ORIGINAL ARTICLE

Combining a WT1 Vaccine (Galinpepimut-S) With Checkpoint Inhibition (Nivolumab) in Patients With WT1–Expressing Diffuse Pleural Mesothelioma: A Phase 1 Study

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ABSTRACT

Introduction: WT1 often presents on the surface of diffuse pleural mesotheliomas (DPMs) and is an ideal therapeutic target. Galinpepimut-S (GPS), a tetravalent, non–human leukocyte antigen–restricted, heteroclitic WT1–specific peptide vaccine was safe and effective in early phase clinical trials and upregulates T-cell suppressive programmed death-ligand 1 in the tumor microenvironment of other malignancies. A randomized phase 2 study of adjuvant GPS in patients with DPM trended toward improved median overall survival.

Methods: To further enhance immunogenicity, we combined GPS with nivolumab, an anti-PD1 monoclonal antibody, in an open-label, single-center phase 1 study, examining tolerability and immunogenicity in patients with previously treated DPM. We enrolled patients with progressive or recurrent DPM treated with at least one course of pemetrexed-based chemotherapy. Patients received two doses of GPS followed by six doses of GPS with intravenous nivolumab every 2 weeks, and up to six additional cycles until disease progression or unacceptable toxicity.

Results: Ten patients were treated; 70% experienced mostly mild treatment-related adverse events; two experienced a grade 3 or higher adverse event. Three of the 10 patients (30%) reported vaccine-specific T-cell responses. There were no partial responses; three patients had prolonged stable disease with up to 17% decrease in tumor volume. Median progression-free survival was 3.9 months and the median overall survival was 7.4 months.

Conclusions: Coadministration of GPS and nivolumab reported a tolerable toxicity profile and induced immune responses in a subset of patients, but initial response and survival benefit were limited possibly owing to the small sample size.

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***Work completed when Drs Lai and Zauderer were at MSKCC**

Drs. Lai and Zauderer were at Memorial Sloan Kettering Cancer Center when the work was completed.

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Introduction

Diffuse pleural mesothelioma (DPM) is an aggressive malignancy with poor outcomes and only two Food and Drug Administration–approved treatment regimens, both in the first-line setting: cisplatin-pemetrexed[1] and ipilimumab-nivolumab.[2] Most patients experience disease progression within the first year and there are no approved treatments in the second line setting.[3–5] No targeted therapies are available, and novel, efficacious treatments are critically needed for patients with DPM.

WT1 is a promising target in DPM. Expression of the *WT1* gene is limited in normal adult tissues but highly expressed in multiple hematologic malignancies and solid tumors including mesothelioma.[6,7] *WT1* was originally described as a tumor suppressor gene, but WT1 protein seems to be involved in tumorigenesis.[8] Although WT1 is a nuclear and cytoplasmic protein, it is processed and the derived peptides are presented on the cell surface, making it an attractive target for immunotherapy.[6] Various peptide sequences from the WT1 antigen have reported immunogenicity and evoke cytotoxic T lymphocyte responses that target and kill WT1-expressing cancer cells.[9] Selective expression on tumor cells coupled with high immune activation potential makes WT1 an ideal candidate for a tumor-selective cancer vaccine.

The WT1 protein is a self-antigen, and overcoming immune tolerance is a potential challenge for effective vaccination. To enhance the immunogenicity of WT1, our Memorial Sloan Kettering Cancer Center research team designed synthetic analog peptides that generate an immune response to the immunizing epitopes and cross-react with the native WT1 peptides known as a heteroclitic response. Two native and two heteroclitic WT1 peptides, which were immunogenic in preclinical studies and pilot human trials,[9,10] were combined into a non–human leukocyte antigen–restricted heteroclitic tetravalent peptide vaccine, Galinpepimut-S (GPS).

A randomized phase 2 trial of GPS in DPM reported a favorable safety profile and a trend toward improved progression-free survival (PFS) and overall survival (OS) in patients who received the vaccine as adjuvant treatment after undergoing multimodality therapy compared with control.[11] GPS also has activity in other malignancies, including multiple myeloma, ovarian cancer, and acute myeloid leukemia.[12–15]

The immune response to vaccines can be attenuated by the up-regulation of T-cell suppressive programmed death-ligand 1 (PD-L1), leading to a paucity of tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment.[16–19] TILs are crucial for effective cytotoxicity and TIL enrichment at the primary tumor site is associated with better prognosis in patients undergoing extrapleural pneumonectomy for DPM.[20,21] Preclinical data reveal that combining immune checkpoint inhibition and vaccines can potentiate immune responses by attenuating immunosuppressive mechanisms and encouraging the action of effector T-cells generated by vaccines.[22] This potential to augment vaccine responses led us to hypothesize that the combination of GPS and nivolumab in patients with previously treated DPM would be feasible, safe, and effective. Herein, we report the phase 1 clinical trial to test this hypothesis.

Materials and Methods

Study Design

This was a single-center, open-label, nonrandomized phase 1 trial of GPS and nivolumab in patients with DPM with progressive or recurrent disease after treatment with pemetrexed-based chemotherapy. This study was approved by the Memorial Sloan Kettering Cancer Center Institutional Review Board (NCT04040231). The study was conducted in accordance with good clinical practice and followed the guiding principles of the Declaration of Helsinki, and local laws and regulations. Patients provided written informed consent for study treatment participation.

Eligibility Criteria

Patients with pathologically confirmed DPM, immunohistochemistry positive for WT1 (clone WT49) in more than 10% of cells, who received at least one prior course of pemetrexed-based chemotherapy and had recurrent or progressive disease were eligible for enrollment. Patients were required to be aged 18 years or older, have a Karnofsky performance status of 70% or higher, have measurable disease on imaging according to modified Response Evaluation Criteria in Solid Tumors (RECIST) for mesothelioma v1.0,[23] and have an adequate hematologic, renal, and hepatic function (absolute neutrophil count $\geq 1000/\mu L$, platelets >100 K/μL, total bilirubin ≤ 1.5 mg/dL, creatinine $\leq 1.5 \times$ upper limits of normal, and aspartate aminotransferase and alanine aminotransferase $\leq 3.0 \times$ upper limits of normal). Patients with prior receipt of DPM-directed surgery or radiation were eligible. Exclusion criteria were pregnancy, prior receipt of checkpoint inhibition, an autoimmune disease requiring treatment with systemic corticosteroids within the past 2 years, receipt of systemic steroids (>10 mg daily prednisone equivalents) at the time of study drug administration, active pneumonitis, known immunodeficiency syndrome, other serious unstable medical illness, or another active cancer.

Treatment Plan

Patients received 800 μg of GPS (SELLAS Life Sciences Group, Inc., New York, NY) administered subcutaneously at a one-to-one ratio with 0.7 mL of Montanide ISA 51 vegetable grade (an immune adjuvant containing a natural oil and refined emulsifier which enhances the immune system's cytotoxic T lymphocyte response against antigens in vaccines and serves to suspend the vaccine peptides to prevent degradation; SEPPIC, Inc., Fairfield, NJ) administered along with the anti–programmed cell death protein-1 immunoglobulin G4 monoclonal antibody nivolumab (Bristol-Myers Squibb, Lawrenceville, NJ) at 240 mg intravenously every 2 weeks over a 16-week period. GPS was administered every 2 weeks on weeks 0 to 14 and nivolumab was administered every 2 weeks on weeks 4 to 14 (Supplementary Fig. 1). Injection sites were primed with 70 μg sargramostim (human granulocyte-macrophage colony-stimulating factor; Partner Therapeutics, Inc., Lexington, MA) administered subcutaneously both 2 days before and on the day of each GPS vaccine. Vaccines were administered in patients' extremities, and vaccination sites were rotated with each dose. Patients who did not have disease progression at the week 16 evaluation were offered a maintenance course of up to six additional doses of GPS plus montanide (after sargramostim priming) and nivolumab. Peripheral blood mononuclear cells (PBMCs) were collected at baseline, before each dose of treatment, and at the time of disease progression. Computed tomography scans of the chest were obtained at baseline, week 8, week 16, and every three months thereafter for up to 1 year in those without disease progression and assessed using the modified RECIST for mesothelioma version 1.0[23] with reference study radiologists. Treatment was discontinued at the end of the maintenance period, at the time of disease progression, or with a dose-limiting toxicity (DLT).

Vaccine Preparation

GPS contains four peptides (Supplementary Table 1) and was prepared as previously reported.[9,15,24–26]

Toxicity Evaluation

Toxicities were graded using the National Cancer Institute Common Toxicity Criteria for Adverse Events version 4.0 and assessments were performed at baseline, weeks 1 and 2, then every 2 weeks until completion of therapy, and subsequently every three months for 1 year. Dose-limiting toxicities were evaluated within 28 days of the first vaccination. Criteria for evaluation are detailed in the study protocol (Supplementary Materials).

Immune Response Evaluation

Detection of antigen-specific T-Cells by intracellular cytokine staining. To investigate antigen-specific T-cell responses, cytokine production after antigenic stimulation was used as a readout by means of intracellular cytokine staining with a T-cell flow cytometry panel on thawed PBMC samples. After initial thaw, cells were rested overnight at 37°C in complete Roswell Park Memorial Institute cell culture medium with 10% pooled human serum and then stimulated with the vaccine antigen pool of four peptide antigens at 2 μg/mL each peptide (Supplementary Table 1) or a cytomegalovirus, Epstein-Barr virus, and influenza virus–positive control peptide pool consisting of 176 major histocompatibility complex class-I and major histocompatibility complex class-II restricted peptides (2 μg/mL each) from various infectious agents (Catalog [Cat.] PM-CEFX-1, JPT Peptide Technologies), along with supplemental interleukin (IL)-2 (Cat. 11011456001, Roche) at 10 U/mL and IL-15 (Cat. 247-ILB-025, R&D Systems) at 10 ng/mL. Complete media with IL-2 and IL-15 was replaced every 2 to 3 days. At the end of the 10-day culture period, cells were counted, washed, and nonstimulated or restimulated with the same initial peptide antigens plus fluorescence-labeled antibody to degranulation marker cluster of differentiation (CD) 107a (H4A3, BioLegend Cat. 328634) for six hours, with the last four hours in the presence of Golgi transport inhibitors brefeldin-a (Cat. 347688, BD Biosciences) and monensin (Cat. 00-4505-51, eBioscience). Nonstimulated cells cultured in parallel were used as negative controls to establish cytokine positivity gates in flow cytometry analyses. After the restimulation period, cells were treated with 2 mM ethylenediaminetetraacetic acid (Cat. 347689, BD Biosciences) for 15 minutes to arrest T-cell activation, washed, and then stored at 4°C overnight in phosphate-buffered saline (D-PBS, Cat. 21-031-CM, Corning) containing 5% fetal bovine serum (Cat. 35-011-CV, Corning). Subsequently, cells were stained with a fixable viability dye (Cat. 423110, BioLegend) and antibodies to surface CD8 (SK1, Cat.560179, BD Biosciences), fixed, permeabilized, washed, and then stained with fluorescence-labeled antibodies to CD3 (UCHT1, Cat. 612940, BD Biosciences), CD4 (SK3, Cat. 612748 BD Biosciences), and the following intracellular cytokines: interferon (IFN)-γ (B27, Cat. 563287, BD Biosciences), IL-2 (MQ1-17H12, Cat. 564166, BD Biosciences), and tumor necrosis factor (TNF)-α (Mab11, Cat. 563996, BD Biosciences). The procedure was optimized to ensure sufficient detection of T-cell subsets by intracellular staining for CD3 and CD4 owing to the potential down-regulation of those receptors after T-cell stimulation. Cells were then washed and resuspended in PBS with 1% fetal bovine serum for

acquisition on a BD Biosciences Fortessa X-20 flow cytometer. After the acquisition, flow cytometry data were analyzed using FlowJo software (FlowJo [RRID:SCR_008520]) to assess antigen-specific polyfunctional T-cell responses in gated CD4 and CD8 T-cell subsets.

Plasma multiplex cytokine measurements. Cytokines were quantitated following manufacturer instructions for the V-PLEX Human Proinflammatory Panel 10-plex kits (for IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ, TNFα, IL-1β, and IL-13) purchased from Meso Scale Diagnostics (Merck Sharp & Dohme [MSD], Cat. #K15049D-1). All reagents were provided with the kit. The standards were reconstituted in the assay diluent provided. Frozen plasma samples were thawed, clarified by brief centrifugation to remove any particulate materials, and diluted twofold in assay diluent. Diluted samples, controls, and standards were added at 50 μL per well into the MSD assay plates pre-coated with 10 capture antibodies per well against the cytokines of interest. The plate was sealed and incubated for two hours at room temperature on an orbital shaker (600 revolutions per minute). At the end of the incubation, the wells were washed three times using 150 μL wash buffer (PBS + 0.05% Tween 20). Detection antibodies conjugated to electrochemiluminescence labels were added at 25 μL per well, and the plate was sealed and incubated for two hours at room temperature on an orbital shaker (600 revolutions per minute). At the end of the incubation, the plate was washed three times as before. Then, 150 μL of the MSD read buffer was added to each well and the plates were read on the MSD QuickPlex SQ 120 imager. The raw data was measured as light intensity detected by instrument photodetectors on application of electricity to the plate electrodes. Data were analyzed using MSD Discovery Workbench software. A four-parameter logistic fit calibration curve was generated for each analyte using standards run in parallel and used to calculate the concentration of each analyte in tested samples. The upper and lower limits of quantification were defined by the highest and lowest concentrations of calibrator run on each plate that exhibited less than 20% coefficient of variability of duplicate wells and 80% to 120% recovery of target values when back calculated by the standard curve regression algorithm.

Statistical Analysis

The primary end point of this phase I study was the safety of nivolumab in combination with the GPS vaccine. The plan was to enroll 10 assessable patients; with a DLT rate of 0.25, there would be a greater than 50% probability of declaring this treatment combination safe. There was an early stopping rule for the first three patients: if two or more developed DLTs in the first 28 days, then the study would have been terminated early. In addition, the study would pause for grade 4 or greater adverse events, at least possibly related to the study drugs, that occurred within 28 days of nivolumab infusion or GPS vaccination (whichever date is later) until further evaluation of the cause of toxicity. Detection of two or fewer DLTs out of the total 10 enrolled patients would deem the combination safe.

The secondary objective was to evaluate the immunogenicity of GPS and nivolumab by assessing WT1-specific cell-mediated immune response and cytokine levels. Descriptive characteristics were used to describe toxicities and immune responses. PFS was measured from the date of consent for the study to the date of progression of the disease by modified RECIST for mesothelioma version 1.0 or death (while in the study). OS was measured from the date of consent for the study to the date of death or last follow-up. PFS and OS were calculated using the Kaplan-Meier method.

Results

Patient and Tumor Characteristics

Ten patients were screened, deemed eligible, and consented to this protocol (Memorial Sloan Kettering Cancer Center protocol #17-654; NCT04040231) between February 10, 2020, and September 28, 2022 (Table 1). The median age at enrollment was 69 years (range 59–80 y) and the median Karnofsky performance status at enrollment was 80% (range 70%–90%). Eight patients (80%) were male individuals. Eight patients (80%) had purely epithelioid tumors, while one patient (10%) had a sarcomatoid tumor and one patient (10%) had a tumor with biphasic histology. Five patients had no or minimally detectable tumoral PD-L1 (clone E1L3N, Cell Signaling) expression (0% or $<$1%), two patients had 80% expression, and one patient each had 10%, 20%, and 30% expression levels. Seven patients (70%) were former smokers and three patients (30%) were never smokers. Two patients (20%) had a history of self-reported classical occupational asbestos exposure. Six patients (60%) underwent surgery: two underwent extended pleurectomy decortication and four underwent partial pleurectomy decortication (partial removal of parietal or visceral pleura or cases with residual gross tumor with R2 resection). All patients received only one line of prior systemic treatment, which consisted of chemotherapy with pemetrexed and platinum. Only two patients (20%) received prior intensity-modulated pleural radiation therapy, one patient (10%) received palliative radiation to the rib, and seven patients (70%) received no radiation before enrollment. The median time from the last cytotoxic treatment to initiation of trial intervention was 107 days (range 18–914 d).

Table 1. Baseline Characteristics of Patients in the Study

Patient Characteristics	n (%)
Median age, y (range)	68.9 (59-80)
Sex	
Male	8 (80)
Female	2 (20)
Stage at initial diagnosis	
I	1 (10)
II	3 (30)
III	2 (20)
IV	4 (40)
Histology	
Epithelioid	8 (80)
Sarcomatoid	1 (10)
Biphasic	1 (10)
Karnofsky performance status at enrollment	
70	1 (10)
80	5 (50)
90	4 (40)
Smoking status	
Former	7 (70)
Current	0 (0)
Never	3 (30)
Asbestos exposure[a]	
Yes	2 (20)
No	8 (80)
Prior surgery	
Extended pleurectomy decortication	2 (20)
Partial pleurectomy decortication	4 (40)
No	4 (40)
Number of prior lines of systemic treatment	
One	10 (100)
Radiation	
Pleural IMPRINT	2 (20)
Other	1 (10)
None	7 (70)

Note: Study population (N = 10).
[a]Asbestos exposure: self-reported classical occupational exposure.
IMPRINT, intensity-modulated pleural radiation therapy.

Table 2. Treatment-Related Adverse Events

Treatment-Related Adverse Event	Any, n (%)	Severe, n (%)
Anorexia	1 (10)	0 (0)
Atrial fibrillation	1 (10)	1 (10)
Constipation	1 (10)	0 (0)
Dizziness	1 (10)	0 (0)
Fatigue	2 (20)	0 (0)
Infusion-related reaction	2 (20)	1 (10)
Injection site reaction	1 (10)	0 (0)
Lung infection	1 (10)	1 (10)
Pelvic pain	1 (10)	0 (0)
Pneumonitis	1 (10)	1 (10)
Skin induration	2 (20)	0 (0)

Note: Severe adverse events included those of grade 3 or higher. Patients per maximum toxicity for treatment-related events (N = 10).

Safety and Tolerability

All 10 patients were included in the safety analysis (Table 2). The most common adverse events were fatigue (20%, all grade 1), infusion-related reaction (20%, grades 1 and 3), and skin induration (20%, all grade 1). There were seven grade 1 toxicities, three grade 2 toxicities, and four grade 3 or higher toxicities. Two patients experienced a grade 3 toxicity attributed to the study intervention. One grade 3 toxicity was atrial fibrillation, possibly attributed to nivolumab, which responded to rate-controlling medications. Another patient developed a grade 3 infusion-related reaction during the administration of nivolumab and the infusion was held; this patient did not receive further nivolumab owing to the progression of the disease on subsequent imaging.

One patient experienced grade 4 toxicities of lung infection and pneumonitis possibly attributed to nivolumab. This was a man aged 73 years with stage III biphasic DPM who had been treated with partial pleurectomy and decortication, six cycles of carboplatin and pemetrexed, and palliative radiation to the rib. After three treatments of GPS and one dose of nivolumab, he developed dyspnea, desaturation with exertion, and chest pain. Imaging was notable for new ground glass opacities in the right lung. At that time, he was also noted to have atrial fibrillation with rapid ventricular rate. Laboratory data was notable for normal white blood cell count, normal troponin, and normal procalcitonin. The infectious workup was unrevealing. The patient's tachycardia was resolved with beta-blockers. The patient was treated with empirical antibiotics and systemic corticosteroids (1 mg/kg) for presumed pneumonitis or lung infection but developed increasing oxygen requirements with hypoxic respiratory failure. The dose of steroids was increased for a few days and the patient experienced improvement in his oxygen requirements. He was discharged from the hospital on a steroid taper. The patient was then re-admitted about 1 week later for acute worsening dyspnea requiring intubation. He was empirically treated with antibiotics and intravenous high-dose corticosteroids. A bronchoscopy was performed with bronchoalveolar lavage and did not reveal any infectious organisms. The patient's clinical condition deteriorated, and he developed a fever, bacteremia, and renal failure, and subsequently expired owing to sepsis from *Enterococcus faecalis* (which was not the presumed organism responsible for his pneumonia).

There were no grade 5 toxicities. Seven of the 10 patients (70%) reported at least one adverse event and five patients (50%) had more than one adverse event.

Immune Response

Fifty-three PBMC samples from eight patients were analyzed for intracellular cytokine production after T-cell stimulation. We examined cellular cytokine responses

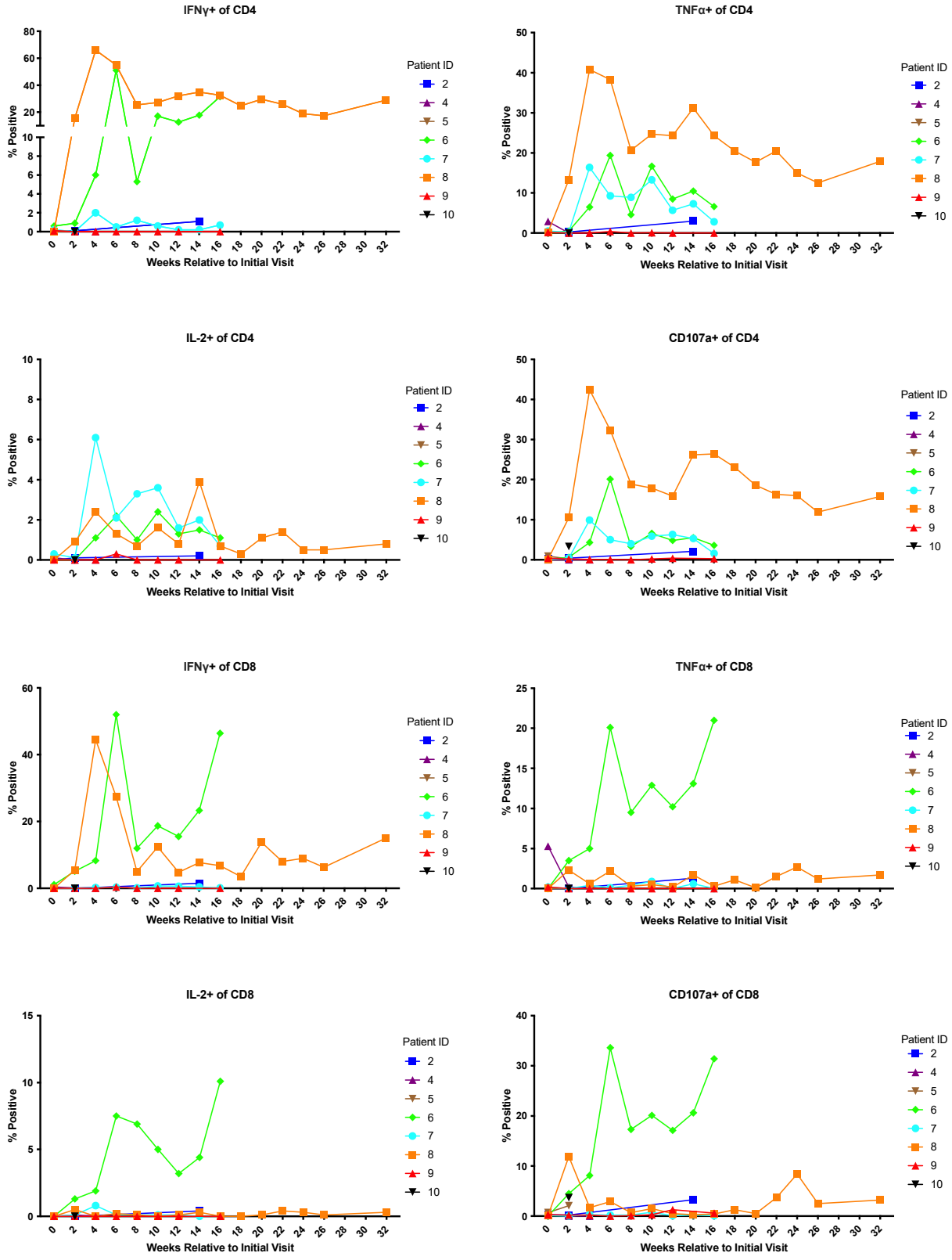

Figure 1. Detection of antigen-specific T-cells after vaccination by intracellular cytokine staining of CD4 and CD8 subsets from PBMCs collected from study patients and stimulated by the WT1 vaccine peptide pool in vitro. Graphs reveal the background-subtracted percentage of CD4 or CD8 T-cells producing each cytokine after an initial 10-day peptide pool stimulation expansion culture followed by six-hour restimulation (or no peptide restimulation as background control) with Golgi transport inhibitors. PBMCs were collected at each patient visit time point after vaccination. CD, cluster of differentiation; IFN, interferon; IL-2, interleukin-2; PBMC, peripheral blood mononuclear cell; TNF, tumor necrosis factor.

(TNFα, IFN-γ, IL-2) and CD107a expression on CD4 and CD8 T-cells stimulated by WT1 peptides and control peptides in vitro (Fig. 1 and Supplementary Figs. 2 and 3). T-cell responses to the WT1 peptide pool could be observed most readily from three patients. Samples from patient 6 displayed both CD4 and CD8 polyfunctional IFN-γ, TNFα, IL-2, and CD107a responses peaking around 6 weeks and continuing beyond. Samples from patient 7 displayed primarily CD4 TNFα and IL-2 responses with some CD107a response at 4 weeks and beyond. Samples from patient 8 displayed primarily CD4 polyfunctional IFN-γ, TNFα, CD107a, and some IL-2 responses and CD8 IFN-γ responses, peaking at week 4 and continuing beyond.

Fifty-six samples from 10 patients were available for plasma cytokine analysis (Fig. 2 and Supplementary Fig. 4). Cytokine levels were most readily observed in the same three patients who reported PBMC responses to stimulation in vitro. Patients 6, 7, and 8 reported elevated levels of IFN-γ and TNFα that tended to peak at week 6. Other noteworthy cytokine elevations included IL-8 in patient 6, IL-6 in patient 7, and IL-8 and IL-10 in patient 8. Measurements of other

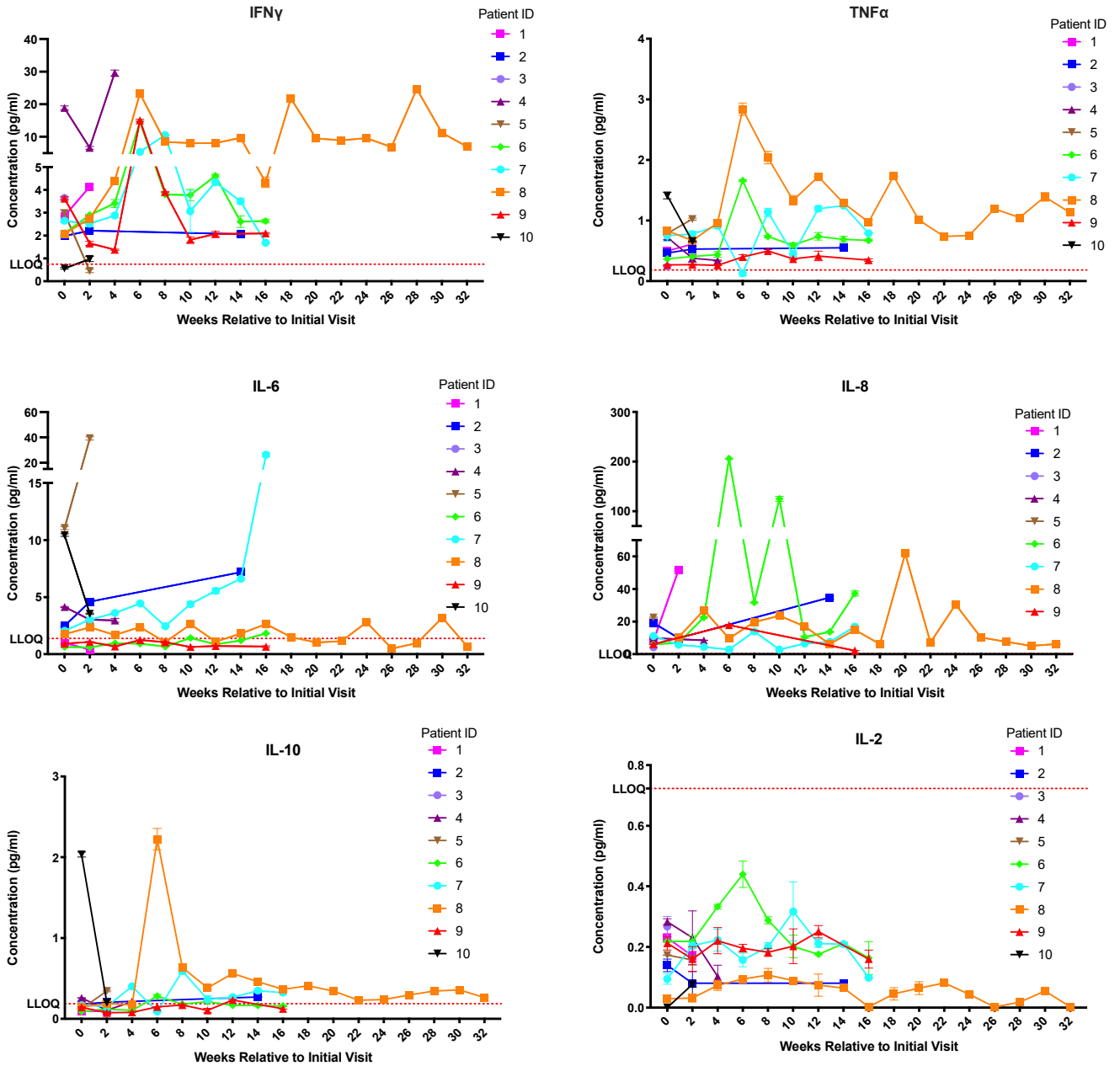

Figure 2. Circulating cytokine concentrations at different time points of treatment as measured with a multiplex panel using plasma samples from study patients. IFN, interferon; IL, interleukin; LLOQ, lower limit of quantitation; TNF, tumor necrosis factor.

cytokines, including IL-2, were at or below the lower level of quantification.

Efficacy Assessment

Exploratory analyses included response to treatment, PFS, and OS. Assessment of best overall response by modified RECIST criteria for mesothelioma was available for eight patients (Fig. 3). There were no partial responses; three patients had prolonged stable disease with up to 17% decrease in tumor volume. The median PFS was 3.9 months, the median OS from the time of enrollment was 7.4 months (Fig. 4), and the median OS from the most recent prior line of therapy was 13.5 months, with a median follow-up of 4.6 months. The median time on treatment was 2.3 months (range 0.9–10.6 mo) (Supplementary Fig. 5).

Discussion

Treatment options for patients with DPM remain limited with no approved agents beyond the first-line setting. This phase 1 trial evaluated the combination of the WT1 peptide vaccine GPS and immune checkpoint inhibitor nivolumab in patients with DPM for progressive or recurrent disease after receiving pemetrexed-based chemotherapy. The study met its primary end point and reported that the combination of the GPS vaccine and nivolumab was safe and well-tolerated.

Treatment-related adverse events were rare and generally grade 1 or grade 2 in severity. The most common adverse events included fatigue, infusion-related reaction, and skin induration, each in 20% of patients. One patient experienced grade 4 pneumonitis and lung infection attributed to nivolumab. There are no previous reports of pneumonitis or lung infections with the GPS vaccine, suggesting that this presentation was most likely attributable to immune checkpoint inhibition alone. The absence of synergistic toxic effects is integral to developing safe combination therapies.

The secondary objective of this study was to evaluate the immunogenicity of GPS combined with nivolumab by assessing the WT1-specific cell-mediated immune response. Three of the 10 patients (30%) reported vaccine-specific T-cell responses, primarily within CD4+ T-cells but also within CD8+ T-cell subpopulations. As in other studies, observed responses peaked between 4 to 6 weeks after treatment initiation.[9,10,24]

Immune response data were difficult to correlate with clinical characteristics and outcomes given the small sample size. Among the three patients who had observable immune responses, all had epithelioid histology and negative PD-L1 expression. Only one patient had a history of tobacco use and none of these patients had self-reported classical occupational asbestos exposure. All these patients had received only one prior line of systemic therapy; two patients received platinum, pemetrexed, and bevacizumab while one patient received platinum and pemetrexed alone; none of these patients underwent prior pleurectomy and decortication or radiotherapy. Time from the last cytotoxic treatment to initiation of trial intervention ranged from 35 to 112 days.

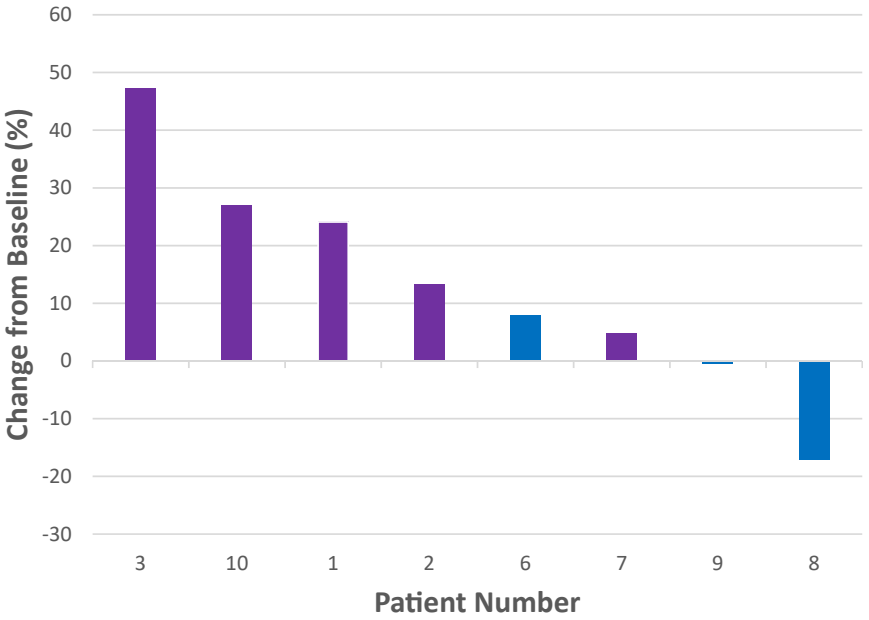

Figure 3. Best overall response based on modified RECIST criteria for mesothelioma. Patients 4 and 5 died before the first protocol CT scan of the protocol and thus could not be evaluated for the best overall response. CT, computed tomography; RECIST, Response Evaluation Criteria in Solid Tumors.

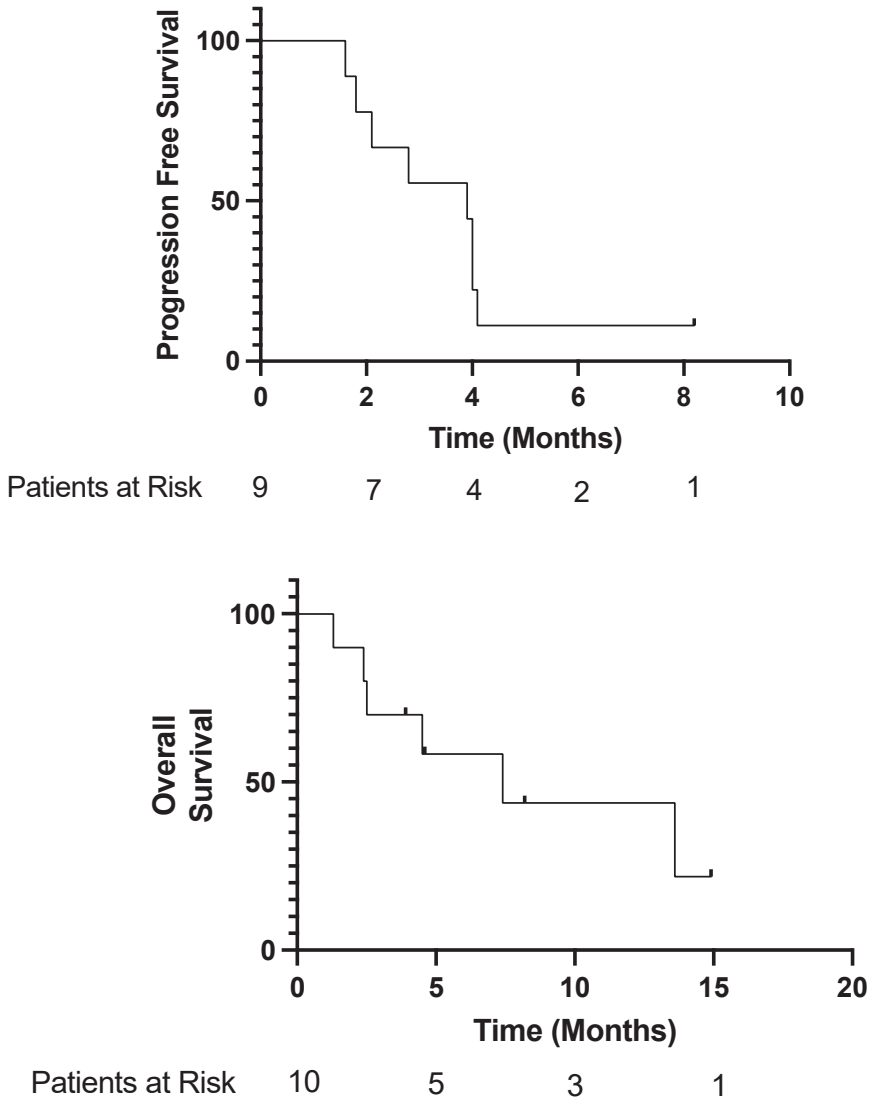

Figure 4. Kaplan-Meier curves demonstrating progression-free survival and overall survival in patients on trial.

Interestingly, two of the patients with readily observed immune responses (patients 6 and 8) also reported prolonged stable disease. Patient 9 also experienced prolonged stable disease and had elevated IFN-γ response at 6 weeks in the multiplex cytokine assay on peripheral blood testing but no other appreciable immune response. On the other hand, patient 7 had an observable vaccine-specific immune response but had a progression of disease in a nontarget lesion at 16 weeks. Thus, immune activation markers may identify a subset of patients with responses, though not all patients demonstrating immune activation achieve a radiographic response.

Our in vitro stimulation results identified WT1-specific immunogenicity, consistent with prior studies of GPS. Subsets of patients in a randomized phase 2 study in DPM and studies in acute myeloid leukemia reported WT1-specific immune responses in vitro with GPS.[11,15] Furthermore, a phase 1 study combining GPS and nivolumab in WT1-expressing epithelial ovarian cancer reported a vaccine-specific immune response in most patients.[27] Optimizing vaccine-specific immunity will be critical for enhancing responses to GPS.

Our results indicate that the combination of GPS and nivolumab stimulated vaccine-specific immunogenicity but not radiographic anticancer activity in patients with DPM. Perhaps these vaccine-induced T-cell populations are necessary, but not sufficient, for disease response owing to additional immunosuppressive pathways. Further analysis of the tumor microenvironment, such as measurement of PD-L1-specific T-cells and vaccine-specific humoral responses, may shed light on the fuller immunologic landscape after treatment with GPS and immune checkpoint inhibition. To better harness the potential of GPS in the treatment of mesothelioma, biomarkers associated with immune activation from GPS

need to be identified to select appropriate patients for potential future studies. Ultimately, combining a cancer vaccine with checkpoint inhibitors may be particularly useful in patients who do not respond to checkpoint inhibitors alone because their tumors are not sufficiently immunogenic to induce T-cell infiltration.[28–30]

The limitations of this study include its small study size, which constrained our ability to correlate disease outcomes with immune response data. In addition, not all patients had sufficient peripheral blood samples to perform immune response testing at multiple time points, which limits our interpretation of this data.

In summary, this phase 1 trial reported that the combination of GPS and nivolumab was safe and well-tolerated in patients with DPM. A subset of patients reported vaccine-specific immune responses, but objective responses and survival outcomes were suboptimal. Further investigation is needed to optimize the role of GPS in the treatment of DPM.

CRediT Authorship Contribution Statement

Prashasti Agrawal: Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing.

Michael Offin: Investigation, Writing - review & editing.

Victoria Lai: Conceptualization, Funding acquisition, Investigation, Methodology, Writing -review & editing.

Michelle S. Ginsberg: Investigation, Writing - review & editing.

Prasad S. Adusumilli: Investigation, Writing - review & editing.

Valerie W. Rusch: Investigation, Writing - review & editing.

Jennifer L. Sauter: Investigation, Writing - review & editing.

Teresa Ho: Investigation, Visualization, Writing - review & editing.

Phillip Wong: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - review & editing.

Marjorie G. Zauderer: Conceptualization, Funding acquisition, Investigation, Methodology, Data curation, Formal analysis, Project administration, Supervision, Writing - original draft, Writing - review & editing.

Disclosure

Dr. Offin has received consulting roles/honoraria with Novartis, Jazz, Pfizer, Targeted Oncology, OncLive, American Society for Radiation Oncology; grant support from the NIH/NCI, Druckenmiller Foundation, and LUNGevity Foundation; and is an uncompensated scientific advisory board member for the Mesothelioma Applied Research Foundation. Dr. Lai received a grant from the Conquer Cancer Foundation of ASCO for this project and has stocks in AstraZeneca. Dr. Adusumilli has consulting/advisory roles at Atara Biotherapeutics, Bio4T2, Carisma Therapeutics, ImmPACT-Bio, Imugene, Johnston & Johnston, Orion, Outpace Bio; research funding from Atara Biotherapeutics and Novocure. Dr. Sauter has stock and other ownership interests in Chemed, Merck, Pfizer, Thermo Fisher Scientific, and a consulting/advisory role at Vivace Therapeutics. Dr. Zauderer has received honoraria/consulting fees for Curis, Ikena, Takeda, GlaxoSmithKline, and Novocure; has received research funding from the Department of Defense, the National Institutes of Health, NCI, Medimmune, Vivace, Precog, GlaxoSmithKline, Epizyme, Polaris, Sellas Life Sciences, Bristol-Myers Squibb, Curis, Atara, Millenium; has received honoraria from CME Medscape and HMP Global; is on data safety monitoring board or advisory board on Roche Diagnostics and Curis, and is chair of the board of directors for the Mesothelioma Applied Research Foundation (uncompensated). The remaining authors declare no conflict of interest.

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Data Availability

The data generated in this study are not publicly available owing to patient privacy requirements but are available on reasonable request from the corresponding author.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2024.100756.

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