Journal for ImmunoTherapy of Cancer

First-in-human phase Ib trial of M9241 (NHS-IL12) plus avelumab in patients with advanced solid tumors, including dose expansion in patients with advanced urothelial carcinoma

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To cite: Strauss J, Deville J-L, Sznol M, *et al*. First-in-human phase Ib trial of M9241 (NHS-IL12) plus avelumab in patients with advanced solid tumors, including dose expansion in patients with advanced urothelial carcinoma. *Journal for ImmunoTherapy of Cancer* 2023;**11**:e005813. doi:10.1136/jitc-2022-005813

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2022-005813).

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Accepted 31 March 2023

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ABSTRACT

Background In preclinical studies, combining M9241 (a novel immunocytokine containing interleukin (IL)-12 heterodimers) with avelumab (anti-programmed death ligand 1 antibody) resulted in additive or synergistic antitumor effects. We report dose-escalation and dose-expansion results from the phase Ib JAVELIN IL-12 trial investigating M9241 plus avelumab.

Methods In the dose-escalation part of JAVELIN IL-12 (NCT02994953), eligible patients had locally advanced or metastatic solid tumors; in the dose-expansion part, eligible patients had locally advanced or metastatic urothelial carcinoma (UC) that had progressed with first-line therapy. Patients received M9241 at 4, 8, 12, or 16.8 µg/kg every 4 weeks (Q4W) plus avelumab 10 mg/kg every 2 weeks (Q2W, dose levels (DLs) 1–4) or M9241 16.8 µg/kg Q4W plus avelumab 800 mg once a week for 12 weeks followed by Q2W (DL5/dose expansion). Primary endpoints for the dose-escalation part were adverse events (AEs) and dose-limiting toxicities (DLTs), and those for the dose-expansion part were confirmed best overall response (BOR) per investigator (Response Evaluation Criteria in Solid Tumors V.1.1) and safety. The dose-expansion part followed a two-stage design; 16 patients were enrolled and treated in stage 1 (single-arm part). A futility analysis based on BOR was planned to determine whether stage 2 (randomized controlled part) would be initiated.

Results At data cut-off, 36 patients had received M9241 plus avelumab in the dose-escalation part. All DLs were well tolerated; one DLT occurred at DL3 (grade 3 autoimmune hepatitis). The maximum-tolerated dose was not reached, and DL5 was declared the recommended phase II dose, considering an observed drug–drug interaction at DL4. Two patients with advanced bladder cancer (DL2 and DL4) had prolonged complete responses. In the dose-expansion part, no objective responses were recorded in the 16 patients with advanced UC; the study failed to meet the criterion (≥3 confirmed objective responses) to initiate stage 2. Any-grade treatment-related AEs occurred in 15 patients (93.8%), including grade ≥3 in 8 (50.0%); no treatment-related deaths occurred. Exposures for avelumab and M9241 concentrations were within expected ranges.

Conclusions M9241 plus avelumab was well tolerated at all DLs, including the dose-expansion part, with no new safety signals. However, the dose-expansion part did not meet the predefined efficacy criterion to proceed to stage 2.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Preclinical studies of concurrent therapy with M9241 plus avelumab resulted in additive or synergistic antitumor effects, suggesting that combining M9241 with avelumab may improve clinical activity.

WHAT THIS STUDY ADDS

⇒ Data from this first-in-human phase Ib trial show that the combination of M9241 plus avelumab was generally well tolerated with a manageable safety profile; however, the dose-expansion part in patients with advanced urothelial carcinoma (UC) did not meet the predefined efficacy criterion to proceed further, which was unexpected and inconsistent with previous avelumab monotherapy studies in advanced UC. Definitive reasons for this negative outcome are unclear but suggest that combining M9241 with weekly avelumab dosing might have reduced antitumor activity compared with avelumab monotherapy every 2 weeks.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These results suggest that combining M9241 with weekly avelumab does not improve antitumor activity in advanced UC, highlighting that further assessment is needed to determine whether M9241 has a role in the treatment of advanced UC.

INTRODUCTION

Immune checkpoint inhibitors have shown antitumor activity across a range of tumor types. Avelumab is an anti-programmed death ligand 1 (PD-L1) monoclonal antibody that is approved in various countries worldwide, including the USA, European Union countries, and Japan, as first-line maintenance treatment for patients with locally advanced or metastatic urothelial carcinoma (UC) that has not progressed with first-line platinum-based chemotherapy, and in the USA, Canada, and Israel for patients with disease progression after platinum-based chemotherapy.[1–5] Avelumab is also approved as monotherapy for patients with metastatic Merkel cell carcinoma and in combination with axitinib for first-line treatment of advanced renal cell carcinoma.[1 2]

Interleukin (IL)-12 is a potent proinflammatory cytokine that promotes effective antitumor immune responses via several mechanisms, including upregulating interferon (IFN)-γ production, promoting differentiation of T-helper 1 cells, and enhancing antibody-dependent cell-mediated cytotoxicity. However, non-targeted IL-12 treatment is associated with toxicity and low levels of IL-12 in the tumor microenvironment.[6 7] M9241 is a novel immunocytokine composed of two heterodimers of IL-12 fused to the heavy chains of the human antibody NHS76.[7 8] NHS76 recognizes DNA–histone epitopes, which can be exposed in necrotic regions of solid tumors when rapid tumor growth outpaces the development of blood vessels.[6 9] M9241 aims to achieve a high concentration of IL-12 within the tumor but a relatively low systemic dose of IL-12, thereby reducing potential toxicity.[6 7] Preclinical studies of M9241 demonstrated that NHS76 targets IL-12 to areas of tumor necrosis, enhancing antitumor activity and decreasing systemic toxicity.[7]

In preclinical models, treatment with a murine version of M9241 (NHS-muIL12) led to changes in the bladder tumor microenvironment, reverting to an immunopermissive environment.[10] Additionally, concurrent therapy with M9241 and avelumab resulted in additive or synergistic antitumor effects.[11 12] In preclinical studies, increased antitumor efficacy also correlated with a higher frequency of tumor antigen-specific $CD8^+$ T cells and enhanced T-cell activation.[7 11] These findings suggest that combining M9241 and avelumab may improve antitumor activity. In a first-in-human, phase I, multiple dose-escalation trial, M9241 monotherapy was well tolerated up to a dose of 16.8 µg/kg and elicited preliminary evidence of clinical benefit in patients with advanced solid tumors.[13] Here, we report the safety, pharmacokinetics, pharmacodynamics, and clinical activity of M9241 plus avelumab in patients with advanced solid tumors, including dose expansion in patients with advanced UC from the phase Ib JAVELIN IL-12 trial.

METHODS

Study design

JAVELIN IL-12 (NCT02994953) was an open-label, multicenter, dose-finding, phase Ib trial with a consecutive parallel-group expansion conducted at 33 sites in North America and Europe. In the dose-escalation part, sequential cohorts of patients received combination therapy with M9241 plus avelumab in a modified 3+3 design to determine the maximum tolerated dose, defined as the maximal dose at which no more than one of six evaluable patients experienced a dose-limiting toxicity (DLT). Patients in the dose-escalation cohorts received one of four ascending dose levels (DLs) of M9241 subcutaneously every 4 weeks (Q4W) plus avelumab 10 mg/kg intravenously every 2 weeks (Q2W). For DL1–DL4, patients received M9241 at 4, 8, 12, and 16.8 µg/kg, respectively. For DL5, patients received M9241 at 16.8 µg/kg Q4W plus avelumab 800 mg once a week (QW) for a 12-week induction followed by Q2W. To limit infusion-related reactions, patients received pretreatment with diphenhydramine and acetaminophen prior to the first four infusions of avelumab. All patients continued treatment until confirmed disease progression, unacceptable toxicity, withdrawal, or loss to follow-up. The dose-expansion part followed a two-stage design; in stage 1 (single-arm part), 16 patients were enrolled and treated with the recommended phase II dose (RP2D) of the combination (DL5) to determine the clinical activity and safety of combination treatment at the RP2D. A futility analysis based on the occurrence of <3 confirmed objective responses by Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1 was planned to determine the futility of the study prior to initiation of stage 2 (randomized part).

Patient eligibility

In the dose-escalation part, eligible patients were aged ≥18 years and had histologically or cytologically proven metastatic or locally advanced solid tumors for which no standard therapy existed; standard therapy had failed; the patient was intolerant of established therapy known to provide clinical benefit for their condition; or standard therapy was not acceptable to the patient. Prior treatment with an immune checkpoint inhibitor was allowed. The dose-expansion part enrolled patients with locally advanced or metastatic UC that had progressed on ≥1 prior line of platinum-based chemotherapy and were anti-programmed death 1/PD-L1 treatment-naive. Additional inclusion criteria for both parts included an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1 and measurable disease per RECIST V.1.1 (except for patients with prostate or breast cancer in the dose-escalation part); tumor tissue for biomarker assessments was required for the dose-expansion part only. Patients were excluded if they had been previously treated with IL-12 or were intolerant to immune checkpoint inhibitor therapy, which was defined as the occurrence of an adverse event (AE) requiring drug discontinuation.

Endpoints and assessments

In the dose-escalation part, primary endpoints were AEs according to the National Cancer Institute's Common Terminology Criteria for AEs V.4.03 and DLTs during

the DLT observation period. Patients were observed for DLTs for the first 3 weeks after undergoing study treatment (one or more injections of M9241 and two infusions of avelumab). A DLT was defined as any grade ≥3 non-hematological AE or grade ≥4 hematological AE that occurred during the DLT observation period and was determined by the investigator or sponsor to be related to either or both study drugs. Any grade 3 autoimmune thyroid-related toxicity that did not clinically resolve to grade ≤2 within 7 days of initiating therapy and any grade ≥3 thrombocytopenia with medically concerning bleeding were also defined as a DLT. The following treatment-related adverse events (TRAEs) were excluded from the DLT definition: grade 4 neutropenia of <5 days' duration; grade 3 infusion-related reaction that resolved within 6 hours of the end of infusion and was controlled with medical management; grade 3 diarrhea or skin toxicity that resolved to grade ≤1 in less than 7 days after medical management (eg, immunosuppressant treatment) was initiated; transient (≤48 hours) grade 3 fatigue, local reactions, influenza-like symptoms, fever, headache, nausea, emesis, and diarrhea; other single laboratory values out of normal range that had no clinical correlate and resolved to grade ≤1 or baseline within 7 days with adequate medical management; and tumor flare phenomenon defined as local pain, irritation, or rash localized at known or suspected tumor sites. Secondary endpoints for the dose-escalation part included pharmacokinetics and confirmed best overall response (BOR) by investigator per RECIST V.1.1.

In the dose-expansion part, the primary endpoints were confirmed BOR by investigator per RECIST V.1.1 and safety. Secondary endpoints included progression-free survival (PFS) by investigator per RECIST V.1.1, overall survival (OS), and pharmacokinetics analyses.

Pharmacokinetics

Concentrations of M9241 and avelumab were measured by a validated immunoassay, with lower limits of quantitation of 1 µg/L and 0.2 mg/L, respectively. Non-compartmental pharmacokinetic analyses were performed using actual doses per patient's weight, actual time points, and actual duration of infusions. Pharmacokinetic parameters of interest were area under the concentration–time curve over the dosing interval (AUC_{tau}), maximum concentration (C_{max}), concentration at the end of dosing interval (C_{trough}), time to maximum concentration (t_{max}), and half-life. In the dose-escalation part, potential pharmacokinetic interaction with avelumab as a 'victim' when coadministered with M9241 was assessed by comparing observed avelumab pharmacokinetic parameters (eg, AUC_{tau} and C_{trough}) with the corresponding median values predicted using an avelumab population pharmacokinetic model.[14] To compare pharmacokinetic parameters for M9241 when combined with avelumab with parameters for M9241 monotherapy, observed M9241 pharmacokinetic data from a phase I trial were used,[13] since no M9241 population pharmacokinetic model is currently available.

Biomarker analyses

In both the dose-escalation and dose-expansion parts of the study, changes in serum levels of cytokines at baseline and during treatment were determined using a validated 10-plex immunoassay (Meso Scale Discovery) following the manufacturer's instructions.

In the dose-escalation part, cryopreserved peripheral blood mononuclear cells (PBMCs) were examined by multicolor flow cytometry to identify 135 peripheral immune cell subsets[15] using the methodology described previously.[16 17] Additionally, in the dose-escalation part, gene expression analyses were performed on total RNA extracted from cryopreserved PBMCs that had been collected from patients before and after 15 days of treatment using the Qiagen RNeasy Plus Minikit (Valencia, California, USA) per the manufacturer's instructions. RNA was analyzed using the nCounter PanCancer Immune Profiling panel (NanoString Technologies, Seattle, Washington, USA), per the manufacturer's protocol. Genes with $p<0.05$ and >1.5-fold change after treatment were analyzed for enrichment of pathways using Ingenuity Pathway Analysis software (Redwood City, California, USA). Changes in immune parameters between two time points were assessed for statistical significance using a Wilcoxon signed-rank test. Blood-based PD-L1 gene expression was assessed in the dose-escalation part using samples from baseline and during treatment; total RNA was extracted using the PAXgene Blood miRNA Kit, and PD-L1 gene expression was detected by using a validated digital droplet PCR method. TaqMan assays containing primer and probe sets were selected based on coverage of the PD-L1 gene and were tested for linearity and efficiency using real-time quantitative PCR. Four reference genes were analyzed in genomic DNA derived from a positive control cell line (A549 treated with IFN-γ).

In the dose-expansion part, immune cell subsets, including T-cell, B-cell, and natural killer (NK)-cell subsets; myeloid-derived suppressor cells; and monocytes from whole-blood samples collected at baseline and during treatment (days 15 and 29) were assessed by flow cytometry using four validated panels of antibodies. Baseline PD-L1 tumor expression in patients with UC was assessed in formalin-fixed paraffin-embedded tissue by immunohistochemistry using the VENTANA PD-L1 (SP263) assay. PD-L1+ status was defined by the following cut-offs: ≥1%, ≥5%, or ≥50% expression in tumor cells or expression in ≥25% of tumor cells; expression in ≥25% of tumor-associated immune cells if the percentage of immune cells present was >1%; or expression in 100% of tumor-associated immune cells if the percentage of immune cells present was 1%.

Statistical analysis

Safety and efficacy were assessed in all patients who received ≥1 dose of any study treatment. Descriptive

Table 1 Baseline characteristics in the dose-escalation and dose-expansion parts

	Dose-escalation part					Dose-expansion part
	Avelumab 10 mg/kg Q2W				Avelumab 800 mg QW→Q2W* plus M9241 16.8 µg/kg (n=7)	Avelumab 800 mg QW→Q2W* plus M9241 16.8 µg/kg (n=16)
	M9241 4 µg/kg (n=9)	M9241 8 µg/kg (n=7)	M9241 12 µg/kg (n=7)	M9241 16.8 µg/kg (n=6)		
Median age, years (range)	60 (41–68)	64 (56–71)	60 (46–75)	55 (47–71)	61 (56–74)	66 (48–79)
Sex, n (%)						
Male	4 (44.4)	5 (71.4)	4 (57.1)	4 (66.7)	5 (71.4)	12 (75.0)
Female	5 (55.6)	2 (28.6)	3 (42.9)	2 (33.3)	2 (28.6)	4 (25.0)
Race, n (%)						
White	7 (77.8)	5 (71.4)	6 (85.7)	6 (100)	6 (85.7)	8 (50.0)
Black	0	1 (14.3)	1 (14.3)	0	1 (14.3)	0
Asian	1 (11.1)	0	0	0	0	0
Other	1 (11.1)	1 (14.3)	0	0	0	0
Not collected	0	0	0	0	0	8 (50.0)
ECOG PS, n (%)						
0	3 (33.3)	3 (42.9)	3 (42.9)	3 (50.0)	3 (42.9)	5 (31.3)
1	6 (66.7)	4 (57.1)	4 (57.1)	3 (50.0)	4 (57.1)	11 (68.8)
Prior lines of therapy for metastatic disease, n (%)						
1	0	1 (14.3)	0	2 (33.3)	4 (57.1)	11 (68.8)
2	1 (11.1)	2 (28.6)	2 (28.6)	1 (16.7)	0	2 (12.5)
3	1 (11.1)	1 (14.3)	2 (28.6)	0	1 (14.3)	0
≥4	2 (22.2)	2 (28.6)	0	1 (16.7)	1 (14.3)	0

*Avelumab QW for 12 weeks then Q2W thereafter.
ECOG PS, Eastern Cooperative Oncology Group performance status; QW, once a week; Q2W, every 2 weeks.

statistics were used to summarize the study results. Time-to-event endpoints were estimated using the Kaplan-Meier method, and 95% CIs for the median were calculated using the Brookmeyer-Crowley method.

RESULTS

Dose-escalation part

Patients

Of a total of 41 screened patients, 36 were enrolled across the five dose groups. At final analysis (data cut-off: November 20, 2020), 36 patients with various solid tumors had received ≥1 dose of M9241 plus avelumab. The primary tumor sites for these patients are reported in online supplemental table S1. Most patients were male and White, and all had an ECOG PS of 0 or 1 (table 1). All patients had discontinued study treatment at the data cut-off. The most common reasons for treatment discontinuation were disease progression (M9241, 52.8%; avelumab, 50.0%) and AEs (M9241, 30.6%; avelumab, 33.3%) (figure 1). One patient (2.8%) who had a complete response with M9241 8 µg/kg plus avelumab discontinued both study drugs per protocol; on disease reoccurrence, this patient subsequently reinitiated both study drugs. Median treatment duration for both drugs across all dose cohorts was 8.0 weeks (range, 4.0–173.1), with a median of two M9241 administrations and four avelumab infusions.

Safety

All 36 patients had ≥1 treatment-emergent AE of any grade; 25 (69.4%) had a grade ≥3 AE (table 2). The most common AEs of any grade (≥25% of all patients) were fever (61.1%), fatigue (58.3%), anemia (52.8%), decrease in lymphocyte count (50.0%), nausea (38.9%), influenza-like illness (36.1%), decreased appetite (30.6%), dizziness (27.8%), chills (25.0%), diarrhea (25.0%), hypoalbuminemia (25.0%), and vomiting (25.0%). TRAEs of any grade occurred in 30 patients (83.3%). Grade ≥3 TRAEs occurred in three patients (8.3%): increased amylase and increased lipase (both grade 3 and considered related to combination treatment), immune-mediated hepatitis (grade 3 and considered related to combination treatment), and decreased lymphocyte count (grade 3 and considered related to M9241).

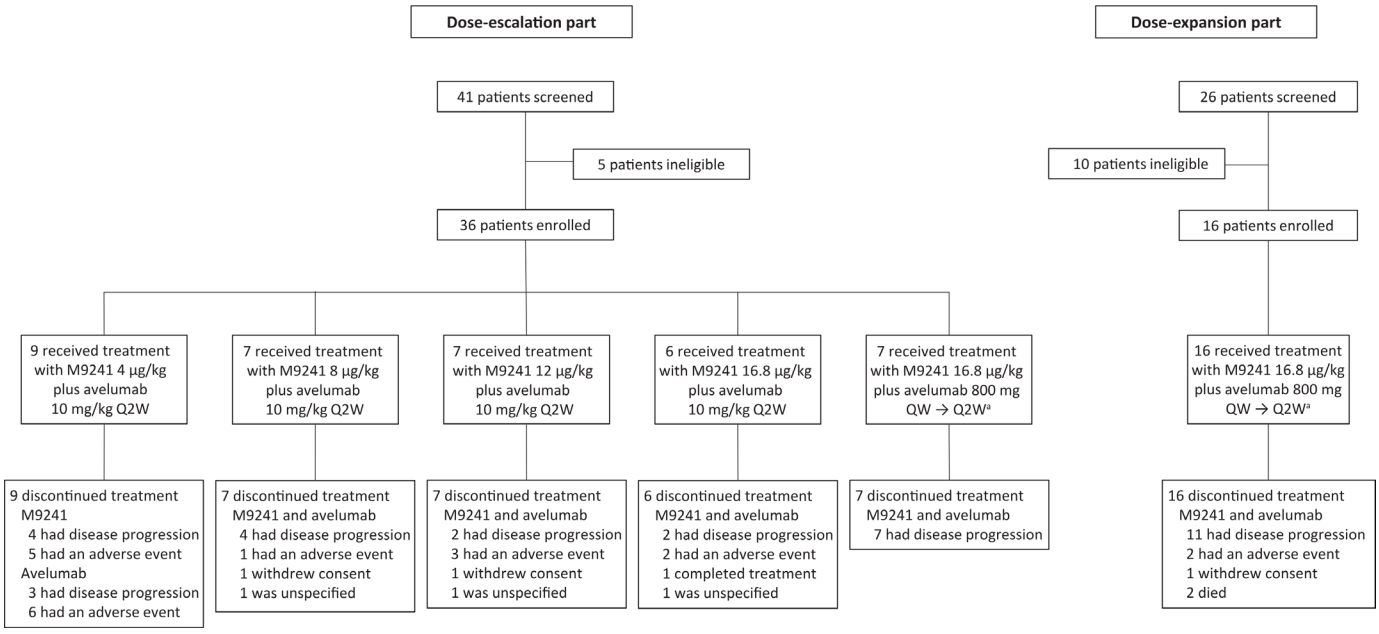

Figure 1 Trial profile for the dose-escalation and dose-expansion parts of the study. [a]Avelumab QW for 12 weeks then Q2W thereafter. QW, once a week; Q2W, every 2 weeks.

The maximum tolerated dose was not formally reached. The maximum administered dose (M9241 16.8 µg/kg Q4W plus avelumab 800 mg QW for 12 weeks followed by Q2W) was declared as the RP2D. In total, 32 patients were evaluable for DLTs. One patient had a DLT of grade 3 immune-mediated hepatitis in the M9241 12 µg/kg plus avelumab cohort, which resolved following steroid therapy. A cytokine release syndrome AE was reported in four patients (11.1%); all were of grade 1 or 2 severity; none led to treatment discontinuation. However, one patient (2.8%) with prostate cancer who received M9241 16.8 µg/kg Q4W plus avelumab 10 mg/kg Q2W had a serious cytokine release syndrome AE of grade 2 severity that was reported on day 2 and had resolved with supportive treatment on day 6; this AE was related to both study drugs but did not lead to drug interruption. Cytokine release syndrome was diagnosed based on clinical symptoms rather than on cytokine measurements; therefore, it is unclear whether these diagnoses were cytokine release syndrome or infusion-related reactions because of overlapping symptomatology and/or timing of treatment.

Clinical activity

Across the dose-escalation cohorts, two patients had prolonged complete responses in the M9241 8 µg/kg Q4W plus avelumab 10 mg/kg Q2W and M9241 16.8 µg/kg Q4W plus avelumab 10 mg/kg Q2W cohorts (table 3 and figure 2A). One patient with immune checkpoint inhibitor–refractory UC (prior atezolizumab treatment) initially achieved a partial response (time to response, 1.8 months) that subsequently deepened to a complete response (duration of response, 24.6 months). This patient discontinued treatment after 24 months (per protocol) and reinitiated treatment due to disease progression after 5 months without treatment; this patient again achieved a partial response that converted to a complete response, which was ongoing at the last reported assessment. One patient with metastatic cancer, with primary site at the anterior wall of the bladder, initially achieved a partial response (time to response, 1.3 months) that subsequently deepened to a complete response that was maintained through to the last reported assessment (duration of response, 29.0+ months). An additional nine patients (25.0%) had stable disease as their BOR.

Pharmacokinetics

Avelumab C_{trough} at cycle 1 day 15 was reduced in patients receiving M9241 16.8 µg/kg Q4W plus avelumab 10 mg/kg Q2W compared with monotherapy at the efficacious dose of 10 mg/kg Q2W[18] (figure 3A). Consequently, an additional DL was introduced, M9241 16.8 µg/kg Q4W plus avelumab 800 mg QW for 12 weeks followed by Q2W. With this dosage, avelumab C_{trough} at cycle 1 day 8 was similar to that for avelumab monotherapy at 10 mg/kg QW[19] (figure 3A). In general, M9241 exposure (specifically AUC_{tau} and C_{max} in cycle 1) tended to increase with increasing M9241 dose. At the M9241 16.8 µg/kg dose, the range of exposures overlapped with those observed in a phase I trial of M9241 monotherapy at the same dose.[13] The t_{max} of M9241 was 1–3 days, and the half-life was variable but was ≈3 days. Compared with other patients at the same DL, the patient with a DLT did not have unexpected/higher exposures.

Biomarker analyses

In total, 27 patients were analyzed for changes in serum analytes, 135 immune cell subsets, and gene expression. A time-dependent induction in serum levels of IFN-γ was observed after M9241 administration (figure 4A). IFN-γ levels increased at day 2 of both cycles 1 and 2 and

Table 2 Safety summary from the dose-escalation and dose-expansion parts

Events, n (%)	Dose-escalation part					Dose-expansion part
	Avelumab 10 mg/kg Q2W				Avelumab 800 mg QW→Q2W** plus M9241 16.8 µg/kg (n=7)	Avelumab 800 mg QW→Q2W** plus M9241 16.8 µg/kg (n=16)
	M9241 4 µg/kg (n=9)	M9241 8 µg/kg (n=7)	M9241 12 µg/kg (n=7)	M9241 16.8 µg/kg (n=6)		
AE, any grade	9 (100)	7 (100)	7 (100)	6 (100)	7 (100)	16 (100)
Grade ≥3	6 (66.7)	5 (71.4)	6 (85.7)	4 (66.7)	4 (57.1)	14 (87.5)
TRAE, any grade	8 (88.9)	5 (71.4)	6 (85.7)	5 (83.3)	6 (85.7)	15 (93.8)
Grade ≥3	0	1 (14.3)	1 (14.3)	1 (16.7)	0	8 (50.0)
Avelumab-related, any grade	2 (22.2)	4 (57.1)	3 (42.9)	4 (66.7)	5 (71.4)	15 (93.8)
Grade ≥3	0	1 (14.3)	1 (14.3)	0	0	8 (50.0)
M9241-related, any grade	8 (88.9)	4 (57.1)	6 (85.7)	5 (83.3)	6 (85.7)	12 (75.0)
Grade ≥3	0	1 (14.3)	1 (14.3)	1 (16.7)	0	6 (37.5)
AE leading to discontinuation of either study drug	6 (66.7)	2 (28.6)	3 (42.9)	2 (33.3)	1 (14.3)	3 (18.8)
TRAE leading to discontinuation of study drug						
Avelumab-related	2 (22.2)	0	1 (14.3)	0	0	0
M9241-related	1 (11.1)	0	1 (14.3)	0	0	0
AE leading to death	0	1 (14.3)	0	1 (16.7)	1 (14.3)	2 (12.5)
TRAE leading to death	0	0	0	0	0	0
Infusion-related reaction	2 (22.2)	2 (28.6)	0	2 (33.3)	2 (28.6)	7 (43.8)
Immune-related AE	2 (22.2)	1 (14.3)	2 (28.6)	0	1 (14.3)	1 (6.3)
Cytokine release syndrome	0	0	0	1 (16.7)	3 (42.9)	3 (18.8)
Avelumab-related	0	0	0	1 (16.7)	3 (42.9)	3 (18.8)
M9241-related	0	0	0	1 (16.7)	3 (42.9)	3 (18.8)
Grade ≥3	0	0	0	0	0	0

*Avelumab QW for 12 weeks then Q2W thereafter.
AE, adverse event; QW, once a week; Q2W, every 2 weeks; TRAE, treatment-related adverse event.

Table 3 Confirmed BOR in the dose-escalation and dose-expansion parts

	Dose-escalation part					Dose-expansion part
	Avelumab 10 mg/kg Q2W				Avelumab 800 mg QW→Q2W** plus M9241 16.8 µg/kg (n=7)	Avelumab 800 mg QW→Q2W** plus M9241 16.8 µg/kg (n=16)
	M9241 4 µg/kg (n=9)	M9241 8 µg/kg (n=7)	M9241 12 µg/kg (n=7)	M9241 16.8 µg/kg (n=6)		
Confirmed BOR, n (%)						
Complete response	0	1 (14.3)	0	1 (16.7)	0	0
Partial response	0	0	0	0	0	0
Stable disease	2 (22.2)	2 (28.6)	2 (28.6)	1 (16.7)	2 (28.6)	2 (12.5)
Progressive disease	6 (66.7)	3 (42.9)	4 (57.1)	2 (33.3)	5 (71.4)	13 (81.3)
Not evaluable	1 (11.1)	1 (14.3)	1 (14.3)	2 (33.3)	0	1 (6.3)

*Avelumab QW for 12 weeks then Q2W thereafter.
BOR, best overall response; QW, once a week; Q2W, every 2 weeks.

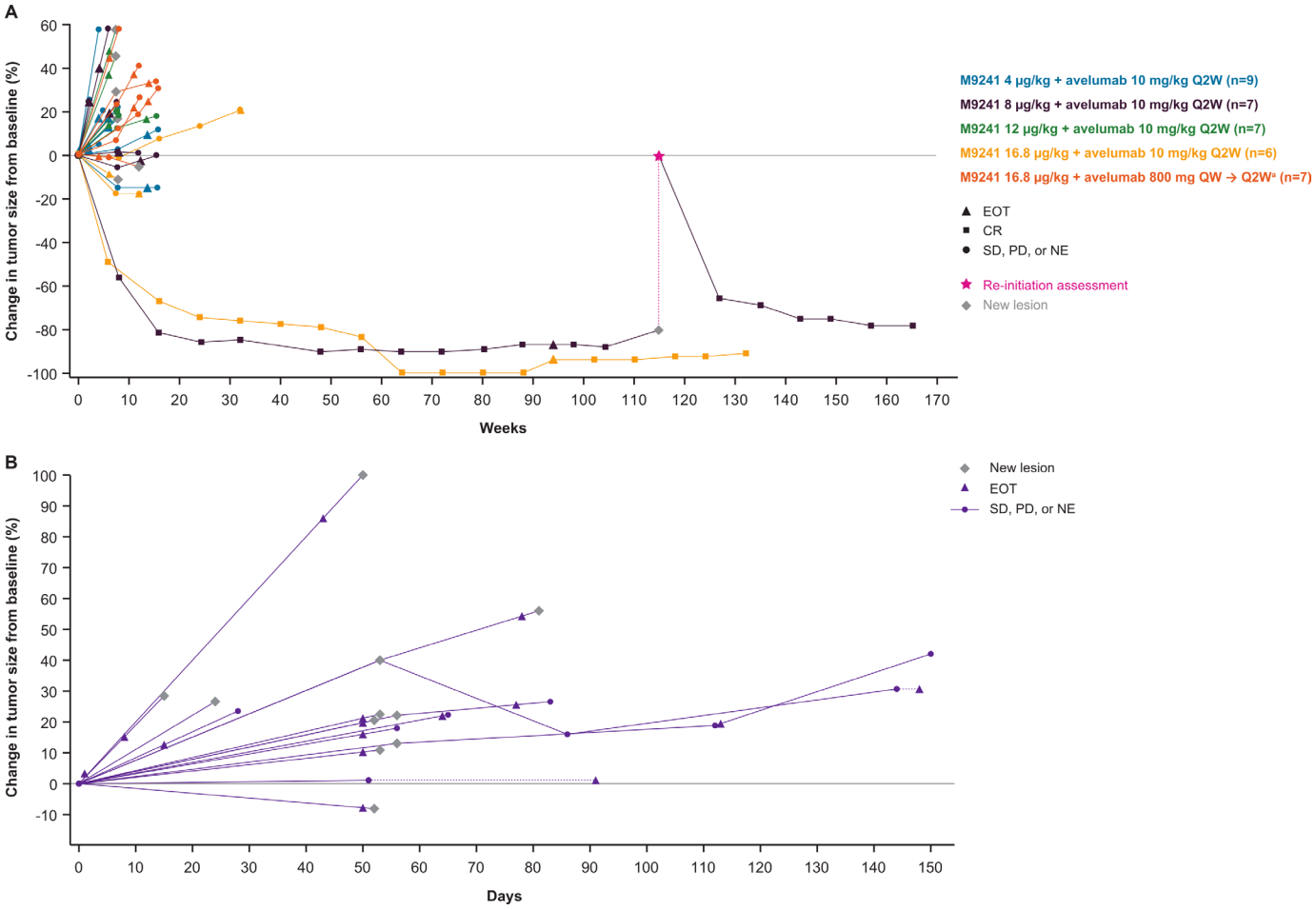

Figure 2 Change in tumor size from baseline in the (A) dose-escalation part (n=36) and (B) dose-expansion part who were treated with M9241 16.8 µg/kg plus avelumab 800 mg QW for 12 weeks then Q2W thereafter (n=15). (B) The dashed lines connect the last reported change in the sum of diameters from baseline to EOT if reported later. One patient was NE because no post-baseline sum of lesion diameters was available. [a]Avelumab QW for 12 weeks then Q2W thereafter. CR, complete response; EOT, end of treatment; NE, not evaluable; PD, progressive disease; QW, once a week; Q2W, every 2 weeks; SD, stable disease.

decreased to near baseline levels after 8–15 days. IFN-γ induction kinetics were consistent with M9241 pharmacokinetic data. Similar trends were observed for IL-10, IL-12p70, and tumor necrosis factor α (online supplemental figure S1).

Analyses of classic PBMC subsets showed a reduction in conventional and plasmacytoid dendritic cells after the first M9241 dose, which returned to baseline levels after the second dose (online supplemental figure S2A). Analyses of refined immune cell subsets reflective of increased $CD8^+$ T-cell and NK-cell activity were also seen (online supplemental figure S2B). No significant dose-dependent differences were observed in immune cell subsets.

A time-dependent induction in PD-L1 gene expression in peripheral blood was observed following M9241 administration in combination with avelumab (data for DL5 are not available because of limited samples) (online supplemental figure S3). PD-L1 gene expression is known to be upregulated by IFN-γ,[20] which is induced by M9241. Accordingly, the induction of PD-L1 gene expression correlated with the induction of IFN-γ (figure 4A and online supplemental figure S3). Changes in expression were analyzed for multiple genes within signaling pathways after the first combined dose of M9241 plus avelumab, which showed notable increases in NK-cell signaling and T-cell exhaustion signaling (online supplemental figure S4). These observed changes were seen independent of the DL of M9241.

Dose-expansion part

Patients

After the RP2D was determined, enrollment began of patients with advanced or metastatic UC in a dose-expansion cohort. Of a total of 26 screened patients, 16 were enrolled. At final analysis (data cut-off: November 6, 2020), 16 patients with advanced UC had received ≥1 dose of M9241 plus avelumab at the RP2D. Of these, six patients (37.5%) had upper-tract tumors and 10 (62.5%) had lower-tract tumors. The median time since first diagnosis was 1.6 years (range, 0.3–12.3); the median time since metastatic diagnosis was 0.7 years (range, 0.1–6.1); and the median time since last disease progression was

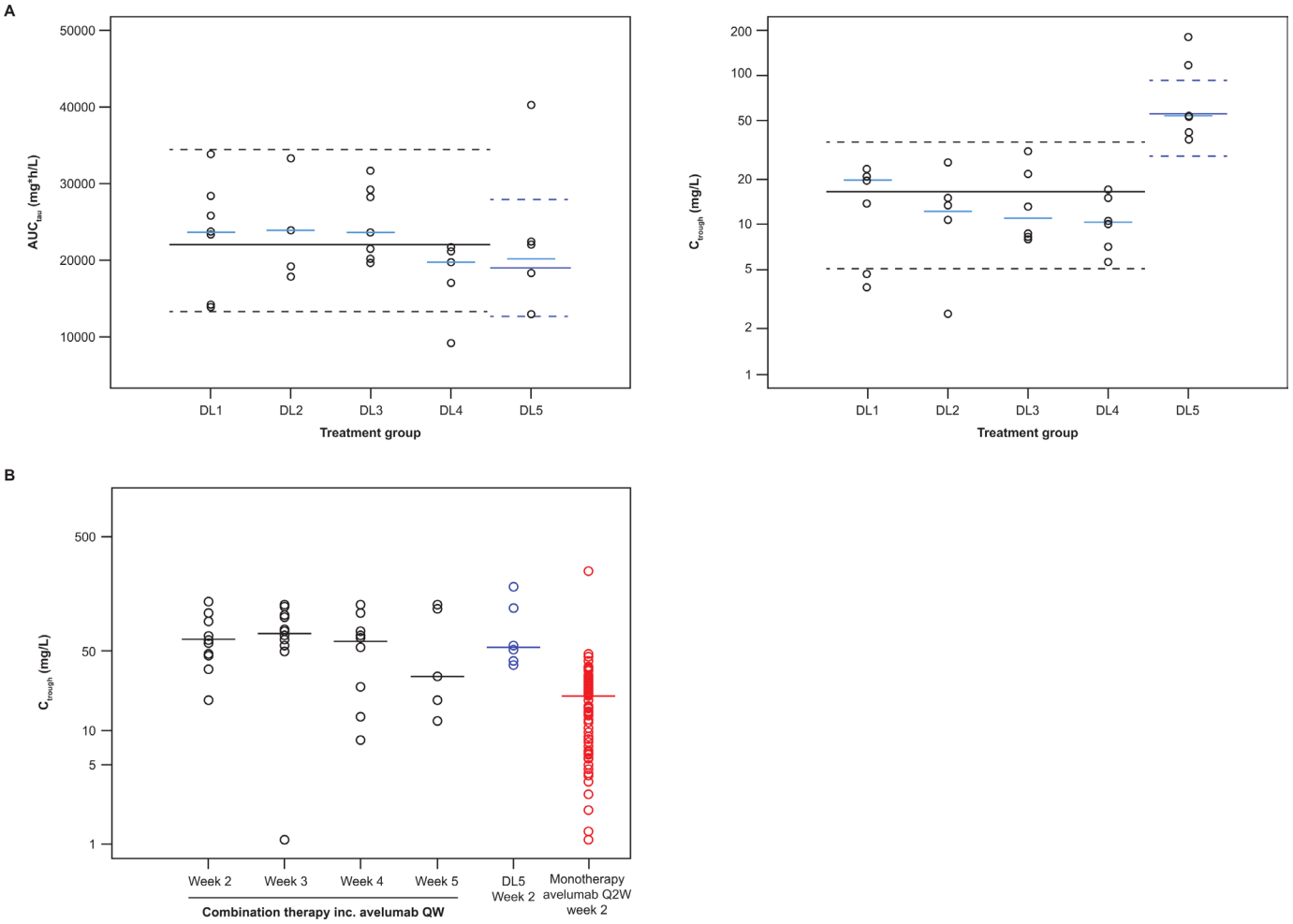

Figure 3 Summary of avelumab exposure in the dose-escalation and dose-expansion parts. (A) Median and individual AUC_{tau} and C_{trough} values after cycle 1 at different DLs in the dose-escalation cohorts. (B) Serum C_{trough} concentrations by week in the dose-expansion part versus the same dose in the dose-escalation part and monotherapy data. (A) The solid lines depict the median (50%) prediction using a population pharmacokinetic model for monotherapy (10 mg/kg Q2W for DL1–DL4, 800 mg QW for 12 weeks then Q2W thereafter for DL5); the dotted lines depict 5% and 95% predictions using the population pharmacokinetic model. (B) The data points show individual patient values, and the horizontal lines show medians; avelumab monotherapy data (red) were taken from the UC cohorts of the JAVELIN Solid Tumor trial (dose, 10 mg/kg Q2W).[18] AUC_{tau}, area under the concentration–time curve over the dosing interval; C_{trough}, concentration at the end of dosing interval; DL, dose level; inc., including; QW, once a week; Q2W, every 2 weeks.

1.5 years (range, 0.9–2.8). Most patients were male and White, and all had an ECOG PS of 0 or 1 (table 1). Baseline PD-L1 status for patients in this part is summarized in online supplemental table S2.

All patients had discontinued study treatment at the data cut-off. The most common reason for treatment discontinuation of both study drugs was disease progression (68.8% for both) (figure 1). Median duration of treatment for both drugs was 8.0 weeks (range, 4.0–25.0), with a median of two M9241 administrations and eight avelumab infusions.

Clinical activity

No complete or partial responses were observed; thus, the study failed to meet the criterion to initiate the next stage (table 3 and figure 2B). Two patients (12.5%) had stable disease as their BOR. Median PFS was 7.6 weeks (95% CI 7.1 to 8.0, online supplemental figure S5A), and median OS was 4.9 months (95% CI 2.3 to 11.8, online supplemental figure S5B).

Pharmacokinetics

Serum concentrations of avelumab (figure 3B) and M9241 were within the expected range compared with the dose-escalation part.

Safety

All 16 patients had ≥1 AE of any grade; 14 (87.5%) had a grade ≥3 AE (table 2). TRAEs of any grade occurred in 15 patients (93.8%); the most common (≥25% of patients) were fever (50.0%), nausea (37.5%), anemia (31.3%), asthenia (31.3%), chills (31.3%), and hyperthermia (25.0%). Grade ≥3 TRAEs occurred in eight patients (50.0%): anemia (18.8%), increase in gamma-glutamyl transferase (12.5%), lymphopenia (12.5%), neutropenia (12.5%), fever (12.5%), hepatocellular injury (6.3%),

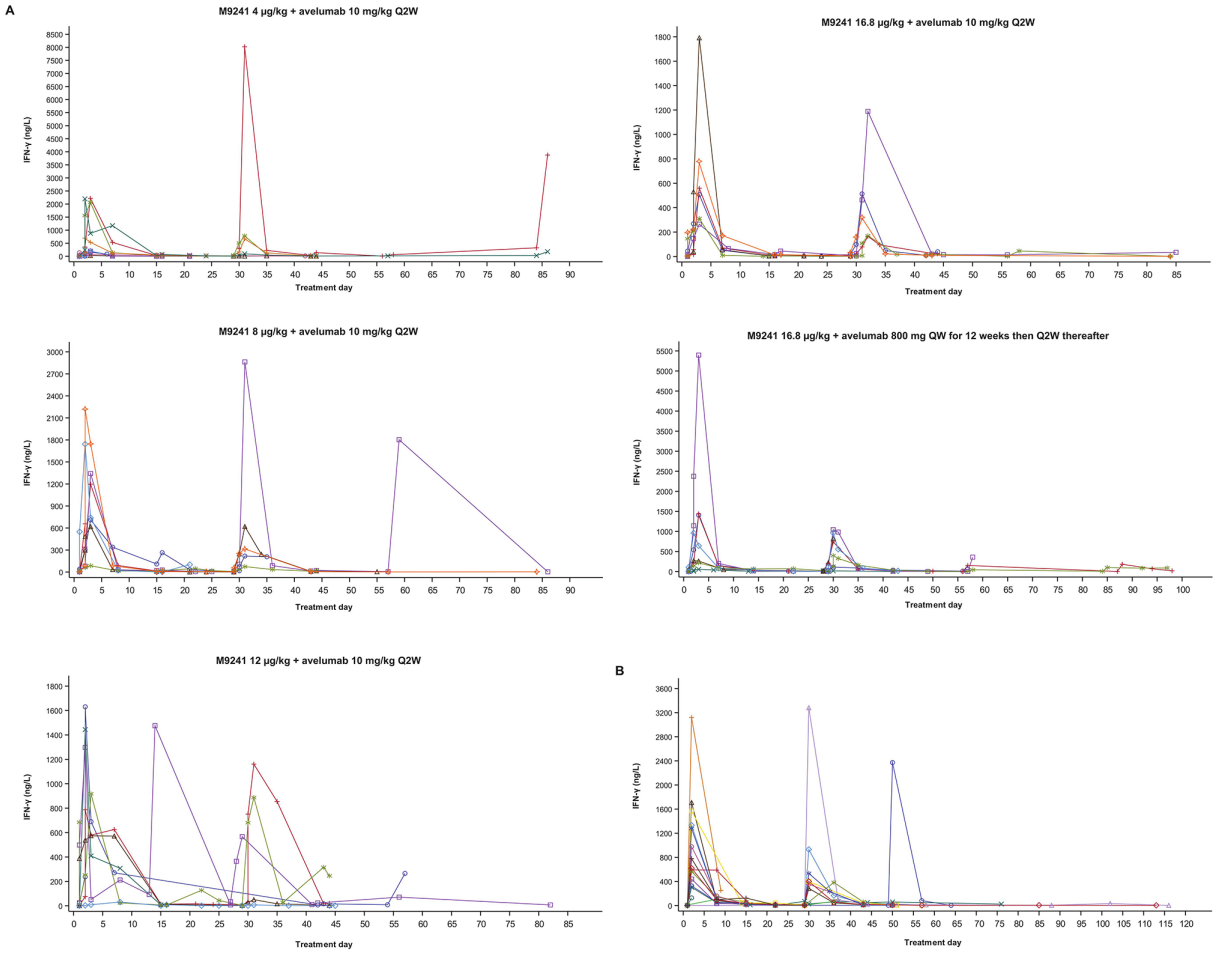

Figure 4 Serum IFN-γ profiles from individual patients in the (A) dose-escalation part and (B) dose-expansion part who were treated with M9241 16.8 µg/kg plus avelumab 800 mg QW for 12 weeks then Q2W thereafter. Patients with a complete response (M9241 8 µg/kg plus avelumab 10 mg/kg Q2W (DL2) and M9241 16.8 µg/kg plus avelumab 10 mg/kg Q2W (DL4)) are depicted with orange lines. IFN, interferon; QW, once a week; Q2W, every 2 weeks.

hyperlipasemia (6.3%), and hypertension (6.3%). No patient had a TRAE that led to treatment discontinuation, and no treatment-related deaths occurred. An immune-related AE, which was a grade 3 acute kidney injury, occurred in one patient (6.3%). Infusion-related reaction occurred in seven patients (43.8%); all were grade 1 or 2. Cytokine release syndrome, as assessed by the investigator, was reported in three patients (18.8%); all were grade 1 or 2. No correlation between increased levels of cytokines in the serum (including IFN-γ) and the occurrence of cytokine release syndrome events could be obtained because of the limited number of patients who experienced cytokine release syndrome in the dose-expansion part.

Biomarker analyses

Similar to findings from the dose-escalation part, a time-dependent induction in serum levels of IFN-γ was observed after M9241 administration in combination with avelumab (figure 4B). IFN-γ levels increased at day 2 of both cycles 1 and 2 and decreased to near baseline level after 8–15 days. Similar trends were observed for IL-10 and IL-12p70 (online supplemental figure S6). A time-dependent increase in NK and $CD8^+$ T-cell proliferation was observed after M9241 administration in combination with avelumab (online supplemental figure S7). Based on a ≥5% tumor cell cut-off (VENTANA PD-L1 (SP263) assay), three patients (18.8%) had PD-L1+ tumors and 12 patients (75.0%) had PD-L1− tumors; one patient (6.3%) was not evaluable (online supplemental table S2).

DISCUSSION

In this first-in-human study, we report the safety, clinical activity, and pharmacokinetics of an anti-PD-L1 antibody plus an IL-12 immunocytokine in patients with locally advanced or metastatic solid tumors, with a dose expansion in patients with advanced UC. The safety profile of M9241 plus avelumab was similar to that observed with individual agents; grade ≥3 TRAEs were reported in 21% of patients across both cohorts with combination therapy vs 20% of patients with M9241 alone[13] and 12% with avelumab monotherapy at 10 mg/kg Q2W.[18] However, the number of patients in the current study was small,

and caution should be used in the interpretation of the results.

Analysis of safety, pharmacokinetics, and pharmacodynamics data from the dose-escalation part led to the selection of M9241 16.8 µg/kg Q4W plus avelumab 800 mg QW for 12 weeks followed by Q2W as the RP2D. Based on non-clinical data and the mechanism of action of M9241, a potential pharmacokinetic interaction between M9241 and avelumab was expected.[12] The observed drug–drug interaction (reduced avelumab exposure with M9241 16.8 µg/kg Q4W plus avelumab 10 mg/kg Q2W vs monotherapy) was likely driven by M9241-mediated IFN-γ induction, causing PD-L1 upregulation in the periphery and tumor, leading to an increased target-mediated clearance of avelumab (sink effect). Consequently, the more frequent avelumab dosing of 800 mg QW for 12 weeks followed by Q2W was introduced to mitigate the sink effect.[19] At the RP2D, pharmacokinetic exposure metrics for M9241 16.8 µg/kg Q4W plus avelumab 800 mg QW were non-inferior to avelumab 10 mg/kg monotherapy Q2W, and concentrations of M9241 were within expected ranges for M9241 monotherapy.[13]

Across the dose-escalation cohorts, two patients had prolonged objective responses (both complete responses) for >24 months, both of which were ongoing at data cut-off. Both patients had advanced bladder cancer, and one patient had received previous immune checkpoint inhibitor treatment. Despite the promising antitumor activity seen in the dose-escalation part with M9241 plus avelumab, no objective responses occurred among 16 patients with platinum-treated advanced UC who received the RP2D of M9241 16.8 µg/kg Q4W plus avelumab 800 mg QW in the dose-expansion part. The study therefore failed to meet the predefined go criterion to proceed to stage 2 (≥3 objective responses), and the study was stopped due to futility at the end of stage 1. These results were unexpected and inconsistent with previous studies of avelumab monotherapy in advanced UC (objective response rate, 16.5%).[18] In addition, median OS in the dose-expansion part was shorter than with avelumab monotherapy (4.9 vs 7.0 months),[18] indicating that patients in this study may have had more aggressive or resistant disease. However, definitive reasons for the negative outcome of the dose-expansion part of this trial are unclear.

Biomarker analyses showed immunological activity of M9241 consistent with that of previous studies[13] and the known function of IL-12[8]; therefore, these analyses did not provide a clear explanation for the limited efficacy that was observed. There was a time-dependent induction of serum levels of cytokines (including IFN-γ, IL-10, and IL-12p70) in the dose-expansion part of the study, consistent with the dose-escalation part. In the dose-expansion part, a time-dependent increase in $CD8^+$ T-cell and NK-cell proliferation in peripheral blood was also observed.

IL-12 may have had an antagonistic effect via its impact on PD-L1 expression and alteration of the overall immunosuppressive state of the tumor microenvironment. IFN-γ induced by IL-12 may have triggered a negative feedback loop to increase expression of PD-L1 and potentially activate additional regulatory mechanisms, for example, by the observed increased expression of immune checkpoint proteins such as LAG-3 (online supplemental figure S4), and other immunoregulatory mechanisms conferring adaptive immune resistance of tumor cells.[20 21] Additional RNA-based studies could help to understand the increase in markers associated with T-cell exhaustion and/or activation; however, we were unable to perform functional experiments due to limited amounts of PBMCs. M9241 was administered subcutaneously and, therefore, first needed to transition from the site of injection to the tumor microenvironment, passing through other tissues and the blood stream, where PD-L1 was then induced. There is a potential that any peripheral sink effect or other regulatory mechanisms may have been avoided with intralesional M9241 administration, which could have also allowed for the use of a lower dose. However, M9241 was not designed as an intralesional drug and instead targets the tumor microenvironment via NHS76 after systemic injection. The avelumab QW treatment schedule may not be able to compensate for higher PD-L1 levels in the tumor or for other regulatory mechanisms not conferred by PD-L1. Therefore, M9241 treatment may have led to peripheral immune exhaustion and an overall immunosuppressive state, potentially due to IFN-γ, that may hinder the antitumor activity of this combination.[22 23] Additionally, in the dose-escalation part, two patients had a complete response with avelumab Q2W dosing, compared with none of the 16 patients in the dose-expansion part with avelumab QW dosing; this suggests that treatment with M9241 in combination with more frequent avelumab QW dosing may lead to T-cell exhaustion.

Additionally, in the dose-expansion part, the proportion of patients with PD-L1− tumors based on a ≥5% tumor cell cut-off was higher than in the JAVELIN Solid Tumor UC cohorts (75.0% vs 54.2%, respectively), although different PD-L1 immunohistochemistry assays were used (VENTANA PD-L1 (SP263) vs Dako PD-L1 73–10 PharmDx, respectively).[18] However, avelumab monotherapy has also shown antitumor activity in patients with PD-L1− urothelial tumors (objective response rate of 12.3% vs 23.8% in patients with PD-L1+ tumors).[18] The proportion of patients with upper tract disease, which is associated with poor prognosis,[24] was also higher in this study than in the JAVELIN Solid Tumor UC cohorts (37.5% vs 23.3%, respectively).[18]

In conclusion, data from this trial show that the combination of M9241 plus avelumab was generally well tolerated with a manageable safety profile. Furthermore, preliminary data, although from a small number of patients, suggest that combining M9241 with avelumab QW might have reduced antitumor activity compared with avelumab Q2W monotherapy and potentially M9241 in combination with avelumab Q2W in patients with advanced UC.

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Acknowledgements The authors thank the patients and their families, investigators, coinvestigators, and the study teams at each of the participating centers.

Contributors Study concept: JSc and JLG; study design: JSc, XW, YV, FB, and JLG; data collection: JSt, AR, MMar, RKP, JSc, RND, Y-TT, XW, and YV; data analysis: JSt, JSc, RND, Y-TT, XW, YV, FB, AS, SC, and JLG; data interpretation: JSt, AR, MMar, RKP, JSc, RND, Y-TT, XW, YV, FB, AS, SC, and JLG. All authors drafted the manuscript and approved the content of the final version of the manuscript. JLG is the guarantor for this article.

Funding This trial was sponsored by Merck (CrossRef Funder ID 10.13039/100009945) as part of an alliance between Merck and Pfizer. This trial was partly funded by the Center for Immuno-Oncology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA. Medical writing support was provided by Sophie Saunders of Clinical Thinking and was funded by Merck and Pfizer.

Competing interests JSt received institutional research funding from Bavarian Nordic, ImmunityBio, Merck, PDS Biotechnology, and Precigen. J-LD received honoraria from Bristol Myers Squibb, Janssen Oncology, and MSD; served as a consultant or advisor for Bristol Myers Squibb, Janssen Oncology, and Sanofi; and was a member of a speakers' bureau for Astellas Pharma. MS served as a consultant or advisor for AbbVie, Adaptimmune, Agenus, Alligator Bioscience, Almac Diagnostics, Anaeropharma, Array BioPharma, AstraZeneca/MedImmune, BioNTech, Boehringer Ingelheim, Boston Pharmaceuticals, Bristol Myers Squibb, Chugai/Roche, Dragonfly Therapeutics, Genentech/Roche, Genmab, Genocea Biosciences, GI Innovation, Immunocore, Innate Pharma, Lilly, Modulate Pharma, Molecular Partners, Nektar, Newlink Genetics, Numab, Pieris Pharmaceuticals, Pierre Fabre, Seattle Genetics, SERVIER, Symphogen, Torque, Verastem, and Zelluna; owns stocks in Actym Therapeutics, Adaptive Biotechnologies, Amphivena, EvolveImmune Therapeutics, Intensity Therapeutics, Nextcure, and Torque; and had other relationships with CEC Oncology, Dava Oncology, Haymarket Media, and Physican's Education Resource. AR received institutional research funding from Ipsen, Merck, and Pfizer; served as a consultant or advisor for AstraZeneca, Bristol Myers Squibb, Eisai, Ipsen, Merck, MSD, Novartis, Pfizer, and Roche; and received reimbursement for travel and accommodation expenses from AstraZeneca, Bristol Myers Squibb, Ipsen, Merck, MSD, Novartis, Pfizer, and Roche. MMar served as a consultant or advisor for Bristol Myers Squibb, Janssen Cilag, Ipsen, Merck, MSD, and Pfizer. RKP received research funding from Exelixis, Janssen Oncology, and Pharmacyclics; served as a consultant or advisor for AstraZeneca, Bayer, Blue Earth Diagnostics, Bristol Myers Squibb, Dendreon, Genomic Health, Jounce Therapeutics, Merck, Pfizer, and Sanofi; was a member of a speakers' bureau for AstraZeneca, Dendreon, Genentech/Roche, Genomic Health, MSD, and Sanofi; and received reimbursement for travel and accommodation expenses from Genentech/Roche. TSG received research funding from Ferring and served as a consultant or advisor for Exelixis, Pfizer, and Merck. MMai received honoraria from Alfasigma, Amgen, AstraZeneca, Bristol Myers Squibb, Merck, MSD, Pierre Fabre, and Roche; served as a consultant or advisor for Alfasigma, AstraZeneca, Bristol Myers Squibb, Merck, MSD, Pierre Fabre, and Roche; owned patents, royalties, and other intellectual properties for 'DNA hypomethylating agents for cancer therapy'; received reimbursement for travel and accommodation expenses from Alfasigma, Amgen, AstraZeneca, Bristol Myers Squibb, Merck, MSD, Pierre Fabre, and Roche; and owned stock in Theravance. LD had no competing interests. JSc received institutional research funding from Bavarian Nordic, ImmunityBio, Incyte, Merck, NextCure, PDS Biotechnology, and Precigen. RND received institutional research funding from Bavarian Nordic, ImmunityBio, Incyte, Merck, NextCure, PDS Biotechnology, and Precigen. Y-TT received institutional research funding from Bavarian Nordic, ImmunityBio, Incyte, Merck, NextCure, PDS Biotechnology, and Precigen. XW and YV are employed by EMD Serono Research & Development Institute, Inc., Billerica, MA, USA, an affiliate of Merck KGaA. FB, JSe, and AS are employed by Merck. SC was employed by Merck at the time of study. JLG received institutional research funding from Bavarian Nordic, ImmunityBio, Incyte, Janssen Oncology, and Merck.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants. The protocol was approved by the institutional review board or independent ethics committee of each center. The authors confirm that the following list is correct and complete: Eticka komise Fakultni nemocnice u sv. Anny v Brne, approval ID 19ML/2018; Eticka komise Nemocnice Rudolfa a Stefanie Benesov a.s., approval ID 718/18; Eticka komise pro multicentricke klinicke hodnoceni Fakultni nemocnice v Motole–Lokalni eticka komise, approval ID EK-70/18; country-level approval for France, Eudra CT Number 2017-002212-13; country-level approval for Germany, approval ID 18/0013–EK 12; country-level approval for Belgium, approval ID 2018/0056; country-level approval for Hungary, approval IDs 18865-0/2019-EKL, 6851-0/2018-EKL, and 48886-0/2019-EKL; Comitato etico degli IRCCS Istituto Europeo di Oncologia e Centro Cardiologico Monzino, approval ID 21/18; Comitato Etico IRCCS Istituto Oncologico Veneto di Padova, approval ID INT 21/18; Comitato Etico di Area Vasta Sud Est, approval ID CE INT 21/18; Comitato Etico Indipendente della Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, approval ID CE INT 21/18; country-level approval for Spain, Eudra CT number 2017-002212-13; Copernicus Group IRB, approval ID QUI1-17-480; National Cancer Institute IRB, approval ID 363629; Washington University in St. Louis IRB, approval ID 201710034; National Cancer Institute IRB, approval ID 532246; WIRB, approval ID 20170751; Copernicus Group IRB, approval ID QUI1-17-480; Copernicus Group IRB, approval ID QUI1-17-480; Integreview IRB, approval ID RM 542; WIRB, approval ID 20170751; Copernicus Group IRB, approval ID 420170480; WIRB, approval ID 20170751; WIRB, approval ID 20170751; and WIRB, approval ID 1149659-3. The trial was conducted in accordance with the ethical principles of the Declaration of Helsinki and the International Council for Harmonisation Guideline for Good Clinical Practice. All patients provided written informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Any requests for data by qualified scientific and medical researchers for legitimate research purposes will be subject to Merck's (CrossRef Funder ID: 10.13039/100009945) Data Sharing Policy. All requests should be submitted in writing to Merck's data sharing portal (https://www.merckgroup.com/en/research/our-approach-to-research-and-development/healthcare/clinical-trials/commitment-responsible-data-sharing.html). When Merck has a coresearch, codevelopment, comarketing or copromotion agreement, or when the product has been out-licensed, the responsibility for disclosure might be dependent on the agreement between parties. Under these circumstances, Merck will endeavor to gain agreement to share data in response to requests.

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