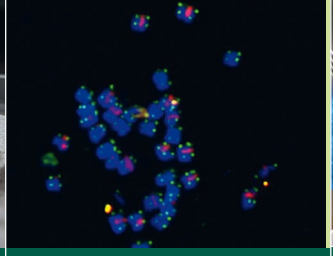
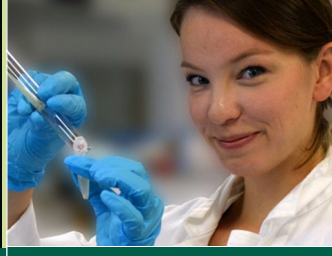
54. Jahrgang Sonderheft 2024 ISSN 0045-205X BLUZAR D 5627

SONDERHEFT
2024





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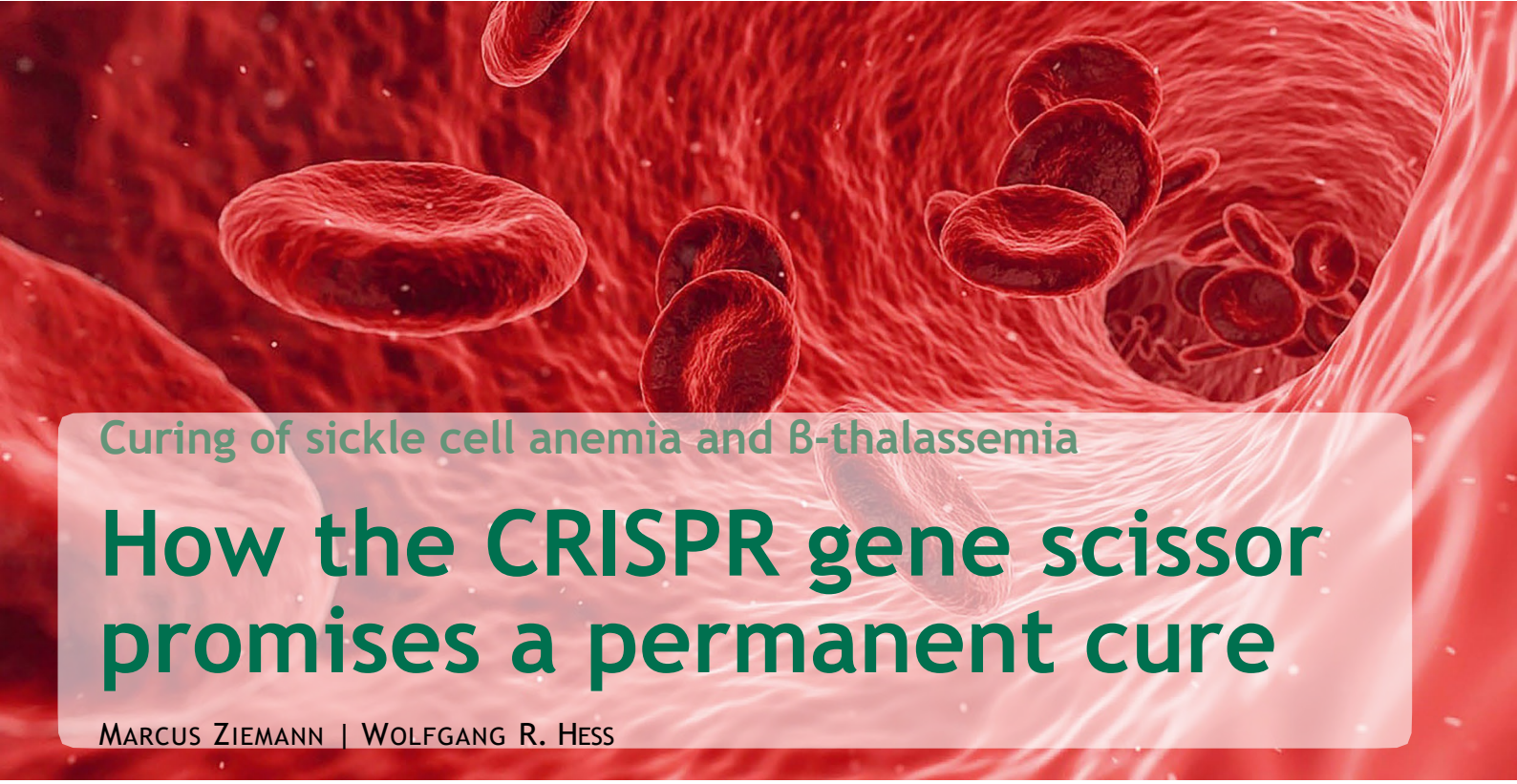


Curing of sickle cell anemia and ß-thalassemia

How the CRISPR gene scissor promises a permanent cure

Marcus Ziemann | Wolfgang R. Hess

Genetic engineering and genome editing have made significant progress in recent decades. Now, another important step forward has been taken with the recent approval of a gene therapy for sickle cell anemia and ß-thalassemia. After just five years of testing, the international team at CRISPR Therapeutics has succeeded in curing both diseases and obtaining medical approval in Great Britain, the USA, the EU and many other countries.

The phenomenon of sickle cell anemia (also known as sickle cell disease) and its genetic basis is perhaps the first example students encounter when they learn about genetic disorders. This is partly because it is a disease that is relatively easy to explain, even at the molecular level and because carriers of this genetic disposition have an increased resistance to malaria, meaning that the disease can also represent an evolutionary advantage in malaria-risk regions.

Brief summary

- Sickle cell anemia is caused by point mutations in the hemoglobin β gene.
- A permanent cure has so far only been possible in rare cases.
- With the help of CRISPR-Cas, the fetal hemoglobin F gene was reprogrammed in sickle cell patients for the first time.
- Symptoms of the disease were reduced or disappeared completely.
- The therapy has already been approved in the USA, the U.K., and other countries.

Many genetic aspects can be explained and understood based on this clinical picture. At the same time, it is also the most widespread inheritable disease, based on just one gene mutation, with over 7.7 million people affected worldwide[1,2].

It is therefore not surprising that sickle cell anemia is now also the target of the first approved CRISPR-based gene therapy[1]. In November 2023, the United Kingdom accepted the new therapy[3], followed by the USA[4] and a conditional EMA approval within the EU[5]. But what kind of treatment is it?

The medical condition of sickle cell anemia

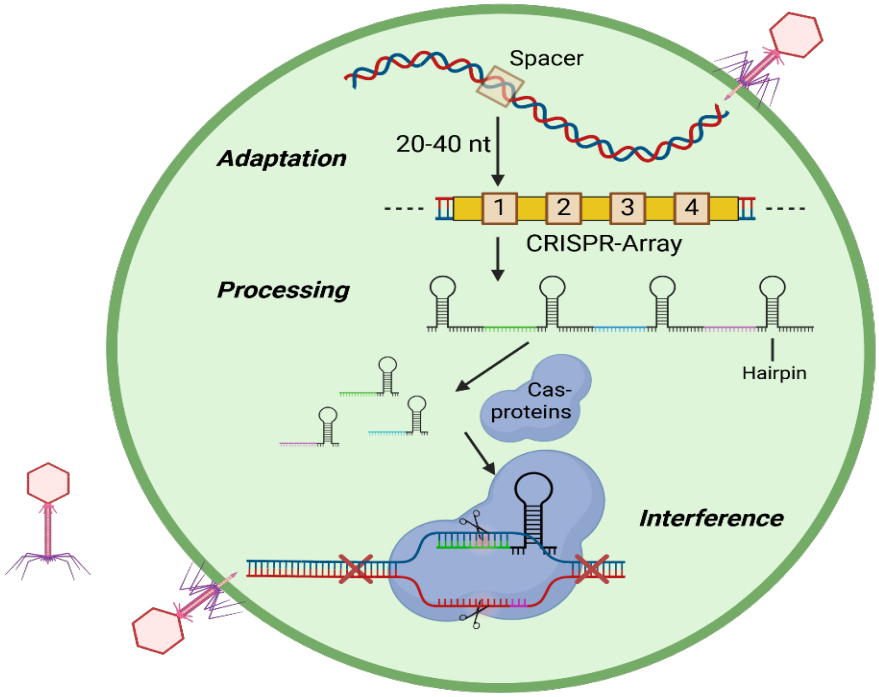
There are various types of sickle cell disease; the most common cause, however, is a point mutation in the gene for the β protein of hemoglobin (E6V)[1,6]. Due to this

mutation, the atypical hemoglobin S (HbS) can polymerize when oxygen is released, causing the red blood cells to deform and create the characteristic sickle shape. These cells can lead to vascular occlusions, manifesting in a lack of oxygen supply to tissues, as well as permanent tissue damage and severe pain[6]. The life expectancy of those affected is often only around 50 years[7].

Hemoglobin is a tetrameric protein in red blood cells and is needed to transport oxygen throughout the body. The structure comprises two α chains (α_2) and a second pair of protein chains. In adults, this is mostly HbA ($\alpha_2\beta_2$)[1]. In fetuses and newborns, we can mainly find the fetal hemoglobin HbF ($\alpha_2\gamma_2$), which is gradually replaced by HbA during the first few months after birth. The reason for this change is that HbF has a higher affinity for oxygen and thus allows the fetus a better oxygen absorption[8]. The sickle cell mutation only exists in the β chain and therefore only affects HbA (also known as HbS when mutated) and starts manifesting itself in children after the third month of life[1]. However, there are also known cases in which HbF is produced in large quantities by adults, which significantly mitigates the effects of sickle cell anemia[9].

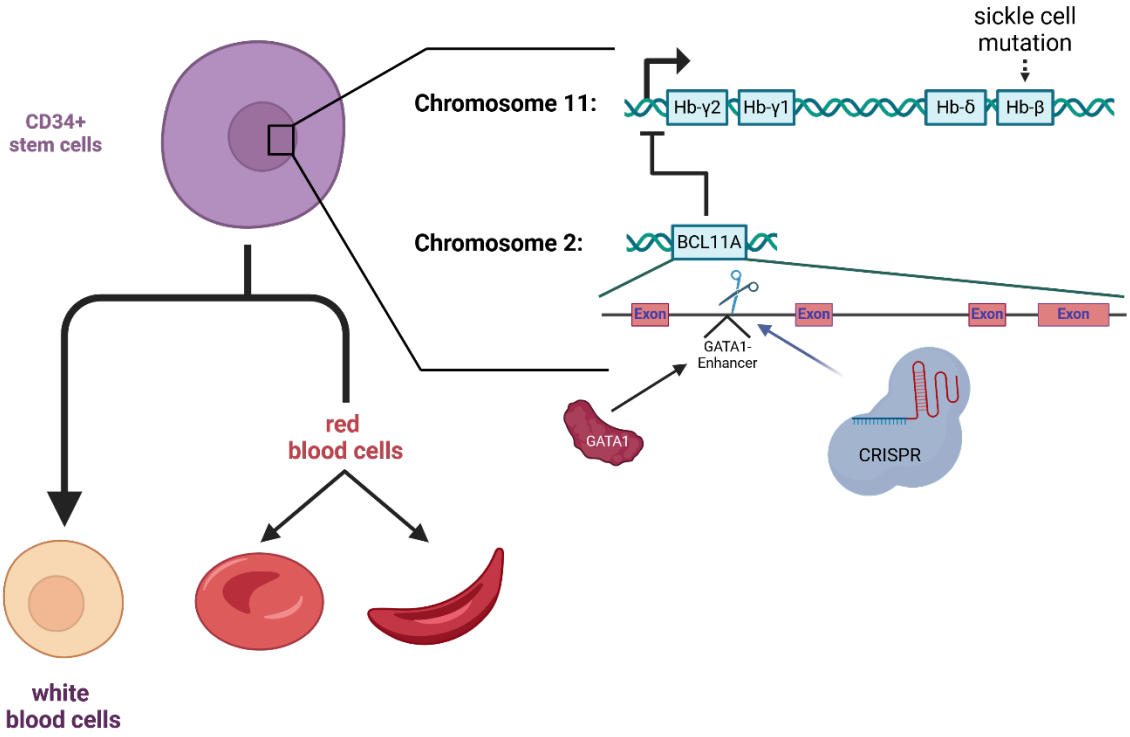
It is important to distinguish between patients with heterozygous and homozygous HbS mutations. People who have only one mutated hemoglobin allele (β^S) form significantly fewer sickle cells because in addition to HbS ($\alpha_2\beta^S_2$), HbA ($\alpha_2\beta_2$) is also formed. HbA does not polymerize and thus blocks sickle cell formation[6,10]. A related condition is β thalassemia, in which too little or almost no hemoglobin β is produced[11]. Patients do not form sickle cells but also suffer from constant anemia and thus develop growth disorders and a weak immune system. The usual treatment for both β thalassemia and sickle cell anemia involves regular blood transfusions or bone marrow transplants[11]. The latter treatment can be a permanent cure, but unfortunately, there is not always a suitable donor available. This is where CRISPR comes into play.

FIGURE 1: CRISPR-CAS SYSTEM IN A BACTERIAL SYSTEM



The bacteriophage infects the cell and the CRISPR-Cas system can incorporate a small part (spacer) of the phage genome into the CRISPR array (adaptation). This array can then be transcribed and processed into short RNAs with the characteristic hairpin structures (processing). Certain enzymes recognize these and cut the RNA sequence into shorter spacer-repeat segments, which then form the CRISPR-Cas complex. If the phage infects again, this complex can then recognize the spacer-matching sequence and destroy the phage DNA (interference). Image created with BioRender.com.

FIGURE 2: REGULATION OF HEMOGLOBIN IN CD34+ CELLS.



The CD34+ stem cell develops into various white and red blood cells. When it develops into red blood cells, it produces hemoglobin. The hemoglobin genes are located mainly on chromosome 11. The formation of fetal hemoglobin ($\alpha_2\gamma_2$) requires the production of γ-hemoglobin (Hb-γ). Its expression in humans is stopped after the neonatal period by BCL11A. This gene is controlled by the universal regulator GATA1, which activates the bcl11A gene by binding to an enhancer region between two exons within the bcl11A gene. This enhancer region is the interface of the Casgevy therapy. Image created with BioRender.com

Gene editing with the CRISPR-Cas9 system

The big breakthrough came with the CRISPR-Cas9 system. CRISPR-Cas systems in nature are a mechanism in bacteria and archaea to defend themselves against bacteriophages or viruses. The system operates in three steps: integration, processing and interaction[12]. The first step occurs after the cell survives the contact with a bacteriophage or virus. It can then incorporate a smaller fragment of the antagonistic DNA, usually 20 to 40 nucleotides long, into its own genome. This step alone is spectacular: the bacterium builds up a genetic memory to "remember" previous infections. These short fragments (called spacers) are stored in segments, where they are separated by short, palindromic repeats (Figure 1). These CRISPR arrays (**c**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats) give the system its name. In the second step, these arrays are expressed in the form of RNA and cut into individual pieces. The repeat elements form characteristic hairpin structures due to their palindromic sequence, which are recognized by specific CRISPR-associated proteins (Cas). From this the so-called CRISPR-Cas complex is formed, consisting of the Cas proteins and the short RNA fragment, which is made up of the cut repeat and the original phage

sequence. In the third step, the complex is now able to recognize new phage infections by comparing the RNA fragment with the DNA in the cell via base pairing. If the complex recognizes this sequence, it cuts the DNA at this position and thus prevents the phage from taking over the cell[12].

The structure of this system allows any DNA target sequence to be specified by inserting a suitable sequence into the CRISPR array. The system can be reprogrammed even more directly if it contains only a single sequence for targeting, in which case it is referred to as a single-guide RNA[1]. There are many areas of application for this reprogrammed CRISPR-Cas system; but the most interesting is probably the modification of genetic material, known as genome editing. For this purpose, the system is given a target gene or a sequence to be cut. The cell's own DNA repair apparatus initially enlarges the resulting gap, but later aligns the adjacent DNA sections back together[13]. However, it is possible to introduce a suitable alternative DNA fragment into the cell, which can then be inserted into the cut area. To do this, the fragment that is supposed to be inserted is embedded between two DNA areas that correspond to the areas around the cutting site[13]. The repair system recognizes the similarity and inserts the section at the location of the original cut[13].

The CRISPR-Cas system exists in many bacteria and archaea, but it is not a universal system. Currently, six types with over 33 subtypes are known, which can also perform alternative functions such as the defense against plasmids or the degradation of RNA[12]. The most well-researched system is type II since its CRISPR-Cas complex consists of only a single protein (Cas9). This system has also been used for sickle cell gene therapy.

The Casgevy gene therapy for the treatment of sickle cell anemia and β-thalassemia

The Casgevy gene therapy (sometimes called Exa-cel) has been developed over the last five years for the treatment of sickle cell anemia and β-thalassemia and is approved for patients up to the age of twelve[3]. For this, the stem cells of the red blood cells (CD34+) are removed and modified outside the body using CRISPR-Cas9-related genome editing[1]. The remaining CD34+ stem cells in the patient are suppressed with medication and the modified cells are given back to the patient. The CRISPR treatment outside the body ensures that no other cells are affected by the change, and that the genetic material in the patient's germ line is not affected, so the treatment only affects the patient himself and not his or her possible offspring.

However, the Casgevy therapy procedure does not alter the mutated β-hemoglobin gene, as might be expected, but instead stabilizes the patient by activating fetal hemoglobin (HbF)[1]. It has long been known that the expression of HbF in sickle cell patients after infancy leads to the suppression of sickle cell formation, and some approved drugs already take advantage of this fact[9]. In order to maintain this effect permanently, CRISPR therapy intervenes in the regulator BCL11A, which is responsible for the repression of γ-hemoglobin and thus also of HbF (Figure 2). More specifically, the GATA1 enhancer, a

regulator region outside of the bcl11A reading frame, is destroyed[1]. This enhancer region is necessary for the BCL11A repressor to be produced in erythrocyte stem cells, but not in other blood cells[14]. This has the advantage that the regulator is retained throughout the body and is only missing in red blood cells. The lack of the repressor allows HbF to be produced and sickle cell formation to be prevented.

In medical studies, 75 patients were treated with this therapy - 44 patients with β-thalassemia and 31 with sickle cell anemia - and followed for a longer period of time (between 1 and 36 months at the time of publication)[15]. None of the sickle cell anemia patients had a vaso-occlusive crisis during this period, which had previously occurred on average nearly four times a year, and only two of the β-thalassemia patients required blood transfusions afterward, although the number of transfusions required was significantly lower. A health-relevant amount of HbF was detected in the blood of all patients[15]. This means that the therapy was successful in all patients.

"How much is that going to cost?"

At the beginning of 2024, there was a lot of media coverage about the high cost of this treatment. Current estimates suggest around 1.5 to 2 million euros per treatment[3]. This sounds absurdly high at first - a treatment that no regular person can afford. But it is important to remember that conventional treatments are not free either. One study concluded that the lifelong medical costs of one sickle cell anemia patient are around 1.6 to 1.7 million US dollars (approx. 1.5 million euros)[7]. Added to this are long hospital stays, a weaker immune system of the patient, which makes them more susceptible to other diseases, frequent and long periods of absence from work, etc. On the other hand, it is also important to remember that there are potential later costs of this therapy that cannot yet be determined. For instance, it is not certain if the amounts of HbF can be maintained over longer periods of time or whether potential long-term side effects still occur. It is also necessary to consider how health insurance companies can compensate for this financial advance. In this respect, it should also be mentioned that this is not just a challenge for Germany. Sickle cell anemia and β-thalassemia are much more widespread in Africa, the eastern Mediterranean and southern India. In the medium term, it is the goal to enable these regions to carry out treatment locally. A clear financial forecast is certainly not easy to make at the moment, but the therapy is not as impossibly expensive as it may seem at first glance.

It cannot be denied that this therapy can help many people - including in Germany - and significantly increase their quality of life as well as their life expectancy. This and the fact that we are only at the beginning of this revolutionary medical technology makes us optimistic for the future.

Summary

Healing sickle cell anemia and β-thalassemia:

Sickle cell anemia and β-thalassemia are diseases caused by point mutations in the hemoglobin β gene. Both diseases significantly affect the health of patients and are widespread worldwide. Using CRISPR-Cas technology, the fetal

hemoglobin F gene was reactivated in CD34+ stem cells, allowing this form of hemoglobin to replace the mutated β variant. The cells were harvested for this procedure and later returned to the patient so that the patient's germline was not affected. All treated patients produced a health-relevant amount of hemoglobin F and showed significantly reduced or no symptoms at all of the disease. The EU, the UK and the USA have already approved this first CRISPR-Cas therapy.

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Marcus Ziemann studied from 2012 to 2019 Biology at the University of Marburg. In his thesis he dealt with the alternative amino acid selenocysteine in the working group of Johann Heider. His master's thesis focused on the self-recognition of type IV CRISPR-Cas systems in the laboratory of Lennart Randau. Since 2019, he has been doing his doctorate at the Albert University of Freiburg in the Department of experimental bioinformatics in the research group of Wolfgang R. Hess and conducts research on CRISPR-Cas systems of cyanobacteria.

Wolfgang R. Hess studied biology at the University of Rostock and the Humboldt University of Berlin from 1982 to 1987. After completing his doctorate (Dr. rer. nat.) in 1990 and his habilitation in genetics in 1999, as well as research stays at the Friedrich Miescher Institute in Basel, the CNRS Institute in Roscoff, France and MIT (Massachusetts Institute of Technology), USA, he moved to the U.S. biotechnology company New England Biolabs in 2003. Hess has been Professor of Experimental Bioinformatics since 2004 and Professor of Genetics at the University of Freiburg since 2008. Email for correspondence:*wolfgang.hess@biologie.uni-freiburg.de*

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