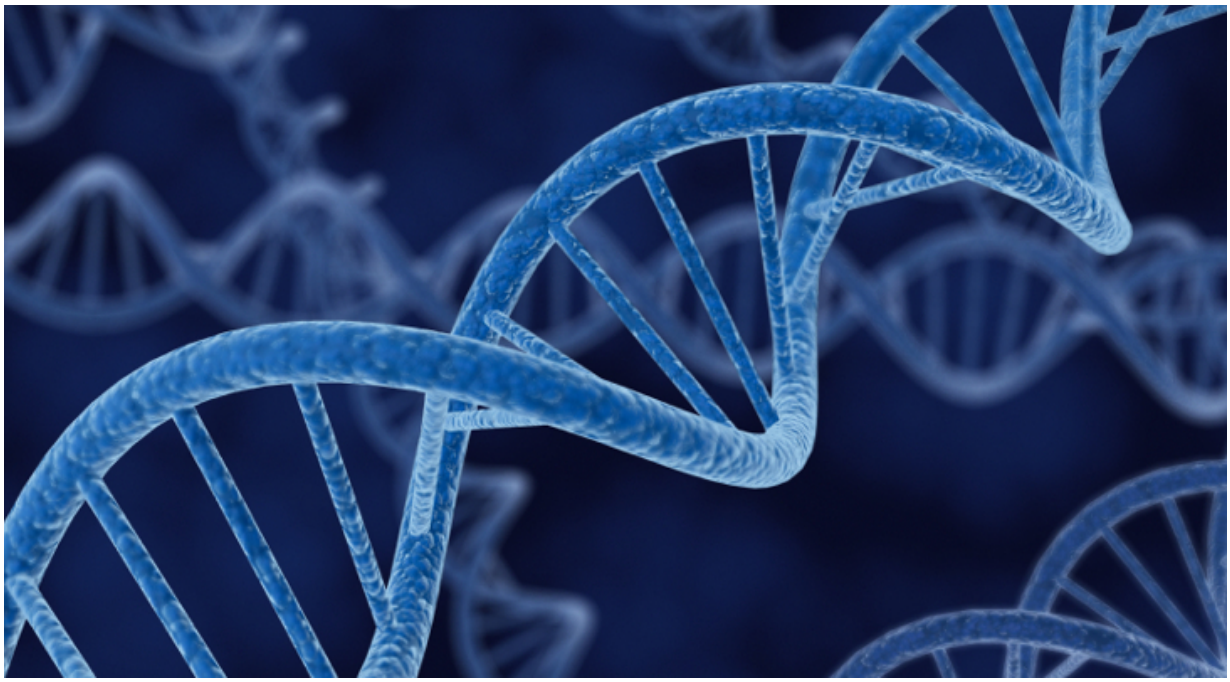
# Gene Therapy



## A technique, which can perform miracles

Aneirin Benedict
Vijiliya Shanmuganathan
Santhana Thamboo

Gymnasium Kirschgarten
2014

# Table of Content

# 1. Preface

From the beginning we on we had agreed to write a report about a technique, which deals with the human body. After researching a lot we read about a method, on how one can cure genetic diseases. It's called gene therapy. Gene therapy has a successful application area in medicine and opens a lot of new treatment methods, to cure diseases, which were incurable until now. So, it gives people more hope to reduce the suffering in our world.

At first, we wondered if gene therapy was successful in Switzerland. On the other hand we wanted to learn more about the different functions of the methods in gene therapy. We were also wondering about the risk and advantages. Gene therapy is not a very developed technique, so we are interested in its new appearing successes [1].

# 2. Introduction

Gene therapy is a technique, where the diseases or genetic problems are fixed at its source, by replacing the defective gene with the corrected version of the gene. With genetic problems one could be dealing with genetic diseases, inherent diseases or acquired genetic diseases like tumors. In gene therapy we can differentiate between somatic and germ-line gene therapy:

- In somatic gene therapy, the therapeutic genes are transferred into the body or somatic cell. Because the genetic defects are corrected in the body cells, the change will not be inherited to the following generations.

- In the germ-line gene therapy the germ cells, that means sperms or egg cells, are genetically changed, which leads to passing of genetic information to the following generations. But because of ethical reasons and insufficient knowledge about any risks for the following generations, this type of therapy on humans is prohibited in many countries.

  A lot of research is necessary for a significant development in the field of gene therapy [2].

## 2.1 Scientific background

In the year 1990 in USA the first gene therapeutic treatment was performed on a human. The 4-year old girl suffered from an inherent immunodeficiency (short SCID) [3]. It also had affected people, who were cured, but after a few years they suffered from another disease, *leukemia.* As well the death of a patient in the USA in 1999 was a response for the gene therapy. But in the beginning the estimate for by-effects was very small.

## 2.2 Reference to actuality

In the meantime, the methods in gene therapy have obvious improved. It also had first successes for the patients, who were treated by gene therapy in hospitals. Additionally it has procedures, in which no Viruses are needed as vectors. Worldwide about 6000 people were treated with gene therapy according to research studies [4]. It's also remarkable that the interest for somatic gene therapy has increased compared to other medical research areas. But the development of the gene therapy methods, for a lot of incurable diseases will still take some years developing. There are also many researches for the ideal vector.

## 2.3 Application area

The originally application area of the gene therapy are inherited diseases. Here the objective for the therapy is to substitute a defect or missing gene. Popular examples are immunodeficiency syndrome like SCID or hemophilia. Nowadays gene therapy is especially used for combat of malicious neoplasm (cancer) [5], because there are often found genetic defects. Additionally gene therapeutic procedures can be oriented of the activation of the immune system against tumor cells or on its direct destruction. Further gene therapy procedures are oriented for example against infectious diseases (especially HIV- Infections) or cardiovascular diseases [6].

## 2.4 Alternatives

In the moment just a few gene therapeutic attempts are durably successful. In the gene therapy viruses are used as transportation (vectors), which possibly could cause side effects.

• **Peptides as alternative**

Scientists of the University in Pittsburgh found a new bioengineering method, to introduce the therapeutic agent directly into the sick cell. Peptides, easily constructed protein compounds, infiltrate medicament.

The scientists assume that every arbitrary molecule can be infiltrated on this way into the cell. The advantage of this procedure is that one can get in in every cell type with minor side effects, because the introduced protein chains can't influence the metabolism. Possible application areas are therapies of diabetes, tumor- or joint diseases [7].

• **Inhibit of calcium pump as alternative**

One hoped to cure mucoviscidosis with gene therapy. Since 1990 it has intensive researches, but without developing an optimal treatment. The disease mucoviscidosis has a new procedure in sight, whereby the gene therapy can be replaced. Mucoviscidosis, also know as cystic fibrosis (CF), is a dysfunction in the mucigenous glands, where the CFTR, a protein in the cell wall, has a defect. Its function is to infiltrate chloride through the cell. The cells of CF-patients produce a little modified CFTR. Because its topology is not normal, it doesn't reach his place in the cell wall. Then the cells ensure that defective proteins are degraded. The chaperones control which molecule can go into the cell and which not. Therefore the chaperones need calcium ions. Scientists of the university Yale found a new possibility for the treatment of CF. The activity of the chaperones should be reduced by the inhibit of calcium pumps, so that more CFTR can reach the cell wall. In an animal experiment the number of CFTR in the cell wall was already increased by the inhibit of calcium pumps, but medical treatment is not possible yet. But the promising results are giving new hope for the treatment of CF, which could be in future an alternative for gene therapy [8].

# 3. Methods and Theory

How we already mentioned in the introduction, the gene therapy is a technique, which can eliminate a disease, by introducing intact genes into the cells.

## 3.1 Different strategies

- Through *substitution* of a defect gene, monogenic determined genetic diseases can be cured. Therefore an intact copy of the corresponding gene exchanges the mutated gene. This is the often-used method.

- Through *inhibition* of a foreign gene, infectious diseases, for example if viruses are located for a longer time in the body, can be cured. A possible application example is AIDS. The genes of the viruses should be eliminated specifically, whereby they are disturbed in their normal functions and the multiplying gets unlikely.

- Through *local gene expression* diseases whose cause are a lot of gene defects, can be cured. It's possible to infiltrate a gene into a cell, if it produces a therapeutic acting protein. The gene will be inserted into the DNA and gives the cell the possibility, to produce the protein on itself [9].

## 3.2 Transduction of genes [10]

There are two ways to transport the gene into the body cell of a patient.

In the **In-vivo** gene therapy, the therapeutic gene is packaged into a suitable vector and is directly delivered into the organism (orange arrow).

In the **Ex-vivo** gene therapy the cells are modified outside of the body. The gene is inserted into the cell and the genetic altered cell is inserted back into the organism (green arrow).

The Ex-vivo gene therapy is more common than the In-vivo gene therapy, because it reduces many risks. The Vectors used in In-vivo gene therapy includes viruses, nanoparticles and more.

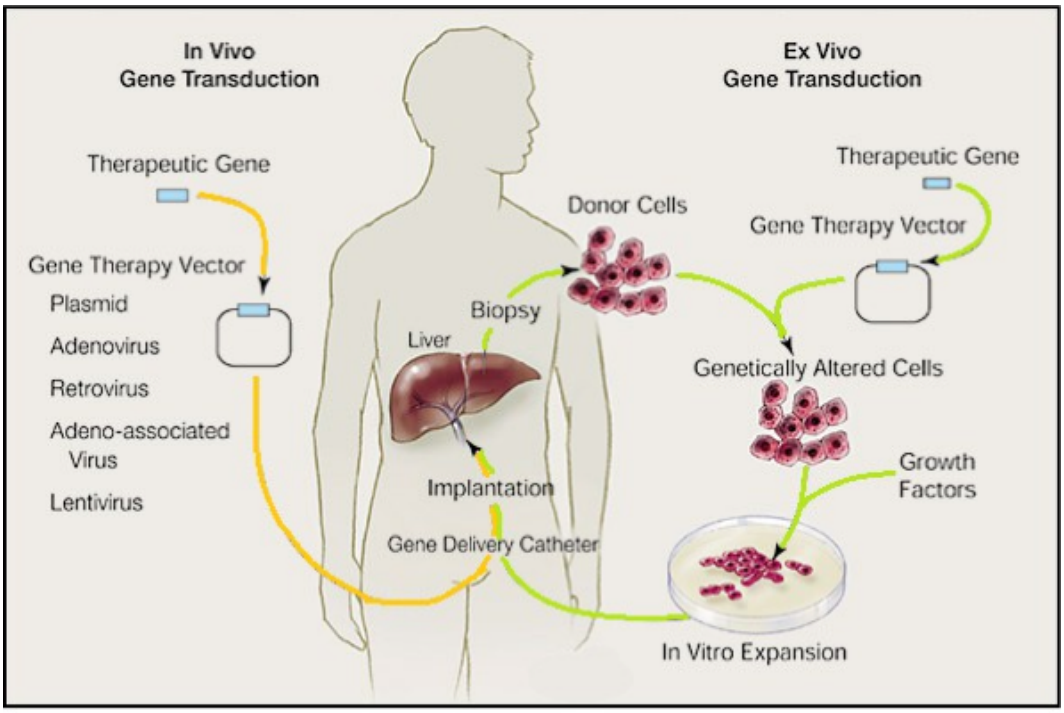



figure 1

## 3.3 Methods for infiltrating genes [11]

The efficient infiltrating of a gene into a targeted cell can be reached with different methods.

**Vectors:** In the In-vivo and ex-vivo method one can work with vectors, which infiltrate the gene into the cell. The gene can be packaged in Liposomes, which merge with the cell membrane. But mostly the gene is packaged in viruses. They enter into the cell and let their genome, the gene that is inserted by human, incorporate with the DNA. **Microinjection:** This technique is very complex and labor intensive but it's one off the most efficient methods. It delivers the DNA directly into the nucleus. A glass pipette with DNA is guided into the nucleus and is injected.

**Electroporation:** The cell and DNA are located between two electrodes and subjected to an electrical pulse. During the electrical pulse the cellular membrane forms holes, whereby the DNA enters.

**Calcium phosphate transfection:** In this solution gene can be better incorporated from the cell into the DNA.

**Gene Gun:** Another method of DNA transfection is particle bombardment or gene gun. DNA is coated onto Wolfram or gold-particles with the Calcium phosphate solution and a thin layer of the gene. This is loaded into a device and fires it on a cell. A few particles hit exactly the cell nucleus.

## 3.4 Actual examples

### 1.Example

In October 2013 it was possible to cure children with the disease severe combined immunodeficiency (SCID) with gene therapy. SCID characterize usually the defect of T-& B-lymphocyte, a part of the leukocytes. This results to serious infections in the first month of life, like pneumonia, meningitis or bloodstream infections. Children with this disease can also become ill from vaccines, which contain viruses. Examples for such vaccines are Chickenpox, measles and Rotavirus etc. This virus may cause life-threatening infections to children with an unhealthy immune system.

There are several ways of SCID. One of these is linked to a deficiency of the enzyme adenosine deaminase (ADA-SCID) SCID is also known as bubble boy disease. In the 1980's a boy with SCID lived for 12 years in a plastic bubble, which was germ-free.

Results for the first children to receive the improved gene therapy were presented at the European Society of Gene and Cell Therapy conference in Madrid in October 2013. The immune system of the children has continued to improve since receiving the treatment is said from the Great Ormond Street Hospital in London.

The little girl Nina was born with ADA-SCID and after one and a half year she was cured through gene therapy. Her Stem cells were harvested from the bone marrow and, before being injected back into body it was given genetically changed ADA genes back. One expected not much for the next 8 month but just after 5 month her white blood cells were nearly doubled and in October 2013 she has the immune system of a healthy baby without any side effects [12 -13].

**2.Example**

In January 2014, six people suffering from Choroideremia improved their sight with a genetically engineered virus. Choroideremia (CHM) is a rare inherited genetic eye disease, which leads to blindness, which is caused by RAB Escort Protein-1 (REP-1). Because Choroideremia is an X-linked recessive disease it occurs almost solely on males. Females have two X-chromosomes. The healthy version of the gene on their other X-chromosome produce REP-1 and that's the reason why females normally doesn't suffer on this disease. Without REP-1, which should usually be formed by the CHM gene, the pigment cells in the retina of the eye stop working and die off. Professor MacLaren's team used a small safe virus to carry the missing CHM gene into the photoreceptors in the retina. The aim is that it starts producing REP-1 and stops the dying of cells. It's better to treat people before too many cell in the retina have been lost. After the results of the six patients with success it's still too early to say if the treatment will last indefinitely. But now they know that passing the gene with a virus doesn't case any damage in the retina. Jonathan Wyatt was the first patient in this gene therapy and said the risk is worth for that [14-15].

# 4. Interview with Dr. Andrea Banfi [16]

**1. What are the advantages of gene therapy compared to other therapies?**

In gene therapy we put a healthy copy of a gene into the body by using a vector. The defect gene gets substituted with a healthy copy. Through this method inherited diseases are easily to cure.

**2. What are the risks and dangers of using gene therapy?**

There are many risks of gene therapy. We use mostly viral vectors as a carrier of the gene. This might cause an unwanted immune system reaction where your body's immune system sees the newly introduced vector as an intruder and attacks it. This can lead to inflammation and organ failure. It's also possible that the virus affects a healthy cell with the mutated gene and causing damage, which can lead to other sickness like cancer.

The virus with the manipulated gene can also remember its abilities of being a virus and attack the cells. The risk of getting cancer is quiet high in gene therapy due to insertion of the gene at a wrong place.

To prevent these risks it's very important to express the gene at the right moment, at the right time, right place and in the right amount. If the gene is over expressed it produces too much proteins of the missing one.

**3. The germ-line therapy is banned in Switzerland. Can you explain us why it is banned and can you tell us about the ethical issues?**

The germ-line therapy is a therapy where the germ cells are manipulated. It's an inheritable change. Therefore only the offspring has an advantage through this therapy and not the patient.

One can make through this therapy designer babies or create new human beings, but that's ethnically not correct.

It's also not a very precise method since these vectors can cause cancer and if that happens in the germ cells then it gets passed through generations.

But nowadays the germ-line therapy is banned nearly everywhere.

4. **When there wouldn't be a ban on germ-line therapy, which therapy do you think is the best? The somatic therapy or the germ-line therapy?**

The somatic therapy is better for the patient because the germ-line therapy doesn't help the patient itself. The germ-line therapy is only good to prevent diseases or correct the diseases for the offspring. And the studies of gene therapy are still too far away from specific manipulation.

5. **Can you explain us the two methods of transmitting these genes into a body cell? The ex-vivo and the in-vivo method.**

In-vivo: you take the gene, which you want and put it in a vector and inject it directly into the tissue of the body, which you want to treat.

Ex-vivo: you take some cells out of the body and culture them in-vitro (in a dish) then you put the genetic manipulation on the cells and replant the cells into the tissue where you have taken it.

The ex-vivo method is safer because you have a better control. The problematic cells can be eliminated from the outside. There is also more manipulation possible. The stem cells, which get treated stay healthy and generate themselves.

6. **In which cases is gene therapy used in Switzerland?**

   *He couldn't answer the question based on Switzerland because he doesn't practice medicine in Switzerland. The answer is in general.*

Today we use gene therapy to treat the following diseases:

- Blood diseases
- Muscular dystrophy
- Eye diseases
- Cardiac diseases (the contraction power is low)
- Tumors
- Cystic fibrosis

These diseases are inheritable. Therefore gene therapy is a possible solution.
There are also some other fields where you can find gene therapy. → www.asgct.org

**7. How do you see the future of gene therapy?**

The future of gene therapy looks brighter now. At the beginning everyone thought that's a good idea and that humans are now able to do anything in the human body. But later they realized that it is a lot more complicated. The side effects like cancer were shocking the people.

But now, 15 years later the scientists found real therapeutic cure for some diseases. The new technologies are also a major part of the new achievements in gene therapy. Through this development we can target the precise gene, which is needed. The genome editing effect was found recently. It helps to develop special enzymes in the human body, which can change any sequence of a genome to any sequence you want.

## 4.2 Visit to the laboratory

On Friday the 14.03.14 we went to the University Hospital Basel
ICFS 407, Hebelstrasse 20. After the interview with Dr. Banfi, he showed us the laboratory where he was working with his group on gene therapy. He showed us the researches about angiogenesis. They are doing a research about the formation of vessels in the body, with genetically modified cells. These cells get implanted into mice or rats. The cells, which stimulate the angiogenesis, produce also the protein VEGF. These get infiltrated into a cell by using retroviruses as vectors. This procedure is an ex-vivo method. After having this protein the body can form again new vessels.
In future this method can be used for humans against wet gangrene, dying of heart parts after having a heart attack.
Dr. Banfi showed us some histological slides of mice ears. We could see through the microscope that the cells were forming new vessels.

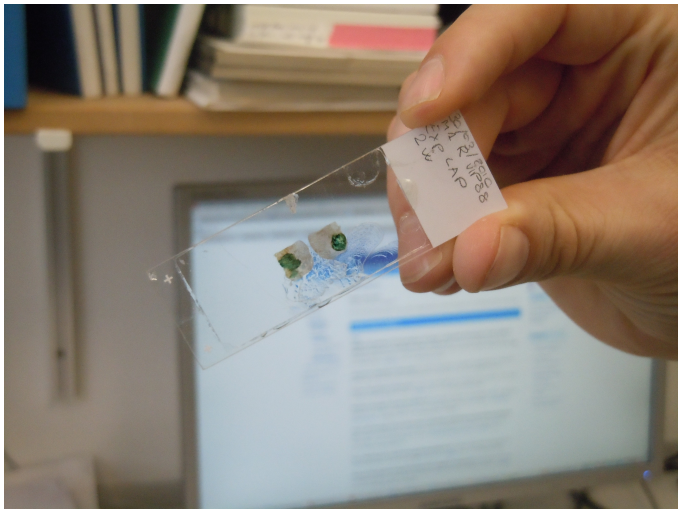


**figure 1**

Here is a picture of the slide.

We could also see human cells in the biosafety room. They put a green fluorescing dye on the human cells, which we could see with the confocal laser scanning microscopy. This was a very interesting experience.

# 5. Discussion

Our discussion is based on the conversation with Dr.Banfi.

## 5.1 Progress and Advantages

In most cases of gene therapy it deals with causal form of treatment. That means the original defect is treated and not its symptoms. Just a causal treatment can lead to a disappearance of a disease, which is not curable with the own body. For many genetic diseases it doesn't have a treatment or just a symptomatically one. But through the correction of the gene defect the concerned people can be cured.

## 5.2 Future of gene therapy

For some immunodeficiency's gene therapy could be a choice for the treatment. For the genetic disease like CHE it has also a positive expectation. In the foreseeable future some strategies for real gene correction (“genetic surgery”) and for the expansion of cells will be so progressed, that we can resign the assembly of viral vectors into the genome for the therapy of genetic diseases. That will minimize the risks for side effects. For cancer diseases, which are very hard to treat, it should have an additional therapy option, which can be appointed in combination with the usual methods.

## 5.3 Germ-line therapy

Because of good reasons, the germ-line therapy is interdicted in the most of the countries, also if it has some theoretical advantages. For example, that the disease would be removed from the germ-line. That means it wouldn't have any risks for concerned people, to pass the disease to offspring. The selective germ-line therapy is, in the view of today, related with many ethical problematic procedures like the gain and consumption of fertilized egg cells. In addition the methods for gene therapy are too risky, for introducing it to germ-line. Possible side effects wouldn't be foreseeable.

## 5.4 Disadvantages and Dangers

For many diseases gene therapy couldn't just express an effective treatment but rather also a gently and lately one of the most cost-effective treatment option. Patient with immunodeficiency are transplanted allogeneic, that means they get hemocytoblast (from the marrow) of a healthy donor.

Such a treatment is only possible for the patient, if a genetically matched donor is found. But even then it is very risky and extravagantly.

Even with ideal matching donors, there is still a high risk, which continues to grow, if the genetic match is not optimal. Most of the patients have after such a transplant hard side effects, not a few even die from the treatment. This is only accepted because children with such severe immunodeficiency don't often reach the adulthood after such transplantation. According to this state of knowledge, gene therapy offers these children a much safer and less burdensome treatment options. Since gene therapy is still far from the ideal of the precise " gene surgery ", they are today introduced with the help of vectors into the cells. These vectors mostly come from viruses. This leads to some theoretical but also practical risks of gene therapy. Theoretically, the viral vectors can be contaminated with wild-type viruses or they can recombine with them after an infection in the patient. Both risks can be excluded with the state of the technique today, that the vectors at the genetic level have hardly anything in common with the original viruses. However, viral proteins are used for the infiltrating of viral vectors by viral mechanisms. Therefore immune responses can occur by a direct transfer of viral vectors into the body. This was, for example, observed in adenoviral vectors, where it leads 1999, because of such immune responses even to a fatality. Therefore, it is now tried to develop vectors for in-vivo administration, which do not trigger acute immune responses. Another viral vector type is derived from so-called retroviruses. These vectors integrate permanently into the target genome and are therefore passed on in cell divisions also to daughter cells. This makes such vectors especially for the correction of genetic diseases in dividing cells (e.g. blood cells) interesting. Actually the vectors were used in the treatment of various immunodeficiencies. Since the blood cells were obtained of the body for the treatment and were genetically modified outside the body, there is virtually no risk of immune reactions. However, it has been shown that the integration of the vectors into the target genome represents a mutagenic incident, which could lead in the worst case to malicious changes. Meanwhile, one tries to optimize the vectors in such a way that the risk of insertion of mutagenesis is minimized. To exclude it completely, it needs a development of precise techniques for the target "microsurgical" correction of the genome. Other risks such as unintended germ-line modification are targeted minimized by corresponding studies and a series of specific measures. Major drawbacks of gene therapy are certainly the very high costs.

## 6.Conclusion

It should be noted that many methods, which are nowadays successfully applied, are over a long development process. There were several solutions to find and there were losses but we can say that gene therapy is a developing technique to cure inheritable diseases. Even if the expectations in 1990 were high we are getting closer to the answers. The scientists still have a lot of work to do since we didn't have a clinical trial yet. Gene therapy is at the moment only a solution for special cases and that might change soon.

## 7. Summary

At the beginning of our paper we describe how we came to the topic of gene therapy and what our questions were. Then we explained that gene therapy is a gene technique where scientists try to heal a disease by manipulating the genes.

The somatic- and germ-line therapy were explained and distinguished. The origin of gene therapy in the 90's and the developing procedures of gene therapy are mentioned. Gene therapy focuses' nowadays on inherited diseases and tumors. Because of the risks we took a look on the alternative versions to heal gene defects. We looked also at the precise procedure of gene therapy, through substitution, gene expression and the in-vivo or ex-vivo method. The different methods to transfer the gene through a vector by microinjection, electroporation and calcium phosphate transfection are described.

We also have some examples that show the efficiency of gene therapy. The article about the bubble boy in 1980 and the girl Nina, born in 2013, were diagnosed with severe combined immunodeficiency (SCID/ADA-SCID). The doctors were able to heal them by manipulating their gene. Another example is the eye disease Choroideremia (CHM). In 2014 were six people treated by transferring a virus with the manipulated gene into the eye.

We went to Dr. Andrea Banfi's office to make an interview. He helped us a lot by answering our questions as precisely as possible. It was a very interesting Interview and we learned a lot. In the Interview and the discussion we talk about the advantages and disadvantages of gene therapy. The ethic question and the future of gene therapy are also included.

And at the end we have our conclusion that gene therapy is still after 15 years at the beginning of its success. We can expect a lot in the future since the technologies are getting better and better. So it might be easier to control the gene therapy.

# 8. Bibliography

[1] http://learn.genetics.utah.edu/content/genetherapy/gtintro/

[2] Prof. Weber U. (2008): Biologie Oberstufe, Gesamtband, Cornelsen Verlag, Berlin.

[3] http://singularityhub.com/2009/02/09/gene-therapy-cures-fatal-bubble-boy-disease/

[4] http://sic.fimea.fi/sic3_11/gene_therapy_targeted_treatment_option.aspx

[5] http://clinicaltrials.gov/show/NCT00001328

[6] http://www.asgct.org/general-public/educational-resources/gene-therapy-and-cell-therapy-for-diseases

[7] http://www.nature.com/gt/journal/v9/n24/full/3301923a.html

[8] http://connection.ebscohost.com/c/articles/9511632/calcium-pump-inhibitors-induce-functional-surface-expression-of-f508-cftr-protein-cystic-fibrosis-epithelial-cells

[9] http://www.medindia.net/articles/genetherapy_treatment.htm

[10] http://genmed.yolasite.com/basics-of-gene-therapy.php

[11] http://members.tripod.com/~dr_venky/med2.html

[12] http://www.newscientist.com/article/mg22029413.200-bubble-kid-success-puts-gene-therapy-back-on-track.html#.U2ESq9zrVd0

[13] http://www.scid.net

[14] http://choroideremia.org/?page_id=26

[15] http://en.wikipedia.org/wiki/Gene_therapy

[16] Dr. Andrea Banfi
Cell and Gene Therapy
University Hospital Basel
ICFS 407, Hebelstrasse 20
4031 Basel (Switzerland)
Tel. +41-61-265 3507
Fax +41-61-265 3990
e-mail: Andrea.Banfi@usb.ch
http://www.icfs-basel.ch/english/cell.htm

figure 1. http://news.softpedia.com/news/Gene-Switched-On-by-Stress-Speeds-Up-the-Spread-of-Cancer-377951.shtml