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# Engineering humoral immunity as prophylaxis or therapy

Cailin E Deal and Alejandro B Balazs

**Purpose of the review**

In this review, we will discuss the field of engineered humoral immunity with an emphasis on recent work using viral vectors to produce antibodies *in vivo*. As an alternative to passive transfer of monoclonal antibody protein, a transgene encoding an antibody is delivered to cells via vector transduction, resulting in expression and secretion by the host cell. This review will summarize the evidence in support of this strategy as an alternative to traditional vaccines against infection and as novel therapeutics for a variety of diseases.

**Recent findings**

Historically, humoral immunity has been engineered through vaccination and passive transfer of monoclonal antibodies. However, recent work suggests that vectors can be used to deliver transgenes encoding broadly neutralizing antibodies to non-hematopoietic tissues and can mediate long-term expression that is capable of preventing or treating infectious diseases. The production of engineered monoclonal antibodies allows for precise targeting and elimination of aberrant self-proteins that are characteristic of certain neurodegenerative disease. This approach has also been successfully used to combat cancer and addiction in several animal models. Despite the wide array of expression platforms that have been described, adeno-associated virus vectors have emerged as the frontrunner for rapid clinical translation.

**Summary**

Recent advances in vector-mediated antibody expression have demonstrated the potential for such interventions to prevent infection and treat disease. As such, it offers an alternative to immunogen-based vaccine design and a novel therapeutic intervention by enabling precise manipulation of humoral immunity. Success translating these approaches to patients may enable the development of effective prevention against previously intractable pathogens that evade immunity such as HIV, influenza, malaria or HCV and may also enable new treatment options for neurodegenerative diseases such as Alzheimer's disease.

**Address**

Ragon Institute of MGH, MIT & Harvard, 400 Technology Sq., Cambridge, MA 02139, United States

Corresponding author: Balazs, Alejandro B (abalazs@mgh.harvard.edu)

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## Introduction

The humoral immune system is one of the first obstacles encountered by invading pathogens, thus playing a crucial role in preventing infection and maintaining human health. Immunological memory, particularly in the form of pre-existing antibodies, has been shown to form the basis of protection for nearly all vaccines in use today [1]. This ability to generate memory against previously encountered pathogens enabled the first practitioners in Asia to engineer immunity against smallpox in the fifteenth century through a process termed variolation [1]. By intentionally exposing patients to the relatively mild variola minor, they were able to induce protective immunity that prevented life-threatening smallpox infection by variola major [1]. Similarly, modern-day vaccines engineer immunity by exposing a patient to inactivated or attenuated whole pathogens, or recombinant components of pathogens that are known to elicit protective immunity. As a result, effective vaccines have been developed against many of the diseases for which natural infection results in immunity against re-infection. This practice has resulted in the global eradication of smallpox [2] and decreased incidence of diphtheria, measles, mumps, pertussis, poliomyelitis, rubella and tetanus [3]. However a number of diseases for which prior exposure is ineffective at preventing subsequent disease have proven more difficult targets. These include intractable pathogens that evade immunity, such as human immunodeficiency virus (HIV), malaria, hepatitis C virus (HCV), and influenza A virus (IAV) as well as complex diseases of self, such as cancer and neurodegenerative disorders.

## Engineered humoral immunity through passive transfer

Ideally, vaccination elicits a protective cellular and humoral response, however the protection raised by most of the currently licensed vaccines is largely antibody-dependent [1,4]. Importantly, neutralizing antibodies (nAb)

alone have been shown to prevent the spread of many diseases within populations [4,5]. This antibody-based protection underlies passive transfer, whereby the administration of sera or purified antibodies into naïve patients transiently confers immunity. Technological advances including the development of hybridoma technology [6] and the 'humanization' of antibodies has spawned a new class of antibody-based drugs which have demonstrated remarkable success in the clinic for a wide range of diseases.

While mAbs have several advantages over conventional small-molecule drugs, there are considerable disadvantages as well. Currently licensed mAbs have a typical half-life ranging from two days to one month *in vivo* [7], necessitating frequent dosing. However, specific mutations in the antibody Fc region have been described that prolong half-life up to five-fold by increasing the affinity of the antibody for the neonatal Fc receptor (FcRn) [8]. Despite the potential for less frequent dosing, high concentrations of most antibodies are necessary to achieve clinical efficacy, resulting in high materials costs [5,9]. Additionally, antibody use is further complicated by the requirement of cold-chain storage and trained medical personnel for administration [7], making such therapy difficult to implement in low-resource areas. For mAb therapy to become a widely administered intervention on a global scale, improved delivery approaches will be required.

## Vectored antibody gene delivery for infectious diseases

One alternative to passive transfer utilizes a vector for the delivery of transgenes encoding previously characterized antibodies. These transgenes direct the production of mAbs in non-hematopoetic cells, which secrete mAbs into the circulation or the local environment. A wide variety of vectors, each with distinct expression profiles, have been considered for this approach (Table 1).

Naked plasmid DNA offers simplicity of use, lack of immunogenicity and ease of large-scale production. Improvements in electroporation techniques has allowed for enhanced transfection of specific tissues *in vivo*. Electroporation of various monoclonal antibody transgenes into muscle has yielded production of mAb light and heavy chains (i.e. Fab fragments), peaking at 50–200 ng/mL in mice and 30–50 ng/mL in sheep [10]. Optimization of the expression plasmid and electroporation conditions yielded 2–3 μg/mL of the VRC01 HIV broadly neutralizing antibody (bNAb) in the plasma of mice 12 days post administration [11•], demonstrating that electroporation of plasmid DNA encoding antibody can be used to rapidly produce mAb *in vivo*. However, the modest concentrations and transient nature of expression obtained with existing protocols limit the clinical potential of this technique.

Viruses have been exploited as vectors for many years owing to their highly evolved mechanisms for efficient delivery of genetic material to host cells. Lentiviral vectors, consisting of an extensively modified HIV-1-derived genome pseudotyped with vesicular stomatitis virus (VSV) G protein, represent an attractive means of integrating transgenes into the host genome, enabling long term gene expression in a wide variety of both dividing and nondividing cells. Such vectors have been used to transduce primary hematopoietic stem cells (HSC) with a transgene encoding the HIV bNAb b12, allowing for their differentiation into plasmablasts that secreted b12 *in vitro* [12]. Using a similar approach, B cells were engineered to secrete HIV bNAb 2G12 in a humanized mouse model, achieving concentrations of approximately 40 ng/mL in plasma, which was sufficient to inhibit HIV infection *in vivo* [13]. However, all of these studies utilized *ex vivo* transduction, making widespread implementation of this approach challenging.

While lentivirus is well suited for long-term expression of mAb, adenoviral vectors have been shown to exhibit transient, but rapid gene expression ideal for responding to infectious disease outbreaks. Adenovirus serotype 5 (Ad5) encoding Palivizumab, a respiratory syncytial virus (RSV) mAb, produced detectable antibody expression as early as four days post-transduction and resulted in a 5.4 fold decrease in RSV titers in the lung four days post-challenge as compared to controls [14]. Ad5 has also been used to express a single-domain antibody specific for H5N1 influenza A virus (IAV) hemagglutinin (HA), which protected mice when administered 14 days prior to, or even 48 h after, infection [15•]. In another study, a mAb targeting the protective antigen of *Bacillus anthracis* was delivered by Ad5 that protected mice from toxin challenge between 1 day and 8 weeks post-administration, but which was no longer protective at 6 months [16].

Adeno-associated virus (AAV) has never been associated with any disease in humans and recombinant vectors derived from AAV (rAAV) result in stable gene expression in the absence of integration through formation of extrachromosomal concatamers of the delivered transgene sequences [17]. The serotype used to package the vector strongly influences its ability to transduce different tissues [17,18] and has been shown to play a significant role in the immunogenicity of the vector in various animal models [19,20]. AAV1 delivering lipoprotein lipase (LPL) was recently approved in Europe as the first ever gene therapy product for humans [21] and recent clinical trials testing AAV8 for the delivery of Factor IX for hemophilia has met with considerable success [22••]. Given their clinical efficacy and favorable expression profile, AAV vectors have been extensively characterized as a platform for the delivery of mAbs *in vivo*. However, the packaging capacity of AAV is limited to 5 kb, presenting a significant obstacle to the efficient expression of both heavy and light chain. As a result, some groups have turned to smaller bivalent single chain antibodies (scFv) or immunoadhesins, chimeric

**Table 1**

**Summary of monoclonal antibody expression vectors**

| | Vector | Ab type | Species of Fc | Isotype | Promoter | Furin/2A cleavage | Animal model | Vector dose | Route | Ab clone | Peak serum concentration | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HIV | Plasmid | Fab | Mouse | IgG1 | CMV | N/A | BALB/c mice | 25 μg | IM | VRC01 | 3.3 μg/mL | Muthumani et al. [11•] |
| | Lentivirus | Full-length | Human | IgG1 | Human μ heavy chain | Y | NSG humanized mice | MOI 1000 | *Ex vivo* B cell transduction | b12 | >1 μg/mL | Luo et al. [12] |
| | Lentivirus | Full-length | Human | IgG1 | hPGK | Y | NSG humanized mice | 100 ng p24 | *Ex vivo* B cell transduction | 2G12 | 40 ng/mL | Joseph et al. [13] |
| | rAAV2 | Full-length | Human | IgG1 | CMV, EF1α | N | Rag-1 mice | $5 \times 10^{11}$ GC | IM | b12 | 8 μg/mL | Lewis et al. [29] |
| | scAAV2 | Immunoadhesin | Rhesus | IgG2 | CMV | N/A | Rhesus macaques | $2 \times 10^{13}$ GC | IM | 4L6<br>5L7 | 400–500 μg/mL<br>200–300 μg/mL | Johnson et al. [30] |
| | rAAV8 | Full-length | Human | IgG1 | CASI | Y | NSG humanized mice | $1 \times 10^{11}$ GC | IM | b12<br>2G12<br>2F5<br>VRC01<br>4E10 | 100 μg/mL<br>>250 μg/mL<br>20 μg/mL<br>>250 μg/mL<br>20 μg/mL | Balazs et al., 2012 |
| | rAAV8 | Full-length | Human | IgG1 | CASI | Y | NSG and BLT humanized mice | $1 \times 10^{11}$ GC | IM | b12<br>VRC01<br>VRC07<br>3BNC117<br>12A12<br>VRC-PG04<br>VRC07G54W<br>NIH45-46G54W<br>PGT121<br>PGT128<br>PG9 | 115 μg/mL<br>100 μg/mL<br>130 μg/mL<br>24 μg/mL<br>23 μg/mL<br>30 μg/mL<br>74 μg/mL<br>40 μg/mL<br>256 μg/mL<br>50 μg/mL<br>390 μg/mL | Balazs et al. [32••] |
| | rAAV8 | Full-length | Human | IgG1 | Human thyroglobulin | Y | NSG humanized mice | $2.5 \times 10^{11}$ GC<br>$2.0 \times 10^{11}$ GC | IV<br>IV | 10-1074<br>3BNC117 | 300 μg/mL<br>20 μg/mL | Horwitz et al. [33••] |
| | scAAV1 | Full-length | Rhesus | IgG2 | CMV | N/A | Rhesus macaques | $2.5 \times 10^{13}$ particles<br>$1 \times 10^{13}$ particles | IM | eCD4-Ig<br>3BNC117<br>PGT121<br>10-1074<br>NIH45-46 | 75–180 μg/mL<br>–<br>–<br>–<br>– | Gardner et al. [34••] |

**Table 1** (*Continued*)

| | Vector | Ab type | Species of Fc | Isotype | Promoter | Furin/2A cleavage | Animal model | Vector dose | Route | Ab clone | Peak serum concentration | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Influenza | Ad5 | Single-domain | – | – | CMV | N | BALB/c | $1 \times 10^8$ PFU<br>$1 \times 10^7$ PFU<br>$1 \times 10^6$ PFU | Intranasal | aHA-7 | – | Tutykhina et al. [15•] |
| | rAAV8 | Full-length | Human | IgG1 | CASI | Y | BALB/c and NSG mice, ferrets | $1 \times 10^{11}$ GC (mice)<br>$5 \times 10^{12}$ GC/kg (ferret) | IM | F10<br>CR6261 | 200 μg/mL (mice)<br>10–100 μg/mL | Balazs et al. [37••] |
| | rAAV9 | Immunoadhesin | Human | IgG1 | CAG | N/A | BALB/c mice, ferret and rhesus macaques | $1 \times 10^{11}$ GC (mice)<br>$1 \times 10^{12}$ GC (ferret)<br>$1 \times 10^{13}$ GC (rhesus) | Intranasal | F16 | 0.5 μg/mL (nose),<br>2.0 μg/mL (lung) | Limberis et al. [38•] |
| Malaria | rAAV8 | Full-length | Human | IgG1 | CASI | Y | C57BL/6NCr | $1 \times 10^{11}$ GC | IM | 2A10<br>2C11 | >1000 μg/mL<br>100–500 μg/mL | Deal et al. [35••] |
| HCV | rAAV9 | Full-length | Human | IgG1 | CASI | Y | Rosa26-Fluc, FNRG humanized mice | $1 \times 10^{11}$ GC | IM | AR3A<br>AR3B<br>AR4A | 1000–3000 μg/mL | de Jong et al. [36••] |
| RSV | Ad5<br>rAAVrh.10 | Full-length | Mouse | IgG1 | CMV | N<br>Y | BALB/c mice | $5 \times 10^{10}$ PFU<br>$1 \times 10^{11}$ GC | IV<br>Intrapleural | Murine palivizumab precursor | – | Skaricic et al. [14] |
| Anthrax | Ad5<br>rAAVrh.10 | Full-length | Mouse | IgG1 | CMV | N<br>Y | C57BL/6 mice | $1 \times 10^{11}$ PFU<br>$1 \times 10^{11}$ GC | IV<br>Intrapleural | 14B7-1H | – | De et al. [16] |
| HD | rAAV2 | scFv intrabody | – | – | CBA | N/A | C57BL/6, BACHD, R6/2, N171-82Q and YAC128 mice | $1 \times 10^{10}$ GC | Intrastriatal | $V_L$12.3 Happ1 | – | Southwell et al. [45] |
| | rAAV1 | scFv intrabody | – | – | – | N/A | B6.HDR6/1 mice | $2 \times 10^{10}$ GC | Intrastriatal | C4 | – | Snyder-Keller [46] |
| AD | rAAV1 | scFv | – | – | CMV | N/A | 3× Tg-AD mice | $1 \times 10^9$ GC | Hippocampal | Aβ-scFv | – | Ryan et al. [48] |
| | rAAV1<br>rAAV2<br>rAAV5 | scFv | – | – | CAG | N/A | C57BL/6 and TgAβPPswe/PS1dE9 mice | $3 \times 10^{10}$ GC | Intracranial | scFv59 | – | Kou et al. [23] |
| | rAAV2 | scFv | – | – | CMV | N/A | APPswe/PS1dE9 mice | $5 \times 10^{10}$ GC | IM, Intraventricular | scFv | – | Wang et al. [24,49,54] |
| | rAAV1 | Full-length | – | – | CMV | Y | Tg2576 mice | $3 \times 10^{10}$ GC | IM | IIA2 | 300 μg/mL | Shimada et al. [50•] |
| ALS | scAAV1 | scFv | – | – | CMV | N/A | $SOD1^{G93A}$, GAP-43-luc/gfp/$SOD1^{G93A}$ mice | $3 \times 10^9$ GC | Intrathecal | D3H5 | – | Patel et al. [25•] |

**Table 1** (*Continued*)

| | Vector | Ab type | Species of Fc | Isotype | Promoter | Furin/2A cleavage | Animal model | Vector dose | Route | Ab clone | Peak serum concentration | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Prion | rAAV9 | scFv | – | – | – | N/A | CD1 mice | $1.4 \times 10^{12}$ GC | Intracranial | D18 | – | Moda et al. [52] |
| | rAAV2 | scFv | –<br>–<br>–<br>– | –<br>–<br>–<br>– | CMV | N/A | Mice | $9 \times 10^{9}$ GC | Intracranial | D18<br>3:3<br>6:4<br>6:6 | – | Wuertzer et al. [26] |
| Cancer | rAAV8 | Full-length | Rat | IgG1 | CAG | Y | NCr nu/nu mice | $2 \times 10^{11}$ GC | IV | DC101 | >8000 μg/mL | Fang et al. [27] |
| | rAAV8 | Full-length | Rat | IgG1 | ZgIL-2P | Y | C57BL/6 mice | $2.5 \times 10^{11}$ GC | IV | DC101 | >1000 μg/mL | Fang et al. [28] |
| | rAAVrh.10 | Full-length | Mouse | IgG1 | CMV | Y | C57BL/6 mice | $1 \times 10^{11}$ GC | IV | 4D5 | 30–40 μg/mL | Wang et al. [24,54] |
| | rAAV8 | scFv | Humanized | IgG1 | CMV | N/A | SCID-BEIGE | $2 \times 10^{11}$ GC | IV | h1567 | 96 μg/mL | Han et al. [56] |
| Addiction | rAAVrh.10 | Full-length | – | – | CAG | Y | BALB/c | $1 \times 10^{11}$ GC | IV | GNC92H2 | – | Rosenberg et al. [58] |
| | rAAVrh.10 | Full-length | – | – | CAG | Y | C57BL/6 mice | $1 \times 10^{11}$ GC | IV | NIC9D9 | 1300 μg/mL | Hicks et al. [57] |

*Abbreviations*: rAAV, recombinant aden-associated virus; scAAV, self-complementary adeno-associated virus; Ad5, adenovirus serotype 5; Fab, fragment-antigen binding; scFv, single-chain variable fragment; CMV, cytomegalovirus; hPGK, human phosphoglycerate kinase promoter; EF1α, elongation factor 1α; N/A, not applicable; MOI, multiplicity of infection; GC, genome copy; PFU, plaque forming unit; IM, intramuscular; IV, intravenous.

antibody-like molecules combining the functional domain with the immunoglobulin constant domain [23,24,25•,26]. However, others have employed heterologous viral sequences such as foot and mouth disease virus-derived 2A self-processing sequence (F2A) to express full-length antibodies from a single open reading frame [27], yielding greater than 1000 μg/mL of sustained mAb serum levels *in vivo* [27,28].

The earliest forms of AAV mediated gene antibody transfer were implemented as a dual-promoter vector whereby the heavy and light chain genes were transcribed independently. This yielded up to 8 μg/mL of biologically active an HIV bNAb for over 6 months in immunodeficient Rag mice [29]. In rhesus macaques, expression of SIV gp120-specific immunoadhesins peaked 3–4 weeks post transduction at 200 μg/mL and was sustained at 20 μg/mL for at least 4 years [30]. Out of nine monkeys challenged with SIV one month after AAV administration, six were completely protected from challenge. The three immunized macaques that became infected were later found to have developed an immune response against the immunoadhesin one week prior to challenge, suggesting that an anti-immunoadhesin response led to the observed failure of protection [30]. AAV vectors were also employed in a similar approach coined 'Vectored ImmunoProphylaxis' (VIP), whereby full-length human IgG bNAbs against HIV were expressed from an optimized transgene that utilized the F2A sequence to allow for the expression of independent heavy and light chains under a muscle-optimized promoter [31]. This transgene was packaged with an AAV8 capsid [19], leading to the production of mAb at serum concentrations greater than 100 μg/mL for at least 52 weeks [31]. Using this system, two different humanized mouse models were protected from either IV challenge with a laboratory strain of HIV [31] or repetitive low-dose vaginal challenge with a more clinically relevant transmitted founder strain (REJO.c) [32••]. Decreasing doses of AAV vector led to dose-dependent antibody expression, enabling a determination of the minimum protective dose *in vivo* for a number of antibodies [31,32••]. AAV-vectored bNAbs have also been shown to work in conjunction with passive mAb transfer and HAART to maintain suppression of HIV replication in humanized mice [33••]. Most recently, a synthetic fusion of CD4-Ig with a small CCR5-mimetic sulfopeptide (eCD4-Ig) was delivered by AAV and protected rhesus macaques from several infectious SHIV challenges suggesting that AAV-vectored synthetic proteins may be able to create effective HIV prophylaxis [34••].

In addition to HIV, successful VIP has also been demonstrated against a number of other infectious diseases. Recently, sterilizing immunity was generated against a murine model of *Plasmodium falciparum* infection, the malaria parasite responsible for the highest mortality in humans [35••], demonstrating the first known example where a parasitic disease was prevented by antibodies alone. Likewise, VIP-mediated expression of bNAbs against HCV conferred protection against viral challenge in humanized mice [36••] and was able to abrogate ongoing HCV infection both *in vitro* and *in vivo*. Additionally, sera taken from mice given VIP expressing different bNAbs targeting IAV hemagglutinin (HA), were able to neutralize all H1, H2 and H5 strains tested, and antibody expression lasted well over a year post-AAV injection [37••]. Interestingly, protection against influenza by VIP did not appear to inhibit the elicitation of novel immune responses, suggesting that VIP may be capable of augmenting immunity without abrogating endogenous immune responses [37••]. Notably, this method also protected immunodeficient and older mice from disease, representing two particularly vulnerable human patient populations who are inadequately protected by traditional vaccination [37••]. While intramuscular (IM) injection of AAV for expression of antibodies has been most common, other routes of administration and target sites have also been used successfully. One study used AAV9 to deliver IAV bNAb F16 by intranasal administration, which resulted in protection at the primary site of challenge [38•]. AAV has also been used to generate long-term expression of an RSV antibody [14]. One study tested a combination of AAV and Ad5 to generate rapid protection against anthrax that lasted at least 26 weeks [16].

## Vectored antibody delivery for neurodegenerative diseases

In addition to engineering immunity against infectious disease, the use of AAV as a means of creating desirable antibody specificities *in vivo* enables targeting of 'self' proteins that would be difficult or impossible to target safely through traditional vaccination. As a result, there has been growing interest in utilizing this approach for the treatment and prevention of neurodegenerative diseases, which represent an increasing share of the healthcare burden in developed nations. For many such diseases, aggregation of misfolded proteins has been suggested as the underlying mechanism, making them ideal targets for mAbs that recognize the misfolded variants and prevent the formation of these aggregates. Small scFv antibody fragments lacking the Fc region, including intrabodies (iAb) that target antigens intracellularly, can distinguish highly homologous proteins, different conformations of the same protein and, in the case of some iAb, target proteins to distinct cellular compartments [39–41]. The genes encoding these scFv have been delivered by AAV vectors, representing a powerful new tool for treating or preventing neurodegenerative disease [42,43].

Huntington's disease (HD) is caused by a mutation in the huntingtin protein (HTT) and is a model for numerous neurodegenerative disorders due to its simple autosomal

dominant inheritance. Several anti-HTT iAbs have been generated [44] and two were tested in transgenic HD models using intrastriatal AAV2 delivery [45]. Of the iAbs tested, Happ1 ameliorated neuropathology in cell lines and conferred significant beneficial effects in a variety of motor and cognitive assays, significantly prolonging life span by 10 weeks in a mouse model of disease [45]. Another group delivering iAb C4 via intrastriatal AAV1 delivery demonstrated that early treatment of an HD mouse model, prevented cells from exhibiting nuclear aggregates and delayed aggregate accumulation [46].

Alzheimer's disease (AD) is a disorder characterized by the diffuse loss of neurons and accumulation of amyloid beta proteins (Aβ) in the brain. Recently, scFvs have been developed that target Aβ, which could reduce Aβ burden and possibly alleviate symptoms [47]. A transgenic mouse model for AD, given an intrahippocampal infusion of AAV1 encoding an Aβ-scFv exhibited lower levels of insoluble Aβ, increased numbers of microglia and demonstrated improved cognitive function [48]. While most studies have administered AAV vectors directly into the brain, this may pose a safety risk as intraventricular delivery of AAV5 led to an increase in hemorrhaging [23]. As an alternate route, IM injection of AAV2 expressing an Aβ-scFv was found to be as effective as intracranial administration in reducing physiologic and behavioral effects of AD without producing detectable inflammatory responses or microhemorrhages in the brain [24,49]. In a separate study, IM injection of AAV1 expressing a full-length Aβ-mAb resulted in sustained anti-Aβ levels above 100 μg/mL in serum that were maintained for up to 64 weeks post-injection [50•]. These levels were found to be effective in decreasing Aβ levels in the brain both prophylactically and therapeutically [50•].

Emerging evidence suggests that misfolding of superoxide dismutase 1 (SOD1) is a common pathogenic event in amyotrophic lateral sclerosis (ALS) [51]. AAV encoding an scFv specific for misfolded SOD1, was injected intrathecally into an ALS mouse model, resulting in reduced neuronal stress, reduced levels of misfolded SOD1 in the spinal cord and attenuation of motor neuron loss (Table 1) [25•]. Overall, this led to delayed disease onset and increased life span that directly correlated with antibody titer.

Similarly, this approach has also been investigated to combat prion disease, which is a neurodegenerative disorder caused by a conversion of cellular prion protein ($PrP^{c}$) into the misfolded, insoluble, $PrP^{sc}$. Mice inoculated peripherally with infectious prions were given an AAV vector expressing scFv targeting $PrP^{sc}$. These mice exhibited decreased $PrP^{sc}$ burden and delayed onset of prion pathogenesis as determined by improvements of clinical signs (Table 1) [26,52].

## Vectored antibody delivery for cancer

As a result of an improved understanding of the molecular basis of cancer, antibody-based drugs have become the standard of care for many types of tumors. Human epidermal growth factor receptor type 2 (HER2) overexpression is associated with reduced survival in cases of human breast cancer. Clinical trials of trastuzumab, an mAb that targets an extracellular region of HER2, have been successful at steady state serum concentrations of greater than 10 μg/mL [53]. The murine precursor to trastuzumab was encoded in an AAV rh.10 vector [54] and administered to C57BL/6 mice, resulting in serum concentrations near 35 μg/mL within twelve weeks that were sustained for at least 56 weeks post injection. A single injection of this vector increased the survival of Balb/c nu/nu mice injected subcutaneously with Calu-3 tumor cells over-expressing HER2, demonstrating its efficacy at inhibiting cancer *in vivo* [54].

Similarly, cutaneous T-cell lymphoma (CTCL), exhibits over-expression of chemokine receptor 4 (CCR4), whose expression is limited amongst non-malignant cells [55]. AAV8 was used to express a humanized anti-CCR4 mAb in a tumor mouse model, resulting in reduced $CCR4^{+}$ tumor growth and increased survival. A single IV injection resulted in 96 μg/mL of mAb in serum, which was able to significantly reduce tumor growth and increase life-span as compared to control animals [56]. While these results have been promising, clinical translation of vectored antibody delivery for cancer will require the use of transient vectors or platforms for regulated mAb delivery.

## Vectored antibody delivery for addiction

Substance abuse creates a substantial healthcare burden and is the target of numerous pharmacological and behavioral interventions. Antibodies that target the addictive substance and prevent receptor signaling could offer a potential treatment, however repeated administration of mAb protein is impractical. Previous studies showed that AAVrh.10 expressing a high affinity anti-nicotine mAb resulted in a serum concentration of 1.3 mg/mL that lasted for at least 18 weeks [57]. AAV-NIC9D9 mice had a majority of serum nicotine bound to the Fab within one minute after challenge with nicotine, resulting in brain concentrations of nicotine that were only 15% of what was observed in naïve controls. Importantly, the expression of this antibody also blocked nicotine-mediated alterations in arterial blood pressure, heart rate and locomoter activity, demonstrating its ability to obviate the physiological effects of nicotine [57]. Using a similar approach against cocaine, AAVrh.10 was engineered to express the high affinity anti-cocaine mAb GNC92H2 [58], leading to the expression of anti-cocaine antibodies for at least 24 weeks after IV administration and resulting in a 31-fold reduction in the ratio of brain to blood cocaine levels and reduced hyperactivity in treated mice as compared to controls [58].

## Conclusion

Successful translation of vectored antibody gene delivery to patients is poised to redefine the landscape of immunological interventions by enabling precise engineering of the specificity and intensity of a desirable humoral response. It goes well beyond the ability of traditional vaccines to enable production of non-natural antibody architectures capable of discriminating between normal and aberrant forms of self-proteins. By circumventing the natural immune system, vectored antibody delivery has the potential to yield protection regardless of immune-status or age, allowing it to reach currently vulnerable populations of patients who cannot respond to vaccines and offering a possible alterative to existing therapies delivered by passive transfer.

## Conflicts of interest

None.

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- • of special interest
- •• of outstanding interest

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