Phase I Study of DMOT4039A, an Antibody-Drug Conjugate Targeting Mesothelin, in Patients with Unresectable Pancreatic or Platinum-Resistant Ovarian Cancer

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Abstract

DMOT4039A, a humanized anti-mesothelin mAb conjugated to the antimitotic agent monomethyl auristatin E (MMAE), was given to patients with pancreatic and ovarian cancer every 3 weeks (0.2–2.8 mg/kg; q3w) or weekly (0.8–1.2 mg/kg). A 3+3 design was used for dose escalation followed by expansion at the recommended phase II dose (RP2D) to evaluate safety and pharmacokinetics. Antitumor response was evaluated per RECIST 1.1 and serum CA19-9 or CA125 declines. Tumor mesothelin expression was determined by IHC. Seventy-one patients (40 pancreatic cancer; 31 ovarian cancer) were treated with DMOT4039A. For the q3w schedule (n = 54), the MTD and RP2D was 2.4 mg/kg, with dose-limiting toxicities of grade 3 hyperglycemia and grade 3 hypophosphatemia at 2.8 mg/kg. For the weekly schedule (n = 17), the maximum assessed dose was 1.2 mg/kg, with further dose escalations deferred because of toxicities limiting scheduled retreatment in later cycles, and therefore the RP2D level for the weekly regimen was determined to be 1 mg/kg. Across both schedules, the most common toxicities were gastrointestinal and constitutional. Treatment-related serious adverse events occurred in 6 patients; 4 patients continued treatment following dose reductions. Drug exposure as measured by antibody-conjugated MMAE and total antibody was generally dose proportional over all dose levels on both schedules. A total of 6 patients had confirmed partial responses (4 ovarian; 2 pancreatic) with DMOT4039A at 2.4 to 2.8 mg/kg i.v. q3w. DMOT4039A administered at doses up to 2.4 mg/kg q3w and 1.0 mg/kg weekly has a tolerable safety profile and antitumor activity in both pancreatic and ovarian cancer. Mol Cancer Ther; 15(3); 439–47. ©2016 AACR.

Introduction

Mesothelin was originally identified as a cancer antigen target via characterization of an antibody raised against ovarian cancer cells, and later named for its presence in normal mesothelial cells (1, 2). Mesothelin is a cell-surface glycosylphosphatidylinositol-linked protein that is largely membrane-bound and highly expressed in 86% to 100% pancreatic ductal adenocarcinomas, 77% ovarian adenocarcinomas, and 89% to 100% mesotheliomas (3–7).

Mesothelin binds to the mucin 16 antigen (8), which is also overexpressed in pancreatic and ovarian cancers (9, 10). Although many studies have examined the possible function of mesothelin, its role in cancer is still unclear and may be cancer-type specific (11). Notwithstanding, with high frequency of expression in pancreatic and ovarian tumors, and expression limited to slowly dividing normal tissues with mesothelial cell lining (pleural, pericardial, and peritoneal surfaces), mesothelin is an attractive antigen target for antibody-based therapies coupled to cytotoxic agents. The target has been clinically validated, with antitumor activity demonstrated in various anti-mesothelin–derived antibody constructs, albeit with modest activity (12, 13), and with a live-attenuated listeria vaccine expressing mesothelin (CRS-207; 14, 15).

DMOT4039A (16) is an antibody–drug conjugate (ADC) composed of the humanized IgG1 anti-mesothelin mAb h7D9 v3 and the potent antitumor agent, monomethyl auristatin E (MMAE), linked through a protease-labile valine-citrulline linker, with a drug-antibody ratio of approximately 3.5. Following antigen-specific binding of the ADC, the complex of mesothelin and the anti-mesothelin ADC can be internalized and MMAE released after the linker has been broken down by intracellular proteases. Free MMAE subsequently results in disruption of the microtubule network, inhibition of cell division and growth to promote tumor cell death by mitotic catastrophe (17–20). DMOT4039A has specific antiproliferative activity against mesothelin-expressing cancer cells in vitro and potent antitumor activity in xenograft cancer models expressing clinically relevant levels of mesothelin.

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(16). Although DMOT4039A does not cross-react with cynomolgus monkey mesothelin, it was similarly well tolerated as a cross-reactive surrogate ADC, with off-target toxicities common to other MMAE conjugates, including neutropenia (21). Importantly, neither ADC caused detectable pleuritis nor pericarditis in cynomolgus monkeys, unlike the anti-mesothelin immunotoxin SS1 (dsFv)PE38 (SS1P; ref. 12); thus, DMOT4039A has the potential to be better tolerated by human subjects. These properties provided the rationale for investigating DMOT4039A for the treatment of frequently mesothelin-positive ovarian and pancreatic tumors.

Materials and Methods

Study design and patient selection

This phase I, multicenter, open-label, dose-escalation study (Clinicaltrials.gov number NCT01469793) was designed to identify the recommended phase II dose (RP2D) of DMOT4039A in patients with unresectable/metastatic pancreatic cancer or platinum-resistant ovarian cancer. The study determined the maximum tolerated dose (MTD) of an every 3-week dosing (q3w), then enrolled an expansion cohort at the q3w RP2D to further characterize the safety and preliminary clinical efficacy of DMOT4039A. A separate cohort of patients was dosed weekly to determine the RP2D level on this schedule with the aim of increasing dose intensity and antitumor activity without incurring additional toxicity.

Patients with pancreatic adenocarcinoma had histologic documentation of incurable, locally advanced unresectable pancreatic adenocarcinoma, or metastatic disease for which no standard therapy exists, including recurrence of previously resected disease. Patients with platinum-resistant ovarian cancer (defined as epithelial ovarian cancer, primary peritoneal cancer, or fallopian tube cancer with radiographic progression or relapse within 6 months of platinum-based chemotherapy) had serous or endometrioid histology. In the expansion cohorts, patients with pancreatic cancer were initially enrolled regardless of mesothelin expression, but the last 10 (of 30) patients were selected for IHC score 2+ to improve the likelihood of detecting response in mesothelin high expressing tumors. For patients with mesothelin IHC score 3+ expression, but the last 10 (of 30) patients were selected for IHC score 2+ or 3+ to improve the likelihood of detecting response in mesothelin high expressing tumors. For patients in the ovarian cancer expansion cohort, all patients were required to have a mesothelin IHC score of 3+. Considering theoretical risk for pericardial toxicity based upon normal mesothelin expression in the pericardium, patients with evidence of pericardial effusions on screening echocardiograms were excluded. Other inclusion criteria were Eastern Cooperative Oncology Group (ECOG) performance score 0 or 1, adequate bone marrow (absolute neutrophil count \( \geq 1.5 \times 10^9/L \), hemoglobin \( \geq 9 \) g/dL, and platelet count \( \geq 100 \times 10^9/L \)), liver (total bilirubin \( \leq 1.5 \times \) upper limit of normal (ULN) and aspartate aminotransferase and alanine aminotransferase \( \leq 2.5 \times \) ULN), and renal function (serum creatinine \( \leq 1.5 \times \) ULN).

The study protocol was approved by Institutional Review Boards prior to patient recruitment and conducted in accordance with International Conference on Harmonization E6 Guidelines for Good Clinical Practice. Each patient provided signed informed consent prior to study enrollment.

Procedures

In the single-agent dose-escalation stage utilizing a 3+3 enrollment scheme, patients were given intravenous DMOT4039A (supplied by Genentech) at 0.2 to 2.8 mg/kg q3w. The infusion period was 90 minutes; however, subsequent infusions over 30 minutes were permitted in the absence of significant infusion-related reactions (e.g., acute adverse events (AE) grade 2 or higher). No premedication was required or recommended prior to DMOT4039A administration.

After identifying the MTD for the q3w schedule, a weekly schedule of DMOT4039A was evaluated. The starting dose of DMOT4039A for the weekly cohorts was one-third of the MTD established for the q3w schedule and was escalated by \( \leq 33\% \) of the preceding dose level.

The RP2D was determined based upon cumulative safety data obtained at all dose levels at the time of cohort expansion, and was required to be at or below the MTD. Dose-expansion cohorts were enrolled at the RP2D to further characterize safety, tolerability, pharmacokinetic variability, and signals of clinical activity of DMOT4039A in 20 patients with pancreatic cancer (10 of whom were enriched with IHC 2+ or 3+) and 8 IHC 3+ selected patients with ovarian cancer (q3w only).

The expansion cohorts were sized to explore response rates, with a larger sample size selected for pancreatic cancer patients anticipating a lower response rate based on historical data. IHC selection for the ovarian cancer expansion was pursued on the basis of a lower predicted expression of mesothelin in this population; modification to select patients on the basis of IHC for the pancreatic cancer expansion was based upon availability of tumor tissue, prevalence of mesothelin during study treatment, and ongoing measures of response during study.

Safety assessment

Safety was evaluated according to NCI CTCAE v4.0. Dose-limiting toxicities (DLT) were defined as any treatment-related grade 3–4 AE occurring within the first 21 days (22, 23) after the first dose of treatment, including grade \( \geq 3 \) nonhematologic toxicity, grade \( \geq 4 \) anemia or thrombocytopenia, grade \( \geq 4 \) neutropenia lasting \( >5 \) days or associated with fever, grade \( \geq 2 \) pericardial or pleural effusion. Patients had echocardiograms performed at screening, after the first cycle of treatment, after cycle 4, and at discontinuation of study treatment. The MTD was defined as the dose at which \( \leq 1 \) of 6 patients experienced a DLT.

Pharmacokinetics and immunogenicity assessments

Serum concentrations of total antibody (antibody with MMAE-to-antibody ratio equal to or greater than zero, including conjugated, partially deconjugated, and fully deconjugated antibody), were determined using a validated ELISA (24). The minimum quantifiable concentration of the assay was 75 ng/mL. Antibody-conjugated MMAE (ac-MMAE) and unconjugated MMAE concentrations were measured in plasma samples using a validated electrospay LC/MS-MS assays with lower limits of quantification of 0.359 and 0.0359 ng/mL, respectively.

Pharmacokinetic parameters for the total antibody, ac-MMAE, and the unconjugated MMAE after the first dose of DMOT4039A in cycle 1 (q3w and weekly schedules) were estimated by standard noncompartmental analysis using WinNonlin 5.2.1 software (Pharsight). At subsequent cycles, the peak and trough (preinfusion) concentrations were summarized using descriptive statistics.

Clinical activity

Objective response rates were estimated for patients with disease measurable by RECIST v1.1 guidelines (25). Tumor
assessed by CT was performed at screening, the last week of every 3-weekly cycle (e.g., cycle 2, 4, etc.), and at study treatment completion. Objective response was defined as a complete or partial response, as determined by investigator and confirmed by repeat assessments ≥4 weeks after initial documentation. Duration of objective response was defined as the time from the initial complete or partial response to the time of disease progression or death. Progression-free survival (PFS) was defined as the time from the first day of study treatment (cycle 1 day 1) to disease progression or death within 30 days of the last study drug administration, whichever occurred first. If a patient did not experience progressive disease or die, or was lost to follow-up, PFS was censored at the day of the last tumor assessment.

Exploratory measures of activity were also assessed by serum CA19-9 in patients with pancreatic cancer or serum CA125 in patients with ovarian cancer. A serologic response was defined as a ≥50% decline in baseline value in patients with baseline values at least two times the ULN.

Mesothelin IHC and soluble mesothelin detection

For determination of mesothelin protein expression in formalin-fixed, paraffin-embedded tissues, a fully automated IHC assay was developed using an anti-mesothelin mouse monoclonal primary antibody (19C3; 16) and Ventana ultraView DAB IHC detection. Mesothelin membranous staining level was scored according the following algorithm, where at least 10% of tumor cells had to be stained to qualify as positive in each category: IHC 3+, the predominant staining intensity is 3+ denoting predominantly strong staining; IHC 2+, predominantly moderate staining; IHC 1+, predominantly weak staining; IHC 0, very weak or no staining in >90% of tumor cells.

To measure shed mesothelin in serum, MaxiSorp plates were coated with 3H2 anti-mesothelin to capture shed serum mesothelin (or mesothelin extracellular domain standards purified from Chinese hamster ovary cells) and binding was detected using biotinylated 19C3 and streptavidin–horseradish peroxidase (Fourie-O’Donohue et al., A novel ELISA for detection of shed mesothelin in ovarian and pancreatic patient serum; manuscript in preparation).

Statistical analysis

Design considerations were not made with regard to explicit power and type I error, but to obtain preliminary safety, pharmacokinetic, and pharmacodynamic information. For safety analysis, all patients who received DMOT4039A were included. For activity analyses, all patients with measurable disease at baseline were included.

Table 1. Patient baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (N = 71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, median (range)</td>
<td>63 (39–75)</td>
</tr>
<tr>
<td>Sex</td>
<td>Female/male</td>
</tr>
<tr>
<td></td>
<td>50/21 (70%/30%)</td>
</tr>
<tr>
<td>Tumor type</td>
<td>Pancreas</td>
</tr>
<tr>
<td></td>
<td>40 (56%)</td>
</tr>
<tr>
<td></td>
<td>Ovarian</td>
</tr>
<tr>
<td></td>
<td>31 (44%)</td>
</tr>
<tr>
<td>ECOG status</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30 (42%)</td>
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<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>41 (58%)</td>
</tr>
<tr>
<td>Prior systemic regimens, median (range)</td>
<td>Pancreas cancer patients (n = 40)</td>
</tr>
<tr>
<td></td>
<td>Ovarian cancer patients (n = 31)</td>
</tr>
</tbody>
</table>

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PROC, platinum-resistant ovarian cancer.

Results

Baseline demographics and treatment exposure

From November 9, 2011 to February 11, 2014, 71 patients (40 pancreatic cancer; 31 ovarian cancer) were enrolled at 6 sites in the United States and the Netherlands. Patient baseline and disease characteristics are shown in Table 1. Fifty-four patients were enrolled in the q3w schedule, and 17 patients were enrolled in the weekly schedule. The cut-off date for analysis was November 3, 2014. For the q3w schedule, the median number of dose administrations was 3 (range 1–15); and for the weekly schedule, it was 6 (range 1–30; Table 2). At the time of the analysis, all patients except one (1%) with ongoing treatment response had discontinued study treatment due to disease progression (73%), lack of response (8%), AE (7%), withdrawal by subject (6%), physician decision (3%), or death due to progression of disease (1%).

Safety

For the q3w regimen (Table 3), two DLTs occurred in 2 patients, hyperglycemia (grade 3), and hypophosphatemia (grade 3), both at the maximum assessed dose of 2.8 mg/kg; therefore, the MTD for DMOT4039A was determined to be 2.4 mg/kg. Additional related serious AEs (SAE) in the q3w regimen occurred in 5 patients treated at the 2.4 and 2.8 mg/kg dose levels, all grade ≤ 3, included pyrexia, gastroapresis, dehydration, hypotension, fatigue, infection, sinus tachycardia, vomiting, and urinary tract infection. Over all dose levels, the most common toxicities were gastrointestinal or constitutional. Cumulative peripheral neuropathy (grades 1–3) occurred in 20% of patients, which is an expected toxicity for microtubule inhibitors (26).

Table 2. DMOT4039A exposure (cycles administered)

<table>
<thead>
<tr>
<th>Extent of exposure to DMOT4039A</th>
<th>Every 3 weeks schedule</th>
<th>Weekly schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2 mg/kg (n = 3)</td>
<td>0.8 mg/kg (n = 3)</td>
</tr>
<tr>
<td></td>
<td>0.4 mg/kg (n = 3)</td>
<td>1.0 mg/kg (n = 7)</td>
</tr>
<tr>
<td></td>
<td>0.8 mg/kg (n = 3)</td>
<td>2.4 mg/kg (n = 6)</td>
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<tr>
<td></td>
<td>1.6 mg/kg (n = 6)</td>
<td>2.8 mg/kg (n = 6)</td>
</tr>
<tr>
<td></td>
<td>2.4 mg/kg (n = 36)</td>
<td>0.8 mg/kg (n = 4)</td>
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<td></td>
<td></td>
<td>1.0 mg/kg (n = 7)</td>
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<td></td>
<td></td>
<td>1.2 mg/kg (n = 7)</td>
</tr>
</tbody>
</table>

*Note: Extent of exposure to DMOT4039A for patients who experienced cumulative peripheral neuropathy (grades 1–3) in the q3w regimen resulted in a dose reduction to 2.4 mg/kg. For the weekly regimen, any patients who experienced cumulative peripheral neuropathy (grades 1–3) had their dose reduced to 2.8 mg/kg.*
The maximum assessed dose for the weekly schedule (Table 4) was 1.2 mg/kg with no occurrence of DLTs. However, further dose escalation on the weekly schedule was not pursued due to cumulative occurrence of poorly tolerated AEs in later cycles. One patient experienced a DMOT4039A-related SAE of ileus (grade 3) and pyrexia (grade 2) on the weekly schedule after the first cycle at the 1.2 mg/kg dose level. In addition, 3 patients experienced a grade 3–related AEs of anemia, rash, and neutropenia and leukopenia, none of which were considered serious. There were no SAEs reported at 0.8 or 1.0 mg/kg dose levels. As a result of these findings, the RP2D level for the weekly schedule was determined to be 1 mg/kg.

On the basis of the expression profile of mesothelin and prior experience with mesothelin-targeted agents, sorosit (defined as pericarditis, pleuritis, and associated effusions) was a potential on-target toxicity. Sorosit was reported in 4 patients (6%) at 2.4 mg/kg q3w level, including 1 patient with a grade 4 pericardial effusion considered related to progression of ovarian cancer and unrelated to study treatment; 3 patients developed pleural effusions (grade 3 in 2 patients; grade 1 in 1 patient) among which only the grade 1 event was considered related to study treatment. Treatment-related sorosit was noted in 1 patient (1%), an 87-year-old female patient with ovarian cancer treated at 2.4 mg/kg q3w. This patient experienced a nonserious grade 1 pleural effusion on cycle 1 day 8 that resolved on cycle 4 day 15, with no action taken with study drug.

### Pharmacokinetics and immunogenicity assessments

For the q3w regimen, the mean ac-MMAE maximum concentration (Cmax) occurred immediately after the infusion, increased with dose and ranged from 63 to 918 ng/mL; ac-MMAE pharmacokinetics showed a multiexponential decline with half-life (t1/2) values ranging from 2.1 to 3.7 days. The ac-MMAE pharmacokinetics was similar to the total antibody with a trend of faster clearance for ac-MMAE than the total antibody analyte. The systemic exposure of unconjugated MMAE was consistently low (approximately 100-fold less than the exposure to ac-MMAE) and exhibited formation-rate–limited kinetics. Minimal accumulation was observed for the ac-MMAE total antibody, and unconjugated MMAE analytes upon repeated dosing on the q3w schedule and steady state appeared to be reached within the first dose in cycle 1. Systemic exposure [maximum concentration (Cmax) and AUC] increased proportionally with dose for the total antibody and the ac-MMAE analytes. The mean clearance (CL) and half-lives were similar across the dose levels tested for the total antibody and the ac-MMAE analytes. The volume of distribution of the total antibody and ac-MMAE analytes approximated the physiologic serum volume and were generally independent of dose, suggesting that the ac-MMAE distribution was dominated by its antibody component. Taken together, the pharmacokinetics of the DMOT4039A total antibody and ac-MMAE analytes was linear across the tested dose range of 0.2 to 2.8 mg/kg for the q3w schedule. Serum total

### Table 3. Most frequent treatment-related AEs occurring in 10% patients on q3w schedule

<table>
<thead>
<tr>
<th>Grade</th>
<th>0.2 mg/kg (n = 3)</th>
<th>0.4 mg/kg (n = 3)</th>
<th>0.8 mg/kg (n = 3)</th>
<th>1.6 mg/kg (n = 3)</th>
<th>2.4 mg/kg (n = 36)</th>
<th>2.8 mg/kg (n = 6)</th>
<th>All patients (N = 54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any patient with AE</td>
<td>—</td>
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<td>—</td>
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<td>—</td>
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<tr>
<td>Fatigue</td>
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<td>—</td>
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<tr>
<td>Nausea</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Diarrhea</td>
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<td>—</td>
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<tr>
<td>Alopecia</td>
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<td>—</td>
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<tr>
<td>Peripheral neuropathy</td>
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<tr>
<td>Anorexia</td>
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<td>Pyrexia</td>
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NOTE: Additional grade 3/4 AEs not included in the table above includes the following: at 2.4 mg/kg: neutropenia (grade 3); hypophosphatemia (grade 3).

### Table 4. Most frequent treatment-related AEs occurring in 10% patients on weekly schedule

<table>
<thead>
<tr>
<th>Grade</th>
<th>0.8 mg/kg (n = 3)</th>
<th>1 mg/kg (n = 7)</th>
<th>1.2 mg/kg (n = 7)</th>
<th>All patients (N = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any patient with AE</td>
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<tr>
<td>Diarrhea</td>
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<tr>
<td>Peripheral neuropathy</td>
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<td>Fatigue</td>
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<tr>
<td>Anemia</td>
<td>—</td>
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<tr>
<td>Hoarse voice</td>
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<td>Neutropenia</td>
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<td>Pyrexia</td>
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NOTE: Additional grade 3/4-related AEs not included in the table above include rash (G3; 1.0 mg/kg), white blood cell count decreased (grade 3; 1.0 mg/kg), ileus (grade 3; 1.2 mg/kg), and bacterial peritonitis (grade 3; 1.2 mg/kg).

*Peripheral neuropathy SMQ terms: peripheral neuropathy, peripheral sensory neuropathy, and paresthesia.*
antibody, plasma ac-MMAE, and unconjugated MMAE concentration–time profiles and the summary of pharmacokinetic parameters after the first dose in cycle 1 for the 2.4 mg/kg q3w cohort are presented in Fig. 1A.

The mean CL and t1/2 values were similar across the doses studied (0.8–1 mg/kg) for the weekly regimen. Pharmacokinetics appeared linear for both the total antibody and the ac-MMAE analytes. The systemic unconjugated MMAE exposure was consistently low and approximately 100-fold less than the exposure to the ac-MMAE analyte. Minimal accumulation was observed for all analytes upon repeated dosing and steady state appeared to be reached within the cycle 1. The cumulative exposure of ac-MMAE and total antibody in cycle 1 after the weekly dose of 0.8 mg/kg was roughly similar to the exposure observed at q3w RP2D (2.4 mg/kg) in cycle 1 (Table 5).

Sixteen (30%) of the 54 tested patients evaluable for anti-DMOT4039A antibodies at any postdose time point were positive for antitherapeutic antibodies (ATA). This response was confirmed by competitive-binding immunodepletion with DMOT4039A. The ATAs were directed predominantly against the antibody rather than the linker or drug. Nevertheless, no differences were observed in the pharmacokinetics, safety profiles, or efficacy outcomes of patients who developed antibodies to DMOT4039A compared with those who did not (data not shown).

**Clinical activity**

**Pancreatic cancer.** Two of 26 (8%) patients with pancreatic cancer on the q3w schedule treated at the RP2D level (2.4 mg/kg) demonstrated confirmed partial responses. One patient demonstrated a partial response lasting 1.4 months, and the other demonstrated a partial response lasting 12.1 months, which was ongoing at the time of manuscript submission (E.G.E. de Vries; personal communication). Both of these patients had normal CA19-9 values at baseline, and therefore were not assessable for biomarker response. Nine additional pancreatic patients (35%) had stable disease as best response (median duration 5.7 months at 2.4 mg/kg), including 5 patients (12%) with stable disease lasting >120 days, including 2 patients with CA19-9 responses (treated at 2.4 mg/kg and 2.8 mg/kg dose levels). The median PFS for pancreatic cancer patients at 2.4 mg/kg was 1.7 months. No RECIST or CA19-9 responses were seen in the 4 pancreatic cancer patients treated on the weekly schedule at any dose level.

**Ovarian cancer.** Three of 10 (30%) ovarian cancer patients on the q3w schedule at the RP2D level (2.4 mg/kg) demonstrated confirmed partial responses, with durations of 2.7, 3.6, and 4.1 months. Each of these patients had concurrent responses in CA125. An additional 3 patients with baseline elevated CA125 treated at the RP2D showed a CA125 response without RECIST response. One of 12 (8%) ovarian cancer patients demonstrated a RECIST response lasting 5.7 months prior to discontinuing for an AE of peripheral neuropathy (grade 2) on the weekly schedule. This patient had normal CA125 at baseline and was not assessable for CA125 response. In addition, 3 patients showed a CA125 response at 0.8, 1.0, and 1.2 mg/kg, without a concurrent RECIST response. The median PFS for ovarian cancer patients at 2.4 mg/kg was 4.9 months.

One female ovarian cancer patient with a response to treatment (Fig. 2B) was a 59-year-old woman previously treated with carboplatin/paclitaxel. This patient received DMOT4039A at a dose of 2.4 mg/kg q3w. Archival tissue showed IHC 3+ staining for mesothelin. She achieved significant clinical improvement in the first cycle, with a decrease in abdominal girth associated with a decline in CA125 from 1,309 to 740. Consistent with observed clinical improvement in cycle 1, a CT scan following cycle 2

![Figure 1.](image-url)

**Figure 1.** DMOT4039A concentration versus nominal time profiles at 2.4 mg/kg.

### Table 5. Pharmacokinetic parameters for the total antibody, ac-MMAE, and unconjugated MMAE analytes in cycle 1 at 2.4 mg/kg

<table>
<thead>
<tr>
<th>DMOT4039A dose (mg/kg)</th>
<th>Cmax mean (% CV)</th>
<th>AUC0-inf mean (% CV)</th>
<th>CL mean (% CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4 q3w (N = 36)</td>
<td>Total Ab (μg/mL)</td>
<td>ac-MMAE (ng/mL)</td>
<td>Total Ab (μg/mL)</td>
</tr>
<tr>
<td>39 (18%)</td>
<td>746 (27%)</td>
<td>4.2 (45%)</td>
<td>162 (38%)</td>
</tr>
<tr>
<td>20 (3%)</td>
<td>27 (22%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: CV, coefficient of variation.
showed a partial response, which was confirmed at cycle 4. Maximum changes in RECIST target lesions showed a 53% decline, with an 89% decline in CA125. She progressed after cycle 6. The AEs experienced by this patient included neutropenia (grade 3), fatigue and dyspnea (grade 2), abdominal pain, nausea, alopecia, and pleura effusion (each grade 1).

Biomarker analysis

Tumor mesothelin protein expression was analyzed by IHC in 30 (75%) patients with pancreatic cancer and 25 (81%) patients with ovarian cancer (Fig. 3). Missing data were due to either lack of available tissue for staining or no evidence of tumor in submitted specimens. Among the evaluable tumors for pancreatic cancer, the distribution of mesothelin membranous IHC staining scores was 0 (17%), 1+ (7%), 2+ (67%), and 3+ (10%); and for ovarian tumors, 0 (4%), 2+ (28%), and 3+ (68%). Although the mesothelin staining frequency is consistent with prior published results (3–6, 16), some bias for higher frequency of expression may exist due to selection of IHC 3+ patients in the expansion cohorts. Representative images of IHC staining in both pancreatic and ovarian tumor specimens are shown in Fig. 3A. As expected, staining appeared less homogenous in pancreatic than ovarian tumors, likely due to the desmoplastic reaction associated with pancreatic cancer (3, 27). Five of 6 (83%) partial responses in the study occurred with tumors IHC scores 2+ or 3+; the 6th responder failed to provide tissue for staining.

Predose soluble serum mesothelin was explored (measured by sMSLN ELISA) as a surrogate biomarker for mesothelin IHC, as obtaining sufficient tumor specimens from the pancreatic cancer cohort was considered a potential risk. Soluble serum mesothelin levels did not correlate with neither tissue mesothelin IHC score nor with clinical activity of DMOT4039A (Fig. 3B).

We evaluated the impact of shed mesothelin levels on pharmacokinetics and no clear impact of predose shed mesothelin levels on acMMAE clearance or volume of distribution (Vss) was observed, including 2 patients with elevated (IHC 2+) shed mesothelin levels.

Discussion

DMOT4039A is a novel anti-mesothelin ADC that is generally well tolerated as a single agent with demonstrated evidence of antitumor activity. The RP2D of 2.4 mg/kg q3w showed responses with a favorable safety profile in patients with pancreatic cancer and ovarian cancer, including those with prior exposure to microtubule inhibitors. In addition, the weekly schedule identified 1.0 mg/kg as an appropriate RP2D level based on overall tolerability and safety. The safety profile was
generally consistent with the class of microtubule inhibitors, and therefore provides opportunities to replace preexisting microtubule inhibitors with a targeted agent with an improved therapeutic index and combine with agents that have nonoverlapping toxicities with DMOT4039A (e.g., cumulative peripheral neuropathy).

The DMOT4039A total antibody and the ac-MMAE analytes demonstrated linear pharmacokinetics across the doses assessed at both the q3w and the weekly schedules. The cumulative exposures for both these analytes were similar regardless of the q3w or the weekly dosing schedules.

The antitumor activity for this anti-mesothelin–MMAE ADC is encouraging, as it appears to have a broader therapeutic index than other targeted therapeutics against mesothelin. This might be due to added selectivity of the microtubule inhibitor for dividing cells relative to other agents that function in a cell cycle–independent manner (e.g., serositis with SS1P; 12). In addition, the activity of DMOT4093A described in this study may have resulted from the enrichment of patients whose tumors expressed mesothelin. Diagnostic selection for auristatin-based ADCs has been consistently associated with increased activity relative to unselected or diagnostically negative patients even in early exploratory settings (28–31).

Despite the similar IHC scores in pancreatic and ovarian cancer, the antitumor activity as assessed by response or stable disease was numerically lower in pancreatic cancer cohort. Potential reasons for this include the inherent variability of assessing small cohorts, the inaccessibility of the ADC to tumor cells due to its relative hypovascular nature, as a result of the dense stromal structure of pancreatic lesions (32). In addition, mesothelin expression in pancreatic cancer may be heterogeneous, inaccessible (i.e., apical) or lost (3, 16). Finally, primary resistance to chemotherapy in pancreatic cancer may be more prevalent. To explore these possibilities, a companion imaging study (NCT01832116) using PET with the anti-mesothelin antibody conjugated to zirconium-89 was conducted, which demonstrated that primary pancreatic
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.D. Weekes, E.L. Lamberts, M.J. Borad, J. Voortman, C.D. Weekes, E.G.E. de Vries, S.J. Scales, D. Samineni, H.M. Verheul, G. Colon-Otero, G. P. Kim, Y. Wang

Disclosure of Potential Conflicts of Interest
C.D. Weekes receives commercial research support from and is a consultant/advisory board member for Genentech. E.G.E. de Vries reports receiving a commercial research grant from Genentech and is a consultant/advisory board member for Synthorx. H.M. Verheul receives commercial research grants from Amgen, Immunoviva BV, Roche, and VIB and is a consultant/advisory board member for Boehringer-Ingelheim. G.P. Kim is a consultant/advisory board member for Genentech. S.J. Scales is a co-inventor of a patent owned by Genentech. G. Colon-Otero reports receiving a commercial research grant from Novartis. No potential conflicts of interest were disclosed by the other authors.

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References


