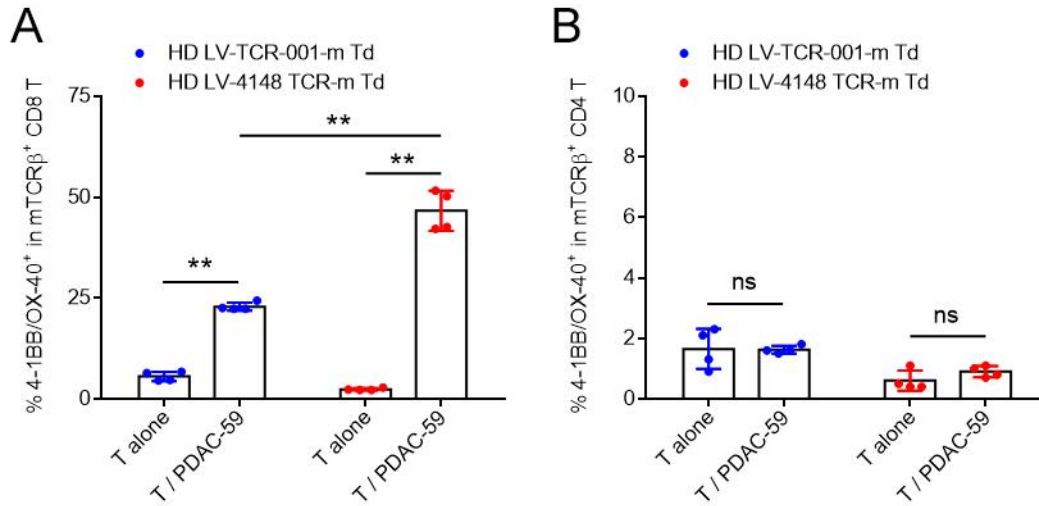


Figure S44. TCR-engineered CD8 T cells specifically recognized pancreatic cancer organoid cells PDAC-59.

(B-(A-B) Healthy donor-derived TCR-001-transduced T cells (HD LV-TCR-001-m Td) or human-mouse hybrid 4148 TCR-transduced T cells (HD LV-4148 TCR-m Td) were cocultured overnight with HLA-A*11:01⁺ KRAS G12V⁺ pancreatic cancer organoid cells PDAC-59 and 4-1BB/OX-40 expression was evaluated by flow cytometry. Summarized data in mTCR⁺ CD8 T cells (A) and mTCR⁺ CD4 T cells (B) are shown. Error bars represent SD of 4 biological replicates. Dots indicate biological replicates. **P < 0.01 by 1-way ANOVA test followed by Tukey's multiple-comparison test.

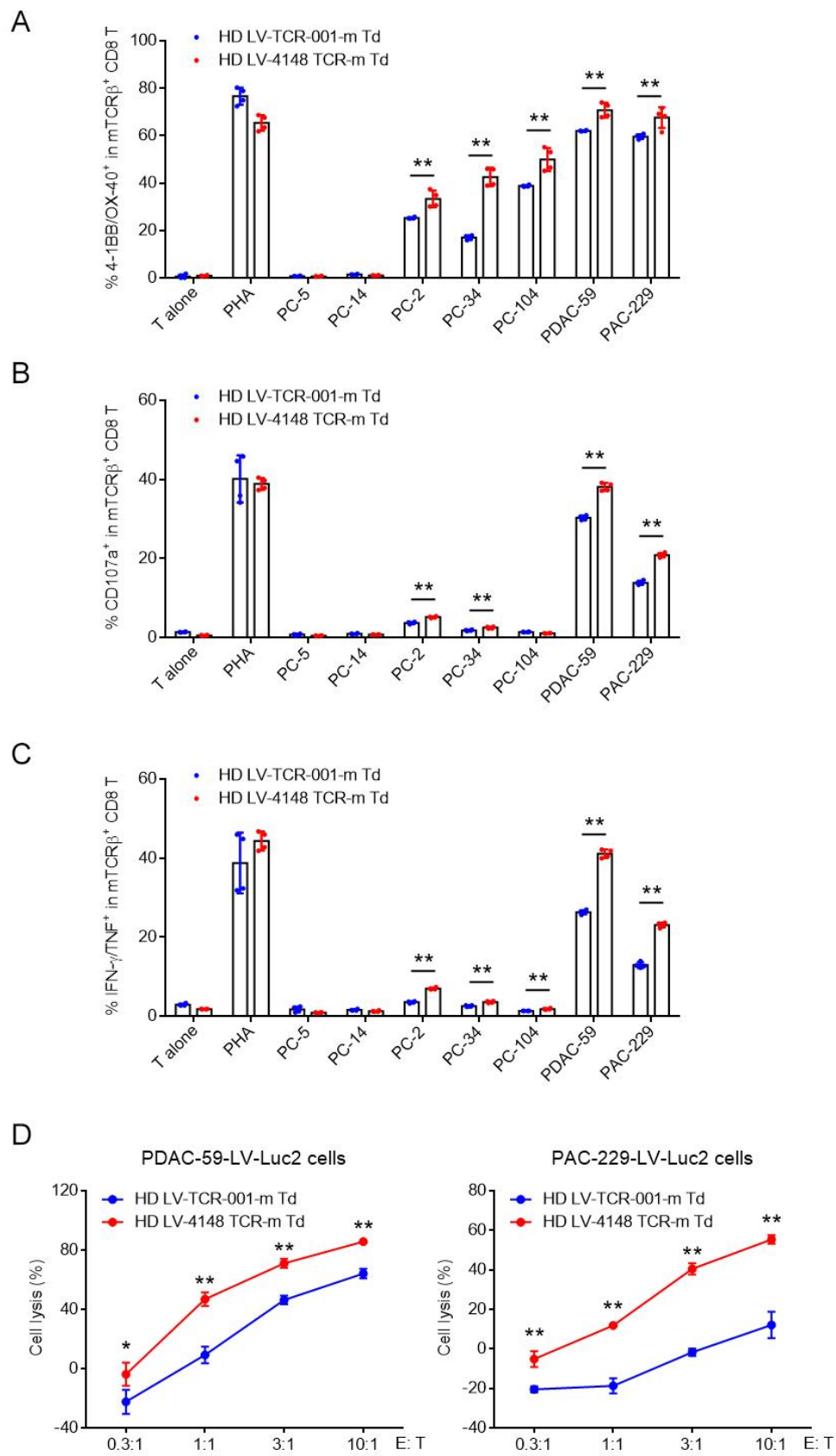


Figure S45. TCR-engineered CD8 T cells specifically recognized and killed pancreatic cancer organoids.

(A-C) Healthy donor-derived TCR-001-transduced T cells (HD LV-TCR-001-m Td) or human-mouse hybrid 4148 TCR-transduced T cells (HD LV-4148 TCR-m Td) were cocultured overnight or 5 hours with various HLA-A*11:01⁺ KRAS G12V⁺ pancreatic cancer organoids (PC-2, PC-34, PC-104, PDAC-59 and PAC-229), HLA-A*11:01⁺ KRAS G12D⁺ pancreatic cancer organoid (PC-5) or HLA-A*11:01⁻ KRAS G12V⁺ pancreatic cancer organoid (PC-14). Flow cytometric analysis of 4-1BB/OX-40 expression (A), CD107a expression (B) and intracellular IFN- γ /TNF production (C) on mTCR β ⁺ CD8 T cells are shown. Error bars represent SD of 4 biological replicates. Dots indicate biological replicates. **P < 0.01 by 1-way ANOVA test followed by Tukey's multiple-comparison test.

(D) HD LV-TCR-001-m Td or HD LV-4148 TCR-m Td cells were cocultured overnight with HLA-A*11:01⁺ KRAS G12V⁺ pancreatic cancer organoids stably transduced with firefly luciferase (PDAC-59-LV-Luc2 and PAC-229-LV-Luc2 cells) at various effector to target ratios. The results of luciferase-based cytotoxicity assays were shown. Error bars represent SD of 3 biological replicates. *P < 0.05 or **P < 0.01 vs. the group at the same effector to target ratio by 1-way ANOVA test followed by Tukey's multiple-comparison test.

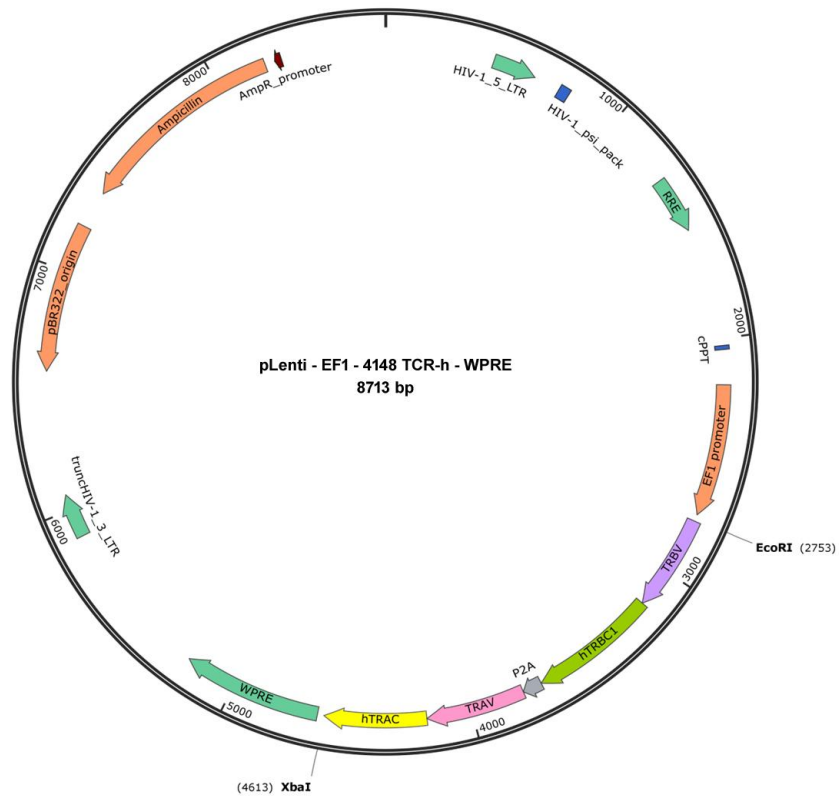


Figure S46. Plasmid map of lentiviral vector expressing 4148 TCR TRBV and TRAV with human TRBC1 and TRAC.

Supplemental Tables.

Table S1. Summary of quality control testing of TCR-transduced T cells prepared for infusion

Tests		Required specification	Pt.003				Pt.005	Pt.006		Pt.007			Pt.008
Test method	Time points		1st	2nd	3rd	4th		1st	2nd	1st	2nd	3rd	
Sterility: Genus mycoplasma qPCR	REP D7	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Sterility: Microbial Culture	REP D7	No growth (72 h)	No growth	No growth	No growth (REP D9)	No growth	No growth (REP D11)	No growth (REP D11)	No growth	No growth	No growth	No growth	No growth
Sterility: Gram's staining	REP D7	Negative	Negative	Negative	Negative (REP D9)	Negative	Negative (REP D11)	Negative (REP D11)	Negative	Negative	Negative	Negative	Negative
Endotoxin: LAL assay	REP D7	≤5 EU/mL	0.078 EU/mL	0.015 EU/mL	<0.01 EU/mL (REP D9)	0.013 EU/mL	<0.01 EU/mL (REP D11)	<0.01 EU/mL (REP D11)	0.013 EU/mL	<0.01 EU/mL	0.096 EU/mL	0.018 EU/mL	<0.01 EU/mL
TCR expression: FACS	REP D7	≥10%	39.0%*	66.7%*	45.7%* (REP D12)	43.0%** (REP D10)	45.7%* (REP D11)	67.8%* (REP D11)	60.9%**	72.1%*	61.1%* [*]	39.1%**	57.8%*
Sterility: Gram's staining	Before cell harvestment	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Endotoxin: LAL assay	Before cell harvestment	≤5 EU/mL	0.035 EU/mL	0.010 EU/mL	0.036 EU/mL	0.017 EU/mL	0.011 EU/mL	<0.01 EU/mL	<0.01 EU/mL	<0.01 EU/mL	<0.01 EU/mL	<0.01 EU/mL	0.032 EU/mL
Cell viability: Trypan blue staining	Infusion product	≥70%	91.2%	97.2%	94.4%	96.0%	84.4%	92.2%	97.1%	91.2%	95.2%	95.0%	94.2%
Total viable cell number: Cell counting by trypan blue staining	Infusion product	≥1e9	1.72E+10	1.72E+10	1.27E+10	1.44E+10	1.52E+10	1.72E+10	1.65E+10	1.65E+10	1.68E+10	1.71E+10	1.71E+10
Sterility: Microbial Culture	Infusion product	No growth (72 h)	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth
Endotoxin: LAL assay	Infusion product	≤5 EU/mL	<0.01 EU/mL	<0.01 EU/mL	<0.01 EU/mL	<0.01 EU/mL	<0.01 EU/mL	0.012 EU/mL	<0.01 EU/mL	0.019 EU/mL	<0.01 EU/mL	<0.01 EU/mL	0.015 EU/mL

RCL: VSV-G qPCR	Infusion product	Negative	Neg ative	Nega tive	Negativ e	Nega tive	Negativ e	Negativ e	Negativ e	Neg ative	Neg ativ e	Neg ativ e	Neg ativ e	Neg ativ e
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Notes: "*" indicates the percentage of mTCR β^+ cells in CD8 T cells and "***" indicates the percentage of TCR V β 5.1 $^+$ cells in CD8 T cells.

Table S2. Characteristics of KRAS G12V A11 TCR-T cells, lymphodepletion regimen and anti-PD1 dose for therapy

Patient	No. of cell infusion	TCR-Td cell	Cell numbers (×10 ⁹)	CD8 T (%)	CD4 T (%)	mTCRβ ⁺ in CD8 T (%)	mTCRβ ⁺ in CD4 T (%)	TCR Vβ5.1 ⁺ in CD8 T (%)	TCR Vβ5.1 ⁺ in CD4 T (%)	TCR ⁺ CD8 T cell numbers (×10 ⁹)	Lymphodepletion regimen	Anti-PD1 dose	TCR-Td persistence <i>in vivo</i>
Pt.003	1st	LV-TCR-001-m Td	17.2	98.9	0.062	40.5	-	-	-	6.9	Cy, 500 mg/m ² /D & Flu, 30 mg/m ² /D, D-4~D-2	-	28 days
	2nd	LV-TCR-001-m Td	17.2	94.3	0.77	62.0	77.7	-	-	10.1	Cy, 500 mg/m ² /D & Flu, 30 mg/m ² /D, D-4~D-2	-	1 day
	3rd	LV-4148 TCR-h Td	12.7	86.6	10.7	-	-	45.7	70.1	5.0	-	200 mg, D1	7 days
	4th	LV-4148 TCR-h Td	14.4	88.3	9.31	-	-	43.0	68.0	5.5	Cy, 500 mg/m ² /D & Flu, 30 mg/m ² /D, D-4~D-3*	200 mg, D1	1 day
Pt.005	1st	LV-TCR-001-m Td	15.2	64.6	30.6	48.6	52.8	-	-	4.8	Cy, 500 mg/m ² /D & Flu, 30 mg/m ² /D, D-4~D-2	-	7 days
Pt.006	1st	LV-TCR-001-m Td	17.2	58.2	38.7	72.1	76.3	-	-	7.2	Cy, 500 mg/m ² /D & Flu, 30 mg/m ² /D, D-4~D-2	200 mg, D71, D92	>111 days
	2nd	LV-4148 TCR-h Td	16.5	29.4	68.3	-	-	55.6	57.8	2.7	Cy, 500 mg/m ² /D & Flu, 30 mg/m ² /D, D-4~D-2	200 mg, D1, D53, D116, D143, D165	>190 days
Pt.007	1st	LV-TCR-001-m Td	16.5	82.1	7.09	78.3	87.5	-	-	10.6	Cy, 500 mg/m ² /D & Flu, 30 mg/m ² /D, D-4~D-2	200 mg, D45, D66	33 days
	2nd	LV-4148 TCR-h Td	16.8	83.8	4.13	-	-	62.5	76.9	8.8	Cy, 500 mg/m ² /D & Flu, 30 mg/m ² /D, D-4~D-2	200 mg, D2, D56	7 days
	3rd	LV-4148 TCR-h Td	17.1	49.3	48.3	-	-	38.0	65.9	3.2	Cy, 500 mg/m ² /D & Flu, 30 mg/m ² /D, D-4~D-3*	-	3 days
Pt.008	1st	LV-TCR-001-m Td	17.1	82.8	14.5	61.6	78.1	-	-	8.7	Cy, 500 mg/m ² /D & Flu, 30 mg/m ² /D, D-4~D-2	200 mg, D14, D35, D56	>46 days

Notes: Cy, Cyclophosphamide; Flu, Fludarabine; Anti-PD1, Tislelizumab. “-”, indicates no usage. “*”, indicates that patients didn’t receive the third usage of Cy and Flu due to fever.

Table S3. Number and percentage of patients from screening to TCR-T cell therapy

	Number of patients (%)
Screened ¹ (n=9)	9 (100%)
Peripheral blood collection (100 mL) ²	9 (100%)
TCR-transduced T cells preparation	9 (100%)
Intention-to-treat	6 (66.7%)
Total treated with TCR-transduced T cell product	5 (55.6%)
Total treated with ≥ 2 infusions of TCR-transduced T cell product	3 (33.3%)

Notes: ¹Patient population from the screening protocol as of July 31, 2023. ²Includes peripheral blood collection for patients with planned or ongoing manufacturing.

Table S4. Summary of treatment-related adverse events by patients

Adverse events	Pt.003				Pt. 005	Pt.006		Pt.007			Pt. 008	All cases (n=11)				
	1st	2nd	3rd*	4th		1st	2nd	1st	2nd	3rd		Gr1 (%)	Gr2 (%)	Gr3 (%)	Gr4 (%)	Total (%)
Hematological																
Lymphopenia	Gr4	Gr4	—	Gr4	Gr4	Gr4	Gr4	Gr4	Gr4	Gr4	Gr4	0 (0)	0 (0)	0 (0)	10 (91)	10 (91)
Aneamia	Gr2	Gr2	Gr3	Gr3	Gr2	Gr1	Gr2	—	Gr1	Gr2	Gr1	3 (27)	5 (45)	2 (18)	0 (0)	10 (91)
Neutropenia	—	Gr1	—	—	Gr1	Gr3	Gr2	—	—	Gr1	Gr1	4 (36)	1 (9)	1 (9)	0 (0)	6 (55)
Leukopenia	—	Gr3	—	—	Gr2	Gr3	Gr3	—	—	Gr3	Gr1	1 (9)	1 (9)	4 (36)	0 (0)	6 (55)
Thrombocytopenia	—	—	—	Gr2	Gr3	—	—	Gr1	—	Gr2	—	1 (9)	2 (18)	1 (9)	0 (0)	4 (36)
Other																
Cytokine release syndrome	Gr1	Gr2	—	Gr1	Gr1	—	Gr1	—	Gr1	Gr1	Gr1	7 (64)	1 (9)	0 (0)	0 (0)	8 (73)
Chills/Pyrexia	Gr1	Gr2	—	Gr2	Gr2	—	Gr1	—	Gr1	Gr2	Gr1	4 (36)	4 (36)	0 (0)	0 (0)	8 (73)
Hypotension	—	Gr1	—	—	—	—	—	—	—	—	—	1 (9)	0 (0)	0 (0)	0 (0)	1 (9)
Fatigue	—	—	—	—	Gr1	—	—	—	—	—	—	1 (9)	0 (0)	0 (0)	0 (0)	1 (9)
Erythema	—	—	—	—	—	—	—	—	—	—	Gr1	1 (9)	0 (0)	0 (0)	0 (0)	1 (9)
Neurotoxicity	—	—	—	—	—	—	—	—	—	—	—	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Notes: Reported within 28 days after cell infusion based on Common terminology criteria for adverse events (CTCAE) edition 5.0. "***" indicates that the patient didn't receive lymphodepletion. "—" = No adverse events reported.

Table S5. List of the used key resources in this study

REAGENT or RESOURCE	SOURCE	IDENTIFIER	CLONE TYPE (For Antibodies)
Antibodies			
Anti-human CD3 Pacific blue	Biolegend	Cat#317314	Clone: OKT3
Anti-human CD8 PE/Cy7	Biolegend	Cat#344712	Clone: SK1
Anti-human CD4 APC/Fire™ 750	Biolegend	Cat#344638	Clone: SK3
Anti-human CD137 APC	Biolegend	Cat#309810	Clone: 4B4-1
Anti-human CD134 FITC	Biolegend	Cat#350006	Clone: Ber-ACT35 (ACT35)
Anti-human CD45RO FITC	Biolegend	Cat#304242	Clone: UCHL1
Anti-human CD62L APC	Biolegend	Cat#304810	Clone: DREG-56
Anti-human Fas Percp Cy5.5	Biolegend	Cat#305630	Clone: DX2
Anti-human PD1 FITC	Biolegend	Cat#329904	Clone: EH12.2H7
Anti-human CD3 (OKT3)	Biolegend	Cat#317326	Clone: OKT3
Anti-human CD-107a PE	Biolegend	Cat#328608	Clone: H4A3
Anti-human IFN- γ APC	Biolegend	Cat#506510	Clone: B27
Anti-human TNF- α Percp Cy5.5	Biolegend	Cat#502926	Clone: MAb11
Anti-mouse TCR- β FITC	Biolegend	Cat#109206	Clone: H57-597
Anti-mouse TCR- β PE	Biolegend	Cat#109208	Clone: H57-597
Anti-human IgG Fc PE	Biolegend	Cat#410708	Clone: M1310G05
Anti-TCR V β 21.3-FITC	Beckman Coulter	Cat#IM1483	Clone: IG125
Anti-TCR V β 5.1-PE	Beckman Coulter	Cat#IM2285	Clone: IMMU 157
TCR V β Repertoire Kit	Beckman Coulter	Cat#IM3497	
Chemicals, peptides, and recombinant proteins			
MACS® GMP T Cell TransAct™	Miltenyi Biotec	170-076-156	4 mL
Monensin solution (1,000X)	Biolegend	Cat#420701	
Brefeldin A solution (1,000X)	Biolegend	Cat#420601	
Custom peptides (HPLC)	Sangon	Custom order	
Recombinant Human GM-CSF	Novoprotein	Cat#GMP-CC79	
Recombinant Human IL-4	Novoprotein	Cat#GMP-CD03	
Recombinant Human Interleukin-2 (¹²⁵ Ala)	SL Pharm	Cat# 1000000 IU	
Commercial assay kits			
Human IFN- γ ELISPOT assay kit	Dakewe	Cat#2110005	

Human IFN- γ ELISA assay kit	Dakewe	Cat#1110002	
Steady-Lumi™ II Firefly Luciferase Assay Kit	Beyotime Biotechnology	Cat#RG059M	