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Phase I dose-escalation and pharmacodynamic study of STING agonist E7766 in advanced solid tumors

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ABSTRACT

E7766 is a novel stimulator of interferon genes (STING) agonist, capable of potent activation of immune cells and generating strong antitumor response in preclinical murine tumor models. Here we present the safety, efficacy, and biomarker results of the first-in-human phase I/Ib study of intratumoral E7766 in patients with advanced solid tumors. Eligible patients with relapsing/refractory cancers (n=24) were enrolled in dose-escalating cohorts to receive intratumoral injections of E7766 from 75 to 1000 µg. The most frequent treatment-related treatment-emergent adverse events were chills (50.0%; 85.7%), fever (40.0%; 85.7%), and fatigue (30.0%; 35.7%) in patients who received non-visceral and visceral injections, respectively. Eight patients (33.3%) achieved stable disease as their best response per modified Response Evaluation Criteria In Solid Tumors version 1.1 with variability between injected and non-injected lesions. Plasma levels of IFN-α, IFN-β, IFN-γ, TNF-α, IL-6, IP-10, MCP1, and MIP1b transiently increased in all evaluable patients within 10 hours postinjection, then dropped to baseline levels. Levels of blood and tumor gene expression increased in most interferon-related and STING genes tested. Further increases in programmed death ligand 1 and cluster of differentiation 8 expression at both the RNA and protein levels were also observed in some patients across dose levels. In total, E7766 generated on-target pharmacodynamic effects in patients with solid tumors. Further exploration in a homogeneous patient population is necessary to assess efficacy.

INTRODUCTION

Advancements in immunotherapy have significantly improved clinical outcomes for patients with various tumor types[1]; however, most patients do not benefit, and some acquire treatment resistance.[2] One potential approach to overcome resistance to cancer immunotherapy is the induction of de novo antitumor immune responses via the generation of type I interferons (IFNs).[3] Substantial interest in the field has focused on the cyclic GMP–AMP synthase–stimulator of IFN genes (STING) pathway as a critical human mechanism for sensing non-self-cytosolic DNA.[4] Activation of STING induces host immunity via type I IFN and other pro-inflammatory cytokines.[4 5]

The STING pathway plays a critical role in bridging the innate immune system and adaptive immunity via regulation of type I IFN and canonical NF-kB pathways.[6] Stimulation of STING via synthetic STING agonists generates a potent antitumor response in multiple non-clinical models,[7] and multiple STING agonists are in various stages of clinical development.[3 8]

E7766, a potent macrocycle-bridged STING agonist, comprises two deoxyadenosine moieties linked by two phosphorothioates and one olefin linker between two purine bases.[9 10] In non-clinical studies, E7766 demonstrates potent antitumor activity by inducing a robust and effective, innate and adaptive antitumoral immune response.[11] Moreover, E7766 activation of the STING pathway results in activation of the IFN pathway and infiltration of T and natural killer cells, leading to antitumor activity.[12]

In this first-in-human, open-label, multicenter phase I study, we report the safety and efficacy of intratumoral E7766 as well as present a series of assays exploring tumor and peripheral blood biomarker correlates associated with E7766 treatment.

METHODS

Study design and patients

This clinical trial pursued dose-escalation that was conducted using an improved modified toxicity probability interval design[13 14] to determine the maximum tolerated dose (MTD) for E7766. E7766 was administered intratumorally at doses ranging from 75 to 1000 µg/mL on days (D) 1, 8, and 15 of cycle (C) 1 (induction cycle) and on D1 of each subsequent 21-day (Q3W)

cycle (maintenance cycle). E7766 was diluted with saline to achieve a final injection volume of 1 mL. Tumor response was assessed by investigators according to modified Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1[15] followed by modified RECIST for immune-based therapeutics, iRECIST.[16]

Eligible patients were adults (≥18 years of age) with solid tumors that were advanced, non-resectable, or recurrent and progressing since the last antitumor therapy, and for which no alternative standard therapy exists. Patients were required to have a minimum of one injectable lesion (cutaneous or deep visceral lesion) that was also accessible for biopsy, and if available, one other measurable lesion also accessible for biopsy, and an Eastern Cooperative Oncology Group Performance Status of 0 or 1. The longest axial tumor diameter was required to be ≥1 to 3 cm or ≥1.5 to 3 cm by short axis diameter in the case of a nodal lesion. Tumor size was determined based on the estimated drug dose required to achieve a homogenous response and a meaningful and tolerable clinical outcome. Study objectives are included in online supplemental material (the protocol is provided as supplemental file 2).

This study (NCT04144140) was performed in compliance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and all applicable local Good Clinical Practice guidelines and regulations. A list of institutional review boards/ethics committees is provided in online supplemental table 1. Informed consent was obtained after the study had been fully explained to each patient and before the conduct of any screening procedures or assessments.

Biomarker analyses

Tissue-based biomarkers

Tumor biopsies were collected during pretreatment and on C2D1. Tumor biopsies were obtained from injected lesions and one predefined non-injected lesion (if available), to examine the potential intratumoral STING pathway modulation and antitumor immune response enhancement following E7766 treatment at both local and systemic levels.

Formalin-fixed paraffin-embedded tumor tissue samples were processed for RNAseq using ImmunoID NeXTplatform (Personalis, California, USA). The transcripts per kilobase million (TPM) values were calculated for genes related to the STING pathway, immune activation, and immune infiltration. Protein expression of programmed death ligand 1 (PD-L1) and cluster of differentiation 8 (CD8) was evaluated by immunohistochemistry-based assays (Neogenomics, Florida, USA).

Peripheral blood biomarkers

Blood biomarker samples were collected before the first administration of E7766 and during the treatment process. Plasma samples were analyzed for immune cytokine expression using electrochemiluminescence-based multiarray assays (TNF-α, IFN-γ, IL-6, IP-10, MCP1, MIP1B) or Simoa assays (IFN-α, β, γ).

Peripheral blood mononuclear cells (PBMCs) were subjected to gene expression profiling using a custom assay panel that includes the top upregulated genes in PBMCs, tumors, and blood that were observed in preclinical models post E7766 treatment, and upregulated genes in PBMCs of human patients with STING gain-of-function mutation.[17]

Statistical analysis

All safety analyses were performed on the Safety Analysis Set defined as the total number of patients who received at least one dose of the study drug. Efficacy analyses were performed on patients who received at least one dose of the study drug and had a baseline tumor assessment.

RNA levels from 26 genes curated from the literature as associated with the STING pathway (labeled as pharmacodynamic genes) and eight housekeeping genes were selected to assess the effective concentration of E7766. Log2 fold change (FC) at each visit was calculated against the corresponding 0-hour value.

To analyze plasma chemokine/chemokine data, values lower than the lower limit of qualification (LLOQ) of the assay were replaced by LLOQ/2 for that marker; values larger than the upper limit of qualification (ULOQ) were replaced by ULOQ×2. For gene expression data from PBMC or tumor samples, log2 FC at each visit time was calculated against the corresponding value at 0 hours for each day or at screening time.

To analyze the association of baseline STING TPM with tumor response, two study groups were assigned based on the size of the first injected lesion: stabilized (for those patients with a maximum tumor growth of <20%) and progressed (for those patients with a maximal tumor growth of ≥20%). A two-sample Wilcoxon rank-sum test between the two groups was performed.

RESULTS

Patients

Of the 24 patients in the full analysis set, 9 (37.5%) were ≥65 years of age, and 14 (58.3%) were male (table 1). The study included a heterogeneous group of patients with advanced solid tumors who received either 1 (n=1 (4.2%)), 2 (n=5 (20.8%)), or ≥3 (n=18 (75%)) prior anticancer regimen; 2 (8.3%) patients received treatment for locally advanced disease, and 19 patients (79.2%) for metastatic disease. The highest dose of E7766 tested was 1000 µg. MTD and recommended phase 2 dose (RP2D) were not defined.

Safety

The dose-limiting toxicity (DLT) analysis set included 23 patients. Of these patients, three (13.0%) had DLTs during the first cycle; two experienced DLTs at the 600 µg dose (grade 2 tachycardia, grade 3 fatigue, and grade 2 dyspnea exertional in one patient; and grade 3 confusion

Table 1 Baseline characteristics and treatment information of patients in the dose-escalation phase

Characteristic	E7766 (75–1000 µg) N=24
Median age, years (range)	60.5 (32, 78)
≥65 years, n (%)	9 (37.5)
Sex, n (%)	
Male	14 (58.3)
Race, n (%)	
White/Black or African American	23 (95.8)/1 (4.2)
ECOG PS, n (%)	
0/1	13 (54.2)/11 (45.8)
Number of previous anticancer regimens, n (%)	
1/2	1 (4.2)/5 (20.8)
≥3	18 (75.0)
Type of previous therapy*, n (%)	
Adjuvant/neoadjuvant	7 (29.2)/4 (16.7)
Locally advanced/metastatic	2 (8.3)/19 (79.2)
Maintenance	4 (16.7)
Patients with previous anti-CTLA-4 therapy, n (%)	
Yes/no	5 (20.8)/19 (79.2)
Patients with previous anti-PD-L1/PD-1 therapy, n (%)	
Yes/no	12 (50.0)/12 (50.0)
Patients with target lesions, n (%)	
Lymph node/non-lymph nodes	11 (45.8)/19 (79.2)
Patients with non-target lesions, n (%)	
Yes/no	20 (83.3)/4 (16.7)
Location of injected lesion†, n (%)	
Visceral/non-visceral	10 (41.7)/14 (58.3)
Number of injected lesions per patient, n (%)	
1/2/3	18 (75.0)/5 (20.8)/1 (4.2)
Median size of injected lesion at screening, range (mm)‡	
Short axis diameter/longest diameter	21.0 (16.0, 39.0)/25.0 (10.0, 78.1)
Median total dose administered, range (µg)	2400 (525, 6240)

*Patients may be counted in multiple categories.
†Visceral injections include "musculoskeletal–soft tissue–trunk," "abdomen/pelvis," "chest," "liver mass," "lung mass'; non-visceral injections include "lymph node–neck adenopathy," "lymph node–abdominal and pelvic adenopathy," "lymph node–thoracic adenopathy," and "skin–extremity."
‡Target lesion is measured according to short axis diameter in malignant lymph nodes and longest diameters in other solid tumors.
CTLA-4, cytotoxic T-lymphocyte associated protein 4; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

in another patient), and one patient experienced a DLT (grade 3 fatigue) at the 780 µg dose.

Overall, 70% of patients with non-visceral injections (n=10) and 100% of patients with visceral injections (n=14) experienced treatment-related treatment-emergent adverse events (TRAEs) of any grade (table 2). TRAEs of grade ≥3 occurred in 20% of patients with non-visceral injections and 42.9% of patients with visceral injections.

The most common TRAEs in patients who received non-visceral or visceral injections were chills (50.0%; 85.7%), fever (40.0%; 85.7%), and fatigue (30.0%; 35.7%) (table 2). Of note, both chills and fever started at a median of 1 day after dose administration (range 1, 9); the median duration of the first occurrence of chills was 1.5 days (range 1, 4); and the median duration of the first occurrence of fever was 1 day (range 1, 3).

Efficacy

In the full analysis set of the dose-escalation phase, the median progression-free survival was 1.25 months (95% CI 1.18 to 2.53), and the median overall survival was 13.96 months (3.98–not estimable). Stable disease was reported as the best tumor response in eight patients (33.3%) with no observed trend of association overall between injected dose and overall modified RECIST version 1.1 outcome. No responses by modified RECIST version 1.1 were observed. Of eight patients with stable disease, five patients (62.5%) received previous anti-PD-L1/programmed cell death protein 1 therapy, and two (25.0%) received previous anti-CTLA4 therapy (online supplemental table 2).

The patient with the greatest degree of overall tumor reduction had gastro-esophageal cancer and was administered E7766 at 75 µg into a cervical lymph node mass. Consequently, this patient experienced a reduction in locoregional cervical and supraclavicular disease as well as non-injected (pulmonary metastasis) tumor shrinkage (figure 1A,B).

All target lesion evaluations were collected in the electronic database. Although some patients had multiple lesions injected, only the reduction of the first injected lesion was shown in figure 1C. The reduction in the sum of non-injected target lesions and of all target lesion diameters are represented for each patient in figure 1D,E, respectively.

In an exploratory analysis, RNAseq data at baseline were analyzed for STING gene expression in relationship to the maximal tumor growth of the first injected lesion: the first injected lesion stabilized if maximal tumor growth was <20% versus the first injected lesion progressed if maximal tumor growth was ≥20% (figure 1F). Data were available from injected sites in 15 patients and non-injected sites in 11 patients. Baseline STING TPM values appeared to be greater in patients whose first injected lesion stabilized whether these lesions were non-injected (p=0.082) or injected (p=0.016) (figure 1F). Of note, no statistically significant correlations with clinical outcomes could be identified with germline states.

Biomarkers

Plasma levels of IP-10, MIP-1b, MCP-1, IFN-α, IFN-γ, IL-6, TNF-α, and IFN-β were available for all 24 patients. Plasma biomarker levels started to increase around 4 hours, reached maximum levels around 6–10 hours postdosing on C1D1 and C1D15 in most patients, and

Table 2 Treatment-related treatment-emergent adverse events occurring in ≥20% of patients in either injection group—dose-escalation phase (Safety Analysis Set)

	Non-visceral* Injection (n=10)		Visceral† injection (n=14)	
Preferred term, n (%)	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**
Patients with any TRAEs	7 (70.0)	2 (20.0)	14 (100.0)	6 (42.9)
Chills	5 (50.0)	0	12 (85.7)	0
Fever	4 (40.0)	0	12 (85.7)	2 (14.3)
Fatigue	3 (30.0)	1 (10.0)	5 (35.7)	2 (14.3)
Nausea	3 (30.0)	0	2 (14.3)	0
Injection site pain	2 (20.0)	1 (10.0)	5 (35.7)	1 (7.1)
Injection site reaction	2 (20.0)	0	4 (28.6)	0
Vomiting	2 (20.0)	0	3 (21.4)	0
Tachycardia	2 (20.0)	0	3 (21.4)	0
Hypotension	0	0	3 (21.4)	1 (7.1)

Percentages are based on the total number of patients in the Safety Analysis Set within the relevant group.
Medical Dictionary for Regulatory Activities (MedDRA) preferred terms "Neoplasm Progression," "Malignant Neoplasm," and "Disease Progression" that are unrelated to the study drug are excluded.
Adverse events were coded using MedDRA V.25.0.
Patients with two or more TRAEs reported in the same preferred term are only counted once.
*Non-visceral injections include "lymph node–neck adenopathy," "lymph node–abdominal and pelvic adenopathy," "lymph node–thoracic adenopathy," and "skin–extremity."
†Visceral injections include "musculoskeletal–soft tissue–trunk," "abdomen/pelvis," "chest," "liver mass," "lung mass."
TRAE, treatment-related treatment-emergent adverse event.

then dropped quickly to almost baseline levels 24 hours postdosing (figure 2).

Levels of IP-10 and IFN-β at 6 hours were significantly higher than baseline ($p<0.05$) in patients who received E7766 at 600 µg or 780 µg on C1D1 and in patients who received E7766 at 600 µg on C1D15 (online supplemental figure 1). The level of cytokines detected at 780 µg increased similarly to those levels at 600 µg suggesting that cytokine levels are not linked to the dose of E7766 injected.

Twenty-one patients had samples available for PBMC-gene expression analysis and 11 patients had samples available for tumor-tissue gene expression analysis (online supplemental figures 2 and 3). Of the 26 PBMC-derived genes tested, 15 showed consistent increase, with 50% having log 2FC>1 (online supplemental figure 2). None of the eight housekeeping genes showed changes associated with treatment. RNA gene expression was also analyzed in tumor tissue extracted between screening and C2D1. Among most patients who had paired samples at baseline and C2D1, TPM values increased post-treatment in most selected target genes and were observed in all tumor samples from injected sites (online supplemental figure 3).

RNAseq data related to PD-L1 and CD8 gene expression was available in paired tumor tissue samples at baseline and on C2D1 in 11 patients. Among these 11 patients, 7 had paired samples from injected sites and 9 had paired samples from non-injected sites. Of patients with paired samples available in injected and non-injected sites, CD8 RNA expression levels and percentage of CD8-positive lymphocytes increased post-treatment on C2D1 compared with baseline in both injected and non-injected sites in two patients who received 150 and 300 µg (online supplemental figure 4A,B). PD-L1 RNA levels and PD-L1 protein expression based on combined positive score increased post-treatment in all three patients who were treated with E7766 at doses from 75 to 300 µg and had paired samples available (online supplemental figure 4C,D). Of four patients treated with E7766 at 600 µg, three showed an increase in PD-L1 RNA levels at drug-injected sites; one patient showed a mild increase in PD-L1 RNA levels at the non-injected site. One patient treated with E7766 at 780 µg showed no changes in PD-L1 RNA levels in the injected site and a decrease in PD-L1 RNA levels and CPS score in the non-injected site.

DISCUSSION

In this phase I study, E7766 was tolerated at a dose of up to 780 µg and demonstrated target engagement, as indicated by peripheral blood and tumor cell gene expression profiling centered on interferon signaling. The most common TRAEs were chills and fever, which are consistent with AEs reported previously in other IFN pathway agonists.[8 18 19]

Following treatment with E7766, the systemic expression of pro-inflammatory cytokines such as IP-10, IFN-α, IFN-β, and IL-6, increased when E7766 was administered at doses >300 µg. Interestingly, two patients who received the lowest dose level at 75 µg demonstrated stable disease, and one patient appeared to benefit with demonstrable tumor regression in

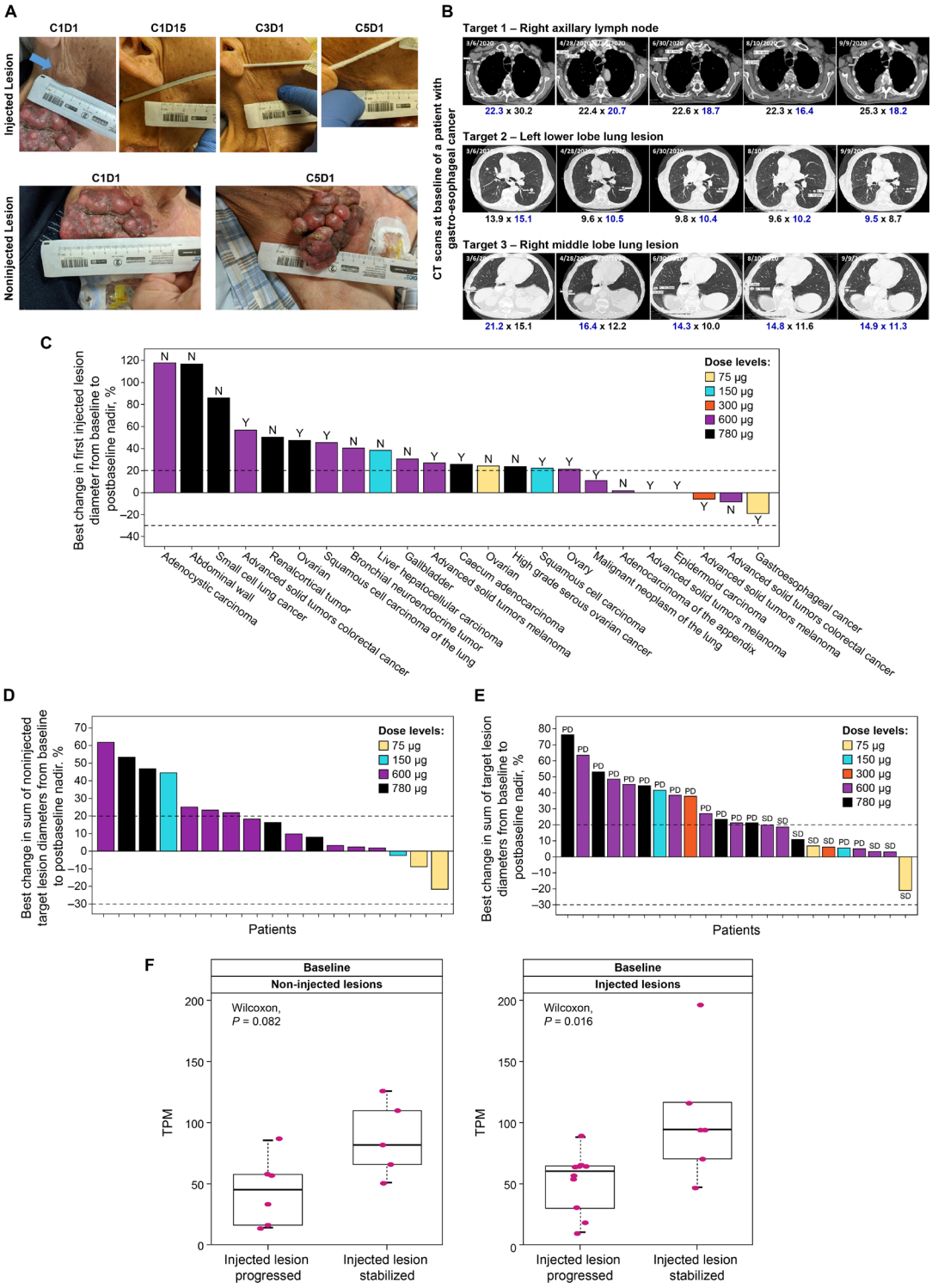

Figure 1 Tumor response in a patient with gastro-esophageal cancer (A, B)[a]; percentage change in the first injected lesion diameter (C[b,c,d]); sum of non-injected target lesion (D[c,d,e]), and all target lesion (E[d]) diameters from baseline to postbaseline nadir by dose level; and baseline STING expression[f] by maximal tumor growth of the first injected lesion (F[g]). [a]Photographs depict the response of injected tumor of a patient with gastro-esophageal cancer: injected with E7766 at 75 µg (first row) and an adjacent non-injected tumor lesion (second row); [b]Y/N on top of each bar indicate the presence (Y) or absence (N) of previous immunotherapy history; [c]by investigator assessment, per modified RECIST version 1.1; [d]include patients with both baseline and ≥1 postbaseline measure of target lesion diameters; [e]patients who received E7766 at 300 µg did not have non-injected target lesions; [f]baseline STING RNA expression was evaluated by measuring the expression of *TMEM173*; [g]patients were divided into two groups based on the size of the first injected lesion: injected lesion progressed if the maximum tumor growth of the first injected lesion was ≥20%, and injected lesion stabilized if the maximum tumor growth of the first injected lesion was <20%. C#D#, cycle # day #; PD, progressive disease; N, no; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; STING, stimulator of interferon genes; TPM, transcripts per kilobase million; Y, yes.

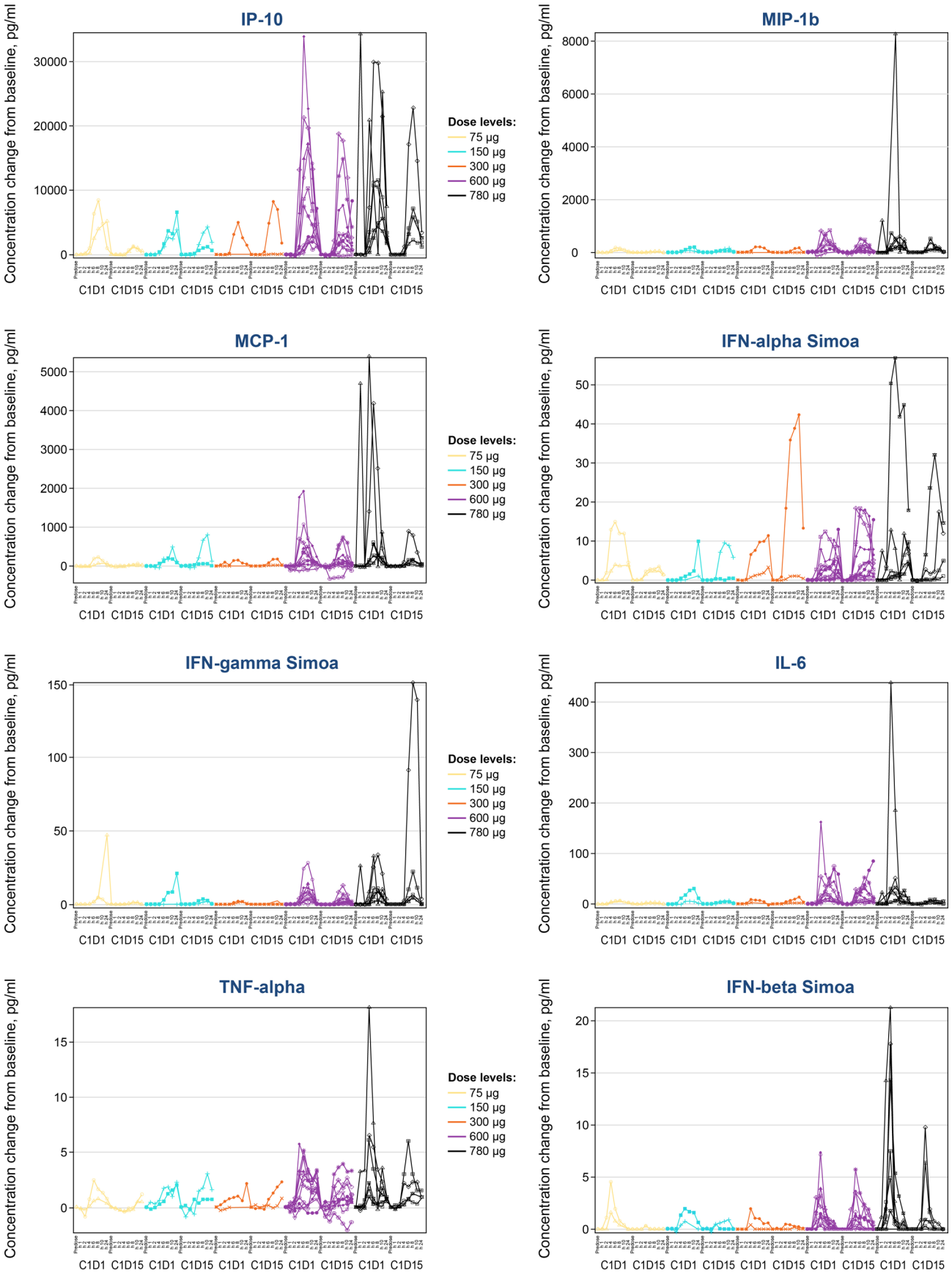

Figure 2 Changes from baseline in plasma levels of different biomarkers in all dosing cohorts of patients receiving E7766. C#D#, cycle # day #; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant; TNF, tumor necrosis factor.

non-injected lesions and systemically (figure 1A,B). Cytokine levels increased after treatment with E7766 at 600 or 780 µg but these changes did not appear to be dose-dependent overall, which is consistent with results seen in prior STING agonist clinical trials.[8] Changes in PBMC and tumor tissue RNA expression also did not appear to be dose-dependent. Of note, levels of CD8+ effector cells increased in patients treated with low doses of E7766. This result is consistent with data reported in some preclinical studies that suggested that lower doses of STING agonists may have similar or greater potency than high doses of STING agonists due to improved expansion of tumor-specific CD8+ effector T cells as well as the induction of vascular normalization within the tumor microenvironment.[20 21]

As the pharmacodynamic effects of E7766 are not dose-dependent, it is difficult to adjust the dose of the study drug in clinical settings to achieve the desired therapeutic effect. The complexity of the STING pathway lies in its ability to both enhance antitumor activity through increased proinflammatory cytokines and facilitate tumor growth by allowing immune cell evasion.[22] Thus, regulating the STING pathway requires a delicate balance between activating (turning on) and inhibiting (turning off) multiple molecular interactions to ensure an appropriate immune response without excessive inflammation.

Choosing the optimal dose of STING agonists as an anticancer therapy is an ongoing challenge in the field. In our study, no MTD was reached and the RP2D was not defined as the trial was discontinued by the sponsor. However, changes in IFN-associated PBMC gene expression and increased PD-L1 expression at either RNA or protein levels in injected and non-injected lesions in some patients across dose levels suggest the potential of E7766 to drive de novo antitumor responses and modulate the tumor microenvironment. These data emphasize the potential benefit of studying intratumoral STING agonism in a dedicated cohort of patients with predominantly cutaneous malignancies. This has yet to be pursued in the field despite multiple agents entering clinical trials.[3]

An area of controversy in the field of anticancer STING agonism is to determine which cells in the tumor microenvironment could be targeted by therapeutic agents. Some studies have proposed that myeloid and dendritic cells are the major immunological mediators, whereas others have proposed that retention of wildtype or non-epigenetic silencing of STING in tumor cells is required for antitumor effects.[23–25] In our study, STING gene expression in tumor cells appeared to be greater in patients whose first injected lesion stabilized at a maximal tumor growth of <20% following E7766 treatment.

In summary, intratumoral administration of the STING agonist E7766 demonstrated manageable safety in the tested doses and demonstrated peripheral blood and intratumoral target engagement. Several limitations to this study include the heterogeneous patient population recruited with different tumor types, the small patient population of a phase I study, potential issues relating to varying injection site techniques (eg, choice of the lesion, cutaneous vs deep visceral lesions), and the inability to assay for intratumoral pharmacokinetic distribution. However, the data overall emphasize the ability of STING agonism to modulate the tumor microenvironment and highlight the need to identify optimal patient populations for both intratumoral and systemically administered STING agonists in the future.

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