



HAL
open science

Recent advances using genetic therapies against infectious diseases and for vaccination

Anne Galy, Ben Berkhout, Karine Breckpot, Chantal Pichon, Kristie Bloom, Hans-Peter Kiem, Michael D Mühlebach, Joseph M Mccune

► **To cite this version:**

Anne Galy, Ben Berkhout, Karine Breckpot, Chantal Pichon, Kristie Bloom, et al.. Recent advances using genetic therapies against infectious diseases and for vaccination. *Human Gene Therapy*, 2023, 10.1089/hum.2023.123 . hal-04197730

HAL Id: hal-04197730

<https://hal.science/hal-04197730v1>

Submitted on 6 Sep 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

Recent advances using genetic therapies against infectious diseases and for vaccination

Anne Galy^{1#}, Ben Berkhout², Karine Breckpot³, Chantal Pichon⁴, Kristie Bloom⁵,
Hans-Peter Kiem⁶, Michael D. Mühlebach⁷, Joseph M. McCune⁸

¹ ART-TG, Inserm, Corbeil-Essonnes, France (anne.galy@inserm.fr)

² Department of Medical Microbiology Laboratory of Experimental Virology Amsterdam UMC, AMC, University of Amsterdam, Amsterdam, the Netherlands (b.berkhout@amsterdamumc.nl)

³ Department of Biomedical Sciences, Laboratory for Molecular and Cellular Therapy, Vrije Universiteit Brussel, Brussels, Belgium (karine.breckpot@vub.be)

⁴ Centre de Biophysique Moléculaire, CNRS, ART-ARNm, Inserm, Orléans and Institut Universitaire de France, Paris, France (chantal.pichon@inserm.fr)

⁵ Wits/SAMRC Antiviral Gene Therapy Research Unit, Infectious Diseases and Oncology Research Institute (IDORI), Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa (kristie.bloom@wits.ac.za)

⁶ Translational Science and Therapeutics Division, Fred Hutchinson Cancer Center, Seattle, USA (hkiem@fredhutch.org)

⁷ Division of Veterinary Medicine, Paul-Ehrlich-Institut, Langen, Germany (Michael.Muehlebach@pei.de)

⁸ HIV Frontiers, Global Health Accelerator, Bill & Melinda Gates Foundation (mike.mccune@gatesfoundation.org)

corresponding author : Anne Galy, PhD, ART-TG Inserm US35, 30 rue H. Desbruères, 91100 Corbeil-Essonnes, France anne.galy@inserm.fr

Abstract

The development of prophylactic or therapeutic medicines for infectious diseases is one of the priorities for health organizations worldwide. Innovative solutions are required to achieve effective, safe, and accessible treatments for most if not all infectious diseases, particularly those that are chronic in nature or that emerge unexpectedly over time. Genetic technologies offer versatile possibilities to design therapies against pathogens. Recent developments such as mRNA vaccines, CRISPR gene editing, and immunotherapies provide unprecedented hope to achieve significant results in the field of infectious diseases. This review will focus on advances in this domain, showcasing the cross-fertilization with other fields (e.g., oncology), and addressing some of the logistical and economic concerns important to consider when making these advances accessible to diverse populations around the world.

Keywords : gene therapy, infectious diseases, HIV, HBV, HCV, mRNA, vaccines, LNP, CRISPR, CAR-T cells, bNAbs, B cells, HSC, adenoviral vectors

Introduction

Worldwide, hundreds of millions of individuals suffer from severe or chronically debilitating infectious diseases for which there is no cure, satisfactory treatment, or effective vaccination. These infectious diseases include those caused by the human immunodeficiency virus type 1 (HIV), hepatitis viruses, *Mycobacterium tuberculosis*, flaviviruses, parasites, or newly emerging pathogens. Though various genetic technologies have been developed to treat or prevent infectious diseases, the field is faced with extraordinary challenges. Well-tolerated and cost-effective therapies or vaccines must be administered to a broad population of individuals, many of them living in resource-constrained geographies. High levels of efficacy must persist in the face of pathogen escape mutation pressure. Recent developments and progress in the field of gene therapy provide new hope for the development and deployment of highly effective and safe treatments against infectious diseases. As one case in point, the recent COVID-19 pandemic demonstrated how recombinant viruses and mRNA-containing lipid nanoparticles (LNPs) can provide rapidly-developed, effective, and safe vaccines for millions of individuals. The COVID-19 vaccine experience will no doubt provide insights into the treatment or prevention of many other diseases and, by the same token, progress in other domains of gene therapy [e.g., CAR-T cells in oncology or RNA interference (RNAi) drugs for rare diseases] will no doubt inform the design of innovative approaches against infectious diseases. Not all of these genetic strategies are defined as gene therapy medicinal products according to drug regulations in the US and in Europe. In fact, only a minority of gene therapy products are being developed for infectious diseases as opposed to monogenic disorders or cancer (1). Gene therapy medicines are biological products that deliver genetic sequences to replace, repair, or regulate the function of cells for therapeutic benefits. Gene therapy medicines exclude vaccines that have a different goal (prevention), mode of action, and clinical development path. Genetic vaccines deliver small amounts of nucleic acids to trigger protective immune responses by effector immune cells that are otherwise not gene-modified. Gene therapy medicinal products also exclude drugs made from nucleic acids in chemical form such as synthesized small interfering RNAs (siRNAs) because their chemical nature does not undergo the same pharmaceutical considerations as biological products. Regardless of their status as medicines, all kinds of genetic technologies can be used to seek

therapeutic or prophylactic effects against infectious diseases that target the host, the pathogens, or the environment (**Figure 1**). While environmental strategies such as genetically-engineered mosquitoes are discussed elsewhere (2), this review will focus on the most recent approaches used in humans, discussing how new developments (in particular new mRNA vaccines or immunotherapies) can be used against pathogens; how the field benefits or advances other domains of gene therapy; and solutions to consider to make these therapies accessible to all.

1. Novel genetic vaccination strategies against infectious diseases or cancer

Conventional vaccine approaches have largely failed to provide long-lasting protection against challenging viruses that cause chronic or repeated infections, such as HIV, herpes simplex virus (HSV), or respiratory syncytial virus (RSV). In the case of rapidly mutating viruses such as influenza, conventional vaccines are also faced with logistical challenges to adapt each year to newly emerging viral variants. When acute viral diseases emerge, as illustrated by the 2014–2016 outbreaks of the Ebola and Zika viruses and by the more recent COVID-19 pandemic, it is crucial to establish vaccine platforms that are more rapid and versatile than those used conventionally. All these challenges can be addressed by genetic technologies, including mRNA vaccines and viral vector vaccines.

1.1. Non-replicating mRNA vaccines

Thirty years after the first demonstration that *in vitro* transcribed (IVT) mRNA can be used to express proteins in animal models (3), more than one billion doses of mRNA vaccines against the SARS-CoV-2 spike protein were administered worldwide during the COVID-19 pandemic, including the administration of 800 million doses of the Pfizer/BioNTech and Moderna vaccines in the EU (4). This extraordinary achievement was made possible by decades of research and of technological improvements in mRNA therapies and mRNA vaccines (5), including engineering the mRNA sequence and codon usage, optimizing the 5' and 3' untranslated regions (UTRs), and incorporating the correct 5' cap structure and polyadenylation sequences to improve mRNA translation and stability. In addition, the use of purification methods and of efficient and non-toxic RNA carriers enabled prolonged antigen expression *in vivo*. A major advance promoting the *in vivo* efficacy of mRNA vaccines has been their formulation with cationic lipids and polymer-containing lipid nanoparticles (LNPs) decorated with carbohydrates or glycomimetics. These formulations are well

tolerated *in vivo* and facilitate endocytosis, improving specificity and internalization in dendritic cells (DCs) for better immune responses and dose reduction (6). The first demonstration of protective immune responses by a non-replicating mRNA vaccine against an infectious pathogen (influenza A) was published ten years ago (7). mRNA vaccines induce strong CD8⁺ and CD4⁺ T cell responses and, unlike DNA immunization, generate potent neutralizing antibody responses in animals after only one or two low-dose immunizations. Protective responses were obtained in macaques with as little as 50 µg of LNP-formulated mRNA encoding Zika virus prM-E protein, modified with 1-methylpseudouridine and purified using FPLC (8). Phase I clinical trials in humans showed the immunogenicity and safety of a nucleoside-modified non-replicating mRNA–LNP vaccine encoding the influenza H10N8 HA protein (ClinicalTrials.gov ID NCT03076385), demonstrating that it induced neutralizing antibodies and had a reactogenicity profile similar to that of traditional protein vaccines. In addition, a Zika virus vaccine was evaluated in a combined phase I/II trial (ClinicalTrials.gov ID NCT03014089) and showed protective efficacy (9). When the COVID-19 pandemic erupted in 2020, these and other results set the stage for the rapid design, production, and distribution of SARS-CoV-2 spike protein mRNA vaccines that induced robust immunity in recipients, protecting them from SARS-CoV-2 disease progression for a period of several months. Three consecutive spike antigen vaccine doses were found to generate increasing neutralization capacity and a gradual increase in antibody avidity that provided protection against variants of concern such as omicron (10). Driven by the needs of the pandemic response, the COVID-19 mRNA vaccines also spearheaded production and distribution supply chains that might now be applied to the use of mRNA vaccines against this and other pathogens in the future. At present, there are many novel ongoing clinical studies with mRNA non-replicating vaccines in a variety of infectious diseases, e.g., those caused by *Mycobacterium tuberculosis* (ClinicalTrials.gov ID NCT05537038), Nipah virus (ClinicalTrials.gov ID NCT05398796 and ClinicalTrials.gov ID NCT05178901), varicella zoster virus (ClinicalTrials.gov ID NCT05703607), HIV (ClinicalTrials.gov ID NCT05217641 and NCT05414786), cytomegalovirus (ClinicalTrials.gov ID NCT05085366). In the latter case, an mRNA vaccine is being tested in recipients of allogeneic hematopoietic stem cell (HSC) transplants, in hopes that it might prevent serious life-threatening complications caused by this virus in immunosuppressed patients (ClinicalTrials.gov ID NCT05683457). Studies in

participants older than 60 years of age are also ongoing with mRNA vaccines targeting RSV (ClinicalTrials.gov ID NCT05127434). Several studies are testing mRNA vaccines against influenza virus in healthy young and older individuals (ClinicalTrials.gov ID NCT05829356), including a randomized dose-escalation study (ClinicalTrials.gov ID NCT05446740) and a phase 2 comparison with protein-based vaccines (ClinicalTrials.gov ID NCT05606965). Finally, combination mRNA multicomponent vaccines are being tested against RSV and influenza virus in older individuals (ClinicalTrials.gov ID NCT05788237) as well as against influenza and SARS-CoV-2 (ClinicalTrials.gov ID NCT05827926) in healthy adults. Results from these clinical studies will provide information about whether and to what extent mRNA vaccines might represent a broadly-applicable solution in our approach against various infectious diseases.

The success of mRNA vaccines in infectious diseases is providing further impetus for their use in the field of oncology, where ultrapersonalized compositions with patient-specific tumor mutations are thought to be needed. In this setting, however, anti-tumor cytolytic effector mechanisms may best be elicited using combination strategies, e.g., associating an LNP-mRNA vaccine with an immune check point inhibitor, and studies of liposomal formulations of mRNA vaccines associated with anti-PD1 antibody therapy are ongoing in melanoma (ClinicalTrials.gov ID NCT05264974) and in prostate cancer (ClinicalTrials.gov ID NCT04382898). RNA-lipid particle vaccines are also being tested in newly-diagnosed pediatric high-grade gliomas and adult glioblastomas (ClinicalTrials.gov ID NCT04573140), in advanced solid tumors (ClinicalTrials.gov ID NCT05202561), and in recurrent pulmonary osteosarcoma (ClinicalTrials.gov ID NCT05660408).

1.2 Self-amplifying mRNA vaccines

In addition to the non-replicating mRNA vaccines described above, self-amplifying mRNA (SAM) vaccines are being developed, with potentially important applications in the fields of oncology and infectious diseases, as reviewed recently (11, 12). Whereas conventional mRNA-based vaccines encode the antigen of interest and contain 5' and 3' UTRs, SAM vaccines also encode viral replication machinery (including those from alphaviruses or vesicular stomatitis virus) enabling intracellular RNA amplification and leading to abundant, extended protein expression. SAM vaccines create their own adjuvants in the form of dsRNA structures, replication intermediates, and other pathogen associated motifs that appear to

contribute to their increased immunogenicity and potency. That said, such adjuvants may also contribute to the reactogenicity of SAM vaccines, limiting repeated applications. Additionally, sequence size constraints apply. Yet, SAM vaccines are being developed for a variety of infectious diseases and cancers. Since their safety appears to be acceptable, they are also being tested in less severe conditions, e.g., a herpes zoster SAM vaccine to prevent shingles (ClinicalTrials.gov ID NCT05871541). Another promising strategy is based on trans-amplifying RNA (taRNA), a split-vector derivative of SAM comprising a non-replicating mRNA encoding an alphaviral replicase and a transreplicon RNA (TR) coding for the antigen. Interestingly, transreplicon expression is higher (≥ 100 -fold) than SAM (13) providing an efficient vaccine against Chikungunya virus infection (14). This system can be optimized by shortening the TR (STR) and selecting evolved versions to optimize gene expression (eSTR) (15). The minimal efficient dose of STR is at least 10-fold less than that of TR without impacting taRNA expression *in vitro*. Moreover, STR-immunized mice developed higher antibody titers against taRNA-encoded influenza HA than did TR-immunized mice. As for SAM vaccines, remaining challenges include the optimization of formulations and the taming of innate immunity.

1.3 Use of viral vector vaccines

Though a variety of different viruses have been previously engineered by recombinant DNA technology to serve as vaccines, the COVID-19 pandemic underscored the fact that adenoviral vector vaccines with high potency and acceptable tolerance can be rapidly developed and distributed (16). About 180 million doses of the AstraZeneca and Janssen/Johnson&Johnson vaccines were administered throughout the world during COVID-19 (4). The advantages of using adenoviral vectors include their large cargo capacity enabling the expression of complex antigenic epitopes. In addition, adenoviral vector vaccines elicit innate immune responses that alleviate the need for adjuvants. Adenoviral vector vaccines elicit durable antibody, CD8⁺ T cell, and CD4⁺ T helper 1 (Th1) cell responses. They are used and developed as vaccines against several pathogens other than SARS-CoV-2. For instance, a chimp adenoviral vaccine is currently being tested against Marburg fever (ClinicalTrials.gov ID NCT05817422). Adenoviral vector vaccines have also been approved for use against Ebola virus (Ad26ZEBOV/ MVA BN Filo). Disadvantages of using adenoviral vectors include high levels of seroprevalence against certain serotypes (e.g., Ad5) in some geographies, which

may interfere with vaccine efficacy ; the induction of neutralizing antibodies ; and unwanted, if uncommon, reactions such as the vaccine-associated thrombotic thrombocytopenia syndrome observed in the COVID-19 vaccination campaigns. The problem of inducing anti-vector immune responses can be circumvented by the use of heterologous prime boost regimens. For instance, a safety and immunogenicity study is undergoing in HIV infection using an Ad26.Mos4.HIV prime and a boost with Modified Vaccinia Ankara (MVA)-BN-HIV in combination with broadly neutralizing antibodies (bNAbs) (ClinicalTrials.gov ID NCT04983030).

Among the viral vectors more recently developed as vaccines, arenaviruses present interesting properties for the priming of strong cellular immunity. These enveloped bi-segmented negative strand RNA viruses target antigen-presenting cells (*e.g.*, DCs) and have developed different strategies to evade host innate immune recognition. An ongoing clinical study is testing an HBV recombinant arenavirus vaccine in healthy participants (ClinicalTrials.gov ID NCT05770895). Lentiviral vectors are also suitable as *in vivo* viral vaccines. A study is ongoing to develop a therapeutic hepatitis C virus (HCV) vaccine using a lentiviral vector in chronic HCV patients (ClinicalTrials.gov ID NCT04318379). Measles vaccine virus is another viral vector that has successfully completed phase II clinical trial against Chikungunya virus (ClinicalTrials.gov ID NCT02861586 and NCT03101111) and is under development against a broad range of other infectious diseases (17) for its high immunogenicity in animal models and well tolerability. However, a SARS-CoV-2 vaccine based on this vector system did not successfully pass clinical development (with pre-formed anti-vector immunity suspected, though not yet shown conclusively, to have been at play).

1.4. DC vaccines

Professional antigen presenting cells such as DCs play a particularly important role in anti-tumor immunity and vaccines targeting these cells developed for indications in the field of oncology may prove important also in the setting of chronic infectious diseases. Though DCs prime tumor antigen-specific CD8⁺ T cells to mount effective anti-tumor immunity, the process is often impaired by a prevailing immunosuppressive tumor micro-environment. Consequently, extensive efforts are ongoing to improve the immunogenicity and efficacy of *ex vivo*-generated DC vaccines. While the results of many DC vaccination studies in cancer

have been disappointing, novel strategies are emerging (as reviewed in (18)), including the use of mRNA-loaded DC and of combination therapies with chemo- or radiotherapy as well as with CAR-T cells. Promising DC-based vaccine strategies include the *in vivo* delivery of antigens and of co-stimulatory signals via DC-targeted proteins, nucleic acids, nanoparticles or viral vectors as reviewed with the development of DC-based vaccines for HIV infection (19).

2. Immunotherapies against infectious diseases : the example of HIV research

Among chronic infectious agents of worldwide importance, HIV (affecting approximately 38 million people which 1.7 million more newly infected each year) represents a case study for challenges in global health and therapeutic innovation. The emergence of combination antiretroviral therapy (ART) has made a strong impact in the management of the disease, suppressing viral replication below the limit of detection, reducing mortality and morbidity, and preventing viral transmission. However, most ART-suppressed HIV-infected individuals who interrupt therapy will experience rebound of HIV viremia within 2-3 weeks, demonstrating that ART does not eradicate the reservoir of replication-competent virus, i.e., it does not cure HIV infection. Since such eradicated cures have only rarely been achieved (primarily by transplantation of hematopoietic stem cells in which the viral receptor, CCR5, is deficient), there is much interest to test the ability of immune and/or gene-based therapies to generate a state of durable ART-free viral suppression. The recent and rapid emergence of various genome editing technologies (e.g., CRISPR/Cas, base editing, and prime editing), targeted *in vivo* gene delivery systems, and cell-based immunotherapies for hemato-oncological diseases have changed the landscape of immunotherapy and of possible drug development for the field of HIV research. Elite HIV controllers who are able to spontaneously suppress HIV to low levels (<50 HIV RNA copies/ml) in the absence of any ART have provided key insights on critical factors that control HIV infection in man (20) and have led to the identification of pertinent targets for novel immune therapies (21), e.g., CCR5 deficiency and the presence of bNAbs capable of blocking entry of a wide array of HIV strains.

Recently, chimeric antigen receptor (CAR)-T cells directed against the HIV gp120 envelope glycoprotein have been used to target HIV-infected cells, showing promising results (22). Thus, in primate models, adoptively transferred, SHIV-specific CD4 CAR-T cells can expand

in response to an antigenic boost and control viremia after interruption of ART (23). By engrafting primates with HSCs expressing a CD4 CAR-T construct and following SHIV infection, it was possible to observe the trafficking to and active proliferation of multilineage CD4 CAR-T cells in various reservoir sites, e.g., lymphoid germinal centers (24), even though the latent virus reservoir was not completely eliminated by this strategy. A dual CAR-T cell approach, targeting two separate gp120 epitopes via two separate CARs, could represent an even more efficient approach to eradicate HIV reservoir cells (25). The duo CAR has a minimized CD4 domain that binds to gp120, exposing the m36 epitope recognized by a second CAR which blocks interactions with the co-receptor, CCR5. In an ongoing trial, two patients have safely received this LVgp120duoCAR-T cell product (ClinicalTrials.gov ID NCT04648046) (26). Alternatively, since CD4 is the main entry receptor for HIV, it might be possible to eradicate or to at least strongly reduce the HIV reservoir by transient elimination of CD4⁺ cells using CD4-specific DARPIn-targeted CAR-T cells, though this concept remains to be tested *in vivo* (27). At this time, major hurdles remain for the use of CAR-T cell therapy in HIV, including identification of the correct antigenic epitopes to direct the CARs, minimization of the risk of viral escape, effective removal of infected cells, and prevention of the generation of newly-infected cells that could augment the existing HIV reservoir. Similarly, and as discussed above, the CAR-T cell technology developed in the field of oncology appears transferrable for the treatment of HIV and possibly also for other pathogens. It has been hypothesized, for instance, that LMP1-specific CAR-T cells might benefit those with Epstein-Barr virus-associated hematological malignancies (ClinicalTrials.gov ID NCT04657965). For intracellular pathogens bearing oncogenes that cannot be recognized by CARs, TCR-engineered T cells recognizing antigen-specific peptides can be developed and a trial to test E7-specific TCR-T cells is open for human papillomavirus-associated cancers (ClinicalTrials.gov ID NCT02858310). Various types of natural killer cell-based therapies are also under study against HIV, as reviewed recently (28). Considering the logistics and costs involved, the use of ex vivo-generated CAR-T cells, while an established practice in the field of oncology, is unlikely to be available to most people living with HIV in resource-constrained geographies. As a result, there is interest in exploring approaches enabling *in vivo* delivery of CAR-T cell gene therapies, and various platforms have now been

developed with receptor-targeted lentiviral vectors, adeno-associated virus (AAV) vectors, or LNPs that could become useful for HIV immunotherapy in the future (29).

Another avenue for HIV immunotherapy is to exploit the properties of bNAbs to both block HIV infection of target cells and to focus cytolytic effector functions against cells that are already infected. Administration of bNAbs has been tested in the setting of HIV prevention (30) and treatment (31), and there is particular interest in understanding their impact when provided at the time of ART initiation (32). Since this approach may be limited by logistical and cost issues, attempts are also being made to explore various genetic modalities including vectored immunoprophylaxis strategies, e.g., using intramuscular injection of AAV gene to express high levels of bNAbs (33). Recent studies in macaques with AAV1 or AAV8-delivered bNAbs have shown promising results, including a prolonged suppression of viremia in a macaque infected by SHIV and receiving AAV1-mediated gene delivery of HIV bNAbs (34), and protection against an intrarectal SIV challenge in macaques injected with AAV8 to express anti-SIV env mAbs (35). However AAV-mediated mAb delivery is limited by the induction of anti-drug antibodies (ADA) e.g. antibodies against the AAV-delivered bNab. ADAs could be reduced by optimizing the transgene cassette (36) or using liver-directed gene delivery. A prime boost protocol in macaques using AAV8 intravenously followed by AAV1 intramuscularly, induced about 200-300 $\mu\text{g}/\text{mL}$ bNAbs for several months with low levels of ADA (37). Based on promising results in animal models, two clinical trials were conducted in humans. The first study conducted in healthy human volunteers given intramuscular injections of AAV1 encoding the PG9 bNAb, failed to achieve quantifiable serum levels of PG9 bNAbs (below 2.5 $\mu\text{g}/\text{mL}$), a result attributable to the detection of ADA in 10 out of the 16 participants and anti-AAV1 capsid antibodies in all participants (38). A second trial was a dose escalation study conducted in 8 participants infected with HIV and stable on ART, who were injected intramuscularly with AAV8 encoding the VRC07 bNAb showing good tolerance and the production of biologically-active VRC07, but serum levels were low (in the range of 1-4 $\mu\text{g}/\text{mL}$ at best) and most participants demonstrated the presence of ADAs (39). Despite these problems, vectored immunoprophylaxis maybe further developed to provide a means to safely produce Abs in humans when it is not possible with current vaccines. In addition to bNAbs, AAV has been used to deliver eCD4-Ig, a fusion of CD4-Ig with a small CCR5-mimetic peptide, to macaques, protecting them from

multiple infectious challenges with SHIV (40). Besides AAV, bNAbs has also been delivered via mRNA or through B cell engineering (33). Indeed, recently, CRISPR/Cas9 genome editing of B cells has been used to precisely insert HIV-binding bNAb paratopes into the IgH and IgK loci in human or murine B cells (41-43). A single CRISPR/Cas9 insertion site enabled the introduction of a DNA template encoding the V_H and V_L variable regions of the bNAb that were spliced into the native Ig locus for physiological expression, maintaining the physiologic opportunity for heavy chain class switching and somatic hyper mutation to increase antibody affinity through CDR3 diversity (41, 44). Following adoptive transfer of the gene-edited B cells, functional HIV-neutralizing bNAbs were produced *in vivo* in mice. Of course, like CAR -T cells, the adoptive transfer of *ex vivo* generated HIV bNAb-specific B cells may provide an important proof of concept while also being an approach that is difficult to implement for millions of individuals. To circumvent this issue, *in vivo* targeting of B cells for bNAb reprogramming is also being explored, e.g., with the injection of two separate AAV vectors to deliver CRISPR sgRNA/Cas9 or the bNAb template, yielding about 2 µg/mL of the 3BNC117 HIV bNAB in mice following several antigenic restimulations (44). One risk of this approach is the mutagenic potential of Cas9 systemic delivery with AAVs, a risk that might be minimized upon limitation of Cas9 expression in B cells using a CD19-specific promoter (44). It remains to be determined whether or not *in vivo* approaches such as this will be safe, unhampered by the induction of anti-drug antibodies, and amenable to long-term HIV protection. Beyond HIV, proof of concept antibody reprogramming in B cells has already been demonstrated for other pathogens, including RSV, influenza virus, and Epstein-Barr virus (42), and may also be considered for cancer.

3. Interfering with the pathogen life cycle

As another approach to attack HIV, host cell gene expression has been targeted to induce either gene silencing at the RNA level (e.g., by RNAi) or to prevent infection by targeting receptor proteins like CCR5 (e.g., with genome editing using CRISPR/Cas9). While the use of RNAi against HIV has been extensively investigated as a gene therapy approach, multiple shRNA constructs are required to avoid mutational escape and these remain difficult to design and to deliver (45). CRISPR gene editing approaches which cause definitive gene DNA mutations in the viral genome or in genes that encode critical cellular co-factors that support virus replication, are more difficult for pathogens to escape than RNAi. Delivery, however,

remains difficult for HIV as it is likely that most or even all of the viral reservoir needs to be inactivated. That said, a team including investigators at Temple University and Excision BioTherapeutics has initiated a clinical trial in aviremic HIV-infected adults on stable ART to test the possibility that CRISPR/Cas9 gene editing can diminish the reservoir to the point of reaching a cure (ClinicalTrials.gov ID NCT05144386).

3.1 Disabling viral replication : The example of hepatitis virus research

World Health Organization estimates from 2019 indicate that close to 300 million people worldwide are living with hepatitis B virus (HBV) and 58 million people with HCV. Though current pharmacological therapies for HBV or HCV are effective, they have limitations - and chronic HBV and HCV infections still account for the majority of cases of cirrhosis and hepatocellular carcinoma. HBV persistence involves replication intermediates comprising covalently closed circular DNA (cccDNA) that are not disabled by the current therapies. Preclinical work shows that CRISPR/Cas technology can target and disable cccDNA sequences as well as other viral genes, affording the potential to overcome infection with HBV and HCV. Different strategies using CRISPR/Cas to target the HBV surface, core, polymerase, or X protein open reading frames (ORFs), alone or in combination with anti-viral drugs, have been explored and have been reviewed recently (46). Targeting HCV at the RNA stage with CRISPR/Cas isolated from *Francisella novicida* expands the use of CRISPR therapies and may have therapeutic utility. Significant challenges remain for the use of CRISPR against chronic hepatitis infections, including ensuring specificity for viral targets and efficient delivery of the gene editing machinery to virus-infected cells without toxicity. As a proof of concept, increased methylation of HBV DNA at CpG islands can be induced by TALE repressors and such epigenetic strategies disabling viral genes may potentially address the problem of unintended mutagenesis of gene editing (47).

3.2 Preventing infection

Antiviral strategies based on genetically engineering viral resistance in host cells are attractive concepts that can be implemented in the hematopoietic system. In the case of HIV, a virus that infects cells of hematopoietic origin, it may be possible to genetically-engineer HSC (e.g., with CRISPR/Cas9) to generate the CCR5 Δ 32 mutation associated with a functional cure for HIV infection (48). Proof of concept results have shown that the ablation of the HIV co-receptor CCR5 or CXCR4 using CRISPR/Cas9 renders cells resistant to HIV

infection *in vitro*. While autologous CCR5-deleted HSCs can be transplanted to patients, the translation of this approach is limited by the high risk (especially in immunocompromised patients) of myeloablative conditioning to ensure the engraftment of gene-modified HSCs. However, a clinical study has been proposed in China to ablate CXCR5 in CD34⁺ HSCs in HIV-infected subjects with hematological malignancies and needing HSC transplants; the status of this study is unknown (ClinicalTrials.gov ID NCT03164135). Beyond niche indications, the use of *ex vivo* cell and gene therapies will not be broadly applicable for people living with HIV in resource-limited parts of the world. Rather, *in vivo* gene delivery strategies may be required – and work is ongoing exploring viral and non-viral vectors to deliver appropriate genetic machinery to long-lived cells of the hematopoietic system, e.g., HSCs. (49).

Decoy receptor strategies, for instance soluble CD4 against HIV, have long been developed to block virus entry. A recent *in vivo* gene therapy protocol has shown efficacy by expression and secretion of such an envelope antagonist (eCD4-Ig) from a helper dependent adenoviral vector in mice and monkeys (50). To achieve *in vivo* gene transfer of eCD4-Ig, HSCs were first mobilized from the bone marrow into the peripheral blood stream by the administration of G-CSF and CXCR4 agonists, followed by intravenous injection of an integrating, helper-dependent adenovirus (HDAd5/35⁺⁺) vector to stably express the transgene together with the O6-methylguanine-DNA methyltransferase (mgmtP140K) gene for subsequent *in vivo* selection with BCNU. A similar *in vivo* protocol was also recently developed to protect against SARS-Cov2 infections (51). In this case, the therapeutic concept was based on the continuous secretion by erythrocytes of the sACE2-Ig decoy protein. Since ACE2 is the virus primary receptor, the strategy is unlikely to induce the selection of escape variants. The stable expression of the transgene, driven by a strong erythroid beta-globin LCR promoter, and followed by *in vivo* selection with BCNU, generates high levels of sACE2-Ig produced by erythrocytes. Levels between 500–1,300 ng/mL of sACE2-Ig were obtained in the serum of mice, protecting them against a deadly dose of SARS-CoV2 (51). Such a protocol was safely implemented not only in mice but also in rhesus macaques, showing that sACE2-Ig expressed from erythroid cells does not affect erythropoiesis. While future optimization is needed, including the design of non-immunogenic high affinity decoys as well as the long-term safety evaluation of SB100X-mediated adenovirus integration, this *in*

in vivo HSC gene delivery platform may be applicable to other *in vivo* gene therapy strategies based on HSCs.

Conclusions

Rapid progress in research on, and clinical translation of, interventions based on gene therapies, gene-based immunotherapies, and genetic vaccines provides growing confidence that these approaches will prove to be applicable and beneficial in the field of infectious diseases. The challenges facing the development and global deployment of safe, efficacious, and accessible interventions must not be underestimated, especially since these novel strategies affect the human immune system and it is difficult to predict adverse reactions without data from clinical trials in various geographical regions. Even still, there are reasons to be optimistic that the fields of mRNA vaccines and of gene therapies will become ever more clinically translatable and beneficial with the advent of improved *in vivo* delivery tools, including LNPs as well as targeted viral and non-viral vectors. Most likely, these strategies will be employed as combination therapies, either with existing pharmacological agents or with other biotherapeutics. Their potential to address unmet clinical needs is tangible, a promise that warrants continued exploration in the future.

Author contribution statement :

AG, BB, KBr, CP, KBI, H-PK, MM, JM : all authors have equally contributed to the writing and editing of the manuscript. AG : conceptualization, writing of initial draft and corresponding author.

Disclosure statement :

None

Funding statement :

The following authors would like to acknowledge funding for this work : AG is funded by the french ANRS (project HIV-BVAC, 21011) ; CP is funded by Ligue Nationale Contre le Cancer (MucORNAvac project : LNCC N° AAPARN 2021.LCC/ChP) ; KBr is funded by the EC HORIZON-HLTH-2022-DISEASE (BAXERNA project : code EUAR135) ; KBI is supported by the South African Medical Research Council (SAMRC) through its Division of Research Capacity Development, funded by the South African National Treasury.

References

1. Informa Aa. Gene, Cell and RNA therapy landscape. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://asgct.org/global/documents/asgct-citeline-q1-2023-report.aspx; Q1 2023.
2. Burki T. Genetically modified mosquitoes. *Lancet Infect Dis.* 2020;20(12):1375-6.
3. Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A, et al. Direct gene transfer into mouse muscle in vivo. *Science.* 1990;247(4949 Pt 1):1465-8.
4. <https://ourworldindata.org/grapher/covid-vaccine-doses-by-manufacturer>. covid-vaccine-doses-by-manufacturer2023.
5. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. *Nat Rev Drug Discov.* 2018;17(4):261-79.
6. Midoux P, Pichon C. Lipid-based mRNA vaccine delivery systems. *Expert Rev Vaccines.* 2015;14(2):221-34.
7. Petsch B, Schnee M, Vogel AB, Lange E, Hoffmann B, Voss D, et al. Protective efficacy of in vitro synthesized, specific mRNA vaccines against influenza A virus infection. *Nat Biotechnol.* 2012;30(12):1210-6.
8. Pardi N, Hogan MJ, Pelc RS, Muramatsu H, Andersen H, DeMaso CR, et al. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature.* 2017;543(7644):248-51.
9. Richner JM, Himansu S, Dowd KA, Butler SL, Salazar V, Fox JM, et al. Modified mRNA Vaccines Protect against Zika Virus Infection. *Cell.* 2017;168(6):1114-25 e10.
10. Wratil PR, Stern M, Priller A, Willmann A, Almanzar G, Vogel E, et al. Three exposures to the spike protein of SARS-CoV-2 by either infection or vaccination elicit superior neutralizing immunity to all variants of concern. *Nat Med.* 2022;28(3):496-503.
11. Bloom K, van den Berg F, Arbuthnot P. Self-amplifying RNA vaccines for infectious diseases. *Gene Ther.* 2021;28(3-4):117-29.

12. Lundstrom K. Self-Replicating RNA Viruses for Vaccine Development against Infectious Diseases and Cancer. *Vaccines (Basel)*. 2021;9(10).
13. Beissert T, Perkovic M, Vogel A, Erbar S, Walzer KC, Hempel T, et al. A Trans-amplifying RNA Vaccine Strategy for Induction of Potent Protective Immunity. *Mol Ther*. 2020;28(1):119-28.
14. Schmidt C, Haefner E, Gerbeth J, Beissert T, Sahin U, Perkovic M, et al. A taRNA vaccine candidate induces a specific immune response that protects mice against Chikungunya virus infections. *Mol Ther Nucleic Acids*. 2022;28:743-54.
15. Perkovic M, Gawletta S, Hempel T, Brill S, Nett E, Sahin U, et al. A trans-amplifying RNA simplified to essential elements is highly replicative and robustly immunogenic in mice. *Mol Ther*. 2023;31(6):1636-46.
16. McCann N, O'Connor D, Lambe T, Pollard AJ. Viral vector vaccines. *Curr Opin Immunol*. 2022;77:102210.
17. Ebenig A, Lange MV, Muhlebach MD. Versatility of live-attenuated measles viruses as platform technology for recombinant vaccines. *NPJ Vaccines*. 2022;7(1):119.
18. Laureano RS, Sprooten J, Vanmeerbeerk I, Borrás DM, Govaerts J, Naulaerts S, et al. Trial watch: Dendritic cell (DC)-based immunotherapy for cancer. *Oncoimmunology*. 2022;11(1):2096363.
19. Espinar-Buitrago M, Muñoz-Fernández MA. New Approaches to Dendritic Cell-Based Therapeutic Vaccines Against HIV-1 Infection. *Front Immunol*. 2021;12:719664.
20. Hartana CA, Yu XG. Immunological effector mechanisms in HIV-1 elite controllers. *Curr Opin HIV AIDS*. 2021;16(5):243-8.
21. Gaiha GD, Rossin EJ, Urbach J, Landeros C, Collins DR, Nwonu C, et al. Structural topology defines protective CD8(+) T cell epitopes in the HIV proteome. *Science*. 2019;364(6439):480-4.

22. Alfageme-Abello O, Porret R, Perreau M, Perez L, Muller YD. Chimeric antigen receptor T-cell therapy for HIV cure. *Curr Opin HIV AIDS*. 2021;16(2):88-97.
23. Rust BJ, Kean LS, Colonna L, Brandenstein KE, Poole NH, Obenza W, et al. Robust expansion of HIV CAR T cells following antigen boosting in ART-suppressed nonhuman primates. *Blood*. 2020;136(15):1722-34.
24. Barber-Axthelm IM, Barber-Axthelm V, Sze KY, Zhen A, Suryawanshi GW, Chen IS, et al. Stem cell-derived CAR T cells traffic to HIV reservoirs in macaques. *JCI Insight*. 2021;6(1).
25. Anthony-Gonda K, Ray A, Su H, Wang Y, Xiong Y, Lee D, et al. In vivo killing of primary HIV-infected cells by peripheral-injected early memory-enriched anti-HIV duoCAR T cells. *JCI Insight*. 2022;7(21).
26. Carvalho T. First two patients receive CAR T cell therapy for HIV. *Nat Med*. 2023;29(6):1290-1.
27. Patasic L, Seifried J, Bezler V, Kaljanac M, Schneider IC, Schmitz H, et al. Designed Ankyrin Repeat Protein (DARPin) to target chimeric antigen receptor (CAR)-redirected T cells towards CD4(+) T cells to reduce the latent HIV(+) cell reservoir. *Med Microbiol Immunol*. 2020;209(6):681-91.
28. Perera Molligoda Arachchige AS. NK cell-based therapies for HIV infection: Investigating current advances and future possibilities. *J Leukoc Biol*. 2022;111(4):921-31.
29. Michels A, Ho N, Buchholz CJ. Precision medicine: In vivo CAR therapy as a showcase for receptor-targeted vector platforms. *Mol Ther*. 2022;30(7):2401-15.
30. Miner MD, Corey L, Montefiori D. Broadly neutralizing monoclonal antibodies for HIV prevention. *J Int AIDS Soc*. 2021;24 Suppl 7(Suppl 7):e25829.
31. Gaebler C, Nogueira L, Stoffel E, Oliveira TY, Breton G, Millard KG, et al. Prolonged viral suppression with anti-HIV-1 antibody therapy. *Nature*. 2022;606(7913):368-74.

32. Lee MJ, Collins S, Babalis D, Johnson N, Falaschetti E, Prevost AT, et al. The RIO trial: rationale, design, and the role of community involvement in a randomised placebo-controlled trial of antiretroviral therapy plus dual long-acting HIV-specific broadly neutralising antibodies (bNAbs) in participants diagnosed with recent HIV infection-study protocol for a two-stage randomised phase II trial. *Trials*. 2022;23(1):263.
33. Joshi LR, Galvez NMS, Ghosh S, Weiner DB, Balazs AB. Delivery platforms for broadly neutralizing antibodies. *Curr Opin HIV AIDS*. 2023;18(4):191-208.
34. Martinez-Navio JM, Fuchs SP, Pantry SN, Lauer WA, Duggan NN, Keele BF, et al. Adeno-Associated Virus Delivery of Anti-HIV Monoclonal Antibodies Can Drive Long-Term Virologic Suppression. *Immunity*. 2019;50(3):567-75 e5.
35. Welles HC, Jennewein MF, Mason RD, Narpala S, Wang L, Cheng C, et al. Vectored delivery of anti-SIV envelope targeting mAb via AAV8 protects rhesus macaques from repeated limiting dose intrarectal swarm SIVsmE660 challenge. *PLoS Pathog*. 2018;14(12):e1007395.
36. Gardner MR, Fetzer I, Kattenhorn LM, Davis-Gardner ME, Zhou AS, Alfant B, et al. Anti-drug Antibody Responses Impair Prophylaxis Mediated by AAV-Delivered HIV-1 Broadly Neutralizing Antibodies. *Mol Ther*. 2019;27(3):650-60.
37. Fuchs SP, Martinez-Navio JM, Rakasz EG, Gao G, Desrosiers RC. Liver-Directed but Not Muscle-Directed AAV-Antibody Gene Transfer Limits Humoral Immune Responses in Rhesus Monkeys. *Mol Ther Methods Clin Dev*. 2020;16:94-102.
38. Priddy FH, Lewis DJM, Gelderblom HC, Hassanin H, Streatfield C, LaBranche C, et al. Adeno-associated virus vectored immunoprophylaxis to prevent HIV in healthy adults: a phase 1 randomised controlled trial. *Lancet HIV*. 2019;6(4):e230-e9.
39. Casazza JP, Cale EM, Narpala S, Yamshchikov GV, Coates EE, Hendel CS, et al. Safety and tolerability of AAV8 delivery of a broadly neutralizing antibody in adults living with HIV: a phase 1, dose-escalation trial. *Nat Med*. 2022;28(5):1022-30.

40. Gardner MR, Fellingner CH, Kattenhorn LM, Davis-Gardner ME, Weber JA, Alfant B, et al. AAV-delivered eCD4-Ig protects rhesus macaques from high-dose SIVmac239 challenges. *Sci Transl Med.* 2019;11(502).
41. Hartweger H, McGuire AT, Horning M, Taylor JJ, Dosenovic P, Yost D, et al. HIV-specific humoral immune responses by CRISPR/Cas9-edited B cells. *J Exp Med.* 2019;216(6):1301-10.
42. Moffett HF, Harms CK, Fitzpatrick KS, Tooley MR, Boonyaratanakornkit J, Taylor JJ. B cells engineered to express pathogen-specific antibodies protect against infection. *Sci Immunol.* 2019;4(35).
43. Voss JE, Gonzalez-Martin A, Andrabi R, Fuller RP, Murrell B, McCoy LE, et al. Reprogramming the antigen specificity of B cells using genome-editing technologies. *Elife.* 2019;8.
44. Nahmad AD, Lazzarotto CR, Zelikson N, Kustin T, Tenuta M, Huang D, et al. In vivo engineered B cells secrete high titers of broadly neutralizing anti-HIV antibodies in mice. *Nat Biotechnol.* 2022;40(8):1241-9.
45. Herrera-Carrillo E, Gao Z, Berkhout B. CRISPR therapy towards an HIV cure. *Brief Funct Genomics.* 2020;19(3):201-8.
46. Moyo B, Bloom K, Scott T, Ely A, Arbuthnot P. Advances with using CRISPR/Cas-mediated gene editing to treat infections with hepatitis B virus and hepatitis C virus. *Virus Res.* 2018;244:311-20.
47. Bloom K, Kaldine H, Cathomen T, Mussolino C, Ely A, Arbuthnot P. Inhibition of replication of hepatitis B virus using transcriptional repressors that target the viral DNA. *BMC Infect Dis.* 2019;19(1):802.
48. Atkins AJ, Allen AG, Dampier W, Haddad EK, Nonnemacher MR, Wigdahl B. HIV-1 cure strategies: why CRISPR? *Expert Opin Biol Ther.* 2021;21(6):781-93.
49. McCune JM, Turner EH, Jiang A, Doehle BP. Bringing Gene Therapies for HIV Disease to Resource-Limited Parts of the World. *Hum Gene Ther.* 2021;32(1-2):21-30.

50. Li C, Anderson AK, Wang H, Gil S, Kim J, Huang L, et al. Stable HIV decoy receptor expression after in vivo HSC transduction in mice and NHPs: Safety and efficacy in protection from SHIV. *Mol Ther.* 2023;31(4):1059-73.
51. Wang H, Li C, Obadan AO, Frizzell H, Hsiang TY, Gil S, et al. In Vivo Hematopoietic Stem Cell Gene Therapy for SARS-CoV2 Infection Using a Decoy Receptor. *Hum Gene Ther.* 2022;33(7-8):389-403.

Figure legend.

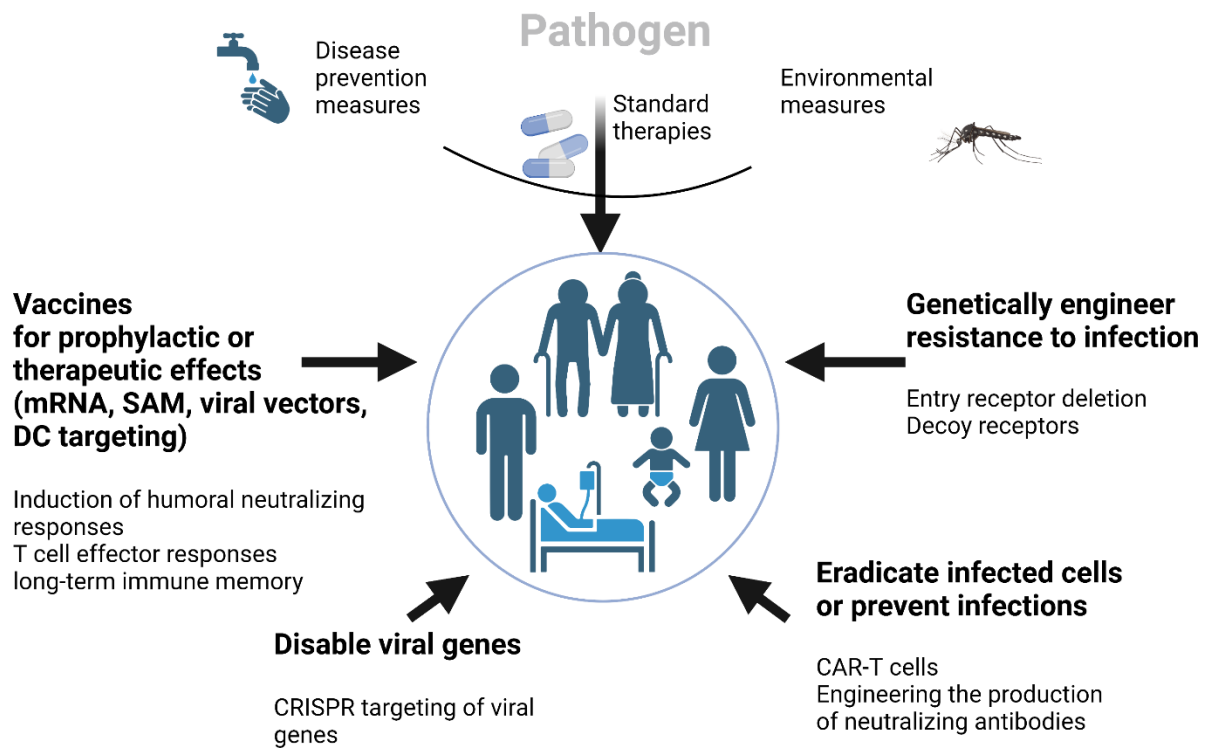


Figure 1 : Different strategies based on genetic technologies to vaccinate, prevent or treat infectious diseases. The figure was created with BioRender.com.