Phase I Study of SS1P, a Recombinant Anti-Mesothelin Immunotoxin Given as a Bolus I.V. Infusion to Patients with Mesothelin-Expressing Mesothelioma, Ovarian, and Pancreatic Cancers

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Abstract

Purpose: To determine the toxicities, maximum tolerated dose (MTD) and pharmacokinetics of the recombinant immunotoxin SS1P (anti-mesothelin dsFv-PE38) in patients with mesothelin-expressing cancers.

Experimental Design: SS1P given as a 30-min i.v. infusion every other day (QOD) for six or three doses was administered to 34 patients with advanced mesothelioma ($n = 20$), ovarian ($n = 12$), and pancreatic ($n = 2$) cancer.

Results: The initial cohort of 17 patients received SS1P QOD × 6 doses and the MTD was 18 μg/kg/dose. Dose-limiting toxicities (DLT) included grade 3 uticaria (one patient) and grade 3 vascular leak syndrome (two patients). To allow further SS1P dose escalation, 17 patients were treated on the QOD × 3 schedule and the MTD was 45 μg/kg/dose. The DLT was grade 3 pleuritis and was seen in two of two patients treated at a dose of 60 μg/kg and in one of nine patients treated at a dose of 45 μg/kg. At the MTD of 45 μg/kg, the mean C_{max} of SS1P was 483 ng/mL and half-life was 466 min. Of the 33 evaluable patients treated, 4 had minor responses, 19 had stable disease (including 2 with resolution of ascites), and 10 had progressive disease.

Conclusions: SS1P is well tolerated with pleuritis as the DLT at the highest dose level. Evidence of clinical activity was noted in a group of heavily pretreated patients. Phase II clinical trials of SS1P are being planned for malignant mesothelioma and other mesothelin-expressing malignancies.

Mesothelin is a 40-kDa cell surface protein that is present on normal mesothelial cells that line the pleura, pericardium, and peritoneum (1, 2). The mesothelin gene encodes a precursor protein of 69 kDa that is processed to a 30-kDa shed protein called megakaryocyte potentiating factor and a 40-kDa fragment, mesothelin, which is attached to the cell membrane by a glycosylphosphatidylinositol anchor (3). Megakaryocyte potentiating factor was isolated from the culture supernatant of a pancreatic cancer cell line, and in mouse, bone marrow cultures stimulated the megakaryocyte colony-forming activity of interleukin-3 (4). The normal biological function of mesothelin is not known. However, recent studies suggest that it may play a role in the i.p. spread of ovarian cancer by serving as the receptor for CA-125 (5, 6).

Mesothelin is an attractive candidate for targeted therapy given its limited expression on normal tissues and high cell surface expression in several tumors especially malignant mesothelioma, ovarian cancer, pancreatic cancers, as well as some squamous cell carcinomas (7–12). To target mesothelin, a recombinant immunotoxin, SS1P [SS1(dsFv)PE38], was developed consisting of an anti-mesothelin Fv (SS1) fused to PE38, a 38-kDa portion of *Pseudomonas* exotoxin A (13–15). After binding to mesothelin, SS1P is internalized by endocytosis and kills cells by arrest of protein synthesis and initiation of programmed cell death (16). SS1P causes complete regression of A431/K5 (human epidermoid carcinoma cell line expressing mesothelin by transfection) tumor xenografts in athymic nude mice (3). In addition, SS1P is cytotoxic to tumor cells obtained directly from patients with mesothelioma and ovarian cancer (17, 18).

Before the initiation of the clinical trial, toxicology studies of SS1P were conducted in cynomolgus monkeys, which have a similar tissue distribution of mesothelin as humans (2). These cynomolgus monkeys were treated with different doses and schedules of SS1P, including a 250 and 1,000 μg/kg every other day (QOD) × 3 schedule. Decreased appetite and physical activity was observed only in animals treated at the 1,000 μg/kg

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Table 1. Patient demographics and clinical characteristics

Characteristics	Patients (*N* = 34)
Sex	
Males	10
Females	24
Age (y)	
Median (range)	60 (40-78)
Diagnosis	
Pleural mesothelioma	7
Peritoneal mesothelioma	12
Inguinal mesothelioma	1
Ovarian cancer	12
Pancreatic cancer	2
Schedule of treatment	
SS1P QOD × 6 doses	17
SS1P QOD × 3 doses	17
No. treatment cycles	
1	31
2	3

dose level. Based on these studies, a starting SS1P dose of 8 μg/kg was felt to be safe for human clinical trials. Necropsy studies of SS1P-treated cynomolgus monkeys showed dose-related, microscopic inflammatory reactions of serosal membranes, suggesting that pleuritis and/or pericarditis could be dose limiting. As described below, only pleuritis was seen in patients treated at the higher dose levels and was the dose-limiting toxicity (DLT) of SS1P.

Given the high expression of mesothelin in many different cancers and limited expression in normal human tissues, a phase I study of SS1P, given as a bolus i.v. infusion, was done. This report summarizes the findings of this trial.

Patients and Methods

Patient eligibility. Patients with nonmucinous epithelial ovarian cancer, epithelial or biphasic malignant mesothelioma, pancreatic adenocarcinoma, and squamous cell cancer of lung, cervix, and head and neck were eligible for the study if they had failed therapy considered standard for their cancer. Patient's tumor tissue had to be positive for mesothelin expression by immunohistochemistry. Other eligibility criteria included age ≥18 years; an Eastern Cooperative Oncology Group performance status ≤2; estimated life expectancy ≥12 weeks; adequate hematopoietic function (absolute neutrophil count ≥1,500/mm^3 and platelet count ≥75,000/mm^3); serum creatinine, calcium, and total bilirubin ≤ upper limit of normal; aspartate aminotransferase and alanine aminotransferase <2.5 × upper limit of normal; serum albumin ≥3.0 gm/dL; baseline oxygen saturation >93% on room air; pulmonary function tests (for patients with pleural mesothelioma and as clinically indicated), including FEV_1, TLC, DL_{CO}, and VC ≥50% of predicted value; and measurable or assessable disease. Exclusion criteria included any of the following: any known central nervous system or spinal cord involvement by tumor; antibodies to SS1P as measured by serum neutralizing activity to SS1P at 200 ng/mL of >75%; New York Heart Association grade II to IV cardiovascular condition or clinically significant pericardial effusion; any infection requiring parental antibiotics, history of human immunodeficiency virus infection, or seropositivity for hepatitis B and hepatitis C; and pregnant or nursing females. Written informed consent was obtained from patients before treatment according to federal and local institutional guidelines.

Study design. This study was done at the University of Oklahoma Health Sciences Center (Oklahoma City, OK), University of Chicago (Chicago, IL), and at the National Cancer Institute (Bethesda, MD). The clinical protocol was approved by the Institutional Review Board at all three institutions. Patients were enrolled in the study from November 2000 to January 2006.

This was a phase I, dose escalation study. Three patients were enrolled at each dose level, and if no patient developed a DLT, subsequent patients were enrolled at the next dose level. However, if one patient developed DLT, dose escalation did not proceed until at least three additional patients without DLT were enrolled at the current dose level. The maximum tolerated dose (MTD) was defined as the highest dose level where zero to one patient of six had DLT. SS1P treatment could be repeated after 4 weeks for a maximum of two additional courses if there was no evidence of progressive disease; no DLT; no allergic hypersensitivity grade ≥3 or grade 2 with respiratory component (i.e., asymptomatic bronchospasm) related to prior treatment; recovery from any drug-related toxicity to grade <1; adequate hematologic and organ function documented <1 week before retreatment; and patients whose serum after first or second cycle caused <75% neutralization of SS1P activity *in vitro* at a SS1P concentration of 200 ng/mL. Initially, patients were treated with SS1P QOD × 6 doses. After the MTD of this schedule was established, patients were treated with SS1P QOD × 3 doses to allow further dose escalation.

Drug formulation and administration. SS1P was produced by ABL and supplied in glass vials in PBS and stored at -70°C. Patients received SS1P diluted in 100 mL of saline containing 0.2% human serum albumin over a period of 30 min. At least 30 min before the first infusion, every patient received a test dose of 10 μg SS1P. If no hypersensitivity reaction was observed, the patients received the SS1P infusion. Patients also received 500 mL D5/half-normal saline before and after SS1P infusion. Premedications to decrease the risk of allergic reactions were not routinely administered when the clinical trial started, but patients treated on the QOD × 3 schedule received antiallergic prophylaxis consisting of 25 mg hydroxyzine, 150 mg ranitidine, and 650 mg acetaminophen administered orally 1 h before and 8 h after each dose.

To determine whether steroids could decrease the pleuritis associated with SS1P administration, three patients were treated at the MTD of SS1P (45 μg/kg/dose QOD × 3 doses) with prednisone. Prednisone (0.5 mg/kg) was administered orally every day × 7 days starting the day of SS1P treatment and then tapered to half the original dose × 2 days and then to one fourth of the original dose × 2 days before being stopped.

Table 2. Dose escalation scheme of SS1P and DLTs

Dose level	Dose (μg/kg/dose)	No. treated patients	Patients with DLT
SS1P QOD × 6 schedule			
1	8	3	—
2	12	3	—
3	18	6	1/6
4	25	5	2/5
SS1P QOD × 3 schedule			
1	25	3	—
2	35	3	—
3	45	9*	1/9
4	60	2	2/2

* Includes three patients treated with SS1P at 45 μg/kg/dose plus prednisone.

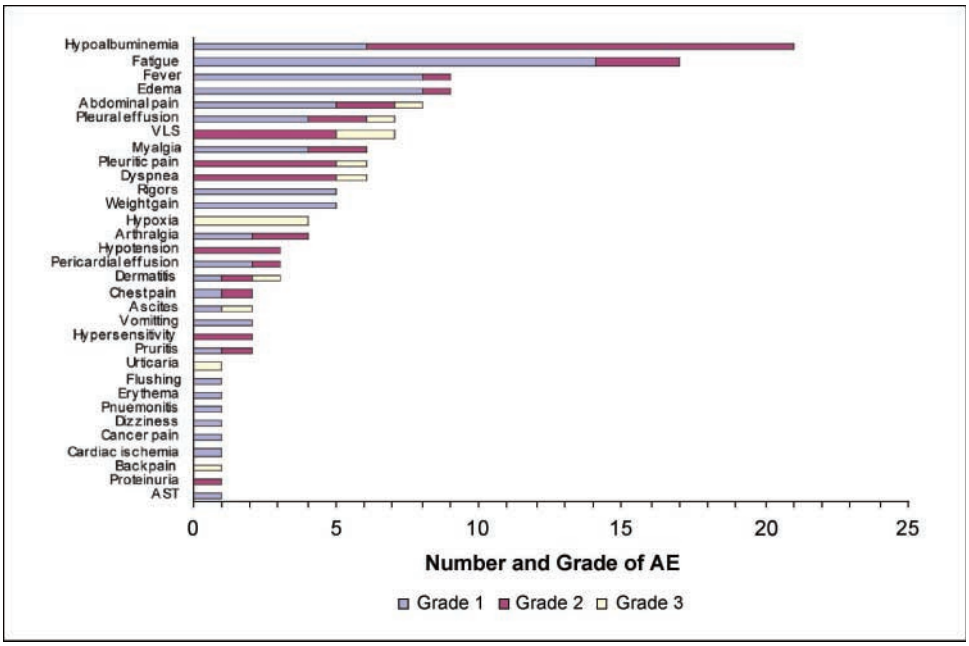

Fig. 1. Toxicity of SS1P. SS1P-related adverse events (*AE*) that were possibly, probably, or definitively related to SS1P. VLS, vascular leak syndrome.

Baseline and treatment assessments. Toxicity was graded using the CTC version 2.0 criteria. For vascular leak syndrome, we used a grading system that was prospectively included in the protocol. Vascular leak syndrome was graded as grade 2 (grade ≥1 hypoalbuminemia with either grade ≥1 weight gain or asymptomatic pulmonary edema), grade 3 (grade ≥1 hypoalbuminemia with either hypotension requiring fluid support of ≥20 cc/kg/h for at least 30 min or symptomatic pulmonary edema), or grade 4 (grade ≥1 hypoalbuminemia with either hypotension requiring pressor support or pulmonary edema requiring ventilatory support).

Tumor evaluation was done at baseline and following treatment. Baseline images were obtained ≤4 weeks before the start of treatment. Tumor measurements were done at day 29 of each course (every 4 weeks). A complete response was defined as complete regression of all radiographically detectable malignant tumor. A partial response was defined as ≥50% and <100% regression of all tumor masses (measured when possible in two diameters) in the absence of any new lesions. A minor response was defined as decreased tumor area ≥20% but <50% from baseline examination with no new tumor. Stable disease was defined as an increase or decrease of tumor of <20% from baseline with no new tumor. Progressive disease was defined as an increase in the tumor area of ≥20% from baseline measurement.

Evaluation of tumor mesothelin expression. All patients had their tumor samples examined for mesothelin expression by immunohistochemistry, initially with anti-mesothelin monoclonal antibody K1 and later using the commercial anti-mesothelin monoclonal antibody 5B2 (Novocastra). This immunohistochemical analysis for mesothelin expression has been described previously (9). Specimens in which ≥30% of tumor cells had cell surface expression of mesothelin were considered positive and eligible for the study. Given the limitations of immunohistochemistry especially in paraffin-fixed tissues, this cutoff was used to make sure that patients to be treated had mesothelin expression in majority of tumor cells. Expression of tumor antigen by ≥30% of tumor cells as a criteria for study entry has also been used in studies of other immunotoxins (19).

Serum antibody and pharmacokinetic assays. Serum neutralizing antibody response to SS1P and SS1P blood levels were measured using bioassays as described previously, except that the immunotoxin used was SS1P and the cell line tested was A431/K5 that expresses mesothelin (20). Patients whose serum caused ≥75% neutralization of SS1P activity *in vitro* at a SS1P concentration of 200 ng/mL were ineligible for participation in the protocol or for retreatment. This cutoff was established because the presence of neutralizing antibody response could result in low SS1P blood levels. Pharmacokinetic variables were calculated from the serum concentration data using a one compartment pharmacokinetic model. For each patient, regression techniques were used to fit an exponential distribution to the serum concentration data using the exact sampling times to calculate the time postinfusion for each sample.

Table 3. SS1P pharmacokinetics

Dose (μg/kg/dose)	No. patients	C_{max} (ng/mL)	AUC_{0-t} (μg/mL min)	*t* 1/2 (min)
8	3	191	124	447
12	3	317	173	377
18	5	338	331	709
25	8	442	343	827
35	3	330	372	773
45	9	483	292	466
60	2	602	279	309

NOTE: Mean SS1P C_{max}, AUC_{0-t}, and *t* 1/2 after C1D1 infusion. Pharmacokinetic data of patients treated with SS1P at 25 μg/kg/dose on the QOD × 6 schedule or QOD × 3 schedule have been combined for analysis. Similarly, pharmacokinetic data from patients treated with SS1P at a dose level of 45 μg/kg/dose with or without prednisone have been combined.

Results

Patients. Thirty-four patients, 24 females and 10 males, were enrolled (Table 1). The median age was 60 (range, 40-78 years). Twenty patients had mesothelioma, 12 had epithelial ovarian cancer, and 2 had pancreatic adenocarcinoma. The first 17 patients received SS1P QOD × 6 doses and the other 17 were treated with SS1P QOD × 3 doses.

Dose levels and DLT. Table 2 summarizes the various dose levels evaluated and number of DLTs at each dose level. Because two of five patients treated with 25 μg/kg/dose SS1P on the QOD × 6 schedule had DLT, the 18 μg/kg/dose level was expanded to treat three more patients, and one of six patients had DLT. Therefore, the MTD of SS1P given QOD × 6 is 18 μg/kg. The DLT was a grade 3 allergic reaction (in one patient treated at 25 μg/kg/dose) and grade 3 vascular leak syndrome (in one patient each treated at the 18 and 25 μg/kg/dose). All patients recovered from their DLT. Because all the DLTs were observed after the fourth dose of SS1P, the protocol was amended to allow treatment of patients with three doses given QOD, which allowed further dose escalation. Using this schedule, both patients treated at 60 μg/kg/dose had DLT and the 45 μg/kg/dose cohort was expanded to treat three more patients and one had DLT. Therefore, the MTD of SS1P given QOD × 3 is 45 μg/kg/dose. Pleuritis was the DLT observed in two of two patients at the 60 μg/kg/dose level and one of six patients at the 45 μg/kg/dose level.

After determining the MTD of SS1P given QOD × 3, three additional patients were treated at the 45 μg/kg/dose QOD × 3 dose schedule with prednisone to determine if prednisone could reduce the SS1P-induced pleuritis and inflammation by measuring serum C-reactive protein levels. No DLTs were observed in the three patients treated with the combination of SS1P plus prednisone. Slight elevation in C-reactive protein level was noted in one of the three patients and none of the patients developed pleuritis.

Toxicity. The toxicities related to SS1P are summarized in Fig. 1. The majority of toxicities were grades 1 and 2. No grade 4 toxicity was observed. The most commonly reported adverse events were hypoalbuminemia in 21 (61.8%) patients and fatigue in 19 (55.9%) patients. The most common grade 3 adverse event was severe hypoxia, reported by four (11.8%) patients. The DLT of pleuritis was characterized by fever, hypoxia, pleural effusion, and pain. Pleuritis developed after the second dose of SS1P in one patient and after the third dose in two patients. The first presentation was pleuritic pain leading to hypoxia due to hypoventilation. Chest X-ray showed unilateral or bilateral pleural effusion. Patients were treated with supplemental oxygen and narcotics for pain relief. Pain and hypoxia resolved within 3 to 9 days. Pleural effusion improved over time and did not require drainage.

Pharmacokinetics of SS1P. The SS1P pharmacokinetics are summarized in Table 3. Peak SS1P levels were dose related with a mean C_{max} of 483 ng/mL at the MTD of 45 μg/kg/dose. SS1P also exhibited prolonged half-life with a mean half-life of 466 min at the MTD. It is known that a small amount of mesothelin is shed into the blood of some patients with mesothelioma and ovarian cancer (21, 22). Our results suggest that this shed mesothelin has very little effect on SS1P blood levels. The SS1P pharmacokinetics on C1D10 (after the fifth dose on the QOD × 6 schedule) and on C1D5 (after the third dose on the QOD × 3 schedule) were similar to C1D1 (data not shown).

Immunogenicity. Thirty of the 34 (88%) patients developed neutralizing antibodies by day 29 of cycle 1 and were not eligible for retreatment. Three patients received a second cycle of SS1P. The majority of patients (62%) developed neutralizing antibodies to SS1P by day 15 and another 18% developed antibodies by day 22. Only two (6%) patients developed antibodies by day 8 to day 11, and one (3%) patient developed neutralizing antibodies by day 29. These results suggest that development of neutralizing antibodies to SS1P is infrequent before day 15. There was no correlation between the development of antibodies to SS1P with dose level.

Antitumor activity of SS1P. Of the 34 patients, 33 had evaluable disease. Of these 33 patients, 4 (12%) patients, 2 with ovarian cancer and 2 with mesothelioma, had minor responses as defined by decreased tumor area ≥20% but <50% from baseline, and lasting ≥4 weeks. Ten (29%) patients had

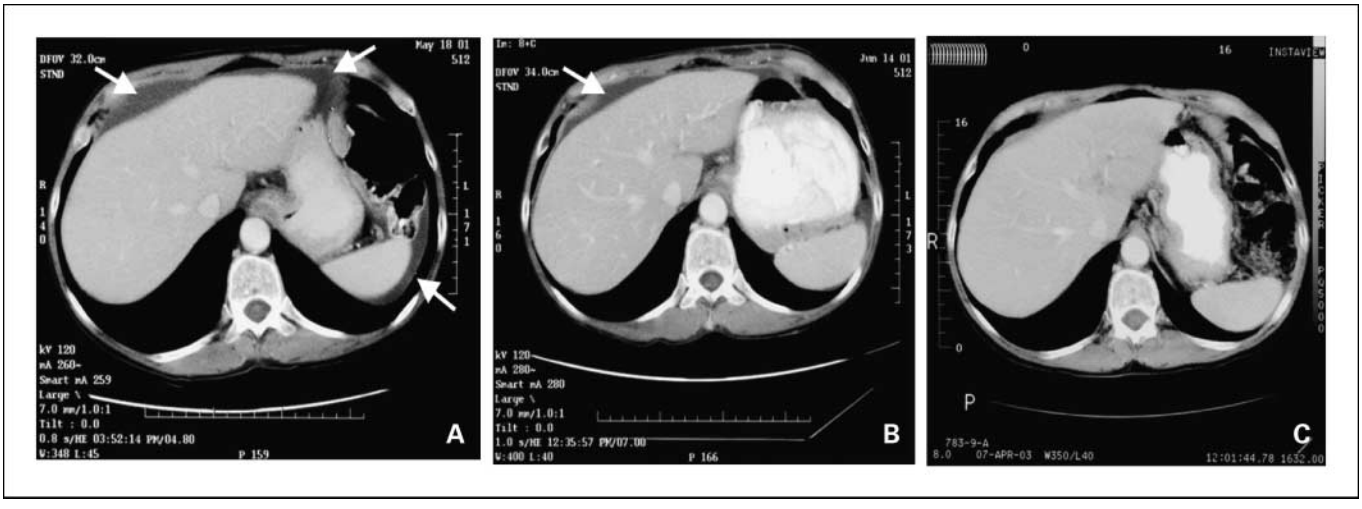

Fig. 2. Abdominal computed tomography scan of a 53-year-old female with peritoneal mesothelioma before treatment (*A*), 4 wks after initiation of SS1P therapy (*B*), and 23 mo after initiation of SS1P therapy (*C*). Patient had complete resolution of ascites and died >5 y after SS1P treatment with no intervening therapy for her mesothelioma.

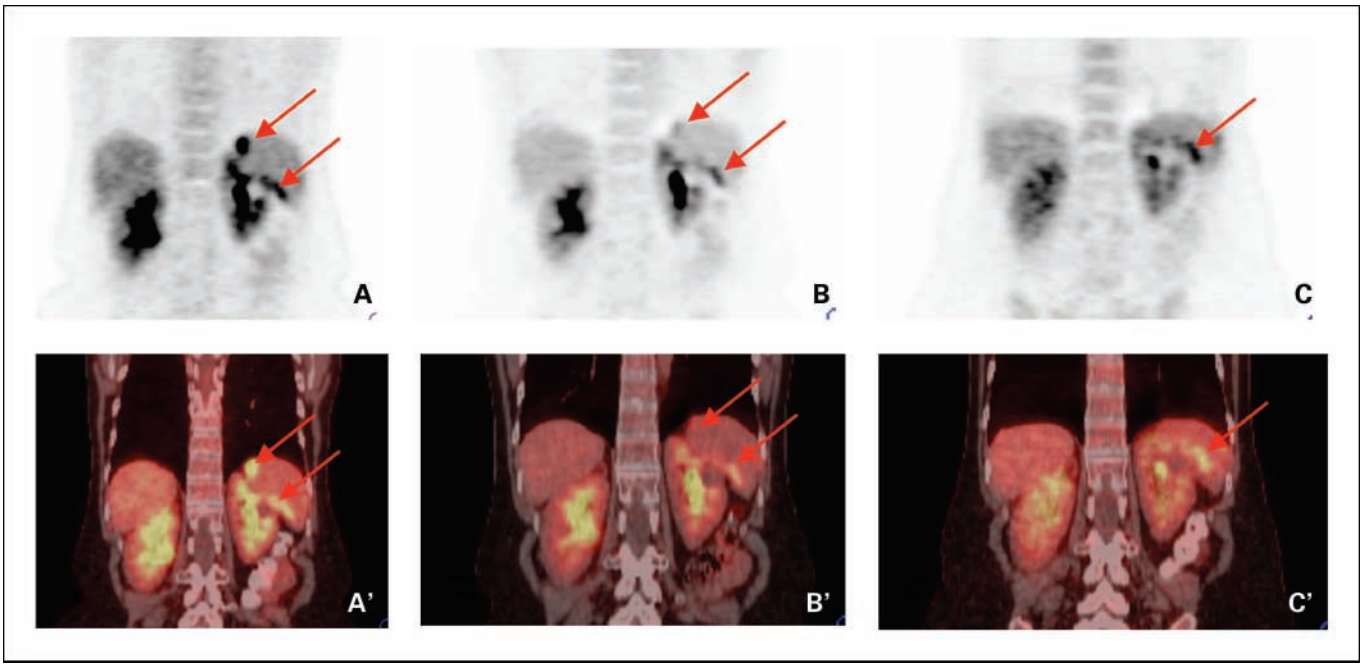

Fig. 3. Positron emission tomography (*top*) and positron emission tomography-computed tomography (*bottom*) in a 53-year-old female with peritoneal mesothelioma before (*A* and *A′*), after first cycle (*B* and *B′*), and after second cycle (*C* and *C′*) of SS1P therapy. After first cycle, there was a decrease in the standard uptake values in the tumors on left anterior surface of liver and around the spleen. After the second cycle, there was no fluorodeoxyglucose uptake in the tumor on the left anterior surface of the liver.

progressive disease, and 19 (56%) patients had stable disease through their first course. Two patients (one ovarian cancer and one peritoneal mesothelioma) with stable disease had complete resolution of their ascites. Stable disease or minor response was seen in 14 of 19 mesothelioma patients, 8 of 12 ovarian cancer patients, and 0 of 2 pancreatic cancer patients with evaluable disease. In the patients treated on the QOD × 6 schedule, minor response was seen in one patient each at the 8 and 12 μg/kg dose level. In the patients treated on the QOD × 3 schedule, there was a suggestion of a possible dose response effect because both the minor responses occurred in patients treated at the 45 μg/kg dose level.

Patient 107 was a 53-year-old female who after her diagnosis of peritoneal mesothelioma was treated with cisplatin and gemcitabine. After six cycles of chemotherapy, laparoscopy showed diffuse studding of the peritoneum with mesothelioma with positive cytology. Computed tomography scan before initiation of SS1P therapy showed abdominal ascites and a pelvic mass. A computed tomography scan done 4 weeks after initiation of SS1P therapy showed resolution of ascites around the spleen and some decrease in ascites around the liver. Computed tomography scan at 23 and 36 months showed complete resolution of ascites with a stable pelvic mass (Fig. 1). The patient died of unrelated causes 5 years after SS1P therapy with no intervening treatment for her mesothelioma. Figure 2 shows tumor response in patient 432 with peritoneal mesothelioma who had undergone cytoreductive surgery and intraoperative hyperthermic chemotherapy at the time of diagnosis and also at the time of tumor recurrence 19 months later. She had disease recurrence after the second surgery and was enrolled in the SS1P study. Her main site of disease was in the left anterior surface of the liver and around the spleen. As shown in Fig. 2, there was a marked decrease in fluorodeoxyglucose uptake on positron emission tomography scan in both the sites of disease after the first cycle. After the second cycle of SS1P, there was no uptake in the metastatic lesion in the left anterior surface of the liver and decreased uptake in the lesions around the spleen.

Discussion

We report here the results of a phase I dose escalation study with a recombinant immunotoxin, SS1P, which targets mesothelin-expressing cancers. In this study, we have established the MTD of SS1P to be 45 μg/kg given i.v. QOD × 3 and the dose-limiting side effect to be pleuritis. Minor tumor responses were noted in 4 patients and stable disease in 19 patients that included 2 patients with resolution of ascites.

Several previous studies with immunotoxins targeting solid tumors have been reported using both chemical conjugates and recombinant immunotoxins. Pai et al. (19) reported disappearance of a single lymph node in a patient with breast cancer and >50% shrinkage of retroperitoneal nodes in a patient with colon cancer treated with LMB-1, a chemical conjugate immunotoxin targeting the Lewis Y tumor antigen. In another study of patients with solid tumors using BR96(sFv)-PE40, a recombinant anti–Lewis Y immunotoxin, very little antitumor activity was seen with only one minor response among the 42 patients treated (23). Similarly, IL4(38-37)-PE38KDEL, an immunotoxin that targets the interleukin-4 receptor showed minimal activity in solid tumors that expressed the interleukin-4 receptor (24). Compared with these previous studies of immunotoxins for the treatment of solid tumors that showed limited activity, our results with SS1P in treating patients with advanced cancer who have failed standard therapies are encouraging.

Self-limited pleuritis was the DLT and is due to SS1P binding to mesothelin expressed on normal pleural mesothelial cells

leading to an inflammatory response as evidenced by elevated C-reactive protein levels. To allow further dose escalation, we treated three patients at the MTD of 45 μg/kg SS1P with prednisone. None of these patients developed pleuritis and slight elevation of C-reactive protein levels was seen in one patient, suggesting that further dose escalation may be possible with concurrent steroid use. We did not observe any significant pericardial toxicity despite the fact that mesothelin is expressed on normal pericardial cells. This suggests that either the pericardial mesothelial cells are less sensitive to SS1P or other factors prevent their damage.

At the 45 μg/kg dose level, the mean C_{max} of SS1P was 483 ng/mL with a half-life of 466 min. In previous studies, the half-life of immunotoxins in humans ranged from 120 to 240 min (20, 25). We were surprised to observe such prolonged half-life of SS1P, ranging from 309 to 827 min, as well as large area under the concentration curves in the current study especially because SS1P is the same size (molecular weight, 63 kDa) as other recombinant immunotoxins. Small amounts of mesothelin are shed into the serum but the levels are rarely over 25 ng/mL and cannot account for the long half-life (22). We have searched for high molecular weight complexes of SS1P bound to some serum protein but were not able to detect such complexes.[6] It has been shown previously that tumor cells obtained from patients with ovarian cancer and mesothelioma are very sensitive to SS1P with IC_{50}s of 1 to 5 ng/mL (17, 18). Therefore, these high and prolonged blood levels of SS1P should have been sufficient to kill a large fraction of the tumor cells if they were readily accessible. It is well documented that antibodies and other protein molecules penetrate tumors slowly and this is probably one factor contributing to the small number of responses (26). In the one patient with peritoneal mesothelioma who had small volume disease with peritoneal studding, a prolonged clinical response was seen, and in other patients, a decrease of ascites was also documented.

The majority of patients treated on this study received one cycle of therapy because patients whose posttreatment serum caused ≥75% neutralization of SS1P activity *in vitro*, at SS1P concentration of 200 ng/mL, were excluded from retreatment. However, our data suggest that this exclusion criteria might have been more stringent for patients treated at the higher dose levels because their peak SS1P blood levels were ~500 ng/mL. Patient 432 treated at a dose of 45 μg/kg had good blood levels of SS1P during the second cycle despite the presence of >75% neutralization of SS1P activity *in vitro* at the end of the first cycle. In addition, this elevated SS1P level was associated with antitumor activity as shown in Fig. 3. These results suggest that at the higher dose levels of SS1P, the presence of low levels of neutralizing antibodies may not interfere with SS1P pharmacokinetics. Similar results have been noted in case of another immunotoxin (denileukin diftitox) that is Food and Drug Administration approved for treatment of CTCL (27).

We have reported recently that synergistic antitumor activity was noted when tumor-bearing mice were treated with SS1P in combination with several different chemotherapies (28). We now plan to carry out phase II studies in mesothelioma and ovarian cancer combining SS1P with chemotherapy. In addition, SS1P administered i.p. could be potentially useful for treatment of ovarian cancer or peritoneal mesothelioma.

[6] Unpublished data.

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