Published in final edited form as:

Hematol Oncol Clin North Am. 2011 February ; 25(1): 75–87. doi:10.1016/j.hoc.2010.11.006.

Purified Hematopoietic Stem Cell Transplantation: The Next Generation of Blood and Immune Replacement

Agnieszka Czechowicz, PhD* and Irving L. Weissman, MD

Institute of Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Lorry I. Lokey Stem Cell Research Building, G3165, 265, Campus Drive, Stanford, CA 94305-5323, USA

Severe combined immunodeficiency (SCID), systemic lupus erythematosus (SLE), and type 1 diabetes share one commonality: these diverse disorders can all be attributed to faulty immune effector cells largely caused by genetic mutations that alter hematopoietic cell-intrinsic function. These defective immune cells inherit their genetic deficiencies from hematopoietic stem cells (HSCs) as they differentiate. Thus, each of these unique diseases should be theoretically curable through the same strategy: replacement of patients' HSCs carrying the problematic mutation with normal HSCs from disease-free donors, thereby generating entire new, healthy hematolymphoid systems. Replacement of disease-causing stem cells with healthy ones has been achieved clinically via hematopoietic cell transplantation (HCT) for the last 40 years, as a treatment modality for a variety of cancers and immunodeficiencies with moderate, but increasing success. This modality has traditionally included transplantation of mixed hematopoietic populations that include HSCs and other cells, such as T cells. This article explores and delineates the potential expansion of this technique to treat a variety of inherited diseases of immune function, the current barriers in HCT and pure HSC transplantation, and the up-and-coming strategies to combat these obstacles.

ADVANTAGES OF PURIFIED ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

HSCs are the only cells within the body that at a clonal level have the ability to self renew for life as well as give rise to all the different distinct mature effectors cells that comprise the blood and immune system.¹ These two properties give HSCs the sole responsibility for the proper lifelong maintenance of hematopoietic homeostasis. However, genetic abnormalities within HSCs can result in diseases such as immunodeficiency, autoimmunity, hemoglobinopathies, or hematologic malignancies, as these defects are passed down from the HSCs to their mature cell progeny, which then generate the diseased blood or immune system.

*Corresponding author. aneeshka@stanford.edu.

Conflict-of-Interest

A.C. declares no financial or commercial conflict of interest.

The first successful hematopoietic cell transplant involving reconstitution of an infant immunologic deficiency was accomplished by Good and colleagues in 1968.² Since then, HCT has been employed as an effective strategy to treat a multitude of hematolymphoid diseases. This procedure, more commonly known as allogeneic bone marrow transplantation, replaces mutant HSCs with functional ones from donor bone marrow grafts, which thereafter give rise to a complete normal hematolymphoid system that if stably engrafted persists for life.³ Although allogeneic HCT can be an effective cure for most hematopoietic-intrinsic blood or immune diseases, it is rarely performed clinically except for life-threatening diseases and in near-death scenarios because of the toxicity of the procedure. Under current practices, allogeneic HCT has a transplant mortality rate of approximately 10% to 20%, far too high to justify its routine use in most nonmalignant settings.⁴

One of the most frequent and dangerous complications associated with allogeneic HCT is graft versus host disease (GvHD).⁵ GvHD is a complex, immunologically mediated, host-directed, inflammatory response that is attributed to transplanted donor cells genetically disparate to their host. During GvHD, grafted mature T cells, having undergone tolerization on donor rather than host thymic epithelium, upon infusion into the host result in a violent immunologic response and particularly react against host lymphoid organs, skin, liver, and gut.^{6,7} Although the likelihood and severity of GvHD can be minimized by transplantation from donors that are a close histocompatible match,⁸ the risks and effects of GvHD remain unacceptably high and dramatically limit HCT.

Based on presentation of symptoms, GvHD has historically been classified into two distinct classes: acute and chronic. Acute GvHD is rapid, occurring within 100 days of HCT and presenting as a syndrome of dermatitis, enteritis, and/or hepatitis.⁷ Chronic GvHD occurs at later time points and differs drastically from acute GvHD, often consisting of an autoimmune-like syndrome combining impairment of multiple organs or organ systems.⁷ To these two commonly studied subsets of GvHD is added a third important subtype, subclinical but immunosuppressive GvHD (see later discussion).⁹ Although T cells have been shown to play a dominant role in these severe complications of HCT, the exact molecular and cellular mechanisms underlying each subtype remain largely unknown.¹⁰

Despite a lack of complete understanding of the pathogenesis of GvHD, one potential solution to prevent its occurrence is to transplant purified HSCs. Often the terms hematopoietic stem cell transplantation (HSCT) and HCT/bone marrow transplantation (BMT) are used interchangeably in the literature, but in reality the clinical methodology differs dramatically. Although the efficacy of BMT relies on the activity of HSC, bone marrow is composed of a heterogeneous mixture of cells, including stem, multipotent progenitors, and mature blood cells, all of which are transferred to the patient in BMT. In contrast, HSCT refers to transfer of a highly purified population of strictly HSCs obtained from the donor bone marrow. The inclusion of cell populations other than HSCs and their resulting effects are what differentiate HCT/BMT from HSCT.

HSCs are defined as cells that can give rise to long-term multilineage reconstitution, as demonstrated when they are transferred into a hematolymphoid-depleted, irradiated host. Separation based on expression of discrete phenotypic cell surface markers and verification

of their functionality in this manner led to identification and isolation of human¹¹ and murine HSCs.¹ HSCs are exceedingly rare cells, making up less than 0.1% of a bone marrow graft. Based on the efforts of multiple scientific groups, the HSC population has been prospectively isolated and refined to purity. All long-term HSC activity in adult mouse bone marrow is believed to be contained within a population marked by the composite phenotype of c-Kit⁺, Thy-1.1^{lo}, lineage marker^{-/lo}, Sca-1⁺, Slamf1⁺, Flk2⁻, and CD34⁻.^{1,12-16} Similarly, the phenotypic profile of human HSCs was validated to consist of CD34⁺ and Thy-1⁺, in addition to lacking CD38⁻, CD45RA⁻, and mature lineage markers.^{11,17,18} Cells with these specific phenotypes are capable of giving rise to lifelong hematopoiesis on transplantation at the single mouse-cell level into congenic myeloablated mice,^{17,19-21} and at the 10 human-cell level in xenogeneic models with myeloablated immunodeficient mice.¹⁸ Validation of in vivo human HSC activity with cells of this phenotype was confirmed in several phase 1 clinical trials, which showed autologous HSC-rescued blood formation in myeloablated recipients and provided sustained, prolonged hematopoiesis.²²⁻²⁴

Isolation of HSCs based on the cell surface markers listed above can be accomplished by combining magnetic bead selection and fluorescence activated cells sorting (FACS) methods, yielding purified HSCs that are depleted of other polluting hematopoietic populations such as T cells.¹ Prospective isolation of HSCs in this manner is the only effective way to completely purge grafts of contaminating, unwanted populations from clinically transplantable HSC populations. In the case of autologous transplantation to treat malignancy, human HSCs purified in this manner provide long-term hematology repopulating activity and are free of contaminating resident or metastasized cancer cells.²² However, in allogeneic transplantation for malignancies, HSC purification eliminates T cells that may function against the cancer and be responsible for the beneficial graft versus tumor (GvT) effect.²⁵ In allogeneic HCT for nonmalignant diseases, purification of HSCs can be profoundly beneficial and can lead to significantly diminished procedure-related toxicity. Purified HSCT decreases the adverse outcomes of HCT/BMT; because removal of T cells from allografts completely eliminates GvHD.²⁶ Purification of HSCs from a graft eliminates the possibility of cotransplantation of host-reactive mature donor T cells, which are often contained within a graft and are primarily responsible for both acute and chronic GvHD.¹⁰ In addition to the gross lesions associated with transplantation of T cells, low doses of T cells within a graft also contribute to underappreciated subclinical GvHD. In HCT, delays in immune reconstitution can be observed even in the setting where GvHD is not readily recognized, attributable to subclinical GvHD. Even after transplantation of grafts containing minimal contaminating T cells, donor T cells attack host lymphoid tissue and destroy tissue architecture, leaving the recipient vulnerable to opportunistic infections. Transplantation of purified HSCs eliminates subclinical GvHD and results in significantly accelerated immune reconstitution,⁹ further increasing transplantation safety. As such, the complications and toxicities of BMT and HSCT are distinct, and further advocate for the transplantation of purified hematopoietic stem cells especially in nonmalignant settings.

APPLICATION OF HSCT: CURING A VARIETY OF NONMALIGNANT HEMATOLYMPHOID DISEASES

Toxicity associated with HCT has dramatically restricted its current practice to life threatening disorders such as hematologic malignancies and bone marrow failure states, where few other therapeutic options exist. However, HCT has other important potential applications beyond its current uses if HCT-associated toxicity could be eliminated. HCT has been shown to effectively reverse nonmalignant genetic hematologic disorders such as sickle cell anemia and β -thalassemia, as well as primary immune deficiencies,²⁷ if sufficient hematopoietic chimerism is achieved. In addition, early experimentation in rodents revealed that marrow transplantation could not only protect against irradiation death and prevent hematopoietic failure, but in the process could induce immune tolerance, resulting in the creation of hematopoietic chimeras that would accept skin grafts from the donor or host strain.²⁸ These and subsequent studies opened the opportunity to expand this technique as a therapeutic modality for a variety of immunologic diseases, and provided a potential alternative to lifelong administration of immunosuppressive drugs following organ transplantation—the aims of transplant biologists and clinicians now for over half a century.^{29–32}

This phenomenon of permanent transplant tolerance is attributable to the elimination of donor-reactive T cells, primarily through negative selection in the thymus of developing T cells with donor-reactive antigen receptors. Transplantation of donor HSCs results in new immune cell generation on a chimeric microenvironment, leading to deletion of reactive immune effector cells against both host (via the thymic medullary epithelium) and donor (via donor derived thymic dendritic cells).^{6,33} Recent studies illustrate that allotransplantation of purified HSCs either before or concurrent with transplantation of matched donor heart tissue precludes injury and subsequent rejection of donor organs.³⁴ Due to cotransplantation of either tissue organs and/or tissue stem cells with HSCs, long-term immune tolerance to donor tissues by the host can be achieved and the need for hazardous lifelong immunosuppression eliminated, as best illustrated in recent trials of kidney/bone marrow transplant patients.^{30,35} The use of HSCT in this manner may significantly abrogate complications of solid organ transplantation, extending organ longevity and decreasing infection susceptibility. Future cotransplantation of HSCs and solid organ tissue generated in vitro from the same embryonic or induced pluripotent stem cell may be possible, expanding the pool of transplant candidates.

The concept of induced immune tolerance by HSCT can additionally be extended to the treatment of autoimmune diseases. HCT and HSCT have been demonstrated to have utility in blocking disease pathogenesis of a wide variety of autoimmune disorders such as diabetes mellitus type 1,³⁶ multiple sclerosis,³⁷ and SLE.^{38,39} These autoimmune diseases are complex, multifactorial diseases often containing an environmental component; however, they also bear a genetic element and involve HSCs predisposed to generating self-reactive T-cell and/or B-cell clones that can react against and attack host tissues.⁴⁰ Transplantation of the disease can be achieved by transplantation of HSCs from donors predisposed to or bearing the disorder into otherwise healthy recipients.⁴¹ Conversely, allogeneic

transplantation of normal donor HSCs into diseased recipients generates tolerance and prevents attack of otherwise reactive tissues.

Cure of these diseases can be achieved by elimination of the host's reactive T cells, and subsequent generation of a new nonself-reactive T-cell compartment from the disease-resistant donor. Current transplantation procedures eliminate host immune cells and thus at least initially suppress the autoimmune disease, regardless of whether autologous or allogeneic HCT is performed. However, in these autoimmune disorders the HSCs are defective and predisposed to generating self-reactive immune cells, thus autologous transplantation as illustrated in mouse models of type 1 diabetes, allergic encephalomyelitis, and SLE is not curative. As such, syngeneic transplantation of purified HSCs in a mouse model of spontaneous autoimmune diabetes mellitus provides no long-term survival benefit.⁴¹ Conversely, transplanted allogeneic HSCs are predisposed to generating nonself-reacting immune cells, and indeed in the same model completely prevent diabetes development throughout life.⁴¹ Clinical data regarding autologous transplantation for autoimmune diseases is variable. In such settings, a naïve immune system is transplanted and, depending on environmental factors, may not always result in rapid re-creation of the diseased state. Some patients show excellent and long-lived clinical remission of disease, whereas others enjoy initial symptomatic benefit with subsequent relapse.⁴²

Autologous transplantation reintroduces the host's defective HSCs, and therefore may not result in long-term cure. As the molecular basis for various monogenic hematolymphoid diseases is determined, gene therapy may become a realistic strategy to correct autologous HSCs before transplantation. On transplantation, these few modified HSCs could reconstitute a complete, corrected hematolymphoid system that persists for life. This strategy would be instrumental in the treatment of immune diseases, in addition to genetic and acquired nonmalignant blood diseases, such as sickle cell anemia for which currently allogeneic HCT is occasionally performed. In addition, gene therapy for HSCs may play a pivotal role in generating HSCs that produce immune cells predisposed to attacking tumors. However, to date gene therapy is individual specific, and is limited by the current inability to achieve reliable and rapid gene transduction with vectors that do not by insertional mutagenesis induce diseases such LMO2-activated acute lymphocytic leukemia.⁴³

Furthermore, transplantation of HSCs generating mature cells resistant to infectious agents may prove an effective strategy to combat a magnitude of viral agents. Case reports of human immunodeficiency virus (HIV)-infected patients transplanted with HSCs from donors resistant to the disease resulted in at least preliminary cure of these patients. In these select scenarios, transplanted donor HSCs generated donor T cells bearing CCR5 defects, making them impenetrable to HIV.⁴⁴ Long-term outcome of these studies is unknown and the feasibility of such treatments using currently available transplantation strategies is questionable. However, these studies illustrate a potential new therapeutic use of HCT if other hurdles such as supply of resistant matched donor cells are overcome.⁴⁵

HCT has been repeatedly confirmed to be the singular curative therapy for this plethora of blood and immune diseases. To date, however, HCT has not been routinely applied in these manners to treat the hundreds of thousands of patients who suffer from these ailments,

primarily because of concerns regarding the morbidity and mortality of allografting procedures. With elimination of GvHD by transplantation of purified HSCs that are debulked of reactive T cells, therapy of this nature may become a mainstream reality.

BARRIERS TO EXPANSION OF HSCT

Continued improvements in the control of regimen-related toxicities are necessary to expand the applications of HCT. Current HCT methods hold exorbitant risk to the patient in terms of the transplant procedure-related morbidity and mortality, providing a major impediment to extrapolation of these practices to a multitude of conditions.

Although GvHD may be eliminated by transplantation of purified HSCs, much toxicity of HCT is also attributable to the conditioning regimens necessary to enable HSC engraftment. Current conditioning methods include irradiation and cytotoxic drugs such as high-dose chemotherapy, which can cause infertility, secondary malignancies, endocrine dysfunction, and organ damage.⁴⁶ Whereas in the malignant settings this conditioning serves the dual purpose of tumor eradication as well as preparation of the host, in the nonmalignant disease setting these regimens lead to inexcusable, nonbeneficial toxicity. Despite the ability of BMT/HSCT to cure many nonmalignant diseases, they have seldom been employed in the treatment of nonlife-threatening yet debilitating diseases, largely due to these associated risks. This situation necessitates the need for more specific and less toxic methods to allow efficient HSC engraftment.

Stable, robust chimerism is necessary in the treatment of these diseases, with disorders such as sickle cell anemia requiring approximately 20% chimerism to ameliorate the side effects of the disease.^{47,48} In the absence of myeloablative therapy, this can be difficult to achieve.⁴⁹ In addition, engraftment of purified HSCs in the absence of other facilitator populations in the bone marrow poses an even larger engraftment challenge. Various facilitator populations that augment HSC engraftment, including in mice CD8 T cells or CD8⁺ TCR⁻ dendritic cells, have been identified in bone marrow; however, many of these cells may also contribute to GvHD and therefore their transplantation should be avoided.⁵⁰ Moreover, the identification and subsequent purification of non-T-cell facilitator populations in humans has not been executed, further limiting our ability to enhance the engraftment of HSC.

Historical clinical data have shown that T-cell depletion results in increased graft failure.^{51,52} T-cell depletion is a major impediment to transplantation of purified HSCs, thwarting the current practice and consequently exposing patients to GvHD. Various “nonmyeloablative” protocols have been developed to permit engraftment of donor cells with attenuated conditioning regimens⁵³; however, although these protocols are not completely myeloablative they are still nonspecific, ablate the bone marrow, and have severe regimen-related toxicities, instigating the need for better preparative regimens.

However, transplanting HSCs without traditional conditioning has been difficult.⁵⁴ Traditional myeloablative conditioning is thought to play a role in immune suppression as well as creating space for transplanted donor HSCs.⁵⁵ HSCs are thought to reside in specialized microenvironments in the bone marrow that can serve as fixed tissue niches for

HSCs, thereby regulating HSC numbers and behavior. Although the precise identities of the niche cells are still largely unknown and controversial, there is a large amount of data indicating that HSC niches exist and are critical to HSC maintenance.⁵⁶

HSCs require specific and special growth factors and cytokines to preserve their unique state. How they receive these signals has been a growing field of research and controversy. In 1978 Schofield⁵⁵ proposed that an HSC site-specific niche must exist to provide these signals and in this way oversee HSC numbers, by regulating an HSC's decision to undergo self-renewal, differentiation, or apoptosis. In a setting of finite numbers of such niches, transplantation of HSCs in excess of these spaces would be predicted to be futile; initial experimentation performed by Micklem and colleagues⁵⁷ supports this hypothesis. Others have since argued that space is not an important factor to donor HSC engraftment, and have shown that in unirradiated recipients, transplantation of whole donor bone marrow readily displaces endogenous host marrow. Rather than by specialized sites, the argument can be made that HSC number is regulated by availability of diffusible factors and thus conditioning need not be done to ensure HSC engraftment.^{58–60} However, these experiments were performed with whole bone marrow, and the conclusions about broad HSC behavior and purified HSC engraftment ability must be taken into consideration in this context.

Using purified HSC transplants, the authors have recently shown that in normal and immunodeficient mice, at any one point only a small number of HSC niches are readily available for transplanted donor HSCs, and transplants without conditioning lead to very low donor HSC chimerism (0.5%).⁶¹ Regardless of the number of HSCs transplanted, once the available HSC niches are saturated additional engraftment cannot be obtained.⁶² Of importance, only HSCs can saturate these niches and cotransplantation of 1000 fold-excess of progenitors does not affect HSC engraftment, arguing that HSCs occupy discrete niches from their downstream progeny.⁶² These data mimic those observed by clinical transplanters who, even in the absence of immune barriers, observe similar very low levels of donor HSC chimerism on transplantation of hematopoietic cells enriched for human HSCs into immunodeficient patients not receiving conditioning.⁶³ The low level of HSC engraftment in these patients is sufficient to restore immune function transiently through proliferation and expansion of immune progenitors; however, over time these few engrafted HSCs encounter exhaustion and loss of the graft is occasionally observed, thereby necessitating ways to increase initial HSC engraftment even in the immunodeficiency setting.⁶⁴

Taken together, these studies suggest that in the absence of conditioning or facilitator populations, in both humans and mice donor HSC engraftment is limited by the availability of appropriate niches. Endogenous HSCs occupy appropriate, otherwise transplantable HSC niches, and therefore one strategy to enhance donor HSC engraftment may be to deplete host HSCs. The development of reagents that specifically displace host HSCs, rather than myeloablative conditioning techniques currently in use, could lead to safer transplantation-based therapies for hematological and nonhematological disorders.

UP-AND-COMING STRATEGIES TO IMPROVE HSCT

HSCs are migratory cells.⁶⁵ Under homeostatic conditions they can be found in blood circulation in addition to bone marrow, albeit at very low but physiologically relevant frequency.⁶⁶ Recent studies have shown that HSCs enter the blood stream via division-independent egress from the bone marrow, leaving behind empty HSC niches available for transplantation, and explaining why low levels of engraftment are observed in nonconditioned settings.⁶⁶ HSCs continually egress from the marrow and enter the blood, suggesting that additional HSC niches may become available over time. Concordantly, saturation of engrafted HSC niches is transient and indeed, repeat rounds of HSCT transplantation lead to additional donor HSC chimerism.^{61,66} This strategy may be one important path through which donor HSC engraftment can be increased with ease.

The natural vacancy of HSC niches admittedly is very slow, and therefore one proposed strategy to increase the competition between the donor and host HSCs is to augment the vacancy of the HSC niches through mobilizing endogenous host HSCs out of their marrow microenvironments and into circulation. This goal may be accomplished with reagents such as AMD3100, which cause significant mobilization without noteworthy proliferation.⁶⁷ Limited murine studies have shown such drugs to function as effective nontoxic conditioning therapeutics.⁶⁸ However, even in the setting of HSC mobilization, transplanted donor HSCs must still compete with displaced host HSCs for HSC space. Therefore, alternative strategies to enhance engraftment by eliminating endogenous competing HSCs are desired.

HSCs rely on a variety of signals for survival and maintenance of their stem cell state. Specifically, HSCs have been shown to require continual c-kit ligand (SCF) for survival, and inhibition of this signal results in apoptosis.⁶⁹ The authors have recently shown that ACK2, an antagonistic monoclonal antibody to the murine c-kit receptor⁷⁰ in immunodeficient mice, eliminates murine HSCs and creates vacant HSC niches available for transplantation.⁶² Donor HSC engraftment efficiency is significantly increased with such conditioning, without any toxic side effects other than transient graying (as c-kit is additionally present on melanocytes). Transplantation of high doses of HSCs or multiple rounds of ACK2 followed by HSCs result in very high levels of mixed chimerism (>90%).⁶² Translation of such strategies, targeting human HSCs, may result in nonmyeloablative regimens that promote donor HSC engraftment with minimal toxicity, thereby significantly decreasing the morbidity and mortality currently experienced with present conditioning regimens.

Such novel conditioning strategies may be effective at obtaining high levels of HSC engraftment. However, conditioning methods including irradiation and cytotoxic agents not only play a role in the creation of incoming space for HSC, but additionally act as immune suppressants and play a role in immune-mediated HSC resistance. In immunodeficient patients, such novel “space-creating” strategies in conjunction with purification of HSCs may be sufficient to eliminate entirely the current toxicities associated with HCT. However, in immunocompetent settings additional reagents will need to be explored to inhibit the host’s immune system, thereby preventing rejection of the incoming transplanted cells. T

lymphocytes and natural killer (NK) cells classically are considered the primary immune mediators of allogeneic HSC resistance.⁷¹ When transplant pairs are fully matched at the major histocompatibility complex (MHC) loci, T-cell immunity predominates. However, if MHC disparities exist, as in, for example, haploidentical transplantations, NK cells also play an important role. Thus, reagents to eliminate the engraftment barrier must deplete or significantly impair the function of both types of lymphoid cells. Monoclonal antibodies may play a significant future role, as they may be used to transiently deplete host T cells and host NK cells before donor cell infusion. Multiple immunosuppressive monoclonal antibodies to human lymphocytes currently exist, including anti-CD2, -CD52, -CD3, -CD4, and -CD8, facilitating the generation of purely antibody-based nontoxic conditioning.

REVOLUTIONIZING HCT

Almost 60 years has passed since the early dismal but promising transplants performed by Thomas and colleagues,⁷² and since then we have learned much about the biology of blood and immune transplantation. Yet today we still face many of the same hurdles faced by our predecessors, namely, the competing challenges of (1) complications arising from GvHD syndrome and (2) toxicities associated with preparative regimens necessary for cell engraftment.

Recent data suggest we may be bordering on developing therapies that overcome these obstacles. By combining these strategies, we may be at the tipping point of changing the practice and therefore application of HCT. If the strategies outlined in this article, or others in their stead, are employed successfully, we may witness a new exciting wave of HCT, and an expansion of the use of HCT from primarily for patients with rapidly lethal diseases to those with a variety of other hematolymphoid diseases for which HCT is currently unacceptable.

From the beginning of clinical HCT, immunodeficiency has been a good initial disease target because it allows for separation of the immune transplant barrier from the other transplantation obstacles, affording scientists and clinicians the ability to sequentially optimize individual treatment components. In this manner, SCID will likely be the first disease treated with the modalities outlined herein before they are extended to other applications. Moving forward, purified HSCT and novel conditioning strategies should allow for better treatment of SCID, obtaining higher donor engraftment without GvHD. Addition of antibody-based immunodepletion will subsequently allow for the combating of nonmalignant blood diseases. Thereafter, transplant tolerance may be achievable using such strategies as cotransplantation of HSCs and tissues/organs, and similarly, autoimmunity may be treated. The final goal is to treat patients whose organs have already been destroyed during autoimmune attacks, such as insulin-dependent type 1 diabetics lacking islet cells and, as has been shown in mice, concurrently transplant them with new organs as well as HSCs that impede rejection of the organ graft, prevent subsequent autoimmunity, and do not lead to GvHD.⁴¹ Such dreams may become a reality in the distant future; meanwhile the incremental successes in any of these realms will allow for the gradual expansion of HCT as a therapeutic option for thousands of patients suffering from the diverse diseases of the blood and immune system.

Acknowledgments

The authors thank D. Bhattacharya and M. Howard for insightful review of the manuscript.

Funding

This investigation was supported by National Institutes of Health grants R01CA086065 and R01HL058770 (to I.L.W.). A.C. is supported by the Medical Scientist Training Program at Stanford University School of Medicine, as well as a grant from The Paul and Daisy Soros Fellowships for New Americans. The program is not responsible for the views expressed. Affiliations that might be perceived to have biased this work are as follows: I.L.W. cofounded and consulted for Systemix, is a cofounder and director of Stem Cells, Inc, and cofounded and is a former director of Cellerant, Inc.

REFERENCES

1. Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. *Science*. 1988; 241(4861):58–62. [PubMed: 2898810]
2. Gatti RA, Meuwissen HJ, Allen HD, et al. Immunological reconstitution of sexlinked lymphopenic immunological deficiency. *Lancet*. 1968; 2(7583):1366–1369. [PubMed: 4177932]
3. McCulloch EA, Till JE. The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice. *Radiat Res*. 1960; 13:115–125. [PubMed: 13858509]
4. Michlitsch JG, Walters MC. Recent advances in bone marrow transplantation in hemoglobinopathies. *Curr Mol Med*. 2008; 8(7):675–689. [PubMed: 18991653]
5. Barnes DW, Loutit JF, Micklem HS. “Secondary disease” of radiation chimeras: a syndrome due to lymphoid aplasia. *Ann N Y Acad Sci*. 1962; 99:374–385. [PubMed: 13969359]
6. Keever CA, Flomenberg N, Brochstein J, et al. Tolerance of engrafted donor T cells following bone marrow transplantation for severe combined immunodeficiency. *Clin Immunol Immunopathol*. 1988; 48(3):261–276. [PubMed: 3042210]
7. Ferrara JL, Deeg HJ. Graft-versus-host disease. *N Engl J Med*. 1991; 324(10):667–674. [PubMed: 1994250]
8. Schierman LW, Nordskog AW. Influence of the B blood group-histocompatibility locus in chickens on a graft-versus-host reaction. *Nature*. 1963; 197:511–512. [PubMed: 13991668]
9. Tsao GJ, Allen JA, Logronio KA, et al. Purified hematopoietic stem cell allografts reconstitute immunity superior to bone marrow. *Proc Natl Acad Sci U S A*. 2009; 106(9):3288–3293. [PubMed: 19223585]
10. Sprent J, Miller JF. Fate of H2-activated T lymphocytes in syngeneic hosts. II. Residence in recirculating lymphocyte pool and capacity to migrate to allografts. *Cell Immunol*. 1976; 21(2): 303–313. [PubMed: 769995]
11. Baum CM, Weissman IL, Tsukamoto AS, et al. Isolation of a candidate human hematopoietic stem-cell population. *Proc Natl Acad Sci U S A*. 1992; 89(7):2804–2808. [PubMed: 1372992]
12. Kiel MJ, Yilmaz OH, Iwashita T, et al. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*. 2005; 121(7):1109–1121. [PubMed: 15989959]
13. Whitlock CA, Tidmarsh GF, Muller-Sieburg C, et al. Bone marrow stromal cell lines with lymphopoietic activity express high levels of a pre-B neoplasia-associated molecule. *Cell*. 1987; 48(6):1009–1021. [PubMed: 3493849]
14. Christensen JL, Weissman IL. Flk-2 is a marker in hematopoietic stem cell differentiation: a simple method to isolate long-term stem cells. *Proc Natl Acad Sci U S A*. 2001; 98(25):14541–14546. [PubMed: 11724967]
15. Matsuoka S, Ebihara Y, Xu M, et al. CD34 expression on long-term repopulating hematopoietic stem cells changes during developmental stages. *Blood*. 2001; 97(2):419–425. [PubMed: 11154218]

16. Adolfsson J, Borge OJ, Bryder D, et al. Upregulation of Flt3 expression within the bone marrow Lin⁽⁺⁾Sca1⁽⁺⁾c-kit⁽⁺⁾ stem cell compartment is accompanied by loss of self-renewal capacity. *Immunity*. 2001; 15(4):659–669. [PubMed: 11672547]
17. Osawa M, Hanada K, Hamada H, et al. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science*. 1996; 273(5272):242–245. [PubMed: 8662508]
18. Majeti R, Park CY, Weissman IL. Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. *Cell Stem Cell*. 2007; 1(6):635–645. [PubMed: 18371405]
19. Matsuzaki Y, Kinjo K, Mulligan RC, et al. Unexpectedly efficient homing capacity of purified murine hematopoietic stem cells. *Immunity*. 2004; 20(1):87–93. [PubMed: 14738767]
20. Wagers AJ, Christensen JL, Weissman IL. Cell fate determination from stem cells. *Gene Ther*. 2002; 9(10):606–612. [PubMed: 12032706]
21. Camargo FD, Chambers SM, Drew E, et al. Hematopoietic stem cells do not engraft with absolute efficiencies. *Blood*. 2006; 107(2):501–507. [PubMed: 16204316]
22. Negrin RS, Atkinson K, Leemhuis T, et al. Transplantation of highly purified CD34⁺Thy-1⁺ hematopoietic stem cells in patients with metastatic breast cancer. *Biol Blood Marrow Transplant*. 2000; 6(3):262–271. [PubMed: 10871151]
23. Vose JM, Bierman PJ, Lynch JC, et al. Transplantation of highly purified CD34⁺Thy-1⁺ hematopoietic stem cells in patients with recurrent indolent non-Hodgkin2019s lymphoma. *Biol Blood Marrow Transplant*. 2001; 7(12):680–687. [PubMed: 11787531]
24. Michallet M, Philip T, Philip I, et al. Transplantation with selected autologous peripheral blood CD34⁺Thy1⁺ hematopoietic stem cells (HSCs) in multiple myeloma: impact of HSC dose on engraftment, safety, and immune reconstitution. *Exp Hematol*. 2000; 28(7):858–870. [PubMed: 10907648]
25. Ito M, Shizuru JA. Graft-vs.-lymphoma effect in an allogeneic hematopoietic stem cell transplantation model. *Biol Blood Marrow Transplant*. 1999; 5(6):357–368. [PubMed: 10595813]
26. Shizuru JA, Jerabek L, Edwards CT, et al. Transplantation of purified hematopoietic stem cells: requirements for overcoming the barriers of allogeneic engraftment. *Biol Blood Marrow Transplant*. 1996; 2(1):3–14. [PubMed: 9078349]
27. Barth E, Malorgio C, Tamaro P. Allogeneic bone marrow transplantation in hematologic disorders of childhood: new trends and controversies. *Haematologica*. 2000; 85(11 Suppl):2–8. [PubMed: 11268319]
28. Main JM, Prehn RT. Successful skin homografts after the administration of high dosage X radiation and homologous bone marrow. *J Natl Cancer Inst*. 1955; 15(4):1023–1029. [PubMed: 13233946]
29. Starzl TE, Demetris AJ, Murase N, et al. Cell migration, chimerism, and graft acceptance. *Lancet*. 1992; 339(8809):1579–1582. [PubMed: 1351558]
30. Scandling JD, Busque S, Dejbakhsh-Jones S, et al. Tolerance and chimerism after renal and hematopoietic-cell transplantation. *N Engl J Med*. 2008; 358(4):362–368. [PubMed: 18216356]
31. Millan MT, Shizuru JA, Hoffmann P, et al. Mixed chimerism and immunosuppressive drug withdrawal after HLA-mismatched kidney and hematopoietic progenitor transplantation. *Transplantation*. 2002; 73(9):1386–1391. [PubMed: 12023614]
32. Alexander SI, Smith N, Hu M, et al. Chimerism and tolerance in a recipient of a deceased-donor liver transplant. *N Engl J Med*. 2008; 358(4):369–374. [PubMed: 18216357]
33. Shizuru JA, Weissman IL, Kernoff R, et al. Purified hematopoietic stem cell grafts induce tolerance to alloantigens and can mediate positive and negative T cell selection. *Proc Natl Acad Sci U S A*. 2000; 97(17):9555–9560. [PubMed: 10920206]
34. Gandy KL, Weissman IL. Tolerance of allogeneic heart grafts in mice simultaneously reconstituted with purified allogeneic hematopoietic stem cells. *Transplantation*. 1998; 65(3):295–304. [PubMed: 9484743]
35. Kawai T, Cosimi AB, Spitzer TR, et al. HLA-mismatched renal transplantation without maintenance immunosuppression. *N Engl J Med*. 2008; 358(4):353–361. [PubMed: 18216355]

36. Nikolic B, Takeuchi Y, Leykin I, et al. Mixed hematopoietic chimerism allows cure of autoimmune diabetes through allogeneic tolerance and reversal of autoimmunity. *Diabetes*. 2004; 53(2):376–383. [PubMed: 14747288]
37. van Gelder M, Kinwel-Bohre EP, van Bekkum DW. Treatment of experimental allergic encephalomyelitis in rats with total body irradiation and syngeneic BMT. *Bone Marrow Transplant*. 1993; 11(3):233–241. [PubMed: 8467289]
38. Traynor AE, Schroeder J, Rosa RM, et al. Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and haemopoietic stem-cell transplantation: a phase I study. *Lancet*. 2000; 356(9231):701–707. [PubMed: 11085688]
39. Smith-Berdan S, Gille D, Weissman IL, et al. Reversal of autoimmune disease in lupus-prone New Zealand black/New Zealand white mice by nonmyeloablative transplantation of purified allogeneic hematopoietic stem cells. *Blood*. 2007; 110(4):1370–1378. [PubMed: 17435112]
40. Todd JA, Bell JI, McDevitt HO. HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature*. 1987; 329(6140):599–604. [PubMed: 3309680]
41. Beilhack GF, Scheffold YC, Weissman IL, et al. Purified allogeneic hematopoietic stem cell transplantation blocks diabetes pathogenesis in NOD mice. *Diabetes*. 2003; 52(1):59–68. [PubMed: 12502494]
42. Krauss AC, Kamani NR. Hematopoietic stem cell transplantation for pediatric autoimmune disease: where we stand and where we need to go. *Bone Marrow Transplant*. 2009; 44(3):137–143. [PubMed: 19597421]
43. McCormack MP, Rabbitts TH. Activation of the T-cell oncogene LMO2 after gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med*. 2004; 350(9):913–922. [PubMed: 14985489]
44. Hutter G, Nowak D, Mossner M, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med*. 2009; 360(7):692–698. [PubMed: 19213682]
45. van Griensven J, De Clercq E, Debyser Z. Hematopoietic stem cell-based gene therapy against HIV infection: promises and caveats. *AIDS Rev*. 2005; 7(1):44–55. [PubMed: 15875660]
46. Ferry C, Socie G. Busulfan-cyclophosphamide versus total body irradiation cyclophosphamide as preparative regimen before allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia: what have we learned? *Exp Hematol*. 2003; 31(12):1182–1186. [PubMed: 14662323]
47. Walters MC, Patience M, Leisenring W, et al. Stable mixed hematopoietic chimerism after bone marrow transplantation for sickle cell anemia. *Biol Blood Marrow Transplant*. 2001; 7(12):665–673. [PubMed: 11787529]
48. Iannone R, Luznik L, Engstrom LW, et al. Effects of mixed hematopoietic chimerism in a mouse model of bone marrow transplantation for sickle cell anemia. *Blood*. 2001; 97(12):3960–3965. [PubMed: 11389040]
49. Storb R, Yu C, Sandmaier BM, et al. Mixed hematopoietic chimerism after marrow allografts. Transplantation in the ambulatory care setting. *Ann N Y Acad Sci*. 1999; 872:372–375. [discussion: 375–6]. [PubMed: 10372139]
50. Gandy KL, Domen J, Aguila H, et al. CD81TCR1 and CD81TCR" cells in whole bone marrow facilitate the engraftment of hematopoietic stem cells across allogeneic barriers. *Immunity*. 1999; 11(5):579–590. [PubMed: 10591183]
51. Kernan NA, Bordignon C, Heller G, et al. Graft failure after T-cell-depleted human leukocyte antigen identical marrow transplants for leukemia: I. Analysis of risk factors and results of secondary transplants. *Blood*. 1989; 74(6):2227–2236. [PubMed: 2804361]
52. Marmont AM, Horowitz MM, Gale RP, et al. T-cell depletion of HLA-identical transplants in leukemia. *Blood*. 1991; 78(8):2120–2130. [PubMed: 1912589]
53. Maloney DG, Sandmaier BM, Mackinnon S, et al. Non-myeloablative transplantation. *Hematology Am Soc Hematol Educ Program*. 2002:392–421. [PubMed: 12446434]
54. Tomita Y, Sachs DH, Sykes M. Myelo suppressive conditioning is required to achieve engraftment of pluripotent stem cells contained in moderate doses of syngeneic bone marrow. *Blood*. 1994; 83(4):939–948. [PubMed: 7906567]
55. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells*. 1978; 4(1-2):7–25. [PubMed: 747780]

56. Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell*. 2008; 132(4):598–611. [PubMed: 18295578]
57. Micklem HS, Clarke CM, Evans EP, et al. Fate of chromosome-marked mouse bone marrow cells transfused into normal syngeneic recipients. *Transplantation*. 1968; 6(2):299–302. [PubMed: 5654091]
58. Saxe DF, Boggs SS, Boggs DR. Transplantation of chromosomally marked syngeneic marrow cells into mice not subjected to hematopoietic stem cell depletion. *Exp Hematol*. 1984; 12(4):277–283. [PubMed: 6370711]
59. Stewart FM, Crittenden RB, Lowry PA, et al. Long-term engraftment of normal and post-5-fluorouracil murine marrow into normal nonmyeloablated mice. *Blood*. 1993; 81(10):2566–2571. [PubMed: 8098231]
60. Wu DD, Keating A. Hematopoietic stem cells engraft in untreated transplant recipients. *Exp Hematol*. 1993; 21(2):251–256. [PubMed: 8425562]
61. Bhattacharya D, Rossi DJ, Bryder D, et al. Purified hematopoietic stem cell engraftment of rare niches corrects severe lymphoid deficiencies without host conditioning. *J Exp Med*. 2006; 203(1):73–85. [PubMed: 16380511]
62. Czechowicz A, Kraft D, Weissman IL, et al. Efficient transplantation via antibody based clearance of hematopoietic stem cell niches. *Science*. 2007; 318(5854):1296–1299. [PubMed: 18033883]
63. Muller SM, Kohn T, Schulz AS, et al. Similar pattern of thymic-dependent T-cell reconstitution in infants with severe combined immunodeficiency after human leukocyte antigen (HLA)-identical and HLA-nonidentical stem cell transplantation. *Blood*. 2000; 96(13):4344–4349. [PubMed: 11110711]
64. Cavazzana-Calvo M, Carlier F, Le Deist F, et al. Long-term T-cell reconstitution after hematopoietic stem-cell transplantation in primary T-cell-immunodeficient patients is associated with myeloid chimerism and possibly the primary disease phenotype. *Blood*. 2007; 109(10):4575–4581. [PubMed: 17272510]
65. Wright DE, Wagers AJ, Gulati AP, et al. Physiological migration of hematopoietic stem and progenitor cells. *Science*. 2001; 294(5548):1933–1936. [PubMed: 11729320]
66. Bhattacharya D, Czechowicz A, Ooi AG, et al. Niche recycling through division independent egress of hematopoietic stem cells. *J Exp Med*. 2009; 206(12):2837–2850. [PubMed: 19887396]
67. Broxmeyer HE, Orschell CM, Clapp DW, et al. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med*. 2005; 201(8):1307–1318. [PubMed: 15837815]
68. Chen J, Larochelle A, Fricker S, et al. Mobilization as a preparative regimen for hematopoietic stem cell transplantation. *Blood*. 2006; 107(9):3764–3771. [PubMed: 16439683]
69. Domen J, Weissman IL. Hematopoietic stem cells need two signals to prevent apoptosis; BCL-2 can provide one of these, Kitl/c-Kit signaling the other. *J Exp Med*. 2000; 192(12):1707–1718. [PubMed: 11120768]
70. Ogawa M, Matsuzaki Y, Nishikawa S, et al. Expression and function of c-kit in hemopoietic progenitor cells. *J Exp Med*. 1991; 174(1):63–71. [PubMed: 1711568]
71. Murphy WJ, Kumar V, Bennett M. Acute rejection of murine bone marrow allografts by natural killer cells and T cells. Differences in kinetics and target antigens recognized. *J Exp Med*. 1987; 166(5):1499–1509. [PubMed: 3316472]
72. Thomas ED, Lochte HL Jr, Lu WC, et al. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. *N Engl J Med*. 1957; 257(11):491–496. [PubMed: 13464965]