

Sickle cell disease today: a 75-year journey from “first molecular disease” to “first gene-editing therapy”

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Introduction

The purpose of this communication is to briefly review the clinical manifestations of sickle cell disease and its potentially devastating consequences and to present a few selected insights on haemoglobin structure, function and biosynthesis.

Knowledge of sickle cell disease is essential for fully understanding the rationale behind the development of exa-cel, a novel one-time gene-editing therapy that promises to dramatically change the lives of patients with this disease [1]. Exa-cel (CASGEVY™Vertex), the first CRISPR-based treatment available for a human genetic disease, was approved in the UK (November 2023) and the US (December 2023) for treating sickle cell disease patients 12 years of age or older with recurrent vaso-occlusive crises [2]. Exa-cel has also been approved for patients with transfusion-dependent β -thalassaemia, which is not discussed further here.

In Switzerland, about 220 young patients with sickle cell disease, a 67% increase from 2012, are currently being followed by the five paediatric university hospitals.

Whereas exa-cel will soon be a treatment option for people with severe sickle cell disease, it has an extremely high price [2]. Thus, it is essential to ensure that its benefits are not limited to patients in high-income countries. Efforts should be directed towards making this treatment or other relevant gene-editing therapies accessible in countries of the Global South where they are most needed. Addressing this challenge will be a central issue in years to come.

Clinical picture

Vascular occlusion following acute haemolysis is the key pathophysiologic mechanism in sickle cell disease [3, 4]. Unpredictable and recurrent vaso-occlusive crises cause intense acute pain due to organ ischemia or infarction that can last for several days. Chronic pain affects over one-third of adults with sickle cell disease. Clinical manifestations are highly variable, as any organ that is vascularized

can be involved. Frequent complications in patients suffering from sickle cell disease include:

- acute chest syndrome
- pulmonary hypertension
- cholelithiasis due to chronic haemolysis
- bacterial infections due to functional asplenia
- stroke
- osteonecrosis
- nephropathy
- proliferative retinopathy
- priapism
- cutaneous ulcers
- venous thrombosis

Some patients have severe sickle cell disease that requires frequent hospitalizations, while others are much less symptomatic. Symptoms during childhood and adult life are often quite different. In the last 60 years, simple or exchange transfusion of red blood cells (RBCs) has become standard therapy in some patients with the most severe complications. However, alloimmunization and iron overload can develop in patients receiving repeated RBC transfusions. Chronic organ dysfunction following vaso-occlusive-induced acute organ ischemia is the rule. The haemolytic anaemia of sickle cell disease is usually well tolerated, but aplastic crises, often parvovirus induced, are not infrequent. As a consequence of these pathologies, sickle cell disease patients in the US have a life expectancy that is reduced by 20 years compared to that of the normal population [3, 4].

Early observations

Sickle cell disease was first described in the Western medical literature in 1910 by James Herrick [5]. A 20-year-old Black student from Grenada, West Indies, was seen because of a febrile respiratory problem. He had a 10-year history of medical problems, including skin ulcers and abdominal pain attacks. On physical examination, the patient was febrile at 38 °C and icteric, and the mucous membranes were pale. There were signs of pulmonary conden-

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sation, several large and painless lymph nodes and many scar-like skin lesions. Lab evaluation revealed severe anaemia with an RBC count reduced to 2.6×10^{12} per litre. Blood smear examination showed that a large fraction of his RBCs had thin, elongated, sickle-shaped and crescent-shaped forms [5]. To summarize, he had an unusual medical history with a peculiar blood picture.

A few patients and a few years later, Vernon Mason proposed that people with these clinical and RBC morphological characteristics had a new clinical entity, which he termed “sickle cell anaemia” [6]. Mason also concluded that the condition was restricted to people of African origin. The notion that sickle cell disease is confined to a specific group of people was underlined in a JAMA editorial in 1947, which stated that “its occurrence depends entirely on the presence of Negro blood, even though in extremely small amounts”, a statement not supported by observational or experimental evidence [7]. Preconceived ideas about the relationship between physical appearance, ethnicity and medical diagnoses were common. This is still the case today [8].

Sickling test

In 1917, Victor Emmel reported studies of RBCs from a 21-year-old “mulatto” patient suffering from recurrent ankle skin ulcers; he also studied her apparently healthy father [9]. The patient was the youngest of four children; the three others died with severe anaemia between the ages of 7 and 22 years. She was anaemic with RBC counts ranging between 1.8 and 3.1×10^{12} per litre over a one-year period. About one-third of RBCs in her freshly collected blood displayed an elongated and sickle-like morphology. Additional studies showed that the shape changes seen in mature erythrocytes were not detected in nucleated erythroid precursors, indicating that sickling was occurring in circulating RBCs. The patient’s blood was then stored and examined at various times for morphological changes. A time-dependent increase in the proportion of sickle-shaped RBCs was observed: almost all of them displayed the abnormal morphology after 24 hours of incubation at room temperature. The experiment was repeated several times over one year. On occasion, fresh blood morphology was essentially normal, and it took several hours of *ex vivo* storage for sickle-shaped erythrocytes to become detectable. This test was used to further characterize patients with the clinical entity identified by Herrick and Mason. Importantly, the patient’s father, who was aged 54 and appeared healthy, had an entirely normal RBC morphology in freshly collected blood; however, numerous sickled erythrocytes, identical to those observed in his daughter, were detected in smears performed after his blood was stored for 24 hours. Thus, Emmel found a way to identify patients with sickle cell disease as well as apparently healthy people whose RBCs could be induced to sickle, i.e. people with the sickle cell trait [9].

First molecular disorder

Linus Pauling, one of the most eminent chemists of the 20th century, contributed substantially to the notion that covalent bond formation is a key mechanism for linking atoms to other atoms. How did he get involved in sickle cell dis-

ease research? In 1944, on a train between Denver and Chicago, Pauling met William Castle, the Harvard haematologist who had identified the pathogenesis of pernicious anaemia [10]. They had a conversation about the nature of sickle cell disease. A major topic of the discussion was the relationship between the tendency of sickle cell disease RBCs to sickle and oxygen partial pressure. RBCs of sickle cell disease patients sickle in venous blood but not arterial blood. In other words, sickling is observed when RBCs are deoxygenated. Pauling envisioned that a possible explanation for this phenomenon could be that RBCs from sickle cell disease patients contain an abnormal form of haemoglobin which, when deoxygenated, acquires the potential to link with itself to form long chains and eventually needle-shaped crystals. He decided to compare haemoglobin from individuals with sickle cell disease, those with sickle cell trait and normal individuals by examining their electrophoretic properties. Single migration peaks were observed for haemoglobin from both sickle cell disease patients and normal individuals. However, the migration distance of haemoglobin from sickle cell disease patients differed significantly from that of haemoglobin from healthy people. As expected, when a 1:1 mixture of haemoglobin from sickle cell disease patients and normal individuals was examined, a two-peak electrophoretic pattern that combined the characteristics of both sickle cell disease and normal haemoglobin emerged. Most interestingly, the same two-peak electrophoretic pattern was also evident in the electrophoresis of haemoglobin from individuals with sickle cell trait [11]. These results led to the following conclusions. First, the distinction between normal and sickle cell anaemia haemoglobin molecules is a consequence of differences in ionizable groups present in the two haemoglobins. This difference is best explained by variation in the amino acid composition of their globin domains. Second, sickle cell disease is a recessive condition, as people with the sickle cell trait are clinically asymptomatic despite their RBCs containing approximately equal amounts of sickle cell disease haemoglobin and normal haemoglobin. Third, it is fundamentally important to recognize that sickle cell disease is a molecular disease. This notion is obvious today, as it is well established that many disease states result from clearly identified molecular lesions.

Haemoglobin structure and function

Haemoglobin molecules consist of four subunits, two α -globin proteins and two β -globin proteins, each of which contains a haem component that binds and releases oxygen via a single central ferrous iron atom [12]. These globin proteins are encoded by α - and β -globin genes. The genes encoding β -globin proteins are located in a linear cluster on chromosome 11q. This cluster also includes sites for regulatory elements like BCL11A, a transcription factor that represses transcription of γ -globin genes; γ -globin is the β -globin family member in foetal haemoglobin (HbF). Within the β -globin gene family, various gene products and the various haemoglobins formed when they bind α -globins include ϵ -globin (embryonic Hb-Gower), γ -globin (HbF), δ -globin (adult HbA₂) and β -globin (adult HbA). Their production is regulated depending on developmental stage. The major adult haemoglobin, HbA, has a structure

of $\alpha_2\beta_2$, while HbF has a structure of $\alpha_2\gamma_2$ [12]. A four-subunit haemoglobin structure ensures cooperativity between the four haemoglobin subunits, such that oxygen molecules are bound in the lungs and delivered and released in the periphery, as evidenced by the sigmoidal shape of the dissociation curve for oxygen bound to haemoglobin. A linear dissociation curve would be observed with a single-unit oxygen transporter, but such a compound would be incapable of delivering oxygen in a regulated fashion, a crucial requirement for the survival of multicellular organisms.

HbS, the haemoglobin variant responsible for sickle cell disease

It took some time to identify the amino acid changes in haemoglobin that Linus Pauling believed were involved in the polymerization of haemoglobin in patients with sickle cell disease. In 1956, Vernon Ingram developed a two-dimensional system of electrophoresis followed by chromatography to isolate tryptic digests of HbA and HbS. A clear difference in the migration patterns of HbA and HbS was observed for one of approximately thirty cleavage products. Further studies showed that this difference is caused by a Glu6 to Val point mutation in the globin β -chain [13]. This change provides an immediate explanation for the sickling reaction: glutamic acid (Glu), a polar residue, is replaced by the highly hydrophobic valine (Val) at the surface of β -globin. Valine-valine interactions between adjacent haemoglobin molecules cause the formation of HbS polymers and sickled erythrocytes and eventually cause vaso-occlusion. Thus, Glu6Val in β -globin is a prototypical gain-of-function mutation. Finally, it should be mentioned that, apart from HbS, several other haemoglobin mutants can cause RBC sickling. Therefore, one needs to keep in mind that, when referring to sickle cell disease, a homozygous Glu6Val mutation in the haemoglobin β -chain is not always the cause of the problem. For example, haemoglobin variants that contain one Glu6Val β -globin chain associated with another mutated β -globin chain can also cause the clinical manifestations of sickle cell disease [3].

Geography

More than 8 million people worldwide, primarily in tropical sub-Saharan African and South Asian countries, live with sickle cell disease. In 2021, more than 500,000 people were born with this condition. Without proper medical intervention, over half of them will not survive beyond the age of 5. The prevalence of cultural biases in high-income countries against individuals from these regions has contributed to the neglect of sickle cell disease, making it one of the most overlooked noncommunicable health issues [8].

In 1954, Allison reported the frequency of the sickle cell gene in a sample of African children in Kampala, Uganda [14]. His findings revealed that 30% of the children with sickle cell trait he examined also had malaria, while 46% of children without it were infected by the parasite. This finding led to the conclusion that carrying the gene for sickle cell disease offers partial protection against malaria, a proposition later supported by many investigations. In-

deed, the shape of sickle cells makes it more difficult for malaria parasites to invade and survive within them. In addition, sickle cells are more prone to haemolysis, which further limits the ability of the parasites to complete their life cycle. Allison also emphasized that resistance to malaria in people with the sickle cell trait provided a selective advantage over those without the trait.

In Europe, sickle cell disease is considered rare, as it affects less than 5 in 10,000 people [15]. In France, a newborn screening program in certain regions, early diagnosis and improved prophylactic measures have led to a significant increase in the life expectancy of patients with sickle cell disease, from 36 years during the period from 1995–2010 to 58 years during the period from 2012–2018 [16]. A study involving 4,270 patients with severe sickle cell disease between 2012 and 2018 showed that 89.1% had vaso-occlusive crises, 54.6% had acute chest syndrome, 18.0% developed gallstones, 11.3% experienced sepsis or meningitis and 4.4% had cerebrovascular symptoms. Chronic complications observed during the follow-up period included osteonecrosis (15.5%), cardiovascular disease (15.4%), pulmonary thrombosis (9.3%), chronic kidney disease (7.9%), iron overload (7.9%), cerebrovascular sequelae (6.8%) and pulmonary hypertension (5.1%). Moreover, 97.9% of the patients with severe sickle cell disease were hospitalized one or more times. Most of these patients required disease-modifying treatments, such as chronic transfusion programs and hydroxyurea [16]. Finally, the European Commission is developing a plan to improve the overall approach to the management of individuals with sickle cell disease. Twelve EU member states are actively involved in the European Reference Network on Rare Haematological Diseases (ERN-EuroBloodNet). Efforts to ameliorate the current situation include establishing a central patient registry for the availability of patient data; organizing multidisciplinary expert teams; and mapping demographics, survival rates, clinical manifestations, genetic data and diagnostic and treatment methods in these twelve states. Additionally, plans are underway to expand newborn screening, improve education and patient involvement in sickle cell disease and promote basic and clinical research in the field. Achieving these goals will require collaboration with the World Health Organization and other relevant stakeholders [15].

Switching between HbF and HbA

HbF is the main haemoglobin form in RBCs between three months after conception until about six months after birth. Subsequently, the major haemoglobin forms are HbA in healthy individuals and HbS in individuals homozygous for the Glu6Val mutation. Consequently, the initial symptoms of sickle cell disease may appear in children at six months of age [12]. This timing indicates that switching from HbF to HbA has major adverse clinical consequences and that preventing this switch or restoring HbF production could be a potential approach for treating sickle cell disease [3, 17]. As mentioned above, individuals with the sickle cell trait are essentially symptom-free, even though the mutated Glu6Val β -globin represents about half of their β -globin. Therefore, a moderate reduction in β -globin and its substitution by γ -globin could be sufficient to signif-

icantly improve the clinical manifestations of sickle cell disease.

Support for the notion that there is a reciprocal relationship between elevated HbF and the clinical manifestations of sickle cell disease is provided by the following observations:

Hereditary persistence of HbF is a genetically determined, asymptomatic condition. Lifelong HbF levels up to 30% of total haemoglobin are observed. This condition is heterogeneous, and several molecular abnormalities of *BCL11A* that are responsible for it have been identified. *BCL11A* is the major repressor of γ -globin gene expression. Therefore, inhibiting this transcription factor could promote long-term continuous production of HbF. Most importantly, people with hereditary persistence of HbF who also are homozygous for HbS do not present with the clinical manifestations of sickle cell disease, supporting the protective value of high HbF levels as a therapeutic strategy for sickle cell disease [17].

Hydroxyurea has been employed for decades to treat myeloproliferative disorders, particularly chronic myeloid leukaemia, so its pharmacological properties and toxicities are well established. The main side effect is dose-related myelosuppression. Hydroxyurea inhibits ribonucleotide reductase, an enzyme involved in DNA synthesis. In 1984, this compound was shown to increase HbF levels in two patients with sickle cell disease. Hydroxyurea creates a stress erythropoiesis state that is associated with delayed DNA synthesis, resulting in macrocytosis and an increase in the fraction of reticulocytes containing HbF [18]. In the years following this observation, hydroxyurea emerged as a major disease-modifying treatment for sickle cell disease. In adults, it reduces the numbers of painful crises, acute chest syndrome episodes and hospitalizations. In children, hydroxyurea has substantial beneficial clinical effects, including being more efficacious than blood transfusions for stroke prevention, without long-term toxicities [3].

These data establish without ambiguity that increasing HbF levels improves the clinical manifestations of sickle cell disease.

Exa-cel

The CRISPR-Cas9 method of genome engineering became a viable option for clinical use when it became clear that abolishing *BCL11A* from erythroid precursors could be an effective method for treating sickle cell disease. The characteristics of the CRISPR-Cas9 technology are presented in a review article by Jennifer Doudna and Emmanuelle Charpentier [19]. For conceiving of and developing this method of gene editing, Doudna and Charpentier were awarded the 2020 Nobel Prize in Chemistry. Cas9, an endonuclease, uses RNA-guided sequences to create site-specific breaks in any DNA sequence of interest. Subsequently, the cell's endogenous machinery repairs the double-strand breaks in the DNA created by Cas9. Vertex Pharmaceuticals indicates that exa-cell is a non-viral CRISPR-Cas9 gene-edited cellular therapy in which a patient's own CD34⁺ haematopoietic progenitor cells are edited *ex vivo* at the erythroid-specific enhancer region of *BCL11A* through a precise double-strand break [1]. Hay-

dar Frangoul and his collaborators reinfused these modified cells into sickle cell disease patients after they had been pre-treated with busulfan conditioning chemotherapy. The participants in this study were 12–35 years of age and had a history of two or more severe vaso-occlusive crises per year in the previous two years [20]. The primary efficacy endpoint was the proportion of patients free of severe vaso-occlusive crises for at least 12 months (VF12). The secondary efficacy endpoint was the proportion of patients free from hospitalization for severe vaso-occlusive crises for at least 12 months (HF12). Among 30 patients in the primary efficacy set, 29 achieved VF12, and all of them achieved HF12. Moreover, all patients had early and sustained increases in total haemoglobin and HbF. Durable improvements in haemolysis and patient-reported outcomes were observed in all patients. Adverse events occurred during the first three months, with rates decreasing over time [20].

Conclusion

Removing *BCL11A* from the genome of red cell precursors in patients with sickle cell disease increases HbF levels and effectively alleviates the clinical manifestations of this debilitating condition. The outcomes achieved with this transformative therapy serve as a compelling example of the power of gene editing in treating disease states with well-defined molecular pathways.

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Potential competing interests

All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflict of interest related to the content of this manuscript was disclosed.

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