# Supplementary Appendix

Supplement to: Leidner R, Sanjuan Silva N, Huang H, et al. Neoantigen T-cell receptor gene therapy in pancreatic cancer. N Engl J Med 2022;386:2112-9. DOI: 10.1056/NEJMoa2119662

This appendix has been provided by the authors to give readers additional information about the work.

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## SUPPLEMENTARY CLINICAL DETAILS

**Clinical Treatment Protocol - IND 27501 (CRI-4483)**

Schedule of Events

| **Study Day** | **-5** | **-4** | **-3** | **-2** | **-1** | **0** | **1** | **2** | **3** | **4** | **FU visits q4wks** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tocilizumab | X | | | | | | | | | | |
| Cyclophosphamide | X | X | | | | | | | | | |
| T-cell infusion | | | | | | X | | | | | |
| Aldesleukin | | | | | | X | X | X | X | | |
| Filgrastim | | | | X | X | X | X | X | X | X | |
| TMP/SMX | X | X | X | X | X | X | X | X | X | X | |
| Valacyclovir | | | | X | X | X | X | X | X | X | |
| Fluconazole | | | | X | X | X | X | X | X | X | |
| Entecavir | X | X | X | X | X | X | X | X | X | X | |

| Schedule for blood draws to analyze serum proteins, white blood cells and infused T cells | |
|---|---|
| **Time of blood draw** | **Tube type, number of tubes, and volume** |
| Prior to conditioning regimen | CPT (5), 8 ml each, and SST (1), 4 ml |
| Prior to cell infusion | SST (1), 4 ml |
| 1. Post cell infusion<br>2. Post cell infusion, once lymphocyte counts > 200/$mm^3$ | 1. SST (1), 4 ml, daily until end of hospitalization<br>2. CPT (5), 8 ml each, once after lymphocyte count > 200/$mm^3$, then every 48-72h for 5 days, then weekly thereafter. If lymphocyte count does not reach 200/$mm^3$, blood will be drawn prior to discharge. |
| Follow up visit(s) | CPT (6), 8 ml each, and SST (1), 4 ml |

Conditioning Regimen:

Tocilizumab, Cyclophosphamide (Fludarabine omitted due to prolonged neutropenia/thrombocytopenia following prior TIL therapy in 2020 at UPMC). Administration of diuretics, mesna, electrolyte replacement, hydration and monitoring of electrolytes is to be performed as clinically indicated. Clinical dose adjustments of chemotherapy, immunotherapy and supportive medications may be necessary per investigator discretion.

Day -5: Tocilizumab:

- Tocilizumab 600mg IVPB in 70 mL NS over 1 hour.

Day -5 and -4: Cyclophosphamide:

- Begin hydration with 0.9% Sodium Chloride infusion containing 10 meq/L of potassium chloride at 125 ml/hr starting upon arrival pre-cyclophosphamide and continue until 24 hours after the end of cyclophosphamide infusion.
- At any time during the conditioning regimen, if urine output is <1.5 ml/kg/hr or if body weight increases >2 kg over pre-cyclophosphamide value, furosemide 10-20 mg IV may be administered. Serum potassium is to be monitored and treated as indicated following administration of furosemide.

Anti-emetic premedications:

- Palonosetron 0.25 mg IV once on day -5
- Fosaprepitant 150 mg IVPB once on day -5

Chemotherapy:

- Cyclophosphamide 30 mg/kg/day for 2 days IV in 250 ml D5W over 2 hours
- Mesna 30 mg/kg/day for 2 days IV over 2 hours (given concurrent with cyclophosphamide through a separate bag).
- Mesna infusion at 3 mg/kg/hour IV, diluted in normal saline over 22 hours after each cyclophosphamide dose.

Day 0: Cell Infusion:

Cells are to be administered at a dose of between $1x10^{10}$ to $5x10^{10}$ lymphocytes. The patient's T cells are to be delivered to the patient care unit by a staff member from the Human Applications Laboratory (HAL). Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), an identification of the product and documentation of administration are entered in the patient's chart, as is done for blood banking protocols. The cells are to be infused intravenously over approximately 30-45 minutes via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

Aldesleukin (IL-2):

The patient is to receive 600,000 IU/kg (based on total body weight) as an intravenous bolus over a 15 minute period approximately every eight hours (+/- 1hr), starting not less than 1 hour but within 24 hours of cell infusion, continuing for up to 3 days (maximum 6 doses). Doses are to be held per standing biotherapy program SOP. Concomitant medications to control side effects of therapy may be given according to existing institutional biotherapy program protocols.

Supportive Care:

The patient is to receive the fixed combination of trimethoprim and sulfamethoxazole (TMP/SMX) as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) orally three times a week on non-consecutive days, beginning the first Monday, Wednesday, or Friday after the first dose of chemotherapy. Pentamidine may be substituted for TMP/SMX-DS in patients with sulfa allergies, to be administered IV at 4 mg/kg (up to a maximum dose of 300 mg) within one week of chemotherapy start and monthly thereafter.

The patient is to receive valacyclovir orally at a dose of 500 mg daily, starting the day after chemotherapy concludes, or acyclovir, 250 mg/$m^2$ IV every 12 hrs if the patient is not able to take medication by mouth.

The patient is to receive entecavir orally at a dose of 0.5 mg daily starting with chemotherapy.

Prophylaxis with TMP/SMX, valacyclovir, and entecavir (as above) is to continue for 6 months post chemotherapy. If the absolute lymphocyte count (ALC) is less than 500 cells/mm3 at 6 months post chemotherapy, prophylaxis is to continue until ALC is greater than 500 cells/mm3.

The patient is to receive fluconazole orally at a dose of 400 mg daily, starting the day after chemotherapy concludes and continue daily until the absolute neutrophil count is > 1000/mm3. Fluconazole may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in patients unable to take it orally.

The patient is to receive filgrastim, or biosimilar, subcutaneous injection 5 mcg/kg/day (rounded to nearest of 300 or 480 mcg/day), starting the day after chemotherapy concludes and continue daily until the absolute neutrophil count following nadir is greater than 1000/$mm^3$ for 3 days, or exceeds 5000/$mm^3$. Pegfilgrastim 6mg subcutaneous may be substituted for filgrastim.

Using daily CBCs as a guide, the patient is to receive platelets and packed red blood cells (PRBCs) as needed. Attempts are to be made to keep Hgb >8.0 g/dl, and plts >15,000/$mm^3$, or per provider discretion. All blood products are to be irradiated. Leukocyte filters are to be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBCs and decrease the risk of CMV infection.

The patient is to start on broad-spectrum antibiotics, either a 3rd or 4th generation cephalosporin or a quinolone for fever of 38.3°C or above, or for two temperatures of 38.0°C or above at least one hour apart, and an ANC <500/$mm^3$. Infectious disease consultation is to be obtained if the patient has an unexplained fever or any infectious complications.

Medical Evaluations:

- Daily during the conditioning regimen
- Complete Blood Count with differential.
- Chemistries: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/AST, Total Bilirubin, LD, Total Protein, Uric Acid, CRP, and procalcitonin.
- Urinalysis with micro, if indicated on days -5 and -4.
- Culture of the ACT product on day -3 (±1 day), and again on day 0.
- Gram stain of the ACT product pre- and post- harvest on day 0.

After Cell Infusion:

- Vital signs are to be monitored hourly (+/- 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated.

Daily until past nadir or provider discretion:

- A review of systems and physical exam
- Complete Blood Count with differential
- Chemistries, as above
- Other tests are to be performed as clinically indicated.

Follow up visits:

- Every 4 weeks ± 1 week for first three months post-cell infusion, then every 3 months for first year post-cell infusion; thereafter per standard of care.
- If a patient is seen more frequently due to changing clinical circumstances, then 2 additional optional blood draws can be obtained during the 12-month period.
- Each visit will include a history, review of systems and physical exam. Other tests will be performed as clinically indicated.

**Clinical Course**

Day (-7): Pre-admission clinic visit:

Patient well in recent weeks. She reports occasional dull pains in her left costophrenic area and her mid-back radiating to the right side, both of which are relieved with acetaminophen. She takes diphenhydramine at bedtime for insomnia. She denies HIV or TB exposure but was diagnosed with hepatitis B virus infection in 1984, since cleared. The only prescription medication she reports taking is CREON (lipase-protease-amylase 24,000-76,000-120,000 units per capsule), 1-2 capsules with meals. Aside from pancreatic adenocarcinoma, her past medical history is otherwise unremarkable. She walks daily and maintains excellent performance status. Her appetite is intact, no recent weight loss, nausea, or vomiting. She has no cough, chest pain or shortness of breath. No symptoms of cold or influenza like illness, no known exposure to COVID-19 and fully vaccinated (two doses of Moderna mRNA vaccine). No other recent medical events. Screening blood tests showed WBC 7.5, Hg 11.8, MCV 93.9, PLT 119k (baseline ~110k), ALC 3,100, ANC 3,700, normal liver and kidney indices (Cr 0.8, eGFR > 60), magnesium 1.6 but otherwise normal electrolytes, blood glucose 96, procalcitonin (PCT) <0.05, CRP <0.5, HBV sAb positive (index 10.14).

Day (-5): Admission to hospital:

Baseline ECG showed sinus bradycardia with 1st degree A-V block, rate 59, PR interval 210 ms, corrected QT interval 403 ms. Labs showed: ALC 2,600, ANC 2,600, Hg 11.0, PLT 115k, normal liver and kidney indices, PCT <0.05, CRP <0.5.

Bedside US guided placement of triple lumen, pressure injectable, central venous catheter (5F PICC SOLO Power) via basilic vein, right upper extremity.

Baseline CT chest/abdomen/pelvis with contrast, demonstrating 16 lung nodules scattered in both lungs, ten $\geq$ 5 mm diameter, three $\geq$ 10mm (see Figure 1c), two RECIST 1.1 target lesions: 17 mm diameter in right middle lobe, and 15 mm diameter in left lower lobe; abdomen and pelvis clear aside from post-operative changes without evidence of local recurrence; stable 36 mm left thyroid nodule (benign, prior FNA).

At ~4 pm: started conditioning regimen with Tocilizumab and anti-emetic premedications, followed by Cyclophosphamide and Mesna (per treatment protocol above); well tolerated, no infusion reactions.

Day (-4): Completed conditioning regimen, no nausea or hematuria.

Day (-3): Complained of headache, stomachache, bone discomfort, mouth and tooth pain, backache, and dyspepsia; no nausea, hematuria, or dysuria. PPI increased to twice daily, with baclofen prn hiccups, loratadine for bone pain, and furosemide prn edema.

Day (-2): Flu-like aches resolved, good appetite; no nausea or hematuria. Started filgrastim, valacyclovir, and fluconazole per protocol.

Day (-1): Back to dry weight. Constipation treated with senna and prn laxative (Miralax).

Day (0): Cell infusion ~1 pm, 14.8 x 10e9 cells in 199 mL over 33 minutes; premedication with acetaminophen and diphenhydramine, no infusion reaction. ALC 100, ANC 6,700, Hg 10.2, PLT 47k, Cr 0.7, ALT 70, AST 77, Tbili 0.28, PCT <0.05, CRP <0.5.

Day (1): ALC 0, ANC 1,200, Hg 9.6, PLT 28k, Cr 0.80, ALT 59, AST 59, Tbili 0.39, PCT 0.06, CRP <0.5. Started schedule acetaminophen per IL-2 protocol, and at ~7 am received first dose IL-2, followed by rigors about 1 hour later, requiring IV meperidine with subsequent vomiting x1. Transfused 1 unit platelets, without reaction. At ~3 pm received second high dose IL-2, again with rigors requiring IV meperidine. At ~5:45 pm developed fever Tmax 39.4, remained hemodynamically stable. Blood cultures sent (all eventually negative), chest x-ray unremarkable. Treated empirically for febrile neutropenia with Cefepime 2 g IV q8h (through to discharge on Day 11). Next IL-2 dose held at 11 pm.

Day (2): ALC 0, ANC 0, Hg 9.0, PLT 37k, Cr 0.8, ALT 271, AST 476, Tbili 1.21, PCT 2.28, CRP 1.0. Appetite intact, no nausea, pains, fatigue, or bleeding. Acetaminophen discontinued. IL-2 dose was held at 7 am due to abrupt grade 3 transaminitis. Repeat liver indices in the afternoon stabilized (ALT 270, AST 369, Tbili 0.95) and she proceeded to receive 3 pm and 11 pm IL-2 doses, accompanied by rigors approximately 1 hour after each dose.

Day (3): ALC 0, ANC 0, Hg 9.2, PLT 14k, Cr 1.0, ALT 285, AST 361, Tbili 1.09, PCT 1.58, CRP 1.4. Onset of fatigue, but appetite intact; no nausea, pains, or bleeding. Onset of hypotension overnight, not improved with IV NS bolus 250 mL x3 (total 750 mL), started phenylephrine pressor support ~6:50 am, with peak dose 65 mcg/min IV by ~8:50 am, weaning off completely by ~2:30 pm. Transfused 1 unit platelets, without reaction. At ~3pm received fifth (final) dose of IL-2, again with rigors, followed by hypotension requiring pressor support ~8:50 pm, with peak dose 65 mcg/min IV by ~10:55 pm, gradually weaned off by ~8:25 am the next morning. No further IL-2 was given (5 of 6 total planned doses).

Day (4): ALC 0, ANC 0, Hg 9.1, PLT 21k, Cr 0.9, ALT 255, AST 297, Tbili 0.96, PCT 1.24, CRP 2.5. Increased fatigue and declining appetite; no nausea, pains, or bleeding. Transfused 1 unit of platelets, without reaction.

Day (5): ALC 100, ANC 0, Hg 8.3, PLT 24k, Cr 0.7, ALT 173, AST 156, Tbili 0.66, PCT 0.64, CRP 2.3. Fatigue resolving and appetite a bit improved with new pruritis on scalp and back; no bleeding. Circa 3 pm, after eating lunch, she complained of abrupt abdominal pain in the left upper quadrant, accompanied by nausea and vomiting. The pain radiated to her mid-back and was reminiscent of past episodes of pancreatitis. She was afebrile and hemodynamically stable, with a benign abdominal exam. Stat labs, including amylase/lipase, were unremarkable, as was stat non-contrast CT abdomen/pelvis. Pain was relieved with oxycodone and resolved completely with bowel rest and IV fluid support over the next 24 hours. Aside from this isolated and self-limited episode, she did not experience abdominal pain during hospitalization. Transfused 1 unit of platelets, without reaction.

Day (6): ALC 400, ANC 0, Hg 8.5, PLT 31k, Cr 0.7, ALT 142, AST 87, Tbili 0.66, PCT 0.31, CRP 1.7. Diet advanced from clear liquids. Reported continued pruritis; no fatigue or bleeding.

Day (7): ALC 1,000, ANC 0, Hg 8.1, PLT 18k, Cr 0.6, ALT 100, AST 55, Tbili 0.46, PCT 0.17, CRP 1.2. Residual pruritis and new sore neck; no nausea, pains, or bleeding. Transfused 1 unit of platelets, without reaction. Began ambulation with supervision.

Day (8): ALC 1,300, ANC 0, Hg 7.6, PLT 20k, Cr 0.6, ALT 76, AST 38, Tbili 0.39, PCT 0.07, CRP 0.6. Sore neck treated with lidocaine patch, ongoing pruritis. Transfused 2 units of PRBC and 1 unit of platelets, without reaction. Increasing ambulation with standby assist.

Day (9): ALC 1,700, ANC 0, Hg 9.1, PLT 22k, Cr 0.5, ALT 55, AST 29, Tbili 0.52, PCT <0.05, CRP <0.5. Arthralgia of neck/occiput and new lower back, treated with lidocaine patches and prn oxycodone. Transfused 1 unit of platelets, without reaction. Increasing activity level.

Day (10): ALC 1,600, ANC 0, Hg 8.9, PLT 13k, Cr 0.6, ALT 48, AST 31, Tbili 0.44, PCT <0.05, CRP <0.5. Transfused 1 unit of platelets, without reaction. Appetite not yet fully recovered, ongoing pruritis and arthralgias from neck to lower back.

Day (11): Discharged home after transfusion of 1 unit of platelets, without reaction. ALC 1,300, ANC 100, Hg 8.5, PLT 16k.

Her only prescription medication on admission was CREON; she was discharged with the following additional medications: TMP/SMX (Bactrim), valacyclovir, entecavir, fluconazole, levofloxacin, omeprazole, prochlorperazine, senna, magnesium oxide, calcium carbonate, oxycodone, lidocaine 5% patch, ammonium lactate 12% lotion. Daily outpatient follow-up was scheduled for filgrastim subcutaneous injection, IV hydration and electrolytes (primarily magnesium), and blood product transfusions as needed.

Day (12): ALC 800, ANC 100, Hg 9.5, PLT 25k. Outpatient visit for daily filgrastim subcutaneous injection and IV hydration. Reported fatigue; no nausea or pain, good appetite. No transfusions indicated.

Day (13): ALC 1,200, ANC 100, Hg 9.3, PLT 18k. Outpatient visit for daily filgrastim subcutaneous injection. Reported feeling well, declined IV hydration. Transfused 1 unit of platelets, without reaction.

Day (14): ALC 1,100, ANC 200, Hg 9.1, PLT 34k. Outpatient visit for daily filgrastim subcutaneous injection. Reported feeling well, declined IV hydration. No transfusions indicated.

Day (15): ALC 700, ANC 200, Hg 8.5, PLT 22k. Outpatient visit for daily filgrastim subcutaneous injection. Reported feeling well, declined IV hydration. Transfused 1 unit of platelets, without reaction.

Day (16): ALC 800, ANC 400, Hg 8.9, PLT 35k. Outpatient visit for daily filgrastim subcutaneous injection. Reported feeling well, declined IV hydration. No transfusions indicated.

Day (17): ALC 1,400, ANC 600, Hg 9.3, PLT 29k. Outpatient visit for daily filgrastim subcutaneous injection. Reported feeling well, declined IV hydration. No transfusions indicated.

Day (18): ALC 1,000, ANC 700, Hg 9.1, PLT 21k. Outpatient visit for daily filgrastim subcutaneous injection. Reported feeling well, declined IV hydration. Transfused 1 unit of platelets, without reaction.

Day (19): ALC 1,100, ANC 1,200, Hg 9.1, PLT 47. Outpatient visit for daily filgrastim subcutaneous injection. Reported fatigue and reduced appetite; no fever, nausea, diarrhea, lightheadedness, or pains. Received IV hydration. No transfusions indicated.

Day (20): ALC 500, ANC 1,300, Hg 8.2, PLT 27. Outpatient visit for daily filgrastim subcutaneous injection. Reported fatigue, reduced appetite, and postural lightheadedness; no fevers, falls, nausea, diarrhea, or pains. Orthostatic hypotension corrected with IV hydration. No transfusions indicated.

Day (21): ALC 600, ANC 1,600, Hg 8.1, PLT 22k. Outpatient visit for daily filgrastim subcutaneous injection. Reported fatigue, reduced appetite, and postural lightheadedness; no fevers, falls, nausea, diarrhea, or pains. Orthostatic hypotension corrected with IV hydration. No transfusions indicated. Started fludrocortisone 0.5mg po daily. Fluconazole and levofloxacin discontinued.

Day (22): ALC 700, ANC 2,300, Hg 8.2, PLT 39k. Filgrastim discontinued after 3 consecutive days ANC > 1,000. Reported starting to feel better. No transfusions indicated. No further IV hydration required.

Day (24): ALC 600, ANC 1,000, Hg 8.0, PLT 34k. No new complaints. Transfused 1 unit of PRBC and 1 unit of platelets, without reaction.

Day (28): ALC 600, ANC 900, Hg 9.3, PLT 69k. IV fluids only

Day (31): ALC 800, ANC 1,000, Hg 9.0, PLT 69k. Seen in clinic follow-up to review Day 30 CT chest/abdomen/pelvis showing 62% decrease in target lesions versus baseline: 6 < 17 mm diameter in right middle lobe, and 6 < 15 mm diameter in left lower lobe, without new lesions (total: 12 mm / 32 mm; RECIST 1.1). Postural lightheadedness resolved; fludrocortisone discontinued. Arthralgia long since resolved, not requiring oxycodone or lidocaine patches. Appetite recovered, energy level improving, ECOG PS 1; only complaint trace pedal edema from fludrocortisone. Labs showed normal chemistries.

Day (85): Seen in clinic follow-up to review Day 85 CT chest/abdomen/pelvis showing 78% decrease in target lesions versus baseline: 1 < 6 < 17 mm diameter in right middle lobe, and 6 < 6 < 15 mm diameter in left lower lobe, without new lesions (total: 7 mm / 32 mm; RECIST 1.1). Feeling well, active, ECOG PS 0, no pains, no new complaints. Labs showed blood counts at baseline: WBC 4.9, ANC 2,800, ALC 1,300, Hg 10.2, PLT 110k, and normal chemistries.

Day (176): Seen in clinic follow-up to review Day 176 CT chest/abdomen/pelvis showing stable 72% decrease in target lesions versus baseline: 2 < 1 < 6 < 17 mm diameter in right middle lobe, and 7 < 6 < 6 < 15 mm diameter in left lower lobe, without new lesions (total: 9 mm / 32 mm; RECIST 1.1). Feeling well, ECOG PS 0, no pains, no new complaints. Labs stable: WBC 4.9, ANC 2,600, ALC 1,600, PLT 109k, and normal chemistries. Stopped entecavir, valacyclovir, TMP/SMX (Bactrim). Only remaining prescription medication is CREON with meals.

## SUPPLEMENTARY MATERIALS AND METHODS

Vector manufacture

The two TCRs used in this report are HLA-C*08:02-restricted TCRs that specifically target either the 9 amino acid long KRAS G12D neoepitope GA**D**GVGKSA ("9mer TCR") or the 10 amino acid long KRAS neoepitope GA**D**GVGKSAL ("10mer TCR"). These two TCRs were originally isolated from the patient described in Tran et al.[1] and the 9mer TCR is referred to as "TRBV5-6 (B) TCR" and the 10mer TCR is referred to as "TRBV10-02 TCR" in that report. Each TCR was codon optimized, synthesized, and cloned into the MSGV1 plasmid by GenScript. See Supplementary Figure S1 for MSGV1 plasmid schematic.

Gammaretroviral vector for each TCR was produced under GMP by the Vector Production Facility at Cincinnati Children's Hospital Medical Center by transient transfection of 293T cells with plasmids encoding gag/pol, envelope RD114, and the TCRs. Supernatants were harvested and underwent safety testing as required by the FDA.

Generation of the infusion product

PBMC was obtained by leukapheresis from the patient through an IRB-approved tissue procurement protocol. Cell manufacture was performed in our cleanroom facility (Cell Production Facility, Human Applications Lab). PBMC ($1x10^6$/mL, 2 mL per well of 24-well plate) were stimulated with 50 ng/mL GMP-grade anti-CD3 antibody (clone OKT3, Miltenyi Biotec) in AIM-V CTS media (Gibco) containing 5% heat inactivated human AB serum (Valley Biomedical), 300 IU/mL IL-2 (Proleukin), 30 ng/mL IL-7 (PeproTech, GMP-grade), 15 ng/mL IL-21 (Cellgenix, GMP-grade), and 5 ng/mL TGF-β (Cellgenix, GMP-grade), and then two days later transduced with the gammaretroviral vector encoding the TCRs. For the transduction, non-treated tissue culture plates were coated with 10 ug/mL GMP-grade Retronectin (Takara/Clontech) the day prior to transduction. On the day of transduction, each of the retroviral supernatants (4 mL of undiluted stock per well) was centrifuged into separate wells of the Retronectin coated 6-well plates, excess supernatant was removed, and then the activated T cells (2e6 per well) were centrifuged onto the plate. The following day, the cells were transferred to a G-Rex 6-well plate and further cultured in AIM-V media containing the above cytokines. At 10 days after the initial stimulation, aliquots of the cells were cryopreserved. See Supplementary Figure S2A for a schematic.

To generate the infusion product, each of the two TCR-transduced T-cell populations were thawed and separately underwent a two-week rapid expansion protocol similar to a previous study[2] using AIM-V CTS media (Gibco) containing 5% heat inactivated human AB serum (Valley Biomedical), lethally irradiated PBMC feeder cells, 30 ng/mL anti-CD3 (clone OKT3, Miltenyi), and 3,000 IU/mL IL-2 (Proleukin) in G-Rex500 flasks (Wilson Wolf), but with the incorporation of 15 ng/mL IL-21 (Cellgenix, GMP-grade), and 5 ng/mL TGF-β (Cellgenix, GMP-grade). After the rapid expansion, the TCR-transduced T-cell cultures were pooled and harvested for patient treatment. See Supplementary Figure S2B for a schematic.

Characterization of the infusion product

Flow cytometry was used to evaluate transduction efficiency and expression of select cell surface markers. Our TCR constructs contain the mouse TCR-alpha and beta chain constant regions (Supplementary Figure S1) and thus transduction efficiency can be evaluated by measuring expression of the mouse TCR-beta constant chain using the anti-mouse TCR-beta constant chain antibody. The following, anti-human flow cytometry antibodies were used in this report: CD3-AF700 (SK7), CD4-APC-Fire750 (SK3), CD8-PE-Cy7/BV510 (SK1 and RPA-T8), 4-1BB-PE/APC

(4B4-1), mouse TCRβ constant-FITC/PE/APC (H57-597), CD103-FITC (Ber-ACT8), CD69-APC-Fire750 (FN50), CD49a-AF647 (TS2/7), PD1-PE-Cy7 (EH12.2H7), TIM3-PE (344823), CD28-FITC (CD28.2), CD27-APC-Fire750 (M-T271), CD62L-APC (DREG-56), CD45RO-PE-Cy7 (UCHL1), TCR Vβ5.2-PE (36213). All antibodies were from BioLegend except for the TCR Vβ5.2-PE (Beckman Coulter).

For preparation of cells for flow cytometry, cells were harvested, and then resuspended in FACS buffer (1X PBS containing 1% FBS and 2 mM EDTA) and incubated with titrated fluorochrome-conjugated antibodies for about 30 minutes in the fridge and the dark. Cells were then washed with FACS buffer, and resuspened with FACS buffer followed by data acquisition with either the BD LSRII or Beckman Coulter CytoFLEX flow cytometers. Flow cytometry data was 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 peripheral blood monocytes using the plastic adherence method. Briefly, patient PBMC were thawed, washed, resuspended in AIM-V media (Gibco) and then transferred to tissue culture flasks and incubated at approximately 1e6 cells/$cm^2$ in a incubator at 37°C, 5% $CO_2$. After 90 min, the non-adherent cells were collected and the adherent cells in the flasks 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 flasks were then vigorously washed again with RPMI and then incubated with DC media which is comprised of RPMI containing 5% human serum, 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco), 1:100 Glutamax (Gibco), 800 IU/ml GM-CSF (Leukine) and 200 U/ml IL-4 (Peprotech). DCs were used in experiments after day 4 of initiation of the cultures.

The cocultures comprised of infusion product T cells ($1x10^5$/well of 96-well plate) incubated with DCs ($0.5x10^6$/well) that were separately pulsed for 2h with 1 ug/mL of the HPLC-purified KRAS peptides (GenScript) wild-type (WT) 9mer (GA**G**GVGKSA), mutated (MUT) 9mer (GA**D**GVGKSA), WT 10mer (GA**G**GVGKSAL), and MUT 10mer (GA**D**GVGKSAL), or electroporated with 80 ug/mL *in vitro* transcribed RNA encoding full length WT KRAS or KRAS G12D. IVT RNA was generated using the mMESSAGE mMACHINE T7 Ultra transcription kit (Ambion), and electroporation of DCs was done using the BTX-830 square wave electroporator with a 2 mm gap cuvette and 150 V, 10 ms, and 1 pulse as previously described[3]. The cells were cocultured overnight in 50/50 media which consisted of a 1 to 1 mixture of AIM-V media with complete media (CM). CM is comprised of RPMI supplemented with 10% human AB serum, 1:100 Glutamax, 25 mM HEPES, 5 µg/mL Gentamicin, and 100 U/ml penicillin and 100 µg/ml streptomycin. No exogenous cytokines were added to the coculture. After an overnight incubation, supernatants were harvested and frozen in a -20°C freezer and cells were collected for flow cytometric analysis of T-cell activation (4-1BB expression). The LegendPlex (CD8/NK Panel, BioLegend) multiplex bead-based assay was used according to manufacturer's instructions to evaluate levels of various cytokines and cytotoxic proteins in the coculture supernatants.

<u>Serum cytokine and protein analysis</u>

Blood was collected in serum separating tubes (SST) before and after cell infusion and serum was harvested and stored in a -80°C freezer. The Immune Monitoring 65-Plex Human ProcartaPlex Panel (Invitrogen, ThermoFisher) was used according to manufacturer's instructions to evaluate concentrations of various cytokines and proteins in the serum.

*In vivo* persistence of engineered T cells

Whole blood was collected in BD Vacutainer CPT mononuclear cell preparation tubes and PBMC were isolated according to manufacturer's instructions. The transduced T cells were tracked using flow cytometric analysis of mouse TCRβ expression as described above. T cells expressing the 9mer TCR could in large part be distinguished from T cells expressing the 10mer TCR since the 9mer TCR contains TRBV5-6 (also known as Vβ5.2) and thus T cells co-expressing mouse TCRβ and Vβ5.2 were categorized as the 9mer transduced T cells. Note that a small percentage of T cells co-expressing mouse TCRβ and Vβ5.2 could represent the 10mer TCR transduced T cells since there is an endogenous T-cell population in the patient that expresses Vβ5.2 and were transduced to express the 10mer TCR.

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

We used IFN-γ ELISPOT and intracellular cytokine staining (ICS) with flow cytometryic analysis to evaluate function of the transduced T cells circulating in the PBMC at about 3 months 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. PBMC were rested in an ultra-low attachment 24-well plate and the infusion product was rested in a GRex 24-well plate. For IFN-γ ELISPOT, in brief, about $2x10^5$ PBMC and $1x10^5$ infusion product per well of a 96-well ELISPOT plate were stimulated overnight with 1 ug/mL of the HPLC-purified wild-type or mutated KRAS 9mer and/or 10mer peptides described above. After overnight culture, the plate was processed and developed to visualize IFN-γ spots as previously described[3].

For ICS, about $3x10^5$ PBMCs and $2x10^5$ infusion product cells were seeded per well of a 96-well round bottom plate. The cells were washed three times with 200 uL 50/50 media. The PBMCs were then transferred to a 96-well flat bottom plate and stimulated with 1 ug/mL of an HPLC-purified KRAS peptides described above. PE-conjugated anti-CD107a antibody (H4A3, Biolegend), Brefeldin A (Biolegend), and Monensin (Biolegend) were added at the onset of stimulation. After five hours of stimulation in an incubator at 37°C and 5% CO2, the cells were filtered through a 40 um mesh filter and transferred to a 96-well round bottom plate. Cells were washed once with 200 uL of FACS buffer, and then stained with BV510-conjugated anti-CD8 antibody (RPA-T8), PE/Cy7-conjugated anti-CD3 antibody (UCHT1), AF700-conjugated anti-CD4 antibody (SK3), and APC/Cy7-conjugated anti-mTCRb antibody (H57-597) (all from Biolegend). The cells were incubated in the dark at 4°C for 30 minutes and then washed once with 200 uL of FACS buffer. The PBMCs were then incubated in fixation buffer (Biolegend) in the dark at room temperature for 15 minutes. The PBMCs were washed once with 200 uL of FACS buffer, then twice with 200 uL of permeabilization buffer (Biolegend). The cells were stained with APC-conjugated anti-IL2 antibody (MQ1-17H12), FITC-conjugated anti-IFNg antibody (4S.B3), and PerCp/Cy5.5-conjugated anti-TNFa antibody (MAb11) (all from Biolegend). After a 30 minute incubation in the dark at room temperature, the PBMCs were washed once with 200 uL of permeabilization buffer. The cells were re-suspended in FACS buffer and analyzed on a BD LSRII flow cytometer.

## SUPPLEMENTARY FIGURES

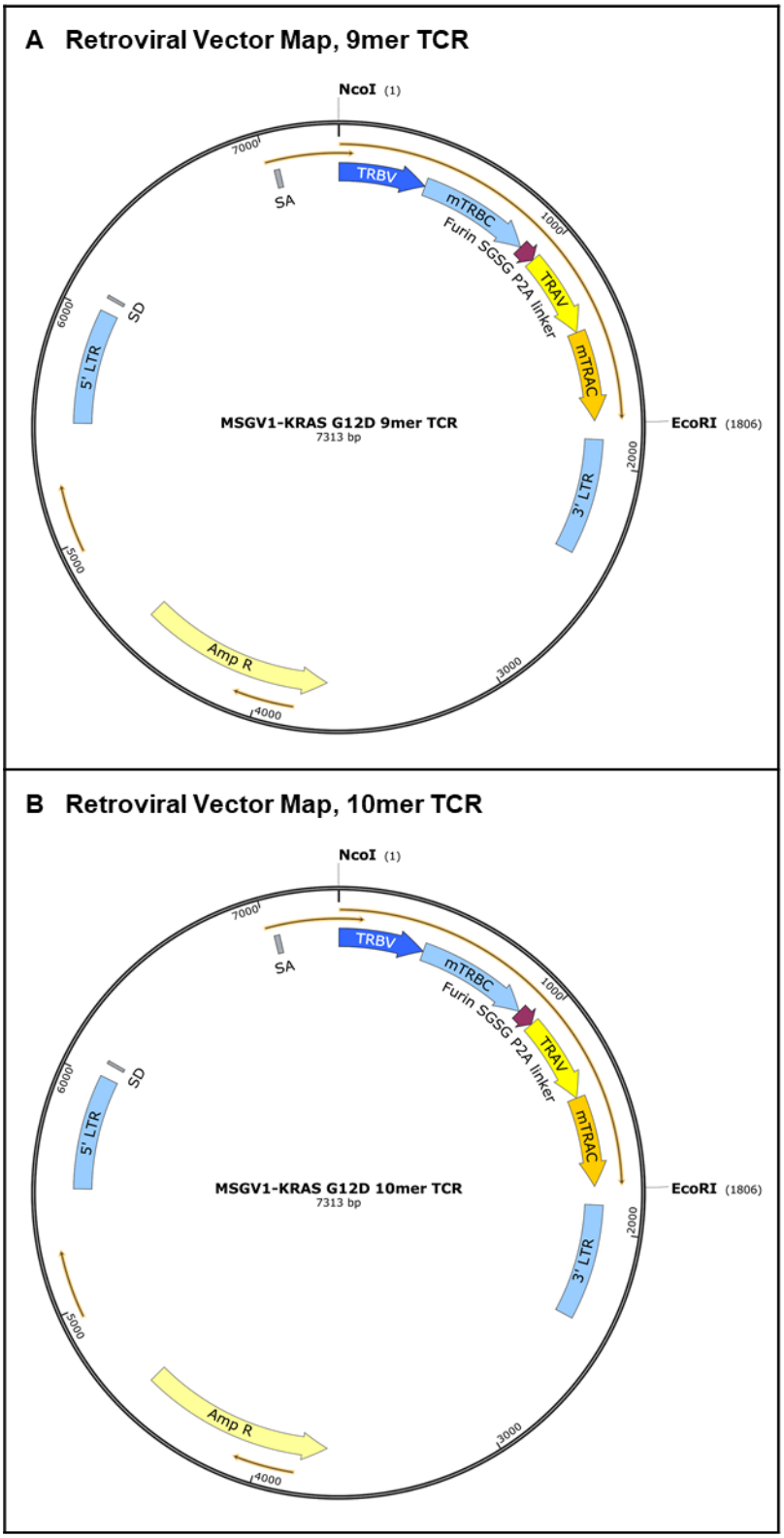


**Figure S1. Retroviral Vector used to Transduce the Patient's T cells.** Panel A and Panel B show the plasmid maps for the KRAS G12D 9mer and 10mer peptide-reactive TCRs, respectively, used to generate cells for therapy. The 9mer and 10mer TCRs were originally isolated from the patient described in Tran et al. (PMID: 27959684) and refer to the TCRs named "TRBV5-6 (B) TCR" and "TRBV10-02 TCR", respectively, in that report.

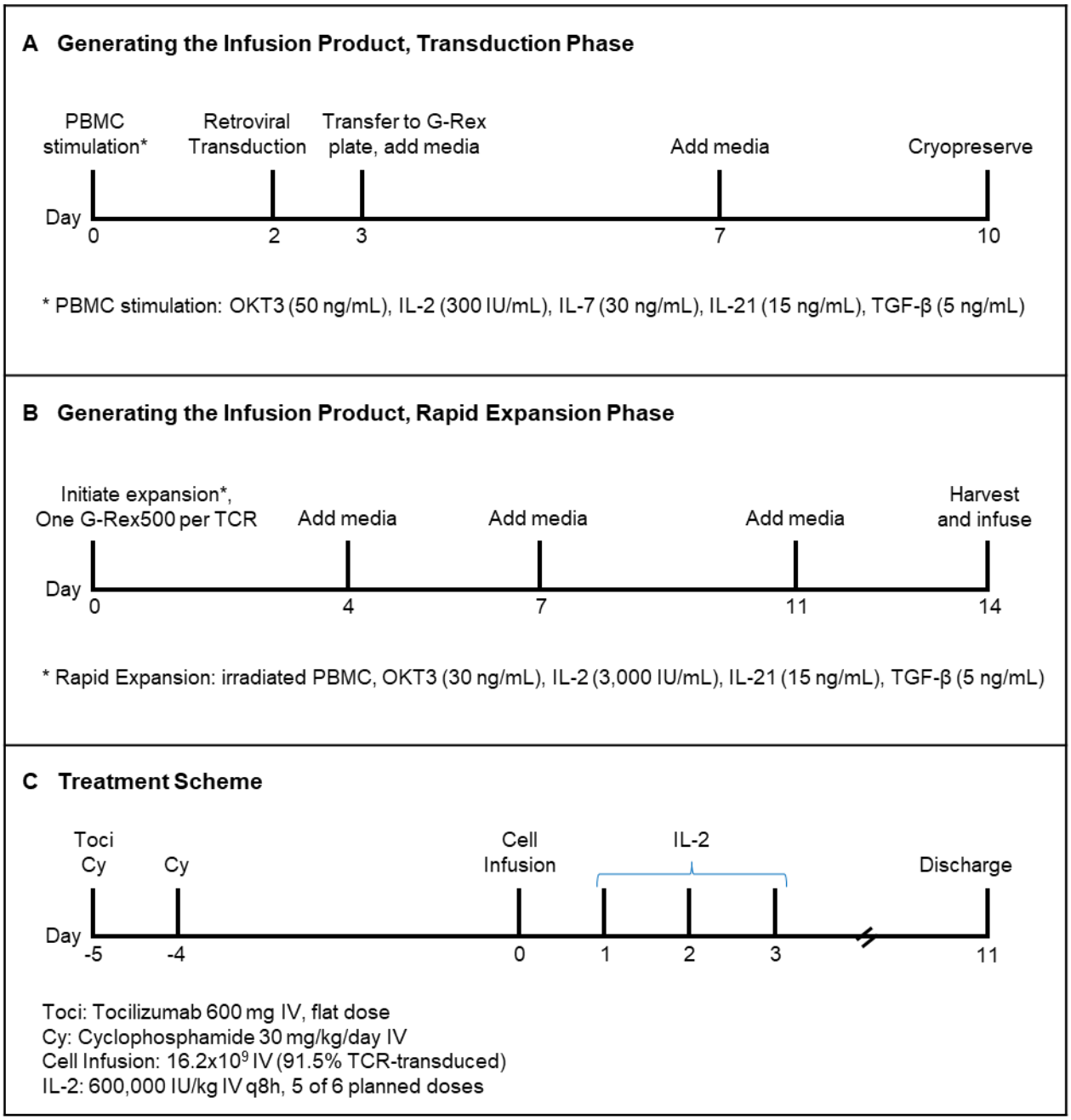


**Figure S2. Cell Therapy Manufacture and Treatment Scheme for Patient CRI-4483.** Panel A shows the T-cell transduction phase and Panel B shows the T-cell rapid expansion phase. Additional details are described in the Supplementary Materials and Methods section. Panel C shows the treatment scheme.

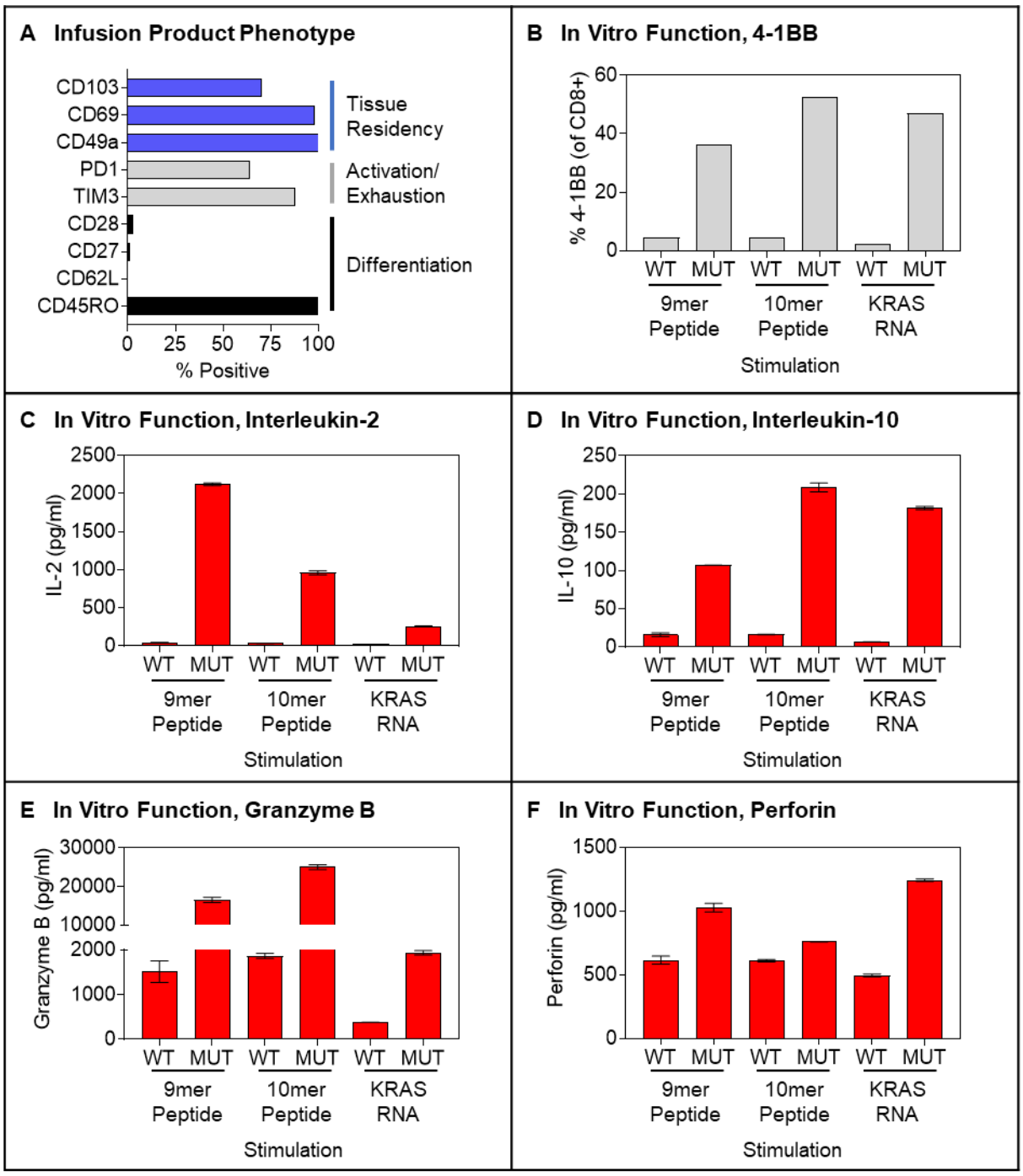


**Figure S3. Additional Characterization of the Infusion Product used to Treat Patient CRI-4483.** Panel A shows flow cytometric analysis of select cell surface proteins expressed on the Infusion Product. Data are gated on CD8 cells. Panel B shows 4-1BB expression on CD8 T cells after an overnight co-culture of the Infusion Product with autologous DCs pulsed with the indicated wild-type (WT) or mutated (MUT) KRAS G12D peptide, or transfected with RNA encoding WT or MUT KRAS G12D. Panels C through F show concentrations of the indicated analyte secreted into the supernatant after the overnight co-culture described in Panel B. Bars represent standard error.

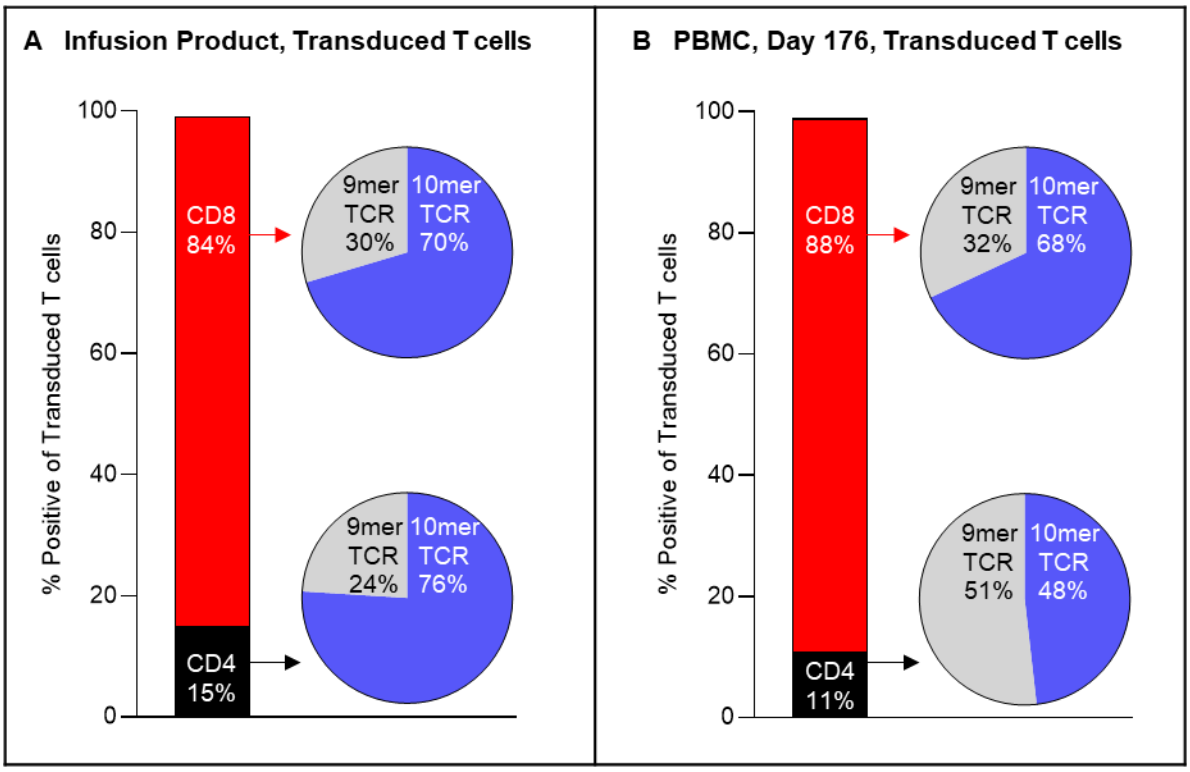


**Figure S4. Evaluation of CD4/CD8 Ratio and Frequency of 9mer and 10mer TCR-Engineered T cells within the Transduced T-Cell Population of the Infusion Product and PBMC 176 Days after Cell Infusion.** Panel A shows flow cytometric analysis of CD4 and CD8 (bar graph) and frequency of the 9mer and 10mer TCR within the transduced CD8+ or CD4+ T-cell population (pie charts) of the Infusion Product. Panel B shows the same as Panel A except the cells are from the patient's PBMC 176 days after cell infusion. N.B., the 9mer TCR is functional in CD4+ T cells (i.e., displays co-receptor independent reactivity against KRAS G12D), while the 10mer TCR does not function well in CD4+ T cells.

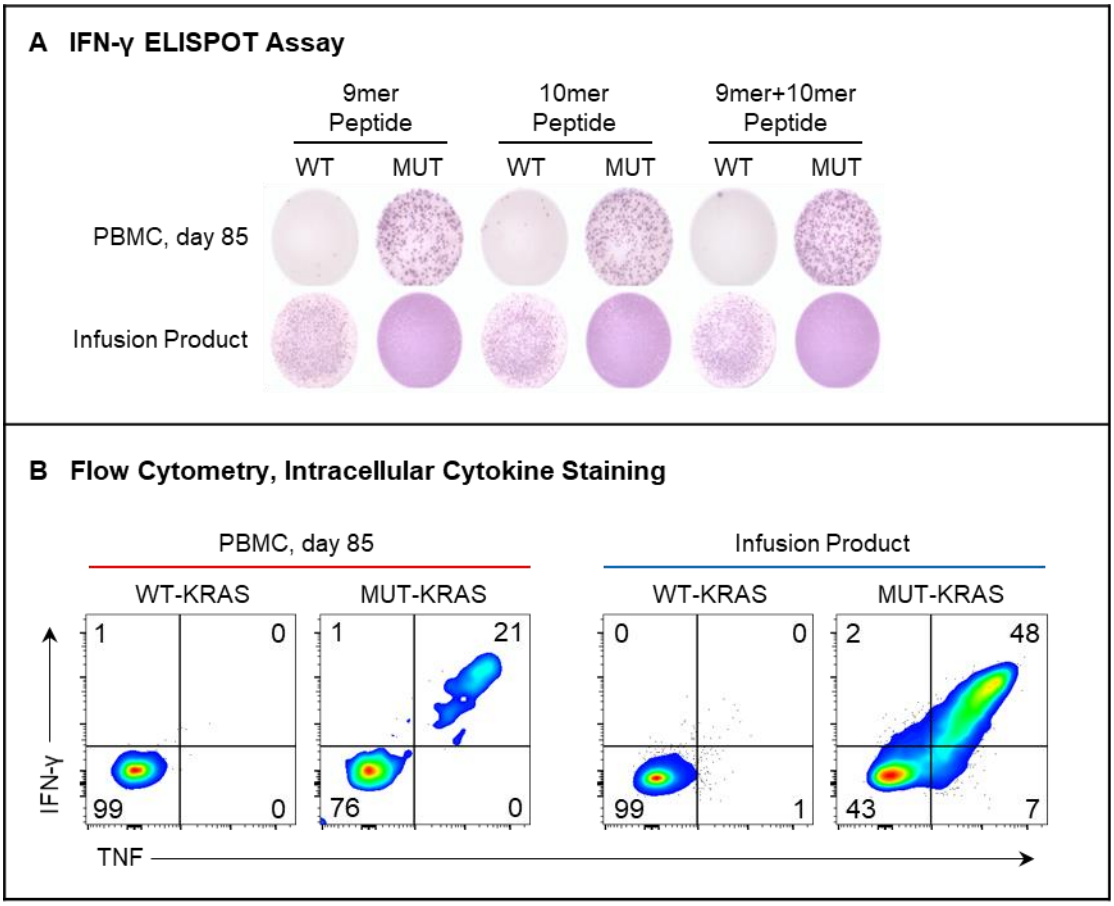


**Figure S5. Evaluation of *In Vitro* Effector Function of the TCR-Engineered T Cells Persisting in the PBMC 85 Days after Cell Infusion for Patient CRI-4483.** Panel A shows IFN-γ ELISPOT images of the day 85 PBMC and Infusion Product after overnight culture with 1 ug/mL of the indicated wild-type (WT) or mutated (MUT) KRAS peptides. Approximately $2x10^5$ PBMC and $1x10^5$ Infusion Product cells were plated per well. Panel B shows the flow cytometric analysis of the day 85 PBMC and Infusion Product after 5h stimulation with WT or MUT KRAS peptides (1 ug/mL each of the 9mer and 10mer peptides combined) and intracellular cytokine staining for IFN-γ and TNF. Data are gated on CD3+mTCRβ+ transduced cells. All cells used in these assays were thawed and rested overnight in media without exogenous cytokines prior to assays. The assays also did not include exogenous cytokines.

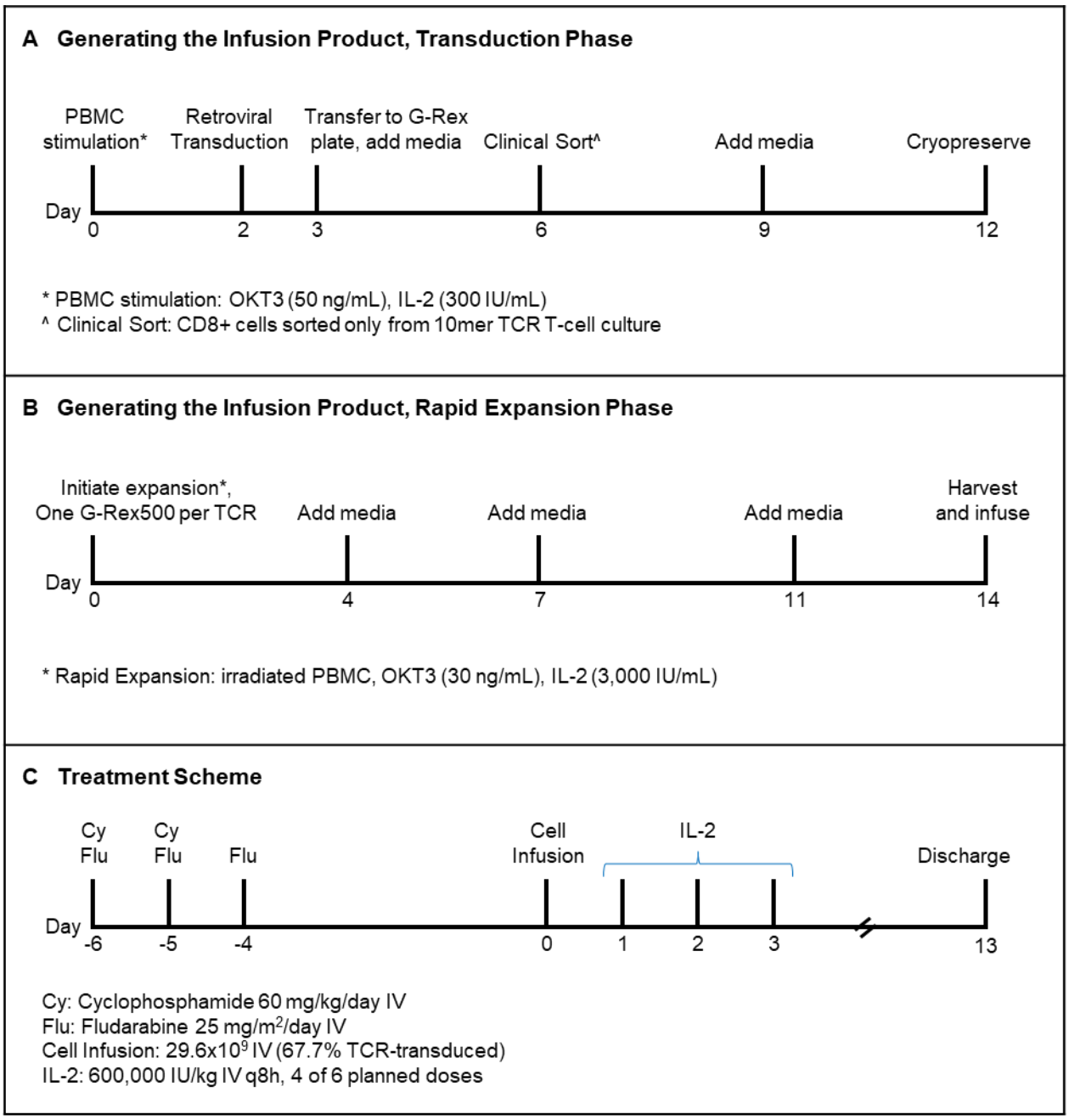


**Figure S6. Cell Therapy Manufacture and Treatment Scheme for Patient CRI-3061.** Panel A shows the T-cell transduction phase and Panel B shows the T-cell rapid expansion phase. Panel C shows the treatment scheme.

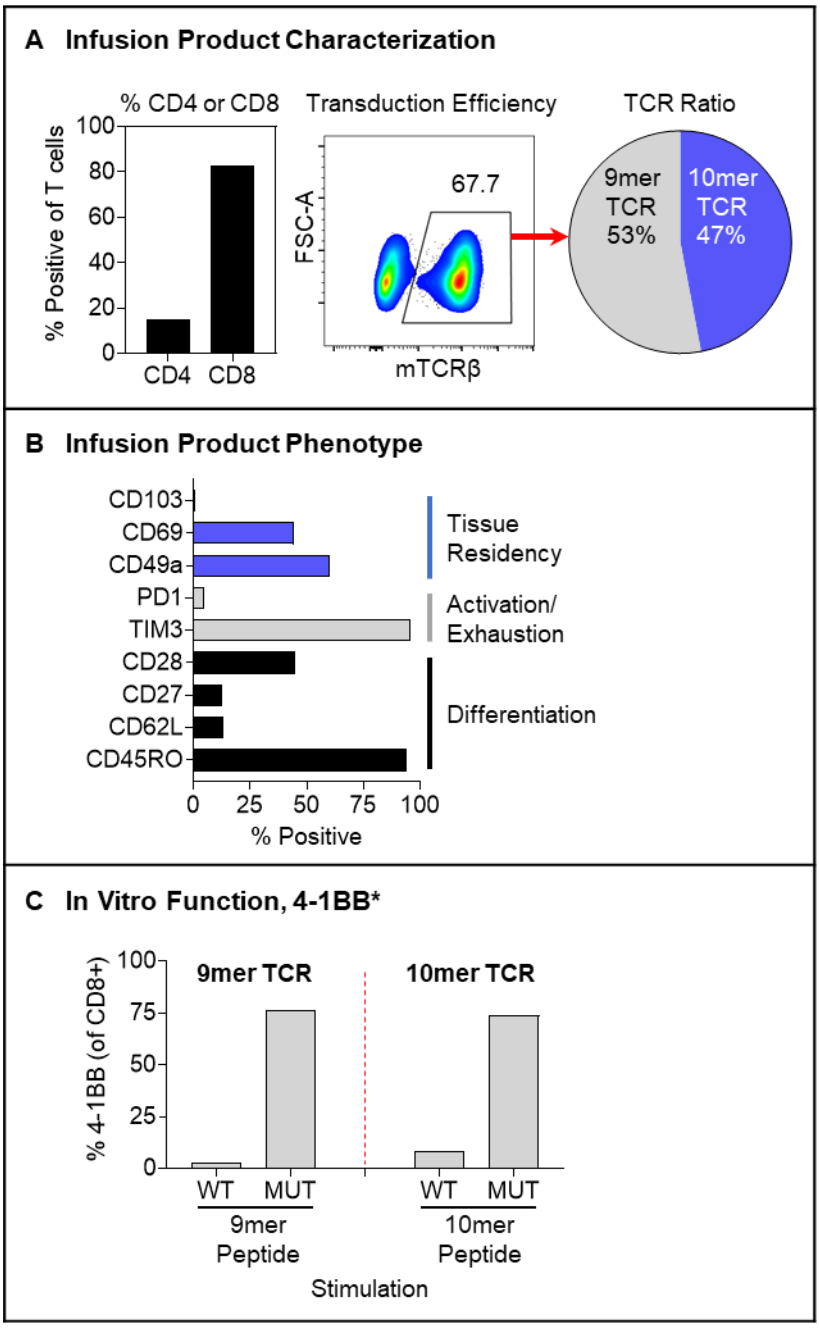


**Figure S7. Characterization of CRI-3061 Infusion Product.** Panel A shows the frequency of CD4 and CD8 T cells (left), transduction efficiency as measured by detection of mouse TCRβ constant chain which is engineered into the TCRs (middle), and ratio of the 9mer and 10mer TCR-transduced T cells (right) in the patient's Infusion Product, as determined by flow cytometry. Panel B shows flow cytometric analysis of select cell surface proteins expressed on the Infusion Product. Data are gated on CD8 cells. Panel C shows 4-1BB expression on CD8 T cells after an overnight co-culture of the 9mer TCR (left) or 10mer TCR (right) T cells with autologous B cells pulsed with the indicated wild-type (WT) or mutated (MUT) KRAS G12D peptide. *Note: T cells used in this assay were from after the first stimulation phase (i.e., Fig. S6A), prior to rapid expansion and pooling into the Infusion Product.

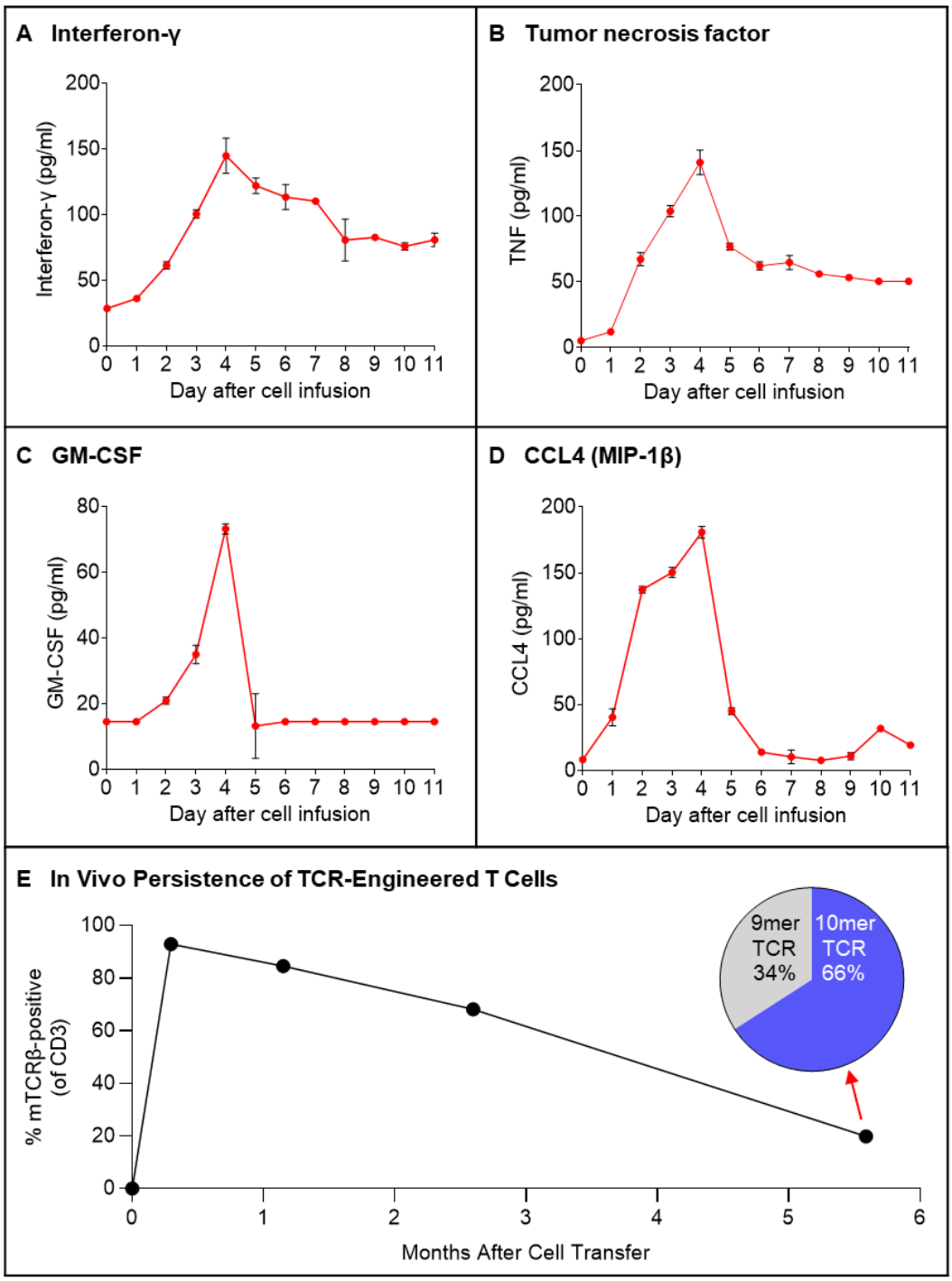


**Figure S8. Serum Cytokine Levels and *In Vivo* T-Cell Persistence in Patient CRI-3061.** Panels A through D show the concentration of the T-cell related cytokines Interferon-γ, TNF, GM-CSF, and CCL4 in the serum before and after cell therapy. Bars represent standard error. Panel E shows the *in vivo* persistence of the transferred TCR-engineered T cells in the peripheral blood as determined by flow cytometric analysis of mouse TCRβ. Pie chart (inset) represents ratio of 9mer and 10mer TCRs within the transduced T cells at day 170 after cell therapy. Data are gated on CD3+ lymphocytes.

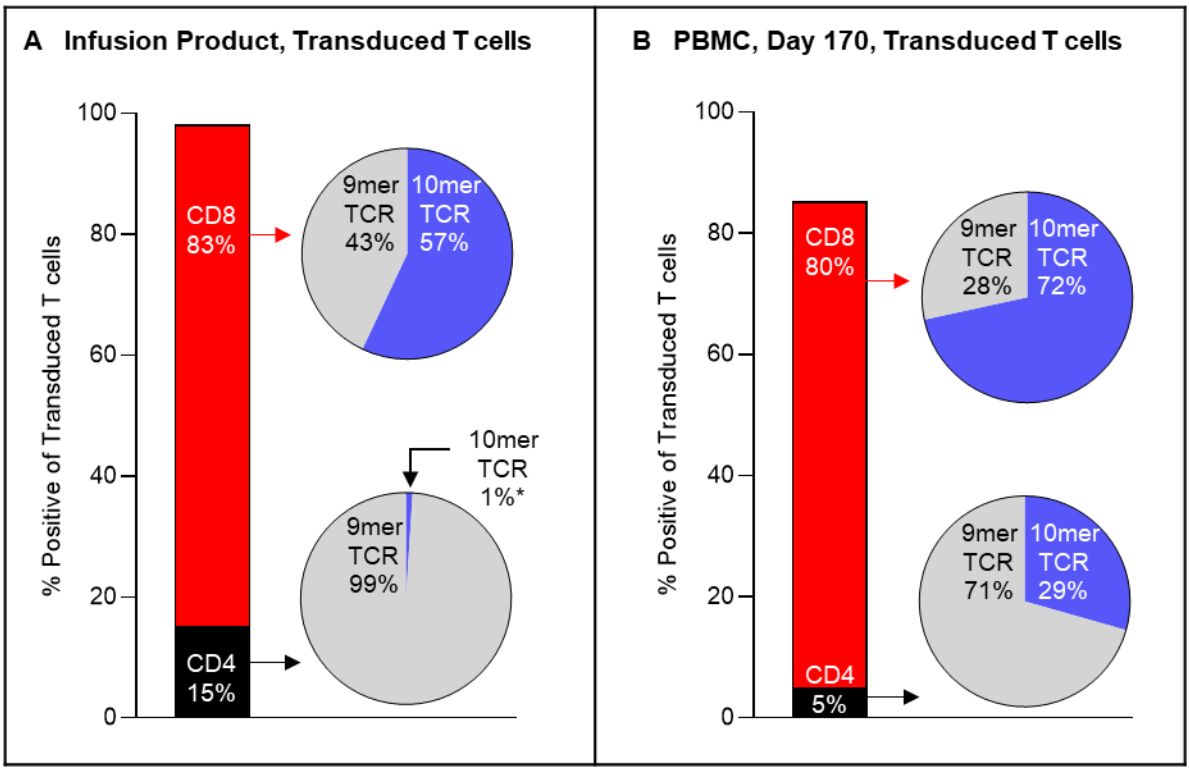


**Figure S9. Evaluation of CD4/CD8 ratio and Frequency of 9mer and 10mer TCR-Engineered T cells within the Transduced T-Cell Population of the Infusion Product and PBMC 170 Days after Cell Infusion for Patient CRI-3061.** Panel A shows flow cytometric analysis of CD4 and CD8 (bar graph) and frequency of the 9mer and 10mer TCR within the transduced CD8+ or CD4+ T-cell population (pie charts) of the Infusion Product. *Note: the 10mer TCR T-cell culture was CD8 enriched as described in Fig. S5A since the 10mer TCR is not very functional in CD4+ T cells (data not shown). Panel B shows the same as Panel A except the cells are from the patient's PBMC 170 days after cell infusion.

## REFERENCES FOR SUPPLEMENTARY APPENDIX


1. Tran E, Robbins PF, Lu YC, et al. T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer. N Engl J Med 2016;375(23):2255-2262. DOI: 10.1056/NEJMoa1609279.
2. Jin BY, Campbell TE, Draper LM, et al. Engineered T cells targeting E7 mediate regression of human papillomavirus cancers in a murine model. JCI insight 2018;3(8). DOI: 10.1172/jci.insight.99488.
3. Tran E, Ahmadzadeh M, Lu YC, et al. Immunogenicity of somatic mutations in human gastrointestinal cancers. Science 2015;350(6266):1387-90. DOI: 10.1126/science.aad1253.