

## Phase I Trial of Continuous Infusion Anti-Mesothelin Recombinant Immunotoxin SS1P

Robert J. Kreitman, Raffit Hassan, David J. FitzGerald, and Ira Pastan

**Abstract Purpose:** To conduct a phase I trial of recombinant immunotoxin SS1P given by continuous infusion in chemoresistant solid tumors expressing mesothelin.

**Experimental Design:** Eligible patients had mesothelioma, ovarian, or pancreatic cancer, which was recurrent or unresectable despite standard therapy, and were mesothelin positive by immunohistochemistry. SS1P was given by continuous infusion for 10 days, and cycles could be repeated at 4-week intervals in the absence of neutralizing antibodies or progressive disease.

**Results:** Twenty-four patients, five with peritoneal mesothelioma, nine with pleural mesothelioma, two with pleural-peritoneal mesothelioma, seven with ovarian carcinoma, and one with pancreatic carcinoma, received 4, 8, 12, 18, and 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$ . The maximum tolerated dose was 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$ , where one of six patients had dose-limiting toxicity due to reversible vascular leak syndrome. Immunogenicity was observed in 18 (75%) of 24 patients, and five (21%) received a second cycle. Constant plasma levels of SS1P were maintained for most of the 10-day infusion time, with median peak levels of up to 153 ng/mL. One patient had a partial response. Nonmajor responses included cessation of ascites and independence from paracentesis, resolution of masses by positron emission tomography, and improved pain and range of motion.

**Conclusions:** As a single agent by continuous infusion, recombinant immunotoxin SS1P was well tolerated up to 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$  and showed evidence of modest clinical activity. Continuous infusion showed no significant advantage over bolus dosing, and further clinical development of SS1P is proceeding by bolus dosing in combination with chemotherapy. (Clin Cancer Res 2009;15(16):5274–9)

Mesothelin is a 40-kDa glycosylphosphatidylinositol-anchored membrane glycoprotein (1, 2). Mesothelin was originally identified by the monoclonal antibody K1 produced by immunization of mice with the OVCAR3 cell line. Mesothelin is made as a 69-kDa precursor protein and then processed into the 30-kDa megakaryocyte potentiating factor and the 40-kDa mesothelin (3). Mesothelin has also been shown to be important for binding CA-125 and probably has a role in malignant invasion (4). Mesothelin is expressed by a variety of solid tumors including nonmucinous ovarian cancer (5), epithelial and mixed but not sarcomatous mesothelioma

(5), squamous cell cancers arising in lung, head and neck, cervix, or esophagus (6), adenocarcinoma of the lung (7), and pancreatic adenocarcinoma (8, 9). Although normal mesothelial tissues express mesothelin, no reactivity is detectable on liver, heart, brain, kidney, bone marrow, cervix, prostate, stomach, esophagus, or skin (10).

Monoclonal antibody K1 was shown to target human mesothelin-positive tumors in mice (11) and had antitumor activity when chemically conjugated to truncated *Pseudomonas* exotoxin (PE; ref. 12). To target mesothelin with a recombinant immunotoxin, mice were DNA-immunized with mesothelin and an Fv expression library screened by phage display to yield SS(Fv)-PE38 (13). PE38 is a truncated form of PE that is missing its binding domain and can be directed by a ligand to bind, internalize into, and kill target cells by ADP-ribosylation and inactivation of elongation factor 2 and apoptosis (14). To improve its affinity for mesothelin, somatic mutational "hotspots" in the hypervariable regions were randomized and selected by phage display to result in the high-affinity recombinant immunotoxin SS1(Fv)-PE38 (15–17). This immunotoxin was stabilized by conversion of the Fv to a disulfide-stabilized form called SS1(dsFv)-PE38 or SS1P (18, 19). SS1P is cytotoxic toward primary cultures of human ovarian and cervical cancer cells, mesothelin-expressing cell lines (20–22), and toward human mesothelin-expressing tumors grown as xenografts in mice (3).

**Authors' Affiliation:** Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland  
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**Requests for reprints:** Robert J. Kreitman, Laboratory of Molecular Biology, National Cancer Institute, 9000 Rockville Pike, Building 37, Room 5124b, Bethesda, MD 20892-4264. Phone: 301-496-6947; Fax: 301-576-3920; E-mail: kreitmar@mail.nih.gov.

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## Translational Relevance

This manuscript describes clinical results of a phase I trial in which the recombinant anti-mesothelin immunotoxin SS1P was given by continuous infusion to patients with mesothelin-positive solid tumors, most commonly mesothelioma. A phase I trial of this agent given by bolus infusion has recently been reported. Recombinant immunotoxins contain an Fv fragment of a monoclonal antibody genetically fused to a truncated bacterial toxin. These agents (~63 kDa) are much smaller than chemical conjugates of whole antibody and toxin, and their plasma lifetimes are much shorter. This manuscript is the first to document that significant plasma levels of recombinant immunotoxin can be maintained in patients by continuous infusion. The results will be useful for understanding and predicting the pharmacokinetics of other proteins of similar type or size. Moreover, this manuscript is an important part of the clinical development of SS1P, which is now undergoing phase II testing.

To determine its clinical activity, SS1P was given in a phase I trial to 34 patients with mesothelin-expressing solid tumors as a 30-minute infusion every other day (QOD; ref. 23). The maximum tolerated dose (MTD) in 17 patients treated QOD  $\times 6$  was 18  $\mu\text{g}/\text{kg}$   $\times 6$  and in 17 patients treated QOD  $\times 3$  was 45  $\mu\text{g}/\text{kg}$  QOD  $\times 3$ . There were four minor responses in 33 evaluable patients, and in addition, resolution of malignant ascites was documented. Immunogenicity by day 29 of cycle 1 was observed in 88% of patients, and plasma levels showed a mean half-life of 466 minutes at the MTD.

Despite excellent antitumor activity achievable in mice with recombinant immunotoxins delivered by continuous infusion (24, 25), this method of administration of recombinant immunotoxins has not been reported in patients. Several clinical trials of larger (~200 kDa) immunotoxin chemical conjugates have been reported (26–28). Although obvious benefit was not observed relative to bolus dosing, these large monoclonal antibody-containing chemical conjugates already had prolonged half-life in the plasma. Because solid tumors are closely packed together making tumor penetration a limiting factor for efficacy (29–31) and because the smaller immunotoxins may lack sufficient time in the plasma to achieve significant penetration, we reasoned that maintenance of constant drug levels in the plasma might improve therapeutic efficacy. We therefore assessed the safety and clinical activity of SS1P given by continuous infusion over a 10-day period. A wide variety of tumor types were included to optimally explore its biological activity in mesothelin-expressing malignancies (5–9).

## Patients and Methods

**Eligibility.** Diagnoses included mesothelioma, ovarian cancer, squamous cell cancer of the head and neck, lung or cervix, or pancreatic cancer. Disease had to be unresectable after standard therapy and mesothelin positive by immunohistochemistry. Patients could not have had treatment for  $\geq 4$  wk before SS1P. Age of  $\geq 18$ , life expectancy of

$\geq 12$  wk, and performance status of 0 to 2 on Eastern Cooperative Oncology Group were required. Laboratory results required included ANC of  $\geq 1,000$ , platelets of  $\geq 75,000$ , creatinine of  $< 2$ , normal bilirubin, and aspartate aminotransferase and alanine aminotransferase of  $< \text{grade } 2$ . Albumin needed to be at least 3, and oxygen saturation  $\geq 92\%$ . Central nervous system tumor was disqualifying. Eligibility for retreatment required absence of high levels of neutralizing antibodies, defined as  $> 75\%$  neutralization by patient serum of 200 ng/mL of the cytotoxic activity of SS1P toward target A431-K5 cells, and also absence of progressive disease.

**Study design.** SS1P was given by continuous infusion for 10 d. Doses of 4 to 25  $\mu\text{g}/\text{kg}/\text{d}$  were diluted to 250 mL using 0.9% NaCl containing 0.2% albumin and infused by a portable pump at a rate of 10 mL/h. The beginning dose of 4  $\mu\text{g}/\text{kg}/\text{d} \times 10$  was chosen because the total dose of 40  $\mu\text{g}/\text{kg}/\text{cycle}$  was similar to the total of the dose per cycle of other recombinant immunotoxins which were associated with some efficacy and without significant toxicity (32–34). Also, continuous infusion doses up to 400  $\mu\text{g}/\text{kg}/\text{d} \times 7$  d were nonlethal in BALB/c mice. Retreatments of patients was allowed in the absence of progressive disease or high levels of neutralizing antibodies. Retreatments cycles were 4 to 6 wk apart. To prevent allergic reactions and fever, patients received oral hydroxyzine (25 mg) and ranitidine (150 mg) 1 h before and 8 h after each dose, and acetaminophen (650 mg) every 6 h  $\times 4$  beginning 1 h before each dose. The dose of treatment in new patients was escalated if zero of three or one of six patients at the previous dose level had dose-limiting toxicity (DLT). The MTD was defined as the highest dose level that caused DLT in zero to one of six patients. No inpatient dose escalation was allowed. DLT was defined as grade of  $\geq 3$  toxicity, and exceptions were made for grade 3 fever, nausea, vomiting, transaminase elevations, grade 4 hematologic toxicity lasting for  $< 5$  d, and grade 3 proteinuria of 3.5 to 10 g/d without creatinine elevation or lasting for  $< 2$  wk. Excessive interruption or failure to complete a cycle of treatment was also considered DLT. Standard response criteria were used as defined previously (23). Neutralizing antibodies were measured by incubating serum with purified SS1P in a 90:10 mixture with a final concentration of 200 ng/mL of SS1P and determining the percentage neutralization of cytotoxicity on A431-K5 cells as described (23). Plasma levels were determined before (day 1) and after beginning the infusion on days 3, 5, and 8 and then before and after stopping the infusion on day 11. Levels were quantified by measuring cytotoxic activity of

**Table 1.** Patient characteristics

Diagnosis	No. cases	Prior Tx, range (median)*
Peritoneal mesothelioma	5	1-3 (2)
Pleural mesothelioma	9	0-4 (2)
Pleural-peritoneal mesothelioma	2	1-3 (2)
Ovarian cancer	7	2-9 (6)
Pancreatic carcinoma	1	1
Total	24	0-9 (2)
Dose levels of SS1P	No. enrolled	No. patients with DLT
4 $\mu\text{g}/\text{kg}/\text{d} \times 10$	3	0
8 $\mu\text{g}/\text{kg}/\text{d} \times 10$ (3)	3	0
12 $\mu\text{g}/\text{kg}/\text{d} \times 10$ (3)	6	1
18 $\mu\text{g}/\text{kg}/\text{d} \times 10$ (3)	6	0
25 $\mu\text{g}/\text{kg}/\text{d} \times 10$ (3)	6	1
Ages: 31-69, median 60		
Males: 13, females: 11		

\*Number of prior chemotherapy regimens per patient.

Dose level (ug/Kg/d ×10):	4	4	4	8	8	8	12	12	12	12	12	12	18	18	18	25	25	25	25	25	25	18	18	18	% patients	
Toxicity Patient #:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	with toxicity	
Edema																									71	
Albumin																										62
Fatigue																										62
Weight gain/VLS																										54
Nausea																										46
Fever																										46
Hypotension																										38
Allergy/Rash																										33
Myalgia																										29
Dizziness																										29
Pleural Effusion																										29
Vomiting																										25
Pleuritic Pain																										25
Appetite																										21
Hypoxia																										17
Headache																										17
Proteinuria																										17
Pericardial Effusion																										17
Dyspnea																										17
Diarrhea																										12
Abdominal pain																										12
Sinus Tachycardia																										12
Arthralgia																										8
Pruritis																										8
Dysgeusia																										8
Sweating																										8
Supraventricular tachycardia																										8
Dyspepsia																										8
SGPT (ALT)																										4
SGOT (AST)																										4
Constipation																										4
Flatulence																										4
Skin changes																										4
Palpitations																										4
Hematuria																										4
Acidosis																										4
Confusion																										4
Ascites																										4

Fig. 1. Toxicity of SS1P by continuous infusion. Adverse events shown were grade 1 (green), grade 2 (orange), grade 3 (red), or grade 4 (black).

dilutions of plasma compared with a standard cytotoxicity curve using purified SS1P, as described (23).

## Results

**Patients and dose escalation.** A total of 24 patients were treated (Table 1), including five with peritoneal mesothelioma, nine with pleural mesothelioma, two with pleural-peritoneal mesothelioma, seven with ovarian carcinoma, and one with pancreatic carcinoma. Compared with ovarian cancer, patients with mesothelioma had fewer prior therapies (median 2 versus 6,  $P = 0.004$ , Wilcoxon), probably due to mesothelioma being more aggressive and less treatable. Groups of three patients received 4 and 8  $\mu\text{g}/\text{kg}/\text{d} \times 10$ , and six patients each received 12, 18, and 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$ .

**SS1P safety and dose escalation.** As shown in Fig. 1, toxicities were usually grades 1 and 2 and the most common adverse events were edema (71%), hypoalbuminemia (62%), fatigue

(62%), weight gain/vascular leak syndrome (54%), nausea (46%), fever (46%), hypotension (38%), and allergy/rash (33%). Grade 3 transaminase elevations and proteinuria were observed in one patient each and were not considered dose limiting by protocol. The 4 and 8  $\mu\text{g}/\text{kg}/\text{d} \times 10$  dose levels were completed with three patients each. The 12  $\mu\text{g}/\text{kg}/\text{d} \times 10$  dose level was expanded to six patients because the first patient stopped treatment after 5 days due to pleuritic chest pain without evidence of cardiopulmonary origin and did not resume the infusion. Groups of three patients each then received 18 and 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$ . Pleuritic chest pain not due to a cardiopulmonary cause was observed in three of six patients at 12  $\mu\text{g}/\text{kg}/\text{d} \times 10$  and two patients at 18  $\mu\text{g}/\text{kg}/\text{d} \times 10$  and typically involved the normal lung in patients with pleurodesis on the contralateral side. One patient at the 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$  dose level had grade 3 proteinuria not associated with symptoms or with a creatinine elevation, resolving several days later with the next 24-hour urine. Although this event was not considered DLT, three

additional patients were enrolled at the 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$  dose level. The last patient had baseline pulmonary hypertension and diastolic dysfunction and, with SS1P plus exogenous fluid, developed large pleural effusions and respiratory failure, resolving with aggressive diuresis. An additional three patients were treated at the 18  $\mu\text{g}/\text{kg}/\text{d}$  dose level without DLT. Dose escalation beyond 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$  was not attempted. Thus, zero of six at 18 and one of six at 25  $\mu\text{g}/\text{kg}$  QOD  $\times 3$  had DLT, defining the higher dose level as the MTD.

**Immunogenicity.** High levels of neutralizing antibodies, defined as  $>75\%$  neutralization of 200 ng/mL of SS1P, were observed in three of three patients at 4 and 8  $\mu\text{g}/\text{kg}/\text{d} \times 10$ , three of six at 12 and 18  $\mu\text{g}/\text{kg}/\text{d} \times 10$ , and six of six at 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$ . Five patients received two cycles, with the second cycle of SS1P at the same dose level as cycle 1. Thus, the immunogenicity rate on cycle 1 was 18 of 24 patients (75%).

**Pharmacokinetics.** Plasma levels of SS1P were determined by incubating dilutions of plasma from treated patients and comparing cytotoxicity on A431-K5 cells to a standard curve of cytotoxicity generated simultaneously by known concentrations of purified SS1P. Plasma levels were measured before the infusion and on days 3, 5, 7, 9, and 11 before and after the infusion ended. Plasma levels typically reached a peak at day 5 and declined before day 11 when patients made neutralizing antibodies early. As shown in Fig. 2, median peak levels of SS1P were 64, 95, 119, 146, and 153 ng/mL at 4, 8, 12, 18, and 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$ , respectively. The medians of both peak concentrations and median area under the curve (AUCs) (the latter determined by the trapezoid method on the cytotoxicity curves) were directly proportional to dose ( $r^2 = 0.88-0.89$ ,  $P = 0.016-0.018$ ).

**Efficacy.** All 24 patients were considered evaluable for response. One patient had a partial response, 12 had stable disease, and 11 had progressive disease. The patient with partial response had ovarian cancer and received 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$ . She had a 15  $\times$  26 mm hepatic lesion, which decreased in size to 10  $\times$  19 mm by C1D29 and to 7  $\times$  16 mm by C1D64. Her CA-125 decreased from 384 to 392 pretreatment to 309 by C1D90 and 243 by C1D119. Another patient at this dose level with peritoneal mesothelioma had severe ascites before SS1P requiring frequent paracentesis and had hypoalbuminemia due to the removal of albumin in the fluid. After finishing

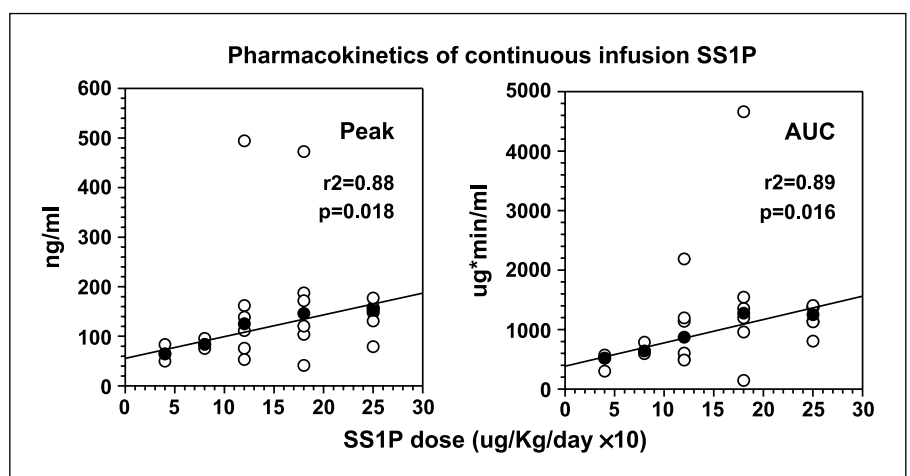
the SS1P infusion, the patient did not require paracentesis for several months and was able to return to jogging. One patient with thoracic mesothelioma had severe chest pain requiring high-dose narcotics and was unable to move his arms without pain. By the end of the infusion of 18  $\mu\text{g}/\text{kg}/\text{d} \times 10$ , the patient's pain had resolved; he regained full range of motion of his arms without pain and was taken off of narcotics. His computed tomographies showed a slight decrease in the size of the chest mass. Another patient at 18  $\mu\text{g}/\text{kg}/\text{d} \times 10$  with mesothelioma had complete disappearance of a supraclavicular lymph node by positron emission tomography scan, which seemed slightly smaller but present on computed tomography. Finally, one patient at 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$  had a significant decrease in the uptake of an abdominal ovarian cancer lesion by positron emission tomography. Overall, 1 of 24 patients had a partial response based on improvement of the CT, which was required by protocol, although other patients not making computed tomography response criteria responded by positron emission tomography.

## Discussion

We found that SS1P given by continuous infusion was well tolerated at doses up to 25  $\mu\text{g}/\text{kg}/\text{d} \times 10$ . The major toxicities included pleuritic pain and third spacing, which are both reversible. Immunogenicity after one cycle was observed in 75% of patients. The median peak plasma level and AUC correlated with dose level, with significant variability between patients. One major response was documented, and several patients had less protocol-defined partial response but evidence of anti-tumor activity.

**Mesothelial targeting of SS1P.** Whereas the adverse events related to vascular leak syndrome, including edema, hypoalbuminemia, weight gain, and hypotension are common to other recombinant immunotoxins and suggest nonspecific toxicity (32, 34), the pleuritic pain observed with SS1P is unique to this recombinant immunotoxin (23) and, thus, suggests direct targeting of normal mesothelial cells. Because pleuritic chest pain typically involved normal lung rather than the side that had undergone pleurodesis, the cause of pain may be inflammation of normal mesothelium lining in the thoracic cavity. Typically the pain subsided spontaneously by days 7 to 9 of infusion even

**Fig. 2.** Pharmacokinetics of SS1P by continuous infusion. The peak levels reached during infusion (left) and the total calculated AUCs (right) for each patient are in open circles, and the medians of each dose level are in closed circles.



**Table 2.** Comparison of continuous infusion and bolus dosing of SS1P

	Continuous infusion	Bolus (30 min) infusion
MTD	25 µg/kg/d × 10	45 µg/kg QOD × 3
MTD (total dose)	250 µg/kg	135 µg/kg
Immunogenicity	18 (75%) of 24	30 (88%) of 34
AUC (median at MTD)	1800 µg min/mL	590 µg min/mL

when significant plasma levels were present at that time. This suggests that the process of mesothelial inflammation or toxicity was self-limited. Although this toxicity was not a major dose-limiting event in this trial, it is potentially a problem and will be addressed in further SS1P development possibly through antiinflammatory therapy.

**Comparison of continuous infusion and bolus dosing.** As shown in Table 2, the total dose of SS1P delivered at MTD (250 µg/kg) is slightly higher than that achieved by bolus dosing of three doses of SS1P (23). Although immunogenicity of SS1P, when given by continuous infusion, was slightly lower than that observed by bolus dosing, it represented a major potential limitation in response. At the MTD, the median AUC over the 10 days of continuous infusion (1,800 µg min/mL) was ~3-fold higher than the median-estimated AUC by bolus dosing (590 µg min/mL). The total AUC by bolus dosing was obtained by multiplying by three the AUC determined on the first of three bolus doses.

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**Clinical development of SS1P.** Response was not dramatically different on this trial compared with the phase I trial of bolus SS1P (23). Antitumor activity was modest despite median plasma levels of up to 153 ng/mL, although concentrations of SS1P at 1 to 10 ng/mL were sufficient to kill mesothelin-expressing cells lines (20, 22) and tumors in organotypic culture (21). It is possible that high levels of soluble mesothelin, within the tumors of patients, interfered with delivery of SS1P to the tumor cells (35) and that chemotherapy, along with SS1P, would decrease the soluble receptor within tumors, facilitating effective targeting to all tumor cells (35-37). To test this hypothesis, a phase II trial is now under way pretreating mesothelioma patients with pemetrexed-cisplatin before SS1P, beginning with an SS1P bolus dosage of 25 µg/kg QOD × 3. If chemotherapy can allow even distribution of SS1P to tumor cells, then bolus dosing, which can achieve peak SS1P levels of nearly 500 ng/mL, with mean half-lives of nearly 8 hours, should be adequate to result in major response in several types of mesothelin-expressing malignancies.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Robert J. Kreitman, Raffit Hassan, David J. FitzGerald, et al.

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