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Review

Block-And-Lock Strategies to Cure HIV Infection

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Abstract: Today HIV infection cannot be cured due to the presence of a reservoir of latently infected cells inducing a viral rebound upon treatment interruption. Hence, the latent reservoir is considered as the major barrier for an HIV cure. So far, efforts to completely eradicate the reservoir via a shock-and-kill approach have proven difficult and unsuccessful. Therefore, more research has been done recently on an alternative block-and-lock functional cure strategy. In contrast to the shock-and-kill strategy that aims to eradicate the entire reservoir, block-and-lock aims to permanently silence all proviruses, even after treatment interruption. HIV silencing can be achieved by targeting different factors of the transcription machinery. In this review, we first describe the underlying mechanisms of HIV transcription and silencing. Next, we give an overview of the different block-and-lock strategies under investigation.

Keywords: HIV; latency; cure; block-and-lock

1. Introduction

Despite significant improvements in clinical outcome, the HIV/AIDS pandemic remains an important threat to public health. Although combination antiretroviral therapy (cART) suppresses plasma viral load to undetectable levels, removal of therapy leads to a viral rebound from a highly stable reservoir of latently infected cells [1]. This reservoir mainly consists of resting memory CD4 T cells and can be found in many different anatomical compartments such as brain, liver, bone marrow and lymphoid tissues [2]. These latently infected cells escape the immune system and are not eliminated by current antiretroviral treatments [3]. Hence, the persistence of these latent reservoirs is the major obstacle towards a cure for HIV-1 infection.

The potential for an HIV cure was highlighted by the long-term HIV remission of two infected individuals (the Berlin and London patient) following an allogeneic stem cell for either leukemia or lymphoma, respectively [4,5]. Both patients received stem cell transplants from donors with a homozygous CCR5Δ32 mutation, rendering the resulting CD4+ T cells resistant to HIV infection by R-tropic strains that use the CCR5 co-receptor for infection. Notably, another patient treated with such CCR5Δ32 stem cells suffered viral rebound from a minority X-tropic strain, which uses the CXCR4 co-receptor, in his reservoir [6,7]. Other patients who received allogeneic stem cell transplantations lacking this mutation rebounded as well [8]. In short, the significant mortality risk, the low chance of finding a HLA-matching donor with CCR5Δ32 and the possibility of rebound even with such a donor mean this treatment is not scalable for the vast majority of HIV-infected individuals. Significant effort has been directed towards the development of potential cures that eliminate the latent reservoir. Studies are ongoing to remove HIV-1 provirus from latent cells using gene-editing strategies [9–11]. However, delivery of gene editing constructs to all reservoir cells in vivo remains a formidable hurdle and gene-editing strategies suffer from unknown off-target risks [12].

Alternatively, the shock-and-kill strategy aims to eradicate the reservoir by repeated reactivation of latent cells that are subsequently killed by the immune system or viral cytopathic effects [13]. Initial clinical trials with several latency reversing agents (LRAs) showed induction of viral RNA production in patients, e.g., by disulfiram and the HDAC inhibitors vorinostat, panobinostat or romidepsin. However, these LRAs did not reduce the size of the latent reservoir [14–16]. Besides low efficacy in the clinic, other limitations of many LRAs are their side effects and toxicity by affecting cellular homeostasis. Moreover, studies show that only a minor fraction of the reservoir is reactivated upon treatment with LRAs, indicating that a combination of multiple LRAs is required [17,18]. Combination approaches, in which LRAs from multiple mechanistic classes are combined, are now investigated to obtain a more effective shock [19–21]. Still, reactivation of latently infected cells is not sufficient to reduce the size of the reservoir. Shan et al. showed in a primary cell model that latently infected cells survive despite viral cytopathic effects and the presence of cytotoxic T cells [22]. The infected cells were only killed upon antigen-specific stimulation of the cytotoxic T cells [22]. Therefore, the 'kill' phase requires optimization by improving immune responses and stimulating apoptosis of infected cells [23,24]. The immune response can be stimulated by TLR agonists [25], immune checkpoint inhibitors [26], therapeutic vaccines [27] and broadly neutralizing antibodies [28,29]. Currently several pro-apoptotic compounds are tested for their capacity to kill latently infected cells, e.g., SMAC (second mitochondria-derived activator of caspase) mimetics [30–32] and inhibitors of the regulator protein B cell lymphoma 2 (Bcl2) [33,34] and PI3K/Akt pathway [35].

The limited success of eradication strategies has caused scientists and clinicians to re-evaluate the definition of HIV cure. The ultimate outcome would indeed be the complete eradication of all replication-competent HIV. However, such a sterilizing cure will be challenging to achieve. A more feasible outcome might be long-term HIV remission or a functional cure. A functional cure could be achieved by durably silencing the latent provirus in infected cells and thereby preventing viral rebound [36]. This so-called block-and-lock strategy prevents HIV transcription and reactivation in latently infected cells. In this review, we will first discuss the HIV transcriptional machinery and determinants leading to transcriptional silencing. Secondly, we will give an overview of various block-and-lock HIV cure strategies acting on different determinants of HIV transcription.

2. HIV Transcription and Silencing

Viral latency is classified in two forms, based on the integration status of the viral DNA: pre- and post-integration latency. Pre-integration latency occurs when the replication cycle is interrupted prior to integration [37]. Unintegrated DNA can be linear or circularized, but generally has a short half-life of approximately one day [38]. Although they may persist longer in macrophages or the brain, they are clinically less relevant [39,40]. Post-integration latency takes place when the virus is stably integrated in the host genome but fails to express proteins. As such, the provirus can persist for the lifespan of the infected cell. Post-integration latency can be sustained by blocks in nuclear export of viral RNA and translation, but often HIV is silenced at the transcriptional level. In this review, we will elaborate on HIV transcription as many of the current block-and-lock strategies act on various determinants of HIV transcription.

HIV transcription is a complex machinery involving many viral and cellular factors. During productive infection, HIV initially produces short completely spliced transcripts encoding transactivator of transcription (Tat) and regulator of virion expression (Rev). When the amount of Tat protein reaches a certain threshold, it binds to the TAR RNA stem loop in the HIV LTR promoter stimulating transcription elongation (Figure 1a) [41]. Tat recruits the positive transcription elongation factor complex (P-TEFb), consisting of cyclin-dependent kinase 9 (CDK9) and Cyclin T1, to the transcription start site (TSS) in the LTR promoter [42]. Next, CDK9 releases and activates RNA polymerase II (RNAPII) that is sequestered by negative elongation factor (NELF) and DRB sensitivity inducing factors (DSIF) [43]. Furthermore, Tat recruits the active chromatin remodeling PBAF

(polybromo-associated BRG-associated factor) complex promoting open chromatin [44]. Changes in these factors can silence HIV transcription resulting in latent infections.

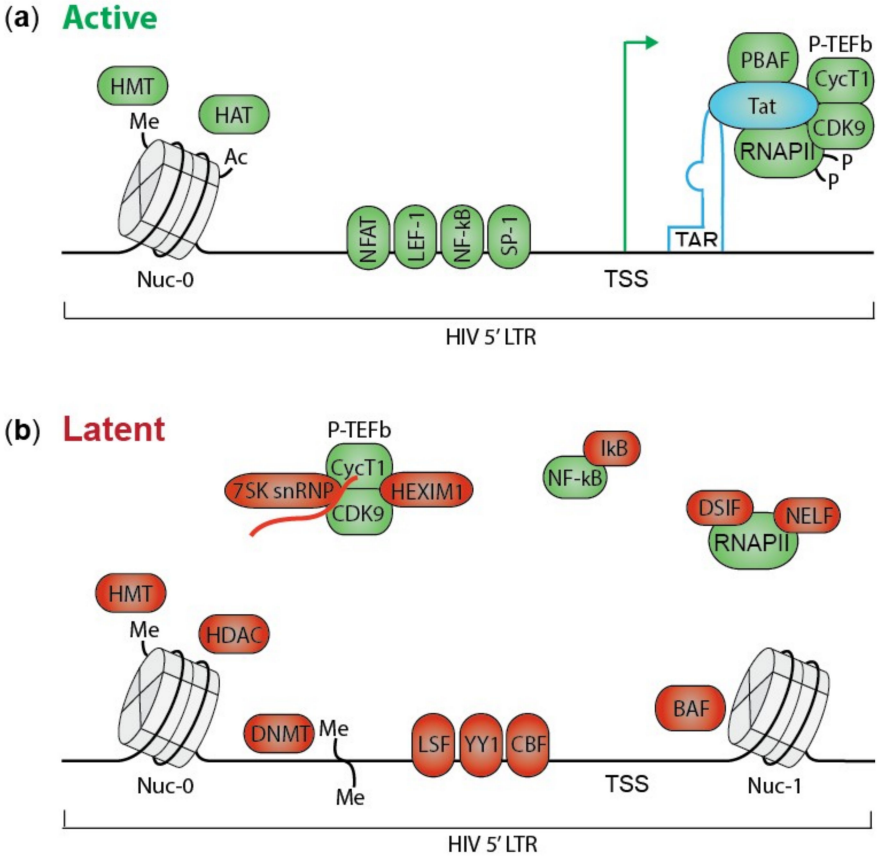

Figure 1. The HIV LTR promoter in active and latent state. (**a**) HIV Tat induces active transcription by binding the TAR RNA element in the LTR promoter and recruiting several transcription activating proteins. P-TEFb releases and activates RNAPII by CDK9-mediated phosphorylation. PBAF ensures open nucleosome-free chromatin. NF-κB and other transcription factors as NFAT, LEF-1 and SP-1 bind to the HIV LTR. An active chromatin landscape is maintained by histone methyl transferases (HMT) and histone acetyl transferases (HAT). (**b**) In latent cells, HIV transcription is inhibited by multiple mechanisms. Several proteins required for effective transcription are sequestered in an inactive state. For instance RNAPII is sequestered by DSIF and NELF, NF-κB by IκB, and P-TEFb by 7SK snRNP and HEXIM1. Furthermore, transcriptional repressors as LSF, YY1 and CBF bind the LTR promoter. A repressive chromatin landscape is formed by HMT, histone deacetylases (HDAC) and DNA methyl transferases (DNMT). Finally, BAF positions Nuc-1 downstream of the TSS, inhibiting transcription elongation. Tat; transactivator of transcription, TAR; transactivation response RNA, P-TEFb; positive transcription elongation factor b, RNAPII; RNA polymerase II, CDK9; cyclin dependent kinase 9, PBAF; polybromo-associated BAF, NF-κB; nuclear factor kappa b, NFAT; nuclear factor of activated cells, LEF-1; lymphoid enhancer-binding factor 1, SP-1; specificity protein 1, HMT; histone methyl transferase, HAT; histone acetyl transferase, DSIF; DRB sensitivity inducing factor, NELF; negative elongation factor, IκB; inhibitors of NF-κB, 7SK snRNP; 7SK small nuclear RNA, HEXIM-1; Hexamethylene bisacetamide-induced protein, LSF; late SV40 factor, YY1; yin yang 1, CBF; C-promoter binding factor, LTR; long terminal repeat, HDAC; histone deacetylase, DNMT; DNA methyl transferase, BAF; BRG-associated factor, Nuc-1; nucleosome 1, TSS; transcription start site. Green symbols represent factors promoting active transcription, while red symbols are transcriptional repressors.

Suboptimal concentrations or post-translational modifications of Tat hamper Tat-induced transcription elongation. For instance, phosphorylation of Tat by CDK2 results in inhibition of transcription [45]. Moreover, in latent cells P-TEFb is retained in an inactive form by hexamethylene bisacetamide-induced protein (HEXIM-1) and 7SK small nuclear RNA (7SK snRNA) (Figure 1b) [46,47]. Tat and bromodomain containing protein 4 (BRD4) can release P-TEFb by disrupting the inactive complex [48]. BRD4 represses HIV transcription by competing with Tat for the P-TEFb binding site [48,49].

Transcription is also affected by the absence or presence of host transcription factors and transcription repressors. Nuclear factor kappa B (NF-κB) is a transcription factor involved in T cell activation that activates HIV transcription even in the absence of Tat [50]. However, in resting cells NF-κB is sequestered in the cytoplasm by inhibitors of NF-κB (IκB). Nuclear factor of activated T-cells (NFAT), another transcription factor, is phosphorylated and resides in the cytoplasm of resting cells [51,52]. NF-κB and NFAT are two key factors for initiation of HIV transcription; for more examples of transcription factors involved in HIV latency please see following review articles [53–56]. Additionally, some repressive host factors like yin yang 1 (YY1), late SV40 factor (LSF) [57] and C-promoter binding factor (CBF) [58] recognize binding sites in the LTR promoter and limit transcription by recruiting histone deacetylases (HDAC) (Figure 1b) [59].

The chromatin and epigenetic landscape also affects HIV transcription [60,61]. The chromatin structure is defined by the presence of nucleosomes, consisting of eight core histones that can be epigenetically modified. Histone acetylation, induced by histone acetyl transferases (HATs), is associated with active transcription; acetylation loosens the chromatin making it more accessible to interacting proteins [62]. H3K36me3 is typically found in the body of active genes [63], while tri-methylation of H3K27 and H3K9, established by histone methyl transferases (HMTs), are associated with transcriptional silencing [64]. Additionally, DNA methylation at CpG dinucleotides in the LTR promoter contributes to latency and restricts reactivation in cell lines and patient samples by hampering the access of transcription factors to the DNA [65,66]. Regardless of the site of integration, the viral LTR promoter is occupied by two nucleosomes, nuc-0 and nuc-1. Nuc-1 is positioned downstream of the transcription start site (TSS) by the ATP-dependent chromatin remodeler BAF (BRG1-associated factor), where it blocks transcription elongation in latent cells (Figure 1b) [67].

Next to a block in transcription initiation or elongation, additional blocks may exist at the level of distal transcription and multiple splicing as postulated by the Yukl group [68]. They used reverse transcription droplet digital PCR to quantify different HIV transcripts in patient-derived cells. Short TAR RNA was most abundant, followed by RNA elongated beyond the LTR promoter, full length poly-adenylated RNA and finally multiple spliced RNA was the least common [68]. Interestingly, the transcription profiles differed in cells from blood compared to gut [69]. These results indicate blocks at transcription elongation, completion and RNA splicing that are tissue specific and might be important in HIV latency. Therefore, all steps involved in viral gene expression require further investigation.

The three-dimensional nuclear organization of chromatin affects the function of the DNA as well [70]. The genome organization within the nucleus is not random; transcriptionally active and inactive regions are physically distinguishable as decondensed euchromatin and condensed heterochromatin, respectively [71]. HIV preferentially integrates in open chromatin close to the nuclear pore, while heterochromatic chromatin in lamina-associated domains (LADs) or the inner nucleus is disfavored [72]. Moreover, nuclear processes like transcription, DNA-replication and -repair are located in certain structural compartments [70]. These facts indicate that the nuclear topography of HIV affects viral transcription.

Finally, the HIV integration site might be an important determinant of viral transcription as it defines both the epigenetic landscape and the 3D nuclear localization of the eventual provirus. HIV integration is not random; the virus has evolved in such a way that it hijacks cellular cofactors to tether its integration to active transcription units [73,74]. Depletion of the cofactors lens epithelium derived growth factor (LEDGF/p75) and cleavage and polyadenylation specificity factor 6 (CPSF6) were

shown to decrease integration in active genes [75–80]. Moreover, proviruses that were retargeted by interfering with LEDGF/p75 were more latent and refractory to reactivation [76]. Finally, several studies suggest that integration in certain (onco-)genes supports clonal expansion of the infected cell contributing to viral persistence [81–86].

3. Block-And-Lock Strategies

Many viral and cellular proteins are involved in HIV transcription and silencing, and hence represent potential targets for future block-and-lock approaches. Several research groups have described mechanisms acting on different factors of HIV transcription in light of a block-and-lock strategy. We list these different block-and-lock strategies below.

3.1. Tat Inhibition by Didehydro-Cortistatin A

Currently the most advanced block-and-lock approach employs a Tat inhibitor, didehydro-cortistatin A (dCA), to silence HIV transcription. The viral Tat protein is an important factor for stimulation of HIV transcriptional elongation by recruiting and activating RNAPII (Figure 1) [87]. HIV Tat represents an interesting target since it is the first viral protein to be expressed upon infection and it has no cellular homolog. dCA is a potent Tat inhibitor that blocks HIV transcription and reactivation by different LRAs in cell lines and primary CD4+ T cells [88,89]. In 2017, the Valente lab used patient-derived cell models and bone marrow/liver/thymus (BLT) mouse latency models to show that prior treatment with dCA delayed and reduced viral rebound [90]. The BLT mice were co-treated with dCA and ART for four weeks prior to treatment interruption. Ten days later, all eight control mice displayed viremia, while the dCA treated mice showed a viral rebound only at day 19. Furthermore, dCA induced a high nucleosomal occupancy at the Nuc-1 region of the LTR promoter potentially explaining its long term effects [90]. In 2019 the Valente group elaborated on this mechanism by showing that dCA promotes tight nucleosome/DNA association by increasing deacetylated histone 3 occupancy at Nuc-1 [91]. Moreover, dCA enhanced the recruitment of the repressive BAF complex while the activating chromatin remodeling complex PBAF was inhibited. In line with these results, less RNAPII was detected at the transcription start site, even upon stimulation with LRAs. The specificity of dCA for Tat was confirmed by the lack of effect on Tat-TAR defective proviruses [91]. Altogether, these results show that dCA inhibits Tat-dependent transcription and induces a repressive epigenetic landscape that hampers HIV reactivation upon treatment interruption.

3.2. LEDGINs

In 2010, structure-based drug design identified the first small molecule inhibitors of the interaction between HIV integrase (IN) and the cellular chromatin-thethering factor LEDGF/p75 [92]. Inhibitors belonging to this class of antivirals [92–96], named 'LEDGINs', are unique due to their multimodal mechanism of action affecting both early and late stages of HIV-1 replication. LEDGINs inhibit HIV-1 integration and allosterically inhibit IN catalytic activity [92,97]. Moreover, LEDGINs enhance IN oligomerization during late stages of the replication cycle resulting in defective progeny virions [98–101]. Viral particles produced in the presence of LEDGINs display morphological defects and are less infectious [98–101]. The idea of using LEDGINs for a functional HIV cure arose when the Debyser lab started investigating their effect on integration sites and latency in 2016 [76,102]. Vranckx et al. showed that viruses capable of integrating in the presence of LEDGIN treatment during infection were retargeted out of active genes. The 3D localization of the provirus was closer to the inner nucleus [76]. Moreover, by using several reporter viruses and cell lines, they showed that these retargeted proviruses were more often in a latent state and refractory to reactivation by LRAs. These results were also confirmed in primary cells [76]. More recently, Vansant et al. showed that infection of cells with virus produced in the presence of LEDGINs also resulted in provirus with a more latent phenotype [103]. Based on these data, the Debyser group postulated that LEDGINs might be useful in a block-and-lock strategy by inhibiting viral integration and retargeting residual

proviruses that manage to integrate in the presence of LEDGINs to sites that are less susceptible to reactivation [102]. In such a cure strategy, ideally LEDGINs are administered as soon as possible after acute infection to affect the formation of the reservoir. Although initial infection represents an interesting niche with over 1.7 million newly infected patients in 2018 [104], it is unclear whether a functional cure strategy with LEDGINs will also be useful in chronically infected patients for instance after treatment interruption. Before this concept moves on to clinically more relevant models such as humanized mice and eventually patients, further research on the mechanism of action of LEDGINs, specifically in the context of HIV latency, is required.

3.3. FACT Inhibition by Curaxin CBL0100

Another regulator of HIV transcription is the 'facilitates chromatin transcription complex' (FACT) that consists of suppressor of Ty16 (SUPT16H) and structure-specific recognition protein (SSRP1) [105]. FACT acts as a histone chaperone and promotes RNAPII driven transcription by destabilizing the nucleosomal structure [106]. In 2011, Gasparian et al. showed that the anticancer compounds named curaxins inhibit FACT and suppress NF-κB mediated transcription [107]. This finding led to the hypothesis that curaxins can promote HIV latency via inhibition of FACT. Indeed, in 2017 Jean et al. was able to block HIV replication and reactivation by using curaxin CBL0100 [108]. There was less reactivation of latent provirus, both in cell lines and in primary cell models, in the presence of CBL0100 compared to reactivation in the presence of DMSO. Curaxin CBL0100 inhibited RNAPII mediated transcription elongation in a Tat-dependent manner [108]. However, the effect was independent of NF-κB binding to the 5′ LTR promoter in contrast to its anti-tumor activity reported before [107,108]. The authors hypothesize that addition of CBL0100 to cART regimens might lead to a faster control of viremia and reduced HIV reactivation that eventually locks the virus in a latent state even upon treatment interruption.

3.4. RNA-Induced Epigenetic Silencing

An alternative approach to silence HIV transcription is by using short interfering (si) or short hairpin (sh) RNA to maintain the repressive heterochromatic landscape at the HIV 5′ LTR promoter. The Kelleher group designed two siRNAs, 143 and Prom A, which target transcription factor binding sites in the LTR promoter [109,110]. siRNA 143 binds upstream of Nuc-0 where binding sites for transcription factors AP-1 (activator protein 1) and COUP (chicken ovalbumin upstream promoter) are located. siRNA Prom A targets a unique NF-κB binding site situated between Nuc-0 and Nuc-1. These siRNAs epigenetically silence HIV transcription by recruiting Argonaute 1 (AGO1), histone deacetylase 1 and histone methyl transferases [111]. AGO1 is an essential component of the RNA-induced silencing complex (RISC) that binds siRNAs and cleaves the mRNA, a process termed RNA interference (RNAi). Both siRNAs reduced reactivation of the latently infected J-Lat cells by two- to three-fold when challenged by different LRAs [111]. Thus, transcriptional gene silencing by siRNAs might be useful in a HIV block-and-lock functional cure via a gene therapy application. The siRNAs could be delivered to cART-treated patients via retroviral vector transduced autologous CD4+ T cells or CD34+ cells in the absence of cART [110]. However, further extensive preclinical evaluation is required.

3.5. HSP90 Inhibitors

Heat shock protein 90 (HSP90) is a cellular chaperone protein that helps folding and stabilizing other proteins. Heat shock proteins protect cells when stressed by high temperatures. They are also required for the production of viral proteins. Upon HIV infection, the expression of HSP90 increases in mononuclear cells and T cells [112,113]. Indeed, HSP90 inhibitors suppress HIV transcription and replication [114,115]. Moreover, HSP90 is involved in HIV reactivation by stimulating Tat-mediated HIV transcription and NF-κB, NFAT and STAT5 (signal transducer and activator of transcription) signaling [116,117]. Hyperthermia also enhances HIV transcription and reactivation [114,118]. Multiple HSP90 inhibitors have been reported to suppress HIV-1: GV1001, a peptide vaccine designed

to induce T cell immunity, and specific HSP90 inhibitors such as AUY922 and 17-AAG [115–117,119]. AUY922 and 17-AAG are in clinical development as anticancer compounds [120–123]. In 2016, Joshi et al. showed that humanized BLT mice pretreated with a reverse transcriptase inhibitor (EFdA) and AUY922 or 17-AAG did not rebound up to 11 weeks after treatment cessation [119]. Upon heat shock or activation of the cells, replication competent virus was recovered from PBMC's and the spleen, indicating that these cells were latently infected [119]. Thus, addition of HSP90 inhibitors to current treatment regimens might lead to long-term remission and potentially a functional cure of HIV infection.

3.6. Jak-STAT Inhibitors

Homeostasis of memory T cells, the major contributor of the latent reservoir, is regulated by cytokines that activate the Jak (Janus kinase)-STAT pathway. The Jak-STAT pathway was shown to be involved in HIV persistence and reactivation as two FDA approved Jak inhibitors, ruxolitinib and tofacitinib, were able to block HIV reactivation in primary CD4+ T cells [124]. Ruxolitinib and tofacitinib are approved for the treatment of hematologic conditions (myelofibrosis and polycythemia vera) and auto-immune conditions (rheumatoid arthritis, psoriatic arthritis and ulcerative colitis) respectively, and have strong anti-inflammatory effects. Latent cells were pre-treated with the two inhibitors for 30 min followed by reactivation in the presence of the compounds for 24 h. Ruxolitinib displayed the strongest inhibitory effect with more than 50% inhibition of reactivation measured by intracellular viral p24 [124]. Moreover, the anti-inflammatory effect of the Jak inhibitors reduced activation of T cells limiting transmission of HIV to other cells. Additionally, they reduced surface expression of the CCR5 co-receptor and decreased the number of CD4+ T cells harboring HIV provirus [125]. Currently, the anti-inflammatory effects of ruxolitinib in HIV infection and seeding of HIV reservoirs are evaluated in a phase 2 clinical trial (NCT02475655).

3.7. BRD4 Modulators

Another important regulator of HIV transcription is the bromodomain-containing protein 4 (BRD4). BRD4 is an epigenetic reader that interacts with various proteins to stimulate gene expression [48,126]. On the other hand, BRD4 inhibits HIV transcription by competing with Tat for binding to the P-TEFb [49]. Recently, Niu et al. identified a small molecule, ZL0580, that binds bromodomain 1 of BRD4 and suppresses HIV transcription [127]. ZL0580 inhibited Tat transactivation and transcription elongation. Additionally, the BRD4 modulator induced a repressive chromatin environment at the LTR promoter [127]. The small molecule delayed time to viral rebound after therapy cessation in PBMCs from aviremic HIV-infected patients. PBMCs treated with ART alone rebounded HIV replication after 2.4 ± 1.3 days, while virus rebounded only after 15 ± 6.1 days in PBMCs treated with ART plus ZL0580 [127]. Moreover, ZL0580 blocked spontaneous HIV replication in PBMCs of aviremic patients that were not treated with ART and blocked PHA stimulated reactivation [127]. As such, BRD4 modulators represent a new class of compounds that can be used for a block-and-lock functional cure strategy. Since the small molecule delayed but not prevented viral rebound, the authors speculate that probably a combination of approaches will be required to durably silence all HIV-1.

3.8. mTOR Inhibitors

To identify novel mechanisms contributing to HIV latency, the Verdin lab performed a genome wide analysis with a shRNA screen [128,129]. They transduced a latent J-Lat cell line with a vector encoding a mCherry fluorescent protein and a shRNA library targeting each protein coding gene [130]. Cells stably transduced with the shRNA express mCherry. The J-Lat cells contain one integrated latent HIV provirus per cell that expresses green fluorescent protein (GFP) upon activation. By characterizing mCherry and GFP double fluorescent cells, they identified three pathways important for HIV latency: transforming growth factor β (TGF- β), actin remodeling and mammalian target of Rapamycin (mTOR) signaling [128]. These pathways are linked as mTOR acts downstream of TFG- β signaling and

upstream of actin remodeling. Inhibition of mTOR suppressed HIV reactivation in primary CD4+ T cells and patient cells by downregulating CDK9 phosphorylation and impeding NF-κB signaling [128]. The authors suggest that mTOR inhibition together with other latency promoting agents such as Tat and HSP90 inhibitors might be useful in a future 'block a lock' functional cure.

3.9. Kinase Inhibitors

Starting from the hypothesis that signaling pathways play a central role in HIV latency, Vargas et al. screened a library of kinase inhibitors targeting a wide range of signaling pathways in the latent 24ST1NLESG cell line [131]. They screened the kinase inhibitors in the absence or presence of different LRAs and found 12 inhibitors that blocked HIV-1 reactivation irrespective of the used LRA [131]. The four most potent compounds are PF-3758309, danusertib, AZ628 and P276-00 that target PAK, Aurora, Raf and CDK kinases, respectively. These compounds had IC_{50} values ranging from 0.0001 to 9.4 μM for blocking latency reversal by different LRAs in 24ST1NLESG cells. Additionally, they inhibited latency reversal in resting CD4+ T cells from HIV-infected donors that were challenged by the anti-CD3/CD28 monoclonal antibody [131]. Further studies are ongoing to evaluate whether these inhibitors could be useful in a block-and-lock functional cure strategy.

3.10. Triptolide

Triptolide is a diterpenoid epoxide derived from a Chinese herb that possesses anti-inflammatory, immunosuppressive and anti-tumor activities [132]. In 2014, Wan et al. first reported on the antiviral activity of triptolide [133]. Triptolide inhibited HIV-1 replication in vitro at the level of viral transcription. More specifically, triptolide hampered Tat-induced LTR activation by stimulating proteasomal degradation of Tat [133]. Currently, the effect of triptolide on the HIV-1 reservoir is being tested in phase III clinical trials in treatment-naive HIV-infected patients (NCT02219672). Whether triptolide has a beneficial effect in chronically and latently infected patients still needs to be studied.

4. Discussion

As described in this review, HIV transcription and latency are driven by complex interactions between cellular and viral proteins. Along with a growing understanding of these mechanisms, the potential targets for a functional block-and-lock cure increase. To evaluate the potential of the block-and-lock strategies, we must consider various factors such as effectiveness, advantages, scalability, potential side effects and challenges. Since patients on antiretroviral therapy have nearly normal lifespans these days and the side effects of newer cART combinations are reduced, new treatment modalities will need to clear a high bar.

With regard to efficacy, an important factor is the duration of the achieved HIV remission. Ideally, such a remission would be lifelong. Even though the idea of a 'block and lock' strategy arose only recently, this review listed the growing body of evidence showing that HIV transcription can be significantly downregulated. Though clinical data is still lacking, all of the strategies above impeded viral transcription and reduced reactivation in the presence of various LRAs in cell lines and/or primary cell models. More advanced studies in BLT mice showed that the Tat inhibitor dCA delayed viral rebound up to 19 days [90], while HSP90 inhibitors could delay rebound up to 11 weeks [119]. Yet so far, none of the investigated strategies has led to complete, long term suppression of virus in all cell and/or animal models. As many of these compounds are still in the early days of development and testing within the HIV field, newer compounds with improved efficacy could still be developed. Then again, HIV transcription can be stimulated in Tat dependent and independent manners, meaning blocking only one transcription pathway may not be enough to completely silence all proviruses. It could be that, as for the shock-and-kill approach, a combination block-and-lock treatment will be needed. It remains to be investigated whether the compounds can be administered during initial infection in

combination with cART for a short period of time (induction therapy) or whether repeated dosing will be required (maintenance therapy).

The block-and-lock strategy holds great promises for the HIV cure field. First, since the approach targets HIV transcription in general, it is likely to affect not only the replication competent, but also the translation competent reservoir [134]. As most proviruses in the patients are defective, they cannot produce infectious progeny viruses, yet some do produce viral proteins, contributing to immune activation [135,136]. Hence silencing both replication competent and defective proviruses may have a positive effect on the patients' health. Secondly, though the idea of living with an inactivated retrovirus may be scary to patients, scientifically speaking, this is not unprecedented. In fact, simian immunodeficiency viruses, HIV's closest relatives, are non-pathogenic in many cases, meaning they have adapted to not affect the lifespan of their host [137]. In addition, fossils of past retroviral infections are spread throughout the human genome as endogenous retroviruses, some of which have played critical roles in human evolution [138]. These precedents hint that taming HIV through a block-and-lock strategy may in the end be more feasible than full eradication.

Another major advantage of the block-and-lock strategy compared to other approaches such as gene therapy and allogeneic stem cell transplantation is the scalability. Most block-and-lock strategies are based on small molecules and/or existing compounds and many of them are likely to be administered in an ambulatory setting, resulting in a relatively low cost. Hence, they can be deployed in the limited resource settings that form the heart of the current HIV epidemic. In contrast, gene therapy and stem cell transplantation are more expensive and require highly specialized and advanced technologies. In the end, if any HIV cure is to affect the global HIV epidemic, we must insist that it can be made available to as many people living with HIV as possible, including those without access to highly advanced medical technology. To be scalable worldwide, a functional cure would have to be effective against a broad range of viral strains. dCA has been shown to bind Tat of subtypes A, B, C, D and E, but virus strains resistant to dCA have been developed in vitro [139,140]. LEDGINs also target a viral protein, HIV integrase. Even though LEDGINs are active against a broad range of HIV strains, specific integrase mutations can induce LEDGIN resistance [97].

Targeting a cellular protein could help to avoid the problem of viral diversity and resistance, but this may come at the cost of more side effects. To our knowledge, curaxin is only in early clinical development (NCT03727789) as an anticancer drug, so data on side effects are scarce. The HSP90 inhibitor AUY922 has been tested in phase I and phase II clinical trials, which showed fatigue, diarrhea and visual disturbances as side effects [120–122]. Trials with 17 AAG have shown hepatotoxicity, headaches and gastro-intestinal burden as potential side effects [122,123]. Ruxolitinib and tofacitinib are linked to hematological abnormalities and skin cancer. Additionally tocafitinib increases the risk of opportunistic infections and gastro-intestinal perforations [141,142]. Further, the use of kinase inhibitors is impeded by their lack of specificity leading to damage in myocytes [143]. Finally, triptolide was shown to have toxic effects on the reproductive tract [144,145]. Though serious side effects may be acceptable in the fields where these treatments are currently used (cancer, myeloproliferative disorders, auto-immune disease), the risk benefit ratio for using them in virally suppressed HIV-infected patients with a normal life span for obtaining a functional cure will be different. Much will depend on the duration of the remission induction phase of treatment and/or the frequency of these treatments if the remission is not lifelong and repeats are necessary. Still, the side effects mentioned above are minor compared to the risks associated with allogeneic stem cell transplantations.

As with all cure strategies, including shock-and-kill and gene editing, the main challenge lies in reaching and affecting each cell containing replication competent provirus. If not each infected cell is targeted, HIV replication will eventually rebound. Although it is challenging to permanently silence all provirus with a block-and-lock approach, extending the time to viral rebound from a few weeks to months or years may already lead to a significant benefit for the patient. This will reduce the need and costs for cART and visits to clinic, and hence improve the quality of life of HIV-infected patients. However, along with this strategy comes a new challenge: since HIV reactivation seems to be

a stochastically regulated process, how can we reliably predict a safe interval wherein no viral rebound will occur so patients can maintain their undetectable and non-transmissible HIV status? To answer this question we will need new tools to predict and/or quickly detect viral rebound. For cure research in general, we need better diagnostic tests to evaluate HIV reservoir size.

Currently there is no satisfactory cure for HIV and any strategy that could help us obtain a cure is worth investigating. As for the block-and-lock strategy, the next step will be bringing more of these treatments to in vivo models and clinical trials to investigate their effect on the latent HIV reservoirs and potential side effects in vivo. Though 35 years of HIV research have not resulted in a cure yet, the medical and scientific advances made by the field are unprecedented. As our knowledge and technology progresses, new insights will eventually lead to new breakthroughs, inside the field of HIV and beyond. If nothing else, research into a block-and lock-strategy will provide valuable knowledge on HIV transcription for the future of all HIV cure research.

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