

# Allogeneic Stem Cell Transplantation: A Historical and Scientific Overview

Anurag K. Singh and Joseph P. McGuirk

## Abstract

The field of hematopoietic stem cell transplant (HSCT) has made ground-breaking progress in the treatment of many malignant and nonmalignant conditions. It has also pioneered the concepts of stem cell therapy and immunotherapy as a tool against cancer. The success of transplant for hematologic malignancies derives both from the ability to treat patients with intensive chemoradiotherapy and from potent graft-versus-leukemia (GVL) effects mediated by donor immunity. Additionally, HSCT has been a curative therapy for several nonmalignant hematologic disorders through the provision of donor-derived hematopoiesis and immunity. Preclinical and clinical research in the field has contributed to an advanced understanding of histocompatibility, graft-versus-host disease (GVHD), GVL effect, and immune reconstitution after trans-

plant. Improved donor selection, tailored conditioning regimens, and better supportive care have helped reduce transplant-related morbidity and mortality and expanded access. The development of unrelated donor registries and increased utilization of cord blood and partially matched related donor transplants have ensured a donor for essentially everyone who needs a transplant. However, significant barriers still remain in the form of disease relapse, GVHD infectious complications, and regimen-related toxicities. Recent developments in the field of cellular therapy are expected to further improve the efficacy of transplant. In this review, we discuss the current science of HSCT from a historical perspective, highlighting major discoveries. We also speculate on future directions in this field. *Cancer Res*; 76(22); 6445–51. ©2016 AACR.

Over the last 50 years, more than one million hematopoietic stem cell transplants (HSCT) have been performed (1). During these years, HSCT has evolved from a highly experimental technique to the standard of care for many malignant and nonmalignant hematologic diseases. HSCT provides a fascinating intersection of concepts that are of considerable interest to the scientific community, such as the dose-response relationship of chemoradiotherapy and cancer eradication, stem cell therapy, cancer immunotherapy, personalized medicine, and supportive care in cancer medicine. As the focus in cancer medicine shifts to immunotherapeutic strategies and personalized medicine, it is fitting to remember that the field of stem cell transplant pioneered both of these concepts. There are two essential types of HSCTs: (i) autologous stem cell transplant, where a patient receives his/her own stem cells as a rescue strategy from otherwise lethal chemoradiotherapy given to eradicate the underlying malignancy and (ii) allogeneic stem cell transplant, where hematopoietic stem cells and the immunological repertoire from a donor are infused into a patient to establish donor-derived hematopoiesis and immunity.

The focus of this review is to provide a historical overview and describe seminal scientific contributions to the development of allogeneic stem cell transplantation as a therapeutic modality.

Division of Hematologic Malignancies and Cellular Therapeutics, University of Kansas Medical Center, Westwood, Kansas.

**Corresponding Author:** Anurag K Singh, Division of Hematologic Malignancies and Cellular Therapeutics, University of Kansas Medical Center, 2330 Shawnee Mission Pkwy, Westwood, KS 66205. Phone: 913-588-6029; Fax: 913-588-4085; E-mail: asingh3@kumc.edu

**doi:** 10.1158/0008-5472.CAN-16-1311

©2016 American Association for Cancer Research.

## The Beginning

An improved knowledge of the biology of hematopoiesis and the possible use of HSCT as a rescue strategy for radiation-induced bone marrow injury surged after the detonation of nuclear weapons in the Second World War. In a pivotal experiment, Jacobsen and colleagues documented that hematopoiesis was preserved in mice after lethal irradiation if the spleen was shielded (2). Lorenz and colleagues later demonstrated that mice recovered from radiation injury when infused with bone marrow after a lethal dose of radiation (3). Subsequent work established bone marrow as the source of "cellular elements" required for hematopoietic recovery (4). The observation of dose-dependent toxicity of radiation on bone marrow supported the concept of using lethal doses of radiation to kill leukemia cells and using donor marrow cells to repopulate the host marrow. In 1956, Barnes and colleagues reported on the successful treatment of murine leukemia with a supralethal dose of radiation, which was followed by bone marrow grafting (5). In 1957, Thomas and colleagues reported treatment of acute leukemia in humans using supralethal radiation followed by bone marrow infusion from fetal and adult cadavers (6). Only two out of six patients demonstrated hematopoietic recovery and all patients died within the first 100 days. Importantly, in both the experiments mentioned above, the authors alluded to a possible immunological reaction mediated by the graft against the leukemia cells.

Similar experiments performed in the early 1960s in humans were not successful because of either subsequent relapsed disease or significant immunological reactions in the host. A review of all human bone marrow transplants performed up until 1969 demonstrated very sobering results with no long-term survivors (7). Clearly, more preclinical work needed to be done before transplant would be ready for clinical use.

## Progress in Murine and Canine Models

Despite disappointing results in humans, research continued in murine and canine models, leading to several key observations:

- Intravenous infusion of bone marrow cells could lead to hematopoietic recovery after marrow ablation (8).
- Billingham and colleagues described an immune reaction characterized by rash, diarrhea after bone marrow infusion, which they called "Runt disease" or what is now known as graft-versus-host disease (GVHD; ref. 9).
- Uphoff and colleagues showed that this immune reaction was likely mediated by genetic factors (10).
- Snell and colleagues described the presence of histocompatibility antigens in the mouse model that influenced graft tolerance (11).
- Mannick and colleagues described prompt hematopoietic recovery in dogs who were exposed to radiation three times the lethal dose followed by infusion of autologous bone marrow cells (12).
- Storb and colleagues reported that cyclophosphamide could be used in place of radiation for conditioning prior to transplant (13).
- Epstein and colleagues described evidence of a dog leukocyte antigen (DLA) system, which was crucial in the determination of risks of graft failure and GVHD after transplantation. In many DLA-matched dogs, long-term engraftment was observed after transplant (14).
- GVHD was still seen in several DLA-matched dogs, which could be prevented by prophylaxis with methotrexate (15).

Progress in animal models fueled a renewed interest and attempt to translate these findings into humans. The development of initial techniques to perform human leukocyte antigen (HLA) typing facilitated this translation into clinical experiments (16).

## Understanding the HLA Barrier

It is now well understood that an individual's immunological identity is expressed in cell-surface proteins encoded by the HLA system, which is located on chromosome 6 and contains over 200 genes forming the major histocompatibility complex (MHC). These proteins play a key role in the reciprocal immunological reactions when allogeneic hematopoietic stem cells are transplanted. In the setting of allogeneic transplant, the most important HLA molecules include HLA-A, HLA-B, and HLA-C (class I), which bind to CD 8<sup>+</sup> T cells and HLA-DP, HLA-DQ, and HLA-DR (class II), which bind to CD 4<sup>+</sup> T cells and cause alloreactivity (17).

## Renewed Interest in Human Transplants

In 1968, Gatti and colleagues reported the first successful allogeneic transplant in an infant with severe combined immunodeficiency (SCID) using bone marrow cells from an HLA-matched sibling donor (18). As it was a nonmalignant condition, no conditioning chemotherapy was considered necessary and no posttransplant immunosuppression was given. However, subsequent studies demonstrated that even patients with severe immunodeficiency require a conditioning regimen to

avoid graft rejection and posttransplant immunosuppression to avoid GVHD. Thomas and colleagues published one of the first reports of HLA-matched sibling donor transplants for hematologic malignancies in 1971 (19). For the next several years, the use of allogeneic transplant was limited to congenital and acquired bone marrow failure syndromes, immunodeficiencies, and advanced refractory leukemia. In 1977, Thomas and colleagues published their results of allogeneic transplantation for 100 consecutive patients with acute leukemia and demonstrated, for the first time, that a small percentage of patients could be cured of this otherwise lethal disease (20). This study established another important observation: patients who were transplanted earlier in their disease course had better outcomes than those with advanced disease.

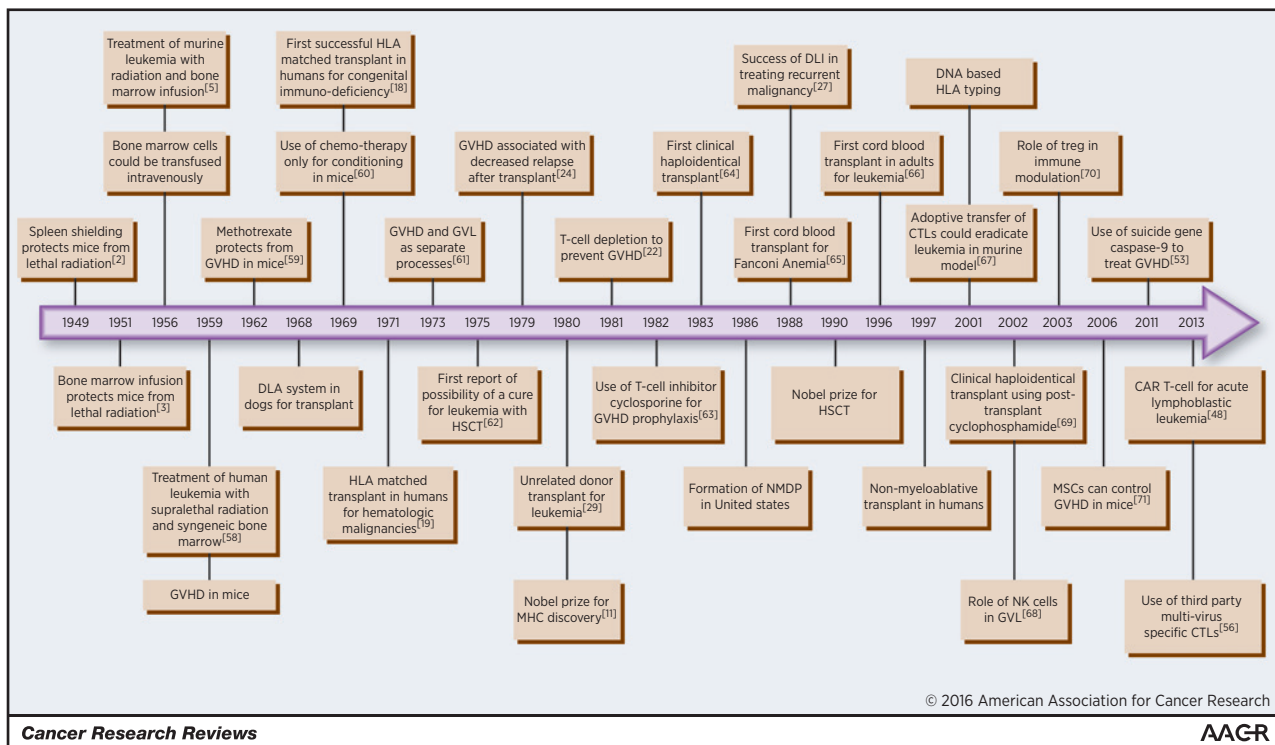
## GVHD and GVL

Preclinical experiments continued to provide further insight into transplant-related processes such as GVHD. As the role of T cells in the pathogenesis of GVHD became established, specific anti-T-cell therapy was tested for GVHD prophylaxis in canine models (21). As a result, drugs that suppressed T-cell cytokine production and proliferation, such as calcineurin inhibitors and the antiproliferative agents, e.g., methotrexate, respectively, were applied in the clinical setting and now represent standard GVHD prophylaxis in humans. Methodologies to deplete an allogeneic graft of T cells prior to infusion as a GVHD prophylactic strategy were also developed during this time (22). Subsequently, the pathogenesis of acute GVHD became understood as involving a three-step process: (i) tissue damage and inflammation resulting from the conditioning regimen, (ii) priming and differentiation of donor T cells, and (iii) target tissue destruction mediated by cellular and inflammatory factors (23). Another form of GVHD (chronic GVHD), less well understood, generally appears later in the clinical course and has rather protean manifestations resembling an autoimmune disease. GVHD, both in its acute and chronic forms, continues to represent a major barrier to successful transplantation.

However, the development of GVHD also focused attention on a phenomenon that was previously hinted at but was not yet definitively established, i.e., the graft-versus-leukemia (GVL) effect. In 1979, Weiden reported that the risk of leukemia relapse was 2.5 times lower in patients with GVHD than in those without GVHD (24). The evidence of this immune-mediated GVL effect was also supported by studies demonstrating:

- An increase in disease relapse rate when *ex vivo* T-cell depletion was used as prophylaxis for GVHD (25).
- The induction of remission in patients who relapsed after transplant by withdrawal of immunosuppression (26).
- Success of donor lymphocyte infusions (DLI) to treat recurrent leukemia after transplantation (27).
- A greater incidence of relapse in recipients of syngeneic donor transplants compared with other HLA-matched transplants (28).

Based on these reports and others, it became increasingly clear that the efficacy of allogeneic transplant in the treatment of hematological malignancies went far beyond the direct leukemia killing brought about by intensive chemoradiotherapy.



**Figure 1.**  
Milestones in the field of allogeneic transplant.

## Expansion of Indications and the Nobel Prize

As only 30% of patients had HLA-matched siblings, a search for HLA-matched unrelated donors led to the first unrelated donor transplant for acute leukemia in 1979 (29). By 1980, the curative potential of HSCTs had encouraged its use in malignancies previously considered "incurable," such as chronic myelogenous leukemia. Allogeneic HSCT was also increasingly utilized as curative therapy not only for severe aplastic anemia, but also for other severe nonmalignant conditions, such as thalassemia, sickle cell anemia, and inborn errors of metabolism as well. Another important development during this time period was the discovery of the physiological presence of CD34<sup>+</sup> hematopoietic stem cells in the peripheral blood at very low frequencies and the subsequent demonstration that their numbers could be greatly expanded through the administration of mobilizing cytokines, allowing for the collection of a stem cell product through venous access rather than through a bone marrow harvest (30, 31). This important finding resulted in a much greater utilization of "mobilized" peripheral blood stem cells, such that this has become the most common source of stem cells for transplantation (32). This strategy provides a larger number of stem cells and faster hematopoietic recovery, but has resulted in a greater incidence of chronic GVHD (33). By 1990, close to 10,000 transplants were being performed annually worldwide for various indications. Many researchers and institutions worldwide have contributed to the development of allogeneic HSCT as a therapeutic modality; however, the Seattle transplant team, under the leadership

of Dr. E. Donnall Thomas (Nobel prize in Medicine, 1990) in particular, deserves immense credit for their work in the pre-clinical and clinical studies leading to this success.

## The Rise of Reduced Intensity Regimens

For the first 30 years, allogeneic transplant was based on the premise of using maximally intensive myeloablative radiation with or without chemotherapy to perform three functions: (i) eradicate cancer cells, (ii) suppress the recipient immune system to prevent rejection of the graft, and (iii) create a "space" in the bone marrow to facilitate donor stem cell engraftment (20). Santos and colleagues introduced a radiation-free conditioning regimen to achieve these goals by combining busulfan and cyclophosphamide (34). These maximally intense chemoradiotherapy and chemotherapy-only regimens still carried significant acute and long-term toxicities and could not be used in the elderly where, ironically, the greatest incidence of hematologic malignancies is seen. An increasing understanding that a GVL effect could be sufficient to eradicate malignant disease led to the development of reduced intensity conditioning regimens in canine models where they demonstrated reliable engraftment, reduced toxicity, and potent antitumor effects (35). Subsequently, different versions of reduced conditioning regimens were utilized in human transplants as well with successful results (36). These regimens often resulted in mixed chimerism (presence of both donor and recipient cells) after transplant followed by subsequently increasing conversion to a donor-derived hematopoietic stem cell population and T-cell repertoire. The ability to successfully

perform transplant using these approaches has circumvented the historic age limit for allogeneic transplant. Currently, over 20% of allogeneic transplants are being performed in those over 60 years of age (32). However, relapse continues to be a challenge with this approach and myeloablative conditioning still remains standard in younger patients with rapidly proliferating malignancies (37).

## Overcoming the HLA Barrier

The HLA system is highly polymorphic; however, haplotypes are conserved in ethnic populations through a phenomenon known as linkage disequilibrium. This understanding of the HLA system in combination with improved HLA typing techniques has resulted in the development of large unrelated donor registries, such as the National Marrow Donor Program (NMDP) in the United States. However, there is a large variability in ethnic representation in these registries. For example, the odds of finding a full match for a Caucasian patient is approximately 70%, whereas this number is 18% for African Americans. One or two antigen mismatch transplants have been done in this situation, but the incidence of graft failure and GVHD remains problematic. Every HLA antigen mismatch between donor and recipient has been shown to adversely affect the success of allogeneic transplant (38).

This problem could be addressed if one could successfully overcome HLA barriers that would otherwise be associated with unacceptable rates of graft rejection and GVHD.

Cord blood and partially HLA-matched related (haploidentical) HSCTs have emerged as viable options in this regard.

Cord blood stem cells require less strict HLA matching and may be associated with a lower risk of severe GVHD because of an immunologically naïve donor-derived T-cell repertoire (39). The limitations of cord blood HSCTs include slower hematopoietic recovery and delayed immune reconstitution because of a limited number of progenitor cells in each unit. Use of double cord blood transplantation has been used as a strategy to overcome this challenge in adults.

Haploidentical stem cells are easily accessible as they can be collected from a biological parent, child or sibling. The most significant barrier with this approach historically has been an intense bidirectional alloreactivity of T cells leading to a high incidence of both graft failure and GVHD (40).

A pioneering approach to address this barrier involved the prevention of graft rejection by intensive conditioning chemotherapy followed by the transplantation of "mega doses" of CD 34<sup>+</sup> stem cells and prevention of GVHD by T-cell depletion of the stem cell product (41). Severe regimen-related toxicities and delayed immune reconstitution with an associated high incidence of opportunistic infections is a serious problem with this approach. Nevertheless, the goal of reliably securing engraftment with limited GVHD was accomplished.

An alternative approach pioneered by the Johns Hopkins group has been based on murine models demonstrating that cyclophosphamide administration after stem cell transplant preferentially targeted alloreactive T cells while sparing stem cells and the peripheral memory T cells. Early after transplant, alloreactive T cells are susceptible to alkylating agent-induced deletion while relatively quiescent hematopoietic stem cells and the nonalloreactive T-cell compartment are protected because of both their nondividing quiescence and their ability

to metabolize cyclophosphamide to inactive metabolites through the increased expression of aldehyde dehydrogenase, which is lacking in alloreactive T cells (42, 43). Subsequent studies in humans have shown that a nonmyeloablative regimen along with T-cell replete bone marrow stem cells is very well tolerated and results in reliable engraftment, limited GVHD, and potent antitumor effects (44). A U.S. randomized trial is currently comparing haploidentical transplant with post-transplant cyclophosphamide to double unit cord blood transplantation.

These modern approaches to donor selection have made it possible to have a donor for essentially all patients who need a transplant. However, disease relapse, GVHD, and infectious complications continue to be major challenges.

## GVL and the Dawn of Cellular Therapy

It is clear that maximizing the GVL effect while minimizing GVHD is the holy grail of transplant immunology.

Initially considered to be mediated solely by donor-derived T cells, the GVL effect is now considered to be multifactorial. The principal cytotoxicity is mediated by T cells and natural killer (NK) cells with ancillary roles played by dendritic cells, B cells, and minor histocompatibility antigens. In HLA-matched transplantation, genetic polymorphism results in expression of different endogenous protein products on HLA molecules. The alloreactivity resulting from these minor histocompatibility antigens appears to be one of the central mediators of GVHD and GVL effects.

DLIs are the simplest way to provide GVL; however, this strategy is commonly associated with the development of severe GVHD and is of limited efficacy in rapidly proliferating acute leukemias.

NK cells are an integral part of innate immunity and increasingly are recognized for their GVL properties, as well. Their detection system includes a group of activating and inhibitory receptors, which can recognize stress-related acquired antigens and absence of self-MHC class I antigen (45). Killer cell immunoglobulin-like receptor (KIR)-ligand mismatches (ligand present on donor hematopoietic cells and absent on recipient tumor cells) play a major role in the GVL effect of NK cells and have been exploited to optimize a GVL effect (46).

Alternatively, T cells could be genetically engineered to express antigen-specific receptors through MHC-dependent or independent presentation. In one such approach, T-cell receptor engineering involves isolation of T cells from the patient, *ex vivo* expansion followed by genetic modification to express a TCR with specificity for tumor peptide presented on MHC (47). Some of the disadvantages of TCR therapy include HLA restriction, potential of alloimmunity with mispairing of  $\alpha$  and  $\beta$  chains, and inability to recognize nonpeptide antigens.

Another strategy has involved the use of adoptive cellular therapy using synthetic, engineered chimeric antigen receptors T cells (CAR-T cell) to target cell surface molecules. These receptors are transduced into T cells using viral vectors (48). Unlike the physiological T-cell receptors, they do not require APCs for antigen presentation and are not HLA restricted, thus circumventing the problem of downregulated HLA expression by tumor cells. They can bind not only to peptides but also to carbohydrates, ganglioside, and proteoglycans. CD19, which is expressed on most B-cell lymphomas but not on hematopoietic stem cells,

was one of the first successfully targeted antigens (49). Remaining challenges to engineered T-cell constructs involve the identification of tumor cell–restricted antigens, more reliable control of on target, off tumor toxicity, and contending with other significant potential toxicities, such as cytokine release syndrome.

### Breaking the Cycle of GVHD

Principal strategies to contend with GVHD have involved prophylactic measures, such as securing optimal HLA-matched donors, *in vivo* and *ex vivo* TCD methodologies, the use of T-cell inhibiting drug therapies and novel drug therapeutics, such as post-HSCT cyclophosphamide and molecules that interfere with the homing of alloreactive T cells to target tissues. Despite these efforts, GVHD remains a common and all too frequent life-threatening complication after HSCT. Treatment strategies of established GVHD are often associated with significant toxicities and poor efficacy. Several recent approaches hold promise for improving outcomes of GVHD. For example, there is increasing evidence that the intestinal microbiome plays an important role in APC/ T-cell cross-talk and commensal bacterial diversity can protect from GVHD-related mortality (50). Another important advance has been represented by a better understanding of the role in immune modulation played by regulatory T cells (Treg). Tregs participate in T-cell self-regulation and have been shown to preferentially suppress alloreactive T cells without increasing the risk of disease relapse (51). This has led to strategies using peritransplant Treg infusions to prevent GVHD. Another cellular based approach to GVHD has involved the recognition of mesenchymal stromal cells (MSC) as mediators of immune suppression. MSCs are multipotent stem cells present in the bone marrow stroma and other tissues that have profound immunomodulatory properties and have shown promising results in both preclinical and clinical GVHD studies (52).

Finally, suicide gene therapy is a promising strategy allowing for more safe administration of DLI to prevent or treat post-HSCT relapse. Di Stasi and colleagues treated pediatric patients with haploidentical T cells engineered through retroviral transduction of an inducible *caspase-9* (*icasp-9*) suicide gene that causes rapid cell death when activated by an otherwise bioinert chemical dimerizer (53). Four of the five patients developed acute GVHD, which was promptly reversed by a single dose of a chemical dimerizer.

### Immunodeficiency and Resultant Infectious Complications

Severe immunodeficiency is universal after allogeneic HSCT and is commonly persistent for months after the procedure. The extent of immunodeficiency and subsequent kinetics of immunologic recovery is dependent upon many variables, including patient and donor characteristics, graft source, HLA disparity, graft manipulation, and development of GVHD (54). A comprehensive understanding of patient vulnerabilities to specific infectious organisms at different time points of immune reconstitution has been achieved. As a result, major improvements in transplant outcomes have been realized through improved early detection of infection as well as prophylactic and preemptive antimicrobial drug strategies. Despite these advances, infection continues to be a major cause of morbidity and mortality after allogeneic HSCT. In particular, viral infections such as cytomeg-

alovirus (CMV), Epstein–Barr virus (EBV), adenovirus, polyomavirus (BK) and community-acquired respiratory viruses contribute significantly to transplant-related morbidity and mortality. Although drug therapies are available for the treatment of some of these viral infections, issues related to lack of efficacy and drug toxicities are common. An improved understanding of endogenous control of viral infections through acquisition of cytotoxic T-lymphocytes (CTL) cells that target viral antigens has led to attempts to generate *ex vivo* virus-specific CTLs and their use as a therapeutic strategy post-transplant. In an interesting approach, multi-virus CTLs were generated when donor mononuclear cells were transduced with a chimeric adenoviral vector expressing CMV antigen for initial stimulation followed by stimulation with EBV-lymphoblastoid cell lines (55). The success of this approach has expanded to generation of third-party multi-virus CTLs that could be available readily for "off the shelf" use (56).

### Collaboration to Move Science Forward

The HSCT community understood the importance of scientific collaboration in advancing the field. Therefore, soon after the first successful transplant, the International Bone Marrow Transplant Registry (IBMTR) was born in 1972, through the visionary efforts of Mortimer M. Bortin, to share outcomes data from individual transplant centers. In 2004, the IBMTR joined hands with the research program of NMDP to form what is now known as the Center for International Blood and Marrow Transplant Research (CIBMTR). The CIBMTR collaborates with the global transplant community to support observational and prospective research through a database of over 425,000 patients and by providing scientific and statistical support. Other international organizations, such as the European Society for Blood and Marrow Transplantation (EBMT), the Asia-Pacific Blood and Marrow Transplantation Group (APBMT), and the Worldwide Network for Blood and Marrow Transplantation (WBMT), have similarly been instrumental in promoting scientific research and increased access to HSCT.

### Conclusions and Future Perspective

This report is certainly not intended as a comprehensive review of scientific accomplishments that led to the development of allogeneic transplant but rather as a broad overview of some of the most important concepts in the field. It is clear that the story of HSCT is one of the most important success stories of science in the 20th century. HSCT in 2016 is significantly safer, more effective, and is available for a wider variety of indications. This success supports the use of allogeneic HSCT as an attractive platform for testing promising cancer therapeutics. In the future, an improved understanding of the genetics and molecular basis of hematologic diseases will enable us to switch from traditional chemoradiotherapy to more specifically targeted immunotherapeutics. The identification of new targets for CAR-T cells coupled with modulation of costimulatory pathways will enhance their efficacy and expand their role in both pre- and posttransplant settings. In a hypothetical situation, a patient with newly diagnosed acute leukemia would be treated with CAR-T cells that target antigens present on leukemia stem cells as well as normal hematopoietic stem cells. These CAR-T cells could then be eradicated

through an apoptosis-inducing suicide gene. T-cell-depleted donor-derived hematopoietic cells would then be infused to generate hematopoiesis. Immune reconstitution would then be facilitated using limited aliquots of donor-derived DLL, also controlled using a suicide gene strategy in the event of GVHD. Specific multi-valent CTLs would be infused in the event of viral reactivations. MSCs and Tregs would be utilized as a back-up strategy in the event of breakthrough GVHD. Finally, improved molecular techniques will allow early detection of leukemia relapse, which could then be treated with CAR-T cells targeted at leukemia-specific antigens. The use of HSCT to facilitate tolerance in solid organ transplant is another area of active clinical research. Further, the clinical use of

"induced pluripotent stem cells" (generated from somatic cells after gene induction for hematopoiesis) to generate blood-specific lineage holds great promise as it could potentially eliminate the need for a stem cell donor (57). Finally, the ongoing research in the use of hematopoietic stem cells for nonhematopoietic tissue repair could usher in a new era of regenerative medicine.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received May 18, 2016; revised July 26, 2016; accepted July 27, 2016; published OnlineFirst October 26, 2016.

#### References

- Gratwohl A, Pasquini MC, Aljurf M, Atsuta Y, Baldomero H, Foecken L, et al. One million haemopoietic stem-cell transplants: a retrospective observational study. *Lancet Haematol* 2015;2:e91-100.
- Jacobson LO, Marks ER, Robson MJ, Gaston EO, Zirkle RE. The effect of spleen protection on mortality following X-irradiation. *J Lab Clin Med* 1949;34:1538-43.
- Lorenz E, Uphoff D, Reid TR, Shelton E. Modification of irradiation injury in mice and guinea pigs by bone marrow injections. *J Natl Cancer Inst* 1951;12:197-201.
- Main JM, Prehn RT. Successful skin homografts after the administration of high dosage X radiation and homologous bone marrow. *J Natl Cancer Inst* 1955;15:1023-29.
- Barnes DWH, Corp MJ, Loutit JF, Neal FE. Treatment of murine leukaemia with X-rays and homologous bone marrow. *BMJ* 1956;2:626-7.
- Thomas ED, Lochte HL Jr, Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. *N Engl J Med* 1957;257:491-6.
- Bortin MM. A compendium of reported human bone marrow transplants. *Transplantation* 1970;9:571-87.
- van Bekkum DW, Vos O, Weyens WWH. Homo- et heterogreffes tissues hematopoietiques chez la souris. *Rev Hemat* 1956;11:477-85.
- Billingham RE, Brent L. Quantitative studies on tissue transplantation immunity. IV. Induction of tolerance in newborn mice and studies on the phenomenon of runt disease. *Phil Trans R Soc Lond B* 1959; 242:477.
- Uphoff DE. Genetic factors influencing irradiation protection by bone marrow. I. The F1 hybrid effect. *J Natl Cancer Inst* 1957. 19:123-5.
- Snell GD. The Nobel Lectures in Immunology. Lecture for the nobel prize for physiology or medicine, 1980: studies in histocompatibility. *Scand J Immunol* 1992;36:513-26.
- Mannick JA, Lochte HL Jr, Ashley CA, Thomas ED, Ferrebee JW. Autografts of bone marrow in dogs after lethal total-body radiation. *Blood* 1960;15:255-66.
- Storb R, Epstein RB, Rudolph RH, Thomas ED. Allogeneic canine bone marrow transplantation following cyclophosphamide. *Transplantation* 1969;7:378-86.
- Epstein RB, Storb R, Ragde H, Thomas ED. Cytotoxic typing antisera for marrow grafting in littermate dogs. *Transplantation* 1968;6:45-58.
- Storb R, Epstein RB, Graham TC, Thomas ED. Methotrexate regimens for control of graft-versus-host disease in dogs with allogeneic marrow grafts. *Transplantation* 1970;9:240-6.
- Rood JIV, Leeuwen AV. Leukocyte grouping. A method and its application. *J Clin Invest* 1963;42:1382-90.
- Neeffes J, Jongsma ML, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol* 2011;11:823-36.
- Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. *Lancet* 1968;2:1366-9.
- Thomas ED, Buckner CD, Rudolph RH, Fefer A, Storb R, Neiman PE, et al. Allogeneic marrow grafting for hematologic malignancy using HLA-matched donor-recipient sibling pairs. *Blood* 1971;38:267-87.
- Thomas ED, Buckner CD, Banaji M, Clift RA, Fefer A, Flournoy N, et al. One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. *Blood* 1977;49:511-33.
- Deeg HJ, Storb R, Weiden PL, Raff RF, Sale GE, Atkinson K, et al. Cyclosporin A and methotrexate in canine marrow transplantation: engraftment, graft-versus-host disease, and induction of intolerance. *Transplantation* 1982;34:30-5.
- Reisner Y. Transplantation for acute leukaemia with HLA-A and B nonidentical parental marrow cells fractionated with soybean agglutinin and sheep red blood cells. *Lancet* 1981;2:327-331.
- Ferrara JL, Cooke KR, Pan L, Krenger W. The immunopathophysiology of acute graft-versus-host-disease. *Stem Cells* 1996;14:473-89.
- Weiden PL. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med* 1979; 300:1068-73.
- Marmont AM. T-cell depletion of HLA-identical transplants in leukemia. *Blood* 1991;78:2120-30.
- Higano CS, Brixey M, Bryant EM, Durnam DM, Doney K, Sullivan KM, et al. Durable complete remission of acute nonlymphocytic leukemia associated with discontinuation of immunosuppression following relapse after allogeneic bone marrow transplantation. A case report of a probable graft-versus-leukemia effect. *Transplantation* 1990;50: 175-7.
- Kolb HJ. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 1990;76: 2462-5.
- Horowitz MM. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990;75:555-62.
- Hansen JA, Clift RA, Thomas ED, Buckner CD, Storb R, Giblett ER, et al. Transplantation of marrow from an unrelated donor to a patient with acute leukemia. *N Engl J Med* 1980;303:565-7.
- Berenson RJ, Andrews RG, Bensinger WI, Kalamaz D, Knitter G, Buckner CD, et al. Antigen CD34<sup>+</sup> marrow cells engraft lethally irradiated baboons. *J Clin Invest* 1988;81:951-5.
- Dührsen U, Villeval JL, Boyd J, Kannourakis G, Morstyn G, Metcalf D. Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. *Blood* 1988;72: 2074-81.
- Pasquini MC, Zhu X. Current uses and outcomes of hematopoietic stem cell transplantation: CIBMTR Summary Slides. 2015: <https://www.cibmtr.org/referencecenter/slidesreports/summaryslides/Pages/index.aspx#CiteSummarySlides>.
- Anasetti C, Logan BR, Lee SJ, Waller EK, Weisdorf DJ, Wingard JR, et al. Peripheral-blood stem cells versus bone marrow from unrelated donors. *N Engl J Med* 2012;367:1487-96.
- Santos GW. Busulfan (Bu) and cyclophosphamide (Cy) for marrow transplantation. *Bone Marrow Transplant* 1989;4:236-9.
- Storb R. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood* 1997;89:3048-54.

36. Giralt S. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood* 1997;89:4531–36.
37. Scott BL, Pasquini MC, Logan B, Wu J, Devine S, Porter DL, et al. Results of a phase III randomized, multi-center study of allogeneic stem cell transplantation after high versus reduced intensity conditioning in patients with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML): blood and marrow transplant clinical trials network (BMT CTN) 0901. *Blood* 2015;126:LBA-8-LBA-8.
38. Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood* 2007;110:4576–83.
39. Garderet L, Duphy N, Douay C, Chalumeau N, Schaeffer V, Zilber MT, et al. The umbilical cord blood alphabeta T-cell repertoire: characteristics of a polyclonal and naive but completely formed repertoire. *Blood* 1998;91:340–6.
40. Beatty PG, Clift RA, Mickelson EM, Nisperos BB, Flournoy N, Martin PJ, et al. Marrow transplantation from related donors other than HLA-identical siblings. *N Engl J Med* 1985;313:765–71.
41. Aversa F, Terenzi A, Tabilio A, Falzetti F, Carotti A, Ballanti S, et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. *J Clin Oncol* 2005;23:3447–54.
42. Luznik L, Jalla S, Engstrom LW, Iannone R, Fuchs EJ. Durable engraftment of major histocompatibility complex-incompatible cells after nonmyeloablative conditioning with fludarabine, low-dose total body irradiation, and posttransplantation cyclophosphamide. *Blood* 2001;98:3456–64.
43. Kastan MB, Schlaffer E, Russo JE, Colvin OM, Civin CI, Hilton J. Direct demonstration of elevated aldehyde dehydrogenase in human hematopoietic progenitor cells. *Blood* 1990;75:1947–50.
44. Luznik L, O'Donnell PV, Symons HJ, Chen AR, Leffell MS, Zahurak M, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant* 2008;14:641–50.
45. Yokoyama WM, Plougastel BF. Immune functions encoded by the natural killer gene complex. *Nat Rev Immunol* 2003;3:304–16.
46. Ruggeri L. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. *Blood* 1999;94:333–9.
47. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood* 2009;114:535–46.
48. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor–modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013;368:1509–18.
49. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor–modified T cells in chronic lymphoid leukemia. *N Engl J Med* 2011;365:725–33.
50. Jenq RR, Ubeda C, Taur Y, Menezes CC, Khanin R, Dudakov JA, et al. Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. *J Exp Med* 2012;209:903–11.
51. Edinger M, Hoffmann P, Ermann J, Drago K, Fathman CG, Strober S, et al. CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. *Nat Med* 2003;9:1144–50.
52. Le Blanc K, Ringdén O. Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 11:321–334.
53. Di Stasi D, Tey S-K, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C, et al. Inducible Apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med* 2011;365:1673–83.
54. Krenger W, Blazar BR, Holländer GA. Thymic T-cell development in allogeneic stem cell transplantation. *Blood* 2011;117:6768–76.
55. Leen AM, Myers GD, Sili U, Huls MH, Weiss H, Leung KS, et al. Monoculture-derived T lymphocytes specific for multiple viruses expand and produce clinically relevant effects in immunocompromised individuals. *Nat Med* 2006;12:1160–66.
56. Leen AM, Bollard CM, Mendizabal AM, Shpall EJ, Szabolcs P, Antin JH, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. *Blood* 2013;121:5113–23.
57. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663–76.
58. Thomas ED, Lochte HL Jr, Cannon JH, Sahler OD, Ferrebee JW. Supralethal whole body irradiation and isologous marrow transplantation in man. *J Clin Invest* 1959;38:1709–16.
59. Lochte HL Jr, Levy AS, Guenther DM, Thomas ED, Ferrebee JW. Prevention of delayed foreign marrow reaction in lethally irradiated mice by early administration of methotrexate. *Nature* 1962;196:1110–11.
60. Santos GW, Owens AH. Allogeneic marrow transplants in cyclophosphamide treated mice. *Transplant Proc* 1969;1:44–46.
61. Bortin MM, Rimm AA, Saltzstein EC, Rodey GE. Graft versus leukemia. III. Apparent independent antihost and antileukemic activity of transplanted immunocompetent cells. *Transplantation* 1973;16:182–8.
62. Thomas ED, Storb R, Clift RA, Fefer A, Johnson FL, Neiman PE, et al. Bone-marrow transplantation. *N Engl J Med* 1975;292:832–43.
63. Deeg HJ. Cyclosporin A and methotrexate in canine marrow transplantation: engraftment, graft-versus-host disease, and induction of tolerance. *Transplantation* 1982;34:30–35.
64. Powles RL, Morgenstern GR, Kay HE, McElwain TJ, Clink HM, Dady PJ, et al. Mismatched family donors for bone-marrow transplantation as treatment for acute leukaemia. *Lancet* 1983;1:612–5.
65. Gluckman E. Transplantation of umbilical cord blood in Fanconi's anemia. *Nouv Rev Fr Hematol* 1990;32:423–5.
66. Laporte J-P, Gorin N-C, Rubinstein P, Lesage S, Portnoi M-F, Barbu V, et al. Cord-blood transplantation from an unrelated donor in an adult with chronic myelogenous leukemia. *N Engl J Med* 1996;335:167–70.
67. Fontaine P, Roy-Proulx G, Knafo L, Baron C, Roy DC, Perreault C. Adoptive transfer of minor histocompatibility antigen-specific T lymphocytes eradicates leukemia cells without causing graft-versus-host disease. *Nat Med* 2001;7:789–94.
68. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002;295:2097–2100.
69. O'Donnell PV, Luznik L, Jones RJ, Vogelsang GB, Leffell MS, Phelps M, et al. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant* 2002;8:377–86.
70. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells. *Nat Immunol* 2003;4:330–6.
71. Yañez R, Lamana ML, García-Castro J, Colmenero I, Ramírez M, Bueren JA. Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. *Stem Cells* 2006;24:2582–91.