Published in final edited form as:

Expert Opin Orphan Drugs. 2015 ; 3(6): 675–689. doi:10.1517/21678707.2015.1039511.

Recombinant adeno-associated virus vectors in the treatment of rare diseases

Eric Hastie[a] **[Postdoctoral Researcher]** and **R. Jude Samulski**[a,*,b] **[Director]**

[a]Gene Therapy Center, University of North Carolina, Chapel Hill, North Carolina, USA, The University of North Carolina at Chapel Hill, 7119 Thurston Bowles Building (104 Manning Drive), Campus Box 7352, Chapel Hill, NC, 27599-7352, United States

[b]Department of Pharmacology, University of North Carolina, Chapel Hill, North Carolina, USA, The University of North Carolina at Chapel Hill, 7119 Thurston Bowles Building (104 Manning Drive), Campus Box 7352, Chapel Hill, NC, 27599-7352, United States

Abstract

Introduction—An estimated 25 million Americans are living with rare diseases. Adeno-associated virus (AAV)-mediated gene therapy is an emerging therapeutic option for the more than 7,000 identified rare diseases. This paper highlights the benefits of AAV therapy compared to conventional small molecules, discusses current pre-clinical and clinical applications of AAV-mediated gene therapy, and offers insights into cutting edge research that will shape the future of AAV for broad therapeutic use.

Areas covered—In this review the biology of AAV and our ability to generate disease-specific variants is summarized. Limitations of current therapy are reviewed, with an emphasis on immune detection of virus, viral tropism and tissue targeting, and limitations of gene expression. Information for this review was found using PubMed and clinicaltrials.gov.

Expert opinion—Currently the scope of clinical trials of AAV gene therapy is concentrated in an array of phase I/II safety trials with less than two dozen rare diseases featured. Pre-clinical, translational studies are expanding in number as developments within the last decade have made generation of improved AAV vectors available to more researchers. Further, one bottleneck that is being overcome is the availability of disease models, which will allow for improved preclinical testing and advancement of AAV to more clinical applications.

Keywords

Adeno-associated virus; AAV vectors; AAV; Gene therapy; Orphan disease; Rare disease

*Corresponding author, rjs@med.unc.edu, Phone: 919.962.3285. Fax: 919.966.0907.
ehastie@med.unc.edu

Financial and competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

1. Introduction to AAV-mediate gene therapy

The Orphan Drug Act of 1983 defined rare diseases as those affecting less than 200,000 Americans. However, with more than 7,000 monogenic rare disease listed by the National Genetic and Rare Disease Information Center (GARD), more than 25 million Americans are affected illustrating the significance of these collective disorders. Historically pharmacological small molecule treatments have been the only option for managing a few rare diseases, but these efforts have a history of off target effects, need to be taken indefinitely, or have reduced efficacy over time as a result of dose tolerance. Protein therapy has filled a number of niches and continues to look promising. However with the advent of gene transfer technology, new options are on the horizon. Emerging as a promising therapeutic, adeno-associated virus (AAV) vectors provide a convenient packaging system to deliver disease-specific, long-term therapies based on virus-mediated delivery of corrected genes or endogenous gene knockdown to abrogate disease phenotypes. To date approximately 50% of the genes responsible for rare diseases have been identified, with genotyping being the limiting factor [1]. However, next-generation sequencing is quickly identifying target genes and it is expected that the genetic mutations causing most rare diseases will be known in the next decade [1]. This review is organized to first discuss the biology of AAV and current strategies to improve AAV gene therapy vectors, while the second half will discuss individual diseases and current AAV research, focusing on pre-clinical studies and clinical trials (Table 1).

2.1 Biology of AAV

AAV is a non-autonomous single-stranded (ss) mammalian DNA virus (family *Parvoviridae*, genus Dependovirus) that requires helper functions, usually provided by associated viruses (such as adenovirus) to complete its life cycle. Thirteen major human and non-human primate (NHP) serotypes have been identified, with more than 100 serotypes identified from NHPs and several other species. The approximate 4.7kb AAV genome is flanked by 145 nucleotide inverted terminal repeats (ITRs) and contains three identified open reading frames (ORFs) encoding eight proteins required for virus propagation [2]. At 25nm, the mature AAV virion consists only of capsid proteins and a ssDNA genome, with both positive and negative DNA strands being incorporated equally [2]. Although, AAV is ubiquitous in nature and it is estimated that more than 70% of humans are seropositive for one or more serotypes, AAV has not been directly implicated in human or animal diseases [3, 4].

More than 30 years ago it was demonstrated that the ORFs of AAV could be supplied in trans allowing the replication and capsid packaging of transgenic DNA flanked by the AAV ITR sequence (termed recombinant AAV or rAAV). In conjunction, with a helper virus free production system, rAAV has been exploited in the basic sciences and as a therapeutic drug in >100 clinical trials for diverse diseases [5]. Most important for safety concerns and therapeutic applications, all recombinant AAV (rAAV) replication, capsid, and packaging protein sequences are replaced with expression cassettes encoding the therapeutic transgene of interest. This allows for generation of replication defective recombinant AAV (rAAV) vectors that have limited antigenic properties and low risk of host cell genome integration.

Significantly, production of research-grade rAAV has been optimized with a scalable format that achieves yields of purified rAAV of 10^{13} to 10^{15} DNase-resistant particles (DRP)/ml[6-8].

2.2 rAAV vector development strategies

The ability of rAAV to deliver therapeutic genes for correction of rare disease phenotypes makes it an exciting vector for clinical use. Challenges for rAAV therapies are being addressed in current research, including: 1) evasion of immune detection (for both the vector capsid and transgene product), 2) manipulating rAAV tissue tropism, and 3) enhancing the transgenic expression cassettes. These research areas aim to overcome premature clearance/immune response to treatment, restrictive targeting of rAAV to specific tissue types and/or to improve transduction of cells that are currently refractory, and to optimize therapeutic gene expression for increased efficacy at low vector doses in clinical applications.

2.3 Evasion of rAAV immune detection

Pre-existing as well as adaptive immunity against AAV is a significant challenge to current therapies and has been reviewed in detail [9-12]. Briefly, development of AAV capsid specific memory B and T cells occurs in childhood in much of the population [13]. Early response to AAV results in production of neutralizing antibodies (NAbs) and memory immune cells that can prevent later rAAV transduction and eliminate transduced cells, respectively. Clinical trials have offered many insights into the humoral responses to rAAV in addition to cell-mediated clearance of transduced cells.

Understanding the NAb response to rAAV is especially important as therapeutic efficacy may depend on the treatment of rare diseases shortly after birth or in early childhood. There does appear to be an optimal treatment window during the first year following birth where maternal NAbs diminish prior to self-made NAbs increasing to a plateau in adolescence [3, 14]. However, even with delivery of high concentrations of rAAV particles, clearance of virus by NAbs can greatly reduce therapeutic efficacy by preventing transduction and limiting long-term gene expression [15]. It should be noted that the prevalence of NAbs has not been examined for all AAV serotypes. One comprehensive study examined NAbs against some of the most common serotypes used for gene therapy, rAAV1, 2, 7, and 8, focusing on prevalence worldwide [4]. The highest prevalence of NAbs was against AAV2, followed by AAV1, with lower NAb prevalence detected for AAV 7 and 8 [4]. Interestingly, another study with nonhuman primates (NHPs) suggests that even non-NAbs can result in premature clearance of AAV8 and that therapy is best in NHPs without pre-existing immunity [16]—unfortunately in humans, this is only 30% of the population.

As rAAV vectors encode no viral genes, the humoral and cell-mediated immune responses are directed against the vector capsid as well as expressed transgenes. Perhaps the most obvious solution would be co-therapy with immunosuppressive drugs, however this will not remove already circulating NAbs, may require extended application, and may not be applicable in rare disease patients with weakened immunity. Further, NAb depletion may result in the removal of antibodies that are cross-reactive for other pathogens, which also could be potentially harmful to immunosuppressed patients. Consequently, other methods of immune evasion are being researched.

Different rAAV serotypes are being explored for gene therapy use, but capsid homology may limit this approach. Antibody cross reactivity was seen against rhesus monkey derived AAVrh10 compared to human serotypes AAV9 and 2 in mice [17]. In another study, AAV2 NAbs were cross-reactive for AAV 5 and 8, and limited the alternative serotype approach in pediatric patients with hemophilia [18]. One group demonstrated the effectiveness of goat-derived rAAV with 94% homology to human AAV5 that was able to evade NAbs, with epitope difference predicted at the capsid surface or spike-like protrusions [19].

Current research is developing additional NAb-resistant rAAVs mainly through capsid protein modification. Directed evolution using random mutagenesis generates capsid protein sequence libraries that require screening for NAb-resistance [20]. More specific to the antigenic regions of the capsid proteins, site-directed mutagenesis is being explored to select for rAAV with reduced NAb binding [21-23]. Further, the use of bioinformatics is allowing for generation of high-resolution maps of sequences required for structure versus NAb recognition sites that may lead to rational design of Nab resistant capsids [24]. In addition to genetic modification, chemical immunoshielding of rAAV capsids with polyethylene glycol or immunoshielding with natural extracellular vesicles has been attempted, with the later showing 4000-fold increase in transduction efficiency [25-27]. Even more, aptamer immunoshielding has shown promise for other viruses and may improve rAAV evasion of NAbs [28].

Following rAAV evasion of NAbs is a need to understand cell-mediated clearance of transduced cells. In fact, prior to early clinical trials, animal models failed to predict $CD8^+$ T-cell as a limiting factor in rAAV therapy, possibly occurring because of pre-existing immunity to AAV [29]. Proteasomal processing of vector capsids or expressed transgenes results in antigen presentation and recognition by cytotoxic T cells. As rAAV is non-replicating, detection of capsid antigen in transduced cells should be limited following treatment. Surprisingly, depending on the vector or target tissue, capsid antigen was detectable weeks to years post treatment [29, 30]. Here again, site-directed mutagenesis of the capsid has been attempted to evade the cell-mediated responses. Specific tyrosine residues were replaced to prevent ubiquitination, proteasomal degradation, and subsequent major histocompatibility complex (MHC) presentation [31]. Regarding expressed transgenes, like the capsid, proteasomal degradation is followed by MHC presentation. However, many factors may influence the cell-mediated response. Of note from numerous clinical observations is prior exposure the therapeutic protein, the route of administration, the target tissue, and even the disease state of the organ, as reviewed previously [12]. Important for clinical use, a direct correlation between dosage and cell-mediated immunity has been observed. Interestingly, the kinetics of T cell activation and clearance of transduced cells may be serotype dependent [29, 32].

Less explored is a role for cellular innate immunity and its effect on adaptive immunity against rAAV. The cytosolic sensor, toll-like receptor 9 (TLR9), identifies the rAAV genome and promotes type I interferon antiviral signaling and inflammation that may enhance the adaptive immune response against rAAV [33, 34]. Additionally, as TLR9 detects CpG sequences, depletion of CpG ligands from the rAAV genome reduces adaptive immune responses and improves transduction [35]. Deficiency in MyD88, an effector molecule of

TLR9, results in decreased NAb responses to rAAV [36]. Though it is unclear if other cellular proteins promote immune responses like TLR9, promyelocytic leukemia protein (PML) or the DNA damage complex Mre11/Rad50/Nbs1 (MRN) were shown to inhibit rAAV second strand synthesis and/or inhibit gene expression independent of rAAV DNA synthesis through an unknown mechanism [37-39]. These studies highlight the need for continued investigation of cellular host anti-viral responses and a link to humoral and cell-mediated immunity against rAAVs.

2.4 rAAV capsid modification for targeted tropism

Cell surface attachment of rAAV is serotype dependent and numerous cell surface receptors have been identified. In general, cell surface receptors like heparin sulfate proteoglycan [40], O- and N-linked sialic acids [41, 42], galactose [43], and ganglioside GM1 [44] contribute to rAAV serotype tropism. Mutagenesis of rAAV capsids allows for attachment to other cell receptors, like chondroitin sulfate [45] and αvβ8 integrin [46], which may help with targeting refractory cells. Importantly, rAAV must be able to infect and transduce target cells where therapy is required. Depending on the rare disease, the therapeutic need could be localized or systemic. The ability to generate designer rAAVs that home to specific tissue types has been describe in numerous reviews about gene therapy of the central nervous system (CNS) [47-50], eye [51, 52], heart [53, 54], lungs [55, 56], ear [57], liver [58], bones and joints [59], muscle [60, 61], or adipose [62, 63] tissue. Many of the ongoing clinical trials of rAAV are exploring rare diseases that require tissue specific treatment (Table 1).

Direct targeting involves inserting small peptide or ligand sequences into the capsid sequence [45]. This approach has improved targeting to tissues including muscle [24] or lungs [39], but is limited in that it may inhibit binding of the rAAV to natural cell surface receptors or generate new epitopes for immune response. Another approach involves using capsid sequences from different serotypes to generate mosaic or chimeric capsid protein libraries. These libraries can be used to produce rAAVs with tropism different from the parental vector and can be easily screened to determine tissue specificity and examine transduction efficiency [64, 65].

Indirect targeting has also being explored. Here, a mediator molecule interacts with the rAAV vector and a specific cell receptor. Examples include bispecific antibodies [66] or biotin [67, 68]. Additionally, chemically directed tropism can be achieved using chemicals that block natural rAAV receptors or prevent virus capsid ubiquitination and are reviewed for rAAV2 [69]. These approaches allow for targeting without modification to the capsid, which may inhibit transduction efficiency.

2.5 Gene expression from rAAV vectors

While the previous two sections focus on steps that are critical for the refinement of future rAAV application, long-term gene expression is the most important aspect of gene therapy vector. The limited packaging size of rAAV vectors is a major limitation when considering gene length options and has been reviewed [70]. Additionally, the rAAV ITR sequences (which have natural promoter activity or contain cellular transcription factor binding sites) can influence transduction efficacy and gene expression levels. Different methods are being

examined to improve rAAV packaging size as well as identify promoter and cellular transcription factor binding sequences within the rAAV genome.

Most therapies are based on single stranded rAAV genomes where a transgene cassette is placed between 2 ITR sequences, but other vectors exist. For instance, the self-complementary AAV (scAAVs) genome was intentionally produced based on a replication intermediate that allows self-annealing of the ssDNA, thus producing a duplex molecule capable of transcription [71]. When tested, scAAV mediates faster and more robust transgene expression. Unlike ssAAV, host mediated second-strand synthesis of the scAAV genome is not required for transgene production. However, due to the requirement for self-annealing of scAAV genomes, scAAV-based cassettes need to be less than half of the approximate 5kb capsid capacity restricting their use to the treatment of diseases requiring large DNA delivery [72]. This method is useful for smaller transgenes and can be applied to rare diseases that require knockdown of mutant genes through shRNA, miRNA, or similar methods.

Expression of larger transgene cassettes is possible using several genetic strategies relying on host-mediated reconstruction of the larger, desired, cassette. Currently, rationally designed overlapping vectors and several types of concatemerization dependent vectors have been described which mediate large gene transduction. In addition, the attempted packaging of cassettes >5kb results in encapsidated rAAV genome fragments (termed fragment AAV or fAAV) that are reconstructed into the intended larger cassette. In the literature, the efficiency of these different approaches is inconsistent, although, it is generally agreed upon that transduction efficiency is substantially decreased when compared to intact ssAAV. No AAV large gene delivery strategies have been approved for clinical application. Importantly, as the efficiency of transgenic DNA reconstruction likely correlates to tissue type and cell cycle status, further investigation is needed to refine AAV large gene delivery. Furthermore, as host replication and DNA repair machinery may be affected in some rare diseases, particular large gene strategies may prove ineffective.

Focusing on current vectors, it is not surprising that transduction efficacy and gene expression can be influenced at the rAAV genome level. Numerous promoters of constitutive gene expression have been studied and compared for rAAV transgene expression: human β-actin, human elongation factor-1α, a chicken β-actin variant, cytomegalovirus (CMV), simian virus 40, and herpes simplex virus thymidine kinase [19]. While this approach is excellent for systemic delivery and expression, in rare disease that may require targeted expression, tissue specific promoters are being explored. In just one of many examples, the use of liver specific promoters can influence gene expression profiles [73]. Here bioinformatics was utilized to identify cis-acting regulatory modules (CRMs) that enhance liver specific gene expression 10 to 100-fold[74].

Interestingly the rAAV genome flanking ITR sequences have very low promoter activity [75]. Even more, the ITRs contain sequences that may act as binding sites for host suppressor proteins. In one instance, a 20-nucleotide ITR sequence that contained homology to a NF-kB-repressing factor binding site was substituted with a sequence for transcription factor binding and resulted in enhanced transduction [76]. Future identification of other regulatory

sequences in the rAAV genome, ITR or otherwise, will allow for rational design of promoters or sequence modification for disease specific application.

3. Rare disease and current rAAV pre-clinical and clinical studies

Gene therapy has been explored for more than 40 years, but to date only one rAAV vector, Glybera, for treatment of the rare disease lipoprotein lipase deficiency (LPL), is approved for use in the European Union. Following a rocky start to clinical trials in 1999, gene therapy is finding its stride and currently rAAVs account for 5.6% of the approximate 2076 gene therapy trials (http://www.abedia.com/wiley/vectors.php) [77]. The list of rare diseases being studied in clinical trials with rAAV therapies in table 1 is promising, with several even in phase III trials. Additionally, rare diseases currently being addressed by other gene therapy vectors may be treatable with rAAV. Combined data from preclinical and clinical investigations are generating an abundance of information to guide researchers in choosing optimal vector-disease combinations. It is clear that the rAAV serotype as well as the dose and route of administration impact therapeutic outcomes. Current clinical trials (Table 1) inject rAAV via many routes: intravenous, intramuscular, intrapleural, into specific brain regions during surgery, subretinal, intranasally, intrahepatic, peripheral vein infusion, or convection-enhanced delivery (CED) to the putamen, intravitreal, or intracerebral. The vectors most often used are based on the most characterized serotype AAV2, but vectors designed from rAAV1, rAAV8, rAAV9, and rAAVrh.10 have also entered the clinic due to broader or enhanced transduction. Results of preclinical and clinical trials offer insights into safety, immune reactions, disease response to treatment, and length of therapeutic efficacy.

An abundance of reviews describe rAAV rare disease gene therapy, including psychiatric disorders, neurodevelopmental disorders, lysosomal storage diseases (LSDs), amyotrophic lateral sclerosis, glycogen storage diseases, inborn metabolism errors, Duchenne muscular dystrophy, and epilepsy to name a few of the most recent in the literature. Several major approaches for using rAAV-mediated gene therapy of rare diseases are being explored: 1) expression of wild type proteins to correct for mutant genes, 2) expression of smaller, functional variants of wild type proteins not amendable to the restrictions of rAAV packaging, or 3) gene silencing through expression of small RNAs. Due to the recent explosion of rAAV research and rare disease, this review groups the rare disease below and highlights important historic milestones as well as the most recent findings in pre-clinical research and clinical trials.

3.1 Retinal diseases

3.1.1 Choroideremia (CHM)—Mainly affecting males, X-linked CHM results in loss of photoreceptors, retinal pigment, and choroidal vessels and leads to blindness by middle age. Because rAAV8 can be used in a ten-fold lower dose than rAAV2 and transgene expression reaches maximal levels sooner [78], the authors explored rAAV8 encoding the Rab escort protein 1 (REP1), demonstrating safety and significant stalling of degeneration and long term rescue of retinal-cortical function in $Chm^{null/WT}$ mice [79]. Optimistic results with gain of visual acuity came from a phase I/II trial with rAAV2 encoding the REP1 protein for

treatment of choroidermia. Patients administered subretinal rAAV2 exhibited improved rod and cone function with a direct correlation of dosage to improved retinal sensitivity [80].

3.1.2 Leber congenital amaurosis (LCA)—This group of heritable retinal dystrophies is characterized by loss of visual function in childhood caused by mutations of more than 15 known genes. Significantly, following reversal of blindness in animal models, clinical trial results have demonstrated long-term safety and markedly increased visual sensitivity in multiple patients with LCA2, caused by retinal pigment epithelium-specific-65-kDa (RPE65) deficiency [81]. Of the current ongoing rare disease clinical trials, Rep65 rAAVs make up the largest cohort, with 10 studies in phase I, II, or III. Additionally, in preclinical studies of LCA12, administration of scAAV8 Y733F capsid mutant expressing retinal degeneration protein 3 (RD3) from a photoreceptor-specific promoter results in photoreceptor cell survival. Rd3 expression from the scAAV8 vector is seen 1 week following treatment in mice, compared to 4 to 6 weeks for previously studied rAAV5 [82, 83].

3.1.3 Retinitis pigmentosa (RP)—RP is a general term for a group of inherited diseases that exist as autosomal-recessive, autosomal-dominant, or X-linked diseases, with multiple causative genes. In a rare autosomal-recessive form of RP, mutation of the human receptor tyrosine kinase MER (MERTK) gene causes a loss of photoreceptors. Subretinal injection of AAV2-CMV-Merkt into Royal College of Surgeons (RCS) rats resulted in a 2.5-fold higher number of functional photoreceptors compared to controls up to 9 weeks post treatment [84]. Similar results were seen in treatment of an autosomal dominant RP where disease arises from mutation to the rhodopsin (RHO) gene. RHO augmentation using rAAV2/5 in $Rho^{-/-}$ mice preserves the survival of rod cells [85]. Addressing a dominant RP cone-rod dystrophy caused by mutated guanylate cyclase-activating protein 1 (GCAP1), scAAV2/8 encoding allele specific shRNA against GCAP1 was injected subretinally in mouse models. Expression of the shRNA was strong1 week post injection and gene silencing lasted 1 year with treatment enhancing photoreceptor survival and delaying onset of degeneration [86]. Lastly, a common X-linked form of RP (XLRP) is caused by mutation to the RP GTPase regulator (RPGR) gene. No treatment is available to date, but in two canine models rAAV2/5-mediated expression of RPGR in rods and cones rescues photoreceptor blindness and prevents disease onset at an early age [87].

3.1.4 Age related macular degeneration (AMD)—Unlike the retinal diseases above, there is no causative mutation known for AMD. However, rAAV therapy may still prove effective. Here, localized inflammation induced by interleukin (IL)-17 may be retinotoxic [88]. Use of rAAV2 encoding the soluble IL-17 receptor prevented retinopathy in mice and fewer lesions and reduced photoreceptor atrophy were observed [88]. One phase I clinical study is ongoing and is based on rAAV2 expressing a chimeric soluble Fms-Related Tyrosine Kinase 1 (Flt1) receptor that suppresses the proangiogenic vascular endothelial growth factor (VEGF). Preclinical data with intravitreal injection of the same vector in mice, rats, and monkeys demonstrated inhibition of pathological neovascularization as well as tolerated therapy and long-term gene expression [89].

3.2 Nervous system related diseases

3.2.1 Spinal muscular atrophy (SMA)—At least four different mutated genes are known to cause neuron degeneration, progressive paralysis, and childhood death in SMA. When expressed at low levels, the survival of motor neuron 1 (SMN1) protein is a causative agent and has been studied in rAAV therapies. Delivery of scAAV9 encoding SMN1 to mice and NHPs results in widespread transgene expression in spinal cord motor neurons and complete rescue of SMA phenotype in mice. Even more, the study noted that a ten-fold lower dose was required for the same expression profile in NHPs when delivered to cerebral spinal fluid compared to intravenous injection [90]. It has also been noted that phosphatase and tensin homolog (PTEN) protein depletion leads to increased neuron survival in SMA. Treatment of mice with rAAV6 encoding shRNA against PTEN or scAAV9 encoding siPTEN resulted in a 3-fold increase in lifespan, suggesting that continued investigation may be beneficial [91]. One phase I trial using scAAV9 encoding an enhanced CMV-chicken beta actin hybrid promoter and SMN has not reported any results.

3.2.2 Aromatic L-amino acid decarboxylase (AADC) deficiency—Mutation to the AADC gene impairs biosynthesis of neurotransmitters like serotonin or dopamine that are required for signaling between cells of the central nervous system. In neonatal mice, following intracerebroventricular injection of rAAV9 encoding human ADDC, dopamine and serotonin levels rose from 25% and 15% to 100% and 40%, respectively, with improved growth rate and survival as well as partially corrected behavioral abnormalities [92]. Currently one clinical trial is exploring the use of rAAV2 encoding AADC for AADC therapy.

3.2.3 Alternatively, a 2010 phase I clinical trial used rAAV2 encoding AADC for treatment of Parkinson's disease and patients have demonstrated improved motor performance [93]—Parkinson's can be caused by multiple gene mutations, but progressive loss of AADC occurs in most cases. Another phase I trial with the rAAV2-hAADC-2 vector is ongoing. Additionally, two clinical trials in phase I and I/II are using rAAV2 to express neurotropic growth factors, glial cell derived neurotrophic factor (GDNF) and neurturin (NRTN), respectively, in an effort to support dopamine producing cells. Further as oral therapies L-DOPA may show some benefits, long-term treatment has unwanted side effects. Recently, it was demonstrated that rAAV5 encoding the tyrosine hydroxylase (TH) or GTP cyclohydrolase 1 (GCH1), enzymes that overcome a rate-limiting step in dopamine production, can be used to produce dopamine in Sprague-Dawley rats [94]. Significantly, the group demonstrated that a destabilized dihydrofolate reductase (DD) domain on the N terminus of GCH1 could be used to control expression in a patient specific manner. In this method, addition of activating ligand trimethoprim (TMP), that crosses the blood-brain barrier, prevents GCH1 expression and, in a dose dependent manner, inhibits dopamine production in rAAV-transduced cells [94].

3.2.4 Fragile X syndrome (FXS)—Mutation to the FMR1 gene encoding the fragile X mental retardation protein (FMRP) results in delayed neurodevelopment and a range of intellectual disability. In the first proof of principle study of rAAV and FXS, intracerebroventricular injection with rAAV9 encoding a major isoform of FMRP improved

neonatal mouse behavior. Physiologically, FMRP expression reached approximately 50% of wild type levels at 56 days post injection [95].

3.2.5 Friedreich's ataxia (FRDA)—A mitochondrial disease, FDRA is characterized by neurodegeneration as well as diabetes and hypertrophic cardiomyopathy, with the later being the primary cause of mortality. FRDA is cause by reduced levels of frataxin (FXN), a protein required for synthesis of iron-sulfur clusters. Intravenous application of AAVrh10, a rhesus monkey derived vector, encoding FXN demonstrates high levels of FXN expression in cardiac tissue and prevents onset of cardiac disease in mice [96]. Significantly, when the vector was used after the onset of heart failure, cardiomyopathy was completely reversed within a few days.

3.2.6 Familial amyloidotic polyneuropathy (FAP)—Mutation of the transthyretin (TTR) gene causes extracellular deposition of amyloid fibrils in the peripheral nervous system and currently, the only treatment is liver transplantation. Treatment of TTR V30M mice with scAAV8 encoding the TTR T119M variant with trans-suppressor activity leads to less destabilized monomers of TTR, more functional TTR tetrameric protein, and reduced non-fibrillar aggregates [97].

3.2.7 Huntington disease (HD)—The autodsomal dominant HD is caused by expansion of the CAG repeat in exon 1 of the huntingtin (HTT) gene and results in neuron degeneration as a result of increased polyglutamine residues in the Htt protein. The disease is fatal and presents at 35 years or older, with patients living approximately 15 years following diagnosis. Intracranial injection of mice with rAAV2/1 encoding miRNA against the Htt transcript results in more than 80% transduction of striatum cells with significant improvements in behavior and reduction of striatal Htt aggregates [98].

3.3 Lysosomal storage diseases

3.3.1 Metachromatic leukodystrophy (MLD)—Accumulation of fats in cells of the nervous system occurs as a result of mutation to the arylsulfatase A or B (ARSA/B) or prosaposin (PSAP) genes. Jugular injection of rAAV9 encoding ARSA and GFP into newborn MLD mice resulted in significant inhibition of accumulation of sulfatide fat in the brain and spinal cord. Importantly, mice had improved balancing abilities, where affected individuals lose motor skills and become unresponsive over time [99]. Also encoding ARSA, AAVrh.10cuARSA is in a phase I/II clinical trial for MLD. Results in MLD mice demonstrated that within 2 months following intrastriatal injection correction of brain sulfatide storage. Interestingly, axonal transport as well as transduction in neurons and oligodendrocytes was improved compared to rAAV5 encoding ARSA that was shown to alleviate most long-term disease manifestations in mice [100].

3.3.2 Mucopolysaccharidosis type I (MPS I)—Deficiency in the lysosomal enzyme α-liduronidase (IDUA) causes glycosaminoglycans accumulation in tissues and results in neurological disease as well as ocular, skeletal, and cardiac diseases. A MPS I feline model treated with cephalic vein injection of rAAV8 encoding feline IDUA caused enzyme activity at approximately 30% to an excess of normal levels, with the affect lasting for 6 months [101].

In a mouse model of MPS VI, retro-orbital injection of rAAV2/8 encoding thyroxine binding globulin (TBG) fused to the human ARSB gene, as in MLD above, results in improved motor performance [102]. This study compared AAV to conventional enzyme replacement therapy (ERT) and noted similar outcomes and reduced animal stress with rAAV gene therapy.

3.3.3 Pompe disease—Also called Glycogen storage disease type 2, Pompe disease is an LSD characterized by a lack of α-1,4 glucosidase (GAA) and an inability to break down glycogen. Cardiopulmonary failure leads to death in infancy and ERT therapy with GAA has shown improved survival. Cotherapy of rAAV9 or rAAV8 encoding GAA and non-depleting CD4 antibodies suppress anti-GAA responses and results in significant reduction of glycogen accumulation [103]. In a phase I/II trial for chronic respiratory failure, treatment with rAAV1 encoding α-glucosidase (GAA) results in a 425% increase in periods of unassisted breathing, with no detectable T-cell mediated immune response to the vector [104].

3.4 Muscle related diseases

3.4.1 Limb-girdle muscular dystrophy (LGMD)—Affecting males and females equally, LGMD results from different recessive, as well as dominant, inheritance patterns. To address the dominant mutation of myotilin (MYOT) in LGMD type 1A, rAAV6 encoding micro RNAs targeting MYOT was administered in the lower limbs of mice. Increased muscle strength and significant functional correction was seen up to 9 months after treatment [105]. In a phase I trial of LGMD type 2D, rAAV1 encoding the alpha-sarcoglycan (αSG) gene with a muscle specific promoter was injected into patient's extensor digitorum brevis (EDB) muscle [106]. Persistent αSG expression was seen for at least 6 months in two out of three patients. Currently an ongoing phase I/II trial is using a scAAVrh74 vector encoding the same promoter-transgene combination for treatment of LGMD type 2C and results have been reported.

3.4.2 Limb-girdle myasthenia (LGM)—A familial disease, mutation to the downstream of kinase 7 (DOK7) gene results in formation of smaller than normal neuromuscular junctions (NMJs). Intravenous administration of rAAV encoding human DOK7 fused to green fluorescent protein (GFP) in Dok-7 transgenic mice caused larger NMJs and longer lifespan [107].

3.4.3 Duchenne muscular dystrophy (DMD)—Mutation to the dystrophin (DMD) gene causes progressive muscle wasting and death and mainly affects males. A golden retriever model of DMD was treated with rAAV8 encoding U7 small nuclear (sn) RNA that promotes exon skipping to restore a functional in-frame DMD transcript. Treatment was tolerated, with approximately 80% of myofibers expressing truncated, yet functional, dystrophin at the highest dose [108]. A phase I trial with rAAV2.5 (a chimeric AAV2 capsid with 5 mutations from AAV1) injected into the patient's bicep demonstrated no immune response to the vector as well as a safe and tolerated therapy [109]. For **Becker muscular dystrophy (BMD**), a variant of DMD, the same absence of a T-cell mediated immune response to rAAV1 was observed in a phase I/II trial. Significantly, rAAV1 encoding an

alternatively spliced follistatin demonstrated improvement in 4 out of 6 BMD patients in a six-minute walk test [110].

3.5 Others rare diseases

3.5.1 Smith-Lemli-Opitz syndrome—Mutation to the 7-dehydrocholesterol reductase (DHCR7) gene greatly reduces cellular production of cholesterol and causes systemic issues including learning disabilities, malformed organs, weakened muscles, and many physical abnormalities. Use of rAAV2 or rAAV8 encoding DHCR7 results in disease improvement in mice, with greater efficacy in the rAAV28 treated animals. Significantly, greater therapeutic outcomes were seen in newborn mice versus juvenile animals [111], highlighting again that timing may be crucial for treatment of rare diseases that present in childhood.

3.5.2 Lipoprotein lipase deficiency (LPLD)—Following the European Medicines Agency approval of Glybera (rAAV1 encoding human lipoprotein lipase (LPL) for LPLD treatment in 2012, the use of AAV for gene therapy moved into reality. In a recent phase II study of LPLD it was found that treatment with rAAV1 encoding a gain of function LPL variant (S447X) did not elicit immune responses following intramuscular administration. The therapy did not impact safety and found that preexisting antibodies did not effect transgene expression up to 52 weeks after treatment [112].

3.5.3 α-1 antitrypsin (AAT)—Results from a phase II trial (NCT0105433) for AAT disease show transgene expression for more than 1 year without immunosuppression [113]. In this trial, following rAAV1 encoding the α-1 antitrypsin gene (SERPINA1, AAT) treatment, it was found that intramuscular delivery induces regulatory T-cells that attenuate cell-mediated clearance of transduced cells and allows for ongoing transgene expression. Interestingly, immunomodulation may affect initial transduction levels, but may not significantly impact transgene expression afterward [113]. Ongoing phase I and II clinical trials are using rAAV1 encoding AAT with a CB promoter (a cytomegalovirus immediate early enhancer/chicken β-actin promoter with a hybrid chicken β-actin/rabbit β-globin intron), while a phase I trial is using rAAV2 with the same construct. The phase II study reported that all subjects developed anti-AAV antibodies and no subjects developed antibodies against AAT [114]. The authors noted that serum levels of AAT > 20 ug/ml were achieved, but that further development will be needed to achieve the required therapeutic levels [114].

3.5.4 Hemophilia B—Impressively, in a phase 1 trial, it was found that intravenous treatment with scAAV8 encoding a codon optimized coagulation factor IX (FIX, F9) with a liver-specific promoter resulted in patient FIX expression for more than 3 years with more than 90% reduction in bleeding episodes [115]. Additionally, a long term follow up study is ongoing and will examine patients treated with intrahepatic injection of rAAV2 encoding FIX with an AAT liver-specific promoter in a phase I trial between 2001 and 2004 [29]. The study is to be completed in 2019. Importantly, previous trials demonstrated a vector dose-dependent inflammation and loss of transduced hepatocytes by cell-mediated immunity. A phase I/II using rAAV8 encoding FIX aims to overcome the dose-dependent inflammation seen in other trials, where rAAV8 at lower doses allows for FIX expression sooner and

stronger than other serotypes [116]. Also promising is preclinical data showing that the use of the scAAV8 encoding FIXR338L, a gain-of-function FIX variant, produced a greater than 6-fold FIX activity compared to wild type FIX without generation of anti-FIX antibodies or CD8+ T cell infiltrates in the liver [117]. These data demonstrate a safety and efficacy and support an ongoing scAAV8.FIXR338L phase I/II clinical trial.

3.5.5 Phenylketonuria (PKU)—An autosomal recessive inheritable phenylalanine hydroxylase (PAH) deficiency results in toxic levels of phenylalanine (Phe) in the blood that leads to severe brain damage. Current treatments include diet modification and sapropterin dihydrochloride that helps break down phenylalanine. Significantly, intraperitoneal injection of scAAV8 encoding murine PAH into a PKU mouse model caused reduction in blood Phe to near normal levels. Importantly, this complete phenotypic correction was seen in mice of both genders and lasted more than one year [118].

3.5.6 Glycogen storage disease type Iα (GSDIα)—Buildup of glycogen in the cells of the body impairs function of many tissue types including: liver, kidneys, and small intestines and results in severe hypoglycemia. A naturally occurring canine model of GSDIα was treated with rAAV2/8 and rAAV2/1. Results demonstrated a transient effect with rAAV2/8, with the dogs showing improvement two weeks post treatment, but no longer able to maintain glucose homeostasis two months post treatment. Using the same animal, portal vein injection of rAAV2/1 resulted in maintained glucose homeostasis two months post treatment, lasting up to 23 months. Measurement of lactate levels indicated that a complete phenotype reversal was not achieved, but that a significant improvement encouraged continued investigation for future treatments [119].

3.5.7 Very long-chain acyl-coA dehydrogenase (VLCAD)—Lethargy, muscle weakness, and hypoglycemia result from an autosomal recessive inheritance of VLCAD that prevents fat to energy conversion, specifically mitochondrial fatty acid oxidation. VLCAD-deficient mice were treated via tail vein injection with rAAV9 encoding VLCAD. A significant drop in long-chain fatty acyl accumulation was observed from 2 weeks to 20 weeks post injection. Correction was not achieved in liver tissue, but cardiac tissue showed significant reduction in long-chain metabolites. Following a fasting cold challenge, treated mice maintained body temperature and euglycemia compared to controls that became lethargic and hypoglycemic and had to be euthanized [120].

4. EXPERT OPINION

Gene therapy using AAV for treatment of rare disease is rapidly emerging as a major therapeutic option. Advances in vector generation and purification allows for scalable, economic production of clinical quality vectors. Additionally, identification of limiting factors like neutralizing antibodies, vector genome length, and transgene expression are being overcome with multiple solutions. One of the biggest limitations to the study of rAAV gene therapies for rare disease is rooted in a lack of animal models. Pre-clinical testing with *in vitro* models is commonplace, but translation to animals has not kept up with the rate of disease identification. Beyond the traditional Cre/LoxP method to generate disease models in animals, the recent creation of CRISPR-Cas9 mice is a novel method for targeted genome

editing [121]. Coupled with next-generation sequencing to identify disease specific genes and the scientific community is empowered with a system to facilitate generation of rare disease models. With promising clinical data suggesting that FDA approval of rAAVs is imminent, the issue of tumorigenicity must be addressed. While numerous trials have demonstrated safety, future research must focus on methods of understanding and preventing nonspecific rAAV genome integration, as the tumorigenicity of the vector in a clinical setting is still not completely understood. However, the recent demonstration that rAAV-mediated promoterless gene targeting without nucleases generates site specific integration in the albumin locus may allow for safe integration in future therapies [122]. Additionally, further research into cellular host responses and rAAV transduction levels may allow for improved therapies. For example, the recently described cyclic GMP-AMP synthase (cGAS) molecule also detects DNA in the cytoplasm and has not been explored in regard to rAAV detection and influence on gene therapy. Additionally, RNA polymerase III (Pol III) is known to link cytosolic DNA detection via DNA-dependent activator of interferon regulatory factor (DAI) to the RNA detection retinoic acid-inducible gene 1 (RIG-I) pathway and promote induction of type interferon antiviral responses. Inhibition of Pol III has not been explored in a rAAV therapeutic context. It is unclear if other host cell proteins might also be targeted to improve rAAV gene therapy.

The continued research of rAAV for rare disease gene therapy will no doubt bring improved quality of life to the millions of affected individuals if vectors are approved for use. However, in some cases where less than 2,000 persons are affected, it may be difficult to find human patients for clinical trials. Additionally, as with any new therapeutic, it is unclear what long term transgene expression may do to an individual, though some trials have seen expression for more than 6 years with no adverse outcomes. Without a doubt, AAV treatment of rare disease is no longer in its infancy and will soon be a useful tool to improve the lives of countless individuals in the future.

Acknowledgments

We would like to acknowledge funding support to R.J.S from University of Pennsylvania ODC (MPSI-13-010-01) and NIH (R01 AI080726, R01 DK084033, P01 HL112761, R01 AI072176, R01 AR064369).

Bibliography

Papers of special note have been highlighted as either of interest

(•) or of considerable interest

(••) to readers.

1. Boycott KM, Vanstone MR, Bulman DE, MacKenzie AE. Rare-disease genetics in the era of next-generation sequencing: discovery to translation. Nature reviews Genetics. Oct; 2013 14(10):681–91.
2. Berns KI, PC. Parvoviridae.. In: Howley, DKaP, editor. Fields Virology. Lippincott Williams & Wilkins; New York: 2007. p. 2437 - 77.
3. Calcedo R, Morizono H, Wang L, McCarter R, He J, Jones D, et al. Adeno-associated virus antibody profiles in newborns, children, and adolescents. Clinical and vaccine immunology : CVI. Sep; 2011 18(9):1586–8. [PubMed: 21775517]

4•. Calcedo R, Vandenberghe LH, Gao G, Lin J, Wilson JM. Worldwide epidemiology of neutralizing antibodies to adeno-associated viruses. The Journal of infectious diseases. Feb 1; 2009 199(3): 381–90. [PubMed: 19133809] [Potential for AAV neutralizing antibodies in patients.]

5. Hastie E, Samulski RJ. AAV at 50: A golden anniversary of discovery, research, and gene therapy success, a personal perspective. Human gene therapy. Mar 26.2015

6. Clement N, Knop DR, Byrne BJ. Large-scale adeno-associated viral vector production using a herpesvirus-based system enables manufacturing for clinical studies. Human gene therapy. Aug; 2009 20(8):796–806. [PubMed: 19569968]

7. Kotin RM. Large-scale recombinant adeno-associated virus production. Human molecular genetics. Apr 15; 2011 20(R1):R2–6. [PubMed: 21531790]

8. Thomas DL, Wang L, Niamke J, Liu J, Kang W, Scotti MM, et al. Scalable recombinant adeno-associated virus production using recombinant herpes simplex virus type 1 coinfection of suspension-adapted mammalian cells. Human gene therapy. Aug; 2009 20(8):861–70. [PubMed: 19419276]

9. Louis Jeune V, Joergensen JA, Hajjar RJ, Weber T. Pre-existing anti-adeno-associated virus antibodies as a challenge in AAV gene therapy. Human gene therapy methods. Apr; 2013 24(2):59–67. [PubMed: 23442094]

10. Selot RS, Hareendran S, Jayandharan GR. Developing immunologically inert adeno-associated virus (AAV) vectors for gene therapy: possibilities and limitations. Current pharmaceutical biotechnology. 2014; 14(12):1072–82. [PubMed: 24678652]

11. Calcedo R, Wilson JM. Humoral Immune Response to AAV. Frontiers in immunology. 2013; 4:341. [PubMed: 24151496]

12. Basner-Tschakarjan E, Mingozzi F. Cell-Mediated Immunity to AAV Vectors, Evolving Concepts and Potential Solutions. Frontiers in immunology. 2014; 5:350. [PubMed: 25101090]

13. Jiang H, Couto LB, Patarroyo-White S, Liu T, Nagy D, Vargas JA, et al. Effects of transient immunosuppression on adenoassociated, virus-mediated, liver-directed gene transfer in rhesus macaques and implications for human gene therapy. Blood. Nov 15; 2006 108(10):3321–8. [PubMed: 16868252]

14. Erles K, Sebokova P, Schlehofer JR. Update on the prevalence of serum antibodies (IgG and IgM) to adeno-associated virus (AAV). Journal of medical virology. Nov; 1999 59(3):406–11. [PubMed: 10502275]

15. Mingozzi F, Meulenberg JJ, Hui DJ, Basner-Tschakarjan E, Hasbrouck NC, Edmonson SA, et al. AAV-1-mediated gene transfer to skeletal muscle in humans results in dose-dependent activation of capsid-specific T cells. Blood. Sep 3; 2009 114(10):2077–86. [PubMed: 19506302]

16. Wang L, Calcedo R, Bell P, Lin J, Grant RL, Siegel DL, et al. Impact of pre-existing immunity on gene transfer to nonhuman primate liver with adeno-associated virus 8 vectors. Human gene therapy. Nov; 2011 22(11):1389–401. [PubMed: 21476868]

17. Thwaite R, Pages G, Chillon M, Bosch A. AAVrh.10 immunogenicity in mice and humans. Relevance of antibody cross-reactivity in human gene therapy. Gene therapy. Nov 20.2014

18. Li C, Narkbunnam N, Samulski RJ, Asokan A, Hu G, Jacobson LJ, et al. Neutralizing antibodies against adeno-associated virus examined prospectively in pediatric patients with hemophilia. Gene therapy. Mar; 2012 19(3):288–94. [PubMed: 21697954]

19. Damdindorj L, Karnan S, Ota A, Hossain E, Konishi Y, Hosokawa Y, et al. A comparative analysis of constitutive promoters located in adeno-associated viral vectors. PloS one. 2014; 9(8):e106472. [PubMed: 25170953]

20. Bartel M, Schaffer D, Buning H. Enhancing the Clinical Potential of AAV Vectors by Capsid Engineering to Evade Pre-Existing Immunity. Frontiers in microbiology. 2011; 2:204. [PubMed: 22065962]

21. Gurda BL, Raupp C, Popa-Wagner R, Naumer M, Olson NH, Ng R, et al. Mapping a neutralizing epitope onto the capsid of adeno-associated virus serotype 8. Journal of virology. Aug; 2012 86(15):7739–51. [PubMed: 22593150]

22. Moskalenko M, Chen L, van Roey M, Donahue BA, Snyder RO, McArthur JG, et al. Epitope mapping of human anti-adeno-associated virus type 2 neutralizing antibodies: implications for

gene therapy and virus structure. Journal of virology. Feb; 2000 74(4):1761–6. [PubMed: 10644347]

23. Tseng YS, Agbandje-McKenna M. Mapping the AAV Capsid Host Antibody Response toward the Development of Second Generation Gene Delivery Vectors. Frontiers in immunology. 2014; 5:9. [PubMed: 24523720]
24. Adachi K, Enoki T, Kawano Y, Veraz M, Nakai H. Drawing a high-resolution functional map of adeno-associated virus capsid by massively parallel sequencing. Nature communications. 2014; 5:3075.
25. Carlisle RC, Benjamin R, Briggs SS, Sumner-Jones S, McIntosh J, Gill D, et al. Coating of adeno-associated virus with reactive polymers can ablate virus tropism, enable retargeting and provide resistance to neutralising antisera. The journal of gene medicine. Apr; 2008 10(4):400–11. [PubMed: 18220318]
26. Le HT, Yu QC, Wilson JM, Croyle MA. Utility of PEGylated recombinant adeno-associated viruses for gene transfer. Journal of controlled release : official journal of the Controlled Release Society. Nov 2; 2005 108(1):161–77. [PubMed: 16125817]
27. Gyorgy B, Fitzpatrick Z, Crommentuijn MH, Mu D, Maguire CA. Naturally enveloped AAV vectors for shielding neutralizing antibodies and robust gene delivery in vivo. Biomaterials. Aug; 2014 35(26):7598–609. [PubMed: 24917028]
28. Labib M, Zamay AS, Muharemagic D, Chechik A, Bell JC, Berezovski MV. Electrochemical sensing of aptamer-facilitated virus immunoshielding. Analytical chemistry. Feb 7; 2012 84(3): 1677–86. [PubMed: 22242920]
29••. Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nature medicine. Mar; 2006 12(3):342–7. [One of the earliest AAV gene therapy trials for hemophilia demonstrating long term efficacy (also see reference 115).]
30. Stieger K, Le Meur G, Lasne F, Weber M, Deschamps JY, Nivard D, et al. Long-term doxycycline-regulated transgene expression in the retina of nonhuman primates following subretinal injection of recombinant AAV vectors. Molecular therapy : the journal of the American Society of Gene Therapy. May; 2006 13(5):967–75. [PubMed: 16442848]
31. Zhong L, Li B, Mah CS, Govindasamy L, Agbandje-McKenna M, Cooper M, et al. Next generation of adeno-associated virus 2 vectors: point mutations in tyrosines lead to high-efficiency transduction at lower doses. Proceedings of the National Academy of Sciences of the United States of America. Jun 3; 2008 105(22):7827–32. [PubMed: 18511559]
32•. Mingozzi F, Maus MV, Hui DJ, Sabatino DE, Murphy SL, Rasko JE, et al. CD8(+) T-cell responses to adeno-associated virus capsid in humans. Nature medicine. Apr; 2007 13(4):419–22. [Innate and adaptive immunity and AAV (also see references 33 - 39).]
33. Zhu J, Huang X, Yang Y. The TLR9-MyD88 pathway is critical for adaptive immune responses to adeno-associated virus gene therapy vectors in mice. The Journal of clinical investigation. Aug; 2009 119(8):2388–98. [PubMed: 19587448]
34. Zaiss AK, Liu Q, Bowen GP, Wong NC, Bartlett JS, Muruve DA. Differential activation of innate immune responses by adenovirus and adeno-associated virus vectors. Journal of virology. May; 2002 76(9):4580–90. [PubMed: 11932423]
35. Faust SM, Bell P, Cutler BJ, Ashley SN, Zhu Y, Rabinowitz JE, et al. CpG-depleted adeno-associated virus vectors evade immune detection. The Journal of clinical investigation. Jul 1; 2013 123(7):2994–3001. [PubMed: 23778142]
36. Zhang P, Luo X, Bird A, Li S, Koeberl DD. Deficiency in MyD88 Signaling Results in Decreased Antibody Responses to an Adeno-Associated Virus Vector in Murine Pompe Disease. BioResearch open access. Jun; 2012 1(3):109–14. [PubMed: 23514839]
37. Mitchell AM, Hirsch ML, Li C, Samulski RJ. Promyelocytic leukemia protein is a cell-intrinsic factor inhibiting parvovirus DNA replication. Journal of virology. Jan; 2014 88(2):925–36. [PubMed: 24198403]
38. Cervelli T, Palacios JA, Zentilin L, Mano M, Schwartz RA, Weitzman MD, et al. Processing of recombinant AAV genomes occurs in specific nuclear structures that overlap with foci of DNA-

damage-response proteins. Journal of cell science. Feb 1; 2008 121(Pt 3):349–57. [PubMed: 18216333]

39. Lentz TB, Samulski RJ. Insight into the Mechanism of Inhibition of Recombinant Adeno-Associated Virus by the Mre11/Rad50/Nbs1 Complex. Journal of virology. Oct 15.2014
40. Summerford C, Samulski RJ. Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. Journal of virology. Feb; 1998 72(2):1438–45. [PubMed: 9445046]
41. Kaludov N, Brown KE, Walters RW, Zabner J, Chiorini JA. Adeno-associated virus serotype 4 (AAV4) and AAV5 both require sialic acid binding for hemagglutination and efficient transduction but differ in sialic acid linkage specificity. Journal of virology. Aug; 2001 75(15):6884–93. [PubMed: 11435568]
42. Walters RW, Yi SM, Keshavjee S, Brown KE, Welsh MJ, Chiorini JA, et al. Binding of adeno-associated virus type 5 to 2,3-linked sialic acid is required for gene transfer. The Journal of biological chemistry. Jun 8; 2001 276(23):20610–6. [PubMed: 11262413]
43. Akache B, Grimm D, Pandey K, Yant SR, Xu H, Kay MA. The 37/67-kilodalton laminin receptor is a receptor for adeno-associated virus serotypes 8, 2, 3, and 9. Journal of virology. Oct; 2006 80(19):9831–6. [PubMed: 16973587]
44. Schmidt M, Chiorini JA. Gangliosides are essential for bovine adeno-associated virus entry. Journal of virology. Jun; 2006 80(11):5516–22. [PubMed: 16699032]
45. Geoghegan JC, Keiser NW, Okulist A, Martins I, Wilson MS, Davidson BL. Chondroitin Sulfate is the Primary Receptor for a Peptide-Modified AAV That Targets Brain Vascular Endothelium In Vivo. Molecular therapy Nucleic acids. 2014; 3:e202. [PubMed: 25313621]
46. Sallach J, Di Pasquale G, Larcher F, Niehoff N, Rubsam M, Huber A, et al. Tropism-modified AAV vectors overcome barriers to successful cutaneous therapy. Molecular therapy : the journal of the American Society of Gene Therapy. May; 2014 22(5):929–39. [PubMed: 24468915]
47. Dayton RD, Wang DB, Klein RL. The advent of AAV9 expands applications for brain and spinal cord gene delivery. Expert opinion on biological therapy. Jun; 2012 12(6):757–66. [PubMed: 22519910]
48. Murlidharan G, Samulski RJ, Asokan A. Biology of adeno-associated viral vectors in the central nervous system. Frontiers in molecular neuroscience. 2014; 7:76. [PubMed: 25285067]
49. Bourdenx M, Dutheil N, Bezard E, Dehay B. Systemic gene delivery to the central nervous system using Adeno-associated virus. Frontiers in molecular neuroscience. 2014; 7:50. [PubMed: 24917785]
50. Lentz TB, Gray SJ, Samulski RJ. Viral vectors for gene delivery to the central nervous system. Neurobiology of disease. Nov; 2012 48(2):179–88. [PubMed: 22001604]
51. Willett K, Bennett J. Immunology of AAV-Mediated Gene Transfer in the Eye. Frontiers in immunology. 2013; 4:261. [PubMed: 24009613]
52. Day TP, Byrne LC, Schaffer DV, Flannery JG. Advances in AAV vector development for gene therapy in the retina. Advances in experimental medicine and biology. 2014; 801:687–93. [PubMed: 24664759]
53. Zacchigna S, Zentilin L, Giacca M. Adeno-associated virus vectors as therapeutic and investigational tools in the cardiovascular system. Circulation research. May 23; 2014 114(11): 1827–46. [PubMed: 24855205]
54. Asokan A, Samulski RJ. An emerging adeno-associated viral vector pipeline for cardiac gene therapy. Human gene therapy. Nov; 2013 24(11):906–13. [PubMed: 24164238]
55. Gruntman AM, Mueller C, Flotte TR, Gao G. Gene transfer in the lung using recombinant adeno-associated virus. Current protocols in microbiology. Aug.2012 Chapter 14:Unit14D 2.
56. Keeler AM, Flotte TR. Cell and gene therapy for genetic diseases: inherited disorders affecting the lung and those mimicking sudden infant death syndrome. Human gene therapy. Jun; 2012 23(6): 548–56. [PubMed: 22642257]
57. Luebke AE, Rova C, Von Doersten PG, Poulsen DJ. Adenoviral and AAV-mediated gene transfer to the inner ear: role of serotype, promoter, and viral load on in vivo and in vitro infection efficiencies. Advances in oto-rhino-laryngology. 2009; 66:87–98. [PubMed: 19494574]

58. van der Laan LJ, Wang Y, Tilanus HW, Janssen HL, Pan Q. AAV-mediated gene therapy for liver diseases: the prime candidate for clinical application? Expert opinion on biological therapy. Mar; 2011 11(3):315–27. [PubMed: 21204741]

59. Evans CH, Ghivizzani SC, Robbins PD. Progress and Prospects: genetic treatments for disorders of bones and joints. Gene therapy. Aug; 2009 16(8):944–52. [PubMed: 19675584]

60. Miyagoe-Suzuki Y, Takeda S. Gene therapy for muscle disease. Experimental cell research. Nov 1; 2010 316(18):3087–92. [PubMed: 20580709]

61. Wang D, Zhong L, Nahid MA, Gao G. The potential of adeno-associated viral vectors for gene delivery to muscle tissue. Expert opinion on drug delivery. Mar; 2014 11(3):345–64. [PubMed: 24386892]

62. Liu X, Magee D, Wang C, McMurphy T, Slater A, During M, et al. Adipose tissue insulin receptor knockdown via a new primate-derived hybrid recombinant AAV serotype. Molecular therapy Methods & clinical development. Feb 5.2014 :1. [PubMed: 26015941]

63. O'Neill SM, Hinkle C, Chen SJ, Sandhu A, Hovhannisyan R, Stephan S, et al. Targeting adipose tissue via systemic gene therapy. Gene therapy. Jul; 2014 21(7):653–61. [PubMed: 24830434]

64. White SJ, Nicklin SA, Buning H, Brosnan MJ, Leike K, Papadakis ED, et al. Targeted gene delivery to vascular tissue in vivo by tropism-modified adeno-associated virus vectors. Circulation. Feb 3; 2004 109(4):513–9. [PubMed: 14732747]

65. Stachler MD, Bartlett JS. Mosaic vectors comprised of modified AAV1 capsid proteins for efficient vector purification and targeting to vascular endothelial cells. Gene therapy. Jun; 2006 13(11):926–31. [PubMed: 16482202]

66. Bartlett JS, Kleinschmidt J, Boucher RC, Samulski RJ. Targeted adeno-associated virus vector transduction of nonpermissive cells mediated by a bispecific F(ab'gamma)2 antibody. Nature biotechnology. Feb; 1999 17(2):181–6.

67. Arnold GS, Sasser AK, Stachler MD, Bartlett JS. Metabolic biotinylation provides a unique platform for the purification and targeting of multiple AAV vector serotypes. Molecular therapy : the journal of the American Society of Gene Therapy. Jul; 2006 14(1):97–106. [PubMed: 16624620]

68. Ponnazhagan S, Mahendra G, Kumar S, Thompson JA, Castillas M, Jr. Conjugate-based targeting of recombinant adeno-associated virus type 2 vectors by using avidin-linked ligands. Journal of virology. Dec; 2002 76(24):12900–7. [PubMed: 12438615]

69. Nonnenmacher M, Weber T. Intracellular transport of recombinant adeno-associated virus vectors. Gene therapy. Jun; 2012 19(6):649–58. [PubMed: 22357511]

70••. Wu Z, Yang H, Colosi P. Effect of genome size on AAV vector packaging. Molecular therapy : the journal of the American Society of Gene Therapy. Jan; 2010 18(1):80–6. [PubMed: 19904234] [AAV gene packaging limitations.]

71••. McCarty DM, Monahan PE, Samulski RJ. Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. Gene therapy. Aug; 2001 8(16):1248–54. [PubMed: 11509958] [Self-complimentary AAV (scAAV) for enhanced transgene expression.]

72. Martino AT, Suzuki M, Markusic DM, Zolotukhin I, Ryals RC, Moghimi B, et al. The genome of self-complementary adeno-associated viral vectors increases Toll-like receptor 9-dependent innate immune responses in the liver. Blood. Jun 16; 2011 117(24):6459–68. [PubMed: 21474674]

73. Xu Z, Ye J, Zhang A, Xie L, Shen Q, Xue J, et al. Gene Therapy for Hemophilia B with liver-specific element mediated by Rep-RBE site-specific integration system. Journal of cardiovascular pharmacology. Oct 7.2014

74. Chuah MK, Petrus I, De Bleser P, Le Guiner C, Gernoux G, Adjali O, et al. Liver-specific transcriptional modules identified by genome-wide in silico analysis enable efficient gene therapy in mice and non-human primates. Molecular therapy : the journal of the American Society of Gene Therapy. Sep; 2014 22(9):1605–13. [PubMed: 24954473]

75. Flotte TR, Afione SA, Solow R, Drumm ML, Markakis D, Guggino WB, et al. Expression of the cystic fibrosis transmembrane conductance regulator from a novel adeno-associated virus promoter. The Journal of biological chemistry. Feb 15; 1993 268(5):3781–90. [PubMed: 7679117]

76. Ling C, Wang Y, Lu Y, Wang L, Jayandharan GR, Aslanidi GV, et al. Enhanced transgene expression from single-stranded D-sequence-substituted recombinant AAV vectors in human cell lines in vitro and in murine hepatocytes in vivo. Journal of virology. Oct 29.2014

77. Somia N, Verma IM. Gene therapy: trials and tribulations. Nature reviews Genetics. Nov; 2000 1(2):91–9.

78. Vandenberghe LH, Bell P, Maguire AM, Cearley CN, Xiao R, Calcedo R, et al. Dosage thresholds for AAV2 and AAV8 photoreceptor gene therapy in monkey. Science translational medicine. Jun 22.2011 3(88):88ra54.

79. Black A, Vasireddy V, Chung DC, Maguire AM, Gaddameedi R, Tolmachova T, et al. Adeno-associated virus 8-mediated gene therapy for choroideremia: preclinical studies in in vitro and in vivo models. The journal of gene medicine. May-Jun;2014 16(5-6):122–30. [PubMed: 24962736]

80•. MacLaren RE, Groppe M, Barnard AR, Cottriall CL, Tolmachova T, Seymour L, et al. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. Lancet. Mar 29; 2014 383(9923):1129–37. [PubMed: 24439297] [Gain of visual acuity in patients.]

81. Jacobson SG, Cideciyan AV, Ratnakaram R, Heon E, Schwartz SB, Roman AJ, et al. Gene therapy for leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. Archives of ophthalmology. Jan; 2012 130(1):9–24. [PubMed: 21911650]

82. Molday LL, Djajadi H, Yan P, Szczygiel L, Boye SL, Chiodo VA, et al. RD3 gene delivery restores guanylate cyclase localization and rescues photoreceptors in the Rd3 mouse model of Leber congenital amaurosis 12. Human molecular genetics. Oct 1; 2013 22(19):3894–905. [PubMed: 23740938]

83. Ku CA, Chiodo VA, Boye SL, Goldberg AF, Li T, Hauswirth WW, et al. Gene therapy using self-complementary Y733F capsid mutant AAV2/8 restores vision in a model of early onset Leber congenital amaurosis. Human molecular genetics. Dec 1; 2011 20(23):4569–81. [PubMed: 21880665]

84. Smith AJ, Schlichtenbrede FC, Tschernutter M, Bainbridge JW, Thrasher AJ, Ali RR. AAV-Mediated gene transfer slows photoreceptor loss in the RCS rat model of retinitis pigmentosa. Molecular therapy : the journal of the American Society of Gene Therapy. Aug; 2003 8(2):188–95. [PubMed: 12907141]

85. Palfi A, Millington-Ward S, Chadderton N, O'Reilly M, Goldmann T, Humphries MM, et al. Adeno-associated virus-mediated rhodopsin replacement provides therapeutic benefit in mice with a targeted disruption of the rhodopsin gene. Human gene therapy. Mar; 2010 21(3):311–23. [PubMed: 19824806]

86. Jiang L, Frederick JM, Baehr W. RNA interference gene therapy in dominant retinitis pigmentosa and cone-rod dystrophy mouse models caused by GCAP1 mutations. Frontiers in molecular neuroscience. 2014; 7:25. [PubMed: 24778606]

87. Beltran WA, Cideciyan AV, Lewin AS, Hauswirth WW, Jacobson SG, Aguirre GD. Gene Augmentation for X-Linked Retinitis Pigmentosa Caused by Mutations in RPGR. Cold Spring Harbor perspectives in medicine. Oct 9.2014

88. Ardeljan D, Wang Y, Park S, Shen D, Chu XK, Yu CR, et al. Interleukin-17 retinotoxicity is prevented by gene transfer of a soluble interleukin-17 receptor acting as a cytokine blocker: implications for age-related macular degeneration. PloS one. 2014; 9(4):e95900. [PubMed: 24780906]

89. Maclachlan TK, Lukason M, Collins M, Munger R, Isenberger E, Rogers C, et al. Preclinical safety evaluation of AAV2-sFLT01- a gene therapy for age-related macular degeneration. Molecular therapy : the journal of the American Society of Gene Therapy. Feb; 2011 19(2):326–34. [PubMed: 21119620]

90•. Meyer K, Ferraiuolo L, Schmelzer L, Braun L, McGovern V, Likhite S, et al. Improving single injection CSF delivery of AAV9-mediated gene therapy for SMA - a dose response study in mice and nonhuman primates. Molecular therapy : the journal of the American Society of Gene Therapy. Oct 31.2014 [Complete rescue of phenotype seen in Non-human primates.]

91. Little D, Valori CF, Mutsaers CA, Bennett EJ, Wyles M, Sharrack B, et al. PTEN Depletion Decreases Disease Severity and Modestly Prolongs Survival in a Mouse Model of Spinal Muscular Atrophy. Molecular therapy : the journal of the American Society of Gene Therapy. Nov 5.2014
92. Lee NC, Chien YH, Hu MH, Liu WS, Chen PW, Wang WH, et al. Treatment of congenital neurotransmitter deficiencies by intracerebral ventricular injection of an adeno-associated virus serotype 9 vector. Human gene therapy. Mar; 2014 25(3):189–98. [PubMed: 24251946]
93•. Muramatsu S, Fujimoto K, Kato S, Mizukami H, Asari S, Ikeguchi K, et al. A phase I study of aromatic L-amino acid decarboxylase gene therapy for Parkinson's disease. Molecular therapy : the journal of the American Society of Gene Therapy. Sep; 2010 18(9):1731–5. [PubMed: 20606642] [Patients demonstrated improved motor performance.]
94. Cederfjall E, Broom L, Kirik D. Controlled striatal DOPA production from a gene delivery system in a rodent model of Parkinson's disease. Molecular therapy : the journal of the American Society of Gene Therapy. Jan 16.2015
95. Gholizadeh S, Arsenault J, Xuan IC, Pacey LK, Hampson DR. Reduced phenotypic severity following adeno-associated virus-mediated FMR1 gene delivery in fragile x mice. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. Dec; 2014 39(13):3100–11. [PubMed: 24998620]
96. Perdomini M, Belbellaa B, Monassier L, Reutenauer L, Messaddeq N, Cartier N, et al. Prevention and reversal of severe mitochondrial cardiomyopathy by gene therapy in a mouse model of Friedreich's ataxia. Nature medicine. May; 2014 20(5):542–7.
97. Batista AR, Gianni D, Ventosa M, Coelho AV, Almeida MR, Sena-Esteves M, et al. Gene therapy approach to FAP: in vivo influence of T119M in TTR deposition in a transgenic V30M mouse model. Gene therapy. Oct 2.2014
98. Stanek LM, Sardi SP, Mastis B, Richards AR, Treleaven CM, Taksir T, et al. Silencing mutant huntingtin by adeno-associated virus-mediated RNA interference ameliorates disease manifestations in the YAC128 mouse model of Huntington's disease. Human gene therapy. May; 2014 25(5):461–74. [PubMed: 24484067]
99. Miyake N, Miyake K, Asakawa N, Yamamoto M, Shimada T. Long-term correction of biochemical and neurological abnormalities in MLD mice model by neonatal systemic injection of an AAV serotype 9 vector. Gene therapy. Apr; 2014 21(4):427–33. [PubMed: 24572788]
100. Piguet F, Sondhi D, Piraud M, Fouquet F, Hackett NR, Ahouansou O, et al. Correction of brain oligodendrocytes by AAVrh.10 intracerebral gene therapy in metachromatic leukodystrophy mice. Human gene therapy. Aug; 2012 23(8):903–14. [PubMed: 22642214]
101. Hinderer C, Bell P, Gurda BL, Wang Q, Louboutin JP, Zhu Y, et al. Liver-directed gene therapy corrects cardiovascular lesions in feline mucopolysaccharidosis type I. Proceedings of the National Academy of Sciences of the United States of America. Oct 14; 2014 111(41):14894–9. [PubMed: 25267637]
102. Ferla R, Claudiani P, Cotugno G, Saccone P, De Leonibus E, Auricchio A. Similar therapeutic efficacy between a single administration of gene therapy and multiple administrations of recombinant enzyme in a mouse model of lysosomal storage disease. Human gene therapy. Jul; 2014 25(7):609–18. [PubMed: 24725025]
103. Han SO, Li S, Brooks ED, Masat E, Leborgne C, Banugaria S, et al. Enhanced Efficacy from Gene Therapy in Pompe Disease Using Co-receptor Blockade. Human gene therapy. Nov 8.2014
104. Smith BK, Collins SW, Conlon TJ, Mah CS, Lawson LA, Martin AD, et al. Phase I/II trial of adeno-associated virus-mediated alpha-glucosidase gene therapy to the diaphragm for chronic respiratory failure in Pompe disease: initial safety and ventilatory outcomes. Human gene therapy. Jun; 2013 24(6):630–40. [PubMed: 23570273]
105. Liu J, Wallace LM, Garwick-Coppens SE, Sloboda DD, Davis CS, Hakim CH, et al. RNAi-mediated Gene Silencing of Mutant Myotilin Improves Myopathy in LGMD1A Mice. Molecular therapy Nucleic acids. 2014; 3:e160. [PubMed: 24781192]
106. Mendell JR, Rodino-Klapac LR, Rosales XQ, Coley BD, Galloway G, Lewis S, et al. Sustained alpha-sarcoglycan gene expression after gene transfer in limb-girdle muscular dystrophy, type 2D. Annals of neurology. Nov; 2010 68(5):629–38. [PubMed: 21031578]

107. Arimura S, Okada T, Tezuka T, Chiyo T, Kasahara Y, Yoshimura T, et al. Neuromuscular disease. DOK7 gene therapy benefits mouse models of diseases characterized by defects in the neuromuscular junction. Science. Sep 19; 2014 345(6203):1505–8. [PubMed: 25237101]

108. Le Guiner C, Montus M, Servais L, Cherel Y, Francois V, Thibaud JL, et al. Forelimb Treatment in a Large Cohort of Dystrophic Dogs Supports Delivery of a Recombinant AAV for Exon Skipping in Duchenne Patients. Molecular therapy : the journal of the American Society of Gene Therapy. Nov; 2014 22(11):1923–35. [PubMed: 25200009]

109. Bowles DE, McPhee SW, Li C, Gray SJ, Samulski JJ, Camp AS, et al. Phase 1 gene therapy for Duchenne muscular dystrophy using a translational optimized AAV vector. Molecular therapy : the journal of the American Society of Gene Therapy. Feb; 2012 20(2):443–55. [PubMed: 22068425]

110. Mendell JR, Sahenk Z, Malik V, Gomez AM, Flanigan KM, Lowes LP, et al. A Phase 1/2a Follistatin Gene Therapy Trial for Becker Muscular Dystrophy. Molecular therapy : the journal of the American Society of Gene Therapy. Oct 17.2014

111. Ying L, Matabosch X, Serra M, Watson B, Shackleton C, Watson G. Biochemical and Physiological Improvement in a Mouse Model of Smith-Lemli-Opitz Syndrome (SLOS) Following Gene Transfer with AAV Vectors. Molecular genetics and metabolism reports. 2014; 1:103–13. [PubMed: 25024934]

112. Ferreira V, Twisk J, Kwikkers K, Aronica E, Brisson D, Methot J, et al. Immune responses to intramuscular administration of alipogene tiparvovec (AAV1-LPL(S447X)) in a phase II clinical trial of lipoprotein lipase deficiency gene therapy. Human gene therapy. Mar; 2014 25(3):180–8. [PubMed: 24299335]

113. Mueller C, Chulay JD, Trapnell BC, Humphries M, Carey B, Sandhaus RA, et al. Human Treg responses allow sustained recombinant adeno-associated virus-mediated transgene expression. The Journal of clinical investigation. Dec 2; 2013 123(12):5310–8. [PubMed: 24231351]

114. Flotte TR, Trapnell BC, Humphries M, Carey B, Calcedo R, Rouhani F, et al. Phase 2 clinical trial of a recombinant adeno-associated viral vector expressing alpha1-antitrypsin: interim results. Human gene therapy. Oct; 2011 22(10):1239–47. [PubMed: 21609134]

115••. Nathwani AC, Reiss UM, Tuddenham EG, Rosales C, Chowdary P, McIntosh J, et al. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. The New England journal of medicine. Nov 20; 2014 371(21):1994–2004. [PubMed: 25409372] [Long term safety and efficacy for AAV-mediated treatment of hemophilia.]

116. Cao Z, Zheng P, Lin Y. A comparative study of hFIX expression mediated by rAAV8 and rAAV1 administrated intramuscularly. Cytotherapy. 2007; 9(6):593–9. [PubMed: 17882724]

117. Monahan PE, Sun J, Gui T, Hu G, Hannah WB, Wichlan DG, et al. Employing a gain-of-function factor IX variant R338L to advance the efficacy and safety of hemophilia B human gene therapy: Preclinical evaluation supporting an ongoing AAV clinical trial. Human gene therapy. Nov 24.2014

118. Yagi H, Ogura T, Mizukami H, Urabe M, Hamada H, Yoshikawa H, et al. Complete restoration of phenylalanine oxidation in phenylketonuria mouse by a self-complementary adeno-associated virus vector. The journal of gene medicine. Feb; 2011 13(2):114–22. [PubMed: 21322099]

119. Weinstein DA, Correia CE, Conlon T, Specht A, Verstegen J, Onclin-Verstegen K, et al. Adeno-associated virus-mediated correction of a canine model of glycogen storage disease type Ia. Human gene therapy. Jul; 2010 21(7):903–10. [PubMed: 20163245]

120. Keeler AM, Conlon T, Walter G, Zeng H, Shaffer SA, Dungtao F, et al. Long-term correction of very long-chain acyl-coA dehydrogenase deficiency in mice using AAV9 gene therapy. Molecular therapy : the journal of the American Society of Gene Therapy. Jun; 2012 20(6):1131–8. [PubMed: 22395529]

121••. Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, et al. CRISPR-Cas9 Knockin Mice for Genome Editing and Cancer Modeling. Cell. Oct 9; 2014 159(2):440–55. [PubMed: 25263330] [Novel methods for generation of rare disease models.]

122. Barzel A, Paulk NK, Shi Y, Huang Y, Chu K, Zhang F, et al. Promoterless gene targeting without nucleases ameliorates haemophilia B in mice. Nature. Jan 15; 2015 517(7534):360–4. [PubMed: 25363772]

highlights

- Overview of adeno-associated virus (AAV) biology
- Strategies to generate recombinant AAV vectors to evade immune clearance, improve tissue tropism, and enhance transgene expression
- Historical and current pre-clinical and clinical challenges for AAV-mediated gene therapy
- Ongoing clinical trials with AAV for treatment of rare diseases
- Highlighted pre-clinical and clinical results from AAV studies of retinal, nervous system, lysosomal storage, muscle, and other rare diseases

This box summarizes key points contained in the article

Table 1

Current clinical trials using AAV vectors for treatment of rare disease

Effected tissue / Rare Disease	AAV used	Phase	Status	Sponsor	Trial ID
Retinal					
Choroideremia	rAAV2.REP1	I	Not yet open	Ian M. MacDonald	NCT02077361
Choroideremia	rAAV2.REP1	I/II	Recruiting	University of Oxford	NCT01461213
Leber Congenital Amaurosis	AAV2-hRPE65v2	I	Ongoing	Spark Therapeutics, LLC	NCT00516477
Leber Congenital Amaurosis	rAAV2-hRPE65	I	Recruiting	Hadassah Medical Organization	NCT00821340
Leber Congenital Amaurosis	AAV2-CBSB-hRPE65	I	Ongoing	University of Pennsylvania	NCT00481546
Leber Congenital Amaurosis	rAAV2/4.hRPE65	I/II	Ongoing	Nantes University Hospital	NCT01496040
Leber Congenital Amaurosis	AAV2-hRPE65v2	I/II	Ongoing	Spark Therapeutics, LLC	NCT01208389
Leber Congenital Amaurosis	rAAV2-CB-hRPE65	I/II	Ongoing	Applied Genetic Technologies Corp	NCT00749957
Leber Congenital Amaurosis	rAAV 2/2.hRPE65p.hRPE65	I/II	Ongoing	University College, London	NCT00643747
Leber Congenital Amaurosis	AAV2-hRPE65v2	III	Recruiting	Spark Therapeutics, LLC	NCT00999609
Leber Congenital Amaurosis	tgAAG76 (rAAV 2/2.hRPE65p.hRPE65)	I/II	Ongoing	University College, London	NCT00643747
Leber Congenital Amaurosis	AAV2-hRPE65v2	III	Recruiting	Spark Therapeutics, LLC	NCT00999609
Leber Hereditary Optic Neuropathy	scAAV2-P1ND4v2	I	Recruiting	John Guy	NCT02161380
Leber Hereditary Optic Neuropathy	rAAV2/2-ND4	I/II	Recruiting	GenSight Biologics	NCT02064569
Leber Hereditary Optic Neuropathy	rAAV2-ND4		Recruiting	Bin Li	NCT01267422
Macular Degeneration	AAV2-sFLT01	I	Ongoing	Genzyme, a Sanofi Company	NCT01024998
MERTK-associated Retinitis Pigmentosa	rAAV2-VMD2-hMERTK	I	Recruiting	Fowzan Alkuraya	NCT01482195
Central Nervous System					
Alzheimer's Disease	CERE-110	II	Ongoing	Ceregene	NCT00876863
Aromatic L-amino Acid Decarboxylase	AAV2-hAADC	I/II	Recruiting	National Taiwan University Hospital	NCT01395641
Parkinson's Disease	AAV-hAADC-2	I	Recruiting	Krystof Bankiewicz	NCT01973543
Parkinson's Disease	AAV2-GDNF	I	Recruiting	National Institute of Neurological Disorders and Stroke	NCT01621581
Parkinson's Disease	CERE-120	I/II	Ongoing	Ceregene	NCT00985517
Late Infantile Neuronal Lipofuscinosis / Batten Disease	AAV2CUhCLN2	I	Ongoing	Weill Medical College of Cornell University	NCT00151216
Late Infantile Neuronal Lipofuscinosis / Batten Disease	AAVrh.10CUhCLN2	I	Recruiting	Weill Medical College of Cornell University	NCT01161576

Effected tissue / Rare Disease	AAV used	Phase	Status	Sponsor	Trial ID
Late Infantile Neuronal Lipofuscinosis / Batten Disease	AAVrh.10CUhCLN2	I/II	Recruiting	Weill Medical College of Cornell University	NCT01414985
Pompe Disease	rAAV9-DES-hGAA	I	Not yet open	University of Florida	NCT02240407
Pompe Disease	rAAV1-CMV-GAA	I/II	Ongoing	University of Florida	NCT00976352
Spinal Muscular Atrophy 1	scAAV9.CB.SMN	I	Recruiting	Jerry R. Mendell	NCT02122952
Blood					
Acute Intermittent Porphyria	rAAV2/5-PBGD	I	Ongoing	Digna Biotech S.L.	NCT02082860
Hemophilia B	AAV2-hFIX16	I	Ongoing	Spark Therapeutics, LLC	NCT00515710
Hemophilia B	scAAV 2/8-LP1-hFIXco	I	Recruiting	St. Jude Children's Research Hospital	NCT00979238
Hemophilia B	AAV8-hFIX19	I/II	Recruiting	Spark Therapeutics, LLC	NCT01620801
Hemophilia B	AskBio009 (AAV8 - Factor IX)	I/II	Recruiting	Asklepios Biopharmaceutical, Inc.	NCT01687608
Liver and Lung					
Alpha-1 Antitrypsin Deficiency	rAAV1-CB-hAAT	I	Ongoing	University of Massachusetts, Worcester	NCT00430768
Alpha-1 Antitrypsin Deficiency	rAAV2-CB-hAAT	I	Ongoing	University of Massachusetts, Worcester	NCT00377416
Alpha-1 Antitrypsin Deficiency	AAVrh.10halpha1AT	I	Not yet open	Weill Medical College of Cornell University	NCT02168686
Alpha-1 Antitrypsin Deficiency	rAAV1-CB-hAAT	II	Ongoing	Applied Genetic Technologies Corp	NCT01054339
Cystic Fibrosis	AAV-CFTR	I	Unknown	National Institute of Diabetes and Digestive and Kidney Diseases	NCT00004533
Systemic					
Familial Lipoprotein Lipase Deficiency	AAV-Human Lipoprotein LipaseS447X	II/III	Ongoing	Amsterdam Molecular Therapeutics	NCT01109498
Familial Lipoprotein Lipase Deficiency	AAV-Human Lipoprotein LipaseS447X	II/III	Ongoing	Amsterdam Molecular Therapeutics	NCT00891306
Metachromatic Leukodystrophy	AAVrh.10cuARSA	I/II	Recruiting	Institut National de la Santé Et de la Recherche Médicale, France	NCT01801709
Muscle			Enrolling by Invitation		
Becker Muscular Dystrophy	rAAV1.CMV.huFollistatin344	I		Nationwide Children's Hospital	NCT01519349
Limb girdle muscular dystrophy type 2C	scAAVrh74.tMCK.hSGCA	I/II	Ongoing	Jerry R. Mendell	NCT01976091