Overcoming Deficiencies in Gene Therapy Delivery with Chimeric and Inducible Vectors

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Abstract

Viral gene therapy intends to bring genetic materials to the cell using a modified virus. This delivery system is called a viral vector. The viral vector often takes the property of the virus in regards to delivery, so the choice of virus leads to various outputs. Viruses differ from one another depending on their tropism, hitting certain tissues better than others; immunogenicity; and size. This review compiles evidence of recent innovations in the delivery of gene therapy using viral vectors with a specific emphasis on inducible and chimeric viral vectors that address long-standing limitations of traditional viral vectors. Conventional viral vectors, despite being the current standard for gene therapy delivery, pose risks and have significant flaws. Many elicit severe immunogenic responses and often hit unintended cells and tissues. This review discusses two new categories of viral vectors: chimeric vectors, which are a combination of two or more vectors, and inducible viral vectors, which use spacio-temporal awareness to improve efficiency and targeting. Chimeric vectors adopt key features from multiple different vectors to create a hybrid. These hybrid vectors can target new cell types, lower immunogenicity, and make downstream purification easier.Meanwhile, inducible vectors rely on external stimuli to dictate their expression. Inducers include small molecules, RNA, and light. While concerns remain around scalability of these therapies, the next level targeting capabilities make them promising for further use in the gene therapy space.

Introduction

Viral vectors are engineered viruses that deliver genetic material into cells, making use of a virus's natural ability to infect cells. There is a broad scope of potential applications for viral vectors including vaccinations, CRISPR gene editing, gene replacement, and cell therapy [1], [2], [3]. This review focuses on gene therapy in particular.

Despite being the most prevalent gene therapy delivery method today, there remain a variety of issues within this field [4] The most commonly used viruses in gene therapy are adeno-associated viruses, retroviruses , and adenoviruses. Some less common alternatives include herpes simplex viruses, oncolytic viruses, and measles viruses. Each has unique strengths and weaknesses which make them better suited to various purposes.

Components of a Viral Vectors

A viral vector is composed of several key components. The vector genome, which consists of either DNA or RNA, expresses genetic cargo and its size determines the payload size of the vector [5]. The capsid and envelope proteins are crucial for determining which types of cells the virus can affect and protect it from an immune response.

Adeno-associated viruses

Adeno-associated viruses (AAV's) are small viruses that carry a meaningful but relatively small immunogenic response compared to lentiviruses and adenoviruses, making them particularly versatile [6]. They can infect both dividing and non-dividing cells [7]. This makes

them useful in muscle cells, which do not divide. Its usage is broad but primarily lies in the respiratory system, eye, gastrointestinal system, and muscles.

AAV is non-integrative, which means, as opposed to integrating permanently in the genome, it is actually expressed as a completely separate construct called an episome. This diminishes the risk of being permanently imprinted in the wrong parts of the body or integrating faulty genetic code found in integrating viruses such as retroviruses. For tissues like muscle, where the majority of mature cells do not replicate, allowing for long-lasting expression of the transgene. However, the lack of integration means the gene will be lost in cell division and filtered out. This limits their utility for long-term projects as they are somewhat temporary.

In the context of CRISPR-Cas gene editing, AAV vectors are frequently used for in vivo delivery. Their durable transgene expression and ability to transduce non-dividing cells makes them a strong candidate for in vivo gene editing.

New research contradicts the previous notion of AAV being minimally immunogenic. In fact, AAV has been shown to induce antibodies towards the virus [8]. The virus is treated as a foreign substance and is attacked similarly to an illness via a natural innate immune response. TLR-9 and TLR-2, two specific known antibodies, innately sense the vector's capsid protein which triggers the immune response [9]. This is a struggle to avoid as it is innate within the virus which is used.

Retroviruses

The most used type of retrovirus for gene therapy is lentivirus. Lentivirus, like other retroviruses, permanently integrates into the host genome [10]. This is both a cause for concern and a benefit. Integration is often risky which is why retroviruses are usually not used for in vivo delivery. The risk is associated with insertional mutagenesis which would lead to permanent integration of a faulty genome. Additionally, it may hit certain unwanted tissues and permanently insert there as well.

Retroviruses are also known for their large payload compared to AAV. The payload is nearly double that of AAV [6]. On the other hand, lentivirus is considerably more toxic than AAV. Lentiviral vectors are considered more toxic than AAVs because their integration into the host genome can disrupt essential genes and trigger stronger immune responses, whereas AAVs are largely non-integrating and less immunogenic. Still, it has been chosen as one of the primary viruses for CRISPR delivery, particularly in vitro (out of body) applications [11]. As opposed to inside of the body where it is difficult to view and flaws in the integration, in vitro applications can verify that there were no harmful integrations in the delivery prior to putting the edited cells in the body.Though there is risk of mutations due to integration, the size capacity has proven beneficial in CAR-T therapy and HIV therapy.

Adenoviruses

Adenoviruses are notable for their high payload, which comes at the cost of an elevated immunogenic response. AAV can only carry 4.7 kilobases (kb) worth of information whereas adenoviruses can carry up to 36 kbs. Adenoviruses carry double stranded DNA, whereas AAV carries a single strand which is later duplicated. They are incredibly immunogenic and are known for their extreme risk. Adenovirus naturally causes respiratory infections, so the immune system recognizes it as an extreme threat [12].

Like AAV, Adenoviruses are non-integrative which means it is good for short-term gene expression. Its primary use has been in vaccinations and cancer therapy [12]. It cannot be used in sensitive tissues like the eye or brain as it is far too immunogenic and would elicit too large of a response. One example of its use was the Johnson & Johnson COVID-19 vaccine [3]. The FDA had to severely restrict the use of this vaccine due to the potentially fatal thrombosis which was linked to the vector.

Adenoviral vectors are capable of carrying CRISPR machinery due to their large payload capacity, which exceeds that of many other viral vectors. This ability allows them to deliver complex or multiple genetic elements in a single vector. However, their strong immunogenic response remains a significant challenge, often limiting their clinical applications despite their efficiency in gene transfer.Other viruses are being explored for potential use in gene therapy,, but the three listed above are currently considered the gold standard.

Limitations of Current Viral Vectors

Despite the variety of viral vectors available, they each have unique limitations. The primary faults include a lack of precision, size constraints, and immunogenicity concerns.

While there have been efforts to engineer each viral vector to hit a specific organ or cell type, vectors generally broadly target cells based on the properties of their capsid proteins.

Another issue concerns the size-constraints. The least immunogenic response comes from AAV, but it also has the smallest payload. In vivo delivery of larger Cas variants such as SpCas9 are plausible but difficult as the capacity for AAV is 4.7 kb. spCas9 takes up 4.2kb plus some, admittedly quite small, gRNA [13]. Additionally, there are genes that are blatantly impossible to fit in current AAV such as Dystrophin which is 11 kb which can be used to regulate Duchenne Muscular Dystrophy [14] . Instead, multiple doses of dystrophin are sent with only part of the gene to fit it inside AAV. If AAV could pack a larger payload, there would be no more need for adenoviruses as the payload issue would be solved with a less immunogenic solution.

On the other hand, if the immune response for adenovirus was lessened, it would become much more practical. Moreover, AAV also carries some risk which, if mitigated, can expand in vivo gene therapies' role as a whole.

New Innovations

This review outlines a new vision for the future of viral vectors. There have been recent developments in viral engineering which attempt to overcome the shortcomings of standard viral vectors. Two main approaches will be discussed. First are chimeric vectors, which are defined as vectors that utilize the favorable properties of multiple vectors, manipulating the advantages and disadvantages of each [15]. Chimeric vectors primarily address immunogenicity and payload size. Alternatively are inducible vectors, which use an external stimulus to dictate their expression. They primarily address the specificity of the virus to target certain organs or cells.

Chimeric Viral Vectors

Chimeric viral vectors are one approach to overcome the limitations of traditional vectors. A chimeric viral vector is a vector engineered by combining various components from two or more different viruses [15]. Viral vectors consist of a capsid protein that makes up the outer shell, the envelope protein, a liquid membrane layer, and the packaged genetic material [16]. Chimeric vectors mix and match these parts to achieve a particular goal, for example a larger

payload for AAV to overcome its small size [17]. Chimeras often combine different types of viruses, but in some cases, variants of the same virus are combined to create a superior alternative.

While observing the payload capacity of AAV contrasted with the immunogenic response of adenovirus, it is clear no one virus mitigates both issues. Moreover, the risks of genome integration posed by lentivirus are also cause for concern. Chimeric designs could increase the transgene size that can be delivered, while reducing immune response. They could also improve safety through non-integrative delivery and enable delivery of complex systems like CRISPR/Cas.

A chimeric viral vector is made by combining various genetic elements using DNA recombination [18]. The manufacturing of recombinant adeno-associated viral vectors involves designing the vector, producing it in cultured cells, purifying the viral particles, and performing quality control to ensure safety and efficacy for clinical use.

AAV-Based Chimeras

AAV comes in numerous different variations called serotypes. Each carries their own individual strengths and weaknesses [19]. Different serotypes have different tissue tropism, meaning they preferentially infect different tissues. For example, AAV10 is efficient at infecting the central nervous system, but has an inconsistent immune response [20]. On the other hand, AAV2 is very versatile, but has a consistent high immune response associated with it. Further variation is seen with AAV5 which triggers a limited immune response, is more inefficient making it an unreliable alternative [21]. The primary use of combining AAV serotypes is to improve transduction to specific tissues.

Table 1. AAV by Serotype Chart

Serotype	Tropism	Immunogenicity	Efficiency
Most Used AAV Serotypes			
AAV 1	Skeletal Muscle, CNS, Lung, Retina, Pancreas	Moderate	Moderate-Low
AAV 2	Liver, Smooth Muscle, Skeletal Muscle, CNS	High	Moderate
AAV 5	Skeletal Muscle, CNS, Liver	Low	Moderate-High
AAV 8	Liver, Skeletal Muscle, Retina, CNS	Moderate	High
AAV 9	Liver, Heart, Brain, Lungs, Skeletal Muscle	Low	High
AAV 10	Liver, CNS	Moderate	High

*Note: CNS refers to the Central Nervous System

Due to the variation in AAV serotypes (see Table 1), chimeras have been made from multiple serotypes to enhance the benefits of each. One of the earliest examples of a chimeric vector was the AAV1/2 vector - a combination of AAV1 and AAV2. AAV2 had been the primary serotype used for in vivo experimentation, especially in the liver,, but AAV1 was better in the muscular system [22].

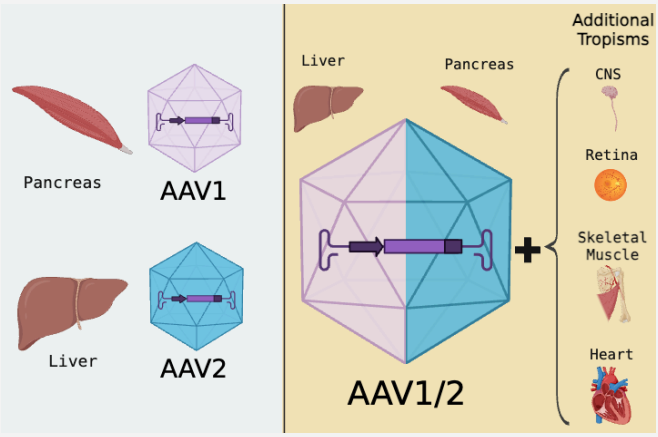

Fig. 1. AAV1/2 Vector Diagram. The change in tropism is observed when combining AAV1 and AAV2 into the chimera AAV1/2. While keeping the primary initial tropism, AAV1/2 can target other tissues - the CNS, the retina, skeletal muscle, and the heart in addition to the liver and pancreas.

AAV1/2 takes capsid proteins from both serotypes and combines them (Fig. 1). The resulting vector is a significantly more versatile option than either AAV1 or AAV2 and can be used throughout the body. AAV2 also carries a much more significant immune response in humans due to many people already having antibodies against AAV2. This makes this chimera more effective than AAV2 as it carries the lessened immune response associated with AAV1.

Shen et al. combined AAV2 and AAV8 to make the AAV2/8 vector, by taking both capsid proteins and dividing them into seven sections and swapping them each to see what traits worked best to transduce the liver (see Fig. 2) [23].

Fig. 2. AAV2/8 Vector Diagram

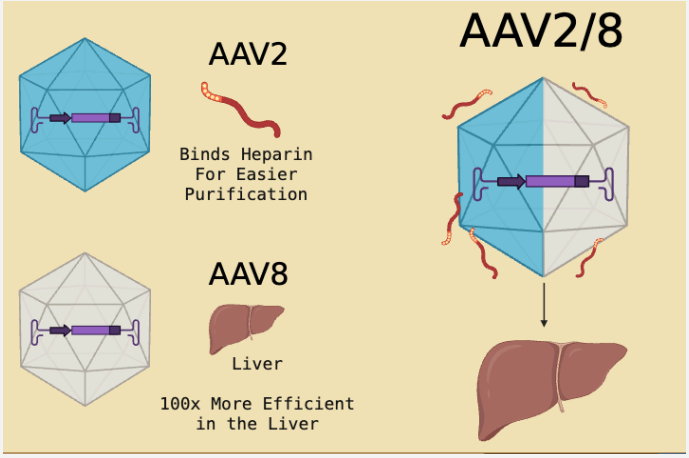

Fig. 2. AAV2/8 Vector Diagram. AAV2/8 chimeras combine the benefits of the purification of AAV2 and the transduction of AAV8 to the liver. Heparin is notated as the red and yellow curve.

AAV8 was already very efficient in the liver, and has fewer neutralizing antibodies in the human body which makes it significantly less immunogenic than AAV2. On the other hand, the AAV2 capsid binds to heparin sulfate proteoglycan. This interaction makes purification of the vector easier. So, ultimately AAV2/8 takes the positive traits of AAV2 in purification and applies it onto AAV8 and its high liver tropism resulting in a better product for human trials (see Table 2).

Table 2. AAV by Serotype Chart with Chimeric Variants

Serotype	Tropism	Immunogenicity	Efficiency
Traditional AAV Serotypes			
AAV 1	Skeletal Muscle, CNS, Lung, Retina, Pancreas	Moderate	Moderate-Low
AAV 2	Liver, Smooth Muscle, Skeletal Muscle, CNS	High	Moderate
AAV 5	Skeletal Muscle, CNS, Liver	Low	Moderate-High
AAV 8	Liver, Skeletal Muscle, Retina, CNS	Moderate	High
AAV 9	Liver, Heart, Brain, Lungs, Skeletal Muscle	Low	High
AAV 10	Liver, CNS	Moderate	High
Chimeric Variants			
AAV 1/2	Target-Specific	Moderate	High
AAV 2/8	Liver, Skeletal Muscle, CNS	Low	High

Adenoviral-Based Chimeras

Like AAV, adenovirus also comes in various serotypes. Notable for their larger payload capacity and high immunogenic response, adenovirus is often refrained from being used despite the potential upside. Often the goal with viruses that have a large immunogenic response is not only to mitigate the response but to improve tropism so minimal collateral damage occurs. This is done by replacing specific capsid proteins with those of other serotypes [24]. Adenovirus serotype 5 (Ad5) has a very large immunogenic response, but it is so versatile that it is the most used. Most people have pre-existing neutralizing antibodies to Ad5 [25]. To circumvent this, chimeric vectors can be made by taking proteins from other serotypes that have less frequent pre-existing immunity to bypass the immune system and hit the target cells.

One chimera replaced the proteins of Ad5 with that of Ad35 making Ad5/35, improving its ability to infect specific cells in mice (see Fig. 3) while maintaining an exceptionally large payload of 8.8 kb from Ad5 [26]. Shayakhmetov et al. with the same chimera found that the transduction efficiency was greater than 50%, compared to 25% without the replaced capsid [27].

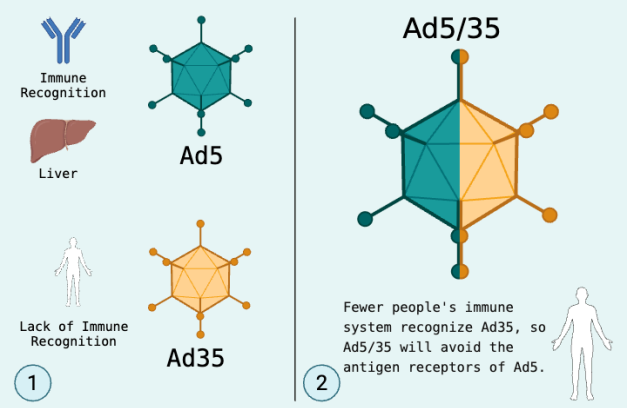

Fig. 3. Ad5/35 Vector Diagram. Ad5 targets the liver extremely efficiently and with a large payload. Many people have antibodies which renders it useless as it never reaches the cell. The proteins on Ad35 bypass the immunoreceptors by changing the capsid protein structure.

Kul et al wanted to address Ad5's failure to enter Purkinje cells in the brain [28]. Many neurological disorders require large payloads that only adenovirus could fulfill. To overcome this, they swapped the external fibers of Ad21, Ad25, and Ad50 to see if the resulting chimera could bypass the immunoreceptors. All three trials were able to successfully enter the cells. Swapping the external fibers of adenovirus is a promising solution to increase the potential usages of adenovirus (see Table 3)

Table 3. Adenovirus by Serotype Chart with Chimeric Variants

Serotype	Tropism	Pre-Existing Immunity	Efficiency
Traditional Adenovirus Serotypes			
Ad 5	Respiratory Tract, Liver, CNS	High	High
Ad 21	Respiratory Tract, Endothelial Cells	Varies	Moderate-High
Ad 25	Tumor Cells, Liver, CNS	Varies	Moderate-High
Ad 26	Stem Cells, Hematopoietic Cells	Low	High
Ad 35	Endothelial Cells, Hematopoietic Cells	Low	High
Ad 50	Hematopoietic Cells, Liver	Varies	High
Chimeric Variant			
Ad5/35	Respiratory Tract, Liver, CNS	Low	Varies

AAV-Peptide Chimeras

Opposed to every vector discussed thus far, peptide vectors are non-viral. They involve a long chain of amino acids to facilitate transportation. These molecules offer a potentially safer alternative to viral vectors as a whole [29]. Moreover, non-viral vectors have been praised for their flexibility with regards to payload uptake and precise cellular targeting [30]. Peptides have one more significant advantage in their ability to target specific cells. Often, they are designed to bind specific receptors so they can only infect a certain type of cell, mitigating side effects. On the other hand, their application in in vivo experimentation has been poor thus far due to low efficiency.

AAV-Peptide vectors are built through peptide insertions into the virus which helps to evade antibodies and reduce the immune response [31]. Moreover, they use the binding attributes of the peptide to hit specific target cells. Bennet et al. developed AAV2.m8, which changes the surface of the vector by adding 7 amino acids specifically targeted towards the retina. A second advantage of AAV2.m8 is that the amino acids block many of the regions that are identified by antibodies against AAV2 (See Table 4).

Table 4. All Serotype and Chimeric Vector Chart

Serotype	Tropism	Immunogenicity	Efficiency
Traditional Vectors			
AAV 1	Skeletal Muscle, CNS, Lung, Retina, Pancreas	Moderate	Moderate-Low
AAV 2	Liver, Smooth Muscle, Skeletal Muscle, CNS	High	Moderate
AAV 5	Skeletal Muscle, CNS, Liver	Low	Moderate-High
AAV 8	Liver, Skeletal Muscle, Retina, CNS	Moderate	High
AAV 9	Liver, Heart, Brain, Lungs, Skeletal Muscle	Low	High
AAV 10	Liver, CNS	Moderate	High
Ad 5	Respiratory Tract, Liver, CNS	Very High	High
Ad 21	Respiratory Tract, Endothelial Cells	Low	Moderate-High
Ad 25	Tumor Cells, Liver, CNS	Low	Moderate-High
Ad 26	Stem Cells, Hematopoietic Cells	Moderate	High
Ad 35	Endothelial Cells, Hematopoietic Cells	Moderate	High
Ad 50	Hematopoietic Cells, Liver	Moderate	High
Chimeric Variants			
AAV 1/2	Target-Specific	Moderate	High
AAV 2/8	Liver, Skeletal Muscle, CNS	Low	High
Ad 5/35	Respiratory Tract, Liver, CNS	Low	Varies
AAV2.m8	Retina, CNS, Liver	Low	Very High

Chimeric vectors clearly have numerous applications that have already begun to be implemented in practice (see Table 4). AAV serotypes have been combined to effectively target new cells with different capsid proteins, to lower immunogenicity, and to make the purification of vectors easier. Adenoviral serotypes were combined to target different cells and improve

transduction rate in order to justify a greater immunogenic response. Lentivirus and AAV were combined to bring lentiviral integration with a reduced immune response. Lastly, peptides can be added to the outer ring of the capsid to escape antibodies in the body and to target specific cells better. Overwhelmingly, weaknesses of each virus are being overcome by chimeric vectors.

Inducible Viral Vectors

Inducible viral vectors are a new method that uses spacio-temporal awareness to improve efficiency and targeting. Inducible viruses use external stimuli outside of the vector to dictate its expression. Often this is done to target specific cells or tissues to avoid collateral damage. A new level of precision could allow viruses to be used in significantly smaller amounts, lowering risk.

Many immunogenicity concerns could potentially be mitigated if vectors were precise and higher efficiency. Efficiency, a major concern, could be largely improved upon with a level of spacio-temporal awareness. A more controlled targeting system would require less virus to achieve a similar therapeutic result.

Inducible vectors contain regulatory elements that respond to a specific stimulus, such as small molecules, light, or temperature. They are typically built by integrating DNA sequences which encode stimulus-responsive elements such as ligand-binding domains, synthetic promoters, or riboswitches. When the plasmid is initially created, these sequences are inserted in front of the gene to regulate it [32]. When a stimulus satisfies the initial prerequisite, the vector is activated.

miRNA Regulated Vectors

MicroRNAs (miRNAs) are small RNAs that bind mRNA, blocking the expression of certain genes [33]. miRNA can be used in viral vectors as well to block expression of the added genome in some tissues. ‘A miRNA regulated vector contains DNA with short miRNA target sequences that miRNAs in the body will bind to and inhibit transgene expression (see Fig. 4). The target sequences chosen in the transgene align with specific miRNAs that are located in different tissues. Therefore, the inclusion of them can block transgene expression in particular tissues where the transgene is not needed.. miRNAs are often tissue specific and naturally regulated, making them ideal regulatory switches for broad use.

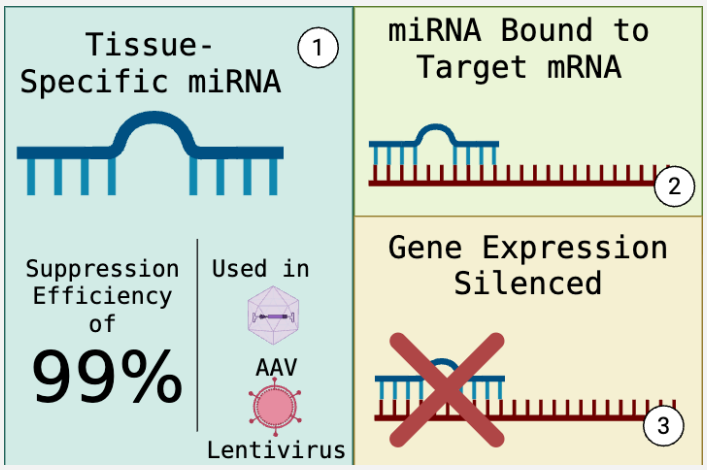

Fig. 4. miRNA-based Silencing. Fig. 4. visualizes miRNA targeting. The blue single-stranded RNA is miRNA. The broad use and effectiveness then explains the silencing of a gene corresponding with the pairs in the used miRNA. 99% of genes are suppressed with miRNA and it is used in multiple different vectors including AAV and Lentivirus.

miRNA is incredibly effective with regards to targeting and accuracy, with a suppression efficiency of 99% in unwanted tissues [34].

Its application is also broad, enabling use in many types of viruses. It initially was used with lentivirus only but has expanded to AAV, adenoviral vectors, and even oncolytic viruses that are specifically geared towards infecting cancer cells. [35]. In terms of disease areas, this technology has been applied to cancer therapy, neurodegenerative disorders, and liver disease.

So et al. used miRNAs in AAV to improve targeting and clarify tropism [19]. AAV9 often hits peripheral tissue including the liver and heart while attempting to enter the brain, which both reduces the intended effect in the CNS and comes with a variety of side effects. The goal was to repress transgene expression in peripheral tissues using miRNAs.

First, they selected their miRNAs, choosing miR-122, which blocked expression in the liver, and miR-1, which blocked expression in cardiac and skeletal muscle tissue [36]. They engineered a version of AAV9 that included target sequences for one of these miRNAs. While both miRNAs proved to be effective and showed reduced expression, miR-122 showed exceptionally reduced expression in the liver. Moreover, no change was found in CNS expression, which meant functionality was preserved in the target tissue. This was a pivotal proof-of-concept for detargeting.

Tet-On Systems

Tet-On has been the long-time standard for inducible viral vectors. There are two primary components: reverse tetracycline-controlled transactivator (rtTA) and tetracycline response element (TRE). rtTA is a protein that binds DNA at a TRE sequence, specifically in the presence of the drug doxycycline (Dox) [37]. In the presence ofDox, the rtTA protein activates expression

of the target gene (see Fig 5). Once Dox is unbound from the rtTA, the cell stops expressing the gene.

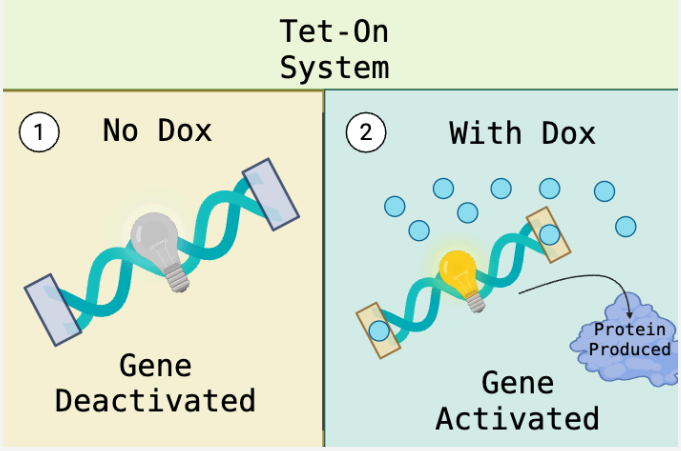

Fig. 5. Tet-On Based Activation. The blue circles represent the antibiotic doxycycline. The presence of the drug recruits proteins which activate the gene. The gene is represented by the DNA and activation is denoted with the lightbulb. When Dox is not present, the gene is inactive.

Still, there are notable risks: Dox often can have off-target effects, since it is an antibiotic. Alterations in the gut have been observed [38]. There may be a trade-off despite the precise genetic control. Still, it has been used in diverse applications with positive outcomes.

S. Goverdhana et al. highlighted the Tet-Off system as an alternative to Tet-On [39]. In Tet-Off, the rtTA normally binds the Tet response element (TRE) to express the gene. However, when Dox is present, it binds to rtTA, preventing its interaction with the TRE and thereby switching off gene expression. This offers a precise "off" switch for gene therapy applications. An advantage of this approach is that continuous drug dosing needs to be administered only when turning expression off. This may reduce many qualms with the drug-related side effects.

Other Chemically-Induced Vectors

The Tet-on system is the most widely used form of molecular-induced vectors, but there are others that are prevalent as well. Komatsu et al. attempted to design a failsafe switch using a Sendai virus [40]. The vector was engineered to both deliver a therapeutic transgene like normal and carry a gene called HSV-TK as a built-in safety feature. Under normal conditions, the gene is expressed no different than any other viral vector. On the other hand, if the drug ganciclovir (GCV) was administered separately, the vector turns HSV-TK into a toxic compound within the cells. The HSV-TK works as a suicide gene which will kill the cell once in contact with GCV which will be administered if sever side effets occur. In the absence of the inducer, GCV, the gene is simply expressed as normal.

Kim and Yokobayashi sought to create a safer method of introducing genes into embryonic stem cells - a method that avoided genomic integration that could be chemically turned on and off. They used a riboswitch based system stimulated by guanine (GuaM8HDV) [32]. A riboswitch is a regulatory segment of RNA that can change its structure when it binds to a certain molecule controlling whether the downstream gene is expressed. This means it is part of the RNA sequence in the vector similar to Tet-On. But the guanine, in particular, acts as an "off" switch. When the guanine is absent, the RNA takes a specific shape and deactivates the gene.

S. Cheng et al. also attempted to refine the AAV-Gene-Switch system. This system relies on mifepristone, a synthetic steroid, as the inducer. An initial draft of this system included variable responsiveness and inconsistencies in their "on" and "off" states [41]. When mifepristone is present, the system is activated. When this occurs, the system results in a therapeutic protein made by the cells. When mifepristone is removed expression turns off and reverts to its baseline state.

Light-Induced Vectors

Light offers a unique solution compared to chemical inducers, which offer temporal control but do not have innate spatial precision. Light can be used to guide the vector spatially without the need for any external control, and has the benefit of reduced side effects.

Light induced vectors rely on light-sensitive proteins that change shape based on the light that hits them [42]. Different wavelengths of light are absorbed by different tissues, which determines how the proteins act in relation with various spacious conditions. For example, blood absorbs blue and green light [43]. The shape then controls gene expression in the same way binding a protein onto RNA works in Tet-On and the molecular-induced vectors.

Hörner et al looked at controlling the delivery of AAV by red light down to an individual cell [44]. Normally, gene delivery is specified to an entire organ, whereas this methodology can differentiate individual cells. Unlike general light-induced viral vectors, the Opto-AAV System that was used makes the AAV itself light sensitive. Light can be shone from outside the body with a laser or LED to pinpoint the location where it needs to be expressed. The spacio-temporal control achieved makes this vector extremely effective in vivo for applications like targeting neurons in the brain precisely, selectively correcting diseased cells, or specifically looking at cancerous cells.

Wang et al attempted to create a lentiviral vector that could be activated or deactivated based on exposure to ultraviolet (UV) light (see Fig. 6) [45].

Fig. 6. UV-Light Based Activated Lentivirus Diagram

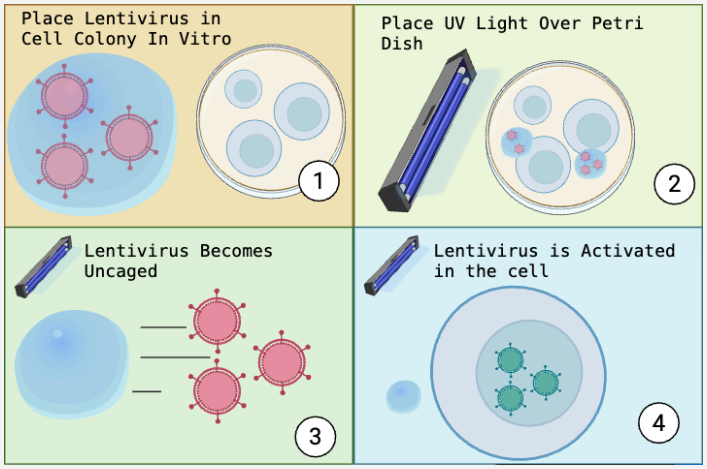

Fig. 6. UV-Light Based Activated Lentivirus Diagram. It lays out the exact procedure for using UV-light based activated lentivirus. THe black and purple lighting strip is the UV light. The bubble from which the lentivirus escapes notes the caging and uncaging of lentivirus. The green lentivirus shows its activated state versus the red deactivated lentivirus.

They were successfully able to separate the lentivirus from the cells using a caging technique. Caging involves encapsulating lentiviral vectors within a protective material to shield them from immune clearance and enable controlled, localized gene delivery. Once the caged vectors reach the target tissue, the light of a specific wavelength, removes the cage. Moreover, photo-switchable lentiviral vectors enable precise temporal control on top of spatial control.

Inducible viral vectors can take many forms, all achieving unique goals while reiterating similar patterns. The spatio-temporal control achieved by these viral vectors is revolutionary. These applications have already had significant successes, from the efficiency of miRNA repressing transgenes in unwanted tissues to light-induced vectors targeting at a single cell resolution. Despite some risks being associated even with the studies presented such as introducing antibiotics, inducible vectors overall improves the efficiency and effectiveness of each viral vector.

Discussion

Across these systems, research has focused on improving control over gene expression by addressing challenges such as toxicity, immunogenicity, off-target effects, limited flexibility, and innate antibodies. There are many benefits of chimeric and inducible vectors, including avoiding or catering to certain organs such as the liver, spine, brain, and retina. Chimeric

vectors are especially effective at this. They also can bypass the immune response by escaping antibody detection, which is a key challenge with any viral gene therapy.

Alternatively, when a controlled dosage of a vector is needed, inducible vectors are a favorable option. Controlled inducers such as small molecules or light can be used to target vectors to certain areas very precisely or it can control the actual dosage given using an on/off command.

Limitations

There are practical concerns that limit the utility of these vectors, despite their promise. In preclinical experiments where only a small volume of virus is required, there is no practical limitation regarding scalability. However, bringing these next-generation vectors to market has considerable limitations associated with it.

Even in its current form, viral vector production is extremely costly and labor intensive. The current per-dose costs for AAV therapies often exceed $10,00,000 putting a huge burden on governments, especially in developing countries, or insurance companies [46]. On the other hand, the size of the dose may decrease with these new vectors due to the improved efficiency. Manufacturing costs may increase due to the increase in vector complexity, however.

In regards to manufacturing, the environments in which viral vectors are made must be extremely controlled, and there are very few recognized facilities that are authorized to produce these for human trials. The upfront cost for a company often adds up to 10 million dollars to even get a facility [47]. Inducible systems that require a second drug product like Dox add further costs. Though Dox itself is quite cheap and FDA approved, it is an additional component which must undergo approval, be manufactured, and be shipped.

Additionally, regulatory agencies such as the FDA and EMA remain cautious toward synthetic control mechanisms and require comprehensive evaluation of safety, efficacy, and long-term stability, due to concerns over safety, off-target effects, and long-term stability [48]. Moreover, nonclinical safety studies often reveal uncertainties around the long-term effects and off-target activity of gene regulation technologies, complicating the pathway to approval and requiring extensive preclinical validation [49]. These challenges may extend development timelines and increase costs, highlighting the need for new strategies that can satisfy regulatory bodies.

Alternative Applications of New Viral Vectors

While this review focused on the use of viral vectors in gene therapy, their use is not limited to that. For example, Tet-On systems have been used in cell therapy. CAR (chimeric antigen receptor) T-Cell therapy, a treatment for blood cancers such as leukemia and lymphoma [2]. CAR T-Cell therapy works by taking out immune cells of the body, editing them to recognize specific cancer cells, then multiplying them in lab to finally put them back in in greater amounts. CAR-T is a very effective technology when it comes to combating large-scale projects and illnesses in the body.

Yet, notably this has come with a few side effects including neurotoxicity and the release of cytokines. When CAR T-Cells attack healthy cells, these side effects come about, which underscores the need for more effective targeting or a fail-safe mechanism. The latter is used to ensure that the process can be reversed in the event that there are severe side effects.

Gu et al. designed a system where the gene that recognizes cancerous cells is only expressed once Dox is administered. Without Dox, there was nearly no expression of the CAR

gene. With Dox, the CAR proteins were expressed, leading to killing of the cancer cells. Additionally, the CAR genes were significantly more precise which emphasizes the success of the on/off component.

Specialized viral vectors can also be used as vaccines. In past trials, vaccines have been largely inefficient when using large vectors such as adenovirus. Efficiency rates could be improved upon with adenoviral based inducers. In individuals who are immunocompromised, a controlled dose through vaccines which can be turned on and off may alleviate side effects and make life-saving vaccines more accessible.

Conclusion

Chimeric and inducible vectors have begun to see use in preclinical research, but the ability to expand is critical. The ability to scale these methods and demonstrate efficacy in human trials is now required in future research. Moreover, future research must evaluate whether the added complexity of chimeric and/or inducible designs translate to measurable advantages.

Despite the theoretical advantages, it may be possible some additions provide unsatisfactory or minimal positive change. While inducers have shown spacio-temporal control, it is unclear how the vector will react with a foreign inducer. Patients may also experience new effects due to the combination of multiple treatments. In experiments thus far, minimal side effects have been observed, but long-term monitoring will be required to ensure safety. Human trials will have significantly tighter regulation which may make testing not completely feasible.

Particular attention should be given to diseases that demand precise regulation of gene activity. In cancer therapy, for example, inducible expression of cytotoxic genes can limit damage to healthy tissues, while in autoimmune disorders, the ability to transiently modulate immune signaling could reduce systemic inflammation. Rare genetic diseases, such as enzyme deficiencies or metabolic disorders, often require tightly titrated therapeutic protein expression, where both the timing and tissue specificity of vector activity are essential for efficacy and safety.

Chimeric and inducible systems have been proven to have meaningful improvements on the current limitations of viral vectors by, for example, bypassing antibodies and specifying tropism. If we can address their remaining limitations, they may prove to be the future of viral vector therapy in specialized fields or even, potentially, in mainstream use.

Figures Created in https://BioRender.com

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