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# Gene Therapy: An Overview of Approaches and Issues

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## An Overview of Approaches and Issues

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*For over 25 years, scientists have been researching and testing gene therapy techniques, but this work has only resulted in a single FDA-approved therapy: Kymriah™ from Novartis. Kymriah™ was approved in the U.S. in August 2017, and genetically modifies a patient's own immune cells to seek out and destroy abnormal blood cells. Although clinical trials continue to test and refine different gene therapy approaches, understanding and evaluating the risks associated with treatment may be overwhelming to patients and caregivers alike. This article attempts to provide readers with an introduction to gene therapy so that anyone considering treatment or caring for someone that is being treated can better understand how gene therapies works, and some of the issues we may have to deal with before gene therapy can become more common.*

## Overview

Gene therapy is still considered an experimental technique (NIH 2017). For some patients, gene therapy represents a last shot at a potential cure for a debilitating or life-threatening disease. For others, gene therapy has caused additional complications and even death. The first gene therapy treatment was approved for commercial distribution in the U.S. in August 2017, which may open the door for other treatments to obtain approval as well (FDA 2017). Patients, healthcare providers, and supporting individuals and organizations need to better understand the technology, underlying science, and associated risks in order to make decisions and take appropriate actions before, during and after treatment. This article provides an overview of current gene therapy methods and discusses associated issues, in order to assist people in assessing treatment options.

## Gene therapy defined

The National Institutes of Health (2017) defines gene therapy as "an experimental technique that uses genes to treat or prevent disease," while the American Society of Gene and Cell Therapy (2017) defines gene therapy as an "approach to treating disease by either modifying the expressions of an individual's genes or correction of abnormal genes." Although both definitions broadly state that gene therapy involves manipulating genes, the ASGT definition includes the statement that genetic ex-

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pression may be modified, rather than the gene itself. In addition, the definition of "gene" has undergone multiple revisions and alterations over time. Currently, NIH (2017) defines a gene as "the basic physical and functional unit of heredity." Much of the high-level discussion of genes revolves around protein products; however, most of the human genome is categorized as "noncoding," which is to say, no protein product or RNA transcript is produced. Noncoding regions (especially those that are highly conserved) have a crucial role in regulating gene expression and cell cycles (Hardison 2000, 369). Gene therapy can involve inserting new genetic sequences into one's cells, correcting existing ones, or regulating the activity of a specific sequence. It is important to understand which situation applies to one's treatment, because each approach comes with a unique set of considerations and consequences.

## Gene therapy approaches

The most effective approach to treatment will depend on a number of factors, including disease type, progression, age, and patient condition. Treatment may be applied directly to the body (in-vivo), or to extracted cells (ex-vivo) which are then reintroduced into the body. One example of ex-vivo therapy is Kymriah™ from Novartis, which utilizes cells extracted from the patient and genetically engineered to form Chimeric Antigen Receptor (CAR) T Cells (FDA 2017). Kymriah™ (tisagenlecleucel) is the first gene therapy product approved by the FDA. The treatment itself may consist of DNA or RNA, and can be delivered to cells via viral or non-viral vectors. Each delivery method has its strengths and weaknesses, and human immune reactivity is an especially important consideration (Lee 2014, 188).

A number of different viral vectors are utilized to deliver genetic information, and each has its own strengths. A retroviral vector contains an RNA payload; once it enters cells, the RNA must be converted to DNA before being inserted into the genome, generally in a random location. This can cause additional problems by disrupting functional genes. Unlike retroviruses, an adenoviral vector infects both replicating and non-replicating cells equally well, and does not integrate into the host cell genome. Adeno-associated viruses integrate into a specific location of the genome (>95%) and have the added benefit of being less prone to immune responses, unlike retroviruses and adenoviruses. However, adeno-associated viruses cannot self-replicate and require "helper-viruses" for this function. The Herpes Simplex Virus (HSV) only targets the nervous system but can carry two to three times the payload of other virus types. Non-viral vectors such as liposomes and naked DNA do not have a maximum payload size, but are far less effective at entering and integrating into the genome. Liposomes have the added drawback of potentially triggering an immune response as well (GSLC 2012).

Some medical conditions demand systemic (whole body) treatment, which generally entails the introduction of substances into the blood. All cells in the body require the blood's function of maintaining the cellular environment via gas and nutrient exchange (Pitman 2011), so blood infusion can be an effective way to reach all cells. In other cases, single tissue or organ types may be targeted for treatment. Furthermore, germline editing may be required, so that future offspring do not inherit a genetic disorder or a predisposition to conditions such as autism, Alzheimer's, and schizophrenia.

## Gene editing technologies

After the DNA or RNA payload is delivered, the subsequent action of the genetic material must be carried out. One approach involves nucleases, proteins that can cut DNA, which seek out and edit specific locations of the patient genome. Zinc Finger Nucleases (ZFN) and Transcription Activator-Like Effector Nucleases (TALENs) are two types of nucleases that are commonly utilized. CRISPR-Cas is a gene editing technique originally discovered in bacteria that has gained much attention recently, due to its specificity and far-reaching applicability.

### CRISPR-Cas

Clustered Regularly Interspaced Palindromic Repeats are effectively DNA memory banks that bacteria use as part of an organism's immune system against viral invaders (Barrangou et al. 2007, 1709). In 2015, Chinese scientists reported that the CRISPR-Cas editing mechanism could be used in humans, as they demonstrated by successfully editing the HBB (β-globin) gene (Liang et al. 2015, 363). This experiment has clinical significance because a mutated form of HBB is largely responsible for β-thalassemia (Hill et al. 1962), a rare but potentially life-threatening condition characterized by non-functioning hemoglobin in red blood cells affecting 1 in 100,000 people worldwide (NORD 2017). Liang (2015) concluded that although the CRISPR-Cas mechanism successfully targeted and edited the HBB gene in human tripronuclear zygotes (3PN, one egg fertilized by two sperm; byproducts of in-vitro fertilization that are usually discarded), much work has to be done to increase the efficiency of homologous recombination directed repair (HDR) resulting from Cas-mediated double-stranded breaks (DSBs). Because DSBs stimulate the cell's internal HDR machinery, homologous strands of DNA are introduced into the cell to be used as repair templates. However, in Liang's case, HBD, a gene similar to HBB, competed with the exogenous DNA during HDR, causing unwanted repair products. Although Liang's experiment caused an uproar over using human zygotes for testing (3PN as they were), it was not long before other scientists followed suit (Vog 2017).

## Gene therapy issues

Gene therapy presents an exciting and positive new medical development in its potential to effectively cure genetic diseases, as well as prevent future generations from inheriting fatal or debilitating mutations. However, as the commercial release of Kymriah™ and other gene products has demonstrated, we are now faced with the exorbitant price of treatment costs: $475,000 USD in the case of Kymriah™. Also, due to licensing, patent, and other restrictions, treatment may only be available in a select few locations, forcing some patients to cross one or more geographic, political and cultural boundaries. Lastly, the underlying science is not fully understood, which is not particularly new as far as science and medicine are concerned, but patients and medical institutions must have an adequate understanding of the technology in order to assess the short and long-term risks involved with treatment.

### Cost

Glybera, the first genetic therapy treatment made available in Europe was over €1,000,000 Euros (Burger and Hirschler 2014), causing frustration for patients and doctors attempting to acquire the treatment. Kymriah™, the first gene therapy approved in the U.S., carries a $475,000 USD price tag. As regulators, insurance providers, doctors, pharmaceutical companies, and patients struggle to negotiate the terms of payment, a number of potential payment schemes are being analyzed. One payment scheme that seems to be gaining in popularity allows for yearly payments as long as the therapy continues to provide a positive benefit. Although this scheme may lessen the initial financial burden, it is conceivable that some patients may end up having to pay for gene therapy indefinitely, even though they are no longer receiving treatment.

### Availability

A search on *clinicaltrials.gov* returns a number of organizations currently recruiting or conducting clinical trials for a wide range of genetically-affected conditions, including cancer. However, the selection criteria can be extremely prohibitive, and obtaining regulatory approval is an arduous, multi-year process. Obtaining gene therapy is not simple, even if the treatment is publicly available. In Glybera's case, only one patient was treated before the product was turned over to a third party. Some countries in the European Union would not cover the cost of treatment, effectively placing the treatment out of reach for their citizens. As more and more gene therapies are approved for use, companies will need to tackle availability issues on a global scale. Regulatory agencies and politicians should also be coordinating internationally so that the citizens they represent are afforded the opportunities to obtain life-saving treatments.

### Risk

Verma (2000) reported that over the previous ten-year period, more than 400 gene therapy clinical trials were conducted, involving over 4000 participants. Only one patient death occurred due to the gene therapy treatment being given to the patient: Jesse Gelsinger, an 18-year-old participant in a trial conducted by the University of Pennsylvania. A search for trials on *clinicaltrials.gov* during the period between 2000 and 2017 returned over 1500 trials with over 315,000 participants. With the explosion of novel gene therapy discoveries and associated clinical trials, there is still cause for concern over whether participants are properly informed before giving consent. In the Gelsinger case, the family's lawyers charged that serious adverse effects were experienced by other patients, and three monkeys had died of complications after being injected (Sibbald 2001). None of this information was provided to Gelsinger, although it was reported to NIH.

As more therapy options and trials are introduced, it is critical for participants to obtain all relevant information related to the risks involved, including potential side effects and adverse events from previous studies. In addition, we should be cognizant of the fact that all trials are endeavors to prove that the therapy under investigation is safe and effective at certain dosages. Adverse events are expected, and not all will be mitigated in time.

## Conclusion

Although gene therapy has been investigated and tested for over twenty-five years, there is still much to be learned, and the gene delivery and editing technologies currently available remain experimental. Among other threats, viral vectors can produce a fatal immune response, and CRISPR-Cas editing produces off-target DNA breaks and unwanted homologous repairs. In addition to short-term effects, the long-term effects of DNA manipulation have yet to be discovered. These risks, along with socioeconomic factors, need to be confronted by doctors, pharmaceutical companies, insurance organizations, and governments as well as patients.

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