OPINION ARTICLE

A new hypothesis on HIV cure [v1; ref status: indexed, http://f1000r.es/3qj]

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Abstract

In this opinion article, I provide the rationale for my hypothesis that nucleoside reverse transcriptase inhibitors (NRTIs) may prevent human immunodeficiency virus (HIV) cure by promoting the survival of cells with integrated provirus. If correct, we may be closer to a cure than we realize.

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Introduction

Current antiretroviral treatment (ART) is extremely effective in controlling replication of human immunodeficiency virus (HIV) and in many patients suppresses the number of virions measurable in peripheral blood, i.e., the HIV viral load, to undetectable levels. Nevertheless, whenever ART is stopped, HIV levels rebound and the disease returns. This lack of eradication is attributed to a stable latent reservoir of HIV-1 in resting $CD4^+$ T lymphocytes and perhaps other susceptible cell types such as macrophages[1]. These cells harbor HIV in the form of replication-competent proviruses that are integrated into the host genome. During effective ART this reservoir decays so slowly that it would theoretically require treatment for 60 years or longer to eliminate it[2].

For this reason, HIV-related research efforts are increasingly being devoted to understanding the nature of this latent virus reservoir and how to eradicate it. Two aspects of the latent virus reservoir have emerged as crucial in maintaining infection. First, HIV is not transcribed or translated from latently infected cells, allowing them to escape detection from the immune system. Second, cells with integrated provirus persist and even expand despite continuous ART[3–5]. To circumvent viral persistence, "kick and kill" strategies have been proposed that attempt to reactivate HIV with latency-reversing agents and then destroy these cells with the help of targeted active or passive immunization strategies. Unfortunately, reactivating cells from infected individuals *ex vivo* has thus far not shown promising results[6,7] and the hurdle for effective immune control of cells reactivated from latency may be high[8,9].

Why cells carrying HIV proviruses continue to expand during ART without expressing viral proteins in the process remains an unresolved paradox. To solve this conundrum tremendous effort is going into characterizing the latent virus reservoir and understanding ongoing immune activation during ART. It is hoped that a synthesis of findings in both areas may provide important clues about navigating available and newly arising treatment options toward a cure.

Mucosal effects of tenofovir

A third area that has been little considered is the effect of ART drugs, both on viral latency and immune activation. Modern antiretroviral combination therapy provides tremendous clinical benefits for HIV-infected patients, dramatically improving quality of life and prolonging life expectancy. Thus, the possibility that a component of ART could paradoxically decrease the chance of a cure being effective had never crossed my mind when we initiated a systems biology evaluation of an ART drug topically applied to the rectal mucosa in a phase I clinical safety trial. This trial, MTN-007 (www.clinicaltrials.gov/ct2/show/NCT01232803), tested the safety and tolerability of a gel containing 1% tenofovir, a phosphonated nucleoside reverse transcriptase inhibitor (NRTI) in development for potential use to prevent rectal HIV transmission[10]. A gel containing 2% nonoxynol-9 (N-9) (a temporary mucosal toxin) was included as a positive control arm, and hydroxyethyl cellulose gel and no gel served as negative controls. Our original hypothesis was that the effects of 1% tenofovir gel on the mucosal transcriptome would be negligible whereas N-9 would activate inflammatory genes. However, upon unblinding of the microarray data, we were surprised to find that tenofovir caused many more genes to change than N-9, more often suppressing than enhancing gene expression[11].

Tenofovir caused three particular changes that bear potential relevance to the HIV cure agenda. First, it strongly inhibited the transcription of a large number of nuclear transcription factors; second, it inhibited the anti-inflammatory function of mucosal epithelial cells; and third, it stimulated signatures of increased cell proliferation and viability. Results obtained from rectal biopsies were replicated in primary vaginal epithelial cells, which also proliferated significantly faster in tenofovir's presence. In addition to the breadth of transcriptional changes, individual effects caused by tenofovir could be large. For example, both *in vivo* and *in vitro*, the drug blocked transcription and protein production of interleukin 10 (IL-10) in the range of 90%[11].

An emerging hypothesis

From these data grew my first suspicion that tenofovir, and perhaps more generally NRTIs, could have unappreciated effects on HIV latency, and may in fact prevent HIV cure by promoting the survival of cells with integrated provirus (Figure 1). Before developing this concept below, I want to caution that many of the statements are preliminary and/or hypothetical, intended to serve as a stimulus to the field for further investigation and verification.

Based on the pronounced inhibitory activity of tenofovir on the transcription of many genes, I hypothesize that it also inhibits transcription of provirus integrated into such genes. Host gene transcriptional activity has been shown to be an important determinant of integrated HIV transcription[12]. This 'integrated virus transcription inhibitor' (IVTI) effect of tenofovir and other NRTIs could explain the transcriptional silence of integrated provirus during ART, since nearly all patients receive an ART regimen containing not just one but two NRTI drugs. Tenofovir's IVTI activity is supported by the preliminary finding that genes reported in two recent studies to be

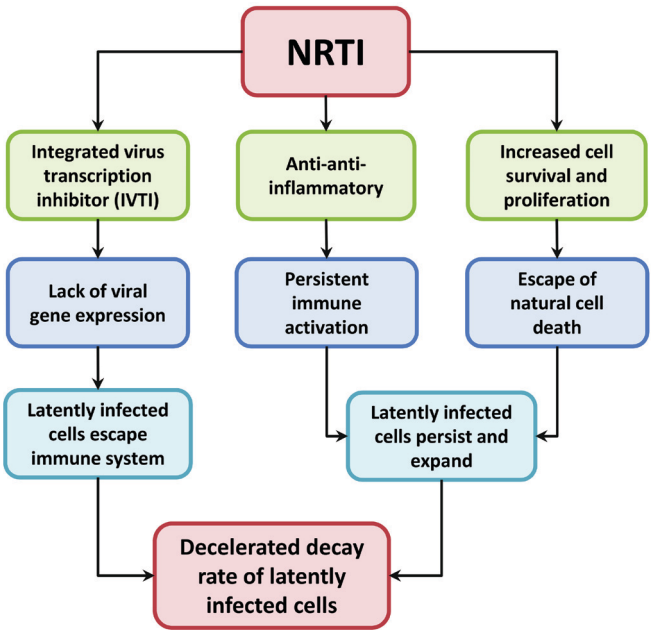

Figure 1. Hypothesized effects of nucleoside reverse transcriptase inhibitors (NRTI) on HIV latency.

preferential sites of HIV integration after periods of ART appear to overlap with genes inhibited in our studies by tenofovir[3,4]. A preliminary analysis of the lists of genes highlighted in the two papers and those found to be strongly inhibited by tenofovir in the rectum showed considerable overlap, including CREBBP, IL6ST, KIF1B, FBXW7, DDX6, IKZF3, ZNF652, DST, CLIC5, GRB2, CEPT1, TAOK1 and PAK2. No overlap was found with genes inhibited by N-9. If true, this overlap would imply that over time NRTIs select for cells in which latent HIV survives because of the drugs' inhibitory effects on transcription of genes hosting integrated provirus.

The IVTI function of NRTIs could be complemented in favoring latency by their inhibitory effect on the immune system's anti-inflammatory circuits. In our study, tenofovir was not directly inflammatory, but its strong inhibition of IL-10, as well as of pathways downstream of the immune homeostatic factor TGF-β, indicated that once inflammation is triggered by an outside event, which could be HIV infection itself, it could be prolonged or perpetuated in the presence of tenofovir. I call this the 'anti-anti-inflammatory' action of tenofovir.

In our cohort of individuals at low risk for inflammation and HIV infection, we did not detect overt inflammation, although tenofovir did significantly increase the density of $CD3^+$ and $CD7^+$ lymphocytes in the rectal mucosa. The participants in CAPRISA 004 (www.clinicaltrials.gov/ct2/show/NCT00441298), an efficacy trial that demonstrated an overall 39% protective effect of vaginal 1% tenofovir gel[13], were at much higher risk for inflammation and HIV infection. This may have uncovered an interesting paradoxical effect of tenofovir: unpublished data in a subset of CAPRISA 004 participants suggest that in the presence of inflammation the risk of HIV infection increased markedly more in the tenofovir than the placebo arm (personal communication). Further analyses by CAPRISA 004 investigators are ongoing. If confirmed, I would hypothetically attribute this effect to tenofovir's anti-anti-inflammatory action.

Thus, the anti-anti-inflammatory effect of NRTIs could explain why the massive immune activation caused by primary HIV infection never completely reverses despite effective ART. Interestingly, a similar persistence of immune activation is observed in HSV infection treated with acyclovir, a nucleoside analogue related to NRTIs, which also inhibits DNA synthesis by terminating the growing strand. That HSV-induced local immune activation does not resolve well with acyclovir treatment has been identified as a possible reason why the HSV-associated increase in HIV susceptibility does not reverse when women with genital HSV infection receive acyclovir[14–16]. Perhaps acyclovir has some of the same anti-anti-inflammatory properties as tenofovir.

Residual immune activation perpetuated by NRTIs could drive the ongoing expansion of cells harboring integrated provirus, and their IVTI function could simultaneously limit transcription of these proviruses. Indeed, our analysis so far indicates that the genes generally turned on by cell activation and the HIV-hosting genes inhibited by tenofovir are different, potentially explaining this apparent paradox. Additionally, the direct cell proliferation- and viability-enhancing effects of tenofovir could contribute to the persistence and expansion of latently infected cells.

ART and anatomic sites of HIV latency

In theory, the HIV latency-inducing effects of NRTIs would likely be strongest where drug concentrations are highest *in vivo*. Studies on tenofovir's biodistribution after oral administration show that it highly enriches in gut tissues[17–19], which is believed to harbor a major portion of the latent HIV reservoir[20]. Estimates also indicate that the rectal concentrations of tenofovir diphosphate, the active intracellular metabolite, are comparable after seven days of oral tenofovir dosing or a single dose of intrarectal 1% tenofovir gel (personal communication, Dr. Craig Hendrix, Johns Hopkins University). Thus, it is likely that some of the effects we observed in the rectal mucosa after topical application also occur after oral dosing, in particular with years of administration and in combination with a second NRTI.

Of note, after oral dosing, NRTI drug concentrations may be even higher in the small intestine than in the colon and rectum, because in the upper gastrointestinal tract locally dissolving drug likely adds to drug distributing from the blood stream. If NRTIs do indeed promote latency, then high drug concentrations would make the small intestine favorable for HIV latency, consistent with the observation that within the gut the duodenum and ileum were preferential sites of residual HIV DNA and unspliced RNA in ART-suppressed patients[20,21]. In fact, if NRTIs did not enhance latency, it would be difficult to explain why residual HIV is found precisely where antiretroviral drug concentrations are highest.

Two special cases of cure without ART

Circumstantial evidence suggests that pharmacological ART is not required to cure HIV/simian immunodeficiency virus (SIV) infection. The only adult patient ever cured of HIV infection, the "Berlin patient" Timothy Brown, received a stem cell transplant from a donor homozygous for a 32-bp deletion in the CCR5 allele, which provides resistance against HIV-1 infection[22]. He took suppressive ART until the point of his first stem cell transplant, at which point he stopped all ART and never resumed it. Of course, he received a powerful alternative to pharmacological ART in the form of two CCR5-deficient stem cell transplants, carried out about one year apart. However, he did not achieve complete chimerism for some time after transplantation, because CCR5-expressing macrophages were still present in rectal biopsies 5.5 months following the stem cell transplants[22,23], and thus potential HIV target cells were not completely eliminated at that point. This could have provided a hold for residual HIV. Perhaps removing the hypothetical latency-favoring activity of the NRTI drugs could have contributed to his cure.

In contrast, two HIV-1-infected patients in Boston who also received stem cell transplants continued ART in the peri- and post-transplantation period, and were not cured[24]. Notably, though, these two patients did not receive CCR5-negative stem cells, which provided a less favorable scenario than in the Berlin patient's case.

The only animals ever cured from a highly pathogenic SIV infection were rhesus macaques who had been vaccinated before SIV challenge with SIV-protein-expressing rhesus cytomegalovirus vectors[25]. Although the vaccinated rhesus macaques all showed signs of ongoing systemic infection for weeks or months after challenge, protected monkeys lost all indications of SIV infection over time, consistent with immune-mediated clearance of an established lentivirus infection. None of these animals ever received ART.

While it was suggested that establishment of a latent SIV reservoir might have been prevented by the persistently high frequencies of vaccine-induced SIV-specific $CD8^+$ T lymphocytes, early on many of these animals showed clear signs of productive infection, which requires viral integration. Thus, a latent reservoir was likely established. However, in the absence of the latency-prolonging effects of NRTIs the decay rate of provirus-containing cells could hypothetically have been accelerated, due to faster natural cell death, less cell expansion, and higher expression of viral proteins, allowing immune recognition by the SIV-specific cytolytic T cells. No viral blips were detected in any animals beyond 70 weeks, perhaps offering a clue as to the time frame required to eradicate a latent reservoir in the absence of NRTIs. However, the pool of latently infected cells was likely small in these animals, and eradication of a larger reservoir may take longer.

Conclusion and outlook

In summary, given that (1) NRTIs may prevent immune detection of latently infected cells by inhibiting transcription of integrated virus, (2) NRTIs may increase persistence of cells with integrated virus by perpetuating inflammation and enhancing cell proliferation, (3) the only monkeys ever cured of SIV infection never received ART, and (4) the only adult patient ever cured of HIV infection discontinued ART before initiating another powerful antiviral therapy, I hypothesize that effectively suppressing HIV with a strategy that does not contain an NRTI component has curative potential.

Only a few years ago, finding a similarly suppressive alternative to an NRTI-containing ART regimen would have posed a dilemma[26]. Today, powerful second-generation integrase inhibitors and non-NRTI drugs (NNRTIs) are entering early human clinical trials[27–29]. Active vaccination, passively infused neutralizing antibodies and vector-expressed CD4/CCR5 co-mimetics show promise as therapeutic immune interventions[25,30–34], and even more complex strategies such as HIV receptor deletion and specific destruction of integrated viral DNA sequences are progressing[35]. We are thus moving into a phase where effective NRTI-sparing strategies are becoming reality and could offer hope for a cure.

One phase IIb trial (www.clinicaltrials.gov/ct2/show/NCT02120352) is registered to switch HIV-1-infected patients who are initially suppressed with an NRTI-containing regimen to an NRTI-free combination of GSK744 LA, a long-acting injectable formulation of the novel integrase inhibitor GSK1265744[27], and TMC278 LA, a long-acting injectable formulation of the novel NNRTI TMC278 (ripilvirine)[29]. Though not designed to test a cure, this regimen may in fact have curative potential. The study sponsors should consider adjusting their design for that purpose.

Competing interests

No competing interests were disclosed.

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This work by Hladik addresses one of the most important problems of HIV research: the persistence of a “latent reservoir” in spite of the virtually complete suppression of viral replication. Indeed, despite dramatic progress in the “functional” cure of HIV infection, we have failed to eradicate virus from the infected organism. The author suggests that the nucleoside reverse transcriptase inhibitors (NRTIs), in particular tenofovir, have a side effect: they suppress a natural anti-inflammatory activity, thus, probably indirectly, facilitating inflammation. Also, ART does not prevent cell proliferation and in some cases even facilitates it, thus increasing the number of cells with the integrated provirus. This proliferation may contribute essentially to the establishment and increase of the HIV reservoir.

The hypothesis suggested in this paper was not tested directly, but the author presents significant arguments in favor of it. His arguments are based on his and others’ research on the effect of tenofovir on tissue explants. One of the strongest effects of tenofovir on human tissues is a blockade of transcription and protein production of IL-10. Moreover, analysis of gene activation in rectal tissue of patients under ART led the author to imply that because of the drugs’ inhibitory effects on transcription of genes hosting integrated provirus, NRTIs select over time for cells in which latent HIV survives.

Finally the author discusses the case of a Berlin patient as well as the curing of non-human primates (rhesus macaques) who have been vaccinated before SIV challenge with SIV protein-expressing rhesus cytomegalovirus vectors. In none of these unique examples of cures was ART used.

In summary, the author suggests that ART suppresses anti-inflammatory responses, indirectly promoting inflammation. This may be true not only for tenofovir and HIV but also for acyclovir and HSV, as these drugs, despite suppressing HSV facilitate HIV in the treated individuals.

This is an original and interesting hypothesis that may explain some aspects of HIV infection that are not understood yet. By the way, it may explain why HIV-1 patients under ART never return to normal even if their virus, which has been suppressed for years, is undetectable, while the immune activation persists, probably leading to various AIDS-unrelated diseases and to premature aging. The author may further develop his hypothesis to cover the area of age-related diseases in functionally cured patients under ART

On the other hand, it is important for the author to emphasize more strongly the success of modern ART as a universal treatment. Even if the author’s hypothesis be proved, the effects he described would be at most the side-effects of the successful treatment, although very important ones. Moreover, in my opinion,

the establishment and persistence of the HIV reservoir hardly can be explained by only one factor.

Finally, the association of the cure with the lack of ART application in just three cases is of course anecdotal, and this fact should be emphasized more strongly.

All these critical remarks are rather of an editorial nature, and Hladik's original hypothesis certainly deserves to be indexed and to be tested in targeted basic and epidemiological research.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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In the absence of a highly effective preventative vaccine to protect against HIV infection, and with a growing burden of HIV-1 infected individuals currently on or soon to be eligible for antiretroviral treatment, there is a major research effort underway to seek approaches that might yield a functional cure for the disease. As Florian Hladik points out in this Opinion Article, a major challenge and obstacle to the goal of developing an HIV Cure is the fact that, despite years of suppressive antiretroviral therapy, a stable latent virus reservoir persists that can seed the rapid rebound in HIV-1 replication. An event that is consistently observed whenever treatment of chronically infected individuals is interrupted.

In this Opinion Article, Hladik draws on recent research from his laboratory, in which a systems biology approach was taken to understand the impact of a topically applied nucleoside reverse transcriptase inhibitor (NRTI), tenofovir, on gene expression in the rectal mucosa. The results of this study showed that topical tenofovir inhibited the transcription of a large number of nuclear transcription factors, inhibited anti-inflammatory functions of mucosal epithelial cells, in particular IL-10 expression, and stimulated signatures of increased cell proliferation and enhance viability. Similar results were observed in primary vaginal epithelial cells in culture (author reference 11).

Based on this research, Hladik hypothesizes that tenofovir might paradoxically enhance formation and maintenance of the latent reservoir by inhibiting the transcription of proviruses integrated into the suppressed genes, and that such transcriptional silencing could explain the lack of HIV expression in such cells. Support for this theory comes from the fact that genes shown recently to be preferential sites for HIV-1 integration (refs) overlap with those inhibited by tenofovir. Moreover, Hladik proposes that by inhibiting anti-inflammatory functions, tenofovir and other NRTIs could perpetuate residual immune activation and drive ongoing expansion of cells harboring an integrated provirus. An effect that could be exacerbated by the cell proliferation and enhancement of viability also observed following administration of the drug. Because NRTIs are generally delivered orally, the author argues that the above effects would be expected to be strongest where drug concentrations are highest, consistent with observations that residual viral DNA is preferentially found in the duodenum and ileum.

Given the strong base to this interesting hypothesis, it is unfortunate that the author uses somewhat anecdotal examples of situations, where non-NRTI approaches have resulted in apparent “cure” of the latent reservoir, to support his theory. While the mechanism of latent virus clearance has not been defined, in the first of these, the Berlin patient Timothy Brown, did stop ART, but as pointed out by the author, underwent two CCR5-deficient stem-cell transplants that could through a combination of resistant cells and graft versus host reaction have cleared residual latent cells. The second example of apparent cure from ongoing early infection is that observed with cytomegalovirus SIV vaccine vectors. While approximately 50% of infected vaccinated animals do appear to clear SIV infection, the mechanistic basis for this is unknown, and it would seem to be a stretch to argue that this is simply because the animals were not ART treated.

The hypothesis put forward in this Opinion Article – that drugs highly effective in suppressing viral replication might actually play a role in sustaining, in a latent state, the very virus that it so effectively inhibits – seems at first counter-intuitive. Nevertheless, the gene expression data from the phase I clinical trial of tenofovir as a rectal microbicide, do provide a plausible underpinning for the hypothesis and it is one that should be tested experimentally. Indeed, given the diversity of treatment regimens that are ongoing for patients, it seems likely that existing clinical samples may well be available to allow such a study to be performed. Moreover, as noted by the author, clinical trials that have or propose to switch patients from an NRTI-based ART regimen to one lacking NRTIs would provide clinical samples with which to test the hypothesis.

Although not discussed by the authors in the eLife manuscript, if the effect of tenofovir on gene expression is reversible – something that could be tested *in vitro* – then one might anticipate that individuals switching from a NRTI-based regimen to a combination of NNRTI, protease inhibitor, or integrase inhibitor, might be expected to exhibit a more rapid decline in the latent reservoir compared to those remaining on an NRTI regimen. Moreover, it should be possible using RT-SHIVs to test whether non-NRTI suppressive antiretroviral regimens result in a reduced latent virus reservoir compared to those on a tenofovir-based regimen.

A minor point – in the Abstract the author states “(NRTIs) may prevent human immunodeficiency virus (HIV) cure”. This should be softened to “may reduce the likelihood of a cure for human immunodeficiency virus”, since one cannot predict the efficacy of future “cure” approaches.

Finally, while the hypothesis is novel and if proven may provide clues to limiting the latent viral reservoir in patients on ART, it is important to note that tenofovir and a second NRTI, Emtricitabine (FTC) form the base of the 3 drug regimen that is the low-cost mainstay of initial HIV-1 treatment across the continent of Africa and other developing countries. While the long-term goal of a cure for HIV, along with an effective preventative vaccine, will likely be key to controlling the epidemic, it is equally important not to undermine confidence in these highly effective components of current therapy.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.