Treatment of steroid-refractory acute graft-versus-host disease with anti-CD147 monoclonal antibody ABX-CBL

H. Joachim Deeg, Bruce R. Blazar, Brian J. Bolwell, Gwynn D. Long, Friedrich Schuening, John Cunningham, Robert M. Rifkin, Sunil Abhyankar, Adrienne D. Briggs, Richard Burt, John Lipani, Lorin K. Roskos, J. Michael White, Nancy Havrilla, Gisela Schwab, and Helen E. Heslop

ABX-CBL, an immunoglobulin M murine monoclonal antibody, recognizes CD147 and initiates cell killing through complement-mediated lysis. In a dose-finding trial, 27 patients with steroid-refractory acute graft-versus-host disease (GVHD) received ABX-CBL at 0.01 (presumed no effect dose), 0.1, 0.2, or 0.3 mg/kg per day, and an additional 32 patients were given ABX-CBL at 0.2 or 0.15 mg/kg per day. All patients had undergone allogeneic transplantation for malignant or nonmalignant disorders and received GVHD prophylaxis, generally with methotrexate- and cyclosporine-containing regimens. None responded to methylprednisolone, given for a minimum of 3 days. ABX-CBL was started 20 to 236 (median, 47) days after transplantation; it was given for 7 consecutive days and was followed by 2 infusions per week for 2 more weeks. Among 51 patients evaluable for efficacy, 26 (51%) responded, including 13 with complete responses (CR) and 13 with partial responses (PR). CR lasting 14 days or longer or PR lasting 7 days or longer occurred in 21 (41%; 8 CR, 13 PR) patients, including 19 of 43 (44%) patients who received 0.1 to 0.3 mg/kg ABX-CBL and 2 of 8 (25%) patients given 0.01 mg/kg per day. Myalgias at doses 0.2 mg/kg or greater were dose limiting and resolved without sequelae. Causes of death included organ failure, progressive GVHD, and infection. No death was attributed to ABX-CBL. At 6 months after the initiation of ABX-CBL therapy, 26 (44%) patients were surviving. These results are encouraging. Further studies on the use of ABX-CBL in the management of GVHD are warranted. (Blood. 2001;98:2052-2058)

Introduction

Acute graft-versus-host disease (GVHD) is a major complication of allogeneic hemopoietic stem cell transplantation (HSCT), generally developing 10 to 30 days after transplantation.[1-3] GVHD is triggered by donor T lymphocytes that recognize recipient tissues as foreign. Resultant cell activation and cytokine release lead to the destruction of host tissue and the clinical syndrome of GVHD.[4,5] The main target organs of GVHD are the immune system, skin, liver, and gastrointestinal tract.[1,6-8] Despite in vivo pharmacologic prophylaxis of GVHD, acute GVHD requiring additional therapy develops in as many as 30% (with human leukocyte antigen [HLA]-identical donors) to 75% (with unrelated donors) of transplant recipients.[1-3]

The current standard for initial therapy of acute GVHD is methylprednisolone at doses of 1 to 2 mg/kg per day. With this approach, 20% to 30% of patients achieve complete responses (CR)—more so with transplants from related than from unrelated donors)[2,3]—and 40% to 60% of patients require secondary therapy.[9,10] Steroid-resistant GVHD often develops in patients with initial diagnoses of more severe disease. In one study, 75% of patients requiring second-line therapy had acute GVHD of grades III to IV.[3] Various agents, including antithymocyte globulin and monoclonal antibodies, have been used for the treatment of steroid-refractory acute GVHD.[2,3,11-13] ABX-CBL is a murine immunoglobulin M (IgM) monoclonal antibody that recognizes CD147 on the cell surface. Human CD147, also known as neurothelin or EMMPRIN, is a member of the immunoglobulin superfamily. It is a 251-amino acid, type 1 transmembrane glycoprotein with an approximate molecular weight of 27.4 kd.[14] CD147 is expressed weakly on human leukocytes, granulocytes, red blood cells, and several other cell types. On activation, CD147 is up-regulated on T and B lymphocytes.[15] Activated T cells ($CD4^+$ and $CD8^+$) and B cells, as well as resting and activated monocytes and dendritic cells, are depleted by ABX-CBL in vitro, whereas resting lymphocytes remain unaffected. ABX-CBL inhibits the in vitro mixed lymphocyte reaction by depleting monocytes, dendritic cells, and activated lymphocytes through a complement-dependent cytotoxic mechanism.[16]

Earlier studies showed that the murine IgM ascites formulation,

From the Fred Hutchinson Cancer Research Center, Seattle, WA; Fairview University Medical Center, Minneapolis, MN; Cleveland Clinic Foundation, OH; Duke University Medical Center, Durham, NC; University of Wisconsin Hospital and Clinic, Madison; St Jude Children's Research Hospital, Memphis, TN; Rocky Mountain Cancer Center, Denver, CO; Center of Cancer Treatment and Research, Palmetto Richland Memorial Hospital, Columbia, SC; Arizona Cancer Center, Tucson; Northwestern University, Chicago, IL; Abgenix, Inc, Fremont, CA; J. M. White Associates, Mt Vernon, WA; Baylor College of Medicine, Houston, TX.

Submitted January 3, 2001; accepted June 1, 2001.

N.H., L.K.R., and G.S. are employees of Abgenix and have a financial interest in the company. J.L. was an employee of Abgenix at the time of the research described in the paper and held a financial interest at that time. J.M.W. is a consultant to Abgenix and receives financial compensation.

Supported by Abgenix and by grants CA18029, HL36444, and CA87948 from the National Institutes of Health (H.J.D.).

Reprints: H. Joachim Deeg, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave North, D1-100, PO Box 19024, Seattle, WA 98109-1024; e-mail: jdeeg@fhcrc.org.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

CBL-1, from which ABX-CBL was derived was effective in treating kidney graft rejection.[17,18] In a pilot study of 10 patients with steroid-refractory acute GVHD treated with CBL-1, Heslop et al[19] observed 5 CR and 4 partial responses (PR). The current report describes treatment with ABX-CBL in 59 patients with steroid-refractory acute GVHD.

Patients and methods

Patients

Patients were eligible to participate in the study if they were at least 1 year of age, underwent allogeneic hemopoietic stem cell transplantation (HSCT) within the past 100 days (see exceptions), had an absolute neutrophil count greater than 0.5×10^9/L, had an International Bone Marrow Transplant Registry (IBMTR) GVHD index[20] of B or higher, and showed no improvement after at least 3 days of treatment with corticosteroids. Steroid dose was at the discretion of the investigator. Some patients were enrolled in this study after showing no improvement from steroid treatment or second-line therapy with other immunosuppressive agents (in addition to prophylaxis). The GVHD prophylaxis regimen was not restricted. Patients were excluded if they had undergone allogeneic HSCT more than once, received an investigational agent (other than antifungal drugs) within 30 days of enrollment, had chronic GVHD or multiorgan failure, required ventilator or vasopressor support, had a serum creatinine level of more than 2.4 mg/dL, or were positive for human antimouse antibody (HAMA). Karnofsky Performance Status (in patients at least 16 years of age) or the Lansky Scale (in younger patients) was assessed at screening, throughout the treatment period, and at follow-up visits. All patients gave written informed consent using forms approved by the Institutional Review Board of the respective institution.

Study design

The first part of this trial was a dose-escalation study involving ABX-CBL at doses of 0.01, 0.1, and 0.3 mg/kg. At each dose level, the complete dose cohort of at least 6 patients was to be treated before advancing to the next higher dose level. The 0.01 mg/kg dose group was the presumed no-effect dose. Because of unacceptable toxicity at 0.3 mg/kg, a complete cohort was not accumulated at that level; instead, another cohort of patients was treated at 0.2 mg/kg. Of the first 27 patients treated, 14 were randomly assigned to have blood samples drawn for pharmacokinetic (PK) studies.

In the second part of the trial, intra-patient dose titration at doses of 0.15 and 0.2 mg/kg per day was tested, and additional patients were enrolled at the 0.2 mg/kg per day dose to augment safety data. All patients who received any amount of ABX-CBL were to be followed up in a long-term program for up to 10 years after treatment.

Dose regimen

ABX-CBL infusions were given over 2 hours daily for 7 consecutive days (induction regimen) followed by a maintenance regimen of 2 infusions per week for 2 weeks, for a total of 11 infusions in 3 weeks.

Assessment of efficacy

Efficacy was assessed by evaluating changes in the overall IBMTR index and in the 4 target organs—skin, liver, lower gastrointestinal tract (LGI), and upper gastrointestinal tract (UGI). Assessments were performed daily for the first 7 days, twice weekly for the next 2 weeks, weekly for the next 4 weeks, and then 4 weeks after the last weekly assessment.

The modified IBMTR index of A was defined as stage 1 skin rash without visceral organ involvement. Index B included stage 2 skin or stage 1-2 visceral organ involvement. Index C was defined as stage 3 skin, gut, or liver involvement (whichever was worse). Index D included stage 4 skin, gut, or liver involvement (whichever was worse). An index of 0 was assigned if there were no clinical manifestations of acute GVHD. Individual organ involvement was scored in skin, UGI, LGI, and liver. Skin severity was categorized by extent of rash (less than 25% in stage 1, 25%-50% in stage 2, more than 50% in stage 3) and the presence of bullae/desquamation (stage 4). Liver function was scored by total bilirubin (3.5-7.9 mg/dL in stages 1-2, 8.0-15.0 in stage 3, more than 15.0 in stage 4). LGI involvement was scored by volume of diarrhea (500-1500 mL/d in stages 1-2, more than 1500 mL/d in stage 3, and severe pain and ileus in stage 4). For pediatric patients, the LGI criteria were 7 mL/kg or greater but less than 14 mL/kg for stage 1, 14 mL/kg or greater but less than 21 mL/kg for stage 2, 21 mL/kg or greater but less than 28 mL/kg for stage 3, and 28 mL/kg or greater for stage 4. UGI was ranked only as stage 1-2 (nausea/vomiting/epigastric pain) or stage 3 (positive histology).

Assessment of response

Response to therapy was determined in all patients who had received at least 4 infusions of ABX-CBL. Patients were assessed by one of the investigators, and objective measurements were verified by chart review.

Overall response was defined in 2 ways: as *extended response*, which was required to have a minimal duration, and as *best response*, which was the maximum improvement over any duration. The latter was intended to be a measure of biologic activity of the antibody even if no lasting response was achieved. CR required reduction of the IBMTR index to 0 for at least 14 days (extended response) or for any duration (best response). PR was an improvement of IBMTR index by at least 2 indices for 7 or more days (extended response) or for any duration (best response). All other changes were considered nonresponses. A flare of acute GVHD in a patient with an extended response of CR or PR was defined as a worsening from the lowest IBMTR index achieved by at least 2 indices that lasted at least 4 successive days.

In addition, responses were assessed for each organ system. An assessment of CR was assigned if the patient had stage 0 for any duration. Responses were *improved* if the severity decreased by at least 1 stage for the specific organ, *stable* if the stage for the organ system remained unchanged, and *progressive* if the organ system worsened by 1 stage or more. Any organ system uninvolved at baseline and remaining uninvolved during the study was considered *uninvolved*, as were organ systems assessed as stage 0 at baseline that worsened at any time during the study but returned to stage 0 before study completion. Patients whose disease fluctuated in one or more target organs throughout the study were categorized by best response. For example, a patient who had stage 1 skin involvement at study entry and whose condition temporarily worsened to stage 3 but then returned to stage 1 was considered stable.

Assessment of the development of chronic graft-versus-host disease

The development of chronic GVHD, defined by established criteria,[21] was assessed at each study visit beginning with study day 9. When patients completed or withdrew from the study, this assessment was completed as part of the long-term follow-up evaluation (telephone call to the patient every 6 months). No data were collected on the extent of chronic GVHD (limited vs extensive).

Assessment of safety

Safety was assessed in all patients who received any amount of ABX-CBL. Physical examinations, vital signs, and laboratory studies (complete blood cell counts, blood chemistries, and urinalysis) were performed at screening; just before treatment (baseline or day 0); on study days 1 through 6, 9, 13, 16, 20, 23, 30, 37, 44, and 72; and 100 days after HSCT. Blood was drawn for T- and B-cell analyses ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and CD4-CD8 ratio) at baseline, daily for 7 days, on study days 9 and 16, and at week 4, 6, and 10 visits. Creatine phosphokinase (CPK) III, the isoenzyme indicative of skeletal muscle damage, was assessed at baseline and again at any time a patient had myalgia. Patients were monitored for HAMA responses by enzyme-linked immunosorbent assay using blood samples drawn at baseline, day 9, and weeks 4, 6, and 10. A sample positive for HAMA was defined as a sample that showed 3 times the background optical density of normal human serum (0.25 OD or less) at a sample dilution of 1:20.

Pharmacokinetics

During the dose-finding portion of the study, in a randomly selected subset of patients (planned to be at least 5 patients in each cohort or at least 20 total), blood samples for PK analysis were obtained just before infusion and after completion of infusion at 15 and 30 minutes, 1, 2, 4, 12, 18, and 24 hours (before the second infusion), and 4 hours after completion of day 9 and day 20 infusions. An enzyme-linked immunosorbent assay was developed by Abgenix to measure levels of ABX-CBL in human serum. ABX-CBL was captured onto 96-well plates coated with goat anti–mouse IgM. ABX-CBL was detected by horseradish peroxidase conjugated to goat anti–mouse IgM. Tetramethylbenzidine was used as the chromogenic substrate, and absorbance was measured at 450 nm. The assay matrix contained 10% human serum in buffer. Serum concentrations of ABX-CBL were determined from a calibration curve fitted to a 4-parameter logistic function. The limit of quantification was 137 ng/mL serum. Analytical recovery ranged from 70% to 130%, with precision less than or equal to 30%.

Statistical methods

Efficacy endpoints of interest were response rate and survival. GVHD responses were assessed for all patients who received at least 4 infusions of ABX-CBL, as prescribed by study design. All other analyses were based on the intent-to-treat—ie, they included all patients who received at least one infusion of ABX-CBL. Because of the small numbers of patients in any dose group except 0.2 mg/kg, the data were summarized descriptively, and no formal analyses were made to compare the dose groups. Summaries comparing the presumed no-effect dose group (0.01 mg/kg) and the pooled 0.1 to 0.3 mg/kg dose groups (presumed active dose groups) are provided. The effects on survival of baseline IBMTR index and other demographic and disease characteristics were determined with a proportional hazards regression model using a significance level of 0.05 ($\alpha = 0.05$). Time-to-event data were summarized using Kaplan-Meier methods. For each patient, linear trends in the measurements of T- and B-cell function over time were calculated during the induction, maintenance, and posttreatment periods. Mean slopes during each period were analyzed using one-sample *t* tests for each dose group. Values are presented as mean ± SD unless otherwise stated. Statistical summaries and analyses were performed using SAS (Statistical Analysis System, Cary, NC) version 6.12 or higher.

Results

Patient and transplantation characteristics are summarized in Table 1. Actual duration of steroid therapy before the institution of ABX-CBL was 4 to 42 (median, 22) days. ABX-CBL treatment was started 20 to 236 (median, 47) days after transplantation. Three patients (all administered 0.2 mg/kg/d) received ABX-CBL therapy starting more than 100 days after HSCT. The mean number of ABX-CBL infusions was 8.6 (median 11, range 1-11). Forty-five (76%) patients completed the induction regimen of 7 infusions, and 36 (61%) patients received all 11 infusions prescribed.

Efficacy

Fifty-one (86%) patients received at least 4 infusions of ABX-CBL and, therefore, were included in the response assessment (Table 2).

Table 1. Patient and transplantation characteristics

Characteristic	ABX-CBL dose group (mg/kg)					All patients
	0.01	0.1	0.15	0.2	0.3	
No. patients	9	7	5	35	3	59
Median age, y (range)	43 (12-49)	32 (5-46)	31 (14-53)	37 (1-59)	27 (1-43)	36 (1-59)
Primary diagnoses (no. patients)						
Acute leukemia	4	2	2	13	2	23
Chronic leukemia	3	3	1	8	0	15
Lymphoma	1	0	1	2	0	4
Other	1	2	1	12	1	17
Conditioning regimen (no. patients)						
Contained TBI	6	4	5	20	2	37
No TBI	3	3	0	15	1	22
GVHD prophylaxis (no. patients)*						
CYA + MTX	6	5	3	16	2	32
FK506+	2	1	2	4	0	9
CYA+	0	0	0	10	1	11
MTX+	0	1	0	4	0	5
Other	1	0	0	1	0	2
Source of stem cells (no. patients)						
Marrow	7	7	3	31	3	51
PBSC	2	0	2	2	0	6
Cord blood	0	0	0	2	0	2
Donor (no. patients)†						
Related: HLA =	5	1	4	14	2	26
HLA ≠	1	1	0	2	0	4
Unrelated: HLA =	2	4	1	12	1	20
HLA ≠	1	1	0	7	0	9
IBMTR index (no. patients)						
B	2	2	1	7	2	14
C	3	2	2	14	1	22
D	4	3	2	14	0	23

TBI indicates total body irradiation; PBSC, peripheral blood stem cells; CYA, cyclosporine; MTX, methotrexate.
*CYA+, CYA plus other agent(s) except MTX; MTX+, MTX plus other agent(s) except CYA or FK506; FK506+, FK506 plus other agent(s).
†HLA-matching criteria used were those accepted at every institution at the time patients underwent transplantation.

Table 2. Treatment response by ABX-CBL dose

	ABX-CBL dose group (mg/kg)					
	0.01	0.1	0.15	0.2*	0.3*	All patients
Enrolled	9	7	5	36	2	59
Analyzed for efficacy	8	6	5	30	2	51
Extended response						
CR	1	1	0	4	2	8
PR	1	2	2	8	0	13
Best response						
CR	1	3	1	6	2	13
PR	2	1	1	9	0	13

*One of the patients originally assigned to receive ABX-CBL at 0.3 mg/kg had severe myalgia and received all subsequent doses at 0.2 mg/kg and, therefore, was evaluated with the 0.2 mg/kg dose group for survival and response.

Extended PR or CR occurred in 21 (41%; 8 CR, 13 PR) patients, including 19 of 43 (44%) patients who received 0.1 mg/kg or higher, and 2 of 8 patients (25%) who received 0.01 mg/kg. A best response of PR or CR occurred in 23 of 43 patients (53%) who received 0.1 mg/kg or higher, and 3 of 8 patients (38%) who received 0.01 mg/kg. Among patients who received at least 4 infusions of ABX-CBL at doses of 0.1 mg/kg or higher, those who responded by best-response criteria included 6 of 11 (55%) patients who had a baseline IBMTR index of B (all CR), 9 of 16 (56%; 3 CR, 6 PR) who had a baseline index of C, and 8 of 16 (50%; 3 CR, 5 PR) who had a baseline index of D. The probability of response (CR or improved) was highest in the skin and lowest in the liver (Table 3). Of the 21 patients who had an extended CR or PR, 5 had flares of GVHD (1 in the 0.1 mg/kg group and 4 in the 0.2 mg/kg dose group). In 2 of these patients, the IBMTR indices rose to pretreatment levels during flare but decreased again before the end of study.

Median survival time among patients given the presumed no-effect dose of 0.01 mg/kg was 20 days after the initiation of therapy with ABX-CBL compared with 117 days among patients treated with 0.1 to 0.3 mg/kg. One hundred, 180, and 450 days, respectively, after the start of ABX-CBL therapy, 28 (47%), 26 (44%), and 17 (29%) patients were surviving (Table 4). Kaplan-Meier estimates of survival among the 0.1 to 0.3 mg/kg cohorts were higher than among patients treated with 0.01 mg/kg ($P = .046$ by generalized Wilcoxon test) (Figure 1). There were no apparent dose-related trends among the 0.1 to 0.3 mg/kg dose groups in terms of responses or survival. Characteristics associated with survival were younger age ($P < .001$), unrelated (vs related) donor ($P = .006$), better baseline (day 1, preinfusion) Karnofsky-Lansky performance status ($P = .019$), and lower baseline total bilirubin ($P < .001$). Not significant were the underlying disease and the patient's baseline IBMTR index of GVHD. All but one of the 17 surviving patients have chronic GVHD.

Table 3. Response by organ system

	Skin		Liver		Upper GI		Lower GI	
Dose group*	0.01	0.1-0.3	0.01	0.1-0.3	0.01	0.1-0.3	0.01	0.1-0.3
Patients evaluated	5	27	4	21	5	26	5	21
CR								
n	1	14	0	4	1	16	2	13
%	20	52	0	19	20	61	40	62
Improved								
n	2	8	0	2	0	1	0	4
%	40	30	0	9	0	4	0	19
Stable								
n	1	3	2	6	4	6	1	3
%	20	11	50	29	80	23	20	14
Progressed								
n	1	2	2	9	0	3	2	1
%	20	7	50	43	0	12	40	5

*ABX-CBL dose group, mg/kg.
n indicates number of patients.

Table 4. Survival by 100, 180, and 450 days after the first infusion of ABX-CBL

	ABX-CBL dose (mg/kg)					
	0.01	0.1	0.15	0.2*	0.3*	All doses
No. patients treated	9	7	5	36	2	59
No. (%) patients alive						
at 100 days	2 (22)	4 (57)	3 (60)	18 (50)	1 (50)	28 (47)
at 180 days	2 (22)	3 (43)	2 (40)	18 (50)	1 (50)	26 (44)
at 450 days	2 (22)	3 (43)	1 (20)	10 (28)	1 (50)	17 (29)

*One of the patients originally assigned to receive ABX-CBL at 0.3 mg/kg had severe myalgia and received all subsequent doses at 0.2 mg/kg; the patient was, therefore, evaluated with the 0.2 mg/kg dose group for survival and response.

None of the deaths were considered related directly to the study drug. Immediate causes of death were mostly multi-organ system failure (11 of 42) and GVHD with or without infection (14 of 42) (Table 5). Among 37 patients who survived beyond day 100 after transplantation, 20 subsequently died, 7 with and 13 without clinical evidence of chronic GVHD. Overall, 10 deaths occurred among 21 patients with CR or PR, 25 among 30 patients who did not respond, and 7 among 8 patients who were not evaluable for response.

Safety

All but one patient had one or more adverse events. In 39 of these 58 (66%) patients, the investigator considered at least one event to be possibly or probably related to ABX-CBL. Thirty (51%) patients reported 34 serious adverse events, of which one (liver failure) was thought to be related to ABX-CBL, and 6 (all myalgias) were thought to be probably related. Severe myalgia was the dose-limiting toxicity necessitating the discontinuation of enrollment into the 0.3 mg/kg cohort and was the reason for a decrease in the dose of the last cohort to 0.2 mg/kg. Three patients (1 at 0.1 mg/kg and 2 at 0.2 mg/kg) withdrew from the study because of myalgia. One patient enrolled in the 0.3 mg/kg dose group had severe myalgia and, therefore, received all subsequent infusions at 0.2

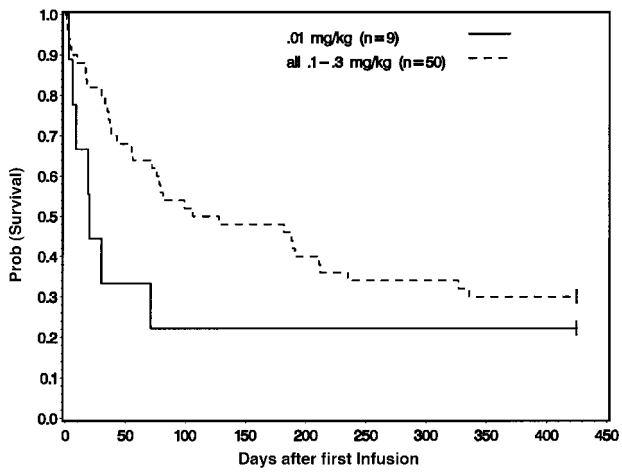

Figure 1. Kaplan-Meier estimate of survival after start of ABX-CBL therapy. Patients treated at 0.01 mg/kg per day (solid line) versus patients given doses of 0.1 to 0.3 mg/kg per day (broken line).

Table 5. Primary causes of death

Cause of death	No. patients			
	Responder	Nonresponder	Unevaluable for response	Total
Multiorgan system failure	1	7	4	12
GVHD (+infection)*	3 (1)	14 (4)	0	17 (5)
Hemorrhage/coagulopathy	2	2	1	5
Pneumonia	2	1	0	3
Relapse	2	0	0	2
Undetermined	0	0	2	2
PTLD	0	1	0	1

PTLD indicates posttransplant lymphoproliferative disorder.
*Seventeen patients died of GVHD; 5 of these also had systemic infections.

mg/kg. Myalgia characteristics are summarized in Table 6. All myalgias resolved without sequelae. Incidence, severity, and duration of myalgia increased as the dose of ABX-CBL was increased, and the onset to myalgia was earlier as the dose increased. All CPK III values were within the normal range (0-175 U/L), except for one patient in the 0.2 mg/kg group, who had myalgia on day 0 and had a CPK-III of 193 U/L on day 1. Based on protocol-specified definitions of the maximum tolerated dose as the highest dose with an observed incidence of dose-limiting toxicity of 25% or less, the maximum tolerated dose was determined to be 0.1 mg/kg.

Other adverse events reported included fever, hypotension, abdominal cramps, tachypnea, leg weakness and fatigue, increased heart rate, headache, chills, increased lactate dehydrogenase, edema, nonspecific numbness, and diaphoresis. Each of these events occurred in 3 or fewer patients. Details are provided in Table 7. No patient experienced a HAMA response. Fifty-one infections of various causes, ranging from mild to life-threatening, were reported in 25 patients. Infections in conjunction with worsening GVHD were considered primary causes of death in 5 patients; in 3 other patients, pneumonia was considered the primary cause of death (Table 5).

Analysis of lymphocyte subsets

Lymphocyte subset data were determined in 38 patients. The spectrum of expression of CD147 in healthy control subjects is described elsewhere.[15] No clinically significant trends were observed in T- and B-cell numbers for any dose group during any study period (induction, maintenance, posttreatment). No apparent dose-response effect on lymphocytes was observed, and no substantial decrease in any lymphocyte subset during induction or increase after treatment occurred during the study. Conceivably, the preceding and concurrent therapy with glucocorticoids modulated such an effect. No apparent differences among the dose groups were noted in the proportion of patients with $CD3^+$, $CD4^+$, $CD8^+$, or $CD19^+$ counts below cut-off values of clinical interest during any of the 3 study periods. Among patients treated with ABX-CBL at 0.2 mg/kg (the largest cohort), $CD4^+$ values decreased during induction in 6 of 27 (22%) patients and after treatment in 4 of 13 (31%) patients. No apparent ABX-CBL–related changes were observed overall in any of the 4 subpopulations of lymphocytes.

Pharmacokinetics

Sufficient blood samples were collected for pharmacokinetic analysis in 12 of 14 patients in the dose-finding portion of the study (Table 8). After infusion, serum ABX-CBL exhibited bi-exponential decay. Terminal half-life was 15 to 19 hours. The pharmacokinetics of ABX-CBL appeared linear across the dosing range. To assess the accumulation of ABX-CBL during the twice-weekly regimen, the 4-hour postdose concentrations on day 20 were compared with those on day 0. An accumulation ratio of approximately 2 was observed in individual patients. Mean ($\pm$ 1 SD) accumulation ratios based on the 4-hour postinfusion concentrations were 2.2 ± 0.93 ($n = 2$), 1.6 ± 0.21 ($n = 2$), and 2.3 ($n = 1$) for the 0.1, 0.2, and 0.3 mg/kg doses, respectively. With the small numbers of patients studied, no correlation of PK parameters and clinical events could be established, though there was a suggestion that myalgias developed more rapidly in patients given higher doses of ABX-CBL.

Discussion

Acute GVHD is a major cause of morbidity and mortality after allogeneic stem cell transplantation.[1,5] Acute GVHD requiring therapy develops in at least 30% of patients who received transplants of hemopoietic stem cells from HLA-identical sibling donors and in 50% to 80% of all patients who received cells from unrelated donors or HLA-mismatched related donors, despite GVHD prophylaxis with immunosuppressive agents such as methotrexate and cyclosporine.[1-3,12] The primary treatment for acute GVHD is corticosteroids, which results in improvement or resolution of the disease in approximately 20% to 50% of patients, depending on the organ systems involved.[3,10] Remaining patients require further treatment, and many die, frequently of infections associated with GVHD and immunosuppressive therapy.[2,3,9,10,21]

No therapeutic standard for steroid-refractory acute GVHD has been established. Treatment modalities include, among others, polyclonal (antithymocyte globulin) and monoclonal antibodies.[2,10-13] Despite initial response rates of 25% to 45%, durable

Table 6. Myalgia characteristics

	ABX-CBL dose in mg/kg (no. patients)					All patients (n = 59)
	0.01 (n = 9)	0.1 (n = 7)	0.15 (n = 5)	0.2 (n = 35)	0.3 (n = 3)	
Patients with myalgia (any severity)	0	3 (43%)	2 (40%)	22 (63%)	2 (66%)	29 (49%)
Mild	0	1 (14%)	0	1 (3%)	0	2 (3%)
Moderate	0	1 (14%)	1 (20%)	5 (14%)	0	7 (12%)
Severe	0	1 (14%)	1 (20%)	16 (46%)	2 (66%)	20 (34%)
Duration (min),	—	85	—	135	—	120
median (range)		(50-120)		(50-8446)		(50-8446)
		(n = 2)		(n = 9)		(n = 11)
Time to onset (minutes),	—	49	105	60	25	52.5
median (range)		(38-60)	—	(5-120)	(20-30)	(5-120)
		(n = 2)	(n = 1)	(n = 17)	(n = 2)	(n = 22)

Table 7. Nonmyalgia infusion-related adverse experiences

	ABX-CBL dose (mg/kg)				
	0.01	0.1	0.15	0.2	0.3
Cohort size	9	7	5	35	3
No. patients reporting an event	1 (11%)	2 (29%)	1 (20%)	13 (37%)	1 (33%)
No. events reported	1	2	1	17	1
Body system					
Cardiac	—	—	—	1, Increased heart rate (moderate)	1, Edema (severe)
Gastrointestinal	—	—	1, Abdominal cramp with diarrhea (moderate)	1, Abdominal pain after infusions (moderate)	—
General	1, Fever with chills (moderate)	1, Fever (moderate)	—	1, Chills (mild) 1, Weak legs and fatigue (moderate) 2, Fever with infusion (moderate) 1, Fever (severe)	—
Hepatobiliary	—	—	—	1, Including lactate dehydrogenase (mild) 1, Liver failure (maximum)	—
Neurologic	—	—	—	1, Headache (mild) 1, Nonspecific numbness (mild)	—
Respiratory	—	—	—	2, Tachypnea with infusion (moderate) 1, Chest tightness (moderate)	—
Vascular	—	1, Hypotension (moderate)	—	1, Transient drop in blood pressure (moderate) 1, Hypotension (moderate)	—
Skin and subcutaneous tissue	—	—	—	1, Severe diaphoresis with infusion (moderate)	—

None of these events occurred in more than 3 patients.
Myalgias are summarized in Table 6.

responses are less frequent.[2] Complete responses, however, are important because long-term survival among patients with sustained CR is superior to that in patients with PR or in nonresponders.[1-3,10]

ABX-CBL recognizes CD147, an antigen that is highly expressed on the surfaces of activated T and B lymphocytes and macrophages, monocytes, and dendritic cells.[15] Furthermore, the binding of ABX-CBL to $CD147^+$ cells induces complement-dependent cytolysis of these cells.[15] Activated T cells and antigen-presenting cells, however, play a central role in allogeneic interactions both in vitro and in vivo.[4,5] In fact, the murine IgM ascites formulation, CBL-1, from which ABX-CBL was derived, was developed as an antibody against activated T cells.[22] ABX-CBL inhibits the in vitro–mixed lymphocyte reaction that has long served as a model for GVHD.[16] The fact that ABX-CBL also depleted monocytes and dendritic cells suggests the possibility that ABX-CBL may prevent further lymphocyte activation by eliminating antigen-presenting cells. On this basis, ABX-CBL was an attractive agent to be tested for its activity in acute GVHD.

Among 51 patients evaluable for efficacy, extended PRs or CRs were observed in 21 (41%). Using the best response criteria, 26 (51%) patients responded. Organ failure, progressive acute GVHD, and infections associated with GVHD were the major causes of death, as they were in other GVHD therapy trials, recent or remote.[23-25] However, flares of GVHD generally responded to reinstitution of ABX-CBL therapy; 44% of patients were still living 6 months after the start of ABX-CBL therapy. These response rates and survival figures are comparable to those reported recently with a humanized monoclonal antibody directed at the α-chain of the interleukin-2 receptor.[13]

The dose-limiting toxicity of ABX-CBL was myalgia. Incidence, severity, and duration increased, and the time to onset of myalgia decreased with increasing doses of ABX-CBL. Such a finding may reflect the peak concentration of ABX-CBL reached, the area under the curve (AUC) of the antibody, or other parameters that affect pharmacodynamics. PK data suggest that daily dosing was appropriate. One might speculate that reducing the rate of ABX-CBL infusion would reduce the likelihood of myalgias. Although myalgias resolved without sequelae in all patients, they caused considerable morbidity, and treatment with narcotics was required. The mechanism responsible for myalgias is not understood. CD147 is expressed on endothelial cells of the blood–brain barrier, and an interaction between vascularization and neuronal differentiation has been suggested.[26,27] Although the occurrence of myalgias was traumatic for unprepared patients, informing the patient of this possibility before infusion of ABX-CBL and providing premedication with agents such as fentanyl rendered this complication manageable in most patients.

As discussed above, ABX-CBL eliminated $CD147^+$ cells. Surprisingly, however, lymphocyte subsets were not significantly altered during therapy. Presumably, activated cells expressing high levels of CD147 were lysed and were replaced by cells expressing only low levels of CD147, which were not susceptible to ABX-CBL. Such an explanation would certainly be plausible in patients whose GVHD resolved during therapy. However, all these patients were severely immunosuppressed before they received ABX-CBL, and any changes (or lack of changes) were likely to have been multifactorial in origin.

Table 8. Pharmacokinetic parameters

Dose group (mg/kg)	Value	C_{max} (ng/mL)	T_{max} (h)	AUC (0-∞) (ng/h · mL)	V_{ss} (mL/kg)	CL (mL/h · kg)	$t_{1/2}$ (h)
0.01 (n = 5)*	Mean	113	2.11	—	—	—	—
	SD	107	0.47	—	—	—	—
0.1 (n = 2)	Mean	304	3.46	5 713	377.2	27.39	14.6
	SD	90	1.47	4 854	28.7	23.27	12.1
0.2 (n = 4)	Mean	1087	2.31	15 952	342.7	13.20	18.5
	SD	716	0.25	3 914	153.3	3.68	4.0
0.3 (n = 1)	Individual	988	4.00	14 559	478.6	20.61	16.7

*In the 0.01-mg/kg dose group, C_{max} and T_{max} were determined in 5 subjects; data were insufficient to determine $AUC_{0-\infty}$, V_{ss}, CL, and $t_{1/2}$ for the 0.01 mg/kg dose.

In this high-risk population, an overall response rate of 53% among patients treated with ABX-CBL at doses greater than or equal to 0.1 mg/kg was encouraging, as were the day 100 and day 180 survival probabilities. ABX-CBL appears to be an effective agent for the treatment of steroid-refractory acute GVHD. Although myalgias were dose limiting, they were manageable with narcotics, and the drug appeared to be effective at levels below this dose-limiting toxicity. Based on the promising findings in this dose-finding trial, a randomized, prospective study to compare ABX-CBL with antithymocyte globulin in patients with steroid-refractory acute GVHD has been initiated.

Acknowledgment

We thank Terry Furlong for patient evaluation and data collection.

References

1. Deeg HJ, Yamaguchi M. Acute graft-versus-host disease. In: Atkinson K, ed. Clinical Bone Marrow and Blood Stem Cell Transplantation. Cambridge, United Kingdom: Cambridge University Press; 2000:681-699.
2. Martin PJ, Schoch G, Fisher L, et al. A retrospective analysis of therapy for acute graft-versus-host disease: secondary treatment. Blood. 1991; 77:1821-1828.
3. Weisdorf D, Haake R, Blazar B, et al. Treatment of moderate/severe acute graft-versus-host disease after allogeneic bone marrow transplantation: an analysis of clinical risk features and outcome. Blood. 1990;75:1024-1030.
4. Ferrara JL, Levy R, Chao NJ. Pathophysiologic mechanisms of acute graft-vs.-host disease [review]. Biol Blood Marrow Transplant. 1999;5: 347-356.
5. Blazar BR, Korngold R, Vallera D. Recent advances in graft-versus-host disease (GVHD) prevention. Immunol Rev. 1997;157:109-14493
6. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18:295-304.
7. Hill GR, Crawford JM, Cooke KR, Brinson YS, Pan L, Ferrara JL. Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. Blood. 1997;90:3204-3213.
8. Thomas ED, Storb R, Clift RA, et al. Bone-marrow transplantation. N Engl J Med. 1975;292:832-843, 895-902.
9. Hings IM, Severson R, Filipovich AH, et al. Treatment of moderate and severe acute GVHD after allogeneic bone marrow transplantation. Transplantation. 1994;58:437-442.
10. Martin PJ, Schoch G, Fisher L, et al. A retrospective analysis of therapy for acute graft-versus-host disease: initial treatment. Blood. 1990;76: 1464-1472.
11. Hebart H, Ehninger G, Schmidt H, et al. Treatment of steroid-resistant graft-versus-host disease after allogeneic bone marrow transplantation with anti-CD3/TCR monoclonal antibodies. Bone Marrow Transplant. 1995;15:891-894.
12. Khoury H, Kashyap A, Brewster C, et al. Anti-thymocyte globulin (ATG) for steroid-resistant acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation: a costly therapy with limited benefits [abstract]. Blood. 1999;94(suppl 1):668.
13. Przepiorka D, Kernan NA, Ippoliti C, et al. Daclizumab, a humanized anti–interleukin-2 receptor alpha chain antibody, for treatment of acute graft-versus-host disease. Blood. 2000;95:83-89.
14. Guo H, Majmudar G, Jensen TC, Biswas C, Toole BP, Gordon MK. Characterization of the gene for human EMMPRIN, a tumor cell surface inducer of matrix metalloproteinases. Gene. 1998;220:99-108.
15. Koch C, Staffler G, Huttinger R, et al. T cell activation-associated epitopes of CD147 in regulation of the T cell response, and their definition by antibody affinity and antigen density. Int Immunol. 1999;11:777-786.
16. Kinukawa T, Terasaki PI. Clonal deletion by a monoclonal antiblast antibody following primary mixed lymphocyte culture. Transplant Proc. 1985; 17:993-998.
17. Oei J, Cicciarelli J, Terasaki PI, Hardiwidjaja S, Mendez R. Treatment of kidney graft rejection with CHAL1 and CBL1 monoclonal antibodies. Transplant Proc. 1985;17:2740-2743.
18. Takahashi H, Okazaki H, Terasaki PI, et al. Follow-up on initial trials of kidney transplant rejection reversal by a monoclonal antiblast antibody. Transplant Proc. 1985;17:69-71.
19. Heslop HE, Benaim E, Brenner MK, et al. Response of steroid-resistant graft-versus-host disease to lymphoblast antibody CBL1. Lancet. 1995;346:805-806.
20. Rowlings PA, Przepiorka D, Klein JP, et al. IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. Br J Haematol. 1997;97:855-864.
21. Sullivan KM. Graft-versus-host-disease. In: Thomas ED, Blume KG, Forman SJ, eds. Hematopoietic Cell Transplantation. 2nd ed. Boston, MA: Blackwell Science; 1999:515-536.
22. Billing R, Wells J, Zettel D, Terasaki PI. Monoclonal and heteroantibody reacting with different antigens common to human blast cells and monocytes. Hybridoma. 1982;1:303-311.
23. Goldman JM, Gale RP, Horowitz MM, et al. Bone marrow transplantation for chronic myelogenous leukemia in chronic phase: increased risk of relapse associated with T-cell depletion. Ann Intern Med. 1988;108:806-814.
24. Hale G, Cobbold S, Waldmann H. T cell depletion with Campath-1 in allogeneic bone marrow transplantation. Transplantation. 1988;45:753-759.
25. Martin PJ, Hansen JA, Buckner CD, et al. Effects of in vitro depletion of T cells in HLA-identical allogeneic marrow grafts. Blood. 1985;66:664-672.
26. Schlosshauer B. Neurothelin: molecular characteristics and developmental regulation in the chick CNS. Development. 1991;113:129-140.
27. Schlosshauer B, Herzog KH. Neurothelin: an inducible cell surface glycoprotein of blood-brain barrier-specific endothelial cells and distinct neurons. J Cell Biol. 1990;110:1261-1274.