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MONOGRAPH

REMESTEMCEL-L, THE FIRST CELLULAR THERAPY PRODUCT FOR THE TREATMENT OF GRAFT-VERSUS-HOST DISEASE

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SUMMARY

Acute graft-versus-host disease (GVHD) is a cause of substantial morbidity and mortality following allogeneic stem cell transplantation. Complete responses to steroid-based front-line treatment occur in 25-40% of patients, and results of second-line treatment are unsatisfactory. This review examines the biological effects of mesenchymal stromal cells (MSCs) in relation to GVHD and describes the clinical results of GVHD treatment with cultured MSCs and the proprietary cryopreserved MSCs known as remestemcel-L.

Key words: Mesenchymal stromal cells – Steroid-refractory graft-versus-host disease – Allogeneic stem cell transplantation

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INTRODUCTION

Allogeneic stem cell transplantation remains the treatment of choice for patients with relapsed or high-risk hematological malignancy. Despite over five decades of

experience with this treatment, however, graft-versus-host disease (GVHD) remains a major cause of concern. It is the main cause of death of patients in remission after allogeneic transplantation, and many patients who survive acute GVHD struggle with severe disability and chronic illness for years. In order to minimize the risk of GVHD, donors are carefully selected based on high-resolution human leukocyte antigen (HLA) typing and clinical factors such as age, gender and parity. Even in transplants between HLA-matched siblings, differences in minor histocompatibility antigens may lead to the activation of alloreactive T lymphocytes, which then results in tissue injury and the clinical manifestations of GVHD.

PATHOPHYSIOLOGY AND CLINICAL MANIFESTATIONS OF ACUTE GVHD

The pathophysiology of acute GVHD has been well described and extensively reviewed elsewhere (1, 2). Briefly, the events leading to the development of clinically apparent GVHD begin during pretransplant conditioning, during which transplant recipients receive high-dose chemotherapy and/or radiotherapy (Fig. 1). The tissue damage that occurs as a result of high-dose therapy results in activation of host antigen-presenting cells (APCs), upregulation of major histocompatibility antigen on the APC surface and presentation of host antigens. Donor T lymphocytes, infused with the stem cell graft, respond to antigenic differences in this milieu by clonal expansion, tissue migration and direct cell–cell cytotoxicity. High levels of proinflammatory cytokines, particularly tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-2 (IL-2), and abundant host antigen lead to an inflammatory cascade that may result in severe tissue damage, organ dysfunction and death.

Acute GVHD is graded according to the degree of dysfunction of its three main target organs, the skin, liver and gastrointestinal tract. When GVHD is clinically mild, it may consist of a localized skin rash (< 50% of body surface area), mild elevation in total bilirubin or low-volume diarrhea (Table I). More severe GVHD (Grade 3 or 4) may be characterized by a generalized bullous dermatitis, severe elevations of total bilirubin (> 100 µmol/L) or severe diarrhea (> 1000 mL/day) with or without abdominal pain or ileus. Bloody diarrhea, although not considered separately in the Consensus grading system, carries an especially poor prognosis. Since many conditions can mimic GVHD, diagnosis generally requires histological confirmation secured through biopsy of an affected organ. Acute GVHD may occur at any point after allogeneic stem cell transplant but typically occurs in the first 2-6 weeks.

Once established, acute GVHD has a high mortality rate and is a cause of significant morbidity and mortality among stem cell transplant recipients. Both pharmacological and immunological methods have been developed to prevent acute GVHD. The most widely used strategy involves the prophylactic administration of a calcineurin inhibitor and an agent to prevent T-lymphocyte proliferation. Both ciclosporin and tacrolimus have been used in this context. Methotrexate is the most commonly used antiproliferative agent for GVHD prophylaxis, and despite its tendency to increase mucositis rates, several studies have demonstrated improved outcomes with its use (3, 4). Mycophenolate mofetil has been given in combination with ciclosporin for GVHD prophylaxis to recipients of reduced-intensity or so-called non-myeloablative transplants (5-7). Immunological methods of GVHD prophylaxis employ ex vivo or in vivo depletion of T lymphocytes. Results of this approach have been mixed, with high rates of rejection, fatal viral infection and relapse reported following depletion of these cells to very low levels (8). Ex vivo T-lymphocyte depletion using antibodies with narrow specificities (9) or administration of lower doses of T-lymphocyte depleting antibodies to patients undergoing transplantation appears to be of some benefit for prevention of both acute and chronic GVHD (10, 11).

Despite the use of effective prophylaxis, clinically significant (grade 2-4) acute GVHD occurs in 30-50% of recipients of sibling transplants and 50-75% of recipients of transplants from unrelated donors (12-15). Corticosteroids remain the cornerstone of treatment of acute GVHD and patients who fail to respond to frontline treatment experience high mortality and poor outcomes. A typical steroid regimen for treatment of acute GVHD consists of methylprednisolone 2 mg/kg/day, often given in divided doses. Corticosteroids are typically added to ongoing calcineurin inhibitors during treatment of acute GVHD. Treatment is continued for 1-2 weeks after a complete remission is achieved, followed by a taper at a rate of no more than 10% of the initial dose per week until discontinuation (16). More rapid tapers may be required for patients who experience severe infection or toxicity related to high doses of corticosteroids. Complete responses to corticosteroids occur in 25-40% of patients (17, 18). Higher response rates are generally observed in cutaneous than in visceral (gastrointestinal or hepatic) GVHD, and response rates are lower among patients with clinically more severe dis-

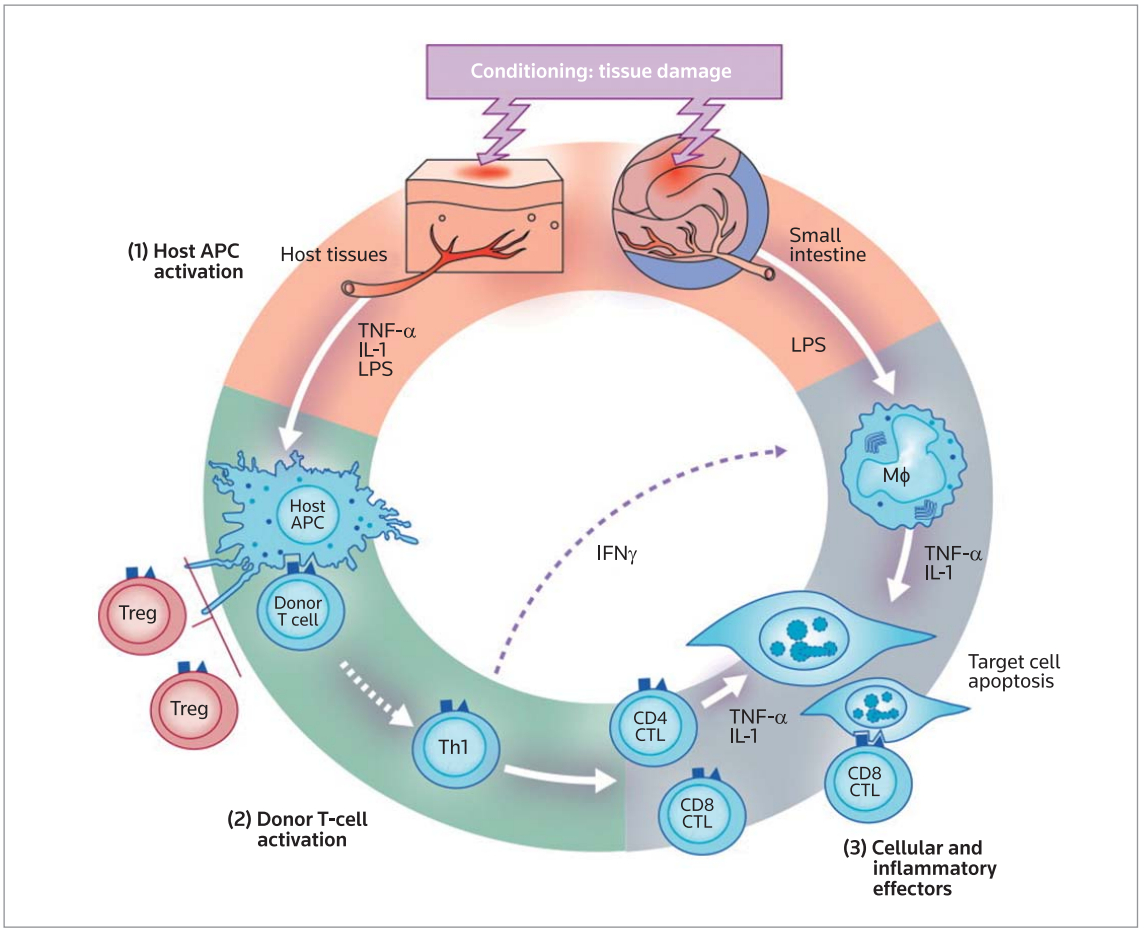

Figure 1. Pathophysiology of acute graft-versus-host disease (GVHD). Host antigen-presenting cells (APC) are activated by conditioning-related tissue damage (1). Donor T lymphocytes encounter host antigen and respond to antigenic differences between the donor and the host (major or minor histocompatibility antigens) by clonal expansion and activation (2). Activated donor T lymphocytes induce tissue damage through cell-mediated and effector-mediated immune responses (3). LPS, lipopolysaccharide; CTL, cytotoxic T lymphocytes. (Reprinted from Ferrara, J.L.M. et al. *Graft-versus-host disease.* Lancet 2009, 373(9674): 1550-1561 (1), used with permission of Elsevier.)

ease (19). Treatment-related mortality is substantially increased (46% vs. 16%, $P = 0.007$) among nonresponders whose methylprednisolone dose is increased from 2 to 5 mg/kg/day, and most centers add an additional agent rather than increasing the steroid dose (20, 21).

THE DILEMMA OF STEROID-REFRACTORY ACUTE GVHD

There is no generally agreed-upon definition of steroid-refractory acute GVHD. One accepted approach is to define GVHD that worsens after 3-5 days of steroid treatment, that does not improve after 5-7 days or that fails to remit completely after 14 days as steroid-refractory. It is important to identify steroid-refractory patients as early as possible in order to avoid unnecessary steroid exposure and delay of more effective therapy.

Second-line treatments of steroid-refractory acute GVHD are at best only moderately effective, and survival is poor due to the substantial toxicity of these approaches. The goal of treatment is to induce a complete remis-

Table I. *Organ-specific staging and overall grading of acute graft-versus-host disease (66).*

Stage	Skin	Liver	Gut
0	No rash	Total bilirubin: < 34 µmol/L	No diarrhea
1	Maculopapular rash < 25% body surface area	Total bilirubin: 34-50 µmol/L	Diarrhea 500-1000 mL/day; nausea and emesis with positive gastric biopsy
2	Maculopapular rash 25-50% body surface area	Total bilirubin: 51-100 µmol/L	Diarrhea 1000-1500 mL/day
3	Maculopapular rash > 50% body surface area	Total bilirubin: 101 to 250 µmol/L	Diarrhea 1500-2000 mL/day
4	Generalized exfoliative, ulcerative or bullous dermatitis	Total bilirubin: ≥ 250 µmol/L	Diarrhea > 2000 mL/day; or severe abdominal pain or ileus

	Stage				
Grade	Skin		Liver		Gut
0	0	and	0	and	0
1	1-2	and	0	and	0
2	3	or	1	or	1
3			2-3	or	2-4
4	4	or	4		

sion of GVHD-related tissue damage to allow organ function to return to normal and prevent further clinical deterioration. This is generally accomplished by inducing profound immunosuppression with antilymphocyte globulins and immunotoxins (alemtuzumab, various antithymocyte globulins [ATG], OKT3, denileukin diftitox) (22-29), anti-cytokine agents (etanercept, infliximab) (30, 31), agents that bind to cytokine receptors (basiliximab, daclizumab) (32-35) or immunomodulatory chemotherapeutic agents (pentostatin, mycophenolate mofetil) (36, 37). Clinical improvement (either complete or partial remission) is seen in 32-94% of cases but complete remissions are uncommon (see Table II). Extracorporeal photopheresis has also been used to treat steroid-refractory acute GVHD with similar outcomes (38, 39). As rates of opportunistic infection are high even among patients who do not respond, treating patients who fail second-line treatment with additional immunosuppressive agents is often challenging. Overall survival has been disappointing, with only approximately one-third of patients reported in phase II trials surviving (range: 4-70%). Follow-up in many of these studies has been short, and clinicians appreciate that these patients face substantial challenges and high rates of mortality that may continue for years. The failure of such diverse immunosuppressive approaches to result in high rates of survival suggests that a new paradigm is required for steroid-refractory GVHD, and cellular therapy has been suggested as a novel method of delivering the benefits of immunosuppressive therapy without the negative effects of additional systemic immunosuppression.

MESENCHYMAL STROMAL CELLS

Mesenchymal stromal cells (MSCs) constitute a rare population of non-hematopoietic cells in the bone marrow. These cells can be identified by their capacity to differentiate into cells of mesodermal origin and are commonly described as having the following properties (40, 41): i) Adherence to plastic in in vitro culture; ii) surface antigen expression of CD105, CD73, CD90 and negativity for hematopoietic lineage markers; and iii) the capacity to differentiate in vitro into osteoblasts, adipocytes and chondroblasts demonstrated by staining in vitro cultures. While these cells can be most easily cultured from bone marrow, stromal cells derived from fat, muscle and cartilage share similar properties.

MSCs provide important physical and growth factor support to hematopoietic stem cells and developing blood elements within the bone marrow microenvironment. They also have the capacity to dampen inflammatory responses through their immunomodulatory properties (42). Extensive in vitro studies have shown that MSCs can prevent entry of T lymphocytes into the cell cycle, thus preventing the clonal expansion of activated lymphocytes necessary for an effective immune response (43). MSCs have also been shown to promote secretion of IL-4 by T helper type 2 (Th2) lymphocytes, impair secretion of interferon-gamma (IFN-γ) by Th1 lymphocytes and enhance development of regulatory T lymphocytes in culture (44). In addition to effects on T lymphocytes, MSCs also alter cytokine secretion by mature

Table II. *Results of representative trials of immunosuppressive treatment of steroid-refractory acute graft-versus-host disease.*

Agent	N	Overall response	Complete response	Survival
ATG (22)	29	Skin 72% GI 38% Liver 38%	Not reported	12% 1-year
ATG (23)	69	41% Grade 2 24% Grade 3-4	14%	4%
ATG (24)	79	54%	20%	32% 1-year
OKT3 (25)	43	69% (12% durable)	12%	Median 80 (2-2474+) days
OKT3 + high-dose MP vs. high-dose MP (28)†	40 vs. 40	53% vs. 33% (P = 0.06)	Not reported	45% vs. 36% (P = 0.6)
Visilizumab (29)	44	32%	14%	32% 180-day
Daclizumab (32)	20	40%	20%	2/20 (10%) (529 and 649 days)
Daclizumab (33)	12 acute	8/12 (66%)	1/12 (8.3%)	2/12 (16%) (458 and 459 days)
Daclizumab (once or twice weekly) (34)††	24 weekly, 19 twice weekly	51%	29% weekly regimen, 47% twice weekly regimen	29% weekly, 53% twice weekly regimen, 120 days
Basiliximab (35)	23	82.5%	17.5%	45% 1-year
Alemtuzumab (26)	18	15/18 (83%)	6/18 (33%)	55% (median follow-up 11 months)
Alemtuzumab (27)	18	17/18 (94%)	5/18 (28%)	33% (median follow-up 36.5 weeks)
Infliximab (30)	32	59%	19%	41% (median follow-up 449 days)
Etanercept (31)	13 acute	6/13 (46%)	4/13 (31%)	9/13 (69%) (median follow-up 317 days)
Pentostatin (36)	23	78%	64%	26% overall
Mycophenolate mofetil (37)	36	72%	Not reported	37% 5-year (acute and chronic)

ATG, antithymocyte globulin; OKT3, muromonab-CD3; MP, Methylprednisolone; †Randomized multicenter trial; ††Sequential group comparison of two regimens of daclizumab.

dendritic cells, prevent differentiation of monocytes into dendritic cells and prevent upregulation of CD1a, CD40, CD80, CD86 and HLA-DR upon activation (44, 45). Monocytes cultured in the presence of MSCs fail to enter the cell cycle upon exposure to granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4, an effect attributed to downregulation of cyclin D2 in the cultured monocytes (46). MSCs appear to require priming by a combination of IFN-γ and one of TNF-α, IL-1α or IL-1β to exert these effects, suggesting that there is important bidirectional cross-talk between immune cells and the local microenvironment. The mediators responsible for the immunomodulatory effects of MSCs appear to include nitric oxide, as MSCs from mice deficient for inducible nitric oxide synthase (iNOS$^{-/-}$) do not impair proliferation of T lymphocytes in response to anti-CD3

antibody to the same degree that MSCs from wild-type mice do (47). Other immunomodulatory mechanisms proposed for these cells include tryptophan degradation by indoleamine-2,3-dioxygenase, prostaglandin E_2, soluble HLA-G and polarization of APCs to more tolerogenic phenotypes (reviewed in Siegel et al. [42]).

Murine models have demonstrated that MSCs can affect the development and course of experimental GVHD. Chung et al. demonstrated that BALB/c (H-2^d) mice cografted with bone marrow and MSCs from HLA-mismatched (H-2^k) C3H/He mice experienced higher survival and lower GVHD scores than mice grafted without MSCs, or mice grafted with bone marrow supplemented with splenocytes (primarily T lymphocytes). They conclude that MSCs suppress the development of GVHD, and that this effect is dependant on the ratio of MSCs and T lymphocytes (48). Joo et al. used a similar experimental design to demonstrate that infusion of C3H10T1/2 cells (commercially available cultured C3H murine MSCs) 10-15 minutes before infusion of bone marrow and splenocytes results in enhanced expression of FoxP3 mRNA levels in thymus and mesenteric lymph nodes, changes associated with enhanced levels of regulatory T lymphocytes in these tissues. They also noted a reduction in histological GVHD and improved survival of pretreated mice, with an effect that depended on the dose of MSCs infused (49). When the migration patterns of MSCs and splenocytes are tracked separately using cells tagged with fluorescent proteins in co-transplantation experiments they follow very similar paths and ultimately home to regional lymph nodes after 37 days. Histologically, the infused stromal cells and splenocytes show close approximation within lymph nodes (50). While not all studies of MSC transplantation in experimental models of GVHD have been positive (51-54), there is plausible biological rationale and reasonable laboratory evidence of safety and efficacy to justify human studies.

In 2004, LeBlanc and coworkers (55) reported the case of a 9-year-old boy who developed steroid-refractory GVHD 11 days after undergoing transplantation from an HLA-matched, unrelated donor for relapsed acute lymphoblastic leukemia. By day 70 post-transplant he had grade 4 acute GVHD despite multiple second- and third-line agents and he was given 2×10^6 MSCs per kg, cultured from a sample of maternal bone marrow, on day 73. Over the next 2 weeks the patient showed rapid and complete resolution of diarrhea and hyperbilirubinemia consistent with a major response to MSC treatment. A brief flare of GVHD after withdrawal of ciclosporin again resolved with MSC infusion. The safety of allogeneic MSC transplantation was demonstrated in 46 patients who received donor MSCs expanded in tissue culture in conjunction with hematopoietic stem cell transplantation. Low rates of grade 3/4 acute GVHD were reported and there was no immediate toxicity associated with infusion of the cultured cells. Adverse events in this group of patients did not differ substantially from those of hematopoietic stem cell transplant recipients in general (56). A phase II trial conducted by the European Bone Marrow Transplant Group confirmed the safety of MSC treatment for steroid-refractory GVHD and demonstrated a response rate of 71% in a cohort of 55 patients (57). Children responded to MSC treatment for steroid-refractory acute GVHD more frequently than adults did (84% vs. 60%, $P = 0.07$). There were no acute or delayed side effects of MSC infusions and treatment-related mortality was substantially lower among responders than among nonresponders (37% vs. 72%, $P = 0.002$). MSCs from third-party donors were as effective as MSCs cultured from the bone marrow donor or family members of the recipient. The latter observation suggests that banked, cryopreserved MSCs may be useful clinically, a feature that has been exploited in the development of remestemcel-L.

REMESTEMCEL-L

Remestemcel-L is a cellular therapy product marketed under the trade name Prochymal® by Osiris Therapeutics. Remestemcel-L consists of cultured, cryopreserved MSCs derived from the bone marrow of healthy donors. Although the production is proprietary, donors are screened and tested according to FDA guidelines for Blood and Tissue Based Products. Initial samples of bone marrow are processed to remove unwanted cells and cultured in medium containing 10% fetal bovine serum (58). Adherent cells form fibroblast-like colonies and are replated for a total of five passages prior to harvest. All reagents used in manufacture of this product are GMP grade. Cells from the final culture and in-production lots are tested for safety (absence of contaminating viruses, bacteria and fungi), purity (absence of hematopoietic cells), identity (expression of such appropriate MSC markers as CD105, CD73, CD90), potency (expression of TNF receptor 1 [TNFR1] and inhibition of IL-2 receptor alpha [IL-2-RA] expression by activated T lymphocytes) and viability. Harvested cells are brought to a final concentration of 100×10^6 cells per 15 mL in PlasmaLyte A supplemented with human serum

albumin and 10% dimethyl sulfoxide (DMSO) prior to cryopreservation (59, 60). Remestemcel-L is thawed and further diluted with PlasmaLyte A prior to intravenous infusion.

HUMAN STUDIES

In 2009, Kebriaei and colleagues reported the results of a randomized, phase II study of two different dose levels of remestemcel-L in combination with corticosteroids for treatment of newly diagnosed acute GVHD. Thirty-two patients aged 18-70 years with untreated grade 2-4 acute GVHD were randomized to receive two infusions of 2×10^6 or 8×10^6 cultured human MSCs per kilogram in addition to methylprednisolone 2 mg/kg or an equivalent dose of prednisone. MSC cultures for this study were prepared from six unrelated, unmatched donors between the ages of 18-30 years. A total of 32 patients were enrolled, but results on only 31 patients are described as 1 patient withdrew consent after 10 days of treatment. The response rate was 94% (77% complete response rate), and the majority of responders maintained their response for at least 90 days. Response rates in gastrointestinal GVHD were particularly high (82%). There was no difference in the response rate between the two dose levels studied. Treatment with remestemcel-L was safe: There were no acute toxicities during administration and screening CT scans failed to demonstrate ectopic tissue formation related to engraftment and differentiation of MSCs. Infectious complications were similar to infections in non-MSC-treated patients with acute GVHD and included 12 grade 3 and 3 grade 4 infections. The authors conclude that remestemcel-L represents a promising new strategy for the treatment of acute GVHD (59).

A compassionate-use multicenter protocol for pediatric refractory acute GVHD was carried between July 2005 and June 2007. Twelve patients with acute GVHD refractory to steroids and at least one additional line of immunosuppressive therapy were given eight infusions of remestemcel-L over 4 weeks. Patients who achieved a partial or mixed response were eligible for four additional doses over a 4-week period. The median age of patients on this study was 6 years, and all had failed two to five (median three) prior immunosuppressive therapies. Treatment was well tolerated, with no acute toxicity observed during the infusions. One patient with malignant osteopetrosis developed ectopic tissue; no MSCs of donor origin were detected in this ectopic tissue by DNA analysis. At completion of therapy, complete and partial responses were seen in 58% and 17% of patients, respectively. Two-year overall survival was 42%, and survival was higher for patients who achieved a complete response. In this report remestemcel-L appeared to be a safe and effective treatment for pediatric steroid-refractory GVHD. The lack of overlapping toxicity enabled heavily pretreated patients to tolerate additional treatment with remestemcel-L (61).

A randomized, placebo-controlled study of remestemcel-L in steroid-refractory acute GVHD has been reported in abstract form. In this study, 244 patients were randomly assigned in a 2:1 ratio to receive either an accepted second-line treatment plus remestemcel-L or second-line treatment plus placebo (PlasmaLyte A plus DMSO) (62). Eight infusions of remestemcel-L or placebo were given over 4 weeks and an additional four weekly infusions could be given to patients with a partial response. The primary endpoint was durable (≥ 28 days) complete response rate, which did not differ between the two groups (35% vs. 30%, $P = 0.3$) in the intent-to-treat population. When the analysis was limited to patients who were treated per protocol, durable complete responses were observed more frequently in patients who received remestemcel-L than in patients who received placebo (40% vs. 28%, $P = 0.08$). Overall responses were observed in 82% of patients who received remestemcel-L compared with 73% of patients who did not ($P = 0.12$). Visceral GVHD responded well to remestemcel-L (odds ratio [OR]: 3.6; 95% confidence interval [CI]: 1.1-11.2; $P < 0.05$ for liver, and OR: 2.2; 95% CI: 1.1-4.4; $P < 0.05$ for lower gastrointestinal GVHD). High response rates of cutaneous GVHD to standard therapy (77% with standard treatment vs. 78% with standard treatment plus remestemcel-L, $P = 0.9$) may have masked the effect of remestemcel-L therapy in this subgroup of patients. Rates of infection and infusional toxicity did not differ between the arms and more patients in the placebo group discontinued treatment due to adverse effects (4.6% vs. 0.6%).

The manufacturer of remestemcel-L has also completed a randomized study in de novo acute GVHD (63), which enrolled 184 patients with untreated CIBMTR grade B-D acute GVHD. The results of this study have yet to be published.

CONCLUSIONS

On May 17, 2012, Osiris Therapeutics received conditional marketing approval (Notice of Compliance with conditions, NOC/c) to distribute Prochymal-brand remestem-

cel-L in Canada for the indication of steroid-refractory acute GVHD in children (64). This approval marked the first approval of a commercial cellular therapy for systemic administration for any indication by a regulatory agency. The approval was based on the drug's relatively benign safety profile and promising evidence of efficacy in the subgroup of patients for which it was approved. As part of the marketing approval, Health Canada required Osiris to provide postmarketing confirmatory studies as well as a registry of treated patients. Similar marketing approval was received from the New Zealand Medicines and Medical Devices Safety Authority (Medsafe) on June 14, 2012 (65). Prochymal-brand remestemcel-L is the only agent to receive regulatory approval for treatment of steroid-refractory GVHD.

Acute GVHD is a serious condition with high mortality if it fails to respond to front-line therapy. Available second-line treatments do not improve overall survival and result in high rates of opportunistic infection. Treatment of steroid-refractory acute GVHD with cultured human MSCs has been shown to be safe, and most series suggest that infection rates are relatively low. In refractory acute GVHD, responses to MSC treatment are reported in 71- 82% of patients, with frequent complete responses seen. Remestemcel-L represents an important proof of the concept that "off-the-shelf" cellular therapy of GVHD is feasible and that regulatory agencies will license these products for therapeutic use. While studies with remestemcel-L suggest that its use in the treatment of steroid-refractory acute GVHD is relatively safe, treatment benefit has not been conclusively demonstrated. Treatment appears to be feasible, and may be effective in a pediatric subgroup of patients but the failure of the pivotal study to meet its primary endpoint makes it difficult to draw firm conclusions about its place in the therapeutic arsenal. The answers to basic questions about the biodistribution and fate of MSCs after infusion may help to optimize treatment with remestemcel-L. Longer follow-up of treated patients and more randomized, controlled studies are necessary to conclude that remestemcel-L represents the most effective therapy for steroid-refractory GVHD. Nonetheless, the era of cellular therapy for acute GVHD has begun, bringing hope for a cure for this otherwise devastating complication of cancer treatment.

DISCLOSURES

A. Daly has been a paid consultant for Osiris Therapeutics, Columbia, MD.

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