

## Improving combination antiretroviral therapy by targeting HIV-1 gene transcription

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Review

**Improving combination antiretroviral therapy by targeting HIV-1 gene transcription**

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**Abstract:**

Introduction: Combination Antiretroviral Therapy (cART) has not allowed the cure of HIV. The main obstacle to HIV eradication is the existence of quiescent reservoirs. Several other limitations of cART have been described, such as strict life-long treatment and high costs, restricting it to Western countries, as well as the development of multidrug resistance. Given these limitations and the impetus to find a cure, the development of new treatments is necessary.

Areas covered: In this review, we discuss the current status of several efficient molecules able to suppress HIV gene transcription, including NF- $\kappa$ B and Tat inhibitors. We also assess the potential of new proteins belonging to the intriguing DING family, which have been reported to have potential anti-HIV-1 activity by inhibiting HIV gene transcription.

Expert opinion: Targeting HIV-1 gene transcription is an alternative approach, which could overcome cART-related issues, such as the emergence of multidrug resistance. Improving cART will rely on the identification and characterization of new actors inhibiting HIV-1 transcription. Combining such efforts with the use of new technologies, the development of new models for preclinical studies, and improvement in drug delivery will considerably reduce drug toxicity and thus increase patient adherence.

**Key words:** cART, HIV transcription, Tat, NF-KB, DING

## Article highlights

- Adding to cART drugs which block the production including the virus transcription could be a significant improvement since this could prevent cART-related side effects like multidrug resistance.
- Intensifying cART with transcriptional inhibitors will help to reduce the size of latent reservoirs, to reduce the prevalence of HAND and to prevent brain injuries in strategies aiming to reactivate and eliminate the brain reservoirs.
- NF- $\kappa$ B and Tat are the two main targets for the improvement of cART. These targets control HIV progression by activating transcription.
- Most of the potential transcriptional inhibitors still await preclinical and clinical trials.
- Improvement of cART relies on identification of new targets, development of adequate animal models and introduction of new technologies.
- In the next 10 years, new drugs and methods to increase bioavailability and bio-distribution may allow at least a functional cure including strategies aiming at the reduction of the pool of latent reservoirs.

## 1. Introduction

Introduction of combination Antiretroviral Therapy (cART) in 1996 has dramatically improved the management of HIV-1 infection and decreased both morbidity and mortality. However, despite initial hopes to cure HIV, the treatment is unable to eradicate the virus [1,2]. Very sensitive methods always detect a residual viremia in patients on cART [3,4]. Moreover, HIV RNAs return to a measurable plasma level when cART is interrupted [5,6]. The origin of the persistent viremia is still debated [7–9]. In the latent phase, HIV proviruses persist in long lived cells such as the central memory CD4+ T-cells, hematopoietic stem cells, dendritic cells and cells from the monocyte-macrophages lineage including the microglial cells, i.e. the resident macrophages of the central nervous system (reviewed in [10]). For example, latently infected cells reactivated by pro-inflammatory cytokines that produce HIV particles at low levels (well known as “Blips”) could explain persistent viremia. An alternative theory, the cryptic ongoing replication states that HIV persists in cells continuously producing low levels of HIV despite cART. The inefficient treatment of ongoing replication supporting cells could either be due to poor drug penetration into sanctuaries as the brain [11] or to cell-to-cell transfer of the virus [12]. The resistance against drugs in cART arising during ongoing replication has profound therapeutic implications. We therefore expect that intensification of cART reduces persistent viremia.

The various limitations of the current antiretroviral therapy makes cART unable to achieve a cure. The combination of drugs used in cART acts at different stages of the viral life cycle; cART needs lifelong drug adherence since ultimately HIV rebounds after cART interruption; there are severe side effects of the treatment including neurocognitive disorders, hyperlipidemia, nephrotoxicity, hepatotoxicity, lipodystrophy. Finally, the cost of the treatment is still excessive and therefore access is limited in poor countries. The latter is a major problem; more than 7000 new HIV infections occur every day and the number of newly diagnosed infections remains far greater than the number of people who have access to cART. Another major concern is the emergence of drug resistance that arises from the

extreme mutability of the virus [13–16]. Overall, the life expectancy of infected patients on cART is still reduced compared to non-infected individuals [17]. Indeed, chronic inflammation from residual viremia leads to several non AIDS related complications i.e. cardiopathy, nephropathy, faster progression towards hepatitis C complications and cancer [18]. Moreover, extracellular Tat resulting from residual viremia is responsible for Tat-associated cancer and HIV-associated neurocognitive disorder (HAND) [19].

In view of these limitations, new classes of drugs which preferably act on new targets of HIV replication should replace or partially substitute the existing ones. Original strategies focusing on the purge of quiescent reservoirs are also needed to completely eradicate the virus (reviewed in [20]). It was suggested that intensifying cART effects residual viremia and prevents the development of non-AIDS events. Except in one case [21], the benefit of the intensification of cART on residual viremia was not conclusive in the studies (reviewed in [9]).

Recently cART has been somewhat improved. New antiretroviral drugs and drug combinations, with good tolerability and efficiency against drug resistant viruses are currently tested in clinical trials [22]. Drugs used in combinations in cART target four stages of the cycle: viral entry, reverse transcription of the viral genome, integration into the genome of the host cell and maturation of viral proteins. However, to date, no drug developed so far targets the first step of the production phase, HIV gene transcription. Besides transcription other processes of viral gene expression i.e. splicing, polyadenylation, RNA nuclear export, translation, packing, dimerization etc, also deserve attention (Figure 1). Targeting these processes can all inhibit virus production or produce non-infectious particles. A recent study suggests that the drug ABX464 targeting the viral protein Rev involved in RNA export has a sustained antiviral effect in a humanized mouse model [23]. This striking observation needs however further investigation; one cannot exclude that it arises from an artefact of the animal model (comments in [24]).

We focus in this review on HIV transcriptional inhibitors. We believe that targeting HIV-1 gene transcription is an alternative approach that could overcome cART-related issues

such as the emergence of multidrug resistance or the progression to HAND and therefore should be seriously considered. After a brief description of the mechanisms involved in HIV-1 transcription, we discuss recent advances in the discovery of HIV inhibitors of the two main targets for HIV-1 transcription control i.e. NF-KB and Tat. We then discuss a new family of promising targets, the DING protein family. These drugs repress HIV-1 transcription and have anti-inflammatory properties. Finally, we discuss routes to improve cART, with a special focus on drugs targeting HIV-1 transcription. The new therapeutic methods might reinforce current cART and at the same time prevent chronic inflammation and some pathologies related to extra cellular Tat such as HAND and Tat-related cancer.

## **2. Regulation of HIV-1 gene transcription**

Once the HIV provirus is integrated into the host cell genome, virus transcription can occur (Figure 1). HIV-1 gene transcription is a critical step in infected cells where the virus exploits the cellular environment in order to maximize gene expression. Transcription is regulated by the interplay of distinct viral and cellular transcription factors that bind the Long Terminal Repeat (LTR) region of HIV-1 (reviewed in [25,26]). This LTR region comprises four functional regions related to HIV-1 transcription regulation: (i) the Transactivator Response (TAR) binds Tat; (ii) the core promoter contains a TATA-box, an initiator and three Sp1 binding sites; (iii) the core enhancer contains NF-AT and NF-KB motifs. According to previous reports, the three SP1 binding sites and the two NF-KB motifs play important roles in the regulation of HIV-1 transcription. (iv) The modulatory region harbors numerous target sequences for cellular transcription factors such as c-Myc, COUP-TF, NF-IL6, Nuclear Hormone responses [26]. Deletion of this region enhances HIV-1 transcription and replication suggesting the presence of a negative regulatory element (NRE) [26,27].

Infected cells have two fates depending on the local state of chromatin structure and on the cellular environment [10]. On the one hand, in the relaxed state of chromatin i.e. euchromatin there is an active transcription and infected cells are productive. On the other

hand, in the compact and very structured state of chromatin i.e. heterochromatin there is no transcription and infected cells become latent and/or quiescent.

**i. Transcriptional status in productively infected cells**

**The early phase of transcription**

Once the HIV-1 promoter is open to the transcriptional machinery, a cellular-dependent early phase complex initiates gene transcription (figure 1 and 2). Multi-spliced RNAs encoding viral regulatory proteins including Tat, Rev and Nef are then transcribed. Reference [26] reviews the mechanism by which transcription of the viral genome adjusts to specific cell types. NF-KB and NF-AT translocate into the nucleus upon activation in T-lymphocytes [28]. Binding of NF-KB and NF-AT to the promoter is a prerequisite for activation of transcription. In contrast, Sp1 and other general transcription factors bind to the core region. Additional factors such as NF-IL6, CREB, USF and Ets are however required for full activation [26]. On the other hand, in microglial cells the core region and the NF-KB sites are sufficient for transcription. The inhibition of NF-KB activation and its signaling pathway offers therefore a promising strategy for anti-HIV therapy [29].

**The late phase of transcription**

The late phase of transcription is under the control of the viral protein Tat. Tat amplifies gene expression by affecting the initiation and elongation phases of transcription (figure 1 and 2). The scheme of Tat induced gene expression is as follows: Tat together with cyclin T1 bind to the TAR RNA structure. This complex subsequently recruits the cyclin dependent kinase 9 (Cdk9) to form the positive transcription factor b (p-TEFb) [30,31]. The p-TEFb complex induces the phosphorylation of the carboxyl-terminal domain (CTD) of RNA polymerase II and the phosphorylation of the negative elongation factors NELF and NSIF therefore circumventing their inhibitory effect [32,33]. Interestingly, Tat exists in two P-TEFb-associated complexes [34]. Tat-com1 includes MLL fusion partners and the PAF1 complex



and potentiates Tat-mediated transactivation, while Tat-com2 contains P-TEFb, the 7SK snRNA, LARP7, MEPCE and represses Tat functions. Indeed association of the small nuclear RNA 7K and the RNA-binding proteins HEXIM1, HEXIM2, LARP7 and MEPCE to the p-TEFb complex reversibly inhibits p-TEFb activity [35–38]. This large, inactive form of p-TEFb (in contrast to its free, active form) is thought to be involved in HIV-1 transcription. HIV-1 evades normal cellular control since Tat selectively recruits free p-TEFb complexes from the large pool of inactive p-TEFb complexes [39–42]. In turn the active p-TEFb promotes the transition of RNA pol II from abortive to productive elongation. Tat may also play an important role in the initiation of transcription and in the assembly of the general transcription factors [43]. In fact, ChIP analyses revealed that Tat increases the binding of several general transcription factors, including RNA pol II, TBP and TFIIB [44]. Tat also requires the presence of Sp1 and NF-KB sites and direct interaction with Sp1 protein for transactivation [45–47]. Moreover, Tat can modulate the expression of various cellular factors involved in the regulation of the HIV-1 gene transcription such as the cytokines TNF $\alpha$ , TGF $\alpha$ , IL2, IL6 and IL10 [48,49]. Recently, a genome-wide binding map of Tat has been performed. It showed that Tat was associated to several promoters involved in immune response and T-cell functions with important implications for the HIV-1 life cycle [50–52]. The precise mechanism of action of Tat on the regulation of cellular gene is not fully understood. A recent work using a global analysis of chromatin shed light on how Tat could modulate cellular gene expression. They showed that Tat is recruited on many promoter sites occupied by the transcription factor ETS1 [53]. Apart from its role in HIV-1 and cellular gene expression, many other functions of Tat have been described [54]. Notably, extra cellular Tat has been involved to the development of HIV-associated neurocognitive disorders [19]. The precise mechanism of HIV-1 Tat neurotoxicity remains unclear. It involves deregulation of calcium homeostasis, oxidative stress, altered mitochondrial biogenesis [19], alteration of autophagy function [55], deregulation of pathway that involved p53 and p73 [56]. Extracellular Tat constitutes a key molecule in HIV pathogenesis even if the exact mechanism of action inducing neuronal dysfunction is still debated. Furthermore, inhibition of extracellular Tat

activity might also help to reduce Tat-associated pathologies such as AIDS-associated cancer and HAND.

All in all, the remarkable advances in recent years in the comprehension of Tat functions make it a potential therapeutic target for HIV-1.

## ii. **Transcriptional status in abortively infected cells**

The switch from active to repressive transcription of HIV depends on chromatin-modifying agents. These molecules modify the epigenetic marks of DNA and histone proteins. Indeed, heterochromatin is associated with CpG islands methylation and histone lysine trimethylation such as H3K9me3 and H3K27me3 [57–59]. In microglial cells a new level of eukaryotic gene regulation was suggested. It was shown that LSD1 and Bcl11b (CTIP2) were associated with the epigenetic marks H3K4me3 and H3K9me3 [60]. Numerous cellular factors have been characterized in the establishment and persistence of HIV latency in the main cellular reservoirs including CD4+ T-cells and microglial cells [61]. In this latter, our laboratory has demonstrated the importance of Bcl11b in inducing both establishment and persistence of HIV latency [reviewed in (62,63)]. Briefly, this protein serves as a platform anchoring a protein complex which include HDAC1, HDAC2 and the histone methyltransferase SUV39H1, which are involved in the establishment of HIV latency [59,64]. Preventing reactivation of HIV Bcl11b enables the persistence of HIV [65]. Bcl11b also interacts with the inactive p-TEFb complex containing HMGA1 and the CDK9 kinase activity of p-TEFb is inhibited by Bcl11b. Furthermore, HMGA1 and Bcl11b working in synergy repress gene transcription [66]. It is fundamental to understand the molecular mechanisms involved in these processes and deserves far more attention since it is a prerequisite for the development of alternative strategies i.e. (1) the reactivation of the reservoirs in “shock and kill” strategy and (2) the inhibition of HIV transcription. In strategy 1, the identification and characterization of new cellular targets involved in the reactivation of HIV transcription depends much on the understanding of the mechanisms. This strategy which might lead to

functional cure, known as “shock and kill”, aims to purge or at least to shrink the cellular reservoirs. This method implies the reactivation of HIV transcription (shock) followed by an intensive cART (kill) [67]. Other promising approach to purge the reservoirs is gene therapy that target the core receptor CCR5 discussed elsewhere [20]. Several recent reports suggested that new inhibitors used alone or in combination could be efficient for reactivating quiescent/latent HIV both *in vitro*, *ex vivo* and *in vivo* ([68] and reviewed in [69]).

In the following sections, we focus on the second strategy which aims to control HIV-1 progression by transcriptional inhibitors.

### **3. Transcriptional inhibitors to control HIV-1 progression**

The control of HIV-1 progression by transcriptional inhibitors is of great interest since no drugs have been developed to date to target this step. To achieve this, one should design drugs targeting cellular cofactors involved in the activation of transcription. The strategy has to by-pass drug-resistance problems that arise with viral proteins. Numerous transcriptional inhibitors have been studied and demonstrated their efficacy in suppressing HIV transcription and/or inducing latency [70]. Strategies which effect transcriptional activators such as NF-AT or NF-IL6 also deserve attention. It has been shown that NF-IL6 is crucial for HIV-1 transcription in the monocyte-macrophage cell lineage including microglial cells [71]. C/EBP $\beta$ , a truncated form of NF-IL6, acts as a transdominant negative repressor of HIV transcription (figure 2). Activation of C/EBP $\beta$  might be interesting and deserves further evaluation. Nevertheless, NF- $\kappa$ B and Tat are the two main targets that control HIV progression by inhibiting transcription. Transcriptional gene silencing based on RNA interference (RNAi) is an alternative strategy [72,73]. Combinatorial gene therapy using 3 siRNAs targeting tat and rev holds promise in achieving a functional cure. A nucleolar TAR decoy has been tested as part of this initial combinatorial gene therapy but didn't provide yet additional benefit in inhibiting HIV replication [73]. This RNAi based approach provides a new way to combat HIV-1 infection and merits more attention.

### 3.1 NF- $\kappa$ B inhibitors

Among other candidates, the inducible transcription factor NF- $\kappa$ B deserves attention since it initiates the early phase of HIV transcription leading to the synthesis of the Tat transactivator. Inhibition of the NF- $\kappa$ B signaling pathway by various molecules has been reported in the literature [74]. However to date none of these molecules have been approved for clinical trials due to their ineffectiveness and/or potential toxicities. A major limitation of these drugs is that they target the NF- $\kappa$ B signaling pathway upstream to I $\kappa$ B degradation. Development of new inhibitors that target the late steps of the NF- $\kappa$ B signaling pathway, i.e. I $\kappa$ B degradation and DNA binding of NF- $\kappa$ B, is in progress. Among these molecules, quinoline derivatives are of interest since some of these inhibit HIV transcription [75–78]. Among 18 tested quinoline-based compounds, 3 were very active *in vitro* and *ex vivo*. Moreover, they indirectly affected HIV transcription by blocking the nuclear translocation of NF- $\kappa$ B and inhibiting Sp1 [79]. A new series of natural and substituted olean-18-ene triterpenoids were recently isolated from *Celastraceae* species and their structures elucidated. These displayed potent antiviral activities [80]. Interestingly, the molecules inhibited HIV-1 transcription by targeting both Sp1 and NF- $\kappa$ B, as reported for the quinolone derivatives as well. Another newly discovered NF- $\kappa$ B inhibitor, dehydroxymethylepoxyquinimycin (DHMEQ) is also promising since it decreases HIV-LTR transcriptional activity mediated by external stimuli such as TNF $\alpha$  or Tat [81]. DHMEQ inhibits NF- $\kappa$ B by specifically binding to it, and inhibiting its subsequent nuclear translocation [82,83]. Natural compounds are another source of drug discovery [84]. A screening program to isolate natural compounds with anti-HIV activities has identified the molecule denbinobin from a variety of *Cannabis sativa* [85]. This phenanthrene (1,4-phenanthrenequinone) molecule inhibits HIV transcription by preventing NF- $\kappa$ B binding to DNA and by affecting I $\kappa$ B phosphorylation and its subsequent degradation [86]. Lignins and small molecules having lignin-like structural functional groups also inhibit NF- $\kappa$ B and HIV-1 transcription [87]. A high-

throughput RNA interference screening has recently identified a deubiquitinase named CYLD which has the property to repress HIV-1 transcription in a NF-AT/NF-KB dependent manner [88]. Finally, a specific myeloid cell factor, COMMD1/murr1 has been recently described as a new inhibitor of the HIV-1 transcription factor [89]. This factor inhibits NF- $\kappa$ B activity by stabilizing I $\kappa$ B thus promoting HIV-1 latency. All these newly identified NF-KB inhibitors constitute potential anti-HIV-1 drugs and can pave the way to the development of new classes of drugs. Moreover, targeting NF-KB possibly reduces chronic inflammation (non-AIDS associated pathologies) [90,91].

However, some limitations of the NF-KB inhibitors should be mentioned. One may expect side effects when targeting cellular factors involved in numerous cellular processes. Furthermore, according to reports, HIV-1 subtypes differ in the basic transcription factor binding sites and this may affect their responsiveness to drugs. Indeed, the conversion of the NF-KB binding site into a GABP binding site has been described in subtype E of HIV. This caused severe loss of NF-KB binding [92]. Response to these NF-KB inhibitors may not work in patients infected with this subtype of HIV-1.

### **3.2 Tat inhibitors**

The viral protein Tat associated to the elongation factor pTEF-b plays a critical role in transcription and constitutes a major therapeutic target of the HIV replication [93,94]. The recent determination of the crystal structure of Tat-pTEFb has given new insights of the mechanism of Tat activation and may help in the design of new Tat inhibitors [95,96]. Targeting pTEFb could also lead to the inhibition of HIV transcription and the decrease of resistance to the virus. There are several ways to inhibit Tat functions: (i) targeting the elongation complex pTEFb mainly by inhibiting the catalytic subunit CDK9 of pTEFb (ii) targeting the Tat-pTEFb interaction, (iii) directly targeting Tat, (iv) or targeting the Tat-TAR association.

(i) Targeting pTEFb

Several inhibitors of components from the pTEFb complex i.e. cyclin T1 and the catalytic subunit CDK9 that includes the nucleotide analogue DRB, flavopiridol and Roscovitine have been described [97]. However, due to the low therapeutic index, these molecules need further optimization before clinical trials. A recently designed derivative of 2-phenylquinazolinone, a potent CDK9 inhibitor, has shown interesting properties [98]. The probable side effects are another major limitation to the development of pTEFb inhibitors since pTEFb is a master regulator of gene transcription.

(ii) Targeting the Tat-pTEFb interaction

Targeting specifically the Tat-pTEFb interaction appears more promising. Several inhibitors of the Tat-pTEFb interaction including celastrol [99] and cyclopentenone prostaglandin 15d PGJ<sub>2</sub> have been described [100]. These two inhibitors interact with Tat and induce covalent bond formation with the cysteine thiols of Tat preventing pTEFb recruitment. However, their therapeutic indexes were too low, essentially due to high toxicity. Structure-based analysis of CDK9 along with published Tat derivatives showed that short Tat peptides can prevent Tat-CDK9 interaction. New drug mimetics inhibiting CDK9 and inhibitors targeting the Cyclin T1-Tat interaction were found by *in silico* analysis [101,102]. Finally, recent advances such as Nuclear Magnetic Resonance (NMR) and Circular Dichroism (CD) have allowed the synthesis of a series of new Tat/TAR inhibitors [103]. A series of pentameric "poly amino acids" (PAAs) molecules have been shown to interact with Tat on the same TAR binding site. However, according to a comparative thermodynamic study of PAA/TAR, there are two different TAR binding modes for Tat (the competitor and the non-competitor modes). Fragment-based screening is another new way to design drugs (reviewed in [104]). This approach aims to target the Tat-TAR interaction. Protein fragments bound to a target (the TAR RNA for example) are screened by various methods such as X-ray crystallography, Nuclear Magnetic Resonance (NMR), Surface Plasmon Resonance

(SPR) and Differential Scanning Fluorimetry (DSF). These methods alone or in combination have already led to the discovery of novel inhibitory sites within HIV protease, reverse transcriptase and integrase. The fragment-based screening is now used to explore new inhibiting sites of the Tat/TAR interaction [105]. Although in its early stage, these studies illustrate the efficacy of modern spectroscopic technologies for the design new drugs.

### (iii) Targeting Tat

A derivative of coumarin was found to inhibit HIV-1 Tat-mediated transcription. The inhibitory mechanism involves the repression of the Akt pathway which leads to the decrease of p300 stability, a protein known to modulate Tat activity by acetylation [106]. Another very promising Tat inhibitor has been recently isolated from the plant *Tripterygium wilfordii* [107]. The molecule named triptolide is a diterpenoid epoxide that inhibits both HIV replication and transcription by increasing the proteasomal degradation of Tat. This molecule is currently in phase III of a clinical trial. Another natural product derivative, tanshinone from the root of *Salvia miltiorrhiza* deserves attention. It inhibits Tat-regulated transcription by inhibiting the AMPK/Nam1/Sirt1 pathway [108]. Using a high-throughput screening assay, a new Tat inhibitor named 6 bromoindirubin 3' oxime (6BIO) has been recently isolated [109]. This small molecule and its derivatives named 18BIOder inhibit a GSK-3 $\beta$  kinase. Interestingly this kinase is found in the GSK-3 $\beta$  complex only in HIV infected cells. 18BIO derivatives are very promising since they inhibit HIV-1 Tat-dependent transcription at the nanomolar level with less toxicity than the original molecule 18BIO. These molecules also confer neuronal and microglial protection by reducing both Tat production and release and prevent or reduce HIV-associated neurocognitive disease (HAND).

### (iv) Targeting Tat-TAR interaction

A much more promising approach is to inhibit binding of Tat on the RNA TAR which is unique for lentiviruses such as HIV-1. However, although many candidates were tested, all failed in clinical trials [110–116]. A series of linear polypeptide analogues have been shown

to impede the Tat-TAR interaction [117,118]. Unfortunately, these molecules also bound cellular host RNAs. Constrained cyclic peptidomimetics derived from HIV-1 Tat have been used to reduce the non-specific binding to RNAs, which is mainly due to a conformational flexibility. Using this method, a series of competitive inhibitors of the Tat-TAR interaction have been identified and tested *in vitro* and *ex vivo* [119–122]. It appears that these molecules play a dual role as they inhibit both HIV gene transcription and HIV reverse transcription [123]. The dual inhibitory mechanism of these compounds could increase their efficiency and selectivity compared to other molecules and therefore deserves further attention. Finally, dCA, a chemical derivative of corticostatin, a natural steroidal alkaloid isolated from a marine sponge is also very promising [124]. This drug prevents Tat-TAR interaction at a very low concentration with a very high therapeutic index (over 8000). These results raise the possibility of the design of new drugs belonging to the dCA family that are capable to inhibit transcription of the virus in latent reservoirs. Importantly, a reduction of deleterious effects of chronic inflammation associated with residual viremia in patients on cART are expected [125]. Moreover, one study shows that dCA pretreated infected cells were never reactivated when treated with latency-reversing agents (LRAs) [126]. This recent work needs further investigation since dCA might induce the formation of new epigenetic modifications that mediate a very strong state of latency called “deep latency”[127].

All these promising molecules need far more investigation before inclusion in current drug regimens. Preclinical and clinical trials are needed to assess the mode of action and to define the adverse clinical effects that might preclude their use in treatments aiming to manage HIV infection.

#### **4. DING proteins: Potential therapeutic agents against HIV-1**

Proteins from the DING family have potential anti-HIV-1 activities. These proteins are involved in HIV gene transcription inhibition [128–132]. DING proteins are widely distributed in prokaryotes and eukaryotes [133,134]. Their name comes from the well conserved N-



terminus region beginning DINGG [135]. Surprisingly, genes encoding DING proteins are lacking from eukaryotic data bases [136]. In human, DING proteins have been associated with several pathologies including rheumatoid arthritis, lithiasis, atherosclerosis and cancer [136]. The DING protein p27<sup>sj</sup>, isolated from the plant *Hypericum perforatum*, inhibits the HIV-1 gene transcription [128]. This protein inhibits LTR-driven transcription in several human cell lines, including microglial cells, by inhibiting the interactions of two well-known activators of HIV gene transcription, the cellular factor NF-IL6 and the viral transactivator Tat. Darbinian et al. reported that p27<sup>sj</sup> exhibits phosphatase activity dephosphorylating the CTD domain of RNA polymerase II [137,138]. Another DING protein, named X-DING CD4, isolated from human sera also has anti-HIV activity. This protein has been characterized as a human resistant factor (HRF) secreted by HIV-1 resistant cells [139,140]. Furthermore, the purified X-DING CD4 protein was able to block LTR-driven expression of a reporter gene as well as HIV-1 replication [129]. The Human Phosphate Binding Protein (HPBP), a further member of the human DING protein family, has also been identified as a potent anti-HIV-1 factor [130]. HPBP strongly inhibits HIV-1 replication and the IC50 value is in the range of 5 nM while the index of selectivity is in the range of 30 to 40. In this range of concentration, HPBP is also a potent anti-HIV-1 molecule in Peripheral Blood Lymphocytes and in primary macrophages, unlike other anti-HIV-1 drugs. Interestingly, HPBP not only targets transcription, it is equally effective against drug resistant HIV strains and wild-type strains. It will be important to investigate these molecules and their peptide derivatives in animal models for drug resistance. Recently, a DING protein from *Pseudomonas aerobacter* PA14 was identified and its structure solved [141]. This protein also suppresses HIV-1 transcription and replication in microglial cells. The inhibition of the interaction between NF-KB and the HIV-1 promoter DING proteins is a common mechanism of action of all these DING proteins [142,143] that could also be used to treat chronic inflammation leading to non-AIDS events [144,145]. As suggested for X-DING-CD4 [129], their functions could also uncover a role in the innate response to infections including HIV-1 [146].

However, results obtained with DING proteins are still preliminary and await preclinical and clinical trials to assess their specificity of action and the adverse clinical effects. A pharmacophore model could be useful in the design of peptide derivatives of DING proteins. We discuss these aspects in more details in the following sections.

## **5. How can one improve cART?**

To date, vaccination is unavailable and cART is the only efficient treatment for HIV infection until eradication of latent reservoirs is not possible. Introduction of cART in 1996 has profoundly changed prospects of HIV infection; it transformed the lethal disease into a chronic pathology that enables infected patients a nearly normal life span. The development of drug resistance remains however one of the main problems encountered with cART. It is estimated that resistance to at least one drug of the cART cocktail develops in 10-17% of new infections [147]. Further improvement of cART remains crucial, even if some improvements are available to prevent virus resistance (mainly a better treatment adherence as a result of once a day regimen). One way would be to target HIV transcription. Although some new drugs were studied for their efficiency to inhibit HIV transcription and others are under investigation, currently no drug is available on the market. One might promote discovery and design of transcriptional inhibitors by efforts in the following areas: (i) new target identification, (ii) introduction of new technologies, (iii) development of new models to test drugs and (iv) improvement of drug delivery.

### **j. Identifying new targets**

Deciphering the molecular mechanisms underlying transcriptional regulation is fundamental since it allows the characterization of potential targets that regulate HIV-1 transcription. We already pointed out the importance of this approach. Indeed, several cellular factors are involved in the establishment and the persistence of HIV-1 latency [61]. Targeting these factors reinforces the state of latency. An alternative strategy is to target

these factors in order to reactivate HIV-1 transcription and replication. Here the idea is to deplete HIV-1 from latent cells and concomitantly eliminate HIV by cART and immunotherapy [61,148,149]. Numerous cellular factors have been involved in HIV-1 transcription (for example ZACS1 [150] and members of the SEC complex [34,151,152]) and many others remain to be discovered. Indeed all these known and not yet discovered factors constitute potential targets to silence HIV-1 transcription. A strategy using miRNA combined with cell-based gene therapy also deserves attention. [153,154]. This strategy permit to specifically target cellular factors involved in the control of HIV transcription in infected cells. The knock-out of the cellular cofactor Tat-SF1, that is known to decrease HIV-1 replication in a T-cell line, is an example for its application [155].

ii. **Exploiting new technologies**

An increasing number of new technologies such as bioengineering, high-throughput screening, computer-aided drug design and combinatorial chemistry completed with NMR, SPR or CD measurements (reviewed in [104]) will considerably improve the chances for discovery of new drugs. New bioengineering processes based on engineered zinc finger transcription factors are now emerging. These proteins are attractive candidates for antiretroviral therapy since they repress LTR activity by binding to HIV-1 LTR in a sequence specific manner [156,157].

*In silico* screening of peptides or small molecules targeting newly identified cellular factors involved in HIV-1 transcription may also help to identify new inhibitors of HIV-1 transcription. Freely available data bases of small molecules and peptides will ease the selection and the design of new antiretroviral agents [158]. This approach has allowed the identification of new inhibitors targeting the cyclin T1-Tat interaction in the pTEFb complex [102] and a HIV-1 transcription inhibitor peptide targeting the cellular phosphatase PP1 [159]. Small molecule inhibitors of HIV-1 gene expression identified by elegant cell-based and fluorescence-based high-throughput screening assays are promising antiretroviral drugs [160,161].

A major limitation of peptide derivatives is that they have to be injected. Alternatively these potent HIV peptides could be used in emergency e.g. in patients on cART infected with multidrug resistant strains.

The pharmacophore model might also help to design rational treatment and allow synthesis of new active drugs. This approach has been used in cancer and HIV-1 therapy. Indeed, this new technology allowed the development of HIV-1 integrase inhibitors [162] and might be extended to other transcriptional inhibitors. Substituted guanidine indole derivatives, also designed by the pharmacophore model, are potential inhibitors of HIV-1 transcription [163].

In addition, several inhibitors of specific protein-protein interactions have been discovered by computer-aided design which could be used in HIV-1 treatments as well [164]. A new class of CDK9 inhibitors, 2 phenyl fragments, has been identified and characterized by this technique [98]. Optimization of these new compounds is necessary.

Another interesting approach is to use combination of existing active molecules. Such combinations include a quinolone derivative identified as a Tat-mediated transcription inhibitor together with the tricyclic core of nevirapine and non-nucleoside reverse transcriptase inhibitors (NNRTIs). Among all the combinations tested, a transcriptional inhibitor in combination with a NNRTIs efficiently inhibited HIV-1 reactivation [165]. Further investigations are needed to obtain powerful derivatives.

Finally, screening natural products is also an option in the design of transcriptional inhibitors since several natural compounds repress HIV transcription. Natural products are one of the most important sources of new drugs [166,167]. Once an effective natural compound is identified, potent derivatives might be synthesized by combinatorial chemical approaches.

Exploring all these new resources helps to select and design new anti-HIV agents. Translational approaches using computational tools and pharmacophore models are very promising and are used more and more to design rational treatments.

### iii. **Development of new models**

Drug resistance to HIV-1 subtypes is a major problem in HIV-1 treatment. [168,169]. A T-cell line has been selected to test drugs on various HIV subtypes [170]. The T- cell line has also been used to test the safety and the efficacy of RNAi based gene therapies but should be extended to alternative strategies.

Rhesus macaque monkeys and mice are largely used in animal models for preclinical trials [171]. However, studies with monkeys are time-consuming and very costly. On the other hand, HIV-1 is not infectious in mice because the virus envelope glycoprotein does not bind to the CD4 receptor and the CCR5 coreceptor [172,173]. Moreover, in mice Tat induced LTR transcription is 10 to 25 fold less active than in humans, since the transactivator Tat is unable to recruit the elongation factor P-TEFb on the TAR-RNA [174,175]. The development of humanized mouse models, such as severe combined immunodeficient (SCID) mice, provides a powerful way to circumvent these problems. Several improvements have been made in the last twenty years in the development of such humanized models [176,177]. In BLT mice, the best model used to date, the thymus, liver and hematopoietic stem cells are humanized by a triple xenograft procedure [178]. This is currently one of the best model for HIV infection and allows to study HIV pathogenesis and to test drugs in preclinical trials (reviewed in [179]). Interestingly these mouse models may also be used for the “shock and kill strategy” [180]. However these models have to be generated *de novo* for each experiment. Moreover, their handling in specific facilities is difficult and therefore costly. In addition, only some parts of the human system can be reconstituted in these mice and this limits their use. For example, human myeloid cells cannot be fully reconstituted in mice. While lymphoid CD4+ cells are the main reservoir of HIV, cells from the myeloid lineage, e.g. the microglial cells are also important reservoirs. Although existing mouse models cannot be used to evaluate treatments targeting these cells [177,181], several improvements of the humanized mouse models have been developed recently [182]. Producing transgenic mice expressing CD4, CCR5 and cyclin T1, which render mouse cells (both lymphoid and myeloid cells) susceptible to HIV infections, should help to overcome these limitations and greatly facilitate preclinical studies [183].

#### iv. **Improving drug delivery**

Tolerance and patient adherence have significantly increased with the introduction of the single-tablet regimens. More efficient drug delivery and thus increasing patient adherence is another method to increase treatment efficiency and tolerance. Several advances have been made in this field, e.g. intracellular delivery of peptides that inhibit the Tat-TAR interaction [184].

The use of 1-1000 nm nanoparticles constitutes a promising tool to reduce toxicity and facilitate treatment adherence [185,186]. HIV therapy may improve by specifically targeting infected cells and tissues with the possibility to reach sanctuaries e.g. in the brain. The nanotechnology-based delivery system could also permit to modulate drug release and to prevent drug degradation. These emerging approaches improve bioavailability and therefore reduce toxicity [187–189]. Nanotechnology allowed several advances in the combat against HIV-1[188,190]. The treatment of HIV in the brain is still a major challenge since most antiretroviral drugs cannot cross the blood brain barrier [187,190,191]. Several nano-vehicles of drugs such as liposomes, dendrimers, synthetic polymeric nanoparticles, micelles, inorganic nanoparticles and natural polymers are available [187,190–195]. A summary of antiretroviral drugs used in HIV-1 nanotherapeutics is presented in a recent review [196]. Still in the early stage, nanotechnologies await preclinical trials in animal models and clinical trials in humans before utilization in HIV treatment.

The adherence to treatment also depends significantly on good oral bioavailability of the drug. *In silico* models to test the human oral bioavailability of drugs are under development. These computational chemical approaches work as filters to optimize high-throughput screening of libraries. This method is able to predict the pharmacokinetics (Absorption, Distribution, Metabolism, Excretion) and toxicities of a drug [197]. Although still in its initial phase, it will certainly improve cART by increasing patient adherence to therapy and drastically reducing the drug development cost.

## 6. Expert opinion

There are several reasons to be optimistic about achieving a cure for HIV infection, even if not in a short term [20]. The introduction of cART in 1996 drastically improved the management of HIV-1 infection. Unfortunately, the treatment requires lifelong adherence, quiescent reservoirs constitute the obstacle for a full HIV eradication. Side effects to cART and multidrug resistance against all drugs used in cART emerge because of lifelong adherence. Moreover, the development of a residual viremia, whatever its origin, is associated with a chronic inflammation that leads to non AIDS-associated pathologies and a shorter life expectancy for infected patients compared to healthy individuals. Taken together, the remedy of all these issues relies on the development of new drugs improving cART. Challenges and requirements for a modified cART to better manage HIV infection are discussed in this review and elsewhere [17,198,199].

We believe that new classes of drugs should target new molecular mechanisms involved in the regulation of viral gene expression. This may lead to the absence of virus production or to the production of non-infectious particles. We focused in the review on transcriptional inhibitors since these can prevent the synthesis of viral proteins such as rev and Tat. The design of new treatments is essential for at least three purposes:

- (i) Will improve cART and hence by intensifying cART which is initiated early during acute HIV infection may contribute to reduce the size of latent/quiescent reservoirs.
- (ii) By preventing synthesis of neurotoxic viral proteins it will reduce the outcome of milder form of HAND (reviewed in [200])
- (iii) Reactivation of the virus with LRA will lead to the synthesis of neurotoxic viral proteins and is often associated with CNS inflammation through macrophage/microglial activation. Targeting transcription may prevent the outcome of brain injuries associated with this “shock and kill strategy” (comments in [201]).

There are currently several candidates with potent anti-HIV activities that inhibit gene transcription *in vitro* and *ex vivo*, which deserve attention. However, further investigations are needed to test them *in vivo*. To date, all molecules, except one, targeting HIV transcription have failed or did not reach clinical trial. No doubt, the new approaches discussed in this review will optimize the potential candidates capable to inhibit specifically HIV transcription. The main challenge for the next 10 years will be to assess these new transcriptional inhibitors and to find methods to deliver them in preclinical and clinical trials. For example, the application of nanotechnologies in trials is presently in an early stage, their wider use will improve both bioavailability and bio-distribution of drugs.

Finally, we point out that several potential drugs targeting HIV transcription have been isolated or derived from natural compounds like P27<sup>SJ</sup> from the plant *Hypericum perforatum*, PA14 from the bacteria *Pseudomonas aerobacter* and corticostatine from the marine sponge *Corticium simplex*. Natural compounds are still a major source of new drugs; they benefit from technical advances in the field of high-throughput screening [202]. As a corollary, this is one more good reason to preserve biodiversity.



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\* Of interest

\*\* Of considerable interest

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Figure 1: HIV-1 replication cycle.

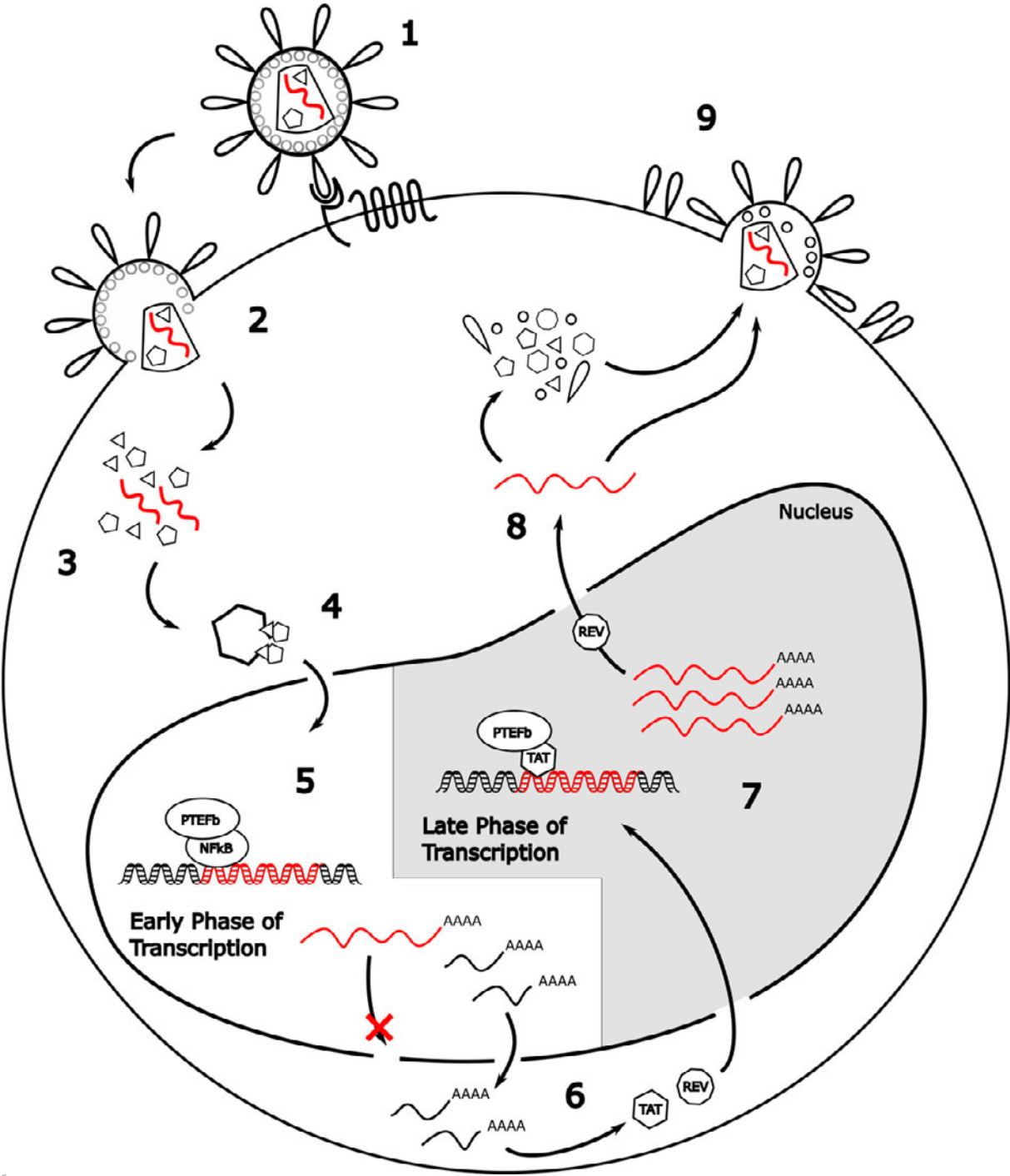


Figure 2: Early and late transcription phase of HIV-1

