REVIEW

Delivery platforms for broadly neutralizing antibodies

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Purpose of review

Passive administration of broadly neutralizing antibodies (bNAbs) is being evaluated as a therapeutic approach to prevent or treat HIV infections. However, a number of challenges face the widespread implementation of passive transfer for HIV. To reduce the need of recurrent administrations of bNAbs, gene-based delivery approaches have been developed which overcome the limitations of passive transfer.

Recent findings

The use of DNA and mRNA for the delivery of bNAbs has made significant progress. DNA-encoded monoclonal antibodies (DMAbs) have shown great promise in animal models of disease and the underlying DNA-based technology is now being tested in vaccine trials for a variety of indications. The COVID-19 pandemic greatly accelerated the development of mRNA-based technology to induce protective immunity. These advances are now being successfully applied to the delivery of monoclonal antibodies using mRNA in animal models. Delivery of bNAbs using viral vectors, primarily adeno-associated virus (AAV), has shown great promise in preclinical animal models and more recently in human studies. Most recently, advances in genome editing techniques have led to engineering of monoclonal antibody expression from B cells. These efforts aim to turn B cells into a source of evolving antibodies that can improve through repeated exposure to the respective antigen.

Summary

The use of these different platforms for antibody delivery has been demonstrated across a wide range of animal models and disease indications, including HIV. Although each approach has unique strengths and weaknesses, additional advances in efficiency of gene delivery and reduced immunogenicity will be necessary to drive widespread implementation of these technologies. Considering the mounting clinical evidence of the potential of bNAbs for HIV treatment and prevention, overcoming the remaining technical challenges for gene-based bNAb delivery represents a relatively straightforward path towards practical interventions against HIV infection.

Keywords

adeno-associated virus, B cell editing, broadly neutralizing antibodies, DNA-based therapies, HIV, mRNA-based therapies, vectored immunoprophylaxis

INTRODUCTION

Since their initial discovery, broadly neutralizing antibodies (bNAbs) targeting diverse HIV-1 isolates have fundamentally altered the landscape of HIV prevention and treatment research [1]. Given their promise, significant effort has been focused on the development of vaccine approaches capable of eliciting bNAbs [2]. Yet passive transfer of bNAb proteins has shown significant effects in studies of either prevention [3–5] or treatment [6–9] of HIV infection. The relatively short half-life of passively transferred monoclonal antibodies necessitates regular infusions to maintain functional circulating titers, hindering the utility of bNAb passive transfer for both treatment and prevention of HIV [5]. This shortcoming has stimulated multiple lines of investigation into alternative approaches for the delivery of bNAbs in more convenient or longer-lived formats. These approaches include genetically encoding bNabs for delivery as plasmid DNA, modified mRNA, or adeno associated virus vectors, with the most recent efforts aimed at genetically engineering host B cells. In this article, we review prior

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KEY POINTS

- Different gene-based delivery approaches have been developed for the expression of broadly neutralizing antibodies (bNAbs).
- The administration of bNAbs through DNA-based platforms has proven to be effective in animal models due to its simplicity, rapid manufacturing, and the lack of vector-directed immune responses.
- The COVID-19 pandemic resulted in rapid translation of mRNA-mediated gene delivery for vaccination; this technology is now being tested for bNAb expression.
- AAV mediated bNAb delivery has achieved long-term antibody expression in humans and is the furthest developed approach.
- Lentiviral- and CRISPR-mediated engineering of bNAb expression by B cells leads to class switch recombination and further affinity maturation.

studies describing the use of each technology as a means of delivering monoclonal antibodies, with specific emphasis on approaches used for HIV bNAb delivery.

DNA for delivery of broadly neutralizing antibodies

The *in vivo* expression of recombinant proteins by exogenous nucleic acid injected into skeletal muscle was reported for the first time in the early 1990s [10,11]. In this approach, protein-coding expression transgenes are encoded in plasmids or other DNA forms for expression *in vivo*. Originally, DNA was advanced as a delivery platform for diverse vaccine and immunization strategies [12,13]. Numerous clinical trials have been performed and several are currently in progress evaluating DNA immunization against infectious diseases and cancers (NCT04090528, NCT03110770, NCT04131413, NCT04251117). During the recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, India granted emergency authorization to license ZyCoV-D (coronavirus disease DNA vaccine developed by Cadila healthcare), a plasmid DNA vaccine delivered by Jet injection for use in adults and children of 12 years and older [14] for prevention of SARS-CoV-2 infection. However, based on improved for *in vivo* delivery, this approach is also being studied. Conceptually, DNA biologics exhibit important features relevant for globally distributable products, including temperature stability, an excellent safety profile and lack of vector-induced immune responses allowing for repeat delivery. A single DNA vector backbone can be re-administered repeatedly for the delivery of either the same or different genes without the induction of vector-specific immunity. The approach has yielded months-long expression from a single inoculation, with a focus on intramuscular (i.m.) or intradermal (i.d.) delivery to simplify use in the field. A major goal for improvement of DNA platforms has been to increase their expression levels and immunogenicity when delivering vaccine antigens. Approaches such as codon and RNA optimization, improved leader sequences, improved delivery formulations and delivery methods are under investigation [14,15]. These approaches include needle-based injections of naked DNA or ballistic DNA, forms of jet delivery, multiple electroporation approaches, sonoporation, and photoporation among others, which can improve *in vivo* expression from gene-encoded DNA cassettes [12,14].

Studies over the past decade have shown continued improvement in the expression levels achieved with DNA-encoded monoclonal antibodies (DMAbs) in animal studies [12,13,15–29]. DMAbs hold promise to accelerate the deployment of new therapeutic interventions and provide preclinical tools for rapid evaluation of biological products. DMAbs have been tested for IgG production targeting prevention and treatment of diverse infectious diseases [15,20,22,23,25,28–36,37▪,38▪▪], and cancer [39–45] (Table 1. Current approaches for DNA delivery of antibodies). For example, DV87.1, a dengue-specific neutralizing antibody, was encoded as a DMAb for delivery of multiserotype neutralizing antibodies produced *in vivo* upon i. m. injection that was sufficient for protection in animal challenges [15]. Studies have reported the delivery of combinations of DMAbs resulting in protection against viral or bacterial disease models. For example, the concomitant use of two broadly neutralizing DMAbs for H1 or H3 influenza viruses resulted in a total of 3 μg/ml of immunoglobulin G (IgG) circulating antibodies, which were able to protect against a lethal dose challenge with either influenza strain, independently [23]. Studies also focused on the engineering of V regions (among others) as an important approach for increasing serum concentrations from DMAb delivery. This was first described using a panel of anti-Ebola monoclonal antibodies (mAbs), which were redeveloped for enhanced *in vivo* expression using a pDNA delivery format [24]. After enhancement, Patel *et al.* demonstrated that a single injection with DMAbs resulted in months of expression, achieving 48 μg/ml peak serum concentration and providing single-dose protection against Ebola challenge in animals. As a response to Zika infection, a potent

Table 1. Current approaches for DNA delivery of antibodies

Type of disease	Disease	Target	Antibodies expressed	Organism tested	Comments	References
Proof of concept	N/A	Human thyroglobulin	Tg10	Mice	• i.m. electroporation of naked DNA allows both constitutive and regulatable expression	Perez *et al.*, 2004
		Mouse CD25 and Human CTLA-4	PC61 5.3 and A3.6B10	NA	• An expression vector system developed for expression of fully functional and antigen-specific human antibodies with correct isotype specificities • RNA and Codon optimized leader sequence demonstrated increased secretion correct isotype specificities	Morrow *et al.*, 2009
Infectious pathogen	Influenza	Hemaglutanin	pHA mAb	Mice	• Multiple delivery of single DMAb at different sites enhances expression and neutralization • Protection against IFV lethal challenge	Yamazaki *et al.*, 2011
			C179, S139/1, 9H10	Mice	• First report- enhanced expression (32 weeks) • Multiple mAb delivery in same animal. • Oligoclonal mAb protection • Heterosubtypic immunity	Andrews *et al.*, 2017
			2-12C	Mice, Pig and Sheep	• Reduction in viral load and lung pathology after pandemic H1N1 influenza challenge at a prophylactic dose • Reduction in only lung pathology at lower dose • mAbs based on bovine Abs demonstrate durable circulating serum concentrations for ~75 days in mice • Peak levels of 7-12 μg/ml for 7–14 days in sheep, with no ADA responses	McNee *et al.*, 2020
			FluA, FluB	Mice	• Single dose protects against lethal challenge • Coordinated delivery of mAb resulting in exceptionally broad protection against both influenza A and B	Elliott *et al.*, 2017
	HIV	Envelope protein	VRC01	Mice	• First report of use of strategies to enhance Fab expression for antibody-encoding plasmids, like codon optimization and improved EP conditions • A peak serum conc of 2–3 μg/ml at day 12 postinjection • Single EP enhanced administration results in rapid production of Fabs *in vivo*, which neutralized a panel of different viral tier 1 and 2 isolates.	Muthumani *et al.*, 2013
			VRC01, PGT151, PGDM1400, PGT121, PGT145, 3BNC117, 10-1074	Mice and Rhesus macaque	• First report- DMAb platform for delivering bnAbs • Delivery of multiple DMAbs to a single animal neutralized the entire global panel of HIV-1. • High peak-circulating levels and broad neutralization activity. • 6–34 mg/ml expression levels in NHPs	Wise *et al.*, 2020

Table 1 (*Continued*)

Type of disease	Disease	Target	Antibodies expressed	Organism tested	Comments	References
	Dengue	E Protein, DIII	DV87.1	Mice	• First report- protection against multiple serotypes into any animal model • Single dose- prevents Antibody-dependent enhancement (ADE)	Flingai *et al.*, 2015
	Chikungunya	Envelope protein	CVM1	Mice	• First report of DMAb and DNA vaccine • Single dose- Protects against lethal challenge	Muthumani *et al.*, 2016
	Pseudomonas aeruginosa	PcrV and Psl exopolysaccharide	αPcrV, MEDI3902	Mice	• Bispecific DMAb- (MEDI3902) exhibits enhanced protective activity with antibiotic treatment in a lethal pneumonia model	Patel *et al.*, 2017
	Ebola	EBOV GP	ZMapp (2G4, 4G7,13C6)	Mice	• Sustained expression for 15 weeks • oligoclonal and antiebola mAbs protection	Andrews *et al.*, 2017
		EBOV GP Fusion Loop and Heptad Repeat 2	DMAb-11, DMAb-34	Mice	• Fully human DMAb confers 100% protection	Patel *et al.*, 2018
	Borrelia burgdorferi	OspA	319–44	Mice	• First report- DNA transfer as a delivery system for antibodies that block transmission of Borrelia in animal models (HuMAb) • Protection against an acute challenge by Borrelia-infected ticks	Wang *et al.*, 2019
	Zika	E protein	DMAb-ZK190	Mice and Rhesus macaque	• First report- infectious disease control in NHPs following *in vivo* delivery of a nucleic acid-encoded antibody • Expression levels persisting >10 weeks in mice and >3 weeks in nonhuman primate (NHPs) • Protection against infectious challenge in NHPs	Esquivel *et al.*, 2019
		Env protein	1C2A6, 1D4G7, 2B707, 3F12E9, 4D6E8, 5E6D9, 6F9D1, D10F4, 8A9F9, 9F7E1	Mice and Rhesus macaque	• First report- *in vivo* expression of anti-ZIKV antibodies from a vector system • Single dose protects against infectious challenge • co-formulated with an anti-ZIKV DNA vaccine provides immediate and persistent anti-ZIKV immune responses	Choi *et al.*, 2020
	Respiratory syncytial virus (RSV)	fusion protein (F)	pGX9369	Mice and Cotton rat	• Single administration of single-chain fragment variable-constant fragment (scFv-Fc) RSV-F DMAb demonstrated long-lasting immunity and effective biodistribution • *In vivo* protection in viral challenge	Schultheis *et al.*, 2020
	Nisseria gonorrhoeae	Oligosaccharide on Lipooligosacchride	2C7	Mice	• Complement-engaging variants facilitated rapid clearance following primary challenge with longer duration of protection	Parzych *et al.*, 2021
	Plasmodium falciparum	Circumsporozoite Surface Protein	human mAb clones CIS43, 317, and L9	Mice	• Long-term serological expression • *In vivo* efficacy of CIS43 and 317 (germ line modified variants) in mosquito bite challenge	Tursi *et al.*, 2022 [37▪]

Table 1 (*Continued*)

Type of disease	Disease	Target	Antibodies expressed	Organism tested	Comments	References
	SARS-CoV-2	Spike protein	COV2-2196 and COV2-2130	Mouse, Hamster	• First report- cryo EM structure of polyclonal *in vivo* produced DMAbs • High peak DMAb serum titers and long-term expression. DMAbs exhibited prolonged kinetics relative to protein IgG • Reduction in lung viral burden by >4–6 logs in a lethal challenge. • Protection was similar to protein mAbs	Parzych *et al.*, 2022 [38▪▪]
Cancer	Breast Cancer	HER2	mumAb4D5	Mice	• High and sustained expression • Effective inhibition of tumor growth	Kim *et al.*, 2016
			Anti-HER2	Mice	• Expression for several months, boosting expression by pDNA re-dosing • Complete tumor regressions	Hollevoet *et al.*, 2018
	Ovarian Cancer	HER2	Anti-HER2	Mice	• High serum expression for long duration • Ovarian tumor control and prolonging survival	Perales-Puchalt *et al.*, 2019
		FSHR	Anti-FSHR (D2AP11)	Mice	• D2AP11 can identify resistant ovarian cancer cell lines • Development of T cell engagers.	Bordoloi *et al.*, 2022
	Prostate Cancer	PSMA	Anti-PSMA	Mice	• First application of enhanced synthetic DNA for *in vivo* production of human mAb for cancer immunotherapy • Robust expression, controlled tumor growth and prolonged survival	Muthumani *et al.*, 2017
	Fibrosarcoma	CTLA-4	Anti CTLA-4	Mice	• Single dose-expression for several months • high serum levels and tumor regression	Duperret *et al.*, 2018
	Colorectal cancer (along with many others)	Cancer embryonic antigen	OVAC	Sheep	• Robust and prolonged *in vivo* production • Dose dependent response observed	Hollevoet *et al.*, 2022
	Glioblastoma Multiforme	DBTE, EGFRvIII and HER2	EGFRvIII-targeting DBTE and HER2	Mice	• First as a monotherapy for direct *in vivo* treatment for GBM in both peripheral and orthotopic challenge animal models • Durable *in vivo* expression and demonstrated potent tumor regression and clearance in mice	Park *et al.*, 2023

bNAbs , broadly neutralizing antibodies.

anti-Zika MAb, ZK-190, was studied first in mice and then in nonhuman primates (NHPs) and was shown to protect against viral challenge [26]. Through a collaborative academic/industry partnership, two potent SARS-CoV-2 mAbs were redesigned for DNA delivery and advanced rapidly into clinical studies as DMAbs. Administration of these DMAbs to BALB/c mice induced peak expression of 5–50 μg/ml of circulating human IgG within 21 days and recapitulated the immune phenotype of the parental mAbs [38▪▪]. Expression from a single i.m. administration was confirmed for over 200 days, showing a prolonged slope of decay. These animals were protected from SARS-CoV-2 challenge, to a similar degree as animals given passive transfer [38▪▪]. Clinical testing of this dual DMAb approach has moved into a human clinical trial (NCT05293249). This study could provide important information on the safety, deliverability, and expression levels of the DMAb approaches for dual mAb delivery.

An important area for HIV research is the delivery of cocktails of broadly neutralizing antibodies as a means of preventing infections or as therapy. Wise *et al.* designed a large panel of DMAbs optimized for *in vivo* expression and evaluated whether these antibodies could be delivered in combinations to neutralize a global viral HIV panel. Specific combinations of 2–4 DMAbs (PGDM1400, PGT121, VRC01, and PGT151) selected from a larger panel in DMAb formats were tested in combinations of DMAb and found that cocktails were able to neutralize the 12-member global panel. A dramatic increase in neutralization breadth was described, with IC_{50} levels below 0.1 μg/ml for all 12 global panel viruses [29]. Two anti-HIV-1 DMAbs, PGDM1400 and PGT121 alone or in combination, were also tested in NHPs, with peak serum concentrations ranging between 6–34 μg/ml and no safety concerns being reported in NHPs. These studies demonstrate that the *in vivo* produced DMAbs retained neutralization properties equivalent to the original bnAbs [29]. These studies serve as a stepping stone for further development of bnAbs as DMAbs. Future efforts are needed to enhance their immunological properties as well as their half-life, which would improve the pharmacokinetic profile and reduce the need for frequent infusions.

DNA as a gene delivery platform has unique advantages, including its inherent stability and simplicity of production, as well as the ease of dissemination worldwide. The simplicity of combining multiple therapeutic proteins/antibodies is likely to be important for the successful application of this approach, particularly for HIV and other infectious diseases. Recent studies using engineered forms of biologics, such as DMAb encoded bispecific T cell engagers (BiTEs) to treat diverse cancers, further support applications of the DNA platform [42,46▪,47]. Future investigations focusing on the development and use of next-generation DNA in the context of HIV therapy and other infectious diseases are potentially of global importance.

Modified mRNA for delivery of broadly neutralizing antibodies

The administration of exogenous mRNA for the expression of proteins by the host has made great strides in the last few years [48]. Notably, the COVID-19 pandemic greatly accelerated the clinical development and widespread use of mRNA-based vaccines to induce the expression of SARS-CoV-2 spike antigen, which stimulated an effective immune response against this virus [49]. Rapid and high-level expression, proven scalability, and an inability to integrate into the host genome, are among the significant advantages that mRNA has over other platforms [50▪▪,51]. However, if delivered alone, mRNA can induce the activation of Pattern Recognition Receptors (PRRs) [52], such as toll-like receptor (TLR)-3, -7, and -8 [53]; retinoic acid-inducible gene I (RIG-I) [54]; melanoma differentiation-associated protein 5 (MDA-5) [55]. In addition, mRNA molecules are degraded by intra- and extra-cellular ribonucleases and cannot easily enter the host cell [56], requiring the incorporation of modified nucleosides to side-step these challenges [57]. Multiple mRNA formats and routes of administration are currently being studied [58].

The use of mRNA to encode antibody transgenes has been tested for a multitude of indications, including pathogens [51,59–74], toxins [65,75,76], and cancers [65,77–82] (Table 2. Current approaches for mRNA delivery of antibodies). Despite these promising reports, most studies of mRNA relating to HIV have been focused on the expression of recombinant viral antigens to promote adaptive humoral immunity rather than to produce bNAbs. The first report on mRNA administration to induce the expression of bNAbs was published in 2017 by Pardi *et al.* [59]. Therein, the expression of VRC01, a bNAb targeting the CD4 binding site of the HIV envelope (Env), was achieved *in vivo* by lipid nanoparticle (LNP)-encapsulated and nucleoside-modified mRNA. The antibody levels achieved by a single injection of 30 μg of mRNA resulted in higher levels of circulating antibodies than those detected for a single administration of 600 μg of recombinant VRC01, culminating in the

Table 2. Current approaches for mRNA delivery of antibodies

Type of disease	Disease	Target	Antibodies expressed	Organism tested	Comments	References
Infectious pathogen	Chikungunya	E2 glycoprotein	CHKV-24 (mRNA-1994)	Mouse, NHP, and humans.	First ever clinical trial for an mRNA-expressed bNAb. Preclinical data shows protection against infection upon mRNA administration and antibody expression.	Kose *et al.*, 2019 and August *et al.*, 2021
	Hepatitis B virus	HBV surface antigens	G12-scFv, G12-scFv-Fc, and G12-IgG	Mouse	The expression of these three antibodies upon i.v. injection of the mRNA to C57BL/6 mice led to long-term clearance of HBV antigens from circulation.	Chen *et al.*, 2022
	HIV	Envelope protein	VRC01	Mouse	The i.v. mRNA administration led to over 170 μg/ml of antibodies in BALB/c or BLT mice.	Pardi *et al.*, 2017
				BHK cells	The mRNA-expressed antibody exhibited neutralizing potency comparable to recombinant protein *in vitro*	Thran *et al.*, 2017
			PGT121	Sheep and NHP	There was a marked antibody expression upon aerosol delivery of the mRNA either as full-length or only heavy chain in the reproductive tract	Lindsay *et al.*, 2020
			N6, PGDM1400, and PGT121	Mouse	The i.v. administration of the mRNA mixture led to the concomitant expression of scFv-Fc antibodies retaining neutralization potency in Tg32	Narayanan *et al.*, 2022
	Influenza A	Influenza A antigen	Human IgG anti-Influenza A	NHP	The i.v. infusion of improved LNP led to increased antibody expression from the mRNA cargo	Sabnis *et al.*, 2018
		M2e and FcγRIV	RiboBiFE	Mouse	The expression of bispecific nanobody (VHHs) with protective features against viral infection was reported upon i.t. administration of the mRNA in BALB/c and C57BL/6	Van Hoecke *et al.*, 2020
	Influenza B	HA	CR8033	Mouse	The i.v. mRNA administration led to up to 2 μg/ml of circulating antibody in Swiss-Albino mice. However, this therapy could not protect from lethal infection.	Thran *et al.*, 2017
	Rabies	G glycoprotein	S057	Mouse	The i.m. injection of the mRNA led to the expression of the antibody as early as 2h in Swiss-Albino mice, which protected them from viral infection even as postexpsosure prophylaxis.	Thran *et al.*, 2017
	P. aeruginosa	Psl (Biofilm component)	CAM003	Mouse	The expression of this antibody as an hIgA1 protein was achieved upon i.v. administration of the mRNA. This therapy led to similar levels of protection as those reported for the recombinant protein in BALB/c mice.	Deal *et al.*, 2023
	Poxviruses	Enveloped virions (EV) and mature virions (MV)	c7D11 (anti-L1 MV), c8A (anti-B5 EV), and c6C (anti-A33 EV)	Rabbits	All three antibodies could be expressed concomitantly upon i.m. injection of the mRNA mix. However, based on empirical projections, the therapy in its current state was unlikely to protect against viral infection	Mucker *et al.*, 2022

Table 2 (*Continued*)

Type of disease	Disease	Target	Antibodies expressed	Organism tested	Comments	References
	RSV	Fusion glycoprotein	Secreted Palivizumab (sPali) and RSV-neutralizing VHH camelid antibody (RSVaVHH)	Mouse	The prophylactic i.t. administration of the mRNA to BALB/c mice reduced viral loads and prevented severe disease.	Tiwari *et al.*, 2018
	Salmonella enterica Typhimurium	O5-antigen of LPS	Sal4	Mouse	The i.v. administration of the mRNA led to the expression of this antibody as an hIgA2, which protected BALB/c mice from infection to similar levels as those reported for the recombinant protein.	Deal *et al.*, 2023
	SARS-CoV-2	Spike protein (RBD)	CB6	Mouse	Testing of self-replicating mRNA i.n. administered. Exhibited protection against viral infection in BALB/c.	Li *et al.*, 2021
			HB27	Mouse and Hamster	This antibody (not the mRNA-expressing therapy) is currently being tested in human trials. i.v.-administration of the mRNA led to long-term protection from infection in BALB/c mice and Syrian hamster.	Deng *et al.*, 2022
			COV2-2832 and DH1041	Hamster	The nebulized prophylactic administration of the mRNA significantly protected Syrian hamsters from SARS-CoV-2.	Vanover *et al.*, 2022
		hACE	3E8	Hamster	This self-replicating mRNA i.n.-administered to Syrian hamster protected against Beta, Delta, Gamma, and Omicron variants.	Zhang *et al.*, 2023
	Zika	Envelope protein	ZIKV-117	Mouse	The pre or postexposure i.m. administration of self-amplifying mRNA to C57BL/6 protected from lethal dose.	Erasmus *et al.*, 2020
Cancer	Nonhodgkin lymphoma	CD20	Rituximab	Mouse	There was a significant deceleration or abolishment of tumor growth in NOD/SCID mice upon i.v. administration of the mRNA.	Thran *et al.*, 2017
	Lymphoblastic leukemia	CD3 and tumor associated proteins (CLDN6, CLDN18.2, and EpCAM)	Three different RiboMAbs	Mouse	The i.v. administration of mRNA encoding for these bispecific ScFv to NSG mice cleared advanced tumor as effectively as the recombinant protein.	Stadler *et al.*, 2017
	Breast cancer	HER-2 (ERBB2)	Trastuzumab	Mouse	The i.v. injection to C57BL/6 mice of the mRNA led to high levels of expression of the antibody and a selective reduction of HER2-positive tumors, with improved survival.	Rybakova *et al.*, 2019

Table 2 (*Continued*)

Type of disease	Disease	Target	Antibodies expressed	Organism tested	Comments	References
	Hepatocellular carcinoma	CCL2 and CCL5	BisCCL2/5i	Mouse	The i.v. administration of the mRNA coding for this bispecific single-domain VH antibody led to long-term survival of CD57BL/6 and BALB/c mice with primary, colorectal, and pancreatic metastasized hepatocellular carcinoma.	Wang *et al.*, 2021
	Colon carcinoma	PD-1 and PD-L1	XA-1	Mouse	A marked expression of this bispecific antibody was seen upon i.v. injection of the mRNA coding in C57BL/6 and NOD/SCID mice. There was also a marked tumor growth inhibition of MC38 cells implanted to NOD/SCID mice.	Wu *et al.*, 2021
		PD-1	Pembrolizumab	Mouse	The i.v. administration of the mRNA coding for this antibody effectively reduced intestinal tumor growth and improved C57BL/6 NOD/SCID mice survival.	Wu *et al.*, 2022
	Acute myeloid leukemia and Subcutaneous melanoma	CD3 and B7H3	B7 homolog 3 protein (B7H3 or CD276) and CD3 bispecific T-cell engaging (BiTE) antibody	Mouse	The i.v. administration of the mRNA coding for the BiTE antibody led to high levels of its expression, with extended half-life compared to the recombinant protein. This therapy also resulted in a marked and long-lived antitumor efficacy in NSG mice.	Huan *et al.*, 2023
Toxins	*Escherichia coli* (O157: H7)	Shiga toxin 2 (Stx2)	VNA-Stx2	Mouse	The mRNA expression of the VNA protected cell viability against Shiga Toxin 2 (Stx2) and can be expressed in CD1 mice.	Thran *et al.*, 2017
	Clostridium botulinum	Botulinum neurotoxin serotype A (BoNTA)	VH domain-based neutralizing agent (VNA)-BonTA	Mouse	The mRNA-expressed VNA neutralized BoNTA *in vitro* to levels comparable to the recombinant protein and can be expressed in CD1 mice.	Thran *et al.*, 2017
		BoNTA, BoNTB, and BoNTE	Heterohexamer VHH	Mouse	The i.m. administration of the mRNA-expressing heterohexamer expressed efficiently and protected CD1 mice from lethal toxin doses for all serotypes of toxins tested.	Mukherjee *et al.*, 2022
		BoNTA	B11-Fc	Mouse	The i.m. administration of the mRNA-expressing nanobody protected BALB/c mice from high lethal doses of serotype A toxin.	Panova *et al.*, 2023

AAV, adeno-associated virus; bNAbs , broadly neutralizing antibodies.

protection of two humanized mouse models from infection with HIV-1 [59]. Antibody levels following mRNA administration peaked at 24 h, with a gradual decrease over five days and a sharp decrease on day 7. Subsequently, a report by Lindsay *et al.* [60] showed that aerosol administration of synthetic mRNA coding for an optimized PGT121, a bNAb targeting the V3-glycan of HIV Env, led to high expression levels in the reproductive tract of sheep and rhesus macaques. This was sufficient to protect against simian-human immunodeficiency virus (SHIV) infection, with antibody expression lasting up to 28 days [60]. A recent study by Narayanan *et al.* [61] sought to induce the simultaneous expression of three bNAbs, PGDM1400, PGT121, and N6, which also target HIV Env, using an mRNA-LNP platform. Simultaneous expression of multiple antibodies could lead to mismatched combinations of heavy- and light-chains, thereby yielding aberrant antibodies [83]. To prevent this, the authors engineered single-chain (sc)Fv-Fc molecules in which the heavy- and light-chain variable domains from each antibody were bound by flexible linkers. This construct was linked to an Fc constant region to eliminate potential mismatches. While *in vitro* expressed PGDM1400 and PGT121 scFv-Fc proteins exhibited similar neutralizing potency as natural antibodies, this strategy was not always effective, requiring full-length IgG sequences for the N6 bNAb to retain activity. *In vivo* administration of an mRNA cocktail led to high expression levels of all three antibodies in human neonatal Fc receptor (FcRn) transgenic mice (Tg32), a model chosen to more accurately predict the pharmacokinetics of human antibodies [61].

Of note is the first report of LNP-mRNA encoding IgA isotype antibodies targeting antigens from *Salmonella typhimurium* and *Pseudomonas aeruginosa* [50■■]. The report by Deal *et al.* [50■■] confirms the successful production of dimeric IgA antibodies by mRNA injection, which preferentially accumulates at mucosal surfaces and exhibits a longer half-life relative to its recombinant counterpart. This valuable proof of concept demonstrates the versatility of mRNA for antibody delivery. Clinically, an ongoing phase 1 study for LNP-encapsulated mRNA encoding for CHKV-24, a monoclonal neutralizing antibody against the Chikungunya virus, is of particular interest [63]. This first-ever clinical trial of *in vivo* expression of a monoclonal antibody through the mRNA platform shows that administration of this mRNA is safe and well tolerated, resulting in therapeutically relevant concentrations and robust neutralizing activity of the circulating antibody [63]. Translating this progress to the clinical use of mRNA to encode HIV-targeting bNAbs is clearly warranted.

Adeno associated virus for delivery of broadly neutralizing antibodies

Vectored antibody delivery, or vectored immunoprophylaxis (VIP), has emerged as a promising tool for the delivery of broadly neutralizing antibodies. Adeno-associated virus (AAV) remains one of the most widely used vectors for antibody delivery (Table 3. Current approaches for AAV delivery of antibodies). AAV-mediated antibody delivery has several advantages over other approaches, including very high expression levels, persistence of expression lasting for years, and clinically proven safety and tolerability. The first demonstration of antibody delivery using AAV was by Lewis *et al.* [84]. After administering a single intramuscular injection of rAAV vector expressing the HIV Env-binding antibody b12, they observed HIV-neutralizing activity in the sera of mice for over 6 months. Important contributions were made by Fang *et al.*, who significantly optimized expression from AAV-based antibody delivery systems [85,86]. They showed that antibodies could be expressed via a single open reading frame by linking heavy and light chains with a picornavirus-based 2A self-processing peptide sequence [85]. We further improved this strategy to achieve higher levels of bNAb using a muscle-optimized CASI promoter and added a posttranscriptional regulatory element (WPRE) downstream of the transgene [87]. This optimized AAV expression cassette resulted in several fold higher levels of bNAb expression (20–250 μg/ml) compared to nonoptimized vectors in both immunocompetent and immunodeficient mouse models [87]. We have shown that VIP is capable of protecting humanized mice from intravenous [87] as well as repeated vaginal challenges with diverse HIV strains [88,89■■]. Others have shown that six out of seven humanized mice could sustain suppression of HIV-1 using AAV8 to express 10-1074, an antibody targeting the V3 glycan in Env [90].

The efficacy of VIP has also been evaluated in NHP models by several groups. Johnson *et al.* [91] showed that a single intramuscular injection of an AAV1 encoding antibody-like immunoadhesin molecules in monkeys resulted in long-term (>1 year) expression of the biologically active protein that blocked SIV challenge. Saunders *et al.* [92] were the first to demonstrate delivery of an HIV bNAb (VRC07) in the NHP model, however these efforts yielded short-lived expression and significant antidrug antibodies (ADA) targeting the simianized VRC07 transgene. Immunosuppression with Cyclosporine during VIP was found to improve expression and reduce ADA responses [92]. When NHP-derived immunoadhesins (5L7 and 4L6) were converted to authentic IgG1 and delivered to rhesus monkeys

Table 3. Current approaches for AAV delivery of antibodies

Disease	AAV seroype	Antibody	Animal model	Route	AAV dose (vector genomes; vg)	Antibody concentration achieved	Antidrug antibody response	Comments	References
HIV	AAV2	B12	Rag1 Mice	Intramuscular	5E+10 to 5E+11	0.5–8 μg/ml			Lewis *et al.*, 2002
	AAV1	Immunoadhesins (4L6, 8S, 5L7, 3V0	Rhesus macaques	Intramuscular	2.00E+13	4L6 (100–190 μg/ml); 5L7 (50- 175 μg/ml)			Johnson *et al.*, 2009
	AAV8	B12	NSG, B6 and Balb/C	Intramuscular	1.00E+11	20–250 μg/ml			Balazs *et al.*, 2011
	AAV8	3BNC117, 10-1074	Humanized mice		2.50E+11	200 μg/ml			Horwitz *et al.*, 2013
	AAV8	multiple anitbodies including PG9, VRC07, 3BNC117	NSG and BLT mice	Intramuscular		0.05–390 μg/ml		Mice receiving AAV-VROC7 were completely resistant to repetitive intravaginal challenge	Balazs *et al.*, 2014
	AAV8	Simianized VRC07	Rhesus macaques	Intramuscular		30?	All animals unless Cyclosporin	First to show that immunosuppression could alleviate ADA responses	Saunders *et al.*, 2015
	AAV1	Immunoadhesin with rhesus IgG1	Rhesus macaques	Intramuscular	0.8E+13 to 2.5E+13	1–270 μg/ml	9/ 12 animals	In one animal, the concentration of antibody was 270 μg/ml and the levels persisted for 2 years	Fuchs *et al.*, 2015
	AAV1	eCD4-Ig	Rhesus macaques	Intramuscular	2.50E+13	17- 77 μg/ml		Rhesus eCD4-Ig was less immunogenic than rhesus forms of bNAbs	Gardner *et al.*, 2015
	AAV1	4L6, 5L7, 1NC9, 8ANC195, 3BNC117	Rhesus macaques	Intramuscular	1.6E+13 to 3E+13	NA	17/20 animals		Martinez-Navio *et al.*, 2016
	AAV8	anti-SIV Env mAb ITS01 and ITS06.02	Rhesus macaques	Intramuscular	1.00E+13	8–21 μg/ml	ADA in 20% animals		Welles *et al.*, 2018
	AAV1 and AAV8	10E8, 3BNC117, 10-1074	Rhesus macaques	Intramuscular	2E+12 vg/kg	2–200 μg/ml	Varying magnitude of ADA present in most animals	Viremia undetectable in one monkey for over 3 years	Martinez-Navio *et al.*, 2019
	AAV1	3BNC117, NIH45–46, 10–1074 and PGT121	Rhesus macaques	Intramuscular	2E+11 to 1E+13	3–69 μg/ml	12/ 12 animals	Antibodies isotyped with IgG2 were found to be less immunogenic than IgG1 isotyped antibodies	Gardner *et al.*, 2019
	AAV1 and AAV8	4L6	Rhesus macaques	Intramuscular / intravenous	0.25E+12 vg/kg	Intramuscular: 1–7 μg/ml Intravenous: 0.3-2.3 μg/ml AAV8 priming and AAV1 boost: 186–302 μg/ml	ADA in 9/9 animals in intramuscular group; no ADA detected (0/3) in intravenous group	Muscle-specific or liver-specific promoters were used	Fuchs *et al.*, 2019
	AAV1	PG9	Human	Intramuscular	4E+12 to 1.2E+14	Undectectable (<2 μg/ml)	10/16 detectable ADA	First human clinical trial	Priddy *et al.*, 2019
	AAV8	VRC07	Human	Intramuscular	5E+10 to 2.5E+12 vg/kg	<1–3.3 μg/ml	3/8 detected ADA (2/8 lost transgene)	Clinical trial ongoing	Casazza *et al.*, 2022
	AAV8	VRC07 containing Fc region of different human IgG subclass	huPBMC and BLT mice	Intramuscluar		<1–70 μg/ml		VRC07-IgG2 exhibited redued protection *in vivo* relative to other IgG subclasses.	Brady *et al.*, 2022

Table 3 (*Continued*)

Disease	AAV seroype	Antibody	Animal model	Route	AAV dose (vector genomes; vg)	Antibody concentration achieved	Antidrug antibody response	Comments	References
Cancer	AAV1	anti-EGFR antibody 14E1	A431 xenograft tumor model	Intramuscular	1E+11 to 5E+11	>1000 µg/ml			Ho *et al.*, 2009
Malaria	AAV8	2A10, 2C11	Mice [C57BL/6 (6NCr)]	Intramuscular	1.00E+11	50–1000 µg/ml			Deal *et al.*, 2014
Clostridioides difficile	AAV6.2FF	actoxumab, bezlotoxumab	Mice and Syrian Hamsters	Intramuscualar	Mice: 1E+11 Hamsters: 1E+12	90–195 µg/ml			Guilleman *et al.*, 2021
Parkinson's Disease	AAV8	anti-Synuclein (NAC32)	Rats (DAT-Cre)	Intracerebral	2E+12 vg per injection site				Chen *et al.*, 2021
Ebola	AAV6.2FF	2G4, 5D2 (murine IgG2a ebola virus mAbs) EBOV mAb 100, 114, FVM04, ADI-15876, CA45 (as human IgG1)	Mice (BALB/c)	Intramuscular	8E+9 to 4E+11	Dose dependent (<1 to 900 µg/ml)		Sustained expression in mice for more than 400 days. Minimum serum antibody level of 2 µg/ml was found to be protective.	Leishout *et al.*, 2022
	AAV9	2G4, 4G7, c13C6	Mice	Intramusclar/ Intranasal	1.00E+11	9 µg/ml in serum; 3 µg/ml in BALF		Humanization of mouse antibodies improved expression profile	Limberis *et al.*, 2016
	AAV9	c2G4, c4G7, c13C6	Mice	Intramuscular/ Intravenous/ Intranasal	2.7E+10 to 3E+11	Intramuscular: <1–26 µg/ml Intravenous: 5.3–33 µg/ml intranasal: not detected			Robert *et al.*, 2018
Herpes simplex virus (HSV)	AAV8	CH42, CH43, E317 (HSV mAbs targeting gD)	Mice (C57BL/6)	Intramuscular	1.00E+11			Passive transfer of HSV-specific mAbs delivered via AAV from dams to their offspring	Backes *et al.*, 2022
RSV	AAV6.2FF	Palivizumab, hRSV90	Mice	Intramuscular / Intranasal	1E+11	174–397 µg/ml (on day 70)		Antibody detected in the serum and at various mucosal surfaces. Maternal passive transfer of antibodies observed.	Rghei *et al.*, 2022
Keratitis ichthyosis deafness	AAV8	abEC1.1	Cx26G45E mouse	Intravenous	1.25E+12	50 µg/ml			Peres *et al.*, 2023
SARS-CoV-2	AAV8 and AAV9	NC0321	hACE2-expressing mice	Intramuscular/ Intranasal	1.00E+11	AAV8 given i.m.: 3.9 µg/ml in serum; 18 µg/ml in BALF AAV9 given IN: 0.9 µg/ml; 65 µg/ml in BALF			Du *et al.*, 2022
Influenza	AAV8	F10, CR6261	Mice (BALB/c and NSG)	Intramuscular	1.00E+11	F10: 100–200 µg/ml CR6261: 0.1–100 µg/ml			Balazs *et al.*, 2013
	AAV9	FI6	Mice	Intranasal	1.00E+11				Adam *et al.*, 2014
	AAV9	MD3606	Mice	Intranasal	4E+7 to 5E+9				Laursen *et al.*, 2018
	AAV8	R1a-B6	Mice	Intramuscular	1.00E+11	0.5–1100 µg/ml			Del Rosario *et al.*, 2020

AAV, adeno-associated virus; bNAbs , broadly neutralizing antibodies; BALF, bronchoalveolar lavage fluid.

using AAV, persisting levels of antibodies were achieved ranging from 1–270 μg/ml [93], but almost all animals exhibited ADA responses against at least one of the two antibodies. Notably, the monkey with the highest level of antibody (270 μg/ml of 5L7) in serum completely resisted six successive HIV i.v. challenges. Recently, the authors reported that this monkey maintained 240–350 μg/ml of 5L7 antibody for over 6 years and still remains protected despite receiving multiple SIVmac239 challenges [94]. Reports from NHP have shown that host ADA can limit the concentration of delivered antibodies. The ADAs bind both heavy and light chains, but they predominantly target variable regions of delivered antibodies [95▪▪,96]. It has also been demonstrated that the magnitude of the ADA response correlates with the degree of sequence divergence of the delivered antibody to the germline sequence [96]. Interestingly, the isotype of the antibody has also been shown to influence the ADA response, as IgG2-Fc isotyped bNAbs induced significantly lower ADA and better protection against SHIV-AD8 challenges than their IgG1-Fc counterparts in the NHP model [97]. However, a recent study reported that VRC07-IgG2 exhibited reduced protection compared to other IgG subclasses in BLT mice. In fact, VRC07-IgG1 provided better protection relative to other IgG subclasses against vaginal challenge of HIV in BLT mice [89▪▪]. Additionally, intravenous administration of AAV8 using a liver-specific promoter to direct expression of the transgene in the liver has been reported to mitigate ADA response in macaques [98]. In addition to bNABs, AAV has been used to deliver eCD4-Ig, a fusion of CD4-Ig with a small CCR5-mimetic sulfopeptide to rhesus macaques [99]. Stable expression of rhesusized eCD4-Ig (17–77 μg/ml) was obtained in these animals, which were protected from multiple infectious challenges with SHIV-AD8. In a follow-up study, the authors reported that AAV1 inoculation of rh-CD4-Ig provided complete protection of macaques from intravenous challenge with SIVmac239 [100]. However, animals eventually succumbed to infection when the challenge dose was escalated. Other studies have also demonstrated long-term virologic suppression using AAV-mediated bNAb delivery. Martinez-Navio *et al.* showed in rhesus monkeys infected with SHIV-AD8, that a combination of AAV1s encoding three bNAbs (3BNC117, 10–1074, and 10E8), resulted in one monkey exhibiting 50–150 μg/ml of 3BNC117 and 10–1074 for over 2 years. Impressively, plasma viremia remained undetectable in this monkey for over 3 years. The authors then extended this study with 12 monkeys using different combinations of antibodies and vectors. Long-term virologic suppression was observed in two monkeys that received a cocktail of four bNABs (N6, 35022, PGT128, and PGT145) delivered using AAV [101]. Overall, findings from the aforementioned NHP studies highlight the possibility of achieving a continued viral suppression from a single AAV-bNAb administration.

Based on the promising results of vectored immunoprophylaxis obtained from preclinical studies in mouse and NHP models, two Phase I clinical trials have been conducted to evaluate its safety and efficacy in humans. In the first human clinical trial, 16 healthy men aged 18–45 years were given an i.m. injection of AAV1 expressing PG9 [102]. Four different doses of AAV1-PG9 were tested, the lowest being 4×10^{12} vector genome copies and the highest being 1.2×10^{14} vector genome copies. No severe reactions or adverse effects were observed in these individuals indicating that antibody-expressing vectors are safe in humans. Although PG9 antibody was not detectable in the serum of these individuals by quantitative ELISA, the serum from four individuals showed detectable neutralizing activity against HIV pseudovirus. It is worth noting, however, that 10 out of 16 (62.5%) recipients in this study developed anti-PG9 antibodies, which could potentially have contributed to low expression or clearance of the transgene. It is also important to note that the lower limit of quantification of the assay used in this study was 2.5 μg/ml. It is plausible that some individuals may have exhibited PG9 levels below the detection limit and were therefore not measurable by the assay.

The results from a second Phase I clinical trial (VRC 603), which utilized AAV8-VRC07 have recently been published [95▪▪]. In this study, eight adults living with HIV on a stable antiretroviral regimen were enrolled and remained on ART throughout the study period. The participants received one of the three doses 5×10^{10} or 5×10^{11} or 2.5×10^{12} vector genome copies/kg intramuscularly. All eight individuals produced measurable amounts of serum VRC07, and in three individuals, the VRC07 concentration was >1 μg/ml. One participant receiving 5×10^{12} μg/kg achieved a VRC07 concentration of 3.3 μg/ml 1.5 years after AAV administration. In six of eight individuals, VRC07 concentrations remained stable near maximal concentration for up to 3 years of follow-up. The neutralizing activity of VRC07 in the serum was found to be equivalent to that of VRC07 produced *in vitro*, indicating that antibodies produced *in vivo* retained full biological activity. ADA responses were observed in three of the eight participants (38%), with responses primarily targeting the Fab portion of VRC07. Interestingly, one of these individuals continued to express VRC07 despite ADA, suggesting that these are not necessarily directly responsible for the loss of transgene expression. As in preclinical NHP studies, the choice of vector and transgene can influence ADA responses, perhaps explaining the less frequent ADA observed in the VRC 603 trial that employed AAV8-VRC07 as compared to the IAVI trial that used AAV1-PG9. Although challenges associated with host immune responses remain, these two human trials have clearly demonstrated the feasibility of vectored immunoprophylaxis as a means of producing long-lived bNAb expression in humans.

B cells for delivery of broadly neutralizing antibodies

Recent advances in genome engineering, largely stemming from the widespread use of lentiviral vectors and CRISPR-mediated gene targeting, have created new avenues for the delivery of antibodies by engineering the genome of B cells (Table 4. Current approaches for B cell-mediated delivery of antibodies).

In 2009, Luo *et al.* [103] reported the transduction of *in vitro* matured human B cells with lentiviruses coding for one of the first-identified bNAbs, b12. This transduction led to the secretion of over 1 μg/ml of b12 *in vitro*. In 2015, Fusil *et al.* [104] demonstrated that *ex vivo* lentiviral transduction of B cells, and the subsequent adoptive transfer of these cells into NSG mice, led to high levels of a hepatitis C virus-specific antibody. Although these early steps were promising, the efficiency of this approach improved dramatically with the emergence of CRIPSR-mediated gene targeting. In 2017, Hung *et al.* reported the first *ex vivo* transduction of proliferating B cells with CRISPR-Cas9 editing techniques, leading to significant secretion of the recombinant protein and the differentiation of these cells into plasma cells [105].

These findings, along with several others in hematopoietic stem cells, as well as primary human T, and B cells [106–110], led to the first reports of engineered human and mouse B cells expressing HIV bNAbs through CRISPR editing [111–113]. Hartweger *et al.* [111] achieved the expression of 3BNC60 and 10–1074, both anti-HIV bNAbs, in primary human and mouse B cells through CRISPR-Cas9 editing. The adoptive transfer of these cells back into B6 mice resulted in high serum concentrations of these antibodies that retained significant neutralizing capacities. Nahmad *et al.* and Huang *et al.* took these approaches a step further and demonstrated the establishment of long-lasting plasma cells, exhibiting affinity maturation, isotype switching, and clonal selection after the adoptive transfer of B cells engineered to express 3BNC117 or VRC01 [112,113]. These cells accumulated in germinal centers and, upon exposure to their antigen (HIV gp120), showed high rates of class switch recombination and affinity maturation, an adaptive immune response that was improved from that originally conferred to the adoptively transferred mice. This milestone for the induction of an evolving humoral response opened new possibilities for the adaption of bNAbs and B cells into *in situ* enhanced therapies [113,113].

Given that *ex vivo* transduction and adoptive transfer of engineered B cells into humans presents significant barriers to translation, Nahmad *et al.* [114] used two different AAVs, one coding for the *Staphylococcus aureus* Cas9 and a guide RNA targeting the IgH locus, and the other containing the sequence for 3BNC117 flanked by homology arms matching the IgH locus. This strategy led to the expression of 3BNC117 as a membrane-bound BCR of the transduced B cells. Joint administration of these AAVs promoted the clonal expansion and differentiation of bNAb-expressing B cells into memory and plasma cells in C57BL/6 mice. Upon immunization with the HIV gp120 antigen, circulating 3BNC117 reached up to 2 μg/ml. However, to achieve these levels of transduction, B cells had to be previously primed to induce their activation. Of note, the authors reported unwanted cleavage of off-target genome sites with this approach, albeit at low frequency [114]. Finally, of significant note is the lentiviral-mediated B cell transduction strategy reported recently by Vamva *et al.* [115] to express the eCD4-Ig immunoadhesin. Through the use of an optimized lentivirus containing the B cell-specific promoter EμB29, the authors achieved efficient expression of this protein in human B cells, capable of neutralizing HIV *in vitro*. Further studies are needed to test the feasibility of this approach *in vivo* and its protection efficacy [115]. While significantly less mature than other platforms, the field of B cell engineering is making rapid advances towards clinical translation of these technologies.

CONCLUSION

Given the promise of bNAbs for HIV prevention and therapy, multiple efforts are under development to efficiently and conveniently deliver these proteins to patients. Although each of the reviewed approaches has intrinsic benefits, they will all need to achieve certain parameters to be clinically useful. A successful approach must be capable of eliciting sufficiently high titers of antibodies to be clinically useful. Recent studies of antibody-mediated prevention in humans have suggested that this could require a steady-state concentration of as much as 10 μg/ml of VRC01 [5]. Similarly, a successful

Table 4. Current approaches for B cell-mediated delivery of antibodies

Type of therapy	Antibody/protein targeted/expressed	Study type	Cell/organism targeted	Comments	References
Lentivirus	b12 (anti-HIV bNAb)	*In vitro*	HSPCs-derived human B cells	A secretion of over 1 μg/ml was registered in culture supernatants upon transfection of these cells.	Luo *et al.*, 2009
mRNA and AAV	CCR5	*Ex vivo*	Primary human T cells and adult mobilized CD34+ PBSCs	The authors modified and optimized the gene editing of the *CCR5* locus as a potential therapy approaches against HIV.	Sather *et al.*, 2015
Lentivirus	ARA3 (anti-HCV antibody)	*Ex vivo*	Primary human B cells	The adoptive transfer of transduced B cells into NSG mice led to high levels of expression of the antibody	Fusil *et al.*, 2015
CRISPR-Cas9	Human factor IX (FIX) or B cell activating factor (BAFF)	*Ex vivo*	Primary human B cells	Through several editions of primary cells, the authors achieved, among others, their differentiation protein-secreting plasma B cells. The expression of BAFF also led to the engraftment of these plasma cells into NSG mice.	Hung *et al.*, 2017
Lentivirus	Targeting of TCR for the expression of several HIV scFv bNAbs (PGT145, VRC07, PGT128, and 10E8)	*Ex vivo*	Primary human T cells	The cells were engineered to express chimeric antigen receptors (CAR) based on HIV bNAbs, leading to its activation and the killing of HIV-infected cells.	Hale *et al.*, 2017
CRISPR-Cas9 and AAV	Human β-globin	*Ex vivo*	HSCs	The collective results from this report set the basis for a CRISPR-based editing therapy for β-hemoglobinopathies.	Dever *et al.*, 2016
Lentivirus	PGT128 and VRC01 (anti-HIV bNAb)	*Ex vivo*	HSPCs	Transduction of cells and engraftment into humanized NSG mice resulted in the expression of these bNAs for the 9 months that this study lasted. PGT128 was also able to reduce HIV viremia and CD4+ T cells decline.	Kuhlmann *et al.*, 2019
CRISPR-Cas9	Targeting of CXCR4 and expression of ozoralizumab (anti-TNF-α nanobody) or adalimumab (anti-TNF-α mAb)	*Ex vivo*	Primary human B cells	The editing performed in this report focused on the homologous recombination of the BCR loci, which actually results in the replacement of the original BCR by the new antibody.	Greiner *et al.*, 2019
CRISPR-Cas9	3BNC60 and 10–1074 (anti-HIV bNAb)	*Ex vivo*	Primary human and mouse B cells	The adoptive transfer into mice led to high antibody titers with marked neutralizing potency.	Hartweger *et al.*, 2019
CRISPR-Cas9 and AAV	3BNC117 (anti-HIV bNAb)	*Ex vivo*	Primary mouse B cells	Immunization with the antigen led to an increased accumulation of engineered cells in the germinal centers and increased rates of class switch recombination. A booster immunization also led to a memory response with a clonal selection pattern.	Nahmad *et al.*, 2020
CRISPR-Cas9	VRC01 (anti-HIV bNAb)	*Ex vivo*	Primary mouse B cells	The adoptive transfer of the engineered cells to immunocompetent mice resulted in the establishment of memory and long-lived plasma cells able to secrete the bNAb properly and even undergo somatic hypermutation.	Huang *et al.*, 2020
CRISPR-Cas9 and AAV	3BNC117 (anti-HIV bNAb)	*In vivo*	Mouse	Through the use of two different AAVs, one for Cas9 and a sgRNA for the IgH locus, and another one for 3BNC117, the expression of the antibody as the membrane-bound BCR was achieved. The bNAb-expressing B cells differentiated into memory and plasma cells in C57BL/6 mice. The *in vivo* expressed antibody exhibited a marked neutralizing potency	Nahmad *et al.*, 2022
Lentivirus	eCD4-Ig immunoadhesin (anti-HIV therapy)	*Ex vivo*	Primary human B cells	Using this optimized lentivirus with a B cell-specific led to the high-efficient expression of this protein, capable of neutralizing HIV *in vitro*	Vamva *et al.*, 2023

AAV, adeno-associated virus; bNAbs , broadly neutralizing antibodies.

approach will need to provide bNAb expression lasting substantially longer than what can be achieved via passive transfer studies. Given the remarkable safety of bNAb passive transfer, any competing approaches will need to demonstrate at least equivalent metrics before they can be deployed widely.

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Conflicts of interest

A.B.B. is a named inventor on patent US9527904B2 held by the California Institute of Technology describing AAV-mediated antibody delivery and holds equity in the following commercial partners: Cure Systems (Founder). D.B.W. has received grant funding, participates in industry collaborations, has received speaking honoraria, and has received fees for consulting, including serving on scientific review committees. Remunerations received by D.B.W. include direct payments and equity/options. D.B.W. also discloses the following associations with commercial partners: Geneos (consultant/advisory board), AstraZeneca (advisory board, speaker), Inovio (board of directors, consultant), Sanofi (advisory board), BBI (advisory board), Pfizer (advisory board), Flagship (consultant), and Advaccine (consultant). The other authors declare that they have no competing interests.

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