New considerations in hematopoietic stem cell transplantation for severe combined immunodeficiency: how did newborn screening change our field, and can we finally brake the glass ceiling for haploidentical transplantation?

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ABSTRACT

Pioneered in 1968, hematopoietic stem cell transplantation (HSCT) first cured a patient with severe combined immune deficiency (SCID) transplanted from a matched sibling, bringing hope for this previously fatal disease. Since then, HSCT has become the standard of care treatment for SCID with thousands of patients transplanted successfully worldwide. Initially successful mainly in patients with a matched sibling donor and in specific easier to transplant types of SCID, nowadays, most patients with SCID undergo successful transplantation due to HSCT technique advances. These include refined human leukocyte antigen (HLA)-tissue typing, use of alternative donors, availability of new stem cell sources such as umbilical cord blood, less toxic chemotherapeutic conditioning, as well as improved graft-versus-host disease (GvHD) prophylaxis. Other factors contributing to the success of transplantation include the improvement of supportive care by molecular detection of viral infections, enabling preemptive antiviral treatment before organ damage occurs. Increased awareness for primary immunodeficiency disorders (PID), leading to earlier diagnosis and referral to specialist centers, has been another important factor in successfully transplanting SCID patients. A major game changer in the last decade has been the implementation of neonatal screening for SCID. This increased early diagnosis, allowing for this disease to be almost universally diagnosed soon after birth in countries which included this test in their newborn screening program. As a result, early and optimal transplant timing and conditions could be achieved. However, very early diagnosis also raised new questions regarding SCID patients with a "leaky" phenotype, as well as dilemmas regarding transplant and conditioning regimens in very young infants. With improved diagnosis and treatment options, overall survival has increased to over 90% for SCID babies with a genoidentical donor and similar results are emerging for matched unrelated donor HSCT. Due to new advances, we hope to achieve similar results for those given HSCT from haploidentical donors as well. This review will focus on the new considerations in HSCT seen in recent years, and examines the effect they have had on treatment options and outcomes for SCID patients.

Statement of novelty: The field of HSCT has advanced considerably since the first successful SCID bone marrow transplant in 1968. However, success rates have been limited due to delayed diagnosis and poor outcome of patients for which a HLA-matched donor could not be found. This review will discuss recent advances occurring in the last decade in HSCT for SCID, and our hopes to bring cure to this once fatal disease.

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History of HSCT for SCID

Severe combined immunodeficiencies (SCID) are heterogeneous primary immunodeficiency disorders (PID) that are characterized by the absence of thymopoiesis, severely impairing T-lymphocyte maturation and function. Without definitive treatment within the first years of life, the condition is invariably fatal. Classic presentation involves persistent viral respiratory or gastrointestinal infection in infancy, failure to clear infections, and persistent and deteriorating symptoms. Multiple pathogens may co-exist and opportunistic infection, such as *Pneumocystis jirovici*, is common. In some areas around the world where routine postnatal immunization with live polio, Bacillus Calmette-Guerin or rotavirus vaccine are given, these can cause persistent and disseminated infection (Patel et al. 2010; Marciano et al. 2014; Heiman et al. 2018).

Due to extensive research and advances in genetic diagnosis, the molecular basis of 75%–80% of SCID types are now understood. Definitive treatment is predominantly by allogeneic HSCT, although gene therapy and enzyme replacement therapy are available for some specific genetic subtypes. Treatment with HSCT has expanded in recent years to include non-malignant disorders such as thalassemia metabolic disorders and many PIDs. Although there is some common ground, there are principals of transplantation for SCID that differ from the treatment of hematological malignancies in which eradication of malignancy is required. The objective of HSCT in patients with SCID is to provide normal hematopoietic stem cells, facilitating correction of the immune defect. The aim is to minimize potential sequelae of treatment, but more importantly, to establish effective lifelong immune function. The outcome of HSCT for SCID is related to a number of different factors, including genotype, pre-existing morbidities and viral infections at the time of HSCT, the type and degree of human leukocyte antigen (HLA) match of the donor, as well as the choice of treatment protocol (Brown et al. 2011; Pai et al. 2014; Gennery 2015).

It is not surprising therefore that there are some unique donor selection factors for patients with SCID. SCID patients tend to come from ethnic minorities, where matched unrelated donors (MUD) are sometimes under represented on the donor registries, and from families with consanguinity (Gennery 2015). The up side of this is a greater chance of finding an HLA-identical donor within the family, due to the fact that in highly consanguineous populations the parents and second degree relatives can often be an identical match. However if no familial donor is found, often there will not be a MUD found in the registries. Another important factor is that despite advances in genetic sequencing and diagnosis of SCID, there are still patients who present with a clinical picture or findings consistent with SCID for whom a genetic diagnosis is not identified. Great care should be taken in transplanting these genetically undefined SCID patients with presumably young healthy family donors. Although it is assumed that most SCID patients have clinical or abnormal bloodwork when evaluated even at a young age, due to "leaky" and variable phenotypes now recognized even within affected members in the same family, without an accurate genetic diagnosis there is a low but possible risk that potential donors could harbor the disease. These factors as well as data from European and North American populations, showing that matched sibling genoidentical donors (MSD) are found for only 15%–25% of patients, facilitated the need to look for other donor sources (Antoine et al. 2003; Grunebaum et al. 2006).

The first attempt to treat SCID with unmodified MUD transplantation was performed in 1973. A patient diagnosed with SCID underwent HSCT from a partial MUD found among 800 normal individuals registered in Denmark. The patient achieved durable engraftment with complete hematopoietic and immunologic reconstitution, but suffered from skin and oral mucosa chronic graft-versus-host disease (GvHD) (O'Reilly et al. 1977). Difficulties finding suitable MUD and the lack of adequate means to control GvHD, together with the increased availability of T-cell depleted haploidentical HSCT in the early 1980s resulted in limited use of MUD HSCT for SCID. The surge in the number of bone marrow donor registries and the development of efficient medications to control GvHD, such as cyclosporine A (CsA), prompted Dr. CM Roifman to systematically explore the use of MUD HSCT for SCID at The Hospital for Sick Children in Toronto, Canada. The HSCT protocol included strict patient isolation, intensive pre-transplant antimicrobial management with intravenous immunoglobulins, *Pneumocystis jiroveci* pneumonia prophylaxis, careful surveillance of herpesvirus groups, and nutritional support. Pre-transplant conditioning consisted of busulfan and cyclophosphamide. This protocol led to improved engraftment and

patient survival rates of 73.3%. All patients had leukocyte engraftment, which were demonstrated to be of 100% donor origin using restriction length fragment polymorphism. Deaths were attributed to GvHD (Grunebaum and Roifman 2011). A follow up study included patients from both Toronto (Canada) and Brescia (Italy) showing similar good survival and engraftment (Mazzolari et al. 2007).

Studies show that all deaths occurring >2 years from transplant involved patients with either chronic GVHD, persistent need for nutritional support, autoimmunity/auto-inflammation, or some combination of these 3 clinical features. Historically, studies looking into outcome after transplantation have shown a favorable outcome for patients for which MSD was found, and to a lesser degree, MUD transplants. However, for patients in which no donor was found and where a haploidentical donor was used, outcomes often reported 60% survival at best until recent years (Heimall et al. 2017).

Significant improvement in survival and outcome of SCID patients occurs with early diagnosis and the ability to prevent infections by very early prophylaxis and isolation measures. Yet, despite the advancement of supportive and infectious surveillance as well as treatment options, the obstacle of GvHD and its grave sequelae still remains our greatest battle to date. Thus, the main factor which determines the success of HSCT nowadays is the degree of donor matching.

Recent advances in HSCT for SCID

Advances in haploidentical transplantation

Although there are more than 30 000 000 donors registered in the World Marrow Donor Association—the world's largest hematopoietic cell database, for over 30% of SCID patients, a MUD or MSD is still not found. Since gene therapy is still rarely available in most centers around the world and exists only for restricted genetic causes of SCID, the option of a haploidentical donor may be the only chance for survival. Advantages of haploidentical donors include their often immediate availability, allowing transplantation to take place very soon after the diagnosis, and the fact that a donor will still likely be available and willing to donate at a later time if needed.

To avoid the risk of severe and usually fatal GvHD, early transplants used MSD only. Following the development of donor blood bank registries, methods to remove alloreactive T-lymphocytes were also generated. Initial techniques using soybean agglutinin and ovine erythrocytes (Reisner et al. 1981) were a particularly successful method for SCID patients, where an absence of cell-mediated immunity reduced the rejection risk by the recipient, particularly in natural killer lymphocyte-deficient phenotypes, and carried a low risk of GvHD (Bertrand et al. 1999). Various alternative methods of T-lymphocyte depletion have been used historically. Additional in vitro depletion methods include physical methods such as counter-flow centrifugal elutriation, using competing centrifugal and counter flow drag forces to separate cells of different size and sedimentation properties (de Witte et al. 1986), and antibody-based methods such as in vitro CAMPATH-1M anti-lymphocyte (anti-CD52) depletion (Gennery et al. 2001). An advantage of in vitro depletion is the ability to accurately determine the CD3 T-lymphocyte dose before the product is infused into the patient. Conversely, administration of in vivo anti-T-lymphocyte antibody directly to the patient prior to infusion of the HLA-mismatched inoculum (using polyclonal rabbit-derived or horse-derived anti-thymocyte globulin (ATG), or alemtuzumab, a humanized monoclonal anti-CD52 antibody) depletes donor and recipient T-lymphocytes, preventing donor T-lymphocyte-mediated acute GvHD and recipient T-lymphocyte-mediated graft rejection. However, intra-individual variation in pharmacokinetics makes this less predictable than in vitro methods. In vivo antibody levels at time of transplantation may also alter the efficacy of T-lymphocyte depletion (Marsh et al. 2016).

Despite advances in general supportive care, survival rates after a haploidentical transplantation for SCID are considerably lower than MSD or MUD. Finally, in the recent decade, new promising methods will likely allow for improved survival.

CD3 TCRαβ CD19 depletion

Depletion methods have been the main technique used to prevent GvHD. However, issues arising from the use of T-lymphocyte depleted grafts include increased risk of rejection and high risk of severe viral infection until thymopoiesis is established. To overcome these issues, partial T-lymphocyte depletion involving removal of T cell receptor (TCR) αβ T-lymphocytes and CD19 B-lymphocytes while retaining TCRγδ T-lymphocytes, is a promising yet expensive solution.

TCRγδ T-lymphocytes exhibit a number of important properties which differ from conventional TCRαβ T-lymphocytes. Although they require the recombination activating genes to arrange the TCR and are considered part of adaptive immunity, TCRγδ T-lymphocytes exhibit innate features including target recognition in a major histocompatibility complex independent manner, and demonstrate a preactivated phenotype, which enables rapid cytokine production, specifically interferon-γ and tumor necrosis factor-α. The absence of HLA restriction reduces the risk of HLA-induced alloreactivity causing acute GvHD, and they are also active against intracellular and extracellular pathogens (Slatter and Gennery 2017).

Reports on the outcome of patients with PID who have received CD3 TCRαβ CD19 depleted products from haploidentical parents or mismatched unrelated donors are encouraging. The overall survival rate of 92% is comparable to that achieved using MSD or MUD. A combination of treosulfan, fludarabine, thiotepa, and either ATG and rituximab or alemtuzumab was sufficient to achieve high level or complete donor chimerism in most cases. The incidence of acute GvHD up to or above grade II in these 2 studies was 13/62 (21%), and only 2 of which (3%) experienced acute GvHD more severe than grade II. Viral infection remains a significant cause of transplant-related mortality, thus, some patients with significant viral disease are administered pre-banked third-party viral specific cytotoxic T-lymphocytes, either pre-transplant or post-transplant, allowing prompt resolution of infection (Balashov et al. 2015; Slatter and Gennery 2017; Shah et al. 2018).

Graft depletion of CD45RA naive T cells

To prevent GVHD and preserve memory T-lymphocytes with potent anti-infective properties, depletion of CD45 naïve T-lymphocytes has also been developed (Touzot et al. 2015). However, these attempts encountered severe infectious sequelae. Adoptive immunotherapy with the injection of donor-derived antiviral T-lymphocytes during the post-transplant period may also speed up the recovery of virus-specific immunity (Bollard and Heslop 2016).

Post-transplant cyclophosphamide (PTCy)

A mouse model to examine the effect of cyclophosphamide (Cy) on skin homograft transplants was first published by Berenbaum and Brown (1964). They observed delayed rejection of the homograft when Cy was given at any time from day 0 to day +4. Animal models demonstrated improved engraftment and decreased GvHD with PTCy in the setting of allogeneic HCT. Although a standard component of cyto-reductive chemotherapy conditioning regimens, Cy is non-toxic to pluripotent hematopoietic stem cells but selectively toxic to recently activated lymphocytes. Therefore, administration of Cy a few days after HSCT would target recently activated previously naïve cells stimulated by recipient alloantigens, but would preserve memory antiviral competence. PTCy also selectively depletes alloreactive naive CD4+ T cells while preserving the regulatory T cells. However this method was not used in transplant protocols until 2012, when a group from The Johns Hopkins Hospital developed a protocol that included early administration of 2 days of high-dose PTCy following fludarabine/low dose Cy/low-dose total body irradiation reduced intensity conditioning (RIC) regimen with bone marrow as the stem cell source. Their results showed graft failure of 13% and a very low incidences of severe acute (5% of grade 3–4) or chronic extensive (5%) GvHD, including PIDs and inherited disorders. Most protocols give 50 mg/kg/day of Cy on days +3 and +4 post-transplantation. Neven et al. (2019) recently published a cohort of 27 patients with PID transplanted with PTCy protocols but their cohort did not include SCID patients. There are some recent successful transplants for Artemis SCID patients picked up by neonatal screening using such protocols (N. Marcus et al., unpublished data).

Advances and challenges posed by newborn screening

A welcomed revolution occurred in 2008 with the pilot neonatal screening test for SCID, first launched in Wisconsin (Baker et al. 2009). During T-lymphocyte receptor development, redundant DNA is excised but remains within the cell and can be used as a marker of thymopoiesis. Patients with SCID (and some other PIDs) lack thymopoiesis and subsequently the redundant DNA (known as a T-cell receptor excision circle, or TREC) is not present. With the blood taken during routine neonatal screening, it is possible to detect TRECs by polymerase chain reaction and thus identify infants with SCID before symptoms develop (Baker et al. 2009; Morinishi et al. 2009). It has previously been demonstrated that the outcome of HSCT for newborn patients with SCID is significantly superior to that of patients presenting with infection (Myers et al. 2002; Roifman et al. 2007). The introduction of newborn

screening (NBS) enables the detection of infants with SCID before they become symptomatic, allowing definitive treatment before they acquire infection. Many states in the USA and other parts of the world have now implemented NBS for SCID (Kwan et al. 2014; Rechavi et al. 2017), enabling early detection and treatment. Another important advantage is that early detection allows for careful donor search not always possible before, due to the patients' often fragile condition at clinical presentation.

Very early diagnosis also leads to new questions and challenges. Some patients detected by NBS have a clinical phenotype of typical SCID with oral thrush or diarrhea and a typical laboratory work up. However, TREC screening also identifies "leaky" SCID due to hypomorphic mutations in known SCID genes; 26% of the SCID cases found by screening were "leaky", as reported in an 11-program study of NBS for SCID in the US (Kwan et al. 2014). There is a worldwide shift in the detection of "leaky" SCID phenotypes following neonatal screening programs, and the percentage of cases of X-linked SCID due to mutations in IL2RG (19%) is lower than expected based on prior studies (~50%) (Thakar et al. 2017). Thus, dilemmas have arisen regarding whether these patients who are picked up by NBS with otherwise near normal immune work up should be transplanted at an early age, or if they should be followed up and transplanted only when laboratory work up supports a SCID phenotype. For example, patients with DCLRE1C (Artemis) mutations have been known to present with SCID but some have a combined immune deficiency not presenting in infancy or a clinical picture similar to CVID (Volk et al. 2015). This occurs in other genetic SCID mutations such as RAG, IL7Rα, and RMRP. Relying on known SCID/CID phenotype course of siblings in the same family is often helpful in favoring a transplant in these cases. Although there are consensus guidelines for treatment of babies picked up by NBS regarding isolation, prophylaxis and IVIG treatment, controversies regarding optimal transplant protocols still exist. This is especially important in NK+ SCID phenotypes which are now increasingly diagnosed following positive NBS. Caution should be taken in giving chemotherapy in very young infants. Some centers have attempted a bridging unconditioned transplant followed by a full transplant later on, however, this exposes patients to some unnecessary complications and is often not successful. In some types of SCID caused by DNA repair defects, such as Artemis deficiency, ionizing radiation and certain conditioning regimens can be dangerous, and treatment should aim to minimize exposure to alkylating agents and ionizing radiation (de Witte et al. 1986). In other cases of SCID, such as IL2Rγ deficiency, HSCT can occur without conditioning and can be performed within the first several months of life. Additionally, if there is a concurrent infection, a decision may be made to proceed to transplant without prior conditioning to reduce the risk of transplant-related mortality. However, if conditioning is used, it is reasonable to wait until 3–6 months of age to allow for further growth, development, and organ maturity (Thakar et al. 2017).

Conclusion

Although there were many advances in SCID molecular diagnosis and treatment over the past 60 years, it was only in the last decade that the glass ceiling for haploidentical transplantation has been broken, allowing for survival rates of patients who do not have a matched donor to trend towards those of the patients who do. NBS for SCID has changed the milieu of molecular causes of SCID to include many "leaky" forms of SCID, such as NK+ and other less classic SCID phenotypes, introducing new challenges and dilemmas but also providing curative treatment options for these patients. It is yet for the next 60 years to determine the best treatment protocols for these patients as well as to determine whether other treatment options such as gene therapy or gene editing will replace HSCT for SCID.

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