Published OnlineFirst December 5, 2018; DOI: 10.1158/1078-0432.CCR-18-2722

Clinical Trials: Immunotherapy

Clinical Cancer Research

A Phase II Study of Tumor-infiltrating Lymphocyte Therapy for Human Papillomavirus–associated Epithelial Cancers

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Abstract

Purpose: Cellular therapy is an emerging cancer treatment modality, but its application to epithelial cancers has been limited. This clinical trial evaluated tumor-infiltrating lymphocyte (TIL) therapy for the treatment of patients with metastatic human papillomavirus (HPV)-associated carcinomas.

Patients and Methods: The trial was a phase II design with two cohorts, cervical cancers and noncervical cancers. Cell infusion was preceded by a lymphocyte-depleting conditioning regimen and followed by systemic high-dose aldesleukin.

Results: Objective tumor responses occurred in 5 of 18 (28%) patients in the cervical cancer cohort and 2 of 11 (18%) patients in the noncervical cancer cohort. Two of the responses in cervical cancer were complete and are ongoing 67 and 53 months after treatment. Responses in the noncervical cancer cohort were in anal cancer and oropharyngeal cancer. The HPV reactivity of the infused T cells correlated with clinical response. Peripheral blood repopulation with HPV-reactive T cells also correlated with clinical response.

Conclusions: These findings support the concept that cellular therapy can mediate the regression of epithelial cancers, and they suggest the importance of predictive biomarkers and novel treatment platforms for more effective therapies.

Introduction

Human papillomavirus (HPV)-associated cancers are common epithelial malignancies that account for approximately 5% of all cancers worldwide (1). They occur at varied anatomic sites including the uterine cervix, anus, vagina, vulva, penis, and oropharynx (2–5). Although it is hoped that this family of cancers will be eliminated by preventive HPV vaccines in the future, they currently cause more than 300,000 deaths each year globally and an estimated 12,500 deaths each year in the United States (6, 7). Advanced-stage HPV-associated cancers are difficult to treat. Combination chemotherapy plus bevacizumab offers some clinical benefit (8), and anti-programmed death 1 receptor (PD-1) therapy has shown clinical activity (9–12), but these malignancies generally are incurable and better treatments are needed.

Adoptive T-cell therapy (ACT), the systemic infusion of therapeutic T cells, is an emerging cancer treatment modality that can induce complete tumor responses in some patients with B-cell malignancies or metastatic melanoma (13). We sought to test whether ACT could mediate the regression of HPV-associated epithelial cancers. We established a method to generate independent tumor-infiltrating lymphocyte (TIL) cultures from fragments of a resected metastatic tumor deposit (14). Because HPV-associated cancers constitutively express the HPV E6 and E7 oncoproteins, immunologically foreign viral proteins that are attractive targets for immunotherapy (3) and cultures with HPV-oncoprotein reactivity were selected preferentially for administration to patients. We have previously reported an in-depth study of the landscape of target antigens in two patients who experienced complete responses on this clinical trial (15). TILs administered to each patient targeted HPV antigens. However, the predominant target antigens were a cancer germline antigen in one patient and mutant neoantigens in another patient. Here, we present a completed clinical trial with long-term follow-up: 18 patients with metastatic cervical cancer (including 9 patients reported previously; ref. 14) and 11 patients with other cancers. In addition, we also report a predictive biomarker and immunologic correlates for the clinical trial.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-18-2722

Published OnlineFirst December 5, 2018; DOI: 10.1158/1078-0432.CCR-18-2722

Translational Relevance

Here, we report that cellular therapy can mediate the regression of HPV-associated cervical cancer, oropharyngeal cancer, and anal cancer, including durable, complete regression of cervical cancer. The findings support expanded research to discover and develop cellular therapy treatments for epithelial cancers.

Patients and Methods

Study design

The trial was a single-center, phase II study (ClinicalTrials.gov NCT01585428) that was designed to determine whether HPV-TILs could mediate regression of advanced HPV-associated cancers. Patients were treated in two disease cohorts (cervical cancers and noncervical cancers) between April 24, 2012 and August 1, 2016. The protocol was approved by the NCI Institutional Review Board at the NIH Clinical Center, and informed consent was obtained from all patients. Patients were treated with a nonmyeloablative chemotherapy conditioning regimen (cyclophosphamide 60 mg/kg i.v. daily for 2 days and fludarabine 25 mg/m^2 daily for 5 days), followed by a single intravenous infusion of HPV-TILs. After cell infusion, aldesleukin was administered as an intravenous bolus at 720,000 IU/kg/dose every 8 hours to tolerance or a maximum of 15 doses. Tumor responses were determined using RECIST version 1.0. The primary objective of the study was to determine the objective tumor response rate and duration in patients with metastatic HPV-associated cancers. The secondary objectives were to determine the toxicity of this treatment regimen and to study immunologic correlates associated with this therapy.

Patients

Patients ≥18–70 years of age with a pathologically confirmed diagnosis of metastatic HPV-associated cancer were eligible for screening and metastectomy for generation of TILs. All patients had received prior platinum-based chemotherapy or chemoradiotherapy. Patients with three or fewer brain metastases that were less than 1 cm in diameter and asymptomatic were permitted to participate. An Eastern Cooperative Oncology Group performance status of 0 or 1 was required.

Generation of HPV-TIL cell products

The HPV type of metastatic tumors was determined by RT-PCR with HPV16 and HPV18 type–specific primer-probe sets. TIL cultures were initiated from tumor fragments and expanded using IL2-containing culture media as described previously (14). Briefly, cultures with lymphocyte outgrowth were tested for reactivity against HPV16 or HPV18 E6 and E7, when applicable. Flow cytometric analysis of each culture was performed using antibodies specific for CD3, CD4, CD8, and CD56 (BD Biosciences). TIL cultures were selected for further expansion and administration to the patient based on HPV-oncoprotein reactivity, rapid growth rate, high T-cell frequency, and high CD8$^+$ T-cell frequency. If HPV reactivity was absent or could not be assessed, the other criteria were used. After treatment of 22 patients (14 cervical cancer and 8 noncervical cancer), the protocol was amended to only treat patients whose tumors were HPV16$^+$ or HPV18$^+$, and who also had three or more TIL cultures with HPV-oncoprotein reactivity (as measured by IFNγ release). The rationale for this amendment was based on the observed correlation between HPV-reactivity and clinical response in the first nine patients with cervical cancer (14). The criteria for positive reactivity was defined as IFNγ release of at least two-fold background and greater than 200 pg/mL (14).

Immunologic and phenotypic analyses

Immunologic assays were performed as described previously (14). Briefly, T cells were cocultured with autologous immature dendritic cells loaded with 1 μmol/L peptide pools (15-mer peptides overlapping by 11 amino acids) spanning E6, E7, or glycoprotein 100 (gp100, negative control; Miltenyi Biotec. For peripheral blood T-cell assays, CD3$^+$ T cells were isolated using pan T-cell Isolation Kit (Miltenyi Biotec) prior to coculture. Dendritic cells were generated from the adherent fraction of peripheral blood mononuclear cells (PBMC) or from CD14$^+$ cells isolated from PBMCs with CD14 magnetic beads (Miltenyi Biotec). For IFNγ production assays, the concentration of IFNγ in the supernatants was determined by ELISA (R&D Systems, Bio-Techne Corp, or Thermo Fisher Scientific). IFNγ enzyme–linked immunospot (ELISpot; Mabtech) assays were performed according to the manufacturer's instructions. CD137 upregulation assays were performed by flow cytometric analysis. After overnight coculture, cells were stained with fluorescent antibodies against CD137, CD3, CD4, and CD8 (BD Biosciences and BioLegend) and counterstained with propidium iodide or 4′,6-diamidino-2-phenylindole (BD Biosciences). Phenotypic analysis of infused TILs was performed using fluorescent antibodies against CD3 (Biolegend), CD4 (BD Biosciences), CD8 (Biolegend), CCR7 (Biolegend), CD45RA (Biolegend), PD-1 (Biolegend), CD27 (Biolegend), LAG-3 (Enzo Life Sciences, Farmingdale, NY) and TIM-3 (R&D Systems) in conjunction with live/dead fixable staining (BD Biosciences). Data were acquired with a FACSCanto II Flow Cytometer (BD Biosciences) or Novocyte (Acea BioSciences Inc) and analyzed with FlowJo Software (Treestar Inc).

Statistical considerations

For each of the two cohorts, the trial used a two-stage optimal design with alpha = 0.05 (5% probability of accepting a poor therapy) and beta = 0.10 (10% probability of rejecting a good therapy). Initial enrollment was planned at 18 patients per cohort. Expansion to 35 patients was planned if three or more of the first 18 patients experienced responses of longer than 4-month duration (this measure was intended to consider both response rate and durability). If fewer than 6 of 35 patients in a cohort had a clinical response, then the treatment was to be considered inadequate for further investigation. Under the null hypothesis (10% response rate), the probability of early termination was 73%. Accrual to the noncervical cohort was terminated because of limitations in cell manufacturing.

The Mann–Whitney *U* test was used to test for correlations between HPV reactivity and clinical responses (GraphPad Prism 7). Reported *P* values are two tailed and not adjusted for multiple comparisons, and $P < 0.05$ was considered statistically significant.

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Table 1. Characteristics of patients and administered T cells

Patient	Age (years)/gender	Histology	HPV type	Sites of disease	Prior systemic treatment	Cells ($\times 10^9$)	Within CD3$^+$ (%)		No. of IL2 doses	Response	
							CD4$^+$	CD8$^+$		Type	Duration or TTP (months)
Cervical cancer											
1	30/F	ASC	18	Iliac lymph nodes, lung, lung hilum, retroperitoneum, vaginal cuff	Cisplatin	101.4	29	72	7	PD	1
2	53/F	SCC	18	Bone, liver, lung, lung hilum, mediastinum, pelvis	Cisplatin, carboplatin, paclitaxel, topotecan, ixabepilone, dimethane sulfonate	126.0	10	90	3	PR	3
3	36/F	SCC	16	Iliac lymph nodes, lung hilum, mediastinum, retroperitoneum	Cisplatin, vincristine, bleomycin, gemcitabine, paclitaxel, topotecan	152.0	21	83	2	CR	67+
4	55/F	SCC	16	Axilla, breast, liver, omentum, pleura, soft tissue	Cisplatin, carboplatin, paclitaxel, fluorouracil, irinotecan, dovitinib, pemetrexed	80.1	23	76	7	PD	2
5	44/F	SCC	18	Brain, mediastinum, supraclavicular nodes	Cisplatin	90.0	66	29	5	PD	2
6	36/F	AC	18	Abdominal wall, liver surface, paracolic, pelvis, retroperitoneum	Cisplatin	74.7	14	86	8	CR	53+
7	59/F	AC	18	Abdominal wall, lung	Cisplatin, paclitaxel, carboplatin, bevacizumab	33.4	36	58	8	PD	1
8	31/F	ASC	18	Pelvis, perihepatic mass	Cisplatin, paclitaxel	46.1	64	29	9	PD	2
9	37/F	AC	18	Axilla, bone, lung, mediastinum, pelvis, retroperitoneum	Cisplatin, carboplatin, paclitaxel, ipilimumab	70.2	33	59	6	PD	1
10	39/F	SCC	not 16/18	Adrenal, retroperitoneum	Cisplatin, paclitaxel, bevacizumab	100.0	8	92	5	PD	2
11	31/F	SCC	16	Cervix, iliac lymph nodes, retroperitoneum	Carboplatin, paclitaxel, bevacizumab	77.0	57	41	1	PD	2
12	48/F	SCC	16	Bone, inguinal lymph nodes, lung, mediastinum, retroperitoneum	Cisplatin, paclitaxel, bevacizumab, listeria-based vaccine trial	70.6	93	4	0	PR	3
13	30/F	SCC	18	Cervix, inguinal lymph nodes, lung, mediastinum	Cisplatin, brachytherapy	101.3	67	27	3	PD	3
14	49/F	SCC	not 16/18	Gastro-esophageal junction, mediastinum, retroperitoneum	Cisplatin, carboplatin, paclitaxel, topotecan, bevacizumab	68.8	53	41	2	PD	2
15	61/F	AC	16	Bone, inguinal lymph nodes, lung	Carboplatin, docetaxel, cisplatin, topotecan, ifosfamide, adriamycin, etoposide	73.9	84	16	1	PD	3
16	51/F	SCC	18	Liver, pelvic, peripancreatic, spleen	Cisplatin, gemcitabine, carboplatin, paclitaxel, bevacizumab	115	71	29	0	PD	2
17	63/F	SCC	18	Lung, lung hilum	Cisplatin, carboplatin, paclitaxel, bevacizumab	112.0	78	22	4	PD	5
18	35/F	NE	18	Lung, lung hilum, liver	Cisplatin, etoposide, topotecan, paclitaxel, bevacizumab	9.0	67	34	3	PR	3
Noncervical cancer											
19	55/M	Tonsillar SCC	16	Axilla, lung, subcutaneous tissue	Cisplatin, fluorouracil, taxotere, carboplatin, cetuximab	89.1	96	2	1	PD	2
20	60/M	Head/neck SCC	16	Axilla, bone, liver, peripancreatic, periportal lymph node, pleura	Cisplatin, capecitabine, carboplatin	150.0	29	64	6	PD	2
21	58/F	Anal SCC	16	Lung, mediastinum, pleura	Cisplatin, fluorouracil, carboplatin, paclitaxel, cetuximab, irinotecan	31.5	47	50	2	PD	3
22	50/F	Anal SCC	16	Mediastinum, retroperitoneum	Cisplatin, fluorouracil, mitomycin C, paclitaxel, carboplatin	69.0	75	16	5	PD	8

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Table 1. Characteristics of patients and administered T cells (Cont'd)

Patient	Age (years)/gender	Histology	HPV type	Sites of disease	Prior systemic treatment	Cells ($\times 10^9$)	Within $CD3^+$ (%)		No. of IL2 doses	Response	
							$CD4^+$	$CD8^+$		Type	Duration or TTP (months)
23	58/F	Anal SCC	16	Iliac lymph nodes, liver, retroperitoneum	Cisplatin, fluorouracil, mitomycin C	47.5	86	9	1	PD	2
24	60/M	Tonsillar SCC	16	Lung, mediastinum	Cisplatin, docetaxel, bevacizumab, cetuximab, fluorouracil, gemcitabine	130.9	41	51	3	PR	5
25	49/F	Anal SCC	16	Pleura	Cisplatin, fluorouracil, rigosertib, capecitabine	133.0	67	20	5	PD	2
26	48/F	Anal SCC	16	Lung	Cisplatin, fluorouracil, mitomycin C, capecitabine	18.4	47	50	5	PR	4
27	52/M	Head/neck SCC	16	Axilla, chest wall, lung hilum, mediastinum, subcutaneous tissue	Cisplatin, fluorouracil, taxotere	125.0	97	3	5	PD	2
28	60/M	Head/neck SCC	16	Lung, lung hilum, mediastinum, para-aortic lymph node, pleura	Cisplatin, carboplatin, fluorouracil, cetuximab, pembrolizumab	102.0	6	94	4	PD	3
29	56/F	Vaginal AC	16	Lung, lung hilum, scapula, para-spinal	Cisplatin, paclitaxel, carboplatin, pemetrexed	107.0	46	55	5	PD	4

Abbreviations: AC, adenocarcinoma; ASC, adenosquamous cell carcinoma; F, female; M, male; NE, neuroendocrine; SCC, squamous cell carcinoma; TTP, time to progression.

Additional details on the Patients and Methods are presented in the Supplementary Data.

Results

Patient characteristics

Twenty-nine patients with metastatic HPV-associated cancer were treated (Table 1 and Supplementary Fig. S1, online only). Eighteen patients were diagnosed with cervical cancer. Eleven patients were diagnosed with other HPV^+ cancers (oropharyngeal cancer ($n = 5$), anal cancer ($n = 5$), and vaginal cancer ($n = 1$)). The median cell dose was 89×10^9 (range, $9–152 \times 10^9$). The median number of systemic IL2 doses was 4 (range 0–9). Infusion products consisted of a median of 53% $CD4^+$ (range, 6%–97%); and 41% $CD8^+$ (range, 2%–94%) T cells. The median age of patients was 50 (range, 30–63 years). Tumors were squamous cell carcinoma ($n = 21$), adenocarcinoma ($n = 5$), adenosquamous carcinoma ($n = 2$), or neuroendocrine ($n = 1$). Cervical cancers were associated with HPV type 18 ($n = 11$), type 16 ($n = 5$), or other types ($n = 2$). Noncervical cancers were associated with HPV16 ($n = 11$). Twenty-five patients had previously received combination chemotherapy regimens. Two patients had also previously received immune checkpoint blockade directed against cytotoxic T-lymphocyte-associated protein 4 or PD-1.

Clinical responses

Seven of 29 patients attained objective tumor responses, 5 of 18 (28%) patients with cervical cancer, and 2 of 11 (18%) patients with other HPV^+ cancers (Table 1; Fig. 1A). In the cervical cancer cohort, two responses were complete (CR) and are ongoing 67 (patient 3) and 53 (patient 6) months after treatment (Fig. 1B); three responses were partial (PR) and of 3-month duration (patients 2, 12, and 18; Table 1; Fig. 1A and B). In the noncervical cancer cohort, 1 patient with oropharyngeal cancer (patient 24) attained a PR of 5-month duration, and 1 patient with anal cancer (patient 26) attained a PR of 4-month duration (Table 1; Fig. 1A and B). The patient with oropharyngeal cancer was a 60-year-old male who had previously received five systemic anticancer agents (Table 1). At the time of treatment, he had multiple thoracic metastases that were progressing prior to TIL infusion (Fig. 1C). Five months after treatment, he developed a new brain metastasis, which was resected (Table 1; Fig. 1B). However, he experienced complete regression of all other disease sites (Fig. 1B and C) and is without evidence of disease 51 months after treatment (Fig. 1B and C). The patient with anal cancer was a 48-year-old female who had previously received two combination chemotherapy regimens (Table 1). At the time of treatment with HPV-TILs, she had progressing cancer involving both lungs (Table 1; Fig. 1D). Following treatment, she experienced a PR that lasted 4 months (Table 1; Fig. 1B and D).

Adverse events

There were no acute infusion-related toxicities and no autoimmune adverse events. The toxicity profile was consistent with the chemotherapy conditioning regimen and aldesleukin. The most common severe adverse events were the expected hematologic toxicities of the conditioning regimen. Grade 3 and 4 adverse events are summarized in Table 2. No treatment-related mortality occurred.

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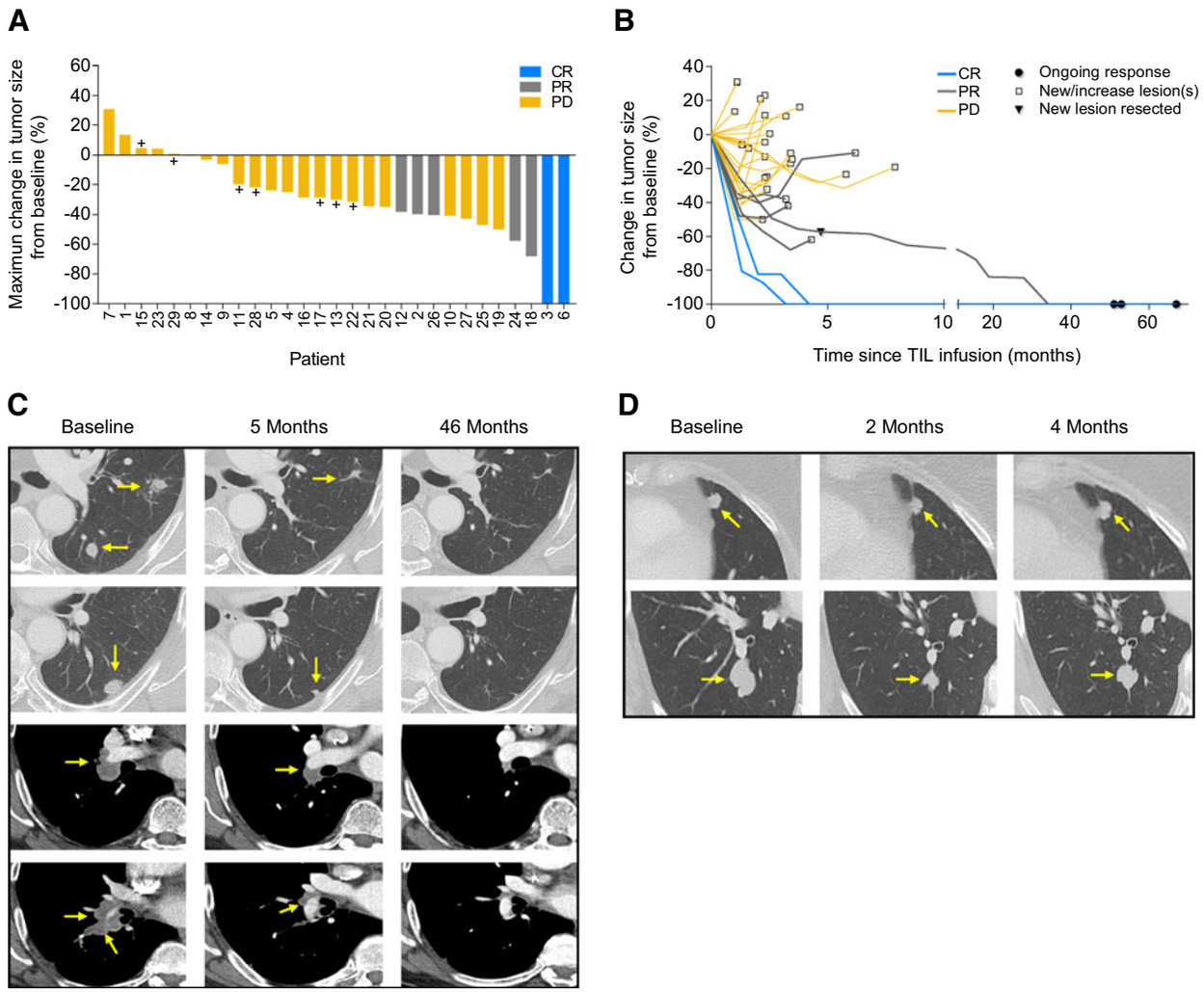

Figure 1.

Clinical responses in patients after HPV-TIL therapy. **A,** Waterfall plot of the maximum change in the sum of target lesions, as compared with baseline measurements, in 29 patients. CR, complete response; PR, partial response; PD, progressive disease; +, stable disease. **B,** Spider plots of the change in the sum of target lesions from pretreatment baseline in 29 patients. Black circles (●) indicate ongoing responses; open squares (□) indicate PD due to either a new lesion(s) or increasing target or nontarget lesion(s). Black triangle (▼) indicates PD in patient 24 with oropharyngeal cancer due to development of a new brain lesion after a PR of 5 months in duration. **C** and **D,** Contrast-enhanced CT scans obtained at baseline and after treatment for patient 24 with oropharyngeal cancer (**C**) and patient 26 with anal cancer (**D**). Tumors are marked by yellow arrows. Patient 24 had disease involving his left lung (first and second row) and mediastinum (third and fourth row). He experienced a PR of 5 months in duration due to a new brain lesion that was surgically excised. He was followed off-protocol, and his target lesions continued to regress, and there was no evidence of disease at most recent follow-up 51 months after treatment. Patient 26 had disease involving both lungs (first and second row). She experienced a PR of 4 months in duration due to increase in her target lesions.

Immunologic correlates of response

Post hoc exploratory analyses were performed to test for immunologic correlates of response. The frequency of infused T cells that responded to HPV E6 and E7 peptide stimulation was determined by flow cytometric measurement of the T-cell activation marker CD137 (Fig. 2A). Cytokine production by infused T cells that responded to HPV E6 and E7 peptide stimulation was measured by IFNγ production assays (Fig. 2B). HPV-TILs displayed greater frequencies of HPV-reactive T cells ($P = 0.0091$) and a higher concentration of HPV-specific IFNγ release ($P = 0.0026$) administered to responding versus nonresponding patients (Supplementary Fig. S2, online only). No differences were detected between responding versus nonresponding patients in the number of administered total T cells ($P = 0.9999$), $CD4^+$ T cells ($P = 0.1097$), $CD8^+$ T cells ($P = 0.7086$), or IL2 doses ($P = 0.4725$; Supplementary Fig. S3, online only). The frequency of HPV-reactive T cells in the peripheral blood before and following treatment was assessed by IFNγ ELISpot assay. Before treatment, minimal, if any, T-cell HPV reactivity was detected (Fig. 2C). One month after treatment, 8 of 22 patients (all patients for whom samples were available) showed HPV reactivity (Fig. 2C). The frequency of HPV-reactive T cells in peripheral blood 1 month after treatment correlated positively with

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Table 2. Adverse events (grades 3 and 4)

Adverse event	No. of patients (#)
Lymphopenia	29
Neutropenia	29
Thrombocytopenia	29
Anemia	25
Infection[a]	17
Febrile neutropenia	12
Metabolic disorders	12
Hypoxia	8
Nausea/vomiting	6
Dyspnea	4
Diarrhea	3
Fatigue	3
Hypotension	3
Cystitis	2
Hemorrhage[b]	2
Oliguria	2
Renal failure[c]	2
Syncope	2
Ureteral obstruction[d]	2
Dysphagia	1
Confusion	1

[a]Includes positive surveillance blood cultures.
[b]Associated with radiation colitis in 1 patient and with an *in situ* cervical carcinoma in 1 patient.
[c]Associated with progressing pelvic tumor in 1 patient.
[d]Associated with progressing pelvic tumors in 2 patients.

clinical response ($P = 0.0135$, Supplementary Fig. S4, online only). The vast majority of HPV-TILs were within the T effector memory population, as defined by lack of CD45RA and CCR7 surface expression (Supplementary Fig. S5, online only). Low percentages of HPV-TILs expressed CD27 while the majority displayed expression of PD-1, TIM-3 and LAG-3 with no noteworthy differences between responding versus non-responding patients (Supplementary Fig. S5, online only).

No differences in the HPV reactivity of the infused T cells were detected between patients with cervical and noncervical cancer as measured by either immunologic assay ($P = 0.9515$ for flow cytometry; $P = 0.7627$ for IFNγ production; Supplementary Fig. S6, online only). No significant differences were detected between patients with cervical and noncervical cancer in the number of administered total T cells ($P = 0.5208$), $CD4^+$ T cells ($P = 0.3869$), $CD8^+$ T cells ($P = 0.3397$), or IL2 doses ($P = 0.8852$; Supplementary Fig. S7, online only).

Discussion

Cellular therapy is effective for the treatment of certain B-cell malignancies, but its study for the treatment of epithelial cancers has been limited (13). Here, we report the treatment of patients with metastatic HPV-associated squamous cell carcinomas and adenocarcinomas with adoptive transfer of tumor-infiltrating T

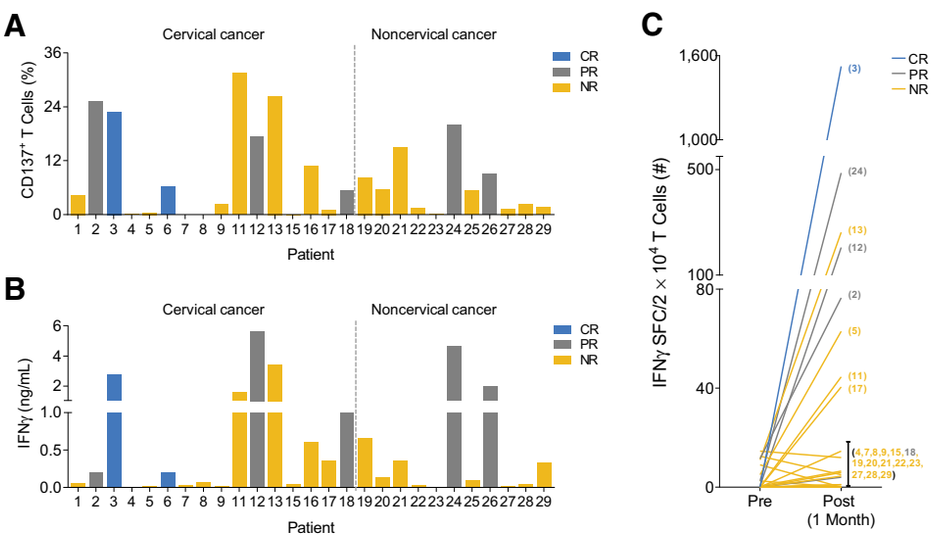

Figure 2.
HPV reactivity of the infused T cells and peripheral blood T cells after infusion. **A** and **B,** Infused HPV-TILs to 27 patients with cervical and noncervical cancer with $HPV16^+$ or $HPV18^+$ tumors were assessed for reactivity against HPV type–specific E6 and E7 oncoproteins using CD137 upregulation by flow cytometry (**A**), and (IFNγ) production assays (**B**). The HPV-type of each patient's tumor is provided in Table 1. Patients 10 and 14 had non-$HPV16^+/18^+$ tumors and were not included in this analysis. Values shown represent sum of HPV-type–specific E6 and E7 reactivity after background subtraction (gp100). CD137 upregulation is depicted for $CD3^+$ T cells. Data are representative of two independent experiments for patients 11 to 13 and 15 to 29, and one experiment for patients 1 to 9 due to unavailability of samples. **C,** Peripheral blood (PB) T cells from before and 1 month after treatment were assessed by IFNγ ELISA for reactivity against HPV-type–specific E6 and E7 oncoproteins in 22 patients (5 responding and 17 nonresponding patients). Patient numbers are indicated in the parentheses. Patients 1, 6, 16, 25, and 26 did not have available samples for this analysis. Patients 10 and 14 had non-$HPV16^+/18^+$ tumors and were not included in this analysis. Values shown represent sum of HPV-type–specific E6 and E7 spot-forming cells (SFC) after background subtraction (gp100). Eight of 22 patients had discernable HPV reactivity (>20 SFC after background subtraction). Data are representative of two independent experiments for patients 11–13, 15, 17–24, and 27–29, and one experiment for patients 2 to 5 and 7 to 9 due to unavailability of samples. CR, responding patient with a complete response; PR, responding patients with a partial response; NR, nonresponding patient.

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cells. Tumor responses occurred in patients with cervical cancer, anal cancer, and oropharyngeal cancer. Responses in 2 patients with cervical cancer were complete and are ongoing 67 and 53 months after treatment.

HPV-associated cancers are typical epithelial malignancies that are difficult to treat when metastatic. Chemotherapy generally consists of combinations of cytotoxic agents, often administered in conjunction with a biological agent (3, 16, 17). These regimens have limited clinical activity and substantial toxicity, and better treatments are needed. Immunotherapy works through different mechanisms than chemotherapy and has been a breakthrough for the treatment of certain malignancies (18). Although targeting HPV oncoproteins with antigen-specific immunotherapy using therapeutic vaccines has thus far been ineffective for metastatic disease, immunotherapy with PD-1–targeted agents has shown clinical activity in cervical (19), anal (10), and oropharyngeal (9, 11, 12, 20) cancer.

This trial represents the first step in the development of cellular immunotherapy for HPV-associated cancers and possibly other epithelial malignancies. It demonstrates the feasibility of the approach and provides evidence of clinical activity including durable, complete tumor regression in some patients. However, this clinical trial has important limitations. The response rate is not well defined because of the small sample size. Although CRs occurred, they were infrequent, and most patients did not appear to benefit from the therapy. In addition, the treatment requires surgery for procurement of a tumor from which to generate TILs, and the generation of high numbers of TILs takes approximately 4 to 6 weeks. In this trial, 45 patients underwent surgery and 29 patients received treatment (Supplementary Fig. S1, online only). Another limitation is the patient-to-patient variability in the HPV reactivity of TILs and the infused cell product. Consistent with reports by others (21), we found that TILs from a number of patients possessed no HPV reactivity or low HPV reactivity (Fig. 2A and B, Supplementary Fig. S1, online only). In the administered T cells, the frequency of HPV reactivity ranged from $\leq$0.1% to 31%, median 5% (Fig. 2A) and the magnitude of IFNγ release ranged from $\leq$0.1 to 5.6 ng/mL, median 0.2 ng/mL (Fig. 2B). One strategy to circumvent surgery and generate a more consistent HPV oncoprotein–targeted cell product may be to administer HPV-specific T cells that are propagated and enriched *ex vivo* from peripheral blood (22). Another strategy may be to administer peripheral blood T cells that are genetically engineered *ex vivo* to target an HPV oncoprotein with a T-cell receptor (13, 23–25). We are presently testing this strategy in an active clinical trial with gene-engineered T cells that target HPV16 E7 (NCT02858310).

HPV-TIL therapy is a personalized treatment in which the characteristics of the adoptively transferred T cells themselves may serve as a mechanism-driven biomarker to predict treatment response and guide patient selection. Consistent with previous findings (14), the magnitude of HPV reactivity of the infused TILs was associated with clinical response. These findings may provide a biomarker of clinical response; however, they do not demonstrate a causal relationship between the targeting of HPV antigens and tumor regression. Indeed, the oncoprotein-reactive T cells represented a relatively small fraction of the infused polyclonal TILs (Fig. 2A, range $\leq$0.1%–31%, median 5%). Furthermore, HPV-associated cancers also harbor somatic gene mutations (mutated neoantigens) and epigenetically dysregulated genes (cancer germline antigens) that may be targeted by the TILs. Indeed, a global landscape analysis of tumor antigen targeting by TILs administered to patients with cervical cancer who had CRs revealed subdominant targeting of HPV antigens but immunodominant targeting of nonviral tumor antigens (15). For patient 3, the primary antigens targeted were mutated neoantigens, and for patient 6 the primary antigen targeted was a cancer germline antigen (15). Thus, the predominant antigens targeted by T cells in HPV^+ cancers may be nonviral.

The grade 3 and grade 4 hematologic toxicities reported in this protocol were an expected consequence of the lymphodepleting conditioning regimen, which consisted of a single cycle of cyclophosphamide and fludarabine. It is uncertain whether the conditioning regimen may have direct antitumor activity in the chemotherapy- refractory cancers treated on this trial. Although cyclophosphamide is not used in the treatment of advanced HPV-associated cancers, its analogue ifosfamide has weak activity in platinum-experienced cervical cancer (response rate of 11%, 1.8–3.1 months duration; ref. 26) and in chemotherapy-experienced head and neck cancer (response rate of 10%; ref. 27). Fludarabine has no clinical activity in cervical cancer (28) or head and neck cancer (29).

Targeting of the oncogenic drivers of cancer with cellular immunotherapy is an attractive treatment strategy (13). HPV-associated cancers are driven by the E6 and E7 oncoproteins, and provide a potential model in which to explore this approach. A clinical trial of T cells genetically engineered with a T-cell receptor that targets the E6 antigen has shown some clinical activity (24). A clinical trial with a higher avidity T-cell receptor that targets the E7 antigen is actively accruing patients (NCT02858310; ref. 25).

In summary, the present study reports a substantial experience with cellular therapy in epithelial cancers. The overall response rate of 24% was modest and may reflect in part the manifold mechanisms of tumor resistance to immunotherapy that are emerging from other studies (30). However, the results support proof-of-principle that ACT can mediate regression of epithelial cancers, including durable complete regression that may be curative for some patients.

Disclosure of Potential Conflicts of Interest

C.A. Klebanoff reports receiving commercial research grants from Gilead, holds ownership interest (including patents) in Gilead, Celgene, Bristol-Myers Squibb, and CVS, and is a consultant/advisory board member for Kite Pharma, Klus Pharma, Bristol-Myers Squibb, RXI, Aleta Biotherapeutics, and G1 Therapeutics. C.S. Hinrichs reports receiving commercial research grants from Kite Pharma. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

We thank the Surgery Branch immunotherapy clinical fellows and nurses for their care of the patients, and the post baccalaureate students and research biologists for experimental assistance. Research support for this study was provided by the Intramural Research Program of the NCI of the NIH.

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Received August 20, 2018; revised October 15, 2018; accepted November 30, 2018; published first December 5, 2018.

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Published OnlineFirst December 5, 2018; DOI: 10.1158/1078-0432.CCR-18-2722

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Clin Cancer Res 2019;25:1486-1493. Published OnlineFirst December 5, 2018.