Risk-Benefit Analysis of the use of Viral Vectors in Gene Therapy

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Abstract

Introduction: The Food and Drug Administration (FDA) is advised by the Recombinant DNA Advisory Committee (RAC) on approvals for gene therapy clinical trials. The RAC reviews the protocols, analyzes the risks and benefits, and reports to the FDA if the ratio of risks to benefits is appropriate for approval.

Materials and Methods: Gene therapy is a procedure that is aimed at replacing, manipulating, or supplementing nonfunctional or malfunctioning genes with a normal copy of the gene. The gene is transferred to the target cell by a vector; the most common vector is the viral vector. The viral vector is based on common replicating viruses. The Adenovirus, Adeno-Associated Virus, and the Retrovirus are the most commonly used vectors in gene therapy clinical trials.

Results: Disadvantages to using the Adenovirus as the vector in gene therapy include non-integration, immunogenicity, replication competence, no targeting, and small insert size. Benefits and advantages to using the adenovirus as a vector in gene therapy include high transduction efficiency, infection of many cell types, and transduction does not require cell division. Risks and disadvantages of the Adeno-Associated Virus include integration, decline in expression over time due to episomal loss by degradation, small packaging capacity, low titers, and a strong immune response. Benefits and advantages of using the Adeno-Associated Virus as the vector in gene therapy trials include integration into host genome, no viral genes, able to transduce cells not actively dividing, and they are non-inflammatory and non-pathogenic. Disadvantages and risks of using the retrovirus as a viral vector in gene therapy include low transduction efficiency, replication competence, insert size, integration, inactivation by complement cascade, and the requirement of cell division for transduction. Benefits and advantages of using the retroviral vector include integration into the host genome, requirement of mitosis for efficient transduction, and no toxicity associated with viral proteins.

Discussion: The risk to benefit analysis is a subjective review of the risks and potential benefits of the procedure/treatment. The risks associated with using the viral vector in gene therapy trials are significant. The potential benefits of the viral vector is amazingly life saving and while they have not been fully realized they are bright on the horizon. Gene therapy researchers are learning from the past trials and tribulations and each new study is showing more and more progress with safety and long term benefits with the use of viral vectors. The risks of using the viral vector in gene therapy do not outweigh the potential benefits.

Introduction

The Food and Drug Administration (FDA) has the job of protecting the public health by assuring the safety and effectiveness of the drugs, devices, and biologics that are used to treat diseases and disorders. Before the FDA will approve a drug/device/biologic for entrance into clinical trials the sponsor must demonstrate that it has a favorable risk to benefit ratio. A benefit is defined as a valued or desired outcome or an advantage. A risk is the probability of harm or injury whether it is physical, psychological, social, or economic that occurs as a result of participation in a research study. The risk in a clinical research trial is evaluated on a scale from minimal to significant. Minimal risk is where the probability and magnitude of harm or discomfort anticipated in the trial are not greater, in and of themselves, than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.[1]

There are many points to consider when analyzing the risks and benefits of a clinical trial. The first and most important is to accurately identify, evaluate, and describe both the risks and anticipated benefits. The next would be to determine where the risks fall on the scale from minimal to significant. It is also important to separate the procedure from the therapeutic agent and analyze its risks and benefits independently. On October 7, 1974 the National Institute of Health (NIH) established the Recombinant DNA Advisory Committee (RAC) in response to public concerns regarding the safety of manipulating genetic material through the use of recombinant DNA techniques.[2] Since its establishment it has served as a federal advisory committee in the approval of gene therapy clinical trials and has been instrumental in analyzing the risks and benefits associated with the proposed trials.

Materials and Methods

Gene Therapy

Gene therapy is a procedure that is aimed at replacing, manipulating, or supplementing nonfunctional or malfunctioning genes with a normal copy of the gene. The therapeutic gene is inserted into the target cell by use of a vector. Vectors that have been used include viruses, liposomes, and naked DNA and RNA molecules. Viruses are the most commonly used vectors as is evidenced by their use in 66.2% of all clinical trials. (Chart 1)

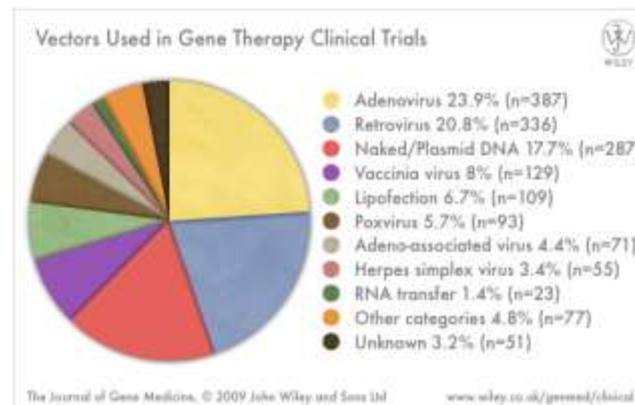

Chart 1: This pie chart shows the percentage of clinical trials in which each vector has been used. [3]

A viral vector is a delivery system that is based on replicating viruses that have the ability to deliver genetic information into a host cell.[4] The virus is a strict intracellular parasite that can only replicated within a host cell and its life cycle is what makes it an ideal candidate for use as a vector. The viral life cycle includes attachment, penetration, uncoating, macromolecular synthesis, and release. A virus attaches to a host cell by recognition of receptors on the cells surface. This receptor recognition system determines the type of cell that the viral vector can transduce since not all body cells have the same receptors at the same levels.

Penetration is the process by which the virus enters the cell; this can be achieved by membrane fusion or endocytosis. Membrane fusion penetration occurs when the viral envelope fuses with the host cell membrane allowing the contents of the envelope to enter the cell. In the

endocytosis method of penetration the virus recognizes receptors on the cell's surface, attaches to these receptors, and becomes engulfed by the cell. Membrane fusion is the method of entry for enveloped viruses, such as the retrovirus. Endocytosis penetration is favored by the non-enveloped viruses, such as the adenovirus or the adeno-associated virus. Once inside the host cell uncoating of the virus occurs. A process necessary for the release of the viral genome and occurs before the viral DNA or RNA can be delivered to the intracellular replication site.

Production of the nucleic acid and protein structures are included in the macromolecular synthesis phase. This includes replication of the viral genome and synthesis of viral structures. The next phase of the viral life cycle is viral assembly where the viral genome gets packaged into the capsid. The enveloped viruses attain their envelope from the host cell membrane through a process called budding. The final phase is the release of the newly formed viruses from the host cell into the organism to travel to and infect other cells and repeat the cycle. Enveloped viruses bud from the cell membrane while the non-enveloped virus lyses the host cell.

This final phase of the viral life cycle is not conducive to most gene therapy protocols. In order to maintain expression of the transferred gene the virus must be modified so that it remains in the latent infection model. Modification starts with the removal of all or some of the viral coding DNA and insertion of the therapeutic gene. The genes that were removed from the wild-type virus that are involved in replication or capsid/envelope proteins are inserted into a packaging construct to assist in trans. The packaging cells are co-transfected with the vector genome and packaging construct to produce recombinant vector particles which can be purified and quantified.[5] (Figure 1)[6]

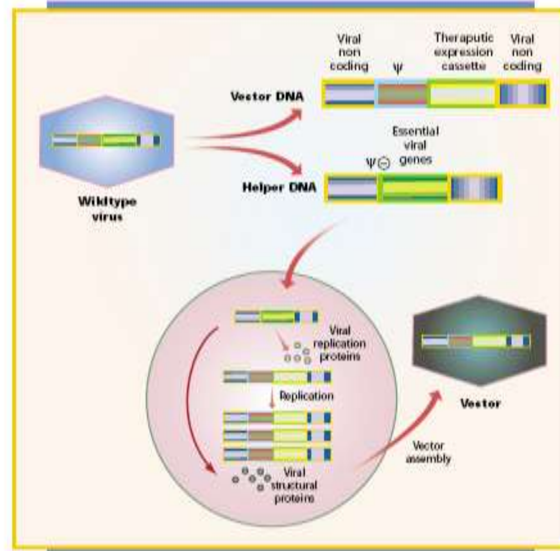

Figure 1: Schematic of the procedure for turning a wild-type virus into a vector for gene therapy.

Adenovirus

The adenovirus is a small non-enveloped icosahedral virus that contains a linear double stranded DNA genome. The coding region of the genome is flanked by inverted terminal repeats (ITRs) and contains two sets of transcription regions. The early transcription region contains five gene regions: E1A, E1B, E2, E3, and E4. The single late transcription region generates a family of five late mRNAs (L1-5). The early and late transcription regions correlate to the two phases of Ad replication cycle. In the early phase the viral DNA gets transported to the nucleus where transcription of the early genes is initiated. The gene products of this phase interfere with the host cell's antiviral defense mechanism and direct the cell to enter into mitosis. The induction of the cell cycle signals the start of the late events in Ad replication. The late phase is characterized by an increase in the gene expression of mRNA regulated by the late promoter resulting in high production of structural proteins and assembly of new virions. The induction of cell lysis releases these virions from the cell.[7] (Figure 2)

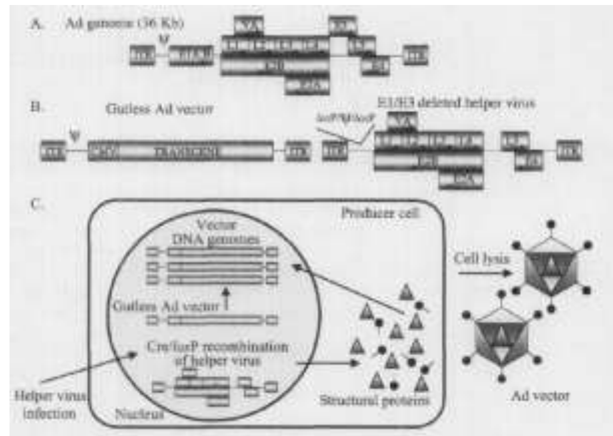

Figure 2: A. Schematic of the genome of a wild-type Adenovirus. B. Schematic of the genome of a gutless Adenoviral Vector and the genome of the helper virus. C. Schematic of the production of the Adenoviral Vector.[7]

Adeno-Associated Virus

The adeno-associated virus (AAV) is a small non-enveloped virus from the family parvoviridae, subfamily dependovirus. The genome is a linear 4.7 kb single-stranded DNA molecule that has two open reading frames, rep and cap. Cap encodes the structural proteins that form the capsid while rep encodes the regulatory proteins. AAV enters the cell through endocytosis and the DNA transported to the nucleus after uncoating. Once in the nucleus the single-stranded DNA is replicated to form double-stranded DNA which then integrates by non-homologous recombination into a specific location on chromosome 19. AAV will remain in a silent or latent infection profile for the life of the cell. The adeno-associated virus is non-pathogenic and cannot replicate independently. However, replication and dissemination of the virus can occur in the presence of a helper virus. It is the gene products of this unrelated helper virus, usually adenovirus or herpes virus, which promotes the replication of the adeno-associated virus.[7] (Figure 3)

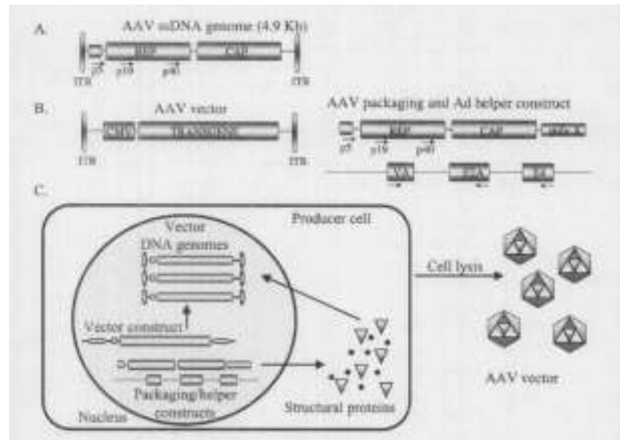

Figure 3: A. Schematic of the genome of a wild-type Adeno-Associated virus. B. Schematic of an Adeno-Associated viral vector and the genome of the packaging and helper construct. C. Schematic of the production of the Adeno-Associated Viral Vector.[7]

Retrovirus

The term retrovirus refers to any of a family of viruses that has an RNA genome and uses the enzyme reverse transcriptase during its life cycle. These viruses are enveloped and contain 2 copies of the single-stranded RNA genome in an icosahedral capsid. The genome contains at least three genes (*gag*, *pol*, and *env*) which are flanked by long terminal repeats (LTRs). The *gag* gene encodes the core proteins, capsid matrix, and nucleocapsid. The *pol* gene encodes the enzymes protease, reverse transcriptase, and integrase and is usually derived from the gag-pol precursor. The *env* gene encodes the envelope glycoproteins that mediate cell entry. Cell entry is achieved by the membrane fusion method. The ssRNA genome, upon uncoating, is converted into double stranded proviral DNA by reverse transcriptase. The proviral DNA is the transported to the nucleus where it is randomly integrated into the host genome mediated by the enzyme integrase. The host cell's transcription factors initiate transcription of the integrated genes and new viral particles are formed. The precursors of *gag-pol* and *gag* assemble together with the two copies of viral single stranded RNA while the *env* glycoproteins are incorporated into the

membrane during the budding process. Virus maturation occurs when the enzyme protease processes the *gag* and *gag-pol* precursors. (Figure 4)

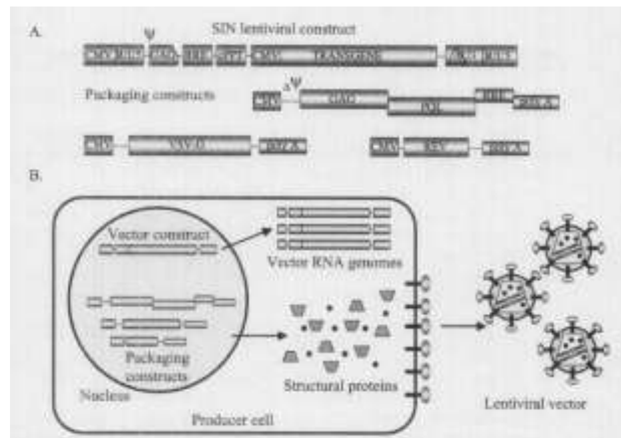

Figure 4: A. Schematic of the HIV-1 based Lentiviral Vector genome and the genomes of the packaging constructs. B. Schematic of the production of the Lentiviral Vector.[7]

This large family of viruses can be sorted into three classes: oncoretroviruses, lentiviruses, and spumaviruses. The oncoretrovirus is the simplest of the three and contains only the gag, pol, and env gene flanked by the LTRs. Examples of oncoretroviruses include the murine leukemia virus (MLV), spleen necrosis virus, Rous sarcoma virus, and avian leucosis virus. The lentiviruses and spumaviruses contain additional viral genes making them more complex than the oncoretroviruses. In the lentivirus sub family there are three to six additional viral proteins that are essential to the replication of the virus and persistence of the infection. Only two of the additional genes, tat and rev, are present in all lentiviruses. These proteins mediate the transactivation of viral transcription and nuclear export of unspliced viral RNA, respectively. An example of a lentivirus is the human immunodeficiency virus type 1. The spumaviruses are also called foamy viruses and contains three open reading frames (ORFs) in addition to the structural proteins. These three ORFs include tas/bel-1, bel-2, and bel-3. The ORF tas/bel-1 has been identified as a co-activator of viral transcription.[7] (Figure 5)

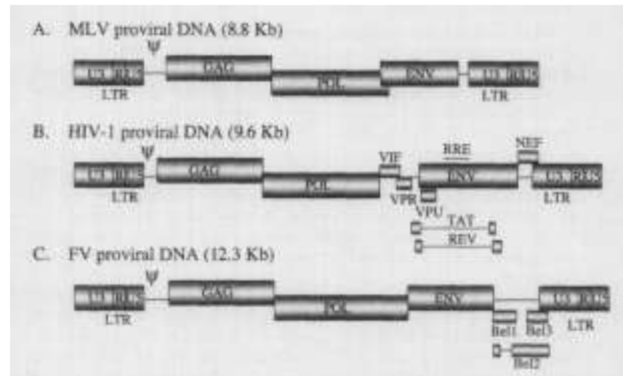

Figure 5: A. Schematic of the genome of the Murine Leukemia Virus (MLV) genome. B. Schematic of the HIV-1 genome. C. Schematic of the Foamy Virus (FV) genome.[7]

Results

<u>Adenoviral Vector</u>

Risks

Disadvantages to using the Adenovirus as the vector in gene therapy include non-integration, immunogenicity, replication competence, no targeting, and small insert size.[8] The Adenovirus genome remains as an episome and does not integrate into the host cell genome. Thus making its effects transient as it is not passed on to daughter cells during mitosis. This also necessitates continuous readministration over the course of a long period of time, possibly as a maintenance drug. Readministration can lead to the development of an immune response. Immunogenicity is the biggest problem with the Adenoviral Vector as it is ubiquitous in the natural population. Also because it can be found in nearly all patients it is possible for the virus to recombine with viral genome existing in the cell to form replication competent virus particles that can then cause a systemic infection in the patient.

The Adenovirus can infect numerous types of human cells which can be both advantageous and disadvantageous because this makes it possible to transduce the wrong cell type. Unintended transduction can cause cell toxicity and cell death. It would also be possible for

the Adenovirus to infect germ line cells which would pass any changes on to the patient's offspring which is prohibited by the FDA. The genome of the adenovirus is approximately 35 kb and a packaging capacity of 8 kb in replication defective vectors up to 30 kb in helper dependent vectors. The average human gene is approximately 3000 base pairs however it varies significantly and can contain up to 2.4 million base pairs (dystrophin). Cystic Fibrosis and Hemophilia are two single gene mutation diseases that are being researched for possible gene therapy trials. Cystic Fibrosis Trasmembrane Conductase Regulator (CFTR) is a 230 kb gene that upon mutation causes the disease Cystic Fibrosis (CF).[9] Hemophilia A is caused by mutations in the F8 gene which spans 186 kb of genomic DNA and has a coding region of 9.1 kb.[10] These are just two examples of how large a human gene can be compared to the packaging capacity of the viral vector.

Gene therapy using viral vectors had its first major setback in 1999 when 18 year old Jesse Gelsinger died as a direct result of the adenovirus that was used to deliver the gene. Jesse had a mild form of ornithine transcarbamylase (OTC) deficiency which is a disorder of nitrogen metabolism which was being treated with a drug and diet regimen. On September 13th 1999 Jesse received an injection of $3.8x10^{13}$ adenoviral vector particles, the largest dose in a gene therapy trial. He died four days later from a systemic inflammatory immune response that caused fever, disseminated intravascular coagulation (DIC), and multiorgan failure.[11]

The immune response seen in Jesse's case is not the only type of response that is possible. In a study evaluating the osteoinductive activity of an adenoviral vector carrying the BMP-2 gene expression of the transgene was only detected in nude, or immunosuppressed rats. Osteoinduction was not seen in the normal immunocompetent population of rats due to a T cell immune response. The immune response did not lead to a systemic life threatening condition as

in the Jesse Gelsinger case however it prevented the adenoviral vector from reaching its target cell.[12]

Researchers have attempted to harness the cytotoxic effects of the wild-type adenovirus by modifying it to conditionally replicate only in tumor. Theoretically, upon infection of the tumor cells the normal life cycle of the virus will take over the cell leading to cell lysis and death of the tumor cell and subsequent infection of the surrounding tumor cells. Onyx-015, an E1b attenuated adenovirus, was injected directly into the tumor of patients with recurrent head and neck cancers in a phase I clinical trial.[13] Nearly all of the patients in the trial (21 of 22) showed an increase in the neutralizing antibody to the adenovirus and only 4 of the 22 patients showed viral replication. No objective responses were observed in any of the patients. These negative results were a result of limited intratumoral spread of the replicating virus due to the presence of the neutralizing antibody.

Human physiology has also been a limitation in the systemic use of adenoviral vectors. The virions have a short half life in the human body due to removal from circulation by the Kupffer cells of the reticuloendothelial system.[14] The Kupffer cell is the macrophage of the liver; it takes up foreign particles that pass through the liver including viruses, toxins, and drugs. Unfortunately, the uptake of the vectors by the Kupffer cells does not result in transgene expression thus decreasing the overall efficiency of the therapy. Additionally, the reticuloendothelial system is part of the immune system and contributes to the innate immune response to the adenoviral vector particles.

Benefits

Benefits and advantages to using the adenovirus as a vector in gene therapy include high transduction efficiency, infection of many cell types, larger packaging capacity, and transduction

does not require cell division.[9] In comparison to other viruses used as vectors, the Adenoviral vector has the largest packaging capacity. Transduction of non-dividing cells is a significant advantage to the use of the adenoviral vector. The majority of cells in the human body are not actively going through cell division, making it disadvantageous for vectors that can only transduce during mitosis. The ability to transduce non-dividing cells is advantageous for the adenovirus because of its inability to integrate into the host genome. In cells that are actively dividing the transgene is often lost because it is not replicated and passed on to daughter cells.

Production of the adenoviral vector can be done with little manipulation and significantly high titers can be achieved. However, it has also been shown that minimal levels of the virus can achieve high levels of transduction of cells and still generate efficient levels of gene expression. Adenoviral vector transgene expression has been documented in several different body tissues including muscle cells[12], pancreatic islet cells[15], respiratory tract epithelia cells[16], and even corneal epithelia cells.[17] Several animal studies have been conducted that were concluded to show safety and efficacy of gene therapy using the adenovirus and the gene delivery vector. Two examples are detailed below.

The Adenovirus has also shown some antitumor effectiveness. In an animal safety and efficacy study on Adenovirus-mediated Interleukin-24 expression in a mouse model showed retarded tumor growth and reduced microvessel density (MVD) and vascular endothelial growth factor (VEGF) expression in tumors. The response seen by the mouse tumors in this study was elicited by Ad-IL-24 and was an induction of apoptosis in the laryngeal cancer cells and inhibition of tumor angiogenesis. Researchers in this study concluded that the Adenoviral vector's potent yet selective killing activity of the cancer cells while sparing the normal cells makes the vector a candidate for cancer gene therapy in humans.[18]

The third most common disease and the fourth leading cause of death in North America is Diabetes. Treatment can range from diet and exercise for borderline Type II diabetics to daily insulin injections for Type I diabetics. Permanent destruction of the insulin-producing beta cells is the characteristic presentation of Type I diabetes. One option for insulin-dependent patients is transplantation of the pancreas. As with any major organ transplantation a regimen of immunosuppressant drugs must be taken for the lifetime of the patient. Transplantation of pancreatic islet cells is safer and less invasive yet has been hindered by graft malfunction and failure. Researchers in an animal study have tested a gene therapy technique involving an adenovirus-mediated TNF (tumor necrosis factor)-related apoptosis-inducing ligand (TRAIL) gene delivered into pancreatic islets to attempt to resist autoreactive T cell assault.[15] The researchers concluded that TRAIL over expression in pancreatic islets extended the allograft survival and function and lead to a therapeutic benefit in the rats.

Adeno Associated Virus

Risks

Risks and disadvantages of the Adeno-Associated Virus include integration, decline in expression over time due to episomal loss by degradation, small packaging capacity, low titers, and a strong cell-mediated immune response. The Adeno-Associated virus is a small virus with a primitive genome that contains only 2 genes. Thus the packaging capacity is also extremely small, approximately 4 to 5 kb. Adeno-Associated Viral vectors are produced in packaging cell lines with the assistance of helper viruses, such as the Adenovirus. This process is inefficient as it does not yield high titers and is frequently contaminated by the helper virus.[8] Titers of AAV vectors seen rarely exceed 10^4 viral particles. Contamination with the helper virus may also lead to an immune response, especially in cases where the adenovirus is the helper. Another major

barrier to the use of the AAV vector is inefficient transduction due to necessary conversion of the single-stranded DNA genome into a double stranded molecule. This not only delays the integration and/or expression of the transgene it can also lead to mutations in the newly formed strand that can lead to an ineffective protein or neoplastic proliferation.

As discussed in regards to the Adenoviral vector, transgenes that remain as episomes instead of integrating into the host genome are at the risk of being lost or degraded when that cell undergoes mitotic division. This is not only a disadvantage of using the AAV as the vector it is also a risk to the patient. If the transgene is lost the cell stops producing the normal protein and the disease or disorder can recur. This necessitates the switch to another form of therapy or readministration of the vector carrying the transgene. In the case of the AAV vector, readministration may cause a cell mediated immune response if memory cells were developed against the vector.

The Adeno-Associated Viral vector is ubiquitous as evidenced by the fact that 80 – 90% of the population becomes infected with it during childhood. It is also thought to be innocuous because it usually remains in the latent infection state unless in the company of a helper virus and has only 2 viral genes. However, 29 different adverse events have been reported in 12 US clinical trials using the Adeno-Associated Virus as the vector for gene delivery, according to the RAC.[19] It has been discovered that while the Adeno-Associated Virus does not cause a humoral immune response as is seen in the Adenovirus it can lead to a cell-mediated immune response.

An animal study conducted to evaluate the use of the Adeno-Associated Virus serotype 7 in gene therapy of the liver. This study was conducted using primates to evaluate the relevance of results seen in the murine liver. A vibrant cytotoxic T cell response to the expressed green fluorescence protein (GFP) that was correlated with hepatitis and loss of expression was

observed in the primate trial that was not seen in the murine trials. It was concluded that under some conditions the primate immune response may react with more aggressive T cell response to the transgene products than was observed in the mice.[20] This would lead a researcher to question the response that might be seen in human patients. Since the human is more physiologically similar to the primate than to the mouse one could hypothesize that the T cell response would emulate that seen in the primate.

Integration can cause a problem in gene therapy if the transgene integrates into an active gene or in a region that can promote the activation of an oncogene. The wild-type AAV has a targeted integration at a site on chromosome 19, however upon removing of the rep gene to insert a therapeutic gene the targeted integration is lost. A recent study has shown that recombinant Adeno-Associated Viruses (rAAV) have a preference for integrating into functional genes. This could be considered an evolutionary tactic developed by viruses because integration into an active gene would most likely ensure replication and spread of infection.

The integration of the rAAV is frequently associated with chromosomal rearrangements and deletions of large segments of the DNA. The integration of the rAAV genome is thought to occur through non-homologous end-joining (NHEJ) of the rAAV free ends to broken chromosome ends that has lead researchers to question if the rAAV causes the breaks or merely takes advantage of pre-existing breaks. Research has suggested that it is the latter of the two theories. Introduction of genotoxic agents that are known to induce double-strand breaks into cell cultures an increase in rAAV integration is observed. It has also been demonstrated that increasing the rAAV vector dose above a threshold level does not increase the number of integrations.[5]

Benefits

Benefits and advantages of using the Adeno-Associated Virus as the vector in gene therapy trials include integration into host genome, no viral genes, able to transduce cells not actively dividing, wide range of host cells, and they are non-inflammatory and non-pathogenic.[5 7] To produce an Adeno-Associated Viral vector, all viral genes are removed to allow room for the therapeutic gene. Removal of all genes creates a space for a larger insert and decreases the risk of an immune response to viral gene products. Transduction of non-dividing cells, as detailed in the adenovirus section, allows for continued expression of transgenes that do not integrate into the host genome. Adeno-Associated Viruses have the distinction of being the only wild-type virus used for gene therapy that is not pathogenic in humans. It is also by itself non-replicating and can only enter the viral life cycle in the case of co-infection with a helper virus.

Approximately 10% of in vivo transduction cases, the transgene will integrate into the host cell genome.[5] This is advantageous when transducing cells that are undergoing mitosis and allows for passing the gene on to daughter cells. The AAV integration is unique in that the wild-type virus will integrate at a specific site on chromosome 19. This feature is advantageous because it means that researchers would know exactly where the virus integrates. However, because this is mediated by the *rep* gene, once the gene is removed the targeted integration does not occur.[21] Leaving the *rep* gene in the vector would retain the targeted integration however it would decrease the insert size which is already quite small. Random integration can still occur even in the absence of the *rep* gene.

Researchers have developed ways to expand the delivering capabilities of the AAV vector. One of these methods is referred to as trans-splicing which is a dual-AAV vector system and involves splitting the transgene into two separate AAV vectors that splice together in the target cell. Researchers in an animal study have harnessed trans-splicing to treat mice with

Duchene Muscular Dystrophy. Body-wide transduction of muscle cells in normal neonatal mice was demonstrated using tsAAV serotype 9. Robust transduction was seen in the heart of these mice and the apparent lack of proactive cellular immune response suggests that the AAV vector may hold great promise for treating human Duchene cardiomyopathy and X-linked dilated cardiomyopathy. [22]

The Adeno-Associated Viral vector is capable of transducing several cell types. One of theses types of cells is the hepatocyte. An animal study was conducted to determine the ability of the Adeno-Associated Viral vector to transduce hepatic cells for use in treatment of diabetes mellitus. This research trial was conducted using diabetic mice which were infected with an AAV vector carrying a gene for insulin expression with a glucose initiated promoter sequence. When the promoter was activated by glucose in the mice's blood the gene for insulin secretion was transcribed. The rAAV efficiently transduced the hepatic cells and the transgene was subsequently expressed resulting in regulation of the blood glucose level in the diabetic mice. This study suggests that the rAAV has the potential for being an effective gene therapy treatment of diabetes mellitus.[23]

Another cell type that the AAV vector can transduce is the muscle cell as was demonstrated in a study designed to investigate the possibility of using gene therapy to inhibit wear debris-induced osteolysis. This is an animal study in which an AAV vector carrying the gene that encodes for osteoprotegerin (OPG) is injected into the muscle cells of mice to stop aseptic loosening of orthopaedic implants secondary to wear debris-induced osteolysis. A single injection of the AAV vector efficiently transduced the myocytes at the injection site and produced high levels of the OPG protein starting at the second day post injection and peaking at the sixth day, efficiently inhibiting wear debris-induced osteolysis and osteoclastogenesis.[24]

Each of the different serotypes of the Adeno-Associated Virus has an affinity for a different cell type. Muscle cells are most easily transduced by recombinant AAV of serotype 1 while serotype 4 is most efficient in infecting the retina. Serotype 5 is quite versatile in that it can infect nervous tissue, airway epithelia, and retina cells efficiently. Serotype 8 is most efficient in transducing hepatocytes. In an animal study for rheumatoid arthritis, serotype 5 transduced the synovial tissue more efficiently than serotypes 1-4.[25]

Retroviral Vector

Risks

Disadvantages and risks of using the retrovirus as a viral vector in gene therapy include low transduction efficiency, replication competence, small insert size, integration, inactivation by the complement cascade, and limited host range due to the requirement of cell division for transduction. The average insert size for a retroviral vector is approximately 8 kb putting the packaging capacity between that of the adenoviral vector and the adeno-associated viral vector. The complement system is a part of the immune system that works by destroying the antigens that are bound by antibodies. Retroviruses have receptors that are antigenic and can attract antibodies and thus can be destroyed by the complement cascade.[8]

The disruption of the nuclear membrane is required for the pre-integration complex to gain access to the chromatin making the effective transduction of the retroviral vector contingent on the target cell entering mitosis shortly after it penetrates. Since a majority of the cells in the human body are not actively dividing the retrovirus is limited in the cells that it can transduce. The necessity of mitosis for effective transduction also leads to the problems associated with insertional mutagenesis. As mentioned above in the discussion on Adeno-Associated Viral vectors, the viral genome has a higher probability of inserting into a functional region of the

genome. Since the retroviral vector is an integrating virus it is not possible to completely avoid the possibility of causing insertional mutations and this should be very clearly explained in all informed consents for gene therapy that uses an integrating vector. It can also lead to problems if the virus should enter a germ-cell as this gene would be passed on to any child born of that cell. Germ-cell therapy is strictly prohibited by the FDA and all participants that are of child-bearing age should be made aware of the risks and any child born of the participant should be tested to determine if the therapeutic gene has infected their genome.[6]

The contamination with replication competent retroviruses is the most significant problem associated with large-scale production of the vector. This contamination does not usually lead to the pathological disease associated with the wild-type virus however it has lead to the development of lymphoma in 3 out of 10 immunocompromised primates in an animal safety study.[26] The pathogenic mechanism was determined to be most consistent with chronic productive retroviral infection that allowed insertional mutagenesis of critical growth control genes. This lead to cell transformation and the development of the cancerous clone.[6]

In April of 2000 the paper, “Gene Therapy of Human Severe Combined Immunodeficiency (SCID)-XI Disease” was released reporting the first definitive cure of a disease by gene therapy.[27] Three young children suffering from the fatal X-linked form of SCID (SCID-XI syndrome) had developed functional immune systems after re-infusion of their own hematopoietic stem cells that had been transduced via ex vivo procedure with a murine leukemia virus (MLV) vector that carried the gene encoding the γ-c chain cytokine receptor. Several more patients were treated with this protocol before it was shown that two of the patients developed a leukemia-like lymphoproliferative disorder. The cancerous T cells that were isolated from these patients are thought to be derived from single transduced cells in which the retrovirus genome

inserted near or in the LIM domain only 2 (LMO2) oncogene thus activating LMO2 expression and causing the neoplastic proliferation.[5]

One animal retroviral gene marking study showed that the transplantation and expansion of a clone of the retrovirally transduced bone marrow cells induced leukemia in mice. The transgene used in the study had growth-promoting properties however; the development of the cancer was most likely to have occurred as a consequence of cooperation between the transgene product and the integration event disrupting the gene encoding a transcription factor that has been implication in the pathogenesis of acute myeloid leukemia.[5]

Another study has shown that retroviral integration may not be as random as was previously thought. Through analysis of hundreds of human immunodeficiency virus (HIV) integration sites in cell cultures it was shown that HIV is more likely to integrate into transcriptionally active genes than into non-coding regions of the host chromatin. In the SCID-XI case there was selection for the transduced cells to proliferate which may have favored the development of the leukemia-like disorder. Clonal expansion required in ex vivo studies is a risk factor for cellular transformation. It is improbable, but not impossible, that the integrating vectors would induce a neoplastic proliferation in non-dividing cells in individuals with a competent immune system or in cases where the proliferation of the cell is not the therapeutic end point.[5]

Benefits

Benefits and advantages of using the retroviral vector include integration into the host genome, requirement of mitosis for efficient transduction, and no toxicity associated with viral proteins.[8] Wild-type retroviruses are associated with chronic infections that are usually well tolerated by the host and cause latent chronic diseases that range from malignancy to

immunodeficiency. Removal of the viral genes that cause the latent disease produces a vector that does not cause an overwhelming immune response. This type of virus is deactivated by the complement system and does not cause inflammation like the Adenovirus. The retroviral vector is the safest in terms of immune adverse reactions. Even in the case that the immune system attacks the retroviral vector it does not cause acute physical harm to the patient. The patient will appear to show no response to the treatment because the vector will have been deactivated before it can reach the target cells.

Integration into the host genome is a risk and a benefit of using the retrovirus. Integration allows for the gene to remain expressed for a longer period of time which is beneficial especially in genetic disorders where the condition continues for the life of the patient. Upon integration the transgene is in a position to be passed on to the daughter cells created from mitotic division. A study was conducted to assess the potential transcriptional interference that is caused by the integration of the retroviral vector in an adenosine deaminase-deficient severe combined immunodeficiency (ADA-SCID) clinical trial.[28] Blood samples were taken from participants in the trial 10 to 30 months after their infusion of genetically corrected CD34+ cells and from healthy volunteers. The expression profiles on ex vivo bulk T-cell populations showed no difference between the participants and the normal healthy volunteers. However, dysregulation of 5.8% of genes in 18.6% of the T-cells from the trial participants was observed as compared to the controls. Fortunately, all of the affected clones retained a stable phenotype and normal in vitro functions. These results affirm the safety of integrating retroviral vectors.

The characteristic of only being able to transduce dividing cells can also be considered an advantage due to the decrease in transduction of non target cells. Mitotically active cells would be considered the target host cell. Such is the case in some cancer gene therapy trials where it is

the tumor cell that is the target cell. Tumor cells are often continuously dividing cells and because of this the introduction of a retroviral vector carrying a suicide gene or pro-drug gene would be beneficial. It may also be possible for the integration of the retroviral gene to insert into the genome in the location of the oncogene that has been activated thus deactivating. It may also integrate to where it would activate or carry in it a tumor suppressor gene.

Long term persistence is the goal of the integrating viral vector. A study was conducted to investigate the long-term outcome of patients that received gene therapy for ADA-SCID.[29] The study followed 10 patients that had received an infusion of autologous CD34+ bone marrow cells transduced with a retroviral vector containing the ADA gene after they had received nonmyeloblative conditioning with busulfan. All patients were still alive after a median follow-up of 4 years, ranging from 1.8 to 8 years. Eight of the ten patients did not require PEG-ADA treatment during the follow-up period and none of the patients received an allogenic bone marrow transplant post therapy. The study determined that the treatment supplied the patients with hematopoietic stem cells that passed a functional ADA gene onto all progeny. There was restoration of immune function in 9 of the 10 patients that were treated in this study. The long-term follow-up of this study revealed patients that had reconstituted immune systems, no toxic side effects, and no neoplastic proliferations. The nonmyeloblative treatment prior to infusion aided the targeting and integration of the vector and the polyclonal pattern of vector integration, T-cell repertoire, and lack of in vivo skewing for potentially dangerous insertion lead to the long term safety, integration, and expression of the transgene in this trial.

The retroviral vector is also being used in cancer clinical trials. In an advanced metastatic melanoma study, 17 patients were infused with genetically engineered lymphocytes. This study was based on a previous treatment that involved removing the patient’s lymphocytes, isolating

the most aggressive tumor-killing cells, multiplying them and reintroducing them into the patient where the cells attack the tumors. This is an autologous lymphocyte transplant and has shown reasonable success. Problems associated with this procedure are that it can only be used for patients with melanoma and only those that already have a population of the aggressive lymphocytes. Gene therapy can solve both of these problems. In the gene therapy clinical trial the patient's lymphocytes are harvested and infected by a retroviral vector to deliver a gene that encodes for T cell receptor proteins (TCRs). The TCRs recognize and bind to specific molecules that are found on the surface of the tumor cells. Researchers have isolated TCRs that recognize other types of cancer including breast and lung.[30]

In the advanced metastatic melanoma trial the patients were split into 3 groups. In the second and third group all of the patients still had 9 to 56 percent of their TCR-expressing lymphocytes. Two of the 17 patients experienced regression of the cancer, sustained high level of genetically engineered lymphocytes, and remained disease-free for over one year. Another major benefit observed in this trial was the fact that there were no toxic side effects attributed to the genetically modified cells in any of the patients. The ex vivo treatment method bypasses the possible immune deactivation of the vector before it reaches the host.

Discussion

Gene therapy has had a very tumultuous past. From adverse reactions to death to inefficiency to cancer, gene therapy has overcome many obstacles to still be researched in clinical trials today. We study the past to learn about and progress in the future and in the case of gene therapy many lessons have been learned. It is because of these lessons that gene therapy using viral vectors is still being conducted and risks minimized with every new trial. The history of the viral vector is just as tumultuous as that of gene therapy. However, through the ups and

downs the vectors have become safer and more reliable. The regulation of gene therapy has come about much like that of drug development and the FDA, out of necessity.

The risk to benefit ratio is a subjective measure of the risks and potential benefits of a clinical trial. The first step to analyzing the risks and benefits of a particular treatment is to first identify and describe them. In the above section an overview of the risks and benefits of each vector type was described. The adenoviral vector has had the most turbulent past of the three vectors due to the death of 18 year old Jesse Gelsinger. Jesse's was the first death to be linked to the vector used in a gene therapy protocol. However, it was also discovered that the FDA was not advised of prior serious adverse reactions and questionable safety data from animal studies. Also, Jesse did not meet the inclusion/exclusion criteria the day that he received his infusion and he received the highest dose of vector that had ever been tested. There were many extenuating circumstances surrounding Jesse's death and since this occurrence regulations on GCP and GMP have been enforced as well as changes in the informed consent procedure.

The immune system is the biggest threat to gene therapy success. Immune responses have been seen with each of the 3 vectors. Jesse's immune response to the adenoviral vector was determined to be his cause of death. The adenoviral vector causes an inflammatory immune response while the adeno-associated viral vector can cause cell mediated immune responses and the retroviral vector can be deactivated by the complement cascade. The risk of an immune response can be overcome by several methods including immune suppression prior to treatment, use of serotypes not previously seen by the immune system, and ex vivo introduction of the viral vector followed by re-infusion of the transformed cells.

Integration is a point of discussion among researchers. While it is beneficial for the long term expression of the transgene it comes at the inescapable risk of insertional mutagenesis. The

wild-type virus has evolved to insert into areas of active transcription to ensure that it will be transcribed and new viruses formed and released to infect more cells. The viral vector has adopted this characteristic and while it is good that the transgene inserts into an area where it is sure to be transcribed and expressed the possibility that it will insert into an active gene is always going to be a risk. Insertion into an active gene can cause many problems including the development of cancerous clones. This risk weighs heavily in the analysis because it is unavoidable so long as the transgene integrates into the host cell genome.

The packaging capacity of viral vectors average around 8 kb and can go as high as 30 kb in gutless adenoviral vectors. The average human gene is approximately 3 kb and can range as high as 2.4 million bases. There are many genes associated with diseases and disorders that just do not fit into the viral vector. Genes can be processed to where only the coding regions are inserted into the vector or in the case of the adeno-associated vector, can be split into two vectors and transpliced upon entrance into the target cell. Packaging capacity limits the use of the viral vector in gene therapy however it does not pose a quantifiable risk to the patient. Diseases whose genes do not fit into viral vectors are treated by using any of the other types of vectors.

Cell targeting is an area of gene therapy that is still being explored. Some viral vectors have a specific host range such as the retroviral vector that can only transduce cells that are dividing. Other vectors can transduce a broad range of cell types such as the adenoviral vector that can transduce most body cells except the hematopoietic cells. The adeno-associated vector falls in the middle of these two categories as the different serotypes have their own host range that can include one type of cell up to three or four types of cells. In each study the vector chosen will be the one that transduces the target cell the most efficiently. Vectors that have a broader host range also have a higher risk of having the vector transduce the wrong cell which can cause

problems such as toxicity, neoplastic proliferation, and decreased concentration at the target cells. Vectors with a narrower host range will be more likely to transduce the proper cell at a higher concentration.

The major ethical issue of gene therapy with viral vectors is the possibility of systemically delivered viral vectors infecting and efficiently transducing germ cells. Germ cell gene therapy has not been approved by the FDA and is of major ethical concern in the integrating viral vectors because the gene will be subsequently passed onto the offspring produced from that cell. The risk of germ cell infection and passage of the transgene onto offspring is solely the risk of integrating vectors such as the retroviral vector and to a lesser extent the adeno-associated vector.

The risks associated with using viruses as vectors in gene therapy are significant however the potential benefits are amazingly life-saving. Gene therapy is currently being developed for potentially fatal diseases and in some cases where no significant options currently exist. Viral vectors carrying beneficial transgenes has saved the lives of children with a fatal immune disorder, is showing progress in fighting advanced metastatic melanoma, diabetes, Duchene's cardiomyopathy, and many other conditions. Gene therapy using viral vectors may have gotten off to a rough start but like the drug development industry it is learning from the past and has a bright future. The potential benefits of viral vectors while not fully realized yet outweigh the risks and many treatments and potential cures will someday be realized and celebrated.

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