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Response of Steroid-Refractory Acute GVHD to α_1-Antitrypsin

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α_1-Antitrypsin (AAT) is a serine protease inhibitor with anti-inflammatory, antiapoptotic, and immunomodulatory properties. It has therapeutic efficacy in animal models of autoimmune diseases, inflammatory disorders, and transplantation. In a phase I/II open-label single-center study, we administered AAT (Glassia; Baxalta/Kamada, New Ziona, Israel) as salvage therapy to 12 patients with steroid-refractory acute graft-versus-host disease (GVHD). AAT was given i.v. at 2 dose levels over a 15-day course. All patients had grades III or IV GVHD with stage 4 gut involvement. After treatment, plasma AAT levels increased in both cohorts and remained within 2 to 4 mg/mL for the duration of treatment. No clinically relevant toxicities attributable to AAT were observed. GVHD manifestations improved in 8 of 12 patients, and 4 responses were complete. Six patients (50%) were alive at last follow-up (>104 to >820 days). These findings show that AAT is well tolerated and has efficacy in the treatment of steroid-refractory severe acute GVHD. Further studies are warranted.

INTRODUCTION

Considerable progress has been achieved with allogeneic hematopoietic cell transplantation (HCT) [1]. However, graft-versus-host disease (GVHD) remains a problem, developing in an acute form in 30% to 70% of patients despite prophylaxis with immunosuppressive agents [2]. The use of post-transplant administration of cyclophosphamide has reduced the incidence but has not eliminated GVHD [3].

Glucocorticoids (steroids), typically methylprednisolone given at doses of .5 to 2 mg/kg/day, have remained frontline therapy of acute GVHD. Approximately 40% of patients achieve satisfactory responses, and steroids can be tapered off without significant flares of GVHD. The likelihood of response depends on the primary organ involvement and the severity of GVHD manifestations [4]. Severe involvement of the gastrointestinal tract, in particular, has proven challenging to treat, with a fatality rate of 80% in steroid-nonresponsive patients [5]. Various agents, including antithymocyte globulin, monoclonal antibodies, extracorporeal photopheresis, and other strategies, have been used to treat steroid-refractory GVHD but have met with only partial success [2,6].

In murine models, we and others have shown that the administration of α_1-antitrypsin (AAT), an abundant serine protease inhibitor with anti-inflammatory properties, is able to suppress the development of GVHD or provide effective therapy of established GVHD [7-9]. Treatment with AAT was associated with marked changes in the expression of proinflammatory cytokines such as IL-1, tumor necrosis factor, and IL-32, which are involved in the pathophysiology and clinical manifestations of GVHD [10]. Furthermore, mice exposed to AAT showed increased numbers of $CD4^+CD25hi$ $FoxP3^+$ regulatory T cells and $CD8^+CD205^+$ dendritic cells in comparison with albumin-treated controls, suggesting a tolerogenic effect [11]. Further, AAT has been used successfully in the treatment of children with recent-onset type 1 diabetes [12] and in patients with myocardial Infarction [13].

Based on those observations, we conducted a prospective phase I/II dose-escalation study to evaluate the clinical tolerability and potential efficacy of AAT in the treatment of

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patients with steroid-refractory acute GVHD. This report describes our experience after treatment of the first 12 patients (cohorts 1 and 2).

METHODS

Study Objectives

The study objectives were to determine the safety and tolerability of AAT as salvage therapy in patients with steroid-refractory acute GVHD, characterize pharmacokinetic and pharmacodynamic effects of AAT on proinflammatory cytokines and the spectrum of peripheral blood T cells, and estimate clinical responses of steroid-refractory acute GVHD to AAT.

Patients

Patients were enrolled from December 2013 through May 2015. Eligible were patients who had undergone allogeneic HCT from related or unrelated donors after various conditioning regimens and who had developed acute GVHD that did not show clinical responses to the administration of i.v. methylprednisolone at 2 mg/kg/day, given for a minimum of 5 days. All patients underwent upper and lower gastrointestinal endoscopies to confirm histologic gut involvement by GVHD and to determine the extent of GVHD. Patients who had received systemic therapy for acute GVHD other than steroids were excluded, as were patients with manifestations of chronic GVHD or acute/chronic GVHD overlap syndrome, recurrent hematologic malignancy, severe organ dysfunction, or uncontrolled infection. All patients provided written consent as required by the Fred Hutchinson Cancer Research Center Institutional Review Board.

Patient characteristics (cohorts 1 and 2 of the study, 6 patients per cohort) are summarized in Table 1. Patients were 26.8 to 73.3 (median 50) years old and weighed 64.0 to 118.9 kg (mean ± standard deviation, 85.9 ± 19.6 kg). Nine patients had received their first transplant and 3 patients their second transplant. Eight patients were transplanted from unrelated donors and 4 from HLA-matched siblings. In 10 patients the source of stem cells was peripheral blood (granulocyte colony-stimulating factor mobilized), whereas 2 patients received HLA nonidentical cord blood cells.

Treatment Plan and Protocol Design

This was a single-center, open-label, dose-escalation study of AAT, which enrolled 6 patients at each dose level, subject to predetermined dose escalation, de-escalation, and stopping rules for toxicity. The protocol design and dosing scheme drew on information derived from the Immune Tolerance Network and the treatment of patients with other diseases, in particular, type 1 diabetes [14]. In cohort 1 the loading dose of AAT (Glassia; Baxalta/Kamada, New Ziona, Israel) was 90 mg/kg on day 1, followed by maintenance doses of 30 mg/kg/day on days 3, 5, 7, 9, 11, 13, and 15. In cohort 2 the loading dose was 90 mg/kg on day 1, followed by 7 maintenance doses of 60 mg/kg/day on the same schedule. AAT was administered i.v. at a rate of .04 mL/kg/min.

Escalation to a higher dose level was considered if ≤2 patients experienced toxicity. If ≥3 of 6 patients met toxicity criteria in cohort 1 but there was evidence of clinical improvement of GVHD, the dose of AAT was de-escalated (cohort 0) as follows: 60 mg/kg on day 1 and 15 mg/kg for each subsequent dose. If toxicity met stopping criteria (toxicity in ≥3 patients) and there was no evidence of clinical efficacy in cohort 1, the trial was to be closed. If ≥3 (of 6) patients experienced toxicity at *any* dose level, the next lower dose was to be the maximum tolerated dose. Dose-limiting toxicities and events that would prompt study closure were defined in the protocol.

During protocol treatment administration of GVHD *prophylaxis* according to the patient's primary transplant protocol continued. Steroids, given as initial *therapy* for GVHD, were also continued through completion of the AAT course, but the dose was allowed to be tapered at a rate to be determined by the attending physician. Antibiotic prophylaxis and other supportive care were given according to institutional practice guidelines. The study is registered as NCT01523821.

Assessment of Response and Toxicity

Responses to AAT were assessed sequentially, in 2 ways, with a *final* assessment on day 28 after initiating therapy with AAT. First, overall responses were measured using criteria established by the Center for International Blood and Marrow Transplant Research (CIBMTR) [15]. Second, using criteria derived from the Acute GVHD Activity Index [16], responses in the gastrointestinal tract were determined. By CIBMTR criteria a complete response was defined as a GVHD score of zero in all assessable organs. A partial response was defined as improvement in 1 or more organs without progression in others. Improvement was defined as at least a 1-point reduction in the organ stage. For a response to be scored as sustained complete response or partial response, the patient could not have received systemic treatment for acute GVHD other than steroids and AAT before study day 28.

In determining responses of gut GVHD, patients were considered to have achieved a complete response if they were able to receive sufficient calories by mouth, not requiring parenteral nutrition, and passed primarily formed stools. A partial response was defined as a decrease in the requirement of parenteral nutrition to less than 50% of needed calories or a reduction of stool volume by greater than 50% for patients with baseline volumes more than 500 mL/day and without ileus.

All patients were scored for response regardless of receiving all 8 doses of AAT therapy or not (intent to treat analysis). Patients were monitored for adverse events throughout treatment and until day 28 after initiation of AAT therapy. Adverse events were to be scored for severity using the criteria defined in the Common Terminology Criteria for Adverse Events, Version 4.0. Specific labs were collected to monitor for the occurrence of coagulopathies and hepatic dysfunction. A Data Safety Monitoring Board provided study oversight.

Pharmacokinetic and Correlative Studies

Pharmacokinetics

Blood samples. AAT blood levels were determined at 24 and 48 hours after the infusion of AAT. AAT concentrations were determined by ELISA using 96-well polystyrene plates coated overnight at 4°C with .5 μg/mL mouse anti-human AAT (R&D Systems, Minneapolis, MN) in 50 mM Na carbonate, pH 9.5.

Stool samples. Stool content of AAT was determined before initiation of treatment and on days 3, 7, 11, and 15. For each collection time point, stool volume was determined over a 24-hour interval. AAT concentrations were measured by a standard nephelometric technique (Children's Hospital, Seattle, WA). AAT clearance (C) was calculated as follows: $C = F \times W/p$, where F is the fecal AAT concentration (mg/g), W is the daily stool weight,

Table 1
Patient Characteristics, Diagnoses, and Transplant Regimens

Patient ID	Number of Transplant	Age (yr)	Sex	Diagnosis	Donor	HLA Match	Source of Stem Cells	Conditioning Regimen	GVHD Prophylaxis
Cohort 1									
1	1	36.5	M	MDS	NR	Y	PBSC	CY, H-TBI (1200)	TAC+MTX
2	1	57.7	M	AML	Sibling	Y	PBSC	CLOFAR, TBI (200)	CSP+MMF
3	1	58.3	F	CLL	NR	Y	PBSC	FLU, TBI (200)	CSP+MMF
4	2	59.3	M	NHL	NR	Y	PBSC	FLU, TBI (200)	CSP+Siro+MMF
5	1	35.7	M	ALL	NR	N	Cord	CY, LI (400), FLU, H-TBI (1320)	CSP+MMF
6	2	50.2	F	ALL	NR	N	Cord	CY, TEPA, FLU, F-TBI (400)	CSP+MMF
Cohort 2									
7	1	49.9	M	AML	NR	Y	PBSC	BU, CY	TAC+MTX
8	1	73.3	M	AML	NR	Y	PBSC	BC8SA, RDB, FLU, TBI (200)	CSP+MMF
9	2	26.8	M	NHL	Sibling	Y	PBSC	FLU, TBI (200)	CSP+MMF
10	1	29.0	M	ALL	NR	Y	PBSC	CY, H-TBI (1200)	TAC+MTX
11	1	69.7	M	MDS	Sibling	Y	PBSC	TREO, FLU	CSP+MMF
12	1	44.4	M	AML	Sibling	Y	PBSC	TREO, FLU, TBI (200)	TAC+MMF

ALL indicates acute lymphocytic leukemia; AML, acute myeloid leukemia, BC8SA, streptavidin-conjugated anti-CD45 antibody; BU, busulfan; CLOFAR, clofarabine, CSP, cyclosporine; CY, cyclophosphamide; F, female; FLU, fludarabine; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; H-TBI, hyperfractionated total body irradiation; LI, localized irradiation; M, male; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MTX, methotrexate; NHL, non-Hodgkin lymphoma; PBSC, peripheral blood stem cells; RDB, radio-labeled DOTA-biotin; siro, sirolimus; TAC, tacrolimus; TBI, total body irradiation; TEPA, thiotepa; TREO, treosulfan; Y, yes; N, no; Tx, transplant; 1, first; 2, second.