Published in final edited form as:

Expert Opin Biol Ther. 2015 June ; 15(6): 845–855. doi:10.1517/14712598.2015.1035645.

Strategies to circumvent humoral immunity to adeno-associated viral vectors

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Abstract

Introduction—Recent success in gene therapy of certain monogenic diseases in the clinic has infused enthusiasm into the continued development of recombinant adeno-associated viral (AAV) vectors as next-generation biologics. However, progress in clinical trials has also highlighted the challenges posed by the host humoral immune response to AAV vectors. Specifically, while pre-existing neutralizing antibodies (NAbs) limit the cohort of eligible patients, NAb generation following treatment prevents vector re-dosing.

Areas covered—In this review, we discuss a spectrum of complementary strategies that can help circumvent the host humoral immune response to AAV.

Expert opinion—Specifically, we present a dual perspective, that is, vector versus host, and highlight the clinical attributes, potential caveats and limitations as well as complementarity associated with the various approaches.

Keywords

adeno-associated virus; capsid; humoral immune response; neutralizing antibody; recombinant adeno-associated viral vectors

1. Introduction

Adeno-associated viruses (AAVs) are non-enveloped, single-stranded, DNA viruses with a 4.7 kb genome encapsulated in a 25 nm icosahedral capsid. The capsid is composed of 60

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Declaration of interest

A Asokan is a consultant to Genzyme and Voyager Therapeutics and an inventor on patents licensed by the University of North Carolina to Asklepios Biopharmaceutical, Inc. Funding was received from the National Institute of Health (NIH). 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.

copies of viral proteins 1, 2 and 3 (ratio of 1:1:10). Wild-type AAV (wtAAV) was first discovered as a contaminant in adenoviral preparations, but was later ascribed to a unique genus of *Dependoparvovirus*, belonging to the Par-voviridae family [1–3]. wtAAVs are unable to undergo a productive viral life cycle without a helper virus. As such, the wtAAV genome is normally dormant, integrated at a site-specific locus in human chromosome 19, AAVS1 site [1,4,5]. In the presence of helper viruses, such as adenovirus or herpes simplex virus; or genotoxic agents, such as UV irradiation; wtAAV genomes can reactivate and initiate a lytic life cycle and spread throughout the host [6–8]. The non-pathogenic nature and the ability to package different transgenes make AAV an ideal candidate for gene therapy [9]. Briefly, transgenes flanked by inverted terminal repeats are packaged efficiently inside the capsid, when Ad helper genes, AAV replication (*rep*) and structural (*cap*) proteins are provided in *trans* [7]. The resulting recombinant AAV vectors are excellent reagents for delivering transgenes that mediate sustained long-term gene expression in episomal form within the nucleus [10,11].

To date, hundreds of different natural AAV subtypes have been isolated from humans and animals, from non-human primates to avian species [12,13]. Despite their similarity at the genetic level, several AAV isolates have demonstrated unique tropisms *in vitro* and *in vivo*. As a result, multiple strains ranging from AAV serotypes 1 through 9 and Rh.10 are under active development as gene therapy vectors for different clinical indications [14]. In 2012, after several decades of research and development, the first AAV-based therapeutic, alipogene tiparvovec (Glybera), produced by UniQure was recommended for approval by European Medicines Agency. This treatment involves delivery of the lipoprotein lipase (LPL) transgene using AAV1 vectors to cure a rare genetic disorder resulting from LPL deficiency and characterized by severe hypertriglyceridemia in patients. Several other AAV-based gene therapy treatments for diseases such as a1-anti-trypsin deficiency, Leber's congenital amaurosis, hemophilia B, Parkinson's disease, spinal muscular atrophy, muscular dystrophy, lysosomal storage disorders, Pompe disease and congenital heart failure are in clinical trials and expected to be marketed as drugs in the future [15].

Despite these early successes and promising future, AAV-based gene therapies face several challenges that remain to be effectively addressed. One such extrinsic factor that limits the clinical impact of AAV-based gene therapy is the prevalence of pre-existing neutralizing antibodies (NAbs) to AAVs in the human population due to natural infection or cross-reactivity of NAbs between different AAV subtypes [16,17]. NAbs bind to antigens with high affinity, thereby blocking interactions required for normal function. In the case of viral infection, NAbs play a pivotal role in controlling or stopping initial virus infection at various step of the virus life cycle (pre- and post-entry). NAbs also opsonize viral particles and facilitate their uptake by phagocytic cells, thereby mediating their rapid clearance from the circulation. Multiple preclinical studies and clinical trials have shown conclusively that pre-existing NAbs adversely affect the gene transfer efficiency of recombinant AAV vectors. Serum NAb titers as low as 1:5 are sufficient to abolish transduction in the liver of mice and non-human primates [18–20]. Furthermore, serological studies reveal a high prevalence of NAbs in the human population worldwide with about 67% of people having antibodies against AAV1, 72% against AAV2 and about 40% against AAV5 – 9 [15]. As a result,

patients are now pre-screened for pre-existing NAbs against AAV before enrollment into clinical trials. Contingent on the AAV subtype to be used in the clinical trial, such screens can prevent as much as 50% of patients from being eligible [21–23]. The epidemiology of NAbs to different AAV subtypes in different patient cohorts has been reviewed in detail elsewhere [16].

Another significant and related challenge is the potential need for AAV vector re-administration in some indications. This situation might be particularly relevant in case of: i) patients receiving low-dose vector treatment in a Phase I trial; ii) in case of children/neonatal patients who will experience growth and proliferation of tissues and iii) patients with degenerative disorders (e.g., muscular dystrophy). The latter two situations are likely to result in dilution or loss of vector genomes accompanied by a decrease in transgene expression to subtherapeutic levels. Taken together, NAbs clearly present a significant barrier to the broad application of AAV-based gene therapies. In the current review, we discuss the advantages and disadvantages of different strategies being developed to evade the pre-existing humoral immune response to AAV vectors as well as allow vector re-dosing in gene therapy clinical trials (Table 1).

2. The vector perspective

2.1 Route of administration

The route of AAV administration can influence the impact of NAbs on transduction efficiency [24,25]. Intravenous (i.v.) injection of recombinant AAV exposes the vector to significantly more NAbs than local administration with a target organ/tissue. For instance, NAbs have been shown to exert minimal effects on transduction efficiency of intramuscularly administered AAV vectors in early clinical trials for α1-anti-trypsin deficiency and hemophilia B [26–28]. In one study, contact of vector and NAbs is minimized by saline flushing of the liver followed by direct injection in portal vein. Such a strategy allows efficient human Factor IX (FIX) transduction by AAV8 in the liver of macaque when NAb titer is up to 1/28 [24]. Other routes of administration are currently being evaluated for delivery of AAV vectors into different tissues by bypassing the circulating system, such as intrathecal delivery to target the CNS [29–31]. At least one study has shown that cerebrospinal fluid (CSF) may carry less NAbs, when compared with serum and intra-CSF administration of rAAV9 in dogs is not completely affected by pre-existing-NAbs [32]. In clinical trials of Leber's congenital amaurosis, AAV-mediated delivery of the RPE65 gene by subretinal injection and successful correction of vision with no adverse effect after 5 years has been reported [33–36].

Although promising, it should first be noted that these approaches do not provide alternatives for gene therapy modalities requiring gene expression in systemic organs such as the liver or heart. Second, such alternative routes of administration can elicit more robust immune responses targeting the transgenes. For instance, intramuscular inoculation of AAV2/hFIX elicits a strong T-cell-dependent antibody response against the FIX transgene product in contrast to hepatic portal vein administration [37–39]. Furthermore, intramuscular injection of AAV1 delivering LPL induces both $CD4^+$ and $CD8^+$ immune reaction to AAV capsid in 50% of tested subjects of human clinical trial [40]. In addition, such a scenario

poses additional challenges and complications in case vector re-administration becomes essential in certain clinical indications as outlined earlier. Certain recent studies have demonstrated the presence of circulating NAbs as a potential barrier to effective transduction in primate models regardless of the route of administration, either cisterna magna infusion or i.v. injection [41–43].

2.2 Discovery of new AAV isolates

The past decade has witnessed the discovery of over 100 AAV variants isolated from different mammalian and non-mammalian species [14]. Of these, only about 10% of the total numbers of AAV strains have been evaluated in preclinical and clinical gene therapy efforts. One potential approach to reach the full potential of AAV-based gene therapies is to carry out a comprehensive evaluation of the epidemiology of NAbs against different natural isolates. Early studies indicate that only 2% of the human population carry NAbs against certain rhesus isolates, AAVrh32.33 [16]. However, the latter strain is also highly immunogenic, which could restrict applications in the gene therapy context [44]. It should also be noted that human IgG prevalence as high as 49% accompanied by a lower NAb seroprevalence of 21% has been reported against certain promising rhesus isolates such as AAVrh.10 [45]. Moreover, cross-reactivity between different AAV isolates regardless of the species of origin, for example, AAV2/AAV9 versus AAVrh.10, has been reported [45]. Nevertheless, it is conceivable that antigenically distinct strains that will expand the patient cohort in clinical trials exist within the pool of previously discovered natural isolates or new(er) AAV that continue to be discovered in different animal species.

While a comprehensive evaluation of the epidemiology of NAbs against all known natural AAV isolates might be challenging, recent advances suggest that *in silico* approaches might be promising. The latter strategies hinge on phylogenetic analysis of AAV genome sequences and structural approaches to define antigenic diversity. For instance, due to the likely history of AAV infection during the course of human evolution, potential reconstruction of functional AAV genomes from ancestral strains has recently been proposed [46]. Another approach is to utilize structural modeling tools to select antigenically distinct AAV strains for further epidemiological analysis [47–49]. Thus, the discovery and continued evaluation of novel AAV vectors derived from natural isolates that can evade pre-existing NAbs in the human population is likely to remain a promising strategy for expanding patient enrollment in clinical trials.

2.3 Engineering new AAV variants

A goal-oriented approach towards tackling the hurdles posed by anti-AAV NAbs is to engineer synthetic AAV strains by modifying antigenic epitopes on the AAV capsid [15,49]. Regions of the AAV capsid important for antibody binding have been identified by multiple approaches, including peptide scanning or insertion, *in silico* modeling and structural analysis. Peptide scanning involves the use of ELISA to identify linear epitopes (short peptides) that binds to NAbs [50] while peptide insertion identifies conformation epitopes by disrupting the NAb epitopes with short amino acid insertions [51]. *In silico* docking of murine-IgG2a to AAV2, and further confirmation through systematic mutagenesis and

disruption of NAb binding, lead to the identification of residues that are accessible to antibodies [48].

Structural analysis has also been used to map out regions of the capsid that are important for NAb binding. In particular, cyro-electron microscopy of AAV capsids bound by monoclonal antibodies has revealed several shared epitopes present in multiple AAV subtypes [47]. These shared regions are present within the threefold protrusion and the two/fivefold wall on AAV1, 5 and 6 [47,52,53]. As structures of different AAV–NAb complexes continue to be solved, our knowledge of immuno-dominant as well as cross-reactive antigenic footprints on the AAV capsid continues to evolve. Using this information, specific regions of the capsid can then be mutated using different protein engineering approaches to potentially create NAb evading AAV variants. In addition to these rational approaches, combinatorial strategies that hinge on applying evolutionary pressure using human sera to evolve NAb escape mutants from diverse AAV capsid libraries have been proposed [54,55]. These approaches have also been reviewed in detail elsewhere [47,56,57].

While capsid engineering shows promise towards developing next-generation AAV vectors that can escape pre-existing NAbs, several important caveats associated with this approach should be noted. First, it is possible that certain antigenic/immunodominant epitopes on the AAV capsid overlap with domains essential for AAV-receptor interactions, cellular uptake, uncoating or other viral trafficking events. This aspect can make engineering/evolving NAb evading AAV mutants particularly challenging. Second, as with natural isolates, it is likely that engineered AAV capsids might still be recognized by cross-reactive NAbs in some patient sera. Third, the current path guiding AAV vectors to the clinic is expensive and lengthy, often requiring toxicity and biodistribution studies in different preclinical models. The latter aspect is particularly relevant when substitution of one AAV strain for a less immunogenic strain is being considered and could require additional toxicity/biodistribution bridging studies from a regulatory perspective.

2.4 Chemical approaches

PEG has been used extensively to increase the half-life and reduce the immunogenicity of drugs in pharmacology [58]. Chemical modification of AAV can successfully mask AAV from NAbs [59,60]. Unlike drugs, considerable attention must be paid to linkage chemistry and stoichiometry of PEG onto AAV to maximize protection while minimizing the adverse effects on transduction efficiency. For instance, linking PEG on AAV using tresyl chloride reactive group provides more effective protection from NAbs than linking chemistry using succinimidyl succinate chemistry and cyanuric chloride [61]. Moreover, when the ratio of lysine:PEG is too high (1:1000) during conjugation, PEG is believed to sterically hinder AAV receptor engagement and reduce AAV transduction efficiency [59]. Other kinds of polymers such as polysaccharides and poly-*N*-(2-hydroxypropyl) methacrylamide have also been conjugated onto adenovirus vectors to shield from NAbs [62,63].

As an alternative, instead of directly grafting polymer onto the capsid, strategies have also developed to encapsulate viral vectors inside a polymer gel that degrades progressively [60]. This method increases the half-life of viral vectors in circulation without hindering receptor engagement on target cells. For example, 60% of adenoviruses are still infectious in the

present of NAb when encapsulated inside poly-lactic glycolic acid [64]. Another polymer, alginate has also been shown to protect intranasally and intraperitoneally delivered adenoviruses from NAbs in mice [65]. Another approach for evading NAbs is to encapsulate AAV vectors in extracellular vesicles. The latter approach enabled the generation of AAV vector formulations that were over 100-fold more resistant to NAbs without adversely affecting transduction efficiency [66].

Although pilot studies indicate that chemical modification or encapsulation of AAV vectors is feasible, several major challenges remain. First, although established in the context of other therapeutic proteins, scale up of polymer – capsid conjugation chemistry could require additional quality control measures with regard to viral infectivity. Second, variability in polymer type/length and chemistries as well as capsid protein chemistry might prevent development of standardized procedures for multiple AAV formulations. Third, the presence of anti-PEG antibodies in some patients might pose additional concerns and challenges in the development of PEG-based conjugates [67]. Fourth, encapsulation of AAV vectors in polymer or lipid formulations eliminates the advantages afforded by different capsids and their respective tissue tropisms. Additional/alternative surface modifications of the formulation might be essential to compensate for this potential drawback.

2.5 Capsid decoys

Another promising approach to evade NAbs is to develop decoys that competitively inhibit capsid neutralization. For instance, co-administration of receptor binding/cell entry-deficient and ‘empty’ AAV2 capsids as decoys along with the therapeutic vector has recently been demonstrated [68]. In this scenario, increasing amounts of empty virions that competitively blocked circulating NAbs resulted in a dose-dependent restoration of transgene expression in non-human primates. Although amenable to scale up, the latter approach poses some concerns. First, empty virions in AAV vector formulations have been shown to reduce transduction efficiency, increase antigenic burden and exacerbate vector-related immunotoxicity presumably indicated by increase in serum levels of liver transaminases [69]. In this regard, an earlier study has shown that both empty virions and full AAV capsids are capable of flagging hepatocytes for cytotoxic T-lymphocyte (CTL)-mediated destruction, thereby underscoring the importance of reducing antigenic burden by removing empty virions from the formulation [70]. Third, it is unclear whether truly empty AAV virions can be generated due to their inherent ability to package non-viral genomic fragments [71–73]. Nevertheless, these studies serve as a model for developing strategies that focus on blocking or removing NAbs as an orthogonal approach towards expanding the patient cohort eligible for gene therapy clinical trials.

3. The host perspective

3.1 Plasmapheresis

In addition to modifying vectors to evade NAbs, attempts have been made to limit the immune response within the host. One such example is plasmapheresis, a clinical procedure involving removal of patient blood, treatment for NAb removal and infusion back into the patient. Briefly, this procedure involves separation of blood into plasma and blood cells,

followed by the removal of a majority of antibodies from the plasma by centrifugation or filtration. The antibody-depleted plasma then returns back to the patient's body, supplemented with albumin and saline to compensate for the loss of protein [74]. This method has been validated for treatment of patients with acute buildup of pathologic antibodies in autoimmune diseases such as Guillain-Barré syndrome and lupus [75,76]. Although the transient nature of plasmapheresis hinders its use in treating autoimmune diseases, this limitation does not preclude use in conjunction with gene therapies. This approach is feasible, since a short window of low NAb concentration is sufficient for vector administration and transduction. In a non-human primate model, after six plasma volumes of plasmapheresis over a 2-day period, sero-positive animals had similar transduction efficiencies as sero-negative animals. In contrast, non-pheresed sero-positive animals showed fourfold reduction of transgene expression [77]. However, removal of IgG from circulation by plasmapheresis suffers from 'rebound', a phenomenon where IgG level returns to or above the original level in a short period of time after treatment. A clinical study found multiple sessions of plasmapheresis required to reduce NAbs to a threshold level for gene therapy due to this 'rebound' phenomenon of IgG [78]. Nonetheless, plasmapheresis provides an effective method for enhancing the effectiveness of gene therapy vectors.

As with all other medical procedures, there are potential risks and complications that can occur with plasmapheresis. First, the effectiveness of plasmapheresis is inversely related to the initial amount of NAbs present within a patient [78]. Thus, it is conceivable that multiple rounds of plasmapheresis in patients will be required to reduce NAb levels for effective gene therapy. Second, complications arising from bacterial infections of the i.v. catheter due to prolonged insertion are possible. Third, it is difficult to implement plasmapheresis on a global scale, especially in developing countries, where resources to perform the procedure on a regular basis might be limited. Last but not least, after plasmapheresis, patients are transiently immunocompromised and susceptible to secondary infection(s) due to the loss of circulating antibodies.

3.2 Targeting B-cell activation and apoptosis

Understanding and manipulating the mechanisms underlying NAb generation by activated B cells might provide insights into how to evade the humoral immune response. B-cell activation is a complex process that takes place within the germinal center and requires multiple steps and signals [79]. The first signal required for B-cell activation is binding of an antigen. Although both soluble and membrane-bound (complexed with MHC class II molecule) antigens are able to bind to the transmembrane immunoglobulin or the B-cell receptor (BCR), AAV-mediated B-cell activation is primarily triggered by membrane-bound AAV fragments displayed by antigen-presenting cells [80]. Binding of antigen to the BCR initiates downstream signal transduction [81]. After initial engagement of antigen by the BCR, aggregation of BCRs recruit intracellular signaling molecules that allow formation of a BCR microcluster [82,83]. When enough microclusters condense, contraction of the B-cell surface allows endocytosis of the antigen via cytoskeleton rearrangement. The antigen is then digested and complexed with MHC II on the B-cell surface for presentation to CD4 helper T cells. Subsequent steps in B-cell activation trigger proliferation and migration to

the germinal center resulting in isotype switching and somatic hypermutation. The majority of the activated B cells proliferate into effector plasma cells and secrete large amount of NAbs, while a small portion will become long-lived, memory B cells and for response to future infections. In the sections below, we discuss certain pharmacological interventions that interfere with the NAb production process and can potentially be adapted to tailor gene therapy modalities. It should be noted that a subset of these approaches might be more suited for addressing challenges associated with AAV vector re-administration rather than evade pre-existing humoral immunity. The topic has also been reviewed in detail by Basner-Tschakarjan *et al*. [84].

Disruption of any step prior to B-cell activation could lead to a collapse of the humoral immune response, which in turn would be beneficial for AAV vector re-administration. For instance, bortezomib is a US FDA-approved protease inhibitor that targets plasma B cells and is used for treating multiple myeloma. Administration of bortezomib after vector injection has been shown to decrease NAb titer by 8 – 10 folds in naive mice, when compared with mock treatment [85]. This decrease in NAb titer is likely caused by inhibiting antigen processing by the proteasome, which potentially reduces subsequent presentation on B cells and contributes to increased transduction efficiency [86–88]. However, this decrease in NAb titer does not reach sufficiently low levels to support vector re-administration.

Another way to inhibit humoral immune response is to reduce the number of activated B cells. By inducing B-cell apoptosis immediately prior to gene therapy, the amount of circulating NAbs can be reduced, potentially allowing for vector re-administration. B-cell apoptosis is a naturally occurring process for both immature (bone marrow) and mature B cells (secondary lymphoid organ) that target self-antigens [89]. Transient immunosuppression by targeting B cells is a way to reduce the number of NAbs in circulation. For instance, the anti-CD20 antibody, rituximab is used extensively in rheumatoid arthritis (RA) patient to reduce patient $CD20^+$B-cell populations (pre-B cell and mature B cell) that play a central role in RA pathogenesis [90]. Rituximab can induce $CD20^+$ B-cell apoptosis directly or through indirect mechanisms. These indirect methods involve either antibody-dependent recruitment of natural killer cells and macrophages that kill the antibody-bound B cells or complement-dependent B-cell lysis through C1q binding and activation of the complement cascade [91]. Rituximab has been shown to effectively reduce circulating antibodies in the blood, including AAV NAbs, for at least 24 weeks after two i.v. infusions of rituximab treatments in small portion of patients [92]. A primate study showed rituximab treatment in combination with cyclosporine A (CsA), a calcineurin inhibitor which blocks T- and B-cell activation can reduce NAbs against both AAV vector and FIX (transgene), which enables long-term transgene expression and vector re-administration [93]. However, immunosuppression is likely to be much less efficient in pre-immunized animals carrying high levels of circulating NAbs.

In a clinical case study of AAV-mediated gene therapy, administration of rituximab has been shown to transiently reduce the number of antibody producing B cells [94]. In this study, rituximab was used in conjunction with long-term daily administration of sirolimus (rapamycin), which binds to mTOR, a protein kinase important for T- and B-cell activation

[95]. The study involved a 40-month treatment of rituximab- and sirolimus-depleted B cells in a 45-month patient with Pompe disease and successfully mitigated an immune response to the AAV capsid [94]. Although a promising approach, it should be noted that triggering B-cell apoptosis has certain potential disadvantages in the context of clinical gene therapy applications. For instance, after rituximab treatment, an average of 6 – 12 months can be needed for B cells to be replenished to normal levels [96]. As a result, patients are immunocompromised for an extended period of time and can be vulnerable to opportunistic pathogens. For instance, such a scenario has been reported in case of two rituximab-treated patients who developed progressive multifocal leukoencephalopathy after activation of normally dormant human polyomavirus John Cunningham virus [97]. Another possible caveat is that rituximab only reduces levels of $CD20^+$ B cells where plasma effectors B cells are not affected [98]. Therefore, rituximab treatment might require plasmapheresis in conjunction to subsequently reduce the amount of NAbs in circulation. Ongoing preclinical studies in non-human primates are likely to further guide the application of such immunodepletion strategies for AAV-mediated gene therapy. Further, it should be noted that such strategies are potentially more relevant for clinical trials that require vector re-administration and might not specifically address the issue of pre-existing circulating NAbs.

Induction of B-cell tolerance can also potentially be applied to prevent a humoral response towards AAV capsids and allow vector re-administration. One such example is antigen-specific immunotherapy, which is used to treat allergy and, to a lesser extent, autoimmune disease [99]. Such approaches can decrease B-cell activation indirectly by inducing regulatory T cells (Treg) for a specific antigen [100,101]. Another approach to induce immune-tolerance is to directly target B-cell activation. This approach can hinge on targeting CD22 or sialic acid binding Ig-like lectin, which are inhibitory co-receptors for B-cell activation [102]. Under normal circumstances, CD22 is excluded from the BCR microcluster. However, forcing CD22 to stay inside BCR microcluster by crosslinking it with antigen inhibits B-cell activation [103]. In such a scenario, inhibition of B-cell activation has been achieved by immunizing mice using beads crosslinked with both an antigen of interest and a CD22 ligand, resulting in immune tolerance to the crosslinked antigen [104]. In another example, immunization of mice with nanoparticles conjugated to human FVIII and CD22 ligand has been demonstrated to induce tolerance to FVIII and suppression of anti-FVIII antibody production [105]. Whether these approaches can be extended to the gene therapy clinic and enable vector re-administration remains to be seen.

3.3 Targeting T-cell activation

Another approach is to target T cells, which can indirectly affect the humoral immune response to the AAV vector. As a key player in B-cell activation, CD4 helper T cells are the primary target for immunosuppression. Depletion of $CD4^+$ T cells through the use of anti-CD4 antibodies can abolish NAbs production against AAV capsids [106]. Alternatively, non-depleting CD4 antibodies can be useful towards targeting $CD4^+$ T cells and facilitate survival of Tregs to induce antigen-specific immune tolerance [107]. A recent study showed non-depleting CD4 mAbs can prevent antibody formation against AAV capsids and the transgene product up to 6 weeks after i.v. administration in mice [108]. A more specific strategy to enrich Tregs population is the use of tregitopes, an Fc fragment of IgG which

specifically activates natural Tregs [109]. A study showed that co-incubation of tregitopes and AAV epitopes can modulate the CTL response against AAV capsids and induce Treg expansion [110]. Further, blockage of T-cell-dependent B-cell activation signals, such as CD40-CD40L or CD28-CD80/86 interactions using antibodies or fusion proteins has been shown to induce immuno-logical hyporesponsiveness against AAV vectors delivered to the mouse lung and consequently allow re-administration [111]. Another T-cell immunosuppressive drug, CsA, has been utilized to decrease AAV-vector-mediated humoral responses. When co-administered with AAV8 vectors, CsA and non-depleting anti-CD4 antibodies can prevent induction of the primary humoral immune response and decrease NAb production by 20-fold [112]. Several challenges are associated with T-cell depletion in the context of gene therapy clinical trials. First, the usefulness of prolonged antibody-mediated depletion of $CD4^+$ T cells as with B cells in the clinic can be limited, as patients will be immunocompromised for an extended period of time. Second, although capable of decreasing NAb production, it is unclear whether such strategies can indeed reduce pre-existing anti-AAV NAbs to levels that would help expand the clinical cohort of eligible patients.

4. Expert opinion

Recombinant AAV vectors delivering therapeutic transgenes are poised to expand the spectrum of biologics/drugs available for treating monogenic diseases in the clinic. As clinical trials continue to advance the field, two key challenges i) preexisting humoral immunity to the AAV capsid and ii) the potential need for vector re-administration have been identified. A spectrum of approaches both from the vector development perspective as well as modulating the host immune profiles are currently being evaluated/developed towards tackling the aforementioned challenges. For instance, the route of vector administration is a simple parameter that can influence the impact of NAbs on AAV transduction. Thus, it is likely that AAV-mediated gene transfer to immune-privileged sites such as the eye and CNS will continue to progress rapidly in the clinic. Exploitation of immune-privileged sites for AAV-mediated expression of secreted transgene products in this regard might be feasible. However, systemic, multi-organ diseases will require additional solutions. For instance, development of next-generation AAV capsids that can evade NAbs might help expand the eligible patient cohort. These approaches can involve isolation of new strains or reengineering existing AAV strains by rational or combinatorial engineering. While several vector candidates have been evaluated successfully in a preclinical setting, the NAb profile for new strains in the human population is still largely unknown. Further, it is noteworthy to mention that evaluation of such vectors in later stage trials is likely to require bridging studies that address toxicity/biodistribution profiles. As an alternative strategy, chemically modified AAV with PEG and other polymers using established methods might enable efficient NAb evasion. However, challenges pertaining to formulation, heterogeneity and quality control of chemically modified AAV vectors manufactured on a clinical scale would need to be addressed. A more recent approach focused on employing capsid decoys to ‘sponge’ NAbs presents an interesting paradigm. However, antigenic overload of AAV capsids is a significant concern that requires to be carefully addressed.

At the other end of the spectrum, clinical interventions such as plasmapheresis and pharmacological modulation of the immune system offer promising, alternative solutions to address pre-existing immunity and/or vector re-administration. Plasmapheresis has proven useful in autoimmune disease to remove pathogenic antibodies from patients and might be beneficial in cases of low NAb titer. Pharmacological modulation of the host immune response might prevent generation of NAbs and hence enable vector re-administration. Although clinical caveats are well documented, strategies to deplete both B and T cells can prove effective. In addition to the aforementioned aspects, efforts to dissect the host immune response to AAV and assess the NAb profile face significant challenges. Specifically, variability in the host immune response and NAb profile against AAV between different preclinical animal models as well as the distinct transduction profiles displayed by AAV strains on different cell/tissue types pose significant hurdles to assay development. Efforts to standardize NAb assays and comprehensive assessment of preclinical as well as clinical data are likely to address the latter issues. With regard to circumventing the humoral immune response, each approach described above offers certain advantages and faces distinct drawbacks or additional challenges. However, it is important to realize that they clearly complement each other and have the potential to expand the patient population that can benefit from next-generation gene therapy products.

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Pharmacological modulation of T cell mediated humoral immunity

Highlights

- The host immune response to adeno-associated viral (AAV) vectors poses a significant clinical challenge.
- Pre-existing neutralizing antibodies (NAbs) preclude vector administration and generation of NAbs following AAV vector administration precludes vector re-dosing.
- Strategies to circumvent the humoral immunity to AAV can be classified from the perspective of the vector development or modulation of host immune response.
- Vector development approaches include routes of administration, isolation of novel strains, reengineering existing capsid templates, chemical modifications of AAV capsids and the use of decoys to evade pre-existing NAbs.
- Plasmapheresis and pharmacological modulation of B and T cells can assist with removal of pre-existing NAbs and mitigate generation of NAbs following vector administration, respectively.
- Strategies from the vector and the host perspective are complementary and likely to help address challenges pertaining to both pre-existing NAbs as well as vector re-dosing, if employed together.

Table 1

Comparison of different strategies to circumvent humoral immunity to AAV vectors.

Strategy	Advantages and clinical attributes	Potential clinical caveats
Vector perspective		
Alternative route of vector administration	Allows immediate translation; can address the issue of pre-existing Nabs	Only certain peripheral administration routes are viable; focused on immune-privileged organs; not suitable for systemic administration
Discovery of new AAV natural isolates	Feasible through next-generation sequencing technologies; can address pre-existing NAbs	Can be labor intensive and time consuming; change in clinical vector candidate/re-dosing will require toxicity/biodistribution data for every new isolate discovered
AAV capsid engineering	Goal-oriented; can address both pre-existing NAbs	Can be labor intensive and time consuming; potential changes in tissue/host tropism and transduction efficiency should be taken into account; change in clinical vector candidate/re-dosing will require toxicity/biodistribution data for every new engineered variant
Chemically modified AAV	Viable formulation approach; previously established for several approved biologics; can potentially address both pre-existing NAbs and vector re-dosing issues	Quality control, potential variability in scale up conditions for different AAV strains; potential effects on tropism and transduction efficiency; pre-existing NAbs against polymers such as PEG
AAV capsid decoys	Easy scale up; broadly applicable to multiple AAV strains; can address both potentially pre-existing NAbs and vector re-dosing issues	Empty AAV shells tend to package host DNA fragments; potential antigenic overload in humans leading to immunotoxicity; potential effects on transduction efficiency
Host perspective		
Plasmapheresis	Established clinical procedure; can potentially address both pre-existing NAbs and vector re-dosing issues	Transient immunosuppression; prolonged clinical procedure that requires multiple rounds of treatment; efficacy dependent on Nab levels in different patients
Targeting B-cell activation and apoptosis	Adjuvant therapy type approach with the US FDA-approved drugs; can potentially address vector re-dosing issues	Immunosuppression; might not effectively reduce pre-existing NAbs; does not reduce plasma effectors or memory B cells
Targeting T-cell activation	Adjuvant therapy type approach with FDA-approved drugs; can potentially address vector re-dosing issues	Indirectly affects humoral response; immunosuppression; might not effectively reduce pre-existing NAbs
Inducing tolerance to capsid antigen	Selective for each capsid; can potentially address vector re-dosing issues	Requires additional clinical intervention and pre-clinical validation; does not reduce pre-existing NAbs

AAV: Adeno-associated viral; NAbs: Neutralizing antibodies.