Chapter 25
Hematopoietic Cell Transplant and Cellular Therapies for Sickle Cell Disease

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Introduction

Sickle cell disease (SCD) is an inherited blood disorder that was first described by James Herrick in 1910 [1]. In 1957, Vernon Ingram, PhD, described the underlying genetic mutation as a single point mutation in codon 6 of β-globin chain and its resultant expression of mutated hemoglobin S that triggers erythrocytes to take a characteristic sickled conformation [2]. SCD now includes a group of different genetic conditions that result in same pathology such as homozygous hemoglobin S [HbSS], sickle hemoglobin C disease [HbSC], sickle β0-thalassemia [HbSβ0-thalassemia], sickle β+-thalassemia [HbSβ+-thalassemia], and other genotypes. SCD affects nearly 100,000 residents of the USA with estimates that it affects hundreds of thousands more worldwide. Annually there are 2000 new children in the United States (US) affected by SCD [3].

The abnormal sickle-shaped erythrocytes disrupt blood flow in small vessels and can cause vaso-occlusion in small vessels, which leads to distal tissue ischemia and inflammation with symptoms defining the acute painful sickle cell crisis. Repeated ischemia-reperfusion episodes are responsible for many of the acute and chronic complications affecting all major organs (anemia, hemolysis, acute splenic sequestration, stroke, cerebral silent infarcts, cognitive impairment, retinopathy, avascular osteonecrosis, leg ulcers, priapism, proteinuria, renal failure, cholelithiasis, hepatocholangiopathy, pulmonary hypertension, etc.), resulting in substantial morbidity and contributing to early mortality [4].

The health and survival of children with SCD have improved considerably after implementation of newborn screening, penicillin prophylaxis, pneumococcal immunization, chronic transfusion, hydroxyurea (Hydrea®) utilization, and

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education about disease complications. However, unfortunately, the average projected life span of affected adults has not improved beyond the fifth decade [5, 6]. Thus far, hematopoietic cell transplantation (HCT) remains the only curative option, which could improve both quantity and quality of life. This chapter will review challenges and outcomes of HCT in patients with SCD, utilizing different conditioning regimens and different donor types as well as briefly promising new cellular therapies such as gene therapy that could offer a curative option.

HLA-Matched Related Donor Transplant (MRD) in Children

1. The first reported case of using HCT to cure SCD was in 1984 in an 8-year-old female, who also had acute myeloid leukemia (AML); her 4-year-old HLA-matched brother was her donor.
    a. Myeloablative (MA) conditioning regimen with cyclophosphamide 60 mg/kg/day × 2 doses and total-body irradiation (TBI) 11.5 Gy.
    b. Graft-versus-host disease (GvHD) prophylaxis consisted of methotrexate and prednisone.
    c. This regimen resulted in a cure of both her AML and SCD.
    d. Despite this successful case, widespread use of HCT to treat SCD has been limited due to its highly variable clinical outcomes.
2. Many studies have attempted to identify patients at risk for progressive organ damage with associated long-term poor outcomes.
    a. This includes patients with debilitating clinical events, such as stroke, recurrent acute chest syndrome, and recurrent painful vaso-occlusive crises, which contribute to the high morbidity and early mortality among patients with sickle cell disease.
3. Since the first reported case, multiple studies have been published using HCT for patients with SCD, supporting this therapy as a curative option.
    a. The first multicenter study included 22 children with symptomatic SCD, who underwent an MA regimen using busulfan 16 mg/kg, cyclophosphamide 200 mg/kg, and antithymocyte globulin (ATG) 90 mg/kg with an HLA-matched sibling donor. At the 4-year follow-up, disease-free survival (DFS) was reported at 73% and overall survival (OS) was 91% [8].
    b. Following that study, many groups have described a series of patients transplanted with an HLA-identical sibling with reported OS that varies between 91% and 100% and event-free survival (EFS) that varies between 73% and 100% [9].
    c. The addition of ATG resulted in a decrease in the 5-year cumulative incidence of graft rejection from 22.6% to 2.9%. Furthermore, its use was associated with an increased frequency of mixed but stable chimerism.

d. GvHD was the principal complication, accounting for four deaths. Twenty percent of patients developed grade II or higher acute GvHD (aGvHD), 8.1% of whom developed grade III to IV aGvHD. The cumulative incidence of chronic GvHD (cGvHD) was 12.6% [10].
e. The largest retrospective study published the results of 1000 HLA-identical transplants, performed between 1986 and 2013, and reported to the European Society for Blood and Marrow Transplantation (EBMT), Eurocord, and the Center for International Blood and Marrow Transplant Research (CIBMTR).
    i. Five-year EFS and OS of 91.4% (95% CI 89.6–93.3%) and 92.9% (95% CI 91.1–94.6%), respectively [11].
    ii. A multivariate analysis of age at the time of transplant was associated with improved OS and EFS.
        - Five-year OS was 95% (95% CI, 93–97%) and 81% (95% CI, 74–88%) for patients aged <16 years and those aged ≥16 years, respectively ($P < 0.001$).
        - The corresponding EFS was 93% (95% CI, 92–95%) and 81% (95% CI, 74–87%; $P < 0.001$).
        - Five-year probability of GvHD-free survival was 86% and 77% for patients aged <16 years and ≥16 years, respectively ($P < 0.001$).
        - The indications for HCT in most of these studies are summarized in Table 25.1.
f. Another landmark clinical trial is the DREPAGREFFE (NCT01340404).
    i. A multicenter, prospective trial between 2010 and 2013 with a 3-year follow-up.
    ii. Enrolled patients with SCD aged <15 years, who were receiving chronic transfusions due to a history of abnormal transcranial Doppler (TCD).
    iii. Children with HLA-matched donors underwent HCT, while those without a suitable donor continued chronic transfusion.

Table 25.1 Summary of clinical indications for HCT in HLA-matched donors for SCD

Children with sickle cell disease (Hgb SS or Hgb SB thalassemia) ≤ 18	*Adults* with sickle cell disease (Hgb SS or Hgb SB thalassemia) >18
Stroke or central nervous system event lasting >24 hours	Stroke or central nervous system event lasting >24 hours
Abnormal MRI/MRA vasculopathy (silent stroke)	Impaired neuropsychological function with abnormal MRI/angiography
Recurrent acute chest syndrome	Recurrent acute chest syndrome
Recurrent vaso-occlusive painful episodes or recurrent priapism	Recurrent vaso-occlusive painful episodes
	Evidence of end-organ damage: Pulmonary hypertension Osteonecrosis Renal insufficiency
	Red-cell alloimmunization

iv. Thirty-two children were enrolled on each arm of the trial, and comparison between the two arms was analyzed using both genetic randomization and propensity-score matching as a sensitivity analysis.
v. The primary end point was the velocity measure at 1 year. Secondary end points were the incidence of stroke, silent cerebral infarcts and stenosis, cognitive performance in comparison with siblings, alloimmunization, and iron overload.
vi. There were no strokes or deaths in either group.
vii. Highest TCD velocities at 1 year were significantly lower on average in the HCT group (129.6 cm/s) vs the chronic transfusion group (170.4 cm/s; $P < 0.001$).
viii. Of the 25 analyzed secondary end points, four showed significant differences.

- The highest TCD velocity at 3 years of 112.4 cm/s in the HCT group vs 156.7 cm/s in the chronic transfusion group; difference, −44.3; $P = 0.001$
- Normalization rate at 1 year of 80.0% in the HCT group vs 48.0% in the chronic transfusion group; difference, 32.0%; $P = 0.045$
- Ferritin levels at 1 year of 905 ng/mL in the HCT group vs 2529 ng/mL in the chronic transfusion group; difference, −1624; $P < 0.001$
- Ferritin levels at 3 years of 382 ng/mL in the HCT group vs 2170 ng/mL in the chronic transfusion group; difference, −1788; $P < 0.001$

ix. Additionally, children who underwent HCT reported better quality of life (QOL) than those receiving chronic transfusion only at 3 years (84.8 vs 73.2, respectively; difference, 11.6; $P = 0.001$), while their parents reported improved QOL at 1 year (88.3 in the HCT group vs 69.7 in the chronic transfusion group; difference, 18.6; $P < 0.001$) and 3 years (84.0 in the HCT group vs 73.1 in the chronic transfusion group; difference, 11.0; $P = 0.01$) [12, 13].
x. *In summary*: Matched sibling donor HCT was associated with greater improvements in TCD velocities and many secondary end points without unexpected toxicity when compared with the chronic transfusion group. One important observation is that at the 3-year follow-up, three children receiving chronic transfusions developed new silent infarcts and two developed stenosis, while no patients in the HCT group developed either of these abnormalities. Although these differences were not statistically significant, this suggests a possible benefit of HCT in halting progression of cerebrovascular disease and vasculopathy.

4. These studies collectively demonstrate:

a. Patients with symptomatic SCD who undergo HCT with an HLA-matched sibling donor have excellent outcomes.

b. Patient age at transplant is important, supporting the notion that early transplant before end-organ damage occurs is fundamental to treatment success [14]
c. DREPAGREFFE trial is a clear evidence of the advantage of early intervention before end-organ damage occurs, similar to studies in thalassemia major where transplant is performed as soon as a matched sibling donor is identified.

5. Studies in the US also show that HCT leads to substantial reductions in healthcare expenditures over time for SCD patients compared to SCD patients who receive supportive therapy alone, with the largest benefit noted among patients with MRDs and those who were younger at the time of transplantation.

   a. Merged data for 176 patients showed that the median total adjusted transplant cost per patient was $467,747.
   b. Healthcare utilization was lower among recipients of matched sibling donor HCT and those with low severity disease compared to those with other types of donor and disease severity types ($P < 0.001$ and $P = 0.022$, respectively).
   c. HCT early in the disease course was associated with significant reductions in admissions ($P < 0.001$), length of stay ($P < 0.001$), and cost ($P = 0.008$).
   d. Reduced posttransplant inpatient healthcare utilization indicates that HCT may provide a sustained decrease in healthcare costs over time [15].

6. Between 2011 and 2015, only 116 HCTs per year were performed on patients with SCD within the US, which some would consider a remarkably low number given the prevalence of this disease and the data accumulated to date.

   a. Multiple factors contribute to the low rates of HCT in this patient population.

      i. One of the major barriers to increased use of this therapy is limited donor availability. Studies assessing donor availability in the SCD population have found that only 14–25% of SCD patients have an HLA-matched related sibling [16]. However, even with less than one-third of patients potentially having a HLA-matched sibling donor, donor availability alone fails to fully account for the low utilization of HCT in this patient population of approximately 100,000 SCD in the US.
      ii. Sociocultural factors, both patient and provider related, may also contribute to this phenomenon [11, 17, 18].

         - Parents of children with SCD and adult patients affected by the disease are willing to accept relatively high risk of mortality to achieve cure of the disease [19]
         - Among healthcare providers, there are variable perceptions of acceptable up-front risk vs the opportunity for long-term cure [20].
         - These observations suggest that clinician attitudes about and clinical practices of discussing HCT with families may play a role in the underutilization of this therapy in the SCD patient population [21].

HLA-Matched MRD Transplant in Adults

Most of the studies described above focused on HCT in children with SCD where MA HCTs proved to be a curative option. However, the potential toxicity of MA transplants may be prohibitive for adults, thus leading to the study of non-myeloablative (NMA) HCT with different degrees of reduced-intensity conditioning (RIC). RIC regimens have traditionally been associated with a higher incidence of graft rejection and GvHD. Reviewed below are several studies that successfully employed increased immunosuppression instead of MA conditioning, resulting in curative treatment option for adult SCD patients with comorbidities.

1. A minimally toxic regimen was first developed by the John Hopkins group using pretransplant fludarabine 150 mg/m^2 and TBI 200 cGy in seven patients. This approach was safe with no mortality and little or no aGvHD. However, after initial engraftment, all patients lost their graft after withdrawal of immunosuppression and experienced autologous recovery with disease recurrence [22].
2. This approach was modified by the group at the National Institutes of Health (NIH).
    a. A pilot study enrolled ten SCD patients (age range 16 to 45 years), who received a NMA conditioning regimen of alemtuzumab (Campath®) 1 mg/kg in divided doses and TBI 300 cGy followed by infusion of G-CSF (Neupogen®)-mobilized peripheral blood stem cells (5.5–31.7 × 10^6 cells/kg) from an HLA-matched sibling. Sirolimus (Rapamune®) was used for GvHD prophylaxis [23].
    b. An additional 20 patients were accrued (for a total of 30 patients evaluated, aged 16–65 years), who were transplanted between 2004 and 2013 with the same NMA regimen [24].
        i. Twenty-nine patients (96%) survived with a median follow-up of 3.4 years. One patient died from intracranial bleeding after graft failure.
        ii. Twenty-six patients (87%) had long-term stable donor engraftment without acute or chronic GvHD.
        iii. The mean donor T-cell chimerism was 48% (95% CI, 34–62%); myeloid chimerism 86% (95% CI, 70–100%).
        iv. Fifteen patients engrafted and discontinued immunosuppression medication with continued stable donor chimerism and no GvHD.
        v. Additional findings in this study included the resolution of hemolysis among engrafted patients, stabilization in brain imaging, a reduction of echocardiographic estimates of pulmonary pressure, and the ability to perform phlebotomy to reduce hepatic iron.
        vi. Another importance healthcare utilization finding was the significant decrease in the mean annual hospitalization rate from 3.23 (95% CI, 1.83–4.63) the year before HCT to 0.63 (95% CI, 0.26–1.01) the first year

Table 25.2 Selected studies of non-myeloablative conditioning (alemtuzumab + TBI 300 cGy) with HLA-matched sibling HCTs for patients with SCD

	Number of patients	Age (years)	Overall survival	Acute GvHD	Sustained engraftment
University of Chicago [26]	*N* = 13	17–40	100%	0%	92%
Saudi Arabia [27]	*N* = 51 17 children 34 adults	27 (14–39) 8.8 (4–14)	Adult 97% Peds 100%	Adult 0% Peds 12%	Adult 90% Peds 100%
Alberta Children's Hospital-Canada [28]	16 children	12 (3–18)	100%	0%	100%

GvHD graft-versus-host disease; *Peds* Pediatric

after. This trend continued to further decrease to 0.19 (95% CI, 0–0.45) in the second year after and subsequently down to 0.11 (95% CI, 0.04–0.19) the third year after HCT.

vii. Another important observation in RIC HCTs where mixed chimerism is common is that patients with myeloid chimerism ≥20% remained free of SCD symptoms, due to the greatly shortened red blood cell (RBC) life span in sickle cells and improved RBC survival of the donor cells. A minority of donor cells is adequate to reverse the sickling phenotype [25].

c. This RIC regimen was replicated at other transplant centers as summarized in Table 25.2. These data suggest that alemtuzumab + low-dose TBI conditioning creates adequate space in the bone marrow and depletes recipient lymphocytes to overcome the risk of graft rejection and facilitate donor engraftment. Additionally, the prolonged half-life of alemtuzumab contributes to in vivo depletion of donor alloreactive T cells, decreasing GvHD risk with very little transplant-related mortality or toxicity.

Alternative Donor Sources

One of the biggest challenges of expanding HCT to the SCD population is the lack of an HLA-matched family donor. In a cohort of 113 children with SCD receiving chronic RBC exchange transfusion therapy, only eight (7%) had identified an unaffected HLA-matched sibling [29] and only three patients (<3%) underwent HLA-matched HCT. In another collaborative study among 22 centers where 4848 patients with SCD were followed, only 14% were likely to have a HLA-identical sibling donor [30]. These data illustrate the important role of alternative donors to expand the access to this life-saving therapy. While several other stem cell sources such as mismatched unrelated bone marrow, umbilical cord blood, and haploidentical stem

cells from a parent or sibling are potential alternative options for HCT in SCD patients, these options are associated with increased risk of graft rejection and/or GvHD.

1. Matched unrelated donor (M-URD)
    a. The National Marrow Donor Program (NMDP) reports African Americans have low probability (16% to 19%) of finding an appropriate 8/8 HLA-matched donor [31].
        i. An important prospective phase II multicenter trial study, BMT CTN 0601 (SCURT: Sickle Cell Unrelated Transplant), aimed to evaluate the role of unrelated donors in SCD.
            - Twenty children with a median age of 14 years (range 4–19 years).
            - Preparative regimen consisted of distal alemtuzumab [Campath®] on days −23, −22, −21, and −20 followed by fludarabine 30 mg/m2/daily on days −8, −7, −6, −5, and −4 and melphalan 140 mg/m2 on day −3.
            - GvHD prophylaxis was a calcineurin inhibitor, short methotrexate 7.5 mg/m2 on days +1, +3, and +6, and methylprednisolone 1 mg/kg per day IV through day +28 [32]
            - The 1-year OS was 86% and 1-year DFS rate was 75%. The regimen was associated with 28% aGvHD (grade II to IV) and 38% chronic extensive GvHD.
            - Six patients died of GvHD, and one patient died following a second transplant.
        ii. Another approach has been CD34+ cell-selected, T-cell-depleted peripheral blood stem cell transplantation in the M-URD setting using an RIC reduced-intensity conditioning regimen including melphalan, thiotepa, fludarabine, and rabbit ATG.
            - A study by Gilman et al. [33] reported outcomes of ten patients (age 5–23 years); the 2-year OS was 90%, and EFS was 80%. This approach enabled stable myeloid engraftment (mean donor chimerism was 99%) with low GvHD rate; however, Epstein-Barr virus (EBV)-related post-transplant lymphoproliferative disorder (PTLD) occurred in three patients and one patient died as a consequence of treatment of PTLD.
        iii. A recent multicenter study reported HCT outcomes in adults with SCD using RIC regimen with busulfan 13.2 mg/kg, fludarabine 175 mg/m^2, and rabbit ATG 6 mg/kg.
            - Twenty-two patients with a median age of 22 years, range 17–36.
            - Seventeen patients had MRDs, and five patients received marrow from an 8/8 HLA-allele-M-URD.
            - One patient died from graft failure; OS was 80% with DFS at 3 years of 60%.

iv. Currently there is another multicenter phase II clinical trial (BMT CTN #1503) testing busulfan, fludarabine, and ATG reduced-toxicity conditioning regimen in both HLA-matched sibling donor and HLA-M-URD bone marrow transplant (BMT) in adults with SCD. At the time of publishing, this clinical trial (NCT02766465) is recruiting and will compare BMT to the standard of care in individuals without a suitably HLA-MRD or M-URD.

2. Umbilical cord blood transplant (UCBT)

a. Given the limited availability M-URD, it is reasonable to consider cord blood as an alternative donor source for HCT.

i. Historically outcomes of UCBT in patients with SCD have been complicated by graft rejection as seen in the SCURT trial, which included a cohort of eight patients, who received unrelated UCBT [34].

- Patients were conditioned with alemtuzumab, fludarabine, and melphalan.
- GvHD prophylaxis consisted of cyclosporine A (CSA) or mycophenolate mofetil (Cellcept®, MMF) and tacrolimus (Prograf®).
- All patients engrafted neutrophils; however, five patients had autologous hematopoietic reconstitution equivalent to 62% graft rejection; the remaining three patients had sustained donor engraftment.
- One-year EFS was 37.5%; therefore, study enrollment into the UCBT cohort was prematurely suspended due to high rates of graft rejection.

ii. Another study also reported a high incidence of graft rejection using a conditioning regimen of busulfan, fludarabine, and alemtuzumab, where only four out of eight patients engrafted with DFS of 50% [35].

iii. The outcome following UCBT from an HLA-MRD is acceptable with 5-year EFS of 86% from a study by Soni et al. [36], who reported outcomes of 22 children with median age 5.2 years (range 1.8–11.7 years).

- Most patients received an MA regimen of busulfan, cyclophosphamide, and ATG.
- Three patients died from infectious complication of transplant, 5% developed aGvHD, and no chronic GVHD was reported.
- The author also investigated co-infusion of bone marrow cells from the same donor as the umbilical cord blood donor in 13 patients as a way to increase the cell dose and enhance engraftment.

  – Neutrophil engraftment occurred at a median day +17, which was 8 days less than UCBT group, none of the patients experienced graft failure, and the EFS was 100% after a median follow-up of 66 months (range: 33–91 months) [36].

iv. More recent data suggest that the addition of thiotepa to the previous RIC regimen of fludarabine, melphalan, and alemtuzumab could improve outcomes in the setting of unrelated UCBT.

- Abraham et al. [37] reported outcomes of nine patients with median age of 4 years (range 3–10 years).
- One-year OS was 100%, and DFS was 78%.
- Of note the median total nucleated cell (TNC) dose was 5.9×10^7/kg (range 3.9–8.5), which was higher than the TNC dose in SCURT trial (median 4.5×10^7/kg with a range of $2.1–6.3 \times 10^7$/kg) and could be a contributing variable.
- This small patient study will need to be validated in larger trial before unrelated UCBT could be used more widely in SCD.

3. Haploidentical HCT (haploID)

a. HaploID donors have increased the access to life-saving HCT therapy in many malignant disorders, especially with the success of T-cell-replete HCT products with posttransplant cyclophosphamide (PTCy) as the method for immune tolerance and prevention of GvHD [38]. A majority of patients will have parents, children, or siblings who can serve as donors.
b. The John Hopkins regimen using T-cell-replete HCT with PTCy served as a platform for many studies that investigated the safety and efficacy of haploID HCT in patients with SCD.

i. A study by Bolaños-Meade et al. [42] used a regimen consisting of rabbit ATG, fludarabine, cyclophosphamide, and TBI 200 cGy.
ii. GvHD prophylaxis consisted of PTCy and mycophenolate mofetil (Cellcept®, MMF) with either tacrolimus or sirolimus.
iii. Seventeen patients were transplanted using bone marrow as the stem cell source; 14 from HLA-haploID donors and three from HLA-MRDs.
iv. With a median follow-up of 711 days, 11 patients had sustained engraftment (EFS = 65%) with no mortality. No cGvHD was reported in the patients who had sustained engraftment.
v. This study provided evidence that haploID HCT is safe and feasible; however, this strategy was associated with a high rate of graft failure (43%).
vi. A modified Hopkins regimen was investigated at the University of Illinois [39], increasing the TBI dose to 300 cGy (instead of 200 cGy) and using peripheral blood stem cells (PBSCs) instead of bone marrow.

- Eight patients were evaluated with a reported DSF of 75% and OS of 87.5%.
- Two patients developed aGvHD; only one patient experienced cGvHD but died later.

c. Another approach was adding thiotepa to the conditioning regimen, which consisted of ATG 0.5 mg/kg on day −9 and 2 mg/kg on days −8 and −7 (total dose 4.5 mg/kg), fludarabine 30 mg/m^2 on days −6 to −2 (total dose 150 mg/m^2), cyclophosphamide 14.5 mg/kg on days −6 and − 5 (total dose 29 mg/kg), and TBI 200 cGy on day −1 [40].

   i. GvHD prophylaxis included PTCy 50 mg/kg on days +3 and + 4, mycophenolate mofetil on days +5 to +35, and sirolimus instead of tacrolimus or cyclosporine to decrease the incidence of neurological complications such as PRES. Sirolimus target levels were 5–15 ng/mL for 1 year.
   ii. Outcomes were very encouraging with 93% (14 of 15) of patients experiencing >95% stable donor engraftment at 6 months and 100% OS. Two patients had grade III–IV aGvHD, one patient had mild chronic GvHD, and 86% of patients (6 of 7) were off immunosuppression therapy by 1-year posttransplantation.

d. Other studies have utilized a similar RIC haploID HCT regimen with thiotepa or increased TBI dose to 400 cGy with excellent EFS of 88% and OS of 100% [41, 42].

e. The preconditioning phase of RIC haploID HCT has also proved critical. Investigators have used hydroxyurea 30 mg/kg for 60 days prior to start of conditioning therapy or pulses of fludarabine and dexamethasone [43]. There is no clear advantage of one therapy over the others, and some therapies may be associated with increased mortality due to infection or macrophage activation syndrome as reported when hydroxyurea, hypertransfusion, and azathioprine (Imuran®) are used as preconditioning therapy [44].

   BMT CTN 1507 (NCT03263559) is an ongoing prospective phase II multicenter trial to evaluate the efficacy and toxicity of haploID BMT in children and adults with SCD after preconditioning with hydroxyurea and a conditioning regimen of ATG, fludarabine, cyclophosphamide, thiotepa, and TBI 200 cGy. Of note this study enrolls both adults and children but has different indications for each group. See Table 25.3 for indications for enrollment.

   i. This study, which aims to enroll 40 patients in each stratum with the primary end point or EFS at 2 years posttransplant, is expected to complete enrollment by the end of 2021.

f. Another approach for haploID HCT by the NIH group is based on their success with NMA platform used with matched sibling donors that was discussed earlier (see section "HLA Matched MRD Transplant in Adults" a, b) [45].

   i. Conditioning consists of alemtuzumab and TBI 400 cGy total followed by infusion of a haploID product.
   ii. GvHD prophylaxis was sirolimus and dose escalation of PTCy.

Table 25.3 Indications for enrollment in BMT CTN 1507

Indication criteria for adults Ages 15–45.99 years at the time of enrollment	Indication criteria for children Ages 5–14.99 years at the time of enrollment
Clinically significant neurological event (stroke) or any neurological deficit lasting >24 hours	Evidence of overt stroke ischemia based on neuroimaging or clinical evidence of permanent neurological injury lasting for 24 hours, or both
History of two or more episodes of ACS in the 2-year period preceding enrollment despite the institution of supportive care measures (i.e., asthma therapy and/or hydroxyurea)	Abnormal TCD measurement with a timed average maximum mean velocity of ≥200 cm/sec in the terminal portion of the internal carotid or proximal portion of middle cerebral artery or if the imaging TCD method is used >185 cm/sec plus evidence of intracranial vasculopathy
History of three or more severe vaso-occlusive pain crises per year in the 2-year period preceding enrollment despite the institution of supportive care measures (i.e., a pain management plan and/or treatment with hydroxyurea); painful episodes related to priapism, osteonecrosis, or any sickle-related complication are acceptable	Silent cerebral infarct defined as an infarct-like lesion based on an MRI signal abnormality at least 3 mm in one dimension and visible in two planes on FLAIR or T2-weighted images (or similar image with 3D imaging) and documented neurological examination performed by a neurologist, demonstrating the participant has a normal neurological examination or an abnormality on examination that could not be explained by the location of the brain lesion(s)
Administration of regular RBC transfusion therapy, defined as receiving ≥8 packed RPB transfusions per year in the 12 months before enrollment to prevent vaso-occlusive clinical complications (i.e., pain, stroke, and ACS)	
An echocardiographic finding of TRJV ≥2.7 m/sec	

ACS acute chest syndrome, *TCD* transcranial Doppler, *MRI* magnetic resonance imaging, *RBC* red blood cell, *TRJV* tricuspid valve regurgitant jet velocity

iii. It is notable there were few patients in their cohort with significant disease complications including cirrhosis, dialysis, and pulmonary hypertension. Despite the severe organ damage, patients tolerated the conditioning regimen with all patients alive at day +100.
iv. PTCy improved donor engraftment with 83% engraftment at the 100 mg/kg dose compared with 33% in the patients who did not receive PTCy.

Gene Therapy

The concept that gene therapy could ameliorate human genetic diseases first emerged in the 1970s. This concept involves the delivery of a functional copy of the defective gene into a patient's own stem cells or manipulation of regulatory genes that are known to influence disease phenotype. This correction is achieved either via gene editing, gene silencing, or gene insertion/addition.

Because SCD arises from single amino acid substitution in "adult" βA-globin (Glu6Val) as a result of a single base substitution (A → T) in the first exon of the human βA-globin gene (HBB), SCD is an attractive target for curative approaches using gene therapy. Table 25.4 below summarizes the available clinical trials in the USA for gene therapy in SCD.

a. Gene transfer and addition strategies have significantly improved over the past decade and became more precise and efficient.
b. The first successful report of gene therapy for a patient with SCD was reported in 2017 [46].

   i. At age 13, this patient underwent bone marrow harvest. The bone marrow-enriched CD34+ cells were transduced with LentiGlobin BB305 vector.

      - The patient received MA conditioning with intravenous busulfan (total AUC was 19,363).
      - After a 2-day washout period, transduced CD34+ cells (5.6×10^6 CD34+ cells/kg) were infused.
      - Red-cell transfusions were continued after transplantation until a large proportion of HbAT87Q (25 to 30% of total hemoglobin) was detected.
      - A level of therapeutic anti-sickling globin (HbAT87Q) of ~50% with biological parameters typical of SCD trait was rapidly achieved.
      - Subsequent multicenter clinical trials HGB-205/206 with the same vector demonstrated the importance of several factors especially the number of the transplanted CD34+ hematopoietic stem cells (HSCs) and transduction protocol [47].
      - Initial studies showed importance of high CD34 and recommended infusing > an average of 7×10^6 CD34+ cells/kg with a vector copy number (VCN) ≥2 for BB305 LV. To achieve this number of CD34+ cells, recent studies used PBSC instead of marrow and along with plerixafor (Mozobil®) mobilization; collections yielded up to 24.5 x 10^6 CD34/kg [48, 49].

c. Gene editing (GE) studies, on the other hand, can be divided into those intended to elevate HgbF to therapeutic levels and those repairing the underlying sickle β^S-globin mutation.

Table 25.4 Current gene therapy trials for SCD in the USA

Clinical trial #	Phase	LV/nuclease	Site/sponsor
NCT02140554 (HGB206)	1/2	BB305 LV	BlueBird bio
NCT02186418	1/2	sGbG LV	Children's Hospital Medical Center, Cincinnati, OH
NCT02247843	1	βAS3-FB LV	University of California Children's Hospital
NCT03745287	1	CRISPR/Cas9 (BCL11A enhancer)	Vertex Pharmaceuticals Incorporated and CRISPR Therapeutics

i. GE relies on the application of engineered nucleases with programmable specificity via zinc-finger nucleases and transcription-activator-like effector nucleases (TALENs) or CRISPR-Cas9 systems.
ii. In 2019, Erica Esrick, MD, presented an abstract at the American Society of Hematology (ASH) conference, reporting the outcomes of feasibility of gene therapy study NCT 03282656 that used BCH-BB694-transduced autologous CD34+ cells in three adult patients aged 21–26 years.

- BCH-BB694 is lentiviral vector (LVV) encoding a shRNA targeting BCL11A embedded in a microRNA scaffold (shmiR), allowing erythroid-specific knockdown to induce γ-globin expression and concomitantly and coordinately repress β-sickle globin expression [50].
- While early data suggest an acceptable safety profile, validation of BCL11A as effective target for HgbF induction in humans was shown. High numbers of F cells in circulation containing high levels of HgbF per F cell were seen, mitigating the cellular pathology of SCD.

d. Although these approaches seem very promising, challenges remain that need to be addressed:

i. Conditioning regimens used in conjunction with autologous gene therapies include MA doses of busulfan. These regimens carry risk for both early transplant-associated toxicity and late effects such as infertility and second malignancies. Therefore, novel regimens that could promote engraftment without such risks are needed to extend autologous therapies more broadly especially in adults with comorbidities.
ii. Access to therapy: The expected high cost of gene therapy is a barrier to a widespread utilization of this potentially transformative therapy especially in Africa where the burden of this disease is the highest.

Conclusion

Allogeneic HCT, especially from an HLA-matched sibling, has a long track record of an ability to cure SCD with limited toxicities. This treatment strategy has been utilized mostly in the severe phenotype of SCD; however, the recent data by DREPAGREFFE study encourage hematologists and transplant physicians to offer MSD HCT early in the disease course before patients suffer end organ or other severe disease manifestation. Only a limited percentage of patients have HLA-matched siblings; therefore, alternative sources of stem cells are needed. The data emerging regarding haploID HCT with PTCy for GvHD prophylaxis are especially exciting as this procedure will expand the donor pool for SCD patients. Additionally, haploID HCT studies have demonstrated sustained engraftment even with RIC regimens, which make this option especially attractive for adult SCD patients who have many comorbidities. Finally, gene editing and gene addition studies to replace the

abnormal gene or augment fetal hemoglobin production are ongoing; however, for these studies to be successful, gene transfer to the hematopoietic stem cell population must be efficient and provide long-term gene expression and cure.

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