BRIEF ARTICLES

Alemtuzumab as Treatment of Steroid-Refractory Acute Graft-versus-Host Disease: Results of a Phase II Study

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We conducted a phase II trial to investigate the safety and efficacy of alemtuzumab in treating steroid-refractory acute graft-versus-host disease (aGVHD) grade II or higher after stem cell transplantation. Ten adult patients (6 with aGVHD grade III and 4 with aGVHD grade IV) were included in the study. Nine patients had gastrointestinal tract involvement, 7 had skin involvement, and 5 had liver involvement. Five patients responded to treatment, 2 with complete response and 3 with partial response. Eight infectious events (4 of grade 3-4) and 7 cytomegalovirus (CMV) reactivations were observed. Six patients had grade 3-4 cytopenia. All 10 patients died (7 resulting from aGVHD progression, 2 from severe infection, and 1 from to leukemia relapse), at a median of 40 days (range, 4 to 88 days) after alemtuzumab treatment. Overall, our findings suggest that steroid-refractory aGVHD may be improved by treatment with alemtuzumab, but that this treatment does not overcome the dismal prognosis of patients with severe aGVHD, demonstrating the need for alternative therapies to treat this complication.

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INTRODUCTION

Acute graft-versus-host disease (aGVHD) remains a major problem in allogeneic stem cell transplantation (SCT), especially in patients who do not respond to standard initial treatment with corticosteroids [1,2]. Steroid-refractory aGVHD is associated with a high rate of morbidity and mortality, primarily from infection and/or multiorgan failure. Although various agents for treating steroid-refractory aGVHD have been tested, including high-dose steroids, antithymocyte globulin (ATG), mycophenolate mofetil (MMF), tacrolimus, sirolimus, etanercept, pentostatin, and several monoclonal antibodies (mAb), none has been established as an effective salvage therapy [1,2].

Alemtuzumab (Campath-1H) is a humanized, unconjugated IgG1 kappa mAb that is specific for CD52 receptors present on mature T and B lymphocytes, monocytes, monocyte-derived dendritic cells (DCs), macrophages, and eosinophils [3,4]. It is well known that the induction of aGVHD requires the presentation of host antigens to donor T cells by antigen-presenting cells (APCs), such as DCs [5-8]. It also is known that alemtuzumab prevents the development of aGVHD and chronic GVHD (cGVHD) in SCT by depleting T cells and DCs from both the donor and the recipient [9-13]. Little data are available on the efficacy of alemtuzumab for treating established aGVHD, however. Here we report a prospective clinical phase II trial conducted to evaluate the safety and efficacy of alemtuzumab as salvage treatment for patients with aGVHD failing primary steroid therapy.

METHODS

Eligibility Criteria

This study was a multicenter phase II trial. The primary endpoint was overall response rate; secondary

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endpoints were toxicity, incidence of infections and posttransplantation lymphoproliferative disorders, and overall survival (OS). Patients were eligible if they were aged ≥18 years, had undergone allogeneic SCT from either a sibling or unrelated donor (matched or mismatched, bone marrow or peripheral blood stem cells [PBSCs], with a myeloablative or reduced-intensity conditioning [RIC] regimen), and had a diagnosis of grade II or higher steroid-refractory aGVHD. Patients who had an uncontrolled infection or a relapse of primary hematologic disease, had received alemtuzumab as part of GVHD prophylaxis 30 days before the diagnosis of steroid-refractory aGVHD, or had been treated with another savage therapy were excluded from this study. Ethical approval was obtained from each participating institution before the trial began. Every participant provided written informed consent before entering into the study.

Diagnosis of Steroid-Refractory aGVHD and Evaluation of Response

The assessment and grading of aGVHD were based primarily on clinical findings. Diagnosis was supported by skin, liver, or gastrointestinal (GI) tract biopsy results whenever indicated and clinically possible. Symptoms were graded based on standard clinical criteria and on the International Bone Marrow Transplant Registry severity index [14-16]. Steroid-refractory aGVHD was defined as progression of aGVHD in the first 72 hours, lack of improvement after 7 days of steroid therapy at a dosage of 2 mg/kg/day, or incomplete response after 14 days of this steroid therapy. Patients who achieved a complete response (CR) or a partial response (PR) after steroid therapy and experienced a relapse with the first taper of steroids also were eligible for the study.

Response to alemtuzumab therapy was evaluated and recorded prospectively on days 7, 14, 21, and 28, and at the last follow-up after initiation of therapy. Organ stage scores, overall clinical grade, and relevant differential diagnosis were recorded at each evaluation. CR was defined as the complete resolution of all aGVHD symptoms in all organs. To be considered a CR, this score had to be maintained for 28 days. PR was defined as durable (≥ 28 more days) improvement in aGVHD stage in all initial target organs without complete resolution and without worsening in any other aGVHD target organ. No response (NR) was defined as maintenance of the same grade of aGVHD or progression of aGVHD in any organ. Progression was defined as worsening aGVHD in one or more organs with or without amelioration in any organ.

Alemtuzumab Treatment and Anti-Infective Prophylaxis

Alemtuzumab was administered i.v. at a dose of 10 mg/day for 5 consecutive days, followed by 10 mg/day once weekly on days 8, 15, and 22 if CR was not achieved. Acetaminophen, diphenhydramine, and methylprednisolone were given as prophylaxis for the secondary effects of alemtuzumab. The administration of cyclosporine (CsA) and/or MMF continued during the alemtuzumab treatment, whereas corticosteroids were slowly tapered in those patients achieving response (2 mg/kg on days 1 to 7, 1.5 mg/kg on days 8 to 15, and a 10% reduction every week thereafter).

Anti-infective prophylaxis was administered according to the policy of each individual center; however, the following drugs were strongly recommended: trimethoprim-sulfamethoxazol or nebulized pentamidine for *Penumocystis jiroveci* pneumonia; fluconazol, voriconazol, or itraconazol for fungal infections; and acyclovir for herpes virus reactivation. Cytomegalovirus (CMV) infection was monitored once or twice weekly by CMV pp65 antigenemia and/or quantitative CMV polymerase chain reaction testing; if the results were positive, then the patient was treated preemptively with gancyclovir, valgancyclovir, or foscarnet.

RESULTS

Patient Characteristics

Ten patients from 6 Spanish centers were enrolled in this trial between March 2007 and April 2008. Patient characteristics are summarized in Table 1. All patients received peripheral blood as the stem cell source, and 6 received allogeneic SCT from unrelated donors. GVHD prophylaxis consisted of CsA in combination with methotrexate (MTX) for the patients receiving a myeloablative conditioning regimen (n = 3) or in combination with MMF for those receiving a fludarabine (Flu)-based RIC regimen (n = 7). The median time from allogeneic SCT to diagnosis of aGVHD was 27.5 days (range, 9 to 144 days). Two 2 patients developed acute manifestations of GVHD beyond day 100 posttransplantation. All patients had severe aGVHD (6 with grade III and 4 with grade IV). Nine patients had GI tract involvement, 7 had skin involvement, and 5 had liver involvement. Eight patients had involvement of 2 or 3 organs (Table 2). Nine patients received alemtuzumab therapy because of a failure of steroid therapy according to the definition of refractory aGVHD, and only 1 patient received alemtuzumab because of aGVHD flare after tapering of steroid therapy. The median time from diagnosis of aGVHD to the initiation of alemtuzumab therapy was 8 days (range, 4 to 22 days). One patient received only the first 4 daily doses of alemtuzumab, because of aGVHD progression and death. Nine patients received at least the first 5 doses of alemtuzumab; 4 received 6 doses, and 2 completed the scheduled treatment of 8 doses. The primary reasons for incomplete treatment were progression of aGVHD and clinical impairment.

Table 1. Patient Characteristics

Characteristic	n
Age, years, median (range)	57 (19-65)
Sex, females/males	3/7
Diagnosis	
Acute leukemias	4
Lymphoproliferative disorders	6
Donor	
HLA-identical sibling	4
HLA-identical unrelated	5
HLA-mismatched unrelated	1
Conditioning regimen	
Myeloablative	3
Reduced intensity	7
Source of stem cells	
Peripheral blood	10
GVHD grade	
III/IV	6/4
B/C/D	3/2/5
Organs involved	
Skin	7
GI	9
Liver	5
Eastern Cooperative	
Oncology Group (ECOG) score	
1-2	5
3-4	3
Not reported	2

Clinical Response, Safety, and Outcomes

One patient died of gut and hepatic aGVHD on day 4 after initiation of alemtuzumab therapy and could not be evaluated for response. Two CRs and 3 PRs were observed, for a clinical response rate of 55%. The 2 patients who achieved CR died, 1 of *Klebsiella pneumoniae* sepsis at 43 days after alemtuzumab treatment and 1 of acute myelogenous leukemia (AML) relapse at 188 days after alemtuzumab treatment. The 3 patients who achieved PR also died, 2 from aGVHD and multiorgan failure at 29 and 93 days after alemtuzumab treatment and 1 from invasive fungal infection at 44 days after alemtuzumab treatment (Table 2). No correlation was observed between the number of doses of alemtuzumab and response (*t*-test and linear regression; $P > .05$); however, the small number of patients included in this study, and the early death of some, precludes us from drawing any conclusions regarding alemtuzumab dosing. All nonresponding patients died of aGVHD progression after a median of 20 days (range, 15 to 42 days). A total of 8 infectious events (4 of which were grade 3-4) and 7 episodes of CMV reactivation were reported. Grade 3-4 anemia, neutropenia, and lymphopenia occurred in one patient each, and thrombocytopenia occurred in 2 patients. One patient presented with grade 3 pancytopenia. No patient had a lymphoproliferative disorder. After obtaining these results, we decided to close the trial prematurely.

DISCUSSION

To date, only a few reports have investigated the efficacy of alemtuzumab in treating steroid-refractory aGVHD. Carella et al. [17] reported successful treatment of steroid-refractory aGVHD with alemtuzumab in 3 patients. One of these patients, a 53-year-old female with grade II-III aGVHD of the skin, GI tract, and liver, treated with 4 doses of alemtuzumab, demonstrated improvement in all organs evaluated; however, she died of renal and cardiac failure (with the renal failure attributed to ganciclovir therapy) on day 106 after undergoing SCT. The second patient, a 46-year-old male with grade III-IV aGVHD involving the GI tract and liver, achieved CR after 4 doses of alemtuzumab. The third patient, a 44 year-old female, developed grade III aGVHD of the liver and achieved PR after 5 doses of alemtuzumab. Wandroo et al. [18] reported 2 patients, with steroid-refractory grade IV aGVHD with liver involvement, who responded to 5 doses of alemtuzumab (10 mg/day).

To the best of our knowledge, to date only one prospective study has addressed the use of alemtuzumab to treat steroid-refractory aGVHD. Gómez-Almaguer et al. [19] reported 18 patients treated with alemtuzumab administered s.c. at a dose of 10 mg/day for 5

Table 2. Results of Alemtuzumab Treatment in Patients with Steroid-Refractory aGVHD

Patient	Donor	Days to Initiation of Alemtuzumab*	aGVHD Stage	Skin Stage	Liver Stage	GI Stage	Response to Alemtuzumab	Death	Cause	Overall Survival†
1	MUD	4	III/C	+	+	+++	PD	Yes	GVHD	16
2	MUD	22	IV/D	0	0	++++	CR	Yes	Bacterial infection	43
3	MUD	6	IV/D	0	++++	0	PR	Yes	GVHD + liver failure	39
4	MSD	10	III/B	+	0	++	PD	Yes	GVHD	24
5	MMUD	12	IV/D	++++	0	+	PR	Yes	GVHD + multiorgan failure	93
6	MSD	4	III/D	0	+	++++	NE	Yes	GVHD	4
7	MUD	8	III/B	++	0	++	PD	Yes	GVHD	42
8	MSD	8	III/B	++	++	+	PR	Yes	IFI	44
9	MUD	9	IV/D	++	0	++++	NR	Yes	GVHD + IFI	15
10	MSD	5	III/C	++	++	+++	CR	Yes	Leukemic relapse	188

aGVHD indicates acute graft-versus-host disease; MUD, matched unrelated donor; MSD, matched sibling donor; MMUD, mismatched unrelated donor; PD, progressive disease; NE, not evaluable; NR, no response; IFI, invasive fungal infection.

*Time from diagnosis of aGVHD to initiation of alemtuzumab.

†Time from alemtuzumab treatment to death.

consecutive days. Eight patients had grade II aGVHD, 8 had grade III aGVHD, and 2 had grade IV aGVHD. The main organs involved were the liver in 4 patients, GI tract in 5, skin in 3, skin and liver in 3, and skin and GI tract in 3. The overall response rate was 83% and the CR rate was 33%, higher than that found in our trial. Ten of the 15 patients who responded to alemtuzumab therapy were alive at a median follow-up of 11 months (range, 3 to 24 months). In our study, 55% of the patients responded to alemtuzumab, but all of the patients because of to aGVHD progression, infection, or relapse. Several differences between our study and that of Gómez-Almaguer et al. [19] may account for these discrepant results; for example, in our trial, the proportion of patients receiving stem cells from an unrelated donor and developing steroid-refractory aGVHD within the first 100 days after SCT was higher (60% vs 80%), and the patients were older (median age, 57 years [range, 19 to 65 years] vs 37 years [range, 1 to 59 years]) and had more advanced GVHD (grade III-IV, 100% vs 56%).

Overall, our findings suggest that although alemtuzumab therapy may improve steroid-refractory aGVHD, it does not overcome the dismal prognosis of patients with severe steroid-refractory aGVHD. However, in view of the small number of patients reported here, our findings do warrant further investigation of alemtuzumab in a larger patient group to better define its application in this clinical situation.

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