Molecular Therapy
Nucleic Acids
Review

Rationale and strategies for the development of safe and effective optimized AAV vectors for human gene therapy

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Recombinant adeno-associated virus (AAV) vectors have been, or are currently in use, in 332 phase I/II/III clinical trials in a number of human diseases, and in some cases, remarkable clinical efficacy has also been achieved. There are now three US Food and Drug Administration (FDA)-approved AAV "drugs," but it has become increasingly clear that the first generation of AAV vectors are not optimal. In addition, relatively large vector doses are needed to achieve clinical efficacy, which has been shown to provoke host immune responses culminating in serious adverse events and, more recently, in the deaths of 10 patients to date. Thus, there is an urgent need for the development of the next generation of AAV vectors that are (1) safe, (2) effective, and (3) human tropic. This review describes the strategies to potentially overcome each of the limitations of the first generation of AAV vectors and the rationale and approaches for the development of the next generation of AAV serotype vectors. These vectors promise to be efficacious at significant reduced doses, likely to achieve clinical efficacy, thereby increasing the safety as well as reducing vector production costs, ensuring translation to the clinic with higher probability of success, without the need for the use of immune suppression, for gene therapy of a wide variety of diseases in humans.

INTRODUCTION

Adeno-associated virus (AAV), first discovered in 1965[1] and considered a "biological oddity,"[2] continued to fascinate only a handful of investigators. Following determination of the complete nucleotide sequence,[3] molecular cloning[4,5] of the wild-type (WT) AAV genome, and the demonstration that the WT AAV possessed the remarkable ability of site-specific integration of the AAV DNA into the long arm of human chromosome 19 (19q13.3),[6,7] although it must be noted that site-specific integration was observed in cell cultures *in vitro*, the first recombinant AAV vectors were subsequently developed by two independent groups.[8,9] Further refinements followed,[10,11] and interest in this vector system has continued to grow exponentially in the past two decades.[12–14]

The first generation of AAV vectors have been or are currently in use in 331 phase I/II/III clinical trials for a wide variety of human diseases, and in some cases, such as Leber's congenital amaurosis,[15–17] lipoprotein lipase deficiency,[18] hemophilia B,[19–26] aromatic L-amino acid decarboxylase deficiency,[27] choroideremia,[28] Leber hereditary optic neuropathy,[29,30] hemophilia A,[31–34] and spinal muscular atrophy,[35] unexpected, remarkable clinical efficacy has also been achieved. Several AAV serotype vectors are now available, which have shown clinical efficacy in a number of human diseases in animal models.[36,37] Thus far, three AAV "drugs"—Luxturna, Zolgensma, and Hemgenix—have been approved by the US Food and Drug Administration (FDA).[38,39]

Despite these remarkable achievements, it has become increasingly clear that the first generation of AAV vectors currently in use are not optimal. For example, despite their efficacy in animal models, these vectors have failed to show clinical efficacy in some cases. In addition, relatively large vector doses are needed to achieve clinical efficacy. The use of high doses has been shown to provoke host immune responses culminating in serious adverse events and, more recently, in the deaths of 10 patients to date. Thus, it has become increasingly clear that there is an urgent need for the development of the next generation of AAV vectors that are (1) safe, (2) effective, and (3) human tropic. Since AAV evolved as a virus, and not as a vector for the purposes of delivery of therapeutic genes, the host immune system cannot distinguish between AAV as a virus versus AAV as a vector. Thus, the use of AAV vectors composed of naturally occurring capsids is likely to induce immune responses, especially at high doses, since the host immune response is directly correlated with the AAV vector dose. Similarly, AAV as a virus does not express its own genes effectively since its single-stranded DNA genome is transcriptionally inactive. Most of the single-stranded AAV (ssAAV) vectors currently in use are also sub-optimal in expressing therapeutic genes. And finally, the tropisms of AAV vectors in animal models do not necessarily translate well in humans, and hence there is a need to identify and further develop human-tropic AAV vectors. This review describes the strategies to overcome each of the limitations of the first

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Table 1. Reported cases of deaths associated with the use of the first generation of AAV vectors

Sponsor	Disease	Serotype	Vector dose (vg/kg)	Number of patients
Audentes Therapeutics	XLMTM	AAV8	3×10^{14} 1×10^{14}	3 1
Lysogene	MPS-IIIA	AAVrh10	7×10^{12}	1
Pfizer	DMD	AAV9	2×10^{14}	1
Novartis	SMA	AAV9	1.1×10^{14}	3
Cure Rare Disease	DMD	AAV9	$1x10^{14}$	1

XLMTM, X-linked myotubular myopathy; MPSIIIA, mucopolysaccharidosis type IIIA; DMD, Duchenne muscular dystrophy; SMA, spinal muscular atrophy.

Table 2. Adverse events/lack of efficacy reported with the use of the first generation of AAV vectors

Sponsor	Disease	Serotype	Vector dose (vg/kg)	Symptoms/outcome
Solid Biosciences	DMD	AAV9	3×10^{14}	thrombocytopenia, renal failure, cardio-pulmonary insufficiency
Pfizer	DMD	AAV9	2×10^{14}	acute kidney injury, atypical hemolytic uremic syndrome-like complement activation, thrombocytopenia
Sarepta Therapeutics	DMD	AAVrh74	2×10^{14}	failed to reach primary clinical endpoint
Amicus Therapeutics	BD	AAV9	5×10^{13}	failed to reach primary clinical endpoint

DMD, Duchenne muscular dystrophy; BD, Batten disease.

generation of AAV vectors and the rationale and approaches for the development of the next generation of AAV serotype vectors. These vectors promise to be efficacious at significant reduced doses, likely to achieve clinical efficacy, thereby increasing the safety as well as reducing vector production costs, ensuring translation to the clinic with higher probability of success, without the need for the use of immune suppression, for gene therapy of a wide variety of human diseases.

Problems associated with the first generation of AAV vectors

Despite their remarkable achievements, it has become increasingly clear that the first generation of AAV vectors that are currently in use in clinical trials in humans are not optimal. For example, in some cases, relatively large vector doses are needed to achieve clinical efficacy.[22,26,31,32,34] It has also become increasingly clear that the currently available first generation of AAV vectors being used at high doses have been shown to provoke host immune responses culminating in serious adverse events and, more recently, in the deaths of 10 patients,[40–46] as shown in Table 1.

Furthermore, the use of the first generation of AAV serotype vectors has also been reported to lead to serious adverse events or has failed to reach the primary clinical endpoints.[47–49] These details are provided in Table 2. Similarly, the use of shuffled AAV vectors derived from directed evolution has also led to serious adverse events that have been reported in gene therapy trials with AAV vectors derived from directed evolution[50–53] (Table S1).

Taken together, the following conclusions can be drawn: (1) the use of the first generation of AAV vectors composed of naturally occurring capsids is likely to induce immune responses, especially at high doses, because the host immune system cannot distinguish between AAV as a virus versus AAV as a vector; (2) the host immune response is directly correlated with the AAV vector dose; and (3) because the WT AAV did not evolve for the purposes of delivery of therapeutic genes, recombinant AAV (rAAV) vectors composed of naturally occurring capsid are unlikely to be optimal in human clinical trials.[54,55]

Thus, it is clear that there is an urgent need for the development of the next generation of AAV vectors that are

- Capable of high-efficiency transduction at lower doses,
- Capable of mediating efficient transgene expression,
- Less immunogenic,
- Capable of obviating the need for immune suppression,
- Capable of transducing primary human cells and tissues, and
- More cost-effective.

Several elegant approaches have been employed by a number of investigators to overcome the limitations of the first generation of AAV vectors. These include the use of directed evolution,[56–60] peptide insertions,[61–66] DNA shuffling,[67–71] rational design,[72–74] ancestral vectors,[75,76] chimeric vectors,[77,78] dual vectors,[79–82] protease activation,[83,84] chemical modifications,[85] and machine learning.[86,87] A brief account of our strategies to achieve most, if not all, of the objectives outlined above follows.

Development of capsid-modified AAV vectors

Since AAV evolved as a virus, and not as a vector, the naturally occurring AAV and the first generation of rAAV vectors appear identical externally. Thus, the host immune system cannot distinguish between the two and targets both equally well. It could be argued that the two are not identical because the naturally occurring AAV contains the WT AAV DNA, whereas the rAAV vector contains a therapeutic gene. However, the immune system cannot "see" what is inside of the virus or the vector and, again, targets them both just the same. In other words, we want our immune system to work perfectly when it comes to viruses but not when we use them as vectors, which is unrealistic. It should be pointed out, however, that the vector genome has been shown to increase the Toll-like receptor 9-dependent immune response, at least in a murine models,[88,89] and that immune response to transgene products can and does occur.[90]

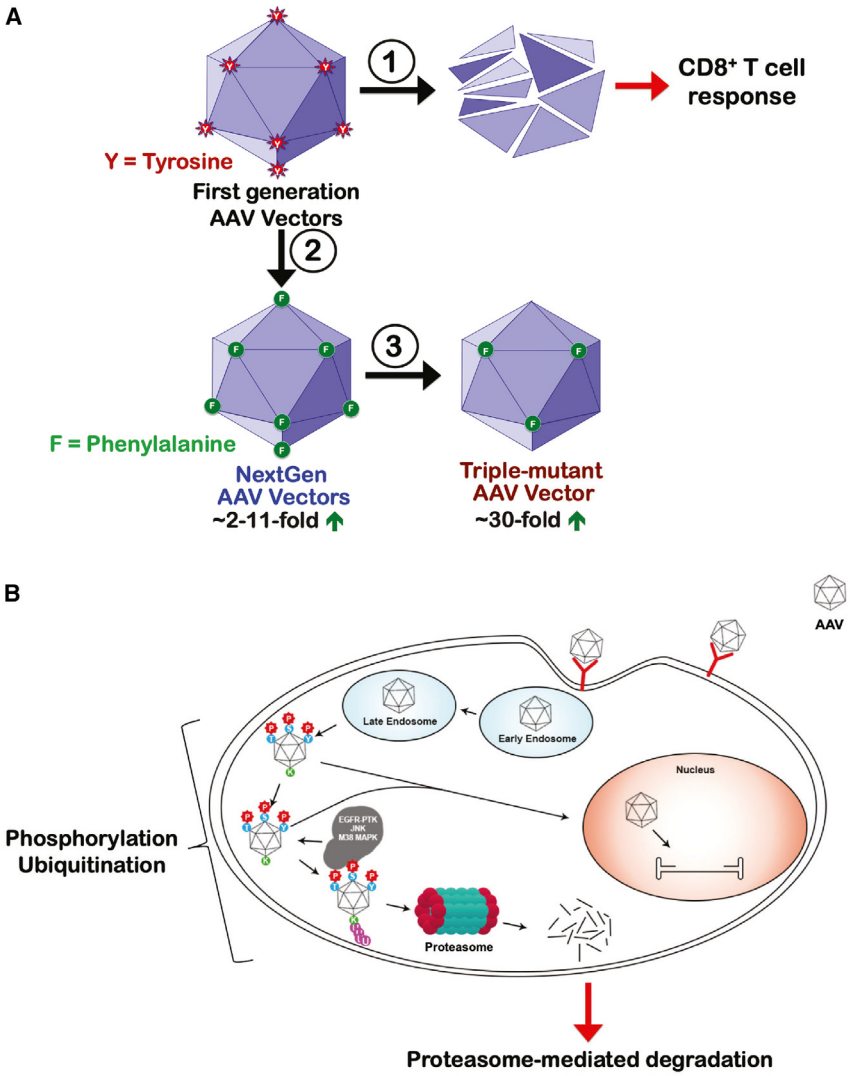

Figure 1. Intracellular fate of the first generation of AAV vectors

(A) Schematic representation of the first generation of AAV2 vectors that contain 7 surface-exposed tyrosine (Y) residues, undergo ① phosphorylation, ubiquitination, and proteasome-mediated degradation in the cytoplasm, and the broken down peptides trigger the MHC class I-mediated $CD8^+$ cytotoxic T cell response. Site-directed mutagenesis ② of each of the Y residues to phenylalanine (F) residues leads to the next generation ("NextGen") of AAV vectors, and the transduction efficiency of each Y-F mutant vector is increased, ranging between ~2- and 11-fold. The combination of the three most efficient mutations ③ into one capsid results in the triple-mutant vector, the transduction efficiency of which is up to ~30-fold higher than the first generation of AAV2 vectors. (B) Schematic representation of the life cycle of the first generation of AAV2 vectors that in addition to 7 Y residues, also contain 15 serine (S), 17 threonine (T), and 10 lysine (K) residues that are surface exposed and undergo phosphorylation and ubiquitination, followed by proteasome-mediated degradation in the cytoplasm. Site-directed mutagenesis each of these residues was also performed, and the transduction efficiencies of the resulting NextGen AAV vectors were documented to be significantly increased.

We nonetheless reasoned that the vector has to be different from the virus. The strategy that led to the development of the next generation ("NextGen") of AAV vectors was reported in 2008[91] and is described briefly as follows.

As depicted schematically in Figure 1A, the first generation of AAV2 vectors have 7 tyrosine (Y) residues that are surface exposed. These Y residues are targeted by cellular epidermal growth factor receptor protein tyrosine kinase (EGFR-PTK)[92] such that a large fraction of incoming AAV vectors become phosphorylated, which serves as a signal for ubiquitination, followed by proteasome-mediated degradation,[93–95] which negatively impacts not only on the transduction efficiency, but the broken down peptides also trigger a cytotoxic T cell response.[96]

Each of the seven Y residues in AAV2 capsids were replaced them with phenylalanine (F) residues to generate 7 different Y-F AAV vectors. By changing just one amino acid on the capsid, it was observed that the transduction efficiency of these vectors could be improved ranging from between ~2- and 11-fold in the mouse liver following tail vein injections.[94] When the three most efficient mutations were combined into one capsid, the resulting triple-mutant vector was up to ~30-fold more efficient than the conventional AAV2 vector in the mouse liver.[97] More specifically, the triple-mutant AAV2 vectors were shown to mediate phenotypic correction in a mouse model of hemophilia B.[97] Thus, it was concluded that the NextGen AAV2 vectors are more efficient than the conventional first generation of AAV2 vectors. Furthermore, the triple-mutant AAV2 vectors were documented to be less immunogenic since they minimize the cytotoxic T cell response by avoiding phosphorylation, ubiquitination, and proteasome-mediated degradation.[91,96,98] The NextGen AAV2 vectors have also been used in a phase I/II clinical trial and have shown clinical efficacy in patients with Leber hereditary optic neuropathy (LHON), with no adverse events.[29,30] More specifically, 14 legally blind patients were enrolled in this trial and were followed for up to 24 months. Thirteen of 14 patients showed improved visual acuity, and 1 patient lost vision because of the course of bilateral visual loss, which is characteristic of LHON.

Since 6 of the 7 tyrosine residues are also conserved in all AAV serotypes, mutagenesis of these residues also increases the transduction efficiencies of all AAV serotype vectors evaluated thus far.[99] In addition to the 7 surface-exposed Y residues, AAV vectors also

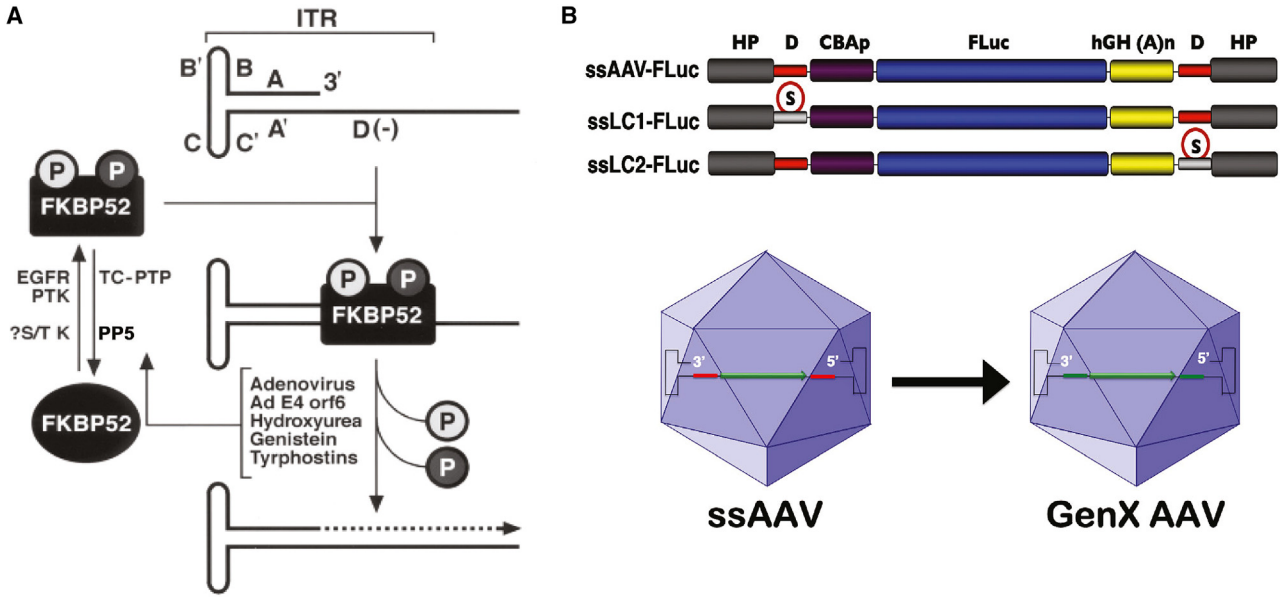

Figure 2. Rate-limiting step of viral second-strand DNAsynthesis of the first generation of ssAAV vectors

(A) Schematic model of inhibition of AAV second-strand DNA synthesis by a cellular chaperone protein, FKBP52, phosphorylated forms of which bind to the single-stranded D-sequence at the 3′ end of the AAV-ITR, which is also the "packaging signal" of the AAV genome and is indispensable. (B) The D-sequence at the 5′ end of the AAV-ITR, on the other hand, is dispensable, deletion of which leads to generation X ("GenX") AAV vectors, transgene expression from which is significantly higher than that from conventional ssAAV vectors.

contain 15 serine (S), 17 threonine (T), and 10 lysine (K) residues that are surface exposed and can be phosphorylated (Y, S, T) or ubiquitinated (K), which leads to proteasome-mediated degradation, as illustrated in Figure 1B. Each of the 15 S residues,[100] 17 of the T residues,[101] and 10 of the K residues[102] were mutagenized. Combination of various permutations and combinations of Y-, S-, T-, and K-mutants led to the identification of the most efficient quadruple mutant (QM) that was observed to be ~80-fold more efficient in the mouse liver.[101]

Thus, the NextGen AAV2 vectors overcome the first major limitation of first-generation AAV2 vectors. Since most, if not all, surface-exposed Y, S, T, and K residues are also highly conserved in all AAV serotypes, the transduction of these vectors can also be significantly increased.[99]

Development of genome-modified AAV vectors

The second major limitation of the first generation of AAV vectors is illustrated in Figure 2. The WT AAV and most of the rAAV vectors contain a ssDNA, which is a problem because there is no host cell RNA polymerase that can transcribe a ssDNA genome.

AAV as a virus does not express its own genes efficiently since viral second-strand DNA synthesis is needed before gene expression can occur. The expectation that AAV as vectors express therapeutic genes to high levels is also unrealistic. Thus, it was reasoned that in the ssAAV vector, the ssDNA genome also needed to be modified to allow viral second-strand DNA synthesis to occur.

This problem has been known since 1997, when it was discovered that a tyrosine-phosphorylated cellular chaperone protein, FKBP52, binds to the single-stranded D-sequence (ssD-sequence) at the 3′ inverted rerminal repeat (ITR) and strongly inhibits AAV second-strand DNA synthesis.[103] Deletion of the D-sequence at the 3′ ITR to prevent FKBP52 binding, thus leading to robust second-strand DNA synthesis, resulted in the failure for the AAV genome to undergo packaging, leading to the conclusion that the D-sequence at the 3′ end is the "packaging signal" for AAV[104,105] and thus could not be deleted.[106,107] However, it was subsequently observed that the deletion of the D-sequence at the 5′ end allowed AAV DNA to undergo successful packaging, which led to the development of the generation X ("GenX") vectors, shown schematically in Figure 3B, the extent of the transgene expression from which was up to 8-fold higher than that from the conventional ssAAV vectors.[108] Thus, GenX vectors overcome the second major limitation of the first generation of ssAAV vectors.

A few additional strategies involving the AAV-ITRs deployed by other investigators include the use of ITRs from different AAV serotypes. For example, Engelhardt and colleagues engineered more efficient vectors using AAV2 and AAV5 ITRs at opposite ends of the vector genome.[109] Interestingly, the hybrid ITR-containing vectors were also more efficient in intermolecular and intramolecular homologous recombination and *trans*-splicing compared with those containing the homologous ITRs. In addition, the use of the hybrid-ITR vectors also improved the delivery of transgenes that exceed the AAV packaging capacity.[110] Samulski and colleagues described the transcriptional activity of AAV ITRs from several

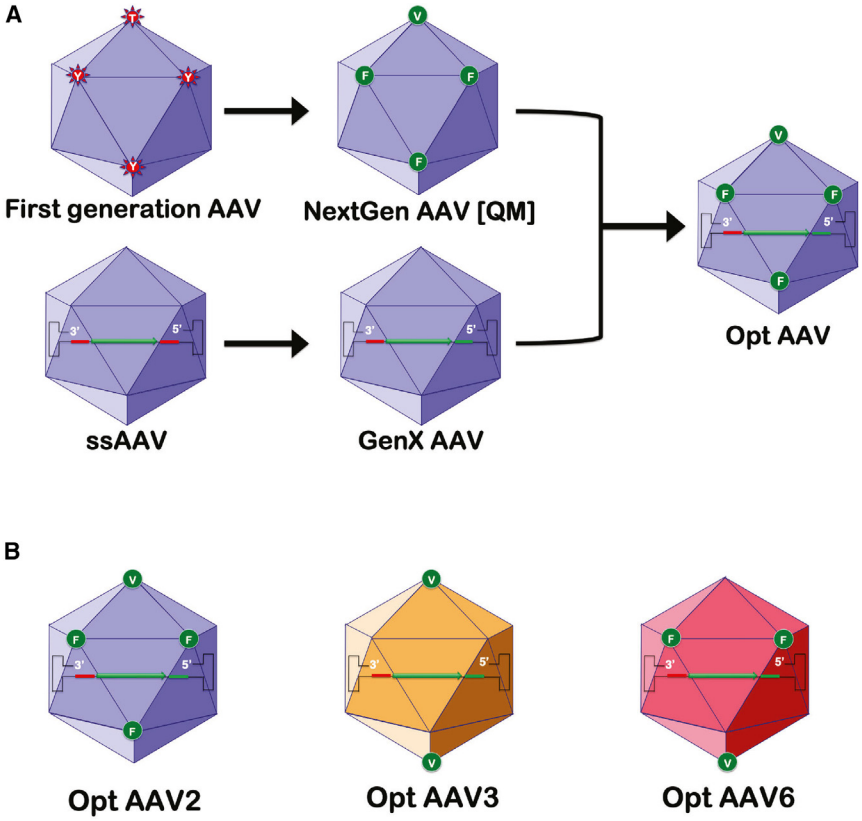

Figure 3. Strategies for the development of capsid-, genome-, and capsid+genome-modified AAV serotype vectors

(A) Schematic structures of first generation, NextGen, ssAAV, GenX, and optimized (Opt) AAV2 vectors. (B) Schematic structures of Opt AAV2, Opt AAV3, and Opt AAV6 serotype vectors.

different serotypes and identified three distinct levels of transcriptional activity independent of the promoter function.[111] Recently, Duan and colleagues reported the development of CpG-free ITRs and documented that although the vector genome encapsidation was not affected, the vector yield was decreased by ~3-fold.[112]

More recently, our own efforts have focused on the development of additional genome-modified AAV vectors, with which it has become feasible to achieve significantly enhanced transgene expression from ssAAV genomes. For example, we observed that the distal 10 nt in the AAV2 D-sequence share partial homology with the glucocorticoid response element (GRE) $^1/_2$ binding site and that the AAV2 genomes in which the distal 10 nt were replaced with the 15-nt consensus full-length GRE site resulted in generation Y (GenY) AAV vectors that mediate up to ~6-fold increased transgene expression.[113] Similarly, we previously reported that AAV second-strand DNA synthesis is strongly inhibited by phosphorylated forms of a host cell chaperone protein, FKBP52, which binds to the D-sequence at the 3′ end,[103] but it has not been possible to delete the D-sequence at the 3′ ITR as it serves as the "packaging signal" for the AAV genome.[104–107] We recently also developed generation Z (GenZ) AAV vectors in which the proximal 10 nt in the D-sequence were replaced with random 10 nt, and one sequence was identified that allowed successful rescue, replication, and packaging of the AAV genome. This sequence was inserted in a rAAV2 genome, replacing the proximal 10 nt in the D-sequence at both ITRs. The extent of the transgene expression from the resulting GenZ ssAAV vectors is up to ~20-fold higher than that from the WT ssAAV vectors.[114] Thus, the GenZ ssAAV vectors not only overcome the problem of viral second-strand DNA synthesis, but they behave more like scAAV vectors but without the size limitation.

Development of optimized AAV vectors

The next obvious question was whether GenX genomes could be packaged into NextGen capsids to achieve even higher efficiency of transduction. As shown schematically in Figure 3A, the packaging of the D-sequence-deleted GenX genomes into NextGen capsids would be expected to lead to optimized (Opt) AAV vectors. Indeed, the development of not only Opt AAV2 vectors but also Opt AAV3 vectors, depicted schematically in Figure 5, was reported, and Opt AAV2 and Opt AAV3 serotype vectors were observed to be ~20- to 30-fold more efficient than the corresponding NextGen AAV2 and AAV3 vectors, respectively.[115] This strategy can and does work with several additional AAV serotype vectors that have been evaluated. Thus, it can be concluded that Opt AAV serotype vectors overcome both major limitations of the first generation of AAV serotype vectors.

Development of strategies to evade the humoral response to AAV vectors

Beyond the cell-mediated immune response, the humoral response to AAV vectors also remains a major challenge since B cells generate anti-AAV antibodies, making repeat vector dosing difficult, and pre-existing neutralizing anti-AAV antibodies preclude patients from being enrolled in clinical trials. This problem has been addressed by the following three distinct strategies: first, by using anti-CD20 antibodies, transient B cell ablation has been used.[116,117] Second, the partial sequence homology between the AAV D-sequence and the major histocompatibility complex (MHC) class II promoter has been exploited to generate AAV vectors that can downregulate the host cell MHC class II promoter function leading to suppression of B cell-helper T cell interaction, resulting in inhibition of differentiation and production of plasma cells that generate antibodies.[118] And third, two independent groups have reported a promising approach to address this problem by using immunoglobulin G (IgG) antibody-degrading enzymes, IdeS[119] and IdeZ.[120] These

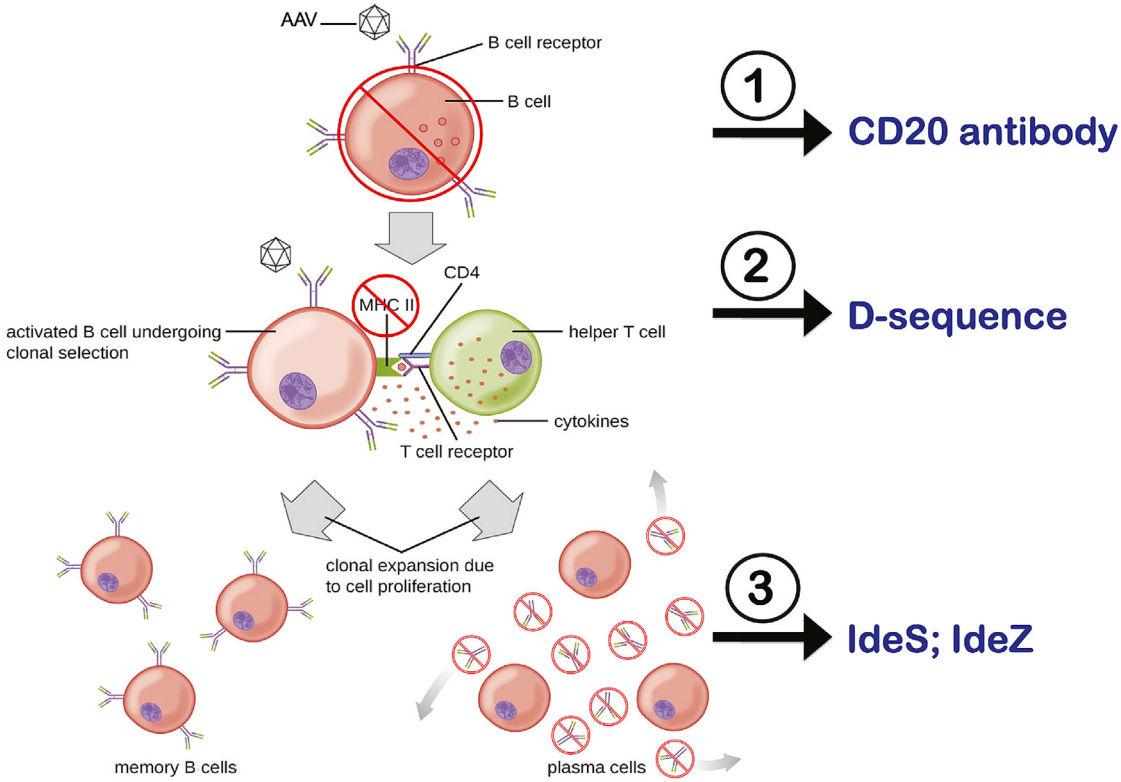

Figure 4. Strategies for dampening the host humoral immune response to AAV vectors

① Transient depletion of B lymphocytes using CD20 antibody, ② AAV D-sequence-mediated suppression of MHC class II gene expression, and ③ transient degradation of pre-existing IgG antibodies using IgG-cleaving IdeS and IdeZ bacterial endopeptidases. Modified image from.[121]

strategies are depicted and summarized in Figure 4.[121] More recently, an additional strategy has been described that utilizes neonatal Fc receptor inhibition to enable AAV gene therapy even in the presence of pre-existing humoral immunity.[122]

Development of human-tropic AAV serotype vectors

Since the tropisms of AAV vectors in animal models do not necessarily translate well in humans, we next wished to identify AAV vectors that specifically and efficiency transduce primary human cell types. In this quest, two human-tropic AAV vectors have been identified that selectively and efficiently transduce primary human cell types. First, of the 10 most commonly used serotypes, AAV3 was observed to be by far the most efficient in transducing primary human hepatocytes,[123,124] and second, AAV6 was identified to be the most efficient in transducing primary human hematopoietic stem cells.[125,126] This is depicted schematically in Figure 5. Several other independent investigators have corroborated these observations for both AAV3 and AAV6 serotype vectors for primary human hepatocytes[127–131] and primary human hematopoietic stem cells,[132–141] respectively. NextGen AAV3 vectors have also been shown to be significantly more efficient in primary hepatocytes in "humanized" mice[142–144] and NextGen AAV6 vectors more efficient in primary human hematopoietic stem cells.[145,146]

However, another important consideration is to develop NextGen AAV vectors that are less liver tropic and instead transduce other organs better for non-liver-specific diseases. This would reduce the risk of severe adverse events (SAEs) associated with liver transduction of most AAV serotype vectors upon systemic administration for non-liver-based diseases.[40–43]

Concerns regarding the use of high doses of AAV vectors

It has been known for more than four decades that infection by the WT AAV2 in infants and young children leads to no significant clinical sequalae.[147] It has also become increasingly clear that the host immune response to AAV vectors directly correlates with the vector dose and that at a dose of 2×10^{11} vg/kg, AAV vectors are only mildly immunogenic.[148] Thus, it was of interest to reexamine the total doses of AAV vectors that were, or are currently, being used in a number of clinical trials in humans. These details are provided in Table 3.

As can been seen, the estimated total number of cells in a human body is ~30 trillion.[149] Thus, it is quite a testament to the remarkable safety of even the first generation of AAV vectors that humans are easily able to tolerate roughly ~140× (4.2 quadrillions) of AAV vectors as there are number of cells in the body. However, serious adverse events and

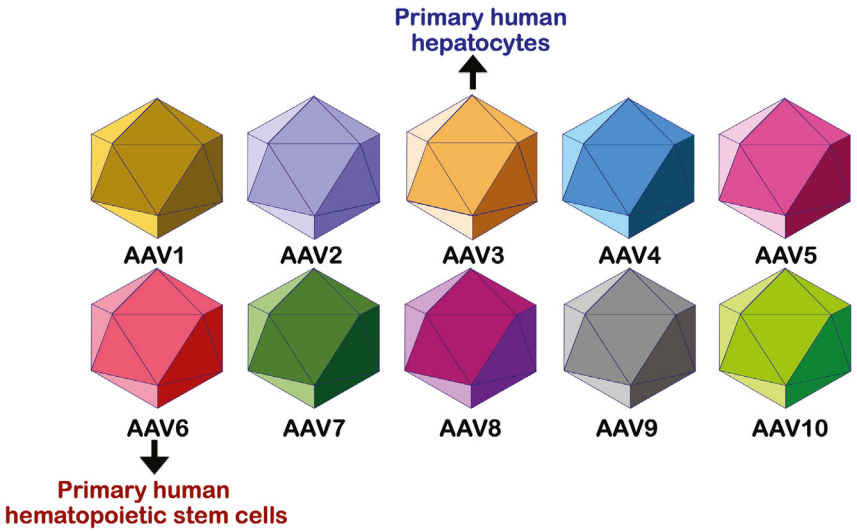

Figure 5. Schematic structures of the 10 most commonly used recombinant AAV serotype vectors
AAV3 and AAV6 vectors have been documented to be selectively tropic for high-efficiency transduction of primary human hepatocytes and primary human hematopoietic stem cells, respectively.

deaths have occurred when the vector doses exceed ~467× (or higher) the number of cells in the body. Thus, again, the use of Opt AAV serotype vectors is likely to be less immunogenic and more effective at significantly lower doses.[55]

Conclusions

Based on the account presented above, the following conclusions can be drawn.

- The use of the first generation of AAV serotype vectors composed of naturally occurring capsids is not optimal, given the induction of the host cell-mediated immune response, especially at high doses.
- The use of the ssAAV genomes with WT ITRs is also not optimal, given the sub-optimal levels of transgene expression.
- The use of NextGen, GenX, and, preferably, Opt AAV serotype vectors in all future clinical trials should be considered.

Epilogue

AAV, as a virus, did not evolve to be used as a vector for the purposes of delivery of therapeutic genes. Thus, the full potential of AAV as a vector will only be realized after its capsid is modified to evade the host immune response and its genome is modified to express optimal levels of the therapeutic transgene. More, the potential use of Opt AAV vectors at significantly reduced doses promises to achieve clinical efficacy, thereby increasing the safety as well as reducing vector production costs, ensuring translation to the clinic with higher probability of success, without the need for the use of immunesuppression, for gene therapy of human diseases. Specifically, Opt AAV serotype vectors are likely to be less immunogenic and more effective at lower doses, thus further increasing the safety as well as reducing vector production costs, ensuring translation to the clinic with a higher probability of success.[150]

Table 3. Total number of AAV particles/cell in the human body

Sponsor	Disease	Vector	Dose (vg/kg)	Total dose (quadrillioin)	AAV particles/cell
uniQure	HB	AAV5	2×10^{13}	1.4	~47
LogicBio Therapeutics	MMA	LB-001	5×10^{13}	3.5	~117
BioMarin	HA	AAV5	6×10^{13}	4.2	~140
Pfizer	DMD	AAV9	2×10^{14}	14.0	~467
Audentes	XLMTM	AAV8	3×10^{14}	21.0	~700

Based on the estimated ~3×10^{13} (30 trillion) total number of cells in the human body.[149] HB, hemophilia B; MMA, methylmalonic acidemia; HA, hemophilia A; DMD, Duchenne muscular dystrophy; XLMTM, X-linked myotubular myopathy.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.omtn.2023.05.014.

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I would like to dedicate this article to the memory of Dr. Nicholas Muzyczka (1947-2023), the Father of AAV vectors.

AUTHOR CONTRIBUTIONS

The author conceptualized and wrote this review article.

DECLARATION OF INTERESTS

The author is a cofounder of, and holds equity in, Lacerta Therapeutics. He is an inventor on several issued/filed patents on rAAV vectors that have been licensed to various gene therapy companies. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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