A Phase I, First-in-Human, Dose-Escalation, Expansion Trial of Cytokine-Encoding Synthetic mRNA Mixture Alone or with Cemiplimab in Advanced Solid Tumors

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ABSTRACT

Purpose: We investigated SAR441000 (mixture of four mRNAs encoding IL-12, single-chain IFN-α-2b, GM-CSF, and IL-15 sushi domain) alone or in combination with cemiplimab in patients with advanced solid tumors.

Patients and Methods: SAR441000 was intratumorally administered weekly in a 4-week cycle in monotherapy and in a 3-week cycle at a predefined dose level with 350 mg cemiplimab (intravenously) every 3 weeks in combination therapy. The primary objective was to determine MTD or maximum administered dose, overall safety, tolerability, and objective response rate of SAR441000.

Results: We enrolled 77 patients previously treated with anticancer therapies [escalation monotherapy: $N = 21$; escalation combination: $N = 15$; and expansion combination (PD-1–refractory melanoma): $N = 41$]. The maximum administered dose at dose level 8 was 4,000 μg. The most common grade ≥3 treatment–related adverse events was fatigue in the escalation phase (monotherapy: 28.6% and combination therapy: 66.7%) and injection-site pain (31.7%) in the expansion phase. In combination therapy, one patient in the escalation phase and two patients in the expansion phase achieved partial responses. At 4,000 μg (highest dose) across all cohorts, the maximum fold change in plasma cytokine concentration was the highest and lowest for IFN-α-2 (74.9-fold) and IL-15 (1.96-fold), respectively. Increased blood IFN-γ and inducible protein-10 levels were observed for most patients.

Conclusions: Intratumoral administration of SAR441000 in combination with cemiplimab was generally well tolerated with antitumor activity in the locoregional disease setting. Anecdotal evidence of pharmacodynamic immunomodulatory effect and distant noninjected lesion antitumor response was observed, without significant effects in patients with advanced solid tumors previously treated with anti–PD-1 therapies.

Introduction

Cytokines are potent immunomodulators that exert their effect in an autocrine and paracrine manner on immune, stromal, and cancer cells within the tumor microenvironment (TME; refs. 1, 2). Systemic recombinant IL-2 and IFN-α-2 are the only available cytokines approved for the treatment of various cancers, including metastatic renal cell carcinoma (3) and melanoma (2, 4). However, low antitumor response and dose-limiting toxicities (DLT) associated with high doses and frequent administration due to shorter half-lives (5, 6) present a challenge in using these immunotherapies and limit clinical development of other cytokines with therapeutic potential.

mRNA-based therapy offers a promising therapeutic approach to transiently express a protein of interest (7), especially in the field of cancer therapeutics (8). mRNA-based therapeutics, such as mRNA vaccines, are well tolerated, with manageable adverse events (AE); they

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Translational Relevance

This phase I trial was the first-in-human study of cytokine-encoding synthetic mRNA mixture (SAR441000) with cemiplimab for patients with solid tumors. A generally acceptable safety profile and anecdotal evidence of pharmacodynamic immunomodulatory effect and distant noninjected lesion antitumor response were observed. However, significant effect was not seen in patients with advanced solid tumors and prior exposure to anti–PD-1 therapies. After weekly SAR441000 intratumoral administration until cycle (C) 3 week (W) 1, no significant accumulation of the four cytokines encoded by SAR441000 was observed in the plasma compared with C1W1. At 4,000 μg (highest dose) across all cohorts, maximum fold change in plasma cytokine concentration was the highest and lowest for IFN-α-2 and IL-15, respectively. Increased plasma levels of IFN-γ and inducible protein-10 were detected in most patients at multiple timepoints [C1 day (D) 1, C1D8, and C3D1], which peaked at 24 hours of SAR441000 administration, suggesting induction of systemic immune modulatory effects.

degrade easily without integrating into the host genome (9, 10) and induce both humoral and cell-mediated immunity (11, 12). Recent studies have optimized mRNA structure, stability, and modes of delivery (9, 10, 13, 14), and the safety and efficacy of these drugs are being assessed in clinical trials (15–17).

SAR441000 is a novel saline-formulated mixture of four chemically modified mRNAs encoding immunomodulatory cytokines—IFN-α-2b, IL-12, GM-CSF, and IL-15 sushi (fusion of the sushi domain from the α-chain of the IL-15 receptor with the IL-15 cytokine). The active principle of each drug substance is a single-stranded, 5′-capped mRNA that is translated into the corresponding protein upon entry into cells in preclinical models (1, 18).

A preclinical study by Hotz and colleagues (1) identified an mRNA mixture encoding four murine cytokines (single-chain IL-12, a genetic fusion of the IL-12p40 and IL-12p35 subunits with a glycine–serine linker between subunits; IFN-α; GM-CSF; and IL-15 sushi) through an iterative *in vivo* screening process for antitumor activity in murine models. Intratumoral administration of this mRNA mixture induced intratumoral IFN-γ expression, immune memory formation, and systemic antigen-specific T-cell expansion and increased granzyme B^+ T-cell infiltration. Furthermore, preclinical studies demonstrated increased antitumor activity and overall survival for intratumoral cytokine mRNA mixture combined with anti–PD-1 therapy compared with treatment with single-agent cytokine mRNA or anti–PD-1 (1, 18, 19).

In this study, we investigated the safety, pharmacokinetics (PK; of the mRNA-encoded cytokines), immunomodulation related pharmacodynamics (PDy; of the downstream cytokines), and antitumor activity of SAR441000 alone and in combination with cemiplimab—a high affinity, hinge-stabilized IgG4P human antibody against the PD-1 receptor, which blocks PD-1–mediated T-cell inhibition and is used as a combination drug to enhance immune response in patients with advanced solid tumors.

Patients and Methods

Trial design

This multicenter, nonrandomized, open-label, first-in-human, phase I study was conducted at 18 centers across six countries (France, Germany, the Netherlands, Belgium, Spain, and the United States). The primary objective was to determine the MTD or maximum administered dose (Supplementary Table S1), overall safety, and tolerability of SAR441000 for dose-escalation (monotherapy and combination therapy) cohorts and objective response rate (ORR) for dose-expansion (combination therapy) cohort. The secondary and exploratory objectives included assessment of PK, PDy, immunogenicity, and preliminary signs of antitumor activity.

The study involved two parts: dose-escalation (monotherapy and combination therapy) cohorts, which included patients with advanced solid tumors, and dose-expansion (combination therapy) cohort, which included patients with advanced melanoma with anti–PD-1/PD-L1 failure (**Fig. 1A**). In the monotherapy cohort, SAR441000 was administered weekly in a 4-week cycle, wherein single-participant dose-escalation was used for the first two dose levels [DL; DL1 (8 μg) and DL2 (24 μg)] in the escalation phase, followed by escalation to higher doses [72 μg (DL3) to 4,000 μg (DL8)] using the Bayesian escalation method with overdose control design. In the combination therapy cohort, SAR441000 was intratumorally administered weekly in a 3-week cycle at the predefined DL; cemiplimab was administered intravenously every 3 weeks at a fixed dose of 350 mg.

This study was terminated by the sponsor after the completion of the melanoma expansion cohort due to nonsafety reasons.

The study was approved by all local institutional review boards and performed in accordance with the Good Clinical Practice and Declaration of Helsinki. The study was registered with ClinicalTrials.gov (NCT03871348). All participants provided written informed consent.

Patient eligibility

Adult patients with advanced solid tumors, a minimum of three lesions at enrollment, and measurable disease (per RECIST v.1.1) were considered; one lesion was considered the target lesion, one was suitable for intratumoral injection and biopsy, and one was suitable for additional biopsy. Only one target lesion must remain untreated. Although most injection sites are nontarget lesions, a target lesion may be injected if others are exhausted. However, at least one target lesion will always be spared. We included mucosal, cutaneous, or subcutaneous lesions ≥0.5 cm and lymph nodes ≥1.5 cm that were suitable for intratumoral injection. The exclusion criteria were Eastern Cooperative Oncology Group performance status >1, central nervous system lymphoma or any new/progressive brain lesion, and moderate-to-severe immune-related AEs (the NCI Common Terminology Criteria for Adverse Events version 5.0) prior to an immunomodulating therapy within 90 days of receiving the first study treatment.

Safety and response analyses

Safety assessments were performed based on DLTs (defined in Supplementary Table S1) during the first 28 days of treatment, AEs (the NCI Common Terminology Criteria for Adverse Events version 5.0), and laboratory abnormalities.

The efficacy endpoints [ORR, disease control rate (DCR), duration of response (DoR), and progression-free survival (PFS)] were assessed as per the RECIST v1.1 and immune RECIST (iRECIST) criteria. Categories of response [complete response (CR), partial response (PR), and stable disease (SD)] were evaluated in patients who were included in the ORR analysis.

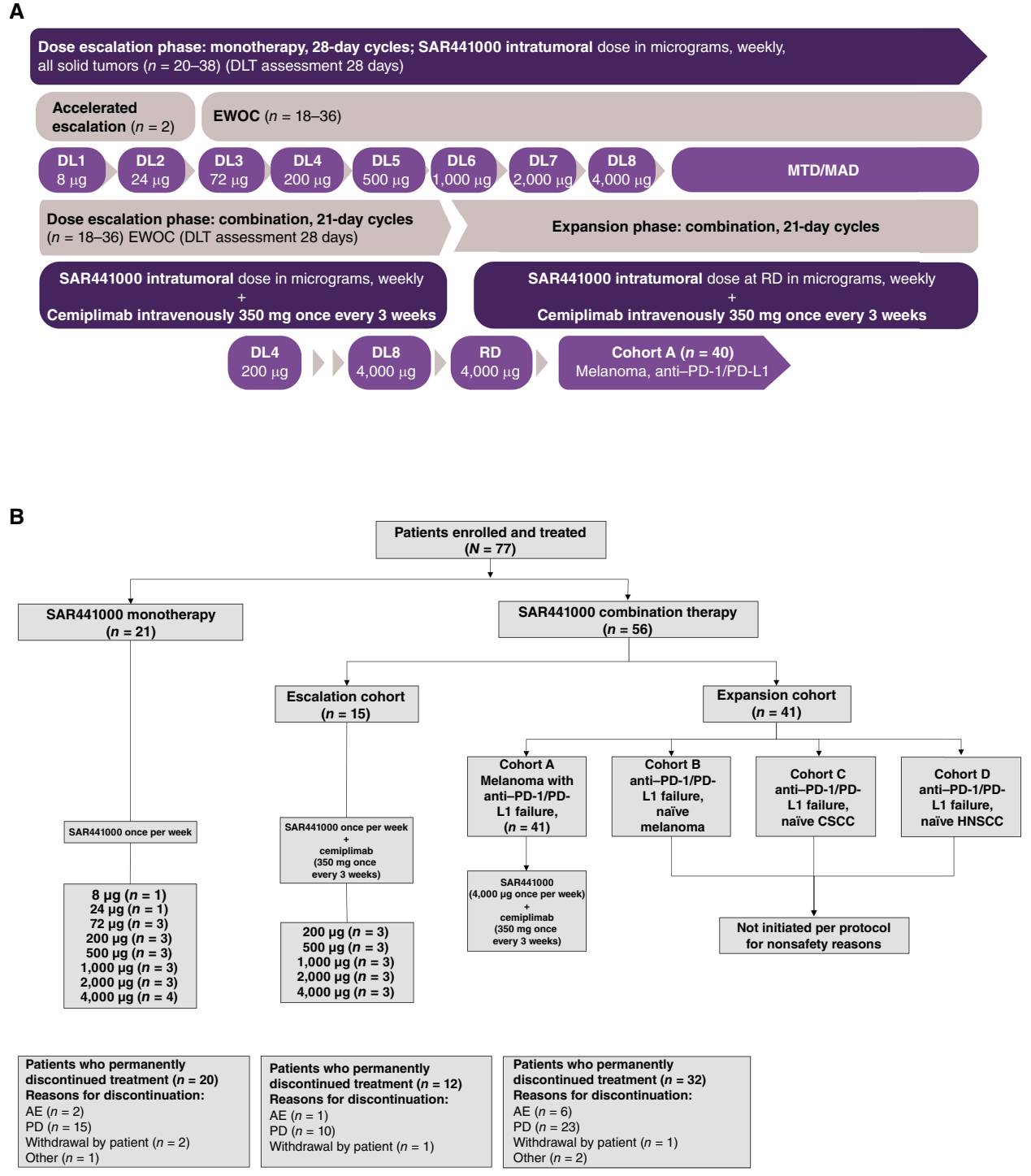


Figure 1.

A, Study design schema. **B,** Patient disposition. CSCC, cutaneous squamous cell carcinoma; EWOC, escalation with overdose control; HNSCC, head and neck squamous cell carcinoma; MAD, maximum administered dose; *N/n*, number of patients; RD, recommended dose.

PK and PDy analyses

Peripheral blood plasma samples were collected pretreatment and posttreatment during cycle 1 and subsequent cycles to assess PK and PDy parameters. Due to intrinsic instability of mRNA in tissues, we could not directly assess SAR441000 PK, thus plasma SAR441000-encoded cytokine levels were used to derive surrogate PK profiles.

Cytokine IFN-γ and the chemokine IFN-γ–inducible protein-10 (IP-10) levels were used to evaluate PDy effects potentially induced by the four cytokines encoded by SAR441000.

Systemic levels of the four SAR441000-encoded cytokines were quantified in plasma K3-EDTA by ultrasensitive ELISA using S-PLEX MSD assay. IFN-γ and IP-10 were measured by a multiplex ELISA using a multispot MSD assay. Cemiplimab levels were measured at Regeneron, using a validated ELISA assay (Supplementary Table S2).

Maximum concentration (C_{max}), AUC during a dose interval (AUC_{tau}), and predose concentration (C_{trough}) were calculated using the plasma levels of SAR441000-encoded cytokines and downstream cytokines; serum cemiplimab concentrations were analyzed using the same method. Additionally, maximal fold change (FC) in cytokine concentrations from the baseline was calculated for each patient, and then, the average values of the FCs were determined. The PK and PDy parameters were further compared based on the status of the best overall response (BOR) with stratification of study cohorts and dose range.

IHC

Tumor biopsies (pretreatment and posttreatment) were collected, and formalin-fixed, paraffin-embedded sections were stained using anti-CD3 (clone 2GV6; Ventana Medical Systems, Inc.; RRID: AB_2335978), anti-CD8 (clone C8/144B; Dako; RRID: AB_2075537), anti-CD68 (clone KP1; Dako; RRID: AB_2314148), and anti–SRY-related HMG-box 10–positive (SOX10; clone EP268; Cell Marque; RRID: AB_2941085) antibodies or matched isotype control antibodies, at NeoGenomics, using fully validated protocols (Supplementary Table S3).

Stained slides were evaluated by qualified pathologists using a standard bright-field microscope. For tumor cells, nuclear staining for SOX10 was considered, whereas for immune cells (CD3 and CD8), membrane staining at any intensity more than the background was taken into consideration.

Targeted RNA sequencing—next-generation sequencing

Details are provided in Annexure 1 (Supplementary Material).

Cell-type deconvolution analysis (microenvironment cell populations counter)

Bulk RNA sequencing data were processed as follows: sequencing reads were mapped to the human reference genome GRCh38 (hg19) using Spliced Transcripts Alignment to a Reference (STAR) aligner (20). Gene expression was measured in transcripts per kilobase million (21), which was $\log_2$-transformed and quantile-normalized for the downstream analysis. We used the microenvironment cell populations counter (MCP-counter) method (22) to estimate the absolute abundance of eight immune and two stromal cell populations in tumor tissues from bulk RNA sequencing data.

T-cell receptor repertoire analysis

Bulk T-cell receptor (TCR) sequencing data were processed using the QIAseq RNA TCR Immune Repertoire Read Processing pipeline on the GeneGlobe platform (QIAGEN) to determine the amino acid sequence of TCR complementarity-determining region 3 and its prevalence. We visualized the changes in the prevalence of major clones using ggplot2 (23).

Immunogenicity analysis

Immunogenicity of SAR441000 and cemiplimab was assessed up to the end of study intervention and during the follow-up period by evaluating the characteristics (preexisting, treatment induced, or boosted) and magnitude (transient, persistent, or indeterminate) of antidrug antibody (ADA) response against SAR441000-encoded cytokine IL-15 sushi and cemiplimab, respectively.

Statistical analysis

The AEs (treatment-emergent period was defined as the time from the first dose of study intervention up to 30 days after the last dose of study intervention), laboratory abnormalities, all relevant PK/PDy measurements, and ADAs were summarized using descriptive statistics. DCR and ORR (per RECIST 1.1 and iRECIST) were summarized with descriptive statistics using a 90% two-sided confidence interval (CI) via the Clopper–Pearson method. DoR and PFS were summarized using the Kaplan–Meier method.

Data availability

Qualified researchers may request access to patient-level data and related study documents including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and dataset specifications. Patient-level data will be anonymized, and study documents will be redacted to protect the privacy of our trial participants. Further details on Sanofi's data sharing criteria, eligible studies, and process for requesting access can be found at: https://www.vivli.org/

Results

Patient disposition and baseline characteristics

Seventy-seven patients were enrolled [escalation monotherapy ($N = 21$), escalation combination therapy ($N = 15$), and expansion combination therapy ($N = 41$)] in the study from January 03, 2019, to July 25, 2022. At the data cutoff date (February 15, 2023), 48 (62.3%) patients had an Eastern Cooperative Oncology Group performance status of 0. Patient demographics and baseline characteristics are summarized in **Table 1**. In escalation monotherapy, escalation combination therapy, and expansion combination therapy cohorts, 15 (71.4%), 10 (66.7%), and 40 (97.6%) patients, respectively, had been exposed to immune checkpoint inhibitors (ICI); 12 (57.1%), 8 (53.3%), and 23 (56.1%) patients, respectively, had received ≥3 prior lines of therapy. Melanoma was the most common type of cancer in all the three cohorts. The representativeness of study participants is assessed in Supplementary Table S4.

At the time of analysis, 64 (83.1%) patients discontinued the treatment due to either progressive disease [PD; 48 (62.3%)], AEs [9 (23.4%)], withdrawal of consent by the patient [4 (5.2%)], or withdrawal for other reasons [3 (3.9%); **Fig. 1B**].

Safety

DLTs

The maximum administered dose of SAR441000 was 4,000 μg at DL8, which was the highest DL included in the dose-escalation phase due to the presumed maximum volume (i.e., 4 mL) that could be injected into a single lesion.

DLTs or serious treatment-related AEs (TRAE), including cytokine release syndrome (CRS), was not reported in the escalation phase in any cohort during the DLT observation period (first 28 days of treatment).

Table 1. Patients' baseline and disease characteristics.

Characteristic	Escalation monotherapy SAR441000 (*N* = 21)	Escalation combination therapy SAR441000 plus cemiplimab (*N* = 15)	Expansion cohort A SAR441000 plus cemiplimab (*N* = 41)
Age (years)			
Median (range)	67 (45–89)	58 (38–73)	62 (32–85)
Male, *n* (%)	10 (47.6)	8 (53.3)	23 (56.1)
Race, *n* (%)			
White	8 (38.1)	14 (93.3)	20 (48.8)
Not reported	13 (61.9)	1 (6.7)	19 (46.3)
Black or African American	—	—	1 (2.4)
Native Hawaiian or other Pacific Islander	—	—	1 (2.4)
ECOG, *n* (%)			
0	14 (66.7)	10 (66.7)	24 (58.5)
1	7 (33.3)	5 (33.3)	17 (41.5)
Type of cancer; *n* (%)			
Melanoma	11 (52.4)	9 (60.0)	40 (97.6)
Breast	4 (19.0)	3 (20.0)	—
Squamous cell	2 (9.5)	—	—
Myxosarcoma	2 (9.5)	—	—
Merkel cell	1 (4.8)	—	—
Basal cell	1 (4.8)	1 (6.7)	—
Parotid	—	1 (6.7)	—
Thyroid	—	1 (6.7)	—
Unknown	—	—	1 (2.4)
Type of prior anticancer therapy, *n* (%)			
Immunotherapy	15 (71.4)	10 (66.7)	40 (97.6)
Others[a]	6 (28.6)	19	29 (70.7)
Number of prior regimens, *n* (%)			
1–2	9 (42.9)	7 (46.7)	18 (43.9)
≥3	12 (57.1)	8 (53.3)	23 (56.1)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; *n*, number of patients.
[a]Other therapies included biologicals, chemotherapy, cryotherapy, targeted therapies, gene therapy, hormonotherapy, radiotherapy, and surgery.

Escalation monotherapy

Of the 21 patients, 19 (90.5%) experienced any-grade AEs, of which the most common were vomiting and fatigue in 6 (28.6%) patients each, followed by hypertension, chills, and pain in extremities in 4 (19.0%) patients each. Nine (42.9%) patients experienced grade ≥3 AEs (**Table 2**). Furthermore, 13 (61.9%) patients reported TRAEs, but none were grade ≥3 or considered serious; grade ≥5 AEs were not reported. Seven deaths occurred during the posttreatment period (>30 days after the last dose of the study drug) due to disease progression, which were not related to the study drug.

Escalation combination therapy

All 15 patients experienced any-grade AEs, with the most common being fatigue (10/15; 66.7%), followed by hypertension and nausea in 5 (33.3%) patients each. Grade ≥3 AEs—hypertension, pyrexia, and anemia—were experienced by one (6.7%) patient each (**Table 2**). Ten (66.7%) patients experienced any-grade TRAEs, with the most common being fatigue ($n = 7$; 46.7%). One (6.7%) patient experienced grade ≥3 syncope (Supplementary Table S5). Six deaths were observed (two at DL4 and one each at DL5–DL8), of which three deaths (one each at DL5–DL7) occurred during the treatment period (within 30 days of the last dose of the study drug), whereas three (two at DL4 and one at DL8) occurred during the posttreatment period. The deaths were attributed to disease progression and were not related to the study drug.

Expansion combination

All 41 patients experienced any-grade AEs, of which injection-site pain ($n = 13$; 31.7%) was the most common all-grade AE, followed by diarrhea, decreased appetite, and asthenia ($n = 11$ each; 26.8%; **Table 2**). Severe (grade ≥3) CRS event was not observed, and two patients (4.9%) experienced low-grade CRS events. Grade ≥3 AEs were experienced by 21 (51.2%) patients and included asthenia, vomiting, fatigue, diarrhea, injection-site pain, and anemia. Four (9.8%) patients experienced grade ≥5 AEs. Thirty-three (80.5%) patients had any-grade TRAEs, of which six (14.6%) patients had TRAEs of grade ≥3 that were diarrhea, injection-site pain, and fatigue (Supplementary Table S5). Seventeen deaths were observed, of which four occurred during the treatment period and 13 during the posttreatment period. All deaths in the expansion cohort were also attributable to disease progression and were unrelated to study treatment.

Efficacy and survival

Of the 21 evaluable patients in the escalation monotherapy cohort, 4 (19.0%) and 13 (61.9%) patients had SD and PD, respectively, whereas 4 (19.0%) were not evaluable (NE) per RECIST 1.1 with a median follow-up time of 30.6 months (**Fig. 2**; Supplementary Table S6).

In the escalation combination cohort, efficacy response was evaluated in 15 patients. One (6.7%) patient achieved PR, 3 (20.0%)

Table 2. AEs: (A) summary and (B) TEAEs occurring in ≥15% of patients.

	Escalation monotherapy	Escalation combination	Expansion combination
A *n* (%)	**SAR441000 (*N* = 21)**	**SAR441000 plus cemiplimab (*N* = 15)**	**SAR441000 plus cemiplimab (*N* = 41)**
Patients with any TEAE	19 (90.5)	15 (100)	41 (100)
Patients with grade ≥3 TEAEs	9 (42.9)	5 (33.3)	21 (51.2)
Patients with grade 5 TEAEs[a]	0	3 (20.0)	4 (9.8)
Patients with any serious TEAE	11 (52.4)	6 (40.0)	26 (63.4)
Patients with any TEAE leading to treatment discontinuation[b]	2 (9.5)	1 (6.7)	8 (19.5)
Patients with any TRAE	13 (61.9)	10 (66.7)	33 (80.5)
Patients with any TRAE of grade ≥3	0	1 (6.7)	6 (14.6)

B	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**
Hypertension	4 (19.0)	1 (4.8)	5 (33.3)	1 (6.7)	0	0
Vomiting	6 (28.6)	0	3 (20.0)	0	10 (24.4)	1 (2.4)
Nausea	0	0	5 (33.3)	0	8 (19.5)	0
Fatigue	6 (28.6)	0	10 (66.7)	0	9 (22.0)	1 (2.4)
Chills	4 (19.0)	0	0	0	0	0
Pain in extremity	4 (19.0)	1 (4.8)	0	0	0	0
Diarrhea	0	0	4 (26.7)	0	11 (26.8)	1 (2.4)
Malaise	0	0	3 (20.0)	0	0	0
Constipation	0	0	3 (20.0)	0	0	0
Dry skin	0	0	3 (20.0)	0	0	0
Pruritus	0	0	3 (20.0)	0	8 (19.5)	0
Pyrexia	0	0	4 (26.7)	1 (6.7)	0	0
Decreased appetite	0	0	0	0	11 (26.8)	0
Cough	0	0	0	0	7 (17.1)	0
Injection-site pain	0	0	0	0	13 (31.7)	1 (2.4)
Asthenia	0	0	0	0	11 (26.8)	2 (4.9)
COVID-19 infection	0	0	0	0	9 (22.0)	0
Anemia	5 (23.8)	2 (9.5)	0	0	7 (17.7)	1 (2.4)

Abbreviations: COVID-19, coronavirus disease 2019; *n*, number of patients; TEAE, treatment-emergent AE.
[a]Grade 5 AEs occurred during the treatment period. None of the grade 5 AEs were considered related to the trial treatment.
[b]Treatment discontinuation was permanent full-intervention discontinuation that included discontinuation of all study drugs.

had SD, and 11 (73.3%) had PD as the BOR. The ORR and DCR were 6.7% (90% CI, 0.3–27.9) and 26.7% (90% CI, 9.7–51.1), respectively, with a median follow-up time of 21.3 months. The median PFS was 2.0 months (90% CI, 1.02–2.12). CR was not reported in any escalation monotherapy or combination therapy patients per RECIST 1.1.

Per iRECIST, patients in the escalation combination cohort achieved an ORR of 13.3% (90% CI, 2.4–36.3). One patient had immune PR (iPR) and one patient had immune CR as the confirmed BOR (Supplementary Table S7). Both patients had malignant melanoma and were either pretreated with anti–PD-1 or anti-CTLA4 monotherapy or combination therapy. The patient with iPR was treated with DL8 and experienced tumor shrinkage in both injected and distant noninjected lesions. The patient with immune CR was treated with DL6 and had locoregional disease of the limb with no distant metastasis at screening. This patient achieved significant shrinkage (>50%) in injected and noninjected lesions at 5 months of treatment.

Of the 41 PD-1/PD-L1–resistant patients treated in the expansion combination cohort, per RECIST 1.1, 2 (4.9%), 10 (24.4%), 24 (58.5%), and 5 (12.2%) had confirmed PR, SD, PD, and NE, respectively. The ORR was 4.9% (90% CI, 0.9–14.6) with a median follow-up time of 8.9 months. The DCR, median DoR, and median PFS were 29.3% (90% CI, 17.8–43.1), 8.9 months (90% CI, 4.99–undefined), and 2.1 months (90% CI, 1.95–2.28), respectively (Supplementary Table S6).

Per iRECIST, 15 (36.6%), 12 (29.3%), and 7 (17.1%) patients had SD, immune-unconfirmed PD, and immune-confirmed progression as the confirmed BOR, respectively, whereas 5 (12.2%) patients were NE. The DCR, median DoR, and median PFS were 34.1% (90% CI, 22.0–48.1), 8.9 months (90% CI, 4.99–undefined), and 5.2 months (90% CI, 2.84–8.40), respectively.

Change in target lesions

In the expansion combination therapy cohort ($n = 41$), 9 (22%) and 10 (24%) patients had injected lesions' responses (>30% tumor size decrease per best percent change) per RECIST v.1.1 and iRECIST, respectively. Thirteen (31.7%) and 16 (39.0%) patients had response in noninjected lesions, of which 9 (22.0%) and 11 (26.8%) patients had response in noninjected target lesions per RECIST v1.1 and iRECIST, respectively. The highest percent change in lesion diameter from the baseline for injected and noninjected (target and nontarget) lesions and the maximum relative change in target and nontarget lesion size data for monotherapy and combination therapy cohorts are provided in Supplementary Fig. S1A and S1B, respectively.

Two and nine patients in the escalation and expansion combination cohorts, respectively, either demonstrated a response or had

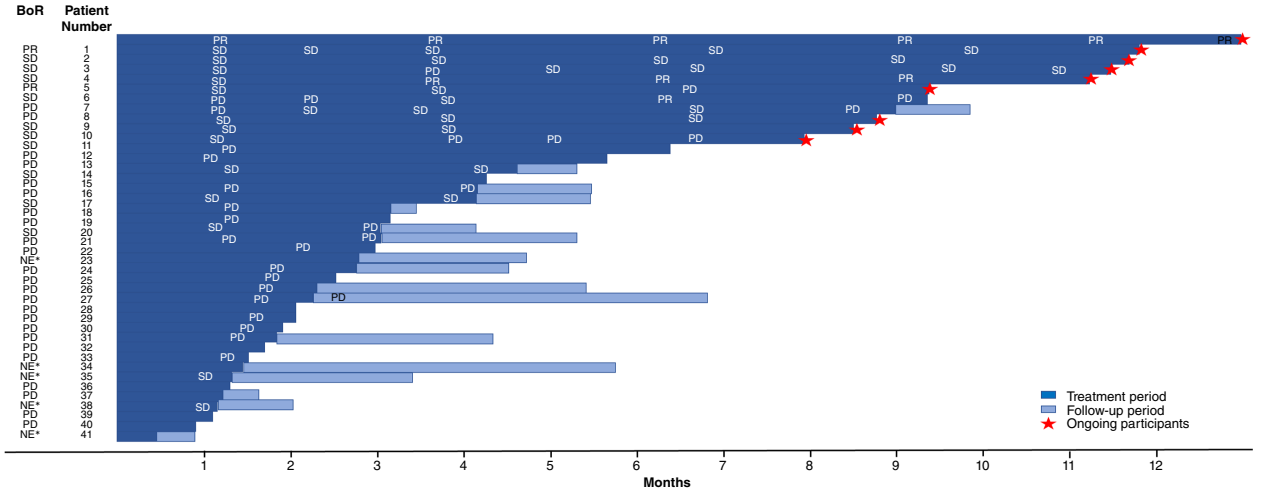


Figure 2.

Patient disease status with confirmed BOR per RECIST 1.1 in expansion combination therapy (SAR441000 plus cemiplimab)*. *, Patients were allowed to continue treatment beyond RECIST 1.1–defined disease progression based on the investigator's discretion.

SD as per the investigator's assessment and were continuing study treatment at the time of data cutoff; per protocol they are allowed for a maximum treatment duration of ≥2 years.

PK analysis

After repeated weekly intratumoral administration of SAR441000 until cycle 3 week 1, significant accumulation of all four expressed cytokines was not observed in the plasma compared with that in cycle 1 week 1. Plasma parameters (C_{max} and AUC) of all four expressed cytokines exhibited large intersubject variabilities and the lack of dose dependency; therefore, FCs in cytokine concentrations from the baseline were considered more appropriate for the evaluation of mRNA expression efficiency of SAR441000. At the highest dose of 4,000 μg (DL8), the average maximal changes for IL-15, GM-CSF, IL-12, and IFN-α-2 across all cohorts were 1.96-, 6.70-, 27.9-, and 74.9-fold, respectively. The profiles between maximal FCs of concentrations for all expressed cytokines and the best overall clinical responses are presented in **Fig. 3A**, and no apparent associations were observed. Furthermore, an association was not observed with low-range (8–500 μg) or high-range (1,000–4,000 μg) doses of SAR441000. Additionally, serum concentrations of cemiplimab at the steady state of the treatment were similar to the reference values reported in the European Union and the United States (24). The median (range) plasma concentrations for all four cytokines for total cohorts are detailed in Supplementary Fig. S2 and Supplementary Table S8.

PDy analysis

The combined effect of SAR441000-encoded cytokines should be that of promoting local and systemic antitumor immune responses. Key mediators of such responses are pro-inflammatory cytokines, such as IFN-γ, and chemokines, such as IP-10 (CXCR10), the latter acting as a chemoattractant for key immune cell populations, including T and NK cells, dendritic cells, and monocyte/macrophages. Thus, posttreatment changes in plasma concentration of downstream cytokines IFN-γ and IP-10 were determined to assess immunomodulation in the blood. Concentration–time profiles of both cytokines revealed high interparticipant variability in the monotherapy and combination escalation cohorts, whereas consistency was observed in the combination expansion cohort. Here, at multiple timepoints (cycle 1 day 1, cycle 1 day 8, and cycle 3 day 1), the concentration of both IFN-γ and IP-10 peaked at 24 hours after intratumoral injection, returning to pretreatment levels within a week (**Fig. 3B**; Supplementary Fig. S3).

An arbitrary fivefold-increase threshold was used to allow quantitative comparison of PDy response among cohorts. A more than fivefold increase in plasma IFN-γ concentration at peak timepoint was observed in 3/21 (14.3%), 4/15 (26.7%), and 25/39 (64.1%) patients in the escalation monotherapy, escalation combination, and expansion combination cohorts, respectively. For IP-10, more than fivefold increase at peak timepoint was observed in 0/21 (0%), 1/15 (6.7%), and 4/39 (10.3%) patients, respectively.

For IFN-γ, maximum FCs in mean concentration from the baseline in escalation monotherapy (DL7 = 2,000 μg, $n = 3$), combination therapy (DL8 = 4,000 μg, $n = 3$), and expansion combination therapy (DL8 = 4,000 μg, $n = 39$) cohorts were 11.15, 17.2, and 8.27, respectively. For IP-10, the maximum FCs of mean concentration from the baseline were 2.57, 4.94, and 1.61 for DL6 = 1,000 μg, $n = 3$; DL8 = 4,000 μg, $n = 3$; and DL8 = 4,000 μg, $n = 39$ in escalation monotherapy, escalation combination, and expansion combination cohorts, respectively.

Overall, an increase in blood IFN-γ and IP-10 concentrations was detected in most participants, suggesting immunomodulatory effect induced by intratumoral SAR441000 administration in combination with cemiplimab. Dose dependency and correlation between plasma concentrations of IFN-γ and IP-10 and the clinical response were not observed (**Fig. 3A**).

PDy effects in the TME were studied in the evaluable paired on-treatment versus pretreatment biopsies, by quantifying changes in the proportion of infiltrating immune cells as measured by IHC. The injected lesions were assessed in six (28.6%), four (26.6%), and nine (21.9%) participants from the escalation monotherapy, escalation combination, and expansion combination cohorts, respectively, whereas noninjected lesions were assessed in zero (0%), 4 (26.6%), and 12 (29.2%) patients, respectively.

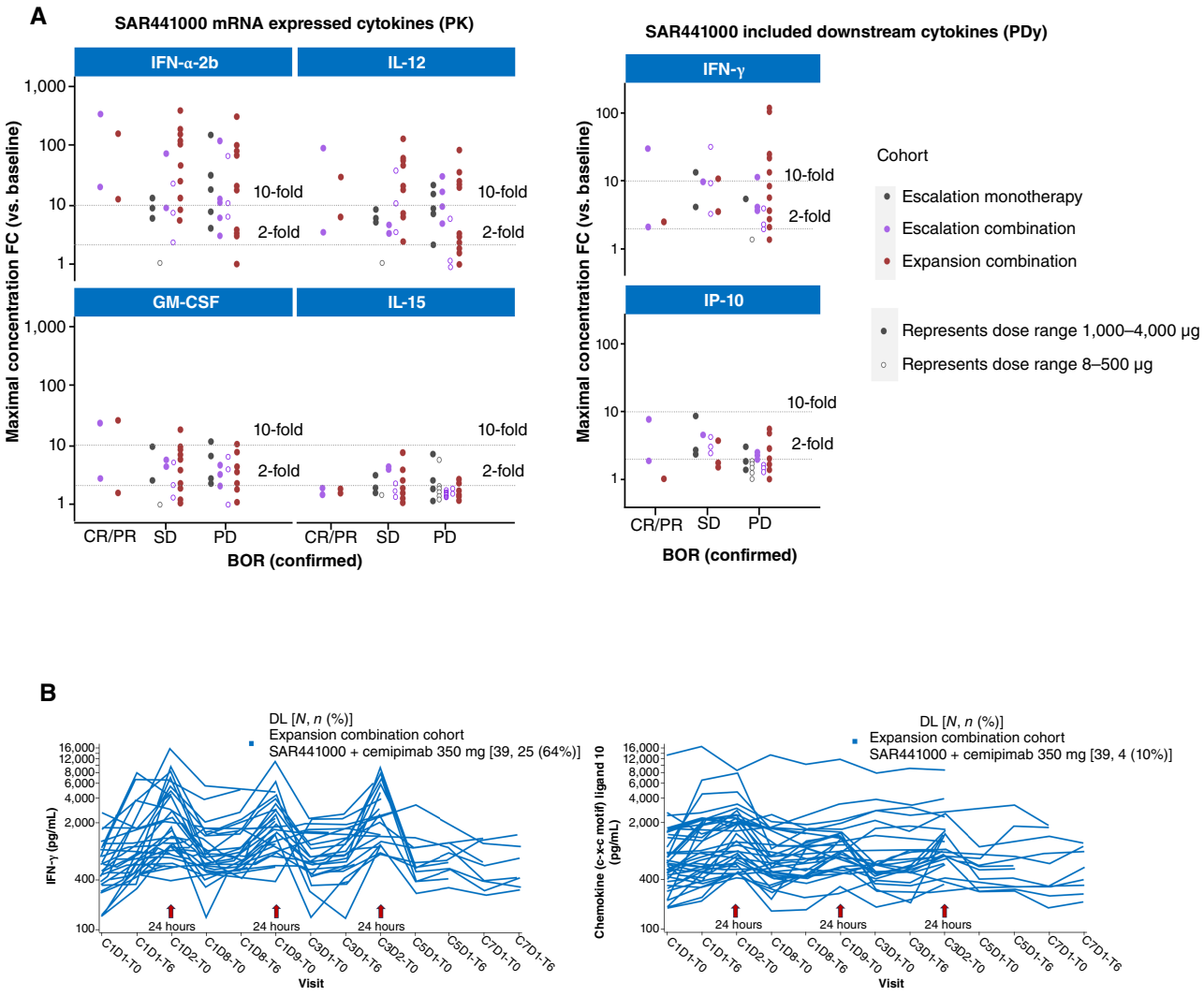
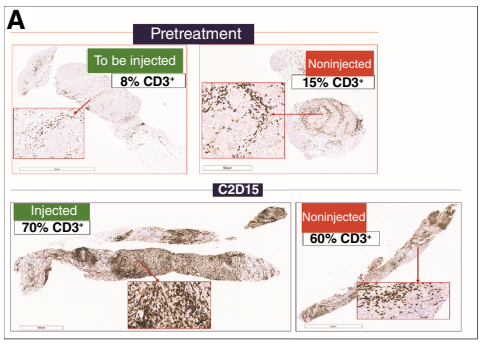


Figure 3.

A, Plasma exposure of mRNA-expressed (PK) and downstream (PDy) cytokines. **B,** Plasma concentration of downstream cytokines IFN-γ and IP-10 after intratumoral injection at multiple timepoints (C1D1, C1D8, and C3D1) in expansion combination cohort. If the value was less than the lower limit of quantification (LLoQ), it was imputed as LLoQ/2, and if the value was more than the upper limit of quantification (ULoQ), it was imputed as ULoQ. C, cycle; D, day; *N*, number of patients; *n* (%), number and percentage of patients with max FC ≥5; T0, 0 hour; T6, 6 hours.

Modulation in the proportion of $CD3^+$ and $CD8^+$ T cells was observed in several patients; however, a consistent trend or relationship with clinical response was not found. Of note, one iPR participant in the escalation combination cohort (DL8) showed dramatic increase in the proportion of $CD3^+$ and $CD8^+$ T cells in both injected and noninjected lesions compared with that at the baseline (**Fig. 4A**), with concomitant reduction in $SOX10^+$ melanoma cells (**Fig. 4B**). In this patient, TCR repertoire analysis of peripheral blood sample revealed early (days 5–6) expansion of multiple clones after SAR441000 and cemiplimab administration, suggesting induction of T-cell–mediated systemic immune response (**Fig. 4C**).

Summary of MCP-counter and IHC evaluable data relative to the CD3 and CD8/cytotoxic cell populations in relation to DL, tumor type, and iRECIST response is shown in Supplementary Fig. S4A (escalation monotherapy and combination) and in Supplementary Fig. S4B (expansion combination). Responses are defined by the criteria specified in the figure legends. Data from monotherapy cohort were inconclusive and have not been included. Increase in the levels of several tumor biomarkers in both injected and noninjected lesions, as per IHC and MCP-counter analyses, was observed in four patients: two in the escalation combination cohort [one with stable disease according to iRECIST (iSD; patient 21) and one with iPR (patient 25); Supplementary Fig. S4A] and two patients in the expansion combination cohort [one with immune-unconfirmed PD (patient 16) and one with iPR (patient 30); Supplementary Fig. S4B]. Interestingly, as all these patients were in the combination arms of the study, it may not be possible to differentiate the contribution of SAR441000 from that of cemiplimab in the observed responses. For three/four of the above patients with



B

Percentage of positive cells/total nucleated cells (tumor + immune cells)

Lesion	Injected		Noninjected	
Timepoint	C1D1	C2D15	C1D1	C2D15
% CD3+	8	70	15	60
% CD8+	5	60	10	50
% CD68+	15	50	20	50
% SOX10+	40	5	30	20

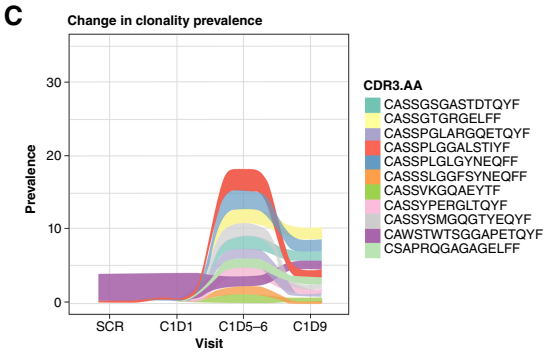


Figure 4.

PDy effects in the tumor of an iPR patient with melanoma from the combination escalation phase. **A,** Posttreatment increase in $CD3^+$ and $CD8^+$ T cells in both injected and noninjected lesions. **B,** Percentage of positive cells/total nucleated cells (tumor plus immune cells) of the exemplified patient. **C,** TCR repertoire analysis in peripheral blood of the same patient. C, cycle; CD, cluster of differentiation; D, day; SCR, screening.

available blood MCP-counter data, tumor biomarker responses did not correlate with those in blood biomarkers, as significant increase was not observed in blood T/CD8/cytotoxic lymphocyte gene signatures (Supplementary Fig. S4A and S4B).

Tumor mutational burden analysis

We analyzed tumor mutational burden (TMB) in four (one at DL7 = 2,000 μg and three at DL8 = 4,000 μg; 26.6%) and seven (DL8 = 4,000 μg; 17.1%) patients from the escalation combination and expansion combination cohorts, respectively. The cutoff criteria were TMB high: ≥13.7 mutations per megabase (mut/Mb); intermediate: ≥5.7 to <13.7 mut/Mb; and low: <5.7 mut/Mb. All patients with TMB-high status had PD, whereas five patients who received clinical benefits, including two PRs from combination treatment, had low/intermediate TMB (Supplementary Table S7).

Immunogenicity

The incidence of treatment-induced ADA was low, with the rate being >10% for both SAR441000-encoded cytokine IL-15 sushi and cemiplimab across all dose-escalation and dose-expansion cohorts (Supplementary Table S9).

Discussion

This study demonstrated that intratumoral injection of SAR441000 was well tolerated in monotherapy and combination therapy in patients with advanced solid tumors. A comparable profile of AEs was noted in both the combination therapy and monotherapy cohorts, with vomiting and fatigue more common in the monotherapy group and injection-site pain, asthenia, decreased appetite, diarrhea, vomiting, and fatigue in the combination expansion group. In the combination cohort, changes in the safety profile of cemiplimab were not observed compared with the known safety profile of cemiplimab and other PD-1 inhibitors (24). These findings were consistent with the AURELIO-03 and MASTERKEY-265 studies. The AURELIO-03 study of SOT101, a superagonist fusion protein of IL-15 and IL-15 receptor α sushi+ domain, in combination with pembrolizumab (anti–PD-1 ICI), did not reveal new safety signals in combination therapy versus monotherapy (25). Most AEs were nonserious and mild to moderate in both monotherapy and combination therapy and were manageable with standard treatment. Treatment-related fatal AEs did not occur during the treatment. Similarly, in the MASTERKEY-265 study, intratumoral administration of talimogene laherparepvec (an oncolytic virus approved in the United States and Europe for treatment of melanoma) in combination with pembrolizumab reported an acceptable safety profile and an encouraging CR rate in patients with advanced melanoma but with higher ORR (48.6% for talimogene laherparepvec–pembrolizumab combination; refs. 26, 27).

In our study, incidences of local reaction, such as injection-site pain, related to intratumoral administration were less common in monotherapy versus combination therapy, suggesting the potentiation of an immune effect because of the combination. However, this finding might also be influenced by the number of patients assessed, the type of lesions injected, and the lack of comparative assessment. Moreover, the risk of systemic inflammatory reactions including CRS is low compared with other systemic immune therapies (28).

SAR441000 treatment as monotherapy may not have been sufficient to illustrate exhaustive tumor responses, but it revealed isolated tumor shrinkage in injected lesions and stable control of disease burden in at least three patients across multiple DLs. The combination therapy induced objective responses in four patients, but SD was achieved in a significantly higher number of patients. The responses were observed in patients with prior treatment with immunotherapies, including ICIs, and across multiple DLs, including the highest tested dose, i.e., 4,000 μg. Most responses observed in the combination therapy cohort were confined to locoregional disease or proximal noninjected lesions. Although systemic responses were induced, they did not translate to measurable distant abscopal effects in most patients. Furthermore, prolonged control of disease (SD) in a substantial number (22.1%) of patients with melanoma was observed. These findings are relevant because they may assist in the development of new therapies for patients with advanced melanoma who have progressed to anti–PD-1/PD-L1–based therapy, who represent a growing unmet need.

The lack of significant objective responses may be attributed to the use of saline-formulated cytokine mRNA and intratumoral mode of administration. Saline-formulated mRNA devoid of carrier material may be safer to administer, as it minimizes off-target toxicity, but it may have resulted in lower-than-expected cytokine concentrations in the tumor. Two recent studies on lipid-based carrier systems have reported the feasibility of intratumoral injection of OX40L mRNA along with mRNA encoding other cytokines or T-cell co-stimulatory factors (29, 30). Thus, lipid-formulated mRNA could offer more stability for potentially higher cytokine expression than naked RNA. Intratumoral administration is a viable approach for direct access to tumors, but the lack of standard administration techniques may have contributed to antitumor activity. Injection volume restrictions and the need to adjust the dose based on evolving tumor size may have limited our ability to deliver a consistent effective dose. The usage of a concentrated drug product and more frequent injections can circumvent certain volume limitations but may not eliminate the problem.

We used the plasma concentration of SAR441000-encoded cytokines as a surrogate PK parameter, with the caveat that these cytokines might not be directly derived from the tumor but be part of a systemic PD response. At the highest dose of 4,000 μg across all cohorts, the average maximum FCs in plasma cytokine concentration were the highest and lowest for IFN-α-2 and IL-15, respectively. A lesser increase in plasma IL-15 levels may be advantageous, as this would limit potential systemic toxicity. The PK profile of cemiplimab in combination with SAR441000 was consistent with the established PK profile of cemiplimab monotherapy (24).

Increased blood PDy marker (IFN-γ and IP-10) levels were detected in most patients, suggesting systemic immunomodulatory effect induced by intratumoral SAR441000 administration, which could have extended to some noninjected lesions. These findings also suggest that the SAR441000-encoded cytokines may have the ability to support putative antitumor T-cell responses. Transcriptomic analysis revealed an increase in blood PDy marker levels in some patients along with parallel increase in T-cell infiltrates in the TME, but without parallel changes in blood T-cell populations. However, a clear relationship between clinical responses and the observed plasma PDy marker changes was not established. Overall, the induced PDy systemic changes observed did not translate into objective clinical activity in most patients. Moreover, an apparent dose–response relationship was not observed, which could be because the saline formulation limited the dose by volume. Furthermore, DLTs were not observed at the highest test dose of 4,000 μg, suggesting that this dose may not be sufficient to produce a desirable treatment effect in the majority of the patients in the study. The other likely reason is tissue heterogeneity because of the different proportions of viable and necrotic cells present in different tumors. The TME has divergent and complex intercellular networks, and the interactions between these networks usually determine tumor development and drug treatment efficacy (31, 32). Therefore, tissue heterogeneity along with other differences in the TME of the injected tumor lesions could have impacted mRNA translation efficiency, leading to heterogeneity in the tumor cytokine expression.

Overall, biomarker data from tumor biopsies were scarce or fragmented; thus sufficient information was not available to investigate tumor antigen-specific T-cell responses in tumor-infiltrating lymphocytes. This was mainly because several biopsies (~30% in the expansion combination cohort) had low tissue quality or tumor content. Furthermore, blood transcriptomic data were unavailable for the monotherapy cohort. This made the correlation of biomarker data with the DL or with antitumor responses to treatment challenging, and the assessment of PDy effects on immune cell populations for monotherapy was inconclusive.

TMB has been correlated with response to ICIs, and high TMB (≥10 mut/Mb) is associated with tumor response to pembrolizumab monotherapy in patients with previously treated, unresectable, or metastatic solid tumors (KEYNOTE-158; ref. 33). Furthermore, improved overall survival and PFS have been observed in several studies in patients with ICI-treated melanoma exhibiting higher baseline TMB than low TMB (34). The analysis of baseline TMB in patients from both combination arms revealed an unexpected trend because all patients with high TMB had PD, whereas the two PR patients had low/intermediate scores. However, the small sample size limited the power of the analysis.

The other limitation of the study was the lack of standard efficacy response assessments for intratumoral drugs and reliance on RECIST 1.1 and iRECIST criteria. Recent novel proposals such as intratumoral RECIST criteria for intratumoral immunotherapies are promising but are yet to be validated (35).

In conclusion, SAR441000 demonstrated a generally acceptable safety profile in both monotherapy and combination therapy cohorts, although a higher number of high-grade AEs were observed in the combination therapy cohort. We did not observe any unexpected safety findings in either monotherapy or combination therapy cohorts, beyond the anticipated risks consistent with the mode of action and preclinical data of SAR441000 and the known safety profile of cemiplimab. The efficacy of SAR441000 treatment as monotherapy was limited to isolated responses in injected lesions and in combination treatment with cemiplimab. Activity was limited to objective responses in a locoregional disease setting and stabilization of disease in some patients. Furthermore, combination therapy demonstrated immune cell infiltration in both injected and noninjected tumors in patients pretreated with ICIs. To conclude, this study did not establish whether SAR441000, an mRNA-based drug, was capable of robust biological activation in most patients but has shown reactive trend in some patients that did not correlate with clinical outcomes. However, the study treatment failed to demonstrate objective response in distant, noninjected lesions, suggesting that the PD changes observed/captured in this study may not be fully reflective of the unique array of factors required for response in melanoma.

Authors' Disclosures

C. Loquai reports personal fees from Bristol Myers Squibb, MSD, Merck, Roche, Immunocore, Novartis, Pierre Fabre, Sanofi, Sun Pharma, Almirall Hermal, Kyowa Kirin, and BioNTech outside the submitted work. S. Champiat reports

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Note

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