Curative options for sickle cell disease: haploidentical stem cell transplantation or gene therapy?

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Summary

Haematopoietic stem cell transplantation (HSCT) is curative in sickle cell disease (SCD); however, the lack of available matched donors makes this therapy out of reach for the majority of patients with SCD. Alternative donor sources such as haploidentical HSCT expand the donor pool to nearly all patients with SCD, with recent data showing high overall survival, limited toxicities, and effective reduction in acute and chronic graft-versus-host disease (GVHD). Simultaneously, multiple gene therapy strategies are entering clinical trials with preliminary data showing their success, theoretically offering all patients yet another curative strategy without the morbidity and mortality of GVHD. As improvements are made for alternative donors in the allogeneic setting and as data emerge from gene therapy trials, the optimal curative strategy for any individual patient with SCD will be determined by many critical factors including efficacy, transplant morbidity and mortality, safety, patient disease status and preference, cost and applicability. Haploidentical may be the preferred choice now based mostly on availability of data; however, gene therapy is closing the gap and may ultimately prove to be the better option. Progress in both strategies, however, makes cure more attainable for the individual with SCD.

Keywords: alternative donor, gene therapy, haploidentical, hematopoietic stem cell transplantation, sickle cell disease.

Sickle cell disease (SCD) describes a group of haemoglobinopathies characterized by chronic anaemia, vaso-occlusive crisis (VOC), chronic pain, cerebrovascular disease, multi-organ damage and early mortality (Paulukonis et al., 2016). Newborn screening, penicillin prophylaxis, and vaccinations have contributed significantly to reduction in early childhood mortality (Lanzkron et al., 2013); however, few therapeutic options currently exist that manage the complications of the disease. While there are numerous targeted therapies under investigation aimed at disease modulation (Morrone et al., 2018), haematopoietic stem cell transplantation (HSCT) remains the only available curative option for the few patients with a matched sibling donor (Walters et al., 1996b; Hsieh et al., 2009; Hsieh et al., 2014), with transplantation from alternative donors and gene therapy being investigated in multiple clinical trials.

Between 1986 and 2013, over 1000 patients received an HLA-identical sibling HSCT and >90% were cured (Gluckman et al., 2017). HSCT is now an established therapeutic option when a patient has a clinical indication and a human leukocyte antigen (HLA)-identical sibling donor. However, <15% of patients have such a donor and only 19% have a matched unrelated donor (MUD) (Walters et al., 1996a; Gragert et al., 2014; Justus et al., 2015). Alternative donor sources offer more patients the chance for cure, though high rates of rejection and graft-versus-host disease (GVHD) currently limit the broad use of these therapies. Reducing rejection rates and transplant-related morbidity and mortality is therefore the aim of ongoing haploidentical donor trials. Transplantation with gene-modified autologous HSCs is theoretically available to all patients who would otherwise qualify for transplantation, and eliminates both graft rejection and GVHD associated with allogeneic HSCT. Although both allogeneic transplantation with expanded donor sources and autologous transplantation after gene modification are theoretically available to all, the reality is that the need for considerable expertise and high costs of these therapies limit their availability to primarily developed countries. Curative therapies in their current form are therefore not a substitute for public health measures that reduce disease burden in the countries where the majority of patients with SCD are born.
After decades of research, both allogeneic HSCT and gene-modified autologous HSCT offer curative options for patients with SCD, the former with robust data from thousands of patients and the latter only just beginning clinical trials (Leonard & Abraham, 2018; Leonard & Tisdale, 2018). Here we describe HSCT options, with a focus on haploidentical HSCT as compared to gene therapy models. As improvements are made in the allogeneic setting and as data emerge from gene therapy trials, many factors discussed here become critical factors in determining the optimal curative strategy for patients with SCD.

**Alternative donor HSCT**

Given the lack of eligible patients with an HLA-matched sibling, optimizing alternative donor HSCT is critical for offering more patients an opportunity for cure (Fitzhugh et al., 2017a; Joseph et al., 2018). Data regarding unrelated cord blood or bone marrow (BM) donors for patients with SCD remain limited, however, particularly given less common, more diverse haplotypes in Africans than the Caucasian population, and an underrepresentation of ethnic minorities in donor registries worldwide (Krishnamurti et al., 2003; Switzer et al., 2013).

The current experience of unrelated donor and mismatched donor HSCT for SCD is limited by small numbers of patients treated, and demonstrates varying results from high rates of rejection and treatment related mortality (Kamani et al., 2012; Shenoy et al., 2016) to 100% overall survival (OS) with high rates of acute GVHD and viral infection (Abraham et al., 2017a). Successful outcomes have been reported (Gilman et al., 2017; Marzollo et al., 2017), but indications and an optimal treatment regimen have not been established and therefore use remains limited.

Haploidentical transplantation promises an expanded donor pool of biological parents, biological children, full- or half-siblings, or even extended family donors. An HLA-haploidentical donor shares exactly one HLA haplotype with the recipient but is mismatched for a variable number of HLA genes on the unshared haplotype. The major challenge of HLA-haploidentical HSCT is therefore the bidirectional alloreactivity leading to high incidences of graft rejection and GVHD. Unlike patients with haematologic malignancies who are pretreated with chemotherapy and irradiation, patients with SCD may have a higher risk of graft rejection due to an intact and robust immune system, a lifetime of anaemia and chronic inflammation, and higher incidence of alloimmunization. To broaden the availability of transplantation to a much larger fraction of patients, improving engraftment without increasing GVHD is the goal, particularly as there is no benefit of graft-versus-tumour effect in non-malignant diseases, and the substitution of one chronic, debilitating disease for another is generally unacceptable.

### Haploidentical transplantation results in sickle cell disease

Success at improving engraftment without increasing GVHD in the haploidentical model would mean expanding a curative option to nearly all patients with SCD (Bolanos-Meade et al., 2012). Novel strategies to accomplish these two goals are focused on T-cell depletion methods that (i) reduce recipient T cells that recognize the disparate HLA of the haploidentical donor cells and cause graft rejection, and (ii) reduce donor T-cell-mediated GVHD. Methods to deplete T cells include in vivo post-transplant cyclophosphamide (PTCy) and ex vivo CD34+ positive selection of donor grafts.

### Post-transplant cyclophosphamide

In contrast to non-selective in vivo T-cell depletion with agents such as anti-thymocyte globulin (ATG) or alemtuzumab that have long half-lives (Bunn et al., 1996; Morris et al., 2003), high-dose PTCy targets depletion of alloreactive T cells while preserving immune reconstitution and protective immunity. In vivo, high-dose CY given on days 3 and 4 post-HSCT is highly cytotoxic to both donor and recipient proliferating T cells (Luznik et al., 2008), yet spares HSCs secondary to their high levels of the enzyme aldehyde dehydrogenase, responsible for metabolizing the drug (Emadi et al., 2009).

The largest initial experience of HLA-haploidentical transplantation in SCD demonstrated that PTCy given on days +3 and +4 was highly effective in reducing GVHD (Bolanos-Meade et al., 2012) (Table I). No patient developed either acute or chronic GVHD, PTCy was well tolerated and there was no transplant-related mortality (TRM); however, rejection remained a critical obstacle (42.8%). Two key possibilities could explain this high rate of rejection: insufficient immunosuppression with immunologically mediated graft rejection, or insufficient myelosuppression with host HSCs outcompeting donor cells. Theoretically both mechanisms could be overcome by increasing total body irradiation (TBI) in the preparative regimen from 200 cGy to 400 cGy and with this change, the rejection rate improved from 42.8% to 6% (n = 1) (Bolanos-Meade et al., 2019). Thirteen (76%) of 17 patients achieved full donor chimaerism, three (18%) had mixed donor-host chimaerism, and all patients were alive at a median follow-up of 705 days. Rates of acute and chronic GVHD were 29% and 18%, with resolution of GVHD in all patients at most recent follow-up, and 88% of patients off immunosuppression. In another study, 23 patients with severe disease (n = 21 with SCD, n = 2 with β-thalassaemia) were treated with non-myeloablative conditioning including 400 cGy TBI, alemtuzumab, escalating doses of PTCy, and sirolimus for GVHD prevention (Fitzhugh et al., 2017c). By eliminating fludarabine (Flu), this regimen could be extended to patients with severe organ damage, including renal failure.
<table>
<thead>
<tr>
<th>Number</th>
<th>Age range (years)</th>
<th>T-cell depletion method</th>
<th>Median CD34+ cell dose (range)</th>
<th>Median CD3+ cell count (range)</th>
<th>Preparative regimen</th>
<th>GVHD prophylaxis</th>
<th>OS</th>
<th>EFS</th>
<th>Rejection</th>
<th>TRM</th>
<th>GVHD (acute/chronic)††</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolanos-Meade et al., 2012</td>
<td>14</td>
<td>15–42</td>
<td>PTCy</td>
<td>5.24 × 10^6/kg (4.4–8.7)</td>
<td>3.61 × 10^5/kg (3.29–6.59)</td>
<td>Flu 30 mg/m², CY 15 mg/kg, TBI 200 cGy, ATG</td>
<td>CY 50 mg/kg × 2, Tacrolimus or sirolimus, MMF</td>
<td>100%</td>
<td>50%</td>
<td>42.3% (n = 6)</td>
<td>0</td>
</tr>
<tr>
<td>Dhedin et al. (2016)</td>
<td>22</td>
<td>3–18</td>
<td>PTCy</td>
<td>3.63 × 10^6/kg (NR)</td>
<td>NR</td>
<td>Azathioprine + HC followed by Flu 150 mg/m², CY 29 mg/kg, TT 10 mg/kg, ATG, TBI 200 cGy</td>
<td>CY 50 mg/kg × 2, sirolimus, MMF</td>
<td>82%</td>
<td>86%</td>
<td>NR</td>
<td>14% (n = 3)</td>
</tr>
<tr>
<td>Marzullo et al., 2017c</td>
<td>23*</td>
<td>20–56</td>
<td>PTCy</td>
<td>12.2 × 10^6/kg (7.0–29.7)</td>
<td>3.8 × 10^5/kg (1.86–8.1 × 10^5)</td>
<td>TBI 400 cGy, alemtuzumab</td>
<td>Sirolimus, CY 0 mg/kg (cohort 1), 50 mg/kg (cohort 2), or 100 mg/kg (cohort 3)</td>
<td>87%</td>
<td>0 cohort 1 25% (n = 2)</td>
<td>65%</td>
<td>0</td>
</tr>
<tr>
<td>Webking et al. (2017)</td>
<td>3</td>
<td>5–20</td>
<td>PTCy</td>
<td>7.5 × 10^6/kg (3.5–8.3)</td>
<td>60 × 10^5/kg (54–251)</td>
<td>Flu 30 mg/m², CY 15 mg/kg, TT 5 mg/kg, treosulfan 14 g/m², alemtuzumab</td>
<td>CY 50 mg/kg × 2, MMF, tacrolimus</td>
<td>100%</td>
<td>100%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fuente (2018)</td>
<td>15†</td>
<td>12–26</td>
<td>PTCy</td>
<td>3 × 10^6/kg (2.5–4.8)</td>
<td>NR</td>
<td>Flu 30 mg/m², CY 14.5 mg/kg, TT 10 mg/kg, TBI 200 cGy, ATG</td>
<td>CY 50 mg/kg × 2, sirolimus, MMF</td>
<td>100%</td>
<td>93%</td>
<td>6% (n = 1)</td>
<td>0</td>
</tr>
<tr>
<td>Frangoul et al. (2018)</td>
<td>4</td>
<td>12–23</td>
<td>PTCy</td>
<td>NR</td>
<td>NR</td>
<td>Flu 30 mg/m², CY 14.5 mg/kg, TT 10 mg/kg, TBI 200 cGy</td>
<td>CY 50 mg/kg × 2, MMF, sirolimus</td>
<td>100%</td>
<td>100%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pavlova et al. (2018)</td>
<td>4</td>
<td>13–23</td>
<td>PTCy</td>
<td>1.86–5 × 10^6/kg</td>
<td>NR</td>
<td>Flu 40 mg/m² + dexamethasone 25 mg/m² followed by BU 120 mg/m², Flu 35 mg/m², ATG</td>
<td>CY 50 mg/kg × 2, tacrolimus or MMF</td>
<td>100%</td>
<td>100%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bolanos-Meade et al., 2019</td>
<td>17‡</td>
<td>6–31</td>
<td>PTCy</td>
<td>4 × 10^6/kg (2.71–6.14)</td>
<td>4.5 × 10^5/kg (2.9–7.1)</td>
<td>Flu 30 mg/m², CY 14.5 mg/kg, TBI 400 cGy, ATG</td>
<td>CY 50 mg/kg × 2, sirolimus or MMF</td>
<td>100%</td>
<td>94%</td>
<td>6% (n = 1)</td>
<td>0</td>
</tr>
<tr>
<td>Dallas et al. (2013)</td>
<td>8</td>
<td>4–17</td>
<td>CD3/CD19 depletion selection</td>
<td>25.4 × 10^6/kg (6–57)</td>
<td>0.07 × 10^6/kg (0.006–0.168)</td>
<td>BU 900 ng/ml, Flu 150–200 mg/m², TT 10 mg/kg, ATG, murine–CD3 (n = 3) or HC + azathioprine followed by BU 900 ng/ml, TT 10 mg/kg, CY 200 mg/kg, murine–CD3 (n = 5)</td>
<td>CY 50 mg/kg × 2, sirolimus or MMF</td>
<td>75%</td>
<td>38%</td>
<td>38%</td>
<td>25% (n = 2)</td>
</tr>
<tr>
<td>Gilman et al. (2017)</td>
<td>8</td>
<td>8–23</td>
<td>CD34 + selection</td>
<td>18 × 10^6/kg (9–25)</td>
<td>&lt;1 × 10^6/kg</td>
<td>Flu 40 mg/m², TT 5 mg/kg, melphalan 140 mg/m², ATG</td>
<td>DLL MTX</td>
<td>88%</td>
<td>88%**</td>
<td>15% (n = 1)</td>
<td>15% (n = 1)</td>
</tr>
<tr>
<td>Marzullo et al. (2017)</td>
<td>2</td>
<td>13–16</td>
<td>CD3 and CD19 depletion</td>
<td>14.5 × 10^6/kg (NR)</td>
<td>NR</td>
<td>None</td>
<td>None</td>
<td>100%</td>
<td>100%</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Notes:
- GVHD: Graft-versus-host disease
- OS: Overall survival
- EFS: Event-free survival
- Rejection: Acute and chronical rejection
- TRM: Treatment-related mortality
- PTCy: Post-transplant cyclophosphamide
- CY: Cyclophosphamide
- Flu: Fluouracil
- ATG: Anti-thymocyte globulin
- MMF: Mycophenolate mofetil
- MTX: Methotrexate
- DLL: Donor lymphocyte infusion
- CD19: CD19 depletion
- CD3: CD3 depletion
Table I. (Continued)

<table>
<thead>
<tr>
<th>Number</th>
<th>Age range (years)</th>
<th>T-cell depletion method</th>
<th>Median CD34 + cell dose (range)</th>
<th>Median CD3 + cell count (range)</th>
<th>Preparative regimen</th>
<th>GVHD prophylaxis</th>
<th>OS</th>
<th>EFS</th>
<th>Rejection</th>
<th>TRM</th>
<th>GVHD (acute/chronic)††</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaziev et al. (2018)</td>
<td>145</td>
<td>3–15</td>
<td>TCRβ, CD19 + depletion</td>
<td>15–7 × 10^6/kg (NR)</td>
<td>NR</td>
<td>Preconditioning with Flu 150 mg/m², HC, azathioprine; BU 14 mg/kg, TT 10 mg/kg, CY 200 mg/kg, ATG CsA, MMF or CsA, methylprednisolone</td>
<td>CaA, MMF or CaA, methylprednisolone</td>
<td>84%</td>
<td>64%</td>
<td>14%</td>
<td>14%</td>
</tr>
<tr>
<td>Fodil et al. (2017)</td>
<td>20</td>
<td>3–31</td>
<td>CD3+/CD19 + depleted (n = 15) TCRβ, CD19 + depletion (n = 5)</td>
<td>13–10 × 10^6/kg (8–78)</td>
<td>14 × 10^7/kg (4–706)</td>
<td>MMF, CaA or tacrolimus</td>
<td>Flu 40 mg/m², TT 5 mg/kg, treosulfan 14 g/m², ATG</td>
<td>90%</td>
<td>90%</td>
<td>NR</td>
<td>10%</td>
</tr>
</tbody>
</table>

ATG, Anti-thymocyte globulin; BU, busulfan; CaA, ciclosporin; CY, cyclophosphamide; DLL, donor lymphocyte infusion; EFS, event-free survival; Flu, fludarabine; GVHD, graft-versus-host disease; HC, hydroxy-carbamide; MMF, mycophenolate mofetil; NR, not reported; PTCy, post-transplant cyclophosphamide; OS, overall survival; SCD, sickle cell disease; TBI, total body irradiation; TCR, T-cell receptor; TRM, treatment related mortality; TT, thiopeta.

* Cohort included a total of 21 patients with SCD and two with β-thalassaemia.
† Cohort who received thiopeta (n = 15), after the stopping rule was met when two of three patients had engraftment failure without thiopeta (n = 3) in the first cohort.
‡ Cohort included a total of 12 patients with SCD and five with β-thalassaemia.
§ Cohort included a total of three patients with SCD and nine with β-thalassaemia.
¶ One patient had 11% chimerism but no symptoms of SCD at follow-up >700 days.
** One patient rejected initial graft but engrafted after second haploidential transplant.
†† GVHD grade II–IV.
Event-free survival (EFS) improved to 50% with two doses of PTCy compared to zero or one. There was no grade II–IV acute GVHD and a single case of mild ocular chronic GVHD. An additional protocol is ongoing which employs pentostatin and oral CY pretransplant to further deplete recipient lymphocytes in an attempt to decrease the rate of graft rejection (NCT02105766).

Another method to potentially improve engraftment in haploidentical HSCT is the addition of thiopeta (TT) which may be done without increasing morbidity or mortality (Table I). The addition of TT to the CY, Flu, TBI, ATG backbone (NCT03240731) decreased the rejection rate from 60% (3 of 5) to 6% (1 of 15) after TT was added, with acute and chronic GVHD rates of 20% and 6%, respectively, and 100% OS after a median follow-up of 13 months (de la Fuente et al., 2019). A second cohort in this study transplanted 22 paediatric patients with azathioprine and hydroxy-carbamide (HC) pretreatment showing 9% graft rejection, 18% acute and chronic GVHD, and an OS of 86-4% (Dhdedin et al., 2016). Other smaller studies with TT reported 100% EFS and OS with no rejection or TRM (Wiebking et al., 2017; Frangoul et al., 2018). One study (n = 4) reported 100% acute GVHD that responded to treatment (Frangoul et al., 2018); otherwise no chronic GVHD was seen in either study. Similar to pretransplant HC, pretransplant immune suppression with Flu and dexamethasone demonstrated an EFS of 100% (Pawlowska et al., 2018); however, 75% developed chronic GVHD (n = 3).

These results of HLA-haploidentical transplantation with PTCy demonstrate high OS, limited toxicity, and effective reduction in acute and chronic GVHD in most studies. The increase in TBI to 400 cGy and the addition of TT to the preparative regimen appears to improve engraftment and is being assessed in an ongoing multicentre trial adding TT to the standard backbone (Flu, Cy, TBI, ATG, with two days of PTCy) plus preconditioning with HC (BMT CTN 1507, NCT03263559).

Ex vivo T-cell-depleted approach

The ex vivo model of CD3+/CD19−/CD19+ (n = 15) or T-cell receptor γδ (TCRγδ) (n = 5) depletion, reporting EFS and OS of 90%, TRM of 10% (n = 2), and acute and chronic GVHD rates of 35% and 20%, respectively (Foell et al., 2017) (Table I). All patients had full donor chimaerism, though high rates of viral reactivation/infection were noted. According to the authors, results were comparable to HLA-matched sibling donors utilizing the same preparative regimen. In a smaller series, 14 patients with thalassaemia (n = 11) or SCD (n = 3) received TCRγδ and CD19− depletion after preconditioning with Flu, HC, and azathioprine followed by conditioning with busulfan (BU), TT, CY, and ATG (Gaziev et al., 2018). EFS and OS were 84% and 64%, respectively, graft failure occurred in two thalassaemia patients (14%), and acute and chronic GVHD was 28% and 21%, respectively. One patient died of chronic GVHD. Viral reactivation was common, including a 23% incidence of Epstein–Barr-virus-related post-transplant lymphoproliferative disorder. Compared to a historical group of patients who received CD34+-selected grafts (n = 32) or CD34+-selected and CD3+/CD19−-depleted grafts (n = 8), TCRγδ/CD19−-depleted grafts were associated with a reduced incidence of graft failure (54% vs. 14%, $P = 0.048$); however, delayed immune reconstitution and associated morbidity and mortality was similarly problematic between groups.
The addition of various T-cell depletion methods after CD34 selection has minimized rejection after HLA-haploidentical transplantation; however, GVHD and delayed immune reconstitution with infectious complications remain problematic. Current and future work to optimize the haploidentical model includes trials investigating pretransplant immunosuppressive therapy (NCT03279094, NCT02757885), non-myeloablative preparative regimens (NCT02678143, NCT01850108, NCT03240731, NCT03263559) with a CD34⁺-selected graft (NCT02165007, NCT01461837), a CD4⁺ T-cell-depleted graft (NCT03249831), a CD3⁵⁺CD19⁻-depleted graft with CD45⁺RA⁻ depletion (NCT03653338), or utilizing peripheral blood stem cells (PBSCs) after non-myeloablative conditioning (NCT03077542).

Historical perspective: gene therapy in sickle cell disease

The concept that gene therapy may ameliorate human genetic diseases emerged in the 1970s after discovery that viruses could serve as a gene delivery system (Rogers & Pfuderer, 1968). Proof of principle and clinical trials emerged throughout the 1990s and early 2000s initially in primary immunodeficiency disorders (PID) (Rosenberg et al., 1990; Cavazzana-Calvo et al., 2000; Hacein-Bey-Abina et al., 2002; Goebel & Dinauer, 2003; Ott et al., 2006; Boztug et al., 2010), with early trials notable for low efficiency of viral transduction, lack of sustained engraftment, incomplete reversal of phenotype, and transformation toward genotoxicity (Malech, 1999; Cavazzana-Calvo et al., 2000; Goebel & Dinauer, 2003; Ott et al., 2006; Hacein-Bey-Abina et al., 2008; Aiuti et al., 2009; Stein et al., 2010; Grez et al., 2011; Candotti et al., 2012; Braun et al., 2014). While effective gene therapy for haemoglobinopathies including β-thalassaemia and SCD has always been an area of significant interest, challenges associated specifically with β-globin expressing vectors, and the need for regulated, lineage-specific, high-level globin expression, pro-longed the transition from the bench to the bedside. By the start of the first clinical trials for haemoglobinopathies in the late 2000s, vector design was safer and more efficient, all while new genome editing techniques were starting to emerge. After identification of the β-globin locus control region (LCR), newer lentiviral constructs utilizing a mutant β-globin gene where glutamine is substituted for threonine at amino acid 87 (βT87Q, LentıGlobin BB305) conferring anti-sickling properties, resolved anaemia, reduced organ damage and expressed up to 20–25% vector haemoglobin with 2-2-3 vector copies per HSC in SCD mouse models (Pawluk et al., 2001). The βASS vector added two additional γ-globin-based substitutions to βT87Q and was similarly able to express 20–25% βASS at 2-2 vector copies per HSC (Levasseur et al., 2003). Other vectors that introduced the γ-globin gene to increase fetal haemoglobin (Hbf) also showed success (Persons et al., 2001; Pestina et al., 2009).

The large size of the β-globin transgene and gene silencing were other initial challenges. For haemoglobinopathies in general, viral vectors were initially insufficient to carry the large globin gene and the regulatory elements required for high-level expression. Vector design has since improved with the use of human immunodeficiency virus (HIV)–based lentiviral vectors and modified LCRs. As for retroviral potential to undergo expression variation and silencing, the addition of insulators such as the chicken hypersensitive site-4 (cHS4), ankyrin (AnkT9W), and FB (βASS-FB) insulators may promote safety and efficiency of gene expression (Aru mugam et al., 2007; Emery, 2011; Breda et al., 2012; Romero et al., 2013).

While early gene therapy methodologies focussed on gene addition strategies, there are now multiple potential strategies for gene therapy in SCD: addition of β-globin to make adult haemoglobin (HbA) or γ-globin to increase Hbf expression, editing of globin regulatory elements, knockdown of Hbf repressors to increase Hbf expression, or direct, site-specific gene editing of the sickle mutation with targeted nucleases. Programmable nucleases such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and meganucleases diminish off-target effects (OTEs) and are able to successfully correct the SCD mutation or induce fetal globin by editing regulatory sequences such as BCL11A, KLF1 and MYB (Tasan et al., 2016). However, programming of these enzymes is difficult, time consuming, and requires significant expertise. The recent discovery of clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9), however, has revolutionized genome editing strategies, introducing a methodology that is easy to design, highly efficient, and inexpensive (Doudna & Charpentier, 2014; Sternberg et al., 2014). A side by side comparison of ZFNs, TALENs, and CRISPR/Cas9 in their ability to modify the β-globin locus demonstrated superiority in the CRISPR/Cas system (Huang et al., 2015). Preclinical studies are promising and have shown therapeutically relevant upregulation of Hbf (Traxler et al., 2016; Antoniani et al., 2018) and successful targeted correction with production of HbA after erythroid differentiation and/or engraftment in immunodeficient mice (Dever et al., 2016; DeWitt et al., 2016; Hoban et al., 2016; Park et al., 2019). Overall, the advantages of targeted gene editing over gene addition include precise gene correction and the ability to significantly reduce or entirely avoid non-specific integration that may lead to insertional oncogenesis. Gene editing also does not have the same risk of losing efficacy due to gene silencing which may occur with gene addition strategies.

Successful gene modification for SCD includes several basic principles: (i) high efficiency gene transfer into HSCs that is safe with minimal genotoxicity, (ii) consistent, integration site-independent, high-level expression of the inserted gene in addition strategies, (iii) phenotypic correction and disease amelioration, and (iv) erythroid lineage-specific and
Table II. Gene therapy results in patients with sickle cell disease.

<table>
<thead>
<tr>
<th>ClinicalTrials.gov identifier</th>
<th>Gene therapy method/study objective</th>
<th>Number</th>
<th>Age range (years)</th>
<th>CD34+ cell dose × 10⁶/kg</th>
<th>DP VCN</th>
<th>Hb (g/l)</th>
<th>Vector-derived Hb (g/l)</th>
<th>OS</th>
<th>Follow-up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribeil et al. (2017), Cavazzana et al. (2017)</td>
<td>To evaluate the safety and efficacy of the LentiGlobin BB305 drug product in subjects with either beta-thalassaemia major or severe SCD (HGB-205)</td>
<td>3</td>
<td>13–21</td>
<td>3–0.5–6</td>
<td>0.5–1.2</td>
<td>Total: 8.8–12.4</td>
<td>1.5–6.1</td>
<td>100%</td>
<td>3.4–31.7*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group A: 16–5-1 Group B: 2-2-3-2 Group C: 3-8</td>
<td></td>
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<tr>
<td></td>
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<td>Group A: 0-3-1-3 Group B: 14-5-0 Group C: 28-5-6</td>
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<td>Total: 7-1-11-4 (Group A)</td>
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<td>Group A: 0.5–1-2</td>
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<td>Group B: 3.2–7-2</td>
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<td>Group C: 32–8-8 (n = 4) Total: 10-9</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Esrick et al. (2018)</td>
<td>To evaluate feasibility of HSC gene transfer for SCD using a lentiviral vector containing a short-hairpin RNA targeting BCL11a gene</td>
<td>3</td>
<td>NR</td>
<td>3-3-6-7</td>
<td>3.3–5-1</td>
<td>HbF/ (HbF + HbS): 29.7%</td>
<td>NR</td>
<td>100%</td>
<td>76 days†</td>
</tr>
<tr>
<td>Malik et al. (2018)</td>
<td>To determine whether transfer of a fetal haemoglobin gene using a Gamma Globin Lentiviral Vector into human blood cells is safe and feasible in patients with SCD</td>
<td>2</td>
<td>25–35</td>
<td>1–6-9</td>
<td>0.22–0.46</td>
<td>HbF*/ (HbF* + HbS): 20–21%</td>
<td>2-1</td>
<td>100%</td>
<td>6–12</td>
</tr>
</tbody>
</table>

BM, bone marrow; HSC, haematopoietic stem cell; NR, not reported; SCD, sickle cell disease.

*As of June 2, 2017,
†As of May 15, 2018,
$Data from one patient,
¶As of July 28, 2018.
developmental stage-specific expression of the inserted gene. After decades of ongoing research, gene therapy for the cure of SCD is now a reality and is being investigated in multiple clinical trials. Gene addition strategies are further along in clinical application, though base editing may ultimately prove advantageous if high efficiency and safety are demonstrated.

### Clinical gene therapy results in sickle cell disease

Most initial clinical approaches to gene therapy in SCD involve virally-mediated gene addition strategies where lentiviral vectors engineered with anti-sickling β-globin or γ-globin cassette become integrated into the host cells’ genome for long-term persistence after the cells are re-transplanted following myeloablative conditioning. As of June 1, 2019, there are eight clinical trials investigating various methodologies for gene therapy in SCD, including various gene addition strategies as well as one study investigating CRISPR/Cas9 editing (NCT02140554, NCT03282656, NCT02186418, NCT02247843, NCT02151526, NCT035653247, NCT03964792, NCT03745287), with preliminary data available from four clinical trials (Table II).

**T87Q gene addition strategies**

HGB-205 (NCT02151526) and HGB-206 (NCT02140554) were the first gene therapy trials opened for patients with SCD (Table II). Both are open-label phase 1 studies designed to evaluate the safety and efficacy of gene therapy by transplantation of autologous CD34⁺ HSCs transduced ex vivo with Lentiglobin BB305 in three patients in France (HGB-205) and up to 50 adults with severe SCD in the US (HGB-206). The first report was a 13-year-old male with HbS homozygous for sickle haemoglobin) treated on HBG-205, reporting a total haemoglobin level of 12.4 g/l 30 months post-gene therapy, with 6.1 g/l (49%) of the patient’s haemoglobin attributable to β_T87Q (Cavazzana et al., 2017; Ribeil et al., 2017). The patient had no clinical symptoms or complications of SCD until, at about 30 months post-treatment, the patient developed pain following an episode of acute gastroenteritis with fever and dehydration and was subsequently hospitalized. Two more patients with SCD have been treated on this protocol, with six and three months of follow-up reported as of 2017 (Cavazzana et al., 2017). At approximately six months post-treatment, one patient was hospitalized for acute chest syndrome. Neither patient had undergone transfusions since Day 21 and 15 post-infusion, with a total Hb of 8:8 and 9:8 g/l and HbA_T87Q of 1:8 and 1:5 g/l, respectively, as of last follow-up.

As of May 15, 2018, 22 patients enrolled on HGB-206 have had HSCs collected, 18 had drug product (DP) made, and 15 have been treated (Tisdale et al., 2018; Mapara et al., 2019). For both HGB-205 and the initial cohorts of HGB-206 (Groups A and B), patient CD34⁺ HSCs were collected via BM harvest (Kanter et al., 2018). In 2016, the HGB-206 protocol was modified for Group B to increase DP vector copy number (VCN), require preharvest transfusions, increase target busulfan levels, and in Group C, explore the use of plerixafor for mobilization and apheresis for cell collection. Reports suggest higher CD34⁺ cells/kg yield after plerixafor mobilization, improved transduction efficiency, and improved HSC quality compared to BM from subjects with SCD (Uchida et al., 2011; Tisdale et al., 2017; Boulad et al., 2018; Essrick et al., 2018; Lagresle-Peyrou et al., 2018; Leonard et al., 2019). In HGB-206, a median CD34⁺ yield was reported of 4.3 (0·1–10·8) × 10⁶ and 10·4 (3·8–21·6) × 10⁶ cells/kg (Mapara et al., 2019). At last visit, HbA_T87Q levels were higher in Group B (3·2–7·2 g/dl) versus Group A (0·5–1·2 g/dl). In four Group C patients at the three-month visit, HbA_T87Q (4·1 [3·2–6·0] g/dl) levels were equal to or exceeded HbS levels (3·3 [2·8–3·8] g/dl). In one Group C patient at the six-month visit, HbA_T87Q was 8·8 g/dl and total Hb was 14·2 g/l. In both HGB-205 and HGB-206, the safety profile post-DP infusion was consistent with myeloablative conditioning and underlying SCD. No adverse events (AEs) related to Lentiglobin BB305 have been reported to date.

Two additional studies (NCT02247843, NCT03964792) are phase 1 studies assessing safety and feasibility of the βAS3 vector. Results have not been published for NCT03964792, whereas NCT03964792 is expected to start September 1, 2019.

**Gene therapy strategies targeting increased fetal haemoglobin expression**

Two studies investigating virally-mediated γ-globin modification in subjects with SCD have published preliminary data in the first several subjects (NCT02186418 and NCT03282656) (Table II). NCT02186418 is a phase 1/2 study investigating the efficacy and safety of γ-globin gene transfer, whereas NCT03282656 is investigating the safety of lentivirally mediated transfer of a short-hairpin RNA (shRNA) targeting BCL11A for increased γ-globin expression. Similar to HGB-205 and HGB-206, patients receiving γ-globin-modified (GM)-HSCs (NCT02186418) had autologous HSCs collected via BM harvest (patient 1 and 2) and/or plerixafor-mobilized collection (patient 2) (Malik et al., 2018). Unlike the Lentiglobin studies where patients received a myeloablative dose of BU, patients received a reduced intensity conditioning (RIC) regimen of a single dose of intravenous melphalan (140 mg/m² BSA) 36 h prior to infusion of GM-HSC. Results for two patients with HbS-b~0~ thalassaemia are currently available with a follow-up of 6 and 12 months, respectively. Patient 1 received 1 × 10⁶ CD34⁺ cells/kg (VCN 0·22), whereas patient 2 received 6.9 × 10⁶ CD34⁺ cells/kg after the addition of plerixafor mobilization to BM harvest (VCN 0·46). AEs were melphalan- and SCD-related, and integration site analysis performed on the infused products demonstrated a highly polyclonal pattern of...
integration. As both patients had been transfused HbA containing RBCs in the initial six months, HbF*/(HbF* + HbS) was calculated (HbF* indicates an additional point mutation in the γ-globin gene allowing distinction from endogenous HbF by HPLC), and was 20% in patient 1 at one year post-transplant, and 21% in patient 2 at Day 180 post-transplant. Both patients had a VCN of 0–2–0.4 detected in all lineages, with an increase of 2-1 g/l HbF* detected in patient 1 (total Hb 10.6 g/l). The authors note that pain, narcotic use, and hospital admissions decreased in both patients post-transplantation.

Erythroid-specific expression of microRNA-adapted shRNAs (shRNamiR) targeting BCL11A effectively induced HbF in human erythroid cells derived from transduced HSCs (Brendel et al., 2016) and is currently being investigated (NCT03282656). In the first cohort of patients ≥18 years old, 3–3.6–7 × 10^6 CD34^-cells/kg were collected in three patients after plerixafor mobilization and apheresis (Esrick et al., 2018). CD34^-cells were transduced with the shRNamiR vector, demonstrating a VCN of 3–3.5–1 copies per cell and >95% vector-positive CD34^-derived colonies. As of July 28, 2018 one subject had received the infusion of gene-modified cells after myeloablative BU conditioning. At a follow-up of 76 days, the patient’s Hb was stable at 10.9 g/l (10.4 pre-transplantation) with an increase in HbF/(HbF + HbS) ratio from 0.8% to 29.7%. The number of F cells had risen to 59.7% with 12 pg HbF/F cell, and in flow-sorted immature erythroid cells γ-globin mRNA was >80% of total β-like globins in the graft-derived population and BCL11A protein was reduced by approximately 90%. AEs were consistent with myeloablative conditioning, and there have been no product-related AEs or SCD-related complications.

CRISPR/Cas9 targeted genome editing in sickle cell disease

Gene editing strategies with CRISPR technology have more unanswered questions, specifically regarding efficiency and specificity of cutting and editing, potential immunogenicity of editing tools or edited cells, and optimal delivery (Demirci et al., 2019). Clinical trials for gene editing will likely focus first on editing HbF expression before trials aim to directly correct the sickle mutation, primarily as the former does not require homology-directed repair. Much of the current research remains in non-human animal models, though the first CRISPR-mediated clinical trial in patients with SCD has recently been opened and is investigating CRISPR-mediated HbF expression. This trial is an open-label, international and multi-site, single-dose phase 1/2 study in up to 12 subjects 18–35 years of age with severe SC disease evaluating the safety and efficacy of autologous CRISPR-Cas9-modified CD34^-human HSC targeting the BCL11A erythroid lineage-specific enhancer (CTX001). This study was posted November 19, 2018, with information regarding recruitment and/or preliminary results not yet published.

Haploidentical transplantation versus gene therapy

As allogeneic transplantation methods such as haploidentical HSCT are being refined, gene therapy for the cure of SCD is now a reality with multiple open clinical trials and preliminary evidence showing early success. As more curative options become available for more patients, the optimal curative strategy must consider multiple factors including transplant efficacy, short- and long-term transplant-associated morbidity and mortality, conditioning regimens, patient disease status, donor/patient match, safety, patient preference, donor availability, cost and applicability. Whether haploidentical HSCT or gene therapy is superior as a curative strategy is based on the considerations discussed below and summarized in Table III.

Efficacy

Initial studies of haploidentical HSCT in patients with SCD were marked by high rates of graft rejection and TRM (Bolanos-Meade et al., 2012; Dallas et al., 2013; Dhedin et al., 2016) (Table I). In the last seven years since the first report of haploidentical HSCT in patients with SCD (Bolanos-Meade et al., 2012), improvements in preparative regimens have decreased the rate of rejection from approximately 50% to 0–6% in the PTCy setting and 0–14% in the ex vivo T-cell depletion setting (Foell et al., 2017; Gilman et al., 2017; Marzollo et al., 2017; Wiebking et al., 2017; Frangoul et al., 2018; Gaziev et al., 2018; Pawlowska et al., 2018; de la Fuente et al., 2019). Follow-up for these most recent studies is between 12 and 60 months.

Preliminary outcome data are currently available for 21 patients treated on four gene therapy trials (Cavazzana et al., 2017; Ribell et al., 2017; Esrick & Bauer, 2018; Kanter et al., 2018; Malik et al., 2018; Mapara et al., 2019) (Table II). All patients treated with LentiGlobin BB305 (n = 18) had a stable increase in vector-derived Hb. T87Q levels were higher (3.2–8.8 g/l) in HGB-206 Groups B and C (n = 6) than in patients in HGB-205 and HGB-206 Group A (0.5–6.1 g/l) who were treated prior to implementation of a refined processing method and plerixafor mobilization. At least five patients (n = 1 from HGB-205, n = 4 from HGB-206 Group C) are producing HbA_T87Q levels equal to or exceeding HbS levels rendering these patients effectively with sickle cell trait. Data from the three patients treated with vectors aimed at increasing HbF report significant increases in HbF expression. The authors report improved haematologic parameters (Kanter et al., 2018), decreased pain (Kanter et al., 2018; Malik et al., 2018) and decreased admissions (Malik et al., 2018) in their respective cohorts, including those with minimal vector-derived Hb where ‘even modest HbA_T87Q production may improve the clinical status of patients with SCD’ (Kanter et al., 2018). Follow-up for these gene therapy studies is between 76 days and ≥2 years.
Though rejection rates for haploidentical HSCT now rival rates of rejection in the matched sibling setting, all recent patients who have received genetically modified autologous HSCs demonstrate engraftment with measurable vector-derived haemoglobin and no signs of rejection of gene-modified cells. Refinements in gene therapy processing methods have improved the amount of vector-derived haemoglobin such that several patients now effectively have sickle cell trait, with many showing clinical improvements in SCD complications after gene therapy.

**Short-term transplant morbidity and mortality**

Though cohort numbers are small, EFS and OS in the most recent haploidentical studies with PTCy are 93–100% and 100%, respectively (Wiebking et al., 2017; Frangoul et al., 2018; Pawlowska et al., 2018; de la Fuente et al., 2019) and 88–100% for both EFS and OS in the ex vivo T-cell depletion setting (Foell et al., 2017; Gilman et al., 2017; Marzollo et al., 2017) (Table I). OS is 100% in all gene therapy patients, with EFS harder to measure in this setting (Table II).

HSC morbidity is significantly influenced by the preparative conditioning regimen regardless of allogeneic versus autologous HSCT methodology. Unlike current haploidentical HSCT protocols, almost all gene therapy studies utilize myeloablative conditioning with BU in order to maximize marrow repopulation with genetically modified cells (exception, NCT02186418). In the allogeneic setting, 20–25% donor myeloid chimaerism is sufficient to reverse the sickle phenotype (Abraham et al., 2017b; Fitzhugh et al., 2017b), and therefore RIC is possible and preferred. RIC allows a greater number of patients access to curative therapies who may have substantial comorbidities and be otherwise unable to tolerate myeloablation. Myeloablative therapy is limited by the short- and long-term toxicities of BU, including but not limited by infertility and gonadal failure, secondary malignancy, and more severe organ toxicity in patients who have impaired organ function pre-HSCT or have been exposed to multiple red blood cell (RBC) transfusion prior to HSCT. One patient from HGB-206 (Group A) developed myelodysplastic syndrome (MDS) within three years of therapy (Mapara et al., 2019), felt to be related to BU and not related to LentiGlobin gene therapy as the patient’s CD34⁺ blasts showed negligible amounts of vector integration versus the CD34⁻ marrow cells (0.02 vs. 0.21 copies per diploid genome) (Mapara et al., 2019). MDS has also been reported in the haploidentical setting, however, likely as a result of TBI and/or PTCy (Fitzhugh et al., 2017c). Though advantages of non-myeloablative versus myeloablative regimens are clear, it is also true that stability of mixed chimaerism in the long term is uncertain under current haploidentical protocols. Current investigations into non-myeloablative monoclonal antibody (MAB) conditioning, whereupon blood counts and immune cell function are preserved, would significantly alter the morbidity of both autologous and allogeneic HSCT (Czechowicz et al., 2019).

**Autologous HSCT with gene-modified cells eliminates the risk of GVHD, a major source of morbidity and mortality in the allogeneic HSCT setting.** While haploidentical T-cell depletion methods have lowered the rates of acute and chronic GVHD, it is not fully eliminated and can lead to significant patient morbidity. Current data suggest superior

### Table III. Summary of considerations: haploidentical transplant versus gene therapy.

<table>
<thead>
<tr>
<th>Transplant factors</th>
<th>Haploidentical transplant</th>
<th>Gene therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Efficacy</strong></td>
<td>Low rejection rate for haploidentical HSCT with refined preparative regimens (0–6% in PTCy, 0–14% in ex vivo T-cell depletion)</td>
<td>Recent recipients of genetically modified autologous HSCs demonstrate improved engraftment with measurable vector-derived haemoglobin and no signs of rejection of gene-modified cells</td>
</tr>
<tr>
<td><strong>Short-term morbidity and mortality</strong></td>
<td>Documented cure in numerous patients</td>
<td>Currently paucity of data in the gene therapy setting</td>
</tr>
<tr>
<td><strong>Long-term safety</strong></td>
<td>Safety in the haploidentical setting relates to HSCT morbidity and mortality, with more robust data to date than gene therapy</td>
<td>Safety data for lentiviral-mediated gene therapy trials currently suggest no risk of AEs related to the DP</td>
</tr>
<tr>
<td><strong>Patient autonomy</strong></td>
<td>Factors such as experience, outcome and safety data, risk to family members in an allogeneic setting, and ethical considerations influence patient decisions</td>
<td>Factors such as ability to safely collect a sufficient number of HSCs for gene therapy, cost, insurance coverage, and expertise limit exportability more so for gene therapy</td>
</tr>
<tr>
<td><strong>Applicability</strong></td>
<td>HLA match considerations and HSCT centre expertise for haploidentical HSCT limit patient participation</td>
<td></td>
</tr>
</tbody>
</table>
GVHD prevention utilizing PTCy over ex vivo T-cell depletion methods, though patient numbers are small in both settings.

**Long-term safety**

Issues surrounding safety in the haploidentical HSCT setting centre around the effects of short-term transplant morbidity as previously discussed. Gene therapy, however, has more unanswered questions regarding long-term safety, particularly given experiences of the past in PID (Malech, 1999; Cavazzana-Calvo et al., 2000; Goebel & Dinauer, 2003; Ott et al., 2006; Hacein-Bey-Abina et al., 2008; Aiuti et al., 2009; Stein et al., 2010; Grez et al., 2011; Candotti et al., 2012; Braun et al., 2014).

The setbacks of the early clinical trials in gene therapy revealed the most significant risk of virally-mediated gene transfer: genotoxicity. In contrast to gammaretroviral vectors that were initially utilized leading to oncogenesis and myelodysplasia by inserting into regulatory regions such as promoters, enhancers, locus control regions, or oncogenes (Ott et al., 2006; Hacein-Bey-Abina et al., 2008; Stein et al., 2010; Braun et al., 2014), lentiviral vectors tend to integrate into the body of genes rather than into regulatory regions thereby lowering the overall risk of neoplastic transformation. Integration site analyses using high-throughput sequencing approaches have not shown preferential integration near oncogenes, nor significant vector-driven clonal expansion (Kohn, 2018). To date, no DP-related AEs, vector-mediated replication-competent lentivirus, or clonal dominance have been reported in any current gene therapy trial for SCD.

The safety of targeted editing with CRISPR/Cas9 is not as clear compared to the safety of lentiviral vectors. The theoretical advantage to site-specific gene correction is the reduction in off-target insertion, the requirement for only transient delivery of the engineered nuclease and repair template to achieve correction, and the lack of permanent insertion of foreign DNA into the genome. Treating cells with CRISPR/Cas9 and a β-globin donor gene for repair may result, however, in multiple genetic outcomes, ranging from healthy (both alleles corrected) to β-thalassaemia major (both alleles disrupted) (Esrick & Bauer, 2018). As precise correction in long-term HSCs is not yet efficient and editing results in reduction in engrafting HSCs (Dever et al., 2016; Hoban et al., 2016), transplantation of mixed culture could be clinically problematic and possible unintended consequences should be addressed before clinical trials.

Safety data for lentiviral-mediated gene therapy trials currently suggest no risk of AEs related to the DP. However, outcome data are in its infancy, with no clinical data utilizing CRISPR/Cas9 editing to date in patients with SCD. Safety in the haploidentical setting relates to HSCT morbidity and mortality, with more robust data to date.

**Patient autonomy**

For those with a clinical indication for HSCT, the importance of a shared decision-making model among providers, patients and their families is imperative. Potential risks and benefits of choosing to either decline or undergo HSCT, and now which type, are essential to preserve patient autonomy and provide informed consent (Nickel & Kamani, 2018).

For some data-driven patients, there is more experience and information regarding outcomes and safety in the haploidentical setting compared to gene therapy. The recent improved outcomes in the haploidentical setting, however, rival cohort numbers similar to those of the gene therapy trials, therefore risks and benefits of the long-term outcomes may be equivalent between options at this current date.

As public awareness of the success of gene therapy grows, the very mention of the term HIV turns patients who historically have a justified mistrust of the medical community away. It is the responsibility of the medical community to clearly and concisely inform patients there is absolutely no risk of HIV transmission. Explaining complex science in a patient-friendly and understandable manner is essential, and applies in all transplant settings where therapy is complex and requires months to years of follow-up, multiple medications, patient compliance, and family and community support. HSCT involves the commitment not only of the patient, but also of family members who support him/her through the process. Gene therapy eliminates the need and risks posed to a family HSC donor, however ethical issues surrounding transplantation in children who cannot consent remain.

**Applicability**

Median HSCT cost per patient is estimated at $467 747 (range: $344 029–$799 219) (Arnold et al., 2017), though this may be nearly 50% lower in patients who receive a non-myeloablative regimen (Saenz & Tisdale, 2015). Costs for gene therapy are less certain and rumoured to be as high as $900 000- $2.1 million (Jensen, 2019), severely limiting real-world applicability. The gene therapy cost model may need to shift from fee-for-service to value-based payment systems (Daniel et al., 2017). One proposal is 80% of the cost of gene therapy is put at risk to prove the value of its treatment. After an initial upfront charge, DP manufacturers would only get paid if the one-time infusion continues to benefit patients. As a chronic disease, SCD management becomes costlier over time with total lifetime charges to an individual living to age 50 exceeding $8 million, rising from patient fees of $200 000 at ages 0–5 to over $7 million for patients 17–50 years of age (Ballas, 2009). Whereas the upfront costs of HSCT and gene therapy are high, quality-adjusted life-years (QALYs) gained and the potential to reduce overall lifetime healthcare costs may render curative therapy cost-effective (Leonard & Tisdale, 2018). Such costs, however, limit these therapies to only developed countries. Non-toxic MAb
conditioning regimens would dramatically change the exportability of HSCT to the non-developed world, though ability to collect donor/patient HSCs will always be required and may be a limitation. Ultimately, expertise in these therapies is required, and at present date given the breadth of experience in the allogeneic setting, exportability may be more suited for allogeneic HSCT.

There are also additional patient factors that may limit either therapy. Patients with a history of antibodies directed against either donor HLA or red cell antigens may preclude a patient from undergoing haploidentical HSCT due to increases in graft rejection and red cell aplasia post-HSCT. Desensitization approaches such as plasmapheresis combined with anti-B cell antibodies (rituximab) and immune modulators (bortezomib) have been used to overcome these concerns in some cases (Ciurea et al., 2009; Ciurea et al., 2018). With regard to gene therapy, success is dependent on safely obtaining a sufficient quantity of autologous SCD patient HSCs capable of lifelong engraftment. In patients with SCD, steady-state BM harvesting is associated with suboptimal HSC quality and yield (Leonard et al., 2019), and PB mobilization with granulocyte colony stimulating factor is contra-indicated (Abbond et al., 1998; Adler et al., 2001; Fitzhugh et al., 2009) though single-agent plerixafor mobilization appears to be safe and effective (Tisdale et al., 2017; Bould et al., 2018; Esrick et al., 2018; Lagresle-Peyrou et al., 2018). Some patients may be unable to tolerate stem cell collection due to high risk of anaesthesia and fluid shifts in a BM harvest or PB collection. Others may ultimately not mobilize a sufficient number of CD34+ cells following plerixafor administration to generate a gene therapy product. It is likely these patient factors will be less of a concern in paediatric patients who have better overall organ function and less exposure to RBC antigens from repeated lifetime RBC transfusions, and is a compelling reason to offer these therapies earlier in the disease course.

Patient factors, the ability to safely collect a sufficient quantity of autologous HSCs, and especially the current proposed cost of gene therapy severely limits the applicability of this therapy. The future of gene therapy may involve direct vector injection, cutting down on the complex and costly manufacturing process and rendering this therapy more affordable and exportable. For widespread applicability of gene therapy in SCD, however, long-term data showing success, improvements in HSC collection and conditioning regimens, increased expertise, changes to insurance payment and dramatic reductions in cost are required which will likely take decades to achieve. Ultimately, however, certain patient factors may limit applicability of either therapy.

Conclusion
While haploidentical HSCT and gene therapy are currently experimental, haploidentical HSCT outcomes are improving and gene therapy trials show promise supporting continued investigation of both approaches. Either strategy may one day be available for most patients, with many factors ultimately determining which will be best for each individual patient. Patients will no longer be limited to a single universal cure available only to a few, but rather several options will be available that are capable of reducing disease burden and improving outcomes and quality of life. In the current day haploidentical transplant with the longer track record for efficacy, safety and applicability is likely to be utilized more but gene therapy is closing the gap and will possibly be the more common option in the future. Given the increased focus for curative therapies in SCD, the ultimate winner here is the patient.

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Conflicts of interest
The authors have no conflicts of interest to disclose.

Author contributions
AL, JT and AA reviewed the data and wrote the paper.

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