Combined approaches for HIV cure

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Purpose of review—A serious effort has begun to develop therapies that may be capable of eradicating established HIV infection in man. Because of the biological complexity of HIV infection that persists despite potent antiretroviral therapy, it is widely believed that if such therapies can be developed they will involve complex, multimodality approaches. We highlight some of the recent studies in this effort.

Recent findings—An inhibitor of histone deacetylase has been demonstrated to disrupt latency in man, and new histone deacetylase inhibitors have been identified. Other potential targets, such as histone methyltransferase, protein kinase C, and BRD4, have been recently studied. Model systems, both in primary cells and in animal models, are beginning to be validated. In the clinic, immune-based therapies to aid in the clearance of persistent infection are also being tested.

Summary—It is too early to know what combination eradication therapies for HIV infection will look like in the future, but candidate therapies and model systems to perform preclinical validation are beginning to take shape.

Keywords
HIV; immune response; latency; resting CD4+ T cells

INTRODUCTION

An increasing understanding of the nature of HIV persistence suggests that the inability to cure HIV infection in the context of suppressive antiretroviral therapy (ART) is a complex biological process characterized by a latent reservoir, persistent viremia (wherein the possibility of cryptic viral replication cannot be excluded), and an anergic and dysfunctional immune response. For many years, it was believed that the central obstacle to curative therapies for HIV-1 infection was the lack of validated approaches to the problem of quiescent but replication-competent provirus in resting CD4+ T lymphocytes or proviral latency. A significant emphasis has therefore been placed on identifying small-molecule chemical agents that can abort the state of proviral quiescence, forcing these cells to express HIV genes or proteins with the hope that these cells will become susceptible to the cytopathic effects of viral replication, or clearance by HIV-specific host immune responses.

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Conflicts of interest
D.J.H. is an employee of Merck Research Laboratories, who hold the license for the clinical HDAC inhibitor, vorinostat.
Although we now have the first direct demonstration that such agents can disrupt HIV latency in vivo, it is unlikely that this or any single therapeutic approach will address the multiple mechanisms now believed to fuel HIV persistence. Therefore, as with ART, eradication will almost certainly require a combination approach. Combination strategies aimed at eradication can be broadly considered in two distinct and complementary approaches:

1. combinations of antilatency reagents to effectively and completely induce the expression of replication-competent proviral genomes within biologically diverse cellular reservoirs in vivo, and

2. strategies to augment the immune response and speed up the clearance of infected cells.

In the laboratory, efforts are focused on identifying and evaluating antilatency molecules with different mechanisms in an expanding array of models of latent proviral HIV infection, with the hope of developing more effective combinations. In the clinic, translational human studies are underway to assess both antilatency therapies as well as various immune augmentation strategies.

**DISRUPTING PROVIRAL LATENCY: MECHANISM-DIRECTED STRATEGIES**

A constellation of molecular mechanisms have been demonstrated to contribute to the establishment and maintenance of proviral latency within resting CD4+ T cells (reviewed in Ref. [1]). Integrated proviral genomes may enter and remain in latency because of the entry of the host cell into the resting state, a critical shortage of cellular cofactors required for HIV expression and of the viral transactivator Tat, the establishment of epigenetic marks on chromatin about the long terminal repeat (LTR) promoter such as deacetylation or methylation, and a negative influence of the expression of host transcriptional units in vicinity of the site of viral integration (transcriptional interference). Which of these influences is the most critical and influential during the establishment of latency, and which later plays a key role in maintaining proviral quiescence are not yet fully understood.

Although each of these mechanisms may present a therapeutic opportunity, it is the inhibition of histone deacetylase (HDAC) that has advanced the farthest into clinical testing. Archin et al. [2] directly measured the activity of the class I-selective HDAC inhibitor vorinostat (SAHA) in ART-suppressed patients. A 400mg dose of SAHA was administered, and the change in the expression of HIV-1 mRNA within resting CD4+ T cells was measured during a window of 4–7h during drug exposure. A mean 4.8-fold increase of unspliced HIV-1 gag RNA expression was observed and was significantly increased compared with pre-exposure expression over baseline in all eight patients. This finding directly demonstrates that a single dose of an HDAC inhibitor in vivo can disrupt latent infection, at least within a detectable proportion of the reservoir of persistent infection within resting CD4+ T cells. Multiple-dose clinical studies of SAHA are underway, seeking to define the potential of the drug to deplete latent infection and to affect a substantial proportion of latently infected cells. A major question with respect to this approach, recently highlighted by an in-vitro modeling study [3], is whether expression of HIV will be sufficient to eliminate these cells. Previous studies of the global HDAC inhibitor valproic acid have not demonstrated an ability to deplete latent infection [4-8]. Recently, Routy et al. [9] completed a long and careful cross-over clinical study that found no consistent depletion of latent infection in patients who received 16 or 32 weeks of ART with 500mg of valproic acid given twice a day.

The broad interest in the potential for HDAC inhibitors to disrupt HIV latency in man has stimulated the study of novel and selective HDAC inhibitors. Oxamflatin, a hydroxamate-
Based agent with clinical applications in certain malignancies, as well as analogs, has been shown to induce the expression of the HIV-1 LTR in the cell line models of latency [10,11]. Another newly synthesized and novel class I-selective HDAC inhibitor with HIV-inducing properties in a cell line model, NCH-51, has also been reported [12]. Lastly, Huber et al. [13] have suggested that droxistostat, an inhibitor with some selectivity for HDAC 3, was more effective at inducing HIV LTR expression in a cell line model and hypothesized that selective targeting of HDAC 3 might be preferred. Although these inhibitors are similar in structure to the hydroxamic acid inhibitor SAHA, they exhibit increased HDAC selectivity and increased reactivation of HIV-1 in cell models of latency. However, it is important to note that there is not yet any known correlation between the potency of induction of HIV LTR expression as measured in such model systems and the ability of agents to induce viral expression from the resting CD4+ T cells of patients.

The combinatorial use of other epigenetic mechanisms such as methyltransferase inhibitors with HDAC inhibitors has been suggested for some time, but clear evidence that such a combination would be clinically beneficial, well tolerated, and effective is lacking. Although older studies had suggested that DNA methylation of the HIV LTR might play a role in latency [14], recent studies of methylation in vivo in CD4 cells obtained from patients failed to find evidence of frequent DNA methylation at the HIV promoter [15].

Histone methylation at nucleosomes about the HIV promoter may play a more significant role than DNA methylation. However, the key enzymes that mediate this epigenetic mark – and therefore the best targets for drug inhibitors – are still unclear. Bernhard et al. [16] presented the evidence that the methyltransferase Suv29H1 may direct silencing, although the model system used for testing involved the use of a Jurkat T-cell line recently infected with a reporter viral vector and an inhibitor of low specificity. Conversely, Friedman et al. [17] suggested that the methyltransferase EZH2 might be a more important target for inhibitors designed to disrupt latent infection. Finally, Bouchat et al. [18] studied both chaetocin and BIX-01294, two inhibitors of Suv39H1 and G9a, respectively. They found that exposure of either inhibitor was associated with the detection of HIV RNA within the pools of peripheral blood mononuclear cells or resting CD4+ T cells obtained from ART-treated patients. These effects were enhanced by the simultaneous exposure of cells to either the HDAC inhibitor SAHA or the nontumor-promoting NF-κB inducer prostratin. Again, as with earlier studies, given the imperfect selectivity of some of the inhibitors used, and the experimental conditions tested, it is not yet clear that the exposures achieved in vitro can be safely maintained in vivo, and whether off-target effects that could lead to clinical toxicity are required to mediate the effects observed in the model systems studied. These are the critical questions that require further study.

Another therapeutic approach toward the disruption of HIV latency is the induction of pathways that are downregulated in latently infected cells, including transcription and the mobilization of NF-κB or signaling through the protein kinase C pathway. Previously identified as a potential inducer of latent HIV expression [19], bryostatin has been found by Pérez et al. [20] to synergize with HDAC inhibitors in the disruption of HIV latency. However, bryostatin is a natural product, difficult and costly to isolate, creating a significant challenge to clinical testing and drug development. Therefore, the reporting of fully synthetic analogs of bryostatin with significantly increased potencies may substantially simplify the further testing of these approaches [21].

Finally, several screening programs seeking cellular factors that could be required for HIV expression identified the human factor BRD4 as a potential inhibitory factor [22,23]. This factor is generally associated with gene activation. When several groups tested a novel and potent small-molecule inhibitor of BRD4, JQ1, a potential to disrupt HIV-1 latency and
induce proviral expression was found [24,25,26]. In some studies, JQ1, when used in combination with the NF-κB activator prostratin or PHA, enhanced the in-vitro reactivation of latent HIV-1 in primary T cells. However, the effect seen in resting CD4 cells obtained from HIV+ patients was almost absent [24]. It remains to be seen whether this approach can progress to practical application in man.

DISRUPTING PROVIRAL LATENCY: SCREENING STRATEGIES TO IDENTIFY NOVEL COMPOUNDS AND TARGETS

The use of chemical library screening provides an alternative to identify novel antilatency strategies. This approach requires the use of model systems to represent the biology of latent proviral HIV infection, a phenomenon that primarily occurs in resting CD4+ T cells. Using industrialized, high-throughput assays, the response of a proviral reporter gene construct can be tested against large libraries of chemical entities and siRNA libraries to identify novel compounds and targets for further optimization and biological validation.

Several such efforts have recently been presented, each relying on different cellular assay formats which all have distinct advantages as well as limitations. Among these, the Siliciano laboratory reported the results of a screen was performed in primary CD4+ T cells transduced with Bcl-2 for longterm survival, infected with HIV-1, and allowed to enter latency. In this screen, disulfiram an inhibitor of aldehyde dehydrogenase used to treat alcoholism was found to induce the expression of quiescent genomes in this primary cell model [27]. Disulfiram has many metabolic products, and the active moiety of disulfiram that mediates an antilatency effect is still under study, but one report in the U1 promyelocytic cell line model suggested that disulfiram reactivated latent HIV-1 expression via reduction in PTEN protein, resulting in Akt phosphorylation and activation of the Akt signaling pathway [28]. Although a pilot clinical trial [29] has thus far failed to convincingly demonstrate the disulfiram perturbs latent HIV infection in vivo, understanding whether pharmacologically relevant levels of the active moiety were achieved is required to interpret the results of this study. Encouragingly, the same assay also identified two classes of quinolines, adducts of 5-chloroquinolin-8-ol, and quinolin-8-yl carbamates that induce HIV-1 expression without activating T-cells [30]. A better understanding of the mechanism of action of these novel compounds could speed further rational development.

More commonly, cell line model systems have been used for such small-molecule screening campaigns [30,31,32]. AV6 was identified as an antilatency compound in a high-throughput screen that utilized a SupT1 immortalized T-cell line [31]. AV6 required nuclear factor of activated T cells (NFAT) to induce HIV-RNA expression, lacked HDAC activity, and induced expression in several clonal T-cell line HIV model systems and in pools of infected primary CD4+ T-cells, albeit not latently infected ones. Interestingly, induction mediated by AV6 was increased by the HDAC inhibitor valproic acid, suggesting that combination use with an HDAC inhibitor could be of potential benefit. Although several other screens have been reported, validation of these early hits in primary cell model systems is lacking, and it is unclear whether any or all of these compounds merit designation as lead candidates for future drug development.

Although the number of agents that have demonstrated the capacity to disrupt latent HIV infection in vitro is increasing, efforts to understand whether combining these agents will enhance either the breadth or level of induction are still quite limited. The scientific rationale to support the use of combinations of agents targeting distinct mechanisms that enforce HIV latency – whether to purge latent provirus more rapidly or more completely – to date remains largely theoretical. There is some data in vitro (e.g. Ref. [18]) that such approaches may be of increased efficacy; however, it is not yet clear that any tissue culture
model system can accurately reflect the in-vivo pharmacokinetics and biological effects of combination antilatency therapy or that such increased efficacy will have a clinically meaningful benefit. Combined approaches will likely require validation in animal model systems. Some combinations currently under investigation in animal models include HDAC inhibitors and methyltransferase inhibitors, and HDAC inhibitors and protein kinase C agonists such as prostratin or bryostatin [1].

**COMBINED APPROACHES TO KICK AND KILL LATENT HIV: MEDICINES AND IMMUNOLOGY**

Although we now have evidence that it is possible to perturb the latent reservoir and induce HIV expression in latently infected cells in HIV-infected patients, as noted above, it is not clear that these cells will either produce sufficient HIV gene products to be eliminated by viral cytopathic mechanisms or to be recognized and then eliminated by the host immune system. In addition to latency, a hallmark of HIV infection is the persistent immune dysfunction observed in HIV-infected patients even after many years of suppressive ART. Therefore, another combination approach needed for the eradication of HIV infection may be the use of various immune-based therapies to enhance the clearance of latently infected cells which have been induced to express HIV antigens [3].

Immune-based strategies such as therapeutic vaccines to augment immune-mediated clearance of virus-producing cells could be combined with antilatency therapy. Vaccines that enhance dendritic cell function may be a promising approach [33]. These approaches might also be combined with adjuvants or cytokine-based agents, such as interleukin-7 (IL-7), that could potentiate cytotoxic T-cell activity or restore CD4+ T-cell homeostasis [34]. At least one clinical trial (NCT01019551), Therapeutic Intensification Plus Immunomodulation in HIV-infected Patients (ERAMUNE-01), with IL-7 is ongoing. However, Bosque et al. [35] raised concerns about the end result of the use of IL-7 in this setting as they found that the expansion of CD4 cells induced by a combination of IL-2 and IL-7 in a laboratory model system lead to only a partial reactivation of latent HIV-1 and a greater proliferation of latently infected cells.

Another approach that has received significant interest, but not yet tested in the clinic, is the use of agents that can reverse the exhaustion of the anti-HIV immune response [36]. The failure to cure HIV-1 infection is believed to be the result of latent, replication competent provirus in quiescent CD4+ T lymphocytes as well as T-cell dysfunction stemming from persistent immune activation. Insights into the cellular mechanisms that control HIV-1 gene expression and chronic immune activation have suggested that targeting negative regulators of immune activation on T-cells may accomplish both of these goals. The best studied of these is the negative regulator PD-1. PD-1+ T-cells constitute a preferential reservoir for HIV in vitro, the interaction between PD-1 and its ligand PDL-1 has been shown to suppress HIV-1 production in primary T-cells in vitro and, conversely, blocking PD-1 engagement with its ligand allows for increased viral replication. Importantly, chronic viral infections, including HIV-1 infection, upregulate PD-1 expression and drive immune senescence. Toward this end, anti-PD1 antibodies are currently being tested in nonhuman primate models of HIV infection [37], but results to date have suggested positive effects on immune function as well as viral replication in viremic SIV-infected rhesus macaques.

Finally, there is rationale for combining the various immune-based approaches. Targeting multiple negative regulators to more effectively reverse the exhausted T-cell phenotype is an approach that is being explored in oncology and has merit for consideration in the context of an approach to eradicating HIV. Targeting negative regulators to restore HIV T-cell function can also potentially augment response to therapeutic vaccination.
CONCLUSION

The failure to cure HIV-1 infection is believed to be the result of low level, viral production/replication and the presence of latent, replication competent provirus in quiescent CD4+ T lymphocytes as well as T-cell dysfunction stemming from persistent immune activation. None of the currently available antiviral therapies will address the long-lived reservoir of latently infected cells or immune dysregulation. Insights into the cellular mechanisms that control HIV-1 gene expression, HIV pathogenesis, and immune dysfunction have suggested several rational approaches that together may address the problem of HIV persistence and provide a basis for evaluating combination approaches to eradicate the virus.

Acknowledgments

This work was supported by the National Institutes of Health grants AI082608, MH085597, and DA030156 to D.M.M., R00046 to the UNC CTSA, and AI50410 to the UNC CFAR.

REFERENCES AND RECOMMENDED READING

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

■ of special interest
■■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 252).


Curr Opin HIV AIDS. Author manuscript; available in PMC 2014 May 01.


**KEY POINTS**

- Histone deacetylase (HDAC) inhibitors have emerged as a viable reagent to disrupt latent HIV infection and are most likely to be included in combination therapies to eradicate HIV infection in the near future.

- Other targets to purge quiescent proviral reservoirs have been identified and are under study, and high-throughput screening efforts are likely to discover new targets.

- Immune augmentation strategies to assist in the clearance of persistent HIV infection are likely to play an important role in combination approaches of the future.