Main Submission

Vectors Have Both Magnitude and Direction: Considerations for Viral versus Non-Viral Vectors in Gene Therapy

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Introduction

In February 2017, a Canadian clinical team introduced functional genes into a patient to treat Fabry disease, a rare genetic disorder characterized by abnormalities in lysosomal storage, which can ultimately lead to life-threatening kidney and heart complications (1). This case received worldwide media attention as the first recorded gene therapy for the disease. Gene therapy is characterized by the therapeutic delivery of nucleic acids into a patient's cells, in order to induce functional changes into the genetic code (2). The patient's medical team, led by Dr. Aneal Khan, used a lentivirus to insert altered genes into stem cells harvested from the patient's bone marrow (1). The cells were subsequently injected back into the patient, and the patient underwent careful immune monitoring to ensure that the lentiviral method of gene therapy did not cause a systemic inflammatory response. This clinical breakthrough prompted discussion in the scientific community regarding the possible challenges with different gene therapy vectors. Somatic gene therapy treatments can deliver DNA into nucleated cells through recombinant viruses or non-viral DNA complexes (3). Both options possess significant advantages and challenges, which require further investigation.

Considerations Regarding Viral Vectors

Viral vectors have been a source of controversy since September 1999, when an 18-year-old patient died of complications in a viral-based clinical trial for gene therapy (4). Jesse Gelsinger was injected with an adenoviral vector carrying a functional gene in order to treat ornithine transcarbamylase deficiency. He died of immune-related complications four days after injection, with subsequent investigations from the Food and Drug Administration concluding that the trial was in violation of research ethics. However, viruses remain the most common vector in gene therapy (Figure 1), as viruses efficiently introduce their genetic material into host cells with the goal of replication (4,5). Limitations of viral vectors include insertional mutagenesis, difficulty in production, as well as immunogenicity due to the patient's immune response (6). Depending on the location within the host's genome, mutations can have varying effects on the cell. For example, lentivirus-based viral vectors, such as that used in the Fabry trial, possess the risk of augmenting cancer, as lentiviruses can spontaneously insert sequences at unplanned locations in genes involved in apoptosis or cellular replication (7). This was evident in a retroviral gene therapy trial in 2002, in which

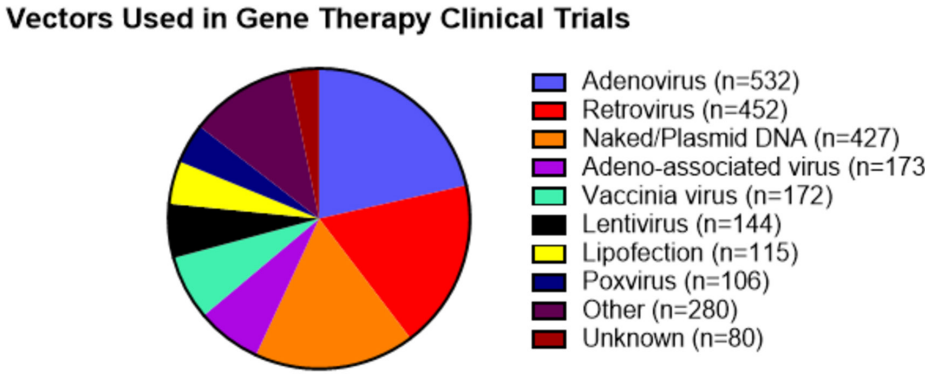

Figure 1: Prevalence of vectors used in gene therapy clinical trials. Adapted from (5).

four patients unfortunately developed leukemia following their lentiviral treatment (7).

Considerations Regarding Non-Viral Vectors

As a result, non-viral gene therapies have gained attention from researchers as a potential alternative. Non-viral vectors are comprised of synthetically produced biological particles, in which the plasmid DNA (pDNA) carrying therapeutic genes is encapsulated or bound to a synthetic chemical compound (8). Upon delivery, the vector is then released at the target site in order to induce changes in the genome. Examples of non-viral vectors include lipoplexes, inorganic nanoparticles, and the injection of naked DNA directly into the host cell (9). In contrast to viral-derived vectors, non-viral systems are relatively easy to mass-produce, and the risk for inflammatory complications is significantly lower (4,7). Furthermore, non-viral vectors pose advantages; in addition to pDNA, they are also capable of delivering synthetic compounds, such as short interfering RNA. However, limitations of non-viral vectors include decreased extracellular stability of the delivery complex, reduced internalization and cellular trafficking of the vector, and unsustainable expression of the therapeutic gene. Ultimately, while recent technological breakthroughs have attempted to mediate these challenges, the transfer efficacy of non-viral gene therapies remains greatly reduced in comparison to viral vectors (4). Further research must be conducted in order to increase the transfer efficacy and bioavailability of non-viral vectors.

Alternative Vectors

When faced with viral and non-viral options for vectors, recently developed "hybrid vectors" also remain a viable option for gene therapy (4,10). Hybrid vectors are comprised of a viral vector, which is conjugated to a synthetic biocompatible polymer, resulting in ablation of the native virus and enhanced transduction towards host cells (10). While this option could still elicit a potential immunologic response to the viral constituents, the risk of inflammatory complications is significantly decreased. One relatively promising example of a hybrid vector uses adeno-associated viruses to encapsulate potent genes in a bacteriophage capsid, and offers sustained gene expression (4). However, depending on the type of hybrid vector, the production process can be cumbersome (10). It is also important to note that alternative vectors may pose an additional risk of oncogenesis, depending on the vector used, the therapeutic gene, and the cell type targeted (11).

Conclusions

In conclusion, both viral and non-viral vectors offer significant advantages and obstacles in effective gene therapy (Table 1). It is important for clinical researchers to tailor vectors to specific applications of gene therapy, in addition to considering alternative options such as hybrid vectors. While cases such as the recent Fabry disease trial present the promising capabilities of gene therapy, the technology is not without risks that must be carefully considered. Further research must be conducted in order to develop an "ideal" gene therapy vector that balances transduction efficiency with the safety profile and ease in production of the vector. ■

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Table 1: A comparison of viral, non-viral, and alternative vectors.

Vectors	Advantages	Limitations
Viral	High transduction efficiency Sustained transgene expression	Insertional mutagenesis Immunogenicity Difficulty in production
Non-viral	Low toxicity and immunogenicity Relatively easy to mass-produce	Reduced extracellular stability Low transduction efficiency and specificity Limited duration of transgene expression
Alternative	Sustained transgene expression Decreased risk of inflammatory complications	Possible immunogenicity and insertional mutagenesis Complicated production

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