

Complete regression of metastatic cervical cancer following treatment with HPV-targeted tumor-infiltrating T cells

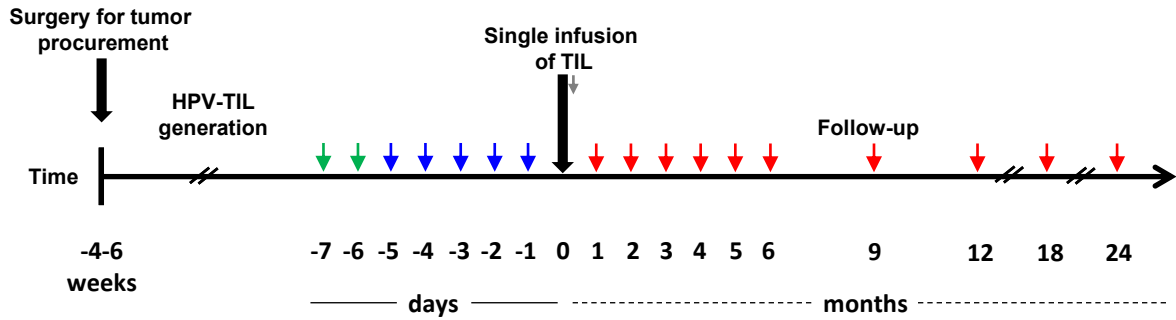
Stevanović, et al.

DATA SUPPLEMENT

Figures and Tables

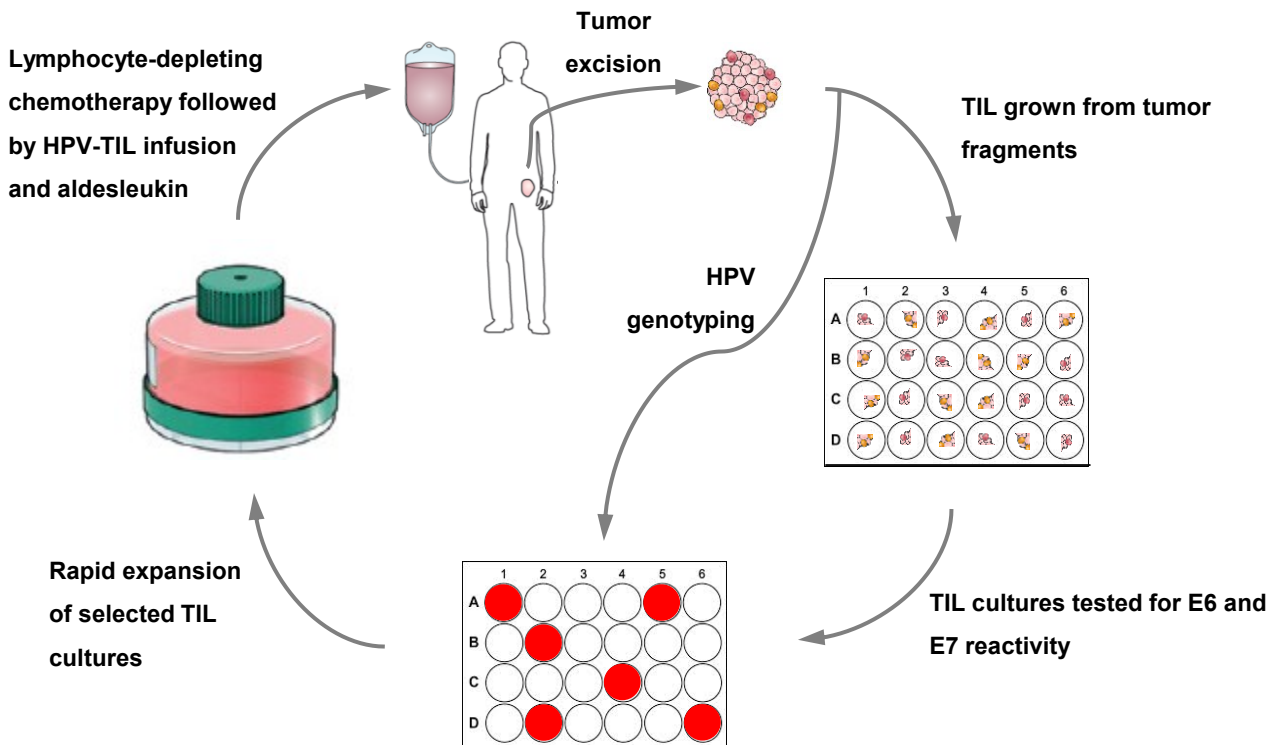
Data Supplement, Figure 1

A



- ↓: cyclophosphamide 60 mg/kg/day
- ↓: fludarabine 25 mg/m²/day
- ↓: aldesleukin 720.000 IU/kg every 8 hours, dosed to tolerance up to 15 doses
- ↓: evaluation for tumor responses and treatment toxicity

B



Data Supplement, Figure 1

Figure 1: Timeline for the HPV-TIL protocol and schema for infusion product generation and administration. (A) A tumor that can be removed with minimal surgical morbidity is excised. The HPV-TIL cell product is generated as shown in (B). Lymphocyte-depleting chemotherapy is administered for the seven days prior to HPV-TIL infusion. HPV-TIL is administered in a single infusion. Aldesleukin is given as tolerated. The number of cells and doses of IL-2 administered to each patient are provided in Table 1. Patients are evaluated at scheduled intervals for treatment response and toxicity. Follow up assessments of tumor responses and adverse events were conducted as indicated. Patients were removed from the study upon disease progression. (B) TIL cultures are initiated from 2 mm fragments and cultured in media containing IL-2. After initial expansion of two to three weeks, individual TIL cultures are tested for HPV-type specific E6 and E7 reactivity. Selected TIL cultures (red wells) are further expanded using a rapid expansion protocol. Expanded TIL are administered to patients.

Data Supplement, Figure 2

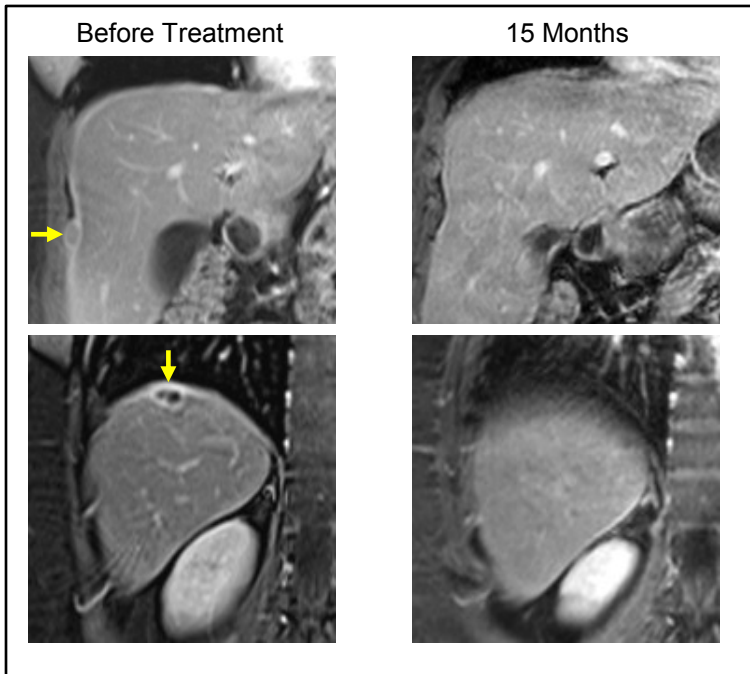


Figure 2: Complete regression of tumors involving the liver surface in Patient 6. Delayed gadolinium-enhanced T1-weighted magnetic resonance imaging coronal views depicting two tumors on the liver surface before treatment. Neither tumor was present 15 months following treatment. Arrows indicate the locations of the tumors.

Data Supplement, Figure 3

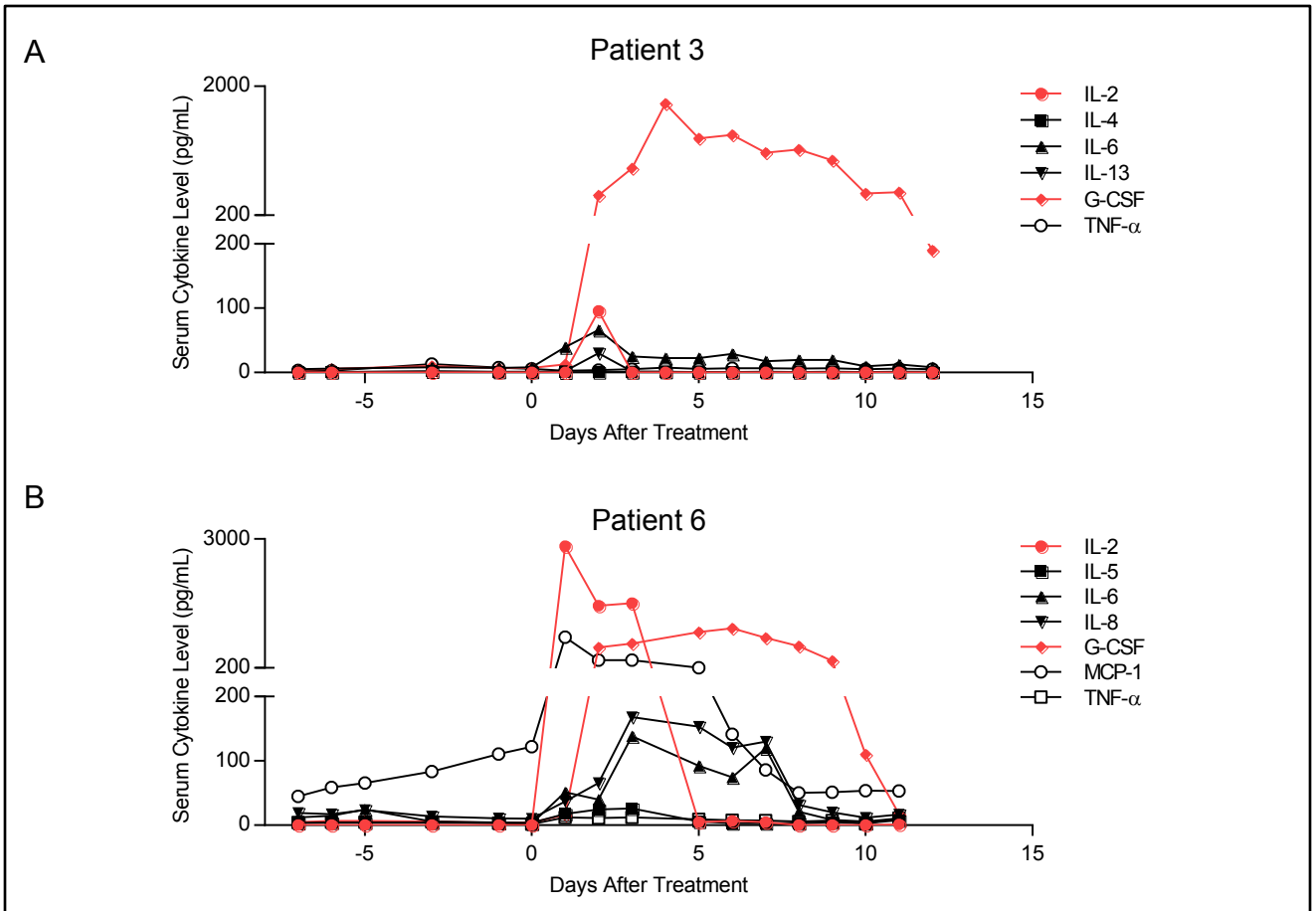
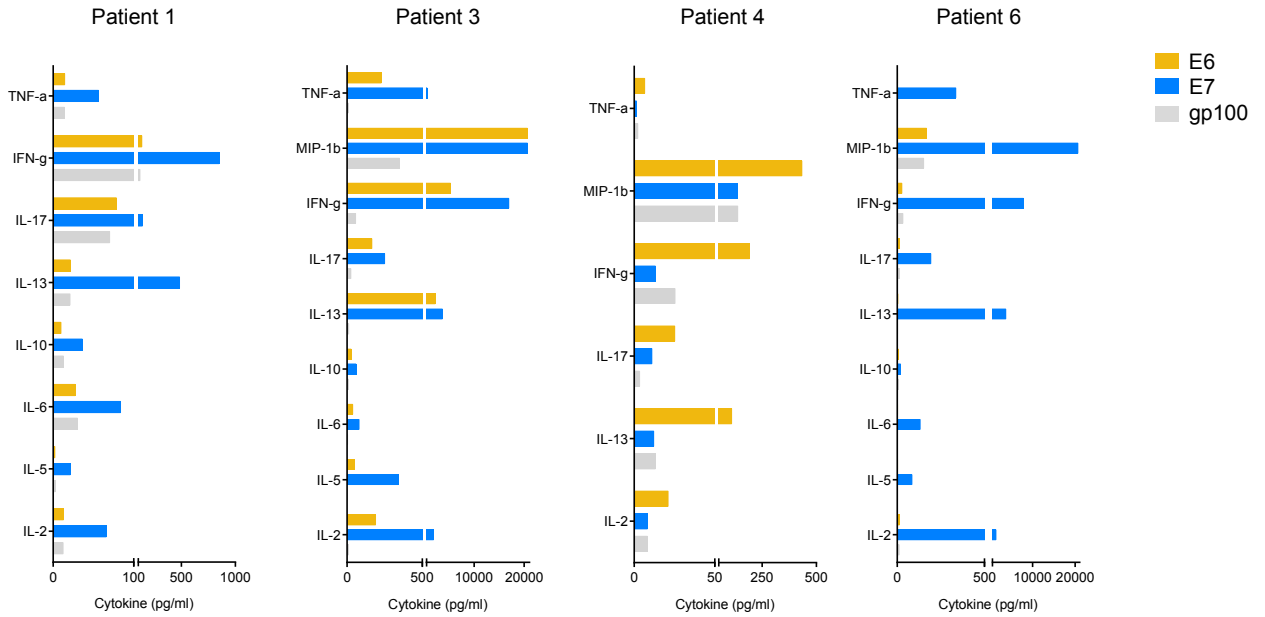


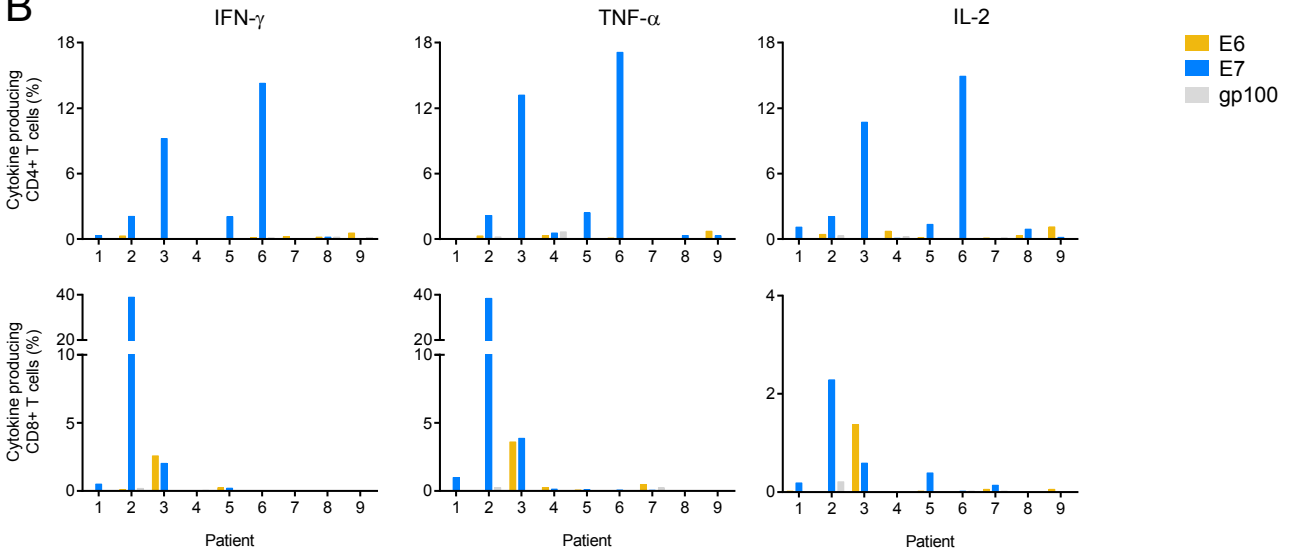
Figure 3: Serum cytokine levels for Patient 3 and Patient 6 following treatment with HPV-TIL. The levels of cytokines in cryopreserved serum were determined as described in Data Supplement. Testing was for the following cytokines: IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, G-CSF, GM-CSF, IFN- γ , MCP-1, MIP-1 β , and TNF- α . Cytokines with levels greater than two-fold baseline on two consecutive measurements are displayed. Cytokines that were administered to patients are in red. Aldesleukin (IL-2) was dosed every eight hours after cell infusion (Patient 3 received two doses and Patient 6 received eight doses). G-CSF was administered daily beginning the day after cell infusion and continued until neutrophil counts recovered (Patient 3 received 11 doses and Patient 6 received nine doses).

Data Supplement, Figure 4

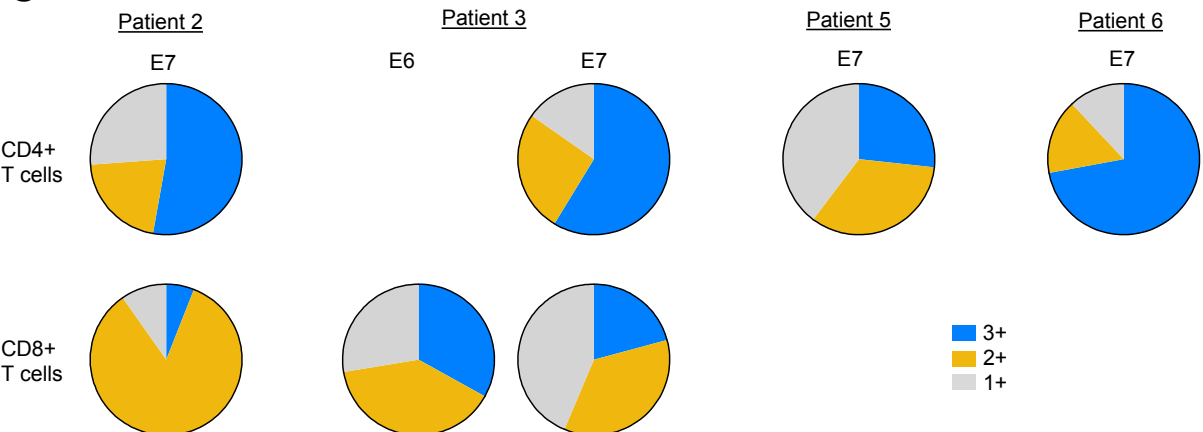
A



B



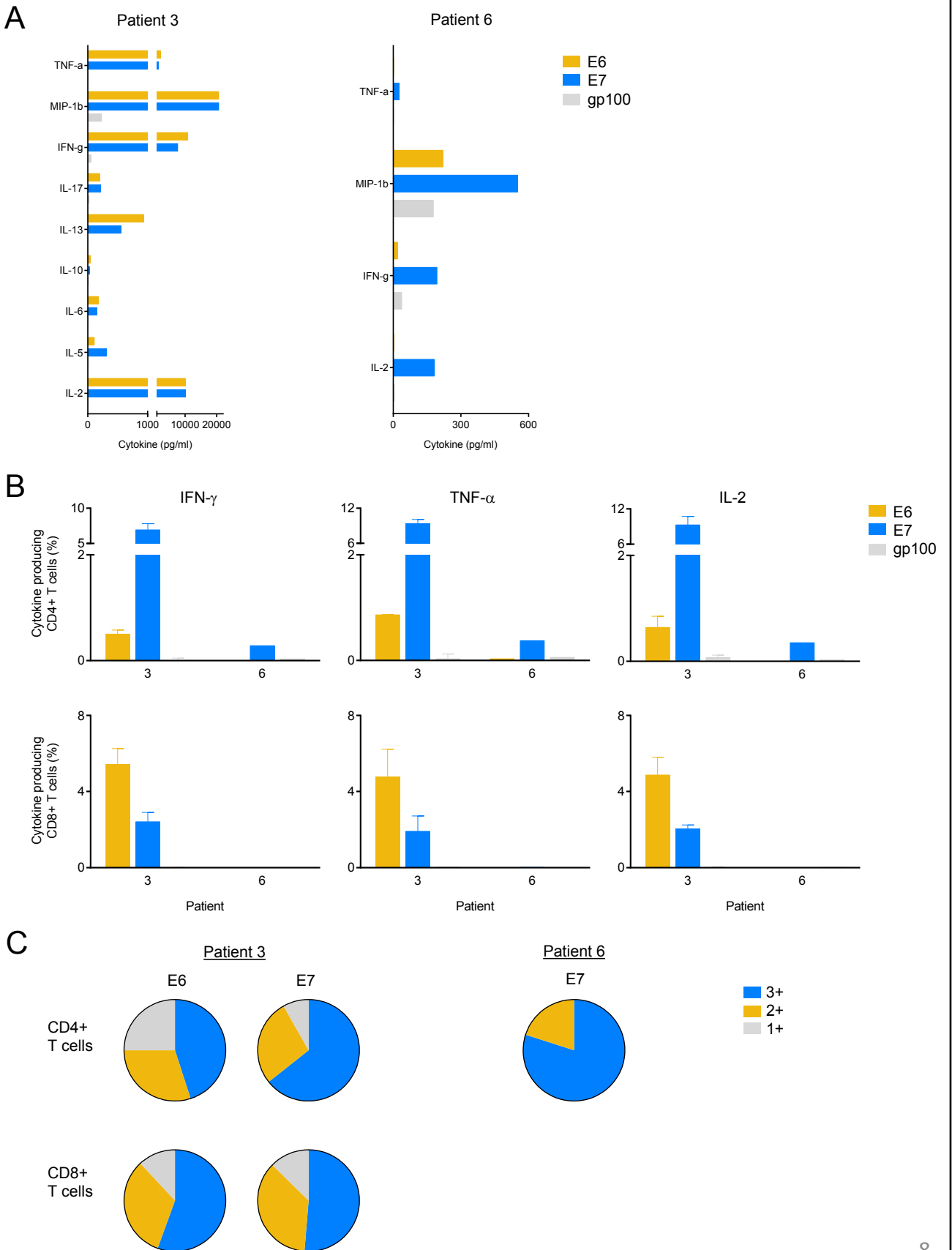
C



Data Supplement, Figure 4

Figure 4: Cytokine production by HPV-reactive T-cells in HPV-TIL infusion products. HPV-TIL infusion products of Patients 1, 3, 4, and 6 were cocultured overnight with autologous dendritic cells loaded with peptide pools spanning the antigen indicated in the figure legends. For Patient 3 and 4, HPV-16+ oncoprotein peptide pools were used, and for Patient 1, 2, 5, 6, 7, 8 and 9 HPV-18+ oncoprotein peptide pools (Table 1). (A) Production of a panel of T-cell associated cytokines (IL-2, IL-5, IL-6, IL-10, IL-13, IL-17, IFN- γ , MIP-1 β , and TNF- α) was determined. Cytokine levels were measured by Bio-Plex Pro Human Cytokine 17-plex assay. Values for cytokines that were two-fold greater in the E6 or E7 sample than in the negative control (gp100) and >20 pg/mL are shown in the graphs. (B) The frequency of HPV-reactive HPV-TIL CD4+ (upper row) and CD8+ (lower row) T-cells that demonstrated intracellular production of IFN- γ , TNF- α , and IL-2 as determined by intracellular cytokine flow cytometry. (C) The proportion of cytokine producing CD4+ (upper) and CD8+ (lower) T cells making the indicated number of cytokines in response to E6 and E7 is shown.

Data Supplement, Figure 5



Data Supplement, Figure 5

Figure 5: Cytokine production by HPV-reactive peripheral blood T-cells approximately one month following treatment with HPV-TIL. CD3⁺ cells from PBMC of Patient 3 and Patient 6 were cocultured overnight with autologous dendritic cells loaded with peptide pools spanning the antigen indicated in the figure legends. For Patient 3, HPV-16+ oncoprotein peptide pools were used, and for Patient 6 HPV-18+ oncoprotein peptide pools (Table 1). (A) Production of a panel of T-cell associated cytokines (IL-2, IL-5, IL-6, IL-10, IL-13, IL-17, IFN- γ , MIP-1 β , and TNF- α) was determined. Cytokine levels were measured by Bio-Plex Pro Human Cytokine 17-plex assay. Values for cytokines that were two-fold greater in the E6 or E7 sample than in the negative control (gp100) and >20 pg/mL are shown in the graphs. (B) The frequency of HPV-reactive peripheral blood CD4⁺ (upper row) and CD8⁺ (lower row) T-cells that demonstrated intracellular production of IFN- γ , TNF- α , and IL-2 as determined by intracellular cytokine flow cytometry. (C) The proportion of cytokine producing CD4⁺ (upper) and CD8⁺ (lower) T cells making the indicated number of cytokines in response to E6 and E7 is shown.

Data Supplement, Table 1

Data Supplement Table 1. HPV Reactivity of Infused HPV-TIL									
Patient No.	IFN- γ ELISPOT*			CD137 upregulation [†]			IFN- γ production [‡]		
	E6	E7	gp100	E6	E7	gp100	E6	E7	gp100
1	1.3	5.5	1.1	2.5	5.9	2.6	59	1087	27
2	0.1	6.8	0.0	1.1	34.6	1.0	30	5841	0
3	5.3	7.9	0.1	7.6	17.3	0.4	9108	23419	0
4	0.3	0.0	0.0	0.3	0.2	0.2	204	20	0
5	0.1	0.6	0.0	3.9	5.2	3.2	81	456	1
6	0.0	4.2	0.0	0.2	11.7	0.2	16	11253	0
7	0.0	0.0	0.0	0.2	0.2	0.2	22	88	0
8	0.1	0.2	0.1	0.9	1.0	0.8	105	189	37
9	1.0	0.2	0.0	2.7	2.0	1.5	463	117	0

* Frequency of IFN- γ -forming T cells (%)

† Frequency of CD137+ T cells (%)

‡ Concentration of IFN- γ (pg/mL)

Data Supplement, Table 2

Data Supplement Table 2: HPV-specific T-cell reactivity in infused TIL and HPV peptides recognized.					
Patient	HPV type	Reactivity	Responding T-cell ¹	Recognized HPV peptides ²	
				CD4+ T cells	CD8+ T cells
1	18	E7	CD4/CD8	E7:13-27, 37-51, 41-55	E7: 49-63
2	18	E7	CD4/CD8	E7:13-27	E7: 13-27, 17-31
3	16	E6/E7	CD4/CD8	E7: 5-19, 9-19, 73-87	E6: 129-143; E7: 73-87
4	16	E6	CD4	#	-
5	18	E7	CD4	#	-
6	18	E7	CD4	E7: 5-19	-
7	18	nd	-	-	-
8	18	nd	-	-	-
9	18	E6	CD4	#	-

¹ Responding T-cells in infused TIL were identified by flow cytometry based on antigen-specific expression of CD137 after co-culture with autologous dendritic cells pulsed with HPV-type specific E6 and E7 antigens.

² Recognized HPV-peptides by infused TIL were identified by flow cytometry based on antigen-specific expression of CD137 and IFN- γ ELISA after co-culture with autologous EBV-LCL pulsed with individual HPV-type specific 15-mer HPV peptides.

#: analysis performed, but recognized HPV peptides could not be determined due to low frequencies of responding T cells.

nd: not detected. Additional details are provided in the Data Supplement.

Data Supplement, Table 3

Data Supplement Table 3. Primer and Probe Sequences for HPV E6 and E7 Gene Expression Assays

HPV-16 E6

Forward primer	GAGAACTGCAATGTTTCAGGACC
Reverse primer	TGTATAGTTGTTTGCAGCTCTGTGC
Probe	CAGGAGCGACCCAGAAAGTTACCACAGTT

HPV-16 E7

Forward primer	CCGGACAGAGCCCATTACAAT
Reverse primer	ACGTGTGTGCTTTGTACGCAC
Probe	TGTTGCAAGTGTGACTCTACGCTTCGGT

HPV-18 E6

Forward primer	CTATAGAGGCCAGTGCCATTTCG
Reverse primer	TTATACTTGTGTTTCTCTGCGTCG
Probe	CAACCGAGCACGACAGGAACGACTCCA

HPV-18 E7

Forward primer	GA CTCAGAGGAAGAAAACGATGAAA
Reverse primer	GTGACGTTGTGGTTCGGCT
Probe	TGGAGTTAATCATCAACATTTACCA