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Phase 1 Study of the Antimesothelin Immunotoxin SS1P in Combination With Pemetrexed and Cisplatin for Front-Line Therapy of Pleural Mesothelioma and Correlation of Tumor Response With Serum Mesothelin, Megakaryocyte Potentiating Factor, and Cancer Antigen 125

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Abstract

BACKGROUND: The primary objective of this study was to determine the safety and maximum tolerated dose (MTD) of the antimesothelin immunotoxin SS1(dsFv)PE38 (SS1P) (a recombinant antimesothelin immunotoxin consisting of a murine antimesothelin variable antibody fragment [Fv] linked to PE38, a truncated portion of *Pseudomonas* exotoxin A) in combination with pemetrexed and cisplatin in chemotherapy-naive patients with advanced malignant pleural mesothelioma (MPM). Secondary objectives included tumor response, SS1P pharmacokinetics, and serum biomarkers of response.

METHODS: Chemotherapy-naive patients with stage III or IV, unresectable, epithelial or biphasic MPM and normal organ functions were eligible. Pemetrexed (500 mg/m^2 on day 1) and cisplatin (75 mg/m^2 on day 1) were administered every 3 weeks for up to 6 cycles with escalating doses of SS1P administered intravenously on days 1, 3, and 5 during cycles 1 and 2. Tumor response was evaluated every 6 weeks.

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CONFLICT OF INTEREST DISCLOSURES

Dr. Thomas was an employee of the US Federal Government during the conduct of this study. Dr. Pastan is an inventor on SS1P patents (7,018-581; with licenses held by Roche and Morphotek) and has assigned all of his rights to the National Institutes of Health.

RESULTS: Twenty-four patients received SS1P at 4 dose levels from 25 to 55 mcg/kg. Grade 3 fatigue was dose-limiting in 1 patient at 55 mcg/kg. The MTD of SS1P was established as 45 mcg/kg. Other grade 3 toxicities associated with SS1P included hypoalbuminemia (21%), back pain (13%), and hypotension (8%). Of 20 evaluable patients, 12 (60%) had a partial response, 3 had stable disease, and 5 had progressive disease. Of 13 patients who received the MTD, 10 (77%) had a partial response, 1 had stable disease, and 2 had progressive disease. Objective radiologic responses were associated with significant decreases in serum mesothelin (P=.0030), megakaryocyte potentiating factor (P=.0005), and cancer antigen 125 (P <.0001).

CONCLUSIONS: SS1P given with pemetrexed and cisplatin is safe and well tolerated and exhibits significant antitumor activity in patients with unresectable, advanced pleural mesothelioma. Serum mesothelin, megakaryocyte potentiating factor, and cancer antigen 125 levels correlated with objective tumor responses.

Keywords

mesothelin; pleural mesothelioma; SS1P; immunotoxin

INTRODUCTION

Malignant pleural mesothelioma (MPM) is an aggressive disease with a poor prognosis. Although patients with limited tumor burden may benefit from surgical resection, most patients with MPM present with advanced disease at diagnosis and are not candidates for cytoreductive surgery. On the basis of results from a randomized phase 3 trial that demonstrated a survival benefit over single-agent cisplatin, combination chemotherapy with cisplatin and pemetrexed is the standard first-line therapy for patients who are ineligible for surgery.[1] However, response rates to combination chemotherapy are modest at 41%, and the median overall survival 12.1 months, underscoring the need for new therapies.

An attractive tumor-specific therapy target for mesothelioma is the tumor differentiation antigen mesothelin, a 40-kilodalton (kDa) glycoprotein present on normal mesothelial cells that line the pleura, peritoneum, and pericardium. Although the normal biologic function of mesothelin is unknown, growing evidence suggests that it may play a role in tumorigenesis and metastasis in certain malignancies.[2] Mesothelin is highly expressed in many human epithelial malignancies, including epithelial and biphasic malignant mesothelioma and ovarian, pancreatic, gastric, and lung adenocarcinomas.[3–6] The limited expression of mesothelin on normal human tissue and high expression in many cancers makes it an excellent target antigen for antibody-based immunotherapy, and several such agents are currently in clinical trials.[7] The mesothelin gene encodes a 70-kDa precursor protein that is cleaved into a soluble 31-kDa fragment, megakaryocyte potentiating factor (MPF), and a membrane-bound 40-kDa mesothelin.[8] Both MPF and membrane-bound mesothelin can be detected in the serum and are promising biomarkers for mesothelioma.[9–13]

SS1(dsFv)PE38 (SS1P) is a recombinant antimesothelin immunotoxin that consists of a murine antimesothelin variable antibody fragment (Fv) linked to PE38, a truncated portion of *Pseudomonas* exotoxin A. In a phase 1 clinical trial of patients with advanced Mesothelin-expressing cancers who had failed standard therapies, bolus administration of

SS1P on alternate days for 3 doses was well tolerated. Pleuritis was the dose-limiting toxicity (DLT), and the maximum tolerated dose (MTD) was 45 mcg/kg.[14] The most commonly reported adverse events (AEs) in the phase 1 trial were hypoalbuminemia and fatigue. In that group of heavily pretreated patients, SS1P had limited antitumor activity. One reason for a lack of activity of single-agent SS1P could be the limited tumor penetration from the site density barrier caused by close packing of tumor cells, high interstitial pressure within tumors, and lack of functional lymphatics.[15] In mice with mesothelin-expressing human tumor xenografts, SS1P had modest antitumor activity by itself; however, when combined with chemotherapy, remarkable synergy was observed.[16,17] We demonstrated that, by killing tumor cells, chemotherapy disrupted the close packing of tumor, allowing better penetration of immunotoxin into the tumor.[18]

On the basis of these preclinical data, we designed this study to determine whether a similar synergistic antitumor effect could be observed in patients with MPM by combining SS1P with standard chemotherapy. The objectives of this phase 1 study were to assess the recommended phase 2 dose (RP2D), safety and tolerability, and the pharmacokinetics (PK) of SS1P when administered in combination with a standard chemotherapy regimen of pemetrexed and cisplatin. Secondary objectives included assessing tumor response and the effectiveness of serum mesothelin, MPF, and cancer antigen 125 (CA 125) as biomarkers of tumor response.

MATERIALS AND METHODS

Eligibility Criteria

Eligible patients were men and nonpregnant women with histologically confirmed MPM of an epithelial or biphasic subtype (those who had biphasic tumors with a predominantly sarcomatoid component were excluded); aged ≥18 years; a Karnofsky performance status ≥70; and adequate bone marrow, lung, liver, and renal functions (the latter was defined as a serum creatinine clearance ≥60 mL/minute estimated according to the Cockroft-Gault formula). Patients must have had measurable stage III or IV disease that was not amenable to potentially curative surgical resection. Patients who had received prior radiotherapy (with the exception of palliative, extrathoracic, localized radiotherapy within 4 weeks), biologic therapy (within 4 weeks), or systemic chemotherapy for pleural mesothelioma were excluded. Tumor mesothelin expression was not an eligibility criterion, because nearly all epithelial mesotheliomas and the epithelial component of the biphasic mesothelioma express mesothelin.[8] The protocol was approved by the Cancer Therapy Evaluation Program and the Intramural Institutional Review Board of the National Cancer Institute (Bethesda, Md) and was registered with clinicaltrials.gov (National Clinical Trials identifier NCT01445392). All patients provided written informed consent.

Study Design

This was a phase 1 dose-escalation study of SS1P in combination with standard doses of pemetrexed and cisplatin using a classic 3+3 design. Patients received pemetrexed (500 mg/m^2) and cisplatin (75 mg/m^2) on day 1 of a 21-day cycle for a maximum of 6 cycles in the absence of disease progression or unacceptable toxicity. SS1P doses ranging from 25 to

55 mcg/kg were administered intravenously over 30 minutes on days 1, 3, and 5 of cycles 1 and 2 only. SS1P was administered only for the first 2 cycles, because previous studies demonstrated that most patients develop SS1P-neutralizing antibodies after 1 or 2 cycles of treatment.[14]

Assessments

Computed tomography scans were obtained at baseline, after every 2 cycles, and regularly during follow-up. Radiologic tumor response was assessed using the Modified Response Evaluation Criteria in Solid Tumors (RECIST) Criteria for Mesothelioma.[19]

AEs were evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.[20] The following AEs were considered dose-limiting: nonhematologic AEs of grade ≥3 severity that were suspected of a causal relation to SS1P, grade 3 vascular leak syndrome (VLS) (defined as a requirement for fluids >20 mL/kg per hour for at least 30 minutes to treat hypotension), grade 3 hypotension in temporal association with VLS, VLS resulting in symptomatic pulmonary edema requiring supplemental oxygen or a decrease >10% in oxygen saturation, grade ≥4 hematologic AEs except lymphopenia lasting >5 days, grade 2 allergic reactions of bronchospasm or urticaria, or any grade ≥3 allergic reaction in the presence of premedication.

Tumor Mesothelin Expression

Mesothelin immunohistochemistry was performed on tumor samples obtained before therapy using monoclonal antibody 5B2 (Novocastra/Leica, Bannockburn, Ill) in a 1:40 dilution. Incubation with primary antibody was preceded by 20 minutes of heat-induced epitope retrieval in citrate buffer, pH 6.0. Detection was performed using the Ventana Ultra View detection kit (Ventana Medical Systems, Inc., Tucson, Ariz) with 3–3′-diaminobenzidine as the chromogen. Immunohistochemical staining was evaluated by 1 pathologist with special expertise in immunohistochemistry. Positive staining (the strength of labeling) was assessed as negative (no labeling), weak (1+), moderate (2+), and strong (3+). The percentage of positive cells also was estimated. The type of labeling was assessed as either membrane labeling (cell perimeter-positive) or cytoplasmic labeling. Specimens in which ≥30% of tumor cells had cell surface expression of mesothelin were considered positive.[14]

Serum Biomarkers

Mesothelin (nmol/L) and MPF levels (ng/mL) were measured in serum using the Mesomark (Fujirebio Diagnostics, Inc., Malvern, Pa) and a human MPF enzyme-linked immunosorbent assay kit (Medical & Biological Laboratories, Nagano, Japan), respectively. CA 125 levels (U/mL) were measured using an automated commercial assay. All assays were run according to the manufacturer’s instructions and were blinded to patient data. Relative changes in mesothelin, MPF, and CA 125 levels were compared with the patient’s best overall radiologic response during treatment.

SS1P Immunogenicity and Pharmacokinetics

Neutralizing antibodies to SS1P were measured retrospectively using a bioassay as described previously.[14,21] Neutralizing antibodies were determined before treatment, before the second cycle, and on day 21 (±3 days) of the second cycle. Greater than 75% neutralization of SS1P activity in vitro at an SS1P concentration of 1000 ng/mL was established as the cutoff for a positive assay based on previous studies.[14]

Approximately 2 mL of blood were collected for SS1P PK analyses at the following time-points during cycles 1 and 2: for the first dose, before infusion; at the end of the 30-minute infusion (EOI); and at 1, 1.5, 2, 4, 8, and 12 hours; for the second dose, before infusion and at EOI; and, for the third dose, before infusion and at EOI. Samples were centrifuged at × 31000*g* for 15 minutes at 4°C, and the plasma was transferred to cryovials and stored at –80°C.

Statistical Methods

The primary objective of this study was to determine the MTD and the RP2D of the combination of SS1P, pemetrexed, and cisplatin in patients with malignant mesothelioma using a standard phase 1 dose-escalation design. In addition, at the RP2D, an expansion cohort was planned to further characterize the toxicity profile of the combination, to evaluate the changes in the level of serum mesothelin, and to gain preliminary information regarding response rates. The association between the relative changes in biomarker levels and the best overall response was evaluated using the nonparametric Wilcoxon rank-sum test, and associations between the changes in biomarker levels and ordered response categories were assessed using a Jonckheere-Terpstra test for trend.[22] Statistical analyses were performed using the GraphPad PRISM software package (GraphPad Software, Inc., La Jolla, Calif) and SAS version 9.3 (SAS Institute Inc., Cary, NC). A 2-sided *P* value <.05 was considered statistically significant.

RESULTS

Patient Characteristics

Twenty-four patients were enrolled between May 2008 and October 2011 (Table 1). Patients were predominantly men (83%), had good performance status, and had a median age of 67 years.

All patients received at least 1 dose of study drug at 1 of 4 dose levels: 25 mcg/kg per dose (n=5), 35 mcg/kg per dose (n=3), 45 mcg/kg per dose (n=15), or 55 mcg/kg per dose (n=1). Twenty-three patients (96%) completed at least 1 chemotherapy cycle. Of those, 3 patients (13%) completed only 1 cycle, 2 patients (8%) completed 2 cycles, and 4 patients (17%) completed 4 cycles. Fourteen patients (58%) completed all 6 cycles of treatment. Two patients in the 25-mcg/kg per dose cohort withdrew consent and were replaced. One patient at the 45-mcg/kg per dose level in the expansion cohort withdrew consent. Another patient at the 45-mcg/kg per dose level in the expansion cohort died during cycle 1 from grade 5 pneumonia and neutropenic sepsis related to chemotherapy.

Clinical Activity

Of the 24 patients who received treatment, 20 were considered evaluable for response (Fig. 1A). Three patients (2 from dose level 1 and 1 from dose level 3) withdrew from the trial before the response assessment. One patient died while on therapy before the response assessment. Of the 20 patients who were evaluable for response, 12 (60%) had a partial response (PR), 3 (15%) had stable disease (SD), and 5 (20%) had progressive disease (PD). Of the 13 patients who received the MTD, 10 had a PR (77%), 1 had SD (8%), and 2 had PD (15%). The median overall survival of evaluable patients was 13.6 months, and the median progression-free survival was 6.0 months.

Representative examples of tumor responses are illustrated in Figure 1B. Patient 009 was a man aged 69 years with unresectable left pleural mesothelioma. He had significant tumor shrinkage resulting in a PR and resolution of tumor-related symptoms after 6 cycles (Fig. 1B). Patient 018 was a ma aged 72 years with unresectable left pleural mesothelioma. He had a radio-graphic PR and resolution of pleural fluid drainage after 2 cycles (Fig. 1B).

Safety

All patients experienced at least 1 AE. All grade ≥2 drug-related AEs are listed in Figure 2. One patient who received SS1P 55 μg/kg had grade 3 fatigue, which was considered a DLT. One man aged 78 years with known chronic kidney disease, atrial fibrillation, a recent history of clostridium difficile infection, and diabetes died of neutropenic sepsis during the first cycle. His renal function steadily declined from day 3, he developed neutropenia complicated by recurrent clostridium difficile colitis, he also developed bilateral pneumonia, and he died on day 10 of treatment. He received 2 doses of SS1P, and the third dose was held because of worsening renal function.

There were no SS1P-related grade 4 toxicities. The most common grade 2 and 3 SS1P-related AEs were hypoalbuminemia (67%), fatigue (42%), hypotension (33%), edema (29%), back pain (17%), and weight gain (17%). Lower extremity edema was observed mostly during cycle 1 and was self-limiting in most patients, in whom it resolved within 1 week of completing SS1P. Common chemotherapy-related toxicities were lymphopenia (75%), anemia (71%), fatigue (58%), neutropenia (58%), and leukopenia (50%).

Treatment-emergent but nondose-limiting toxicities in the patients who withdrew consent at the 25-mcg/kg dose level included grade 2 fatigue and hypoalbuminemia in 1 patient and grade 2 anorexia, edema, fatigue, anemia, and mucositis and grade 3 hyponatremia in the other patient. The patient who withdrew consent at the 45-mcg/kg dose level developed grade 2 fever and grade 3 hypotension during cycle 2, 1 day after the first dose of SS1P, which responded to intravenous fluids and antipyretics. Because this event could have been related to SS1P, the patient did not receive the day-3 or day-5 SS1P infusions.

Although the protocol allowed additional accrual at the MTD, we chose to expand accrual to the 45-μg/kg dose level, because it was declared the RP2D. That decision was based on the tolerability and responses observed at the 45-μg/kg dose level and taking into consideration the experience with single-agent SS1P, for which 45 μg/kg was the MTD.[14]

Tumor Mesothelin Expression

Eleven of the 12 available tumor samples (92%) stained positive for mesothelin. Figure 3 depicts representative immunohistochemical staining of tumor samples, indicating strong, moderate, and weak staining. The predominant pattern was strong membranous and cytoplasmic mesothelin expression. Strong mesothelin expression in 90% to 100% of tumor cells was observed in 8 tumors. Six of those patients were evaluable for therapy response: 1 had SD, 1 had PD, and 4 had a PR. Strong-to-moderate staining in 35% to 65% of cells was observed in 3 tumors: 1 patient had SD, and 2 had a PR.

Biomarker Correlation With Tumor Response

Mesothelin, CA 125, and MPF measurements were available for the 20 patients who were evaluable for response. For each patient, the relative changes in biomarker levels were compared with their best overall radiologic response (SD, PR, or PD) and were displayed in waterfall plots (Fig. 4). For mesothelin and CA 125 levels, the biomarker change represented the maximal relative change (negative or positive) during therapy compared with baseline. For MPF, only pretherapy and post-therapy levels were available.

Each of the 20 patients had at least 2 data points at which all 3 biomarker values were measured. In those 40 matched samples, mesothelin and MPF levels were strongly correlated (Spearman r=0.90; P < .0001), whereas CA 125 had only a moderate association with mesothelin (Spearman r=0.43; P=.006) and MPF (Spearman r=0.42; P=.007).

The radiologic response of a PR versus less than a PR was associated significantly with changes in levels of mesothelin (P=.0030), MPF (P=.0005), and CA 125 (P < .0001) (Fig. 4). In addition, there was a strong, significant correlation in the relative changes over the 3 ordered response categories (PR>SD>PD) for mesothelin (P=.0004), MPF (P=.0002), and CA 125 (P=.0001). The 5 patients who had PD typically experienced a substantial increase in biomarker levels; whereas, in the 12 patients who had a PR, the levels generally decreased. The 3 patients with SD mostly had a decrease in mesothelin and MPF levels but an increase in CA 125 levels. We also evaluated the accuracy of biomarker changes in reflecting responses to therapy. A 15% threshold was considered to avoid interference with assay variance. Consequently, patients with PD were expected to have an increase in biomarker levels >15%, whereas those who had a PR required a decrease <15%. Patients with SD were required to have no significant change, ie, between 15% and –15%. Overall, mesothelin correctly classified 14 of 20 patients (70% accuracy), MPF correctly classified 15 patients (75% accuracy), and CA 125 correctly classified 12 patients (60% accuracy). These results can be derived from the waterfall plots (Fig. 4). Baseline mesothelin, MPF, and CA 125 levels (Table 1) were not predictive of therapy response (P > .05).

SS1P Immunogenicity and PK Analysis

Before starting treatment, only 1 of the 21 patients (5%) with data available had serum SS1P-neutralizing antibodies. Maximum serum SS1P concentrations (Cmax) were low in that patient (10 ng/mL on day 1 of the first cycle), whereas all patients who did not have neutralizing antibodies (n520; 95%) achieved an SS1P Cmax >150 ng/mL. After the first cycle, all but 2 of the 21 patients (90%) developed SS1P-neutralizing antibodies. SS1P

Cmax values >150 ng/mL were achieved during cycle 2 only by the 2 patients who did not develop SS1P-neutralizing antibodies.

All treated patients were included in the PK analysis (Fig. 5). SS1P Cmax at dose levels 25 μg/kg (n=5), 35 μg/kg (n=3), and 45 μg/kg (n=15) are illustrated in Figure 5A. SS1P Cmax values at different dose levels were not statistically different, probably because of small patient numbers in individual groups, and were 1334 ng/ mL at the MTD. The SS1P area under the receiver operating characteristic curve was 480 μg/mL per minute, and the SS1P t1/2 at the MTD was 727 minutes (Fig. 5B,C). SS1P exposure and t1/2 did not differ statistically between different dose levels ($P > .05$).

DISCUSSION

On the basis of preclinical studies that demonstrated striking synergy when SS1P was combined with chemotherapy, we designed this phase 1 clinical trial of SS1P in combination with pemetrexed and cisplatin in chemotherapy-naïve patients with MPM. Our results indicate that combining SS1P with pemetrexed and cisplatin is well tolerated and produces no overlapping toxicities.

The results from this trial compare favorably with those from the phase 3 trial that led to the approval of pemetrexed and cisplatin for patients with MPM.[1] The pivotal trial reported an objective response rate of 41% in patients who received pemetrexed and cisplatin compared with 17% in patients who received cisplatin alone. The combination of SS1P with cisplatin and pemetrexed resulted in response rates of 60% in all evaluable patients and 77% in patients who received the MTD. Although promising, the response rates reported in this trial should be interpreted with caution, because this was a phase 1 study with a limited number of patients.

Common SS1P-related AEs included weight gain, edema, hypoalbuminemia, and pleuritic pain. Pleuritic chest pain is caused by an inflammatory response from SS1P binding to the mesothelin expressed on normal pleural mesothelial cells. Pleuritic pain was experienced either during or within a few hours of SS1P infusion, was transient, and responded well to narcotic analgesics. Other SS1P-related AEs were because of VLS, which resulted in leakage of fluid from the circulation into the tissues, edema, and a decrease in serum protein levels. However, VLS was mild and was not dose-limiting.

We determined the antibody response to SS1P using a neutralization assay that measured the ability of patient serum to neutralize the activity of SS1P against a mesothelin-expressing cell line in vitro. SS1P-neutralizing antibodies were present in 5% of patients before treatment and in 90% of patients after 1 cycle of treatment. During cycles 1 and 2, the neutralization of SS1P activity correlated with markedly lower serum SS1P levels, suggesting that antibodies neutralized SS1P activity. Development of neutralizing antibodies precluded administration of additional cycles of SS1P. The development of SS1P-neutralizing antibodies at a rate comparable to that achieved with single-agent SS1P administration indicates that concurrent chemotherapy does not affect antibody formation.[14] Strategies being explored to minimize immunogenicity and allow for repeated therapeutic

administration of SS1P include modification of the immunotoxin protein structure to decrease its immunogenicity and/or host immune depletion.[23,24] In a pilot study, we recently demonstrated that host immune depletion can effectively delay antibody formation.[25] Of 10 patients who received pentostatin and cyclophosphamide to deplete T cells and B cells before SS1P, only 2 patients (20%) developed anti-SS1P antibodies at the end of cycle 1. Three patients with extensive disease who had received multiple prior treatments had durable PRs and remained alive >14 months after starting treatment. Future studies of immunotoxins in combination with chemotherapy should incorporate strategies to minimize neutralizing antibody formation.

Objective radiologic assessment of MPM is notoriously difficult,[19] hence the need for biomarkers of response. Previous reports indicated that serum levels of mesothelin, MPF, and CA 125 are potential markers of response in mesothelioma.[26–28] We confirmed those findings and were able to demonstrate that changes in mesothelin and MPF levels are better than changes in CA 125 levels for reflecting tumor response. Validation studies in a larger cohort are warranted to assess the use of serum biomarkers as an adjuvant to radiologic evaluation of therapy response. It is noteworthy that baseline levels of mesothelin, MPF, and CA 125 did not predict response to SS1P and chemotherapy. The same appears to be true for mesothelin tumor staining, although available data were limited.

The results from this phase 1 trial indicate that SS1P can be safely combined with chemotherapy. Although the objective response rate appears to be higher than that reported in historical controls who received chemotherapy alone, the rapid development of antibodies likely hampered the efficacy of SS1P. Ongoing translational efforts are focused on minimizing the immunogenicity of SS1P and optimizing the efficacy of this combination.

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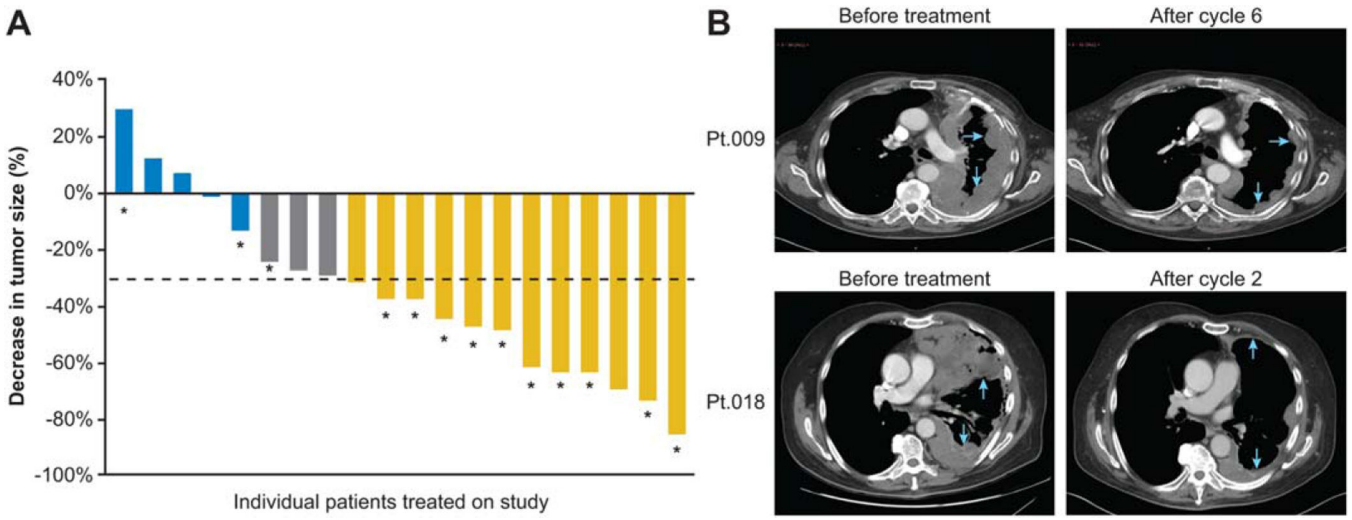

Figure 1.

(A) This waterfall plot illustrates representative radiologic responses and the greatest relative change in the sum of greatest dimensions of target lesions for all evaluable patients (n520). Yellow, gray, and blue bars indicate patients who attained a partial response, stable disease, and progressive disease, respectively. The dashed line at 230% indicates a partial response; asterisks indicate patients who received the maximum tolerated dose. (B) These are axial computed tomographic images of patient 009 before treatment and after 6 treatment cycles and of patient of 018 before treatment and after 2 treatment cycles. Both patients had partial responses to treatment. Arrow indicate areas of tumor involvement.

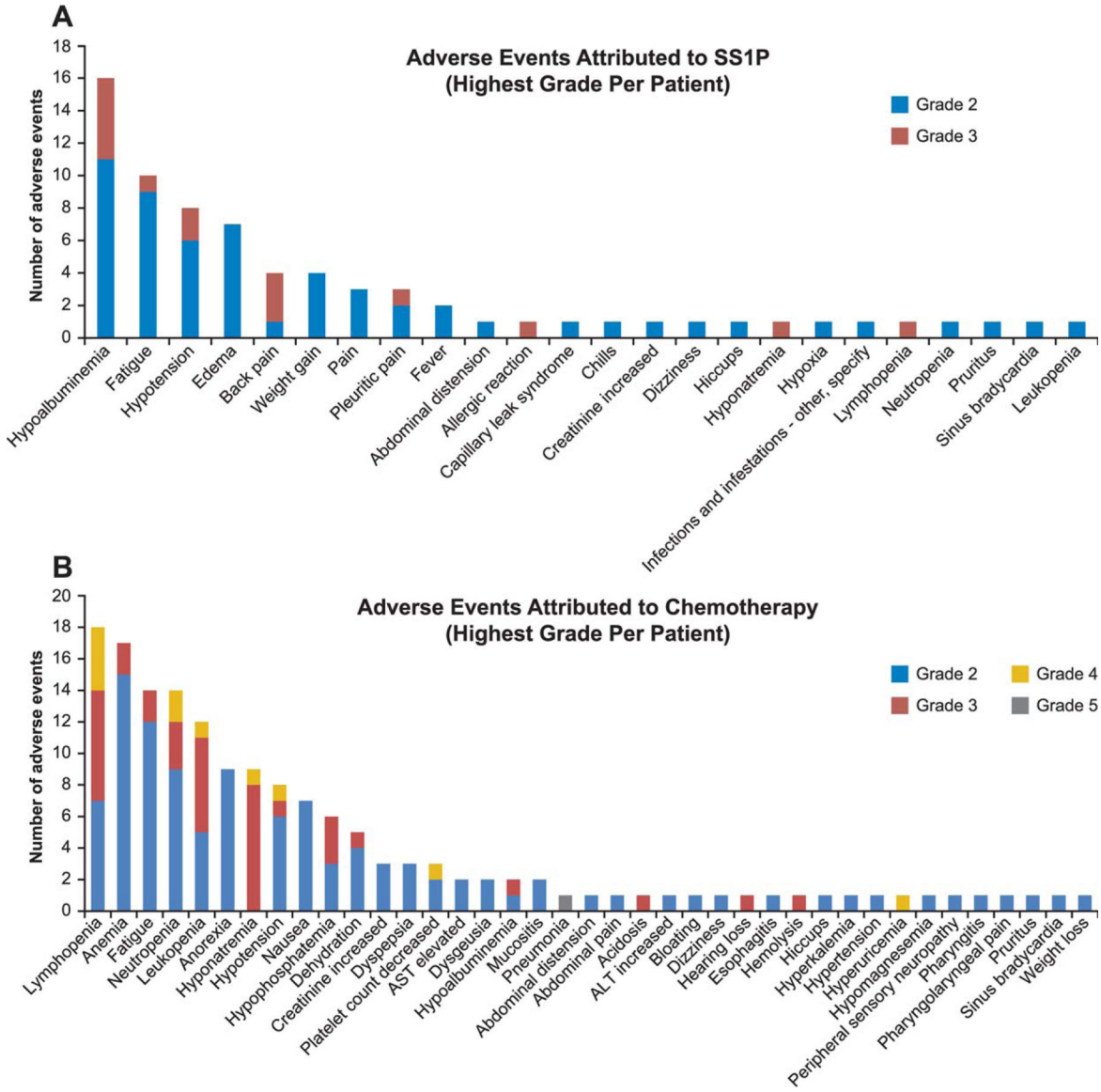

Figure 2.

Grade 2 or higher toxicities related to (A) SS1(dsFv)PE38 (SS1P) (a recombinant antimesothelin immunotoxin consisting of a murine antimesothelin variable antibody fragment [Fv] linked to PE38, a truncated portion of *Pseudomonas* exotoxin A) and (B) and chemotherapy are illustrated. The highest grade for each patient is depicted.

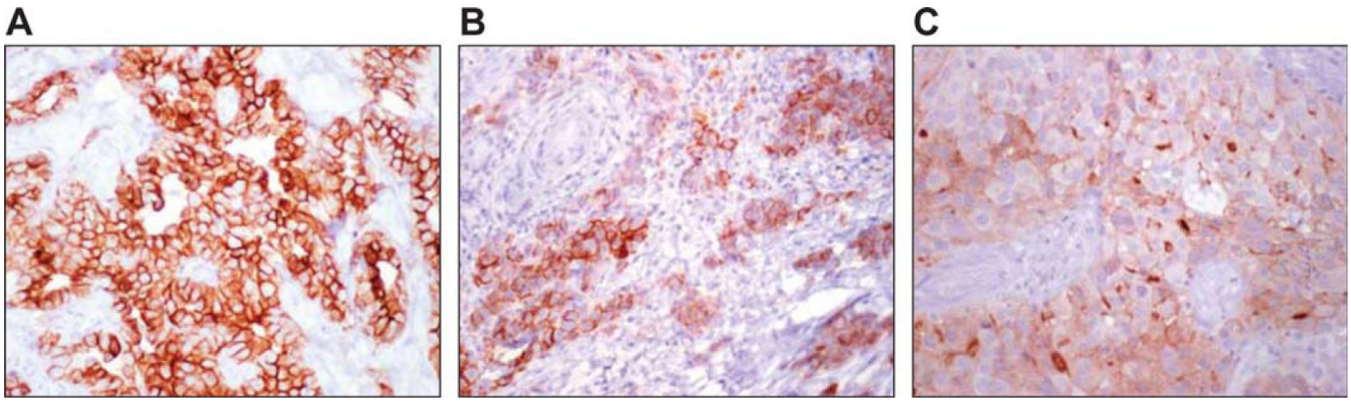

Figure 3.

Representative immunohistochemical staining of tumor samples reveals various intensities of mesothelin staining: (A) strong, (B) moderate, and (C) weak. Areas of brown indicate mesothelin staining of tumor cells.

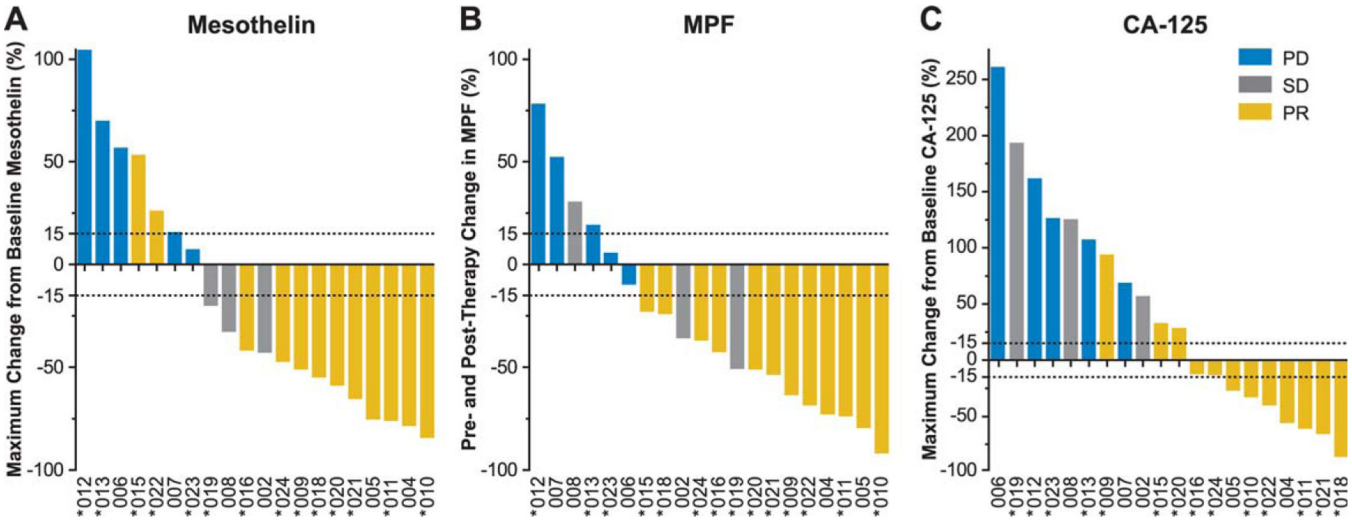

Figure 4.

Waterfall plots depict the relative biomarker changes and the best radiologic response in each of the 20 evaluable patients. For (A) mesothelin and (C) cancer antigen 125 (CA 125), the relative biomarker changes are based on the maximal change during therapy compared with baseline. (B) For megakaryocyte potentiating factor (MPF), the displayed biomarker changes indicate the relative difference between pretherapy and post-therapy samples. Dotted lines at –15% and 15% represent the thresholds for a significant change; asterisks indicate patients who received the maximum tolerated dose. PD indicates progressive disease; SD, stable disease; PR, partial response.

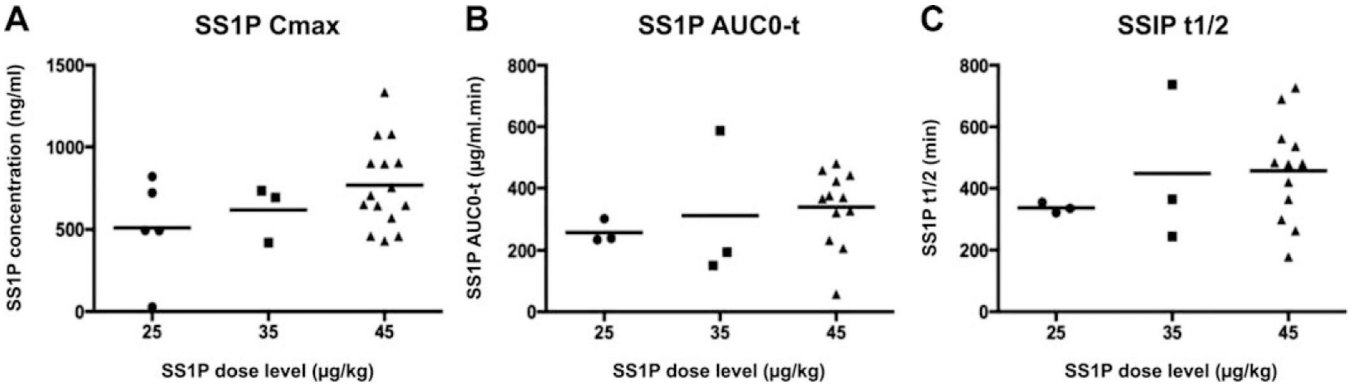

Figure 5.

The pharmacokinetics of SS1(dsFv)PE38 (SS1P) (a recombinant antimesothelin immunotoxin consisting of a murine antimesothelin variable antibody fragment [Fv] linked to PE38, a truncated portion of *Pseudomonas* exotoxin A) are illustrated. Scatter plots illustrate (A) individual patient values for the mean peak concentration (Cmax) (in ng/mL); (B) the area under the receiver operating characteristic curve (AUC) from zero to time *t* (AUC0-t) (in μg/mL per minute); and (C) the half-life (t1/2) (in minutes) of SS1P at the dose levels 25 μg/kg, 35 μg/kg, and 45 μg/kg. Serum SS1P, AUC0-t, and t1/2 values did not differ statistically between the different dose levels. Horizontal bars indicate mean SS1P Cmax, AUC0-t, and t1/2 values.

TABLE 1.

Patient Demographics and Clinical Characteristics

Variable	No. of Patients (%)
Age: Median [range], y	67 [51–78]
Sex	
Men	20 (83)
Women	4 (17)
Karnofsky performance status	
80	4 (17)
90	20 (83)
Baseline biomarker level: Median [range][a]	
Mesothelin, nmol/L	6.0 [0.6–57.6]
MPF, ng/mL	56.8 [10.4–530.0]
CA 125, U/mL	13.3 [4.7–2478.0]
SS1P dose level, mcg/kg/dose	
25	5
35	3
45	15
55	1
No. of chemotherapy cycles administered[b]	
1	3 (12.5)
2	2 (8.3)
4	4 (16.7)
6	14 (58.3)

Abbreviations: CA 125, cancer antigen 125; MPF, megakaryocyte potentiating factor; SS1P, SS1(dsFv)PE38 (a recombinant antimesothelin immunotoxin consisting of a murine antimesothelin variable antibody fragment (Fv) linked to PE38, a truncated portion of *Pseudomonas* exotoxin A).

[a]Serum biomarker levels were available in 20 of 24 patients.

[b]One patient in the 45-mcg/kg dose-expansion cohort failed to complete the first cycle of therapy and died from grade 5 pneumonia and neutropenic sepsis.