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Toward an HIV Cure Based on Targeted Killing of Infected Cells: Different Approaches Against Acute Versus Chronic Infection

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Abstract

Purpose of review—Current regimens of combination antiretroviral therapy (cART) offer effective control of HIV infection, with maintenance of immune health and near-normal life expectancy. What will it take to progress beyond the status quo, whereby infectious virus can be eradicated (a “sterilizing cure”) or fully controlled without the need for ongoing cART (a “functional cure”)?

Recent findings—Based on therapeutic advances in the cancer field, we propose that targeted cytotoxic therapy to kill HIV-infected cells represents a logical complement to cART for achieving an HIV cure. This concept is based on the fact that cART effectively blocks replication of the virus, but does not eliminate cells that are already infected; targeted cytotoxic therapy would contribute precisely this missing component. We suggest that different modalities are suited for curing primary acute versus established chronic infection. For acute infection, relatively short-acting potent agents such as recombinant immunotoxins might prove sufficient for HIV eradication whereas for chronic infection, a long-lasting (lifelong?) modality is required to maintain full virus control, as might be achieved with genetically modified autologous T cells.

Summary—We present perspectives for complementing cART with targeted cytotoxic therapy whereby HIV infection is either eradicated or fully controlled, thereby eliminating the need for lifelong antiretroviral therapy.

Keywords

HIV cure; targeted cytotoxic therapy; recombinant immunotoxins; Fc-mediated effector functions; genetically modified T cells; T cell receptor; chimeric antigen receptor

INTRODUCTION

Once dismissed as a mere pipe dream, the quest for a cure of HIV infection has moved with cautious optimism toward the forefront of HIV treatment research [reviewed in (1–4)]. Initial inspiration came from the “Berlin patient”, an HIV-positive man who developed leukemia and was cured of the infection by allogeneic hematopoietic stem transplantation

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from a CCR5 delta32 homozygous donor (5–7). This success was followed by the “Mississippi baby”, an infected newborn who was treated with aggressive combination antiretroviral therapy (cART) beginning 30 hours after birth and continuing for 18 months; the absence of rebound viremia over the next 12 months after cART discontinuation (8) raised great excitement about the potential for very early treatment to prevent establishment of persistent viral reservoirs. This hope was dashed when HIV rebounded in the child around 2 years after cART cessation (9), thereby casting doubt about the potential for early cART alone to achieve a durable drug-free state of HIV remission. In this review, we extend previous suggestions on the potential value of complementing the replication-inhibiting activity of cART regimens with targeted killing of HIV-infected cells (10, 11). Specifically, we propose that the modes of targeted cytotoxic therapy applicable for achieving an HIV cure differ for primary acute versus established chronic infection. For acute infection, relatively short-term treatment with a transiently active modality might provide sufficient complementation of cART to prevent establishment of long-term viral reservoirs, resulting in eradication of virus and infected cells (i.e. a “sterilizing cure”). For chronic infection wherein persistent viral reservoirs have already taken hold, a highly durable mode of targeted cell killing is required to provide continuous suppression of persisting replication and ongoing surveillance and destruction of newly activated reservoir cells (i.e. a “functional cure”). We emphasize that targeted cytotoxic therapy, like a therapeutic vaccine, is conceptually different from cART intensification with additional inhibitors of the virus replication cycle (12) in that it seeks to eliminate cells that are already infected.

TARGETED CYTOTOXIC THERAPIES: MULTIPLE APPROACHES FOR TREATING CANCER

The cancer field has pioneered development of diverse strategies to kill specific target cell types based on their surface over-expression of a molecule(s) that can be targeted by a specific antibody or ligand. These have been extensively reviewed as indicated in the following sections. In considering potential application to HIV infection, it is helpful to distinguish two concepts for targeted cytotoxic therapy:

Transiently active targeted cytotoxic therapy

Various highly potent targeted killing strategies are capable of functioning for only limited duration due to short biological half-life and activity-impairing immune responses. Anti-cancer efforts have led to diverse approaches in this category, including targeted cytotoxic proteins such as recombinant immunotoxins (RITs) (13), antibody-drug conjugates (14), and radiolabeled antibodies (15), as well as more complex modalities such as oncolytic viruses (16) and targeted nanoparticles delivering cytotoxic components (17). Also of considerable promise are agents that act indirectly by recruiting host cytotoxic mechanisms, e.g. antibodies that promote Fc-mediated innate effector functions (complement-mediated cytolysis, antibody-dependent cellular cytotoxicity) (18) as well as bispecific antibodies that redirect T cells to kill selected cell targets (19). Advances (mostly pre-clinical) have been made in developing such approaches to treat HIV and other viral infections (20–24).

Durably active targeted cytotoxic therapy

Gene therapy strategies are under development for sustained *in vivo* delivery of therapeutic proteins for a variety of pathologies including cancer and viral diseases (25–27). Adeno-associated virus (AAV) vectors have shown particular promise, for their ease of administration (intramuscular injection) and ability to elicit sustained high levels of monoclonal antibodies and related proteins in the circulation. Another relevant gene therapy approach involves adoptive transfer of autologous CD8⁺ T cells genetically modified *ex vivo* to express a targeted killing protein construct, such as a cloned T cell receptor (TCR) or a chimeric antigen receptor (CAR). These technologies are demonstrating great promise in the treatment of certain cancers (28), and have been proposed for use against viruses including HIV (29).

APPLICATION OF TARGETED CYTOTOXIC THERAPIES FOR TREATING HIV: CONTEXT MATTERS

The distinct obstacles to curing acute versus chronic HIV infection suggest that these conditions will require different modes of targeted cell killing. The selected examples described below illustrate how choices can be guided by basic considerations and some experimental evidence.

Acute HIV infection: Transiently active modalities may be suitable

HIV-1 latency in humans is established within a few days or weeks after primary infection, raising doubts about whether acute infection can be cured with very early cART alone (9, 30). A recent study of SIV infection in rhesus macaques modeled this therapeutic challenge: initiation of suppressive cART as early as 3 days post-mucosal infection failed to prevent virus emergence upon cessation of a 24-week treatment period (31)**. This raises the question of whether complementing cART with targeted cytotoxic therapy, even for a short period, would significantly increase the chances for virus eradication before reservoir establishment.

Recombinant immunotoxins (RITs) are fusion protein generated by linking two components with distinct functions: a targeting moiety (typically an scFv or a ligand) with high-affinity for the surface molecule of interest, and a cytotoxic moiety that potently kills when internalized into the cytosol of the target cell. Ribosomal inactivating proteins from a wide variety of bacterial and plant species have been favored sources of the cytotoxic component (32); indeed it has been calculated that a single internalized molecule is sufficient to enzymatically kill a cell. RITs derived from *Pseudomonas aeruginosa* exotoxin A (PE) have yielded highly favorable early phase clinical results against certain leukemias (13). In collaboration with Dr. Ira Pastan and coworkers at NCI, NIH, we have developed and characterized RITs based on *Pseudomonas aeruginosa* exotoxin A (PE) that target HIV-1 gp120 on the surface of infected cells. Figure 1 shows two anti-HIV RITs with different N-terminal targeting motifs: CD4-PE containing the first two ectodomains of human CD4, and 3B3-PE containing the scFv from a mAb directed against the CD4 binding site of HIV-1 gp120. Extensive *In vitro* analyses [reviewed in (20)] illustrated the highly potent and specific targeted cytotoxic activities of both RITs against diverse HIV-1 isolates, replicating

in relevant human cell types (PBMC and monocyte-derived macrophages). Importantly, marked synergy was observed between RIT and reverse transcriptase inhibitor activities. When translated to the thy/liv SCID-hu mouse model (33), complementation was striking for treatment of acute infection (initiated immediately post-challenge). As shown in Fig. 2A, viral load in the thy/liv implant was strongly suppressed by (suboptimal) cART at the end of a 1-month treatment period, but rebounded sharply by 1-month after cART cessation. RIT treatment (CD4-PE or 3B3-PE), which alone had minimal effect during an initial 1-month treatment period (not shown), strikingly enhanced the activity of suboptimal cART as illustrated by the absence of virus rebound after cessation of treatment (Fig. 2A). These results highlight the potent complementary action of cART drugs, which block virus replication, and RITs, which kill cells that are already infected. These cooperative activities were much less evident when tested against established HIV-1 infection in this murine model (33). cART (optimal dose, i.e. sufficient to prevent establishment of infection when administered at the acute stage) suppressed viral load significantly but not fully after 1-month of treatment; addition of the 3B3-PE RIT promoted further reduction, but did not eradicate the infection (Fig. 2B). Parallel findings were obtained recently in the bone marrow-liver-thy (BLT) humanized mouse model (34)*: 3B3-PE significantly augmented cART suppression of established HIV-1 infection in various tissues, but did not eliminate the virus (Fig. 2C). Taken together, these findings suggest considerable potential value of RITs to complement cART in efforts to cure acute HIV infection; utility against chronic infection seems unlikely. This distinction is further amplified by the fact that current RITs include highly immunogenic foreign components that may be tolerated for short-term treatment of acute infection, but not for established infection. The significant recent advances in eliminating B and T cell epitopes from PE-based RITs (35, 36) would not seem to override this concern.

Chronic HIV Infection: Durably acting modalities will be required

Anti-HIV/SIV applications are at the forefront of rapidly advancing technologies for gene-based delivery of therapeutic proteins. AAV vectors engendered very high and persisting circulating levels of recombinant neutralizing antibodies that gave substantial protection against SIV (37) and HIV-1 (38, 39) in rhesus macaque and humanized mouse models, respectively. This technology is readily adaptable to the production of proteins for targeted killing of infected cells, including broadly reactive anti-Env monoclonal antibodies genetically optimized for Fc-mediated effector functions as well as bispecific antibodies to redirect T cell-mediated cytotoxicity.

The alternative approach involving adoptive transfer of genetically modified autologous T cells would mimic the cell-mediated immunity sought by therapeutic vaccination (40) or observed in elite controllers (41), hopefully enabling long-term cessation of cART. Preclinical anti-HIV applications have been described for both alternatives of this approach, i.e. TCRs (42, 43) and CARs (44–51). Several factors should be considered in weighing their potential value against HIV:

1) Minimization of viral escape. For an scFv-based CAR, a broadly neutralizing antibody is the obvious choice. However HIV-1 can readily mutate to escape a single antibody despite

its broad reactivity (52), an scFv-based strategy probably would require (simultaneous) treatment with multiple CARs targeting different epitopes. A CD4-based CAR may circumvent this concern, given the presumed requirement for all viable HIV-1 clinical variants to retain usage of the primary entry receptor. CARs employing CD4 as the targeting motif were tested in clinical studies more than a decade ago (53–55), and a subsequent analysis of peripheral blood samples verified that the CARs persisted and retained expression and proliferative capacity (56). However their anti-HIV efficacies in the hosts were modest; this could be attributed to the use of “first-generation” CAR signaling domains, and possibly also to the susceptibility of the transduced CD8⁺ T cells to HIV infection via the CD4-based transgene. Adoptive immunotherapy using cloned TCRs similarly suffers from the well-known selection for CTL escape mutants in infected hosts (57).

2) Minimization of immunogenicity. Clinical studies in the cancer field have encountered function-impairing antibody responses against scFv-based CAR, primarily against the framework of the murine scFvs currently in use; anti-idiotypic responses against the variable regions have also been noted (58, 59). Presumably this issue is minimized with a CD4 CAR, which, aside from the protein linker and junction segments, consists of all-human, nonvariable components. Similarly human TCR transgenes likely pose minimal risk for eliciting a function-impairing immune responses.

3) Restrictions of activity. A major advantage of CARs is that they are MHC-independent and do not require antigen processing, unlike TCRs on CD8⁺ T cells, which recognize peptide/MHC-I complexes. This latter requirement may prove particularly disadvantageous for treating HIV, given the down-modulation of surface MHC-I in HIV-infected cells (60).

4) Variety and kinetic availability of epitopes for TCRs versus CARs. Anti-HIV CARs are limited to targeting Env, the only virus-encoded protein on the infected cell surface. By contrast, TCRs can target a diversity of HIV-derived peptide/MHC complexes, and the kinetics of their surface presentation during the virus replication cycle influences CTL susceptibility (57). The relatively late expression of Env epitopes, closer to the onset of progeny virus release, has been proposed to compromise the effectiveness of CTL against Env relative to Gag, an effect that is amplified by the ability of Gag-specific CTL to recognize antigen delivered by the incoming virion, before viral protein expression (61). Thus CAR-modified T cells may be at a disadvantage relative to those expressing a TCR against Gag or other viral epitopes expressed earlier in the replication cycle.

5) CAR-mediated susceptibility of transduced CD8⁺ T cells to HIV infection. It has been suggested that a CD4 CAR might render transduced CD8⁺ T cells susceptible to HIV infection (44, 47, 49), since they endogenously express abundant levels of the CCR5 coreceptor (62). Thus additional genetic modifications to the CAR construct may be required to prevent this undesired effect, such as additional sequences (e.g. anti-HIV siRNA) that protect against infection.

6) Mispairing of TCR chains. A TCR transduced into CD8⁺ T cells may be subject to mispairing with the endogenous TCR alpha and beta chains. In addition to reducing surface expression of the transgene, there is the possibility of generating a self-reactive TCR.

An additional concern that may compromise both engineered TCRs and CARs is the emerging notion that follicular CD4⁺ T helper cells (Tfh) in the germinal centers of peripheral lymph nodes appear to be major sites of HIV-1 replication during chronic infection. These sites appear to be sanctuaries protected from influx of CTL (63–67), and

their contributions to virus persistence become particularly prominent in the face of a strong CTL pressure as recently shown (68)**. This new study of SIV-infected rhesus macaques demonstrated that the low level productive infection in elite controllers was confined to Tfh in the B cell follicles of secondary lymphoid tissues, due to elimination of infected CD4⁺ T cells from extra-follicular sites by highly potent CTL, which are excluded from the follicular zone (68)**. The authors note the profound implications of the germinal center sanctuary for immunotherapeutic efforts to clear persisting virus and achieve a functional cure. On the other hand, the fact that this phenomenon was observed in elite controllers may suggest that potent genetically modified T cells suppress infection sufficiently to achieve this desirable status.

CONCLUSIONS

As the HIV cure research agenda advances, targeted cytotoxic therapy is likely to play an increasingly prominent role. That is not meant to understate the many concerns and questions regarding efficacy, risk, and practicality of the approaches highlighted here. Is the kinetic window of opportunity for curing acute infection so short as to limit the potential beneficiaries to vanishingly small numbers? Will the efficacy of vector-delivered proteins be compromised by their restricted access to sanctuary sites of persisting virus (e.g. CNS, testes), and by their potential immunogenicity over the long term involved in maintain suppression of residual chronic infection? Will genetically modified T cells persist and function for sufficient duration, in all the necessary anatomic sites, to enable long-term freedom from cART? Will scalability issues preclude implementation of individualized genetic therapies in global regions where they might otherwise provide the most benefit? The answers to some of these issues will begin to clarify as results from nonhuman primate studies emerge, and as technologies improve in function and cost. Analogous questions proliferated in the years before development of effective cART, and they should not obstruct the path to finding a cure for HIV infection.

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Key Points

- Targeted killing of HIV-infected cells is a rational complement to cART for curing HIV infection.
- Different modalities of targeted cytotoxic therapy are appropriate in efforts to cure acute versus chronic HIV infection.
- For acute infection, transiently active modalities are suggested such as recombinant immunotoxins or antibodies that act indirectly to promote cytotoxic mechanisms.
- For chronic infection, durably active modalities are required such as genetically modified autologous T cells, to provide continuous suppression of persisting replication and destruction of infected cells activated from latent reservoirs.
- Studies in relevant animal models are required to pave the way for clinical trials.

Figure 1. Anti-HIV recombinant immunotoxins based on *Pseudomonas* exotoxin

A. PE is a single chain protein whose domain organization is readily adaptable for RIT design; the native amino terminal domain I is simply replaced with a protein moiety that provides the desired targeting specificity. The HIV-1 gp120-targeting moieties are either CD4 (domains 1 + 2, yielding CD4-PE) or the scFv of monoclonal antibody 3B3 against the CD4 binding site (yielding 3B3-PE). The C-terminal domain III segment catalyzes ADP-ribosylation of elongation factor 2, causing shut-down of protein synthesis and cell death. The central domain II was originally thought to catalyze internalization, although recent studies indicate that for some RITs it is dispensable for function. Figure previously published in ref. (20).

A. Acute: thy/liv SCID-hu mouse

B. Chronic: thy/liv SCID-hu mouse

C. Chronic: BLT humanized mouse

Figure 2. Activities of anti-HIV RITs in murine models of HIV-1 infection

A. Treatment of acute infection. Thy/liv SCID-hu mice were infected ip with HIV-1₅₉ (8000 TCID₅₀) and either not treated (None) or immediately started on treatment with suboptimal cART alone, or with either CD4-PE or 3B3-PE. Treatment was continued for 1 month, at which time the implants were sampled for analysis of infectious HIV-1. The treatment was stopped and the implants were sampled again 30 days later. Suboptimal cART corresponded to zidovudine (10 mg kg⁻¹ day⁻¹), lamivudine (10 mg kg⁻¹ day⁻¹), and ritonavir (20 mg

kg⁻¹ day⁻¹). The RITs were administered ip at 50 ng/dose beginning on day 1, 5 doses on alternate days.

B. Treatment of chronic infection. Thy/liv SCID-hu mice infected as in panel A and treatment was begun 3 weeks later with nothing (None), optimal cART (10× the levels in panel A) alone, or with either CD4-PE or 3BG3-PE (same doses as in panel A). Treatment was continued for 1 month, at which time the implants were sampled for analysis of infectious HIV-1. The HIV-1 levels in the untreated animals were the same at the start and end of the treatment period.

C. Treatment of chronic infection. BLT humanized mice chronically infected with HIV-1_{JR-CSF} were either left untreated, treated with cART alone or cART followed by 3B3-PE starting at day 28 after cART initiation. cART (daily ip) consisted of tenofovir disoproxil fumarate, 208 mg/kg; emtricitabine, 240 mg/kg; raltegravir, 56 mg/kg. 3B3-PE was administered ip, 7 total doses on alternate days, 4 doses at 1 microgm/25 g followed by 3 doses at 5 microgm/25 gm.

Panels 2A and 2B: data adapted from ref. (33); Panel 2C: figure adapted from ref. (34).