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Phase I Study of Urate Oxidase in the Reduction of Acute Graft-Versus-Host Disease after Myeloablative Allogeneic Stem Cell Transplantation

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Graft-versus-host disease (GVHD) is a donor T cell driven response against host tissue that can complicate allogeneic hematopoietic stem cell transplantation (HSCT). During acute GVHD, endogenous adjuvants such as uric acid are released by damaged host tissue, activating alloreactive donor T cells. A phase I study was conducted at the Massachusetts General Hospital between 2007 and 2010 to test the hypothesis that reduction of uric acid levels during allogeneic HSCT can modulate the development of acute GVHD. Twenty-one patients with hematologic malignancies in complete remission undergoing myeloablative peripheral blood HSCT received recombinant urate oxidase at .20 mg/kg for 5 consecutive days during conditioning. Results were compared with all patients who underwent allogeneic HSCT at our institution during the same time period who met the same inclusion and exclusion criteria but were not enrolled in the study. The only major adverse event was a case of hemolytic anemia in a patient who had glucose-6-phosphate dehydrogenase deficiency. Primary outcome was the cumulative incidence of grades II to IV acute GVHD, which was significantly decreased in the treatment group in the intention-to-treat analysis (57% [12/21] versus 24% [5/21], $P = .036$) and in the per-protocol analysis ($P = .017$). Patients who developed acute GVHD had a higher level of serum uric acid during the pretransplantation period compared with those who did not ($P < .001$). There was no difference in disease-free or overall survival. Our study suggests that urate oxidase can be safely administered during myeloablative conditioning and may reduce the incidence of acute GVHD.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) offers the best chance to cure many hematologic malignancies and serves to both rescue the recipient bone marrow after conditioning and provide adoptive immunotherapy against residual tumor cells. Although enabling a graft-versus-tumor effect, allogeneic HSCT can also facilitate the development of graft-versus-host disease (GVHD), a donor T cell–driven immunologic response against healthy host tissue that can cause significant morbidity and mortality [1]. The risk of acute GVHD (aGVHD), which is often epidemiologically defined as disease occurring within the first 100 days of transplantation [2], has been estimated to range from 20% to 60%, depending on factors such as intensity of conditioning, type of graft, GVHD prophylaxis regimen, and varying degrees of donor parity, sex matching, and recipient age [3,4].

Molecular insight into the pathogenesis of aGVHD is critical toward the development of novel strategies to prevent or mediate its occurrence. The underlying mechanism of GVHD is complex and is represented by a positive feedback loop culminating in T cell–mediated destruction of host tissue. In the acute setting, the initiation of GVHD is thought to involve the stimulation of antigen presenting cells (APCs) through exposure of the immune system to endogenous "danger signals" from dying cells, which can result from the cytotoxic effects of conditioning regimens, or to pathogen-associated molecular patterns from translocation of gut bacteria [2]. Signaling through APC costimulatory molecules coupled with antigen presentation subsequently induce the expansion of alloreactive donor T cells that recognize mismatched minor histocompatibility complexes and MHCs, leading to T cell–induced apoptosis of host tissue, which causes further release of endogenous adjuvants that aggravates the cycle [1,5].

In this study we wanted to examine the feasibility and potential effect of suppressing the activation of APCs and thereby break the GVHD cycle by targeting an important endogenous "danger signal." It has long been known that even in the absence of exposure to microbial products, immune stimulation can occur when dying cells release certain danger signals that act as endogenous adjuvants. However, only recently has a specific soluble factor been identified to directly mediate this process. By isolating cytosol from dying

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cells and serially examining fractionated subsets for adjuvant activity, Shi et al. [6] identified uric acid as one such critical factor released from injured cells. When co-injected with antigen in vivo, purified uric acid significantly enhanced CD8+ T cell response in splenocytes of mice, whereas elimination of the uric acid through the use of allopurinol or uricase inhibited activation of cytotoxic T lymphocytes [6]. They also demonstrated that in vitro exposure of bone marrow–derived dendritic cells to uric acid can induce upregulation of costimulatory molecules CD80 and CD86. Other studies have further demonstrated the potential of suppressing uric acid to blunt APC activation and T cell response against foreign antigens, in 1 case using tumor xenograft models in mice [7] and in another by using a transgenic system expressing exogenous antigens [8].

Taken in the context of our current understanding of aGVHD, these preclinical results suggest that lowering uric acid levels during the conditioning period before transplantation may suppress the activation of host APCs and therefore alter the course of aGVHD. We sought to test this hypothesis through the administration of rasburicase (Elitek/Fasturtec; Sanofi, Paris, France), a recombinant urate oxidase enzyme that catalyzes the oxidation of uric acid into allantoin, an inactive and soluble metabolite. Rasburicase has been shown in multiple clinical trials conducted in cancer patients to be greater than 98% effective at reducing hyperuricemia [9-11]. The safety profile has been well established in adults at a dose up to .20 mg/kg/day for 5 to 7 consecutive days with minimal side effects except for rare instances of hemolytic anemia and methemoglobinemia, which have occurred in patients with glucose-6-phosphate dehydrogenase deficiency [9]. The use of rasburicase as a potential therapy to prevent aGVHD has not been studied thus far.

Based on these observations, we developed a phase I clinical trial that served as both a feasibility study for using rasburicase in the setting of bone marrow transplantation and to preliminarily test its ability to reduce the incidence of aGVHD among patients undergoing myeloablative allogeneic HSCT. We compared results to matched control subjects obtained from chart record review including all patients in the same time period who were not in the study but who were deemed eligible based on inclusion and exclusion criteria.

METHODS

Institutional review board approval by the Dana-Farber Cancer Institute/Partners Cancer Care was obtained on April 17, 2007. Twenty-one patients with hematologic malignancies in complete remission who received myeloablative preparative regimens followed by granulocyte colony-stimulating factor–mobilized HLA-matched peripheral blood HSCT were enrolled in a pilot trial between 2007 and 2010 at the Massachusetts General Hospital. All transplants were derived from 8/8 HLA allele (A, B, C, DRB1) matched related or 8/8 HLA allele matched unrelated donors.

Conditioning regimens were chosen before study enrollment and based on individual investigator's discretion. These included myeloablative doses of either (1) busulfan (3.2 mg/kg/day from day −7 to day −4) and cyclophosphamide (60 mg/kg/day on day −3 and day −2), (2) busulfan (3.2 mg/kg/day from day −6 to day −3) and fludarabine (40 mg/m^2/day from day −6 to day −3), or (3) cyclophosphamide (60 mg/kg/day on day −3 and day −2) and total body irradiation (13.2 Gy over 8 fractions from day −7 to day −4). GVHD prophylaxis was also chosen based on individual physician's discretion and consisted of cyclosporine or tacrolimus in addition to methotrexate for matched related donor (MRD) transplants and tacrolimus, methotrexate, with or without rabbit antithymocyte globulin (1.5 mg/kg/day on days −3, −2, and −1), for matched unrelated donor (MUD) transplants. In the absence of GVHD, tapering was begun at 100 days post-transplant and discontinued at day 180. Rasburicase was administered intravenously for 5 consecutive days beginning on the first day of conditioning at a dose of .20 mg/kg over 30 minutes.

Outcomes were compared with all patients in complete remission undergoing myeloablative allogeneic HSCT between 2007 and 2010 at this institution but who did not enroll in the trial, identified via medical records chart review. Control patients received 300 mg allopurinol daily from the first day of conditioning to day −1 as part of the institutional guidelines. Adverse events were classified using the Common Terminology Criteria for Adverse Events (CTCAE v4.0, National Institutes of Health). The primary study endpoints included feasibility and cumulative incidence of grades II to IV aGVHD as defined by Thomas et al. [12].

Differences in disease type, gender, percentage of first transplant, donor type, aGVHD prophylaxis, conditioning regimen, and incidence of chronic GVHD (cGVHD) were evaluated using Fisher's exact test or chi-square test. Differences in age, engraftment time, average uric acid levels, and time to aGVHD were evaluated using the Wilcoxon test. Cumulative incidence of aGVHD grades II to IV was estimated in the presence of death as a competing risk, and the difference between curves was compared using Gray's test [13]. Severity of cGVHD was classified according to Filipovich et al. [14]. Overall survival and progression-free survival were calculated using the method of Kaplan and Meier. Statistical analysis was performed using R Statistical Software (version 2.15.2; Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Twenty-one patients were included in both the rasburicase and control groups. Baseline characteristics collected for the remaining patients in each group included age, gender, type of disease, disease status at time of transplant, transplant type, conditioning regimen, and number of prior transplants. There were no statistical differences in any of the baseline characteristics between the 2 groups (Table 1). Engraftment was achieved in all patients, and the median time to combined neutrophil (.5 × 10^9/L) and platelet

Table 1
Baseline Characteristics between Rasburicase and Control Groups

	Rasburicase Group N = 21	Control Group N = 21
Mean age, yr (range)	42.9 (20-59)	45 (21-55)
Male	14 (67)	9 (43)
Disease		
Acute myelogenous leukemia	14 (67)	13 (62)
Acute lymphoblastic leukemia	5 (24)	4 (19)
Myelodysplastic syndrome	—	1 (5)
Non-Hodgkin lymphoma	—	2 (10)
Myeloproliferative disease	2 (10)	—
Chronic myelogenous leukemia	—	1 (5)
Transplant characteristics		
Mean engraftment time (range)	15 (13-28)	15 (14-29)
First transplant	21 (100)	19 (90)
MRD	16 (76)	13 (62)
Cyclosporine + methotrexate	14 (86)	11 (85)
Tacrolimus + methotrexate	2 (14)	2 (15)
MUD	5 (24)	8 (38)
Tacrolimus + methotrexate + ATG	4 (80)	8 (80)
Tacrolimus + methotrexate	1 (20)	2 (20)
Disease status at time of transplant		
Complete remission	20 (95)	21 (100)
Conditioning protocol		
Busulfan + cyclophosphamide/fludarabine*	14 (67)	16 (76)
TBI + cyclophosphamide	7 (33)	5 (24)

ATG indicates antithymocyte globulin; TBI, total body irradiation.
Values are total number of cases, with percents in parentheses, unless otherwise noted. All parameters measured were similar between the 2 groups. Parameters (P): age ($P = .561$), sex ($P = .215$), disease type ($P = .292$), engraftment time ($P = .977$), % first transplant ($P = 1.00$), type of donor ($P = .506$), MRD GVHD prophylaxis ($P = 1.000$), MUD GVHD prophylaxis ($P = 1.000$), disease status at time of transplant ($P = 1.000$), conditioning protocol ($P = .734$).

* Two patients in the rasburicase group received busulfan and fludarabine for the conditioning protocol and the remainder of the patients in both groups received busulfan and cyclophosphamide.

engraftment (20×10^9/L) was 15 days for both groups. The most common type of adverse events in the rasburicase group was mucositis, nausea, diarrhea, and vomiting, although the incidence of these events were no different from that seen in the control group and likely reflect the side effect profile from the conditioning regimen (Supplemental Table 1). The only grades III or IV adverse event attributable to rasburicase was intravascular hemolysis in a patient that occurred after 2 doses of rasburicase. This patient recovered and was later found to have glucose-6-phosphate dehydrogenase deficiency, a condition known to cause a predisposition to rasburicase-induced hemolysis from oxidative stress [9]. The patient did not receive any further doses of rasburicase and was removed from the per-protocol analysis. Another patient in the rasburicase group experienced tongue numbness without swelling on day −1 of transplant that resolved in 2 days with no interference in oral intake.

Comparison of serum uric acid levels showed that patients who received rasburicase achieved a significantly lower level of uric acid from day −7 to day +1 of transplantation compared with the control group (Figure 1), with most patients in the treatment group achieving a serum uric acid level of .0 mg/dL after 1 day of treatment. Of note, patients who were not part of the study received allopurinol per institutional guidelines, which accounts for the decrease in uric acid levels in the control group. aGVHD occurred in 12 of 21 patients in the control group (57%; grade II, 5; grade III, 3; and grade IV, 4) and in 5 of 21 patients in the treatment group (24%; grade II, 2; grade III, 2; and grade IV, 1) (Figure 2). Involvement by organ system is noted in Supplemental Table 2.

The cumulative incidence of grades II to IV aGVHD was higher in the control group compared with the rasburicase group, reaching statistical significance in the intention-to-treat analysis ($P = .036$) and per-protocol analysis ($P = .017$), which excluded the patient who received only 2 doses of rasburicase (Figure 3A,B). Of note, 2 patients in the control group and 1 patient in the rasburicase group experienced aGVHD after donor lymphocyte infusions for evidence of relapsed disease at days 155, 308, and 189, respectively. When these patients were removed from the aGVHD group based on competing risk, statistical significance was maintained in the per-protocol analysis ($P = .030$) and trended toward significance in the intention-to-treat analysis ($P = .064$). Subgroup analysis by transplant type suggested that

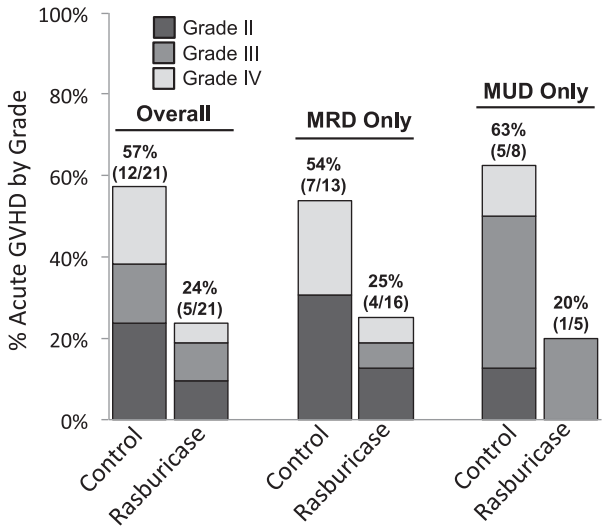

Figure 2. Breakdown of aGVHD by grade in control versus rasburicase groups. All transplant types (control, 12/21; grade II, 5; grade III, 3; grade IV, 4 versus rasburicase, 5/21; grade II, 2; grade III, 2; grade IV, 1), MRD (control, 7/13; grade II, 4; grade IV, 3 versus rasburicase 4/16; grade II, 2; grade III, 1; grade IV, 1), and MUD (control, 5/8; grade II, 1; grade III, 3; grade IV, 1 versus rasburicase, 1/5; grade III, 1).

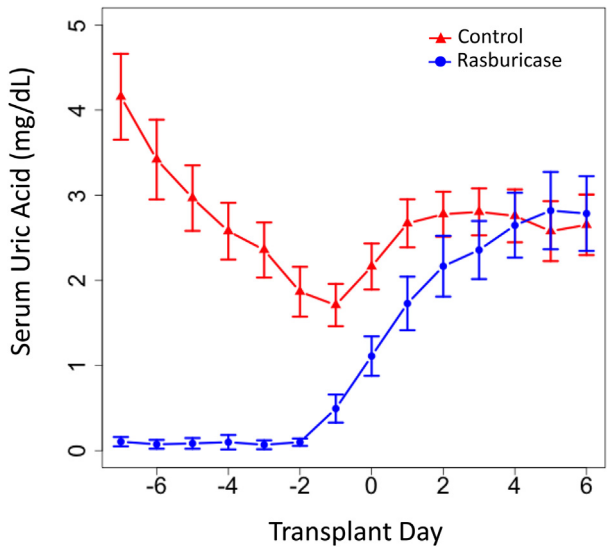

Figure 1. Serum uric acid levels pre- and post-transplant in control (triangle) versus rasburicase (circle) groups from day −7 to day 6. The rasburicase group had significantly higher levels of serum uric acid ($P < .050$) than the control group for each day from day −7 to day 1.

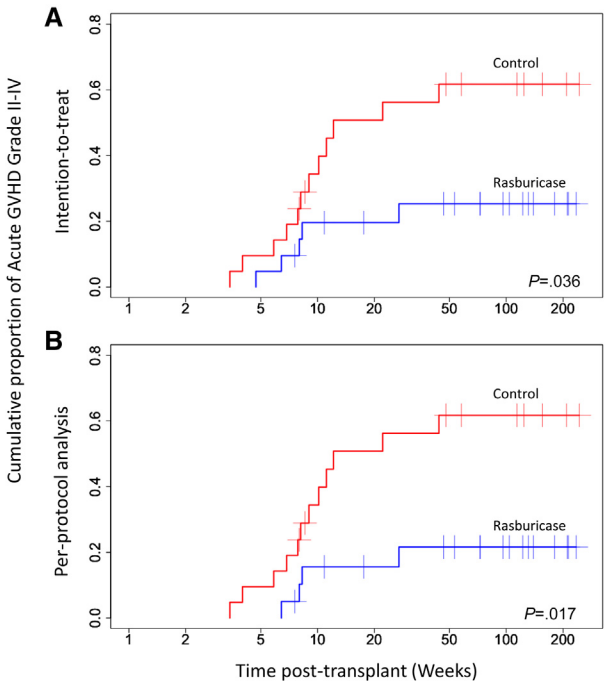

Figure 3. (A) Cumulative incidence of grades II to IV aGVHD in matched control subjects versus rasburicase treated patients, intention-to-treat ($P = .036$). (B) Cumulative incidence of grades II to IV aGVHD in matched control subjects versus rasburicase treated patients, per-protocol ($P = .017$).

the magnitude of our results was more pronounced in MUD transplants (63% versus 20% developed aGVHD) compared with MRD transplants (54% versus 25%), although our study was not sufficiently powered to detect statistical difference between these categories.

There was no difference in the time to aGVHD onset between the 2 groups (mean control, 55.0 days, versus rasburicase, 48.0 days, $P = .531$). There was also no difference in the incidence of cGVHD (control, 12/21, versus rasburicase, 12/21) or the severity of cGVHD (control, 5 mild and 7 moderate-severe, versus rasburicase, 8 mild and 4 moderate-severe, $P = .414$), although there was a trend toward milder cGVHD in the rasburicase group. Two cases of cGVHD in the control group and 2 cases in the rasburicase group were deemed as possible overlap syndrome. In the control group, there was 1 case of mild cutaneous GVHD at day 152 of transplant and moderate hepatic GVHD at day 202 of transplant. In the rasburicase group, there were 2 cases of mild cutaneous GVHD that occurred on transplant days 122 and 155. Of note, if these overlap syndromes were counted as aGVHD, only the case of moderate hepatic GVHD would have been categorized in the grades II to IV category.

There was no difference in progression-free or overall survival between the 2 groups. Nine deaths that occurred in the control group included 7 cases of relapsed disease, 1 case of infection associated with increased immunosuppression from aGVHD flare at transplant day 177, and 1 case of respiratory failure thought secondary to pneumonia and fibrotic lung disease at transplant day 60. Nine deaths also occurred in the rasburicase group, which included 7 cases of relapsed disease, 1 case of H_1N_1 influenza at transplant day 510, and 1 case of metapneumovirus at transplant day 53.

We also determined whether serum uric acid level is associated with development of aGVHD. Of all patients included in the study, those who developed grades II to IV aGVHD had a significantly higher average daily level of serum uric acid during the pretransplant period compared with those who did not ($P < .001$) (Figure 4A). Subgroup analysis suggested a similar pattern for patients receiving MRD transplants (Figure 4B) and MUD transplants (Figure 4C).

DISCUSSION

Our results leave many questions unanswered and have several limitations. First, this is a nonrandomized, single-center study, and thus patient selection in the experimental group may be biased toward positive outcome despite our best efforts at matching control subjects. We also acknowledge that although the timing and duration of rasburicase administration was based on hypotheses generated from preclinical data as well as data on its safety and efficacy [10,11], we have not established the optimal duration of rasburicase administration. Extending the administration of rasburicase may increase its effectiveness because we demonstrated that uric acid levels rise immediately after cessation of treatment. However, it is impossible to determine in the current study if the elevation of uric acid during this period is simply a marker of increased inflammation or if it plays a causative role in the disease development. Another limitation of our study stems from our methodology in measuring uric acid. Serum uric acid may not accurately reflect tissue uric acid levels, which is likely more physiologically relevant and thus could limit our ability to make a more robust association between uric acid levels and GVHD outcome.

It also remains unclear whether prolonged suppression of uric acid could diminish the graft-versus-tumor response. The lack of difference in progression-free survival between the control and treatment group suggests that, at least with this brief administration, there does not seem to be a risk of dampening a graft-versus-tumor effect. Notably, our control patients all received allopurinol before transplantation as a part of institutional guidelines, which is not the standard across all transplant centers and which results in a decrease in serum uric acid levels as well, albeit to a significantly lesser degree than uricolytic agents. However, we do not believe this decreases the validity of our study for 2 reasons. First, the incidence of aGVHD in the control group is similar to that reported in literature, suggesting that the control group provides a reasonable basis for comparison. Second, if decreasing uric acid levels do have a protective effect, then the results we see compared with the control group may be

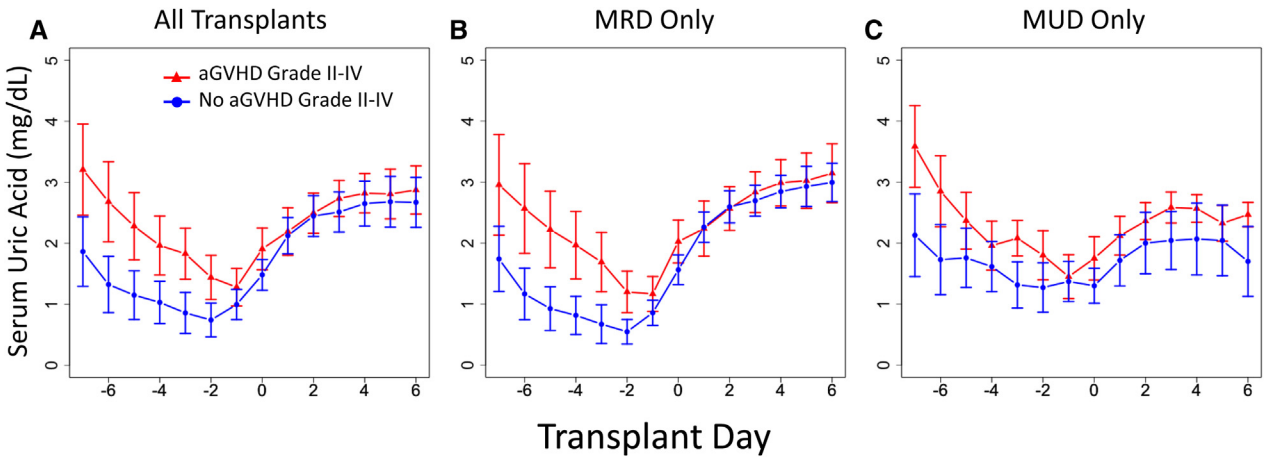

Figure 4. Serum uric acid levels measured from day −7 to day 6 of transplant for all study patients. (A) The average pretransplant (day −7 to day −1) and post-transplant (day 0 to day 6) serum uric acid levels for all patients in the study who developed grades II to IV aGVHD (triangles) compared with those who did not (circles) were as follows: pre, 2.08 mg/dL versus 1.12 mg/dL, $P < .001$; and post, 2.50 versus 2.36, $P = .405$. (B) Average serum uric acid levels for MRD patients only (pre, 1.97 mg/dL versus .93 mg/dL, $P < .001$; post, 2.66 mg/dL versus 2.55 mg/dL, $P = .558$). (C) Average serum uric acid levels for MUD patients only (pre, 2.25 mg/dL versus 1.60 mg/dL, $P = .078$; post, 2.25 mg/dL versus 1.84 mg/dL, $P = .195$).

magnified if those patients were not given allopurinol and thus have a higher baseline of serum uric acid.

In summary, our data so far provide evidence that the use of rasburicase to lower serum uric acid levels has the potential to reduce the incidence of aGVHD in a safe manner in patients without glucose-6-phosphate dehydrogenase deficiency. Although not designed as our primary outcome, the larger reduction of GVHD seen in the MUD transplants compared with the MRD transplants is another piece of evidence that supports our hypothesis, because it is reasonable to hypothesize that the higher degree of unwanted T cell activation caused by more potential antigenic mismatches in the MUD subgroup would benefit more from suppression of uric acid levels. These observations are concordant with the underlying mechanistic framework of aGVHD: the initial cytotoxicity induced by the transplant protocol releases uric acid and other pro-inflammatory soluble factors that potentiate host APCs that then lead to increased CD8+ T cell activity and tissue damage. Based on these data, further work in mouse models is necessary to determine the precise mechanism behind uric acid suppression in aGVHD and to determine the optimal timing and administration of uricolytic agents. A prospective, randomized study is also a logical follow-up to assess the efficacy of uric acid suppression on aGVHD.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.02.003.

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