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

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Abstract

Severe Combined Immunodeficiency (SCID), Systemic Lupus Erythematosus (SLE), and Type I Diabetes share one commonality: these diverse disorders can all be attributed to faulty immune effector cells which are largely caused by genetic mutations that alter hematopoietic cell-intrinsic function. These defective immune cells inherit their genetic deficiencies from hematopoietic stem cells (HSC) 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 50 years, as a treatment modality for a variety of cancers and immunodeficiencies with moderate, but increasing success. This has traditionally included transplantation of mixed hematopoietic populations that include HSC and other cells, such as T-cells. This review 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, as well as the up-and-coming strategies to combat these obstacles.

Keywords

Hematopoeitic Stem Cell Transplantation; Non-Maligant Hematolymphoid Disorders; Non-Myeloablative Conditioning; Immune Tolerance; Autoimmune Diseases

Advantages of Purified Allogeneic Hematopoietic Stem Cell Transplantation

Hematopoietic stem cells (HSCs) are the only cells within the body that at a clonal level have the ability to both 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

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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 HSC to their mature cell progeny, which then generate the diseased blood or immune system.

The first successful hematopoietic cell transplant involving reconstitution of an infant immunological deficiency was accomplished by Good and colleagues in 1968.[2] Since then, hematopoietic cell transplantation (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 due to the toxicity of the procedure. Under current practices, allogeneic HCT has a transplant mortality rate of approximately ~10–20%, far too high to justify its routine use in most non-malignant settings.4

One of the most frequent and dangerous complications associated with allogeneic hematopoietic cell transplantation is Graft vs. Host Disease (GvHD).[5] GvHD is a complex, immunologically mediated, host-directed, inflammatory response which 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 While 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 hematopoietic cell transplantation.

Historically, based upon presentation of symptoms, GvHD has 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 sub-type, which is sub-clinical but immunosuppressive GVHD (see below).[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 sub-type 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 Hematopoietic Cell Transplantation (HCT) / Bone Marrow Transplantation (BMT) are used interchangeably in the literature, but in reality, the clinical methodology differ dramatically. Although the efficacy of BMT relies on the activity of HSC, bone marrow is composed of a heterogeneous mixture of cells, including stem, multi-potent 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 HSC obtained from the donor bone marrow. The inclusion of cell populations other than HSC and their resulting effects are what differentiate HCT/BMT from HSCT.

HSCs are defined as cells which can give rise to long-term multi-lineage reconstitution, as demonstrated when they are transferred into a hematolymphoid depleted, irradiated host.

Separation based upon expression of discrete phenotypic cell surface markers and verification of their functionality in this manner, led to identification and isolation of human [11] and murine HSC.[1] HSC are exceedingly rare cells, making up <0.1% of a bone marrow graft. Based upon the efforts of multiple scientific groups, the HSC population has been prospectively isolated and refined to purify. 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 13 14 15 16] Similarly, the phenotypic profile of human HSC 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 upon transplantation at the single mouse-cell level into congenic myeloablated mice,[17 19, 20 21] and at the ten human-cell level in xenogenic models with myeloablated immunodeficient mice.[18] Validation of in vivo human HSC activity with cells of this phenotype was confirmed in several Phase I clinical trials, which showed autologous HSC rescued blood formation in myeloablated recipients and provided sustained, prolonged hematopoiesis.[22 23 24]

Isolation of HSC based upon the cell surface markers indicated above can be accomplished by combining magnetic bead selection and fluorescence activated cells sorting (FACS) methods, yielding purified HSC that are depleted of other polluting hematopoietic populations such as T-cells.1 Prospective isolation of HSC 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 hematolymphoid 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 vs. Tumor (GvT) effect.25

In allogeneic hematopoietic cell transplantation for non-malignant diseases, purification of HSC can be profoundly beneficial and lead to significantly diminished procedure-related toxicity. Purified HSCT decreases the adverse outcomes of HCT/BMT; since removal of T-cells from allografts completely eliminates GvHD.[26] Purification of HSC from a graft eliminates the possibility of co-transplantation of host-reactive mature donor T-cells, which are often contained within a graft and primarily responsible for both acute and chronic GvHD.[10] In addition to the gross lesions associated with transplantation of T-cells, low dose of T-cells within a graft also contribute to under-appreciated sub-clinical GvHD. In HCT, delays in immune reconstitution can be observed even in the setting where GvHD is not readily recognized, attributable to sub-clinical GvHD. Even post 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 sub-clinical GvHD and results in significantly accelerated immune reconstitution,[9] further increasing transplantation safety. As such, the complications and toxicities of BMT and HSCT are quite distinct, and further advocate for the transplantation of purified hematopoietic stem cells especially in non-malignant settings.

Application of HSCT: Curing A Variety of Non-Malignant 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 non-malignant genetic hematologic disorders such as sickle cell anemia and beta thalassemia as well as primary immune deficiencies,[27] if sufficient hematopoietic chimerism is achieved. Additionally, early experimentation in rodents revealed that marrow transplantation could not only protect from irradiation death and prevent hematopoietic failure, but in the process induced immune tolerance and result 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 immunological diseases and provided a potential alternative to lifelong administration of immunosuppressive drugs following organ transplantation, aims of transplant biologists and clinicians for now over half a century.[29 30 31 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 HSC 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 HSC either prior or concurrent with transplantation of matched donor heart tissue precludes injury and subsequent rejection of donor organs.34 Due to co-transplantation of either tissue organs and/or tissue stem cells with HSC, long-term immune tolerance to donor tissues by the host can be achieved and the need for hazardous life-long immuno-suppression eliminated, as best illustrated in recent trials of kidney/BM transplant patients.35 [30] The use of HSCT in this manner may significantly abrogate complications of solid organ transplantation, extending organ longevity and decreasing infection susceptibility. Future co-transplantation of HSC 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 HSC transplantation have been demonstrated to have utility in blocking disease pathogenesis of a wide variety of autoimmune disorders such as diabetes mellitus type 1 (DM1),36 multiple sclerosis (MS),37 and systemic lupus erythematosus (SLE).38 39 These autoimmune diseases are complex, multi-factorial diseases often containing an environmental component, however they also bear a genetic element and involve HSC 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 HSC from donors predisposed to or bearing the disorder into otherwise healthy recipients.41 Conversely allogeneic transplantation of normal donor HSC 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 non-self-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 HSC 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 systemic lupus erythematosus is not curative. As such, syngeneic transplantation of purified HSC in a mouse model of spontaneous autoimmune diabetes mellitus provides no long-term survival benefit.[41] Conversely, transplanted allogeneic HSC are posed to generating non-self-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 hosts defective HSC, 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 HSC prior to transplantation. Upon transplantation, these few modified HSC 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 where currently allogeneic HCT is occasionally performed. Additionally, gene therapy of HSC may play a pivotal role in generating HSC that produce immune cells that are 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 HSC generating mature cells resistant to infectious agents may prove an effective strategy to combat a magnitude of viral agents. Case reports of HIV-infected patients, transplanted with HSC from donors resistant to the disease, resulted in at least preliminary cure of these patients. In these select scenarios, transplanted donor HSC 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 utilizing 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 that suffer from these ailments primarily subsequent to concerns regarding the morbidity and mortality of allografting procedures. With elimination of GvHD by transplantation of purified HSC, that are debulked of reactive T-cells, therapy of this nature maybe become a mainstream reality.

Barriers to Expansion of Hematopoietic Stem Cell Transplantation

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 HSC, 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 non-malignant disease setting these regimens lead to inexcusable, non-beneficial toxicity. Despite the ability of BMT/HSCT to cure many non-malignant diseases, they have seldom been employed in the treatment of non-life threatening yet debilitating diseases largely due to these associated

risks. This 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 ~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] Additionally, engraftment of purified hematopoietic stem cells in the absence of other facilitator populations in the bone marrow poses an even larger engraftment challenge. Various facilitator populations, including in mice CD8 T-cells or $CD8^+$ TCR^- dendritic cells, have been indentified in bone marrow that augment HSC engraftment, 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 has showed that T-cell depletion results in increased graft failures.[51 52] This is a major impediment of transplantation of purified HSCs, preventing the current practice and consequentially exposing patients to GvHD. Various “non-myeloablative” protocols have been developed to permit engraftment of donor cells with attenuated conditioning regimens,[53] however while these protocols are not completely myeloablative, they are still non-specific, 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 maintance.[56]

HSC 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 Schofield proposed that a HSC site-specific niche must exist to provide these signals and in this way oversee HSC numbers, by regulating an HSC's decisions to undergo self-renewal, differentiation, or apoptosis.[55] In a setting of finite numbers of such niches, transplantation of HSC in excess of these spaces would be predicted to be futile; initial experimentation carried out by Micklem and colleagues supports this hypothesis.[57] Others have since argued that space is not an important factor to donor HSC engraftment, and show 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. However, these experiments were carried out with whole bone marrow, and the conclusions about broad HSC behavior and purified HSC engraftment ability must be taken into consideration in this context.[58 59 60]

Using purified HSC transplants, we 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 Importantly, only HSC can saturate these niches, and co-transplantation of 1,000 fold-excess of progenitors does not affects HSC engraftment, arguing that HSC occupy discrete niches

from their downstream progeny.62 These data mimic that observed by clinical transplanters who even in the absence of immune barriers, observe similarly very low levels of donor HSC chimerism upon transplantation of hematopoietic cells enriched for human HSC 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 HSC 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 non-hematological disorders.

Up-and-coming strategies to improve Hematopoietic Stem Cell Transplantation

Hematopoietic stem cells 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 HSC 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 non-conditioned settings.[66] HSC 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 HSC transplantation lead to additional donor HSC chimerism.[61 66] This may be one important strategy through which with ease donor HSC engraftment can be increased.

Admittedly, the natural vacancy of HSC niches is very slow and therefore one proposed strategy to increase the competition between the donor and host HSC is to augment the vacancy of the HSC niches through mobilizing endogenous host HSC out of their marrow microenvironments and into circulation. This 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 non-toxic conditioning therapeutics.[68] However, even in the setting of HSC mobilization, transplanted donor HSC must still compete with displaced host HSC for HSC space. Therefore alternative strategies to enhance engraftment by eliminating endogenous competing HSC are desired.

HSC rely on a variety of signals for survival and maintenance of their stem-cell state. Specifically HSC have been shown to require continual kit-ligand (SCF) for survival, and inhibition of this signal results in apoptosis.69 We have recently shown that ACK2, an antagonistic monoclonal antibody to the murine c-kit receptor 70 in immunodeficient mice eliminates murine HSC, 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 HSC or multiple rounds of ACK2 followed by HSC result in very high levels of mixed chimerism (>90%).62 Translation of such strategies, targeting human HSC, may result in non-myeloablative 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 creation of incoming space for HSC, but additionally act as immune suppressants and play a role in immune-mediated HSC resistance. In the immunodeficient patients, such novel "space-creating" strategies in conjunction with purification of HSC 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 hosts' immune system thereby preventing rejection of the incoming transplanted cells. Classically, T lymphocytes and natural killer (NK) cells 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, haplo-identical 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 and host NK cells prior to 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 non-toxic conditioning.

Revolutionizing HCT

Almost 60 years has passed since the early dismal but promising transplants performed by Thomas and colleagues, [72] and since 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 1) complications arising from graft vs host disease syndrome and 2) toxicities associated with preparative regimens necessary for cell engraftment.

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

From the beginning of clinical hematopoietic cell transplantation, 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 above 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 combating of non-malignant blood diseases. Thereafter transplant tolerance may be achievable using such strategies by co-transplantation of HSC and tissues/organs and similarly autoimmunity may be treated. The final goal is to treat patients who's 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 hematopoietic cell transplantation as a therapeutic option for thousands of patients suffering from the diverse diseases of the blood and immune system.

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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 sex-linked 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 bloodgroup-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-Hodgkin's 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 375–376. [PubMed: 10372139]
50. Gandy KL, Domen J, Aguila H, et al. CD8+TCR+ and CD8+TCR− 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. Myelosuppressive 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 tranfused 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]