T-cell deplete versus T-cell replete haploidentical hematopoietic stem cell transplantation for sickle cell disease: where are we?

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T-cell deplete versus T-cell replete haploidentical hematopoietic stem cell transplantation for sickle cell disease: where are we?

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ABSTRACT

Introduction: Severe sickle cell disease is associated with progressive end-organ damage and early mortality in adults. While allogeneic hematopoietic cell transplant from a matched related donor is curative, the vast majority of patients do not have a compatible sibling. Accordingly, platforms using haploidentical donors have been developed, which provide near-universal availability. Areas covered: This review focuses on the two commonly used approaches for haploidentical hematopoietic transplants, namely T-cell deplete and T-cell replete, each of which is associated with unique benefits and drawbacks. The purpose of this paper is to facilitate individualized decision-making for patients and providers by reviewing the pros- and cons of these differing approaches. Expert opinion: Individuals with sickle cell disease eligible for a hematopoietic cell transplant can be curative, the vast majority of patients do not have a compatible sibling. Accordingly, platforms using haploidentical hematopoietic cell transplantation (haplo-HCT) with potential for near-universal donor availability and reduced toxicity are being developed.

1. Introduction

Hematopoietic cell transplantation (HCT) has been shown to be curative for children and adults with severe sickle cell disease using matched sibling donors as a source of stem cells [1–6]. While this approach now achieves stable donor engraftment with limited acute and chronic graft versus host disease (GvHD), its application is restricted by donor availability and regimen-related toxicities [7]. To address these limitations, alternative donor platforms such as related haploidentical hematopoietic cell transplantation (haplo-HCT) with potential for near-universal donor availability and reduced toxicity are being developed [8–15].

The main approaches used to overcome the human leukocyte antigen barrier with haplo-HCT includes T-cell deplete (TCD) grafts, a process of T cell removal or reduction in-vitro, which alters the immune system and its response, or T-cell replete (TCR) grafts, where the graft is unmanipulated, using bone marrow or peripheral blood stem cells as graft sources [16–20]. These approaches are increasingly being pioneered to overcome the inherent bidirectional alloreactivity and resultant graft rejection and GvHD associated with haplo-HCT [21–23]. Despite promising results with TCD approaches, uptake is limited due to the requirement for specialized centers with expertise in expensive T-cell depletion technology [16–19]. The TCR haplo-HCT strategy, in contrast, is simple, cheap, and generalizable, and is increasingly being adopted by different transplant centers worldwide, though both approaches are associated with significant post-HCT immunoablation with increased risk of opportunistic infections, including early viral reactivation [21–24].

Given the extensive worldwide burden of sickle cell disease, especially in low resource settings, an urgency exists to develop a cost-effective curative approach for children and adults with severe, progressive disease despite best supportive care [25–28]. In this review article, we aim to highlight the pros and cons of different approaches to haplo-HCT, and its application as a curative modality for severe sickle cell disease, allowing for individualized decision-making for patients and providers.

1.1. Global burden of sickle cell disease

Sickle cell disease, the most common hereditary hemoglobinopathy in the world, is a chronic and previously incurable condition associated with extensive morbidity and early mortality resulting from progressive multi-system end-organ damage [25–28]. Globally, the disease affects millions of people, with over 300,000 births worldwide annually, and approximately 100,000 Americans, including 1/365 African-American births and 1/16,300 Hispanic-American births [29–31].

The pathophysiology results from abnormal precipitation of hemoglobin in red blood cells, leading to chronic hemolysis with progressive small and large vessel vasculopathy [26,28]. Progressive and repeated bouts of ischemia-reperfusion injury contribute to a chronic, systemic inflammatory state with widespread endothelial dysfunction, increased risk of
remote organ injury, and multi-organ dysfunction syndrome [26,28,32]. The most common indication for HCT includes >2 vaso-occlusive episodes per year, overt and silent cerebrovascular accidents with progressive cognitive dysfunction, and life-threatening cardiopulmonary abnormalities such as recurrent acute chest syndrome and pulmonary hypertension (tricuspid regurgitant jet velocity ≥2.7 m/s) [26,28]. Furthermore, sickle cell disease is a major public health concern due to extensive health-care utilization and costs, including frequent hospitalizations, resulting in lost productivity, impaired quality of life, and reduced life expectancy [33,34]. Despite advances in care with improved survival in children, the disease is still plagued with early mortality in adults [25,33].

1.2. Limitations of current management strategies for severe sickle cell disease

The aim of current management strategies for sickle cell disease is to prevent acute complications such as repeated vaso-occlusive crises, acute chest syndrome, and strokes [28,34,35]. Hydroxyurea has been shown to decrease the rate of vaso-occlusive episodes and recurrent acute chest syndrome, which lead to its approval by the Food and Drug Administration in 1998 and the European Medicines Agency in 2007 [36–39]. However, challenges exist, including but not limited to long-term adherence, concerns about fertility, secondary malignancies, and no perceived benefit for mitigating against the age-dependent chronic organ dysfunction [36–39]. Crizanlizumab, a monoclonal antibody that inhibits p-selectin, was recently approved for the reduction of vaso-occlusive pain episodes and recurrent acute chest syndrome when administered alone or with hydroxyurea [41]. However, red blood cell transfusion remains the mainstay of therapy for primary and secondary prevention of ischemic stroke, acute chest syndrome, and acute exacerbations of anemia [42–48]. Blood transfusion therapy decreases, but does not eliminate, infarct recurrence, including silent cerebral infarcts, and is associated with complications such as transfusion reactions, iron overload, catheter-related infection, and red blood cell alloimmunization [42–48]. Long-term adherence remains a concern [43]. Gene therapy approaches involving ex-vivo gene transfer into autologous hematopoietic stem cells, appears promising as an alternative curative approach, and may provide a long term and potentially curative treatment for sickle cell disease, though with notable side effects and unique challenges [49,50].

2. HCT as a curative option for severe sickle cell disease

2.1. Historical perspectives

The only validated curative approach for sickle cell disease is HCT (Table 1) [1–6]. Initial trials included children who could tolerate myeloablative conditioning regimens and who had matched sibling donors [1–6,51]. Early attempts at HCT for sickle cell disease revealed a high rate of CNS complications including cerebral hemorrhage and seizures [1–6]. Modifications to supportive care including standard anti-seizure prophylaxis, transfusions to keep platelets above 50,000/μL to prevent bleeding, maintenance of hemoglobin between 9 and 11 g/dL to avoid cerebral hypoxia and hyperviscosity, respectively, and strict blood pressure control within ± 20% of pre-HCT baseline significantly reduced the number of CNS adverse events [4,8,10,11]. This approach results in 5–10% risk of graft failure, 15–20% incidence of grade II-IV acute GvHD, and 10–20% risk of chronic GvHD, with overall survival rates over 95% [1–6,51]. The long-term complications of potentially impaired growth during adolescence and the risk of infertility highly influence the decision-making process of families weighing the pros and cons of HCT [52,53]. Successful engraftment, however, results in amelioration of sickle cell disease-related symptoms and cessation of progressive organ damage including central nervous system vasculopathy and pulmonary disease, but long-term follow-up has revealed common impairments in pubertal development and fertility, likely a combination of prior sickle cell disease and hydroxyurea administration followed by high dose alkylating agents used in the conditioning regimen [4,8,9,24,52–55]. Reduced toxicity/intensity regimens are now commonly employed by most HCT centers for matched sibling donor transplants to lessen short and long-term toxicities, particularly for adults who often have more advanced end-organ disease and are less likely to tolerate myeloablative conditioning [56]. Hsieh et al. pioneered a non-myeloablative conditioning regimen with total body irradiation and alemtuzumab followed by matched sibling donor transplant using peripheral blood stem cells in 10 adult patients with severe sickle cell disease, with results showing stable donor engraftment at levels to reduce the disease phenotype in 90% (9/10) of patients with no GvHD or mortality at a median follow up of 30 months post-transplant [51]. Despite these promising results, only 10–14% of patients
have a matched sibling donor and registries such as the National Marrow Donor Program are limited in terms of racial and ethnic donors [7,57].

2.2. Unique challenges of transplanting individuals with sickle cell disease

Unique challenges exist with HCT for hemoglobinopathies compared to other hematologic disorders. The first challenge is an increased risk of mixed chimerism and graft rejection, partly due to a fully immunocompetent recipient immune system, hyper-proliferative bone marrow, and alloimmunization related to prior multiple blood transfusions [8,9,11,13,58]. However, unlike malignancies mixed chimerism is tolerable for sickle cell disease given the significant survival advantage of non-sickle erythroblasts and erythrocytes over recipient cells and donor chimerism as low as 25% has been reported to resolve sickle cell disease-related symptoms [58]. Pre-existing sickle cell disease-organ-related complications and transfusional iron overload may alter the pharmacokinetics of conditioning medications and unique strategies, such as conservative busulfan exposure targeting has been suggested to avoid dynamic changes in clearance and high toxicity [54]. GvHD is not considered advantageous for sickle cell disease due to lack of need for a graft-versus-tumor effect [8,9]. However, ethnicity has been shown to influence the risk for GvHD with African-American and Caucasian Americans demonstrating higher rates of acute GvHD in matched sibling donor transplants and African-American recipients having higher rates of high-grade acute GvHD and extensive chronic GvHD compared to Caucasian recipients receiving transplant for aplastic anemia [59,60]. The use of alternate donor sources has also been associated with increased incidence of chronic GvHD in African-American recipients of HCT [60]. In a multi-center phase II study of 29 children with severe sickle cell disease who underwent matched unrelated donor transplants, Shenoy et al. at one year showed a 28% rate of acute grades II-IV GvHD and a 62% incidence of chronic GvHD with 38% of cases being extensive, all of which indicate the need to develop safer protocols with less toxic conditioning regimens, and better GvHD prophylaxis [61]. The process of finding a matched unrelated donor is also labor and time intensive and mainly limited to high resource countries, which precludes most patients from consideration who live in low resource settings [7,57]. The development of other alternative donor platforms such as unrelated umbilical cord blood transplant, has been limited by increased graft rejection, severely delayed immune reconstitution, and associated increased risk of opportunistic pathogens, though strategies are being developed to overcome these barriers [62]. Patients with sickle cell disease are also less likely to proceed to HCT over concern for long-term toxicities. In the United Kingdom, Chakrabarti et al. showed in formal surveys sent to patients and families that the main reason that HCT is declined is a concern for development of chronic GvHD [63].
3. Improving donor options and cure for sickle cell disease: the 50% solution

In attempts to overcome the barriers for engraftment, reduce GvHD, increase donor availability, and long-term toxicities, haplo-HCT has evolved as an alternative curative approach to sickle cell disease [8,9,56]. Historically, haplo-HCT for indications including malignant disorders such as leukemias and lymphomas and non-malignant conditions such as primary immunodeficiency syndromes, bone marrow failure syndromes, and hemoglobinopathies has been limited by severe GvHD and graft rejection stemming from intense bidirectional human leukocyte antigen alloreactivity [64–67]. With improvements in transplant technology, haplo-HCT platforms are now increasingly being explored as a curative modality for severe sickle cell disease due to the main advantage of near-universal donor availability from first-degree relatives [8,9].

To overcome the human leukocyte antigen barrier, TCD and TCR approaches have been developed, utilizing sophisticated methods for graft manipulation in the former and drug-induced immunologic tolerance in the latter to allow for engraftment while minimizing GvHD [16–23]. Importantly, to date, no randomized clinical trial has compared outcomes using TCD versus TCR haplo-HCT approaches for sickle cell disease in children and adults [68]. The goals of the current review article are to outline the various strategies that allow for successful haplo-HCT for sickle cell disease using current TCD and TCR methods and critically analyze transplant-related outcomes in terms of engraftment, graft rejection, regimen-related toxicities, acute and chronic GvHD, infectious complications, and immune reconstitution. In addition, we will discuss the impact of these transplant approaches on disease-related outcomes and provide commentary on future strategies to improve patient outcomes.

3.1. Haplo-HCT using T-cell deplete (TCD) approaches

Substantial translational immunological research has shown that one of the most effective methods for prevention of GvHD is with ex vivo depletion of the stem cell products of T-cells (TCD) [16–19]. This approach has the unique advantage of not requiring prolonged use of immunosuppressive medications, which are associated with potentially extensive morbidity from multi-organ system toxicity [16–19]. Ex vivo TCD can broadly be divided into one of two strategies: CD34+ positive selection and negative selection of T-cells or a subset of T-cells. These techniques differ not only in the laboratory procedure but also in the composition of the product, with CD34+ selection eliminating other populations of mononuclear cells, such as B- and NK-cells, that can impact post-transplant immune reconstitution.

The critical role of T-cells in the pathogenesis of GvHD was initially shown in pre-clinical models in which mice received lethal irradiation followed by bone marrow transplants with spleen cell fractions depleted of small lymphocytes, resulting in 80–100% survival with minimal GvHD compared to mice who received increasing doses of lymphocytes, all of whom died from severe GvHD [17,18]. Thereafter, ex vivo T-cell depletion by means of soybean agglutination followed by E-rosetting and counterflow centrifuge elutriation was developed [16–18]. Aversa and colleagues pioneered positive selection, purification, and infusion of ‘megadoses’ of CD34+ cells, which revolutionized the field of haplo-HCT in the early to mid-1990s after it was essentially abandoned due to unacceptably high non-relapse mortality [69,70]. More nuanced T-cell selection methods have since been developed to improve engraftment and pathogen-specific immunity (Table 2).

3.2. Haplo-HCT using T-cell replete (TCR) approaches

TCR haplo-HCT relies on in vivo strategies to overcome human leukocyte antigen disparity and subsequent bi-directional alloreactivity (Table 3). The use of post-transplant cyclophosphamide has transformed the field of haplo-HCT by allowing for selective deletion of alloreactive T-cells in vivo, which lack expression of the drug metabolizing enzyme, aldehyde dehydrogenase [79–83] (Figure 1, Table 3). Post-transplant cyclophosphamide modulates alloreactivity associated with partially matched donors in animals and humans [20–23]. This has resulted in similar levels of GvHD and long-term immune reconstruction as seen with matched sibling donor BMT [79–83]. Preferential expansion of regulatory T-cells may also contribute to the reduced GvHD seen with the post-transplant cyclophosphamide approach [79–83] (Table 3). These approaches are depicted in Figure 1 and Table 3 [79–83].

4. Outcomes following TCD haplo-HCT approaches for sickle cell disease

Promising transplant outcomes have been demonstrated using TCD grafts [24] (Table 4). Dallas et al. conducted a study using reduced-intensity conditioning with parental haploidentical donor HCT, with granulocyte colony-stimulating factor–mobilized peripheral blood stem cells as donor source in eight children with sickle cell disease with a median age of 9 ± 5 years. The TCD transplant approach used CD34+ selection with CliniMACS (Miltenyi Biotech) ex vivo and CD3+ depletion with muromonab in vivo [24]. The study was conducted with the goal of increasing donor availability, reducing graft rejection and improving long-term outcomes following haplo-HCT for sickle cell disease. The results show that 75% (6/8) of haplo-HCT recipients survived long term, with disease-free survival of 38%, and disease recurrence of 38%. The incidence of acute and chronic GvHD was 50% and 38%, respectively [24]. Importantly though, patients who fully engrafted had a resolution of sickle cell disease-related symptoms.

Foell and colleagues developed a treosulfan (L-treitol-1,4-bis-methanesulfonate) based conditioning regimen with CD3+/CD19+ depleted grafts for children with severe sickle cell disease with a median age of 16 years who failed at least 1 year of hydroxyurea therapy [84] (Table 4). The purpose of the study was to determine whether treosulfan, the prodrug of L-epoxybutane, a water-soluble bifunctional alkylating agent with myeloablative and immunosuppressive properties, could provide effective HCT conditioning with reduced risk of toxicities, particularly sinusoidal obstruction...
Table 2. Pros and cons of available T-cell deplete approaches used in haplo-HCT.

<table>
<thead>
<tr>
<th>T-Cell deplete method</th>
<th>Mechanism</th>
<th>Pros</th>
<th>Cons</th>
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<tbody>
<tr>
<td>General [16–18]</td>
<td>Multiple (listed below)</td>
<td>Conceptually most effective means to prevent acute and chronic GvHD</td>
<td>More effective in children than adults (due to better thymus function in children with associated greater T-cell receptor diversity versus adults, who rely more on peripheral cytokine-mediated T-cell expansion post-transplant)</td>
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<td>Low acute and chronic GvHD</td>
<td>Expensive</td>
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<td>Reduced need for post-transplant immune-suppressive medications</td>
<td>Labor intensive</td>
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<td></td>
<td>Less pulmonary and hepatic toxicity peri-transplant</td>
<td>Specialized expertise required</td>
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<td></td>
<td></td>
<td>Prevent EBV-PTLD (high potential morbidity and mortality) by removing CD19 cells ex vivo</td>
<td>Not available at most stem cell transplant centers</td>
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<td></td>
<td>Higher graft rejection/lower engraftment related to depletion of T-cells (especially gamma/delta), natural killer cells, and hematopoietic progenitors that facilitate engraftment</td>
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<td>Delayed IR with increased risk of opportunistic infections</td>
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<td>CD34 positive selection [19,83,84]</td>
<td>Positive selection of CD34(^+) stem cells by immunoadsorption columns (immunomagnetic beads) combined physical and immunological separation of cells</td>
<td>Beneficial for engraftment (barrier overcome by &quot;megadose&quot; CD34(^+) stem cell infusion)</td>
<td>Loss of cells such as gamma/delta T-cells and natural killer cells that facilitate engraftment, with subsequent increased risk of graft rejection</td>
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<td>Potential for severely delayed IR with increased infectious risk profile</td>
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<td>for many months to years and conceptual risk of relapse of disease (return of sickle cell disease phenotype)</td>
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<td>Myeloablative conditioning used more so (accentuates existing end-organ damage, higher risk of acute and chronic GvHD, higher transplant-related mortality)</td>
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<tr>
<td>CD3(^+) and CD19(^+) [93]</td>
<td>Ex vivo negative selection of CD3 (T-cells) and CD19 (B-cells)</td>
<td>Lower risk of EBV-PTLD (from removing potential EBV-infected CD19 cells in graft)</td>
<td>Aforementioned risks</td>
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<td>Loss of cells that promote engraftment (gamma/delta T-cells and natural killer cells)</td>
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<tr>
<td>T-cell receptor alpha/beta(^+) and CD19(^+) [16–18,74]</td>
<td>Ex vivo depletion of more specific T-cell subsets that drive acute GvHD and B-cells that increase risk of EBV-PTLD</td>
<td>Retain gamma/delta(^+) T-cells (promote IR and provide pathogen specific immunity) and natural killer cells while depleting alloreactive T-cells that cause acute GvHD</td>
<td>Requires even more specialized expertise than CD34 positive selection methods of CD3(^+) and CD19(^+) negative selection</td>
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<td>Less delayed T-cell specific IR</td>
<td>Available at fewer centers</td>
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<td>Preemptively reduce GvHD by infusion of regulatory T-cells, which are anti-inflammatory, potential to change phenotype of infused cells with goal of less alloreactivity</td>
<td>More data for children than adults</td>
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<td>Retain pathogen-specific immunity (fewer opportunistic infections)</td>
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<td>Retain conventional T-cells that facilitate engraftment and reduce disease relapse</td>
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<td>No additional delay in IR</td>
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<td>Still potential to avoid or stop immunosuppressive medications early post-transplant (due to activity of regulatory T-cells)</td>
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<tr>
<td>Regulatory T-cell and conventional T-cell co-infusion [75,76]</td>
<td>Regulatory T-cell infusion prior to transplant, followed by conventional graft infusion on day 0</td>
<td>Achieve 1) engraftment with lower risk of grades II-IV acute GvHD first and then 2) boost IR to reduce risk of opportunistic infections and relapse next Separate GvHD from graft-versus-tumor effect</td>
<td>Not yet tested for non-malignant conditions, i.e. sickle cell disease and beta-thalassemia</td>
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<td>Available at even fewer centers, more sophisticated graft manipulation methodologies, and labor and time intensive than aforementioned strategies</td>
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<td>Conceptual potential for regulatory T-cells to counteract the benefit of conventional T-cells, with delayed engraftment/increased graft rejection and increased risk of opportunistic infections as a result</td>
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<td>CD34(^+) cell enriched with T-cell addback [77,78,95,96]</td>
<td>Positive selection of CD34(^+) hematopoietic stem cells (immunoadsorption techniques) with reduction in alloreactive T-cells and then controlled &quot;addback&quot; of CD3 selected T-cells</td>
<td>Achieve 1) engraftment with lower risk of grades II-IV acute GvHD first and then 2) boost IR to reduce risk of opportunistic infections and relapse next Separate GvHD from graft-versus-tumor effect</td>
<td>Accumulating data for sickle cell disease, mostly in the form of abstracts rather than publications</td>
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<td>Not available at most centers (may require enrollment on a clinical trial, for which enrollment is higher for children than adults)</td>
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<td>Potential for severe acute GvHD with T-cell &quot;addback&quot;, with resultant need for immune suppression</td>
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</tbody>
</table>

Legend: Haplo-HCT (haploidentical hematopoietic cell transplant), TCD (T-cell deplete), EBV (Epstein-Barr virus), PTLD (post-transplant lymphoproliferative disorder), IR (immune reconstitution), TCR (T-cell replete); GvHD (graft versus host disease)
### Table 3. Pros and cons of T-cell replete approaches used in haplo-HCT.

<table>
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</table>
| General [16–18]       | In vivo rather than ex vivo depletion of recipient and donor alloreactive T-cells (with anti-thymocyte globulin or alemtuzumab, with or without total body total body or lymphoid irradiation) | Available at almost all transplant centers in Europe and United States  
Methods more easily replicable  
Conceptually lower cost compared to T-cell depletion methods (due to lack of need for expensive graft manipulation technology) | Need for in vivo T-cell depletion with anti-thymocyte globulin or alemtuzumab, with potential for delayed IR and increased risk of opportunistic pathogens  
Higher GvHD risk with peripheral blood stem cell grafts  
Potential for severe cytokine release syndrome (especially with peripheral blood stem cell grafts) due to rapid activation of T-cells |
| GIAC protocol [79]    | Modulation of alloreactive T-cells with: 1) Granulocyte colony stimulating factor donor priming, 2) Intensive immunosuppression post-transplant, 3) Anti-thymocyte globulin, 4) Combined peripheral blood and bone marrow allografts | Reduce alloreactivity of donor T-cells with granulocyte colony stimulating factor (shift from T-helper 1 to T-helper 2 phenotype) and of both donor and recipient T-cells with anti-thymocyte globulin  
Improved engraftment due to use of peripheral blood stem cells  
No need for graft manipulation  
Protocols easily replicable at different institutions | Morbidity from multiple drugs needed for post-transplant immune  
Increased risk of viral reactivation and opportunistic pathogens in early post-transplant period due to anti-thymocyte globulin  
Unanswered question of non-inherited maternal and paternal antigens (for donor selection)  
Not as extensively studied in setting of SCD |
| Post-transplant cyclophosphamide [21–23,79–83] | Preferential deletion of proliferative alloreactive donor and recipient T-cells due to lack of expression of the enzyme aldehyde dehydrogenase 1  
Reduce host T-cells responding to donor antigens peripherally post-transplant  
Intrathymic deletion of donor-reactive host T-cells (central tolerance) | Reduced acute and chronic GvHD  
Expansion of regulatory T-cells that promote immune tolerance  
Replicable at any transplant center  
Used with either bone marrow or peripheral blood stem cell grafts (compared to GIAC protocol)  
Low likelihood of developing EBV-PTLD  
Potential acute toxicity from high doses of cyclophosphamide, including cardiac (type I agent, with hemorrhagic necrosis and heart failure), lung (pneumonitis and pulmonary fibrosis), bladder (associated with BK virus cystitis), secondary malignancy (chromosome 5 and 7 deletion signature, from alkylating agent exposure)  
Increased viral reactivation  
Increased risk of infertility secondary to additional alkylator therapy |
| Donor lymphocyte infusion [16–18,79] | Example of adoptive immunotherapy  
Boost T-cell dose post-transplant, treat disease relapse or mixed chimerism | Designed to boost engraftment and reduce rejection, i.e. setting of falling chimerism  
Potential for attenuating GvHD with herpes simplex virus thymidine kinase "suicide" gene incorporation into donor T-cells | Potential for severe, acute GvHD  
Questions remain regarding patient population to receive along with timing and dosing of infusions |

Legend: Haplo-HCT (haploidentical hematopoietic cell transplant), TCD (T-cell deplete), EBV (Epstein-Barr virus), PTLD (post-transplant lymphoproliferative disorder), IR (immune reconstitution), TCR (T-cell replete); GvHD (graft-versus-host-disease)
syndrome, and reduce the problem of graft rejection seen in multiple other haplo-HCT protocols [84,85]. At a median follow up of 26 months, all patients were alive and 89% (8/9) and fully engrafted [84]. Despite the regimen being myeloablative, the majority of patients tolerated it well, as evidenced by mild grade 1–2 toxicities without grade 3–4 effects [84]. In terms of GvHD, 56% (5/9) patients had acute GvHD and 11% (1/9) had chronic GvHD [84] (Table 4). The main complication was early viral reactivation, similar to other haplo-HCT approaches. The novel aspect of the Foell et al. study, however, relates to treosulfan, which should be investigated further to facilitate engraftment with reduced toxicity following haplo-HCT.

Gilman and colleagues conducted a phase II study in children and young adults with severe sickle cell disease, median age 14 years (range 5–23 years), to investigate the use of reduced intensity conditioning regimens with CD34 positively selected peripheral blood TCD grafts on outcomes, namely engraftment and GvHD [86] (Table 4). Additionally, patients were screened for a phase I-II study for donor lymphocyte infusion to boost immune reconstitution post-TCD transplant. Of the 10 patients enrolled, 8 underwent haplo-HCT. After transplant, all eight patients engrafted, with incidence of grades II-IV acute GvHD of 20% and one case of chronic GvHD, which occurred in a patient with previous acute GvHD who also received a donor lymphocyte infusion for refractory post-transplant lymphoproliferative disorder [86] (Table 4). At two years, the overall survival was 90% and event-free survival was 80%, suggesting that outcomes with reduced intensity conditioning are improving with the haplo-HCT platform.

Gaziev and colleagues published the result of a single institution, retrospective study of children and young adults with hemoglobinopathies, with a median age of 7 years (range 3–15.2) undergoing myeloablative conditioning followed by haplo-HCT [87] (Table 4). The protocol involved the selective depletion of T-cell receptor alpha/beta+ cells, which was hypothesized to result in less graft rejection, given the growing knowledge of gamma/delta+ T-cells, NK cells, and hematopoietic progenitor cells for engraftment [87]. Of the 14 children included, 3 had sickle cell disease and 11 had thalassemia. The authors assessed outcomes compared to a group of 40 patients with hemoglobinopathies who received CD34+ selected peripheral blood and bone marrow grafts (n = 32) or CD34+ selected peripheral blood and CD3+/ CD19+ depleted bone marrow grafts (n = 8), though both groups were similar in baseline characteristics to the T-cell receptor alpha/beta+ and CD19+ group (87). The 5-year probability of overall survival and disease-free survival was 84% and 69%, respectively, for the T-cell receptor alpha/beta+ and CD19+ group compared to 78% and 39%, respectively, for the CD34+ selected control group [87] (Table 4). Notably, graft failure was significantly less in the T-cell alpha/beta+ and CD19+ group compared to the historical cohort (14% vs 45%, p = 0.048) [87] (Table 4). Outcomes in terms of acute GvHD, chronic GvHD, post-transplant lymphoproliferative disorder, and viral reactivation were not significantly different between the groups. The overall findings show benefit with more refined selective means of TCD. In addition, the study suggests that data from haplo-HCT for transfusion-dependent thalassemia may be applicable to sickle cell disease, which may in part be due to shared pathophysiology in terms of not requiring a graft-versus-leukemia effect for either and the challenges of overcoming a functional recipient immune system for engraftment.
<table>
<thead>
<tr>
<th>Author</th>
<th>Graft source</th>
<th>Conditioning regimen</th>
<th>N (median age</th>
<th>OS</th>
<th>Engraftment (%)</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dallas et al [24].</td>
<td>PBSC</td>
<td>Reduced intensity ATG, fludarabine, thiopeta, busulfan,</td>
<td>8 (median age</td>
<td>75%</td>
<td>63% (5/8)</td>
<td>Acute GvHD – 50% (4/8) Grade II-IV 25% (2/8) Chronic GvHD 38% (3/8)</td>
</tr>
<tr>
<td>CD34* selection</td>
<td></td>
<td>Hydroxyurea and azathioprine for 3 months prior to transplant,</td>
<td></td>
<td></td>
<td></td>
<td>Moderate-severe 25% (2/8) GvHD</td>
</tr>
<tr>
<td>CD3* depletion</td>
<td></td>
<td>busulfan, thiopeta, cyclophosphamide, muromonab (5 patients)</td>
<td>9, range 4–17</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>GVH prophylaxis – MMF</td>
<td></td>
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</tr>
<tr>
<td>Gaziev et al. [87]</td>
<td>PBSC</td>
<td>Hydroxyurea and azathioprine, fludarabine pre-conditioning</td>
<td>3 sickle cell</td>
<td>84%</td>
<td>36% (5/14) acute</td>
<td>4 developed auto-immune disorders</td>
</tr>
<tr>
<td>T-cell receptor alpha/</td>
<td></td>
<td>ATG, busulfan, thiopeta, cyclophosphamide GVH prophylaxis –</td>
<td></td>
<td></td>
<td></td>
<td>infections: Reactivation of CMV and EBV</td>
</tr>
<tr>
<td>beta* CD19 depletion</td>
<td></td>
<td>cyclosporine and methylprednisolone or cyclosporine and MMF</td>
<td></td>
<td></td>
<td></td>
<td>Adenovirus, Bacterial infections, with gram-positive and negative sepsis</td>
</tr>
<tr>
<td>Gilman et al. [86]</td>
<td>PBSC</td>
<td>Reduced intensity ATG, melphalan, thiopeta, fludarabine</td>
<td>8</td>
<td>88%</td>
<td>100% (8/8)</td>
<td>2 engraftment syndrome</td>
</tr>
<tr>
<td>CD34* selection</td>
<td></td>
<td>GVH prophylaxis – none</td>
<td>6–60 months</td>
<td></td>
<td></td>
<td>2 posterior reversible encephalopathy syndrome</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>88% (7/8) alive and without SCD</td>
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<td></td>
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<td></td>
<td></td>
<td>13% (1/8) died from disseminated aspergillosis</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>All survivors in school and/or employed</td>
</tr>
<tr>
<td>Foell et al. [84]</td>
<td>PBSC</td>
<td>Myeloablative ATG, fludarabine, treosulfan GVH prophylaxis –</td>
<td>9</td>
<td>89%</td>
<td>100% (9/9)</td>
<td>4 EBV reactivation (2 with PTLD), 1 CMV enteritis, 6 HHV-6 reactivation</td>
</tr>
<tr>
<td>CD3* and CD19* depletion</td>
<td></td>
<td>cyclosporine, MMF</td>
<td>6–42 months</td>
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<tr>
<td></td>
<td></td>
<td>(median 26 months)</td>
<td></td>
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<tr>
<td>Marzullo et al. [98]</td>
<td>PBSC</td>
<td>ATG, thiotepa, treosulfan, fludarabine GVH prophylaxis – none</td>
<td>2</td>
<td>100%</td>
<td>50% (1/2)</td>
<td>No sickle cell-related complications</td>
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<tr>
<td>T-cell receptor alpha/</td>
<td></td>
<td></td>
<td>1.5–3.9 years</td>
<td></td>
<td></td>
<td>No renal or hepatic impairment</td>
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<tr>
<td>beta* and CD19* depletion</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Normal height, weight, thyroid function</td>
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<td></td>
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<td></td>
<td>1 with secondary gonadal failure</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>100% (2/2) alive and well, free of sickle cell disease related symptoms</td>
</tr>
<tr>
<td>Abikoff et al. [77]</td>
<td>BM</td>
<td>Pre-conditioning hydroxyurea and azathioprine days –59 to –11</td>
<td>6</td>
<td>86%</td>
<td>0% acute or</td>
<td>1 EBV reactivation</td>
</tr>
<tr>
<td>CD34* cell enriched</td>
<td></td>
<td>Conditioning with fludarabine, busulfan, thiotepa, cyclophos-</td>
<td></td>
<td></td>
<td>chronic GVHD</td>
<td>1 death from sinusoidal obstruction syndrome</td>
</tr>
<tr>
<td>with T-cell addback</td>
<td></td>
<td>phosphide, anti-thymocyte globulin, total body irradiation</td>
<td></td>
<td></td>
<td></td>
<td>1 viral infection and no serious bacterial infections</td>
</tr>
</tbody>
</table>

(Continued)
Table 4. (Continued).

<table>
<thead>
<tr>
<th>Author</th>
<th>Graft source</th>
<th>Conditioning regimen</th>
<th>N</th>
<th>OS</th>
<th>GvHD  (%)</th>
<th>Engraftment (%)</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cairo et al. [78]</td>
<td>BM</td>
<td>Preconditioning with hydroxyurea and azathioprine days −59 to −11 Conditioning with fludarabine, busulfan, thiotepa, cyclophosphamide, anti-thymocyte globulin total body irradiation</td>
<td>14</td>
<td>93% (13/14) estimated 1 year survival</td>
<td>11% grades II-IV acute GvHD</td>
<td>1 death from sinusoidal obstruction syndrome 4 CMV, 1 adenovirus</td>
<td></td>
</tr>
<tr>
<td>Talano et al. [96]</td>
<td>BM</td>
<td>Pre-conditioning hydroxyurea and azathioprine Conditioning with fludarabine, busulfan, thiotepa, cyclophosphamide, anti-thymocyte globulin, total lymphoid irradiation</td>
<td>21</td>
<td>88.2% 1 year overall survival</td>
<td>7.1% grades II-IV acute GvHD 20% chronic GvHD</td>
<td>3 deaths from sinusoidal obstruction syndrome, complications of chronic GvHD, and steroid refractory acute GvHD, respectively</td>
<td></td>
</tr>
</tbody>
</table>

Legend: Haplo-HCT (haploidentical hematopoietic cell transplant), RIC (reduced intensity conditioning), PBSC (peripheral blood stem cell), BM (bone marrow), ATG (anti-thymocyte globulin), G-BM (granulocyte colony-stimulating factor primed bone marrow), G-PBSC (granulocyte colony-stimulating factor primed peripheral blood stem cells), MMF (mycophenolate mofetil), GvHD (graft-versus-host-disease), OS (overall survival), EFS (event-free survival), CMV (cytomegalovirus), EBV (Epstein-Barr virus)
5. Outcomes following TCR haplo-HCT approaches for sickle cell disease

Bolanos-Meade and colleagues explored the use of haplo-HCT with post-transplant cyclophosphamide in adults with non-malignant hematologic disorders [13]. Initially, 17 adults with sickle cell disease were screened and 14 participants, median age of 23.5 years, were transplanted using a regimen of anti-thymocyte globulin, fludarabine, cyclophosphamide, and total body irradiation, followed by bone marrow allografts from haploidentical-related donors [13]. All patients had at least one half-matched donor, though two were excluded due to high donor-specific human leukocyte antigen antibodies and inability to undergo bone marrow harvesting for medical reasons, respectively. GvHD prophylaxis was with mycophenolate mofetil, a calcineurin inhibitor, and post-transplant cyclophosphamide, which was given on days 3 and 4 post-transplant. At a median follow up of 711 days, engraftment occurred in 57% (8/14) haplo-HCT recipients, with graft rejection occurring in the remaining 43% (6/14) patients [13] (Table 5). However, the patients who did not engraft all received autologous hematopoiesis. At last follow up, 43% (6/14) haplo-HCT patients had over 95% myeloid chimerism and were off immunosuppression. A total of 14% (2/14) of haplo-HCT patients were mixed chimeras at the study conclusion [13]. The study was notable for the absence of acute or chronic GvHD as well as 100% overall survival [13]. All engrafted patients had amelioration of sickle cell disease-related symptoms, which provided the impetus for further investigation into improving haplo-HCT platform with post-transplant cyclophosphamide.

An unmet need with the haplo-HCT with the post-transplant cyclophosphamide approach remains reducing the high graft rejection rate. Current strategies employed to improve donor engraftment has included granulocyte colony-stimulating factor bone marrow priming to increase T-cell content in the graft, since T-cells have been shown to be important for engraftment, though also drive the pathogenesis of acute and chronic GvHD. Another approach is reducing donor-specific human leukocyte antigen antibodies, which are associated with high graft rejection rates in haplo-HCT recipients [88,89]. While the last three patients on Bolanos-Meade et al. protocol received granulocyte colony-stimulating factor, 33% (1/3) had late graft rejection [13]. While none of the patients in the study had clinically significant anti-donor specific human leukocyte antigen antibodies, it is still possible that graft rejection may have been mediated by alloimmunization from chronic red blood cell transfusion therapy, suggesting that desensitization protocols may be beneficial even if donor-specific antibodies are not detectable [13,88,89].

Fitzhugh and colleagues developed a TCR haplo-HCT protocol with post-transplant cyclophosphamide using granulocyte colony-stimulating factor primed peripheral blood stem cells rather than bone marrow as a graft source [90] (Table 5). The study tested the hypothesis that patients with severe end-organ damage from sickle cell disease could be cured with non-chemotherapy based conditioning. This was a prospective phase I/II haplo-HCT study, of the initial 23 patients, 21 had severe sickle cell disease and 2 with transfusion-dependent thalassemia, with a median age of 36 years (range 20–56 years). The conditioning regimen was non-myeloablative and included low dose total body irradiation and alemtuzumab, designed with three cohorts and escalating doses of post-transplant cyclophosphamide [90]. Patients in cohort 1 (n = 3) did not receive post-transplant cyclophosphamide, patients in cohort 2 received a single dose of 50 mg/kg (n = 8), and those in cohort 3 (n = 12) received 100 mg/kg in divided doses. Long-term engraftment was 0% (0/3), 63% (5/8), and 83% (10/12) in cohorts 1, 2, and 3, respectively [90] (Table 5). Notably, only 25% of patients in the second cohort remained free of sickle cell disease compared to 50% in cohort three, indicating the importance of the higher doses of post-transplant cyclophosphamide. Of the 21 sickle cell disease patients, 86% survived, three patients died, all of which occurred in patients who had graft rejection. In terms of GvHD, grade I acute GvHD developed in two patients, and resolved with systemic and topical steroids, respectively [90] (Table 5). Limited ocular GvHD developed in one patient also, which responded to topical therapy. Similar to the Bolanos-Meade et al. study with TCR grafts and post-transplant cyclophosphamide, graft rejection occurred in 50% (6/12) of patients in cohort three, though the conditioning protocol was weighted towards minimizing GvHD, with the potential cost of rejection as a result. However, the engraftment rate and steady-state donor chimerism were highest in cohort three, in patients who received the highest doses of post-transplant cyclophosphamide, thereby setting a precedent for future studies [90].

Saraf and colleagues published a single institution retrospective study to investigate the optimal conditioning regimen with adults with severe sickle cell disease undergoing haplo-HCT [91] (Table 5). The study included 10 patients with a median age of 28 years, with the first two receiving a non-chemotherapy based conditioning regimen of alemtuzumab and total body irradiation, and the remaining eight receiving the Johns Hopkins protocol utilized by Bolanos-Meade et al. The results indicate the importance of chemotherapy based conditioning, as the first two patients did not engraft, while the remaining eight all engrafted, with one patient experiencing secondary graft rejection one year post transplant [91]. In terms of GvHD, 25% (2/8) had grades II–IV acute GvHD, while one patient had chronic GvHD that resulted in death [92]. The overall survival was 88%. The study is important in terms of showing successful long-term engraftment and reduced chronic GvHD for adult patients undergoing reduced intensity conditioning. In addition, as indicated by Fitzhugh et al., non-chemotherapy regimens may not be sufficient to ensure adequate long-term engraftment [90,91].

In an international, multi-institutional phase II study of haplo-HCT with non-myeloablative conditioning and post-transplant cyclophosphamide for patients with severe sickle cell disease by De la Fuente et al. [93] (Table 5). The primary endpoint of the study was 1-year event-free survival, with events being considered as primary or secondary graft rejection, stroke, or death. A total of 16 patients underwent 18 haplo-BMT with a median age of 20.9 years, and the first three receiving a conditioning regimen similar to the Johns Hopkins platform [13,93]. However, the initial 2/3 experienced graft rejection, which necessitated the addition of thiotepa (Figure 2). Most patients received granulocyte colony-
<table>
<thead>
<tr>
<th>Author</th>
<th>Graft source</th>
<th>Conditioning regimen</th>
<th>N/Age</th>
<th>OS (%)</th>
<th>GvHD (%)</th>
<th>Engraftment (%)</th>
<th>Sickle cell-related and transplant outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolanos-Meade et al. [13]</td>
<td>G-BM (3), BM (11)</td>
<td>Non-myeloablative ATG (12 patients), fludarabine, cyclophosphamide, total body irradiation, GvHD prophylaxis – PTCy, FK, sirolimus, MMF</td>
<td>14/Age (range 15–52 years)</td>
<td>100% (14/14) at 7.5–66 months</td>
<td>0% (0/14) acute GvHD</td>
<td>57% (8/14)</td>
<td>50% (7/14) alive and without sickle cell related symptoms No new strokes, acute chest syndrome, priapism Infectors 3 CMV reactivation, 1 EBV reactivation, 1 with RSV upper respiratory infection, and mycobacterium lung infection</td>
</tr>
<tr>
<td>Fitzhugh et al. [90]</td>
<td>PBSC</td>
<td>Non-myeloablative alemtuzumab, total body irradiation, GvHD prophylaxis – PTCy, sirolimus</td>
<td>12/Age (range 20–56 years)</td>
<td>92% (11/12)</td>
<td>8% (1/8) acute GvHD</td>
<td>70%</td>
<td>No SCD related issues, no sinusoidal obstruction syndrome 2 patients with graft rejection developed high-grade myelodysplastic syndrome with fibrosis 1 patient with pulmonary hypertension and heart failure (died) 1 died from infection post-surgery 50% (6/12) alive and without sickle cell disease associated symptoms Infectors 4 CMV reactivation, 1 CMV colitis, 1 disseminated adenovirus, 3 maintained chronic EBV viremia, 1 EBV-PTLD, 3 were treated for presumed fungal pulmonary nodules and 15 bacteremia</td>
</tr>
<tr>
<td>Webking et al. [99]</td>
<td>BM</td>
<td>Myeloablative alemtuzumab, fludarabine, treosulfan, thiopeta, cyclophosphamide GvHD prophylaxis – PTCy, tacrolimus, sirolimus, MMF</td>
<td>3/Age (range 8.5–20.3 years)</td>
<td>100% (3/3) at 11–30 months</td>
<td>33% (1/3) grades II-IV acute GvHD</td>
<td>100% (3/3)</td>
<td>No central nervous system toxicity Infectors 2 CMV reactivation, 1 VZV reactivation</td>
</tr>
<tr>
<td>Pawlowska et al. [100]</td>
<td>BM (3), PBSC (1)</td>
<td>Pre-transplant immunosuppression (fludarabine and dexamethasone) for two courses Non-myeloablative ATG, busulfan, fludarabine GvHD prophylaxis – PTCy, tacrolimus, sirolimus and ruxolitinib (2 patients), MMF</td>
<td>4/Age (range 13–23 years)</td>
<td>100% (4/4) at range 5–11 months</td>
<td>2.5% (1/4) acute GvHD</td>
<td>75% (3/4) chronic GvHD</td>
<td>100% (4/4) 2 patients had antibody management protocol (for high donor specific anti-HLA antibodies) 1 patient with persistent opioid dependence Infectors 3 HHV-6 viremia, 1 CMV viremia</td>
</tr>
<tr>
<td>De la Fuente et al. [93]</td>
<td>BM</td>
<td>Non-myeloablative ATG, fludarabine, cyclophosphamide, total body irradiation (all), and thiopeta (15 patients) GvHD prophylaxis – PTCy, MMF, sirolimus</td>
<td>18/Age (range12.1–26 years)</td>
<td>100% (16/16)</td>
<td>13% (2/16) grades III-IV acute GvHD</td>
<td>83% (15/18)</td>
<td>1 case sinusoidal obstruction syndrome 2 posterior reversible encephalopathy syndrome 1 new infarct (patient who did not engraft) Suspected MMF induced gastritis, ulcer with bleeding, typhlitis Infectors 6 with EBV reactivation (no PTLD), 3 with CMV reactivation, 1 adenovirus respiratory infection, 1 BK cystitis, 2 cases oral HSV infection, 2 HHV-6 viremia (1 with HHV-6 encephalopathy)</td>
</tr>
<tr>
<td>Saraf et al. [91]</td>
<td>PBSC</td>
<td>Non-myeloablative ATG, fludarabine, cyclophosphamide, total body irradiation GvHD prophylaxis – PTCy, MMF, sirolimus</td>
<td>8/Age (range 20–38 years)</td>
<td>88% (7/8)</td>
<td>63% (5/8) EFS</td>
<td>88%</td>
<td>Oral HSV-1, E. coli urinary tract infection, enterococcus urinary tract infection, coronavirus, influenza, 3 CMV reactivation</td>
</tr>
</tbody>
</table>

(Continued)
Table 5. (Continued).

<table>
<thead>
<tr>
<th>Author</th>
<th>Graft source</th>
<th>Conditioning regimen</th>
<th>N/Age</th>
<th>OS (%)</th>
<th>GvHD (%)</th>
<th>Engraftment (%)</th>
<th>Sickle cell-related and transplant outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patel et al. [97]</td>
<td>BM</td>
<td>Pre-conditioning with hydroxyurea and</td>
<td>23 (Median age 14.8 years)</td>
<td>100% (23/23) overall survival for patients with 1 year immune reconstitution data</td>
<td>8.7% (2/23) grades II-IV acute GvHD</td>
<td>100%</td>
<td>Viral reactivation and/or infection in 70% (16/23) patients CMV 35% (8/23), HHV-6 22% (5/23), polyoma virus 17% (4/23) 3 cases each of HSV-1 stomatitis, parvovirus, EBV reactivation (without post-transplant lymphoproliferative disease)</td>
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<tr>
<td></td>
<td>PTCy</td>
<td>azathoprine 72% (18/23)</td>
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<tr>
<td></td>
<td></td>
<td>Non-myeloablative ATG, fludarabine,</td>
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<td></td>
<td></td>
<td>cyclophamide, total body irradiation,</td>
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<td></td>
<td></td>
<td>thiopeta GvHD prophylaxis – PTCy, MMF,</td>
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<td></td>
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<td>sirolimus</td>
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</tbody>
</table>

Legend: HLA (human leukocyte antigen), RIC (reduced intensity conditioning), PBSC (peripheral blood stem cell), BM (bone marrow), ATG (anti-thymocyte globulin), G-BM (granulocyte colony-stimulating factor primed bone marrow), MMF (mycophenolate mofetil), PTIS (pre-transplant immune suppression), PTLD (post-transplant lymphoproliferative disorder), post-transplant cyclophosphamide (PTCy), GvHD (graft-versus-host-disease), OS (overall survival), EFS (event-freerativeness), CMV (cytomegalovirus), EBV (Epstein-Barrvirus).
stimulating factor-primed bone marrow grafts. Thereafter, of the 15 patients who received the new regimen, including two who had previous rejection, 93% (14/15) had over 95% stable myeloid donor engraftment after at least 6 months of follow up [93]. No mortality was seen. In terms of GvHD, two patients had grades III-IV acute GvHD, one had mild chronic GvHD, and 86% (6/7) of patients were off all immunosuppression by 1-year post-transplant [93] (Table 5). Importantly, the results suggest that the addition of thiotepa to the Johns Hopkins haplo-BMT with the post-transplant cyclophosphamide platform may be an effective strategy to improve engraftment potentially extending this curative modality to individuals with severe sickle cell disease, including older patients [93]. This approach formed the basis for an ongoing multicenter national study in the United States, Blood and Marrow Transplant Clinical Trials Network Protocol 1507 (clinicaltrials.gov as NCT03263559). Table 5 reviews transplant outcomes from published studies using TCR platforms for haplo-HCT for sickle cell disease.

In summary, current TCD and TCR-based approaches appear to address the unmet need for lack of donor availability, reduced regimen-related toxicity, while improving engraftment and minimizing GvHD, especially with the PTCy based approaches. However, challenges remain, and the optimal conditioning regimen and graft source are yet to be defined, including an efficient, safe approach to mitigate against anti-donor specific HLA antibodies which will be encountered in many patients with SCD on chronic blood transfusion therapy as a therapeutic modality. Tables 4 and 5 summarize transplant outcomes from published studies using TCD and TCR-based approaches for haplo-HCT for sickle cell disease.

6. Immune reconstitution following haplo-HCT for sickle cell disease

Immune recovery post-HCT is crucial to avoid short and long-term infectious complications. Sickle cell disease patients have disease-specific infectious risk secondary to splenic dysfunction, although splenic phagocytic recovery has been reported in post-HCT period in some patients [94]. Infectious complications are increased following both TCD and TCR haplo-HCT secondary to intense immunoablation and subsequent delayed immune reconstitution [16–18]. Initial TCD approaches have the potential to result in the elimination of pathogen-specific memory T-cells [16–18]. To combat the problem of increased viral reactivations and/or infections in the setting of delayed immune reconstitution following TCD haplo-HCT, Ayello et al. showed in a preliminary study of haplo-HCT in children and adolescents with severe sickle cell disease using CD34+ positive cell selection followed by mononuclear cell addback with all patients receiving granulocyte colony-stimulating factor primed peripheral blood stem cells [95] (Table 4). The main purpose of the study was to quantitatively and qualitatively characterize immune reconstitution post-transplant. Data on immune cell subsets were collected pre-transplant and then on days +30, +60, +100, and +180 post-transplant [95] (Table 4). The results showed that the median time for neutrophil and platelet engraftment was days +10 and +16, respectively. Median donor chimerism in the erythroid lineage was 95% at day +60, and 90% for both T-cell and NK cell lineages at day +30, respectively. NK and NK T-cell immune reconstitution was rapid and peaked at day +30. The findings suggest that relatively rapid immune reconstitution after TCD haplo-HCT can be achieved with mononuclear cell addback, with the potential...
to obviate delayed engraftment and increased opportunistic pathogen risk with TCD associated delayed immune reconstitution [95]. Similarly, in a study of 21 patients undergoing haplo-HCT, Talano et al. showed median quantitative immune reconstitution at one year of NK, CD3⁺, CD4⁺, and CD8⁺ cells was 204, 795, 408, and 375 cells/μL, respectively, indicating appropriate reconstitution [96] (Table 4).

TCR grafts with post-transplant cyclophosphamide, on the other hand, have been hypothesized to result in selective depletion of donor alloreactive naive T-cells with retention of donor non-alloreactive T-cells, including memory cells, that provide pathogen-specific immunity [21–23,79]. Immune reconstitution is hypothesized to be favorable as a result of peripheral expansion of these T-cells, particularly given the slow functional recovery of T-cells, particularly thymic-derived cells, post-transplant [21–23,79–81]. Fitzhugh et al. evaluated immune reconstitution of B, T, and NK cell subsets following their novel non-myeloablative human leukocyte antigen haplo-HCT platform using peripheral blood stem cells in patients with sickle cell disease in whom such data were available [90]. B cell immune recovery was not impacted at any time points sampled in all cohorts post-transplant. However, mean CD3⁺ recovery was limited in cohort 1, in which no post-transplant cyclophosphamide was given, by slow CD3⁺ CD4⁺ T-cell recovery. CD4⁺ counts normalized within one year in cohorts 2 and 3, in which cyclophosphamide was given for one and two doses, respectively, post-transplant, and after two years in cohort 1. CD8⁺ counts returned to normal range within the first 6 months. NK cells remained within the normal range at all-time points [90] (Table 5). In a cohort of 23 patients with severe sickle cell disease, Patel et al. also showed rapid immune reconstitution in B, T, and NK cell subsets post-transplant in all patients with available data [97] (Table 5).

7. Complications following haplo-HCT approaches for sickle cell disease

7.1. Viral reactivation/infections

One of the major consequences of TCD and TCR haplo-HCT is infection complications.

TCD protocols are hypothesized to result in a greater degree of infectious complications, namely viral reactivation, as a result of delayed and impaired T-cell-mediated immunity. In a study of three sickle cell disease and 11 thalassemia patients, Gaziev et al. found that viral reactivation was common with T-cell receptor alpha/beta⁺ and CD19⁺ depleted grafts, occurring in the vast majority of patients, with reactivation of CMV in 64%, EBV 28%, adenovirus 7%, and BK virus 64%, though no cases of CMV or adenovirus disease were noted [87] (Table 4). BK-associated hemorrhagic cystitis was not uncommon, occurring in 35% of patients [87]. In addition, bacterial and fungal infections were also noted. Similarly, Foell et al. in 2017 noted that viral reactivation was common, with three cases of CMV reactivation without disease, one case of CMV pneumonitis that was treated with donor-derived CMV-specific T-cells, as well as reactivation of EBV, adenovirus, HHV-6, and BK virus [84] (Table 4). In a smaller series of two patients, Marzollo et al. noted only one case of EBV reactivation that was treated with rituximab, though no other cases of viral reactivation despite serial monitoring [84] (Table 4).

Gilman et al. also noted an increased likelihood of viral complications with CD34⁺ selected grafts, with cases of EBV reactivation with post-transplant lymphoproliferative disorder along with CMV enteritis and HHV-6 viremia [86] (Table 4).

Viral reactivation is also problematic following TCR-based approaches. Bolanos-Meade et al. in 2012 reported that three patients had CMV reactivation without disease, one had EBV reactivation that was treated with a single dose of rituximab, and one developed respiratory syncytial virus-related upper respiratory tract infection as well as mycobacterium tuberculosis that was isolated on bronchoscopy [13] (Table 5). This was corroborated by Fitzhugh et al. in 2017, who used peripheral blood stem cell grafts instead of bone marrow grafts and found a similar infectious profile, with four cases of CMV reactivation, one case of CMV disease with colitis, one patient with disseminated adenovirus that resolved spontaneously 1.3 years after transplant, and one case of EBV post-transplant lymphoproliferative disorder that developed 30 days after transplant and resolved after six doses of rituximab [90] (Table 5). Notably, all cases of CMV reactivation or disease resolved after foscarnet therapy [90].

Viral reactivation was notable even with a small sample size of three patients in the Wiebking et al. study from 2017, with two patients developing CMV reactivation and one with VZV reactivation, though none developed disease [99] (Table 5). Cessation of immunosuppression was noted to result in improved immune reconstitution of T-cell subsets, more so for the two pediatric patients compared to the one adult patient, which may influence host immunity to viral pathogens [99] (Table 5). For other TCR protocols, Pawlowska et al. also noted viral reactivation, with three cases of HHV-6 viremia and one of CMV reactivation, which resolved with ganciclovir treatment [100] (Table 5). De La Fuente et al. in the collaborative study also reported viral reactivation in nine patients, six with EBV reactivation but no post-transplant lymphoproliferative disorder treated with rituximab, three with CMV reactivation treated with either ganciclovir and/or foscarnet [93] (Table 5). In addition, cases of adenovirus respiratory infection, BK cystitis, HSV oral infection, and HHV-6 viremia with encephalopathy were noted [93] (Table 5). Saraf et al. identified multiple cases of viral reactivations or infections, including CMV reactivation without infection and oral HSV in addition to cases of coronavirus and influenza [91] (Table 5).

7.2. Bacterial and fungal infections

While viral reactivations/infections occur commonly post-haplo-HCT, bacterial and fungal infections were also documented in the aforementioned studies with both TCD and TCR approaches (Tables 4 and 5). Gilman et al. identified invasive aspergillosis that occurred after transplant, while Fitzhugh et al. showed multiple cases of bacteremia, the majority of which resolved with treatment, and three cases of presumed fungal pulmonary nodules [86,90] (Tables 4 and 5). Data from Saraf et al. also showed two cases of urinary tract infections, with the remainder of infections being viral in origin [91] (Table 5). De La Fuente et al. in their collaborative study identified cases of...
presumed invasive fungal infections and multiple cases of documented bacterial sepsis from both gram-positive and gram-negative pathogens; however, the cases were transient and did not impact overall survival [93] (Table 5).

8. Long-term outcomes following haplo-HCT approaches for sickle cell disease

8.1. TCD-based approaches

Gilman et al. noted that of the 90% (9/10) patients who survived, none had progression of cerebrovascular disease or painful crises related to sickle cell disease [86] (Table 4). No significant differences existed in pre and post-transplant pulmonary function testing [97]. Also, all survivors were either in school or employed at the last follow up, indicative of meaningful quality of life improvement post-transplant [86]. The findings indicate that successful engraftment using this reduced intensity conditioning regimen and TCD grafts resulted in amelioration of sickle cell disease-related symptoms. Mitigating against post-transplant lymphoproliferative disorder is necessary to promote future use of the protocol, which raises the consideration of adding rituximab to the conditioning regimen in select patients [86].

Foell et al. found in there TCD approach that the four patients who had abnormal transcranial Dopplers pre-transplant had normalization of velocities post-transplant [84] (Table 4). Similarly, Marzollo et al. (TCR) found that no sickle cell disease manifestations were noted after transplant in the two haplo-HCT patients in their study [98] (Table 4). One of the evaluated patients had significant reduction of bilateral moderate stenosis on magnetic resonance angiography and stable white matter hyperintensity on magnetic resonance imaging [98]. Similar to the Foell et al. group who used myeloablative conditioning regimens and treosulfan, the data by Marzollo et al. suggest that treosulfan can be safely used for reduced intensity haplo-HCT regimens for effective sickle cell disease treatment, though progress must be made to reduce long-term toxicities such as endocrine abnormalities, especially for younger patients who desire family planning [84,98]. Secondary gonadal failure has been reported among post-transplant patients [84,98].

The most extensive evaluation of organ function and quality of life following haplo-HCT for severe sickle cell disease was conducted by Dallas et al. in 2013 with a TCD-based approach [24] (Table 4). All patients underwent organ function studies, including blood counts and serum chemistries, pulmonary function tests, transthoracic echocardiogram, transcranial Doppler, liver biopsy, endocrine function tests, and bone age assessment, pre- and post-transplant. In engrafted patients, the haplo-HCT protected against progression of neurological dysfunction, including overt and silent infarcts and cognitive decline [24]. All patients who had abnormal transcranial Doppler velocities prior to transplant had normalization post-transplant. While renal function significantly worsened after transplant, pulmonary function remained the same, including assessment of tricuspid regurgitant jet velocity for pulmonary hypertension, suggesting amelioration of hemolysis as a contributing factor [24]. In terms of endocrine outcomes, bone mineral density increased post-transplant, and patients with delayed bone growth prior to transplant had normalization post-transplant in the absence of chronic GvHD [24]. However, infertility was more common with busulfan containing myeloablative regimens [24]. Patients who underwent myeloablative conditioning had progressive decline in renal, cardiac, and pulmonary function over time, compared to the reduced intensive conditioning haplo-HCT group [24]. In summary, the study demonstrates that severe sickle cell disease can be cured using a TCD-based approach, but further investigation is needed to balance toxicities from the conditioning regimen with progressive end-organ damage associated with the underlying disease process.

8.2. TCR-based approaches

Bolanos-Meade et al. noted transplant-related complications, namely that 21% (3/14) patients developed posterior reversible encephalopathy syndrome, though all recovered full neurologic function with continuation of sirolimus rather than tacrolimus [13] (Table 5). Pulmonary infiltrates developed 6 months post-transplant in one patient, which was suspected to be due to either sirolimus toxicity or fungal infection. Fitzhugh et al., in contrast, included patients who had more cumulative end-organ damage prior to transplant, compared to other published clinical trials [90] (Table 5). The majority of patients (61%) had at least three severe sickle cell disease-related co-morbidities at baseline, which is more reflective of the types of patients who are evaluated for HCT [90]. No sickle cell disease-related complications occurred in the peri-transplant period [90]. Unfortunately, two patients who had secondary graft rejection developed high-grade myelodysplastic syndrome with fibrosis, and both died of associated complications [90] (Table 5). Suspected sirolimus-related toxicity occurred in seven patients [90].

In the Wiebking et al. study in which myeloablative conditioning was chosen, only one patient had mild mucositis, with grade 3 toxicity [99] (Table 5). No patient had central nervous system toxicity even while on tacrolimus [99]. Short-term toxicity was minimal, though long-term complications, such as effect on growth and reproductive health, are not determined thus far [99]. Similarly, with the novel platform of pre-transplant immunosuppression developed by Pawlowska et al., no patient developed complications related to fludarabine or dexamethasone, such as neurotoxicity, hypertension, posterior reversible encephalopathy syndrome, or pain crises, suggesting that this regimen can be safely implemented [100]. De La Fuente et al. showed that all engrafted patients had resolution of the sickle cell disease phenotype, though transplant-related complications were noted, namely one case of sinusoidal obstruction syndrome, two cases of posterior reversible encephalopathy syndrome, and one case of new cerebral infarction that developed in a patient with primary graft rejection [93] (Table 5).
9. Expert opinion

The choice between TCD- and TCR-based haplo-HCT for sickle cell disease depends largely on institutional preference and expertise. Though improving overall survival, disease-free survival, and transplant-related mortality have been seen with both modalities (Tables 4 and 5), TCR-based approaches with post-transplant cyclophosphamide offer the potential for lower acute and chronic GvHD, with drug-induced immunologic tolerance. TCD-based approaches are still plagued with delayed immune reconstitution and both are associated with risk of increased opportunistic pathogens, namely viral reactivations and infections [17,18]. TCR-based approaches are also beneficial in terms of easy availability, more affordable, favorable logistics, and not requiring specialized centers and infrastructure for complicated cell depletion methods. While there are no direct cost comparison studies between TCR and TCD-based approaches, the required ex vivo T-cell receptor $\mu^+$/CD19$^+$ depletion is performed using an immune-magnetic method (CliniMACS, Miltenyi Biotech, Bergisch Gladbach, Germany) [17,18]. Donors requiring CD34$^+$ cell collection three to four months before bone marrow harvest. CD34$^+$ cells are then isolated using the ClinMACS device (Miltenyi Biotech) and cryopreserved, adding to the overall cost of the procedure, compared to TCR methodology that requires only post-transplant cyclophosphamide or other methods of drug-induced immunologic tolerance.

Non-relapse mortality associated with regimen toxicity and chronic GvHD with either modality can erode the trust of patients with sickle cell disease and their families, potentially leading to fewer affected individuals seeking haplo-HCT as a curative modality. Regardless of the transplant platform, addressing the increased viral reactivation or infection using novel approaches such as the creation of donor pathogenesis-specific T-cells, directed to CMV, EBV, BK and/or adenovirus, or use of novel infectious prophylactic approaches are urgently needed. Also, preservation of fertility is paramount to help address some of the major concerns of patients and families seeking this curative approach. Furthermore, strategies to boost immune reconstitution post-haplo-HCT following TCD-based approaches must be incorporated when utilizing this transplant approach for sickle cell disease patients.

Given the potential of near-universal donor availability with haplo-HCT, we believe formal criteria for patient selection should continue to be modified to provide such that optimal candidates are referred for haplo-HCT [101,102]. There is also a need for the development and research on the quality of life measures for patients who undergo haplo-HCT, given the higher short-term morbidity and mortality of transplant versus non-transplant patients. While common measures such as organ function and sickle-related events are commonly measured, we advocate for assessing the impact of improved hematologic markers, such as hemoglobin and hemolysis parameters, particularly on cognitive function and development in pediatric patients. Importantly, studies are needed to investigate the feasibility of haplo-HCT in low-income areas, where the burden of sickle cell disease is the highest. A dilemma exists in terms of addressing a major public health dilemma in resource-poor countries with a procedure that is highly resource and labor intensive. Doing so, however, may improve the lives of many more patients than those treated in developed nations alone. Lastly, major advances have been made with gene therapy as a curative modality for monogenic disorders such as sickle cell disease [49,50]. While potential advantages include no requirement for donor availability, no risk of GvHD, or prolonged immunosuppressive therapy, it requires large amounts of CD34+ cells, typically require two to three rounds of stem cell mobilization, and usually requires myeloablative doses of chemotherapy to improve transduction efficiency, which makes it presently intolerable for adults with sickle cell disease. Thus, infertility, late-effects, and concern for off-target gene effects remain problematic.

A major need is to completely abrogate chronic GvHD with novel graft manipulation strategies when using TCD-based approaches, and not trading one chronic disease, namely severe sickle cell disease, for chronic GvHD. TCR-based approaches with post-transplant cyclophosphamide mitigate against this complication, and our current institutional preference is the use of bone marrow rather than peripheral blood grafts due to at least 10-log fewer T-cell content, though the process is more labor intensive, with potential anesthesia-related risks to the donor.

9.1. Five-year view

The use of related haplo-HCT approaches promises to change the landscape for a curative approach for severe sickle cell disease in both children and adults. Not only does it increase donor availability, but also the use of reduced intensity conditioning regimens allows inclusion of individuals with severe sickle cell disease with pre-existing end-organ damage to harness the benefit of this approach. Outcomes following haplo-HCT for sickle cell disease are approaching those for matched sibling donor transplants, though unique barriers exist, namely extensive alloimmunization from prior red blood cell transfusions, with significant donor-specific anti-human leukocyte antigen antibodies and subsequent increased risk of graft rejection or delayed engraftment. Accordingly, the use of validated antibody desensitization protocols will broaden the number of donor options by not excluding haploidentical family members because of donor-specific human leukocyte antigen antibodies [88,89]. Approaches based on solid organ human leukocyte antigen antibody depletion techniques include a combination of modalities such as plasmapheresis, intravenous immune globulin, adsorption using irradiated donor lymphocytes, platelets, and/or staphylococcal protein A columns [92,103]. Institutional preference using these techniques varies, though are often under-utilized, which can result in unacceptable delays for patients eligible for haplo-HCT. Increasing knowledge and expertise are urgently needed in addressing this vexing problem with donor selection, and clinical trials to help compare outcomes using different desensitization protocols, which will improve donor options and reduce the probability of graft rejection.

Other important hurdles include defining optimal conditioning for haplo-HCT that reduces regimen-related toxicities
in patients with pre-existing multi-organ system damage, preserves fertility, reduces infectious complications, especially early viral reactivations and infections, and minimizes late-effects. In our view, more nuanced analyses of immune reconstitution, including naïve and memory T-cell subsets will be performed to better understand factors that influence donor chimerism and pathogen-specific immunity. Additionally, as more children and young adults are considered for haplo-HCT, gonadal function will be more routinely assessed to encourage fertility preservation.

A relative advantage of related haplo-HCT transplant is donor accessibility for advanced cellular therapies, meaning that T-cell addback strategies are more easily possible. The use of cytokines to boost T-cell subsets is being pioneered in allogeneic transplant to address disease relapse and should be developed for non-malignant diseases to improve pathogen-specific immunity and engraftment. The role of natural killer cell killer immunoglobulin-like receptor ligand mismatching is also underexplored in sickle cell disease, though is important given the association between NK cells underlying sickle cell disease pathogenesis [94]. Multi-institution randomized trials comparing TCD and TCR approaches must be performed to truly know which platforms are preferred. Over time, gene therapy may compete with haplo-HCT as a potential curative option. Continued collaboration through learning consortiums is needed to answer these complex questions using these curative modalities.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

• Landmark paper for showing that haplo-HSCT with NMA conditioning regimen can reverse phenotype of severe SCD with minimal transplant-related effects or acute or chronic GvHD. Since published, research has focused on ways to reduce graft rejection, as seen in the study (43%).


• Most comprehensive assessment of organ function pre and post-haplo-HSCT performed to date. Sets the standard for toxicity measurement for other studies.


95. Similar study as the current review, though with focus more broadly on patients with thalassemia also, while our article includes data on both children and adults. Extensive analysis of pros and cons of TCD versus TCR approaches for children.


99. Important study for showing efficacy of non-chemotherapy based conditioning regimen for children with existing multi-organ system disease prior to consideration of HSCT. Shows that haplo-HSCT can lead to excellent survival with minimal GvHD in a non-traditional clinical trial population.


103. Thiopeta based conditioning regimen results in 100% OS, minimal acute and chronic GvHD, and over 83% engraftment rate, suggesting that the drug should be incorporated into more protocols.


- Pioneering concept of pre-transplant immune suppression therapy with NMA conditioning regimen, with excellent outcomes in a small group of patients. Also, discuss importance of developing comfort level with desensitization protocols.

