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Silence the transcriptionally active HIV reservoir to improve treatment outcomes

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Summary:

Persistence of the transcriptionally active HIV reservoir (TAHR) has important implications for people living with HIV (PLWH), including chronic immune activation and inflammation. Supplementing antiretroviral therapy with HIV transcriptional inhibitors could overcome this by silencing the TAHR.

Main Text:

Antiretroviral therapy (ART) has transformed the lives of PLWH; however, it is not curative. Treatment interruption typically results in viral rebound within a few weeks, with subsequent disease progression if ART is not re-initiated. Even PLWH who are virally-suppressed have a higher risk of developing kidney, liver, neurologic, bone or cardiovascular diseases, and cancers among other comorbidities. This has been linked to elevated levels of immune activation and inflammation that persist even after long-term ART in virologically suppressed PLWH relative to people without HIV.

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Defining the TAHR

Although ART generally reduces plasma HIV RNA to undetectable levels using common clinical assays, low-level viremia (LLV) frequently remains detectable through ultra-sensitive assays. Initially, LLV was considered the result of ongoing HIV replication in anatomical sites with suboptimal ART penetration, a potential reason for emergence of mutants resistant to ART. However, LLV remained unaffected by ART intensification, and strong evidence for low-level HIV replication (e.g., evolution of the virus reservoir on ART) has not emerged. This suggests that ART sufficiently suppresses complete cycles of HIV replication in PLWH, and that LLV likely arises from HIV reservoir cells. By targeting many critical steps in the viral life cycle, ART prevents new cycles of infection, but does not block HIV transcription. Although the HIV reservoir has traditionally been described as “silent” or “latent”, as defined by cells harboring integrated proviral DNA but lacking viral RNA or protein expression, evidence is accumulating that the viral reservoir might be “leakier” than initially thought. One could envision the HIV reservoir as a transcriptional gradient between two extremes of true latency and full productive infection, the latter defined by a state where cells express viral RNA and proteins and progeny viral particles are released. We refer to the population of HIV reservoir cells that exhibit varying degrees of HIV transcription and translation in the presence of ART as the transcriptionally active HIV reservoir (TAHR).

The TAHR may comprise a large proportion of the total HIV reservoir. In fact, transcripts containing the HIV TAR RNA, a dynamic stem-loop-bulge secondary structure that forms at the first 59 bp of HIV transcripts, were detected in over 81% of HIV-DNA-positive cells in limiting dilution assays[1]. The majority of those TAR-containing RNAs are short, abortive transcripts originating from transcription initiation events that failed to sufficiently elongate or that were prematurely terminated. Single-genome sequencing coupled with RNA quantification further confirmed the prevalence of transcriptionally active CD4 T-cells in ART-suppressed PLWH. It was found that 31% of all infected CD4 T-cells were positive for any HIV LTR-containing transcript[2] and 2—18% were expressing longer, Gag/Pol-containing HIV transcripts, indicative of the presence of full-length, unspliced HIV RNA[3]. The degree of transcriptional activation varies between individuals and can surpass the typical 1–3 RNA plasma copies of HIV RNA/mL detected with highly sensitive assays, resulting in plasma HIV RNA levels detected by common clinical assays. The phenomenon of ART-insensitive viremia, termed non-suppressible viremia (NSV), is estimated to occur in 1 out of 250 PLWH on ART and can be considered an extreme case of persistent viral transcription. NSV has been extensively studied in a few well-characterized participants. It was revealed that HIV RNA originated from highly expanded, persistent T-cell clones with both intact and defective proviral DNA fueling ART-insensitive viremia in the absence of disease progression.

Potential mechanisms driving immune stimulation by the TAHR

The TAHR may facilitate chronic immune activation and inflammation in PLWH on ART through multiple mechanisms[4] (Fig 1). First, the persistent, low-level generation of viral RNAs can engage innate nucleic acid sensing pathways and trigger type I interferon-mediated immunity. An analogous mechanism has been suggested for sensing of RNAs

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derived from retrotransposons, including the family of endogenous retroviruses, which may contribute to disease pathology in a diverse array of autoimmune diseases, including Aicardi-Goutières Syndrome, Multiple Sclerosis and Alzheimer's disease. Although direct experimental evidence for HIV RNA sensing in HIV reservoir cells is missing, both full-length and short, abortive HIV RNA transcripts are sensed in HIV *ex vivo* infection models[5]. This suggests that cell-intrinsic detection of accumulating HIV RNA may also occur *in vivo*. Second, the TAHR may also generate viral proteins or particles that drive inflammatory responses[6]. The viral proteins Tat, Nef and gp120 are long known to induce hyperactivation of HIV-infected as well as non-infected bystander cells exposed to the secreted proteins and more recently, the plasma levels of gp120 were correlated with immune dysregulation in PLWH with undetectable viremia[7]. Although viral particles and proteins derived from reservoir cells may not establish a productive infection because of ART, they may contribute to the re-occurring exposure of viral proteins to adaptive immunity, which is in line with the reported maintenance of a dysfunctional, exhausted T-cell population in PLWH that persists despite long-term ART adherence. Lastly, the TAHR may also drive the expansion of the viral reservoir by antigen-driven clonal expansion of HIV- and CMV-specific, HIV-infected T-cells, which by promoting HIV persistence can fuel chronic inflammation. HIV preferentially persists in clonally-expanded T-cells with specificity to commonly circulating antigens in PLWH, such as HIV and CMV. The re-occurring presence of HIV antigens may directly drive the expansion of HIV-specific HIV reservoir cells. The key question is how HIV-infected cells persist despite recognition by the immune system? Recent literature suggests that this resilience is in part due to the presence of immune-evasive and pro-survival cellular signatures found in cells harboring HIV DNA[8,9]. These phenotypic adaptations might allow long-term survival of HIV-positive cells despite immune recognition and facilitate persistent HIV RNA and protein expression, which in turn contributes to chronic immune activation and inflammation.

Therapeutic options for targeting the TAHR

It is well established that current ART regimens have minimal impact on chronic immune activation in well suppressed PLWH, yet the lack of mechanistical insight curtails the development of specific immunological or virological interventions. Therapeutics targeting HIV transcription or post-transcriptional events are also currently limited. As HIV transcription depends on the host transcription machinery, the viral factors Tat and TAR and their interactions with P-TEFb are preferential targets for interfering with viral transcription, without affecting host transcription (Fig. 2). Some drugs approved by the FDA for non-HIV related conditions are potent inhibitors of HIV expression, e.g., cyclosporin A, rapamycin, spironolactone, tofacitinib, ruxolitinib, and filgotinib (reviewed here[10]). Best studied among those is spironolactone, an aldosterone antagonist FDA-approved for the treatment of hyperaldosteronism. Spironolactone inhibits HIV-1 transcription by decreasing RNAP II recruitment to the HIV-1 promoter via degradation of the XPB cellular helicase, an element of the TFIIH complex, and blocking transcriptional initiation at the HIV promoter. This inhibits HIV replication and reactivation from latency in *ex vivo* infection models[11,12].

Targeted development of inhibitors specifically for Tat or TAR has been limited so far, although early studies of small molecules inhibiting HIV transcription via Tat or TAR

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interference showed promise[10]. In contrast, studies with synthetic peptide ligands of TAR with nanomolar to picomolar binding affinities, did not effectively interfere with TAR binding to Tat/P-TEFb and only weakly affected transcriptional activation. The naturally derived compound didehydro-Cortistatin A (dCA) has potent anti-Tat activity[13]. dCA binds to the basic region of Tat, disrupting Tat binding to TAR/P-TEFb and the powerful Tat feedback mechanism on HIV transcription and its own production, the absence of which is believed to ultimately culminate in heterochromatinization of the HIV promoter. In both *in vitro* and *in vivo* models of HIV, dCA supplementation of ART accelerates viral suppression to below detection limits and inhibits viral rebound after treatment interruption, even in the presence of latency reversal agents[14]. The success of dCA in HIV transcriptional silencing has propelled development of more cost-effective dCA synthesis protocols, as well as the targeted identification of more Tat inhibitors.

In addition to targeting transcription directly, other drugs that modulate HIV RNA processing and other post-transcriptional events could be considered. For instance, immunomodulatory agents which inhibit HIV RNA splicing (e.g., filgotinib[15]) could be explored as block and lock agents. The Rev/RRE/CRM1 complex is a promising target like the Tat/TAR/Cyclin T1 complex. In short, the viral Rev protein binds the Rev response element (RRE), a cis-acting nuclear export element present in all un-spliced and partially spliced HIV transcripts, which then recruits the cellular export receptor CRM1 to facilitate under-spliced viral RNA export. Although post-transcriptional HIV inhibitors leave viral RNA expression unincumbered and thus may still allow immune sensing, they could synergize with transcriptional inhibitors to decrease viral gene products (both RNA and protein) below a threshold that may otherwise drive chronic inflammation and immune dysfunction.

Conclusions

Together, the cumulative research so far suggests a model where the TAHR is a major contributor to chronic immune activation and inflammation that continues to negatively impact the health of PLWH despite ART. We propose that complementing current ART regiments with HIV transcriptional inhibitors that can synergistically target initiation and elongation, and possibly those that inhibit posttranscriptional steps, is a necessary strategy to close the loophole in current ART therapies. While the benefits of HIV transcriptional inhibition remain to be determined in clinical trials, we speculate that the continuous inhibition of transcription over time may not only suppress remaining immune activation but may also drive the viral promoter into a state of “deep” latency from which HIV transcriptional reactivation is unlikely to occur. Such a transcriptionally repressive chromatin state may permit durable viral control and pave the way for ART-free remission. These therapeutic avenues are, however, currently hampered by the limited availability of drugs that are virus-specific and meet the necessary criteria for large scale production. Thus, continued research efforts are required to identify, develop, and test novel inhibitors for HIV transcription.

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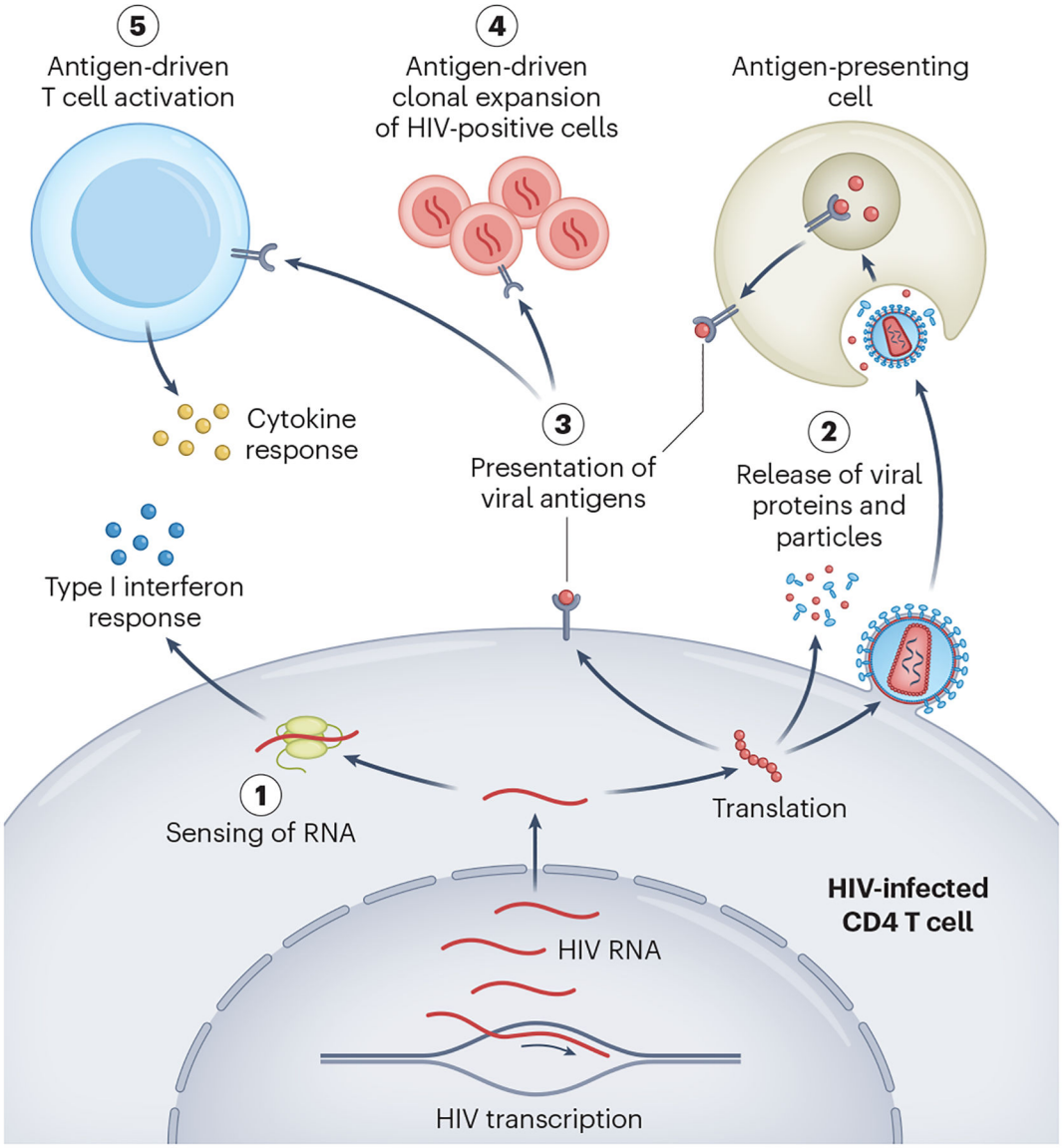

Figure 1. Potential mechanisms of immune stimulation by the TAHR under ART.

HIV RNA derived from integrated proviral DNA can be recognized by cytosolic RNA sensors and trigger a type I IFN-mediated innate immune response (1). HIV RNA also gives rise to proteins that may be directly released or generate progeny virus particles (2). HIV proteins may be processed for presentation on MHC complexes either on the HIV-positive cell or on specialized antigen presenting cells (3). Antigen presentation may trigger antigen-driven clonal expansion of HIV reservoir cells of HIV-specific T-cells (4) and induce T-cell activation and cytokine responses (5).

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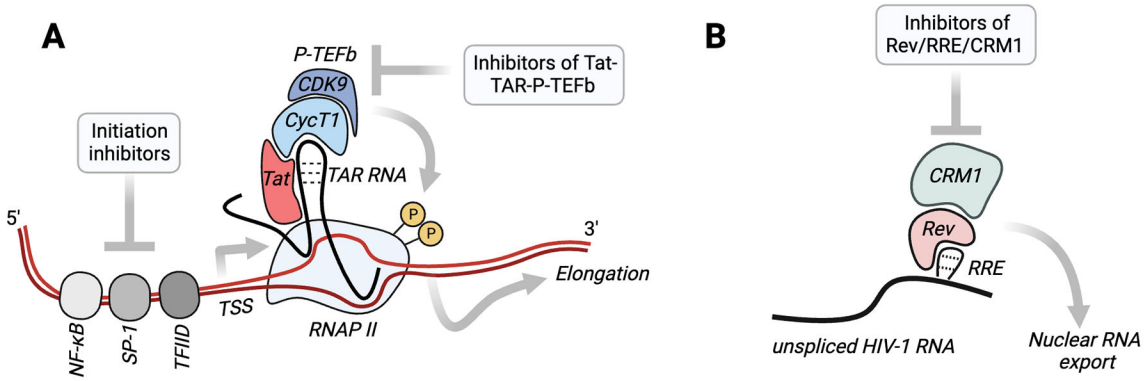

Figure 2. Nuclear targets to inhibit viral transcript production and export.

(A) Upon initiation, RNA polymerase II (RNAP II) pauses downstream of the viral TAR RNA region. To resume viral transcription, Tat recruits P-TEFb (CDK9/CyclinT1) to the transcribed HIV TAR RNA that is present at 5' ends of viral transcripts. P-TEFb recruitment leads to phosphorylation of RNAP II and transcriptional elongation. Blocking transcriptional initiation and elongation by targeting the pre-initiation complex or the Tat/P-TEFb complex, are paths to inhibit viral transcription. (B) Additional targeting of post-transcriptional steps including nuclear export by Rev/CRM1 may reduce residual viral protein and virion production.