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Short-communication

A Simplistic Overview of Gene Therapy

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Short-communication

Gene therapy can be defined as the manipulation of genetic material to prevent, treat, or correct or diseases. One of the most explored gene therapies is that of one that uses a viral vector to deliver a gene to a cell. The process goes something like this.

A virus is chosen to carry the desired gene cargo. The majority of the Viral DNA is removed from the viral shell. The space created by the removal of the viral DNA provides a cargo space for a therapeutic gene. The chosen gene is manufactured in the lab and inserted into the virus cargo area. That virus loaded with the new payload is then cultured and expanded in the laboratory. Once the desired number of loaded viral particles is grown, the product is collected and prepared as a therapeutic agent. The subject of the gene therapy is dosed with the loaded virus. The virus attaches to the cell membrane and injects its cargo into the cell cytoplasm. The usual intercellular mechanisms transport the injected payload through the cytoplasm for insertion into the nucleus. Once in the nucleus, the cargo DNA undergoes transcription, and a gene product is produced by the cellular machinery. The treated cell now produces the product of the cargo DNA.

There are several categories of gene therapy technology and techniques, each with its approach and nuances. The main categories of gene therapy are:

Gene Replacement Therapy

- Introduces a functional copy of a gene to replace a faulty or missing gene.
- The therapeutic gene is delivered into the patient's cells using vectors, such as viral vectors or non-viral methods [1].

Gene Editing

- Directly modifies the DNA within the patient's cells to correct or replace a defective gene.
- Utilizes tools like CRISPR-Cas9 or other gene-editing technologies. CRISPR/Cas9, allows scientists to make

precise changes to the genome of cells or organisms [1].

Gene Addition Therapy

- Augments the function of cells by adding new genes that enhance specific capabilities.
- Introduces extra copies of functional genes to supplement existing genetic material [1].

Gene Silencing Therapy

- Inhibits the expression of a specific gene to reduce the production of harmful proteins.
- Uses RNA interference (RNAi) or antisense oligonucleotides to block or degrade the target gene's messenger RNA (mRNA) [1].

Immunotherapy

- Enhances the immune system's ability to target and destroy non-self.
- Introduces genes that stimulate the immune response, such as chimeric antigen receptor (CAR) T-cell therapy [1].

Non-viral Vector-Based Gene Therapy

- Achieves gene delivery without the use of viral vectors, reducing potential safety concerns in by some methods.
- Utilizes various non-viral methods, such as liposomes, nanoparticles, or electroporation, to transport therapeutic genes into cells.
- percent of cells successfully transduced and gene effect durability may be significantly less and shorter compared to viral vector however substantial cost savings also exist compared to viral vector use [1].

Ex Vivo Gene Therapy

- removing donor/patient cells, laboratory genetic modification of those cells followed by administration of those modified cells to the donor/patient [1].

In Vivo Gene Therapy

- Administers the therapeutic genes directly into the patient's body to target specific tissues or organs without removing cells [1].

A comprehensive list of gene therapy techniques and technology should include the following:

- Meganucleases (MegNs): a naturally occurring cellular entity that can be used to create DNA cleavage mechanisms [1].
- Zinc finger nucleases (ZFNs): site specific gene editing restriction enzymes created in the lab [1].
- Transcription activator-like effector nucleases (TALENs) [1].
- Plasmid gene delivery [2].

For the scope of this communication, the comments are limited to the use of viral vector gene therapy. A separate dedicated communication can address the other vector technologies strengths and weaknesses.

Gene therapy has the potential to provide a one-time, long-lasting treatment for genetic diseases. Many genetic diseases are caused by defects in a single gene, and gene therapy has the potential to correct these defects, potentially providing a permanent cure for the disease. Gene therapies have the potential to be more targeted and specific than traditional therapies. By targeting the specific genes involved in a particular disease,

gene therapy can potentially be more effective at treating the disease, with fewer side effects. Gene therapies not only have the potential to be used to treat a wide range of genetic diseases, but also other conditions including cancer, cardiovascular disease, and neurodegenerative diseases.

Viral Gene therapy, as a field, faces three broad categories of challenges to bring therapy to available medical use.

1. Vector choice
2. Gene choice
3. Immune system issues

Viral vector choice

The greatest danger of gene therapy is the risk of off-target effects. This refers to the possibility that the therapeutic gene may be inserted into the genome at a location that results in the activation of an adjacent chromosomal gene. The unintentional activation of a neighboring gene has several potential consequences. The neighboring gene can be an oncogene, a gene whose activation results in a cancer occurrence.

The neighboring gene can produce a product that has undesirable metabolic or enzymatic properties which will manifest as a new clinical problem. The insertion can result in silencing of an adjacent gene whose absence creates a negative clinical scenario. The chances of these off-target effects are related to the vector itself. Viral vector DNA payload can integrate into the genome to variable degrees and in predictable as well as random locations. The frequency and location of integration are unique to each vector and also equates to the risk of off-target effect. The vector used to deliver the gene must be carefully selected to minimize the risk of adverse effects as there are risks to gene therapy that are directly related to the vector being used and unrelated to the gene being delivered. [5,6,11]

Gene choice

The therapeutic gene itself must be carefully evaluated to ensure that it is unlikely to cause unintended consequences or harm to the patient. The desired known function of the gene is only part of the puzzle. The real challenge is to define all of the effects that a given gene may have on a disease or tissue in the patient that is not the focus of the therapy. Without knowing all the effects of a gene's function, the probability of an undesired effect is real and unacceptable. Sorting out all of the possible functions of a given gene is a very time-consuming and detailed process that can take years [6].

Immunological issues

In the application of Gene Therapy, there is a risk of an allergic reaction, but it is easily dealt with using the same methods developed for avoiding infusion reactions: prophylactic administration of histamine blockers and steroids administered pre-treatment. However, there are some more complex issues with the immune system that impact the efficacy of gene therapy. An aggressive immune reaction can not only put the patient in a life-threatening crisis but also prevent a successful gene transfer thus making the entire gene therapy a fruitless expensive exercise [6,9].

Preexisting antibodies in a gene therapy recipient occur from environmental exposure as well as from iatrogenic exposure to AAV. Regardless of the source of anti-AAV antibodies, their presence requires that measures be undertaken to neutralize the antibodies to preserve the possibility of a successful gene therapy result. [6,9].

The innate and adaptive immune system components both play a role in the body's immune reaction to gene therapy. Together, the innate and adaptive immune systems work in concert to provide a comprehensive defense

against infections [9]. The adaptive immune system response is most consequential regarding the success of gene therapy. The presence of immunological memory and antibodies from prior exposure to a foreign entity poses the most specific and aggressive challenge to successful gene therapy [6,9].

The innate immune system provides rapid but nonspecific response to a wide range of potential threats, including bacteria, viruses, fungi, and other foreign substances. The qualities of the innate immune system make it the foundation of one's immune response. It requires no training as it functions non-specifically, and it is present at birth. Here's an overview of how the innate immune system functions:

1. **Physical and Chemical Barriers:** The skin and mucous membranes act as physical barriers to pathogens while chemical Barriers such as saliva, tears, and stomach acid secretions contain enzymes and chemicals that can inhibit or destroy pathogens [9].
2. **Phagocytosis:** White blood cells called phagocytes, including neutrophils and macrophages engulf and digest pathogens [9].
3. **Inflammation:** A coordinated response to tissue injury or infection. In this process blood vessels dilate and cytokines and chemokines attract immune cells to the site of infection [9].
4. **Complement System:** Serum proteins that, upon activation, lead to the formation of membrane attack complexes (MAC). MAC can directly lyse pathogens. Complement proteins enhance phagocytosis and contribute to inflammation [9].
5. **Natural Killer (NK) Cells:** A type of lymphocyte that recognizes and destroys infected or mutated cells [9].
6. **Interferons:** Viral presence induced signaling proteins with antiviral properties that modulate the immune response by activating other components of the immune system [9].
7. **Cellular and Molecular Pattern Recognition:** Pattern recognition receptors (PRRs) located on and within the cell membrane recognize molecular patterns common to pathogens (PAMPs - pathogen associated molecular patterns). [9].

The adaptive immune system is a complex and highly specialized defense mechanism that provides a targeted response to specific pathogens. Programming this system takes time but results in immunological memory, once its programmed, it can mount a faster and more efficient immune response to a previously encountered pathogen. Here's an overview of the components of the adaptive immune [9].

1. **Antigen Recognition system**: Pathogens have proteins or macromolecules that are unique to a given pathogen. Recognition of these structures stimulates the T and B lymphocytes activity [9].
2. **B Cells and Antibody-Mediated Immunity:** Each B cell has a specific receptor that can bind to a specific pathogen antigen in a process called antibody mediated immunity [9].
3. **T-cell mediated immunity:** Helper T cells (CD^{4+}) and cytotoxic T cells (CD^{8+}) make up the T cell mediated immunity system. Helper T cells release cytokines which activate B cells and cytotoxic T cells. The cytotoxic T cells recognize and directly destroy infected or mutated cells as well as induce apoptosis. T cells recognize antigens presented by antigen presenting cells (APC) [9].
4. **Antigen Presentation:** Macrophage and dendritic cells make up the APC

complex. These cells engulf pathogens and fragment them. These fragments are displayed on the lymphocyte surface using a protein called major histocompatibility complex (MHC) type 1 and 2. Helper T Cells recognize the antigen-MHC1 complex while cytotoxic T cells recognize the MHC 2 complex. [9].

5. **Clonal Selection and Expansion:** Once activated, the B Cell antibody antigen complex differentiates into plasma cells which then become high output antibody factories programmed against that specific pathogen [9].
6. **Immunological Memory:** The hallmark of the adaptive immune e system is the formation of immunological immunity via memory B and T cells. Upon re-exposure to a known pathogen, these memory cells promote a specific rapid immune response [9].

As mentioned above, blunting the innate immune response is rather easy and accomplished by histamine blockers and steroids. Recent research suggests that the contribution of the innate immune system to a gene therapy failure is negligible and inconsequential compared to the adaptive immune system [6,9].

Recent research indicates that the presence of immunological immunity seems to be the most consequential issue regarding the success or failure of a gene therapy. The presence of preprogrammed and pathogen-specific anti-vector antibodies that can be produced in large volumes in a short amount of time poses the greatest threat to the gene therapy process from the time of injection to past the time of therapeutic gene integration into the nucleus of the cell, a process that can take up to two months. Cytotoxic T-cell programming and activation peaks at approximately 5 weeks post-injection. Cells successfully transduced with a functioning para- chromosomal intranuclear gene transfer can be detected and targeted by the adaptive immune system weeks to months after injection, putting those functioning transduced cells at risk forever. [6,9].

Fortunately, in a patient without anti-vector serotype antibodies that match the intended gene therapy vector serotype, the adaptive immune system is not a factor. But for those patients who have anti-vector antibodies, it is a big problem.

Strategies used to address the presence of anti-vector antibodies include immune suppression with a variety of medications including steroids and drugs used in organ transplant science as well as anti-cancer drugs. It seems that these medications are very good at addressing the innate immune response but essentially useless at addressing the adaptive immune response [6,9].

What has been successful at addressing the presence of anti-vector antibodies is the active filtration of these antibodies. Serial plasmapheresis is a shotgun-type method by which to remove the anti-vector antibodies, but it is complicated by the removal of the non-anti-vector antibodies needed to function against the library of known pathogens the subject has and may encounter again. Stripping the Gene Therapy recipient of a significant amount of the developed adaptive immune system leaves them significantly at risk to a serious life- threatening pathogen. In practice, antibody filtration becomes a balance of removing antibodies to blunt the adaptive immune response but not so many that the patient is temporally without an adaptive immune response to all programmed and known pathogens [11,12].

Barriers to gene therapy

There is a need for extensive clinical testing to ensure the safety and effectiveness of the therapy prior to widespread adoption into clinical

practice. This is only overcome with extensive laboratory and translational research. Regulatory approval from agencies such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) is required for introduction into clinical practice. These agencies are responsible for ensuring that new therapies are safe and effective before they can be used in patients. The process of obtaining regulatory approval can be time-consuming and costly, which is a barrier to the development and use of gene therapies. The time required to meet these two major developmental barriers is on the order of years and hundreds of millions of dollars.

Today, Gene therapy is being tested as a treatment for genetic diseases such as sickle cell anemia, hemophilia, and cystic fibrosis, as well as structural-based diseases that have a genetic basis. Several Gene Therapy treatments are now approved for use in clinical practice [2,3,5]. One example is the use of gene therapy to treat inherited retinal diseases, which cause vision loss due to the absence or dysfunction of specific proteins in the retina.

Adeno-associated virus (AAV) is a type of virus that is commonly used as a vector for gene transfer therapy. Gene therapies using AAV vectors have shown promise for the treatment of a wide range of genetic diseases. Historically the AAV vector is the most widely used and thoroughly studied gene therapy vector with approximately 50 years of research and clinical use [2,6].

Several AAV-based gene therapies have been approved for use in clinical practice and include:

- Luxturna uses an AAV vector to deliver a gene that encodes a functional copy of a missing protein to the retina, restoring vision in some patients [5].
- **Zolgensma:** This gene therapy is used to treat spinal muscular atrophy (SMA), a genetic disorder that causes muscle weakness and atrophy. Zolgensma uses an AAV vector to deliver a functional copy of the SMN1 gene to the CNS, which is missing or nonfunctional in patients with SMA [4].
- **Voretigene neparvovec-rzyl:** This gene therapy is used to treat inherited retinal diseases that cause vision loss due to defects in the RPE65 gene. Voretigene neparvovec-rzyl uses an AAV vector to deliver a functional copy of the RPE65 gene to cells in the retina, restoring vision in some patients [5,7].

Some examples of emerging AAV-based gene therapies include:

- **Gene therapies for neurological disorders:** Researchers are developing AAV-based gene therapies for the treatment of neurological disorders, such as Parkinson's disease, Alzheimer's disease, and multiple sclerosis. [5,7,8] These therapies aim to deliver functional copies of genes that are missing or nonfunctional. The therapeutic goal is to improve symptoms and slow or reversing the progression of the disease.
- **Gene therapies for cardiovascular diseases:** AAV-based gene therapies are also being developed for the treatment of cardiovascular diseases, such as heart failure and hypertension. These therapies aim to deliver functional copies of genes involved in the regulation of blood pressure and heart function, to improve heart function and reduce the risk of heart-related complications [5,7,8].
- **Gene therapies for cancer:** AAV-based gene therapies are being explored as a potential treatment for certain types of cancer, including pancreatic cancer and glioblastoma. These therapies aim to

deliver genes that can help to kill cancer cells or inhibit the growth and spread of cancer [10].

There are several reasons why AAV is so popular:

1. AAV is a small virus with a single-stranded DNA genome, belonging to the Parvoviridae family. AAV has a wild serotype that is ubiquitous in our environment. Various studies have found that between 60 and 90 percent of the general population have been exposed to and infected with the AAV virus [5,8,10].
2. AAV infection is an asymptomatic and subclinical infection. Exposure and infection go unnoticed. It is considered non-pathogenic and does not cause disease in humans. Prior infection is determined by the presence of anti-AAV antibodies in the patient's serum [5,8,10].
3. AAV exhibits different serotypes, each with distinct surface proteins, influencing its tissue specificity and the types of cells it can infect. Researchers can select specific AAV serotypes to target different tissues or organs based on therapeutic needs [5,8,10].
4. AAV integration into the nuclear DNA is known to be predictable and reliable regarding incidence (approximately 0.1%) in location (human chromosome 19 at site q13. 4 (AAVS1). This addresses the likelihood of an off-target effect from AAV and is also the reason AAV has such a good safety profile [5,8,10].
5. AAV is known for its low immunogenicity, meaning it typically does not provoke a strong immune response [5,8-10].
6. AAV has a track record of use in the gene therapy field for approximately 50 years so much more is known about AAV as a viral vector than other vectors [5,8-10].

The downside of AAV:

- AAV is a relatively small viral vector. Many human genes are simply too large to fit in an AAV capsid shell [5,8,10].
- Because of the ubiquity of AAV in the environment, many patients may have anti-AAV antibodies circulating [5,8,10].

Non-AAV viral vectors

The use of non-AVV viral vectors brings with it some benefits with the most useful being the potential for a much larger payload carrying capacity. This opens up the possibility of inserting genes too large to fit into the AAV capsid shell thereby widely expanding the reach and scope of actual gene therapy.

Here is a detailed but by no means a comprehensive list of viral vectors and a notable characteristic property two of those viral vectors.

1. Adenovirus (AdV): Adenoviruses are a family of viruses that can infect a variety of tissues. Their use in gene therapy may be limited by immune responses [11].
2. Lentivirus: Lentiviruses, a type of retrovirus, can integrate their genetic material into the host genome allowing stable and long-term expression of the therapeutic gene [11].
3. Herpes Simplex Virus (HSV): The herpes simplex virus family has certain strains that can infect a variety of cell types [11].
4. Sendai Virus (SeV): Sendai virus is a type of paramyxovirus that has the advantage of not integrating into the host genome reducing the risk of insertional mutagenesis [11].

5. Baculovirus: Baculoviruses are insect viruses that have been modified for use in mammalian cells. They are particularly used for transient gene expression and vaccine production [11].
6. Retrovirus: Retroviruses as a class are capable of integrating their genetic material into the host genome but their use, other than Lentiviruses, has declined due to safety concerns [11].
7. Measles Virus: Measles virus has the ability to infect a wide range of cells and carry a large payload [11].
8. Foamy Virus: Foamy virus family have a relatively low pathogenicity and are capable of long-term transgene expression [11].

Each of these viral vectors holds a unique set of positive and negative attributes which determine whether or not a given vector will fit the clinical need to carry a certain gene for a certain clinical problem while avoiding the undesirable off-target effects.

Conclusion

Ongoing research and technological advancements continue to refine and expand the applications of gene therapy. Anti-vector antibody issues remain and must be addressed. New Gene Therapy vectors will have different frequencies, locations, and incidences of genome integrations. Each vector genomic integration characteristics must be defined and mapped to avoid the most undesired off-target effect of gene therapy, integration into the genome that turns on an adjacent cancer-inducing gene (an oncogene).

Addressing the strengths and limitations of the available and emerging gene therapy vectors, identifying the benefits and potential dangers of a particular gene to be used in gene therapy, and overcoming the issues posed by the adaptive immune system represent are and will continue to be the current and future challenges to the field of Gene therapy.

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