original report

T-Cell Receptor Gene Therapy for Human Papillomavirus–Associated Epithelial Cancers: A First-in-Human, Phase I/II Study

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abstract

PURPOSE Genetically engineered T-cell therapy is an emerging treatment of hematologic cancers with potential utility in epithelial cancers. We investigated T-cell therapy for the treatment of metastatic human papillomavirus (HPV)–associated epithelial cancers.

METHODS This phase I/II, single-center trial enrolled patients with metastatic HPV16-positive cancer from any primary tumor site who had received prior platinum-based therapy. Treatment consisted of autologous genetically engineered T cells expressing a T-cell receptor directed against HPV16 E6 (E6 T-cell receptor T cells), a conditioning regimen, and systemic aldesleukin.

RESULTS Twelve patients were treated in the study. No dose-limiting toxicities were observed in the phase I portion. Two patients, both in the highest-dose cohort, experienced objective tumor responses. A patient with three lung metastases experienced complete regression of one tumor and partial regression of two tumors, which were subsequently resected; she has no evidence of disease 3 years after treatment. All patients demonstrated high levels of peripheral blood engraftment with E6 T-cell receptor T cells 1 month after treatment (median, 30%; range, 4% to 53%). One patient's resistant tumor demonstrated a frameshift deletion in interferon gamma receptor 1, which mediates response to interferon gamma, an essential molecule for T-cell–mediated antitumor activity. Another patient's resistant tumor demonstrated loss of *HLA-A*02:01*, the antigen presentation molecule required for this therapy. A tumor from a patient who responded to treatment did not demonstrate genetic defects in interferon gamma response or antigen presentation.

CONCLUSION Engineered T cells can induce regression of epithelial cancer. Tumor resistance was observed in the context of T-cell programmed death-1 expression and defects in interferon gamma and antigen presentation pathway components. These findings have important implications for development of cellular therapy in epithelial cancers.

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INTRODUCTION

Genetically engineered T-cell therapy is emerging as an effective cancer treatment.[1-3] Anti-CD19 chimeric antigen receptor T cells were recently approved by the US Food and Drug Administration for the treatment of certain lymphomas and acute lymphoblastic leukemia. In solid tumors, engineered T-cell receptor (TCR) T cells directed against cancer/testis antigen 1 have clinical activity in melanoma and synovial cell sarcoma.[4] However, data to support this approach in epithelial cancers, which are the most common human malignancies, are limited.[5] Previous exploration of mechanisms of response and resistance to genetically engineered T-cell therapy in epithelial cancers has been confounded by severe toxicities to healthy tissues[6-9] and inconsistent target antigen expression.[10]

Human papillomavirus (HPV)–associated epithelial cancers include squamous cell carcinomas (SCC) and adenocarcinomas of the cervix, oropharynx, anus, vulva, vagina, and penis.[11-14] Advanced HPV-associated cancers are generally incurable and resistant to chemotherapy.[12,15,16] These cancers express the E6 and E7 oncoproteins, which are viral antigens that drive malignancy and are absent from healthy tissues,[12] making them attractive targets to study genetically engineered T-cell therapy in epithelial cancer.[5]

We identified a high-avidity TCR directed against an epitope of HPV16 E6 ($E6_{29-38}$) that is presented by HLA-A*02:01 (E6 TCR).[17] The TCR was identified from a human T cell; complementarity-determining regions were unaltered, thereby reducing the relative risk of unintended autoreactivity.[10] This proof-of-concept

ASSOCIATED CONTENT

Data Supplement
Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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study was undertaken to determine whether genetically engineered T cells expressing the E6 TCR (E6 TCR T cells) can mediate regression of HPV-associated epithelial cancers and to explore potential mechanisms of tumor response and resistance to T-cell therapy.

METHODS

Study Design and Participants

This first-in-human, single-center, single-arm, phase I/II study was conducted at the National Cancer Institute. Patient screening is described in the Data Supplement. Eligible patients were ages 18 to 70 years, HLA-A*02:01$^+$, and had metastatic HPV16-positive cancer from any primary tumor site. Formalin-fixed paraffin-embedded tissue blocks were used to confirm HPV16 positivity (cobas 4800 HR-HPV; Roche Molecular Diagnostics, Pleasanton, CA). Patients had received or declined previous systemic therapy, with more than 4 weeks elapsed since previous systemic treatment. Patients had an Eastern Cooperative Oncology Group performance score of 0 or 1 and adequate hematologic, hepatic, and renal function. See the Data Supplement for additional patient eligibility criteria.

Phase I used an accelerated dose-escalation design, with one patient per dose level at doses of 1×10^9, 1×10^{10}, 1×10^{11}, and 1 to 2×10^{11} cells. If a dose-limiting toxicity (DLT) was observed or escalation to cohort 4 was permitted, this group would expand to six patients. The maximum tolerated dose (MTD) was defined as the highest dose at which no more than one of six patients experienced a DLT, defined as grade 3 or greater toxicities that were not due to the chemotherapy, aldesleukin, or cancer. A full definition of DLTs is provided in the Data Supplement. Phase II had a planned enrollment of up to 21 patients at the MTD. Although no DLTs or safety events warranted early stopping, the study was stopped after 12 patients because of closure of the GMP cell manufacturing facility that was supporting the clinical trial. Additional details on study design are included in the Data Supplement.

Clinical responses were determined per Response Evaluation Criteria in Solid Tumor (version 1.0). Tumor size was evaluated using computed tomography or magnetic resonance imaging at baseline and at follow-up visits every month (first 3 months), every 3 months (for 6 months), every 6 months (for 12 months), and as needed thereafter. Physical examinations, routine laboratory monitoring, and blood tests were conducted at each follow-up visit, and additional leukapheresis was obtained when feasible at the first follow-up appointment. Responses were reviewed and confirmed at a weekly assessment. Patients were taken off study for progressive disease. Retreatment was permitted for patients who received less than the MTD. Duration of response and time to progression were measured from the time of cell infusion until date of progression. Toxicity and adverse event reporting used the Common Terminology Criteria for Adverse Events (version 3.0).

The protocol was reviewed and approved by the National Cancer Institute Institutional Review Board, and the study was conducted in accordance with the Declaration of Helsinki. All patients gave written informed consent. The trial is registered as ClinicalTrials.gov identifier: NCT02280811.

Outcomes

The primary end point of this study was safety, including identification of DLTs and determination of the MTD. Secondary end points were objective tumor response rate and duration of response. Exploratory objectives included immunologic correlates associated with E6 TCR T-cell treatment.

Procedures and Treatment

Patients underwent leukapheresis to acquire peripheral blood mononuclear cells. Peripheral blood mononuclear cells were stimulated with 50 ng/mL soluble anti-CD3 (OKT3; Ortho-Biotech, Bridgewater, NJ) and 300 IU recombinant interleukin-2 (IL-2). The cells were transduced with E6 TCR retrovirus, a gamma retrovirus supernatant manufactured by the National Cancer Institute Surgery Branch Vector Production Facility (Bethesda, MD). Cells were expanded with a rapid expansion protocol.[18] Biospecimens were handled with universal precautions, and laboratory research was conducted with biosafety level-2 practices.

Patients received a nonmyeloablative, lymphodepleting preparative regimen of cyclophosphamide (60 mg/kg daily, intravenous [IV]) for 2 days, followed by fludarabine (25 mg/m^2, IV) daily for 5 days. The next day, E6 TCR T cells were administered as a single, one-time, IV infusion (Data Supplement). Administration of E6 TCR T cells was followed by high-dose bolus aldesleukin (720,000 IU/kg, IV) infusion every 8 hours until development of limiting adverse effects.

Cellular Analyses

Blood samples, infusion product T cells, and tumor samples were collected on an optional basis. Tumor samples were dissociated (gentleMACS Dissociator; Miltenyi Biotec, Auburn, CA). Flow cytometry was conducted on an LSRFortessa (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (Tree Star, Ashland, OR). Flow cytometry was performed using fluorescently conjugated anti-human (CD3-PE-CF594 [BD Biosciences], CD8-BV421 [BioLegend, San Diego, CA], IFN-γ-FITC [eBioscience, San Diego, CA], TNF-α-PE-Cy7 [BioLegend], IL-2-APC [eBioscience], CD107a-APC-H7 [BD Biosciences]), and antimouse (TCR-beta constant chain-APC-Cy7 [mTCRβ; BD Biosciences]) antibodies with live/dead fixable aqua stain (eBioscience). Dissociated tumors were analyzed using CD3-PE-CF594 (BD Biosciences), CD4-PE

(BD Biosciences), PD-1-PE-Cy7 (BioLegend), and CD8-FITC (BD Biosciences), mTCRβ-APC-Cy7 (BD Biosciences), and $E6_{29-38}$ HLA-A*02:01 tetramer-APC (MBL International, Woburn, MA) with live/dead fixable yellow stain (eBioscience). Whole-exome sequencing and transcriptome sequencing of optimum cutting temperature embedded tumor tissue was performed on a HiSeq2500 platform (Illumina, San Diego, CA) by Covance (Princeton, NJ).

Queried genes are shown in the Data Supplement. Immunohistochemistry (IHC) images were captured at 20× using the Nuance System (PerkinElmer, Waltham, MA) on formalin-fixed paraffin-embedded tumor samples using programmed death-1 (PD-1; anti–PD-1 antibody [NAT105]; Abcam, Cambridge, United Kingdom), programmed death-ligand 1 (PD-L1; E1L3N; Cell Signaling Technology, Danvers, MA), CD3 (LN10; Leica Microsystems, Buffalo Grove, IL), and interferon gamma receptor 1 (IFNGR1; Abcam). Multiplex chromogenic IHC was performed using Vulcan Red chromogen (Biocare Medical) against CD8 (C8/144B Agilent; Santa Clara, CA) and 3,3′-diaminobenzidine staining of anti-mouse TCR-alpha constant chain (mTCRα; proprietary clone; Kite Pharma, Santa Monica, CA).

Statistical Analyses

Statistical power was not calculated for the phase I stage; the recommended phase II dose was based on safety. Phase II was statistically powered to detect a response rate of 20% ($P1 = .20$; null $< 5\%$, $P0 = .05$) with a planned enrollment of 21 patients ($\alpha = .05$; $\beta = .10$).

Unpaired two-tailed *t* tests were used to compare patients with and without responses. A P value of $< .05$ was considered significant. Statistical differences in HLA-allele frequency in genomic studies were determined by a Mann-Whitney test. GraphPad Prism (version 7.01; GraphPad Software, San Diego, CA) was used for analyses.

RESULTS

Between October 24, 2014, and March 24, 2016, 12 patients with metastatic HPV16-positive cervical (n = 6), anal (n = 4), oropharyngeal (n = 1), and vaginal (n = 1) cancer were treated with E6 TCR T cells. Three of the cervical cancer tumors were adenocarcinomas; all other tumors were SCCs. Baseline characteristics of each patient and for the E6 TCR T-cell products are listed in Table 1. The median age was 50 years (range, 32 to 70 years). All patients had metastatic cancer and had previously received platinum therapy. Two patients had previously received immunotherapy; patient 10 had received checkpoint blockade with an anti–PD-1 agent, and patient 5 had received adoptive T-cell therapy with tumor-infiltrating lymphocytes. A median of 105×10^9 (range, 1 to 170×10^9) T cells were administered. The median number of aldesleukin doses given was three (range, zero to six doses). As of the March 23, 2018, cutoff, no patients continued to receive treatment and one remained in follow-up.

No autoimmune adverse events or off-target toxicities attributable to E6 TCR T cells or DLTs were observed. No acute toxicities to cell infusion or cytokine storm occurred. Grade 3/4 adverse events are listed in Table 2. As expected, the most common toxicities were transient cytopenias secondary to the lymphocyte-depleting conditioning regimen. Aldesleukin was dosed to tolerance, and its toxicities resolved with discontinuation of the drug. Signs and symptoms of cytokine release syndrome were typical of aldesleukin and did not limit the E6 TCR T-cell dose. IL-6 levels were elevated in some patients after treatment (Data Supplement). One patient (patient 3) required temporary intubation for hypoxia after bleeding from an endobronchial tumor in the setting of thrombocytopenia.

Two of 12 patients (two of nine treated at the highest cell dose) attained objective tumor responses (Table 1; Fig 1A and 1B). Patient 5 was a 48-year old woman with anal SCC who had previously received fluorouracil, mitomycin, and radiation; cisplatin plus capecitabine; and adoptive transfer of tumor-infiltrating lymphocytes. At the time of E6 TCR T-cell treatment, she experienced disease progression in the bilateral lungs. After treatment, she experienced a partial response, with complete regression of one lung tumor and partial regression of two lung tumors, which were each resected on progression. She had no evidence of disease 3 years after treatment (Fig 1C). Patient 10 was a 64-year-old woman with anal SCC previously treated with fluorouracil, mitomycin, and radiation, followed by combination cisplatin plus fluorouracil and then nivolumab. She experienced disease progression involving the hilum, lung, and mediastinum. After treatment, she experienced a partial response lasting 3 months before progression of a nontarget lesion (Fig 1D). Most patients attained tumor regression, although it was of variable depth and duration (Fig 1A).

A median of 60% (range, 45% to 76%) of administered T cells expressed the E6 TCR as measured by flow cytometry. Infusion products were composed of a median of 54% $CD8^+$ T cells (range, 18% to 79%) and 42% $CD4^+$ T cells (range, 19% to 65%; Table 1). There were no statistically significant differences in the number of E6 TCR T cells, $CD8^+$ T cells, or $CD4^+$ T cells between responders and nonresponders (Fig 2A; Data Supplement). To assess the reactivity of the administered T cells against the E6 target, infused T cells were incubated with peptide-loaded target cells or antigen-expressing tumor cells, and a panel of T-cell effector functions was assessed by flow cytometry. Infused T cells responded to 293-A2 cells loaded with $E6_{29-38}$ peptide and 4050 tumor cells that naturally express HPV16 E6, as evidenced by production of interferon gamma (IFN-γ), tumor necrosis factor alpha, and IL-2, and cell surface mobilization of lysosomal-associated membrane protein 1 (LAMP-1/CD107a; Data Supplement),

TABLE 1. Characteristics of Patients and Administered T Cells

Patient	Age (years)	Sex	Diagnosis	Sites of Disease	Prior Systemic Treatment	Dose Level	Cells ($\times 10^9$)	Within CD3+ (%)			No. of Aldesleukin Doses	Response (duration or TTP, months)*
								CD8+	CD4+	E6 T Cells		
1a	50	F	Vaginal SCC	Liver	Cisplatin, paclitaxel, topotecan, carboplatin, gemcitabine, bevacizumab, vinorelbine	1	1.0	56	42	47	7	SD (6)
1b†				Liver, psoas muscle			134.0	44	51	60	4	PD (2)
2	37	F	Cervical SCC	Pelvis, retroperitoneum	Cisplatin, gemcitabine, paclitaxel, bevacizumab	2	10.0	18	60	76	4	PD (2)
3	32	F	Cervical SCC	Bone, lung, mediastinum, spleen	Cisplatin, paclitaxel, carboplatin, bevacizumab, gemcitabine	2	25.0	52	34	62	0	PD (1)
4	46	F	Cervical AC	Omentum, pelvis, peritoneum, retroperitoneum	Cisplatin, gemcitabine, paclitaxel, taxotere, bevacizumab	3	100.0	68	23	51	2	SD (6)
5	48	F	Anal SCC	Lung	Cisplatin, FU, mitomycin, capecitabine, HPV-TIL	4	170.0	58	26	45	2	PR (6)
6	49	F	Cervical AC	Brain, liver, mediastinum, pelvis	Carboplatin, docetaxel, paclitaxel, topotecan, bevacizumab	4	104.0	64	33	71	4	PD (2)
7	62	F	Cervical SCC	Hilum, lung, pleura	Cisplatin, carboplatin, paclitaxel, topotecan, gemcitabine, pemetrexed	4	115.0	35	64	53	2	PD (2)
8	65	M	Head and neck SCC	Axilla, mediastinum, pleura	Cisplatin, FU, cetuximab	4	125.2	79	19	47	1	SD (4)
9	33	F	Cervical AC	Mediastinum, pelvis, retroperitoneum	Carboplatin, paclitaxel, bevacizumab	4	116.0	32	65	71	5	SD (4)
10	64	F	Anal SCC	Hilum, lung, mediastinum	Cisplatin, FU, mitomycin, nivolumab	4	117.0	73	24	51	6	PR (3)
11	50	F	Anal SCC	Hilum, lung, pleura	Cisplatin, FU, mitomycin	4‡	70.7	40	56	61	4	PD (3)
12	70	M	Anal SCC	Liver, penis	Cisplatin, FU, mitomycin	4‡	105.0	54	45	69	0	PD (1)

Abbreviations: AC, adenocarcinoma; FU, fluorouracil; HPV-TIL, human papillomavirus–tumor-infiltrating lymphocyte; adoptive transfer of TILs (protocol NCT01585428); IL-2, interleukin-2; PD, progressive disease; PR, partial response; SCC, squamous cell carcinoma; SD, stable disease; TTP, time to progression.

*Duration in months from T-cell infusion. Follow-up of patients equals duration of response or TTP.

†Patient 1 retreatment.

‡Patients 11 and 12 were treated in the phase II portion of the trial at the maximum tolerated dose defined during phase I (dose level 4).

TABLE 2. Adverse Events (grades 3 and 4)

	Events, No. (%)				
	Dose Level				All Dose Levels
Adverse Event	1	2	3	4	1-4
Lymphopenia	1 (100)	2 (100)	1 (100)	9 (100)	13 (100)
Neutropenia	1 (100)	2 (100)	1 (100)	9 (100)	13 (100)
Thrombocytopenia	1 (100)	2 (100)	1 (100)	9 (100)	13 (100)
Anemia	1 (100)	2 (100)	1 (100)	7 (78)	11 (85)
Febrile neutropenia	—	1 (50)	1 (100)	3 (33)	5 (38)
Infection*	1 (100)	—	—	3 (33)	4 (31)
Diarrhea	—	—	—	1 (11)	1 (8)
Hyperbilirubinemia	—	1 (50)	—	—	1 (8)
Rash	—	1 (50)	—	—	1 (8)
Syncope	—	—	—	1 (11)	1 (8)
Bronchial obstruction	—	1 (50)†	—	—	1 (8)
Hemorrhage (pulmonary)	—	1 (50)†	—	—	1 (8)
Hypoxia	—	1 (50)†	—	—	1 (8)
Pleural effusion	—	1 (50)†	—	—	1 (8)
Prolonged intubation	—	1 (50)†	—	—	1 (8)

*Includes positive surveillance blood cultures.
†Occurred in the same patient.

which indicate T-cell activation. Both responders and nonresponders showed polyfunctional T-cell reactivity against E6.

To assess engraftment of E6 TCR T cells after infusion, peripheral blood was analyzed for E6 TCR expression approximately 1 month post-treatment. E6 TCR T cells represented a median of 30% (range, 4% to 53%) of the total $CD3^+$ T-cell population in 11 patients with available samples (Fig 2A; Data Supplement). To test the functionality of the persisting E6 TCR T cells, post-treatment peripheral blood T cells were cocultured with $E6_{29\text{-}38}$ peptide-loaded target cells. In nine patient samples, production of IFN-γ, tumor necrosis factor alpha, and IL-2, and cell surface mobilization of CD107a (Fig 2B) was observed, indicating that post-treatment T cells recognized $E6_{29\text{-}38}$ peptide-loaded target cells. This response was detected in responders and nonresponders. Although infusion product E6 TCR T cells were predominantly effector memory T cells (T_{EM}, $CD45RA^-/CCR7^-$), post-treatment persisting E6 TCR T cells contained a greater proportion of terminally differentiated effector memory $CD45RA^+$ T cells (T_{EMRA}, $CD45RA^+/CCR7^-$), indicating that E6 TCR T cells differentiated and then expanded after antigen exposure (Data Supplement). PD-1 expression by E6 TCR T cells before infusion was low (median, 2.3%; range, 0.2% to 9.2%). In all patients, less than 10% of engrafted peripheral blood E6 TCR T cells expressed PD-1 (Data Supplement).

Potential mechanisms of response and resistance to treatment were studied in patients with available post-treatment tumor biopsy specimens. Patient 2, who did not respond to treatment, received 25×10^9 cells and had 4% E6 TCR T-cell engraftment after 1 month. No pre-treatment biopsy was performed. Whole-exome sequencing of the post-treatment tumor biopsy revealed a frameshift deletion in *IFNGR1*, which was detected in three of three metastatic tumors from the patient (Fig 3A). IHC analysis showed decreased surface expression of IFNGR1 on tumor cells compared with infiltrating immune cells on the same slide. This finding was not observed in a tumor from patient 5, who responded to treatment (Fig 3B).

Patient 5 had a 6-month partial response after treatment with 170×10^9 T cells. She had 26% peripheral blood E6 TCR T-cell engraftment 17 months after treatment. The engrafted E6 TCR T cells infrequently expressed PD-1 (Fig 4A). IHC analysis of her tumor showed infiltration with $CD3^+$ cells and expression of PD-1 and PD-L1 by infiltrating immune cells (Fig 4B). E6 TCR T cells were only detected in the post-treatment biopsy from patient 5, and PD-1/PD-L1 staining was only performed on this sample. Multiplex immunofluorescence revealed intratumoral presence of E6 TCR T cells (identified by mouse TCRα staining), some of which were $CD8^+$ (Fig 4C). Flow cytometry of a tumor biopsy also demonstrated the presence of E6 TCR T cells. In tumor-infiltrating cells, E6 TCR expression was less intense, and PD-1 was more frequent than in infusion product cells (Fig 4D).

Patient 11 received 70.7×10^9 cells, had 22% peripheral blood engraftment of E6 TCR T cells 1 month post-treatment,

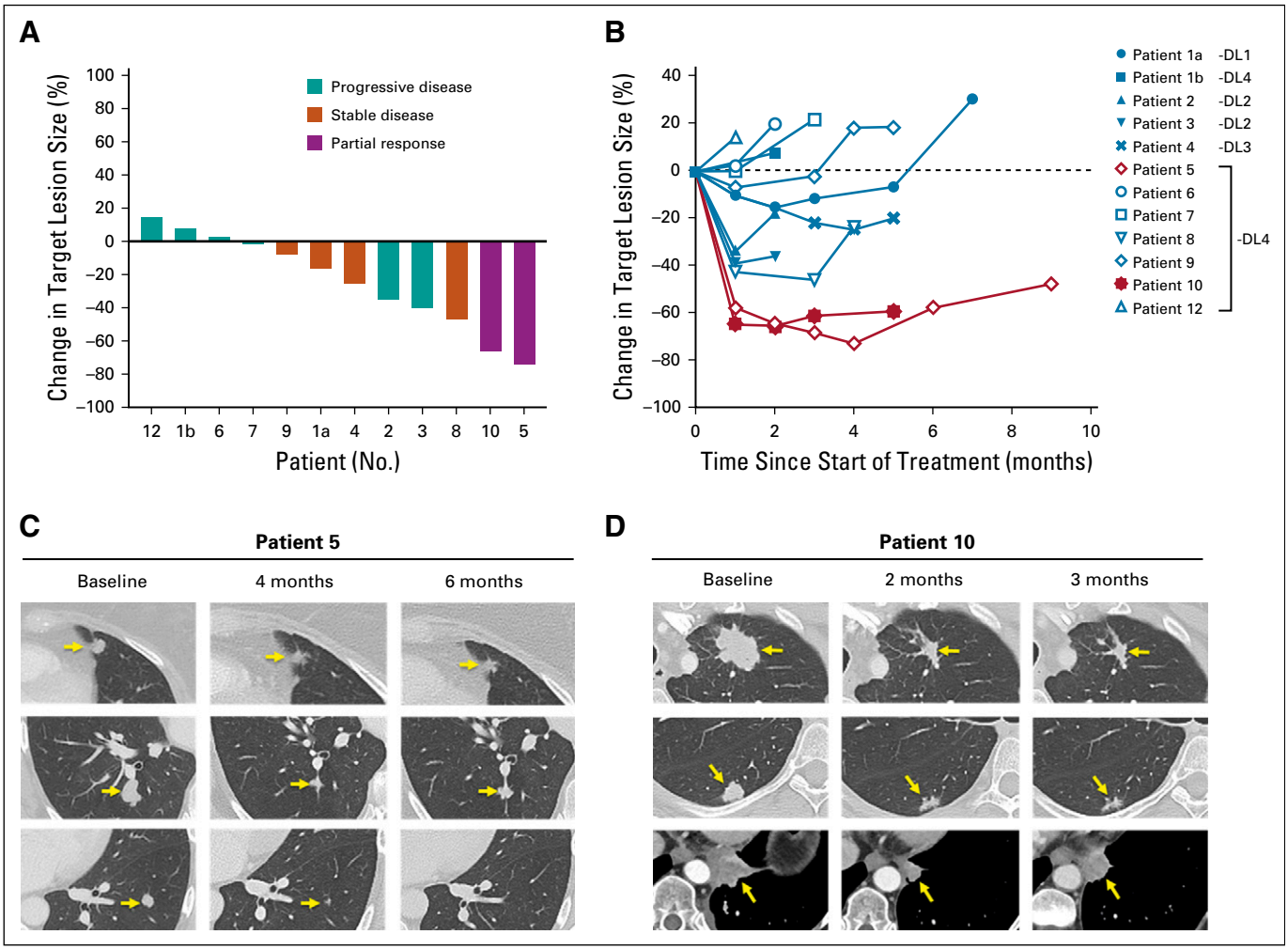

FIG 1. Tumor responses after treatment with E6 TCR T cells. (A) Waterfall plot of the best response of the target lesions for each evaluable patient. (B) Spider plot indicating the change in size of the target lesions for each evaluable patient. The sum of the longest diameters is plotted. (C) Contrast-enhanced computed tomography scans showing tumor responses for patient 5. Two lesions regressed partially (top row and middle row), and one lesion regressed completely (bottom row). (D) Contrast-enhanced computed tomography scans showing tumor responses for patient 10.

and experienced rapid cancer progression. A post-treatment tumor biopsy demonstrated loss of heterozygosity involving the HLA superlocus of chromosome 6p21 (Data Supplement). Analysis of *HLA-A* genomic sequencing revealed allelic imbalance with loss of *HLA-A*02:01* ($P < .001$), the necessary restriction element for E6 TCR T cells (Data Supplement). A tumor biopsy from patient 5 did not display genetic defects in antigen processing and presentation.

DISCUSSION

Adoptive transfer of autologous T cells transduced with a TCR that targets HPV16 E6 resulted in tumor regression and objective responses in patients with chemotherapy-refractory, metastatic HPV16-positive epithelial cancer. Neither on-target autoimmune toxicity[6,7] nor off-target cross-reactivity against healthy tissue[8,19] were observed. These results support the concept that genetically engineered T cells can mediate regression of metastatic epithelial cancer and establish a cell therapy model in which mechanisms of response and resistance to T-cell therapy for epithelial cancers can be explored.

Despite targeting a tumor antigen that is generally considered to be constitutively expressed with a high-avidity TCR and the robust engraftment of the E6 TCR T cells, the response rate was modest, suggesting the presence of resistance to T-cell–mediated recognition and/or attack (Data Supplement). In nonresponding patients, one tumor showed a truncating mutation in *IFNGR1*, a central molecule in T-cell effector function,[20] and another tumor showed loss of *HLA-A*02:01*, the restriction element for E6 TCR. Another possible mechanism of resistance is expression of PD-1 by tumor-infiltrating E6 TCR T cells and expression of PD-L1 by tumor-infiltrating immune cells, which was observed in the residual tumor of a patient achieving partial response. Furthermore, although the HPV

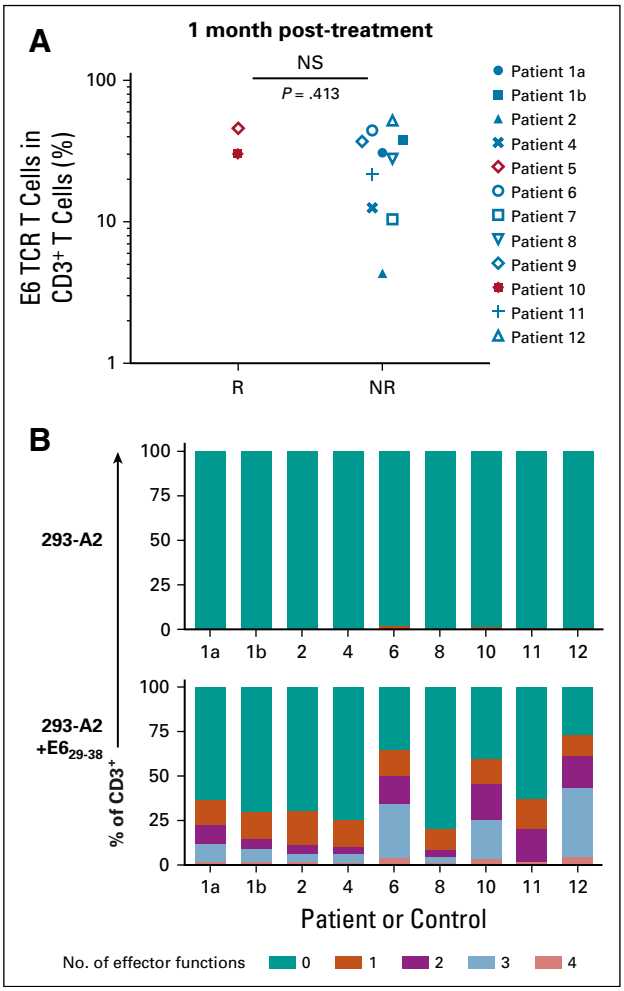

FIG 2. Peripheral blood engraftment of functional E6 TCR T cells. (A) The frequency of E6 TCR T cells in the peripheral blood of responders (R) and nonresponders (NR) with samples available. (B) Functional response of T cells in the peripheral blood against the targeted $E6_{29-38}$ epitope. Post-treatment peripheral blood mononuclear cells were incubated with $E6_{29-38}$ peptide-pulsed 293-A2 cells (positive target) or 292-A2 (negative target). Intracellular interferon gamma, tumor necrosis factor alpha, and interleukin-2 production, as well as cell surface mobilization of the degranulation marker CD107a were assessed by intracellular flow cytometry. Gating was on $CD3^+$ lymphocytes. Data from all patients with samples available are shown. NS, not significant.

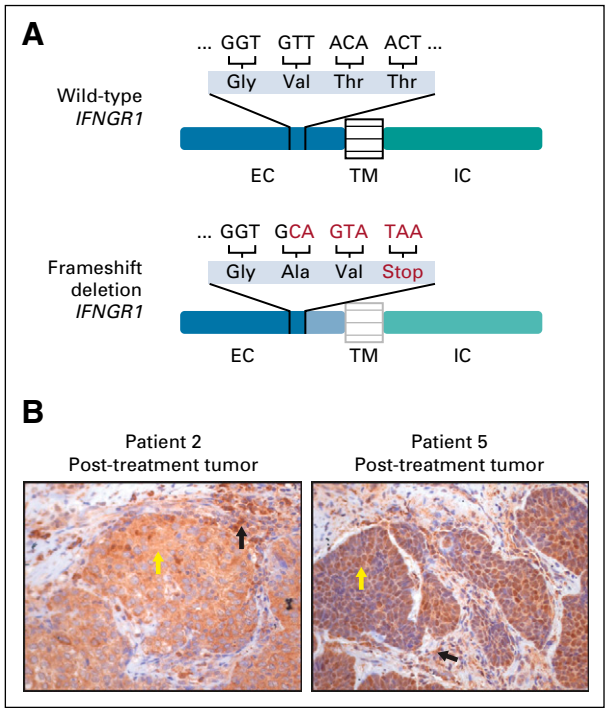

FIG 3. Tumor resistance associated with a truncating frameshift deletion in interferon gamma receptor 1 (*IFNGR1*). (A) Exomic sequencing data from a post-treatment tumor biopsy from patient 2 demonstrated a truncating frameshift deletion in the extracellular domain of *IFNGR1*. (B) Immunohistochemistry on a tumor biopsy from patient 2 (nonresponder with an *IFNGR1* mutation) revealed decreased expression of IFNGR1 by tumor cells but not tumor-infiltrating immune cells. In contrast, a tumor biopsy from patient 5 (responder without an *IFNGR1* mutation) did not show this finding. Black arrows denote infiltrating immune cells; yellow arrows denote tumor cells. EC, extracellular region; IC, intracellular region; TM, transmembrane region.

oncoproteins are generally considered to be constitutively expressed, loss of E6 expression may be possible in some cells and might mediate resistance.

These findings define new challenges in the development of effective engineered T-cell therapies for epithelial cancers. It may be important to develop patient screening assays that detect genomic defects conferring treatment resistance[21,22] or to evaluate T-cell therapy in earlier lines of treatment when tumors are less subjected to selective immune pressure.[20] For cancers with heterogeneous genetic defects, combination therapies and strategies to increase bystander killing may be explored. Several strategies have described preventative inhibition of adoptively transferred T cells by PD-1 signals[23-26]; inhibition of PD-1 may be effective for some patients experiencing partial responses.

Genetic defects that may influence responses to immunotherapy by inhibiting antigen processing and presentation and/or responses to IFN-γ are gaining interest. Mutations in β2-microglobulin (the required β-chain of HLA class I molecules) and JAK1/JAK2 (molecules important in IFN-γ signaling) have been associated with resistance to PD-1 blockade in melanoma.[27] In addition, HLA–loss of heterozygosity was recently shown to be associated with possible immune editing in lung cancer.[28] Tumor escape through loss of an HLA allele responsible for presenting the target antigen was described in a report of tumor-infiltrating T-cell therapy in a patient with KRAS-mutant colorectal cancer.[29] A recent genomics study also found that defects in antigen processing and presentation may frequently contribute to immune evasion in colorectal cancer.[30]

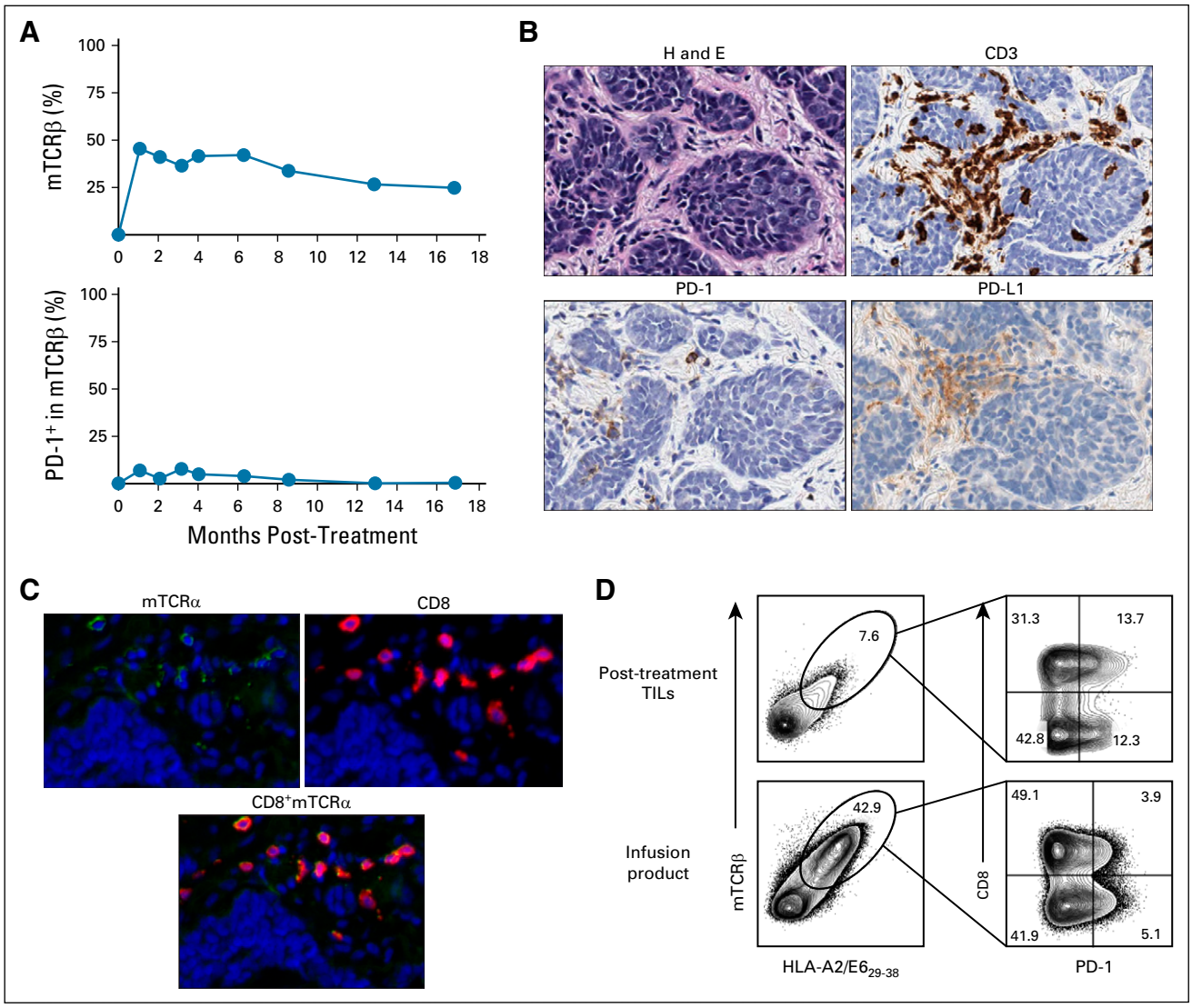

FIG 4. Tumor response associated with tumor infiltration with E6 TCR T cells and expression of programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1) in the tumor. (A) Peripheral blood engraftment of E6 TCR T cells (mTCRβ$^+$) and expression of PD-1 by engrafted E6 TCR T cells from patient 5 as measured by flow cytometry. (B) Hematoxylin and eosin (H and E) and immunohistochemistry staining for CD3, PD-1, and PD-L1 were performed on a residual tumor from patient 5 that was resected 9 months post-treatment. (C) Multiplex immunohistochemistry staining for CD8 (red), mTCRα (green), and nuclear staining (blue) of a tumor from patient 5 that was resected 10 months post-treatment. (D) Flow cytometric analysis of PD-1 expression by E6 TCR T cells (identified by coexpression of mTCRβ and HLA-A*02:01-E6$_{29-38}$ tetramer) from a tumor resected from patient 5 10 months after treatment. Comparison is made with infused T cells, which were analyzed in the same experiment. TILs, tumor-infiltrating lymphocytes.

The limitations of this study include the small size that precluded the determination of a response rate, the small number of post-treatment tumor biopsies obtained, and the lack of an intention-to-treat analysis because of patient screening on a department-wide protocol. Evaluation of these tumors was informative, because the genetic defects in antigen presentation and IFN-γ response in non-responders may explain why these patients did not respond. Although the importance of these genes in tumor immunology is well established, we could not determine whether these defects were responsible for the observed treatment failure. Furthermore, the exploratory examination of tumor-intrinsic genetic defects in this study is hypothesis generating, but did not conclusively identify predictive biomarkers.

In conclusion, these findings support continued investigation of genetically engineered T-cell therapy for epithelial cancers and begin to define future challenges for cellular therapy in epithelial cancers. We are currently conducting a clinical trial for HPV16-positive cancers with a higher avidity TCR that targets E7 (ClinicalTrials.gov identifier: NCT02858310).

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

T-Cell Receptor Gene Therapy for Human Papillomavirus–Associated Epithelial Cancers: A First-in-Human, Phase I/II Study

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