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Editorial

Therapeutic advances in sickle cell disease in the last decade

It is estimated that each year, Nigeria, India and the Democratic Republic of Congo share half the burden of the 300,000 newborns affected by sickle cell disease (SCD)[1]. Here, we focus on the therapeutic advances in SCD that have taken place in the last decade. While hydroxyurea (HU) and blood transfusion remain the two disease-modifying treatment options, evidence for their efficacy from clinical studies and therapeutic trials in the last decade has led to their expanded use. Initially approved on the basis of its efficacy in reducing pain crises in adults with a history of three or more acute pain crises in a year, HU was also noted to reduce acute chest syndrome frequency and improve anaemia, minimizing the need for blood transfusions[2]. Longitudinal studies of patients receiving HU therapy for more than five years now demonstrate important effects on mortality reduction[3,4]. Patients in the Belgian cohort receiving HU showed a significantly better survival compared with either patients receiving no disease-modifying treatment or patients undergoing stem cell transplantation[3]. Moreover, the relative safety of HU in children[5], even in settings with high infectious disease burden[6,7], has increased our confidence in broadening the clinical use of this drug such that some centres initiate HU therapy in infants with SCD as early as nine months of age[8]. However, in one study, the proportion of adult patients with SCD with acute painful crises in the United States (US) receiving HU treatment was reported to be less than 25 per cent[9], suggesting the need for more health systems research to achieve better translation of evidence into practice[10]. In 2016 the Transcranial Doppler (TCD) With Transfusions Changing to Hydroxyurea (TWiTCH) trial was completed[11]. This referred to the multicentre, open-label, phase III non-inferiority trial of HU therapy versus continuation of exchange blood transfusions in children with SCD with no severe vasculopathy detected on magnetic resonance angiography but at risk of stroke as assessed by abnormal TCD flow velocities (>200 cm/sec). Children on prophylactic exchange transfusions for at least two years when transitioned to HU maintained similar average TCD values as those children that continued exchange transfusions[11]. Interim analysis demonstrated non-inferiority of HU based on TCD measurements, but one should bear in mind that the follow up period was less than 24 months. Patients benefiting most from the conclusions of the TWiTCH study are children and young adults; replacing blood transfusion with HU would reduce the frequency of blood transfusion and time taken off from school.

Blood transfusion is effective in preventing and managing many acute and chronic complications but has the attendant risks of alloimmunization and haemosiderosis. While chronic blood transfusion has an evidence base for stroke prophylaxis or treatment, its use in many other medical situations, such as management of acute pain and acute chest syndrome is based largely on observation and clinical experience[12]. Thus, long-term transfusion rates in adults and in children even in well-resourced countries are highly variable (between 0 and 42% for adults and between 1.2 and 19.5% for children in the UK[13]). Surgery and general anaesthesia are associated with an increased rate of sickle-related complications, and many physicians transfuse patients before surgery to reduce perioperative complications. The Transfusion Alternatives Pre-Operatively in Sickle Cell Disease (TAPS) trial provides evidence supporting this practice and recommends that patients with haemoglobin (Hb) SS and HbSβ^0 thalassemia with Hb <9 g/dl undergoing low- and moderate-risk surgery receive pre-operative simple transfusion aiming for a Hb level of 10 g/dl[14]. The risk of alloimmunization persists despite extended red cell phenotyping (standard practice for SCD

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patients in many centres in the UK and US)[15], but the emergence and broader utilization of DNA-based testing offer the possibility of further diminishing this risk[16]. Increasing the use of blood transfusion results in secondary iron overload, and in patients with SCD, the liver is most at risk.

Acute pain crises probably the most frequent complication in sickle cell anaemia, and the most common reason to utilize hospital emergency services are a burden on the healthcare system. Cellular adhesion, coagulation and inflammation, pathways downstream of HbS polymerization, have a profound influence on vascular manifestations of SCD. From this perspective, crizanlizumab, a humanized monoclonal antibody to P-selectin, holds much promise for the prevention of acute vaso-occlusive pain. In a double-blind, randomized, placebo-controlled phase II trial, adult participants (on or off HU) who received the agent intravenously 15 times throughout the course of a year had a 45 per cent reduction in the annualized rate of pain compared to those receiving placebo[17]. Although the 30-min intravenous infusion of crizanlizumab mandates a visit to a medical facility, its relative long life (30 days) offers advantages compared to daily HU therapy. Anticoagulant treatment has also demonstrated activity in a placebo-controlled trial for vaso-occlusive crises[18], but selection bias in this setting may have compromised validity of the results. Surprisingly, the Determining Effects of Platelet Inhibition on Vaso-Occlusive Events (DOVE) trial of prasugrel failed to reduce frequency of acute painful episodes[19]. In addition to platelet inhibition, the observation that genetic or pharmacologic inhibition of the coagulation pathway *per se* reduces organ damage and vasculopathy in murine models of SCD[20] has prompted human studies of pharmacological agents that inhibit the actions of factors Xa and IIa in SCD patients (NCT02072668 and NCT02179177, phase II studies of rivaroxaban and apixaban, respectively). Ischaemia-reperfusion injury is thought to underlie vaso-occlusive crises, and a subset of inflammatory cells known as invariant natural killer T-cells (iNKT) cells is believed to mediate these effects. Patients with SCD have higher iNKT cell numbers and reducing their function with small molecule inhibitors (*e.g.* regadenoson)[21] or immunodepleting them with monoclonal antibodies[22] seems safe.

The last decade also witnessed several clinical trials on agents inhibiting HbS polymerization, the underlying mechanism of the sickle cell pathology. HbS polymerization occurs only under low oxygen tension prompting one approach of using agents to maintain HbS in the oxygenated state, such as GBT440[23,24] and derivatives of 5-hydroxymethyl-2 furfural (*e.g.* AES103), with several others in various stages of development[25]. Although AES103 initially showed promise *in vitro*, this was not successfully translated into clinical use[26,27]. Phase II studies have found GBT440 to be safe and effective in raising Hb almost 1.5 g/dl in support of its anti-polymerization effects that possibly reduce sickle-induced haemolysis[28]. Multicentre phase III clinical studies of GBT440 are now being launched with anaemia reduction as a primary end-point.

Inhibition of HbS polymerization via therapeutic induction or 'de-repression' of HbF has been pursued with numerous agents in clinical trials since the 1980s, but the only successful agent approved by the US Food and Drug Administration is HU. One of the mechanisms by which HU acts is through increasing HbF, but the patient-to-patient response is highly variable and the distribution of the increase in HbF levels is heterocellular. Ideally, an increased HbF level that is evenly distributed among all erythrocytes (pancellular) would be more effective in thwarting HbS polymerization[29]. In recent years, advances in unravelling the molecular mechanisms controlling globin gene expression have led to new generations of agents that fall into two groups - those that affect chromatin regulators (such as decitabine on DNA methylation and histone deacetylase inhibitors) and the others that affect DNA-binding transcription factors. Several trials of HbF-inducing agents are under investigation[30-33]. At least three transcription factors - BCL11A, KLF1 and MYB - important for gamma-globin silencing have been identified[34-39]. Of these, BCL11A is not only the most potent repressor of HbF but also has the safest therapeutic window. MYB has an essential role in haematopoietic stem and progenitor cells, and although KLF1 is erythroid-specific, it has a pleiotropic effect on erythropoiesis. Although BCL11A has important roles in neuronal development and B-cell lymphopoiesis, dissection of the erythroid-specific enhancer down to a small region in the gene offers great hope for the possibility of disrupting this region and specifically targeting erythroid function using genome-editing technology (such zinc-finger nucleases or CRISPR-Cas 9). Another approach of reducing BCL11A levels is knocking down its expression by RNA interference, further enhanced by the development of novel vectors that can restrict the effect to the erythroid lineage. One could also 'de-repress'

gamma-globin expression by forcing an interaction between the β-locus control region and the γ gene using a synthetic DNA-binding protein[40].

The last decade has also seen considerable advances in gene therapy for SCD using lentiviral vectors (anti-sickling β-globin or γ-increasing) as autologous haematopoietic stem cell transplantation (HSCT). The most advanced of these is the anti-sickling β-globin vector containing the HbAT87Q mutation, first tested in a patient with transfusion-dependent HbE/β-thalassemia[41]. The first such treated case of SCD reported therapeutic levels of anti-sickling β-Hb (>50%), absence of crises, correction of disease hallmarks and no evidence of insertional mutagenesis[42]. Interim results from a phase I/II study using this vector revealed therapeutic HbAT87Q expression in all seven patients with SCD[43]. These data should reassure patients of the short-term (and hopefully long-term) safety of gene therapy and encourage participation in future clinical trials.

Allogeneic HSCT has become an acceptable treatment option for SCD; human leucocyte antigen (HLA)-identical sibling donor transplantation in 1000 patients conducted over 1986-2013 revealed excellent outcomes with both children and adults demonstrating 93 percent overall survival (95% confidence interval, 91.1-94.6)[44]. Modifications to the intensity of conditioning have expanded allogeneic transplantation as a treatment option for adult patients with pre-existing organ dysfunction, who would have been otherwise ineligible for transplantation using standard myeloablative conditioning[45]. However, less than 14 per cent of patients with SCD have HLA-matched siblings as donors. Hence, several approaches are needed to make HSCT available to more patients including expanding the sources from which stem cells can be obtained, such as an umbilical cord blood and haploidentical family members or matched unrelated donors combined with less intensive conditioning strategies and better supportive care.

Medical advances become meaningful only when the fruit of such advances reach the majority of patients. For this, we need to ensure access to high-quality care[1,46] for all patients, bearing in mind that the vast majority of patients with SCD come from a disadvantaged group and from geographically resource-limited settings. In India, and for that matter elsewhere, systematic data collection in the setting of clinical care would provide a clearer definition for and guide the expanding role of HU, HSCT and potential novel therapies in this population. Facilitating population-based diagnosis or monitoring of therapy within the framework of accessible primary care for patients with SCD is particularly relevant to patients lacking access to frequent hospital/clinic visits in resource-limited settings. The technology for point-of-care diagnostic devices offers much promise by detecting sickle Hb accurately, requiring <1 µl of whole blood and minimal to no instruments or power sources[47,48]. Finally, the role of collaborative science for this global health problem cannot be overemphasized. Several models exist, but the challenge is for resource-limited countries themselves to determine how best to develop relationships built on trust that improves the health of an already disadvantaged population.

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