Immunotherapeutic restoration in HIV-infected individuals

While the development of combined active antiretroviral therapy (cART) has dramatically improved life expectancies and quality of life in HIV-infected individuals, long-term clinical problems, such as metabolic complications, remain important constraints of life-long cART. Complete immune restoration using only cART is normally unattainable even in cases of sufficient plasma viral suppression. The need for immunologic adjuncts that complement cART remains, because while cART alone may result in the complete recovery of peripheral net CD4+ T lymphocytes, it may not affect the reservoir of HIV-infected cells. Here, we review current immunotherapies for HIV infection, with a particular emphasis on recent advances in cytokine therapies, therapeutic immunization, monoclonal antibodies, immune-modulating drugs, nanotechnology-based approaches and radioimmunotherapy.

KEYWORDS: cytokine therapy ■ HIV ■ immune-modulating drugs ■ immunotherapy ■ monoclonal antibodies ■ nanotechnology-based approaches ■ radioimmunotherapy ■ therapeutic immunization

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Current combined antiretroviral therapies (cARTs) attempt to minimize adverse drug events, such as long-term complications, and to circumvent drug resistance. The development of single-tablet, fixed-dose, combination antiretroviral drugs was intended to improve long-term compliance to cART. While the introduction of cART has dramatically improved life expectancies and quality of life in HIV-1-infected individuals, clinical problems, including various metabolic complications and HIV-associated neurocognitive disorder (HAND), have emerged as important constraints on life-long cART [1]. In addition, immune reconstitution by cART alone can be only partial and heterogeneous, although successful virologic suppression is achieved [2–5]. In addition, cART may result in the recovery of peripheral net CD4+ T lymphocytes, but it could not affect the reservoir of HIV-infected cells [5]. Furthermore, cART does not induce HIV-specific immune responses and HIV-specific immunity decreases during cART. For these reasons, alternative adjunctive immunotherapeutic strategies that complement cART remain active research foci.

The depletion and immunologic dysfunction of T lymphocytes in HIV-infected individuals may result from loss of homeostasis, chronic immune activation by the HIV infection itself or bystander T lymphocyte death by other latent infections [6]. Cytokines that are important in the proliferation, function and regulation of T lymphocytes include IL-2, -7, -15 and -21. However, impaired regulation of cytokine production in HIV-infected individuals, which results in decreases of IL-2 or IL-15 and increases in TNF-α or IFN-γ, may contribute to T lymphocyte dysfunction as well as a defective immune response [2].

Immunotherapy in HIV-infected individuals directly targets the aberrant immune response caused by HIV infection [3]. cART alone cannot completely eliminate noncirculating infected cells and may not restore immunologic health in HIV-infected individuals [7]. Ongoing HIV replication results in immune activation, not only in individuals without treatment, but also during viral load (VL) suppression with cART [7,8]. In particular, immunologic low or nonresponders, defined as patients who have suboptimal immune restoration despite complete control of HIV replication by cART, represent 5–30% of HIV-infected patients receiving cART [9]. The risk of disease progression or death is significantly higher in nonresponders compared with patients who achieve better CD4+ T lymphocyte restoration [9].

Because viral reservoirs in latently infected cells quickly start producing HIV particles when cART is discontinued, the control of viral reservoirs is very important for the eradication of HIV in humans. These problems have strengthened the rationale that new adjuvant immunotherapeutic restoration for the treatment of HIV-infected individuals must be explored. The development of new immunotherapeutic

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strategies for the treatment of HIV infection is critical, especially for immunologic nonresponders to cART and for use in supportive adjuvants for structured treatment interruption (STI) of cART for antiretroviral drug conservation.

In this article, we reviewed up-to-date knowledge regarding various clinical aspects of immunotherapy in HIV-infected individuals. Figure 1 summarizes important research trajectories outlined in this article.

Cytokine immunotherapy

IL-2

IL-2 is a cytokine that is produced by activated T lymphocytes and that regulates the growth, differentiation, survival and death of multiple lymphocyte subsets, particularly $CD4^+$ and $CD8^+$ T lymphocytes [10]. IL-2 enhances cytolytic activity against a number of target cells and improves the function of natural killer (NK) cells [10,11]. HIV infection is characterized by deficiencies in IL-2 caused by the progressive destruction and functional impairment of $CD4^+$ T lymphocytes [12]. Administration of IL-2 to HIV-infected patients leads to expansion of lymphocytes through prolongation of lymphocyte half-lifes, along with increases in naive and central memory cells, and a concomitant decrease in the level of HIV-associated immune activation [13,14].

Since 1995, numerous Phase II, randomized, controlled clinical trials have tested the effects of *Escherichia coli*-expressed and human recombinant IL-2 (hrIL-2) in small samples of HIV-infected individuals at various stages of HIV infection. These studies demonstrated that intravenous or subcutaneous hrIL-2 administration, combined with ART, may result in significant increases in $CD4^+$ T lymphocyte counts compared with the effects of cART alone [15–21]. The following findings were also established:

- Patients tolerate subcutaneous administration of IL-2 better than continuous intravenous injection, although both administration routes demonstrate similar efficacy [22];
- Polyethylene glycol (PEG) hrIL-2 exhibits decreased biologic efficacy [22,23];

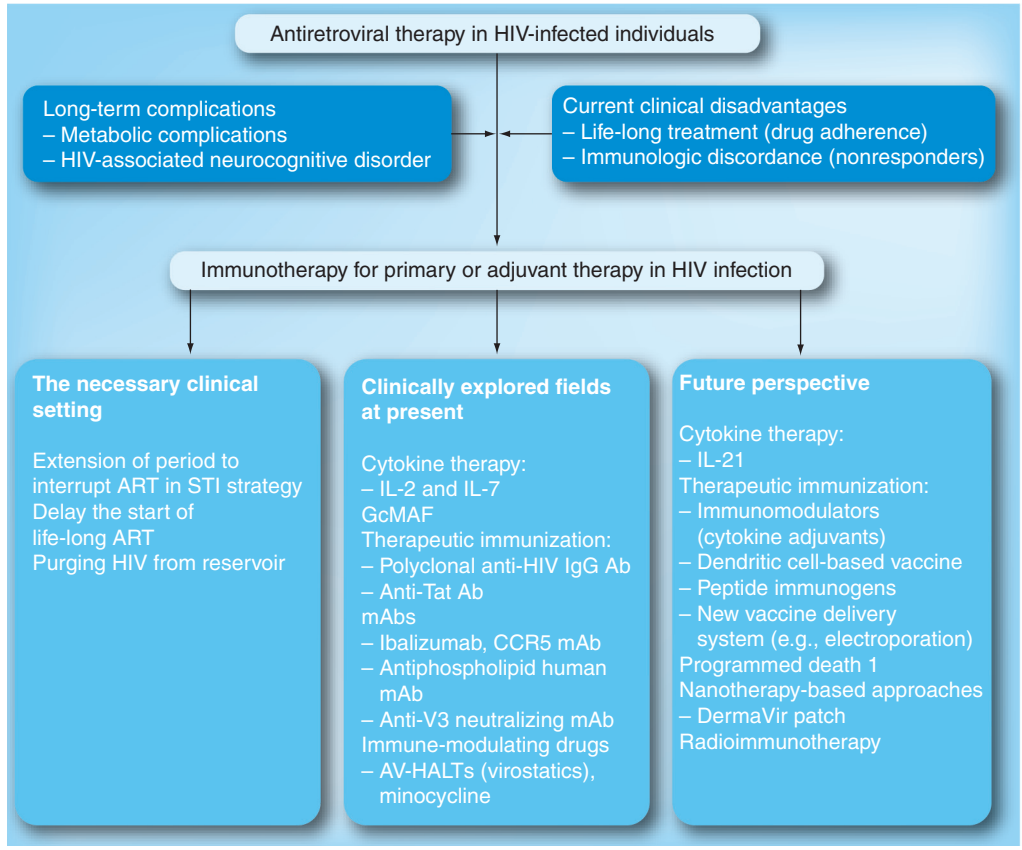

Figure 1. Immunotherapeutic approaches to primary or adjuvant therapy in HIV infection.
Ab: Antibody; ART: Antiretroviral therapy; AV-HALT: Antiviral hyperactivation-limiting therapeutic; cART: Combined antiretroviral therapy; GcMAF: Gc protein-derived macrophage-activating factor; mAb: Monoclonal antibody; STI: Structured treatment interruption.

- Once-daily doses of 3 million IU of subcutaneous hrIL-2 lead to stable increases in CD4+ T lymphocyte counts and are better tolerated by patients [24–26] as are twice daily injections of 4.5 million IU, which lead to equal efficacy and fewer toxic reactions [16,27–29];
- 5-day cycles of intravenous hrIL-2 administered every 8 weeks are more effective for increasing CD4+ T lymphocyte counts than longer and more frequent schedules [21,30];
- The magnitude of CD4+ T lymphocyte expansion is associated with the dose of subcutaneous or intravenous hrIL-2, baseline or nadir CD4+ T lymphocyte counts and plasma HIV VL. Absolute increases in CD4+ T lymphocytes are greater in patients with higher baseline CD4+ T lymphocyte counts [15,17,20,25,31].

The clinical benefits of subcutaneous hrIL-2 therapy were most recently evaluated in two of the largest prospective, randomized, controlled, Phase III, multicenter studies; the Multicenter Randomized Study of the Biological and Clinical Efficacy of Subcutaneous Recombinant, Human Inteleukin-2 in HIV-Infected Patients with Low CD4 Counts under Active Antiretroviral Therapy (SILCAAT), and the Evaluation of Subcutaneous Proleukin in a Randomized International Trial (ESPRIT) [32]. The main aims of these studies were to determine the clinical end point of hrIL-2 therapy, which includes the occurrence of opportunistic infection and death, and to evaluate the safety of hrIL-2 therapy [32]. Table 1 summarizes the most important characteristics and results of these two trials. The patient samples in the SILCAAT and ESPRIT studies included HIV-1 infected adults with low (50–299 cell/mm^3) and high CD4+ T lymphocyte counts (more than 300 cells/mm^3), respectively [32]. Both studies randomized subjects into two groups of equal numbers to receive either hrIL-2 plus cART or cART alone [32]. The results of SILCAAT and ESPRIT were discouraging, owing to the fact that the combination of hrIL-2 with cART yielded no clinical benefits. The primary end point, either the development of opportunistic disease or death from any cause, occurred at similar stages in patients treated with a combination of hrIL-2 as in patients treated with cART alone, despite substantial and sustained increases in CD4+ T-lymphocyte counts in the former group, especially during the early stages of IL-2 administration [32].

Furthermore, many studies have revealed that intermittent subcutaneous hrIL-2 can generate various side effects, ranging from minor gastrointestinal symptoms, fever, myalgia, fatigue and hyperbilirubinemia, to serious symptoms such as psychological disorder or vascular events (such as capillary leakage, shock, myocardial infarction and deep vein thrombosis) [32–38].

IL-7

IL-7 is a principal homeostatic cytokine for T lymphocytes, and is critical for the maintenance of CD4+ and CD8+ naive T lymphocytes [39]. In an animal study, IL-7 administration altered peripheral T lymphocyte homeostasis in both normal and simian immunodeficiency virus (SIV)-infected nonhuman primates and induced immunological improvement in SIV-infected rhesus macaques that were being treated with an antiviral therapy [40,41]. Therefore, IL-7 therapy may lead to the expansion of CD4+ and CD8+ T lymphocyte reservoirs in patients with lymphocyte depletion [39,42–44]. T lymphocyte expansion also results in a significant broadening of circulating T-cell receptor (TCR) repertoire diversity [45]. In a small, nonrandomized, human study, human recombinant IL-7 (hrIL-7) was administered subcutaneously in 16 patients with refractory cancer [45]. Both CD4+ and CD8+ T lymphocyte counts increased, suggesting that hrIL-7 therapy may enhance and broaden immune response, particularly in patients with limited naive T lymphocytes and decreased TCR repertoire diversity, such as HIV-infected patients [45].

CYT 99 007, a first-generation nonglycosylated form of hrIL-7, was evaluated in preclinical and Phase I studies of HIV-infected individuals and found to be well tolerated in repeated dose trials. Administration of CYT 99 007 also triggered long-lasting increases in both CD4+ and CD8+ T lymphocytes [46]. A second-generation glycosylated hrIL-7, CYT 107, was evaluated in the Investigational Study of Placebo versus IL-7 in HAART-Treated Patients (INSPIRE) study, a Phase I/IIa, randomized, placebo controlled, single-blind, multicenter trial of chronic HIV-infected individuals with CD4+ T lymphocyte counts between 101 and 400 cell/mm^3 and plasma HIV VL fewer than 50 copies/ml following 12 months of cART. Three different test dose amounts (10, 20 or 30 µg/kg/week) of CYT 107 were administered subcutaneously thrice weekly in a sample of eight patients, with two additional control patients receiving placebo. Based on an interim analysis that was presented in 2009, three cycles of CYT 107 induced a sustained, dose-dependent increase of CD4+ T lymphocytes [47].

Table 1. Summaries of the SILCAAT and ESPRIT studies of IL-2 therapy in patients with HIV infection.

Important issues	SILCAAT (n = 1695)	ESPRIT (n = 4111)
Characteristics of study participants	Low CD4+ T-cell count (50–299/mm^3) and plasma HIV-RNA ≤10,000 copies/ml IL-2 combined with cART: 849 patients IL-2 alone: 846 patients	High CD4+ T-cell count ≥300/mm^3 IL-2 combined with cART: 2071 patients IL-2 alone: 2040 patients
Schedule of IL-2 administration	Induction phase (within 12 months) 1. One cycle (9 million IU/day, every 12 h, for 5 consecutive days) 2. Six cycles, approximately 8 weeks apart Maintenance phase	Induction phase (within 12 months) 1. One cycle (15 million IU/day, every 12 h, for 5 consecutive days) 2. Three cycles, approximately 8 weeks apart Maintenance phase
CD4+ T lymphocyte counts (median increase in IL-2 combined with cART compared with cART alone according to follow-up duration)	1 year: 99/mm^3 6 years: 38/mm^3 On average: 53/mm^3 (95% CI: 40–66)	1 year: 185/mm^3 6 year: 113/mm^3 On average: 159/mm^3 (95% CI: 145–174)
Primary end point: opportunistic disease or death from any cause	IL-2 combined with cART: 110 (1.94) cART alone: 119 (2.13) HR with IL-2, 0.91 (95% CI: 0.70–1.18); p = 0.47	IL-2 combined with cART: 159 (1.14) cART alone: 165 (1.21) HR with IL-2, 0.94 (95% CI: 0.75–1.16); p = 0.55
Grade 4 adverse events	IL-2 combined with cART: 203 (3.93) cART alone: 186 (3.58) HR with IL-2, 1.10 (95% CI: 0.90–1.34); p = 0.35	IL-2 combined with cART: 466 (3.80) cART alone: 383 (3.09) HR with IL-2, 1.23 (95% CI: 1.07–1.41); p = 0.003

Data are presented as number of patients (rate/100 person-year).
cART: Combined antiretroviral therapy; ESPRIT: Evaluation of Subcutaneous Proleukin in a Randomized International Trial; HR: Hazard ratio; IU: International unit; SILCAAT: Multicenter Randomized Study of the Biological and Clinical Efficacy of Subcutaneous Recombinant, Human IL-2 in HIV-Infected Patients with Low CD4 Counts Under Active Antiretroviral Therapy.
Adapted from [32].

A higher proportion of patients demonstrated CD4+ T lymphocyte counts that were higher than 500 cells/mm^3. Patients receiving injections of 20 µg/kg/week demonstrated a trend towards higher thymic output as well as median increases of CD4+ and CD8+ T lymphocyte counts from 240 to 563 cells/mm^3 (135%) and from 659 to 1210 cells/mm^3 (65%), respectively. All patients tolerated the treatment well, without clinical or laboratory toxicities rated higher than grade 2. However, it remains unclear as to whether the immunologic restoration achieved by hrIL-7 therapy is correlated with improved clinical outcomes such as decreases in morbidity and mortality, or whether increases in CD4+ T lymphocytes continue over the long term, especially in immunologic nonresponders compared with patients who exhibit higher CD4+ T lymphocyte counts following cART. These questions should be addressed by large, well-designed clinical studies.

IL-21

IL-21 was relatively recently discovered to be a significant, multifunctional, immune-enhancing and immune-regulatory cytokine and several *in vivo* animal model studies have revealed that IL-21 plays an essential role in controlling chronic viral infections [48–50]. IL-21 can also promote differentiation of naive CD4+ T lymphocytes into IL-17-producing CD4+ T lymphocytes (Th17 cells), which play an important role in inducing inflammation and controlling invading pathogens [51,52]. CD4+ T lymphocytes are the main producers of IL-21 in humans [53]. IL-21, unlike IL-2, does not support proliferation of anti-CD3-activated regulatory T cells that are involved in the suppression of antiviral immunity [54]. Serum levels of IL-21 were significantly decreased in chronic HIV-infected individuals and were correlated with CD4+ T lymphocyte counts [55,56]. Recently, Iannello *et al.* reported that IL-21 production was compromised early in the course of HIV infection, and maintained normally in only elite controllers [56]. This study also revealed that cART alone partially restored the production of IL-21 and the frequencies of IL-21-producing HIV-specific antigen-experienced CD4+ T lymphocytes were decreased in HIV-infected patients [56]. Furthermore, recombinant human IL-21 prevented enhanced spontaneous *ex vivo* death of CD4+ T lymphocytes from HIV-infected individuals [56]. These findings suggest that IL-21 should be considered for immunotherapy in HIV-infected individuals.

Gc protein-derived macrophage-activating factor

Serum Gc protein, also known as vitamin D3-binding protein, is the precursor of macrophage-activating factor (MAF). In HIV-infected individuals, MAF precursor activity of

the serum Gc protein is lost or reduced, because the Gc protein is deglycosylated by α-*N*-acetylgalactosaminidase that is released from the gp120 of HIV-infected lymphocytes [57,58]. This decrease in macrophage activation may result in immunosuppression [58,59]. Therefore, the administration of Gc protein-derived macrophage-activating factor (GcMAF) may effect immune restoration in HIV-infected individuals. A small, nonrandomized, clinical study performed in 15 asymptomatic treatment-naive Japanese HIV-infected individuals was reported in 2009 [57]. Throughout the 7-year study period, less than 18 weekly intramuscular injections of 100 ng GcMAF led to recovery of low serum α-*N*-acetylgalactosaminidase activities that were equivalent to those of non-HIV-infected controls and the maintenance of $CD4^+$ T lymphocyte counts that were within the normal range (798 ± 213 cells/mm^3). Plasma HIV VL and p24 antigen levels also decreased following GcMAF therapy owing to immunologic restoration [57]. Interestingly, the cessation of GcMAF therapy did not result in rebounds of serum α-*N*-acetylgalactosaminidase activities, HIV VL or p24 antigen levels [57].

Therapeutic immunization

The concept of therapeutic immunization for HIV-1 infection was first raised by Salk in 1987 [60]. The main purpose of therapeutic immunization is to immunize for the induction of HIV-antigen-specific immune response in already HIV-infected individuals, paricularly T-cell immunity that may maintain immunologic control capacity in HIV infection [61]. Therapeutic immunization has several potential indications that complement cART. For example, it may sustain immunologic fitness or HIV control during the early period of HIV infection so that the initiation of cART can safely be delayed, as well as the safe cART-free periods can be prolonged in chronic asymptomatic HIV-infected individuals. Moreover, therapeutic immunization may maintain HIV control and immunologic fitness in HIV-infected patients receiving continuous cART, especially patients who fail to restore or maintain adequate $CD4^+$ T lymphocyte counts. cART, combined with therapeutic immunization, must be able to suppress HIV bursts arising from HIV-1-infected $CD4^+$ T lymphocytes that are activated by immunization [62].

Therapeutic immunization can, theoretically, induce HIV-specific polyfunctional $CD4^+$ and $CD8^+$ T lymphocyte immune response and, therefore, control HIV replication [61]. However, several candidate HIV vaccines (subunit or whole inactivated virus) have proven ineffective for controlling HIV replication. These findings have prompted new investigations evaluating the potential of therapeutic immunization.

Newer approaches utilize common, effective γ-chain cytokine adjuvants as immunomodulators to enhance vaccine-induced HIV-specific, adaptive immune responses. In particular, HIV-infected patients with discordant responses to cART, such as plasma HIV VL that become fully suppressed while $CD4^+$ T lymphocyte counts remain suboptimal, may become important candidates for restorative therapeutic immunization with immunomodulators. These strategies for replenishing destroyed $CD4^+$ memory T lymphocyte populations and augmenting vaccine-induced specific immunities have focused on the roles of cytokines that are part of the common γ-chain cytokine family (such as IL-2, -7, -12 and -15), which regulate proliferation, activation, differentiation and survival of T lymphocytes. The SIV infection model in rhesus macaques has been used to evaluate the effectiveness of immunomodulator cytokines as therapeutic vaccine adjuvants. Several animal studies have revealed that IL-2 and IL-15 adjuvant vaccines preserve peripheral $CD4^+$ T lymphocyte counts and decrease the VL set point [63–67]. However, in order to determine the true efficacies of these cytokines as therapeutic vaccine immunomodulating adjuvants, human trials must be performed in various clinical settings.

Dendritic cell (DC)-based HIV vaccine strategies have recently been investigated [3,68–71]. DCs of myeloid origin are the most potent professional antigen-presenting cells (APCs) in HIV infection [3]. In particular, myeloid DCs have the exceptional ability to activate T-cell immune responses to HIV, which is why these cells have been explored as *ex vivo* and *in vivo* immunotherapies [72,73]. The use of DCs for HIV immunotherapy effectively controls HIV replication by using natural pathways of antigen recognition and processing during HIV-specific immune response. Such therapies could prolong the length of the healthy cART period. Myeloid DCs may be grown from blood monocytes by culturing in IL-4 and GM-CSF, a breakthrough that may clear the method for *ex vivo* immunotherapeutic human trials [74,75]. Several clinical trials have already tested DC-derived vaccines in small samples of HIV-infected patients. Immature autologous or allogenic blood DCs or IL-4/GM-CSF monocyte-derived DCs were generated using various adjuncts to promote DC maturation, including IL-1β/IL-6/TNF-α,

monocyte-conditioned medium, and TNF-α alone [72,76–80]. These studies have yielded promising results, including reduction of plasma HIV VL or maintenance of stable VL after STI [3].

Because naturally occurring human antibodies, developed by specific immune humoral responses to HIV infection, are unable to efficiently neutralize HIV, clear the infection or prevent disease progression, passive immunization with more potent appropriate neutralizing antibodies for HIV may be a solution for immunotherapy in HIV-1-infected patients. Neutralizing antibodies against HIV-1 could result in anti-HIV activity even in cART-experienced patients, and B lymphocytes can be stimulated to generate high titers of broadly cross-reactive neutralizing antibodies against multiple subtypes of HIV.

Polyclonal anti-HIV IgG antibody (^{PE}HRG214)

^{PE}HRG214 is a polyclonal anti-HIV IgG antibody preparation produced by immunization of goats with purified HIV-associated proteins followed by booster immunizations with synthetic peptides to highly conserved HIV epitope regions without recognition by the human immune system [81,82]. ^{PE}HRG214 has a high titer and affinity to multiple unique epitope regions on HIV, including two on gp120 and one each on gp41, p24, p66, p17 and p11. It could potently neutralize and lyse a broad spectrum of primary and reference HIV isolates *in vitro* [83]. In a Phase I study, a single intravenously administered dose of ^{PE}HRG214 was reasonably well tolerated and achieved adequate plasma concentrations, exceeding those found to neutralize HIV in laboratory experiments [81,82]. A HRG2 study (clinical trial NCT00385567) was performed as a Phase II, randomized, controlled, open-label, multidose trial to determine the efficacy, safety, immunogenicity and pharmacokinetic profiles of ^{PE}HRG214 in HIV-infected patients receiving optimized background regimen (OBR) of antiretroviral agents, treated intravenously three-times weekly for up to 16 weeks. The primary objective of this study was to determine the effect of ^{PE}HRG214 on decreasing the plasma VL, as compared with a control group. This study was commenced in January 2007, but has been terminated owing to the difficulty in recruiting eligible patients in a timely fashion [201].

The Thymon University Tat immunogen (TUTI-16)

HIV-1 Tat protein, a virally encoded toxin, is secreted by HIV-1-infected cells and acts on uninfected cells, making them permissive to HIV-1 replication. HIV-1 Tat enhances chronic HIV replication and induces immune suppression. HIV-1 Tat activities can be blocked *in vitro* and *in vivo* by anti-Tat antibodies. Antibodies to Tat inhibit Tat-mediated transcellular activation *in vitro* and minimize chronic plasma viremia. TUTI-16 is a fully synthetic, self-adjuvanting, lipopeptide vaccine that is water soluble and administered by subcutaneous injection. In a preclinical study, a priming dose and a 3-week boost in rats induced a high-titer antibody response to the eight known distinct epitope variants of HIV-1 Tat protein. These antibodies blocked the function of the HIV-1 Tat protein (toxin), which is essential to the maintenance of chronic HIV-1 viremia [84]. Therefore, TUTI-16 has potential as a therapeutic vaccine for HIV-1-infected individuals. The Phase I/IIa clinical study of TUTI-16 in asymptomatic HIV-1-infected and -uninfected subjects will be performed to gather safety and human immunogenecity (anti-HIV-1 Tat antibody titer) data on subcutaneously administrated TUTI-16 [201].

Monoclonal antibodies

Humanized anti-CD4 IgG monoclonal antibody (ibalizumab)

HIV-1 entry into host cells is a multistep process that offers several potential targets for antiretroviral treatment. Ibalizumab, formerly called TNX-355 and Hu548, is a humanized anti-CD4 IgG_4 monoclonal antibody (mAb) of murine origin that interferes with HIV entry. Ibalizumab binds to the interface between domain 1 and 2 of the extracellular region of CD4 [85]. It appears to result in anti-HIV activity due to postbinding conformational effects that prevent CD4-bound gp120 from interacting with CCR5 or CXCR4, instead of directly inhibiting the binding of gp120 to CD4 [86]. Because ibalizumab binds a site away from the MHC class II molecule, it does not cause an immunosuppressive state, unlike other mAbs that target domain 1 of CD4 and competitively inhibit gp120 binding that can interfere with MHC class II immune function [87]. The average half-life of ibalizumab is 3–3.5 days shorter than the 2–3 weeks of IgG under normal physiological circumstances [88].

The first Phase I clinical study to determine the anti-HIV activity and safety of TNX-355 in 30 HIV-infected individuals, who were not on cART or were on a failing regimen with a plasma HIV VL greater than 5000 copies/ml, was performed with the administration of single-dose, intravenous infusions over five doses, ranging from 0.3 to 25 mg/kg. The dose-related reductions

in plasma HIV VL were observed in the 3, 10 and 25 mg/kg groups. In addition, dose-dependent peak increases in $CD4^+$ T lymphocytes were observed within 24 h of treatment. Nonserious adverse events did not cause the discontinuation of the treatment in any subjects [89].

A Phase Ib multidose study for evaluating the anti-HIV activity, pharmacokinetics and safety of ibalizumab in 22 HIV-infected patients, who remained off other antiretroviral drugs or continued a stable failing regimen, revealed that ibalizumab, intravenously administered either weekly or biweekly had anti-HIV activity, good tolerability and safety [88]. There was neither immunogenicity nor severe drug-related adverse events [88]. Treatment with ibalizumab resulted in substantial rapid peak reduction of plasma HIV VL (0.5–1.7 $\log_{10}$) in 20 out of 22 patients following 1–2 weeks of treatment, and did not cause $CD4^+$ T lymphocyte depletion [88]. Interestingly, an *in vitro* study revealed high level synergistic, anti-HIV activity when ibalizumab and the other entry inhibitor, enfuvirtide, were combined together [90]. In addition, in a Phase Ib study, there was no evidence of cross-resistance between the two entry inhibitors [88].

The 24-week analysis of a randomized, double-blinded, placebo-controlled, Phase IIa study was performed and presented [91]. A total of 82 antiretroviral triple classes-experienced patients were randomized to receive ibalizumab (10 mg/kg weekly for 8 weeks, followed by either 10 mg/kg every 2 weeks, or 15 mg/kg every 2 weeks, to complete 24 weeks) versus placebo in addition to an OBR. The plasma HIV VL reductions were significantly greater in the two treatment arms, with a mean 0.95 $\log_{10}$ decrease in the 15 mg/kg arm and 1.16 $\log_{10}$ decrease in the 10 mg/kg arm compared with a 0.20 $\log_{10}$ decrease in the placebo arm.

Furthermore, ibalizumab administrated as an intravenous infusion, displayed promising anti-HIV activity and safety in early clinical trials [88,89]. Until now, the immunosuppressive effects had not been demonstrated with ibalizumab in nonhuman primates and human clinical trials [88,92].

Anti-V3 neutralizing mAb (KD-247)

An antibody response capable of neutralizing not only the homologous, but also the heterogeneous, form of the CXCR4-tropic HIV-1 MNp and CCR5-tropic (R5) HIV-$1_{JR\text{-}CSF}$ was achieved through sequential immunization with a combination of synthetic peptides representing HIV-1 Env V3 sequences from HIV-1 clade B isolates [93]. The anti-V3 mAbs generated from peripheral blood mononuclear cells (PBMCs) of HIV-infected individuals have been shown to contain cross-neutralizing anti-V3 mAbs that neutralize primary HIV isolates [94,95]. Through the sequential immunization of mice with V3 peptides from HIV-1 clade B field isolates, the cross-reactive antisera that strongly bound to V3 peptides from homologous and heterologous primary isolates was inducted [93].

KD-247 is a reshaped, humanized, anti-V3, neutralizing mAb, derived from a *C25* gene that efficiently neutralized primary isolates of HIV-1 [93]. C25 was obtained by immunizing mice sequentially with six V3 peptides, representing clade B HIV-1 viruses [96]. KD-247 was generated by transferring the genes of the complementary determining region of C25 into the genes of the human V region of the antibody [93,97]. The epitope of KD-247 was mapped to six amino acids, IGPGRA, at the tip of the gp120–V3 loop [93,98]. It bound with high affinity to the PGR motif within the HIV-1 Env V3 tip region, and displayed potent cross-neutralizing activity against subtype B, primary HIV-1 isolates [93]. Among the established reference of anti-V3 mAbs, KD-247 most effectively neutralized primary HIV-1 field isolates including R5 viruses possessing the matching neutralization sequence motif, suggesting its promise for clinical applications [93]. The complete protection from challenge of infection by a pathogenic simian/human immunodeficiency virus (SHIV) 89.6 strain was observed when a high concentration of KD-247 was used in this animal model [99]. In addition, KD-247 might not only directly neutralize the HIV, but also maintain $CD4^+$ T lymphocytes in lymphoid tissues [97]. Because KD-247 showed the complete, or strong, inhibition of HIV replication *ex vivo* and the protection of monkeys against the highly pathogenic simian/human immunodeficiency virus, KD-247 could be a promising new immunotherapeutic candidate for HIV-infected individuals [93,96,99].

However, neutralization evasion from anti-V3 mAbs, including KD-247, has been reported and associated with amino acid substitution mutation within the epitope of the V3 loop and outside V3 including V2 region [95,98]. In addition, a clone with a V2 potential *N*-linked glycosylation site insertion and mutation in V3 demonstrated a high level of resistance to KD-247 [100]. Through the acquisition of a potential glycosylation site in V2, HIV can escape a neutralizing anti-V3 mAb. However, one study revealed that high concentrations of KD-247 are needed for viral acquisition

of KD-247 resistance and that the escape variants are more sensitive to CCR5 inhibitors. In addition, there was a strong synergistic effect between KD-247 and CCR5 inhibitors at all concentrations tested [101].

CCR5 mAbs

CCR5 mAb binds to CCR5 and potently inhibits R5 HIV-1 *in vitro*. A few CCR5 mAbs have demonstrated broad and potent anti-HIV activity *in vitro* [102–104]. The most potent CCR5 mAbs showed an IC_{50} in the range of 0.1–1.0 μg/ml, with an approximately 1 log_{10} variation across diverse viral isolates. The mAbs resulted in essentially complete inhibition of HIV replication at higher concentrations [105–107]. CCR5 mAbs have demonstrated similar potencies for HIV strains derived from various stages of disease [108], genetic subtypes [104,109,110] and pediatric/adult infection [111]. CCR5 mAbs efficiently inhibited the CCR5-mediated entry of dual/mixed (R5X4) virus in cell lines that express CCR5 but not CXCR4 [104,111]. However, limited inhibition of R5X4 viruses was observed in cultures of PBMCs [108–110].

Two human CCR5 mAbs of IgG_4 isotype, that is, PRO140 and HGS004, have recently entered clinical trials in HIV-infected individuals with only R5-virus detectable, plasma VL greater than 5000 copies/ml, $CD4^+$ T lymphocytes greater than 250 cells/mm^3 and no concurrent cART [112,113]. These studies have successfully completed proof-of-concept, providing initial information on the potential therapeutic utility of these agents [112,113]. Clinical studies have established CCR5 mAbs as potent antiretroviral agents with prolonged activity following a single dose. Therefore, CCR5 mAbs can represent both a distinct class of CCR5 inhibitor and a novel approach to HIV-1 immunotherapy.

PRO140 is a humanized form of the mouse CCR5 mAb PA14 that binds an epitope spanning extracellular loop 2 (ECL2) and amino-terminal domain and does not antagonize the natural activity of CCR5 *in vitro* [108,111,114]. The potency and breadth of anti-HIV activity for PA14 and PRO140 have been demonstrated in several preclinical studies [108,109,111,114]. The first clinical trial of PRO140 in HIV-infected individuals was a Phase I, randomized, double-blind, placebo-controlled, dose-escalating study in 39 patients with early-stage infection, no cART for 3 months and only R5 HIV-1 detectable, to evaluate the pharmacokinetics, tolerability, receptor occupancy and anti-HIV effects of single, escalating-doses infusion, ranging from 0.5, 2 to 5 mg/kg [113]. In this trial, the single, intravenous infusion of PRO140 was generally well tolerated and no dose-limiting toxicity was observed [113]. In addition, PRO140 demonstrated potent, rapid, prolonged and dose-dependent anti-HIV activity, and a highly-significant reduction in mean VL of tenfold or more was observed within 4 days for each of the dosing groups and persisted for 2–3 weeks after treatment in the 5 mg/kg group [113]. In the 5 mg/kg PRO140 group, there was a trend towards increased $CD4^+$ T lymphocytes over baseline, the significant CCR5 receptor occupancy was observed for 2–4 weeks in all PRO140 groups and there was no depletion of $CCR5^+$ cells following treatment [113]. In another clinical trial of a similar design to that of the first studies, PRO140-administrated subcutaneously also offered the potential for significant and prolonged, dose-dependent, HIV-1 RNA suppression, and demonstrated good tolerability [115]. In the most recently reported Phase IIa study of PRO140, administrated intravenously, the mean maximum reduction from baseline HIV-1 RNA was 1.8 log_{10} for both 5 and 10 mg/kg doses ($p < 0.001$ control to placebo) [116]. VL nadired at day 12 postinfusion and remained significantly reduced through day 29 for both PRO140 dose groups ($p < 0.01$) [116]. Consequently, the single 5 and 10 mg/kg intravenous treatments with PRO140 exhibited potent, long-lived anti-HIV activity and were generally well tolerated [116]. However, further studies in humans must be performed in various clinical settings, including in patients receiving cART, or patients with treatment-experience or advance-stage infection.

HGS004 (CCR5mAb004) is a human mAb that binds ECL2 and inhibits R5 HIV-1 entry and chemokine signaling with similar efficiencies to that of PRO140 [112,117]. The results of a Phase I, single-blind, randomized, placebo-controlled clinical trial to evaluate the tolerability, pharmacokinetics, receptor occupancy and anti-HIV effects of single, escalating doses, ranging from 0.4, 2, 8, 20 to 40 mg/kg HGS004 in 63 HIV-infected subjects, revealed that HGS004 was generally well tolerated and showed meaningful anti-HIV activity when administered to individuals with R5 HIV-1 tropism [112]. Significant reductions in HIV-1 VL were observed at doses of 8 mg/kg and higher and the mean CCR5 receptor occupancy was approximately 80% at day 28 for each of the three highest dose groups [112]. In addition, significant increases in $CD4^+$ T lymphocyte counts were observed in all HGS004 dose groups [112].

CCR5mAbs are distinctly different from small-molecule CCR5 antagonists (e.g., maraviroc, vicriviroc [SCH-417690], aplaviroc, TAK-220, TAK-779, SCH-350581 and SCH-351125 [SCH-C]) in terms of their sites or mechanisms for CCR5 binding and HIV-1 inhibition. CCR5mAbs and small-molecule CCR5 antagonists can be considered distinct classes of CCR5 inhibitors based on their potent anti-HIV synergy and lack of cross-resistance. Despite demonstrating high-level resistance to the small-molecule CCR5 antagonists, HIV remains susceptible, or even hypersusceptible, to inhibition by CCR5mAbs [118–120]. In one study among HIV-infected subjects with treatment failure to a vicriviroc-containing antiretroviral drugs regimen with high-level resistance to vicriviroc and another small-molecule CCR5 antagonist, the susceptibility to inhibition of the HGS004 increased [119]. However, an anti-HIV synergistic effect was revealed for each group for most combinations of CCR5mAbs and small-molecule CCR5 antagonists, and this synergistic effect was attributed to cobinding in the distinct epitopes of the CCR5 receptor [114,121]. The synergistic effect was also observed between CCR5mAb and enfuvirtide [114,121].

Antiphospholipid human mAb

Brown *et al.* described a murine mAb against phosphatidylinositol phosphate that neutralizes HIV-1 in PBMC cultures [122]. Recently, Moody *et al.* reported that four human antiphospholipid mAbs (PGN632, P1, IS4 and CL1) inhibit HIV-1 R5 primary isolate infection of PBMCs with an IC_{80} of less than 0.02 to approximately 10 μg/ml [123]. The mechanism of HIV inhibition involved stimulation of an innate anti-HIV-1 response, including the release of soluble chemokines, such as MIP-1α and MIP-1β, which block HIV-1 entry [123]. These results suggest that anti-phospholipid human mAbs could target immunotherapy in HIV-infected individuals.

F105, anti-gp 120 human IgG1κ mAb

gp120, which is located at the plasma membrane of HIV-1-infected cells and on the surface of HIV particles, provides an excellent means of discriminating HIV-1-infected host cells from non-infected cells. It is a viral glycopeptide that plays a pivotal role in the HIV-entry process. The binding of the HIV envelope, gp120, with the CD4 receptor of the $CD4^+$ T lymphocyte initiates a cascade of events that leads to viral entry. gp120 contains a relatively conserved CD4 binding site that offers an accessible epitope for recognition by mAbs [124]. F105 is a human IgG1κ mAb that was derived from a HIV-1-infected individual. It binds to a discontinuous epitope overlapping, but not entirely congruent, with the CD4 binding region of gp120, and competes with soluble CD4 for gp120 binding. F105 is capable of binding antigen expressed on the surface of a wide range of HIV-1 laboratory strains and primary isolates, thereby neutralizing a subset of these strains. In addition, it has been shown to neutralize the IIIB, SF2 and MN strain of HIV at concentrations readily achievable in humans [124]. In a Phase I dose-escalation clinical study, F105, administered as a single intravenous injection, demonstrated the full anti-gp120 binding activity and was safe, nontoxic and well tolerated [125].

A recently reported study, using a heavy-chain fragment of the mAb F105, fused to protamine for delivering siRNA and targeting the viral gag mRNA, specifically into HIV-1-infected cells. This study demonstrated that HIV production in previously infected $CD4^+$ T lymphocytes could be suppressed by this modality [126]. In addition, the most recent study demonstrated the specificity of binding and entry of F105 into HIV-infected cells. F105 was rapidly taken up into the cell, accumulated in the Golgi apparatus and displayed a higher gp120 affinity. F105 bound exclusively to cells expressing gp120 in a coreceptor-independent manner and did not bind to HIV-uninfected cells. These data further support the potential of mAbs as immunotherapeutic targeting agents and offer new insights into the possibility of F105 as an excellent targeting moiety for the selective delivery of antiretroviral drugs or cytotoxic compounds to HIV-1-infected cells [127].

IgG-reactive antibodies

An immunogen able to drive broadly neutralizing antibodies has yet to be identified for HIV infection. In healthy individuals, a functional immune network contains a wide range of natural autoantibodies, with T and B lymphocyte clones being essential for self-assertion [128]. Recent data has demonstrated that broadly and potently neutralizing anti-HIV-1 human mAbs comprised of B lymphocytes of HIV-1-infected individuals have features of natural autoantibodies [129]. Antibodies directed against carbohydrate moieties of glycoproteins, including those of HIV-1 gp120, are present in normal human serum (NHS) [130]. The affinity chromatography-separated (fractionated) IgG-reactive antibodies (anti-IgG antibody) made from NHS, which also can react with a gp120 V3 loop derived

peptide, prevented human PBMC infection by HIV-1 primary isolates, but unfractionated total NHS IgG did not neutralize HIV-1 infectivity [131]. The recent data have demonstrated the importance of the natural anti-HIV immune response in HIV-infected individuals [132]. In addition, a very recent study showed that anti-IgG antibodies purified from NHS and a GammaBind G Sepharose Flowthrough™ (Amersham Biosciences, NJ, USA) fraction for the removal of IgM and IgG, which contained the IgG_2 dimers, neutralized HIV-1_{BaL} strain with 100% effectiveness at 2 μg/ml concentration, as well as both HIV-1 X4- and R5-tropic primary isolates with an IC_{50} between 0.4 and 1.8 μg/ml [133]. Therefore, anti-IgG antibodies may be useful as a new immunotherapeutic tool in HIV-infected individuals.

Immune-modulating drugs

Antiviral hyperactivation-limiting therapeutics (virostatics)

Antiviral hyperactivation-limiting therapeutics (AV-HALTs) are a newly investigated class of antiretroviral drugs. Unlike other classes of antiretroviral drugs given to suppress HIV replication, AV-HALTs are single or combination drugs designed to reduce the rate of viral replication while, at the same time, also directly reducing the state of immune-system hyperactivation that is believed to drive the loss of $CD4^+$ T lymphocytes leading to disease progression of HIV.

Chronic immune stimulation, owing to persistent HIV replication, induces continuous T lymphocyte activation and proliferation of both HIV-infected and bystander cells, ultimately resulting in the exhaustion of the immune system [134,135]. There is a growing recognition that successful long-term therapy of HIV infection should not only reduce HIV replication, but also ultimately limit the chronic hyperactivation of the immune system. Therefore, AV-HALTs, also called virostatics, are designed to accomplish two goals: the reduction of plasma HIV VL and the reduction of immune system hyperactivation. Virostatics are characterized by the combination of a drug directly limiting HIV replication (viro) and another drug indirectly inhibiting the HIV by reducing cellular proliferation (static).

Antiviral hyperactivation-limiting therapeutics have been developed as two forms of multidrug combined regimens (first generation) or single drugs composed of a single molecule (second generation). First-generation AV-HALTs accomplish this by combining an antiretroviral drug (e.g., didanosine [ddI]) with a cytostatic agent (e.g., hydroxyurea [HU]) as a new class of drugs [136]. The HU–ddI combination has been shown to synergistically limit immune activation and control HIV replication by both antiviral and cytostatic activities. The mechanism of this combined regimen is based on the capacity of HU to reduce the synthesis of intracellular components required for HIV replication, namely deoxyneucleotides (dNTP), in particular, deoxyadenosine trisphosphate (dATP, a ddI competitor), by inhibiting ribonucleotide reductase, a cellular enzyme that transforms ribonucleotides into dNTP [137]. Therefore, by decreasing the availability of the natural substrates (such as dNTP), HU increases the relative concentration of the NRTI, resulting in increased anti-HIV activity. Because the nucleoside analog ddI is the precursor of the dATP analog, HU can help to reduce the cellular pool of dNTP while ddI terminates DNA synthesis. In addition, because the majority of HIV-1 replication occurs in actively dividing cells, HU can limit cellular proliferation, thereby limiting HIV replication in target cells (e.g., $CD4^+$ T lymphocytes) [138].

The HU–ddI combination regimen has several advantages, such as a favorable resistance and anti-HIV profiles, the ability to suppress various resistant quasispecies and to provide the potential for durability, because HU targets are not viral enzymes (e.g., reverse transcriptases and proteases), but are essential cellular proteins. In addition, the HU–ddI combination regimen has also been shown to compensate for resistance to ddI, explaining the long-term anti-HIV efficacy observed with this combination [139]. This combination has immune modulating activity and reduces viral targets (such as $CD4^+$ T lymphocytes), possibly with limited immunosuppressive effects. Regarding the concerns of immunosuppressive effects of HU, several studies have demonstrated that HU blocks cellular proliferation but does not impede cellular activation nor diminish recall response [140,141]. In addition, suppression of immune activation by cytostatic drugs, such as HU, could complement cART. Although, upon treatment with HU and ddI, the $CD4^+$ T lymphocyte increase is blunted and the functionality of the immune system is conserved. Although the earlier studies resulted in concerns regarding the decrease in $CD4^+$ T lymphocyte count, treatment failure and the pancreatic toxicity of high-dose HU treatment (1200 mg/daily) [142], the low-dose HU (600 mg/daily) treatment demonstrated better tolerability, rare adverse events and greater potency in suppressing

HIV replication than high-dose HU treatment [136]. The combination of HU and ddI strikes a balance between viral suppression and drug-related toxicity and could have a role in both delaying cART, by an induction therapy that limits viral replication and loss of CD4+ T lymphocytes, and substituting cART with maintenance therapy. The VS411-C201 study is a Phase IIa randomized, double-blind, five-arm, dose-finding, multicenter study performed with various dosages of VS411, which is the enterically coated capsule composed of low-dose HU and slow-release ddI combination. This study was started in 2008 and the results are expected to be reported soon.

Mycophenolate mofetil (MMF) selectively inhibits the *de novo* synthesis of guanosine nucleotides by competing with inosine monophosphate dehydrogenease; therefore, it would be expected to potentate the effects of guanosine inhibitors of reverse transcriptase such as abacavir. Cyclosporine (CSA) can suppress T-cell activation and may have anti-HIV activity through the interaction with HIV-1 Gag polyprotein to interfere with viral maturation. Therefore, other virostatics such as MMF and CSA can theoretically have some efficacy in controlling HIV and/or reservoirs. However, several small clinical studies with MMF and CSA, combined with various antiretroviral drugs, have demonstrated conflicting results with regard to VL and CD4+ T lymphocyte counts as well as potential adverse events, including immune suppression, in spite of the promising *in vitro* results demonstrating inhibition of HIV replication [143–147].

Minocycline

Minocycline is an antibiotic with immunomodulatory and anti-inflammatory activity that markedly reduces the expression of monocyte chemoattractant protein (MCP)-1, as detected in cerebrospinal fluid in the SIV/macaque model of HIV-1 infection [148]. Therefore, minocycline, as an immunomodulatory drug, could be an effective, low-cost, adjunctive treatment to cART.

Szeto *et al.* recently reported that minocycline can attenuate HIV-1 infection and replication by suppressing the activation of CD4+ T lymphocytes [149]. This study revealed that minocycline decreased single-cycle CXCR4-tropic HIV replication in a dose-dependent manner and decreased intracellular viral RNA levels after *in vitro* infection of primary human CD4+ T lymphocytes. Reactivation of HIV was also decreased in a primary CD4+ T lymphocyte-derived model of HIV latency and in resting CD4+ T lymphocyte reservoirs from HIV-infected patients who had undetectable plasma VL with cART. In addition to these findings, minocycline treatment altered T lymphocyte activation and caused significant blunting changes in expression of activation/proliferation markers and cytokine secretion of CD4+ T lymphocytes in response to activation [149]. Minocycline may mediate anti-HIV activities by altering the cellular environment through reduction of the secretion of inflammatory cytokines (such as IL-2, IFN-γ and TNF-α) rather than via direct effects on HIV-1 replication. Because minocycline may target a step downstream of early T lymphocyte activation events, it may be classified as a new class of anticellular anti-HIV drugs.

In addition, Zink *et al.* demonstrated that minocycline decreases the HIV VL in both plasma and cerebrospinal fluid and decreases viral RNA in the brain, using the advanced SIV/macaque model of HIV-associated neurologic diseases [148]. Minocycline treatment also reduced the severity of CNS disease with anti-HIV activities and anti-inflammatory effects. In addition to the inhibitory mechanism on p38 mitogen-activated protein kinase activity cascade, minocycline may suppress HIV-1 infection by inhibiting HIV-1 integrase [150,151]. These findings suggest that minocycline uses one additional pathway to inhibit HIV-1 replication. More active research is required to reveal the anti-HIV mechanisms and clinical benefits of minocycline. Because minocycline has the advantages of being able to penetrate the blood–brain barrier and neuroprotective activity, it could be effectively used to prevent and/or treat HAND, including asymptomatic neurocognitive impairment. Currently, the efficacy of minocycline in reducing neurologic disorder in HIV-infected individuals is being analyzed in clinical trials [201]. Several questions must be studied to clarify the clinical benefits of minocycline, such as whether normally prescribed doses could achieve serum or cellular levels that would be effective in suppressing HIV-1 replication in humans, and whether it has cross-interactions with other antiretroviral drugs.

Nevertheless, because minocycline has a number of advantages, including the enhanced penetration to tissue, especially brain, low incidence of toxicity with long-term administration, and low cost, the anti-HIV effects of minocycline, which are mediated by altering the cellular environment rather than directly targeting virus, suggest that it could be an anticellular anti-HIV drug.

Programmed death-1

In the setting of chronic infection, such as HIV, the immune regulation function is also able to prevent the deleterious effects of unchecked immune activation caused by the body's response to the persistent antigen. Our immune system has developed various mechanisms to perform this regulatory role, one of which is through the transmembrane immunoreceptor, programmed death-1 (PD-1), which is a negative regulator of activated T lymphocytes. The progressive loss and exhaustion of T lymphocyte function in the setting of various chronic viral infections has been associated with the upregulation of PD-1 [152–154].

In HIV infection, the level of expression of PD-1 on T lymphocytes correlates with decreased HIV-specific T lymphocyte function, and PD-1 expression can be used to predict HIV disease progression in that PD-1 level is positively correlated with increases in HIV-specific $CD8^+$ T lymphocytes and plasma VL, while negatively associated with $CD4^+$ T lymphocyte counts [155]. In addition, PD-1 blockade using PD-1 antibodies resulted in the recovery of HIV-induced T lymphocyte exhaustion through an increase in HIV-specific $CD8^+$ T lymphocyte proliferation and cytokine production *in vitro* [156]. Velu *et al.* recently presented the first *in vivo* study results to demonstrate enhancement of a SIV-specific immune response, which is an expansion of SIV-specific, polyfunctional $CD8^+$ T lymphocyte counts and function, through PD-1 blockade, using an anti-PD-1 antibody, with nine SIV-infected macaques [157]. This study also reported that the enhanced immunologic response, following anti-PD-1 antibody treatment, corresponded with significant reductions in plasma VL and prolonged survival, as well as good tolerability, with no evidence of newly developed autoimmunity [157]. Anti-PD1 antagonists appear to be a novel immunotherapeutic tool in HIV infection. Recently, the administration of CT-011 (a humanized IgG_1 monoclonal PD-1 antibody) in patients with advanced hematologic malignancies demonstrated safety and good tolerability [158].

However, some issues, including the likelihood of the development of autoimmunity with repeated administration of anti-PD1 antagonists, the effect of blocking regulatory T lymphocytes and the presence of multiple inhibitory receptors that coregulate $CD8^+$ T lymphocyte exhaustion in chronic viral infection should be further evaluated to help ensure a successful outcome with anti-PD-1 therapy in HIV-infected individuals [159,160].

Nanotechnology-based approaches for immunotherapy

Although the preclinical studies demonstrated enhancement of immune responses, most of the clinical trials for therapeutic immunization have consistently failed to provide clinical improvements in HIV-infected individuals. Most of these therapeutic vaccine studies have been based on the delivery of the immunogenic factors through viruses or *ex vivo* DCs. The vaccine delivery through viral vectors has various risks and *ex vivo* generation and manipulation of autologous DCs is a difficult therapeutic strategy to utilize widely as it involves very labor intensive, complicated procedures with high costs and multiple procedures for product control at different sites [161]. Therefore, new approaches, using targeted nanotechnology techniques for delivery of immunomodulatory drugs and targeting antigens to DCs surface receptors *in vivo*, could provide very important opportunities [3].

Various polymeric systems have been explored for *in vivo* targeting of DCs and delivery of small molecules, proteins or DNAs showing potential for immunotherapy in HIV infection. Polyethylene glycol (PEG)-stabilized polypropylene sulfide (PPS) polymer nanoparticles accumulate in DCs that exist in lymph nodes (LNs) [162]. Following interstitial injection of PPS nanoparticles, nanoparticle-containing DCs accumulate in the LNs, with levels peaking at 96 h with 40–50% of DCs and other APCs having internalized nanoparticles [162]. In another study, nanoparticles made of poly-D,L-lacticide-coglycolic acid (PLGA) copolymer demonstrated enhanced delivery of antigens to murine bone marrow-derived DCs *in vitro* [163]. More recently, a report demonstrated that HIV p24 protein, absorbed on the surface of surfactant-free anionic poly-D,L-lactide (PLA) nanoparticles, was efficiently taken-up by mouse DCs, resulting in an enhanced cellular and mucosal immune response in mice [164]. However, the clinical study for these nanoparticles has not yet been started.

The most clinically advanced application of nanotechnology for immunotherapy of HIV is the DermaVir Patch that reached Phase II clinical trials [201]. DermaVir, a topical DC-based HIV DNA plasmid vaccine, is a targeted nanoparticle system based on mannose-targeted polyethylenimine polymer, glucose and HIV antigen-coding plasmid DNA formulated into nanoparticles (~100 nm) and administered under a topical patch between the epidermal and dermal skin layers, where large numbers of Langerhans cells and dermal DCs are found [165–167]. The nanoparticles

are delivered to epidermal Langerhans cells that trap the nanoparticles and mature to become highly immunogenic on their way to the LNs. Mature DCs, containing the nanoparticles, present antigens to T lymphocytes inducing cellular immunity. DermaVir is immunogenic and safe in SIV-infected rhesus macaques, and a novel formulation, which includes an IL-15 plasmid intended to enhance HIV-specific memory T-cell production, is being prepared for testing [168,169]. Preclinical studies and Phase I clinical trials demonstrated safety and tolerability of the DermaVir patch, which led the progression to Phase II clinical trials to assess the safety, tolerability, immunogenecity and antiretroviral activity of the DermaVir patch (LC002) in treatment-naive, HIV-1-infected individuals and to investigate the effect of therapeutic immunization on the quantity of HIV-specific T-cell precursors during cART followed by STI as the antiretroviral-sparing concept in HIV-infected patients currently under cART [201]. This is the first nanotechnology-based immunotherapy for HIV that has reached clinical studies and further work in this area is encouraged.

Radioimmunotherapy

Some scientists at the Albert Einstein College of Medicine of Yeshiva University (NY, USA) have piggybacked antibodies onto radioactive payloads in order to deliver radiation that selectively targets and destroys HIV-infected cells. It is important that the development of a delivery system of microbicidal radiation uses highly specific microbe-targeting mAbs. By attaching radioactive material to a particular antibody, radiation can be targeted at specific cells that express the corresponding antigen, minimizing collateral damage to other tissues. This experimental treatment, termed radioimmunotherapy (RIT), could be used to treat HIV infection. This new modality, which targets and kills HIV-1-infected cells when combined with cART, could have a major impact on the treatment of acute HIV infection through elimination of persistent reservoirs of HIV-infected cells. The Albert Einstein team examined the efficacy of RIT for treatment of HIV infection *in vivo* with a HIV envelope-specific human anti-gp41 mAb 246-D radiolabeled with ^{213}Bi or ^{188}Re. Unlike other HIV-related glycoproteins, gp41 antigen usually is not shed into the bloodstream, which would lead many of radioactive-labeled antibodies to miss their target. Human PBMCs, infected with HIV-1_{JR-CSF} were injected into the spleens of SCID mice and the mice were treated intraperitoneally with radiolabeled mAbs 1 h later. Treatment of mice with ^{188}Re-labeled mAb 246-D, administered either before or after intrasplenic injection, dramatically reduced the number of HIV-1-infected cells. Similar results were obtained after the treatment of mice with ^{213}Bi-246-D. However, ^{188}Re-246-D was more effective *in vivo* than ^{213}Bi-246-D. Consequently, their studies showed that RIT could effectively target and kill HIV-1-infected human PBMCs *in vivo* [170]. However, when using a RIT in HIV-infected patients, we must always be concerned about the short-term (e.g., hematologic toxicity) or long-term adverse effects (e.g., neoplasm). In addition, the application of RIT to HIV infection will require optimization of the dose to ascertain and minimize toxic effects.

Future perspective

TABLE 2 summarizes the currently ongoing clinical studies in the field of immunotherapy of HIV infection [201].

The recent, large SILCAART and ESPRIT studies yielded disappointing results for IL-2, which has been the most promising immunotherapeutic tool for HIV infection. Therefore, the modification of current immunotherapeutic approaches for HIV should be considered. The results of *in vivo* studies, including human trials and animal models, or *in vitro* studies that promise immune restoration in HIV infection must also demonstrate clear improvements of clinical outcomes, such as decreases in mortality and/or morbidities caused by HIV infection. The SILCAAT and ESPRIT studies finally proved that IL-2 is too toxic for immunotherapeutic use and focused attention on IL-7. Most recently, IL-7 is one of the new candidates of immunotherapy in HIV infection. Although much fewer clinical data are currently available for IL-7, it seems that IL-7 is superior to IL-2 in terms of safety and tolerability. Furthermore, IL-21, which has been the most actively researched cytokine immunotherapy until now, could be an important target in HIV infection.

One possible strategy to circumvent the problem of the induction of anti-HIV-1 antibodies is to search for antibodies with novel specifications that can inhibit HIV-1 infectivity and that could be potentially used as a vaccine strategy. Synthetic peptide immunogens are amenable to modifications that may improve immunogenicity and reactivity to HIV infection. Peptide immunogens providing beneficial effects when used in therapeutic immunization programs may provide the basis for future HIV vaccines [61]. A clinical study

that will evaluate the therapeutic value of pulsing overlapping HIV peptides onto PBMCs or whole blood in humans is anticipated, owing to the promising results of previous studies in nonhuman primates [171]. New delivery methods for therapeutic vaccines, for example vaccines that will be administered into the intradermal area by electroporation, and new various immunomodulators that will be used as adjuvants to vaccine therapies, are currently being developed and assessed for use as immunorestoration modalities to combat HIV infection.

A neutralizing antibody against HIV-1 is one of the essential elements of the immunotherapeutic restoration in HIV infection. However, primary isolates of HIV-1 are relatively resistant to neutralization compared with variants selected for growth in cell lines [172]. With regard to the role of neutralizing antibody responses in HIV infection, it remains important to determine whether high-titered and cross-reactive neutralizing antibodies will be produced by active immunization with a novel viral antigen.

Among the immunotherapies using mAb, novel CCR5 mAbs have been yielding the most hopeful results. CCR5 mAbs have several important advantages over existing therapies in terms of infrequent (e.g., single) dosing, favorable safety, limited drug–drug interactions, synergistic effects with other antiretroviral drugs, such as small-molecule CCR5 antagonists or enfuvirtide, and no cross-resistance with small-molecule CCR5 antagonists. Further promising results are warranted for CCR5 mAb, especially in combination therapy with small-molecule CCR5 antagonists.

In addition, ibalizumab could be developed as an important novel therapeutic drug in HIV immunotherapy because its mechanisms of action allow anti-HIV activity, regardless of chemokine receptor tropism, and it is likely that the cross-resistance with other classes of antiretroviral drugs is relatively low. However, further, larger studies in various clinical settings, including treatment-experienced patients or combination with standard cART, must be performed to expand its clinical usefulness. In addition, the most recent clinical studies have not shown sustained anti-HIV activity, despite continued treatment [88]. Furthermore, because emerging resistance to ibalizumab was manifest by reduced maximal percentage inhibition [88], the

Table 2. Ongoing clinical immunotherapeutic trials in HIV-infected individuals.

Treatment drugs and modalities	Phase	Study designs and objects	Study participants	Comments
Ibalizumab	IIb	Randomized, double-blinded, 48-week, multicenter, dose–response study of ibalizumab (800 mg every 2 weeks vs 2000 mg every 4 weeks) plus an OBR	Treatment-experienced patients	TMB-202 study
KD-247	I	To evaluate the safety, tolerability and pharmacokinetics of KD-247	Asymptomatic, treatment-naive individuals	–
UB-421 antibody	I	Open-label, single-dose, dose-escalation study to evaluate the safety and pharmacokinetics of UB-421 antibody	Asymptomatic, HIV-1-infected adults	UB-421 is the antibody targeting the HIV-1 receptor on the CD4 molecule (domain 1) of T lymphocytes and monocytes. The neutralizing activity of UB-421 blocks HIV-1 from binding to its receptor on $CD4^+$ cells
IL-7	I	Randomized, placebo-controlled, double-blind study evaluating the safety of subcutaneous, single-dose, recombinant human IL-7	HIV-1-infected subjects who are receiving ART	–
TNX-355	II	Multicenter, randomized, double-blind, placebo-controlled, three-arm study of the anti-CD4 monoclonal antibody TNX-355 with OBR	Treatment-experienced patients	–
Lymphocyte infusion		Immunologic and virologic response in HIV-infected HLA-B*57 progressors after infusion of lymphocytes from HIV-infected HLA-B*57 'elite' long-term nonprogressors	Patients failing ART	To evaluate the effect of giving HLA-B*57 to white blood cells from an individual with a controlled HIV infection to an individual who cannot control HIV infection, as a form of HIV treatment (apheresis)

ART: Antiretroviral therapy; OBR: Optimized background regimen.
Data from [201].

development of resistance to ibalizumab must be overcome. While the Phase IIa study is complete, a 24-week Phase IIb dose-comparison study for ibalizumab is still in progress [201].

Virostatic action may be a novel multipronged approach for attacking HIV. It has primarily been studied in patients with advanced HIV disease as the salvage therapy or as components of trials involving STI. However, virostatics may have a role either in primary treatment or as adjuvant to existing cART. Several critical problems regarding immune-based therapies remain to be answered, including the most proper clinical settings for therapy, the optimum administration schedules (dosing, duration and intervals) and ideal antiretroviral drug combination. Additional cytostatic small molecules, which can result in cell-cycle arrest (e.g., rapamycin, roscovitine and resveratol, among others), could be candidates for virostatics therapy in HIV infection [173,174]. A recent small, randomized, double-blind, placebo-controlled pilot study demonstrated that leflunomide, which was approved by the US FDA for the treatment of rheumatoid arthritis, was effective in reducing immune activation in chronic HIV-infected individuals and was well tolerated with low-grade adverse events [175].

The immunomodulatory effects of minocycline promise to reduce the damaging immunopathogenesis resulting from HIV infection, particularly in tissues such as the brain. The immunomodulatory and anti-HIV effects of minocycline strongly suggest that it would be effective as a novel maintenance anti-HIV agent with cART. Minocycline could prevent reactivation of HIV from the $CD4^+$ T lymphocyte latent reservoir and provide therapeutic immunomodulation by dampening chronic immune activation and inflammatory processes that contribute to pathogenesis. Besides virostatics and minocycline, oleanolic acid, desferrioxamine and CpG oligodeoxyneucleotides are other possible immunomodulatory drugs for the treatment of HIV infection.

Executive summary

- Alternative adjunctive immunotherapeutic strategies, which complement combined active antiretroviral therapy (cART), remain active research foci.
- The development of new immunotherapic strategies for the treatment of HIV infection is critical, especially for immunologic nonresponders to cART and for use in supportive adjuvants for structured treatment interruption of antiretroviral therapy for antiretroviral drug conservation.
- The results of two recent, large studies, The Multicenter Randomized Study of the Biological and Clinical Efficacy of Subcutaneous Recombinant, Human IL-2 in HIV-infected Patients with Low CD4 Counts Under Active Antiretroviral Therapy (SILCAART) and The Evaluation of Subcutaneous Proleukin in a Randomized International Trial (ESPRIT), demonstrated that IL-2, combined with antiretroviral therapy, did not result in clinical benefits when the development of opportunistic disease or death from any cause were considered as outcome indicators.
- The SILCAAT and ESPRIT studies finally proved that IL-2 is too toxic for immunotherapeutic use, and focused attention on IL-7. Most recently, IL-7 has been a new candidate for immunotherapy in HIV infection.
- HIV DNA therapeutic vaccines, including immunomodulators of various cytokines or dendritic cells, are being actively evaluated.
- A neutralizing monoclonal antibody (mAb) against HIV-1, such as ibalizumab, KD-247 or CCR5mAb, is an essential element in immunotherapeutic restoration with HIV infection.
- The emerging clinical and laboratory data support the view that CCR5 mAb offers several potential advantages over existing cART in terms of potency, tolerability, dosing frequency and other factors.
- Immune-modulating drugs, including antiviral hyperactivation-limiting therapeutics and minocycline, are promising drugs for HIV immunotherapy.
- Nanoimmunotherapy, or nanotechnology-based vaccines, are another major treatment option requiring further clinical studies. The results from the Phase II DermaVir patch clinical trials will be available soon.

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