Repairing Damaged Goods: Mesenchymal Stromal Cell Therapy for Secondary Failure of Hematopoiesis Post-Allogeneic Hematopoietic Cell Transplant

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Many high-risk or advanced cancers of the blood, such as acute leukemia and myelodysplastic syndrome, cannot be cured by chemotherapy alone. Allogeneic hematopoietic cell transplantation (HCT) is a procedure that has the potential to cure such cancers not only by replacing a patient’s diseased hematopoietic stem cells with healthy donor cells, but also by harnessing the benefit of a new donor immune system to eradicate any residual malignant cells. Although initial donor stem cell engraftment occurs relatively quickly and reliably [1], the early months after an allogeneic HCT are associated with profound deficits in immune function. Furthermore, many patients will experience a decline in function of their transplanted cells, manifested by secondary cytopenias and life-threatening immunodeficiencies, occurring when post-transplant complications are encountered, principally graft-versus-host disease (GVHD) [2,3].

A deeper understanding of the mechanisms behind secondary post-transplant lymphohematopoietic failure, and new methods to intervene upon this complication, are needed to improve survival in allogeneic HCT recipients. While standard options include administration of growth factors (i.e., G-CSF) or donor lymphocyte infusions, novel cellular therapeutic options under current investigation include the infusion of mesenchymal stromal cells (MSC) as a means to restore allograft hematopoietic function and possibly improve immune homeostasis after complications of allogeneic HCT.

Despite several advances in the care of HCT recipients in recent years, approximately half of all patients undergoing allogeneic HCT do not survive longer than 2 years beyond the transplant procedure [4]. Clinical predictors associated with improved survival after allogeneic HCT include rapid and complete immune reconstitution and normalization of blood counts [2,5]. After HCT, lymphocyte recovery is widely regarded as a surrogate marker for immune recovery [6-16]. We and others have shown that the absolute lymphocyte count (ALC) at approximately three months post-HCT is predictive of survival, regardless of the underlying disease for which the transplant was performed, and regardless of the conditioning regimen (myeloablative versus reduced intensity) [2,9] (manuscript under review). However, we have also shown that the absolute monocyte count (AMC) at the day +100 time point is an important surrogate of long-term survival, with both reduced transplant-related mortality (TRM) [2] as well as a decreased relapse risk in those achieving normal monocyte counts by day +100 (manuscript under review). Another study of 30 patients has shown an association of monocyte recovery at day +90 and decreased chronic graft-versus-host disease (GVHD) and relapse risk [17]. Others have shown an increased risk of invasive fungal infections in those with severe post-transplant monocytopenia [18]. Therefore, emerging data supports that recovery of monocytes may be as important as that of lymphocytes in post-transplant immune reconstitution.

Thrombocytopenia is also increasingly recognized as a poor prognostic finding in the early post-transplant period [3]. In patients with early recovery of platelet counts but subsequent development of severe thrombocytopenia, the phenomenon of secondary failure of platelet recovery (SFPR) has been described. In allogeneic HCT recipients, SFPR has been described at a median of 63 days (21-156) post-transplant, and associated with a hazard ratio for death of 2.6 [19]. Recovery of which specific cell type—lymphocytes, monocytes, platelets, or other yet unspecified subset, or a pattern of multiple cell types—that can best predict post-transplant outcomes at the day +100 time point is currently unknown. It is possible that critical early, post-transplant events may be reflected in day +100 post-HCT cytopenias and serve as indicators for patients at risk for poor outcomes. A variety of factors, including infections, medication effects, GVHD, graft rejection, and impending relapse can result in cytopenias in patients following allogeneic HCT. We are currently working toward defining the severity of secondary cytopenias that place patients at risk for poor outcomes based upon complete blood count parameters at the day +100 evaluation through the Center for International Blood and Marrow Transplant Research. If we can successfully develop a risk-stratification tool based upon post-transplant cytopenias, the natural next step is to develop a clinical trial aimed at improvement of secondary hematopoietic failure in these patients at risk for poor survival. It is unlikely that manipulation of existing immune suppression (predominantly calcineurin inhibitors) would markedly impact hematopoiesis and subsequent outcomes, and we would therefore propose that any clinical trial be aimed at directly addressing the mechanisms behind the described phenomenon.

Mouse models have provided initial insights into the mechanisms behind the profound lymphopenia and hematopoietic failure that can be observed with severe GVHD reactions. Both reduced thymic output...

and perturbation of the peripheral T cell compartment secondary to lymphoid organ microenvironmental damage lead to marked T lymphopenia in mice with GVHD [20]. Additionally, hematopoietic stem cells fail to proliferate due to downregulation of CCND1 and MYC, both negatively regulated by interferon-gamma [21]. Together, these early results suggested that both problems with lymphoid and hematopoietic microenvironment as well as inflammatory cytokines contribute to reduced lymphohematopoiesis. Given the complex intersection between hematopoietic stem cells and their microenvironment, as well as the contribution of cytokine signaling, it has subsequently been theorized that maintenance of stromal integrity and anti-inflammatory cytokine secretion provided by supplemental MSCs could abrogate this effect [22].

MSCs are pluripotent cells with the potential to differentiate into layers of mesoderm (bone, cartilage, fat). They have been theorized to be progenitor cells for the bone marrow stroma, and thus thought to potentially be able to affect hematopoiesis and marrow regeneration after the stroma-damaging chemotherapy and/or radiation conditioning given prior to HCT [23]. NOD/SCID xenograft models have demonstrated that MSCs can support the hematopoietic reconstitution of myeloid, lymphoid, and megakaryocytic lineages [24], as well as facilitate human cord blood stem cell engraftment into NOD/SCID mice, especially at low transplanted doses of cord blood stem cells [25]. Additionally, MSCs have a low immunogenic profile, and have been shown to modulate T cell function in in vitro assays [26,27].

The first clinical trial of HLA-matched sibling-derived MSCs given prior to HCT demonstrated safety and feasibility, although no clear beneficial signal in terms of improved engraftment or reduced GVHD rates compared with historically reported outcomes was identified in this heterogeneous group of patients [28]. However, subsequent clinical trials of alternate, non-HLA matched donor MSC co-transplantation have suggested benefit in the engraftment of hematopoietic stem cells [23,29,30]. For example, in a series of 14 children undergoing haploidentical HCT [29], co-infusion of haploidentical MSCs was associated with improved lymphocyte (especially natural killer cell) recovery and reduced graft failure rates (0% versus 15% in historical controls). No patient died of GVHD and only 2 died of relapse in the MSC-treated cohort, compared to death due to GVHD and relapse in 2 and 7 patients in the historical control cohort, respectively. While early infusion of MSCs may improve engraftment and possibly immune reconstitution, whether their later use can improve secondary cytopenias has not yet been proven in controlled studies. Encouragingly, MSC infusions have been reported to durably rescue four allogeneic HCT recipients from severe cytopenias in a recent case series (3 with refractory thrombocytopenia, 1 with refractory neutropenia) [31]. Additionally, a report of recovery from pure red cell aplasia in ABO-mismatched HCT has recently been published [32].

Given the association of acute GVHD and secondary cytopenias [33-36], it is possible that the marrow is a GVHD target organ amenable to MSC homing and repair. Some studies have shown low level persistence of infused MSCs in damaged organs, including marrow [29]. For example, haploidentical MSCs could be identified in the bone marrow biopsy of a patient with severe aplastic anemia [37]. Additionally, after infusion for steroid-resistant GVHD, third-party donor HLA-mismatched MSC DNA could be identified in organs affected by GVHD but not in healthy tissue [38]. With trials incorporating MSCs for the treatment of steroid-refractory GVHD showing promise [38-40], it seems plausible to focus additional efforts on whether and how MSC infusions could be used to treat established lymphohematopoietic graft-versus-host reactions.

Future studies of the optimal timing of MSC infusions (i.e., as prophylaxis or as treatment of post-transplant complications), coupled with correlative studies of hematopoietic and immune function, will be required to determine the most appropriate application for MSC infusions in allogeneic HCT. Several additional areas of debate surrounding the use of MSC regenerative therapy remain, including the best source of MSCs (i.e., matched sibling, haploidentical, or third-party donor) and the optimum dose of MSC/kg patient body weight necessary to effect a response. Several complexities regarding how best to generate MSCs as a cellular therapy, including the creation of suitable fetal bovine serum-free culture conditions to reduce the risk of zoonoses and allergic reactions, are highlighted in the article presented by Khanna-Jain et al. [41], in this issue of Journal of Stem Cell Research and Therapy. Ongoing research into stromal cell source (marrow, adipose tissue, placenta/umbilical cord blood, dental pulp), the ideal culture conditions, cytokine cocktails, and possibility of priming of MSCs prior to infusion, all contribute to the complexity of MSC therapy. While the regenerative potential of stromal cell therapy in allogeneic HCT is very promising based upon extensive pre-clinical data and early clinical trial results, whether this could be a durable, cost-effective method to repair a damaged lymphohematopoietic system remains to be tested in a controlled fashion.

References
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