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Research Article

Lefitolimod in Combination with Ipilimumab in Patients with Advanced Solid Tumors: A Phase I Trial

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ABSTRACT

Introduction: TLR9 agonists are immunomodulators that have been of interest for combined use with cancer immunotherapy. TLR9 agonists, such as lefitolimod (MGN1703), significantly increased Th1 response in preclinical models and have demonstrated efficacy in early clinical trials. This trial assessed the safety and preliminary efficacy of the combination of lefitolimod and ipilimumab in patients with advanced solid tumors. **Methods:** This was a single-center, open-label, investigator-initiated phase I trial conducted at The University of Texas MD Anderson Cancer Center. Patients received leftolimod either subcutaneously (at escalating doses of 15–120 mg) or intratumorally (at the maximum feasible dose) in combination with ipilimumab (3 mg/kg). Paired biopsy samples were collected before the start of treatment and after two treatment cycles and analyzed by flow cytometry. **Results:** We enrolled a total of 28 patients in this study with a median age of 56 years (range 19–75) in the escalation cohort and 60 years (range 34 –92) in the expansion cohort. The median number of prior lines of therapy was 4 (range 0–12). Eleven patients had at least one treatment-related adverse event (TRAE). The most common TRAEs were skin rash ($n = 4$, 14%), fatigue ($n = 3$, 11%), and pruritis ($n = 2$, 7%). No grade 4 or 5 AEs occurred, and no patients required dose reduction or treatment discontinuation due to AEs. The maximum tolerated dose (MTD) was not reached in this study. Of 28 patients, 21 patients had response-evaluable disease. No patients had a complete or partial response; 8 and 13 patients had stable and progressive disease as the best response, respectively. Paired biopsy samples were obtained from five patients. Increases in intratumoral CD8 T-cell frequency, memory CD8 phenotype ($CD45RO^+$), and proliferation ($Ki67^+$) in four of five patients suggested that the combination of lefitolimod and ipilimumab led to proinflammatory immune conditioning of the tumor microenvironment. **Conclusions:** The combination of lefitolimod (administered subcutaneously or intratumorally) and ipilimumab was safe and well tolerated but demonstrated modest antitumor activity in patients with advanced cancers. **ClinicalTrials.gov ID: NCT02668770**

Keywords: immunotherapy, toll-like receptor, checkpoint inhibitor, intratumoral therapy, cancer therapeutics

INTRODUCTION

Immunotherapy, which is now considered the standard of care in many tumor types, has revolutionized cancer management over the past decade.[1,2] Nevertheless, primary and secondary immunotherapy resistance limits response or its durability; thus, new molecules have been explored with the intent of inducing a more potent activation of the immune system hypothetically, leading to improved clinical benefit.[3] These molecules include toll-like receptor (TLR) agonists, which were recently shown to induce innate and adaptive immunity against tumors,[4–6] especially when administered intratumorally (IT).[7] TLR9 agonists were specifically shown to increase Th1 responses[8] and were found to be active against solid tumors in preclinical models and early-phase clinical trials.[4,9] Lefitolimod (MGN1703), one such TLR9 agonist,[10] is a covalently closed dumbbell-shaped DNA molecule with 116 nucleotides, which has two single-stranded 30-base hairpin loops and a double-stranded 28-base-pair stem.

Preclinical and early clinical data have suggested that using TLR9 agonists with an immune checkpoint blockade could have a synergistic therapeutic effect.[11–15] This could be related to changes in the tumor microenvironment that result from treatment with TLR9. For example, some studies have shown that TLR9 agonists can upregulate PDL1 expression, which has been proposed as a predictive biomarker for sensitivity to immune checkpoint inhibitors.[5,16] Additionally, TLR9 agonists can sensitize poorly immunogenic tumors to an immune checkpoint blockade by promoting CD8+ T-cell responses within the tumor microenvironment.[15]

Therefore, we hypothesized that combining lefitolimod with ipilimumab (an anti–CTLA-4 immune checkpoint antibody) would be safe and clinically effective, possibly overcoming the immunosuppressive environment in advanced malignancies. In this phase I study (Clinicaltrials.gov ID: NCT02668770), we assessed the safety and preliminary efficacy of the combination of lefitolimod and ipilimumab in patients with advanced solid tumors.

METHODS

Study Design

This was a single-center, open-label, investigator-initiated, phase I trial testing the combination of lefitolimod (MGN1703) and ipilimumab. The study was conducted at The University of Texas MD Anderson Cancer Center between March 2017 and February 2020, according to the principles of the Declaration of Helsinki and Good Clinical Practice recommendations. The protocol was approved by the MD Anderson institutional review board. All patients provided written informed consent before participation in the study.

The primary objectives of this study were to evaluate the safety and toxicity profile of the combination of lefitolimod and ipilimumab and to determine the maximum tolerated dose (MTD) and recommended phase II dose (RP2D) for lefitolimod in this combination. Secondary objectives were to determine the antitumor activity of the combination of these drugs in advanced malignancies, to assess the intratumoral T-cell microenvironment in paired pretreatment and posttreatment tumor biopsy specimens, and to assess the relationship between pretreatment immune biomarkers and tumor response. The study was designed to include a dose-escalation phase with lefitolimod given subcutaneously (SQ) according to predefined dose levels (Supplementary Table S1, available online) and a dose expansion phase in three cohorts (arm 1 with lefitolimod, given SQ at MTD defined in dose escalation, arm 2 with lefitolimod, given intratumorally at maximum feasible dose, and arm 3 with lefitolimod, given SQ at MTD defined in dose escalation in patients who had radiation therapy within 2 weeks prior to study initiation). Enrollment in the second expansion arm (the intratumoral arm) was allowed once a dose level was shown to be safe in at least three patients with systemic administration. The study was closed early, and enrollment in expansion arms 1 and 3 was not completed (Supplementary Fig. S1).

Patients

Key inclusion criteria were the presence of histologically confirmed metastatic or locally advanced solid tumor that had failed to respond to or progressed despite standard therapy or for which standard therapy did not exist; Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less (equal to a Karnofsky Performance Scale score $>$ 60%); age of at least 18 years; measurable disease as defined by immune-related Response Evaluation Criteria in Solid Tumors or Response Evaluation Criteria in Solid Tumors 1.1; and adequate organ and marrow function.

Key exclusion criteria were severe autoimmune disease; history of acute diverticulitis, intra-abdominal abscess, gastrointestinal obstruction, abdominal carcinomatosis, or other known risk factors for bowel perforation; and/or previous exposure to TLR agonist therapy.

Treatment

In the dose-escalation cohort, patients were enrolled at escalating doses of lefitolimod (15, 30, 60, and 120 mg) and a fixed dose of ipilimumab (3 mg/kg). In each 3-week cycle, lefitolimod was administered SQ weekly, and ipilimumab was administered intravenously on day 8 of each treatment cycle. Dose reductions of lefitolimod were applied in patients who experienced treatment-related toxicities (Supplementary Table S1). The dose of ipililumab was based on the US Food and Drug Administration–approved dose for melanoma. During the expansion cohort, treatment was given in a similar schedule to the dose-escalation cohort, except that lefitolimod was administered IT at 15 mg, which corresponded to the

maximum volume considered feasible for an IT injection. Patients in the expansion cohort also received ipilimumab (3 mg/kg) every 3 weeks.

Patients received up to four treatment cycles for a total of 12 weeks of treatment. After four cycles, patients received weekly maintenance treatment of single-agent lefitolimod, administered SQ until the progression of the disease or intolerable toxicity.

Study Assessment

Safety was assessed by adverse events (AEs) evaluation and regular blood testing, which was done weekly during ipilimumab and lefitolimod coadministration, and every two cycles during maintenance treatment. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0. A dose-limiting toxicity (DLT) was defined as any AE that happened within the first 21 days with a severity grade of 3 or 4, which was considered possibly, probably, or definitely related to the combination treatment (Supplementary Methods). For considering an AE to be a DLT, the toxicity should have been attributed to the combination of lefitolimod and ipilimumab, not either agent alone. After the DLT period, patients were monitored for AEs until 90 days after maintenance treatment discontinuation.

Efficacy was assessed using positron emission tomography–computed tomography (PET/CT) scans every two cycles and evaluated according to immune-related response criteria, as described by Wolchok et al.[17]

Correlative Analysis

Tumor biopsy samples were collected no more than 2 weeks before the treatment start date and after the completion of two treatment cycles. As previously described, two cores were digested into single-cell suspensions with collagenase H (Sigma) and DNase (Roche).[18] Next, viable tumor-infiltrating immune cells were enriched via Histopaque-1119 (Sigma) density gradient centrifugation. The resulting preparation was then stained with a cocktail of antibodies to assess lymphocyte and myeloid cell tumor infiltration and phenotypes. Analysis was performed on a BD LSR II flow cytometer, and the results were analyzed using FlowJo version 10. For all patients enrolled in the study, peripheral blood mononuclear cells (PBMC) were isolated and purified by ficoll density gradient separation and frozen in aliquots of 1×10^7 cells per vial for subsequent analysis. PBMC analysis was done using flow cytometry for biomarkers of response to therapy. Specifically, PBMCs were analyzed for signs of myeloid activation consistent with the known activating function of lefitolimod. Arginase is an immunosuppressive enzyme prominently expressed by tumor myeloid stroma with the potential to repress tumor-specific T cells. Prior studies have described the potential of TLR9 agonists to reduce the expression of arginase by tumor myeloid cells.[19] We compared pretreatment PBMCs with those collected on day 15 of cycle 1. We grouped patients on the basis of whether they showed a decreased frequency of arginase-expressing $CD11b^+$, $CD11c^-$ myeloid cells (largely macrophages) with therapy, or an increased frequency. We hypothesized that reduced myeloid arginase would correlate with improved clinical responses.

Statistical Design

This protocol used a modified toxicity probability interval design. This adaptive method for determining the dose has been demonstrated to be superior to the traditional 3 + 3 methods for determining MTD. Modified toxicity probability interval software version 2.1 was used to generate the dose assignment table. Dose escalation of MGN1703 was escalated per schema to maximize their dose when given concurrently. At least three patients were enrolled per dose level. All patients enrolled at each dose level were evaluated for DLTs to determine the MTD. For this study, the equivalence interval was calibrated (25%, 35%) to achieve a DLT rate.

RESULTS

Between March 2017 and February 2020, 28 patients were enrolled in our study. The median age was 56 years (range 19–75) in the dose escalation cohort and 60 years (range 34–92) in the expansion cohort. Women comprised 68% of the patients in the escalation cohort and 89% in the expansion cohort. The types of cancers that patients had are summarized in Table 1. The median number of prior lines of therapy was 4 (range 0–12).

Two patients with malignant melanoma received lefitolimod on cycle 1, day 1, but were subsequently removed from the study prior to receiving ipilimumab due to rapid progression of underlying disease. Neither patient experienced significant treatment-related toxic effects.

Safety and Tolerability

All patients experienced at least one AE, and 11 experienced at least one treatment-related AE (TRAE). The most common TRAE was skin rash ($n = 4$, 14%), followed by fatigue ($n = 3$, 11%) and pruritis ($n = 2$, 7%). The reported TRAEs are summarized in Table 2. No grade 4 or 5 AEs occurred, no patients required dose reduction, and treatment was not discontinued due to AEs. Because no patients had DLT, MTD was not reached in this study.

Clinical Efficacy

Of the 28 patients enrolled, seven were not radiologically evaluable for response due to rapid clinical progression before the first restaging ($n = 4$), the finding of new lesions and nonmeasurable target lesions on positron emission tomography–computed tomography scan ($n = 1$), or the withdrawal of consent before the first restaging ($n = 2$). Among those 28 patients, no patients had a complete or partial response. Eight patients had stable disease as the best response, 12 had radiologically progressive disease by immune-related response criteria,

Table 1. Baseline patient characteristics

	Escalation Cohort (*n* = 9)	Expansion Cohort (*n* = 19)
Age, median (range), y	56 (19–75)	60 (34–92)
Sex, *n* (%)		
Female	13 (68)	8 (89)
Male	6 (32)	1 (11)
Malignancy, *n*		
Sarcoma (non-leiomyosarcoma)	3	1
Melanoma	2	3
Leiomyosarcoma	—	2
Cervical squamous cell carcinoma	2	—
Hepatocellular carcinoma	2	—
Mesothelioma	1	1
Skin squamous cell carcinoma	—	1
Breast cancer	—	1
Other*	9	—

*"Other" includes one patient with each of the following: adenoid cystic carcinoma, anal squamous cell carcinoma, bladder/transitional cell carcinoma, lung adenocarcinoma, pancreatic neuroendocrine tumors (high-grade), primary malignant neoplasm of the peritoneum, prostate adenocarcinoma, renal cell carcinoma, and uterine serous carcinoma.

and one had clinically progressive but radiologically stable disease (Fig. 1).

Correlative Analysis

Paired biopsy samples were obtained from five patients in dose expansion and analyzed by flow cytometry. The pre- and posttreatment biopsies for TIL analysis were obtained from the same lesion, and in the case of the intratumoral patient shown as "PT36" (Fig. 2), they were from a noninjected lesion. Increases in intratumoral CD8 T-cell frequency, memory CD8 phenotype ($CD45RO^+$), and proliferation ($Ki67^+$) in four of five patients suggested proinflammatory immune conditioning of the tumor microenvironment by the combination of lefitolimod and ipilimumab (Fig. 2). These beneficial effects may have been counteracted to some degree by the numerical increase in CD8 T-cell expression of the immune checkpoint protein PD-1 seen in all five patients. Although all five patients showed evidence of increased CD4 effector T-cell maturation ($CD45RO^+$), other findings were mixed, with three of five patients showing enrichment in frequency, two of five showing increases in proliferation, and three of five showing increased PD-1 induction.

PBMCs were collected from most included patients and analyzed for possible reduction in myeloid Arginase correlating with improved clinical outcomes. However, there was no significant correlation between progression at first restaging and peripheral changes in Arginase-positive myeloid populations (Fig. 3). Patients exhibiting peripheral myeloid Arginase reduction were more likely to exhibit HLA-DR upregulation (a marker of myeloid activation); however, HLA-DR upregulation was not significantly correlated with clinical response. The patients in whom macrophage Arginase reduction was observed tended to show dendritic cell Arginase reduction; however, the monocyte-enriched population ($CD11b^+c^+$) showed a distinct pattern of pre- to posttreatment Arginase expression. No significant correlations with the lefitolimod dose were observed for myeloid Arginase downregulation or HLA-DR upregulation (Fig. 3), suggesting that either changes in these circulating myeloid markers of activation and/or suppression may not have been reflective of intratumoral myeloid dynamics, or that the drug failed to impact myeloid activation state as preclinical TLR9 agonist studies would have predicted.

For a limited number of patients, we performed similar analyses comparing PBMC samples collected on day 15 of cycle 1 and those collected on day 15 of cycle 2. In this case, patients 18, 20, 39, 44, and 53 exhibited Arginase downregulation in their $CD11b^+$, $CD11c^-$ circulating myeloid cells, whereas patients 10, 35, and 36 exhibited an increase in Arginase expression in the same population (Fig. 4). Reduction in Arginase in the macrophage population tended to correlate with reduction in the proliferation marker Ki67 and the immunosuppressive ligand PD-L1 but not with induction of HLA-DR.

Table 2. Treatment-related adverse events (TRAEs) with lefitolimod and ipilimumab treatment

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Skin rash	3 (10)	1 (4)	—	—	—
Fatigue	2 (7)	—	1 (4)	—	—
Pruritis	1 (4)	1 (4)	—	—	—
Mucositis	—	—	1 (4)	—	—
Anemia	1 (4)	—	—	—	—
Dyspnea	1 (4)	—	—	—	—
Blurred vision	—	1 (4)	—	—	—
Thrombocytopenia	—	—	1 (4)	—	—
Arthritis	—	1 (4)	—	—	—
Hypothyroidism	—	1 (4)	—	—	—
Pain in the tumor	1 (4)	—	—	—	—
% of patients with TRAE	33	20	12	0	0
Number of 28 patients with TRAE	9	5	3	0	0

Values are presented as *n* (%).

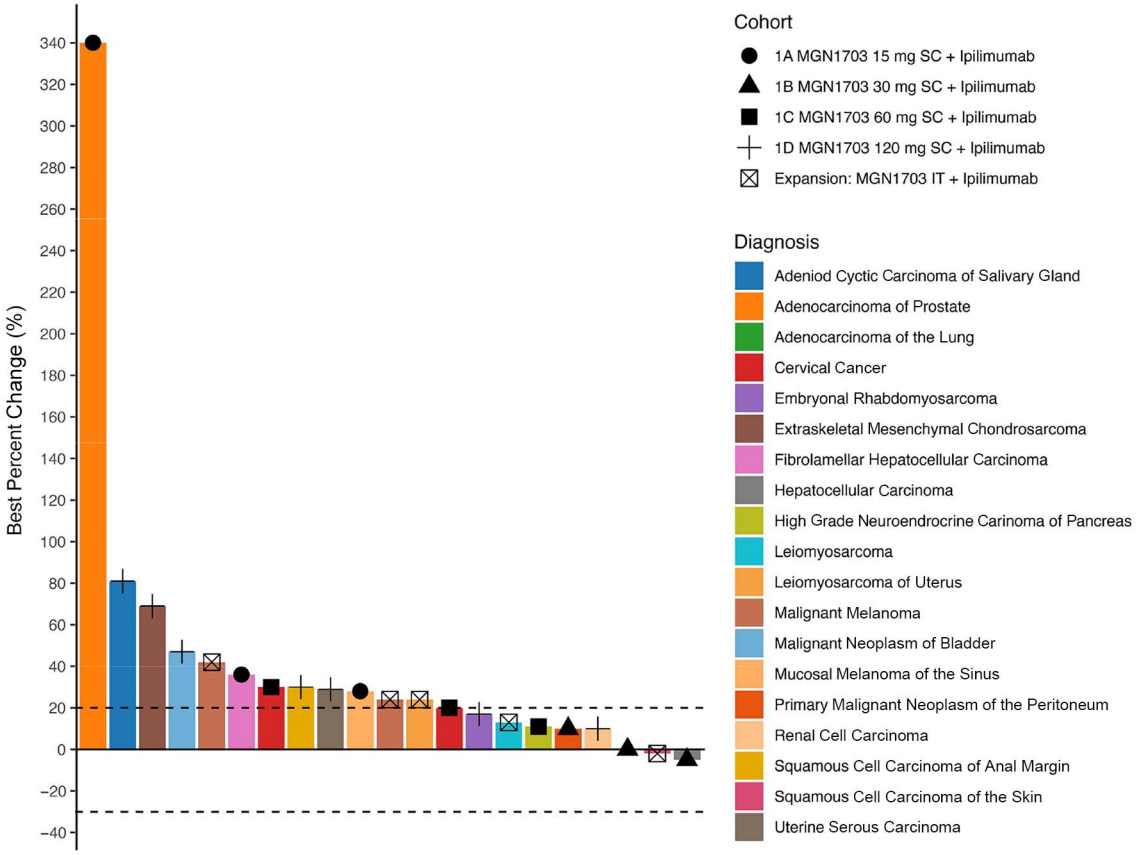

Figure 1. Waterfall plot of best treatment response compared with baseline.

Interestingly, all patients with stable disease showed upregulation of HLA-DR in circulating macrophages, while only one patient with progressive disease showed such upregulation, potentially suggesting HLA-DR may be a biomarker of response worth investigating in a larger cohort. Of note, this trend was not observed in the circulating monocyte and dendritic cell fractions. Across both the pretreatment to cycle 1 and cycle 1 to cycle 2 analyses, changes in Arginase level were consistent in both trajectory and magnitude for monocytes and dendritic cells for Patients 10, 18, 20, 35, 36, and 44, with the only change being a more pronounced increase in Arginase expression in both populations for patient 39 in the cycle 1 to 2 period versus pretreatment to cycle 1 (Figs. 3 and 4).

In contrast, macrophage arginase varied in both trajectory and magnitude for patients 18, 20, 36, and 39 between pretreatment to cycle 1 versus cycle 1 to cycle 2. Changes in Ki67 and PD-L1 expression on therapy, however, were consistent among all three myeloid cell populations. Given the small number of intratumoral patients with serial

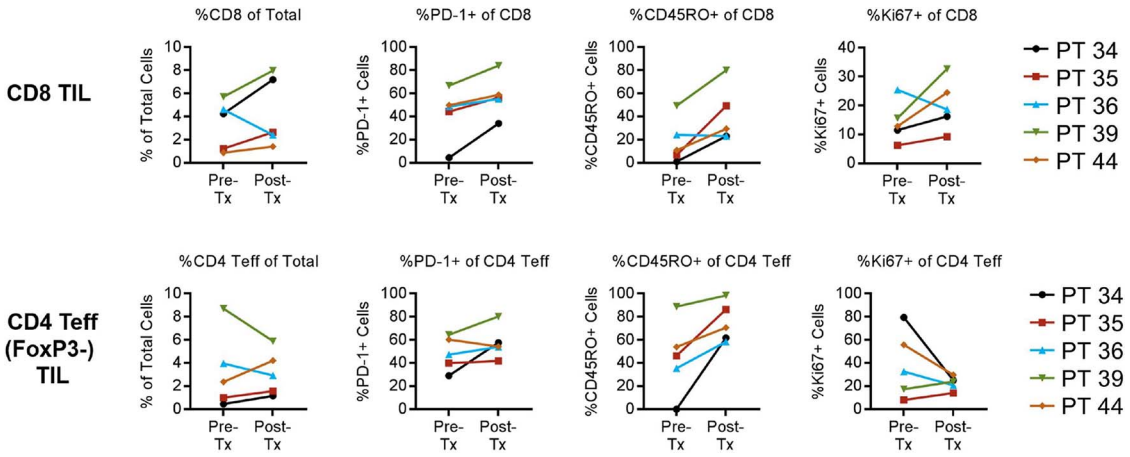

Figure 2. Phenotyping of infiltrating CD8 and CD4 effectors from paired pretreatment and posttreatment biopsy specimens. PT: patient; Teff: effector T cells; TIL: tumor-infiltrating lymphocytes; Tx: treatment.

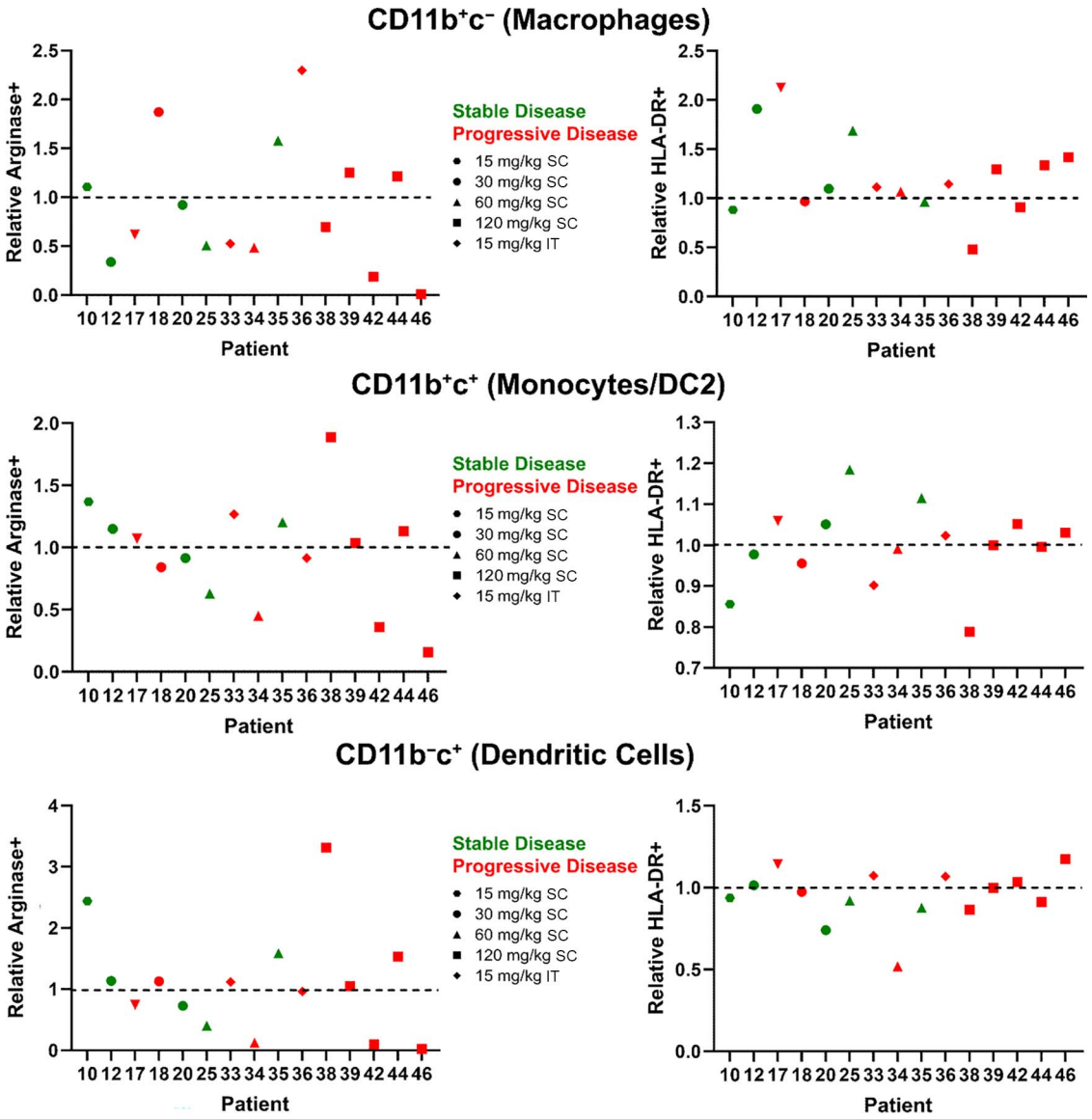

Figure 3. Phenotypic changes in myeloid peripheral blood mononuclear cells (PBMC) cells from before treatment to day 15 of cycle 1.

blood samples available for analysis, it is not possible to comment on potential differences between the impact of SQ and IT therapy on the circulating myeloid biomarkers examined here. Patient 53 did show downregulation of Arginase and PD-L1 and upregulation of HLA-DR between cycle 1 and cycle 2, which might suggest an improvement that was reflected in the circulation that developed over successive cycles of therapy; unfortunately, we did not have a pretreatment blood sample from that patient to assess change triggered by cycle 1.

DISCUSSION

We conducted a phase I clinical trial to test the combination of lefitolimod and ipilimumab in patients with advanced cancers. The dose-escalation phase was conducted with SQ injections of 15, 30, 60, and 120 mg of lefitolimod and a fixed dose of 3 mg/kg of ipilimumab, while the expansion phase was conducted with IT injection of 15 mg of lefitolimod, also in combination with ipilimumab. Skin rash, fatigue, and pruritis were the most common TRAEs. The MTD was not reached in this study. While the combination appeared preliminary safe, clinical activity was modest, with no patients having a RECIST response and a small cohort having stable disease as the best response.

One important finding of our study is that IT administration of the TLR9 agonist lefitolimod was safe and well tolerated. IT administration of immunotherapies is a targeted approach to manipulate the tumor immune microenvironment. Directed treatments, particularly for tumors with rich immune cell infiltrates, are an opportunity for enhanced local activation with reduced off-target (i.e., systemic) toxicities. Our findings suggest IT administration was both safe and feasible and supports future efforts for IT administration.

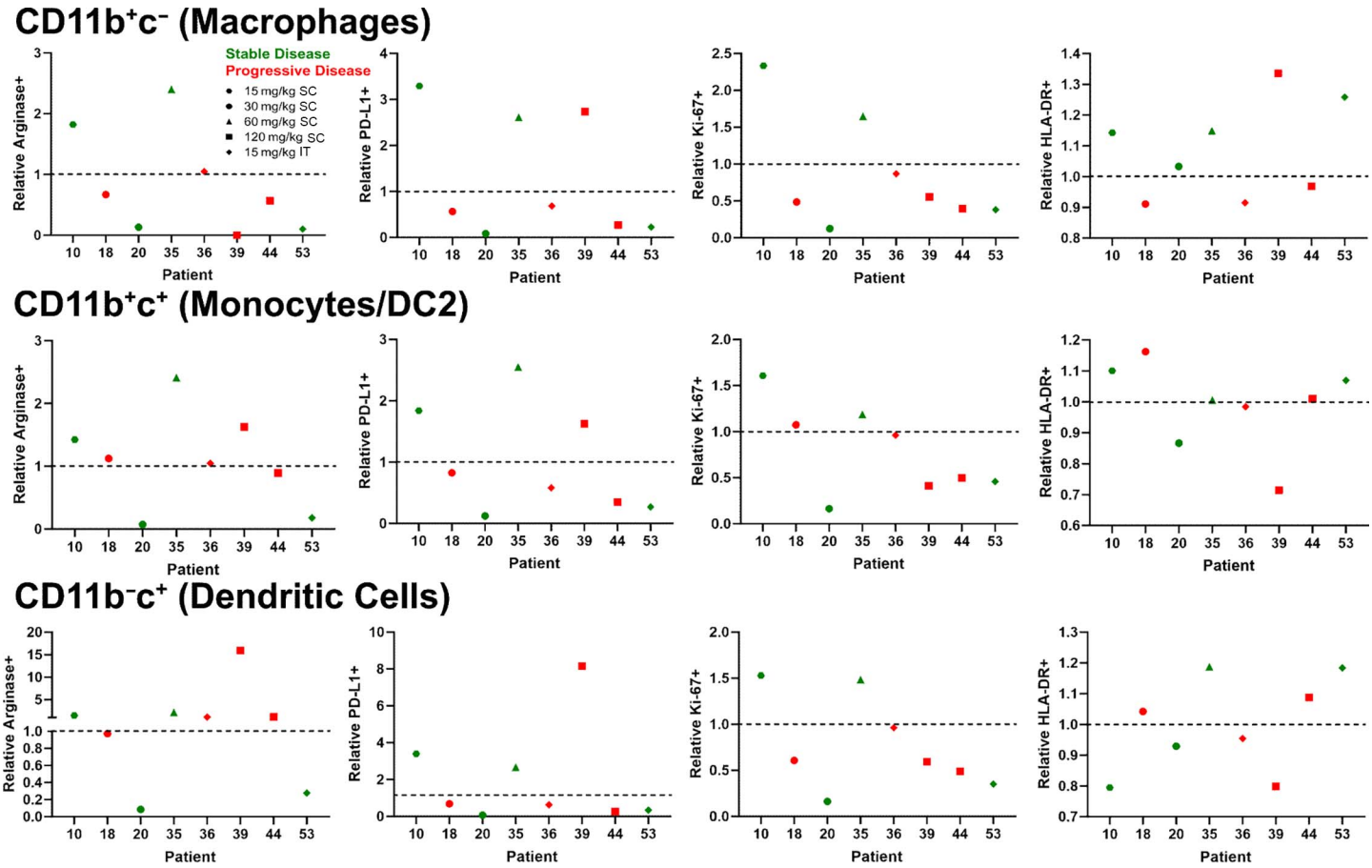

Figure 4. Phenotypic change in peripheral blood mononuclear cells (PBMC) myeloid cells from day 15 of cycle 1 to day 15 of cycle 2 of treatment. IT: intratumorally; SC: subcutaneously.

Immune-mediated AEs are potentially fatal events associated with immunotherapy. In this trial, the combination of lefitolimod and ipilimumab was not associated with a higher incidence of AEs than the incidence previously reported for single-agent ipilimumab.[20] This finding is concordant with data in the literature on the combinations of TLR9 agonists and immune checkpoint inhibitors, in which combination treatments were associated with no more AEs than immune checkpoint inhibitor monotherapy.[21]

In terms of efficacy, stable disease in 8 of 28 patients was the best response that was observed. There were no objective responses, similar to what was observed in previous trials with TLR9 agonists. The response rate described in previous phase I and II trials was at most 20% for single-agent TLR9 agonists.[9,22–26] Similarly, response rates in trials of combinations of TLR9 agonists were not significantly higher than response rates in single-agent trials.[27–30] The trials that tested combinations of TLR9 agonists and immune checkpoint inhibitors demonstrated initial responses.[25,31,32] However, the phase III clinical trial that tested a TLR9 agonist (tilsotolimod) in combination with ipilimumab in melanoma (ILLUMINATE-301) did not meet the coprimary endpoint of response rate.[33] Therefore, TLR9 agonists in combination with immune checkpoint inhibitors have not been proven to be an appealing treatment in terms of efficacy.

We collected tumor-infiltrating lymphocytes and evaluated CD4 and CD8 populations in pre- and post-treatment biopsy specimens. Our findings suggested that this combination improved CD8 T-cell infiltration into the tumor microenvironment but that the therapeutic impact of these cells may have been limited by concomitant upregulation of the immune checkpoint protein PD-1. These findings suggest that a more durable benefit might be possible by adding PD-1 blockade to this regimen; however, a triple combination, if administered concurrently, would carry a significantly higher rate of immune-related AEs. Following therapy, a subset of patients showed downregulation of arginase in their circulating myeloid cells (particularly macrophages) accompanied by other positive signs of TLR9 response, including HLA-DR upregulation and, in some cases, PD-L1 downregulation. These changes did not seem to correlate with clinical outcomes.

As a single-institution phase I clinical trial, this study had limitations. The limited number of patients may have led us to underestimate the frequency of severe AEs. Also, evaluating the combination of lefitolimod and ipilimumab in diverse cancer types, including cancers with varying degrees

of response to immunotherapy, may have obscured signals of efficacy in a specific tumor type.

CONCLUSIONS

The combination of lefitolimod (administered SQ or IT) and ipilimumab is safe and well tolerated, but it had modest antitumor activity in patients with advanced cancers. Further investigations of this combination would need to rely on other data to study efficacy in different disease settings.

Conflicts of Interest

Matthew J. Reilley reports research funding from Adeli-Norte, Astrazeneca, Cardiff Oncology, Deciphera, Macrogenics, Merck, Pfizer, Seattle Genetics, Surface Oncology, Xencor, and ZielBio; and consulting/advisory role for Cardinal Health, Curio Science, Helsinn, Pfizer, and Seattle Genetics. *Sarina A. Piha-Paul* reports research funding (to institution) from AbbVie, ABM Therapeutics, Acepodia, Alkermes, Aminex Therapeutics, BioMarin Pharmaceutical, Boehringer Ingelheim, Bristol Myers Squib, Cerulean Pharma, Chugai Pharmaceutical Co., Curis, Cyclacel Pharmaceuticals, Daiichi Sankyo, Dohme, Eli Lilly, ENB Therapeutics, Epigenetix, Five Prime Therapeutics, F-Star Beta Limited, F-Star Therapeutics, Gene Quantum, Genmab A/S, Gilead Sciences, GlaxoSmithKline, Helix BioPharma, Hengrui Pharmaceuticals, HiberCell, Immunomedics, Incyte, Jacobio Pharmaceuticals, Jazz Pharmaceuticals, Jiangsu Simcere Pharmaceutical Co., Loxo Oncology, Lytix Biopharma AS, Medimmune, Medivation, Merck Sharp, Nectin Therapeutics, Novartis Pharmaceuticals, Nurix, OncoNano Medicine, Pieris Pharmaceuticals, Pfizer, Phanes Therapeutics, Principia Biopharma, ProFoundBio US, Puma Biotechnology, Purinomia Biotech, Rapt Therapeutics, Replimune; Roche/Blueprint, Seattle Genetics, Silverback Therapeutics, Shasqi, Synlogic Therapeutics, Taiho Oncology, Tallac Therapeutics, Tesaro, Theradex Oncology, Toragen Therapeutics, TransThera Bio, Xencor, ZielBio; and consulting for CRC Oncology and Lilly USA.

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Data Availability

Complete study data are available by contacting the corresponding author.

Supplemental Material

Supplemental materials are available online with the article.

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