

Review

THERAPEUTIC CHALLENGES IN CHILDHOOD SICKLE CELL DISEASE PART 1: CURRENT AND FUTURE TREATMENT OPTIONS

Sickle cell disease (SCD) is one of the commonest inherited diseases in the UK, affecting approximately 1 in 4000 live births every year. For the majority of patients, the mainstays of treatment are preventative and supportive. For those children with severe SCD, three major therapeutic options are currently available: blood transfusion, hydroxyurea and bone marrow transplantation. This review focuses on the relative roles of these therapeutic modalities in severe paediatric SCD and assesses the prospects for new treatment modalities, including non-myeloablative stem cell transplantation, short chain fatty acids, membrane active drugs and gene therapy.

CURRENT THERAPEUTIC OPTIONS IN SEVERE SICKLE CELL DISEASE IN CHILDHOOD

After decades with little new to offer patients with sickle cell disease (SCD), there have been several advances in the last 10–15 years which have contributed both to improved quality of life and to an increase in life expectancy, at least for those patients with access to treatment in developed countries. This two-part review outlines the roles of different treatment options in severe paediatric sickle cell disease (SCD). In the first part, we review the three principal current therapeutic modalities [blood transfusion, hydroxyurea and bone marrow transplantation (BMT)] and potential future treatments, and in the second part we describe an evidence-based, problem-orientated approach to some of the major complications of SCD in childhood.

BLOOD TRANSFUSION

Red cell transfusion is widely used in the management of SCD. Approximately 50% of all patients receive a red cell transfusion at some stage in their lives and 5% are on chronic transfusion programmes (Rosse *et al.*, 1990). Nevertheless, few controlled trials have rigorously evaluated either the indications for transfusion in SCD or the best protocols to use. The requirements for red cell transfusion in patients with sickle cell disease have been reviewed elsewhere (Davies & Roberts-Harewood, 1997; Ohene-Frempong, 2001; Vichinsky, 2001). The major indications for transfusion in SCD are summarized in Table I.

Red cell transfusion for acute problems

The main indications for 'top-up' red cell transfusion in children are severe anaemia complicating acute splenic or hepatic sequestration (Emond *et al.*, 1985) and aplastic crises due to parvovirus B19 infection (Serjeant *et al.*, 2001). In this situation, the haemoglobin should be raised to the child's steady state (it should never be raised acutely to >10 g/dl as this may cause an increase in blood viscosity).

For most other severe complications of SCD, exchange rather than top-up transfusion has significant advantages. For severe acute chest crises with hypoxia despite continuous positive airway pressure (CPAP) or mechanical ventilation, exchange transfusion is the treatment of choice to reduce sickling and increase oxygen carriage without an increase in viscosity (Schmalzer *et al.*, 1987; Emre *et al.*, 1995). Despite a lack of controlled studies, acute exchange transfusion has been shown in observational studies to prevent pulmonary complications, shorten the duration of the acute illness and reduce mortality in children with acute chest syndrome (Lanzkowsky *et al.*, 1978; Emre *et al.*, 1995). Exchange transfusion is also used in the acute management of SCD patients presenting with a new stroke or transient ischaemic attacks. Although widely practised and supported on the theoretical grounds of improving perfusion and oxygenation in the region of the infarct, the value of red cell transfusion in the acute management of stroke is unclear as it may not influence long-term neurological outcome (Ohene-Frempong, 1991). Exchange transfusion appears to improve survival in the acute multi-organ failure which occasionally complicates the course of SCD, although there are few data available for children (Hassell *et al.*, 1994). Exchange transfusion, rather than top-up transfusion, is also the preferred option for hyperhaemolysis secondary to malaria (Newton *et al.*, 1997) as it enables the removal of infected and damaged red cells as well as treating the anaemia.

Controversial uses of red cell transfusions for acute episodes in childhood SCD include their role in ameliorating prolonged vaso-occlusive crises and in treating priapism. There is no evidence that red cell transfusion reduces the severity or duration of established painful vaso-occlusive crises. The evidence that red cell transfusion is useful in the management of priapism is anecdotal and mostly derives from studies carried out more than 20 years ago (Ohene-Frempong, 2001). In addition, it is quite frequently ineffective (McCarthy *et al.*, 2000) and may be associated with severe neurological events (Rackoff *et al.*, 1992).

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Table I. Indications for transfusion in children with sickle cell disease.

Top-up
Splenic sequestration*
Hepatic sequestration*
Aplastic crises*
Exchange transfusion
Chest syndrome*
Stroke*
Hepatic failure*
Priapism†
Mesenteric syndrome†
Hypertransfusion
Stroke (to prevent recurrence)*
Renal failure (to prevent/delay deterioration)†
Chronic sickle lung disease†
Osteonecrosis‡
Leg ulcers‡
Surgery§
Selected patients preoperatively (e.g. joint replacement)

*Proven value.

†May help.

‡No proof of value shown yet.

§See text.

Modified from Davies and Roberts-Harewood, 1997.

Peri-operative red cell transfusions

On the basis of anecdotal evidence and reports of small series of patients, many centres give pre-operative red cell transfusions with the aim of reducing complications in patients with SCD undergoing anaesthesia and surgical procedures. The largest study to examine the role of transfusion in the preoperative management of SCD was a randomized study comparing exchange transfusion (to achieve Hb of > 10 g/dl and HbS fraction of < 30%) with simple transfusion (to a Hb of > 10 g/dl) (Vichinsky *et al*, 1995). This showed no difference in the frequency of post-operative sickle chest syndrome, fever, infection or painful crises between the two treatment arms, while allo-immunization and haemolytic transfusion reactions occurred more frequently after exchange transfusion (Vichinsky *et al*, 1995). This indicates that where peri-operative transfusion is required, 'top-up' transfusion to produce a Hb of around 10 g/dl would be the preferred option, exchange transfusion being limited to high-risk surgery (such as organ transplantation, hip/knee replacement and eye surgery) and to patients with a high baseline haemoglobin.

The question of which procedures are safe to carry out in SCD children without pre-operative red cell transfusion remains controversial because of a lack of evidence from controlled clinical trials (Riddington & Williamson, 2001); however, there are data from small series which indicate that, depending on the prior clinical course of the individual patient, minor and straightforward procedures (such as tonsillectomy and cholecystectomy) can often be safely undertaken without transfusion (Griffin & Buchanan, 1993; Hatley *et al*, 1995; Davies & Roberts-Harewood, 1997; Haberkern *et al*, 1997).

Chronic transfusion therapy

The main indications for chronic transfusion in children with SCD are the prevention of recurrent stroke (Pegelow *et al*, 1995) and, more recently, in the prevention of a first stroke in children with abnormal blood flow identified by transcranial Doppler ultrasonography (Adams *et al*, 1998). In children with sickle-related stroke, chronic transfusion reduces recurrence by about 90% (Pegelow *et al*, 1995). Most patients are commenced on a hypertransfusion regimen (aiming to maintain the HbS below 25% and the Hb between 10 and 14.5 g/dl) but exchange transfusion may be used to minimize iron overload (Cohen *et al*, 1992; Kim *et al*, 1994). The optimal duration of therapy has not been determined as recurrence has been reported even after 12 years of transfusions (Wang *et al*, 1991).

Chronic transfusion programmes have also been used to prevent recurrence of acute chest syndrome, to reduce the frequency of painful crises and in chronic heart failure or renal failure, although there are no clinical trials to confirm their efficacy in children (Davies & Olatunji, 1995; Ohene-Frempong, 2001). The strongest evidence for the use of chronic transfusion to reduce painful crises is a prospective randomized trial in pregnant patients with sickle cell disease, in which there was a significant reduction in the frequency of vaso-occlusive crises in the patients who were prophylactically transfused (Koshy *et al*, 1988). Since the introduction of hydroxyurea (see below), chronic transfusion for recurrent crises should be considered only as a last resort in view of the risks associated with long-term transfusions (Castro, 1999).

Complications of transfusion

The most common serious complications of transfusion in children with SCD are iron overload, allo-immunization and transfusion-transmitted infections, though problems with vascular access are also an extremely common practical problem especially for children on chronic transfusion programmes.

As in thalassaemia major, iron overload causes significant morbidity and mortality in SCD patients on chronic transfusion programmes (Ballas, 2001). In the recent study of 371 adult patients with SCD treated at Thomas Jefferson University Hospital in Philadelphia, 50% of the in-patients were transfused and around 10% of these patients were iron overloaded (Ballas, 2001). Mortality was significantly higher in the iron-overloaded patients (64% vs 5%), as was the proportion of patients with organ failure (71% vs 19%) (Ballas, 2001). There are no specific data for the impact of iron overload on morbidity and mortality in children with SCD. Iron overload can be minimized by limiting red cell transfusions to defined indications, by using manual or automated exchange transfusion rather than hypertransfusion (Kim *et al*, 1994), by providing intensive support to encourage compliance with desferrioxamine (Treadwell & Weissman, 2001) and by 'tailored' iron chelation regimens based on accurate assessment of tissue iron-associated damage (Anderson *et al*, 2001; Brittenham *et al*, 2001) and judicious use of the oral iron chelator, deferiprone (Olivieri

et al., 1998; Wonke *et al.*, 1998; Pippard & Weatherall, 2000; Ceci *et al.*, 2002; Maggio *et al.*, 2002).

The development of red cell allo-antibodies, most frequently anti-K, anti-E or anti-C, was reported in 18–36% of SCD patients (Vichinsky *et al.*, 1990). Red cell allo-immunization makes compatibility testing for future transfusions difficult and increases the risk of life-threatening, delayed haemolytic transfusion reactions (Syed *et al.*, 1996). The rate of allo-immunization is related to the number of units of blood received but is reduced by routine administration of phenotypically matched units at least for K, E and C (Vichinsky *et al.*, 1995, 2001). All SCD patients should, therefore, have extended red cell phenotyping, including ABO, Rh, K, Kidd, Duffy, Lewis and MNSs systems, soon after diagnosis and before transfusions are started so that red cells phenotypically matched for ABO, Rh and K can be selected as required (Davies & Roberts-Harewood, 1997; Olujuhunge *et al.*, 2001; Vichinsky *et al.*, 2001).

Children on regular transfusions are also at risk of transfusion-transmitted infections, although the rate of transmission has reduced in recent years, at least in well-resourced countries (Murphy *et al.*, 2001; Vichinsky, 2001). Hepatitis B and C remain the most serious risk: an American study showing that 10% of adults with SCD were infected with hepatitis C (Hasan *et al.*, 1996). The risk of hepatitis B is estimated at 1 in 63 000 units in the USA (Vichinsky, 2001) and 1 in 50 000–170 000 units in the UK (Murphy *et al.*, 2001), making hepatitis B vaccination an important part of management for all children with SCD. Repeated transfusions are also associated with a significant risk of transfusion-related lung injury and post-transfusion hyperhaemolysis characterized by destruction of both autologous and transfused red cells with negative serological findings (Cullis *et al.*, 1995).

HYDROXYUREA

Hydroxyurea exerts its beneficial effects in SCD via a number of mechanisms, including inhibition of intracellular

polymerization of HbS by mixed hybrid molecules ($\alpha_2\beta^S\gamma$) with higher solubility than HbS, modification of red cell-endothelial interactions and the rheological properties of HbS-containing red cells, and via its myelosuppressive effects, particularly on neutrophils. Its development, pharmacology and use in SCD have been the subject of a recent comprehensive review (Halsey & Roberts, 2003). Here we focus on the therapeutic aspects in children with SCD.

Efficacy of hydroxyurea in children with SCD

Clinical trials of hydroxyurea in children with SCD. There have now been more than a dozen published studies of hydroxyurea in children with SCD, although most involved small numbers (Ferster *et al.*, 1996; Jayabose *et al.*, 1996; Scott *et al.*, 1996; Kinney *et al.*, 1999; Koren *et al.*, 1999; de Montalembert *et al.*, 1999; Wang *et al.*, 2001; Ware *et al.*, 2002; see also Halsey & Roberts, 2003). The main findings of the four largest series are shown in Table II.

Ferster *et al.* (2001) recently reported the long-term follow-up on a cohort of paediatric SCD patients treated with hydroxyurea from the Belgian National Registry. There were 93 patients (87 children) with severe SCD (median age of 7 years) with a median follow-up of 3.5 years. The number of admissions to hospital and the number of days of hospitalization both dropped significantly in the patients on hydroxyurea, so that for those who completed 5 years of therapy over half had no significant vaso-occlusive crises during therapy. There was also a reduced frequency of acute chest syndrome (nine events) and splenic sequestration (one event) compared with that expected from historical series. These clinical benefits were paralleled by significant rises in the haemoglobin levels and HbF levels (% HbF), and no important adverse events were noted. The actual dose of hydroxyurea given in the study was usually less than 25 mg/kg/d and, as found in the multicentre study of hydroxyurea in sickle cell anaemia (MSH) in adults, the clinical benefit of hydroxyurea was seen without escalating the dose of hydroxyurea to the maximum tolerated dose (MTD) (Charache *et al.*, 1995).

Table II. Hydroxyurea treatment for childhood sickle cell disease.

	Mean number of VOC/year pre-HU	Mean number of VOC/year on HU	Pre-HU Hb	Hb at max dose	Number of transfusions/patient-year pre-HU	Number of transfusions/patient-year on HU	% HbF pre-HU	% HbF on HU
Kinney <i>et al.</i> (1999) (<i>n</i> = 84)	NA	NA	7.8	9.0 (<i>P</i> < 0.001)	NA	NA	7.3	15.5 (<i>P</i> < 0.0001)
Ferster <i>et al.</i> (2001) (<i>n</i> = 93)	2.76	1.2 (<i>P</i> < 0.05)	8.2	8.7 (<i>P</i> < 0.05)	NA	0.17	7.3	12.9 (<i>P</i> < 0.01)
Jayabose <i>et al.</i> (1996) (<i>n</i> = 14)	2.5	0.87 (<i>P</i> < 0.0001)	7.2	8.5 (<i>P</i> < 0.001)	1.83	0.2	3.9	17.8 (<i>P</i> < 0.0001)
Wang <i>et al.</i> (2001) (<i>n</i> = 28)	NA	NA	8.5	8.8 (<i>P</i> < 0.01)	NA	NA	21.8	20.3 (<i>P</i> < 0.001)*

*Compared with age-matched control subjects.
VOC, veno-occlusive crises; NA, not assessed.

Other studies in children have given similar results, with reductions in hospital admissions and adverse events (Jayabose *et al*, 1996; Koren *et al*, 1999; Maier-Redelsperger *et al*, 1999). Growth and development also appeared to be unaffected (Ware *et al*, 2002). Jayabose *et al* (1996) treated 14 children with a history of three or more painful crises or recurrent chest syndrome in the past year or with a Hb < 7 g/dl with escalating doses of hydroxyurea. The frequency of vaso-occlusive crises fell from 2.5 crises per patient-year prior to hydroxyurea to 0.87 crises per patient-year during treatment (a 65% reduction) with a median rise in Hb of 1.9 g/dl. However, given the open-label nature of both these studies, some of this benefit may have been due to more rigorous supportive care and/or placebo effects. de Montalembert *et al* (1999) have likewise reported a decrease in the number of painful crises in 27/28 children treated with hydroxyurea.

In the larger, multicentre phase I/II trial of hydroxyurea in children with sickle cell anaemia (HUG-KIDS) study (Kinney *et al*, 1999), 84 children between the ages of 5 and 15 years with a history of three or more painful crises in the previous year or recurrent chest syndrome were treated with hydroxyurea. This study did not address clinical efficacy, instead the major end-points were haematological response and safety. Hydroxyurea significantly increased haemoglobin levels, percentage HbF and percentage F-cells. The rise in percentage HbF was greater in children who had a higher baseline HbF level and Hb concentration, and in those who received a higher MTD (Ware *et al*, 2002). The most commonly observed toxicity was transient myelosuppression: no severe adverse effects occurred and there was no effect on growth. A number of other small-scale studies have been conducted in children with similar results (reviewed by Maier-Redelsperger *et al*, 1999). Taken together, these data indicate that hydroxyurea is likely to decrease the frequency of vaso-occlusive crises, acute chest syndrome and transfusion requirements in children at least as effectively as it does in adults.

There is no evidence yet that hydroxyurea reduces the recurrence or development of stroke in children or in adults. Similarly, there is no evidence in children that hydroxyurea reduces the SCD-related mortality. This is perhaps not surprising given the relatively low mortality of SCD in childhood and the fact that a significant reduction in mortality in adults was seen only after several years of follow-up in the MSH study (Steinberg *et al*, 1999).

Hydroxyurea in very young children with SCD. The role of hydroxyurea in this age group remains to be defined. Two small studies have been reported. Hoppe *et al* (2000) treated eight children aged 2–5 years (median 3.7 years) and found no unexpected toxicity, and normal growth and development. However, there were no patients with severe SCD and efficacy could not be assessed in such a small study (Hoppe *et al*, 2000). A second study (Wang *et al*, 2001) treated even younger children (aged 6–28 months) with homozygous SCD or S- β^0 thalassaemia to address whether early use of hydroxyurea might be useful in the primary prevention of organ damage. This study revealed no adverse effects of hydroxyurea in a group of 28 children, and confirmed the

preservation of normal growth and development reported in older children. There was also a possible beneficial effect on preservation of splenic function. Unfortunately, two of the 28 children experienced neurological events despite being maintained on hydroxyurea (Wang *et al*, 2001). Thus, starting hydroxyurea early in life did not prevent major complications of SCD and, until further data from randomized controlled trials are available, there is no indication for the use hydroxyurea in this way in very young children.

Factors that predict response to hydroxyurea. The clinical and haematological response to hydroxyurea is variable both in adults and children. In part this may reflect patient compliance, although this can be difficult to assess. In the HUG-KIDS study, baseline HbF level, baseline Hb level, the MTD and compliance were all significantly associated with a higher rise in percentage HbF in hydroxyurea-treated children (Ware *et al*, 2002), and other studies have shown an association between higher baseline neutrophil and reticulocyte counts and absence of the Bantu (CAR) haplotype with above-average increases in HbF levels. While these studies suggest that, at a population level, selected baseline laboratory parameters, a higher MTD and attention to compliance may be useful in predicting the HbF response to hydroxyurea, the clinical and haematological response to this drug is complex and variable so that these parameters cannot be used to predict a response in an individual patient.

Toxicity of hydroxyurea in children. The short-term side-effects of hydroxyurea in children are the same as those in adults. The commonest side-effects are dose-dependent myelosuppression, which is usually transient but occasionally more prolonged (Vichinsky & Lubin, 1994), nausea and vomiting, and skin rashes (Kinney *et al*, 1999; de Montalembert *et al*, 1999). In the long term, the most worrying potential risk of hydroxyurea in children is that of leukaemogenicity. While data from hydroxyurea-treated children with cyanotic heart disease are reassuring (Triadou *et al*, 1994), two cases of haematological malignancy have been reported in children with SCD treated with hydroxyurea: a 10-year-old with Ph-positive ALL (Ferster *et al*, 2001) and an 8-year-old with Hodgkin's disease (Moschovi *et al*, 2001), although the duration of treatment (7 weeks and 6 months respectively) makes it unlikely that these malignancies were secondary to hydroxyurea therapy. No cases of haematological malignancy have been reported in the adults in the largest series, the MSH study, suggesting that the risk of leukaemic transformation is low. Nevertheless, continued caution and long-term follow-up is essential (de Montalembert & Davies, 2001), and it may be relevant that recent *in vitro* studies have shown an increased mutation rate in children treated with hydroxyurea for 7–30 months, as measured by the VDJ recombination assay (Hanft *et al*, 2000).

BONE MARROW TRANSPLANTATION

Bone marrow transplantation (BMT) remains the only curative therapy for SCD. The role of BMT in managing severe SCD is discussed in detail in *Part 2* of this review:

summarized here are the criteria used to identify the patients most likely to benefit from BMT, the conditioning regimens used and the outcome of BMT.

Patient selection

Patient selection is aimed at identifying individuals with SCD who will benefit most from BMT, while excluding those at an unacceptably high risk of transplant-related morbidity and mortality due to pre-existing organ damage. This underlines the importance of a rigorous pretransplant work-up, including formal assessment of pulmonary, cardiac, hepatic and renal function, as well as detailed neurological assessment with magnetic resonance imaging, angiography and neuropsychometric studies. The current British Paediatric Haematology Forum criteria for selection of patients with SCD for BMT (Davies, 1993) are shown in Table III. The Seattle collaborative study have similar inclusion criteria but specify that patients undergoing BMT for recurrent painful vaso-occlusive crises should have had > 2 episodes per year for 3 years consecutively, and also broaden the indications to include sickle nephropathy with a glomerular filtration rate (GFR) of 30–50%, bilateral proliferative retinopathy and major visual impairment, osteonecrosis of multiple joints, and red cell allo-immuniza-

tion (> 2 antibodies) during long-term transfusion therapy (Walters *et al.*, 1996a).

It is estimated that fewer than 10% of children with SCD fulfil these criteria, of whom only one in five will have a matched sibling donor (Davies & Roberts, 1996). Among 4848 patients that were < 16-year-old reported to the Seattle collaborative study, 315 (6.5%) met the entry criteria, of these only 41% had tissue typing performed (24% had no sibling available) and 14% had a human leucocyte antigen (HLA)-identical sibling (Walters *et al.*, 1996a). Thus in this study, the major barrier to BMT for SCD was lack of an HLA-identical donor rather than parental/physician refusal or lack of financial/psychosocial support. Nonetheless, for those patients who are eligible and do have an available donor, the paediatric haematologist needs to be sensitive to cultural issues, particularly the risk of infertility, which clearly do influence the decision on whether to transplant.

Results of BMT for SCD

Approximately 150 patients with SCD, nearly all < 16 years of age, have been transplanted worldwide. The majority of these have been reported in three major series, the results of which are summarized in Table IV. The Seattle collaborative

Table III. Indications for BMT in childhood SCD.*

Criteria for inclusion	
1.	Age < 16 years and HLA-matched sibling donor
2.	One or more of the following:
	(a) Sickle cell disease related neurological deficit, stroke or subarachnoid haemorrhage
	(b) > 2 episodes acute sickle chest syndrome and stage 1 chronic sickle lung disease
	(c) Recurrent, severe debilitating pain due to vaso-occlusive crises
	(d) Problems relating to future medical care, e.g. unavailability of adequately screened blood products
Exclusions	
1.	Donor with major haemoglobinopathy
2.	One or more of the following: (a) Karnofsky performance score < 70%, (b) Major intellectual impairment,
	(c) Moderate/severe portal fibrosis, (d) Glomerular filtration rate < 30% predicted, (e) Stage III and IV sickle lung disease,
	(f) Cardiomyopathy, and (g) HIV infection

*Paediatric Haematology Forum criteria (Davies, 1993).

Table IV. Results of major published series of BMT for sickle cell disease.

	Walters <i>et al</i> (1996a, 2000) (n = 50)	Bernaudin <i>et al</i> (1997) (n = 26)	Vermynen <i>et al</i> (1998) (n = 50)
Median follow-up (months)	39	55	60
Overall survival	94% (6 years)	92% (8 years)	93% (11 years)
Event-free survival	84% (6 years)	75% (8 years)	82% (11 years)
Graft rejection/autologous reconstitution	10%	18%	10%
Stable mixed chimaerism	8.5%	0%	12.5%
Acute GVHD ≥ grade 2	7.7%	23%	20%
Acute GVHD ≥ grade 3	3.8%	Not available	2%
Chronic GVHD, limited	Not available	7.7%	14%
Chronic GVHD, extensive	3.8%	7.7%	6%

group, which includes the Hammersmith and Birmingham Children's Hospitals in the UK (Walters *et al*, 1996b, 2000), and the French group (Bernaudin *et al*, 1997) have reported a total of 76 children transplanted for symptomatic SCD. In contrast, the Belgian group (Vermylen *et al*, 1998) has reported the results of 36 children transplanted because of previous morbidity, but also 14 asymptomatic patients transplanted at a much younger age because they were to return to countries where medical care was not optimal. All patients received conditioning with busulphan 14–16 mg/kg or 485 mg/m² and cyclophosphamide 200 mg/kg. In the initial French and Belgian patients, no serotherapy was used but, in view of high early rates of mixed chimaerism and rejection, most groups have now incorporated pretransplant antilymphocyte globulin or Campath into their conditioning regimens (Walters *et al*, 1996b; Bernaudin *et al*, 1997).

Survival. The results show projected overall survival of 92–94% and event-free survival of 75–84% at 6–11 years in the three series. Deaths were from complications of acute and chronic graft-versus-host disease (GVHD; $n = 5$), intracranial haemorrhage ($n = 1$) and sudden death 6 years post BMT ($n = 1$). All patients with stable, predominantly donor, engraftment became free of the clinical manifestations of SCD. In the Belgian series, the results with the asymptomatic patients were superior to those transplanted for symptomatic disease, with overall survival of 100% vs 88% and event-free survival of 93% vs 76%: in view of this, they have proposed giving parents the option of early transplantation regardless of symptomatology if a matched sibling donor is available.

Engraftment, rejection and chimaerism. Primary graft failure was uncommon (3% overall), but the cumulative incidence of graft rejection was 10–18%, which in all but one patient was accompanied by autologous reconstitution. Several of the patients who rejected their grafts have developed increased HbF concentrations, which ameliorated their disease severity for several years (Ferster *et al*, 1995). No clear risk factors have been associated with rejection but, in the French series, inclusion of serotherapy in the conditioning regimen decreased the incidence of mixed chimaerism/rejection from 25% to 7%. Both the Seattle collaborative and the Belgian studies have reported a number of patients with stable mixed chimaerism, all of whom remained asymptomatic.

Toxicity. Patients with SCD are at increased risk of neurological complications after BMT, particularly seizures and intracranial haemorrhage (Walters *et al*, 1995). In their initial cohort, the Seattle collaborative study reported neurological complications in seven of 21 patients, with seizures in six patients and intracranial haemorrhage in three patients. The overall incidence of neurological complications was not different in patients with or without pretransplant stroke (this may reflect subclinical vasculopathy in those without a prior history of stroke), but mortality (25% vs 0%) and intracranial haemorrhage (38% vs 0%) were higher in the former. However, since implementing prophylactic measures, including a higher platelet transfusion threshold, and rigorous control of blood pres-

sure, magnesium and cyclosporine levels, there have been no further cases of intracranial haemorrhage although the high incidence of seizures persists (21%). With the exception of neurological complications, post-transplant complications are similar to those seen after BMT for β -thalassaemia. Significant acute GVHD (> grade 2) has been reported in approximately 20% of patients but this was rarely severe (Bernaudin *et al*, 1997; Vermylen *et al*, 1998). Chronic GVHD occurred in 15–20% of patients, was extensive in 6–8% of patients and is clearly a major cause of post-transplant mortality, accounting for four out of a total of seven deaths in the three published series.

Long-term effects and quality of life. The effect of BMT on growth is complicated by growth retardation by SCD itself and transfusion-related iron overload. Based on the experience of patients transplanted with busulphan/cyclophosphamide conditioning regimens for other indications, significant growth impairment is not anticipated (Giorgiani *et al*, 1995). The Belgian group reported continuing growth along the centiles in all but two patients, who received long-term immunosuppression for chronic GVHD. In their late-effects cohort, the Seattle collaborative group observed little improvement in height compared with age and sex-adjusted norms using standard deviation scores, although in our experience growth has improved in all of our patients post BMT. Thyroid function has been normal in almost all patients. With regard to fertility, as might be expected after high-dose therapy with alkylating agents, in both the Seattle collaborative and Belgian studies, the majority of evaluable females over the age of 13 show primary amenorrhoea, delayed sexual maturation and elevated gonadotrophin levels (Vermylen *et al*, 1998; Walters *et al*, 2000). In contrast, the majority of evaluable males have normal sexual development, although some have elevated gonadotrophin and reduced testosterone levels. Fertility has not been assessed, but by analogy with BMT for other indications, is likely to be impaired as a result of the toxicity of busulphan to the germinal epithelium of the testes (Sanders *et al*, 1996).

It is too early to fully assess the risk of secondary malignancy post BMT for SCD, but Vermylen *et al* (1998) have reported a patient with myelodysplasia evolving into refractory acute myeloid leukaemia, 53 months post BMT, arising in the donor cells after prolonged therapy with azathioprine and thalidomide for chronic GVHD.

The quality of life among patients with stable engraftment of donor cells has not been studied in detail. However, for 21/22 patients in the Seattle late effects cohort and 42/45 patients in the Belgian study, Karnofsky or Lansky scores were 100% and those with poorer quality of life measures were the individuals with extensive chronic GVHD.

FUTURE PROSPECTS: NEW APPROACHES TO STEM CELL TRANSPLANTATION (SCT) IN SCD

Volunteer unrelated donor (VUD) BMT

The lack of available donors for the majority of patients has focused attention on the use of alternative sources of

haemopoietic stem cells. With the advent of molecular typing for HLA alleles, and more effective GVHD and antiviral agents, the results for VUD transplants for a number of indications in children are now comparable to those transplanted from a matched sibling donor (Oakhill *et al.*, 1996). The only data in SCD refer to the use of unrelated donor cord blood stem cells (see below). The Seattle collaborative study group have recently approved a protocol for VUD BMT in children with SCD with one or more of the following features: renal insufficiency, red cell allo-immunization and recurrent acute chest syndrome. Unfortunately, however, the ethnic groups at risk of SCD are under-represented on unrelated donor panels.

Cord Blood SCT

Cord blood SCT offers the potential advantages of an apparently lower incidence of GVHD than conventional BMT (Rubinstein *et al.*, 1998), greater tolerance for HLA disparity and the possibility of expansion of the donor pool for patients with SCD by a policy of directed collection from deliveries of mothers from ethnic minorities. Conversely, the risk of graft rejection remains high, particularly when the cell dose is low, and engraftment may be slow (Rubinstein *et al.*, 1998). The series reported by Vermylen *et al.* (1998) included two successful sibling cord blood transplants for SCD and 11 cord blood transplants for SCD (10 successful) have been reported by the Eurocord group (Locatelli *et al.*, 2002). Yeager *et al.* (2000) have also reported two cord blood transplants from unrelated donors in children with SCD, albeit with short follow-up at the time of reporting. Nonetheless, given the limited data and the relatively high incidence of graft rejection/autologous reconstitution in patients with SCD undergoing conventional BMT, cord blood SCT for SCD should be viewed as experimental and should only be performed in the context of clinical trials.

Non-myeloablative conditioning protocols

The available experience from the transplantation of patients with SCD or thalassaemia indicates that stable, mixed haemopoietic chimaerism is sufficient to cure the disease phenotype (Vermylen *et al.*, 1998; Walters *et al.*, 2000). Thus myeloablation per se is not mandatory for the cure of SCD, raising the possibility that reduced-intensity conditioning regimens could be used. Studies in a mouse model of SCD suggest this approach could even be curative in the HLA-mismatched setting (Kean *et al.*, 2002). A number of groups have reported SCT for haematological malignancies with highly immunosuppressive but non-myeloablative regimens, with apparently reduced short-term toxicity (Giralt *et al.*, 1997; Slavin *et al.*, 1998; Amrolia *et al.*, 2000; Kottaridis *et al.*, 2000; McSweeney *et al.*, 2001). Although, in general, the follow-up is too short to assess long-term transplant-related toxicity, it is likely that this will also be reduced and preliminary reports of pregnancies following non-myeloablative SCT are encouraging. Given the reduced toxicity, this seems an attractive approach for patients with SCD, particularly older patients and those with acquired organ toxicity. For children, it may offer the potential to retain gonadal function and fertility.

At the time of writing, there have been three published reports of non-myeloablative SCT in SCD. Krishnamurti *et al.* (2001) have reported the successful matched sibling BMT of a child with homozygous SCD who was transplanted for stroke, recurrent painful vaso-occlusive crises and acute chest syndrome with a fludarabine/busulphan/antithymocyte globulin (ATG)/total lymphoid irradiation regimen. The patient was disease free, without GVHD and with 100% donor engraftment at 14 months post BMT. van Besien *et al.* (2000) have transplanted two adult patients with a fludarabine/melphalan/ATG-conditioned regimen and granulocyte colony-stimulating factor-mobilized stem cells from matched sibling donors. Both patients engrafted with full donor chimaerism by d 30, but both developed acute GVHD and eventually died of GVHD-related complications. Finally, Schleuning *et al.* (2002) described an adult patient transplanted using a fludarabine/cyclophosphamide-conditioned regimen with peripheral blood stem cells from a matched sibling donor and with mycophenolate mofetil in addition to cyclosporine for GVHD prophylaxis. The patient was fully engrafted with no GVHD 300+ d post transplant.

Thus, non-myeloablative stem cell transplantation for sickle cell disease is feasible and, as it offers the possibility of reduced toxicity, should certainly be studied further. A number of questions remain to be answered. In particular, in the setting of malignancy, some reduced-intensity conditioning protocols have been associated with high rates of graft rejection and this may be a major obstacle to this approach given the propensity of SCD patients to reject, even with conventional conditioning regimens. The optimal stem cell source is uncertain: while the increased stem cell dose from peripheral blood stem cells may make rejection less likely, the use of peripheral blood stem cells may be associated with an increased incidence of chronic GVHD. Mixed T-cell chimaerism is likely to be associated with a reduced incidence of acute GVHD (Childs *et al.*, 1999) and it may therefore be advantageous to choose a regimen which induces early mixed chimaerism in the majority of patients, perhaps in association with rigorous post-transplant immunosuppression to stabilize host-donor tolerance. Likewise, it is not clear as to how to manage patients who achieve mixed haemopoietic chimaerism but show declining levels of donor contribution: while withdrawal of immunosuppression is sometimes effective, the use of donor lymphocyte infusions in this setting has been associated with fatal GVHD. Thus, while this approach has the potential for major therapeutic benefits, non-myeloablative transplants for SCD should be performed in the context of well-constructed clinical trials in centres with expertise in BMT for SCD. One such phase 1 trial has recently been designed (Amado & Schiller, 2000) to evaluate the safety and feasibility of non-myeloablative peripheral blood SCT with a pentostatin/cyclophosphamide-based regimen in adult patients with SCD who have evidence of chronic end-organ damage, utilizing the same indications as the Seattle collaborative group.

SHORT CHAIN FATTY ACIDS

Evidence from *in vitro* and animal studies shows that short chain fatty acids, such as butyrate, can modulate HbF levels. Initial trials of intravenous arginine butyrate in SCD were disappointing as the observed increases in HbF and F-reticulocyte counts were not sustained (Perrine *et al*, 1993). Oral therapy with sodium butyrate requires large doses and the responses, in terms of HbF induction, have been inconsistent (Dover *et al*, 1994). More recently, Atweh *et al* (1999) have reported better results with a pulsed regimen in which butyrate was administered intravenously for 4 d once or twice monthly. This regimen was well tolerated and resulted in significant increases in mean HbF levels (from 7.2% to 21%) and mean Hb (from 7.8 g/dl to 8.8 g/dl) after a mean duration of 30 weeks. Although these encouraging results merit further investigation in selected patients with severe disease to assess compliance and clinical efficacy, it seems clear that short chain fatty acid therapy is unlikely to be a feasible therapeutic option for the majority of patients with SCD until better oral formulations are available.

MODIFIERS OF OXYGEN AFFINITY, MEMBRANE ACTIVE DRUGS AND DRUGS INTERFERING WITH ADHERENCE

Several biological modifiers of sickle cells or their interactions with vascular endothelium are currently under investigation in SCD. A number of such agents show promise in preclinical studies and these now need to be evaluated in the clinical setting, both alone and in combination with existing therapies such as hydroxyurea. These include agents which modify the oxygen affinity of sickle red blood cells in transgenic mice, such as nitric oxide which increases the oxygen affinity of HbS in humans both *in vitro* and *in vivo*, although its clinical efficacy and long-term toxicity remain unknown (Trudel *et al*, 1994; Head *et al*, 1997). Strategies for preventing dehydration of sickle red cells have also been investigated, including clotrimazole and magnesium. Pilot studies of clotrimazole, metabolites of which block the red cell membrane Gardos channel and reduce red cell dehydration in mouse models of SCD, reduce red cell density in patients with SCD (Brugnara *et al*, 1996) but the drug is toxic at doses likely to confer clinical benefit. Red cell magnesium levels are abnormally low in sickle erythrocytes (De Franceschi *et al*, 1997) and magnesium is known to modulate the K-Cl co-transport system reducing red cell dehydration. This provides the rationale for studies in SCD which have suggested that magnesium supplementation may be effective in reducing the frequency of painful crises (De Franceschi *et al*, 2000). Other novel agents, such as NS3623 and NS1652, chloride conductance inhibitors, have also been shown to reduce sickle red cell dehydration but their effect on sickling is yet to be determined. Finally, RheothRx, an agent which blocks hydrophobic adhesive reactions and so would be predicted to reduce the adhesion of sickled red cells to endothelium, has been shown to significantly reduce analgesic requirements, pain intensity and duration of hospital stay in some SCD patients with painful crises (Adams-Graves *et al*, 1997).

GENE THERAPY

The transfer of a normal β -globin gene along with key regulatory sequences to correct the genetic basis for SCD in autologous stem cells would be the ideal cure for SCD. Indeed, gene therapy is now a reality for certain genetic defects (Cavazzana-Calvo *et al*, 2000). Despite encouraging results in mouse models (Pawliuk *et al*, 2001), however, gene replacement therapy for SCD has remained elusive. The challenges that have been encountered have been reviewed previously (Persons & Nienhuis, 2000; Tisdale & Sadelain, 2001). A major problem has been the inefficient transduction of haemopoietic stem cells with available vectors. Recent advances in retroviral construction and methods of transduction, the development of alternative vectors such as lentiviruses, and the possibility of a selective advantage to sickle-corrected erythroid progenitors may overcome this hurdle. Another issue is that, in contrast to SCID, the correction of haemoglobinopathies requires long-term, high-level, lineage-specific expression of β -globin genes. Globin gene regulation is highly complex and requires the locus control region, the critical elements of which have now been dissected. However, when these elements are incorporated into retroviral vectors, rearrangements that prevent expression of the transgene have been a major problem. These problems have led some groups to investigate alternative approaches such as transfer of transcription factors which upregulate γ -globin gene expression, which may require lower levels of expression and simpler regulatory sequences. Despite these recent advances, the clinical application of gene therapy for SCD is unlikely to be a reality for at least 5 years.

CONCLUSIONS

In summary, three major therapeutic options are now available for children with severe SCD: transfusion, hydroxyurea and BMT. The advantages and disadvantages of each approach should be discussed for each individual patient. In doing this, the paediatric haematologist needs to take a number of factors into account. These include age, the antecedent history and complications of the patient's disease, the presence of chronic organ damage, the history of compliance, the family history and availability of an HLA-identical donor, the desire to live in a country where medical care is inadequate, and patient/parental choice. Additionally, the physician needs to make an ongoing assessment of the potential future value of novel therapies. In Part 2 of this review, we aim to provide a problem-orientated, evidence-based approach for this decision-making process.

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REFERENCES

- Adams, R.J., McKie, V.C., Hsu, L., Files, B., Vichinsky, E., Pegelow, C., Abboud, M., Gallagher, D., Kutlar, A., Nichols, F.T., Bonds, D.R. & Brambilla, D. (1998) Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. *New England Journal of Medicine*, **339**, 5–11.
- Adams-Graves, P., Kedar, A., Koshy, M., Steinberg, M., Veith, R., Ward, D., Crawford, R., Edwards, S., Bustrack, J. & Emanuele, M. (1997) RheothRx (podoxamer 188) injection for the acute painful episode of sickle cell disease: a pilot study. *Blood*, **90**, 2041–2046.
- Amado, R.G. & Schiller, G.J. (2000) Nonmyeloablative approaches to the treatment of sickle hemoglobinopathies. *Seminars in Oncology*, **27**, 82–89.
- Amrolia, P., Gaspar, H.B., Hassan, A., Webb, D., Jones, A., Sturt, N., Mieli-Vergani, G., Pagliuca, A., Mufti, G., Hadzic, N., Davies, G. & Veys, P. (2000) Nonmyeloablative stem cell transplantation for congenital immunodeficiencies. *Blood*, **96**, 1239–1246.
- Anderson, L., Holden, S., Davis, B., Prescott, E., Charrier, C.C., Bunce, N.H., Firmin, D.N., Wonke, B., Porter, J., Walker, J.M. & Pennell, D.J. (2001) Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *European Heart Journal*, **22**, 2171–2179.
- Atweh, G.F., Sutton, M., Nassif, I., Boosalis, V., Dover, G.J., Wallenstein, S., Wright, E., McMahon, L., Stamatoyannopoulos, G., Faller, D.V. & Perrine, S.P. (1999) Sustained induction of fetal hemoglobin by pulse butyrate therapy in SCD. *Blood*, **93**, 1790–1797.
- Ballas, S.K. (2001) Iron overload is a determinant of morbidity and mortality in adult patients with sickle cell disease. *Seminars in Hematology*, **38**, 30–36.
- Bernaudin, F., Souillet, G., Vannier, J.P., Vilmer, G., Michel, G., Lutz, P., Pouvrier, E., Bordigoni, P., Margueritte, G., Kuentz, M. & Vernant, J.P. (1997) Report of the French experience concerning 26 children transplanted for severe sickle cell disease. *Bone Marrow Transplantation*, **19**, 112–115.
- van Besien, K., Bartholomew, A., Stock, W., Peace, D., Devine, S., Sher, D., Sosman, J., Chen, Y.H., Koshy, M. & Hoffman, R. (2000) Fludarabine-based conditioning for allogeneic transplantation in adults with SCD. *Bone Marrow Transplantation*, **26**, 445–449.
- Brittenham, G.M., Sheth, S., Alen, C.J. & Farrell, D.E. (2001) Non-invasive methods for quantitative assessment of transfusional iron overload in sickle cell disease. *Seminars in Hematology*, **38**, 37–56.
- Brugnara, C., Gee, B., Armsby, C.C., Kurth, S., Sakamoto, M., Rifai, N., Alper, S.L. & Platt, O.S. (1996) Therapy with oral clotrimazole induces inhibition of the Gardos channel and reduction of erythrocyte dehydration in patients with sickle cell disease. *Journal of Clinical Investigation*, **97**, 1227–1234.
- Castro, O. (1999) Management of sickle cell disease: recent advances and controversies. *British Journal of Haematology*, **107**, 2–11.
- Cavazzana-Calvo, M., Hacein-Bey, S., de Saint, B.G., Gross, F., Yvon, E., Nussbaum, P., Selz, F., Hue, C., Certain, S., Casanova, J.L., Bousso, P., Deist, F.L. & Fischer, A. (2000) Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science*, **288**, 669–672.
- Ceci, A., Baiardi, P., Felisi, M., Cappellini, M.D., De Carnelli, V.S.V., Galanello, R., Maggio, A., Maserà, G., Piga, A., Schettini, F., Stefano, I. & Tricta, F. (2002) The safety and effectiveness of deferiprone in a large-scale, 3-year study in Italian patients. *British Journal of Haematology*, **118**, 330–336.
- Charache, S., Terrin, M.L., Moore, R.D., Dover, G.J., Barton, F.B., Eckert, S.V., McMahon, R.P. & Bonds, D.R. (1995) Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. *New England Journal of Medicine*, **332**, 1317–1322.
- Childs, R., Clave, E., Contentin, N., Jayasekera, D., Hensel, N., Leitman, S., Read, E.J., Carter, C., Bahceci, E., Young, N.S. & Barrett, A.J. (1999) Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. *Blood*, **94**, 3234–3241.
- Cohen, A.R., Martin, M.B., Silber, J.H., Kim, H.C., Ohene-Frempong, K. & Schwartz, E. (1992) A modified transfusion program for prevention of stroke in sickle cell disease. *Blood*, **79**, 1657–1661.
- Cullis, J., Win, N., Dudley, J.M. & Kaye, T. (1995) Post-transfusion hyperhaemolysis in a patient with sickle cell disease: use of steroids and intravenous immunoglobulin to prevent further red cell destruction. *Vox Sanguinis*, **69**, 355–357.
- Davies, S.C. (1993) Bone marrow transplant for sickle cell disease: the dilemma. *Blood Reviews*, **7**, 4–9.
- Davies, S.C. & Olatunji, P.O. (1995) Blood transfusion in sickle cell disease. *Vox Sanguinis*, **68**, 145–151.
- Davies, S.C. & Roberts, I.A. (1996) Bone marrow transplant for sickle cell disease: an update. *Archives of Disease in Childhood*, **75**, 3–6.
- Davies, S.C. & Roberts-Harewood, M. (1997) Blood transfusion in sickle cell disease. *Blood Reviews*, **11**, 57–71.
- De Franceschi, L., Bachir, D., Galacteros, F., Tchernia, G., Cynober, T., Alper, S., Platt, O., Beuzard, Y. & Brugnara, C. (1997) Oral magnesium supplements reduce erythrocyte dehydration in patients with sickle cell disease. *Journal of Clinical Investigation*, **100**, 1847–1852.
- De Franceschi, L., Bachir, D., Galacteros, F., Tchernia, G., Cynober, T., Neuberger, D., Beuzard, Y. & Brugnara, C. (2000) Oral magnesium pidolate: effects of long-term administration in patients with sickle cell disease. *British Journal of Haematology*, **108**, 284–289.
- Dover, G.J., Brusilow, S. & Charache, S. (1994) Induction of fetal hemoglobin production in subjects with sickle cell anemia by oral sodium phenylbutyrate. *Blood*, **84**, 339–343.
- Emond, A.M., Collis, R., Darvill, D., Higgs, D.R., Maude, G.H. & Serjeant, G.R. (1985) Acute splenic sequestration in homozygous sickle cell disease: natural history and management. *Journal of Pediatrics*, **107**, 201–206.
- Emre, U., Miller, S.T., Gutierrez, M., Steiner, P., Rao, S.P. & Rao, M. (1995) Effect of transfusion in acute chest syndrome of sickle cell disease. *Journal of Pediatrics*, **127**, 901–904.
- Ferster, A., Corazza, F., Vertongen, F., Bujan, W., Devalck, C., Fondu, P., Cochaux, P., Lambermont, M., Khaladji, Z. & Sariban, E. (1995) Transplanted sickle-cell disease patients with autologous bone marrow recovery after graft failure develop increased levels of fetal haemoglobin which corrects disease severity. *British Journal of Haematology*, **90**, 804–808.
- Ferster, A., Vermeylen, C., Cornu, G., Buyse, M., Corazza, F., Devalck, C., Fondu, P., Toppet, M. & Sariban, E. (1996) Hydroxyurea for treatment of severe sickle cell anemia: a pediatric clinical trial. *Blood*, **88**, 1960–1964.
- Ferster, A., Tahriri, P., Vermeylen, C., Sturbois, G., Corazza, F., Fondu, P., Devalck, C., Dresse, M.F., Feremans, W., Hunnicke, K., Toppet, M., Philippet, P., Van Geet, C. & Sariban, E. (2001) Five years of experience with hydroxyurea in children and young adults with sickle cell disease. *Blood*, **97**, 3628–3632.
- Giorgiani, G., Bozzola, M., Locatelli, F., Picco, P., Zecca, M., Cisternino, M., Dallorso, S., Bonetti, F., Dini, G. & Borroni, C. (1995)

- Role of busulfan and total body irradiation on growth of prepubertal children receiving bone marrow transplantation and results of treatment with recombinant human growth hormone. *Blood*, **86**, 825–831.
- Giralt, S., Estey, E., van Albitar, M.B.K., Rondon, G., Anderlini, P., O'Brien, S., Khouri, I., Gajewski, J., Mehra, R., Claxton, D., Andersson, B., Beran, M., Przepiorcka, D., Koller, C., Kornblau, S., Korbling, M., Keating, M., Kantarjian, H. & Champlin, R. (1997) Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood*, **89**, 4531–4536.
- Griffin, T.C. & Buchanan, G.R. (1993) Elective surgery in children with sickle cell disease without preoperative blood transfusion. *Journal of Pediatric Surgery*, **28**, 681–685.
- Haber Kern, C.M., Neumayr, L.D., Orringer, E.P., Earles, A.N., Robertson, S.M., Black, D., Abboud, M.R., Koshy, M., Idowu, O. & Vichinsky, E.P. (1997) Cholecystectomy in sickle cell anemia patients: perioperative outcome of 364 cases from the National Preoperative Transfusion Study. Preoperative Transfusion in Sickle Cell Disease Study Group. *Blood*, **89**, 1533–1542.
- Halsey, C. & Roberts, I.A.G. (2003) The role of hydroxyurea in sickle cell disease. *British Journal of Haematology* (in press).
- Hanft, V.N., Fruchtman, S.R., Pickens, C.V., Rosse, W.F., Howard, T.A. & Ware, R.E. (2000) Acquired DNA mutations associated with in vivo hydroxyurea exposure. *Blood*, **95**, 3589–3593.
- Hasan, M.F., Marsh, F., Posner, G., Bellevue, R., Dosik, H., Suatengco, R. & Ramani, N. (1996) *American Journal of Gastroenterology*, **91**, 1204–1206.
- Hassell, K.L., Eckman, J.R. & Lane, P.A. (1994) Acute multiorgan failure syndrome: a potentially catastrophic complication of severe sickle cell pain episodes. *American Journal of Medicine*, **96**, 155–162.
- Hatley, R.M., Crist, D., Howell, C.G., Herline, A.J. & Gadacz, T.R. (1995) Laparoscopic cholecystectomy in children with sickle cell disease. *American Journal of Surgery*, **61**, 169–171.
- Head, C.A., Brugnara, C., Martinez-Ruiz, R., Kacmarek, R.M., Bridges, K.R., Kuter, D., Bloch, K.D. & Zapol, W.M. (1997) Low concentrations of nitric oxide increase oxygen affinity of sickle erythrocytes in vitro and in vivo. *Journal of Clinical Investigation*, **100**, 1193–1198.
- Hoppe, C., Vichinsky, E., Quirolo, K., van Warmerdam, J., Allen, K. & Styles, L. (2000) Use of hydroxyurea in children ages 2–5 years with sickle cell disease. *Journal of Pediatric Hematology and Oncology*, **22**, 330–334.
- Jayabose, S., Tugal, O., Sandoval, C., Patel, P., Puder, D., Lin, T. & Visintainer, P. (1996) Clinical and hematologic effects of hydroxyurea in children with sickle cell anemia. *Journal of Pediatrics*, **129**, 559–565.
- Kean, L.S., Durham, M.M., Adams, A.B., Hsu, L.L., Perry, J.R., Dillehay, D., Pearson, T.C., Waller, E.K., Larsen, C.P. & Archer, D.R. (2002) A cure for murine sickle cell disease through stable mixed chimerism and tolerance induction after non-myeloablative conditioning and major histocompatibility complex-mismatched bone marrow transplantation. *Blood*, **99**, 1840–1849.
- Kim, H.C., Dugan, N.P., Silber, J.H., Martin, M.B., Schwartz, E., Ohene-Frempong, K. & Cohen, A.R. (1994) Erythrocytapheresis therapy to reduce iron overload in chronically transfused patients with sickle cell disease. *Blood*, **83**, 1136–1142.
- Kinney, T.R., Helms, R.W., O'Branski, E.E., Ohene-Frempong, K., Wang, W., Daeschner, C., Vichinsky, E., Redding-Lallinger, R., Gee, B., Platt, O.S. & Ware, R.E. (1999) Safety of hydroxyurea in children with sickle cell anemia: results of the HUG-KIDS study, a phase I/II trial. Pediatric Hydroxyurea Group. *Blood*, **94**, 1550–1554.
- Koren, A., Segal-Kupershmit, D., Zalman, L., Levin, C., Abu, H.M., Palmor, H., Luder, A. & Attias, D. (1999) Effect of hydroxyurea in sickle cell anemia: a clinical trial in children and teenagers with severe sickle cell anemia and sickle cell beta-thalassemia. *Pediatric Hematology and Oncology*, **16**, 221–232.
- Koshy, M., Burd, L., Wallace, D., Moawad, A. & Baron, J. (1988) Prophylactic red-cell transfusions in pregnant patients with sickle cell disease. A randomized cooperative study. *New England Journal of Medicine*, **319**, 1447–1452.
- Kottaridis, P.D., Milligan, D.W., Chopra, R., Chakraverty, R.K., Chakrabarti, S., Robinson, S., Peggs, K., Verfuether, S., Pettengell, R., Marsh, J.C., Schey, S., Mahendra, P., Morgan, G.J., Hale, G., Waldmann, H., de Elvira, M.C., Williams, C.D., Devereux, S., Linch, D.C., Goldstone, A.H. & MacKinnon, S. (2000) In vivo CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. *Blood*, **96**, 2419–2425.
- Krishnamurti, L., Blazar, B.R. & Wagner, J.E. (2001) Bone marrow transplantation without myeloablation for SCD. *New England Journal of Medicine*, **344**, 68.
- Lanzkowsky, P., Shende, A., Karayalcin, G., Kim, Y.J. & Aballi, A.J. (1978) Partial exchange transfusion in sickle cell anemia. Use in children with serious complications. *American Journal of Disease in Childhood*, **132**, 1206–1208.
- Locatelli, F., Rocha, V., Reed, W., Bernaudin, F., Ertem, M., Grafakos, S., Brichard, B., Li, X., Nagler, A., Giorgiani, G., Haut, P.R., Brochstein, J.A., Nugent, D.J., Blatt, J., Woodard, P., Kurtzberg, J., Rubin, C.M., Miniero, R., Lutz, P., Raja, T., Roberts, I., Will, A.M., Yaniv, I., Vermynen, C., Tannoia, N., Garnier, F., Ionescu, I., Walters, M.C., Lubin, B.H. & Gluckman, E. (2002) Related cord blood transplant in patients with thalassaemia and sickle cell disease. *Bone Marrow Transplantation*, **29**, s68.
- McCarthy, L.J., Vattuone, J., Weidner, J., Skipworth, E., Fernandez, C., Jackson, L., Rothenberger, S., Waxman, D., Miraglia, C., Porcu, C. & Danielson, C.F.M. (2000) Do automated red cell exchanges relieve priapism in patients with sickle cell anemia? *Therapeutic Apheresis*, **4**, 256–258.
- McSweeney, P.A., Niederwieser, D., Shizuru, J.A., Sandmaier, B.M., Molina, A.J., Maloney, D.G., Chauncey, T.R., Gooley, T.A., Hegenbart, U., Nash, R.A., Radich, J., Wagner, J.L., Minor, S., Appelbaum, F.R., Bensinger, W.I., Bryant, E., Flowers, M.E., Georges, G.E., Grumet, F.C., Kiem, H.P. & Torok-Storb, B., Yu, C., Blume, K.G. & Storb, R.F. (2001) Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood*, **97**, 3390–3400.
- Maggio, A., D'Amico, G., Morabito, A., Capra, M., Ciaccio, C., Cianciulli, P., Di Gregorio, F., Garozzo, G., Malizia, R., Magnano, C., Mangiagli, A., Quarta, G., Rizzo, M., D'Ascola, D.G., Rizzo, A. & Midiri, M. (2002) Deferiprone versus deferoxamine in patients with thalassemia major: a randomized clinical trial. *Blood Cells, Molecules and Disease*, **28**, 196–208.
- Maier-Redelsperger, M., Labie, D. & Elion, J. (1999) Long-term hydroxyurea treatment in young sickle cell patients. *Current Opinion in Hematology*, **6**, 115–120.
- de Montalembert, M. & Davies, S.C. (2001) Is hydroxyurea leukemogenic in children with sickle cell disease? *Blood*, **98**, 2878–2879.
- de Montalembert, M., Begue, P., Bernaudin, F., Thuret, I., Bachir, D. & Micheau, M. (1999) Preliminary report of a toxicity study of hydroxyurea in sickle cell disease. French Study Group on Sickle Cell Disease. *Archives of Disease in Childhood*, **81**, 437–439.

- Moschovi, M., Psychou, F., Menegas, D., Tsangaris, G.T., Tzortzatou-Stathopoulou, F. & Nikolaidou, P. (2001) Hodgkin's disease in a child with sickle cell disease treated with hydroxyurea. *Pediatric Hematology and Oncology*, **18**, 371–376.
- Murphy, M.F., Wallington, T.B., Kelsey, P., Boulton, F., Bruce, M., Cohen, H., Duguid, J., Knowles, S.M., Poole, G. & Williamson, L.M. (2001) Guidelines for the clinical use of red cell transfusions. *British Journal of Haematology*, **113**, 24–31.
- Newton, C.R., Warn, P.A., Winstanley, P.A., Peshu, N., Snow, R.W., Pasvol, G. & Marsh, K. (1997) Severe anaemia in children living in a malaria endemic area of Kenya. *Tropical Medicine and International Health*, **2**, 165–178.
- Oakhill, A., Pamphilon, D.H., Potter, M.N., Steward, C.G., Goodman, S., Green, A., Goulden, P., Goulden, N.J., Hale, G., Waldmann, H. & Cornish, J.M. (1996) Unrelated donor bone marrow transplantation for children with relapsed acute lymphoblastic leukaemia in second complete remission. *British Journal of Haematology*, **94**, 574–578.
- Ohene-Frempong, K. (1991) Stroke in sickle cell disease: demographic, clinical, and therapeutic considerations. *Seminars in Hematology*, **28**, 213–219.
- Ohene-Frempong, K. (2001) Indications for red cell transfusion in sickle cell disease. *Seminars in Hematology*, **38**, 5–13.
- Olivieri, N.F., Brittenham, G.M., McLaren, C.E., Templeton, D.M., Cameron, R.G., McClelland, R.A., Burt, A.D. & Fleming, K.A. (1998) Long-term safety and effectiveness of iron-chelation therapy with deferoxamine for thalassaemia major. *New England Journal of Medicine*, **339**, 417–423.
- Olujohungbe, A., Hambleton, I., Stephens, L., Serjeant, B. & Serjeant, G. (2001) Red cell antibodies in patients with homozygous sickle cell disease: a comparison of patients in Jamaica and the United Kingdom. *British Journal of Haematology*, **113**, 661–665.
- Pawliuk, R., Westerman, K.A., Fabry, M.E., Payen, E., Tighe, R., Bouhassira, E.E., Acharya, S.A., Ellis, J., London, I.M., Eaves, C.J., Humphries, R.K., Beuzard, Y., Nagel, R.L. & LeBoulch, P. (2001) Correction of sickle cell disease in transgenic mouse models by gene therapy. *Science*, **294**, 2368–2371.
- Pegelow, C.H., Adams, R.J., McKie, V., Abboud, M., Berman, B., Miller, S.T., Olivieri, N., Vichinsky, E., Wang, W. & Brambilla, D. (1995) Risk of recurrent stroke in patients with sickle cell disease treated with erythrocyte transfusions. *Journal of Pediatrics*, **126**, 896–899.
- Perrine, S.P., Ginder, G.D., Faller, D.V., Dover, G.H., Ikuta, T., Witkowska, H.E., Cai, S.P., Vichinsky, E.P. & Olivieri, N.F. (1993) A short-term trial of butyrate to stimulate fetal-globin-gene expression in the beta-globin disorders. *New England Journal of Medicine*, **328**, 81–86.
- Persons, D.A. & Nienhuis, A.W. (2000) Gene therapy for the hemoglobin disorders: past, present, and future. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 5022–5024.
- Pippard, M.J. & Weatherall, D.J. (2000) Oral iron chelation therapy for thalassaemia: an uncertain scene. *British Journal of Haematology*, **111**, 2–5.
- Rackoff, W.R., Ohene-Frempong, K., Month, S., Scott, J.P., Neahring, B. & Cohen, A.R. (1992) Neurologic events after partial exchange transfusion for priapism in sickle cell disease. *Journal of Pediatrics*, **120**, 882–885.
- Riddington, C. & Williamson, L. (2001) Preoperative blood transfusions for sickle cell disease. *Cochrane Database System Review*, CD003149.
- Rosse, W.F., Gallagher, D., Kinney, T.R., Castro, O., Dosik, H., Moehr, J., Wang, W. & Levy, P.S. (1990) Transfusion and alloimmunization in sickle cell disease. The Cooperative Study of Sickle Cell Disease. *Blood*, **76**, 1431–1437.
- Rubinstein, P., Carrier, C., Scaradavou, A., Kurtzberg, J., Adamson, J., Migliaccio, A.R., Berkowitz, R.L., Cabbad, M., Dobrila, N.L., Taylor, P.E., Rosenfield, R.E. & Stevens, C.E. (1998) Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *New England Journal of Medicine*, **339**, 1565–1577.
- Sanders, J.E., Hawley, J., Levy, W., Gooley, T., Buckner, C.D., Deeg, H.J., Doney, K., Storb, R., Sullivan, K., Witherspoon, R. & Appelbaum, F.R. (1996) Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. *Blood*, **87**, 3045–3052.
- Schleuning, M., Stoetzer, O., Waterhouse, C., Schlemmer, M., Ledderose, G. & Kolb, H.J. (2002) Hematopoietic stem cell transplantation after reduced-intensity conditioning as treatment of SCD. *Experimental Hematology*, **30**, 7–10.
- Schmalzer, E.A., Lee, J.O., Brown, A.K., Usami, S. & Chien, S. (1987) Viscosity of mixtures of sickle and normal red cells at varying hematocrit levels. Implications for transfusion. *Transfusion*, **27**, 228–233.
- Scott, J.P., Hillery, C.A., Brown, E.R., Misiewicz, V. & Labotka, R.J. (1996) Hydroxyurea therapy in children severely affected with sickle cell disease. *Journal of Pediatrics*, **128**, 820–828.
- Serjeant, B.E., Hambleton, I.R., Kerr, S., Kilty, C.G. & Serjeant, G.R. (2001) Haematological response to parvovirus B19 infection in homozygous sickle-cell disease. *Lancet*, **358**, 1779–1780.
- Slavin, S., Nagler, A., Naparstek, E., Kapelushnik, Y., Aker, M., Cividalli, G., Varadi, G., Kirschbaum, M., Ackerstein, A., Samuel, S., Amar, A., Brautbar, C., Ben, T.O., Eldor, A. & Or, R. (1998) Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood*, **91**, 756–763.
- Steinberg, M.H., Barton, F.B., Castro, O., Koshy, M., Eckman, J. & Terrin, M. (1999b) Risks and benefits of hydroxyurea in adult sickle cell anemia: effects at 6 to 7 years. *Blood*, **94**, 644a.
- Syed, S.K., Sears, D.A., Werch, J.B., Udden, M.M. & Milam, J.D. (1996) Delayed haemolytic transfusion reaction in sickle cell disease. *American Journal of Medical Science*, **312**, 175–181.
- Tisdale, J. & Sadelain, M. (2001) Toward gene therapy for disorders of globin synthesis. *Seminars in Hematology*, **38**, 382–392.
- Treadwell, M.J. & Weissman, L. (2001) Improving adherence with deferoxamine regimens for patients receiving chronic transfusion therapy. *Seminars in Hematology*, **38**, 77–84.
- Triadou, P., Maier-Redelsperger, M., Krishnamoorthy, R., Deschamps, A., Casadevall, N., Dunda, O., Ducrocq, R., Elion, J., Grot, R. & Labie, D. (1994) Fetal haemoglobin variations following hydroxyurea treatment in patients with cyanotic congenital heart disease. *Nouvelle Revue Francais Hematologie*, **36**, 367–372.
- Trudel, M., De Paep, M.E., Chretien, N., Saadane, N., Jacmain, J., Sorette, M., Hoang, T. & Beuzard, Y. (1994) Sickle cell disease of transgenic SAD mice. *Blood*, **84**, 3189–3197.
- Vermylen, C., Cornu, G., Ferster, A., Brichard, B., Ninane, J., Ferrant, A., Zenebergh, A., Maes, P., Dhooge, C., Benoit, Y., Beguin, Y., Dresse, M.F. & Sariban, E. (1998) Hematopoietic stem cell transplantation for sickle cell anaemia: the first 50 patients transplanted in Belgium. *Bone Marrow Transplantation*, **22**, 1–6.
- Vichinsky, E. (2001) Current issues with blood transfusion in sickle cell disease. *Seminars in Hematology*, **38**, 14–22.

- Vichinsky, E.P. & Lubin, B.H. (1994) A cautionary note regarding hydroxyurea in sickle cell disease. *Blood*, **83**, 1124–1128.
- Vichinsky, E.P., Earles, A., Johnson, R.A., Hoag, M.S., Williams, A. & Lubin, B. (1990) Alloimmunization in sickle cell anemia and transfusion of racially unmatched blood. *New England Journal of Medicine*, **322**, 1617–1621.
- Vichinsky, E.P., Haberkern, C.M., Neumayr, L., Earles, A.N., Black, D., Koshy, M., Pegelow, C., Abboud, M., Ohene-Frempong, K. & Iyer, R.V. (1995) A comparison of conservative and aggressive transfusion regimens in the perioperative management of sickle cell disease. The Preoperative Transfusion in Sickle Cell Disease Study Group. *New England Journal of Medicine*, **333**, 206–213.
- Vichinsky, E.P., Luban, N.L., Wright, E., Olivieri, N., Driscoll, C., Pegelow, C.H. & Adams, R.J. (2001) Prospective RBC phenotype matching in a stroke-prevention trial in sickle cell anemia: a multicenter transfusion trial. *Transfusion*, **41**, 1086–1092.
- Walters, M.C., Sullivan, K.M., Bernaudin, F., Souillet, G., Vannier, J.P., Johnson, F.L., Lenarsky, C., Powars, D., Bunin, N. & Ohene-Frempong, K. (1995) Neurologic complications after allogeneic marrow transplantation for sickle cell anemia. *Blood*, **85**, 879–884.
- Walters, M.C., Patience, M., Leisenring, W., Eckman, J.R., Scott, J.P., Mentzer, W.C., Davies, S.C., Ohene-Frempong, K., Bernaudin, F., Matthews, D.C., Storb, R. & Sullivan, K.M. (1996a) Bone marrow transplantation for sickle cell disease. *New England Journal of Medicine*, **335**, 369–376.
- Walters, M.C., Patience, M., Leisenring, W., Eckman, J.R., Buchanan, G.R., Rogers, Z.R., Olivieri, N.E., Vichinsky, E., Davies, S.C., Mentzer, W.C., Powars, D., Scott, J.P., Bernaudin, F., Ohene-Frempong, K., Darbyshire, P.J., Wayne, A., Roberts, I.A., Dinndorf, P., Brandalise, S., Sanders, J.E., Matthews, D.C., Appelbaum, F.R., Storb, R. & Sullivan, K.M. (1996b) Barriers to bone marrow transplantation for sickle cell anemia. *Biology of Blood and Marrow Transplantation*, **2**, 100–104.
- Walters, M.C., Storb, R., Patience, M., Leisenring, W., Taylor, T., Sanders, J.E., Buchanan, G.E., Rogers, Z.R., Dinndorf, P., Davies, S.C., Roberts, I.A., Dickerhoff, R., Yeager, A.M., Hsu, L., Kurtzberg, J., Ohene-Frempong, K., Bunin, N., Bernaudin, F., Wong, W.Y., Scott, J.P., Margolis, D., Vichinsky, E., Wall, D.A., Wayne, A.S., Pegelow, C., Redding-Lallinger, R., Wiley, J., Klemperer, M., Mentzer, W.C., Smith, F.O. & Sullivan, K.M. (2000) Impact of bone marrow transplantation for symptomatic sickle cell disease: an interim report. Multicenter investigation of bone marrow transplantation for sickle cell disease. *Blood*, **95**, 1918–1924.
- Wang, W.C., Kovnar, E.H., Tonkin, I.L., Mulhern, R.K., Langston, J.W., Day, S.W., Schell, M.J. & Wilimas, J.A. (1991) High risk of recurrent stroke after discontinuance of five to twelve years of transfusion therapy in patients with sickle cell disease. *Journal of Pediatrics*, **118**, 377–382.
- Wang, W.C., Wynn, L.W., Rogers, Z.R., Scott, J.P., Lane, P.A. & Ware, R.E. (2001) A two-year pilot trial of hydroxyurea in very young children with sickle-cell anemia. *Journal of Pediatrics*, **139**, 790–796.
- Ware, R.E., Eggleston, B., Redding-Lallinger, R., Wang, W.C., Smith-Whitley, K., Daeschner, C., Gee, B., Styles, L.A., Helms, R.W., Kinney, T.R. & Ohene-Frempong, K. (2002) Predictors of fetal hemoglobin response in children with sickle cell anemia receiving hydroxyurea therapy. *Blood*, **99**, 10–14.
- Wonke, B., Wright, C. & Hoffbrand, A.V. (1998) Combined therapy with deferiprone and desferrioxamine. *British Journal of Haematology*, **103**, 361–364.
- Yeager, A.M., Mehta, P.S., Adamkiewicz, T.V., Olson, E., Hsu, L.L., Kedar, A., Olson, T.A., Boyer, M.W., Ogden, A.K., Wingard, J.R. & Eckman, J.R. (2000) Unrelated placental/umbilical cord blood cell (UCBC) transplantation in children with high-risk sickle cell disease (SCD). *Blood*, **96**, 366b.

Keywords: sickle cell disease, transfusion, hydroxyurea, bone marrow transplantation.