Gene Therapy: The New Weapon Against Diseases until There Difficult To Overcome: Some Current Facts of Gene Therapy and Cases of Sickle Cell Anaemia

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A therapeutic breakthrough to defeat Sickle cell anaemia through gene therapy?

Definition

Sickle cell anaemia is an inherited genetic disease that affects the hemoglobin chains of red blood cell hemoglobin, carrying oxygen less well through the body. It is a rare disease, however, it is the most widespread genetic disease in the world and especially widespread in sub-Saharan Africa.

It causes anemia, painful seizures that affect several organs, it is also called sickle cell anemia, this disease results in a deformation of red blood cells in the form of sickle or a crescent moon, which prevents normal circulation in the blood vessels. This will cause blood flow to be blocked.

It is a disease that is geographically concentrated in certain areas such as Africa, India, Brazil, the Mediterranean Basin, but it is currently found everywhere because of mass migration and has been considered since 2008 by United Nations as a public health priority. Sickle cell disease affects black people and accounts for 50% of deaths in childhood.

A 2005 study published in The Lancet estimated that 4.4 million people worldwide are affected by the disease.

Research is trying to improve the treatment of sickle cell disease, too, through gene therapy, which has already been proven today.

Sickle cell anaemia is the most common genetic disease, yet it is still little known to the public

According to the United Nations, sickle cell anaemia is the most responded genetic disease in France with more than 12,000 people affected and recognized as a public health priority. Sickle cell disease is still poorly known to the public and patients with this genetic disease do not benefit from optimal management: IGNORANCE? In France, DOMs are considered to be areas of risk, which is why screening for the disease is mandatory at birth for all children born in the DOM as well as for babies born in the metropolis but whose parents are from Overseas.

Description of the hemoglobin abnormality responsible for sickle cell anaemia:

The hemoglobin of sickle cell anaemia is partially or completely replaced by hemoglobin S, which deforms the red blood cell into a form called sickle, which can no longer circulate normally and is destroyed with the result that decrease in the number of transporting oxygen into the bloodstream. Sickle cell disease is transmitted through the union of two parents who both carry the genetic abnormality. Parents may be healthy carriers, i.e. they possess as much hemoglobin A as hemoglobin S, they are AS carriers called heterozygous, generally do not suffer any of the symptoms of the disease only, risk transmeding to their children the gene holding the abnormality hence the importance of early detection in children born.

Sickle cell anaemia manifests itself as: vaso-occlusive seizures, infections, (fear of septicemia, meningitis) and worsening anaemia. Sickle deformation of red blood cells causes small vessels to block acute, intense, excruciating acute pain attacks requiring hospitalizations, as well as doses of morphine.

Painful seizures can be so intense that a normal life cannot be possible. Regular medical follow-up is essential to improve quality of life and try to prevent the symptoms of the disease as best as possible. (1,2)

Complications of sickle cell disease include:

Mortality is significant during childhood, secondary to spleen pathologies (thrombosis, haemorrhage, occurrence and recurrence of infections).

The manifestations are also made of stroke, stroke, dysfunction of the spleen. Sickle cell disease also causes heart, eye problems, etc.

To date, many believe that there is currently no treatment for this disease and that it is simply possible to treat and prevent symptoms, treat seizures, alleviate pain with painkillers, reduce the risk of infection with antibiotics, transfusions for severe anaemia, because these are patients who are usually anaemic and therefore bear lower than normal levels, this by chronicity. Blood transfusions are offered when hemoglobin levels are less than 7 g/dL. A folic acid supplement is offered, as well as by iron kelp, in case of frequent transfusions. Anticoagulants or vasodilators can help too.

Adequate hydration on a daily basis, and even through intravenous infusions if necessary. The psychological impact of pain on the child, requires appropriate treatment and put in place quickly.

Bone marrow transplantation is reserved for very severe forms of sickle cell disease. Currently, the development of gene therapies allows us to have hope in the treatment of this disease and others such as cancers, certain chronic diseases, some rare diseases... (3,4,5)
GENE THERAPY

Definition

Gene therapy is a treatment method of using nucleic acid (RNA or DNA) as a pharmaceutical product to prevent, treat or cure a disease. The first successful trials of gene therapy date back some 20 years: in the late 1990s, a medical team was able to successfully treat patients with severe immune deficiency. Since then, many clinical trials have been underway to treat conditions as varied as cancer, cardiovascular disease or certain infectious diseases.

What are the positive impacts of Gene therapy?

The use of gene therapy in its early days concerned diseases due to defective genes (monogenic diseases). Today this is extended to cancers, this by stimulating the immune system of patients against cancer cells. Some tests concern the regeneration of heart tissue, heart disease, neurological, neurodegenerative, infectious, severe immune deficiencies, hematological diseases.

Gene therapy has proven its worth in the treatment of severe immune deficiencies, two gene therapy treatments have been approved, including one used in France since 2012, which has effectively treated the family deficit in lipoprotein lipase. More than a thousand clinical trials are underway. France is one of the world leaders in the field of gene therapy.

Some real facts about gene therapy

Researchers at the University of Oxford in the United Kingdom used gene therapy in mice with retinitis pigmentosa to reprogram the cells at the back of the eye to become sensitive to light again. This degenerative genetic disease of the eye is characterized by a progressive loss of vision. This disease particularly affects people between the age of 10 and 30.

Scientists monitored the guinea pigs for a year, which partially regained sight with a high level of visual perception.

"There are many blind patients in our clinics and the ability to restore their eyesight with a relatively simple genetic procedure is very exciting. Our next step will be to start a clinical trial in men," said Samantha de Silva, author of the study.

A young woman with a genetic disease of the retina, has integrated a clinical trial of gene therapy. She found that her eyesight improved.

According to these words, she had never had a very good view. "When I was a child, I was diagnosed with strabisms very early on. But my mother soon realized that the problem was more serious. I couldn't see the things that were being pointed at me, I was banging everywhere," recalls the young woman, now older.

After a diagnostic wandering, when the girl was 8 years old, doctors discovered that the little girl was suffering from Leber's congenital amaurosis, a genetic disease of the retina whose outcome, blindness, is inevitable. Or at least it was. Because, in 2012, she participated in a clinical trial.

To paraphrase it "I was injected into the left eye, the one with which I saw the least well, the healthy gene," she says. Two months after the operation, the first positive signs appeared: I could see both the left eye and the right eye. And since then, his eyesight has improved and continues to improve by the day.

"My illness is characterized by a very small field of vision. Imagine you see the world through the hole of a straw and you will have an idea of what I was seeing! She discovers the details of the view that I see a lot more details about my environment," she says. This allows me to move more easily and especially by being more confident. She also better distinguishes the details of her interlocutors' clothes, as well as their faces and haircuts: "Before, I tended to imagine what I couldn't see. And sometimes it was a big surprise to discover that a person I had imagined with pepper and salt hair was finally bald!».

In the United States, some of the patients who received similar therapy in both eyes saw their condition improve even more dramatically. "I won't hesitate for a second if I have the opportunity to receive the treatment in the second eye," she says.

After clinical trials in mice, 9 patients participated in this clinical trial, out of the 9 patients, 6 showed an improvement in their vision.

Gene therapy would therefore allow vision to be restored by opting for a reprogramming of cells at the bottom of the eye to become sensitive to light again, according to the results of a study published in the medical journal Science Translationalmedicine. Most causes of blindness occur as a result of the loss of millions of light-sensitive photo-receptor cells in the retina.

Scientists have managed to treat mice with type 1 diabetes. To do this, they reprogrammed cells to make them produce the insulin they lack.

Encouraging trials towards a cure for type 1 diabetes? That's according to a team of U.S. researchers who have tested gene therapy on mice to restore the production of insulin that is lacking in people with this disease. The new study was published January 4, 2018 in the journal Cell Stem Cell.

Scientists are currently testing monkeys and hope to be able to conduct human clinical trials soon.

Gene Therapy and Sickle Cell Anaemia

The first gene therapy trial in a French teenager with sickle cell disease was a success. Two years after therapy, he showed no signs of the disease, and even better, he would have even stopped coming to the hospital on a regular basis for transfusions in order to relieve these occlusive vaso seizures as was the case before therapy Gene.

Researchers at Necker-Children's Hospital in Paris published in the New England Journal of Medicine the results of a first advance in gene therapy in sickle cell patients.

It turns out that researchers at the Institute of Genetic Diseases "Imagine" took stem cells from the bone marrow of a teenager with sickle cell disease (a form of hereditary anaemia). Then they "corrected" the genetic defect of these cells by introducing a missing gene (this is called gene therapy) before reintroducing these stem cells into the young patient's bone marrow.

The patient after two years, is in remission, and asymptomatic of the disease, this one is now in shape and these regular and monthly visits for blood transfusion have ceased. To quote one of the researchers, this patient came every month to get transfused to avoid having huge bone pain. He is no longer transfused at all, he hardly ever comes to the hospital again. It gave it a potential for activity that was impossible until now," explains Dr. Jean-Antoine Ribeil of Necker Hospital, on the Europe 1 antenna, this result confirms the effectiveness of gene therapy according to the researchers.

More than 200 babies are born with sickle cell anaemia each year in France. This genetic disease mainly affects people with dull skin, which is why DOMs are considered a risk zone and in the departments, screenings are carried out on all newborns.
Sickle cell anaemia is an inherited genetic disease characterized by an abnormality of hemoglobin, an essential component of red blood cells, allowing the transport of oxygen in the blood. Symptoms, screening, treatment: discovery.

Sickle cell disease is a recessive autosomal abnormality, affecting both girls and boys, and occurs when the patient carries two genes for the disease. Sickle cell disease is a genetic disease caused by a mutation in the gene encoding the synthesis of hemoglobin. For the disease to be transmitted, parents must both carry the mutated gene S, the normal gene being gene A, the severe form is the SS homozygote form, which is manifested before the age of 2 by jaundice, pale complexion, and abdominal pain. The disease is suspected of chronic anaemia observed on blood samples and confirmed by hemoglobin electrophoresis, an examination to show a high level of hemoglobin S, which is involved in the disease.

Prior to gene therapy, the only alternative until then was a bone marrow transplant after compatibility tests, otherwise there was no cure for sickle cell disease. "For most patients, management is based on prevention of complications and regular medical follow-up," explains INSERM. "The only curative treatment currently available is bone marrow transplantation. This procedure is very cumbersome, expensive, with risks; it is therefore reserved for the most severe forms of the disease. About 20 children benefit from it each year in France." (5,6)

To improve patient care, researchers are working to find therapeutic alternatives. "Studies are underway to identify genetic characteristics responsible for variations in disease manifestations depending on the patient," notes INSERM. Hopes for recovery are also based on gene therapy. The objective is to ‘graft’ a healthy beta-globin gene into hematopoietic stem cells of sickle cells." (4,5).

The Association for the Information and Prevention of Sickle cell disease (APIPD) has just published '10 golden rules' that can help sickle cell patients to be less prone to seizures:

- Keeping a good lifestyle: washing hands with soap and water to limit microbial risk, oral and body hygiene;
- Have a healthy and varied diet, including fresh fruits and vegetables;
- Monitor your temperature: have a thermometer at your fingertips all the time. If it is above 38°C, consult your doctor.
- Drink plenty of water, about 2.5 litres per day;
- Avoid poorly ventilated areas;
- Avoid temperature differences;
- Monitor eye colour and dark urine;
- Avoid anything that can slow or block blood flow: tight clothing, cross-legged;
- Avoid alcohol and tobacco;
- See your doctor regularly.

News that saves millions of lives is rare enough to be mentioned. At the American Society of Hematology (ASH) conference in San Diego from December 1-4, 2018, a study showing the exceptional efficacy of sickle cell disease in an already known, inexpensive and easy-to-administer treatment moved many. Combined with a new, simpler diagnostic test, the fight against the disease is expected to make phenomenal progress.

Hydroxyurea represents the possibility of saving children now condemned in Africa by sickle cell disease, but remains inaccessible for some patients from very poor families. This molecule well known in Europe and the United States has a proven track worth against this genetic disease characterized by a malformation of red blood cells in sub-Saharan Africa. There is frequently no treatment in this area, resulting in complications that can be vital.

The study reached (RealizingEffectivenessAcross Continents with Hydroxyurea) was conducted in several African countries (Angola, Kenya, Uganda, Democratic Republic of Congo). This low-cost, easy-to-administer treatment could help fight the disease that is wreaking havoc in sub-Saharan Africa.
It is estimated that 300,000 children are born with sickle cell disease each year worldwide, with about 75% in Africa. (7)

**Sickle cell anemia: efficacy of gene therapy in the first patient**

Gene therapy has successfully treated a young boy with a severe form of hereditary chronic anaemia, sickle cell disease, which is the first patient in the world to benefit from this innovative treatment, researchers said.

Sickle cell anaemia is characterized by a deformation of red blood cells in the form of sickle.

The patient was treated with gene therapy at the age of 13 in October 2014 at the Necker-Children Sick Hospital and the Imagine Institute in Paris by the team led by Prof Marina Cavazzana (AP-HP, Inserm, Université Paris Descartes).

**A corrective gene leading to complete remission**

The therapy, conducted in collaboration with Professor Philippe Leboulch (CEA France and Harvard University), who developed the transporter vector and the correcting gene, allowed complete remission of the signs of the persistent disease nearly two and a half years later.

**A therapy of the future**

The first results 15 months after the transplant, published in the New England Journal of Medicine, "confirm the efficacy of this therapy of the future," according to the authors.

"He's fine, he doesn't need a monthly transfusion, painkillers or hospitalization," Ms Cavazzana told AFP.

The young patient benefited from this therapy following osteonecrosis, a bone destruction caused by clots of the small vessels, had a bilateral hip replacement.

**A promising alternative**

Gene therapy is an alternative for patients who cannot benefit from a bone marrow transplant due to a lack of a compatible donor in siblings.

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**Description of support**

The first phase of treatment involved taking blood stem cells, which cause blood cells (red and white blood cells, platelets) from the patient's bone marrow.

These stem cells are then "corrected" in the laboratory by inserting the gene-drug using a viral vector, already developed to treat another form of severe anaemia, Beta-thalassemia;

The HIV-derived viral vector made harmless and carrying the correcting gene is now supplied on a large scale by the American company bluebird bio under the name LentiGlobin.

Before re-injecting the corrected cells, the bone marrow is treated with the drug Busulfan to remove abnormal, untreated stem cells.
The corrected cells were then re-injected to the patient. After the transplant, he stayed in the hospital for a month.

**A satisfactory recovery.**

The patient currently has returned to a normal life with a return to school, a normal school life and even participation in sports and physical activities.

Since the first patient with thalassemia treated with gene therapy in France more than a decade ago, there have been technical advances including a highly purified vector, confirms Prof. LEBOULCH. "The production of the therapeutic protein from the vector is remarkably high, its level is twice that which would have been enough to be therapeutic," he says.

"The body normally produces about 300 billion red blood cells a day," notes Prof. Leboulch.

The number of patients treated is increasing

About 20 patients with beta-thalassemia have been treated worldwide, including four, aged 13 to 21, recently in France and 18 in the United States. At least seven sickle cell patients were also treated in the United States with the same LentiGlobin vector. (9)

**Effectiveness and Conclusion**

Gene therapy therefore embodies a hope, the future in the face of this hereditary blood disease that affects 250,000 babies at birth each year worldwide. For the first time in the world, a patient with sickle cell disease has been successfully treated with gene therapy. Published in the New England Journal of Medicine, this breakthrough was obtained by a team from Necker Hospital in Paris and the Imagine Institute (1).

« He no longer has any signs of the disease. Even with two years of hindsight, we prefer to remain cautious and talk about remission rather than healing. But all indications are that his condition will remain stable," explains Professor Marina Cavazzana, head of the biotherapy department at Necker.

**Gene therapy, a hope to cure sickle cell disease**

For the first time in the world, this blood disease has been successfully treated with gene therapy at the Necker Hospital in Paris.

Two years later, the patient is in complete remission

Treatments help alleviate symptoms and reduce the frequency of seizures. The only lasting treatment is a bone marrow transplant. "But more often than not, this transplant is not possible because of the lack of a compatible donor in the family. And then gene therapy can be the solution," says Professor Cavazzana.

"Clinical trials to confirm the value of this approach"

In October 2014, his team applied this treatment to a 13-year-old boy with severe sickle cell disease. Doctors first took stem cells from him before introducing a "therapeutic" gene using a vector developed by Professor Philippe Leboulch (CEA/University Paris-South). Once "corrected," the cells were then re-injected to the patient, who is no longer in need of treatment and has resumed school activities.

At this time, it is difficult to say when this therapy will be widely available. "That's of course the goal. But in the immediate future, we will continue clinical trials to confirm the value of this approach," says Professor Cavazzana. (10)

**Promising but preliminary results and therefore to be confirmed**

The results, 9 months after the transplant, were presented in early December 2015 to the American Society of Hematology; this gene therapy allowed this patient to produce normal hemoglobin, at a rate of 51.5% of the total hemoglobin produced, which freed him from the monthly transfusion exchanges previously necessary.

If these early, very preliminary results are confirmed, this type of gene therapy could become a curative therapeutic for patients who do not benefit from a compatible HLA donor. (11)

Healthy red blood cells in a blood vessel. They carry oxygen in one direction and carbon dioxide in the other through the hemoglobin they contain. In patients with sickle cell disease, they are deformed because hemoglobin is deformed. The only current curative treatment is bone marrow transplantation. Gene therapy could be added.

Year after year, the fight against this disease progresses. Latest breakthrough ? The care of a teenager diagnosed with sickle cell disease in 2014 by Professor Marina Cavazzana (Necker-Sick Children Hospital, AP-HP) and the Imagine Institute. The young patient received gene therapy treatment. Nine months later, more than half of hemoglobin is normal.

In practice, "stem cells were taken from his body, and a normal copy of a hemoglobin gene reintroduced into these cells," the scientists said. "Nine months after receiving this corrected cell transplant . . . the patient is doing well and produces about 51.5% normal hemoglobin."

In view of its results, and if one can move from remission or word "healing", gene therapy could become the future in the management of patients with sickle cell disease, now mainly supported by the treatment of symptoms, and for patients with compatibility of an HLA donor, treated with a bone marrow transplant.

Gene therapy is therefore well on its way to being one of the best treatments for sickle cell disease. This would change the quality and life expectancy of patients.

To date, only two non-curative approaches exist: monthly transfusion and hydroxyurea treatment to increase hemoglobin levels and soothe severe pain). But unlike gene therapy, none of them limit the damage to organs weakened by a lack of oxygen supply.

Sickle cell anaemia: The first patient was successfully treated, started a normal life

The teenager treated at Necker Hospital, who has a very severe form of the disease, no longer has painful seizures and lives normally without a transfusion.

"This boy is in a totally stable situation, and the level of correction obtained gives hope that his remission will continue for years to come," says Marina Cavazzana, who refuses to talk about healing. If these good results are confirmed over time and on a larger scale, this gene therapy could be offered as an alternative to bone marrow transplantation in patients who do not have a compatible donor," she said. That's potentially a lot of people.

**Effectiveness of gene therapy**

"This patient is part of a Phase I-II study involving seven seven patients, all of whom are being treated at Necker Hospital," says Marina Cavazzana. Five - four with thalassemia and our young sickle cell disease - have already been treated, with positive results. Two others, sickle cell disease, are being treated."
In the United States, this same gene therapy is the subject of two Phase I-II clinical trials, one in thalassemia patients and the other in sickle cell adults.

The latter's preliminary results, covering an average of seven patients followed for an average of seven months, are much more modest than in the French patient, according to a paper presented in December 2016 to the American Congress of Hematology.

At the moment, the cost of this therapy is very high, in the order of 500,000 euros per patient, according to Professor Cavazzana. "But this amount is expected to fall sharply in the coming years, with process automation," she says. (12, 13)

The results of this innovative therapy against sickle cell disease have been published in the New England Journal of Medicine

For the first time in history, treatment has allowed complete remission of clinical signs of sickle cell disease. After 15 months of follow-up, the 13-year-old, who benefited from it, is doing well. These results, published March 2, 2017 in the New England Journal of Medicine, confirm the efficacy of this future therapy.

The young patient was treated in October 2014 in a Phase I/II clinical trial with a severe form of sickle cell disease. This severe chronic anaemia causes painful seizures, damage to vital organs (heart, liver, kidneys, brain, bone, etc.), high susceptibility to infections, iron overload and endocrine disorders.

The introduction of a therapeutic gene into blood cells

The first phase of the trial involved the taking of hematopoietic stem cells, which produce all blood cell lines, at the patient's bone marrow level. A viral vector carrying a therapeutic gene was then introduced into these cells to correct them. This lentiviral vector, capable of carrying long, complex segments of DNA, was developed by Professor Philippe Leboulch, CEA's Senior Advisor for Medical Innovation (Harvard Medical School) and is produced on a large scale by the American company bluebird bio.

Left: the sickle cells before treatment. On the right: cells corrected by the introduction of a therapeutic gene. The prospect of larger clinical trials; The treated cells, thus "corrected," were re-injected to the young patient by venous way. The teenager was taken into care during his hospitalization in the pediatric immunohematology department of the Necker-Enfants Sick Hospital in collaboration with prof Stéphane Blanche and Dr Jean-Antoine Ribeil.

Today the young boy has resumed a normal life and all his physical and school activities. "With this approach to gene therapy, we hope to develop future clinical trials and include a significant number of patients suffering from sickle cell disease, in the Ile-de-France and on the national territory," said Prof Marina Cavazzana. (5,12,14,15).

References

1. https://sante.journaldesfemmes.fr/diseases/2540196-drepanocytose-homozygote-heterozygote/
2. Service of Internal Medicine, sickle cell disease reference centre, Georges Pompidou European Hospital, AP-HP, 20 rue Leblanc, 75908 Paris cedex 15, France.
3. https://www.topsante.com/medicine/genetic diseases/other-diseases-rare/la-drepanocytose-a-disease-of-the-pain-101099/(page)/5
4. https://destinationsante.com/drepanocytose-the-guérison-bientot-possible.html
5. Faculty of Medicine, Université Paris Descartes, rue du Faubourg-Saint-Jacques, 75014 Paris, France.
6. Internal Medicine Department, Sickle cell disease reference center, Tenon Hospital, AP-HP, 4 China Street, 75970 Paris cedex 20, France.
7. https://www.centrepresseaveyron.fr/2018/12/10/drepanocytose-a-treatment-simple-and-not-dear-to-save-of-millions-of-lives.5009516.php
8. http://www.rfi.fr/science/20170302-drepanocytose-advance-major-therapie-genique-anemie-chronic-hereditaire
9. https://www.doctissimo.fr/sante/news/drepanocytose-therapie-genique
10. https://www.lacroix.com/Sciences/Sante/Therapie-genique-espoir-pour-guerir-drepanocytose-2017-03-02-1200828856
11. https://www.vidal.fr/actualites/18890/traitemen_t_de_la_drepanocytose_premiers_resultats_encourageants_d_un_essai_francais_de_therapie_genique/Sur VIDAL.frVIDAL Recou ”Drepanocytose” La revue de médecine interneVolume 36, n° SS1pages 53-58 (mai 2015)
12. Département de biothérapie, centre de référence de la drépanocytose, hôpital Necker – Enfants-malades, AP – HP, 149, rue de Sèvres, 75743 Paris cedex, France.
13. https://www.lemonde.fr/sante/article/2017/03/01/drepanocytose-succes-d-une-therapie-genique-chez-le-premier-patient-traite_5087805_1651302.html
14. Site: http://www.aphp.fr Assistance Publique-Hôpitaux de Paris

15. Université Pierre-et-Marie-Curie, 4, Place Jussieu, 75005 Paris, France

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