Hematopoietic Cell Transplantation for Nonmalignant Disorders

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Hereditary disorders that trace their origin to the hematopoietic stem cell have been targeted for allogeneic therapy and were among the first human diseases cured by successful hematopoietic cell transplantation (HCT). More recently, the possibility of treating nonhematopoietic hereditary disorders in which engraftment of hematopoietic cells might ameliorate tissue damage in target organs has also been investigated with encouraging results. As in the malignant hematological disorders, transplantation results have improved over the past 3 decades as a consequence of more refined donor selection and patient risk stratification with modifications to the conditioning regimen. The application of these principles is described in this update about HCT for hereditary marrow failure syndromes and hemoglobin disorders. In addition, a novel indication of HCT for epidermolysis bullosa is presented. Together, these representative disorders illustrate the potential for an expanding role of HCT for nonmalignant disorders.

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HEMATOGENOUS CELLS FOR EXTRACELLULAR MATRIX DEFICIENCY EPIDERMOLYSIS BULLOSA

One of the most fascinating and daunting challenges of current transplantation biology is to harness the potential of stem cells for tissue regeneration. Adoptive transfer of hematopoietic stem cells can, of course, regenerate the lymphohematopoietic system in the recipients of hematopoietic cell transplantation (HCT), but only recently have we and others obtained evidence that bone marrow and cord blood transplantation can mediate tissue repair in the largest extramedullary organ—the skin.

The skin is constantly exposed to environmental insults and requires effective repair tools to maintain its protective function. Skin repair is a highly organized process that integrates the skin cells, skin extracellular matrix, and systemic factors—mainly blood cells and cytokines—into dynamic tissue healing. Keratinocytes and dermal fibroblasts express adhesive proteins that ensure the epidermis remains attached to the skin basement membrane and to the papillary dermis. Congenital deficiency of any of at least 15 such proteins results in a blistering condition called epidermolysis bullosa (EB) [1]. Of the multiple forms of EB, the severest are:

- recessive dystrophic EB (RDEB) caused by mutations in the collagen type VII (C7) gene (COL7A1), and
- junctional EB (JEB) caused by loss-of-function mutations in one of the three genes (LAMA3, LAMB3, and LAMC2) that encode one of three chains in the heterotrimeric protein laminin 332 (L332).

C7 and L332 deficiencies result in severe scarring, contractures, and, ultimately, shortened survival—most often because of infections and aggressive squamous cell carcinoma. Even though suffering is difficult to measure objectively, the painful challenges that individuals with EB endure are extraordinary. EB skin can blister with a touch, and the resulting wounds heal with mutilating scarring. Their upper alimentary tract can blister just as easily. Esophageal strictures develop with aberrant tissue repair, and eating is painful. People with EB are separated from many interactions with their peers, as most common daily activities are impossible for them. Every moment and aspect of their lives is consumed by this debilitating genodermatosis [2].

Remarkably, stem cell transplantation can ameliorate the deficiency of this skin-specific structural
protein in an animal model of RDEB [3]. We found that donor cells at the dermal-epidermal junction produced C7 in the skin of the RDEB mice. Strikingly, paw blisters healed and rudimentary homotrimers of C7, termed anchoring fibrils, were formed [4]. These data were supported by the research of others, who demonstrated the positive effect of in utero infusion of bone marrow cells in RDEB and postnatal infusions in JEB [5,6].

Based on these proof-of-concept studies in murine models that show the replacement of missing C7 and skin repair, a first in-human study was initiated in 2007. After this clinical trial of allogeneic HCT in seven children with RDEB had shown that C7 can increase and ameliorate many of the disease manifestations [7], we hypothesized that children with JEB could respond favorably to allogeneic transplantation as well, and that both groups could benefit from coinfusion of hematopoietic and mesenchymal stem cells (MSCs) from allogeneic marrow.

To investigate this, we treated eight additional children with RDEB and two children with JEB using allogeneic cotransplantation of hematopoietic and MSCs. Altogether, 17 patients have been treated, with survival in 13 and clinical amelioration of the disease in 11. Healthy donor cells from the hematopoietic graft migrated to the injured skin. Simultaneously, there has been an increase in the production of C7 and L332 and increased skin integrity with reduced tendency to blister formation. The results of these trials demonstrate that the infusion of allogeneic hematopoietic cells can result in substantial clinical benefit [8].

Laboratory investigations in EB show that:

1. Unique among proteins critical to skin integrity, C7 function depends on extracellular polymerization. This makes RDEB the ideal human skin condition to explore, and thus illuminate, the mechanisms of cross-correction of structural protein deficiency in extracellular matrix.

2. Depletion of cells with high proliferative potential from skin stem cell niches by hyperactive unproductive tissue repair in EB may facilitate high donor cell engraftment. The empty stem cell niches in EB skin that are depleted of their own stem cells by repeated and futile attempts at skin regeneration, may serve as permissive “docking stations” for donor cells. Such cells may, in turn, release factors that operate on the host or that may differentiate into cells that directly participate in the wound-healing process via release of C7, L332, or other tissue repair proteins. Simultaneously, this further supports the idea of EB as a unique model of skin tissue repair with donor regenerative cells [9].

3. The novel technology of reprogramming skin cells into pluripotent stem cells (iPSCs) by a combination of specific transcription factors can further illuminate the early stages of aberrant skin formation in development and, in principle, provide an inexhaustible supply of EB patient-specific stem cells that can be useful for future stem cell gene therapy strategies [10].

Clinical trials in EB illustrate that:

1. Translation of laboratory observations in relevant animal models to a clinically meaningful intervention is possible within a short period of time (<2 years).

2. Further modifications in the use of stem cell transplantation as a durable source of extracellular matrix proteins may make this regenerative medicine approach effective in other cutaneous and extracutaneous conditions.

3. Only by performing the laboratory and clinical experimentation that contributed to achievement of the original objectives do we learn about the nature of “the growing frontier.” Thus, only in the context of new data do previously unanticipated questions emerge, both biologic, for example, how robust must hematogenous engraftment be to significantly contribute to skin repair?; and ethical, for example, how much preliminary data are enough to substantiate clinical trials in infants with severe phenotype versus adults with a mild form of the same disease?

In summary, significant biochemical and quality-of-life benefits can be derived from allogeneic hematopoietic and mesenchymal cell transplantation for RDEB and JEB, conditions characterized by severely compromised wound healing because of the congenital absence of skin structural proteins. These severe congenital mechanobullous disorders are unique in their capacity to illuminate the mechanisms of cross-correction of a structural protein deficiency in extracellular matrix and regeneration of extramedullary tissues by hematogenous cells in disease and injury states.

### HCT FOR CHROMOSOME SENSITIVITY SYNDROMES

DNA is continuously damaged by endogenous and exogenous (eg, ionizing radiation) mutagens. Repair of DNA damage (DNA double- and single-strand breaks) is a complex process carried out by an array of DNA repair pathways (eg, homologous recombination, non-homologous end-joining). Defects in any of these repair mechanisms or in telomere maintenance can lead to chromosome instability syndromes. The chromosome instability syndromes mainly manifest as congenital bone marrow failure (BMF) syndromes (eg, Fanconi anemia, Shwachman-Diamond syndrome [SDS], dyskeratosis congenita [DC], Bloom syndrome, and Nijmegen breakage syndrome and a variety of primary immune deficiency disorders such as severe...
combined immune deficiency syndromes—DNA ligase IV, Artemis, Cernunnos, etc.).

Congenital BMF syndromes are associated with a range of congenital anomalies, impaired hematopoiesis leading to progressive marrow failure, and cancer predisposition. The typical clinical features of these disorders are generally well known to practicing hematologists, although they are sometimes overlooked in adult practice where they present less frequently. However, it is becoming increasingly apparent that patients lacking characteristic physical stigmata may still harbor a congenital BMF syndrome. In addition, some patients present with clinical symptoms for the first time as an adult. Sensitive and specific diagnostic tests, including identification of mutations in specific genes, are now available for many of these disorders. This is particularly useful while evaluating patients with suspected marrow failure syndrome, and it is essential for screening sibling donors, to ensure that an affected sibling is not used as a stem cell donor. HCT remains the only potentially curative option for many of these disorders. However, the inherent chemotherapy and radiation sensitivity makes transplantation for these disorders challenging.

FANCONI ANEMIA

Fanconi anemia (FA) is a genetic disorder characterized by congenital anomalies, progressive BMF, and predisposition to malignancies. The cellular phenotype of FA is characterized by an abnormally high level of baseline chromosomal breakage along with an increased sensitivity to DNA cross-linking or alkylating agents [11]. The earliest attempts at transplanting patients with FA (1970s and 1980s) used 50 mg/kg of cyclophosphamide × 4 days. These transplantations had high mortality and morbidity. This study provided the first clinical evidence of a special sensitivity of FA cells to alkylating chemotherapy agents. Such hypersensitivity was also observed in vitro when FA cells were incubated with alkylating agents. Gluckman and colleagues [12] were the first to investigate a markedly attenuated conditioning regimen for patients with FA and successfully demonstrated that HCT could be safely performed using low-dose cyclophosphamide, with long-term survival in 75% of patients. They also confirmed the suspected increased radiosensitivity in the majority of patients with FA. Following these results, patients were conditioned with low-dose cyclophosphamide (20 mg/kg) in combination with 5 Gy thoracoabdominal irradiation. The addition of antithymocyte globulin (ATG) to the Gluckman regimen was the next important milestone. The most recent of these reports showed a 10-year actuarial survival rate of 89%, with a significant decrease in acute (23%) and chronic (12%) graft-versus-host disease (aGVHD and cGVHD) in a matched sibling donor HCT setting [13].

Over the last 3 decades, these regimens have been modified significantly, with the goal of limiting toxicity while maintaining engraftment and improving outcomes by decreasing GVHD. An additional important goal is to minimize the development of secondary malignancies in these patients. Kapelushnik and colleagues [14] were the first to report use of a fludarabine-based conditioning regimen for a child with FA in leukemic transformation. This highly immunosuppressive nucleoside analog is well tolerated by patients with FA, as it does not cause direct DNA damage and has allowed for the elimination of radiation in a matched sibling donor setting with good results.

Outcomes of unrelated donor (URD) HCT for FA have been inferior, primarily because of high risk of graft failure, aGVHD, and excessive regimen-related organ toxicity. Before the introduction of fludarabine in the FA-preparative regimen, survival for URD transplants was approximately 30%. Three groups in the United States including Cincinnati Children’s Hospital, University of Minnesota, and Memorial Sloan-Kettering Cancer Center have pioneered regimens that include low-dose total-body irradiation (TBI), cyclophosphamide, ATG, and fludarabine followed by T cell-depleted marrow or peripheral blood stem cell transplants [15-17]. All three centers have had promising results. However, with the small number of patients with FA who undergo allogeneic HCT, results of single-center trials are difficult to interpret in terms of optimal cytoreductive regimens and dosing. Reported survival rates with fludarabine-containing preparative regimens range from 38% to 96%. In an attempt to decrease late secondary malignancies and improve immune recovery in this vulnerable population, the Minnesota group has shown that the TBI dose can be reduced to 300 cGy. However, further reduction led to graft failure.

Most recently with a similar goal, a multi-institutional study is ongoing at four centers, using a chemotherapy-only preparative regimen, containing pharmacokinetically adjusted low-dose busulfan, cyclophosphamide, fludarabine, and rabbit ATG. All grafts are T cell depleted using the CliniMacs CD34 columns (Miltenyi, Auburn, CA). So far, engraftment, early toxicity, and infection data appear to be similar to historical TBI-based protocols, with no graft failures after 15 of a planned 25 transplants. Overall, these results appear promising, and future patients will likely not need radiation.

SDS

HCT is the only known curative treatment for bone marrow failure associated with SDS. Similar to FA, children with SDS tend to have increased toxicity with intensive conditioning regimens. Various case reports/series describe fatal congestive heart failure, neurologic
complications, pulmonary complications, and multiorgan failure with typical ablative regimens containing cyclophosphamide. In one review, more than 50% of the patients succumbed to cardiopulmonary complications in the early posttransplantation phase. Similarly, a review of the European experience with HCT in 26 SDS patients [18], reported an overall treatment-related mortality of 35.5% at 1 year. Interestingly, they found a significantly higher mortality rate in patients receiving a TBI-containing conditioning regimen (67% for TBI versus 20% for non-TBI-containing regimen; \( P = .03 \)). Recent efforts have thus focused on reduced-intensity conditioning (RIC) regimens to ameliorate cardiac and pulmonary toxicities. Sauer and colleagues [19] used fludarabine, treosulfan, and melphalan, and we have previously reported our experience with successful use of an RIC regimen consisting of Campath-1H, fludarabine, and melphalan. These data indicate that HCT with RIC is feasible in patients with SDS and is associated with excellent donor cell engraftment and modest morbidity.

DC

DC is an inherited disorder that usually presents with the clinical triad of abnormal skin pigmentation, nail dystrophy, and mucosal leukoplakia. Noncutaneous manifestations include progressive liver cirrhosis and pulmonary fibrosis. The availability of a simple diagnostic blood test measuring telomere length and genetic testing that is positive in about 50% of patients has greatly increased recognition of this disorder. Outcomes of allogeneic HCT have been poor because of early and late complications. Langstone and colleagues [20] performed transplantation on eight patients with DC and BMF. Six who underwent transplantation using matched sibling donors were conditioned with cyclophosphamide (140-200 mg/kg) with or without ATG, and two with matched URD received cyclophosphamide (120 mg/kg) and TBI (1200 cGy). Three patients died from respiratory failure and pulmonary fibrosis at 70 days, 8 years, and 20 years posttransplantation. Three patients died early from invasive fungal infections, one died from refractory aGVHD on day 44, and one patient was alive at 463 days, who underwent surgical resection of a rectal carcinoma 14 months post HCT. Other reports using myeloablative conditioning showed similar results.

The presence of pulmonary disease in a significant proportion of DC patients before HCT may explain the high incidence of fatal pulmonary complications in the HCT setting. More recent studies have used RIC with encouraging results for successful engraftment with fewer complications, for both related and unrelated allografts [25]. Tolar et al. recently published their experience with a RIC regimen containing cyclophosphamide, fludarabine, alemtuzumab, and a single 200 cGy dose of TBI. Four patients were alive with full donor engraftment at a median follow-up of 26.5 months [21]. At our center, we have adopted RIC with Campath 1H, fludarabine, and melphalan, with the melphalan dose reduced by 50% to avoid excessive regimen-related toxicity and mortality. One of these patients with DC and myelodysplastic syndrome with monosomy 7 is 1 year posttransplantation, with normal hematopoiesis and no evidence of monosomy 7. Of note, this patient did not receive Campath to avoid risk of mixed chimerism and potential relapse of myelodysplastic syndrome. However, long-term follow-up data are not available, and it remains to be seen how these patients ultimately fare. Regardless of the potential reduction in toxicity associated with these regimens, preexisting characteristics of DC (eg, pulmonary and liver disease) may ultimately limit the effectiveness of HCT in these patients.

In summary, progress in improving the outcomes for children with chromosomal sensitivity syndromes has been limited by the rarity of these disorders, as well as disease-specific genetic, molecular, cellular, and clinical characteristics that increase the risks of complications associated with HCT. However, recent progress has been made, as best evidenced by use of various RIC regimens with improved outcomes and by improved diagnostic testing. The rarity of these diseases, coupled with the likely incremental improvements that will result from ongoing research will require prospective multicenter or even international clinical trials to improve the outcome for these children.

HCT FOR HEMOGLOBINOPATHIES

The hemoglobinopathies, which account for the most common hereditary disorders worldwide, are curable by HCT. But unlike rarer hereditary disorders of childhood that are rapidly fatal in the absence of HCT, expanded and improved supportive therapies for hemoglobin disorders now routinely extend the life span of affected individuals well into adulthood [22,23]. As a consequence, in regions of the world where this healthcare is readily available, HCT is pursued infrequently in children with these disorders because of a perception among families and their physicians that the risk-benefit balance has shifted away from HCT [24]. More recently, however, there has emerged a growing consensus that survival into adulthood is accompanied by chronic and life-threatening complications, and this new understanding has caused many to reconsider the role of HCT for these disorders, particularly in young adult patients in whom the quality of life has declined [25]. Thus, transplantation investigators are now challenged by how to make HCT successful in older individuals where risks of GVHD, graft rejection, and
impaired organ function are very likely to have a negative impact on outcomes. In this brief review, we will examine what has and has not been successful in elucidating a successful approach to HCT for hemoglobinopathies and speculate about the best future prospects for expanding the role of HCT.

The curative potential of HCT for hemoglobinopathies was proved first in thalassemia major and later in sickle cell disease, utilizing a myeloablative backbone of busulfan and cyclophosphamide before HLA-identical (ID) sibling bone marrow transplantation (reviewed in [26]). These initial studies showed a very high success rate, with failures caused either by transplant-related deaths or more frequently, graft rejection accompanied by autologous reconstitution and disease recurrence. The risk of disease recurrence in thalassemia was highest in pediatric recipients who had poor-risk features such as hepatomegaly, hepatic fibrosis, or poor adherence to regular iron chelation therapy before HCT. In addition, transplant-related mortality in young adults with thalassemia ranged from 27% to 37% [27]. Together, these clinical series showed that it was possible to treat hemoglobinopathies successfully by HCT and that patient selection affected outcome. However, as most affected individuals lacked an HLA-ID sibling donor, the application of HCT for hemoglobinopathies was not broadly expanded, and for the most part was restricted to children.

Several recent patient series show progress in how to tackle the problems of graft rejection and conditioning regimen toxicity, and in so doing, should help expand HCT beyond the realm of HLA-ID sibling bone marrow transplantation. First, by extending the duration and intensity of pretransplantation immunosuppression by administering azathioprine and hydroxyurea 6 weeks before HCT and by adding fludarabine to the conditioning regimen, it was possible to improve transplantation outcomes in high-risk pediatric thalassemia recipients [28]. Similarly, the replacement of fludarabine for cyclophosphamide in the conditioning regimen resulted in an acceptable toxicity profile and was sufficient for engraftment after unrelated donor bone marrow transplantation in children and young adults [29]. The use of treosulfan in lieu of busulfan also appeared to generate excellent results in the unrelated donor setting [30]. Thus, it appears that modulation of the conditioning regimen in such a manner that might amplify suppression of the host-versus-graft rejection has served to make engraftment of donor cells more likely to occur, even after alternate-donor HCT. Similarly, the use of alternative and novel chemotherapeutic agents in the conditioning regimen might also improve the toxicity profile.

Other obvious strategies to extend transplantation more safely and broadly have also been pursued, with mixed results. There are now several series that report only transient donor engraftment after nonmyeloablative preparation following a minimal toxicity regimen, and this strategy has been dropped for the most part [31]. There has been better success with engraftment after applying reduced-intensity regimens that accomplish immunoablation by incorporating the use of alemtuzumab. One recent trial in adults with sickle cell disease who also received sirolimus for postgrafting immunosuppression experienced stable donor-host chimerism when postgrafting immunosuppression was extended long term after HCT [32]. Several multicenter clinical trials are being conducted to explore the utility of a combination of alemtuzumab with melphalan and fludarabine in children with hemoglobinopathies based upon promising preliminary experience with this regimen in children with nonmalignant conditions. The use of umbilical cord blood (UCB) as a source of donor cells has also been tested, as this source of hematopoietic cells extends a lower risk of GVHD, a complication that carries no benefit in nonmalignant conditions. Initial reports showed that UCB from HLA-ID sibling donors, either alone or in combination with marrow from the same sibling donor, showed results that were very similar to outcomes after bone marrow transplantation, with a reduced risk of GVHD [33]. Attempts to extend these results by using unrelated UCB donors have been disappointing, due in large part to a high rate of graft rejection, particularly after nonmyeloablative preparation [34]. Thus, the future use of UCB from unrelated donors for this indication is uncertain today.

In summary, the investigations and clinical experience reported to date indicate that there is an unusually difficult barrier to allogeneic engraftment after HCT for hemoglobinopathies, which remains incompletely understood. Nonetheless, there is very good empirical evidence that aggressive inhibition of the host immune system is required for engraftment, using either myeloablative or immunoablative therapy for pretransplant conditioning. This is particularly important in the setting of unrelated and HLA-mismatched donor HCT. It is also very likely that donor availability will continue to create challenges, especially in light of the poor results after unrelated UCB transplantation that have diminished enthusiasm for this source of hematopoietic cells. Thus, it is possible that renewed effort will be focused on the use of UCB-transplantation and haploidentical related donors. Of course, this approach also carries a significant challenge of how to overcome the barrier to engraftment, as was highlighted in a recent thalassemia trial [35]. But improved methodologies, such as the use of postgrafting high-dose cyclophosphamide, might provide a novel path to engraftment without significant GVHD [36]. Additional investigations into the causes of graft rejection and clinical trials
of novel transplantation designs that mitigate this risk will be needed to advance alternative donor transplantation for hemoglobinopathies.

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REFERENCES