An Ounce of Tat Prevention Is Worth a Pound of Functional Cure

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Infection by HIV-1 can effectively be suppressed by antiretroviral therapy (ART). However, the persistence of a small, latent virus reservoir impedes viral eradication. Thus, most infected individuals must be treated with ART for life. While efforts to find a sterilizing cure that eradicates the virus are ongoing, an alternative approach deemed a functional cure has remained less explored. A functional cure for HIV is envisioned as long-term control of virus replication in the absence of ART (reviewed in references 1 and 2). Such a functional cure might encompass effective immune interventions, reduction of the reservoir to a highly "controllable" size, and/or induction of a long-lasting suppression of viral gene expression. The Tat viral protein is a strong transactivator of viral gene expression, and viral replication is severely restricted in its absence; thus, it makes a logical antiviral target. A recent article by Mousseau et al. (3) reports on a novel Tat inhibitor that the authors show has the unusual ability to induce a "permanent state of latency." Didehydro-cortistatin A (dCA) is a departure from current FDA-approved antiretroviral drugs in that it inhibits viral gene expression from integrated viruses. For the above reasons, dCA represents a novel inhibitor class.

Cortistatin A (CA) is a steroidal alkaloid from *Corticium simplex*, a marine sponge, which has raised interest based on its antiproliferative effects (4). dCA is a derivative of CA that was reported to be equipotent (5) to the parent compound as well as relatively easy to obtain by chemical synthesis. One of the salient features of CA is its ability to bind to and inhibit cyclin-dependent kinase 11 (CDK11) with nanomolar affinity (6). CDK11 was recently shown by Valente and colleagues to be essential for HIV-1 gene expression by phosphorylating factors required for proper 3′ processing of viral mRNAs (7). Based on these findings, Mousseau and colleagues in the Valente laboratory predicted that dCA, by blocking the activity of CDK11, would in turn hinder HIV-1 transcription (8). dCA proved to be a potent inhibitor of HIV replication (8).

Intriguingly, Mousseau et al. were unable to reproduce CA-mediated CDK11 inhibition when testing dCA and instead demonstrated a different mechanism for dCA's antiviral effects. Mousseau and colleagues suggest that dCA binds to Tat's basic domain, the same domain that mediates localization to the nucleolus and binding to the transactivation response element (TAR) (8). dCA thus prevents recruitment of Tat to the TAR stem-loop structure, presumably hindering the recruitment of P-TEFb (positive transcription elongation factor-b) and transcription elongation (see Fig. 1).

The latest study by the Valente group further explores the effects of dCA (3) by asking whether the inhibitor has an impact on viral latency. This study begins by testing *ex vivo* the effect of dCA on peripheral-blood-derived resting T cells from HIV-infected, aviremic patients. Cells from nine selected patients were activated with anti-CD3 and -CD28 antibodies, in the presence or absence of dCA, and viruses released to the supernatant were measured with an ultrasensitive, quantitative reverse transcription-PCR (RT-qPCR) assay. Addition of dCA along with anti-CD3 and anti-CD28 diminished the release of virus in all patient samples by 92.3% on average. In the face of the powerful stimulation used, this represents a significant blockade of virus reactivation.

Efforts to further understand the dCA effects on latency led Mousseau and colleagues to experiment with several laboratory models of latency. First, the authors used an unpublished model consisting of HeLa CD4 cells latently infected with HIV-1_{NL4-3}, where residual viral gene expression produces about 500 pg/ml of p24 in the culture supernatant. Treatment of these cells with dCA suppressed residual p24 to undetectable levels (<3.1 pg/ml). This was consistent with a simultaneous reduction in viral mRNA production of 97% imposed by dCA. In contrast, conventional ART (lamivudine, raltegravir, efavirenz) failed to reduce the residual p24 levels.

Cessation of ART, both *in vivo* and *in vitro*, leads to a rebound in viral replication. When treatment with dCA of the latently infected HeLa CD4 cells was interrupted at day 100, viral rebound was not observed for the remainder of the experiment (150 days). This finding was quite surprising and, possibly, reveals an additional activity of dCA, which is to induce a "permanent state of latency." Interestingly, an earlier treatment cessation at day 24 did not lead to suppression of residual virus, which suggests that successful induction of the long-term suppressive effect of dCA may require longer exposure to the drug. Similar effects were observed in other cell culture models of latency, demonstrating that the findings are not unique to one model.

To further establish that the inhibitory effect of dCA was at the level of transcription, the authors performed chromatin immunoprecipitation analysis with an antibody to RNA polymerase II (Pol II), which transcribes HIV-1 genes. This analysis revealed that dCA treatment modestly impaired recruitment of the polymerase to promoter-proximal regions (indicative of transcription initiation) and severely impaired recruitment to a distal open reading frame, *vpr* (transcription elongation). Recruitment of Pol II to these viral sequences in response to tumor necrosis factor alpha (TNF-α) stimulation, an NF-κB-mediated event that does not require Tat, was unaffected.

The authors report that dCA, at the concentrations tested, was neither toxic nor cytostatic. Transcription inhibitors, in particular flavopiridol and DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole), which, like dCA block elongation, are known to be cytotoxic (reviewed in reference 9). A critical difference between the mode of action of these compounds and that of dCA is that

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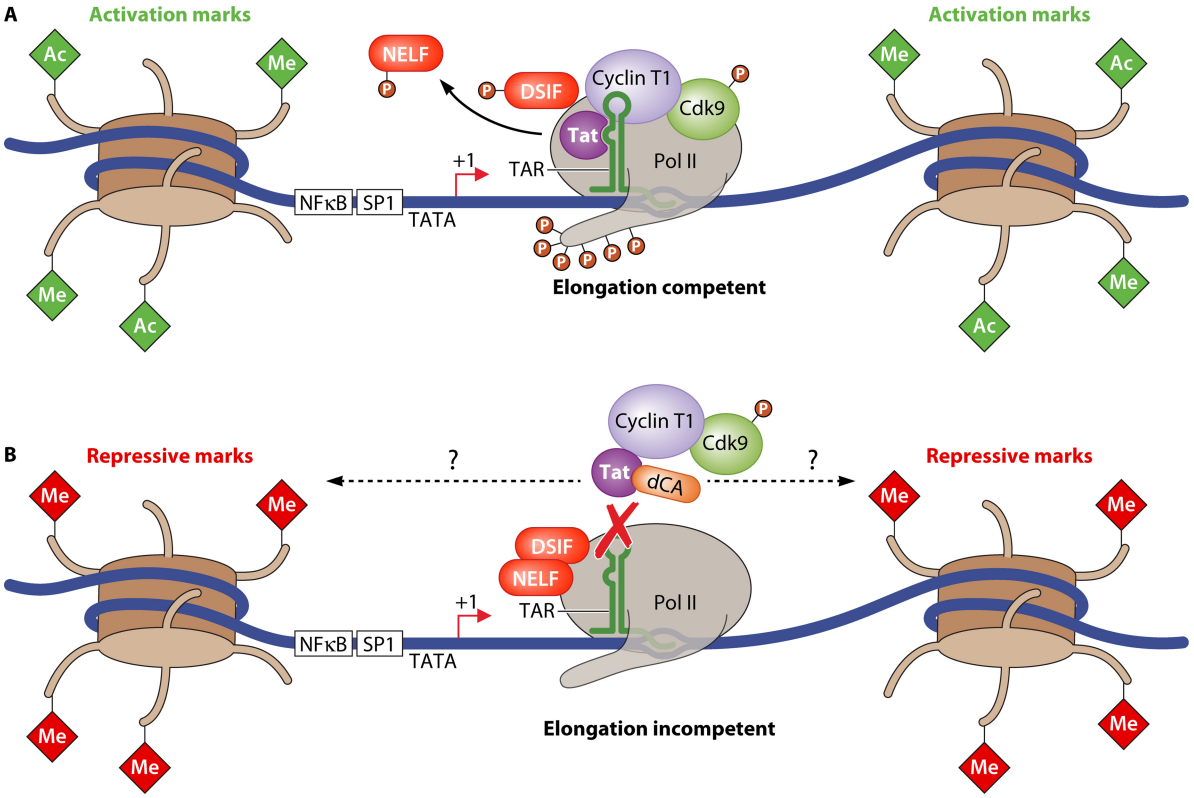

FIG 1 Didehydrocortistatin A establishes of a long-lasting latency state after interfering with Tat function. (A) Successful recruitment of P-TEFb (cyclin T1 plus CDK9) by Tat to the transactivation response element (TAR) within nascent HIV-1 transcripts allows for a switch from the initiation to the elongation mode of RNA Pol II. This is mediated in part by phosphorylation of negative factors, DSIF (DRB sensitivity-inducing factor) and NELF (negative elongation factor), and hyperphosphorylation of the polymerase C-terminal domain. In this context, nucleosomes flanking the transcription initiation site are poised for productive transcription via activating epigenetic marks. Ac, acetyl; Me, methyl. (B) dCA binds to Tat and blocks Tat's ability to deliver P-TEFb to the nascent transcript, prohibiting the RNA Pol II switch to an elongation mode. A long-term consequence of dCA's blockade of Tat activity, as proposed by Mousseau and colleagues (3), is the establishment of a closed chromatin state via repressive epigenetic marks. How Tat inhibition by dCA may lead to generation of a repressive chromatin environment is not presently understood (question marks).

while flavopiridol and DRB target CDK9 and other cellular kinases, dCA directly interacts with Tat but not with CDK9. Therefore, dCA specifically blocks the activity of CDK9 at the viral promoter but not at cellular transcription units.

Since dCA inhibits the role of Tat, the authors predicted that transfection of the HeLa CD4 cells with a plasmid encoding active Tat would rescue HIV-1 from its transcriptional suppression. Although this prediction was indeed substantiated, the rescue (resumption of p24 secretion into the medium) lasted 17 days, at which point viral gene expression spontaneously began to decrease and became undetectable again. Control experiments revealed that (i) mutations in the viral sequences did not account for the loss of gene expression, (ii) the number of cells containing proviruses remained almost constant, and (iii) residual dCA was not detected after drug discontinuation. These results point toward epigenetic changes being the culprit of this unusual latent state.

Could this deep state of latency, well documented in several transformed cell lines, be induced in cells from patients? The *grand finale* experiment, performed *ex vivo* with cells from two aviremic patients, addresses this question. Patient cells were expanded *ex vivo* in the presence of ART and in the presence or absence of dCA. When ART was discontinued, control cultures demonstrated viral rebound. However, in cultures exposed to dCA, the rebound was 93% suppressed. Furthermore, stimulation of these cells with prostratin, a protein kinase C agonist, led to the expected viral reactivation in control cultures, but no reactivation was observed in those previously treated with dCA (reactivation was 99.9% suppressed). Therefore, all evidence seems to indicate that dCA sets up a profound state of HIV-1 latency that is resistant to both passive rebound (ART discontinuation) and active stimulation (with prostratin).

Although the authors logically propose that long-lasting epigenetic marks underlie this type of latency, the only potential mark (among many possibilities) tested in the Mousseau et al. study (3) is the presence of DNA cytosine methylation, for which they report a negative result. Therefore, a complete mechanistic understanding for these exciting observations is not available and represents an obvious future direction. A plausible model proposed by Mousseau et al. emerging from their recent findings (3) is depicted in Fig. 1.

Thus far, the most promising intervention leading to a functional cure has emerged from the Visconti cohort study of early ART (10). What factors determined the long-term virologic control in the Visconti patients is not known. Furthermore, candidate genetic and immunological predictors of control (frequency of HIV-specific $CD8^+$ T cell responses or absence of unfavorable HLA genotypes) did not seem to apply (10). Could epigenetic

regulation of proviruses contribute to virologic control in Visconti patients and perhaps in elite controllers?

The activities of dCA shown by Mousseau et al. begin to carve a new niche within the still-nebulous realm of a functional cure by pointing to an external means to downmodulate the transcriptional activities of integrated viruses into a forced state of latency and to further perpetuate the latent state of viruses already silent. This therapeutic principle could, potentially, be combined with immune effector therapies or even with early ART.

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REFERENCES

1. **Mylvaganam GH, Silvestri G, Amara RR**. 2015. HIV therapeutic vaccines: moving towards a functional cure. Curr Opin Immunol **35:**1–8. http://dx.doi.org/10.1016/j.coi.2015.05.001.
2. **Eisele E, Siliciano RF**. 2012. Redefining the viral reservoirs that prevent HIV-1 eradication. Immunity **37:**377–388. http://dx.doi.org/10.1016/j.immuni.2012.08.010.
3. **Mousseau G, Kessing CF, Fromentin R, Trautmann L, Chomont N, Valente ST**. 2015. The Tat inhibitor didehydro-cortistatin A prevents HIV-1 reactivation from latency. mBio **6**(4):e000465-15. http://dx.doi.org/10.1128/mBio.00465-15.
4. **Aoki S, Watanabe Y, Sanagawa M, Setiawan A, Kotoku N, Kobayashi M**. 2006. Cortistatins A, B, C, and D, anti-angiogenic steroidal alkaloids, from the marine sponge Corticium simplex. J Am Chem Soc **128:**3148–3149. http://dx.doi.org/10.1021/ja057404h.
5. **Shi J, Manolikakes G, Yeh CH, Guerrero CA, Shenvi RA, Shigehisa H, Baran PS**. 2011. Scalable synthesis of cortistatin A and related structures. J Am Chem Soc **133:**8014–8027. http://dx.doi.org/10.1021/ja202103e.
6. **Cee VJ, Chen DY, Lee MR, Nicolaou KC**. 2009. Cortistatin A is a high-affinity ligand of protein kinases ROCK, CDK8, and CDK11. Angew Chem Int Ed Engl **48:**8952–8957. http://dx.doi.org/10.1002/anie.200904778.
7. **Valente ST, Gilmartin GM, Venkatarama K, Arriagada G, Goff SP**. 2009. HIV-1 mRNA 3′ end processing is distinctively regulated by eIF3f, CDK11, and splice factor 9G8. Mol Cell **36:**279–289. http://dx.doi.org/10.1016/j.molcel.2009.10.004.
8. **Mousseau G, Clementz MA, Bakeman WN, Nagarsheth N, Cameron M, Shi J, Baran P, Fromentin R, Chomont N, Valente ST**. 2012. An analog of the natural steroidal alkaloid cortistatin A potently suppresses Tat-dependent HIV transcription. Cell Host Microbe **12:**97–108. http://dx.doi.org/10.1016/j.chom.2012.05.016.
9. **Bensaude O**. 2011. Inhibiting eukaryotic transcription: which compound to choose? How to evaluate its activity? Transcription **2:**103–108. http://dx.doi.org/10.4161/trns.2.3.16172.
10. **Saez-Cirion A, Bacchus C, Hocqueloux L, Avettand-Fenoel V, Girault I, Lecuroux C, Potard V, Versmisse P, Melard A, Prazuck T, Descours B, Guergnon J, Viard JP, Boufassa F, Lambotte O, Goujard C, Meyer L, Costagliola D, Venet A, Pancino G, Autran B, Rouzioux C, ANRS VISCONTI Study Group**. 2013. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. PLoS Pathog **9:**e1003211.

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