Listicle

Gene Therapy – Insights Into Current Applications and Future Prospects

By Neeta Ratanghayra

Gene therapy is the administration of genetic material to modify or manipulate the expression of a gene product or to alter the biological properties of living cells for therapeutic use. The advent of next-generation technologies has led to a revival in gene therapy research with path-breaking therapies approved after years of hard work.[1]

Several *in vivo* gene therapies, oligonucleotide-based therapies and cell therapies are now approved and available. These therapies treat diverse disorders such as inherited blindness, neuromuscular disease and cancer.[2] More than 4000 gene therapy clinical trials are in various phases of development with majority of the trials focused on somatic-alteration diseases, mainly cancer.[3]

This listicle will explore the different approaches for developing gene therapies and the latest applications of gene therapies in modern medicine.

In vivo and *ex vivo* approaches

The two main approaches used in clinical gene therapy are direct *in vivo* administration and *ex vivo* gene therapy. *In vivo* gene therapy involves the direct introduction of a vector containing the therapeutic genetic material into the patient. In the *ex vivo* approach, the patient's cells are taken out of the body and then transduced by a vector in culture to incorporate the therapeutic gene. The gene-modified cells are then transplanted back into the patient.[4]

Both approaches utilize a vector to deliver the genetic material, but there are different types of vectors to choose from. Adenoviral vectors are the mainstay of *in vivo* strategies owing to their high transfection efficiency. However, problems relating to antigenicity and short-term expression of transgenes can restrict their use.

Last-generation adenovirus vectors, or gutless adenovirus, can be used in place of the traditional adenoviral vector as the associated *in vivo* immune response is highly reduced compared to first- and second-generation adenovirus vectors. Last-generation adenovirus vectors are also associated with high transduction efficiency and tropism.[5]

Lentiviral vectors are another extensively used gene delivery vectors. Lentiviral vectors can integrate into both dividing and nondividing cells. They also enable long term expression of transgenes and lack antigenicity, which makes them a unique and ideal tool.[6]

Ex vivo approaches offer the advantage of fully characterizing the genetically modified cells and selecting them for desired phenotypes before using them for therapy. The property of the cells may also be chosen to enhance the therapeutic potential.

Gene therapy techniques

1. Gene replacement therapy

In gene replacement therapy, a fully functioning gene is introduced to replace a mutated gene either directly *in vivo* or through *ex vivo* cell therapy.[7] Gene replacement therapy is a viable strategy that can be used in monogenic diseases resulting from a single defective gene on the autosomes. It has been used in diseases such as cystic fibrosis, hemophilia and spinal muscular atrophy.

2. Gene addition for complex disorders and infectious diseases

Gene replacement is not feasible for disorders caused by the combinatorial effect of multiple genes and environmental factors. These include disorders such as cancer and heart disease as well as infectious diseases. In such complex disorders, gene addition can be used. Gene addition involves incorporating functional copies of a gene to address the underlying genetic cause. Gene addition strategies in complex disorders require a deep understanding of the disease mechanisms.[8]

Gene addition strategies have shown promising results in several cancer clinical studies. One such example is the use of replication-competent herpes simplex virus vectors that replicate specifically in actively dividing tumor cells.[9]

3. Gene knockdown by RNA interference

Gain-of-toxicity mutations lead to the production of toxic gene products that require gene knockdown strategies to treat the disorder. The selective silencing of target genes in specific cell types by RNA interference (RNAi) is a powerful approach to tackle such mutations.[10]

RNAi is a biological process by which double-stranded RNA induces sequence-specific gene silencing by targeting mRNA for degradation. RNAi has been widely used as a tool for knocking down the expression of individual genes post-transcriptionally.[11] The first RNAi based therapy was approved in 2018, nearly 20 years after the researchers first discovered the technique.[12]

4. Gene editing to introduce targeted changes in the host genome

Genome editing technologies, via engineered or bacterial nucleases, have paved the way to directly targeting and modifying genomic sequences. Genome editing can be achieved *in vitro* or *in vivo* by delivering the gene-editing machinery in situ, which can add, ablates and "corrects" genes as well as perform other highly targeted genomic modifications. Genome editing tools such as zinc-finger nucleases (ZNFs), TALENs, and CRISPR/Cas9 are promising tools that are being used to manipulate genomes with unprecedented precision.[13]

Challenges with gene therapy

1. Immune response to gene delivery vectors and products of foreign transgenes

Immune response to gene delivery vectors and products of foreign transgenes is a major obstacle in gene therapy. The engineering of modified AAV capsids that evade pre-existing neutralizing antibodies, methods for temporary clearing of antibodies from circulation and immunosuppression regimens are some possible ways to deal with the immune response.[2]

2. Inefficient gene transfer and lack of viral specificity

Inefficient gene transfer by viral vectors is another challenge. Even vectors with very high transduction efficiency *in vitro* can fail to produce significant infection rates when applied to clinical trials.[6,14]

Another limitation is the lack of viral specificity, which may lead to cells in the vicinity of the target cells being infected. The use of tissue-specific promoters has partially addressed this issue, however, tissue-specific promoters cannot be applied to *all* disease states. There may also be problems with promoter "leakage" from endogenous viral sequences.

3. Issues with controlled gene expression

Controlled gene expression is another overarching issue. For gene therapy to succeed as a strategy, it is important that the gene is correctly regulated. The ability to switch transgenes on and off is critical not only when the therapy is no longer needed but also in the case of the development of adverse side effects to the treatment.[15]

4. Safety issues

Administration of a virus can result in inflammation or active infection. The administration of retrovirus, which incorporates randomly into the genome, can also result in insertional mutagenesis and malignant transformation.[6]

Future prospects

During the last five years, gene therapy has experienced substantial progress for a multitude of indications. Among the clinical trials conducted or currently in progress, most trials have focused on cancer. The other indications include monogenic, infectious and cardiovascular diseases.[16]

Approved therapies lay a foundation for future treatments

Approved gene therapies form the basis on which treatments for many other conditions can be developed.[2] For example, the success of *in vivo* AAV gene transfer to the human retina and central nervous system has facilitated the development of AAV-based therapies to treat hemophilia.[17] Likewise, early technology development in *ex vivo* lentiviral and retroviral gene transfer to T cells has enabled therapy development for sickle cell disease[18] and beta-thalassemia.[19]

Non-viral nanoparticles for gene delivery

Substantial progress has been made in the engineering and profiling of non-viral nanoparticles for gene delivery. Lipid-based nanoparticles, quantum dots, carbon nanotubes, magnetic nanoparticles, silica nanoparticles and polymer-based nanoparticles are some non-viral, multifunctional

nanoparticles explored in gene therapy. Nanoparticles are being studied in gene delivery strategies such as combinational gene therapy, image-guided gene delivery, optically-trackable and optically-activated gene therapy.[20]

Nanoparticles offer the potential to circumvent detection by the immune system. They also enhance gene stability, enable protection from nuclease degradation and allow improved targeting.

Next-generation editing technologies

To improve success with gene therapy, several next-generation editing technologies are being explored. These techniques are seen to improve specificity, accuracy, efficiency, and applicability to different classes of disease.

- Base editing and prime editing have enabled the precise alteration of genomic sequences in the absence of DNA breaks and without the reliance on the activity of endogenous DNA repair pathways.[21]

- RNA editing involves targeting disease-linked mutations in RNA. This enables alterations in gene expression without necessitating permanent changes to genomic sequences; a relatively safe and efficient option.[22]

Another innovation is the use of epigenome editing technologies. The epigenome is dysfunctional in many diseases and disorders. Thus, the ability to precisely edit the epigenome holds promise for enhancing our understanding of how epigenetic modifications function and enabling the manipulation of cell phenotypes for research or therapeutic purposes.

Functional genomics

Another field that will dramatically impact the space of gene therapy is functional genomics. Studies and therapeutic interventions have focused almost exclusively on genes. However, ~ 98% of our genome comprises non-coding DNA that harbors epigenetic regulators responsible for > 90% of susceptibility to common disease. Exploring the non-coding component of the genome will pave the way to a whole new area of disease biology and therapeutic target discovery.[2]

The promise of gene therapy

Gene therapy offers great promise in treating disease for which ineffective modalities exist, or no options are available. The traditional strategies to deliver genetic material are highly variable, and innovative, insightful techniques continue to emerge. The field is progressing rapidly, and varied approaches are being studied in several clinical trials for hard-to-treat disease, including rare genetic conditions.

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