**Multifarious immunotherapeutic approaches to cure HIV-1 infection**

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Immunotherapy in the context of treated HIV-1 infection aims to improve immune responses to achieve better control of the virus. To date, multifaceted immunotherapeutic approaches have been shown to reduce immune activation and increase CD4 T-lymphocyte counts, further to the effects of antiretroviral therapy alone, in addition to improving HIV-1-specific T-cell responses. While sterilizing cure of HIV-1 would involve elimination of all replication-competent virus, a functional cure in which the host has long-lasting control of viral replication may be more feasible.

In this commentary, we discuss novel strategies aimed at targeting the latent viral reservoir with cure of HIV-1 infection being the ultimate goal, an achievement that would have considerable impact on worldwide HIV-1 infection.

**Introduction**

Combination antiretroviral therapy (cART) reduces morbidity and mortality in HIV-1-infected individuals; however, it cannot eradicate the virus and does not enable the immune system to control HIV-1 replication. Viral reservoirs persist and viral rebound is seen following treatment interruption. Immunotherapy in treated HIV-1 infection aims to diminish viral reservoirs by reversing T-cell dysfunction and modifying immune responses to mirror those found in long-term nonprogressors or HIV controllers. Ideally, immunotherapeutic approaches targeting the vulnerable T-cell population would induce and maintain HIV-1-specific responses, increase polyfunctionality, enhance thymic function, reduce levels of immune activation and reverse T-cell anergy and exhaustion.

Novel approaches may further benefit clinical outcomes by providing more sustained viral control, preventing reservoir re-seeding, reducing risk of both HIV-1-related and inflammation-related morbidity and mortality, and most optimistically even eradicating the reservoir.

In this context, the rational design of interventions must take into consideration the sequence, timing, dosage and kinetics of immunotherapeutic agents, in order to optimize the desired immune response. A major target in HIV-1 eradication is to reactivate latent integrated provirus in cART-treated patients, however reports suggest that current latency reversal agents alone are not sufficient to expunge the HIV-1 reservoir. The induction of robust virus-specific cell-mediated responses that will eliminate reactivated provirus is thought to be key. The same immunological principles may apply in the absence of eradication where functional cure is achieved by maintenance of viral control, hence the renewed interest in therapeutic vaccines and immunotherapies to be used in conjunction with candidate latency reversal agents.

**Early Initiation of cART Facilitates Efficacious Immunotherapeutic Intervention**

Studies seeking to optimize the timing and duration of cART have highlighted the advantages of early intervention with antiretroviral drugs. Initiation of cART in early HIV-1 infection results in lower residual viral reservoirs compared to levels observed in individuals treated during chronic HIV-1 infection. Moreover, early treatment with cART restrains the level of immune activation and facilitates...
improved polyfunctionality of HIV-1-specific T-cell responses. Early treatment also prevents the latent viral reservoir from becoming dominated by HIV-1 variants that have mutated to escape cytotoxic T lymphocytes (CTL); this has implications for both therapeutic vaccine design and HIV cure strategies. Furthermore, earlier initiation of cART and higher nadir CD4 T-cell counts have been associated with the preservation of functional recall T-cell responses. In a study investigating 2288 Volume 11 Issue 9Human Vaccines & Immunotherapeutics

specific T-cell responses. 12 Early treatment of HIV-1-infected patients demonstrated marked enhancement of HIV-1-specific responses in patients that had both higher nadir and pre-immunotherapy baseline CD4 T-cell counts. For this reason, patients enrolled in our recent Phase I immunotherapy trial (ClinicalTrials.gov Identifier: NCT01130376) were carefully considered in terms of CD4 T-cell counts and duration of suppressed viremia (Fig. 1). On an individual patient basis, those demonstrating the greatest benefit from immunotherapy (with improved clinical and immunological parameters) received cART at earlier stages following infection, which concurs with a number of reports. There is an emerging consensus that early initiation of cART is an essential aspect of long-term control of HIV-1 and likely to be critical in future latency reversal approaches.

Essential Role of Therapeutic Vaccines to Provide Antigenic Stimulus

To date no therapeutic vaccine has been licensed for use in the treatment of HIV-1 infection. However, immunization in conjunction with cART may lead to a reversal of anergy by selective induction and activation of specific memory T-cell responses against a number of viral proteins. By presenting viral proteins in novel ways, with or without specific adjuvants, or by presenting both immunodominant and subdominant epitopes, specific beneficial memory responses may be induced or augmented, and T-cell anergy reversed. That said, even administered in the context of cART, therapeutic immunization is likely to have little efficacy if used without other concomitant immunotherapies due to the immunocompromised status of chronically HIV-1-infected patients. In a study we recently conducted, we hypothesized that HIV-1-specific responses would be enhanced by the administration of cytokines and recombinant human growth hormone (rhGH) in chronic treated HIV-1 infection. Timing and delivery route of immunization were carefully considered in order to achieve the most robust antigen-specific responses. In this Phase I clinical trial, the FIT Biotech GTU® Multi-HIVB DNA vaccine was administered via 10 intradermal injections resulting in modest immunogenicity, whereas another therapeutic DNA vaccine study reported markedly enhanced HIV-1-specific CD8 T-cell responses following exfoliation of the skin and topical administration of the immunogen with a patch. In vivo electroporation delivery of a DNA vaccine was found to significantly improve cellular immune responses in terms of rate, magnitude, duration and breadth to a number of antigens, which concurred with results from animal models demonstrating improved responses with electroporation. The GTU® Multi-HIVB vaccine used in untreated

| Baseline characteristics | Randomisation |
|--------------------------|--------------|
| Plasma HIV-1 RNA < 50 copies/ml | Arm 1: Therapeutic immunisation |
| CD4 T-cell counts > 400 cells/mm³ blood | IL-2 and GM-CSF |
| Age > 18 years | rhGH |
| On cART ≥ 8 months | Arm 2: |
| | IL-2 and GM-CSF |
| | rhGH |
| | Arm 3: |
| | IL-2 and GM-CSF |
| | rhGH |

Figure 1. Immunotherapy trial treatment schedule overview: A multifaceted approach. In our recent study, patients were eligible to participate if they were over 18 y of age, chronically infected with HIV-1, on a stable cART regimen for ≥6 months, with CD4 T-cell counts >400 cells/mm³ blood, and plasma HIV-1 RNA <50 copies/ml. Subjects could not be receiving or have received immunomodulatory drugs or immunisation. Patients who met the eligibility criteria were randomized into one of 3 arms of the trial: Arm 1) to receive the FIT Biotech Plc, Tamper, Finland) at baseline, followed by administration of IL-2 (Aldesleukin, Proleukin; Novartis, Cambridge, UK) and GM-CSF (Sargramostim, Leukine; Berlin, Germany) at baseline, followed by administration of IL-2 (Aldesleukin, Proleukin; Novartis, Cambridge, UK) and GM-CSF (Sargramostim, Leukine; Berlin, Germany).
HIV-1+ individuals induced antigen-specific responses, with beneficial effects on plasma HIV-1 RNA and CD4 T-cell recovery, particularly following intramuscular administration.20,24 Given the renewed interest in therapeutic vaccines as a component of latency reversal strategies, it is of utmost importance to determine the optimal timing and route of administration, in addition to the choice of immunogen.

**Immunomodulatory Biologic Agents and Timing of their Administration**

While previous immunotherapy studies have had limited efficacy to date, the lessons we have learned from them regarding the agents utilized and timing of their administration must be used to inform the rational design of future clinical trials.3,9 Immunotherapy with cytokines in conjunction with cART has been explored as a way of purging the viral reservoir, and targeting immune dysfunction.25 We have recently shown that administration of IL-2 following immunization as part of an immunotherapeutic program that included granulocyte-macrophage colony-stimulating factor (GM-CSF) and rhGH was safe and well-tolerated. In this recent study, immunization was used to prime specific cellular responses within cART-treated-HIV-1+ individuals followed by the administration of a regime of cytokines/rhGH along with further vaccine boosts designed to augment and sustain memory T-cell responses to HIV-1.3 Although the long-term clinical benefit of IL-2 administration in HIV-1+ subjects has been challenged, IL-2 therapy has been shown to increase CD4 T-cell counts,26 reduce the pool of resting CD4 T cells harbouring replication-competent virus,27 and induce HIV-1-specific T-cell responses.28 In our trials utilizing IL-2 immunotherapy (biweekly subcutaneous administration of 5 × 10⁶ Units), we demonstrated greater clinical and immunological improvements associated with this cytokine.15,17 The potential of this cytokine to augment virus-specific T-cell responses along with therapeutic immunization and antiretroviral drugs warrants further investigation. In our studies, we have shown IL-2 to be safe to administer with side effects limited by pre-administration prophylaxis.15,17,28 This lack of toxicity was also reported for daily low-dose IL-2 therapy in HIV-1 infection.29 Timing of IL-2 administration must be considered with regard to any planned immunization schedule. In animal models, relevant responses were preserved and maintained when IL-2 was administered during the antigen-specific T-cell contraction phase of the immune response.30,31 In contrast, IL-2 given pre-immunization in HIV-1+ cART-treated patients failed to increase specific T-cell proliferation.32,33 In 2 case studies, IL-2 administered following tetanus immunization in HIV-1-infected persons was found to better sustain tetanus-specific T-cell responses than IL-2 given together with vaccine or before immunization.34 Therefore IL-2 is likely to be more beneficial for augmenting vaccine-induced responses when given following immunization. In the context of HIV-1 infection, GM-CSF has been reported to increase CD4 T-cell counts and reduce plasma HIV-1 RNA.35 GM-CSF activates antigen-presenting cells, stimulates macrophage differentiation and proliferation.36,37 and may target the viral reservoir in these cells. In addition, IL-2 and GM-CSF have shown efficacy as adjuvants for DNA- or peptide-based vaccines.38 The use of rhGH to treat HIV-1-associated wasting revealed demonstrable improvements in a number of factors, including quality of life.39 Immunological benefits of rhGH include: reversal of thymic involution;40-43 increase in total and naive CD4 T-cell counts;40,41,43,44 restoration of T-cell responses against HIV-1;44-46 and reduction in expression of activation and apoptosis markers.41 Pharmacological and low doses of rhGH have been found to be effective and confer clinical benefit when administered for 12 weeks or more,40-46 which should be taken into account for future strategies.

The novel combination immunotherapy approach was well-tolerated, and subjects who received immunization, IL-2, GM-CSF and rhGH showed the most marked changes at the final study time point compared to baseline.17 This combination of cytokines, subsequent hormone administration and immunization resulted in a significant increase in CD4 T-cell counts, improved CD4/CD8 ratios, enhanced functional T-cell responses to Gag and a reversal of unfavorable (activated/exhausted) immunophenotypes. In a previous study in which IL-2 was administered during weeks 0, 4 and 8, HIV-1-specific lymphoproliferative responses were not detected until week 24.28 These responses were principally directed against Gag p24, and associated with transient ‘blips’ in plasma HIV-1 RNA levels.28 Such ‘blips’ may provide a form of autoimmunization, which could facilitate greater immune control of the induced latent reservoir. Similarly we reported a delayed Gag-specific response at week 48,17 although the last dose of vaccine was given at week 12, and these responses were not associated with transient viral ‘blips’. Transient ‘blips’ in viremia during immunotherapeutic intervention or latency reversal approaches may not necessarily lead to de novo infection, since this is thought to be prevented by cART.47 However, the pharmacokinetic profiles of the drugs administered appear to be insufficient to enable complete tissue penetration, such that a program of cART intensification should be considered in this setting, for example including CCR5 antagonists.48 Administration of IL-2 and GM-CSF in cART-treated patients with inadequate immune reconstitution despite virological suppression was found to result in HIV-1-specific proliferative responses and IL-2 production.49 Changes observed in our recent trial were most striking for subjects who received all interventions, suggesting a combinatorial outcome of these agents with distinct biological effects. While there was an initial cytokine-induced immune activation, which was short-lived, overall there was a reduction in activation state for individuals who received IL-2 and GM-CSF compared to subjects who did not receive these cytokines. This was accompanied by decreased surface expression of the negative regulator, programmed cell death 1 (PD-1), on both CD4 and CD8 T cells at week 48 compared to baseline for all...
Proviral DNA and Quantification of Replication-Competent Virus: Prospective Biomarkers to Measure Reversal of Latency

Across a number of our studies we have shown reductions in proviral HIV-1 DNA, be it with early cART, rhGH therapy or combined vaccine/cytokine/hormone immunotherapy. In our recent study, on an individual patient basis, subjects who showed the most improved HIV-1-specific responses at week 48 also showed a reduction of proviral DNA, which might be due to the low levels of proviral DNA at baseline, as these individuals started cART in the first month following diagnosis. To date there is no universally agreed method to accurately quantify latent infection, i.e. measurement of the proportion of integrated HIV-1 DNA capable of infectious virion production.

Long-lived resting memory CD4 T cells along with other tissue reservoirs that include the brain, lymphoid tissue (lymph node and gut), bone marrow, and genital tract, may each contribute to viral rebound and are difficult to sample clinically. In some of these tissue reservoirs drug concentrations may be suboptimal. Understanding the generation and maintenance of latent proviruses and latently infected T cells is central to HIV-1 cure strategies, however the mechanisms involved are complex and beyond the scope of this commentary. Currently, at least 2 steps are thought to be essential: HIV-1 induction and expression from latently infected cells; and virus-specific CTL recognition and killing of infected cells. Studies evaluating the extent of latency and potential for its reversal have been varied. The vast majority of viruses in the latent reservoir of patients treated during chronic HIV-1 infection comprise CTL escape mutations, which are rare in the reservoirs of subjects treated during acute infection. Recent work shows that only 12% of proviruses are non-mutated and replication competent. However, measurement of HIV-1 latency reversal ex vivo using a different approach revealed that only 1.5% of proviruses in resting CD4 T cells were able to produce virions following CD3/CD28 activation. Considering the high proportion of defective proviruses in the latent reservoir (88–98.5%), an agreed method to accurately measure potential latency reversal by various candidate agents is urgently needed. The reported failures of various first generation latency reversal agents on their own resulted in modifying such approaches. To date, a number of potential latency reversal agents have failed to upregulate HIV-1 gene expression in resting CD4+ T cells from treated patients, with the exception of the protein kinase C agonist, bryostatin-1. The next generation of candidate agents will hopefully yield more promising results. Ongoing and planned trials of these interventions are incorporating the strategies we have reviewed here, including early cART, therapeutic immunization, cytokines and cART intensification (Table 1). A number of clinical trials are optimizing dosing, sequence and timing, in addition to utilizing multifaceted approaches. Combinations of drugs with pharmacokinetic profiles that achieve comprehensive tissue distribution are needed to target latency, as we fill gaps in our knowledge regarding in vivo latency mechanisms. As mentioned, the methods to measure latency reversal are varied. This is particularly troubling because the emerging consensus is that existing assays either overestimate or underestimate the size of the latent HIV-1 reservoir. The ultimate measure of success for latency reversal strategies will be structured treatment cessation, though this approach must be used with great caution.

Conclusions and Perspectives

Irrespective of the mechanisms responsible for the establishment of HIV-1 latency, it represents a major hurdle for HIV-1 clearance. A number of agents and strategies have been proposed to activate latent HIV-1 in order to deplete the reservoir by a combination of viral cytopathic effects and modulated cell-mediated immunity ultimately resulting in control of HIV-1 replication. Such control is observed in long-term nonprogressors or HIV controllers and forms the foundation for functional cure. Larger studies employing various biological agents and accurate quantification of latency reversal are needed to fully elucidate the clinical benefit of these therapies. Approaches aimed at purging virus by stimulating HIV-1-specific T cells in the absence of potentially detrimental global T-cell activation, need not abandon strategies employing low-dose IL-2 immunotherapy in addition to treatment with other agents described herein. Whether at lower physiological or higher pharmacological doses, current candidate agents have been associated with improved thymic function, enhanced T-cell functionality, lessened immune activation, and reduced proviral DNA; again lending weight to the idea of synergistic approach. Longer-term follow-up would assess whether or not these improvements are maintained. Current endeavors seek to establish consensus on combined therapies, their optimal timing and dosing, given the antigen loss post-cART. There has been an optimistic shift toward the development of...
Table 1. Immunotherapeutic interventions and their potential for HIV-1 latency reversal: A combined approach to induce cure of HIV-1 infection

| Intervention | Immunological benefits | HIV-1 reservoir and latency reversal |
|--------------|------------------------|-------------------------------------|
| None         | Infected CD4+ lymphocytes and cells of the monocytic lineage; dysfunctional APC; dysregulated NK cells; excessive immune activation; immunosuppression; T-cell anergy and unresponsiveness to HIV-1 | Establishment of the latent reservoir |
| cART during chronic HIV-1 infection | Increased numbers of CD4 T cells (including naive); functional improvement in T-cell responses to recall antigens; partial normalization of activation, exhaustion, and regulatory function; some normalization of NK cell and APC function; incomplete reconstitution of fully functional HIV-1-specific CD4 and CD8 T-cell responses | Persistence of latent reservoir not impacted by either cART or immune reconstitution; latent reservoir dominated by CTL escape mutations |
| cART during acute HIV-1 infection | Increased numbers of CD4 T cells (including naive); improved functional multipotency of HIV-1-specific responses; enhanced immune reconstitution and better response to immunisation/immunotherapy | Reduced size of the latent viral reservoir comparable to that observed in LTNP; latent viruses carry fewer CTL escape mutations |
| cART intensification (e.g. CCR5 antagonists) | Favourably adjusts HIV-1 associated T-cell activation and differentiation profiles; prevention of de novo infection of susceptible cells from viral replication or reactivation | Potential to prevent reservoir re-seeding |
| HIV-1 immunogens | Provision of unpathogenic antigenic stimulation; induced/boosted anti-HIV-1 functional responses (new and memory) | Potential to deplete viral reservoirs (or at least reduce these to levels observed in LTNP or elite controllers) |
| Cytokines (e.g., IL-2) | Increased numbers of CD4 T cells; improved T-cell growth, survival, differentiation/maturation; reversal of T-cell anergy; regulation and maintenance of T cells along distinct differentiation pathways; increased frequency and function of T effectors and Tregs, particularly HIV-1-specific CD4 HTL and CD8 T cells | Lower numbers of HIV-1-infected latent CD4 T cells; potential to purge virus through transient viral ‘blips’ |
| Cytokines (e.g. GM-CSF) | Reversal of anergy; increased T effector cells; increased frequency of HIV-1-specific HTL and CTL; enhanced APC and NK cell function | Potential to purge viral reservoirs in cells of the monocytic lineage |
| Hormones (e.g., rhGH) | Increased thymic activity; increased pool of naive T cells; decreased systemic hyperactivation; restored differentiation/maturation, prevention of apoptosis, and promotion of proliferation; increased NK cell function | Possible impact on integrated provirus |
| Latency reversal agents (e.g. protein kinase C agonists) | HIV-1 induction and expression from latently infected cells; proteins/peptides processed and presented to immune cells | Potential to reactivate the latent viral reservoir in infected resting CD4+ cells; however stimulation of HIV-1-specific CTL prior to reactivating latent HIV-1 is thought to be crucial; successful strategies might include HIV-1 immunogens and low-dose IL-2 |
| Immune modulatory drugs (e.g., PD-1/PD-L1 blockade) | Restoration of proliferative and effector function of CD4 and CD8 T cells, respectively; restoration of HIV-1-specific CD8 T-cell effector function due to reversal of exhaustion | Impact on HIV-1 reservoir to be determined |

APC, antigen-presenting cell; cART, combination antiretroviral therapy; CTL, cytotoxic T lymphocyte; GM-CSF, granulocyte macrophage colony stimulating factor; HDAC, histone deacetylase; HTL, helper T lymphocyte; IL-2, interleukin-2; LTNP, long-term nonprogressor; NK, natural killer cell; PD-1, programmed cell death-1; PD-L1, PD-1 ligand 1; rhGH, recombinant human growth hormone; T-reg, regulatory T cell.

Curative strategies, in which the focus of prospective immunotherapy will not only be to induce and maintain increased T-cell functionality, improve immunophenotypes and confer clinical benefit, but also to target the latent reservoir. While eradication of the reservoir is the aim of a sterilizing cure, containment of the reservoir is essential to achieve a functional cure of HIV-1 infection. Successful immunological approaches may require different step-wise novel interventions, grouping of existing and new generation immunotherapeutic and latency reversal agents, and larger patient cohorts, alongside the development of accurate and consensus measurement of latency reversal, to result in curative intervention. Further detailed investigations are required to identify immunological, virological and clinical biomarkers for accurate assessment of end-point efficacy in the approaches described.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
Acknowledgments
The authors thank Dr. Gareth A D Hardy, Dr. Jocelyn S Downey and Dr. Adriano Boasso for helpful discussion and critical comments.

Funding
The authors were funded by grants from the MRC (Grant number G0501957), Westminster Medical School Research Trust and St Stephen’s AIDS Trust.

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