Severe steroid-refractory acute graft-versus-host disease (aGVHD) causes significant morbidity and mortality after allogeneic hematopoietic stem cell transplantation. Early clinical trials of therapy with human mesenchymal stem cells (hMSCs) in pediatric patients with severe aGVHD, resistant to multiple immunosuppressive agents, showed promising results. In this study, we evaluated the risk/benefit profile of remestemcel-L, an off-the-shelf source of culture expanded human mesenchymal stem cells, provided by Mesoblast, Inc. under an expanded access program, as a rescue agent for treatment-resistant aGVHD in pediatric patients. Children with grade B-D aGVHD failing steroids and, in most cases, other immunosuppressive agents were eligible for enrollment. Patients received 8 bi-weekly i.v. infusions of 2×10^6 hMSCs/kg for 4 weeks, with an additional 4 weekly infusions after day +28 for patients who achieved either a partial or mixed response. The enrolled patients compose a very challenging population with severe disease that was nonresponsive to standard of care. One hundred sixty patients (median age, 10 years; 60% male; and 61% Caucasian) were treated in this study. One hundred thirty five patients (84%) received unrelated donor transplant. Graft source was bone marrow in 65 (41%), PBSC in 34 (21%), cord blood in 54 (34%) and donor leukocyte infusion in 5 (3%). At baseline, the distribution of aGVHD grades B, C, and D was 19%, 28%, and 53%, respectively; 81% of the patients had severe aGVHD (grade C or D). Median duration of aGVHD before enrollment was 28 d (range, 1 to 237), and patients had failed a median of 3 immunosuppressive agents. Organ involvement at baseline was 89% gastrointestinal (GI), 50% skin, and 29% liver. Sixty-six patients (41%) had 2 organs involved, and 24 patients (15%) had all 3 organs involved. The rate of overall response (complete and partial response) at day +28 was 74% for aGVHD grade B, 66% for grade C, and 59% for grade D. Overall response for individual organs at day +28 was 62% for GI, 77% for skin, and 53% for liver. Collectively, overall response at day +28 for patients treated for severe refractory aGVHD was 64%, and this response was correlated with statistically significant improved survival at day +100 after hMSC infusion. Patients who responded to therapy by day +28 had a higher Kaplan-Meier estimated probability of 100-d survival compared with patients who did not respond (79% vs. 21%; $P < .0001$). Substantial numbers of patients benefited from additional therapy beyond the initial 4 weeks of treatment. Ninety one patients (57%) received more than 8 infusions, with 48% of those patients experiencing additional improvement by day +100. Remestemcel-L infusions were generally well tolerated, without excessive infusional toxicities or ectopic tissue formation. The results of this study support the use of remestemcel-L for pediatric patients with refractory aGVHD.

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DNase I Treatment Reduces GVHD in Mice

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Graft-versus-host-disease (GVHD) is an alloimmune response complicating allogeneic hematopoietic stem cell transplantation (allo-HSCT). The development of GVHD is thought to involve three phases: T cell activation, followed by proliferation and differentiation of allogeneic T cells into activated effector cells, and finally specific tissue damage. In the activation phase, donor T cells interact with host APCs, leading to activation and differentiation toward the effector pathway and migration of these cells to target tissues affected during acute GVHD. Although donor T lymphocytes and recipient antigen presenting cells (APCs) are the primarily mediators of GVHD, the molecular and cellular basis are not well understood. Thus, the prevention and treatment of GVHD remains a major challenge. Deoxyribonuclease I (DNaseI) is an endonuclease that facilitates chromatin breakdown of apoptotic and necrotic cells. The impaired activity of DNaseI has been associated with the pathogenesis of systemic lupus erythematosus (SLE) and inflammatory bowel diseases (IBD). Using the MHC class I and II disparate model, C57BL/6 (H-2^b) to BALB/c (H-2^d), our studies demonstrate that DNaseI treatment can reduce GVHD mortality and morbidity in mice. In comparison to PBS control, DNaseI-treated mice showed a lower mortality rate. Within 4 weeks post-transplantation, 80% of DNaseI-treated recipients survived, compared with only 30% of PBS control mice. Whereas control recipients had severe GVHD in the skin, intestine, liver, and lung, DNaseI-treated mice exhibited only mild changes in these organs, reflected in their significantly lower GVHD scores. Furthermore, we analyzed donor-derived T cells in mice 7 days post-transplant, and found a significantly lower number of donor-derived $CD4^+$ and $CD8^+$ subsets in the peripheral lymph node (pLN) and Peyer's patches (PP) of DNaseI-treated mice compared to that of PBS control recipients. We further investigated the functional consequences of DNaseI treatment on inflammatory cytokine production in GVHD mice, and found the frequency of IFN-γ (Th1), IL-17 (Th17) and IL-2 producing donor $CD4^+$ cells in the spleen was significantly reduced in DNaseI-treated mice compared to PBS control mice. The results have established the disease-modifying activity of DNaseI in mouse GVHD model, and thus raise the possibility for using this drug as a potential new therapeutic intervention.

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Early Apoptotic Cells (ApoCell) As Prophylaxis of Graft-Versus-Host Disease Is Safe and Effective: 1 Year Follow-up and Mechanism of Action

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Apoptotic cells infusion (ApoCell, by Enlivex Ltd) is a novel cellular therapy that was found to be effective in a variety of mouse models of autoimmune diseases. ApoCell was tested in humans in addition to cyclosporine and methotrexate, as prophylaxis of graft-versus-host disease (GVHD) in HLA-matched myeloablative allogeneic bone marrow transplantation (alloBMT) from a related donor. We conducted a phase I//IIa clinical trial where 13 patients (median age, 37 years; range, 20-59 years) with advanced hematologic malignancies that received conventional myeloablation were