

## Original Article

# The evolving role of statins in hematopoietic stem and progenitor cell transplantation

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**Abstract:** Allogeneic hematopoietic stem cell transplantation is the sole curative modality for a variety of malignant and benign hematological disorders. Despite advances in supportive care and transplant conditioning regimens graft-versus-host disease (GVHD), infectious complications and end organ toxicity remain the leading causes of transplant related mortality (TRM). Development of safe and effective strategies to mitigate these significant complications associated with HSCT, are urgently needed. Statins are lipid lowering drugs, which reduce cholesterol production by inhibiting HMG-CoA reductase, with a well defined toxicity profile. Statins have pleiotropic immunomodulatory effects which are relevant in the context of treating and preventing GVHD. In addition to GVHD statins may possess several other effects that might have clinical benefit in the setting of hematopoietic cell transplantation, such as treatment of bronchiolitis obliterans and antineoplastic activity. Herein we review the emerging role of statins in improving the outcomes of patients undergoing HSCT.

**Keywords:** Statin, atorvastatin, GVHD, HMG-CoA reductase inhibitors, allogeneic stem cell transplantation, multiple myeloma, idiopathic pulmonary fibrosis, graft-versus-host disease

## Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is the sole curative modality for a variety of malignant and benign hematological disorders [1]. Despite advances in supportive care and transplant conditioning regimens graft-versus-host disease (GVHD), infectious complications and end organ toxicity remain the leading causes of transplant related mortality (TRM) [2]. Development of safe and effective strategies to mitigate these significant complications associated with HSCT, are urgently needed. Herein we review the emerging role of statins in improving the outcomes of patients undergoing HSCT.

## HMG-CoA reductase inhibitors

HMG-CoA (3-Hydroxy-3-Methylglutaryl-Coenzyme-A) reductase inhibitors (statins) are prescribed worldwide to prevent and treat dyslipidemia and atherosclerotic vascular disease [3]. The mechanism of action involves

binding to HMG-CoA reductase and displacement of its natural substrate HMG-CoA, leading to inhibition of mevalonate and cholesterol biosynthesis [3]. Atorvastatin, the prototypic statin drug has a proven safety track record [4-6]. Comprehensive assessment of atorvastatin's safety profile using data from 44 clinical trials comprising 16,495 patients demonstrated that the incidence of adverse events with atorvastatin did not increase in the 10 to 80 mg dose range and was similar to that observed with placebo. Moreover musculoskeletal and hepatic adverse events were infrequent and rarely required treatment discontinuation [7].

## Graft-versus-Host Disease

GVHD develops in 30-75% of transplant recipients depending on the degree of histocompatibility between the donor and the recipient, donor's age, ABO compatibility, GVHD prophylaxis used etc. [8, 9]. With standard (calcineurin inhibitor  $\pm$  methotrexate or mycophenolate mofetil-based) prophylactic regimens, GVHD

continues to be a major transplant complication. More intense (and often expensive) GVHD prophylactic strategies employing *ex vivo* T-cell depletion (TCD), and monoclonal or polyclonal antibodies, often display a narrow therapeutic index and are frequently complicated by delayed immune-reconstitution, increased risk of infectious complications and disease relapse [10-13], adding significant burden [14] to already enormous healthcare costs of transplant recipients [15, 16]. To date GVHD remains the major barrier to successful allogeneic HSCT outcomes, underscoring the urgent need for novel strategies designed to effectively prevent the development of this life threatening complication.

### Immunomodulatory effects of Statins and GVHD

Statins possess potent immunomodulatory and anti-inflammatory properties that are critical not only in atherosclerosis-associated inflammation but are also relevant in autoimmune diseases such as multiple sclerosis and rheumatoid arthritis [17-19]. Reduced mevalonate production by statins not only inhibits the synthesis of cholesterol, but it also inhibits production of key isoprenoid intermediate molecules required for the isoprenylation of GTP-binding cell signaling proteins such as Ras, Rho and Rac [3]. By inhibiting this posttranslational modification, statins affect multiple intracellular signaling pathways and cellular functions including differentiation, motility, secretion and proliferation [19, 20].

Statin-mediated inhibition of Ras may lead to a bias towards T-helper type 2 (Th-2) cell development and inhibition of pro-inflammatory Th-1 driven responses [21]. Acute GVHD is thought to be mediated by alloreactive T cells with a Th-1 cytokine phenotype. Interestingly polarized Th-2 alloreactive CD4<sup>+</sup> donor T cells fail to induce experimental acute GVHD [22-24]. Statin mediated changes in intracellular signaling can also affect the development of regulatory T (Treg) cells, as exposure of human CD4<sup>+</sup> T cells to atorvastatin *in vitro* increases expression of forkhead box P3 (FOXP3) [25]. In fact increased numbers of circulating FOXP3<sup>+</sup> Treg cells are present in dyslipidemic patients taking statins [25]. Statins also promote the migration of Treg cells to inflammatory foci in skin by increased expression of CXCL1, the ligand for CCR8, a chemokine receptor that is highly expressed on FOXP3<sup>+</sup> Treg cells [26]. Expansion of Treg cells

is an attractive strategy to prevent acute GVHD [27].

Statins indirectly decrease T-cell activation by inhibiting interferon gamma (IFN- $\gamma$ ) induced expression of major histocompatibility complex class (MHC) II molecules and by blocking up-regulation of a variety of co-stimulatory molecules and cytokines in antigen presenting cells (APCs) [19, 28]. APCs are critical for the pathogenesis of GVHD, and strategies to prevent activation of APCs can potentially abrogate GVHD [29]. In summary, statins demonstrate the ability to suppress T cell-dependent immune responses at multiple levels and hold great promise as a new class of immunomodulatory drugs to prevent acute GVHD.

### Statins and experimental acute GVHD

Statins (atorvastatin and fluvastatin) have shown the ability to prevent acute GVHD in a MHC mismatched murine bone marrow transplant (BMT) model [30]. In a series of elegant experiments, Zeiser et al. demonstrated that pre-treating donor mice with atorvastatin lead to significantly less acute GVHD by: (i) inhibiting donor T-cell proliferation, (ii) decreased Th-1 cytokine (tumor necrosis factor- $\alpha$ , IFN- $\gamma$ ) production and (iii) increased Th-2 cytokine (IL-10, IL-4) production. In contrast to pre-treating donor mice, administering atorvastatin to recipient mice only, also protected against acute GVHD with a long-term survival of 50% compared with 0% survival in control animals. APCs of recipient mice pretreated with atorvastatin or placebo were analyzed at different time points after transplantation. Surface expression of CD80, CD86, CD40, and MHC class II antigens were decreased in animals treated with the atorvastatin as compared with the placebo group. Interestingly pre-treating both donor and recipient mice with atorvastatin produced synergistic protective effects (65% survival), when compared with survival of only donor or recipient pretreated groups (approximately 40% survival). Perhaps the most significant finding was that atorvastatin pretreatment did not interfere with perforin-mediated cytotoxicity or Fas-Fas-ligand interaction-mediated anti-leukemia effects.

The M.D. Anderson group has also shown prevention of acute GVHD by lovastatin mediated inhibition of LFA-1 (leukocyte function-associated antigen-1) activation, thereby pre-

**Table 1.** Studies evaluating the role of statins in preventing GVHD

Author	Journal Year	Study Design	N	Acute GVHD	Chronic GVHD	Comments
Hamadani [41]	Blood 2008	Statin use by recipients only	67	Significant reduction	No effect	Limited by small sample size and retrospective design Included both MS and MUD
Rotta [43]	Blood 2010	Statin use by both recipients and donors	567	Significant reduction	No effect	Included MS only Benefit confined to patients receiving cyclosporine
Rotta [44]	BBMT 2010	Statin use by recipients only	120 6	Significant reduction (in MS only)	Significant reduction	Large, retrospective study Included both MS and MUD Suggest increased risk of disease relapse with statin use

Abbreviations: MUD=matched unrelated donors; MS=matched sibling donors.

venting donor T-cells from homing to lymph nodes and Peyer's patches, in a murine BMT model [31]. Yoon et al. recently evaluated the role of statins in preventing chronic GVHD in a B10.D2→BALB/c murine BMT model [32]. The onset of clinical cutaneous chronic GVHD was significantly slower in pravastatin-treated mice compared to the control group (36 days vs. 25 days, respectively,  $P<0.05$ ). Animals injected with pravastatin also had less submucosal fibrosis in lungs. Pravastatin also significantly reduced protein and chemokine concentrations and number of inflammatory cells in bronchoalveolar lavage fluid. Significantly lower numbers of donor CD11b and CD4 cells were observed in skin and bronchoalveolar lavage fluid after pravastatin treatment. This study suggests that the chronic GVHD protecting effect of statins may involve down-regulation of chemokine production and reduction of collagen synthesis.

In humans, increased Th-2 cytokine levels before allogeneic HSCT are associated with less acute GVHD and improved TRM [33-35]. Atorvastatin has been shown to induce Th-2 polarization when administered to healthy volunteers or patients with acute coronary syndrome or autoimmune disorders [36-39]. Interestingly co-administration of atorvastatin and cyclosporine (an agent commonly used for GVHD prophylaxis in human) in dogs have shown synergistic inhibitory effects on mitochondrial function in activated T-cells [40]. These clinical observations highlight the fact that the reduced acute GVHD seen in murine models [30] by atorvastatin induced Th-2 polarization can be translated into real clinical benefit at the bedside. Moreover, the statin-induced down regulation of co-

stimulatory molecules and MHC-II expression on APCs, responsible for abrogating acute GVHD in recipient mice [30], has also been demonstrated in humans treated with statin drugs [28, 37].

### Statins and clinical GVHD

Prompted by these data, we retrospectively investigated whether statin use at the time of clinical allogeneic HSCT would result in reduced acute GVHD while sparing the beneficial graft-versus-leukemia (GVL) effects (Table 1) [41]. Sixty-seven consecutive patients with acute leukemia undergoing allogeneic transplantation were evaluated. Patients taking any statin drug (at a dose of  $\geq 40\text{mg/day}$ ) for at least one month before and three months after transplantation were compared to those without a history of statin use. The two groups had similar baseline characteristics. Acute GVHD was scored according to the consensus criteria.[42] The rate of grade 2-4 acute GVHD was 10% ( $n=1$ ) in the statin group compared to 40% ( $n=23$ ) in the control group ( $p\text{-value}=0.08$ ). On subgroup analysis of patients with acute myeloid leukemia only ( $n=49$ ), a significantly reduced incidence of grade 2-4 acute GVHD was seen in statin group (0%) compared to the control group (43%) ( $p\text{-value}=0.02$ ). We also analyzed if statin use, while reducing acute GVHD, mitigated the GVL effects. Kaplan-Meier estimates of 3-year progression free survival (PFS) in patients with or without statin use were 54% and 28% respectively ( $p\text{-value}=0.17$ ). This non significant trend of improved PFS suggested that the GVL effects are preserved in patients using statins at the time of ASCT.

Rotta et al. [43] retrospectively analyzed correlation between statin use and risk of acute GVHD in 567 patients undergoing matched sibling allogeneic HSCT (**Table 1**). Compared to transplants where neither the donor nor the recipient were using a statin at the time of transplantation (n = 464), statin use by the donor was associated with a decreased risk of grade 3-4 acute GVHD (hazard ratio, 0.28; 95% CI, 0.1-0.9). Statin use by both the donor and recipient (n=12) was suggestively associated with a decreased risk of grade 3 or 4 acute GVHD (hazard ratio, 0.00; 95% CI, undefined). Risks of disease relapse and TRM were not significantly affected by donor or recipient statin exposure. Interestingly statin use by the recipient only, in contrast to our previous findings [41], did not confer protection against GVHD. Similarly protective effects of statins were more prominent in patients receiving cyclosporine for GVHD prophylaxis, compared to the ones receiving tacrolimus. Recently synergistic activity of atorvastatin and cyclosporine in inhibiting T-cell activation in an animal model has been reported, partially explaining this intriguing synergy between statins and cyclosporine [40]. However the animal model did not address, whether a similar synergy existed between statins and other calcineurin inhibitors.

A recent large retrospective study evaluated the role of statin use by transplant recipients only [44], in preventing GVHD. In line with our original observation, this study reported that statin use in patients undergoing matched sibling transplantation was associated with significantly reduced rates of severe acute GVHD. Of note in contrast to prior publications [41, 43], in this study statin use was also associated significantly reduced rates of extensive chronic GVHD.

In summary, extensive preclinical and retrospective clinical data suggest that statins can prevent acute (and possibly chronic) GVHD without decreasing GVL effects. In order to prospectively validate these observations, a phase-II study evaluating the role of atorvastatin in preventing acute GVHD in patients undergoing matching sibling allogeneic HSCT is currently in advanced stage of patient accrual at our cancer center (NCT01175148; [www.clinicaltrials.gov](http://www.clinicaltrials.gov)). A similar study is in currently in planning stages at Ohio State University, USA (Steven Devine, M.D.; personal communication).

## Anti-myeloma activity of statins

Statins have demonstrated antineoplastic and pro-apoptotic activities in various in-vitro studies involving myeloma cell lines. Van de Donk et al. [45] reported that lovastatin can inhibit membrane-localization and resultant cytosolic accumulation of Ras by inhibition of isoprenylation, leading to markedly decreased viability of plasma cells. The cytotoxic effect of lovastatin on plasma cells is possibly the consequence of interference with the isoprenylation of Ras and its subsequent involvement in signaling cascades thereby inhibiting proliferation and cell survival signals. HMG-CoA reductase is differentially up-regulated in cell adhesion-mediated drug resistant (CAM-DR) and de novo resistant myeloma cell lines [46]. Interestingly co-culturing statins (simvastatin and lovastatin) with melphalan, resistant myeloma cells, and bone marrow stromal cells leads to complete abrogation of CAM-DR [47]. Further experiments with specific inhibitors of components of the HMG-CoA pathway revealed that CAM-DR in multiple myeloma seems to be mediated by activation of the HMG-CoA/Geranylgeranyl Pyrophosphate/Rho-protein/Rho-kinase pathway. Microarray analyses also demonstrated that *de novo* and acquired drug resistance in myeloma cell lines was associated with an increase of HMG-CoA reductase gene expression [47]. In a different study, statins co-cultured with myeloma cell lines reduced mitochondrial membrane potential and inhibited the cytosolic release of mitochondria-derived activator of caspases, leading to activation of caspases 9, 3, 8 and resultant apoptosis [48]. In a small study, addition of simvastatin to bortezomib or bendamustine in a total of 6 patients with refractory myeloma was shown to produce an apparent improvement in response [49].

## Role of statins in autologous transplantation for myeloma

Based on the preliminary evidence that statins may serve as sensitizing agents for other chemotherapeutic agents in multiple myeloma, we evaluated the impact of statin use by myeloma patients at the time of autologous HSCT, on transplantation outcomes [50]. In a cohort of one hundred and forty-six consecutive patients with multiple myeloma undergoing autologous HSCT, outcomes of patients taking statins for at

least one month before and after transplant (n=28) were compared to those without a history of statin use (n=118). The two groups had similar baseline characteristics including age, international stage, number of prior therapies, and percentage of patients with high-risk cytogenetics. A trend towards higher overall response rate post-transplant was seen in the statin group (93% vs. 78%; p=0.07). In patients with high-risk cytogenetics, the overall response rate in the statin group (n=5) was 100% versus 78% for non-statin group (n=14). This better response rate, however did not translate into improved overall survival. It is possible the sample size of the study limited the detection of a statistically significant difference between the two groups. The excellent therapeutic index of statins and their potential anti-myeloma activity, warrant further investigation of these agents in myeloma patients undergoing autologous HSCT.

## Statins as modulators of bronchiolitis obliterans

Bronchiolitis obliterans is a well-recognized non-infectious, late-onset pulmonary complication of allogeneic HSCT, and is associated with substantial morbidity and mortality [51]. Bronchiolitis obliterans typically presents with symptoms of cough, dyspnea and/or airway obstruction. Pathologically, it results from obstruction and/or obliteration of the small airways and is characterized by luminal occlusion of the terminal and respiratory bronchioles by inflammatory and fibrous tissue [51]. Data from lung transplants suggest that statin use by transplant recipients is associated with significantly reduced rates of acute organ rejection, obliterative bronchiolitis and a superior overall survival [52]. These substantial clinical benefits associated with statin use after pulmonary transplantation, may have clinical significance in reducing similar pulmonary complications following allogeneic HSCT. Duncan et al. [53] reported on the safety and tolerability of statins (pravastatin) in pediatric allogeneic HSCT patients with established bronchiolitis obliterans. All participants tolerated the drug without difficulty, with evidence of clinical improvement in pulmonary function and there were no statin-associated adverse effects. Changes in creatine kinase (CK) and transaminases were minimal. One patient experienced increased CK and alanine aminotransferase, and a decrease in platelet count in the setting of severe systemic illness. Statins merit further study in this significant complication of allografting.

## Conclusions

The pleiotropic immunomodulatory effects of statins and their excellent safety profile, make them an ideal yet inexpensive agent, well suited for further study in improving HSCT outcomes. The ongoing phase II clinical trials, will clarify their role in preventing acute GVHD following matched sibling allogeneic HSCT (NCT01175148; [www.clinicaltrials.gov](http://www.clinicaltrials.gov)). If activity of statins in preventing transplant related complications is confirmed in well designed prospective studies, it will have significant effect on offsetting tremendous costs associated with current available strategies for preventing and treating transplant complications.

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