CASGEVY Treatment

„CASGEVY – the drug that put genetic editing into the commercial pharmaceutical world."

(The Lab Mouse, 2024)

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1 Preface

Diseases caused by mutations of haemoglobin, like sickle cell anaemia and thalassemia are carried by nearly 5% of the world population (Tossea, 2018). Sickle cell anaemia is characterized by a point mutation in the gene that encodes the beta-globin chain of haemoglobin which results in sickle shaped blood cells. Sickle cell anaemia is an inherited disorder (Tisdale, 2020). People having sickle cell anaemia suffer from acute and chronic pain, acute chest syndrome and have an increased incidence of stroke to only mention a few. The average life span expectancy of people with this disorder is about 20 years less than the general population's (Kavanagh, 2022).

We consider it necessary to do research on the field of treating people with sickle cell anaemia and other haemoglobin disorders as they are suffering and have a higher risk of stroke. People with an acute stroke or multi-organ failure need blood transfusions. Blood transfusions are associated with risks like an immune response to the donor blood (MAYO Clinic Staff, 2023). The risks of allogeneic transplantations are discussed in the subchapter 3.5 Allogeneic and autologous transplantation.

Concerning the treatment of CASGEVY we find it especially interesting to find out whether long-term side effects could be imaginable and whether all people with sickle cell anaemia are candidates for this form of treatment.

2 Introduction

The CASGEVY treatment was first allowed in the US from the FDA (Food and Drug Administration) on 8 December 2023. It is the first cell-based gene therapy for the treatment of sickle cell disease in people who are over 12 years old. This gene therapy allows patients to use their own stem cells for the treatment. The CASGEVY treatment uses CRISPR/Cas9 for gene-editing. (McCartney, 2024)

2.1 Alternative treatments

Another treatment against sickle cell anaemia is called LYFGENIA (Lovotibeglogene autotemcel). This treatment got approved at the same time as the CASGEVY treatment and is a gene therapy as well. It is, like the CASGEVY treatment, only approved for people of age 12 and older.

LYFGENIA uses a lentiviral vector to modificate the stem cells. They are modified to produce another form of haemoglobin A. Red blood cells containing it have a lower risk of sickling and clogging blood vessels. (FDA, 2023)

The less severely diseased patients get treated with allogeneic transplantations.

3 Theoretical Background

3.1 CRISPR/Cas9

The CRISPR/Cas system originates from a bacterial defense mechanism against viral infections (Transparenz Gentechnik, 2023).

CRISPR/Cas9 is used for genetic engineering. It makes easier, to determine the functions of proteins and DNA sequences, as they only have to be deactivated and then the consequences

have to be observed. It is also used in plants, to make them resistant against special diseases. There also are many other uses for it. (SIMPLY SCIENCE, 2019)

CRISPR/Cas9 is electroporated into the cell with a guide RNA of your choice. The Cas9 works as a gene scissor and the guide RNA tells it where exactly to go and to cut. The guide RNA is 20 base pairs long and the Cas9 protein cuts between two previously determined base pairs. (Hoppe, P., personal communication)

3.2 Stem cells

There are different types of stem cells. Hematopoietic stem cells are the origin of all blood cells (National Cancer Institute). The hematopoietic stem cells sit in the bone marrow niche. In the bone marrow niche, there is very low oxygen, that prevents reaction of the stem cells with their surroundings. The idea of the stem cells is that they are quiescent, so they wait in the bone marrow until they are needed. It's still possible that some stem cells are found in the blood, but the most of them sit in the bone marrow niche. (Hoppe, P., personal communication)

3.3 Foetal and adult haemoglobin

During pregnancy, the foetus has a high expression of foetal haemoglobin. After birth, this changes, and adult haemoglobin is instead present in our bodies (Apostolova, P., personal communication). Foetal haemoglobin consists of two alpha and two gamma globins, whereas the adult form consists of two alpha and two beta globins. The foetal haemoglobin has a higher affinity for oxygen. This is very important during pregnancy as the oxygen can easily be handed over from the mother to the baby. (Hoppe, P., personal communication)

3.4 Sickle cell anaemia

Sickle cell anaemia is an inherited disease that affects the building of haemoglobin. It is a mutation of the beta globin of the adult haemoglobin. These haemoglobin cells have an elongated form and tend to polymerize forming clots, that can clog blood vessels. This reduces the blood flow, leads to a limited oxygen supply of the body, and causes the symptoms of the disease.

A typic symptom of sickle cell anaemia is fatigue because the oxygen supply in the body is reduced. Another symptom can be vision problems, because the fine blood vessels in the eyes get plugged by the clogs of the sickle cell shaped blood cells. Other symptoms are mentioned in the Preface.

Clogging can happen in other tissues as well, for example in the spleen. The spleen is an important organ for the immune system. Due to that people with sickle cell anaemia can be more exposed to infections. Clogging can also happen in the fine blood vessels in the fingers, which causes pain and swelling. (Hoppe, P., personal communication)

3.5 Allogeneic and autologous transplantation

3.5.1 Genetically not modified stem cell transplantations

In the autologous transplantation stem cells of the patient get removed and frozen. After chemotherapy, these stem cells are reinduced into the patient. There is no genetic modification of the stem cells. This treatment is only possible when the stem cells aren't affected by the disease. In aggressive diseases like leukemia, the stem cells are affected, and can't be given back after chemotherapy. In this case an allogeneic transplantation is better. This procedure is done since the 1990s. (Apostolova, P., personal communication)

In the allogeneic transplantation, stem cells from a foreign donor are transplanted into the patient. For treating severe diseases like leukemia, generally the allogeneic transplantation is done.

Nowadays, the stem cells are mostly collected from the blood. This is possible by using the granulocyte colony stimulation factor. The procedure is explained in the subchapter 4.2 Harvest of the stem cells.

With allogeneic transplantation, not only stem cells are transferred, but also immune cells. An advantage of them is that they can identify cancer cells and eliminate them. Allogeneic transplantations are used when the patient can profit from the additional immune effect. (Apostolova, P., personal communication)

There is a risk for immunological complications as the transplanted stem cells are from a foreign donor. Two reactions can result from an allogeneic transplantation. One is the graft versus host disease. In this case, the transplanted stem cells (from the graft) make immune cells and those immune cells attack the patient (host). The other disease is the host versus graft disease. In this case, the remaining immune components in the recipient destroy the stem cells of the donor. Immune suppressive medication is given to avoid these complications, but by suppressing the immune system, the patients are as well more prone to get infections. (Hoppe, P., personal communication)

3.5.2 Genetically modified stem cell transplantations

Genetically modified stem cell transplantations are based on autologous transplantations. The autologous transplantation is beneficial because there are less complications than in the allogeneic transplantation. (Apostolova, P., personal communication)

4 The engineering technique

The CASGEVY treatment is a genetically modified stem cell transplantation. It is approved since the end of 2023 or the beginning of this year in Europe, the UK, and the United States of America. It can cure diseases of haemoglobin like beta thalassemia and sickle cell anaemia. (Barrie, 2024)

The hope is, that this treatment is a lifelong treatment. But up until now, there has been only a clinical trial with low patient numbers, the long-term side effects aren't known yet. The future will tell how efficient this treatment is. (Hoppe, P., personal communication)

4.1 Method

The modification of stem cells is done ex vivo. Stem cells are harvested from the patient, and then get modified in vitro. After chemotherapy, the modified stem cells are reinfused into the patient. The modification is done ex vivo, because only the hematopoietic stem cells get modified and other cells shouldn't be involved. The modification is done by CRISPR/Cas9. (Apostolova, P., personal communication)

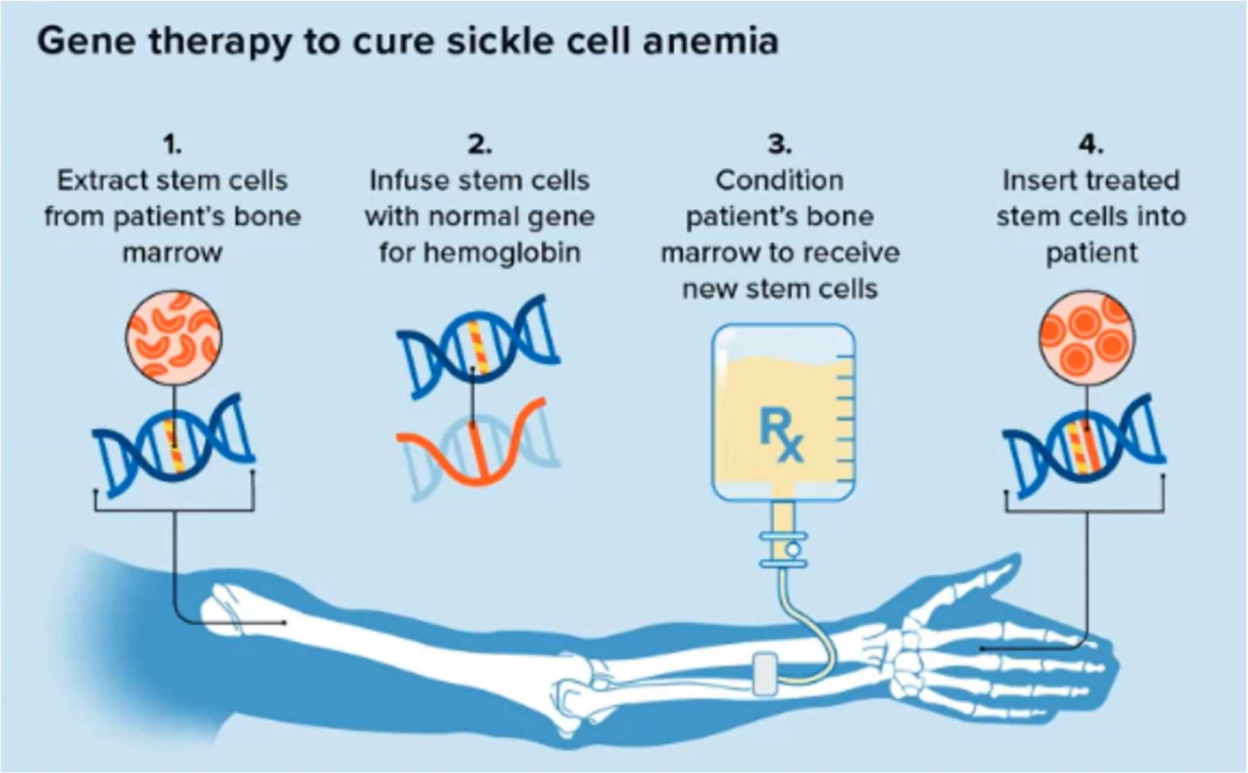

Fig.1: Gene therapy to cure sickle cell anaemia

4.2 Harvest of the stem cells

For the CASGEVY treatment hematopoietic stem cells are needed (see Fig.1). These stem cells are found in the bone marrow and are responsible for the production of blood cells. But it's still possible to collect the cells out of the patient's blood. This is possible by giving the medication "Granulocyte colony stimulating factor". This medication is a growth factor that is produced in the body as well, only in much smaller quantities. When given as a medication, it leads to a high proliferation of stem cells.

The stem cells leave the bone marrow and are then found in the blood, where they are collected by apheresis. (The patient is connected to a machine, the blood from the patient flows through the machine and it collects the hematopoietic stem cells.) (Hoppe, P., personal communication)

4.3 Modification of stem cells

The modification is done by CRISPR/Cas9.

The guide RNA leads the Cas9 to the promoter of a gene called BCL11A. There it induces a double strand break in the genome. The cell then tries to hectically repair this break. While it repairs it, it can happen, that there is an insertion or deletion of a few base pairs. You want that to happen, because then the promoter doesn't work anymore. If that doesn't happen, the strands get repaired correctly and the promoter is intact. Because the CRISPR/Cas is still present in the cell, it will cut again until it is deactivated. By deleting the promoter of BCL11A, it can't get produced anymore.

The gene BCL11A suppresses the production of the foetal haemoglobin. So, the modification doesn't correct the inefficient production of the adult haemoglobin.

With the promoter destroyed, BCL11A can't repress the building of foetal haemoglobin anymore. The modification "inhibits the inhibitor of foetal haemoglobin". This causes patients to produce more foetal haemoglobin. It can get to be up to 50% of the whole haemoglobin pool.

Foetal haemoglobin is a big improvement to the mutated haemoglobin and is almost as good as adult haemoglobin.

In theory, it would be possible to correct the gene that is impaired. CRISPR/Cas9 can correct gene sequences. For the CASGEVY treatment, the inactivation of BCL11A works. It would be interesting for the future to find a way to correct genes or insert healthy ones to cure a multitude of diseases. (Hoppe, P., personal communication)

4.4 Reinduction of the modified stem cells

After chemotherapy, the stem cells are reinduced into the patient's blood by a procedure like blood infusion (Fig.1, 4. picture).

Chemotherapy should kill all hematopoietic stem cells that remained in the body. After the reinfusion of the modified stem cells, there should be modified stem cells only. (Hoppe, P., personal communication)

4.5 Side effects

It is hard to differentiate the side effects of the modified stem cells from the side effects from the chemotherapy.

In the clinical studies up until now there have been side effects like a low count of white blood cells, low count of platelets, headache, vomiting, nausea, and abdominal pain. These symptoms are probably due to the chemotherapy. There are no side effects from the modified stem cells expected.

Because the DNA of the stem cells gets changed, it is possible, that cancer could become a long-term side effect (Hoppe, P., personal communication).

4.6 Costs

In the USA, the treatment costs about 2 million dollars and in the UK, the price is 1 million British pounds (Herper, 2023).

At the moment, the costs will remain very high, because it is a new treatment. In the future, there will be more companies offering CASGEVY treatment, that will lower the costs because of the concurrence on the market. But the treatment will remain expensive. The procedure to generate the modified cells is laborious. Not only specialized laboratories are required, but the treatment is as well labor-intensive, as one has to be very careful not to contaminate the stem cells. (Hoppe, P., personal communication)

4.7 When the CASGEVY treatment isn't an option

Nowadays, the treatment is only approved for certain indications. Patients have to fulfill a certain degree of severity of their disease. Patients with light symptoms won't get approved.

In the current approval, only patients that can't get an allogeneic transplantation can get a CASGEVY treatment. The allogeneic transplantation is much cheaper, but severe side effects can occur because of the foreign stem cells. However, research is more experienced with allogeneic

transplantations than with the CASGEVY treatment. The specialists nowadays have to decide if the gene modified stem cell transplantation, or the allogeneic transplantation is the better option for their patient. (Hoppe, P., personal communication)

5 Interviews with specialists

5.1 Interviews

We chose two researchers to conduct interviews with and prepared questions to discussed.

First, we interviewed Prof. Dr. Petya Apostolova on the 30th of April. She works for the University of Basel in the department of Biomedicine. Her research focuses on tumor immunology, cancer and immune cell metabolism, Allogeneic hematopoietic stem cell transplantation and cancer immunotherapy. We contacted Prof. Dr. Apostolova because we thought that she could tell us more about stem cell transplantations as it is a major topic for the CASGEVY treatment.

In addition, we interviewed Dr. Philipp Hoppe on the 3rd of May. He is Lab Head at the Novartis institute for BioMedical Research in the department Chemical Biology & Therapeutics. His research group works mostly on the CRISPR/Cas9 technology. His interests lie in immunology, and he focuses on questions about stem cells and their development. We contacted Dr. Hoppe as we are interested in the use of CRISPR/Cas9 for the modification of stem cells in the CASGEVY treatment.

5.2 Lab

We could only take pictures of the Lab of the BioMedical center of the University Basel. We weren't allowed to take pictures in the Labs in Novartis. We were shown Prof. Dr. Apostolova's workspace and the room where the cells are kept. Unfortunately, they didn't have any stem cells there, but we saw cancer cells instead (Fig. 5).

Fig.2: Labspace of Prof. Dr. Apostolova

Fig.3: storing room for cells

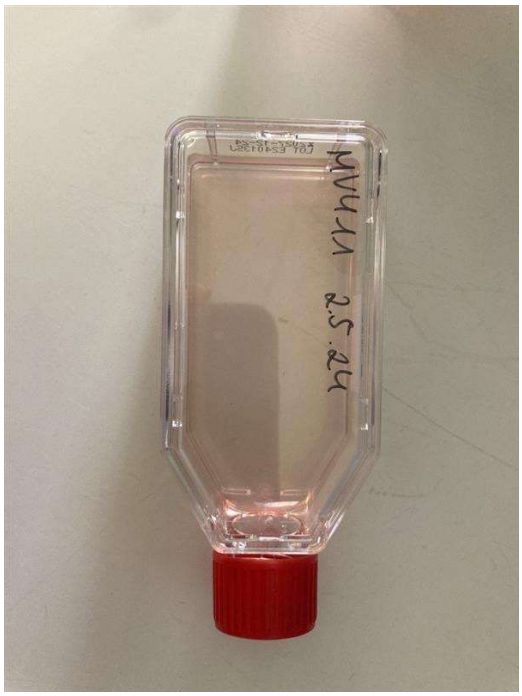

Fig.4: Container of cells

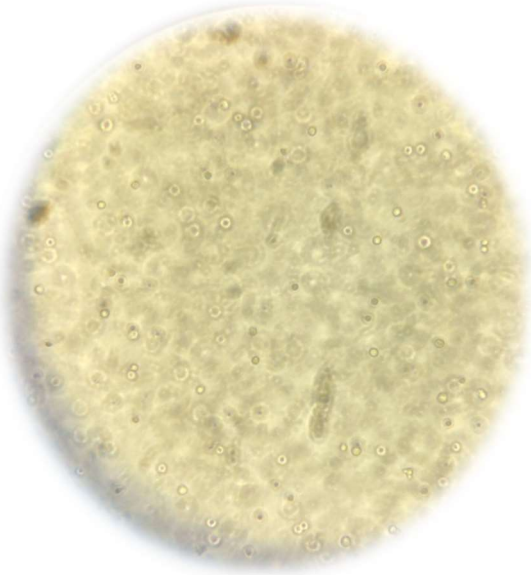

Fig.5: cancer cells under a microscope

6 Discussion

The CASGEVY treatment offers an alternative to the hitherto existing treatments for sickle cell anemia. Some people which received this treatment even claimed to be cured from sickle cell disease (CNBC Television, 2023). The treatment is the first expectedly lifelong treatment against sickle cell anaemia and other similar sicknesses. Before, some patients had to have regular treatments and got non genetically engineered transplantations. The life span of people having sickle cell disease was much shorter than the live span of people without sickle cell disease. The CASGEVY treatment solves many symptoms with the replacement of the adult with the foetal haemoglobin. Unfortunately, it is very expensive and can only be afforded by very few poeple. The most affected poeple live in Africa, and don't have the money to pay it. The CASGEVY treatment is also only allowed for people over 12 years of age. For some people this is already too late.

In the CASGEVY treatment two processes are used: CRISPR/Cas9 and chemotherapy. With CRISPR/Cas9, the hematopoietic stem cells are modified. Chemotherapy is used to remove the remaining stem cells in the bone marrow. Chemotherapy has severe effects. It can cause hairloss, painful mouth sores and infertility. Further disadvantages of this treatment are the long hospital stays. The clinical trial was only made with 31 Patients over a span of two years and was approved a short time ago, so no long-term side effects of the gene manipulation could have been discovered yet. We will have to wait, to see which side effects occur, and how they can be dealt with. We also don't know how high the chances of success are. We can read about the rate of success of the CASGEVY treatment on the website "casgevy.com". As the website acts as advertisement for the treatment, we can't rely on the data presented there. Only 31 people were included in the studies. Two of these 31 people had a VOC (vascular ocular conclusion) within the first 12 months after the treatment. But what happens after these 12 months isn't clear. More studies need to be done to understand the treatment better. Furthermore, the website seems to

encourage people with genetic disorders to go for the CASGEVY treatment, this seems rather unprofessional to us.

Furthermore, it's important that we consider allogeneic transplantation as well. It is done frequently since over 30 years, and we have much more experience with this treatment than with the CASGEVY treatment.

For the future we think it probable that a new technology will be developed, that could be used to treat sickle cell disease. We think it would be interesting to do research in the field of directly correcting the wrong base pair encoding for a wrong protein and then causing a genetic disease. This technique could then also be used to treat other genetic diseases apart from sickle cell anemia.

7 Summary

The CASGEVY treatment is one of the two gene therapies, that were approved in december 2023 to cure sickle cell anaemia. The second treatment is called LYGENIA and uses a lentiviral vector to modificate the hematopoietic stem cell. The cell then produces another type of haemoglobin A, that reduces the clotting of the red blood cells.

Before these gene therapies allogeneic transplantations were used. It has the disadvantage that it generates an immune response in the body. But allogeneic transplantations are still used today because we have more experience with them than with CASGEVY treatment.

The CASGEVY treatment treats patients with sickle cell anaemia. The stem cells are harvested from the blood and modified ex vivo with CRISPR/Cas9. This manipulation deactivates a gene called BCL 11A, that is responsible for the repression of foetal haemoglobin. After chemotherapy the modified stem cells are then reintroduced inside the body.

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9 Appendix

9.1 Interview with Prof. Dr. Petya Apostolova, 30.04.2024

Speaker 1: Nadine, Léa-Sophie, Johanna

Speaker 2: Prof. Dr. Petya Apostolova

Speaker 1 [00:02:24] Okay. So, there are stem cell transplantations where we use stem cells of another person, and then we have the autologous stem cell modification, so when we take our own stem cells when is the autologous stem cell modification better or why is it better?

Speaker 2 [00:02:48] Okay. So, I think here it's important to distinguish. Do we talk about transplantation of non-genetically modified cells or do we talk about transplantation of genetically modified cells. So in principle stem cell transplantation is something that existed for a long time before these treatments grew and is mainly used for treating different types of cancers, where autologous transplantation is like I say, the patient first the, you know, the stem cells from the patients get removed, they get frozen, patients receive chemotherapy, and then the stem cells that have been frozen from the same person get reinfused. This is a procedure we now more most frequently do for multiple myeloma. This is a certain type of B cell malignancy, and for some lymphomas. So basically, for hematological diseases that are not the most aggressive diseases, the most aggressive diseases will be leukemias. And this is where we use allogenic transplantation where we take stem cells from a foreign donor and transplant them in the recipient. The reason for that is that, in leukemia, very often the malignant cell is the stem cell. And so, we don't want to risk transferring these cells is one reason. Another important reason is that when you do an allergenic transplantation, we don't only transfer stem cells, but we also transfer immune cells from the donor to the recipient. And these immune cells are capable of recognizing cancer cells in the patient as foreign and eliminating them in an immunological manner. And so, this is important when we have highly aggressive diseases such as leukemia, where we know that patients benefit from this, additionally new infections. So, we don't have only replacement of the stem cells, but we also have an addition of an immunotherapy. So, this is, what we have been doing so far with non-engineered, hematopoietic stem cells. Now in the context of engineered human hematopoietic stem cells, the current therapies are based on autologous transplantation. So, the cells of the patient will be modified and would be transferred. And this is beneficial because it is less toxic like transferring the stem cells from a foreign donor to a patient also leads to immunological complications that can be very severe. So, I think when we do not want an additional immunological effect, we would go for autologous, transplantation. If we want to have an additional immune effect, then we go for allogenic transplantation. Currently with non-modified stem cell is yeah, I think one more important thing though also for the future would be that for autologous stem cells you don't have what we call off the shelf medication or drug. You can imagine like if if everything was allogenic there could be a bank where it's stored like a little bit like medication in the pharmacy. And then you say, okay, I have this patient, I need this now, and you can take it off the shelf and use it immediately. Whereas for the autologous preference, it takes some time to like to manufacture them. And this means that there is a delay in the treatment of the patient. But still, as I said, like

due to the immunological complications that we have with the allogeneic donors I think, wherever possible, to use the patient's own material that is a good solution.

Speaker 1 [00:06:23] Since when is the autologous stem cell, transplantation? Since when do we do that?

Speaker 2 [00:06:29] You mean with the non-engineered? Well, I would say probably regularly for sure since the 90s 1990s and there because this is also the allogeneic transplantation really increasing frequency in the 1990s. And it has been done the allergenic I know for sure since the 1950s but like really in clinical routine I would say has been the last 30 to 40 years.

Speaker 1 [00:06:55] And the genetically modified ones. Since when or do we already do them?

Speaker 2 [00:07:00] I have not treated a patient with this treatment yet, so it has been approved, I think, in Europe, in the US for the last maybe 4 or 5 months. And last year that the first approval came. So obviously there have been clinical trials before then, but it's really approved the license components.

Speaker 1 [00:07:19] So okay, now we'll talk about, Casgevy treatment. So has this treatment, lifelong effects?

Speaker 2 [00:07:38] I mean, this is hard to say because it has only been around for such a short time. Right? The idea is that it should be. Yeah. This should be a lifelong treatment. It is the idea. But I think only time will show if that works.

Speaker 1 [00:07:55] So the modification is done in vivo, or ex vivo and why.

Speaker 2 [00:08:00] With Casgevy, the modification is done ex vivo, which means that the stem cells are first harvested from the patient, then they're modified in vitro and then they're reinforced in the patient. I think the most important reason for this is that the therapy can be targeted only really to the hematopoietic stem cells, and only they get modified. Because you can imagine if we have a patient and we inject, gene modifying therapy of whatever kind in the patient, we will always have the risk that not only the hematopoietic stem cells get edited, maybe also other cells in the body get edited, which we don't want. And so, by really taking and modifying under the human hematopoietic stem cells, we can make sure that, yeah, only these cells are changed and potentially reduce toxicity.

Speaker 1 [00:08:46] And how is this modification done.

Speaker 2 [00:08:49] It is done with CRISPR. The interesting thing that I found to find about this approach is that, so Casgevy is approved for the treatment of diseases of hemoglobin, so it's, for beta thalassemia and for sickle cell anemia. So, these are both inherited diseases that in some way affect the building of hemoglobin. And by this affect the, I would roughly say the oxygen supply of the body. Right. But instead of like targeting, we're trying to correct the actual defect, like in the hemoglobin chains. What, this therapy does is that it inactivates a gene, which is called BCL11A. And this gene, suppresses the production of fetal hemoglobin. So maybe we need to go back a bit when we have the fetus. Still, during pregnancy, fetuses have a high expression of the so-called fetal hemoglobin, which is a certain type of hemoglobin, when once we are born, changes to what we have as adult hemoglobin. And in these individuals, the adult hemoglobin is in some way defect. There are gene defects that lead to inefficient production, or inefficient function. And so what the Casgevy treatment does is that it impairs or, you know, kind

of edits the gene BCL11A. This gene becomes less active and then it cannot repress. The normal function of this gene is to repress the building of fetal hemoglobin. So, we basically inhibit the inhibitor of the fetal hemoglobin. And this is why these individuals build then more of the fetal hemoglobin. Up to 50% of the whole hemoglobin pool. And the fetal from a globin is actually quite good. It's not as good as the adult, but it can help to, kind of compensate for the defect that these people have in the adult hemoglobin. This is what I kind of find really interesting, that it's not oh, we're going to actually correct the gene that is impaired and restore correct gene sequence, but it says, okay, normal hemoglobin is lost but can't be produced in these people. But we're going to use another way to actually give them the fetal hemoglobin which can replace the function. But basically, the therapy is a CRISPR therapy that inactivates this gene.

Speaker 1 [00:11:23] Okay. And would there as well be the possibility to insert a gene that is able to produce the adult hemoglobin?

Speaker 2 [00:11:33] I mean, in theory, yes. I don't I honestly cannot tell you why this is not the approach that people pursued. I guess this proved like really in clinical practice to be to be more efficient. With CRISPR in theory, it is also possible to correct gene sequences, so to say, okay, like for example, in sickle cell disease, if there's a point mutation that you can like really in principle, correct exactly this sequence. And I think that maybe for other diseases in the future this will be developed where like really correct the original gene or insert like you say, insert the healthy gene.

Speaker 1 [00:12:12] Okay. So those would be the possible developments for this type of research?

Speaker 2 [00:12:18] Yeah. I mean, I think there is still a lot to develop because the clinical trials on which the approval was based, had only very low patient numbers. And so, we still know very little about the long-term effects. Also, the long-term side effects. And so, I think one important future development is really to see how safe is the therapy over a longer time period or like how effective is it? Is it like say okay, has it like lifelong effects? These are important things. And then of course, like picking other diseases, correcting them in a different manner. We like also independently from the human hematopoietic stem cells because we have genetic diseases. Also, a lot of those skeletal system neuronal system that. This will future developments.

Speaker 1 [00:13:11] Okay. So. And the modification of the stem cells. Which stem cells do we use?

Speaker 2 [00:13:20] From Casgevy we use, hematopoietic stem cells that are collected from the blood. So also, for the autologous or the allergenic transplantation, most of the time we don't collect the stem cells from the bone marrow, but we collect them from the blood. And this is possible because the patients receive, injection with a certain medication. It's called, granulocyte colony stimulating factor. This is a growth factor that is also normally produced in the body. But when we apply it as a medication, it leads to like high proliferation of the stem cells. And then the stem cells also leave the bone marrow and are found in the blood. And so, we collect them by a procedure which is called off erases where basically the patient is connected to a machine. The blood flows through the machine. And this certain fraction of them also later with other techniques of hematopoietic stem cells is purified. These hematopoietic stem cells, based on a certain marker on the surface of the cells. It's called CD 34. and so, these CD 34 human hematopoietic stem cells are collected, and they are modified. Of course, like with modifying hematopoietic stem cells, we cannot correct diseases of the brain.

Speaker 1 [00:14:34] So it's only indirectly, taken from the bone marrow.

Speaker 2 [00:14:38] Well, there is no intervention on the bone marrow of the patients. The stem cells leave the bone marrow, then go into the blood and get collected from the blood.

Speaker 1 [00:14:47] And when the modification is done, the chemo is done. Are the stem cells as well go back through the blood?

Speaker 2 [00:14:54] Yes. Yeah. They're infused a bit like a blood infusion.

Speaker 1 [00:14:57] Okay. And is there a risk because there are as well old stem cells before modification. Or are they all killed by chemo.

Speaker 2 [00:15:10] So the chemotherapy that is done, the idea of it is that it should completely eliminate the old bone marrow, including the old hematopoietic stem cells. So then when we get the infusion of the new ones, basically, ideally all of the human hematopoietic stem cells then should be the new ones, which are the gene edited, or at least the, the bigger proportion.

Speaker 1 [00:15:32] Would this therapy be possible without chemo?

Speaker 2 [00:15:37] Not at this time, although there have been a lot of attempts for quite some time to find chemotherapy free ways to deplete the bone marrow niche, for example, with targeted substances like antibodies that would replace the human hematopoietic stem cells or radiation. At the moment it is not possible. But I think also this is a potential development, where we maybe will be able to find chemotherapy free conditioning procedures.

Speaker 1 [00:16:05] Are there any, additional side effects, due to the modification?

Speaker 2 [00:16:12] Yeah, I think it's still hard to say. What was most frequently observed in the clinical trials was that the patient had low counts of white blood cells and low counts of platelets, which, however, I think is more due to the chemotherapy and not just due to the infusion of the modified stem cells. Then I think other frequent things, were like headaches and nausea, vomiting, abdominal pain. I think a lot of this is probably caused by chemotherapy, I think we don't know yet so well, what is caused specifically by these, modified stem cells that it doesn't have, like, because I don't know whether you have heard about Car-T cells, which are chimeric antigen receptor T cells, another type of similar therapy, and they have very specific inflammatory side effects that are really due to this T cell medication that we've given so far for these hematopoietic stem cells. We don't have something where we say, okay, it comes really only from these cells, this particular side effect.

Speaker 1 [00:19:23] How much does this treatment cost?

Speaker 2 [00:19:26] So this is an excellent question. The current costs in the U.S. are about $2 million. I think in the UK, I was reading about a bit more than 1 million GBP.

Speaker 1 [00:19:39] It's quite expensive.

Speaker 2 [00:19:42] So. Yeah, it's quite expensive.

Speaker 1 [00:19:43] Are there any ways to offer this treatment for less expensive?

Speaker 2 [00:19:48] I think at the moment, no. Also, because it's so new. I think this is also an area that will have a lot of developments in the next year where there will come other companies, there will be more competition. And this, of course, will like change the prices on the market. It will remain very expensive because the procedure to like generate these cells is

very laborious. In terms of requirements of the laboratories where such, such, therapeutic products can be, produced. And also, because it's very labor intensive. So, like different. Other than like producing a drug that is a chemical and is very straightforward. This really requires a lot of human working hours. It will get cheaper, but it will not get like super much cheaper very soon.

Speaker 1 [00:20:43] And, when is the Casgevy treatment not possible. Like, are there any, indications where it is not possible?

Speaker 2 [00:20:55] I mean, the thing is that it is only approved for certain indications, right? Like it's not you cannot give it to any patient with sickle cell anemia. They have to fulfill a certain a degree of severity of their disease. So, they have only very light disease symptoms. It will not be approved for such patient. Or I think actually, in the current approval, it's only for patients who are not eligible or cannot undergo an allogeneic transplantation because like this would be the option, right. We talked a bit in the beginning. Okay. You can replace the human hematopoietic stem cells with these edited hematopoietic stem cells. Like we take the cells from the patient, edit them, give them back. But we could also say okay, in the same patient I'm going to transfer allogeneic stem cells even though I don't need an immune effect. What I was saying earlier, I'm going to transfer the hematopoietic stem cells from a completely healthy person. And by this I replace the whatever hemoglobin incorrection there is. And this person will then continue to live. And so, this is the allogeneic transplantation is much, much cheaper. It can have considerable side effects and considerable toxicities. But on the other hand, we have much more experience with it. And so now there is a bit of a discussion in the field which patient is actually a candidate for gene editing therapy versus the much cheaper, much more frequently used allogeneic stem cell transplantation. So, but I can't come up with something like if the patient really falls into this certain category, what are exclusion criteria? I guess if for some reason you cannot connect the stem cells, that will be something.

Speaker 1 [00:22:39] And how much would the allergenic transplantation be in comparison to the Casgevy treatment be?

Speaker 2 [00:22:46] Yeah, let's say about 2 to 300,000.

Speaker 2 [00:22:53] The difference is very big. Although it's also a very expensive procedure. Absolutely. Mostly because it's yeah, it's frequently connected with a long hospital stay. But it is like still like, let's say five to I mean, maybe not quite ten times less. More than five times less.

Speaker 1 [00:23:12] Thank you very much for your time. Do you have anything to add?

Speaker 2 [00:23:27] No, I think these are good and important questions. I think the one particular thing about these where we use CRISPR to correct hematological diseases, one specific consideration will remain. The last thing that I said, like which patients are better helped with such a gene therapy compared to an allogeneic stem cell transplant, where we can really replace their bone marrow with completely healthy bone marrow. I think this is something that we'll continue to devote to kind of, keep us busy. I think for other types of diseases where we cannot so easily replace a certain tissue, like, let's say even for neurological diseases where we can say, okay, I'll replace, in whatever way muscle cells or neural cells with the cells from another donor. I think this is probably where there will be a lot of developments in the news, although we have to say due to the costs, which as I mentioned, will not fall a lot down in any foreseeable future. It will remain therapy that is available only in privileged countries, and it's only to individual patients.

Speaker 1 [00:24:45] Great. Thanks a lot.

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9.2 Interview with Dr. Philipp Hoppe, 03.05.2024

Speaker 1: Nadine, Léa-Sophie, Johanna

Speaker 2: Dr. Philipp Hoppe

Speaker 1 [00:03:29] The hematopoietic stem cells, the ones that produce the blood. And are they only found inside of the bone marrow or also in the blood?

Speaker 2 [00:03:42] It depends. So in, in steady state condition. So in a normal, healthy person like you hopefully, or me, the very classical idea of stem cells or hematopoietic stem cells in this case is that they sit in the bone marrow niche. So that's a kind of a bed for the stem cells where they're in contact with stromal cells. Also, the idea is that there's very low oxygen where the real stem cells sit, because oxygen is a very reactive chemical molecule. And, you can have also radicals builds by oxygen. So by keeping the hematopoietic stem cells in the niche or in the bed relatively far away from blood vessels, you also keep them safe. And normally the idea of a very classical textbook idea of stem cells is that they are quiescent. So mean means quiet and not doing anything. They're just waiting in the bone marrow until they are needed. However, they can also migrate out of their bone marrow, out of the bone marrow niche and really go through the whole blood system. But they express specific molecules. So at some point they will also go back to bed, if you will. And, especially if you want to isolate the blood stem cells, of a patient, either for autologous or also for allogeneic stem cell transplantation, you can mobilize them so you can treat the patients with a drug that's often, GCSF. So granulocyte colony stimulating factor. But this you can mobilize the stem cells and by that they go to the blood. And then it's quite nice because you can take a big blood sample and then isolate the stem cells out of the blood, whereas you'd otherwise you would need to really go into the bone marrow. And that's very painful, as you can imagine, because it goes through, really get to the bone. Usually you do it as in the hip bone, and you really go there with a very strong needle. And that hurts, obviously, as you can imagine.

Speaker 1 [00:15:07] Okay. I think let's move on to blood diseases. How does sickle cell anemia affect a person?

Speaker 2 [00:15:17] So if you look up what anemia means, it's either that you have too few hemoglobin in your blood or, that the red blood cells are not mature enough, or there are not enough red blood cells, or that they are somehow diseased. In the sickle cell disease they have the sickle cell shape. And, I mean, the main symptoms of the patients are so in the end, they don't have enough good red blood cells to say it in a simple way. So what happens is that all organs tend to not get enough oxygen. So the symptoms are they are fatigued. They're very tired usually because of the life of also especially kids. If you think about when kids need to grow or reach puberty, it can also happen that they have delayed development so that they would not would reach puberty later, or rather short and not as well developed. Other things are they can have vision problems because in the eye there are very, very fine blood vessels and because of the sickle cell shape of the erythrocytes, they clock and they start to plug first and the smallest vessels. So that's why they can have, vision problems. Then also other clockings happen, for example, the spleen, which is an important organ for the immune system. That's why you can be

exposed to more, infections, or other symptoms for example pain because. Yeah. If you imagine also in your fingers, for example, they're very thin. The more deep you go into the tissue, the more fine the the vessels are. So you can also have like pain or swelling in, in the fingers or in other organs. So these are main symptoms. But the biggest problematic is the tiredness. The pain plus. Yeah. But the underlying problem is not enough oxygen. Gets where it needs to go.

Speaker 1 [00:17:40] In the sickle cell anemia, the defect is in the form of the hemoglobin. Form affects how it, it gets the oxygen. It's the only thing that's changed by the sickle cell.

Speaker 2 [00:17:58] So the sickle cell anemia is so the most prominent form of it is a very specific point mutation. And so let me go back to hemoglobin. Hemoglobin is the molecule that carries oxygen throughout your body. It's incredible how much oxygen a healthy person has in his or her blood. It's really if you could isolate all the components of blood, hemoglobin would be 20% of it. Something that you can look it up in the internet. It's really you have uncountable amounts of hemoglobin in your body. And one hemoglobin, one hemoglobin molecule in a healthy adult consists of is a is a tetramer consisting out of two alpha globin molecules to beta globin molecules and each of these four globin molecules carries a heme group. Heme group is a nonprotein. It's a chemical structure. It's not part of the protein, but it's linked to the proteins slash globins. And each of these heme groups has an iron in the middle. And that's where the oxygen binds to it. So a healthy hemoglobin molecule, when it's fully saturated can carry four oxygen molecules. Now coming back to the sickle cell anemia. So the very classical textbook sickle cell anemia there's a very specific point mutation in the beta globin and it's glutamic acid to valine point mutation. And what happens is that the the individual hemoglobin molecules tend to stick to each other. And then you have, I don't know how many thousands, tens or hundreds of thousands they make like rod structures because it's very sticky to each other. And all these rod structures of sticky hemoglobin molecules make this sickle cell shape of the erythrocytes. Normal erythrocytes have this donut structure not with a hole, but a 3D shape of a donut. And, sickle cell disease. Erythrocytes have this sickle cell shape. And these sickle cell erythrocytes, they are responsible for clotting the blood vessels. Sorry. It's okay if I accuse anything. I talk quite a lot. Is it too much or is it okay? Good. What was the original question? Sorry. No that was fine. Now, you talked about the. For oxygen can be a healthy one. Yeah. I think you wanted to mention how much can be, transported in the sickle cell.

Speaker 1 [00:21:50] We learned in, a previous interview. That, there is an fetal form of hemoglobin. In the fetus. And, when the fetus is born it changes into the adult form. Yeah. Why exactly is that? And, in the treatment, in the treatments, we can change back to the fetal hemoglobin. Why exactly are there two different ones and what are the differences?

Speaker 2 [00:22:20] Yeah. So the fetal hemoglobin does not consist of two alpha and beta globins but two alpha and two gamma globins. And the reason is as follows. The embryo in the uterus in pregnant mothers they don't have their own lungs obviously, but the, the mother and the fetus are connected through the placenta and through the umbilical cord. And now, the fetal hemoglobin has a higher affinity for oxygen than the adult hemoglobin. And what happens is in the connected bloodstream of the mother and the fetus, there needs to be, handover from the adult hemoglobin of oxygen to the fetal one so that the fetal one stays in the fetal blood circulation. And because the fetal one has a higher affinity for oxygen, it's relatively easy to do this handover of oxygen from the adult mother hemoglobin to the fetal hemoglobin.

Speaker 1 [00:23:28] And why doesn't it just say the fetal hemoglobin. Why does it change afterwards?

Speaker 2 [00:23:44] I'm not entirely sure, but I could imagine that there must be a there must be an evolutionary advantage that there is the adult version of the hemoglobin. Maybe in a in a healthy adult and adult doesn't mean legal adult like above 18 or 21 in the US. But post birth that's already kind of adult in in this context. Probably there's an evolutionary advantage that the affinity of the adult hemoglobin is not as high for oxygen as in the embryos, but it's a bit speculation. Honestly, I don't know the real answer to that actually, I'd try to look it up before, but I also could not find the answer.

Speaker 1 [00:24:36] And so fetal hemoglobin is just able to bind the oxygen, like more densely.

Speaker 2 [00:24:43] Yeah. So if you have a, if you have it in a, in a glass, and if you do an experiment and mix 50, 50% of adult fetal hemoglobin and blow oxygen into this, whatever glass, dish glass you have that it would the oxygen would go more to the fetal hemoglobin because it has a higher affinity that what what it means.

Speaker 1 [00:25:04] It's the reason that the baby gets some oxygen.

Speaker 2 [00:25:09] Yeah. Because if if it was the other way around, if the adult, hemoglobin had a higher affinity, this handover would not happen as easily. And then the embryo would suffer from. Yeah. Low oxygen.

Speaker 1 [00:25:24] There's the drug treatment against sickle cell. And also, beta talassemia. Could this also be with allogenic transplants and not only with their own?

Speaker 2 [00:25:55] Yes. Theoretically, yes. But as I mentioned, like one of the first questions is you can choose, you would take your own stem cells, edit them and give them back to you because then you avoid this again. Graft versus host disease. That means. No, sorry. So this graft is what you would transplant? That's a grapht. The host is the recipient. Graft versus host disease means if you know, if you receive a cell from a donor, it's a stem cell. It can make theoretically the whole blood. And that means. And the blood, I mean component of the blood. So the aretherosites the trhombosites and the white blood cells. So you could get as a very potent stem cell transplanted in your body from any donor. And this stem cell would then make immune cells. And those immune cells would attack you as a host. That's called graft versus host disease. That's one issue. And then you have the host versus graft disease that the remaining immune components or immune cells of the recipient destroys the stem cells and its progeny. So the two things and that's why when you can you would go for an autologous stem cell transplant. I can give you an example where allogenic stem cell transplant is better.

One is imagine you inherited from your parents a mutation that predisposes you to get leukemia. If you know that, and you might want to consider that you want to get rid of your own mutated, not yet leukemias, but predisposed to get leukemia. You want to get rid of your own stem cells and replace them by healthy stem cells. Then you would want to go for allogeneic stem cell transplant. Or another example is and there have been publications described originally it was a bit of a coincidence that it was discovered. But when the researchers followed up they understood why, for HIV. HIV is a disease where the HIV virus hides in the T cells of of patients and the receptor on the cells with the HIV virus docks and binds and goes into the cells is largely, chemokine receptor called CCR5 chemokine receptor number five. And just by luck, originally by luck, I think a HIV positive patient once received the allogenic stem cell transplant, and by a natural occurring mutation, the donor had a mutation in the CCR5 receptor. And over time, that recipient that was HIV positive got turned HIV negative because over time, the donor sample that had some mutation in CCR5 so the HIV could not spread anymore in the T cells of the T cells that originated from the transplanted stem cells it is quite a special case. But that's an example

where it makes sense to go for allogeneic stem cell transplants. The likelihood. So if you're not lucky and have one egg twin, which is quite rare, the likelihood of finding a genetical twin that can donate transplant. Sorry, it can donate, stem cells to you without having any issues with graft versus host or host versus graft disease is 1 in 900,000. So it's a bit it's less likely to win the jackpot in lottery. But it's also I mean 1 in 900,000 is very unlikely. That's why there is a like from Swiss Red cross, for example, these calls to register yourself to determine your HLA subtypes. So whenever someone in Switzerland or I mean I think those things are connected like I'm from Germany, I was once registered in the, in the German blood stem cell bank. So you just get a sample and you, you take some sample from the inner side of your cheeks, put in a tube, send it back, and then they determine if you are, they determine your tissue type, if you will. And then whenever they find, a patient that would need a stem cell transplant from you, they would tell you. But 1 in 900,000 is pretty rare. And often you don't. You're not that lucky, unfortunately. And if you don't have a genetic identical twin, you go for compromise. So you give a stem cell transplant that's most similar as similar as possible as it can be, but then it's not perfect. And because of that, whenever you get an allogenic stem cell transplantation, you often get also immune suppressive medication so that you avoid this graft versus host or host was graft disease. But of course, if you get immunosuppressive medication, you're also more prone to get infections.

Speaker 1 [00:35:23] In the Casgevy treatment, I think the patients, produce, foetal haemoglobin. And as you said before, the foetal haemoglobin has a higher affinity for oxygen how does this affect the patient which receives the Casgevy treatment.

Speaker 2 [00:35:42] Yeah I also looked that up. But I couldn't find a clear answer. I was speculating a bit here so I and I said this earlier. Right. There must be a evolutionary advantage of this adult haemoglobin. The foetal haemoglobin. If you compare an adult that expresses foetal haemoglobin to, sickle cell patient, the foetal haemoglobin is way better than remaining as a sickle cell patient. Probably. But I couldn't find clear answers earlier today when I looked for that. But probably those people could not become professional soccer players or whatever, this kind of thing, so that you really go to the maximum possible of physical examination, these kind of things. But compared to being a sickle cell patient, having the foetal haemoglobin re expressed, or there are also patients fully independently of being sickle cell patients or not, those patients that have other mutations in the in their genes that by themselves have a maintained foetal haemoglobin expressed sometimes it is not even discovered unless you really look for that. So let's answer the question. It's surprisingly mild. There's a short answer.

Speaker 1 [00:37:07] Like in Casgevy treatment, having, the stem cells out of the person. When we do the modification, we inactivate the gene BCL11a. That gene is like, suppressing the gene that is producing foetal haemoglobin. Why do we do that and not correct the sequence from the actual time a globin, which is wrong.

Speaker 2 [00:37:48] Yes. For sure what you're suggesting. So to correct the wrong gene would be, from a scientific point of view, be much more elegant. And that would also be in the dream scenario of any gene therapy that you corrected to normal of the wild type. There's a practical issue, a practical aspects of that. And. That is. And I also had to look this up and it was pretty hard to find. So I had to spend some minutes today to find this out how this really works. So and I assume you know how Cas9 classical Cas9 works, right? You have the Cas9. You have this guide RNA. It's like an address tag. It's a 20 base pair long sequence. And this tells the Cas9 of the gene scissors. It's also called go there and cut exactly here. And you know that it cuts between base pair 17 and 18 of a given sequence. It just needs this so-called Pam sequence. Have you

learned that Pam sequence proto space adjacent motif? That is a, it's a it's any base pair and the two guanines. So n for any and gg and this is the only prerequisite for the very classical first described Cas9 protein. And the way Casgevy works is you take a guide RNA. And the Cas9 protein, and you electroporate this in into isolated hematopoietic stem cells. So you really put them under a very harsh electric shock. And with this applying this electric shock to the cells, they have very transiently holes in their cell membrane. And by that the so-called rnp derival nuclear protein complex gets sucked into the cells. And it's this recombinant Cas9 protein loaded with a guide RNA. And that guide RNA now goes it doesn't go to the coding sequence of BCL 11 A, but it goes to the promoter of another transcription factor called Gata one, that binds to the promoter region that regulates expression of BCL one. So you said it destroys BCL one. I don't know the exact you said, but if you think now really scientifically, where does it bind to which base pair gets cut and what happens at the molecular level? Is that the promoter region of BCL 111 A gets destroyed. And practically this is very easy. And also I mean I work in very early research. I my job is actually to find new drug targets. And we also use Crispr technology to find new drug targets. So my idea is to find the next BCL 11A to, to do anything with it. And only then the people who come after me in the pipeline, they try to find the drug. So my job is to, to find drug targets to make a drug for. So it's very early. It's very far away from manufacturing, from making the selling gene therapy, etc.. So it's really early and, and the Crispr Cas9 is a fantastic tool to do something with DNA. It's really amazing and very well deserved and the two ladies that worked on this for, for decades. And it's a bacterial immune system. So it was no way to foresee that this would be such a game changer for the whole scientific community. And, I'm saying this because I'm doing these simple cuts with Cas9 pretty much every week myself in the lab. So technically it's very easy. You need a cast iron protein, you need a guide RNA and the whole sequence, the whole, genome sequence. So it's very easy to go to the browser, in this case the genome browser. You go, okay, I want to cut this gene. I type in the gene I see really literally I see the sequence on my screen and I design a guide RNA for that. I, I make an order and a few days later I get the guide RNA as a molecule and also a recombinant Cas9 protein and take the cells I'm working with and do electroporation with those cells. So it technically it's very easy. And because it's so easy, it's also quite reliable. The difference is of course if you take now you need to the infrastructure. You need a doctor or team of doctors and nurses to isolate the stem cells. You need, absolutely clean laboratory infrastructure to do something with the cells. And it's really like you can imagine from movies that really work in this space suits with, low pressure, that, and then it's super clean environment. And for this early research where I work is not necessary. So, I mean, I'm supposed to wear a lab coat and safety glasses, but I don't need to be that careful, because whatever I do, it doesn't end up in patients. So technically, this is very easy. If you want to correct a sequence which would be the much more elegant way of solving things you would need. It's not only enough to cut by cutting, you destroy. You would need to repair. So the way you do it is you use the Cas9 to cut the sequence, but to repair, you need to either provide a so-called repair template that the destroyed the side where it's destroyed. It has really a blueprint of okay, it needs to repair in such a way. And this is not as efficient as just cutting and looking the other way. So technically it's much more difficult and also less efficient. So it might be that if I have a thousand cells in my dish and I have done this method, I might have 100 says where this works. If I have 1000 cells and just want to cut, the efficiency is 70, 80%. You still have 20 cells, 20% of cells that. But you don't have to cut as you wanted. But just cutting destroying is technically much easier than repairing. So, you can really have a A to G correction, but then you need to be a bit lucky that the mutation is, some is a T to A that you can rewrite it with a A to T. So, for this base editor things you need to be a bit lucky or you provide the repair template. But that is quite inefficient. So, in that case and one of the next questions, if I may already answer

this earlier was when did the clinical study start for, for CAS, GV and the first one was 2018. I looked it up and at some point, you have to balance a bit. Okay, you have an idea, you draw it on the whiteboard and say, I want to develop a gene therapy, and this is how we do it, then it still takes years. And anyway, in this case, it's quite fast that it's already, reaching patients. You have to have to balance it. What's possible on on the date, basically in the start of the project and waiting for a better solution. Of course, if we now decided today to develop a gene therapy, you would use, maybe a better Cas9 protein that's even more reliable and has less side effects, but it always needs to be, it's a mixture of how beneficial is, is it for the patients? How risky is it? Is it ethically okay to do these kinds of things? Scientifically of course correcting the wrong gene would be much better. Last thing I say about this for sickle cell disease, sickle cell anaemia, you have this very defined mutation, so you know what to do. So, you could do it. But as it's not as efficient and it's more complicated technically you rather go for well, I just try to promote the region and I will get what I need for the beta thalassemia. There is not one mutation. There are hundreds of mutations, none in there if you want to have a product. And in the end, the gene therapy is also a product with very strict protocol. But, imagine here for the sickle cell, it would even work because, you know, this is the mutation. This is you have to this is what you have to repair for beta thalassemia. You have hundreds of mutations. So, you will need to have 100 different products if you want to correct this sequence. And that's simply not doable because, yeah. You need somehow at the end of protocol, it's clear from A to Z. What you have to do? So that's, all things that needs to be considered. When why why you wouldn't why you're not repairing the defect just Gene.

Speaker 1 [00:47:14] But you can use the Casgevy treatment for beta thalassemia.

Speaker 2 [00:47:19] You know, and there's also proof for it. And, the reason is, as you can guess by, by its name, the beta thalassemia is also, disease where the beta globin, the very same beta globin is mutated. And of course, and it's no coincidence that the company added two companies went for BCL 11A in this treatment, because then it can hit, two birds with one stone, because it can be approved for sickle cell anaemia. And also, both of them to see because the principle is very same. Something is wrong with the beta globin. So, you're expressing foetal gamma globin. And by that you have very same. The therapy is the same. You have then the re expression of the fetal module.

Speaker 1 [00:48:07] And so it's enough in if we can cut the promoter like when we've cut, once it's done it's destroyed?

Speaker 2 [00:48:20] I need to go a bit into the detail here. What it's up for, for, for this cutting to happen, you need the Cas9 protein and the guide RNA. And the guide RNA guides the Cas9 protein to the side. And then it induces at the between base pair 17 and 18 a double strand break in the genome. For cells, a double strand break anywhere in the genome makes it go on full alert. It's the very same as when you get a sunburn. Yeah, it's full alert on the cells because sunburn is a two high dose of radiation. And what happens is the cell go in red alert mode and they try to hectically repair the open double strand break and close it. And what happens is it gets closed back to how it was normal. And keep that in your head for a minute. Or it can also happen that there's a little indel. Indel means either insertion or deletion of one, two, or three base pairs, and so you mutate the sequence to different than it was before. And this is what you want. So, you don't want only to cut the DNA because it will the DNA repair mechanism of of the cells kicks in and repairs it and closes it no matter what. So, it will not leave it open. And you want this indels to happen, because if you have a short indels a bit too many more randomly added base pairs or randomly chopped off base pairs removed, the promoter doesn't work in one. This is one. Two

what you have now if the cell. What I ask you to keep in mind quickly. If the cells closest to open double strand break back to normal, there is still guide RNA in Cas9 protein protein around. So, it was again cut the genomic locus open. So, and unless you are unlucky that the Cas9 protein gets degraded in the cells for example, it won't happen anymore. But as long as there's this guide RNA and the Cas9 protein around it will cut the wild type of locus as long as it's around. And because of the cell endogenous cell repair mechanisms and having this short indels at the cut side, that's the reason why this CAS Chevy in this case actually works. It would not work if it just would repair it because then it's same same as before. Then it wouldn't do anything.

Speaker 1 [00:55:00] And what are the chances of success for the Casgevy treatment?

Speaker 2 [00:55:08] Quite high. I looked I mean, I didn't know this, so I looked it up. And if you go on the internet to casgevy.com, you can can read about it. So. And you know, you have to make clinical trials if you ever want a new product. A simple pill or, injection with an antibody or a cell. And gene therapy especially, you need to run clinical trials with patients. And I was very surprised to read that. There were less than 100 patients only. Yeah, exactly. That's how I looked. There were less than 100 patients where they tested the Casgevy treatment on. Because I know. So, for a pill, if you want a new blood pressure pill or for heart failure, you have this. Phase one, phase two, phase three clinical trials. Phase one is in healthy volunteers. Phase two is dose finding. And try to look for side effects. And phase three is usually if you can, at least in non-cancer space. Double blind placebo-controlled study. So double blind means neither the doctor nor the patient knows if they get the placebo or the new drug. And to have enough statistical power, you need to have the drug approved by the authorities. Like in Switzerland, the Swiss medic in the EU is the EMA European Medical Agency in the US, very well known as the FDA. The food and drug Association. And yeah, so classically you run a phase three, phase three clinical trial with thousands of patients. And I was very surprised to read myself today that, for the Casgevy they did it with a few dozens of patients. And when you look there and that's to answer your question. So, on their web page really on casgevy.com, you can read that 93.5 of people. That's a very high percentage. But then an absolute number that is 29 out of 31 people did not have a severe VOC. And VOC was vascular ocular conclusion. So, an issue in the eyes that that was the so-called readout. So, the question is when is this therapy successful? Is it when they don't have an issue in the eye? Is it when they don't have pain? Is it when they're not tired anymore? Is it when they can run a marathon? You're pretty free to define as a as a sponsor of this how it's called in those studies and what you define as a success. And from the home page now it's you can read the other bullet is 100% of people evaluated, which it was. 30 out of 30 were not hospitalized for a severe VOC for 12 months in a row after receiving Casgevy, and then 22.2 months on average for the 29 people who did not have a severe VOC. And that's already who did not have something severe. So, it's already kind of you can read when you read this is any preselecting for people that didn't have another issue. So, you can read that the average length of time without a severe VOC was 22.2 months, measured as a median amount. One person experienced a severe VOC at month 22.8 and required hospitalization. So, I mean, if you and it's really like advertising not 93.50 amazing hundred percent. Oh amazing 22 months, no issues with eye issue issues. So, say it's simple. That's good. But if you're really critical, you say, okay, so wait a minute, that's only 30 people. What about after three years? After four years, what about the five? So, what about in ten years? And I think, if this was there more question to come about side effects, long term effects, these kinds of things, you know.

Speaker 1 [00:59:10] Could one use the principles of the Casgevy treatment to cure all diseases aside from blood diseases?

Speaker 2 [00:59:21] Yes. Of course. The beauty and the great thing about hematopoietic stem cells. Said as earlier, it's a liquid organ. It's so well understood. You can give GCS and mobilize the stem cells. You can get stem cells off the patient. You can do something with them. They are in suspension culture. That means they just swim in your medium and you can give it back to patients. It's technically so easy now. Unfortunately, not every other, not every organ has such a nice, simple stem cell. I can give you some examples. The liver. Maybe you know this from Greek mythology. Who was this guy that was hanging? Yeah, right. He had his liver eaten every day by, whatever badger or whatever bird it was, and it regrew overnight. And then the next day, the bird came again. And a part of the liver. The reason this myth exists is because the liver has such a high regeneration potential by itself, without having a clear stem cell. You can chop away two thirds of your liver, and after a few months it will regrow. That's the link to this Greek mythology. That's absolutely amazing. If you think about it from a scientific point of view, how the cells said, oh, we have two thirds living of our organ. Okay, guys, we need to make more liver. So you you you you regrow, you make tool for the A16 cells. You need to have the right shape again from the liver. And this is scientifically absolutely amazing. But there's no real stem cell. The blood stem cell system you have one blood stem cell. Then you have so-called multiple organ progenitors that can give rise to all the blood cells, but it doesn't have indefinite self-renewal capacity. Then you have kind of the lymphoid common lymphoid progenitor that makes all the B cells and T cells, and the myeloid progenitor, which makes red blood cells the thrombus sites. And this, granulocytes and the monocytes, which are part of the innate immune system. If the innate and adaptive immune system and the hematopoietic stem cell system is very hierarchical. There's one stem cell on top. And you can like what I mentioned earlier with this animal experiments, you can prove that this one single stem cell can reconstitute the whole blood system that's slashing the system of, recipient mouths and the other organs, and they give you the examples of liver. That's not not the case there. The cells have a regenerative potential by themselves without being hierarchical, a stem cell. Another example. Kidneys is also an internal organ like liver. But the kidney the architecture of the kidney is completely different. You have around 1 million nephron units. It's a very small filtering unit that filters the blood and cleans the blood and washes the blood whenever you look for, for whatever reason, if you ever lose one nephron. Now this is one million. It will not come back. If you lose another one, it will not come back if you lose one kidney and you can donate one kidney and still have a normal life, the kidney won't regrow and there is no kidney stem cell. There is certain regenerative capacity in some parts of such a nephron. Such a filtration is damaged, but once it's too much damage, it doesn't come back in brain strokes. If you have a stroke from whatever grandfather grandmother, parents at some point, it's very severe and very often associated with a bad outcome that people cannot speak anymore, cannot work anymore, cannot in the worst case cannot breathe, anyone need a ventilator, etc. that is because there's no brain stem cell where your brain grows back to normal within a few weeks or months there are neural stem cells. But they are very specialized. They sit in the seven-trigger zone of the brain. It's very deep in the brain and in the olfactory bulb. So, if that said, cells that make you smell and since they have certain stem cell activity, but you think of stroke, if you have a stroke, part of the brain that that and will not come back, another tissue that has quite a lot of stem cell capacity is the intestine. How often does the intestine renew itself? What do you think? May you notice?

Speaker 1 [01:03:51] Seven days.

Speaker 2 [01:03:52] Yeah. It's. Yeah, something a few days. It's absolute incredible that your whole several meters long intestine, if you pull it out of your body and put here in the classroom,

regenerates itself within a few days over the whole lifetime of your body there you have a stem cell, and that's again, it's the so-called MGR5 five, a certain transcription factor MGR5 positive stem cell. And, all these things are from blood, from, brain, from intestine. And just to give you a few examples, there are stem cells. The problem is all these mentioned examples. They are not in a liquid organ that deep, deep in your brain, in your intestine, wherever. And then have your very simple practical problem. You cannot take it out, do something with it and give it back. But you would need to whatever you want to develop as, even if it's a pill or a cell gene therapy even more so, you need to somewhere get your I call it not a drug or gene therapy. You need to get it into that stem cell off your tissue. So, this is incredibly difficult. And that's why, of course, that's the future in the long run that we don't treat anymore symptoms but really cure. And the Casgevy treatment is a cure. But this is things that. Yeah, everyone is thinking about, how to also have this curative gene therapy treatments in other tissues rather than not only blood. And it's really fantastic that it works in blood, but because it's also relatively simple, because blood is a liquid organ.

Speaker 1 [01:05:44] We we spoke of the side effects earlier. Do you have some possible long term side effects?

Speaker 2 [01:05:55] Yeah. Very good question. Very difficult question to answer. If so, again from the regulatory authorities, if you want to have a new drug approved you need to show that you don't have side effects. But you cannot now have cast Casgevy. Not yet. It's a preapproval you cannot have because Casgevy and wait 50 years and check in 50 years. If some patient had received such a drug developed leukemias, especially not in 30 people, they would need thousands of participants and then again goes back to the the pharma company. So, if what's often said from pharmaceutical companies. Is from 100 ideas in the lab. Like what I work on. I work on ideas, concepts. I try to find new drug targets, then make a drug for a cell or gene therapy from 100 ideas. Only ten make it to the clinic. And out of those ten that are being tried out on humans, only one makes it to a product on the market. So, whatever is sold as is one out of 100, drugs, the drug slash cell slash gene therapy. This has to finance every other every of those 99 failures. And that's that's incredibly expensive. And then at the same time, pharma companies use in very rare cases do have small family run businesses, but usually they are at the stock exchange, and they have, obligation to their shareholders. So, they also need to earn money with it. That's just as brutal as it is if you think about it, because in the end you want to help patients. The pharma companies also need to earn money. And we're not discussing again, we cannot discuss. We can meet another time and talk about that. But it's. Do you know what Casgevy costs?

Speaker 1 [01:07:55] Like 2 million.

Speaker 2 [01:07:57] 2 million? Yeah. I mean, you go like, what the fuck? It's. Why is it justified? Is it worth. Two million is incredible. Imagine how many I don't know. Antibiotic prescriptions you can give to. How many thousands of people. And then have the two million for this CASGEVY. The reason is, first of all, the pharma companies need to finance all the failed projects. They also have obligation for the shareholders and. It would not work. No. If you think about it, you give all the power to the universities and you have research being done at the universities and published research, and it wouldn't work. So you can think about capitalism. It's now a different discussing what you want or not. But I truly think it would not work if it wasn't this capitalistic thinking behind it. The, the, the, the competition between the pharma companies, etc. this in in the end, it's good that something comes out of it. Sorry is a bit off topic. What was the question? The side effects. Yes. The way I was talking about this, the economic aspect and the money and the prize

for drugs, etc., is that the pharma companies can only earn money with a drug or, syringe or a cell or the gene therapy when it's under patent protection, this can run up to 20 years after that. And you can bet your ass on that. The day after the patent expires, a generic is ready to go on the market. And it's good because they can make the same drug and sell it for much less, which is good for patients, it's good for the health care system, etc.. And now on one hand, it would be good if you could test a drug for 50 years and then only give it to patients. But of course, they're patients already suffering today. And there are millions of people with, with sickle cell disease. So you also want to treat them today. So this is a fine balance between what do we know, what's the state of the art, what's the scientific knowledge of something? And do the health authorities think it's safe enough to give it to the patients? And is the benefit big enough for the patients with the risks that might be there?

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