Published OnlineFirst August 11, 2009; DOI: 10.1158/1078-0432.CCR-09-0062

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 μg/kg/d ×10. The maximum tolerated dose was 25 μg/kg/d ×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 μg/kg/d ×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

Received 1/13/09; revised 5/11/09; accepted 5/15/09; published OnlineFirst 8/11/09.

Grant support: NIH National Cancer Institute Center for Cancer Research Intramural Research Program.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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.

doi:10.1158/1078-0432.CCR-09-0062

Published OnlineFirst August 11, 2009; DOI: 10.1158/1078-0432.CCR-09-0062

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 ×6 was 18 μg/kg ×6 and in 17 patients treated QOD ×3 was 45 μg/kg QOD ×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 ≥4 wk before SS1P. Age of ≥18, life expectancy of ≥12 wk, and performance status of 0 to 2 on Eastern Cooperative Oncology Group were required. Laboratory results required included ANC of ≥1,000, platelets of ≥75,000, creatinine of < 2, normal bilirubin, and aspartate aminotransferase and alanine aminotransferase of <grade 2. Albumin needed to be at least 3, and oxygen saturation ≥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 μg/kg/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 μg/kg/d ×10 was chosen because the total dose of 40 μg/kg/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 μg/kg/d ×7 d were nonlethal in BALB/c mice. Retreatment of patients was allowed in the absence of progressive disease or high levels of neutralizing antibodies. Retreatment 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 ×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 intrapatient dose escalation was allowed. DLT was defined as grade of ≥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 μg/kg/d ×10	3	0
8 μg/kg/d ×10 (3)	3	0
12 μg/kg/d ×10 (3)	6	1
18 μg/kg/d ×10 (3)	6	0
25 μg/kg/d ×10 (3)	6	1
Ages: 31-69, median 60		
Males: 13, females: 11		

*Number of prior chemotherapy regimens per patient.

Published OnlineFirst August 11, 2009; DOI: 10.1158/1078-0432.CCR-09-0062

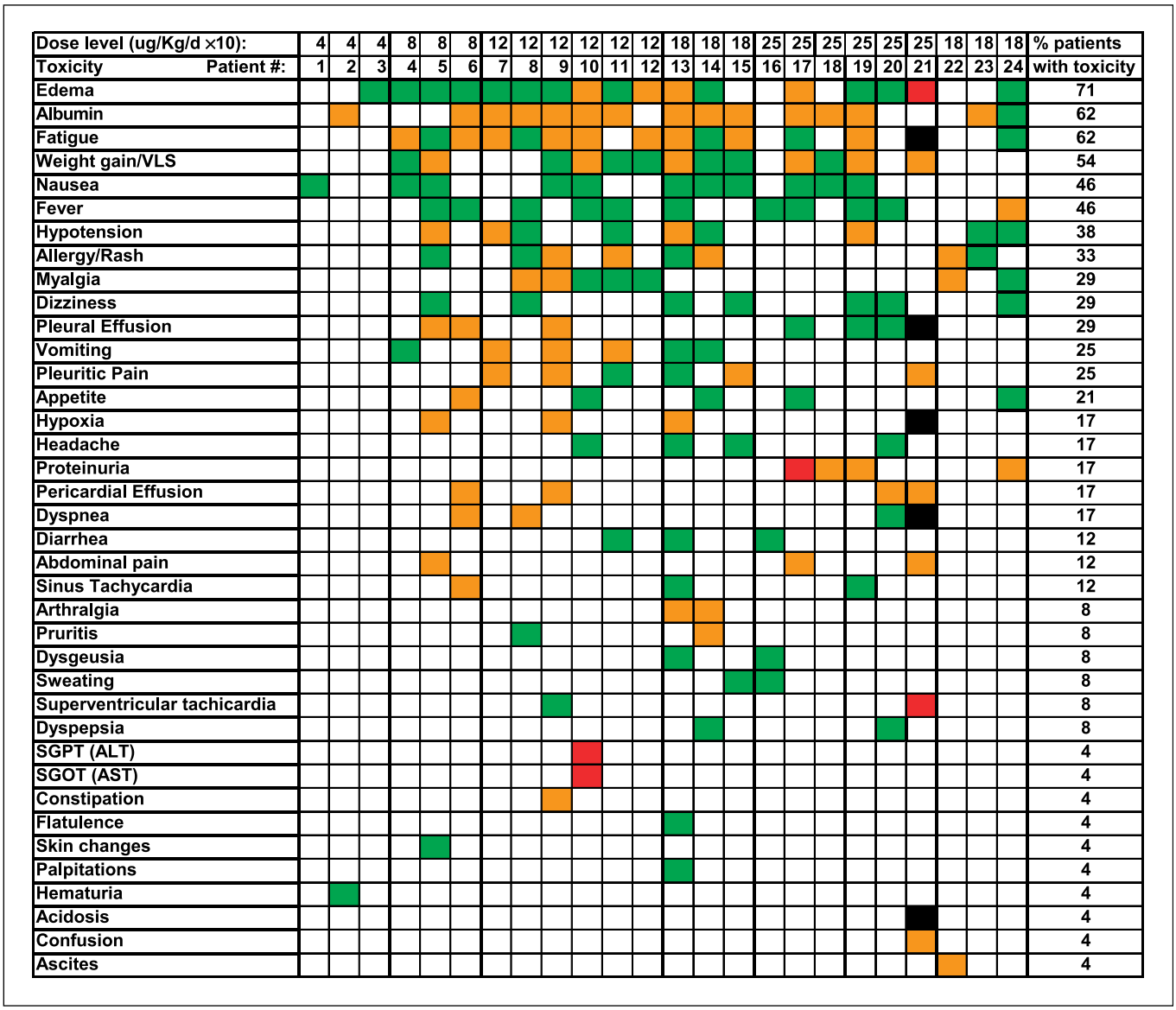

Fig. 1. Toxicity of SS1P by continuous infusion. Adverse events shown were grade 1 (*green*), grade 2 (*orange*), grade 3 (*red*), or grade 4 (*black*).

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 μg/kg/d ×10, and six patients each received 12, 18, and 25 μg/kg/d ×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 μg/kg/d ×10 dose levels were completed with three patients each. The 12 μg/kg/d ×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 μg/kg/d ×10. Pleuritic chest pain not due to a cardiopulmonary cause was observed in three of six patients at 12 μg/kg/d ×10 and two patients at 18 μg/kg/d ×10 and typically involved the normal lung in patients with pleurodesis on the contralateral side. One patient at the 25 μg/kg/d ×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

Published OnlineFirst August 11, 2009; DOI: 10.1158/1078-0432.CCR-09-0062

additional patients were enrolled at the 25 μg/kg/d ×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 μg/kg/d dose level without DLT. Dose escalation beyond 25 μg/kg/d ×10 was not attempted. Thus, zero of six at 18 and one of six at 25 μg/kg QOD ×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 μg/kg/d ×10, three of six at 12 and 18 μg/kg/d ×10, and six of six at 25 μg/kg/d ×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 μg/kg/d ×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 μg/kg/d ×10. She had a 15 × 26 mm hepatic lesion, which decreased in size to 10 × 19 mm by C1D29 and to 7 × 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 μg/kg/d ×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 μg/kg/d ×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 μg/kg/d ×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 μg/kg/d ×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 antitumor 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

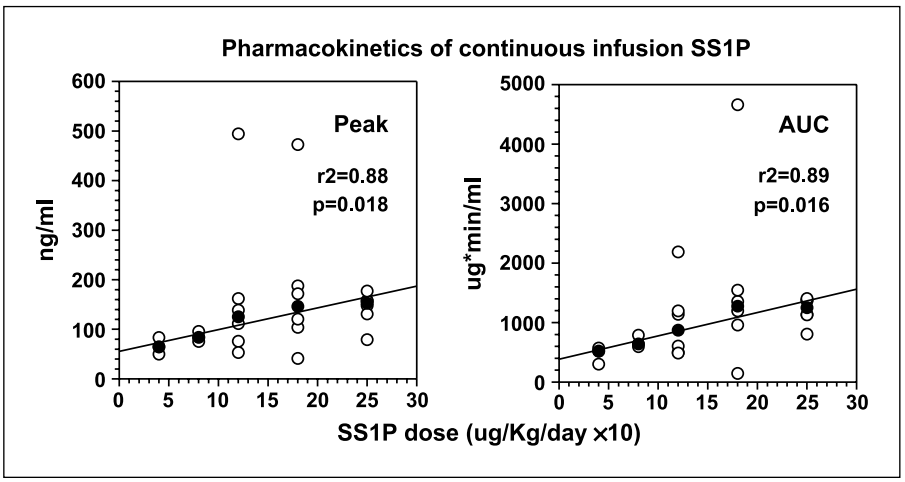

Fig. 2. Pharmacokinetics of SS1P by continuous infusion. The peak levels reached during infusion (*left*) and the total calculated AUCs (*right*) for each patient are in open circles, and the medians of each dose level are in closed circles.

Published OnlineFirst August 11, 2009; DOI: 10.1158/1078-0432.CCR-09-0062

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.

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-cisplatinum 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.

Acknowledgments

We thank research nurses Karen Bergeron, Rita Mincemoyer, Diana O'Hagan, Kelly Cahill, and Michelle Zancan and Dr. David Squires for their work with patients on this trial; Dr. Lee Pai-Scherf for writing a portion of the protocol; Dr. Mark Willingham for performing immunohistochemistry; and National Cancer Institute Medical Oncology Branch fellows and nurses for their efforts.

References

1. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc Natl Acad Sci U S A 1996;93:136–40.
2. Palumbo C, Bei R, Procopio A, Modesti A. Molecular targets and targeted therapies for malignant mesothelioma. Curr Med Chem 2008;15:855–67.
3. Hassan R, Bera T, Pastan I. Mesothelin: a new target for immunotherapy. Clin Cancer Res 2004;10:3937–42.
4. Gubbels JA, Belisle J, Onda M, et al. Mesothelin-MUC16 binding is a high affinity, N-glycan dependent interaction that facilitates peritoneal metastasis of ovarian tumors. Mol Cancer 2006;5:50.
5. Chang K, Pai LH, Pass H, et al. Monoclonal antibody K1 reacts with epithelial mesothelioma but not with lung adenocarcinoma. Am J Surg Pathol 1992;16:259–68.
6. Chang K, Pastan I, Willingham MC. Frequent expression of the tumor antigen CAK1 in squamous-cell carcinomas. Int J Cancer 1992;51:548–54.
7. Kushitani K, Takeshima Y, Amatya VJ, Furonaka O, Sakatani A, Inai K. Immunohistochemical marker panels for distinguishing between epithelioid mesothelioma and lung adenocarcinoma. Pathol Int 2007;57:190–9.
8. Argani P, Iacobuzio-Donahue C, Ryu B, et al. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). Clin Cancer Res 2001;7:3862–8.
9. Ryu B, Jones J, Blades NJ, et al. Relationships and differentially expressed genes among pancreatic cancers examined by large-scale serial analysis of gene expression. Cancer Res 2002;62:819–26.
10. Chang K, Pastan I, Willingham MC. Isolation and characterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. Int J Cancer 1992;50:373–81.
11. Hassan R, Wu C, Brechbiel MW, Margulies I, Kreitman RJ, Pastan I. ^{111}Indium-labeled Monoclonal antibody K1: biodistribution study in nude mice bearing a human carcinoma xenograft expressing mesothelin. Int J Cancer 1999;80:559–63.
12. Hassan R, Viner J, Wang QC, Kreitman RJ, Pastan I. Anti-tumor activity of K1-38QQR, an immunotoxin targeting mesothelin, a cell-surface antigen overexpressed in ovarian cancer and malignant mesothelioma. J Immunotherapy 2000;23:473–9.
13. Chowdhury PS, Chang K, Pastan I. Isolation of anti-mesothelin antibodies from a phage display library. Mol Immunol 1997;34:9–20.
14. Pastan I, Hassan R, Fitzgerald DJ, Kreitman RJ. Immunotoxin treatment of cancer. Annu Rev Med 2007;58:221.
15. Chowdhury PS, Pastan I. Improving antibody affinity by mimicking somatic hypermutation *in vitro*. Nat Biotechnol 1999;17:568–72.
16. Chowdhury PS, Viner JL, Beers R, Pastan I. Isolation of a high-affinity stable single-chain Fv specific for mesothelin from DNA-immunized mice by phage display and construction of a recombinant immunotoxin with anti-tumor activity. Proc Natl Acad Sci U S A 1998;95:669–74.
17. Chowdhury PS, Vasmatzis G, Lee B, Pastan I. Improved stability and yield of a Fv-toxin fusion protein by computer design and protein engineering of the Fv. J Mol Biol 1998;281:917–28.
18. Reiter Y, Brinkmann U, Kreitman RJ, Jung S-H, Lee B, Pastan I. Stabilization of the Fv fragments in recombinant immunotoxins by disulfide bonds engineered into conserved framework regions. Biochemistry 1994;33:5451–9.
19. Pastan I, Hassan R, FitzGerald DJP, Kreitman RJ. Immunotoxin therapy of cancer. Nat Rev Cancer 2006;6:559–65.
20. Onda M, Nagata S, Tsutsumi Y, et al. Lowering the isoelectric point of the Fv portion of recombinant immunotoxins leads to decreased nonspecific animal toxicity without affecting antitumor activity. Cancer Res 2001;61:5070–7.
21. Hassan R, Lerner MR, Benbrook D, et al. Antitumor activity of SS(dsFv)PE38 and SS1(dsFv)PE38, recombinant antimesothelin immunotoxins against human gynecologic cancers grown in organotypic culture *in vitro*. Clin Cancer Res 2002;8:3520–6.
22. Li Q, Verschraegen CF, Mendoza J, Hassan R. Cytotoxic activity of the recombinant antimesothelin immunotoxin, SS1(dsFv)PE38, towards tumor cell lines established from ascites of patients with peritoneal mesotheliomas. Anticancer Res 2004;24:1327–35.
23. Hassan R, Bullock S, Premkumar A, et al. Phase I study of SS1P, a recombinant antimesothelin immunotoxin given as a bolus i.v. infusion to patients with mesothelin-expressing mesothelioma, ovarian, and pancreatic cancers. Clin Cancer Res 2007;13:5144–9.
24. Kreitman RJ, Wang QC, FitzGerald DJP, Pastan I. Complete regression of human B-cell lymphoma xenografts in mice treated with recombinant

Published OnlineFirst August 11, 2009; DOI: 10.1158/1078-0432.CCR-09-0062

anti-CD22 immunotoxin RFB4(dsFv)-PE38 at doses tolerated by Cynomolgus monkeys. Int J Cancer 1999;81:148–55.

25. Benhar I, Reiter Y, Pai LH, Pastan I. Administration of disulfide-stabilized Fv-immunotoxins B1(dsFv)-PE38 and B3(dsFv)-PE38 by continuous infusion increases their efficacy in curing large tumor xenografts in nude mice. Int J Cancer 1995;62:351–5.

26. Grossbard ML, Lambert JM, Goldmacher VS, et al. Anti-B4-blocked ricin: a phase I trial of 7-day continuous infusion in patients with B-cell neoplasms. J Clin Oncol 1993;11:726–37.

27. Stone MJ, Sausville EA, Fay JW, et al. A phase I study of bolus versus continuous infusion of the anti-CD19 immunotoxin, IgG-HD37-dgA, in patients with B-cell lymphoma. Blood 1996;88:1188–97.

28. Grossbard ML, Fidias P, Kinsella J, et al. Anti-B4-blocked ricin: a phase II trial of 7 day continuous infusion in patients with multiple myeloma. Br J Haematol 1998;102:509–15.

29. Sung C, Dedrick RL, Hall WA, Johnson PA, Youle RJ. The spatial distribution of immunotoxins in solid tumors: assessment by quantitative autoradiography. Cancer Res 1993;53:2092–9.

30. Sung C, Youle RJ, Dedrick RL. Pharmacokinetic analysis of immunotoxin uptake in solid tumors: role of plasma kinetics, capillary permeability, and binding. Cancer Res 1990;50:7382–92.

31. Fujimori K, Covell DG, Fletcher JE, Weinstein JN. Modeling analysis of the global and microscopic distribution of immunoglobulin G, F(ab')2, and Fab in tumors. Cancer Res 1989;49:5656–63.

32. Kreitman RJ, Wilson WH, White JD, et al. Phase I trial of recombinant immunotoxin Anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. J Clin Oncol 2000;18:1614–36.

33. Kreitman RJ, Wilson WH, Bergeron K, et al. Efficacy of the anti-cd22 recombinant immunotoxin BL22 in chemotherapy-resistant hairy-cell leukemia. New Engl J Med 2001;345:241–7.

34. Kreitman RJ, Squires DR, Stetler-Stevenson M, et al. Phase I trial of recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) in patients with B-cell malignancies. J Clin Oncol 2005;23:6719–29.

35. Zhang Y, Xiang L, Hassan R, Pastan I. Immunotoxin and Taxol synergy results from a decrease in shed mesothelin levels in the extracellular space of tumors. Proc Natl Acad Sci U S A 2007;104:17099–104.

36. Hassan R, Broaddus VC, Wilson S, Liewehr DJ, Zhang J. Anti-mesothelin immunotoxin SS1P in combination with gemcitabine results in increased activity against mesothelin-expressing tumor xenografts. Clin Cancer Res 2007;13:7166–71.

37. Zhang Y, Xiang L, Hassan R, et al. Synergistic anti-tumor activity of Taxol and immunotoxin SS1P in tumor bearing mice. Clin Cancer Res 2006;12:4695–701.

AACR American Association for Cancer Research

Clinical Cancer Research

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

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

Clin Cancer Res 2009;15:5274-5279. Published OnlineFirst August 11, 2009.