Biol Blood Marrow Transplant 23 (2017) 1295–1302

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Recipient Immune Modulation with Atorvastatin for Acute Graft-versus-Host Disease Prophylaxis after Allogeneic Transplantation

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Article history:
Received 10 March 2017
Accepted 9 April 2017

Key Words:
Atorvastatin
Allogeneic transplantation
Graft-versus-host disease
Immune modulation

A B S T R A C T

Atorvastatin administration to both the donors and recipients of matched related donor (MRD) allogeneic hematopoietic cell transplantation (allo-HCT) as acute graft-versus-host disease (GVHD) prophylaxis has been shown to be safe and effective. However, its efficacy as acute GVHD prophylaxis when given only to allo-HCT recipients is unknown. We conducted a phase II study to evaluate the safety and efficacy of atorvastatin-based acute GVHD prophylaxis given only to the recipients of MRD (n = 30) or matched unrelated donor (MUD) (n = 39) allo-HCT, enrolled in 2 separate cohorts. Atorvastatin (40 mg/day) was administered along with standard GVHD prophylaxis consisting of tacrolimus and methotrexate. All patients were evaluable for acute GVHD. The cumulative incidences of grade II to IV acute GVHD at day +100 in the MRD and MUD cohorts were 9.9% (95% confidence interval [CI], 0 to 20%) and 29.6% (95% CI,15.6% to 43.6%), respectively. The cumulative incidences of grade III and IV acute GVHD at day +100 in the MRD and MUD cohorts were 3.4% (95% CI, 0 to 9.7%) and 18.3% (95% CI, 6.3% to 30.4%), respectively. The corresponding rates of moderate/severe chronic GVHD at 1 year were 28.1% (95% CI, 11% to 45.2%) and 38.9% (95% CI, 20.9% to 57%), respectively. In the MRD cohort, the 1-year nonrelapse mortality, relapse rate, progression-free survival, and overall survival were 6.7% (95% CI, 0 to 15.4%), 43.3% (95% CI, 24.9% to 61.7%), 50% (95% CI, 32.1% to 67.9%), and 66.7% (95% CI, 49.8% to 83.6%), respectively. The respective figures for the MUD cohort were 10.3% (95% CI, 8% to 19.7%), 20.5% (95% CI, 7.9% to 33.1%), 69.2% (95% CI, 54.7% to 83.7%), and 79.5% (95% CI, 66.8% to 92.2%), respectively. No grade 4 toxicities attributable to atorvastatin were seen. In conclusion, the addition of atorvastatin to standard GVHD prophylaxis in only the recipients of MRD and MUD allo-HCT appears to be feasible and safe. The preliminary efficacy seen here warrants confirmation in randomized trials.

INTRODUCTION

Acute graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (allo-HCT). It develops in 30% to 55% of patients undergoing transplantation from either matched related donors (MRD) or matched unrelated donors (MUD) [1]. Acute GVHD is triggered when donor T cells encounter recipient antigen-presenting cells (APCs), leading to activation of these alloreactive donor cells and eventual immune-mediated host tissue damage [2]. This process of donor T cell activation requires costimulation via CD80 and CD86, which are upregulated on APCs during the early phase of acute GVHD. Local proinflammatory cytokines generated by tissue damage from transplantation conditioning, such as TNF-α, IL-1, and interferon-γ, promote T helper (T_H)-1 differentiation of donor T cells [3], enhancing their alloreactivity against host antigens. In both murine models and humans, cytokine release related to the T_H-1 phenotype predicts the incidence and

Financial disclosure: See Acknowledgments on page 1301.
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http://dx.doi.org/10.1016/j.bbmt.2017.04.009
1083-8791/

severity of acute GVHD, whereas patients with high IL-10 production have a lower risk for GVHD [4-6]. The prognosis of patients developing acute GVHD, especially the subset with Minnesota high-risk [7], grade III or IV, or steroid-unresponsive acute GVHD, is poor [8]. Current GVHD prophylactic modalities have modest efficacy and a narrow therapeutic index. Novel strategies to effectively prevent acute GVHD without delayed immune reconstitution and increased risk of disease relapse remain an unmet medical need.

Pharmacological agents called *statins* (or 3-hydroxy-3-methyl-CoA reductase inhibitors) have pleotropic effects on immune system that are relevant in the context of acute GVHD. Statins induce depletion of isoprenoid intermediates, leading to T_H-2 polarization and inhibition of proinflammatory T_H-1 differentiation [9]. Alloreactive T cells with T_H-1 cytokine profile are potent mediators of acute GVHD, whereas T_H-2 cells fail to induce experimental acute GVHD [10-12]. Statins also reduce T cell activation by downmodulating the expression of MHC-II and costimulatory molecules on APCs [13,14]. Considering the key role of APCs in the pathogenesis of GVHD, strategies to prevent their activation may abrogate GVHD risk [15]. Statin-mediated expansion of regulatory T cells is another potential mechanism to prevent acute GVHD with this agent [16,17]. In experimental models, simultaneous statin administration to both the donor and recipient mice showed synergistic protective effects against acute GVHD (compared with administration in donor or recipient mice alone) by inhibiting donor T cell proliferation, inducing donor T_H-2 polarization, and by downregulating MHC-II and costimulatory molecule expression on recipient APCs [18]. Retrospective studies have also suggested the efficacy of statins against GVHD in clinical setting [19-21].

Mirroring the murine model by Zeiser et al. [18], we previously reported a phase II trial that evaluated the addition of atorvastatin to standard calcineurin inhibitor–based acute GVHD prophylaxis to both the donors and recipients of MRD allo-HCT, and we reported low rates of acute GVHD with no significant added toxicity [22]. However, routine administration of atorvastatin prophylaxis to healthy sibling donors, while shown to be safe and effective in our previous report [22], remains a logistical challenge outside the clinical trial setting. In addition, investigation of a dual (donor and recipient) atorvastatin prophylactic approach in the MUD setting is also difficult to implement. A prophylactic strategy of atorvastatin administration to allograft recipients alone is, thus, potentially attractive and if effective, would have broader practice implications for both MRD and MUD transplantation. Based on preliminary retrospective data showing potential benefit of statin administration solely to transplant recipients [19,20,23,24], we hypothesized that atorvastatin administration to just the recipients of MRD and MUD allo-HCT would be a safe and effective method of preventing acute GVHD.

Patients and Methods

This prospective phase II clinical trial (ClinicalTrials.gov number: NCT01665677) was approved by the institutional review boards of participating institutions. Written and signed informed consent was obtained before patient enrollment.

Inclusion and exclusion criteria

Adult patients (≥18 years) with hematological malignancies requiring an allo-HCT with an available HLA-matched sibling or unrelated donor were eligible. Patients with active and uncontrolled infections; abnormal renal (creatinine clearance <40 mL/minute), hepatic (serum bilirubin >2 mg/dL, serum aspartate transaminase and alanine transaminase >3 times upper limit of normal), pulmonary (diffusion capacity of the lung for carbon monoxide or forced expiratory volume in 1 second <40% of predicted), or cardiac (left ventricular ejection fraction <40%) function; poor Karnofsky performance score (<70); or a history of atorvastatin intolerance or allergy were excluded. Patients undergoing an ex vivo or in vivo T cell–depleted allo-HCT were not eligible.

Treatment and GVHD prophylaxis

In both the MRD and MUD cohorts, acute GVHD prophylaxis consisted of tacrolimus (.015 mg/kg intravenously or .03 mg/kg/day orally, starting on day -2), methotrexate (5 mg/m^2 on days +1, +3, +6, and +11), and atorvastatin (40 mg/day orally, starting on day -14). Atorvastatin was continued until any 1 of the following events occurred: discontinuation of all immunosuppressive medications, day +180, the development of grade II to IV acute GVHD, severe chronic GVHD, or grade 3 or 4 adverse events related to atorvastatin use. The dose of tacrolimus was adjusted to a target trough level of 5 ng/mL to 12 ng/mL. Tacrolimus taper commenced after day +100, with the goal of stopping immunosuppression by day +180 in the absence of GVHD. The intensity of the transplantation conditioning regimen was at the discretion of the treating physician. Growth factors to promote neutrophil recovery after transplantation were not routinely administered. All patients received antibacterial (fluoroquinolones), antifungal (fluconazole or voriconazole), antiviral (acyclovir or valacyclovir), and *Pneumocystis jiroveci* prophylaxis.

Endpoints

The primary endpoints were patient safety and the cumulative incidence of grade II to IV acute GVHD at day +100. Secondary endpoints included cumulative incidence of grade III to IV acute GVHD, chronic GVHD, disease relapse, nonrelapse mortality (NRM), progression-free survival (PFS), and overall survival (OS). Assessment of GVHD-free, relapse-free survival (GRFS) was not prespecified in the protocol and was performed post hoc. All suspected cases of acute GVHD were histologically confirmed, at least in 1 target organ. Consensus Conference Criteria [25] and the National Institutes of Health Consensus Development Project Criteria [26] were used for the grading acute and chronic GVHD, respectively. Compliance with atorvastatin prophylaxis was monitored by reviewing patient diaries and by assessing the remaining quantity of the study medication with the patients. Disease risk index (DRI) was assigned as previously described [27] . National Cancer Institute Common Toxicity Criteria for Adverse Events, version 4.0 was used to grade adverse events.

Immune reconstitution and donor cell chimerism

For immune reconstitution assays, peripheral blood mononuclear cells were obtained from EDTA-anticoagulated whole blood samples obtained on days +30, +100, +180, and +365 after transplantation (described in the Supplemental Appendix). For these analyses, $CD4^+$ T cells were defined as $CD3^+CD4^+$, $CD8^+$ T cells as $CD3^+CD8^+$, $CD4^+$ memory T cells as $CD3^+CD27^+CD45RO^+CD4^+$, $CD8^+$ memory T cells as $CD3^+CD27^+CD45RO^+CD8^+$, $CD4^+$ naïve T cells as $CD3^+CD45RA^+CD45RO^-CD4^+$, $CD8^+$ naïve T cells as $CD3^+CD45RA^+CD45RO^-CD8^+$, regulatory T cells as $CD3^+CD4^+CD25^{med\text{-}high}CD127^{low}$, natural killer cells as