



Laboratory test: Bayer researcher Philip Ramsey uses coagulation diagnosis equipment to test blood samples for potential blood clots. His work is aimed at finding out why the balance between coagulation factors and inhibitors is disrupted in some people.

NEW THERAPEUTIC APPROACHES FOR HEREDITARY DISEASES

Treating hemophilia with gene surgery

Hemophilia A is a hereditary disease caused by a defect in an extensively studied gene. The disease is therefore particularly well suited for gene therapy, i.e. targeted intervention in the genome of human cells. Bayer experts are working on ways of treating or even curing the disease with gene therapy. And they are investigating other promising ideas that could make the lives of people with hemophilia A easier.

Photos: Peter Ginter/Bayer AG (5), Sabine Bunge/Bayer AG (1), Peter Himsel/Bayer AG (1), Giovanni Conconi/Foalola (1), Hellbauer & Foretti (1), John Soares (1)

80

percent of people living with hemophilia suffer from a defect in the gene for coagulation Factor VIII.

Source: National Hemophilia Foundation



Targets for treatment: the chromosomes (image left) of patients with hereditary hemophilia A have a defect in the FVIII gene which could be corrected by gene therapy. Dr. Nils Pfaff and his team in Wuppertal (photo right) are working on alternative approaches aimed at inhibiting proteins that act as a brake on the coagulation system.

from 1940

Blood plasma infusions in hospital

For the first time, hemophilia patients with acute bleeding can be treated with blood plasma from healthy donors. The infusions take several hours and often necessitate hospitalization.



“Undoubtedly the greatest opportunity offered by CRISPR/Cas9 is that we could use the technology to treat genetic diseases.”

Professor Emmanuelle Charpentier, Head of the Max Planck Institute of Infection Biology, Berlin

People with hemophilia live in constant danger. Even a cut while shaving or the tiniest of scratches can be a risk. A scrape that healthy people would simply cover with a plaster will continue to drip blood for hours in people affected by hemophilia, because their blood does not clot. Even a small bump can cause major bleeding under the skin of a person with hemophilia A. If the blood vessels remain open, blood can also flow into the joints. Patients who do not receive effective treatment can suffer chronic damage to their knees, ankles and elbows due to this constant bleeding. In these cases, the joints swell and become inflamed, causing severe pain. The consequences are joint disorders, joint erosion and muscle weakness, making patients reliant on walking aids or consigning them to a wheelchair. Bleeding into vital organs like the brain can even be acutely life-threatening.

Approximately 320,000 people worldwide suffer from hemophilia A, the most common form of the disease. Two out of three sufferers have the inherited type (see text box on page 50). Their DNA differs from that of the majority of people in just one small detail: their gene for producing blood coagulation Factor VIII is defective. Without Factor VIII, the blood coagulation system does not work correctly.

Patients today can live with this disease by injecting themselves at regular intervals with coagulation Factor VIII. The factor is injected directly into a vein, daily or several times per week,

for the patient's entire life. As this represents a burden for the patients, researchers are working on developing gene therapies for hemophilia. This could enable them to not only improve the quality of life of patients but also, in the long term, find a cure for the disease.

The principle behind gene therapy involves introducing an intact copy of the defective gene into cells in the patient's body. The inserted gene can then take the place of the defective gene and in this way, in the case of hemophilia A, initiate production of coagulation Factor VIII.

Different methods for repairing genes

This gene is well documented and can be manufactured in the laboratory. But what sounds so simple has so far presented biotechnologists around the world with major challenges. The gene has to be packed into a carrying system, a so-called vector, to be transported into the cells.

Bayer is collaborating with partners on two approaches to achieve the dream of a gene therapy for hemophilia: the conventional approach of introducing an intact gene sequence, and by means of gene editing, i.e. replacement of the defective sequence with a healthy segment using gene scissors (see infographic on page 49).

“The conventional method involves packing the intact gene sequence in a viral envelope. The viral envelope we use belongs to a group of

Two methods of gene therapy

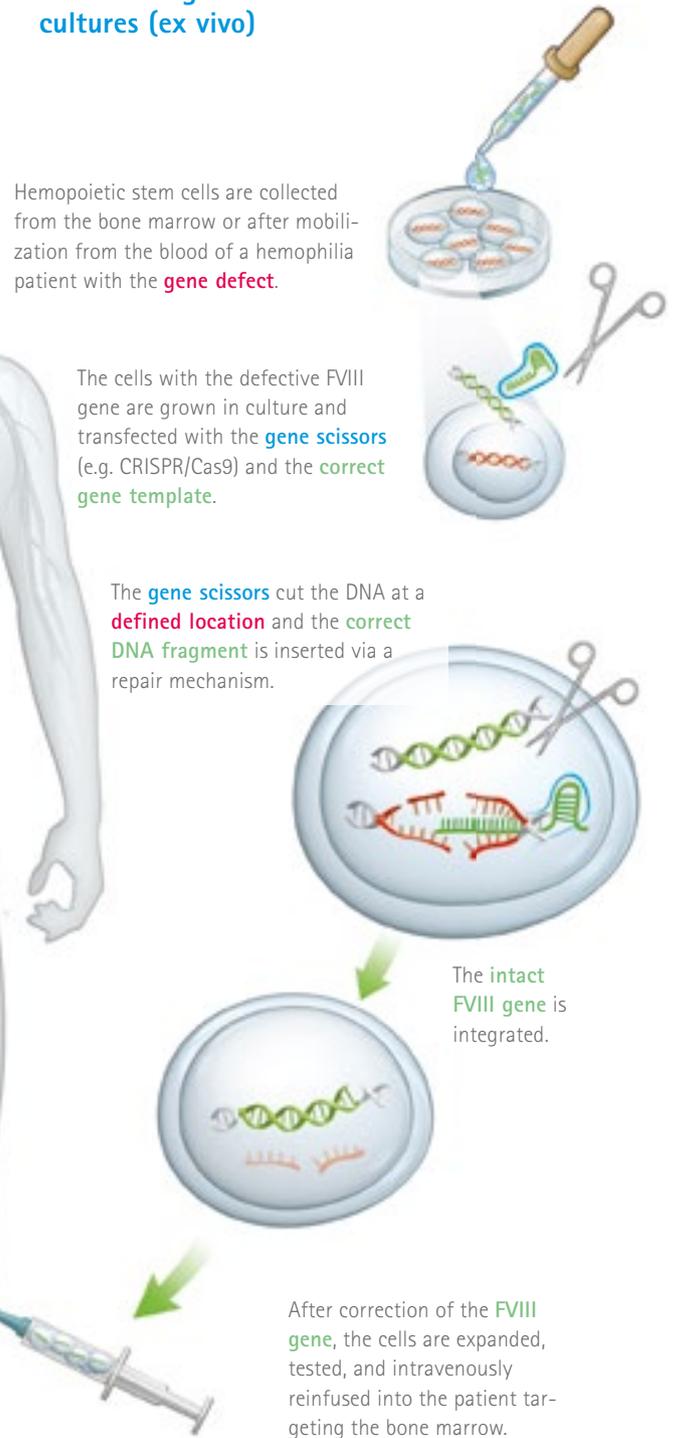
Gene therapy is particularly suitable for patients suffering from a condition that has been thoroughly investigated and is caused by a single genetic defect. The objective then is to introduce a healthy version of the defective gene into the patient's DNA and thus compensate for the defect. The graphic shows two potential approaches which are currently being investigated by Bayer. Both methods are aimed at ensuring that patients with hemophilia A can produce sufficient quantities of coagulation Factor VIII (FVIII) themselves in the future

Vector-based gene therapy in the patient (in vivo)



With the intact FVIII gene, the patient is now able to express FVIII to prevent spontaneous bleeding. The objective of this research is to cure the patient permanently with a one-time treatment.

Gene editing in cell cultures (ex vivo)





Developing gene therapies from scratch: former Bayer postdoc Mamle Quarmyme is now working on novel therapies for hemophilia based on the CRISPR/Cas9 technology at Casebia Therapeutics in San Francisco, a joint venture between Bayer and CRISPR Therapeutics.

1965

Out-patient treatment possible

A new technique known as cryo-precipitation reduces the volume required for plasma infusions and allows out-patient treatment for the first time. In addition, people living with hemophilia can now for the first time undergo surgery.

1970s

Start of home treatment

Doctors are increasingly training their patients to inject themselves with Factor VIII products. This new treatment option primarily improves patients' quality of life.

viruses that are not pathogenic but suitable for delivering genetic materials into cells," explains Dr. Frank Reetz, Global Program Head at Bayer's Pharmaceuticals Division. The virus shuttle constructed in the laboratory is injected into the bloodstream where it penetrates the body cells, in particular the liver cells, and releases the intact gene.

This compensates for the gene defect and the cells are now able, thanks to the introduced gene, to produce functional coagulation Factor VIII themselves. Bayer is collaborating with the U.S. biotech company Dimension Therapeutics to

develop this type of gene therapy (see interview on page 53). The experts already have a lot of experience in the evolving field of gene therapy – from research to the production of a gene agent and early clinical development. "The objective of the collaboration between Bayer and Dimension Therapeutics is to develop a gene therapy for patients with hemophilia A," explains Reetz. He and his colleagues hope that gene therapy will be able to reduce the burden of the disease such as the need for regular intravenous injections and thus increase the patients' quality of life. Another advantage would be that the coagulation factor

Hemophilia, a hereditary disease

Hemophilia A is a hereditary disease that occurs mainly in men, affecting 1 in 5,000 males on average. This is because the clotting Factor VIII gene is on the X chromosome and since men only have one X chromosome, a defect in the Factor VIII gene has a direct impact. Women, on the other hand, have two X chromosomes and consequently two Factor VIII genes. If one of these is defective, the second X chromosome compensates for the defect. Affected women produce enough Factor VIII and do not develop the clotting disorder. They can however pass on the predisposition for the genetic disease to their children. In two-thirds of all cases of hemophilia, the causes of the condition are inherited.

In roughly one-third of patients, there is no known family history of hemophilia. Experts believe that the cause of their disease is a spontaneous mutation.

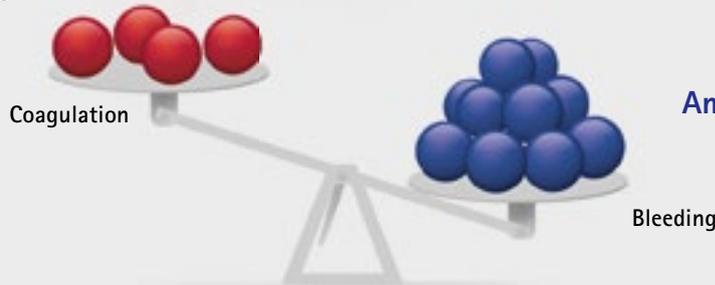
Combating hemophilia directly in the blood

The human body maintains a delicate state of balance which reliably protects it against excessive blood loss but also prevents the formation of blood clots at the wrong location. In hemophilia patients, this balance is out of kilter. Bayer scientists are working on two fundamentally different approaches to restoring this balance.



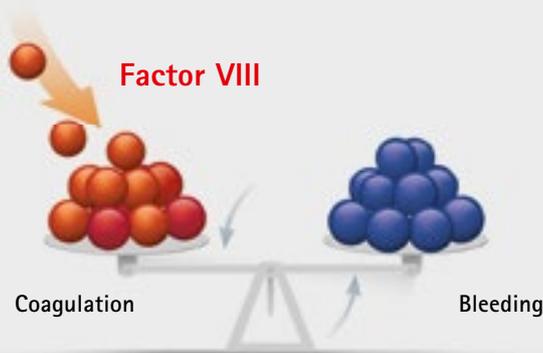
In hemophilia patients, the balance in the blood is not right: the absence of Factor VIII disrupts the balance between **clotting factors** and **anticoagulating factors**. This prevents reliable blood coagulation.

Clotting factors

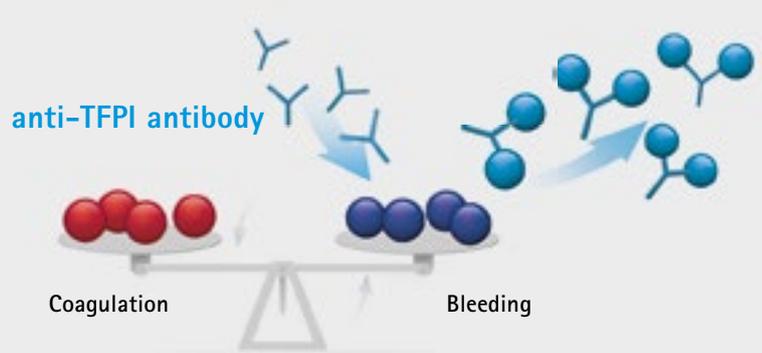


Anticoagulating factors

Replacement treatment with Factor VIII



Inhibition of anticoagulating factor TFPI



In replacement therapy with **Factor VIII**, patients inject the missing **clotting factor** directly into the bloodstream themselves. This restores balance to the coagulation system. Bleeds can be routinely prevented.

Therapy with **anti-TFPI antibody** directly targets the anticoagulating factor side: the antibody intercepts the **anticoagulating factor TFPI** and thus restores balance to the blood coagulation system.





Research alliance: Dr. Peter Nell (photo left) is working on developing gene editing therapies at Casebia Therapeutics. Bayer researchers Jinger Xie and post-doc Ana Pereira test samples (photo right, right to left).

1984

Successful cloning of the Factor VIII gene

For the first time, the gene for the vital clotting factor can be copied in the laboratory. This allows Factor VIII to be produced by genetic engineering and dramatically reduces the risk of viral disease transmission.

would be produced continuously and its concentration in blood would vary less, ensuring better protection for the patients. "But we still have a long way to go," says Reetz, warning against excessive expectations.

Another form of gene therapy is based on the discovery of a method called CRISPR/Cas9, a kind of molecular scissors. Using this method known as gene editing, the researchers plan to remove the defective part of the gene and replace it with an intact new segment – in other words, completely repair the gene.

CRISPR/Cas9 method cuts DNA at precisely defined locations

Bayer has recognized the immense potential of this technology, which could represent a breakthrough in the treatment of genetic disorders, and has therefore established a joint venture called Casebia Therapeutics with the Swiss company CRISPR Therapeutics. Professor Emmanuelle Charpentier, currently Director of the Max Planck Institute for Infection Biology in Berlin, co-developed the CRISPR/Cas9 genome editing tool, an achievement for which she received among other honors the Hansen Family Award (see research 29, "Gene scissors to combat hereditary diseases").

What is revolutionary about the CRISPR/Cas9 method is that the optimized gene scissors can cut DNA at exactly the location in the genome

selected beforehand by the scientists. "This means that for the first time we can specifically target a defective gene sequence and then cut precisely at that sequence," explains Dr. Peter Nell, head of Strategy & Business Development at Casebia Therapeutics in San Francisco, USA. An additionally supplied, healthy gene sequence fills the gap and corrects the gene defect. Nell is remaining realistic, however. "This latest method is fascinating and has incredible potential, but it must first prove its worth in numerous tests before it can be used in patients." He nevertheless feels that there is a high likelihood that it will one day allow faulty genomes – and not only those of people with hemophilia – to be repaired.

In addition to gene therapy, Bayer's researchers are also looking for alternative therapeutic options to improve coagulation. Hemostasis, the natural process that stops bleeding, is the result of a finely balanced interaction between coagulation factors and inhibitors. Bayer researchers are therefore investigating another therapeutic approach to hemophilia A which is currently

Extreme care: the production of hemophilia drugs is subject to stringent standards. Bayer employee Ashiana Ali inspects ampoules of a currently marketed drug product before they are packaged.



"We hope that gene therapy can improve the quality of life of patients in the future."

Dr. Frank Reetz,
Global Program Head, Bayer

being tested in a clinical study. Instead of re-establishing the balance by intravenously administering coagulation Factor VIII, the idea behind this approach involves blocking the body's own coagulation inhibitors that promote the tendency to bleed (see infographic on page 51). Deactivating these inhibitors makes the blood coagulate more easily, halting the bleeding. "Targets for this approach are anti-coagulant factors such as TFPI or Tissue Factor Pathway Inhibitor," says Dr. Nils Pfaff, a research scientist at Bayer Cardiovascular Research. In healthy people, TFPI is one of several anticoagulant factors in the clotting cascade.

Human blood coagulation is a balancing act, requiring precise work from scientists

"By specifically targeting TFPI we are helping to rebalance the clotting system in hemophilia patients, restoring normal clotting levels," explains Pfaff. The research team at Bayer has managed to identify a unique anti-TFPI antibody that binds to two distinct domains on the protein. The therapy is currently in clinical Phase I testing, i.e. undergoing initial tests in hemophilia patients. Pfaff and his colleagues have thoroughly done their homework. "In our research studies, we have implemented biomarkers, in other words molecules that allow monitoring of the efficacy and safety of this mechanism." The study is being performed with the utmost care, as the clotting of blood is, and will remain, a balancing act. If the imbalance tips in favor of coagulation, the risk of unwanted blood clots increases. But through decades of research in this area of expertise Bayer researchers have learned how to walk this fine line. ■

Interview



**Annalisa
Jenkins**

“Objectives for gene therapies”

research spoke to Dr. Annalisa Jenkins, Chief Executive Officer at Dimension Therapeutics, which is collaborating with Bayer to develop a gene therapy for hemophilia A.

What diseases are suitable for gene therapy?

We have carefully and systematically developed criteria for this. At present, we are targeting diseases that are caused by a single gene. In addition, the way in which the disease develops needs to be well understood. Data must also be available from clinical experience or preclinical studies to suggest that restoration of five to ten percent of gene function could in itself be clinically significant and would consequently also be advantageous for patients. In concrete terms, we are working on hemophilia A and inherited metabolic disorders.

Why are you collaborating with Bayer?

Besides the financial agreements, we particularly value Bayer's experience in the field of hemophilia. We also benefit from the existing network of doctors, leading opinion-formers and regulatory bodies when it comes to clinical development.

