Commentary

Gene Therapy: Recent Advancement and Challenges

Comment [A1]: Review of literature

Abstract

Background: Gene therapy involves delivering therapeutic genomic material to a target tissue to modify expression of a protein or induce other characteristic changes. Recent advancements in the field, including FDA-approval of numerous gene therapies, are paving the way for future technological progress. There are two types of gene therapy, somatic and germline, but most research and drug development has been for somatic cells. In this article, we discuss the recent advancements and challenges associated with gene therapy.

Main text: There are multiple types of gene therapies, including hematopoietic stem cell therapy, CAR-T cell therapy, and Crispr/Cas9 gene therapy. Rare diseases and cancers are being researched to determine methods of treatment using gene therapy, and several clinical trials have been performed within the last decade to test the efficacy of new therapeutic drugs, many of them at least somewhat successful. However, there do exist some challenges associated with gene therapy, including large-scale production of vectors, precision of gene delivery to target tissue, and most importantly, immune responses of patients.

Conclusions: The near future is an exciting time for new gene therapy technologies and strategies. Researchers and patients can look forward to new advancements in base editing, prime editing, and RNA-targeted editing technologies. Furthermore, future research can focus on new genetic targets, such as genes whose functions may still be unknown and epigenomic elements.

Keywords: gene therapy, stem cell therapy, rare diseases, gene editing

Background

Gene therapy is breaking barriers in medicine and changing how scientists and medical professionals approach diseases previously thought to be incurable [1-3]. While still a relatively new field, much progress has been made with the research and development of numerous oligonucleotide-based therapies, autologous cell therapies, and in vivo gene therapies, and the FDA approval of over 20 gene therapies [4]. Yet, gene therapy remains a complex subject with many factors to consider, including which cells to target, hurdles in delivering the gene, and efficacy of treatment. Diseases and their genetic links need to be completely understood, a way to effectively distribute gene copies to the cells must be available, and specific cells that need treatment have to be identified and accessible. Currently, gene therapy is divided into two categories. First is somatic cell gene therapy, in which the therapeutic gene is transferred into somatic cells of a patient. The modification is restricted to the treated individual and will not pass on to progeny [5]. Second is germline gene therapy, in which primordial germ cells are modified by inserting a functional gene into the genome, so that modification is hereditary. Most gene therapy research so far has focused on treating individuals through somatic cell gene therapy [6].

As directly inserting the gene of interest into target cells would not result in expression of the gene, a carrier called a vector is engineered to deliver the gene. Frequently, certain modified viruses are used as vectors because they can deliver the gene through infection [7-9]. For example, retroviruses integrate their genetic material (such as the new gene) into the target cell's genome, whereas adenoviruses introduce their DNA into the nucleus of the cell but do not integrate it into a chromosome. There are two ways of administering gene therapy (**Figure 1**). The vector can be given directly into a specific tissue, where it is taken up by individual cells.

Alternatively, a sample of the patient's cells can be removed, and the vector can be administered to those cells *ex vivo*. The modified cells are grown and then transplanted back into the patient.

Main Body

There are various methods of gene therapy. Due to their longevity and the capacity for self-renewal, hematopoietic stem cells have been used as targets for gene transfer. Gene manipulation of stem cells could create induced pluripotent stem cells (iPSCs), which, when reinjected into the patient, will continue to self-renew and give rise to many types of cells with different phenotypes. For example, patients infected with hepatitis B or C may require a liver transplant and may need transplantation of mature hepatocytes or differentiated iPSCs. Gene manipulation can not only convert stem cells to hepatocytes, but because those cells may still be susceptible to reinfection by hepatitis, transferring a vector that encodes a hairpin RNA directed against the virus would provide those cells with resistance from reinfection [10].

CAR-T (chimeric antigen receptor T cell) therapy is a type of immunotherapy that involves manipulation of T cells so they can better recognize and attack tumor cells [11-13]. T cells are collected from a patient, and a viral vector is used to introduce a gene encoding a chimeric antigen receptor (CAR) which is a surface protein [14]. These new T cells are grown *ex vivo* and then infused back into the patient. The CARs allow the cells to attach to specific antigens on tumor cells and destroy them. However, there are certain disadvantages to this approach. CAR-T cells may sometimes identify non-tumor cells that express the target epitope. Additionally, cytokine release syndrome (CRS) can occur, in which CAR-T infusion can induce activation of the immune system, leading to a rapid increase in the levels of inflammatory/damaging cytokines [10].

CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated 9) is an incredibly versatile and useful technique for gene editing. Originating in the adaptive immune system of prokaryotes, the CRISPR technique was developed to enable editing of target-specific DNA sequences of the genome of any organism. A guide RNA strand guides the Cas9 nuclease to the target dsDNA to cleave it. If reparation occurs in the presence of donor DNA, the result is integration of a new gene into the genome. The first example of the success of CRISPR-based gene therapy was shown in clinical trials of gene editing for sickle cell disease and beta-thalassemia [10].

Gene therapy holds promise to treat a wide range of diseases. The various conditions amenable to gene therapy, their symptoms, and gene therapy options available or under development have been summarized in **Table 1**.

There are a number of challenges that must be surmounted when developing gene therapies. The vector needs to be very specific to the target cell, not induce an allergic reaction or inflammatory immune response, and be purified in large quantities and high concentrations so that it can be produced and made available on a large scale. The vector should be capable of expressing the gene for the patient's entire life. The presence of viral genetic material in plasmid can induce an acute immune response and/or a possible oncogenic transformation, so it is a necessity that the new gene is inserted safely and is precisely controlled by the body. There also is a need to find better ways to deliver genes and target them to particular cells. Altered viral vectors may infect cells other than the ones containing mutated genes. This can damage healthy cells, causing other diseases. However, the largest obstacle to the success of gene therapy is human immune response to viral vectors—up to 50% of patients are excluded from AAV-based therapies because of pre-existing resistance to the viral capsids. Some recent technological

advances—such as modified AAV capsids that evade pre-existing neutralizing antibodies, temporary removal of those antibodies from circulation, and/or methods of immunosuppression—have allowed researchers to bypass the problems of pre-existing or adaptive immunity to the vector. This may allow for redosing of the therapy. Another solution may emerge through using non-viral nanoparticles for gene delivery. Aside from the advantage nanoparticles may have in evading detection by the immune system, which restricts viral vector delivery, they may also be uniquely suitable for tissue targeting for in vivo gene transfer [23-25].

Conclusions

With the ongoing rate of technological and biological innovation, the future holds great promise for gene therapy as a topic for research and for ailing patients. The advent of base editing and prime editing have allowed for very specific alterations of genomic sequences without the need for possibly dangerous double-stranded DNA breaks and cellular DNA repair pathway. RNA-targeted editing technologies allow for temporary and reversible changes to gene expression without permanently changing the genome. This is potentially safer and more efficient than alternatives. Epigenome editing technologies are also promising in that they are adjustable, reversible, and may cause heritable improvements. Understanding the functions of more genes and regulatory elements in the genome can also aid scientists in homing in on specific genetic targets. The function of around 6,000 of the over 20,000 human genes is currently unknown. Furthermore, instead of focusing on the genes themselves, more research can be done on non-coding regions of DNA, which account for roughly 98% of the human genome and hold epigenetic regulators that are responsible for over 90% of susceptibility for common diseases [23]. Editing a gene regulatory element to alter gene expression, rather than directly

editing the genetic mutation itself, can be a very useful strategy. While some of these new ideas may be in early stages and gene therapy does pose unique challenges, recent advancements have certainly informed the future of the field. With a wide array of new potential technologies and techniques on the horizon, the possibilities for gene therapy are seemingly endless.

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Table 1: A summary of disorders amenable to gene therapy

Disease	Description/Symptoms	Trials/Therapy	References
Adenosine Deaminase Severe Combined Immune Deficiency (ADA-SCID)	- Caused by genetic defect in ADA gene that causes a person to have no B and/or T lymphocytes - Children are born without an effective immune system and will die without bone marrow transplantation - High risk of developing fatal viral, bacterial, and fungal infections and certain types of cancers	- Single infusion of autologous gene-corrected bone marrow HSCs - 18 ADA-SCID children were treated, survival was 100%, and the majority of patients demonstrated long-term gene correction in T cells - No noticeable side effects in treated patients after immune system reconstitution	(Aiuti A et al., 2017) [15]
Chronic Granulomatous Disorder (CGD)	- Certain white blood cells (phagocytes such as neutrophils and monocytes) don't work properly - Infections in various organs such as lungs (pneumonia is common), skin, lymph nodes, liver, stomach, and intestines	- Five phase I/II clinical trials were performed with 12 patients total using retroviral vectors to deliver functional genes - Patients experienced clinical benefit with resistance to microbial infections However, efficacy progressively decreased with time in all patients, and several patients developed myelodysplastic syndrome (MDS), a cancer in which immature blood cells in the bone marrow do not mature	(Arnold and Heimall, 2017) [16]
Hemophilia	- Patients are not able to form blood clots - Hemophilia A: FVIII protein deficiency - Hemophilia B: FIX protein deficiency - Suffer from potentially fatal external and internal bleeding	- In the first clinical trial for hemophilia A, an AAV (adeno-associated virus) vector was used to transfer a correct gene to the liver. Patients in the highest-dose cohort saw an increase in FVIII levels and a significant reduction in	(Perrin et al., 2019) [17]

		annual bleeding rate. In a different trial, at the highest vector dose, patients saw higher levels of FVIII expression but also toxicities. - Hemophilia B trials similarly used AAVs, but success was more moderate and there was a recurring pattern of transient transaminitis (high levels of transaminase enzymes) in treated patients	
Leber's congenital amaurosis (LCA)	- Rare inherited eye disease associated with nystagmus, slow pupillary response, or blindness	- Ongoing trials show subretinal injection of AAV vectors encoding the correct gene are safe and efficacious for at least 1.5 years after treatment	(Simonelli, 2010) (Chiu et al., 2021) [18,19]
Parkinson's Disease	- Tremors, difficulty with walking and moving - Dopamine deficit - Build up of misfolded proteins in neurons	- Several approaches have been tested - Expression of the growth factor neurturin, a homolog of GDNF (glial derived neurotrophic factor), to try to prevent damage - Expression of the enzyme GAD (glutamate decarboxylase), which produces GABA (gamma-aminobutyric acid), an inhibitory neurotransmitter that reduces neuronal stimulation - Expression of tyrosine hydroxylase (the enzyme that produces L-DOPA, a precursor to dopamine) - Clinical trials demonstrate safety and moderate efficacy, but have not gone past phase II	(Sudhakar and Richardson, 2019) [20]

Huntington's Disease	- Mutation in huntingtin gene leads to build up of toxic huntingtin (mHtt) protein - Problems with mood and cognitive abilities (can decline into dementia) - Motor skill problems, including gait and coordination - Movement disorder called chorea	- Preclinical gene therapy strategies focused on using RNAi to suppress levels of mHtt by targeting and destroying mHtt mRNA (clinical trials began in 2019) - Gene splicing techniques, such as CRISPR/Cas9, are being researched further to directly fix the faulty gene	(Sudhakar and Richardson, 2019) [20]
Alzheimer's Disease	- Degeneration of cholinergic neurons - Problems with mood and language, disorientation - Cognitive decline leads into dementia	- Studies in non-human primates showed nerve growth factor (NGF) improved function of cholinergic neurons - Phase I and II clinical trials were done using AAVs to deliver the NGF gene intracerebrally, but there was no significant difference between the treatment and placebo groups	(Sudhakar and Richardson, 2019) [20]
Mesothelioma	- Rare cancer caused by asbestos exposure that usually affects the lining of the lungs or abdomen	- Clinical trial are focused on delivering an interferon (which has anti-tumor effects) gene via a genetically engineered adenovirus - Results show the therapy is safe and overall survival rate is higher when compared with historical controls (Sterman et al., 2016)	(Sterman, 2016) [21]
Other cancers	- Uncontrolled growth and spread of unregulated, abnormal cells in the body	- Clinical trials with various gene therapy strategies including suicide gene therapy, oncolytic virotherapy, anti-	(Roma-Rodrigues, 2020) [22]

Comment [A2]: Why did you add sickle cell anemia?

		angiogenesis, therapeutic gene vaccines, and more	

Figure 1

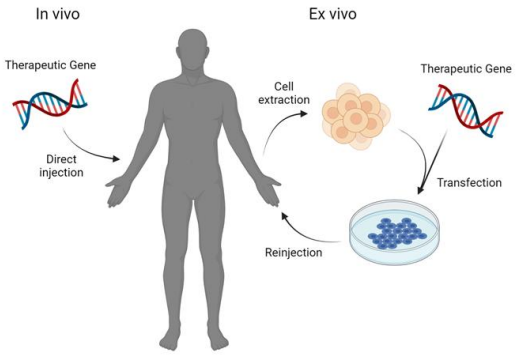

Figure 1: A schematic representation of *in vivo* versus *ex vivo* gene therapy administration.

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