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Purpose of review
Tremendous advances in cell and gene therapy may soon realize the goal of treating and possibly curing HIV disease. These advances rely on new technologies for cell engineering and new strategies for product manufacturing that are targeting the most important immune deficits in HIV and promising to reconstitute protective, antiviral immunity and achieve natural suppression of HIV disease.

Recent findings
We summarize important advances in vectored passive immunity, e.g., directing in vivo expression of protective antibodies or antiviral proteins, B cell engineering to overcome the inadequate humoral immune response to HIV, and T cell engineering that is breaking new ground using viral vector modification of HIV specific T cells. These innovative approaches build on a substantial history of gene and cell therapy research in HIV disease.

Summary
Cell and gene therapy for HIV disease has been an area of tremendous innovation during the nearly two decades since early reports showed evidence for modulating disease. Recent efforts are building on the early experiences, closing gaps in previous approaches, and moving closer to effective treatment. Products approaching or already in clinical trials hold great promise for achieving durable suppression of HIV that will revolutionize therapy and offering hope to infected individuals that disease may be controlled without lifelong dependence on antiretroviral medications.

Video abstract
http://links.lww.com/COHA/A15.

Keywords
B cell engineering, cell and gene therapy, T cell engineering, vectored passive immunity, virus-specific T cell

INTRODUCTION
During more than 20 years of research, cell and gene therapy products were developed and tested as potential treatments or cures for HIV disease. Here, we examine recent progress and highlight the potential for cell and gene therapy to deliver the sought-after cure.

The history of cell and gene therapy for HIV traces back to at least 1994 when Roberts et al. [1] used a retroviral vector to reprogram T cells to express a major histocompatibility complex (MHC)-unrestricted recognition molecule. The recognition molecule was a truncated form of the CD4 glycoprotein receptor for HIV and the engineered cells were expected to detect and destroy infected cells that expressed cell surface envelope glycoprotein. This was an early form of what we know today as Chimeric Antigen Receptor (CAR) mediated and MHC-unrestricted recognition of cellular antigens. In the ensuing years, T cells have been engineered to recognize infected cells using multiple receptors, resist HIV attachment, and inhibit virus replication. Genetic modifications have been made to circulating lymphocytes or hematopoietic stem cell precursor cells (HSCPC) and cells were infused during...
clinical trials to test whether they controlled the rebound of plasma viremia following interruption of therapy. Save for the example of cells edited ex vivo to disrupt the CCR5 gene and infused into a trial participant heterozygous for the CCR5delta32 loss of function allele [2], stable suppression of HIV in the absence of antiretroviral drug therapy has not been achieved.

To understand why so many strategies have fallen short of the goal and whether new products have better prospects, we provide this critical review of the most recent progress in cell and gene therapy for HIV. We intentionally omitted studies on genetic modification of bulk CD4 T cells, principally involving CRISPR/Cas9 editing systems, and recent developments in CAR approaches that are covered elsewhere in this volume.

VECTORED PASSIVE IMMUNITY TO INHIBIT HIV INFECTION IN VIVO

Substantial attention has been focused on improving the poor humoral immune response to HIV wherein neutralizing antibodies arise infrequently and only after many months of infection. One important method for overcoming this lack of protective humoral immunity is the use of vectored passive immunity.Vectored passive immunity is the delivery to tissues of synthetic genes encoding protective antibodies or antiviral proteins. Vectors include recombinant, attenuated viruses and plasmids but will undoubtedly involve naked RNA delivery in the future. Vectored passive immunity may deliver neutralizing antibodies or antibodies capable of mediating antibody-dependent cellular cytotoxicity. A clinical study of adeno-associated viral vector (AAV) expressing the broadly neutralizing antibody PG9 was performed in healthy (HIV-negative) adult men [3]. Participants received recombinant AAV by intramuscular injection with doses ranging from $4 \times 10^{12}$ to $1.2 \times 10^{14}$ vector genomes; roughly $1.4 \times 10^{12}$ vector genomes per kg. The study enrolled 21 volunteers who completed 48 weeks of follow-up with no adverse events related to the product. Serum PG9 was detected by an HIV neutralization assay but could not be detected by ELISA. Anti-drug antibodies were detected in 10 participants from the higher dose groups. In this trial, recombinant AAV1 expressing PG9 monoclonal antibody failed to sustain high serum antibody levels. In contrast, a study of SHIV-infected rhesus macaques inoculated with $2 \times 10^{12}$ vector genomes per kg per vector and receiving two or four different AAV expressing monoclonal antibodies (antibodies 3BNC117 plus 10-074 or antibodies PGT145, 35022, NG and PGT128) for a total AAV dose of $4 \times 10^{12}$ vector genomes per kg with two antibody-encoding vectors or $8 \times 10^{12}$ vector genomes per kg for the group receiving four recombinant AAV. The macaques received an initial dose of recombinant vectors using the AAV8 serotype and received a second dose 25 weeks later using the AAV1 serotype to avoid vector-neutralizing antibodies [4]. Macaques receiving two different AAV showed a trend to lower levels of plasma viremia but results were more dramatic for two of four macaques in the group treated with four different AAV; these animals had large and sustained decreases in plasma viremia despite occasional “blips” out to 55 weeks after the initial AAV injection.

Differences in the AAV serotype, use of one versus two doses, choice of antibody constructs and testing in HIV-negative humans compared to SHIV-infected macaques makes comparisons difficult. It is easy to believe that multiple antibody gene constructs are better than a single construct just like combination antiretroviral therapy exceeded the clinical benefit from individual drugs. The value of AAV8 versus AAV1 may be important and the dose used in the clinical safety study may have been too low. It is also important to note that earlier macaque studies demonstrated in vivo protective effects of AAV-expressed antibodies that were not reflected by assays for serum neutralization [5**] and the problem of anti-drug antibodies that bind and inhibit the antibody encoded on AAV [6*,7**] remains a potential stumbling block to broader clinical implementation. A recent review provides more detailed discussion of antibody therapy and the use of AAV vectors, including detailed discussion of literature published before 2019 [8*].

Direct injection of plasmid DNA, encoding genes for monoclonal antibodies, is an alternative to the use of AAV. Plasmid DNA was formulated with hyaluronidase and injected in mice or nonhuman
primates via the intramuscular route [9**]. High titers of serum neutralizing antibodies were detected in both cases and the levels in nonhuman primates exceeded neutralizing titers that protected macaques from Zika virus infection, which was used as a preliminary target for serum antibody levels [10***]. The use of plasmid DNA has the additional benefit that anti-vector antibodies are not produced and repeat dosing is simplified.

Vecteded passive immunity was also demonstrated in nonhuman primates using the antibody-like molecule eCD4-IG. The eCD4-IG contains domains of CD4 that bind HIV envelope glycoprotein plus a small CCR5-mimetic sulfopeptide fused to the Fc region of IgG [11]. Protection was observed but potency was reduced by the appearance of anti-drug antibodies mostly directed against the sulfopeptide. The use of eCD4-IG may simplify vectored passive immunity by delivering a single soluble molecule instead of multiple antibodies, but clinical trials results have not been reported and the problem of anti-drug antibodies remains.

**GENETIC ENGINEERING OF B CELLS**

Engineered B cells represent an important new opportunity for overcoming the inadequacy of natural humoral immunity in HIV disease. Broadly neutralizing antibodies appear in a minority of infected individuals and then only after 1–3 years of infection. Passive immunization, using direct injection of purified proteins or vectored passive immunity discussed above, demonstrated the clinical impact of potent antibodies. Recent efforts are trying to improve both efficiency and potency of humoral immunity through genetic engineering of B cells.

Most *ex vivo* engineering of lymphocytes involves retroviral or lentiviral vectors but this approach works poorly for human B cells due to low transduction efficiency. An alternate strategy relies on plasmid transfection of B cells with the use of CRISPR/Cas9 editing to replace the chromosomal immunoglobulin gene with a synthetic gene expressing an antibody against the HIV envelope glycoprotein. Mature murine or human B cells received a synthetic antibody gene without an independent promoter, that was expressing a broadly neutralizing antibody. The construct was integrated (knock-in) into the chromosomal heavy chain locus [12**]. Expression of the engineered antibody gene relied on the natural immunoglobulin gene transcriptional promoter. The efficiency of modification was higher for human compared to murine B cells and immunization of reconstituted mice with the cognate antigen elicited serum antibody titers at protective levels. The knock-in occurred primarily within the active heavy chain locus, not on the chromosome silenced by allelic exclusion, and resulted in high-level expression of antibodies on the cell surface.

A related study started with a construct containing the heavy chain (VH) promoter and a gene encoding the broadly neutralizing antibody VRC01. AAV was used to deliver a CRISP/Cas9 ribonucleoprotein complex and guide RNA to direct Cas9 DNA cleavage and plasmid transfection via electroporation delivered a donor DNA strand containing 5' and 3' homology arms plus the antibody expression construct [13**]. Adoptive transfer returned the engineered B cells into mice. The highest titer antibodies were seen after antigen boosting of reconstituted mice and especially for knock-in constructs targeted to the heavy chain locus. Surprisingly, the engineered antibodies demonstrated somatic maturation in V region sequences indicating antigen selection *in vivo*. Engineered B cells homed to germinal centers and predominated over the endogenous response whereas evidencing class switch recombination and memory phenotype [14**].

Engineering of B cells holds substantial promise for expressing broadly neutralizing antibodies independent of the slow and unreliable natural response to HIV. The possibility of undergoing isotype switching, and somatic maturation suggests these engineered genes are substrates for natural maturation of the antibody response, which may allow sufficient antibody gene changes to overcome rapid escape due to virus variation. It will be critical to decide whether the best starting point is to engineer expression of a known neutralizing antibody or to insert a precursor sequence closer to the germ line that might have greater flexibility for responding to a variety of HIV variants or may accelerate in the process of somatic maturation.

**GENETIC ENGINEERING OF T CELLS**

The goals of treating and curing HIV disease have attracted the attention of cell and gene therapy experts leading to the great variety of approaches and clinical trials discussed here and elsewhere in this volume. Missing from most of these strategies is an effort to combine the power of cell programming with the proven utility of immunotherapy. The most significant and durable immune defect in HIV disease is the loss and failure to reconstitute HIV-specific CD4 T cells resulting in a reduced capacity for differentiating new CD8 cytotoxic T cells to match viral evolution and poor B cell responses due to the lack of MHC Class II-restricted
T cell help. When bulk CD4 T cells are engineered ex vivo and infused, the frequency of HIV-specific T cells does not change and the potential for immunotherapy is lost. New efforts are underway that combine the power of cell engineering with existing methods for treating disease with antigen-specific T cells. Treating viral disease with virus-specific T cells (VST) is an established technology used in the context of allogeneic hematopoietic stem cell transplantation in adults (reviewed in [15]).

The use of VST requires ex vivo expansion of antigen-specific T cells followed by infusion. For treatment targeting HIV autologous cells are preferred in contrast to allogeneic cells used in the post-transplant setting. Autologous VST was tested in HIV disease using ex vivo expanded cells generated by isolating and maturing dendritic cells then adding peripheral blood mononuclear cells plus peptide pools representing HIV Gag, Pol, and Nef Proteins [18]. The resulting cells were infused and well-tolerated but there was no meaningful change in the frequency of latently infected T cells in circulation. Importantly, this procedure did not modify CD4 T cells to protect them against HIV infection and the manufactured product contained only a low proportion of CD4 T cells. Ongoing clinical studies (NCT03485963) are assessing this product. Although the modification of bulk PBMC produced HIV-specific CD8 T cells, the product did not provide sufficient amounts of durable HIV-specific CD4 T cells to achieve immune control of HIV.

We know from previous studies that higher levels of HIV-specific CD4 T cells and especially the Gag-specific subset, are associated with natural virus control. Persons who naturally control HIV viremia without antiretroviral drug therapy have higher levels of Gag-specific CD4 T cells compared to non-controllers [19] and higher levels of helper T cells are correlated with greater breadth of neutralizing antibodies [20] possibly due to increased levels and function of CD4 T follicular helper cells [21]. Early induction and maintenance of Gag-specific CD4 T cells is an important feature of persons who control HIV [22,23] and reconstituting this capacity should be an objective for cell and gene immunotherapy of HIV [24]. Cell therapies based on genetic modification of bulk CD4 T cells may provide HIV-resistant cells for what is termed a “replacement immune system” [15] but will not impact the frequency of HIV-specific CD4 T cells, which is vital to reconstituting natural immune control of HIV.

Recently, two groups have reported progress in combining the concepts of VST with the technology of cell and gene therapy. Using a method for generating HIV-specific T cells from uninfected naive donors [25], HIV-specific T cells were expanded and transduced with lentivirus vector encoding a monoclonal antibody capable of binding HIV envelope glycoprotein and directing antibody-dependent cellular cytotoxicity [26]. The engineered cells showed a slight decrease in proliferation rates compared to unmodified cells and produced antibody capable of antibody-dependent cellular cytotoxicity. The use of these cells is imagined to be part of a shock and kill approach wherein potent, polyclonal stimulation of CD4 T cells in vivo will activate HIV protein production among latently infected cells and antibodies expressed from a viral transgene will redirect killing to the infected cells.

Our own efforts have focused on reconstituting CD4 T cell immunity to HIV, making these cells (AGT103-T) resistant to HIV and preventing the release of HIV if they were latently infected prior to genetic modification and expansion. Using a semi-automated GMP-compliant manufacturing process it was possible to enrich Gag-specific CD4 T cells from approximately 0.05% of CD4 T cells in starting material to levels as high as 30% in a final cell product that is >95% CD4 T cells [27]. During manufacturing, Gag-stimulated cells were transduced with a lentivirus vector expressing miRNA capable of decreasing cell surface CCR5 and attacking HIV genomic and mRNA in the Tat and Vif gene regions. Consequently, CCR5 levels are modulated mainly in Gag-specific CD4 T cells, these cells resist both CCR5- and CXCR4-tropic strains of strains, and virus release after inducing latent cells is impaired. Compared to cell infusion with the SB781 bulk CD4 T cell product, comprised of CD4 T cells modified by adenovirus expressing Zinc finger nuclease targeting the CCR5 gene [2], AGT103-T delivers 1,000 times the number of modified, Gag-specific CD4 T cells compared to SB781 or other cell products generated from bulk T cell populations. Our goal is to reconstitute the Gag-specific CD4 T cell population with cells resistant to HIV and expect these cells to improve the activation/differentiation of HIV-specific cytotoxic CD8 T cells to overcome viral escape through mutation, and to increase helper function for B cells that will promote the development of broadly neutralizing antibodies. The AGT103-T cell product is being evaluated in the RePAIR clinical trial (NCT04561258).

CONCLUSION

Genetically engineered T and B cells have potential to treat HIV disease. Potent and durable control of HIV depends on repairing specific immune defects in HIV disease such as engineering B cells to produce protective, HIV-specific antibodies or modifying
HIV-specific T cells to recover the cellular immune response. Reconstituting HIV-specific CD4 T cells with genetically modified cells that resist depletion may repair a key defect caused by HIV infection, mediate a decline in the latent viral reservoir, and provide immune control of HIV disease.

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Conflicts of interest
C.D.P. and K.H. are employees and shareholders of American Gene Technologies and C.D.P. holds patents covering the AGT103-T cell product. J.B. is the Principal Investigator of the RePAIR clinical trial sponsored by American Gene Technologies.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest
■■ of outstanding interest

7. Useful review of earlier work on antibody expression using recombinant AAV.
9. A nonhuman primate study showing protection from SIV challenge after in vivo expression of eCD4-Ig from an AAV vector.

Demonstration that anti-drug antibodies (antibodies against the expressed HIV-specific antibody) limit treatment effectiveness.

13. Important study on programming B cells to produce HIV-specific antibody and increasing antibody expression by cognate antigen immunization.
16. Excellent review on the importance of antigen-specific T cells for therapy.
18. First clinical scale cell and gene therapy product for HIV that is highly enriched for Gag-specific CD4 T cells, which are modified by a lentivirus vector.
20. Nonhuman primate study demonstrating durable antibody expression directed by recombinant AAV and protection from SHIV challenge.
22. Mouse model study demonstrating functionality of genetically engineered B cells producing antibodies specific for HIV.
24. Description of a process for manufacturing cell products enriched with antigen-specific T cells.
27. Demonstration that antigen specific T cells can be programmed to produce anti-HIV antibody and are function in antibody-dependent cellular cytotoxicity.
29. Clinical trial testing the capacity for recombinant AAV to express HIV-specific antibodies in healthy adults.

Mouse and nonhuman primate studies demonstrating serum levels of HIV-specific antibodies produced by transfection with recombinant plasmids.