

Pentostatin in Steroid-Refractory Acute Graft-Versus-Host Disease

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

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ABSTRACT

Purpose

Acute graft-versus-host disease (aGVHD) is a major complication of allogeneic bone marrow transplantation. In steroid-refractory aGVHD, mortality is very high. Pentostatin, a potent inhibitor of adenosine deaminase, induces lymphocyte apoptosis and may be useful in the treatment of this condition.

Patients and Methods

We have conducted a phase I dose escalation study of pentostatin in patients with steroid-refractory aGVHD. Twenty-three patients were enrolled. Starting dose was 1 mg/m²/d by intravenous injection for 3 days.

Results

The maximum tolerated dose was found to be 1.5 mg/m²/d. Late infections at the 2-mg/m²/d dose level were believed to be dose limiting toxicities. Lymphopenia was universal, but the neutrophil count was generally not affected. Fevers associated with neutropenia were not observed. Otherwise, the drug was well tolerated, with only modest elevations of liver function tests and thrombocytopenia, each being observed in a single patient. Twenty-two patients were assessable for response, including 14 complete responses (63%) and three partial responses (13%). Median survival after therapy for the group was 85 days (range, 5 to 1,258 days).

Conclusion

The suggested intravenous dose for a phase II study will be 1.5 mg/m²/d for 3 days. Pentostatin has activity in patients with steroid-refractory aGVHD that is worth exploring in future trials.

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INTRODUCTION

Acute graft-versus-host disease (aGVHD) is a major problem after allogeneic bone marrow transplantation (BMT), with considerable morbidity and mortality.¹ Less than 50% of patients with grades II to IV² will achieve durable responses after initial therapy.³⁻⁶ Steroids produce sustained responses in only 41% of patients receiving a matched-related allogeneic BMT,⁷ and only 24% of those with an unrelated donor.⁸ In patients not responding to steroids, mortality is very high.^{3,9} Survival in this group is stage dependent. Patients

with grade II, III, and IV had median survivals of only 4.1, 3.6, and 2.7 months, respectively, after salvage therapy with antithymocyte globulin (ATG).³ These patients have been heavily treated, and many of them have serious comorbidities.

Pentostatin (deoxycoformycin, Nipent) is a nucleoside analog that is a potent inhibitor of adenosine deaminase.¹⁰ Lymphocytes are especially sensitive to the effects of pentostatin, as it irreversibly inhibits adenosine deaminase, blocking the metabolism of 2'-deoxyadenosine.¹⁰ Patients with inherited adenosine deaminase deficiency

have few T cells and have a form of severe combined immunodeficiency. Pentostatin creates a similar condition.¹¹ Lymphocytes have a high ratio of deoxycytidine kinase to 5-nucleotidase, favoring the formation of 2'-deoxyadenosine 5'-triphosphate (dATP) from 2'-deoxyadenosine.¹⁰ The accumulation of dATP slows lymphocyte growth and causes apoptosis. Pentostatin causes a decrease of T-cell response to interleukin-2 (IL-2) and IL-2 production by T-cells, a reduction in T-cell number and function, antibody and nonantibody dependent cytotoxicity, a decrease on natural killer cell numbers and lymphocyte count.¹²⁻¹⁷ Pentostatin has been used to prevent GVHD in a mouse model of BMT.¹⁸ Mice showed no GVHD and had an increased survival when compared with untreated controls. In humans, when used as part of the conditioning regimen, pentostatin produced excellent results in preventing GVHD. Miller et al reported on 55 patients who received a regimen consisting of extracorporeal photopheresis, pentostatin, and reduced dose total-body irradiation. GVHD prophylaxis consisted of cyclosporine and methotrexate. aGVHD greater than grade II developed in 9%, chronic GVHD in 43%, extensive GVHD in 12%, and limited GVHD in 31%.¹⁹ Purine nucleoside analogs may have a potent effect against GVHD. However, the toxicity profile, particularly the mild hematologic effects, make pentostatin more appealing for treatment of GVHD compared with other nucleoside analogs.²⁰ Therefore, we designed a phase I dose-escalation study to find the maximum tolerated dose of pentostatin in this setting.

PATIENTS AND METHODS

Patients

All patients gave informed consent. The study was approved by the Johns Hopkins institutional review board. Patients with biopsy-proven grade II to IV aGVHD following an allogeneic BMT or a donor lymphocyte infusion (DLI) were enrolled onto this trial if they failed to respond to steroids. We defined steroid-refractory disease as aGVHD that progressed despite 48 hours of treatment with methylprednisolone (≥ 2 mg/kg/d) or that did not improve despite 4 days of methylprednisolone (same dose). Progression was defined as development of aGVHD in an additional organ system, or advancement of aGVHD by at least one stage. Patients required myeloid engraftment (absolute neutrophil count [ANC] $> 1 \times 10^9/L$) and had to have received GVHD prophylaxis with tacrolimus, cyclosporine, ATG, methotrexate, and/or prednisone. DLI recipients had to progress despite steroids and cyclosporine or tacrolimus, and they followed the same criteria for refractoriness. Exclusion criteria included allergy/intolerance to pentostatin, chronic GVHD, more than one allogeneic BMT, pregnancy, HIV infection, hemodialysis or estimated creatinine clearance (CrCl) less than 30 mL/min/1.73 m², mechanical ventilation, and active bleeding. The protocol was amended to exclude patients with infection not responding to therapy or life expectancy of less than 2 weeks, after a patient was enrolled in septic shock while receiving pressors.

Drug Doses

Five patients were to be enrolled in each dose level starting at 1 mg/m²/d by intravenous injection (IV) for 3 days, and the next cohort was scheduled to receive 2 mg/m²/d IV for 3 days. Thereafter, three patients were to be accrued to each of the subsequent dose levels (ie, 3 mg/m²/d IV and 4 mg/m²/d IV for 3 days). National Cancer Institute toxicity criteria (modified for patients following a BMT) were used to define dose-limiting toxicity (DLT). DLT was defined as grade 3 or 4 toxicity using this scale. In the absence of DLT attributable to pentostatin, escalation to the next dose level occurred.

The decision to enter patients into a higher dose group was based on cumulative safety information. Patients were not entered into a higher dose group until all patients entered into the former group had been assessed for safety. Patients who had responded initially to pentostatin and relapsed or showed a partial response could receive additional therapy with the drug if 2 weeks elapsed. The additional doses of pentostatin were given at the same dose as the initial one. Dose modifications for this agent throughout treatment were made based on estimated CrCl. If the CrCl was greater than 50 mL/min/1.73 m², there would be no modification; less than 50 mL/min/1.73 m² and more than 30 mL/min/1.73 m², doses reduced by 50%. If the estimated CrCl was less than 30 mL/min/1.73 m², the drug was not given. Once patients were enrolled, steroids were rapidly tapered at a rate of 25% every 2 days.

Follow-Up

Patients were evaluated for toxicity and response at least weekly (and usually daily) for the first 28 days after completion of pentostatin. The extent of aGVHD was assessed at each visit. Improvement in skin aGVHD was defined as a 25% reduction in surface area involved. Progressive skin aGVHD was defined as an increase in involved surface area by more than 25%. Liver disease was considered to be improved if there was a decrease in serum bilirubin to less than 2 mg/dL for patients with baseline values of 2 to 4 mg/dL, a decrease of more than 2 mg/dL for patients with baseline values of 4 to 8 mg/dL, or a 25% decrease in serum bilirubin for patients with baseline values greater than 8 mg/dL. Progressive liver disease was defined as an increase of serum bilirubin by more than 2 mg/dL for patients with baseline values less than 8 mg/dL or greater than 25% increase in serum bilirubin for patients with baseline values more than 8 mg/dL. Gut GVHD was considered improved if there was a decrease in the 3-day average stool volume by more than 500 mL, with clearing of cramps and bleeding (patients could still have a positive test for occult blood in the stool). Clearing of cramps and bleeding was considered evidence of response in patients with less than 500 mL diarrhea volume, but not in patients with greater than 500 mL diarrhea volume. Progressive gut disease was considered as an increase in 3-day average stool by more than 500 mL, or development of cramps and bleeding. Responses were evaluated at the time of patient's evaluation. Complete response (CR) was defined as resolution of all signs and symptoms of GVHD in all assessable organs without additional treatment. Partial response (PR) was improvement in one organ without deterioration in others. Mixed response (MR) was improvement in one organ with deterioration in another organ. Stable disease was absence of any improvement or progression as defined above.

Pharmacokinetic Assay and Analysis

Pentostatin plasma concentrations were determined using a previously described technique.²¹ Fitting of the concentration-time data was performed using the WINNonlin (Scientific Consultant, Apex, NC) Professional Version 4.0 (Pharsight Corp, Cary, NC) software program. Standard pharmacokinetic parameters were

determined by fitting the plasma concentration–time data into a two-compartment model for multiple dose regimen input. Pharmacokinetic data are reported for the first dose each patient received. The area under the plasma concentration–time curve (AUC) from time zero to infinity ($AUC_{0 \rightarrow \infty}$) was calculated using the linear-log trapezoidal rule with the observed data.

Statistical Analysis

Differences between means of groups of data were evaluated for statistical significance by Student's *t* test. Correlations between continuous variables were evaluated by Pearson correlation coefficients, and correlations involving ordinal variables were evaluated by Spearman correlation coefficients. Coefficients of determination were calculated. All analyses were two-sided, and α less than .05 was considered statistically significant.

RESULTS

Patients

Twenty-four patients were registered, and 23 were eligible and were treated. There were 10 females and 13 males, 20 white patients, two Hispanic patients, and one African American patient. Median age was 43 years (range, 6 months to 63 years).

Patient characteristics are in Table 1. Prior therapy for aGVHD included cyclosporine in 23 patients, tacrolimus in 17 patients, psoralen plus ultraviolet-A irradiation for at least 1 month in two patients, mycophenolate mofetil for at least 3 weeks in seven patients, daclizumab (at least four doses) in five patients, and ATG in four patients. All patients received at least 2 mg/kg of methylprednisolone, and 11 had a pulse ranging from 500 to 1,000 mg/m² (in recipients of mismatched and unrelated donors) to 10 mg/kg (sibling donor). Patients who received ATG were at least 4 weeks after the ATG before entering the trial. All but 6 patients in the study had progression of their aGVHD while on steroids (the 6 remaining did not improve on steroid therapy). For these patients with persistence, the interval between disease diagnosis and treatment with steroids was between 8 and 103 days. For the entire group, the interval between the diagnosis of aGVHD and enrollment in the study was between 5 and 140 days, with a median of 34 days.

Dose Modifications

Three patients required dose modifications due to their CrCl. In the 1-mg/m²/d dose cohort, patients 105 and 116

Table 1. Patient Characteristics and Response to Therapy

Patient No.	Sex	Age	Disease	Donor Type	Dose Level (mg/m ²)	No. of Cycles	GVHD at Diagnosis			Overall Grade	GVHD Response			Overall Response	Survival (days)	$AUC_{0 \rightarrow \infty}$ (h * ng/mL)*
							Skin	Liver	Gut		Skin	Liver	Gut			
101	M	25	NHL	MRD/DLI	1	2	3	3	—	III	CR	CR	CR	CR	1,258	1,094
102	F	28	AML	MMRD	1	1	3	4	1	IV	CR	PR	CR	PR	22	886
103	M	50	NHL	MRD	1	1	—	—	2	III	—	NR	CR	MR	27	1,395
104	M	50	AML	MUD	1	1	2	—	2	III	CR	CR	CR	CR	85	1,679
105	F	49	NHL	MRD	1	1	3	4	—	IV	PR	NR	—	MR	9	737
106	F	10	ALL	MUD	2	1	3	—	2	III	CR	—	CR	CR	9	1,625
107	M	24	AA	MRD	2	2	1	—	3	III	CR	—	PR	PR	> 1,619	1,458
108	F	49	AML	MRD	2	2	1	—	3	III	CR	—	CR	CR	18	2,556
109	M	47	CML	MRD	2	1	—	4	—	IV	Not assessable		Not assessable	5	4,796	
110	F	43	NHL	MRD/DLI	2	1	—	4	—	IV	—	CR	—	CR	92	2,270
111	M	50	NHL	MRD	2	2	—	—	> 1	II	—	—	CR	CR	> 1,044	2,250
112	F	44	MM	MRD/DLI	2	1	—	—	1	II	—	—	CR	CR	21	1,372
113	F	41	CML	MMRD	3	1	2	1	3	III	CR	CR	PR	PR	298	2,400
115	M	9	AML	MMRD	1	1	3	—	—	II	NR	—	—	NR	148	NA
116	M	35	ALL	MRD	1	1	—	4	—	IV	—	NR	—	NR	12	NA
117	M	55	CML	MRD	1	1	3	—	—	II	CR	—	—	CR	> 518	NA
118	F	16	AML/ALL	MUD	1	1	3	—	—	II	CR	—	—	CR	314	NA
119	M	0.6	AML	MMUD	1	1	3	—	—	II	CR	—	—	CR	> 908	NA
120	M	51	ALL	MMRD	1.5	1	3	1	4	III	NR	NR	NR	NR	27	NA
121	M	43	AML	MMRD	1.5	2	2	1	2	III	CR	CR	CR	CR	> 741	NA
122	M	53	NHL	MRD	1.5	2	3	2	4	III	CR	CR	CR	CR	92	NA
123	F	63	AML	MRD	1.5	1	—	—	2	III	—	—	CR	CR	> 83	NA
124	F	3	CML	MUD	1.5	1	3	—	—	II	CR	—	—	CR	73	NA

NOTE. Patients 105, 106, and 116 had a dose modification as explained in the text. Abbreviations: M, male; F, female; NHL, non-Hodgkin's lymphoma; AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; CML, chronic myeloid leukemia; MM, multiple myeloma; AA, aplastic anemia; AML/ALL, biphenotypic leukemia; MRD, matched-related donor; DLI, donor lymphocyte infusion; MMRD, mismatched related donor; MUD, matched-unrelated donor; MMUD, mismatched unrelated donor; CR, complete response; PR, partial response; NR, no response; MR, mixed response; —, no acute graft-versus-host-disease; NA, not available. *Area under the plasma concentration–time curve (AUC) for patients 105 and 109 is calculated for only the first two doses. The dose for patient 106 was reduced by 50% on day 3, hence the AUC is based on 2-mg/m² doses on days 1 and 2, and on a 1-mg/m² dose on day 3. Survival is in days from study enrollment until last visit.

received only a 0.5-mg/m² dose, and in the 2-mg/m²/d dose group, patient 106 received only a 1-mg/m² dose on the third day of the cycle. In the latter patient, it was felt that the change in CrCl was a result of the underlying medical condition rather than a consequence of pentostatin. The dose was de-escalated after two patients receiving pentostatin 2 mg/m²/d developed late infections, and one patient was already receiving 3 mg/m²/d. Then, the study dose was decreased from 2 mg/m²/d to 1 mg/m²/d. It was decided to treat five more patients to gather more toxicity data at that dose and then try an intermediate dose at 1.5 mg/m²/d.

Toxicities

Pentostatin was well tolerated. The DLT was the presence of late (> 3 weeks after pentostatin infusion) infections at the 2-mg/m²/d dose level (patients 110 and 112). Lymphopenia was universal after therapy, but the ANC was generally not affected. No episodes of neutropenic fever were observed. Patient 120 developed thrombocytopenia 5 days after receiving the drug. Patient 123 had an increase in her liver enzymes (less than twice normal) that did not return to normal as of last visit, 2 and a half months after therapy (no liver biopsy was done). No renal, neurologic, or cardiac toxicities were noted. Table 2 presents all toxicities recorded.

Outcome

Twenty-two patients were assessable for response (Table 1), and these included 14 CR (64%), three PR (14%), two MR (9%), and three (13%) with progressive disease. All patients who received a second cycle responded. One patient was not assessable. He was septic and required blood pressure support. During a brief window of several hours while weaned off his pressors, he was placed on study. He was taken off study several hours later for advanced life support. After his entry, the protocol was amended to exclude patients with such limited life expectancy. The patient died of hypotensive shock and liver failure due to sepsis 5 days after his first pentostatin infusion. As of September 29, 2004, or last contact with us, six patients (26%) were alive. Median survival after therapy until their last contact with us for the entire group was 85 days (range, 5 to 1,619 days). One patient developed chronic GVHD of the skin. Table 3 presents the outcome of the patients. Twenty-three patients received one cycle of pentostatin, and six patients were re-treated at the same dose on disease progression.

Table 2. Toxicities Attributable to Pentostatin

No. of Patients on Given Dose	Toxicity	Grade
1 at 1 mg/m ²	Thrombocytopenia	3
1 at 1.5 mg/m ²	Neutropenia	1
1 at 1.5 mg/m ²	Hepatic-liver enzymes 2 to 5× normal	1
2 at 2 mg/m ²	Late infections: polymicrobial sepsis and pulmonary aspergillosis	5

Table 3. Clinical Outcomes of the Patients Enrolled

Patient No.	Current Condition or Cause of Death
101	Died of relapsed lymphoma
102	Died of respiratory failure and adenovirus infection
103	Died of GVHD
104	Died of HHV6/pneumonia and CMV infection
105	Died of GVHD
106	Died of severe diffuse alveolar damage and disseminated adenovirus
107	Alive without GVHD
108	Died of sepsis
109	Died of hepatic failure and shock due to sepsis
110	Died of pulmonary aspergillosis
111	Alive without GVHD
112	Died of polymicrobial sepsis
113	Died of relapsed leukemia
115	Died of bronchiolitis obliterans
116	Died of hepatorenal syndrome and intracranial bleed
117	Alive without GVHD
118	Died of multiorgan failure
119	Alive with chronic GVHD
120	Died of refractory aGVHD
121	Alive without GVHD
122	Died of refractory chronic GVHD, <i>Clostridium</i> sepsis, and aspergillosis
123	Alive without GVHD
124	Died of bronchiolitis obliterans

Abbreviations: GVHD, graft-versus-host disease; aGVHD, acute GVHD; HHV, human herpes virus; CMV, cytomegalovirus.

Plasma Pharmacokinetics

Plasma concentration–time profiles were analyzed for the first 13 patients. Each patient received 0.5 to 3 mg/m² of pentostatin daily for 3 days. Four patients received a second cycle. The pentostatin dose was administered IV over a range of 18 to 50 minutes. The plasma concentration–time profiles exhibited biexponential decay, and the pharmacokinetic parameters were similar to previous reports.^{22–29} Pharmacokinetic parameters are presented in Table 4, and a 3-day pharmacokinetics profile is shown in Figure 1. The experimental data were well described by the two-compartment model, with deviations between observed and predicted concentrations generally less than 10%. No statistically significant differences were noted among the mean pharmacokinetic parameters for patients receiving 1 or 2 mg/m² of pentostatin (Table 4). Therefore, the pharmacokinetics of pentostatin seem to be independent of dose within this range. Interpatient pharmacokinetic variability was extensive, as indicated by an overlap in plasma AUC_{0→∞} between the respective dose groups (Fig 2). There is a statistically significant relationship between the observed plasma AUC_{0→∞} and the dose of the drug administered, as shown in Figure 2. The single patient who received a reduced dose of 0.5 mg/m² due to low CrCl had a pentostatin clearance value of approximately 50% of the mean

Table 4. Pharmacokinetic Parameters of Patients Receiving 0.5 to 3 mg/m² Pentostatin Daily for 3 Days

Parameter	All Patients		0.5 mg/m ²	1 mg/m ²		2 mg/m ²		3 mg/m ²
	Mean	SD		Mean	SD	Mean	SD	
n	13		1*	4		7		1
AUC _{0→∞} (h * ng/mL)	—		737†	1,260	347	1,980	532‡	2,400
AUC _{1st dose} (h * ng/mL)	—		313	310	91.1	960	572	602
Cl _t (L/h/m ²)	3.19	1.17	1.56	3.35	0.952	3.19	1.29	4.19
Vd _{ss} (L/m ²)	26.7	8.55	24.9	27.6	13.8	25.1	5.64	36.4
MRT (h)	10.5	7.52	16.0	9.20	5.39	10.7	9.57	8.68
t _{1/2α} (h)	0.412	0.440	0.188	0.616	0.560	0.357	0.430	0.212
t _{1/2β} (h)	8.14	5.46	11.8	7.66	4.88	8.04	6.71	6.96
α (h ⁻¹)	3.85	2.62	3.69	2.73	2.72	4.59	2.92	3.28
β (h ⁻¹)	0.128	0.0976	0.0585	0.159	0.165	0.123	0.0613	0.100
k ₁₂ (h ⁻¹)	2.46	1.78	2.72	1.45	1.37	3.03	2.09	2.23
k ₂₁ (h ⁻¹)	1.10	0.887	0.740	1.02	1.24	1.27	0.838	0.600
k ₁₀ (h ⁻¹)	0.422	0.261	0.292	0.425	0.339	0.421	0.270	1.27

NOTE. The following parameters are presented: area under the plasma concentration–time curve from time zero to infinity (AUC_{0→∞}), AUC from the first dose administered (AUC_{1st dose}), total body clearance (Cl_t), volume of distribution at steady state (Vd_{ss}), mean residence time (MRT), plasma half-lives during the first (t_{1/2α}) and second (t_{1/2β}) exponential phases, macroscopic rate constants (α and β), and microscopic rate constants (k₁₂, k₂₁, and k₁₀). All parameters were calculated for the first dose of pentostatin administered to each of 13 patients, except for AUC_{0→∞}, which was calculated for all three doses.

*The intended dose for this patient was 1 mg/m² but was reduced to 0.5 mg/m² because of low creatinine clearance.

†Patient received only two doses.

‡Data for five patients who received three doses. The other two patients were not included in the AUC_{0→∞} calculation because one patient received 1 mg/m² on day 3 due to reduced creatinine clearance, and data were collected for only the first two doses for another patient.

clearance value for all patients (Table 4). Patient 109 suffered hepatic failure and showed a reduction in clearance to 27% of the mean.

Pharmacologic and Clinical Correlations

Of the 13 patients who had pharmacokinetic analysis completed, there were seven CR, three PR, two MR, and one patient who was not assessable. While the correlation between response and AUC_{0→∞} was not statistically significant (Fig 3), two of two patients with AUC values less than

1,000 hours * ng/mL did not obtain a CR, and survival was ≤ 22 days. In contrast, nine of 10 patients with AUC greater than 1,000 hours * ng/mL either obtained a CR or survived for ≥ 298 days.

A possible association between AUC and the DLT of late infections was considered. The two patients with late infections had AUC values of 2,270 and 1,372 hours * ng/mL, respectively. The AUC range for all assessable patients was 737 to 2,556 hours * ng/mL.

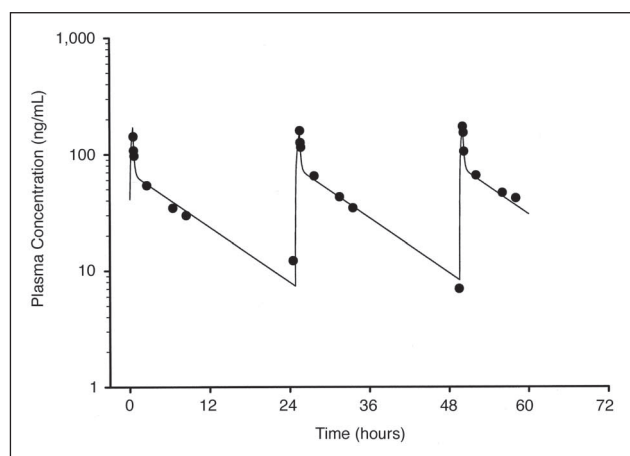


Fig 1. Plasma concentration–time profile of a patient with steroid-refractory acute graft-versus-host disease receiving pentostatin daily for 3 days. Circles represent experimental data, and the solid line represents the calculated profile of the pharmacokinetic analysis. The data presented are from a patient receiving 2 mg/m².

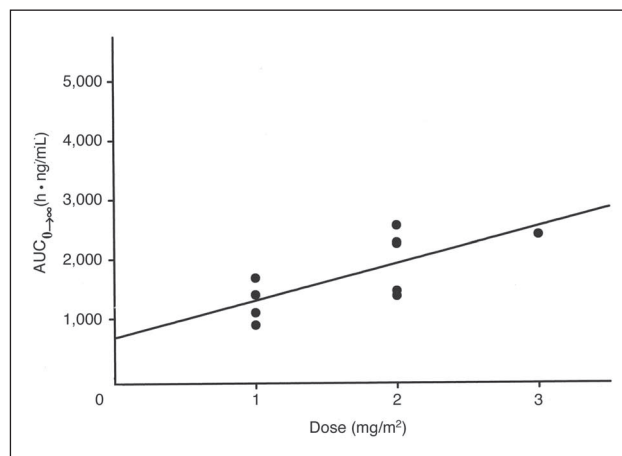


Fig 2. Correlation of the area under the plasma concentration–time curve from time zero to infinity (AUC_{0→∞}). r² = 0.51; P = .02. Patient Nos. 105, 106, and 109 are not included. No. 105 received two doses. No. 106 received a reduced dose on day 3 due to low creatinine clearance. No. 109 suffered hepatic failure, and pentostatin administration was withheld on day 3.

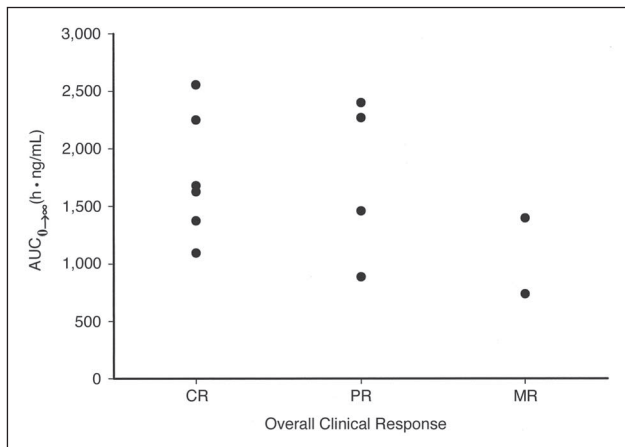


Fig 3. Correlation of overall clinical response with the area under the plasma concentration-time curve from time zero to infinity ($AUC_{0-\infty}$). CR, complete response; PR, partial response; MR mixed response. $r^2 = 0.098$; $P = .32$. Patient No. 109 was not assessable and was not included in this correlation.

DISCUSSION

We describe the first study to evaluate pentostatin in steroid refractory aGVHD. Pharmacokinetic evaluation of pentostatin showed a linear relationship between the dose of the drug and the resultant plasma AUC. While the number of patients studied with pharmacokinetics in this trial is limited, there is clear variability in the observed plasma AUC values at each dose level. The dose-independent pharmacokinetics and the extensive interpatient variability agree with similar findings in previous studies.^{24,25} Previous studies used dose reductions in patients with renal insufficiency, and pharmacokinetic results showed statistically significant correlations between CrCl and total body clearance of pentostatin.^{25,28} A decreased clearance of pentostatin was found in the patient with abnormal renal function where pharmacokinetics could be evaluated. Hence, our results support the use of dose reductions for impaired renal function.

We explored the relationship of the plasma concentration-time profile with clinical response. Previous publications have established that the overall AUC is a better correlate for this type of drug effect than is plasma concentration.³⁰ A correlation between AUC and clinical efficacy was suggested by our finding of an incomplete drug response for AUC values below 1,000 hours * ng/mL, with complete responses or long-term survival for 90% of patients receiving higher AUC values. A positive, but not statistically significant, correlation between AUC and clinical response provided a further suggestion in this direction.

Correlations between pentostatin AUC values and indicators of toxicity or potential toxicity were also evaluated. The patients exhibiting late infections, considered the DLT in this study, had AUCs in the middle of the total range, suggesting a lack of correlation. Long-term survivors (> two times the median survival) had AUC values of 1,094;

1,458; 2,250; and 2,400. To evaluate the potentially important relationship between pentostatin AUC and clinical response or toxicity, pharmacokinetic monitoring during follow-up phase II are needed.

There are major difficulties in the evaluation of new therapies in patients with steroid-refractory aGVHD. First, these patients are quite ill and are on many medications. Attribution of toxicity in this setting is complicated. However, study design should produce a dose that will be well tolerated for use earlier in the course of the disease, when there is a better chance of altering the course of aGVHD. Second, evaluation of response is also difficult in these patients. There is no universally accepted definition of when patients should be considered steroid-refractory or for response in patients with severe end-organ damage. We set our definition based on the fact that patients who do not respond by day 5 of therapy with methylprednisolone have an increased mortality.³¹ Finally, end-organ damage in GVHD may be so severe that it may not be reversible. Hence, a novel agent may control the immune response but have no effect on survival. Although these issues make evaluation of new agents in refractory aGVHD difficult, the poor survival of this group of patients makes the search for new agents imperative.

It was decided to proceed with a 3-day regimen of pentostatin instead of the commonly used single dose of the drug. We were attempting to be conservative with the expectation that some toxicities could be decreased this way as patients with steroid-refractory aGVHD do not tolerate standard doses of purine analogs.³² A major concern was renal toxicity. These patients have received multiple nephrotoxic drugs. By administering the dose over 3 days, it was possible to better monitor renal function. A similar approach was successful in patients with hairy cell leukemia.³³

Patients with aGVHD usually suffer from an intrinsic immune deficiency due to their GVHD, prior agranulocytosis, mucosal breakdown, prior therapy with steroids, and other end-organ toxicities.³⁴⁻³⁶ Many of the patients in this trial had pre-existing infections. The occurrence of two late fatal infections in patients who had received DLI was particularly concerning. These patients had already recovered from the acute toxicity of the transplant and had not received cytotoxic therapy before the DLI. Although it is impossible to attribute these infections solely to pentostatin, we believed that they were worrisome and stopped the dose escalation. The dose was de-escalated from 3 mg/m² after two patients receiving 2 mg/m² developed late infections and one patient was already receiving the higher dose. We de-escalated the dose to 1 mg/m² and treated five additional patients to gather more toxicity data. After confirming that this dose was well tolerated, an intermediate dose of 1.5 mg/m² was examined.

When considering response criteria for this trial, our assumption was that most patients would have end-stage

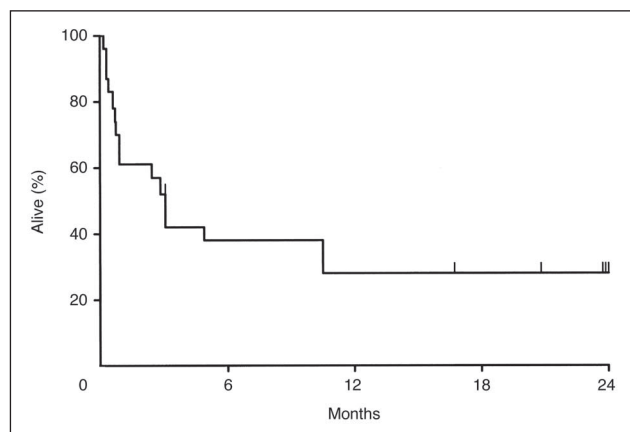


Fig 4. Kaplan-Meier plot for overall survival of patients treated with pentostatin for steroid-refractory acute graft-versus-host disease.

aGVHD, for which there are no accepted response criteria. For example, a patient with a bilirubin of 40, which decreased to 15.1, would still be stage 4 liver disease. Conversely, we also wanted to avoid trivial changes (for example 1,100 mL of diarrhea decreasing to 950 mL, and hence improving from grade III to grade II) should not be considered a response. Patients were graded in real time for their response using both conventional staging and the response criteria described in the protocol. All responders improved in both our criteria and in the standard staging criteria. Overall survival is illustrated in Figure 4.

Comparisons among drugs in phase I trials is difficult. However, the efficacy and favorable toxicity profiles observed with pentostatin in this group of patients warrants further exploration in the treatment of aGVHD. Historically, ATG was used for steroid-refractory disease.⁷ However, the benefit was observed in those with less-advanced GVHD.^{3,37} Our experience using ATG as salvage therapy was disappointing,³ and others have obtained similar results.³⁸ Other therapies have

been used and proven either ineffective^{5,39} or too toxic, despite some encouraging results.^{6,40-42}

There are several potential advantages to the use of pentostatin in treating aGVHD. This agent exploits a targeted pathway not utilized in most prophylaxis regimens for GVHD. The agent is available, and extensive long-term experience exists in patients treated for hairy cell leukemia.³⁴ In this phase I trial, the drug was well tolerated, and no patient discontinued its use because of toxicity.

In conclusion, for a phase II trial, we recommend the dose of 1.5 mg/m²/d IV for 3 days. Dose modification for patients with impaired renal function must be considered. Pentostatin has promising activity in the treatment of patients with steroid-refractory aGVHD. If subsequent phase II trials confirm these findings, this agent should be explored in patients who have received less immunosuppression.

Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Consultant/Advisory Role: Javier Bolaños-Meade, SuperGen; Michael R. Grever, SuperGen. Stock Ownership: Jeffrey Margolis, Eli Lilly. Honoraria: David A. Jacobsohn, SuperGen; Viki Anders, SuperGen; Michael R. Grever, SuperGen; Georgia B. Vogelsang, SuperGen. Research Funding: David A. Jacobsohn, SuperGen; Jeffrey Margolis, SuperGen; John C. Byrd, SuperGen; Michael R. Grever, SuperGen; Georgia B. Vogelsang, SuperGen. For a detailed description of these categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and Disclosures of Potential Conflicts of Interest found in Information for Contributors in the front of each issue.

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