An Introduction to Gene Therapy

What is it?

Genes are strings and strings of nucleotides that govern our bodily functions and make up whom we are today. Not only are the genes themselves important, but the proteins they code for are even more important. If the gene has too many mutations that it disrupts the structure of the protein, the functionality of the protein can be altered, causing it to function poorly or function in a different way that would prove detrimental to other functions in the body. This in turn, causes diseases. Gene therapy is the use of genetics as medication and a cure for diseases. Rather than using pharmaceutical drugs that treat the effects of a disease, gene therapy is used to target the gene that is causing the disease.

There are many approaches to gene therapy. Here are the four most common ways that gene therapy is conducted:

A normal is inserted into the genome to replace the corrupted gene that is causing the disease. Usually the most common approach.

An abnormal gene could be swapped for a normal gene through homologous recombination during meiosis (http://biolog-e.ls.biu.ac.il/faculty/wides/80-855/Capecchi_MR_1989.pdf)

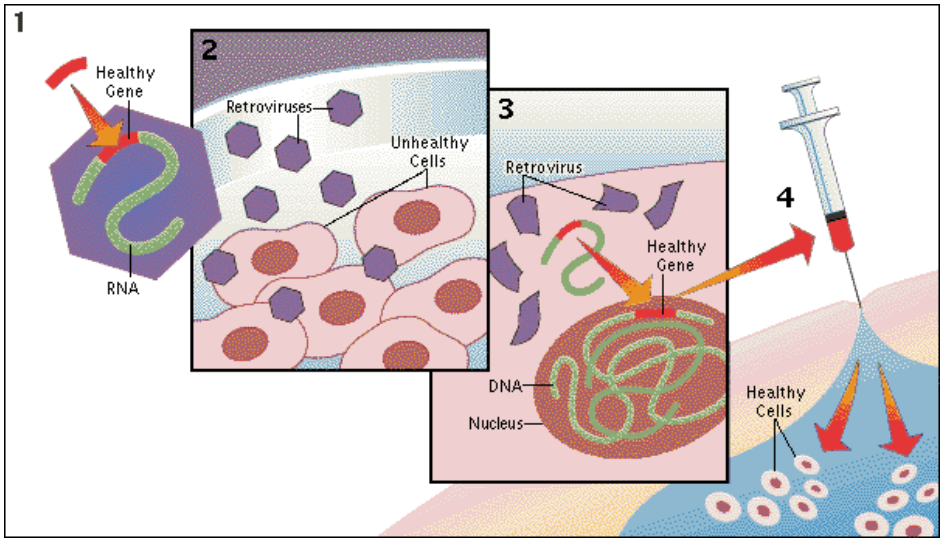
The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.

The regulation (the degree to which a gene is expressed or not expressed) of a particular gene could be altered. (http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml).

After describing these methods, this paper will go on to mention the clinical successes for gene therapy for various diseases, analyze the problems with gene therapy and its success, and address the ethical concerns with gene therapy.

How does it Work?

As mentioned in the introduction, there are four main ways of approaching gene therapy. The first method involves inserting a normal gene into the genome. This is accomplished by inserting the normal DNA into a vector/virus that then "infects" the target/host cell. Soon, the normal DNA is incorporated into the host cell's genome. Now the host cell will reproduce and replicate the new therapeutic DNA along with the old genome.



Many types of viruses are used to infect the target cell (http://www.wiley.com/legacy/wileychi/genmed/clinical/) :

Retroviruses - viruses that creates double-stranded DNA copies of their RNA genomes, through a reverse-transcriptase. These copies of its genome can be integrated into the genome of host cells. Human immunodeficiency virus (HIV) is a retrovirus. (citation)

- **Adenoviruses** - viruses with double-stranded DNA. Typically the common cold is an adenovirus. However this virus has to be reinjected into every cell, it does not replicate. (citation)
- **Adeno-associated viruses** - single-stranded DNA viruses that can insert their genetic material at a specific site on chromosome 19. (citation)
- **Herpes simplex viruses** - double-stranded DNA viruses that infect neurons. (citation) . (http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml). Similarly, gene regulation would make use of inserting a different kind of promotor or a different kind of gene that would yield a protein that take part in over-expressing or under-expressing the gene of interest.

The use of homologous recombination is more common in targeting somatic cells that would eventually turn into germ cells. Many model organisms have been used for this technique, the most prime example remains the mouse model studied by Mario R. Capecchi, Sir Martin J. Evans, Oliver Smithies, who experimented with the mice. (http://www.bio.davidson.edu/Courses/genomics/method/homolrecomb.html). Usually homologous recombination occurs during the cell cycle in somatic cells that are transforming into germ cells—when the chromosomes align along the center in order to separate, some of the genetic materials between the chromosomes switch, thus leading to germ cells that have a combination of DNA from both chromosomes initially. Scientists take this to their advantage, and use these processes in gene therapy. By creating a construct DNA that has the "normal" DNA, they introduce this DNA into a cell. The normal DNA is set on the exact same spot on artificial construct homologous chromosome that is the same length as the chromosome with the nonfunctional DNA that we are trying to replace. Then while going through homologous recombination, these two chromosomes will switch materials, and hopefully the nonfunctional DNA on the actual

chromosome will be replaced with the functional DNA. Then the correct functional DNA chromosomes will be selected for using many different methods. This method was performed by Mario R. Capecchi, Sir Martin J. Evans, Oliver Smithies, who won the nobel prize for their experiment with knock out mice. Essentially, by introducing an engineered DNA construct that would yield a color of interest into germ line cells of mice, they were able to reproduce the color of interest in the next generation of mice.

Problems with Gene Therapy

There are many problems with gene therapy. First of all, gene therapy tends to be very single cell oriented. In other words, you would have to inject a virus into practically a majority of cells to induce the desired effect. One cell having the correct genome isn't enough to take care of the diseases that many other cells with corrupted genome would be inducing. It is indeed hard to rapidly integrate therapeutic DNA into the genome, due to the rapidly dividing nature of cells. We would have to have multiple insertions of DNA which would be difficult given the volume of new cells produced every second. Furthermore, just as with organ transplants, our cells could potentially produce an immune response that rejects the DNA and produces a response that could possibly even kill the cells with the inserted gene. Lastly, gene therapy tends to target single gene diseases, and multi-gene disorders like Parkinson's seem to have less success when it comes to gene therapy.

Milestones in Gene Therapy Research:

Some Successful Experiments

There have indeed been many successful experiments using gene therapy. Thalassaemia, a blood disorder, is caused by mutations in one or both of the genes that code for hemoglobin. Rather than replace the gene, the approach used by

Ryszard Kole and colleagues at the University of North Carolina repairs the dysfunctional messenger RNA produced by the defective genes. According to the abstract and paper published by Dr. Kole, the following mutations of the beta-globin gene, A-->G at nt 110 of the first intron (beta 110), T-->G at nt 705 and C-->T at nt 654 of the second intron (IVS2(705) and IVS2(654), respectively), leads to abnormal splicing of the corresponding pre-mRNA associated with hemoglobin and its function. Aberrant splicing of beta 110 pre-mRNA was efficiently reversed (using the selective reverse mutation) by an oligonucleotide targeted against the branch point sequence in the first intron of the pre-mRNA but not by an oligonucleotide targeted against the aberrant 3' splice site. In both IVS2(705) and IVS2(654) pre-mRNAs, correct splicing was restored. (http://www.pnas.org/content/93/23/12840.short).

In 2008, Maguire et. Al., conducted an experiment in which they used gene therapy to revive visual functions in mammals. One form of the disease, LCA2, is caused by mutations in the retinal pigment epithelium–specific 65-kDa protein gene (*RPE65*). Through using a adeno-associated virus, recombinant AAV2.hRPE65v2 containing *RPE65* cDNA was introduced to target cells in vitro. AAV2.hRPE65v2 that was injected behind the retina of animal models of LCA2 resulted in rapid development of visual function (http://www.nejm.org/doi/full/10.1056/NEJMoa0802315#t=abstract).

Gene therapy has even been used in the cancer field. In an experiment conducted by Porter et al., the researchers designed a vector expressing a chimeric antigen receptor with specificity for the B-cell antigen CD19, coupled with a co-

stimulatory receptor in T cells, and a signal-transduction component of the T-cell antigen receptor signaling domains. They injected these vectors into the cells of a patient with chronic leukemia: the cancer went into remission. http://www.nejm.org/doi/full/10.1056/NEJMoa1103849.

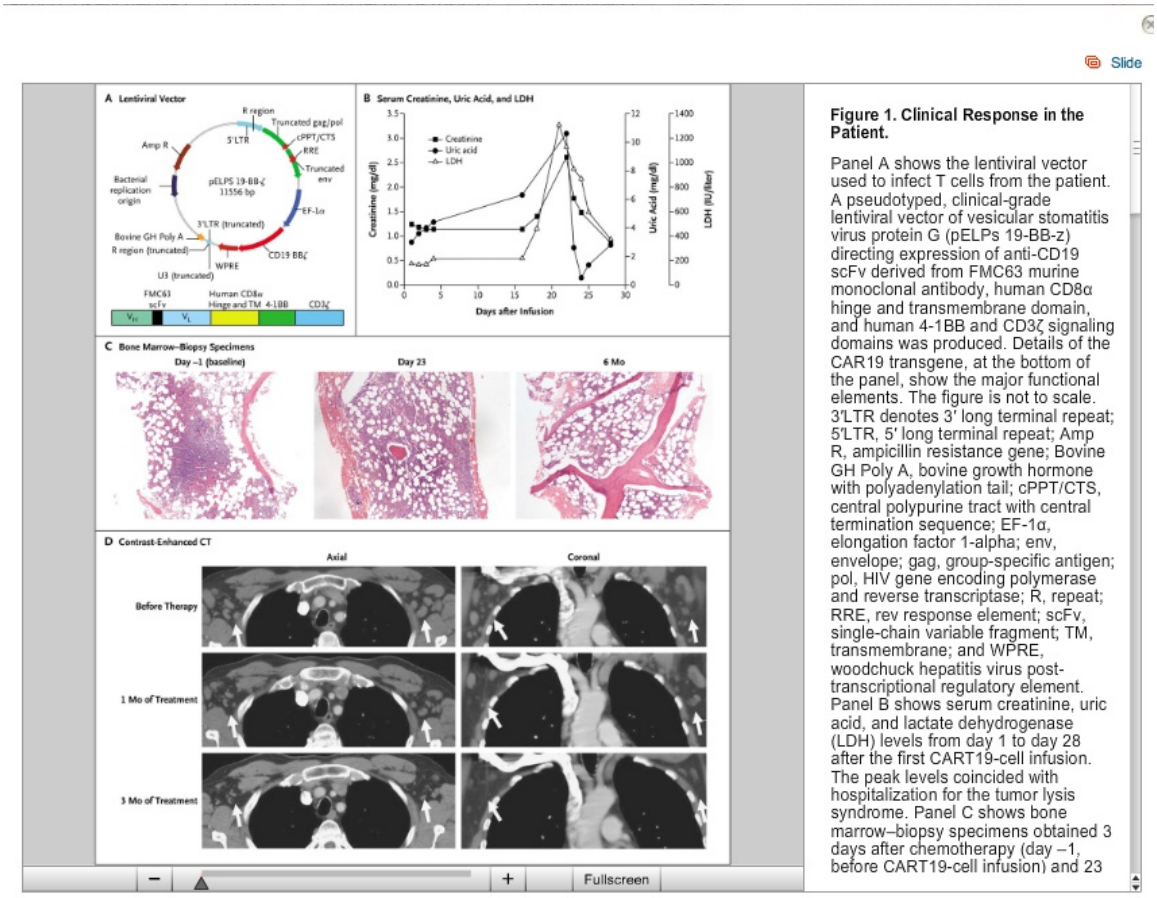


Figure 1. Clinical Response in the Patient.

Panel A shows the lentiviral vector used to infect T cells from the patient. A pseudotyped, clinical-grade lentiviral vector of vesicular stomatitis virus protein G (pELPs 19-BB-z) directing expression of anti-CD19 scFv derived from FMC63 murine monoclonal antibody, human CD8α hinge and transmembrane domain, and human 4-1BB and CD3ζ signaling domains was produced. Details of the CAR19 transgene, at the bottom of the panel, show the major functional elements. The figure is not to scale. 3′LTR denotes 3′ long terminal repeat; 5′LTR, 5′ long terminal repeat; Amp R, ampicillin resistance gene; Bovine GH Poly A, bovine growth hormone with polyadenylation tail; cPPT/CTS, central polypurine tract with central termination sequence; EF-1α, elongation factor 1-alpha; env, envelope; gag, group-specific antigen; pol, HIV gene encoding polymerase and reverse transcriptase; R, repeat; RRE, rev response element; scFv, single-chain variable fragment; TM, transmembrane; and WPRE, woodchuck hepatitis virus post-transcriptional regulatory element. Panel B shows serum creatinine, uric acid, and lactate dehydrogenase (LDH) levels from day 1 to day 28 after the first CART19-cell infusion. The peak levels coincided with hospitalization for the tumor lysis syndrome. Panel C shows bone marrow–biopsy specimens obtained 3 days after chemotherapy (day −1, before CART19-cell infusion) and 23

Conclusions: Ethical Concerns?

From a personal perspective, I feel gene therapy can have a promising future if we can control the risks and problems associated with it. However, there are more pressing ethical concerns that need to be taken into consideration. As mentioned before, there are two approaches to gene therapy: somatic cell line and germ cell line. If mutations are introduced in somatic cells, they are limited to the patient in which the cells reside. However, if the mutations are introduced to germ cells, these mutations could carry on

into the generations. As seen in the “knock-out mouse” nobel prize winning experiment, future generations could express mutations that occurred in the germ cells of the previous generation. This causes concern for many because the question becomes this: how ethical is it to mutate your genome and introduce the possibility of other mutations occurring. Some are against the idea of tampering with embryonic cells in the first place. Others, however, are also against the implications of gene therapy: now we have yet another way we can select the genes that we want our offspring to have, making the creation of human beings a robotic and controllable rather than a natural process. Furthermore, animal testing is needed in order to test the risk of gene therapy. This also brings into question the extent to which we should be allowed to use mice and other animals in such genetic experiments. These are indeed some questions to think about when studying genomics, gene therapy, and its implications for the future.

http://www.nejm.org/doi/pdf/10.1056/NEJM198011273032210