A red blood cell disease meets a company with a plasma protein heritage

CSL Behring

For more than a century, CSL Behring has earned a reputation as an organization driven to care for patients with rare and serious diseases. We are a company known for keeping our promises – to patients, and providers, making us one of the world's top biotechnology companies. CSL Behring's research and development (R&D) focus supports the company's sustainable growth by advancing world-class science, technology and collaboration. These activities aim to address unmet medical needs or enhance current treatments in five therapeutic areas (Immunology, Hematology, Cardiovascular & Metabolic, Respiratory and Transplant) and utilizes expertise across three discovery platforms (plasma fractionation, recombinant technology, and cell and gene therapy).

CSL Behring's dedication of its R&D activities to develop novel therapies for sickle cell disease (SCD) is in line with our mission to provide products, medicines and programmes for the treatment of patients with rare diseases. Being a potentially life-threatening disease with substantial unmet needs and still limited treatment options for patients, SCD is an important focus area of our R&D activities in benign haematology. Even just a decade ago, there were few, if any, therapies available to improve the lives of patients suffering from this disease, and CSL Behring is committed to further innovation to advance the therapeutic landscape in SCD.

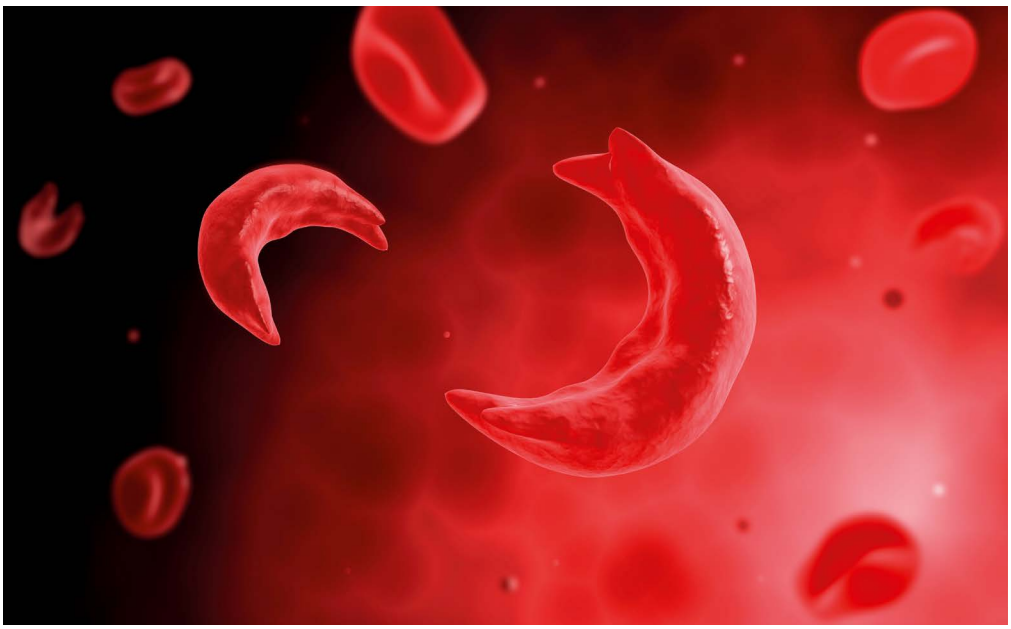

Figure 1. People with sickle cell disease inherit the haemoglobin-S gene, which causes changes in the red blood cell from a smooth rounded shape to one that is sickle shaped, rigid, sticky and has a short life span.

A HISTORY OF SICKLE CELL DISEASE AND PHARMACEUTICALS

Sickle cell disease has been recognized in Africa for centuries under various tribal names characterizing the gnawing pain in bones and joints: *chwechweechwe* (Ga tribe), *nwiiwii* (Fante tribe), *nuidudui* (Ewe tribe), and *ahotutuo* (Twi tribe)[1]. Twentieth century technology changed the scientific perceptions of this blood disease. In 1910, James Herrick first applied the name 'sickle' to describe the unique red cell morphology discovered on peripheral blood smear by his medical intern colleague Ernest Irons (**Fig. 1**)[2]. SCD earned the additional moniker 'first molecular disease' in 1949, when Nobel laureate Linus Pauling and colleagues identified the abnormal sickle haemoglobin by electrophoresis[3]. The amino acid mutation of glutamine-6 to valine in sickle haemoglobin was identified in 1957 by Vernon Ingram[4], and the corresponding single nucleotide mutation was reported in 1973 by Marotta and colleagues[5]. Despite its position in the cutting edge of scientific technology, treatment approaches for SCD lagged far behind. Robert B. Scott, a physician-researcher at the Medical College of Virginia in 1970 noted the high prevalence of SCD among the Black population in the United States, exceeding cystic fibrosis, childhood leukaemia and phenylketonuria. Scott also highlighted the low priority of SCD among biomedical researchers and funding agencies[6].

SCD gained some additional resources with the passage of the National Sickle Cell Anemia Control Act in 1972[7]. Collaborative academic clinical trials funded by the National Institutes of Health (NIH) began to move the needle on SCD. The SCD population gained improvements in health and longevity with the development of penicillin prophylaxis and immunization against pneumococcal sepsis and meningitis, and advancements in red cell transfusions, eventually even easing the scourge of childhood strokes. However, no specific therapies controlled the recurrent episodes of acute pain

or the silent erosion of organ function.

It took another government-sponsored academic collaborative clinical trial to bring approval to the first drug for SCD. Hydroxyurea, repurposed from its origins as an antileukemic drug, was shown to reduce the incidence of vaso-occlusive episodes by a consortium of academic sites led by Johns Hopkins Hospital haematologists Samuel Charache and George Dover and funded by the NIH[8]. Hydroxyurea administered prophylactically also reduced the incidence of the acute chest syndrome and the need for blood transfusions, leading to its approval for adults with SCD in 1998. These results were recapitulated in a trial in young children with SCD[9], leading to approval in 2017 of hydroxyurea for children age two years and older with SCD.

2017 was also the first year that an industry-sponsored study led to approval of a new agent for sickle cell disease. L-glutamine was approved for patients age five years and older with sickle cell disease to reduce severe complications[10]. Two years later, the FDA approved crizanlizumab to reduce the frequency of vaso-occlusive crises (VOCs) in adults and paediatric patients aged 16 years and older with sickle cell disease[11]. Nearly simultaneously, the FDA granted accelerated approval to voxelotor for adults and paediatric patients 12 years of age and older with sickle cell disease[12]. More than a century passed after the description of sickle cell disease in the English medical literature before an industry-sponsored pharmaceutical agent was approved for the condition. Suddenly, in a two-year span, three new prophylactic drugs provided options for treatment for patients suffering from the first molecular disease.

As of July 2021, clinicaltrials.gov listed 40 actively recruiting, industry-sponsored clinical trials for patients with SCD, and another 153 interventional trials funded by the federal government or universities. These trials include health technology, novel small molecules, monoclonal antibodies, haematopoietic cell transplants and gene therapy.

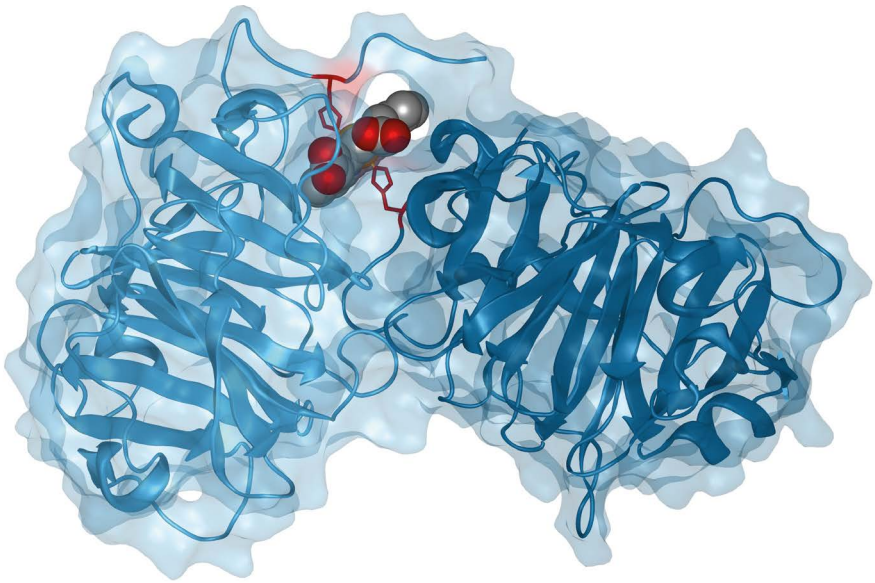

Figure 2. Structure of the haem–haemopexin complex shown as a three-dimensional ribbon diagram with superimposed electrostatic potential map. The haemopexin protein is depicted in blue, highlighted histidine residues in dark red (His 213 and His 266) coordinating and stabilizing the haem molecule within the binding pocket. The haem molecule is represented by the grey and red spheres. Image generated by Thomas Gentinetta at CSL Behring using structural data from Paoli *et al*.[17] PDB database entry: 1QJS. Visualized with Protean 3D, DNASTAR Lasergene v17.

SCD is an important haematologic disease that presents an opportunity to apply the historical strengths of CSL Behring in plasma protein replacement. CSL889 (haemopexin) is CSL Behring's new investigational agent, currently recruiting in a phase 1 trial [ClinicalTrials.gov Identifier: NCT04285827]. Haemopexin is an endogenous plasma protein that neutralizes haem (**Fig. 2**), a breakdown product of haemoglobin released from the fragile red blood cells of SCD. In cell culture and mouse models of SCD, haemopexin has been investigated in pathways involving vaso-occlusion, inflammation, and endothelial cell exposure of P-selectin and von Willebrand factor[13,14,15]. CSL Behring's plasma protein entry into SCD drug development represents its expansion from its longtime identity furnishing replacement protein therapy for rare diseases like haemophilia, congenital immune deficiency, hereditary angioedema and alpha-1-antitrypsin deficiency. Plasma haemopexin depletion in SCD presents a target for haemopexin replacement[15].

HISTORY OF CSL BEHRING

The Commonwealth Serum Laboratories (CSL) was established in Australia in 1916 to service the health needs of a nation isolated by war. Over the ensuing years CSL provided Australians with rapid access to twentieth century medical advances including insulin and penicillin, and vaccines against influenza, polio and other infectious diseases. CSL Limited was incorporated in 1991 and listed on the Australian Securities Exchange (ASX) in 1994.

Since then, CSL has acquired a number of companies including Aventis Behring, which is now known as global biotechnology leader CSL Behring; US plasma collector Nabi, which helped to form the world's premier plasma collection company in CSL Plasma; the Novartis influenza vaccine business, now integrated and known as Seqirus, the world's second largest influenza vaccines company; Calimmune, a leader in gene-modification and cell delivery technology; and Vitaeris, a biopharmaceutical company focused on the development of clazakizumab as a potential treatment option for organ transplant recipients experiencing rejection. Their combined and rich histories make CSL the innovative global leader it is today (**Fig. 3**).

CSL Plasma is the largest collector of human blood plasma in the world, sourcing plasma from hundreds of thousands of donors globally to produce a range of life-saving medicines for critically ill patients.

Seqirus is the second largest influenza vaccine company in the world and a major partner in the prevention and control of influenza globally. It is a

Figure 3. Expanding CSL Behring's R&D facility in Pasadena, California, US, enables the focus on cell and gene therapy that promises to advance new capabilities in this scientific platform.

transcontinental partner in pandemic preparedness and response, and a leading supplier of influenza vaccines to global markets for both northern and southern hemisphere seasons.

CSL Behring is a global biotechnology leader with a broad range of quality medicines in the industry and substantial markets throughout the world with therapies indicated for bleeding disorders, immunodeficiencies, hereditary angioedema, neurological disorders and alpha-1-antitrypsin deficiency (**Fig. 4**).

CSL Behring has linked its strong foundation of science to discovering, developing and delivering novel medicines for unmet patient needs and has established itself as the industry-leading plasma-based biotech company – progressing into multiple new disease areas across its five therapeutic areas and three scientific platforms. We're also making important investments in our global R&D hubs, expanding our capabilities, and building strategic collaborations with academic institutions, industry and researchers to help unlock additional sources of innovation. With an integrated and broad pipeline of therapies, diverse sources of innovation and a science- and business-driven approach – the company is well positioned to deliver innovative medicines for patients with rare or serious diseases in the decades ahead.

DRIVEN BY OUR RESEARCH

CSL Behring's R&D portfolio encompasses a wide variety of activities directed towards the discovery and development of new innovative therapies as well as improvements in our existing products and manufacturing processes. The company's three discovery platforms are a foundation for its research.

Plasma fractionation – Our clinical research activities make products available to patients worldwide and identify novel therapeutic uses for plasma proteins. As a leading manufacturer and developer of therapeutics derived from human plasma, CSL Behring is committed to maintaining the highest product safety standards and to continually improving manufacturing effectiveness. Our product portfolio in haematology covers a broad range of plasma-derived proteins, including plasma-derived FVIII, FVIII-VWF complex concentrate, prothrombin complex concentrate and fibrinogen concentrate. CSL Behring's research programmes are focused on developing novel plasma proteins with improved efficacy and enhanced convenience.

Recombinant technology - We have extensive experience in the production, clinical development and launch of recombinant coagulation factors. We are also focused on the development, production and testing of novel monoclonal antibodies (MAbs) to treat inflammatory diseases (CSL324), hereditary angioedema (CSL312) and target fatty acid metabolism (CSL346). In collaboration with Momenta Pharmaceuticals, we are also developing recombinant Fc multimer proteins to control inflammation associated with autoimmune diseases.

Gene and cell therapy - Through the acquisition of Calimmune Inc., CSL Behring is now focused on the development of *ex vivo* haematopoietic stem cell (HSC) gene therapy, which has the potential to offer a significant advantage to patients suffering from currently incurable genetic diseases.

HAEMATOLOGY RESEARCH CAPABILITIES OF CSL BEHRING

At CSL Behring we are committed to our promise of serving patients' needs by developing new therapies that ease the burden of care and improve quality of life. CSL Behring has a longstanding history as a plasma-based biotechnology company and a strong heritage in benign haematology as one of its core therapeutic areas (TAs) of interest. R&D activities in haematology are designed to support and expand our current portfolio of innovative therapies for the treatment of rare diseases and life-threatening conditions, including congenital coagulation disorders such as haemophilia A, haemophilia B and von Willebrand disease, as well as acquired bleeding disorders in critical care medicine. This includes investigating the potential of 4-factor prothrombin complex concentrate for trauma patients with life-threatening bleeding. We are exploring new indications in haematology, including the development of novel therapeutic approaches for the treatment of patients with subarachnoid haemorrhage and for the prevention and treatment of thrombotic conditions. Moreover, a large portion of CSL Behring haematology R&D is dedicated to the discovery and development of new therapeutic strategies for the treatment of patients with sickle cell anaemia.

CSL Behring's R&D organization has a global footprint, with preclinical and translational research facilities in Australia, Europe and the US (**Fig. 5**). In order to drive our haematology R&D portfolio we utilize internal expertise and a broad network of external collaborators in the deployment

Figure 4. These scientists at the CSL Behring Research Center for Translational Medicine are part of a team of more than 1,700 research and development experts dedicated to developing and delivering new therapies to solve unmet medical needs and save lives.

of three main drug discovery platforms: plasma fractionation, recombinant protein technology and, more recently, cell and gene therapy. With respect to plasma fractionation our research efforts are directed towards identifying novel therapeutic uses for plasma proteins, while our recombinant protein technology capabilities comprise the production of recombinant coagulation factors as well as the discovery, engineering and development of novel monoclonal antibodies. Our activities in cell and gene therapy are focused on the development of *ex vivo* gene modified haematopoietic stem cells (HSC's) for the treatment of patients suffering from currently incurable genetic diseases. To further expand and complement our ongoing cell and gene therapy activities, CSL has recently completed a global commercialization and license agreement with uniQure (NASDAQ: QURE) for etranacogene dezaparvovec (AMT-061), a novel gene therapy for the treatment of haemophilia B currently being investigated in phase 3 clinical trial [NCT03569891][16].

CSL Behring's capabilities in haematology cover the entire R&D value chain from early target discovery, drug engineering and nonclinical pharmacology (including a wide range of *in vitro* assay systems and different disease-specific *in vivo* models), through to translational research and nonclinical and clinical development of drug candidates. Research partnerships with leading academic institutions and small biotech companies are a key element of our R&D strategy and ensure access to both early innovation and relevant skill sets. Our research external innovation strategy in haematology encompasses all three of our technology platforms and includes, for example, our long-term Gene Therapy Alliance with the Seattle Children's Research Institute, as well as more focused collaborations such as our work with the University of Minnesota exploring haemopexin as a supplementation therapy in SCD.

Despite emerging and promising new treatment options, SCD remains the largest rare disease with a high unmet need. Its complex pathophysiology arises from a single point mutation in the beta chain of haemoglobin that causes cellular and membrane abnormalities of red blood cells ultimately converging in erythrocyte sickling, aggregation and chronic haemolysis. Painful vaso-occlusive episodes as well as anaemia represent the most prominent clinical hallmarks of this genetic disease, although end-organ damage and a variety of co-morbidities and complications contribute to the morbidity and early mortality of patients. On a cellular and molecular level these clinical symptoms involve an intricate interplay of multiple pathways, comprising cell–cell interactions and increased endothelial adhesiveness, endothelial dysfunction, sterile inflammation, in particular as a consequence of erythrocyte break-down and the release of cell-free haemoglobin and haem, and a pro-thrombotic milieu.

To account for disease complexity and heterogeneity of patient phenotypes, early drug discovery research at CSL Behring

Figure 5. CSL Behring has established a collaborative, global research and development network and a commitment to funding innovation in new products and improvements in commercialized products.

follows a multi-dimensional approach targeting different patho-mechanisms, patient needs and disease states in SCD. Similar to the therapeutic approaches in haemophilia or hereditary angioedema, we believe SCD patients require and deserve a wide range of different treatment options including: (i) effective therapeutics to treat acute VOC episodes; (ii) improved chronic treatments for the prevention of VOCs, other acute complications and chronic end-organ damage that synergize with available therapies; and (iii) cell and gene therapies that hold the promise of a potential curative treatment correcting the genetic 'root cause' of the disease.

Our ongoing innovative research and clinical trial activities demonstrate our commitment to patients living with SCD.

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