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Fundamentals of GENE THERAPY:

ADDRESSING GAPS IN PHYSICIAN EDUCATION

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Gene therapy is a contemporary therapeutic intervention with recent positive results and regulatory approvals either completed or expected in the next several years for various conditions. The evolving view is that gene therapy will ultimately offer hope across a range of otherwise debilitating or difficult-to-treat conditions. We may soon see several new therapies approved in the United States and Europe for hemophilia and central nervous system disorders.[1,2] With gene therapy entering the clinics, physicians will be tasked with educating and counseling patients on opportunities for participation in clinical trials and adoption of approved gene therapies for treatment of rare diseases. Yet, two recent publications from the hematology space revealed significant deficits in physicians' self-reported and tested knowledge of gene therapy.[3,4] With the rapid evolution of gene therapy potentially outpacing medical curricula, education gaps may persist across other specialties as well. Thus, the purpose of this review is to summarize the general concepts of gene therapy with a specific focus on monogenic rare disease in hematology and central nervous system disorders where burgeoning therapies are currently entering clinical investigations and approaching regulatory approval.

Basic principles of gene therapy: augmentation vs suppression vs editing

Gene therapy, in the broadest sense, is the introduction of foreign genetic material into a cell with therapeutic intent. Augmentation is the process of introducing a working copy of a missing or dysfunctional gene, typically through a viral carrier (vector). In some genetic disorders where a mutation causes an excess of encoded protein, such as in hereditary transthyretin amyloidosis or Huntington's disease (HD), gene therapy can be used to "silence," or suppress the resultant gain-of-function using RNA interference (RNAi).[1,5-8]

Genes introduced into cells (transgenes) may either integrate into the host genome or remain separate (episomal). Vectors are utilized to carry both the transgene and a promotor sequence that drives gene expression, augmenting proper function of the transgene in the appropriate target tissue. Concerns of toxicities due to over-correction can be addressed through engineering of the promoter sequence.[2,9] Integration enables transferred genes to be copied and passed on to daughter cells during division ensuring a more durable, if not permanent change. But the potential for random insertion may lead to mutagenic disruptions, including malignancy. In contrast, non-integrating or episomal transgenes can be lost during cell division, and expression of the transgene can decline with cellular turnover.[6] Episomal loss will vary by tissue, and may not be ideal for dividing target tissues or for patient populations where cell turnover is more rapid during growth and development, such as children.[9,10] Despite this potential limitation, a recent report from a clinical trial in hemophilia B has demonstrated sustained efficacy (increased factor IX [FIX] activity) for up to 8 years after episomal gene augmentation therapy.[11]

For single gene disorders, a model approach would be to edit or replace the defective gene. Recent technologies such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats, with associated protein 9 (CRISPR/Cas9) may allow such an approach.[1,12] Joined with nucleases to break the double stranded DNA, ZFN and TALENS rely on engineered proteins that look for specific genetic sequences. CRISPR/Cas9, on the other hand, employs RNA as a guide to target complementary base pairs, with Cas9 creating the double strand break. Natural repair of the broken ends does occur, sometimes introducing new base pairs that damage the gene. With the above mentioned technologies, donor DNA can be inserted to facilitate a repair that silences or edits a defective gene. Once the genetic material is exposed, the target gene can be deactivated, or corrected using a vector to insert new genetic code.[2,12]

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Aside from gene repair and editing, these tools can direct transgene integration into "safe harbor" sites in the genome, effectively providing the opportunity for stable long-term expression in high-transcription locations where integration would not be predicted to have a deleterious effect.[7,13]

CRISPR/Cas9 offers the advantage of being more cost-effective but will require significant fine-tuning to avoid or limit previously observed off-target mutagensis.[2,12]

In vivo vs *ex vivo* methods of gene transfer

Cells isolated from patients can be genetically modified in culture, expanded, and then returned to the patient. This *ex vivo* approach enables transfection conditions to be controlled and optimized. In addition, unmodified cells can be eliminated prior to transplantation. Effective return of modified cells to the body can, however, be difficult. For transduced blood or hematopoietic stem cells (HSCs) there is little problem as these can be returned directly to the bloodstream, although the patient will require conditioning chemotherapy to allow the transduced cells to engraft. However, it is necessary for other cells to have an appropriate support matrix and vascular supply. Site of implantation would be a major determinant in providing the right local environment for the effective function of transplanted cells.[2,7,14]

Alternatively, modification of cells within the body (*in vivo* modification) minimizes problems associated with transplanting cells. It provides the advantage of apparent simplicity, ease of use, and cost effectiveness. Ideally, it would involve a one-time injection of a vector containing the appropriate genetic material into the bloodstream or targeting tissues that need to be modified. To be functional, *in vivo* systems need to be efficient, not inactivated by pre-existing host antibodies or an induced cellular immune response, and, if injected systemically, targeted to specific tissues. Any host immune response that develops on exposure to the vector itself may also preclude vector readministration. Inadvertent germline gene transfer cannot be excluded after systemic administration. Safety in this regard is an important prerequisite for any *in vivo* system prior to clinical use.[2]

Vector types

Physical gene delivery systems, such as electroporation, direct injection, liposome encapsulation, and receptor-mediated transfer have the benefit of being non-viral modes of transfer, and thus less likely to incite a host immune response, but produce poor transduction rates. Genes transferred using physical methods remain episomal and their perpetuity is thus tied to the rate of cell turnover. For non-viral gene therapies, integration of therapeutic genes into the genome requires vector-mediated transfer utilizing ZFN, TALEN, or CRISPR.[1,12,15]

Viruses, including retroviruses, adenoviruses, parvo/adeno-associated viruses, herpes virus, Sendai virus, spumavirus, and lentivirus (LV), have all been studied as vectors for gene therapy due to their innate ability to infect cells and transfer genetic material as part of the infectious process.[1] Most viruses used for gene transfer have had genes that confer virulence removed, or self-inactivated, with therapeutic genes substituted.[2]

In general, retroviruses allow permanent transfer of genes into the DNA of nondividing cells. Gamma retroviral (γ-retrovirus) vectors were used in early *ex vivo* studies to transfer genes to HSCs but suffered oncogenic setbacks. Though γ-retrovirus remains in use for T-cell engineering, LV has emerged as a safer alternative. LV improves efficiency through enhanced importation into the nucleus and the ability to carry a greater payload (8kb) than γ-retrovirus. Spumavirus appears to offer advantages similar to LV, however it is relatively new to the arena.[2,6,7,16]

Adenoviruses are double stranded DNA viruses frequently studied prior to 2012 due to their ability to carry larger transgenes (35-36 kb) with high levels of gene expression in both *in vivo* and *ex vivo* applications.[1,6] However, most individuals have become naturally immunized to adenovirus, leading to impairment of adenoviral transduction *in vivo*, and host immune response can result in clearance of transduced cells. Adenoviral gene therapy systems have been associated with at least one death due to a massive immune response.[2,12,17]

Adeno-associated viruses (AAV) are 4.7kb single-stranded DNA human parvoviruses packed within a protein capsid and are currently used in the majority of systemic gene therapy trials.[1,9] Recombinant AAV vectors (rAAV) allow a portion of viral coding sequence to be replaced by a transgene. Though not known to cause human disease, most adults are seropositive to one or more AAV serotype, with rates of immunity varying amongst serotypes. AAV demonstrates a reduced immune response as compared to adenovirus, making AAV a more useful vector system. Additionally, AAV serotypes exhibit tissue-specific tropism, making them suitable for *in vivo* use with more targeted biodistribution. However, limits include a smaller transgene capacity, episomally transient expression, and immune-mediated cytotoxicity that can lead to reduction of transgene product. Challenges with immunogenicity are being addressed through vector dose, capsid engineering, serotype choice and selection of AAV-naïve patients.[2,7,18,19] Currently, there are ongoing clinical trials for various diseases using AAV technology, including one FDA approved product addressing a rare inherited retinal disease.[1]

Clinical considerations

Current gene therapy applications largely focus on disorders that are life-limiting (such as spinal muscular atrophy), poorly managed conventionally, or have a significant treatment

burden.[1,7,20] Many gene therapies are targeting monogenic disorders with a clear cause-effect relationship, or where a protein is normally expressed in an accessible tissue such that minimal expression of the transgene makes a significant difference. In these conditions, gene augmentation would theoretically alleviate disease. Hemophilia is a condition where even low expression of the missing clotting factor at 2% to 5% of normal could profoundly reduce treatment need, as normal levels are not essential to life-changing improvements.[19] Lentiviral transduction of HSCs is of interest given the potential to encourage both factor VIII and IX expression in modified platelets.[21] However, in this area systemic AAV-mediated gene augmentation approaches have become more favored. Phase 1/2 clinical trials for hemophilia B have to date established safety for the timeframe in which the products were studied, and many were able to move patients from severe bleeding phenotype to moderate or mild.[22,23,24] Transition to a more potent transgene in current AAV trials now demonstrates increased factor IX activity levels to a range of 14% to 81%.[22,25,26] AAV-based gene therapy for hemophilia A has been more technically challenging due to the size of the factor VIII gene and concerns about development of inhibitors to factor VIII. A recent study has shown lasting benefit using AAV5, with normal or near normal factor VIII levels achieved at 2 years and no inhibitors detected.[27] The potential for cellular immune response with and without associated decreases in transgene/protein expression remains a challenge with some,[22,23,25] but not all,[24,26,27] gene therapies in hemophilia. As a result, some hemophilia A and B trials are now employing the use of prophylactic steroid protocols to protect against a cellular immune response developing.[25,27,28]

In contrast, other single gene disorders that have a clear cause and effect relationship require modification of stem cells, such as sickle cell disease and thalassemia. These are more complex to tackle because gene therapy requires a permanent modification of a selected subgroup of HSCs. Despite difficulties maintaining long-term expression due to HSC turnover, integrating lentiviral augmentation therapies show promise in ongoing trials, and clinical trials of gene editing techniques are beginning.[1,2,7,21,29]

Efficacy of gene therapies for diseases which are not predominantly single-gene disorders, where there is no clear cause and effect relation, or where there is an imperfectly understood molecular mechanism, will be more difficult to predict. Many neurological and neurodegenerative disorders fall into this category yet are still considered potential targets for gene therapy. Animal modeling studies have advanced intraparenchymal administration of rAAV vectors in Alzheimer's disease, Parkinson's disease (PD), complex liposomal storage disorders (LSD), HD, amyotrophic lateral sclerosis (ALS), and spinal muscular atrophy (SMA). Human trials in PD have explored rAAV-mediated delivery of several key genes including L-amino acid decarboxylase (AADC) to promote conversion of L-dopa to dopamine, glial cell line-derived neurotrophic factor (GDNF) and neurturin. AADC augmentation currently shows the greatest promise.[30] In SMA, a fatal infant disease, AAV vectors carrying the survival motor neuron gene 1 given as a single intravenous injection extended survival and improved motor function in a phase 1 clinical trial.[31] In HD, ZFN editing and RNAi strategies are viewed as the most promising gene therapy approaches, and the first AAV gene therapy clinical trial in HD has been cleared by the FDA to begin in 2019.[2,20,32] Clinical trials are also beginning in giant axonal neuropathy (GAN).[9,20] The greatest difficulty with CNS diseases is finding less invasive administration techniques that afford benefit across the blood-brain barrier.[33]

Current approved gene therapy protocols avoid germline cells (egg and sperm) due to ethical concerns. Somatic cells are, therefore, the sole target. However, vector shedding (dissemination of the virus through secretion or excretion) and gene detection in semen have raised concerns over the potential for germline modification.[2,6] Effective barrier contraception is required in current protocols until vector DNA can no longer be detected in semen.

As head-to-head comparison data are not available for current gene therapy programs, it is essential that treaters and patients consider those in development carefully. First and foremost, studies should reflect clinically meaningful outcome measures and a strong safety profile. Data on long-term safety and durability of efficacy will require life-long collection of appropriate data through patient registries. While there are encouraging data on durable efficacy in hemophilia B, we cannot be sure how long gene therapy will last, and the potential need for retreatment is something that must be considered.[11] Consequently, patients should weigh the possibility of an antibody response to viral vectors, which may preclude re-administration. Much work is being undertaken on this issue and how it may be circumvented.[34]

Finally, cost modelling will be a critical element of evaluating the health economic impact of expensive upfront gene therapy costs. Indeed, some early modeling has suggested that gene therapy may well be more cost-effective than life-long conventional therapies.[35]

Conclusion

Though gene therapy can offer the opportunity to significantly improve symptomology or slow progression of non-cancerous blood and/or neurologic disorders, it currently remains in the research arena. As such, those being treated do so with uncertain expectation of the outcome. It will be a number of years before a fully informed position is available, but

clinicians should be thinking about who might benefit most from gene therapy based on extrapolation of early clinical trial data, potential impact on health care costs and patient quality of life.

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