

# Gene therapy – The future of medicine

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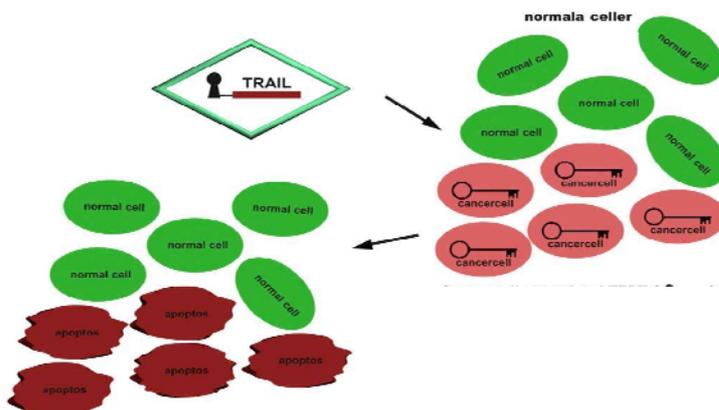
Populärvetenskaplig sammanfattning av Självständigt arbete I biologi VT 2008

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*Gene therapy is a new and revolutionary approach for treating a range of different diseases. It employs viruses as carriers of genetic material into cells with the aim of correcting malfunctioning genes. This technique could possibly allow doctors to treat genetic disorders with unprecedented ease.*

## TRAIL

Cancer is the result of a condition in which cells have lost control over their lifecycle, and become immortal. Normally cells that have been damaged in some way can detect this and initiate a sort of suicide mechanism called apoptosis. Cells are subject to aging just like the organism they reside in. One way of cell aging is that chromosome ends become slightly shorter every time a cell divides. This leads after a certain number of divisions to instability in of the chromosomes. There is an enzyme called telomerase that fixes this problem by adding pieces of DNA to the ends of chromosomes to counteract the shortening in certain cells, but not all. Researchers have discovered that the activity of telomerase is a good indicator of carcinogenic activity. TRAIL is a protein that can initiate a sequence of events leading to apoptosis. Taken together, the fact that TRAIL can induce cell death, and that the enzyme telomerase is up regulated in cancerous cells, researchers saw a way to construct a gene therapy virus that could use these characteristics to target cancer cells. The way they made the gene therapeutic virus was by cutting and pasting genes together. The basis for the cancer cell killing virus construction was an adeno associated virus. Viral genes were replaced by the gene for TRAIL under control of a preceding promoter sequence for a part of the telomerase enzyme. A promoter sequence is a kind of starting point for gene transcription. This means that TRAIL will only be expressed in cells that also express the telomerase enzyme. In other words they constructed a virus that could infect most cells, but only kill those that display characteristics of cancer. This can be thought of as a kind of lock and key relationship. The lock consists of the promoter sequence controlling expression of TRAIL and can only be unlocked by cancer cells. Studies performed in the lab showed promising results. Populations of cancer cells had a mortality rate after infection by the vector ranging from 23 % to 66 %, whereas non-cancerous cells only had about a 5 % mortality rate after infection by the vector. The researchers had thus showed that the vector was effective at inducing cellular apoptosis, and that it had a clear preference to do this in cancerous cells leaving the healthy cell population largely unaffected.

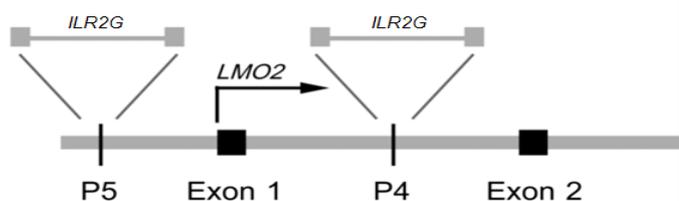


## X-SCID

The first major success with gene therapy was reported in the year 2000, from a children's hospital in France. They reported that they had successfully treated two patients with a disorder called X-linked severe combined immunodeficiency or X-SCID. The condition is caused by one part of a certain receptor on cells in the immune system failing to function correctly due to genetic mutations. This causes signalling between immune system cells to be impaired, leaving the patient with an underdeveloped defence against viruses and bacteria, and thus exceedingly susceptible to infections. The receptor part that is defective in X-SCID patients is a protein called common  $\gamma$  chain, and is involved in many different signalling pathways in the immune system. Most importantly maybe, it receives instructions for a type of cells called T-cells to divide and multiply. T-cells are crucial members of the immune system. They signal to other defence cells, which cells have been attacked by an intruding pathogen, such as a virus or bacteria. They can also kill infected cells. As X-SCID patients lack the receptor that tells their immune system to gear up for an attack, their so called progenitor immune cells never develop in to the mature infection fighting cells they were meant to be. In simple terms one could say that parts of the immune system never gets up to fight because they can't hear their friends calling for help. This affliction can be cured by bone marrow transplantations by introducing T-cell progenitors that have a functioning common  $\gamma$  chain, or in other terms can hear their friends call for help. However if there is no suitable donor the affected child have very little chance of survival.

The French researchers and doctors developed engineered viruses to carry a functioning copy of the defective gene into the patients bone marrow cells. The gene therapeutic virus was constructed from a retrovirus. Retroviruses have the ability to incorporate their genes in the hosts genome. The most famous example of this type of virus is probably HIV. The virus used by the doctors in France was extensively modified to avoid being harmful to the patients, and fitted with the gene that the doctors wanted introduced into the patients. The infection of the patients bone marrow cells were made *ex vivo* which meant that they took the cells out of the patients body and put them in a test tube, where they were infected by the virus. After a short stay in test tubes the bone marrow cells were reintroduced in the patients. Within months after the treatment the patients developed functioning immune systems.

Three years later a shocking discovery was made. Two of the patients that were thought to have been cured developed leukaemia. After intense research into why this had happened it was concluded that the gene therapy virus had inserted its genes in a few cells, near or in an other gene called *LMO2*. The *LMO2* is involved in cell division. When the therapeutic genes were inserted in or near it, they activated the gene, and the cells started to divide in an uncontrolled manner, consistent with the course of events involved in leukaemia. Because of these events the French X-SCID trials are thought to represent both the great possibilities with gene therapy and its inherent dangers.



### *How gene therapy vectors are constructed?*

There are a few criteria that needs to be insured for a virus vector to be viable as a therapeutic agent. It needs to be able to carry genetic material into cells. This is the least problematic feature to construct as viruses have this as an fundamental function of their lifecycle. What is more difficult is to make the virus unable to reproduce on its own. A good analogy to what might happen if this isn't insured is this. Imagine taking a drug in the form of a pill. Once the pill is swallowed one would enjoy the benefits of it, relief of a headache or reduction of a fever. Gene therapeutic viruses can have the same effect but on more complicated deceases, particularly genetic ones. However, when we swallow a pill this is eventually degraded and flushed out of our systems. Viruses, as biological creatures have a survival strategy, which is to infect cells and then hijack its system to reproduce. If this isn't controlled it would be similar to swallowing a pill, and two hours later the pill would have multiplied in the stomach to several hundred pills. The way to control this in viruses is to delete or modify certain parts of their DNA. Once this is done the empty space in the viral DNA can be used to package therapeutic genes.

To do this cells are placed in a test tube and used as factories to create viruses that are to be used for treatment of various deceases. In these cells two different parts of DNA is placed. On part is DNA that a doctor wants to introduce into a patient. The other part is genetic material functioning as a blue print for how the viral structure is to be built. In the therapeutic piece of DNA there is packaging signal that determines whether or not genetic material is to be packaged into the virus particle. The blueprint DNA does not have this signal, and is thus not packaged into this virus. This feature of the production is what makes sure that like the pills in the previous analogy, gene therapeutic viruses are not able to reproduce in the patient. Allowing gene therapy viruses to reproduce uncontrollably in a patient is not good as it will produce immune reactions similar to those of viruses of pathogenic nature. This would both make the patient sick from the treatment and the immune response would also severely reduce any therapeutic effect the virus would have produced.

### *Where does the road of gene therapy lead?*

The idea of gene therapy might make many, if not all, somewhat uneasy as to whether tampering with genetic material is right. It is important to understand thought, that all research and clinical studies approved today, world wide, is directed towards genetic manipulations of somatic cells, and not germ line cells. This means that genetic alterations made in a patient cannot be inherited to its children. Altering genetic expression in somatic cells to treat deceases is not that different from treating it with conventional drugs. The difference is that with gene therapy doctors have the ability to fix a problem at its source, instead of just reducing the symptoms.