Clinical Cancer Research

# Phase I Trial of M7824 (MSB0011359C), a Bifunctional Fusion Protein Targeting PD-L1 and TGFβ, in Advanced Solid Tumors

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## Abstract

**Purpose:** M7824 (MSB0011359C) is an innovative first-in-class bifunctional fusion protein composed of a mAb against programmed death ligand 1 (PD-L1) fused to a TGFβ "trap."

**Experimental Design:** In the 3+3 dose-escalation component of this phase I study (NCT02517398), eligible patients with advanced solid tumors received M7824 at 1, 3, 10, or 20 mg/kg once every 2 weeks until confirmed progression, unacceptable toxicity, or trial withdrawal; in addition, a cohort received an initial 0.3 mg/kg dose to evaluate pharmacokinetics/pharmacodynamics, followed by 10 mg/kg dosing. The primary objective is to determine the safety and maximum tolerated dose (MTD); secondary objectives include pharmacokinetics, immunogenicity, and best overall response.

**Results:** Nineteen heavily pretreated patients with ECOG 0–1 have received M7824. Grade ≥3 treatment-related adverse events occurred in four patients (skin infection secondary to localized bullous pemphigoid, asymptomatic lipase increase, colitis with associated anemia, and gastroparesis with hypokalemia). The MTD was not reached. M7824 saturated peripheral PD-L1 and sequestered any released plasma TGFβ1, -β2, and -β3 throughout the dosing period at >1 mg/kg. There were signs of efficacy across all dose levels, including one ongoing confirmed complete response (cervical cancer), two durable confirmed partial responses (PR; pancreatic cancer; anal cancer), one near-PR (cervical cancer), and two cases of prolonged stable disease in patients with growing disease at study entry (pancreatic cancer; carcinoid).

**Conclusions:** M7824 has a manageable safety profile in patients with heavily pretreated advanced solid tumors. Early signs of efficacy are encouraging, and multiple expansion cohorts are ongoing in a range of tumors. *Clin Cancer Res; 24(6); 1287–95. ©2018 AACR.*



## Introduction

Multiple agents targeting the programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) pathway have received regulatory approval, demonstrating impressive durations of response for multiple tumor types, including melanoma, non–small cell lung cancer, renal cell cancer, and head and neck cancer (1–9). Atezolizumab, avelumab, and durvalumab are anti-PD-L1 antibodies with proven efficacy and regulatory approval (10–13). Unfortunately, not all cancer types seem to respond to these agents, and, even among susceptible cancer types, the percentage of responding patients is usually <20% (14).

To increase the rate of response to these therapies, many ongoing trials are evaluating anti-PD-1/PD-L1 agents in combination with other immunotherapies. However, these combination strategies have limitations, including (at least) additive toxicity, reduced administration-related convenience to patients, and complex and lengthy clinical development paths to regulatory approval (15). Accordingly, novel approaches are required to better serve patients' needs.

Bifunctional antibodies represent an emerging and exciting new therapeutic strategy, whereby two molecular targets are simultaneously inhibited by a single agent containing two distinct functional domains. Importantly, bifunctional strategies have the potential to circumvent the limitations associated with combination immunotherapy cited above (16).

Combined inhibition of PD-L1 and TGFβ is a promising therapeutic strategy because these key pathways have independent and complementary immunosuppressive functions. More specifically, these two pathways have partially nonredundant effects on the adaptive and innate immune systems, including the capacity of TGFβ to impinge upon relevant tumor cell-extrinsic processes that alter the local microenvironment; therefore, their dual inhibition may result in enhanced antitumor activity. Importantly, targeting both PD-L1 and TGFβ in the tumor microenvironment is suggested to be more important than their inhibition

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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## Translational Relevance

Excitement surrounding the durable benefits associated with PD-1/PD-L1–targeted therapy has been tempered somewhat by responses being confined to only a subset of patients. To increase the rate of response, many ongoing trials are evaluating anti–PD-1/PD-L1 agents in combination with other immunotherapies; however, these combination strategies have limitations, and novel approaches are required. M7824 (MSB0011359C) is an innovative first-in-class bifunctional fusion protein composed of a mAb against PD-L1 fused to a TGFβ "trap." We report the first clinical data for M7824, including pharmacokinetics, safety, and efficacy findings, which derive from a phase I dose-escalation study in patients with advanced solid tumors. M7824 saturated peripheral PD-L1 and sequestered any released plasma TGFβ throughout the dosing period at a dose >1 mg/kg. M7824 appeared to have a manageable safety profile, and early evidence of clinical efficacy, including one ongoing confirmed complete response and two durable confirmed partial responses, was demonstrated.

in the peripheral blood, especially for immune-excluded and immune-desert tumors. M7824 (MSB0011359C) is an innovative first-in-class bifunctional fusion protein composed of a human IgG1 monoclonal antibody against PD-L1 fused to the extracellular domain of TGFβ receptor II (TGF-βRII) to function as a TGFβ "trap." The anti–PD-L1 moiety of M7824 is based on avelumab, which has been approved in various countries for the treatment of metastatic Merkel cell carcinoma and in the US for treatment of locally advanced or metastatic urothelial carcinoma that has progressed during or after platinum-containing chemotherapy (11, 12, 17). Consistent with the above-stated hypothesis that concomitant inhibition of the PD-L1 and TGFβ pathways may result in enhanced antitumor activity, preclinical studies in murine models indicated that M7824 had improved antitumor activity compared with either an anti–PD-L1 antibody or TGFβ trap alone, extended survival and conferred long-term protective antitumor activity in cured mice upon tumor rechallenge, and substantially increased $CD8^+$ T-cell and natural killer (NK) cell infiltration while decreasing myeloid-derived suppressor cell (MDSC) infiltration within the tumor compared with an anti–PD-L1 antibody (Lan and colleagues, submitted). Further supporting the possibility of complementary interplay between the PD-L1 and TGFβ pathways, preclinical studies have also shown the ability of M7824, but not an anti–PD-L1 antibody, to reverse the mesenchymalization of carcinoma cells and enhance response to chemotherapy (18).

Based on the above-cited rationale and these preclinical data, this phase I 3+3 dose-escalation trial (NCT02517398) evaluates the safety, pharmacokinetics/pharmacodynamics, and efficacy of M7824 in patients with advanced solid tumors.

## Patients and Methods

### Study design and patients

NCT02517398 is a phase I, open-label, dose-escalation and dose-expansion trial of M7824 in patients with heavily pretreated advanced solid tumors. Using a database cutoff date of March 21, 2017, data from the dose-escalation phase of the study are reported here (the dose-expansion cohort will not be described in this manuscript). Patients were enrolled using a standard 3+3 study design.

Patients had histologically or cytologically confirmed metastatic or locally advanced solid tumors for which no effective standard therapy existed or standard therapy had failed. All patients had an ECOG performance status of 0 or 1, preserved organ function, and evaluable or measurable disease. Patients with a history of autoimmune disease were ineligible, except for those with type 1 diabetes mellitus, vitiligo, alopecia, psoriasis, or hypothyroid or hyperthyroid disease not requiring immunosuppressive treatment. Prior therapy with any antibody/drug-targeting immune checkpoints was not allowed.

The study was conducted in accordance with all applicable regulatory requirements, and the protocol was approved by the Institutional Review Board of the Center for Cancer Research at the National Cancer Institute. Each patient provided signed informed consent before study enrollment.

### Procedures

In the 3+3 dose-escalation component, patients received M7824 as a 1-hour intravenous infusion at dose levels of 1, 3, 10, or 20 mg/kg once every 2 weeks (Q2W); in addition, a cohort received an initial dose at 0.3 mg/kg to establish a pharmacokinetic/pharmacodynamic relationship at low-dose levels where no full-PD effect is present, followed by 10 mg/kg dosing thereafter (a dose within the range of predicted efficacy based on preclinical efficacy data and clinical PD-L1 target occupancy and TGFβ trapping in blood data). Treatment was planned to continue until progressive disease was confirmed by a subsequent scan, unacceptable toxicity, or withdrawal from study. Treatment through an initial progression was recommended until it could be confirmed, and, in general, treatment beyond progression was allowable based on the clinical judgment of the investigator. To mitigate potential infusion-related reactions, premedication with an antihistamine and paracetamol (acetaminophen) approximately 30–60 minutes before each dose of M7824 was mandatory for the first two infusions and was optional and at the discretion of the investigator after the second infusion. If grade $\geq$2 infusion reactions were seen during the first two infusions, premedication should not have been stopped.

### Outcomes

The primary objective of this study was to determine the safety, tolerability, and maximum tolerated dose (MTD) of M7824; secondary objectives included pharmacokinetics, immunogenicity, and best overall response.

### Safety

Safety was evaluated according to NCI CTCAE v4.03. For dose-escalation purposes, a dose-limiting toxicity (DLT) was defined as any grade $\geq$3 toxicity considered by the investigator to be related to M7824 that occurred during the DLT assessment window (21 days after the first administration of M7824), with the following exceptions: transient (<6 hours) grade 3 infusion reaction or fever; grade 3 fatigue, headache, nausea, or emesis resolving to grade 1 or better within 24 hours; grade 3 anemia resolving to $\geq$9 g/dL within 14 days without blood transfusion or use of erythroid growth factor; and the development of a malignant skin lesion that could be locally resected with negative margins. The

3+3 design evaluated MTD in terms of the number of patients experiencing a DLT (vs. the number of DLT events experienced by an individual patient): more than one of six patients within a dose level experiencing a DLT would suggest that the MTD had been exceeded; in such case, the MTD would be defined as the highest dose level at which no more than 1 of 6 patients treated in a cohort and evaluable for DLT determination experienced a DLT.

### Pharmacokinetics/pharmacodynamics

Key pharmacokinetic parameters were derived from standard noncompartment analysis. PD-L1 target occupancy was measured in peripheral blood mononuclear cells (PBMC) using a flow cytometry method developed at EMD Serono then transferred and validated at Quintiles (V162). Whole blood was processed to PBMCs, which were incubated with human IgG to saturate nonspecific binding prior to incubating at 4°C with a cocktail of anti-CD3 Alexa Fluor 488, anti-CD20 PE, and either biotinylated avelumab or biotinylated inactive control antibody unable to bind PD-L1. Streptavidin-APC was subsequently incubated at 4°C to bind the biotinylated antibody. The cell population was gated on the $CD3^+$ cells and target occupancy calculated.

Total TGFβ1, -β2, and -β3 levels in plasma were determined with analytically validated Bio-Plex Pro TGFβ1, -β2, and -β3 assays (Tech note 6127; Bio-Rad). The test was performed following the manufacturer's instruction manual; plasma was collected into EDTA tubes prior to therapy and on days 2, 8, and 15 of the first treatment cycle. Briefly, TGFβ1, -β2, and -β3 were dissociated from the binding proteins via hydrochloric acid treatment for 10 minutes, and were neutralized with an equal volume of neutralization buffer. The samples were subsequently diluted with sample diluent and immediately incubated with capture antibody-coupled beads for TGFβ1, -β2, and -β3. Following the addition of biotinylated detection antibodies and streptavidin-PE detection agent, the formed immune complexes for TGFβ1, -β2, and -β3 were detected with a Bio-Plex MagPix instrument in the presence of calibration standards. The data were analyzed with Bio-Plex Manager Software 6.1 to determine the concentrations of TGFβ1, -β2, and -β3 for each sample tested.

### Clinical activity

On-treatment patients underwent tumor assessment scans or physical examination with photography every 6 weeks unless clinical symptoms warranted earlier imaging or until confirmed disease progression. Objective responses, best overall responses, and duration of responses were assessed. Objective responses, including confirmed responses, and progression were assessed using RECIST v1.1 by the investigator and reviewed by an independent radiologist at the investigational site. Complete response (CR) was defined as complete disappearance of all lesions (whether measurable or not) and no new lesions and all measurable lymph nodes reduced to $\leq$10 mm in short axis. Partial response (PR) was defined as a decrease by $\geq$30% in the sum of the longest diameters of target and new measurable lesions using baseline measurements as a reference. Responses were confirmed by repeat assessment after 6 weeks of follow-up. The duration of objective response was defined as the time from the initial response to the time of disease progression or death. Progressive disease was defined as a $\geq$20% increase in the sum of the longest diameters of target and new measurable lesions, confirmed by repeat imaging at 4 to 6 weeks. Stable disease (SD) was defined as a change in the sum of target and new lesions not meeting the above definitions for CR, PR, or progressive disease.

### Biomarker assessment

If patients had elevated tumor markers (e.g., CEA or CA19-9) prior to enrollment, those tumor markers were followed every 2 weeks during treatment. Samples for peripheral and tumor-based exploratory biomarkers related to response were also collected at various time points; however, no data on response-related biomarkers are reported in this manuscript.

### Peripheral immune cell subset analyses

Multicolor flow cytometry was performed on frozen PBMCs to identify 131 different immune cell subsets, including nine classic subsets [$CD4^+$ and $CD8^+$ T cells, B cells, regulatory T cells (Tregs), NK cells, NK T cells, conventional dendritic cells (cDC), plasmacytoid dendritic cells (pDC), and MDSCs] as well as 122 subsets relating to their maturation/function, as previously described (19–21).

### Statistical analysis

The sample size for the dose-escalation component of the trial followed the well-established 3+3 design for dose-finding studies (more than one of six patients within a dose level experiencing a DLT would suggest that the MTD had been exceeded). Design considerations were not made with regard to explicit power and type I error but rather to obtain safety, pharmacokinetics, and efficacy data. For safety and efficacy analyses, all patients who received M7824 were included. In the peripheral immune cell subset analyses, *P* values were calculated using the Wilcoxon matched pairs signed rank test and, due to the large number of tests performed, were adjusted using Holm's method (step-down Bonferroni); subsets with a potentially biologically relevant change were defined as those with a Holm-adjusted $P < 0.05$, majority of patients >25% change, difference in medians of pretherapy versus posttherapy >0.01% of PBMCs, and a frequency >0.01% of PBMCs.

## Results

### Baseline demographics

From September 1, 2015, to March 21, 2017 (database cutoff for this report), 19 patients were enrolled at the Center for Cancer Research at the National Cancer Institute (Bethesda, MD). Patients received M7824 at a dose of 0.3→10 mg/kg ($n = 3$), 1 mg/kg ($n = 3$), 3 mg/kg ($n = 3$), 10 mg/kg ($n = 3$), or 20 mg/kg ($n = 7$; one patient in the 20 mg/kg cohort was not evaluable for DLTs (received palliative radiation during the DLT assessment period, although this did not result in treatment interruption or any toxicity within the DLT reporting window) and was replaced, resulting in seven patients at this dose level despite no DLTs observed in the first three patients evaluated).

Patient baseline and disease characteristics are shown in Table 1. All patients had an ECOG performance status of 0 or 1, 84% had received $\geq$2 prior anticancer therapies, and a variety of primary tumor types were represented.

### Treatment exposure

At the database cutoff date, the median duration of therapy with M7824 in this 19-patient cohort was 11.9 weeks (range,

**Table 1.** Patient baseline characteristics

| Patient characteristics | Total ($n = 19$) |
|---|---|
| Sex, *n* (%) | |
| Male | 9 (47.4) |
| Female | 10 (52.6) |
| Age, median (range), years | 56 (33–78) |
| Number of prior anticancer therapies, *n* (%) | |
| 0 | 1 (5.3) |
| 1 | 2 (10.5) |
| 2 | 6 (31.6) |
| 3 | 4 (21.1) |
| ≥4 | 6 (31.6) |
| ECOG performance status, *n* (%) | |
| 0 | 10 (52.6) |
| 1 | 9 (47.4) |
| Primary tumor type, *n* (%) | |
| Adenoid cystic carcinoma | 2 (10.5) |
| Anal | 2 (10.5) |
| Appendiceal | 1 (5.3) |
| Bronchopulmonary carcinoid | 1 (5.3) |
| Cervix uteri | 4 (21.1) |
| Chordoma | 1 (5.3) |
| Colorectal | 2 (10.5) |
| Pancreatic | 5 (26.3) |
| Small bowel | 1 (5.3) |

ECOG, Eastern Cooperative Oncology Group.

4.0–41.9 weeks), although this varied substantially between the different dose levels [0.3→10 mg/kg, 11.9 weeks (range, 4.0–29.9 weeks); 1 mg/kg, 13.7 weeks (range, 8.0–34.1 weeks); 3 mg/kg, 23.9 weeks (range, 14.0–27.9 weeks); 10 mg/kg, 13.9 weeks (range, 6.0–41.9 weeks); and 20 mg/kg, 6.0 weeks (range, 4.0–17.7 weeks)].

As of the database cutoff date, one patient (in the 0.3→10 mg/kg cohort) remained on active treatment and the other 18 had discontinued, including three patients who discontinued due to treatment-related adverse events (AE; bullous pemphigoid, colitis, and gastroparesis).

### Safety

As of the database cutoff date, all 19 patients had ≥1 AE. 58% of patients experienced ≥1 serious AE; 26% of patients experienced a treatment-related serious AE (Supplementary Tables S1 and S2).

At the time of this analysis, 47% of patients had ≥1 treatment-related AE, as further described in Table 2 (Supplementary Tables S3 and S4). Grade ≥3 treatment-related AEs occurred in four patients: one patient (3 mg/kg) had grade 2 bullous pemphigoid on day 185 that responded well to corticosteroids but ultimately led to treatment discontinuation, followed by a grade 3 superimposed skin infection diagnosed on day 201 that resolved with antibiotics; one patient (20 mg/kg) developed grade 3 asymptomatic lipase increase without evidence of pancreatitis (days 13 and 41) and subsequently had a grade 2 keratoacanthoma on day 128 (which regressed when M7824 was discontinued); a third patient (20 mg/kg) was formally diagnosed with colitis on day 27 (grade 2) that responded well to corticosteroids but resulted in treatment discontinuation on day 30 (grade 3). Finally, one patient (10 mg/kg) experienced grade 4 hypokalemia on day 294 (grade 3 episode reported on day 308) in the setting of grade 3 gastroparesis diagnosed on day 310 that led to treatment discontinuation. This episode of gastroparesis was medically managed without corticosteroids. It gradually improved and eventually resolved over a 2-month period. Colitis and its secondary events of grade 3 anemia and rectal hemorrhage (which occurred in a previously radiated area) were considered dose limiting.

One infusion-related reaction occurred (grade 2), which was mild and manageable; treatment was not terminated due to the infusion-related reaction.

The MTD was not reached at the highest dose level in this study, 20 mg/kg. As noted earlier, three patients discontinued M7824 treatment due to treatment-related AEs (the cases of bullous pemphigoid, colitis, and gastroparesis described above). No AEs led to death.

**Table 2.** Treatment-related AEs

| *n* (%) | 0.3→10 mg/kg ($n = 3$) | | 1 mg/kg ($n = 3$) | | 3 mg/kg ($n = 3$) | | 10 mg/kg ($n = 3$) | | 20 mg/kg ($n = 7$) | | Total ($n = 19$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Any Grade | Grade ≥3 | Any Grade | Grade ≥3 | Any Grade | Grade ≥3 | Any Grade | Grade ≥3 | Any Grade | Grade ≥3 | Any Grade | Grade ≥3 |
| Patients with any event | 2 (66.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (66.7) | 1 (33.3) | 1 (33.3) | 1 (33.3) | 4 (57.1) | 2 (28.6) | 9 (47.4) | 4 (21.1) |
| Anemia | | | | | | | | | 1 (14.3) | 1 (14.3) | 1 (5.3) | 1 (5.3) |
| Appetite decrease | | | | | | | 1 (33.3) | | | | 1 (5.3) | |
| Bullous pemphigoid | | | | | 1 (33.3) | | | | | | 1 (5.3) | |
| Colitis | | | | | | | | | 1 (14.3) | 1 (14.3) | 1 (5.3) | 1 (5.3) |
| Dermatitis acneiform | | | | | | | 1 (33.3) | | | | 1 (5.3) | |
| Dyspnea | | | | | | | | | 1 (14.3) | | 1 (5.3) | |
| Gastroparesis | | | | | | | 1 (33.3) | 1 (33.3) | | | 1 (5.3) | 1 (5.3) |
| Hyperthyroidism | 1 (33.3) | | | | | | | | 1 (14.3) | | 2 (10.5) | |
| Hypokalemia[a] | | | | | | | 1 (33.3) | 1 (33.3) | | | 1 (5.3) | 1 (5.3) |
| Hypothyroidism | 1 (33.3) | | | | | | 1 (33.3) | | 1 (14.3) | | 3 (15.8) | |
| Infusion-related reaction | | | | | | | | | 1 (14.3) | | 1 (5.3) | |
| Keratoacanthoma | 1 (33.3) | | | | | | | | 1 (14.3) | | 2 (10.5) | |
| Lipase increase | | | | | | | | | 1 (14.3) | 1 (14.3) | 1 (5.3) | 1 (5.3) |
| Nausea | | | | | 1 (33.3) | | 1 (33.3) | | | | 2 (10.5) | |
| Pruritus | | | | | 1 (33.3) | | | | | | 1 (5.3) | |
| Rash maculopapular | 1 (33.3) | | | | 1 (33.3) | | 1 (33.3) | | | | 3 (15.8) | |
| Skin infection | | | | | 1 (33.3) | 1 (33.3) | | | | | 1 (5.3) | 1 (5.3) |
| Skin papilloma | 1 (33.3) | | | | | | | | | | 1 (5.3) | |
| Vomiting | | | | | 1 (33.3) | | 1 (33.3) | | | | 2 (10.5) | |
| Weight decrease | | | | | | | 1 (33.3) | | | | 1 (5.3) | |

[a]Hypokalemia was the only grade 4 treatment-related AE; all other treatment-related grade ≥3 events were grade 3.

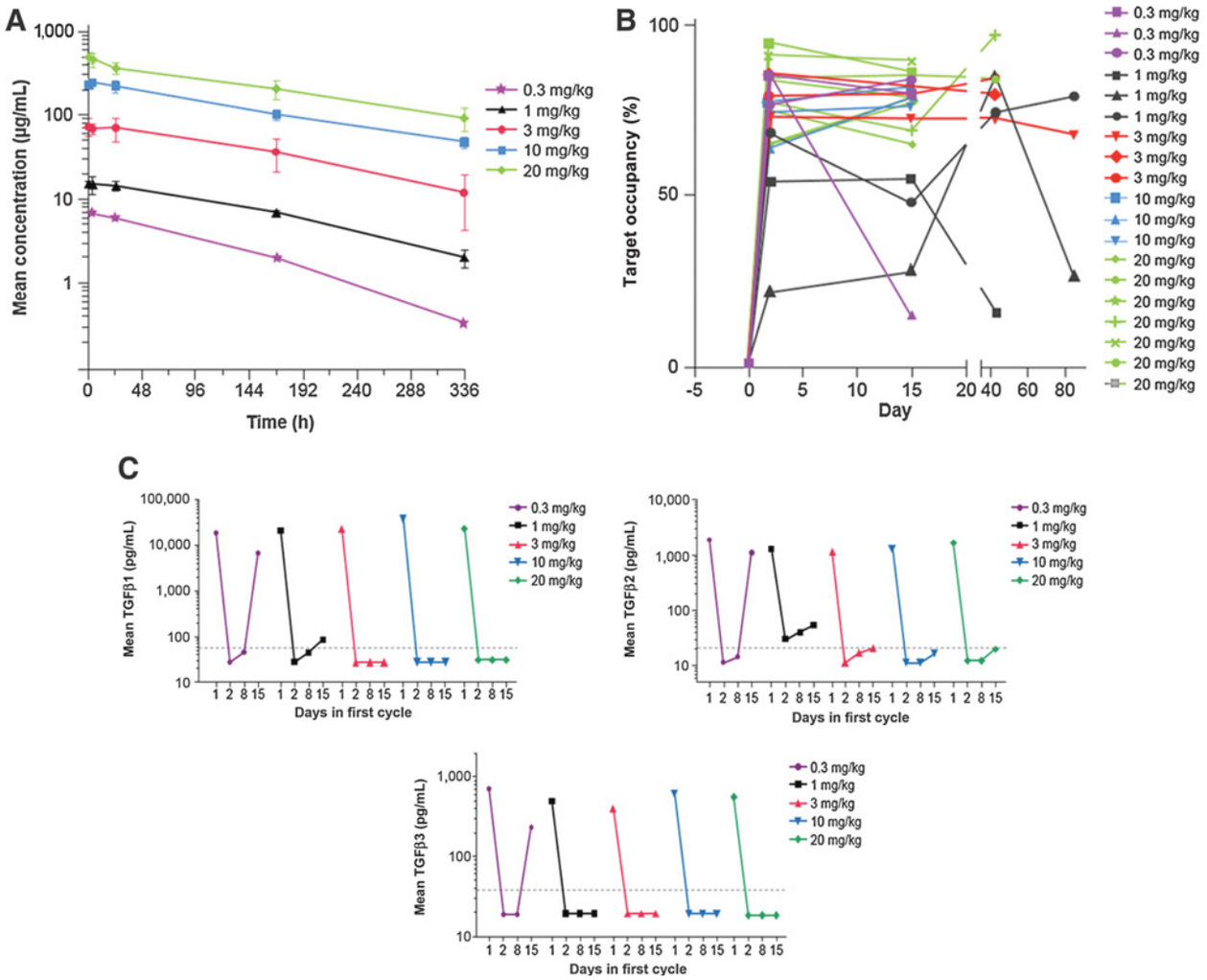


**Figure 1.**

**A,** Concentration versus time profile following intravenous administration of M7824. **B,** PD-L1 target occupancy following intravenous administration of M7824. **C,** Total TGFβ1, -β2, and -β3 plasma concentrations following intravenous administration of M7824.

### Pharmacokinetics and pharmacodynamics

Pharmacokinetics analyses showed a dose-linear increase in exposure starting at 3 mg/kg. The apparent mean terminal half-life was 75 ± 16, 108 ± 5, 140 ± 57, 151 ± 26, and 158 ± 18 hours (mean ± SD) at the 0.3, 1, 3, 10, and 20 mg/kg doses, respectively. This indicates that target-mediated drug disposition may be saturated at dose levels ≥3 mg/kg (Fig. 1A).

PD-L1 target occupancy analyses demonstrated saturation of PD-L1 in PBMCs throughout the entire dosing interval at doses >1 mg/kg (Fig. 1B).

Bio-Plex TGFβ assays (Bio-Rad) were used to demonstrate that M7824 sequestered all released plasma TGFβ1, -β2, and -β3 in a dose-dependent manner with complete sequestration of all three isoforms for the entire dosing period at doses >1 mg/kg (Fig. 1C). In a series of control experiments, we demonstrated that free TGFβ1, -β2, and -β3 were undetectable in drug-naïve donor plasma, but high levels were detectable once released from the latent complex by acid activation; spiking of M7824 (10 µg/mL) into drug-naïve donor plasma, followed by acid activation, prevented the detection of latent TGFβ1, -β2, and -β3 and a dose-titration experiment suggested that the $IC_{50}$ of M7824 for TGFβ1 was 0.37 µg/mL (data not shown). Together, these data demonstrate that our assay detected TGFβ1, -β2, and -β3 released from the latent complex in our patient population and that M7824 reached sufficient levels to completely bind all three TGFβ isoforms in circulation.

### Clinical activity

On the basis of the database cutoff date, the largest changes from baseline in the sum of diameters of target lesions are summarized in Fig. 2. Table 3 presents confirmed best overall response data for each patient. Evidence of clinical activity with M7824 was observed across all evaluated dose levels.

One patient with human papillomavirus (HPV)-positive cervical cancer metastatic to the mediastinal lymph nodes treated at 10 mg/kg has an ongoing CR, despite, as described above, having discontinued treatment at 10.5 months due to gastroparesis (Fig. 3A). The CR was not observed until 7.5 months on study;

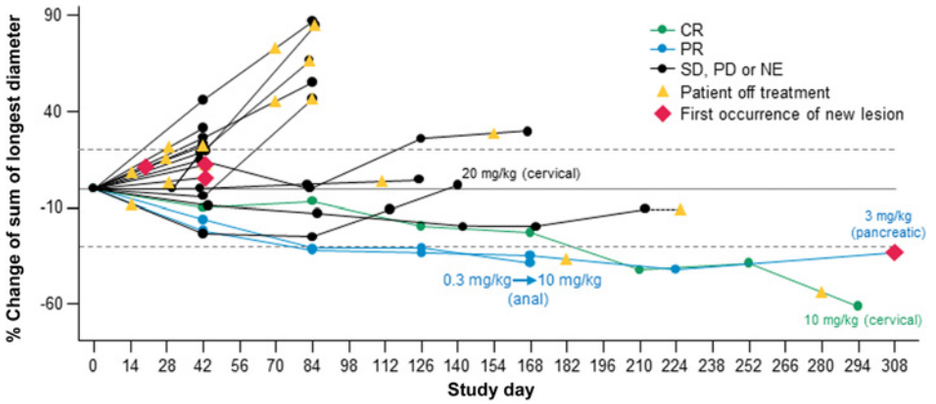


**Figure 2.**
Largest change from baseline in the sum of longest diameters according to RECIST v1.1. The dotted line at −30% indicates the threshold for a PR. Because the patient with a confirmed CR had lymph node–only disease, the sum of diameters does not reach 0. NE, not evaluable; RECIST, Response Evaluation Criteria In Solid Tumors.

however, a marked reduction in the patient's tumor markers was noted much earlier (Fig. 3B).

A second patient, who had locally advanced mismatch repair–deficient pancreatic cancer and received M7824 at 3 mg/kg, had a durable PR that was confirmed after 4.5 months on study and persisted until evidence of disease progression at 10.5 months on study (Fig. 3C). Again, a substantial decrease in the patient's tumor markers was noted weeks before the PR (Fig. 3D). Of note, this is the patient described above who experienced bullous pemphigoid, which ultimately led to treatment discontinuation; however, following disease progression, this patient underwent M7824 retreatment and again developed a PR with decreasing tumor markers. However, 8 weeks after starting retreatment, this patient had a reoccurrence of bullous pemphigoid and his treatment was again held.

An ongoing durable PR, confirmed after 4.5 months on study, has been documented in a patient with HPV-positive anal cancer metastatic to the liver and retroperitoneal and periportal nodes who received M7824 at a dose of 0.3→10 mg/kg (Supplementary Fig. S1A and S1B).

There were also signs of clinical activity in a patient with cervical cancer of unknown HPV status metastatic to the lungs who was treated with M7824 at a dose of 20 mg/kg. This patient, earlier described as having discontinued treatment due to colitis on day 30, had a near-PR after receiving 2 doses of M7824 (Supplementary Fig. S1C).

Two additional patients with growing disease at study entry achieved prolonged SD following treatment with M7824. The first had pancreatic cancer of unknown mismatch repair status, which was growing at the time of study entry, and was treated with M7824 at a dose of 3 mg/kg; this patient experienced 4.5 months of SD. The second patient, who had carcinoid that was growing at the time of study entry and was treated with M7824 at 1 mg/kg, had SD for 8 months, including a 20% reduction in the sum of diameters of target lesions at 6 months on study and reduced tumor burden at treatment discontinuation compared with baseline (M7824 was discontinued to allow other treatments for worsening carcinoid symptoms). Finally, one patient with chordoma treated at the 20 mg/kg dose level had confirmed disease progression on day 85 and treatment was discontinued; at a

**Table 3.** Confirmed best overall response according to RECIST v1.1

| Dose level | Tumor type | Confirmed best overall response | Total number of M7824 Infusions | Still on active treatment | Reason for treatment discontinuation |
|---|---|---|---|---|---|
| 0.3→10 mg/kg | Anal | PR | 14 | Yes | |
| 0.3→10 mg/kg | Cervix uteri | PD | 2 | No | PD |
| 0.3→10 mg/kg | Colorectal | PD | 6 | No | AE[a] |
| 1 mg/kg | Adenoid cystic carcinoma | PD | 4 | No | PD |
| 1 mg/kg | Bronchopulmonary carcinoid | SD | 14 | No | Other |
| 1 mg/kg | Pancreatic | PD | 6 | No | PD |
| 3 mg/kg | Pancreatic | PR | 14 | No | AE |
| 3 mg/kg | Pancreatic | SD | 6 | No | Other |
| 3 mg/kg | Pancreatic | SD | 12 | No | PD |
| 10 mg/kg | Colorectal | PD | 3 | No | PD |
| 10 mg/kg | Appendiceal | SD | 7 | No | PD |
| 10 mg/kg | Cervix uteri | CR | 21 | No | AE |
| 20 mg/kg | Cervix uteri | PD | 3 | No | PD |
| 20 mg/kg | Chordoma | PD[b] | 6 | No | PD |
| 20 mg/kg | Pancreatic | SD | 8 | No | PD |
| 20 mg/kg | Adenoid cystic carcinoma | PD | 3 | No | PD |
| 20 mg/kg | Cervix uteri | SD | 2 | No | AE |
| 20 mg/kg | Anal | PD | 3 | No | PD |
| 20 mg/kg | Small bowel | NE | 2 | No | PD |

Abbreviations: NE, not evaluable; RECIST, Response Evaluation Criteria In Solid Tumors.
[a]Unrelated to study treatment.
[b]Disease progression confirmed on day 85, and treatment was discontinued; however, at a follow-up visit on day 280, a restaging scan showed a 45% decrease in tumor burden despite no further therapeutic interventions after discontinuing M7824.

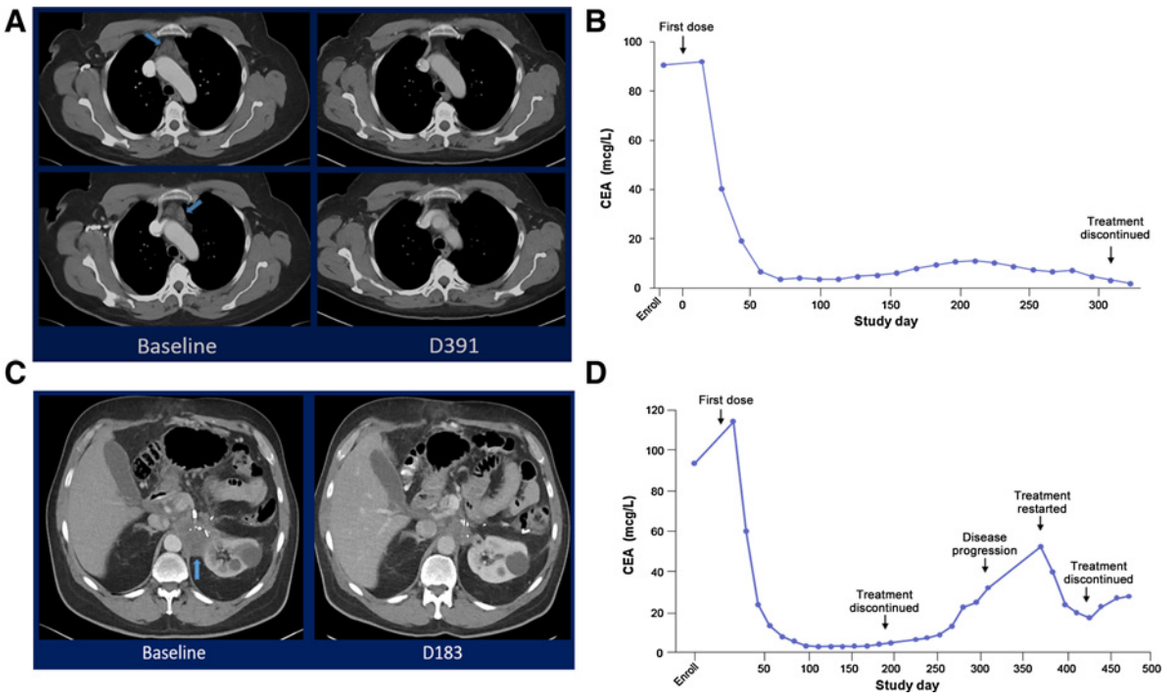


**Figure 3.**

**A,** This 49-year-old woman with metastatic cervical cancer after cisplatin/taxol followed by carboplatin/taxol plus bevacizumab was enrolled with two pathologically enlarging mediastinal lymph nodes (arrows in left side of figure). Restaging scan 7.5 months after enrollment showed reduction in lymph nodes to <1 cm by short-axis measurement, meeting RECIST v1.1 criteria for a CR. CR was durable as of her 13-month restaging scan (right side of figure). **B,** CEA curve for patient with ongoing durable confirmed CR. **C,** This 61-year-old man with locally advanced pancreatic cancer s/p FOLFIRINOX, gemcitabine/abraxane, and XELOX was enrolled with an enlarging tumor in the pancreatic bed (arrow in left side of figure). Restaging scans at 3 months showed a PR, which was confirmed at 4.5 months. Scans at 6 months showed a 49% reduction in his disease by long-axis measurement (right side of figure). **D,** CEA curve for patient with pancreatic cancer with durable confirmed PR. CEA, carcinoembryonic antigen; FOLFIRINOX, leucovorin, fluorouracil, irinotecan, and oxaliplatin; RECIST, Response Evaluation Criteria In Solid Tumors; XELOX, oxaliplatin and capecitabine.

follow-up visit on day 280, a restaging scan showed a 45% decrease in tumor burden despite no further therapeutic interventions after discontinuing M7824.

### Peripheral immune cell subset analyses

We previously described a flow cytometry–based assay in which immune cell subsets can be analyzed from frozen PBMCs (19–21). Here, we analyzed 131 immune cell subsets in PBMCs prior to therapy and 2 weeks after one, three, or six administrations of M7824, as well as at later time points, when samples were available. This analysis showed no statistical differences in any of the 131 immune cell subsets analyzed at any time pre-M7824 versus post-M7824. Although we evaluated a very heterogeneous group of patients and relatively few patients were analyzed ($n = 14/19$), those patients with evidence of clinical benefit showed trends of increases in B cells and $CD4^+$ T cells and decreases in MDSCs expressing CD16 at the time of best overall clinical response versus pre-M7824 therapy levels. There was no depletion of $PD\text{-}L1^+$ immune cell subsets resulting from M7824 treatment.

## Discussion

Elevated plasma TGFβ and PD-L1 expression on tumor cells have been found to play important roles in tumor immune evasion and to correlate with poorer outcomes in many different human cancers (22–29). Agents targeting the PD-1/PD-L1 pathway have gained regulatory approval, displaying impressive durations of response for several tumor types; however, for most tumor types, <20% of patients respond to these therapies (14). Response rates to antibodies (e.g., fresolimumab) and small-molecule inhibitors (e.g., galunisertib) targeting the TGFβ pathway have been similarly modest (30). Combined inhibition of PD-L1 and TGFβ is a potentially promising therapeutic strategy because these key pathways have independent and complementary immunosuppressive functions; therefore, their dual inhibition may result in enhanced antitumor activity. Bifunctional antibodies may afford a particularly attractive strategy for achieving combination immunotherapy (16). Indeed, M7824, an innovative first-in-class bifunctional fusion protein targeting PD-L1 and TGFβ, showed improved antitumor activity in preclinical mouse tumor models compared with an anti-PD-L1 antibody or TGFβ sequestration alone (Lan and colleagues, submitted).

On the basis of this work, we conducted the first-in-human clinical trial of M7824 to determine its safety, pharmacokinetics, and efficacy in 19 patients with heavily pretreated advanced solid tumors using a 3+3 dose-escalation design. As of the database cutoff date, one patient remained on active treatment.

Overall, M7824 had a manageable safety profile. Grade ≥3 treatment-related AEs occurred in four patients (skin infection

secondary to grade 2 bullous pemphigoid, asymptomatic lipase increase, colitis with associated anemia, and gastroparesis with hypokalemia). Colitis and its secondary events of anemia and rectal hemorrhage (in a previously radiated area) were considered dose limiting in one patient, and three patients discontinued M7824 due to treatment-related AEs (bullous pemphigoid, colitis, and gastroparesis). Gastroparesis has not been well described as a toxicity of anti–PD-L1 therapies, yet these agents are known in rare cases to cause mononeuropathies. Given this information and the fact that this patient had no comorbidities (such as diabetes or underlying autoimmune disorders) to explain this event, it was felt most appropriate to attribute gastroparesis to M7824; this patient also had baseline low grade chronic diarrhea (from past radiotherapy), which likely contributed in part to the two episodes of severe hypokalemia. The occurrence of keratoacanthoma in two patients is consistent with prior reports involving TGFβ-depleting therapies and could be related to complex crosstalk between TGFβ and other key signaling pathways (e.g., RAS; ref. 31); notably, when these patients discontinued M7824, their lesions regressed, as observed with earlier TGFβ-blocking agents (31). Aside from the keratoacanthomas, the safety profile of M7824 was similar to that of anti–PD-1/PD-L1 agents (1–13), including anticipated treatment-related AEs that were immune related (e.g., bullous pemphigoid and colitis) and manageable with a course of corticosteroids. Notably, we did not observe uncontrolled cytokine release with M7824 (data on file), in contrast to reports from a phase I study investigating the anti–TGF-βRII mAb LY3022859 (32). The MTD was not reached at the highest dose level in this study, 20 mg/kg. Clinical pharmacokinetics, target occupancy, and clinical efficacy and safety data from this study were used to inform dose and dose-regimen selection for the expansion cohorts. Specifically, exposures at $\geq$3 mg/kg Q2W were in the range of efficacious exposures observed in mouse efficacy models (manuscript in preparation), resulted in complete PD-L1 target occupancy and TGFβ trapping in blood throughout the dosing interval in humans, and were associated with preliminary clinical efficacy and a manageable safety profile. Dose levels of 3 mg/kg, 10 mg/kg, 500 mg, and 1,200 mg Q2W are under investigation in the ongoing expansion cohorts.

The exposures associated with these dose levels appear to be in the active range. The activity of M7824 as a PD-L1 inhibitor and TGFβ trap was confirmed because M7824 was able to both saturate PD-L1 in PBMCs and sequester any released plasma TGFβ1, -β2, and -β3 for the entirety of the dosing period at doses >1 mg/kg.

Evidence of clinical activity with M7824 was observed across all evaluated dose levels, including one ongoing confirmed CR (cervical cancer), two durable confirmed PRs (pancreatic cancer; anal cancer), one near-PR (cervical cancer), two cases of prolonged SD in patients with growing disease at study entry (pancreatic cancer; carcinoid), and one case of late-onset tumor shrinkage after early progression (chordoma). Interestingly, the CR (cervical cancer) and anal cancer confirmed PR occurred in patients with HPV-positive disease, whereas the pancreatic cancer confirmed PR occurred in a patient with mismatch repair-deficient disease. Finally, the delayed kinetics of response observed here (CR and PRs confirmed at 7.5 and 4.5 months, respectively), despite substantial decreases in tumor markers weeks earlier, merit further investigation; so too does the potential utility of M7824 retreatment.

Analysis of 131 immune cell subsets in PBMCs showed statistically nonsignificant increases in B cells and $CD4^+$ T cells and decreases in MDSCs expressing CD16 at the time of best overall clinical response versus pre-M7824 therapy levels in patients with clinical benefit. Similar to what was observed in PBMCs using this assay in patients receiving the anti–PD-L1 antibody avelumab, there was no depletion of $PD\text{-}L1^+$ immune cell subsets resulting from M7824 (19–21). However, the current analysis involved a small sample size and measured immune cell subsets in the peripheral blood (vs. tumor microenvironment); hence, these findings should be considered as trends since definitive conclusions are not possible.

In conclusion, these data from a phase I dose-escalation study show that the innovative first-in-class bifunctional fusion protein M7824 appears to have a manageable safety profile in patients with heavily pretreated advanced solid tumors. In addition, M7824 can saturate PD-L1 in PBMCs and sequester any released plasma TGFβ1, -β2, and -β3 at doses >1 mg/kg. Early evidence of clinical efficacy is encouraging and multiple expansion cohorts are currently ongoing in a range of tumor type, including colorectal cancer and non-small cell lung cancer.

## Disclosure of Potential Conflicts of Interest

I. Grenga is currently an employee of EMD Serono. No potential conflicts of interest were disclosed by the other authors.

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