



## Review

NF- $\kappa$ B sub-pathways and HIV cure: A revisitLilly M. Wong<sup>a</sup>, Guochun Jiang<sup>a,b,\*</sup><sup>a</sup> UNC HIV Cure Center, Institute of Global Health and Infectious Diseases, United States<sup>b</sup> Department of Biochemistry and Biophysics, The University of North Carolina at Chapel Hill Chapel Hill, NC 27599-7042, United States

## ARTICLE INFO

## Article History:

Received 25 August 2020

Revised 16 November 2020

Accepted 19 November 2020

Available online xxx

## Keywords:

HIV latency

Canonical NF- $\kappa$ BNoncanonical NF- $\kappa$ B

PEBP1

Raf1

HIV cure

## ABSTRACT

HIV cure is thwarted by the presence of quiescent yet replication competent HIV-1 (HIV). Antiretroviral therapy (ART) is unable to eradicate reservoirs, and upon cessation of ART, HIV will rebound. This review encompasses the curative strategies of HIV in the context of NF- $\kappa$ B sub-pathways that are currently exploited and demonstrate promise in the disruption of latent HIV. Canonical NF- $\kappa$ B signaling has long been established to drive HIV proviral expression while noncanonical NF- $\kappa$ B signaling, a novel and perhaps more desirable mechanism of latency reversal due to its unique characteristics, has recently been shown to also promote HIV expression from latency. Furthermore, we discuss the previously unrecognized upstream signaling of NF- $\kappa$ B as a new avenue for exploration of a functional cure of HIV.

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## 1. Introduction

Antiretroviral therapy (ART), a first-line defense against human immunodeficiency virus-1 (HIV) replication, has decreased HIV incidence and transmission. Roughly 38 million people worldwide are living with HIV. Despite the declining rate of HIV infection, only 25.4 million people had access to ART in 2019, which indicates limitations that must be addressed on a global scale [1,2]. This includes socioeconomic barriers from costly treatment, social stigma, pill fatigue, patient non-adherence, drug resistance and age-related comorbidities along with potential drug interactions from treatment of underlying diseases [2]. When ART is interrupted, HIV rebounds which promotes transmission and progresses to AIDS [3], indicating that while ART is able to suppress viral replication, replication competent HIV remains in a persistent state, otherwise known as the latent HIV reservoir [4].

Latent HIV reservoirs are products of active CD4<sup>+</sup> T cells that have transitioned to a state of quiescence after stable viral integration. The state of quiescence achieved by HIV is a result of manipulations to mechanisms of transcription [5]. Once the HIV-infected cells are in the resting state, there is minimal transcription whereas the infected cells persist as memory resting CD4<sup>+</sup> T cells, leading to reservoirs with transcriptionally silent HIV provirus. Through quantitative viral outgrowth assay (QVOA), it was previously determined that the half-life of replication-competent HIV is ~3.6–3.7 years and would require consistent treatment for at least 60 years to purge infected cells while

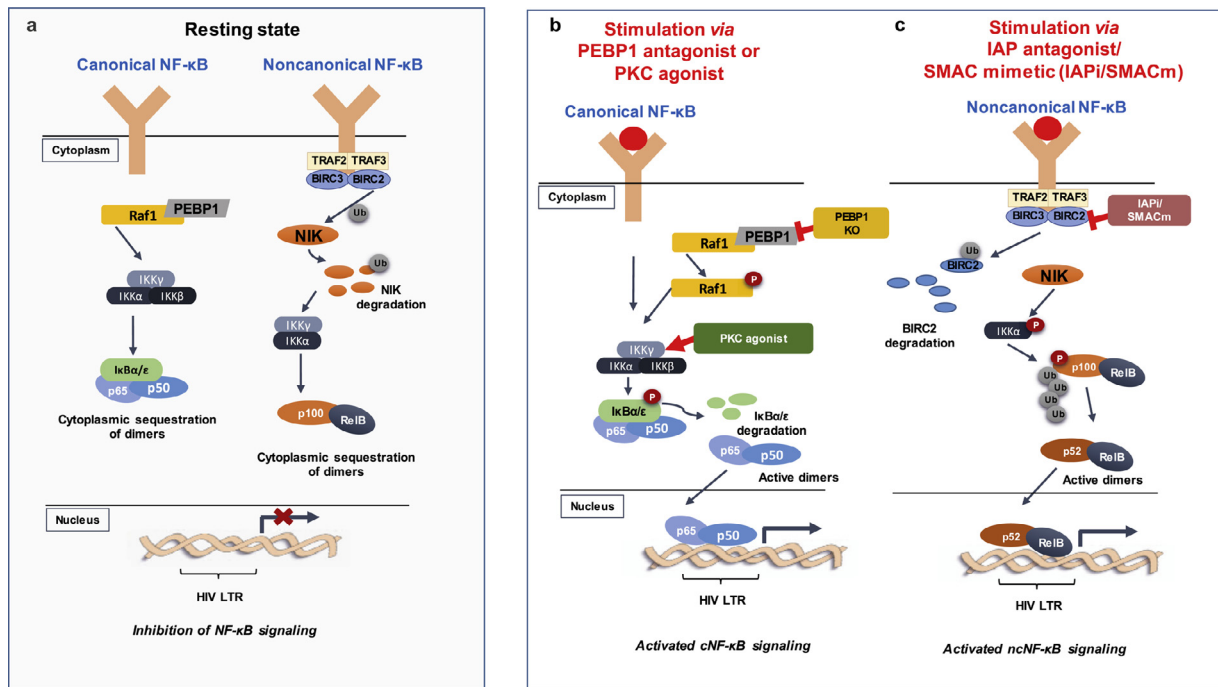
natural decay would take approximately 73 years [4,6]. However, this underestimates the size of the latent reservoir because the sensitivity of QVOA only reflects inducible intact proviruses [7,8]. One example is that some proviruses cannot be effectively reactivated unless there is a second round of stimulation [9]. It has been shown that defective proviruses produce viral proteins [10], indicating a need for improved characterization of the latent reservoir. Intact proviral DNA assay (IPDA) was designed to overcome the limitations of QVOA and PCR of total HIV DNA where IPDA maximally quantifies replication-competent proviruses by analysis of amplicons in *env* and packaging signal regions [11]. Peluso and colleagues used IPDA to determine that the half-life of HIV reservoir was 4.0 years from initiation of ART to year 7, and jumps to 18.7 years after year 7 where intact proviruses decay at a faster rate than defective proviruses [12]. In addition, it has been shown that cells harboring defective virus can be recognized by HIV-1-specific cytotoxic T lymphocytes while cells harboring replication-competent HIV seem resistant to CD8<sup>+</sup> T cells that may need to be addressed to cure infection [13–15]. These issues raise challenges for the eradication of HIV reservoirs.

## 2. To “shock and kill” or “block and lock”

Two central therapeutic approaches, *i.e.* “block and lock” and “shock and kill”, have been proposed for a cure of HIV. The “block and lock” strategy aims to suppress HIV transcriptional machinery to induce a deep silent state, followed by anticipated epigenetic modifications of HIV promoter for induction of a permanently silent transcriptional state so that viral rebound cannot occur or is significantly

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**Fig. 1.** NF- $\kappa$ B signaling pathway is involved in the transcription of HIV which can be exploited for HIV cure studies. A. Canonical and noncanonical NF- $\kappa$ B subpathways at their resting states. B. Canonical NF- $\kappa$ B activation after PEBP1 knockdown (KO) leads to phosphorylation of Raf1 or PKC agonist acts on IKK $\gamma$  to enable I $\kappa$ B $\alpha/\beta$  degradation, leading to the activation of HIV transcription or latency reversal. C. Noncanonical NF- $\kappa$ B signaling is activated by IAPi/SMACm such as birinapant or AZD5582 via p100 cleavage into p52 for subsequent HIV transcription or latency reversal.

delayed if ART is ceased [16,5,17]. It is not known whether deep latency can be achieved and whether provirus integration sites impact the induction of deep latency as interestingly observed in the elite controllers [18]. In contrast, the “shock and kill” strategy utilizes latency reversal agents (LRAs) to reactivate latent HIV through host-dependent mechanisms where cell-induced apoptosis is then elicited by either immune-mediated clearance or by viral-mediated cytopathic effect [19,20].

Among these LRAs, NF- $\kappa$ B stimulators such as PKC agonists (PKCa) have been efficacious in latency reversal both *in vitro* and *ex vivo* [21–23]. Some recent *in vivo* studies demonstrated that the use of PKCa, such as ingenol mebutate (PEP005), *Kansui* and SMAC mimetics, may be a suitable clinical approach by targeting NF- $\kappa$ B signaling pathway [22,24–26] (Fig. 1b-c). PKCa displayed activity of latency reversal to a broad range of memory CD4+ T cell subsets compared with other LRAs [27]. These important and perhaps surprising findings urge us to look further into NF- $\kappa$ B signaling. Although NF- $\kappa$ B pathway has been extensively investigated in the regulation of HIV transcription and latency, a revisit of this molecular signaling pathway may be timely in our current efforts for a cure of HIV.

### 3. Molecular mechanism of HIV transcription and latency

Many gene components are essential for HIV replication in host immune cells, including its long terminal repeat (LTR) located at the 5' end. After HIV cDNA integrates into the host genome in CD4+ T cells, some of these cells remain in a quiescent state, leading to the establishment of latent reservoirs unless reactivated [28]. At LTR where HIV transcription is initiated, there are binding sites of many transcription factors such as NF- $\kappa$ B, nuclear factor of activated T cells (NFAT), and Sp1 on the U3 region [29]. The expression and recruitment of these transcription factors into HIV LTR are essential for the initiation of HIV transcription while HIV Tat, when in the super elongation complex, is critical for efficient transcriptional elongation of HIV [5]. P-TEFb, an enzymatic complex comprised of CyclinT1 and CDK9, regulates Tat transactivation for transcriptional elongation of

HIV mRNA [30]. Impaired expression or mis-translocation of these HIV transcription factors is associated with HIV latency [31,32].

HIV transcription is also controlled at the chromatin level. Histone acetylation and methylation are two established posttranslational modifications (PTMs) of the epigenetic landscape, which target histone tails of HIV LTR. Histone methylation or deacetylation at HIV LTR is related to the establishment and/or maintenance of HIV latency, which enforces a closed chromatin state to silence proviral HIV [33]. Histone deacetylase (HDAC) [34,35] and histone methyltransferase inhibitors [36,37] have been exhibited to reactivate latent HIV. Recently, histone lysine crotonylation (Kcr) was identified as an evolutionarily conserved PTM among several eukaryotic species [38]. Tan and colleagues identified and validated the presence of Kcr on several of the core histones where enrichment of Kcr was observed at sites of active promoters and potential enhancers [38]. This has been linked to the regulation of HIV transcription where ACS2 provides crotonyl-CoA to histones at the HIV LTR for PTM writers of histone crotonylation, such as p300 [39]. When histone decrotonylation occurs at the HIV LTR through ACS2 suppression, HIV tends to quiescence [39].

### 4. NF- $\kappa$ B signaling sub-pathways: Canonical, noncanonical and atypical

NF- $\kappa$ B, a family of evolutionarily conserved transcription factors, is a master regulator of the inflammatory and immune responses. In response to foreign stimuli, NF- $\kappa$ B recruits adaptive and innate immune cells such as macrophages and neutrophils to the infection site and triggers inflammation [40], which can be resolved thereafter. For example, the activation of NF- $\kappa$ B provokes antiviral transcription of interferon and interferon-stimulating genes, which provides protection against bacterial and viral pathogens [28]. However, dysregulated inflammatory responses can also contribute acute or chronic inflammatory diseases, inducing tissue damage. In addition, NF- $\kappa$ B promotes immune cell proliferation, differentiation, and inhibition of apoptosis by proapoptotic or antiapoptotic gene expression [40].

There are five constituents in the NF- $\kappa$ B family: NF- $\kappa$ B 1 (p50), NF- $\kappa$ B 2 (p52), p65 (RelA), RelB, and c-Rel [40]. They are structurally similar in that the members have a Rel homology domain (RHD), a domain responsible for the binding of DNA, dimerization and inhibitor of  $\kappa$ B (I $\kappa$ B) interaction [41]. These five constituents are sequestered in the cytoplasm by upstream inhibitory proteins of the I $\kappa$ B family by which NF- $\kappa$ B activity is regulated [40]. Vital proteins of the I $\kappa$ B family contains an ankyrin repeat domain, a repeating structural motif made of roughly 33 amino acids, which facilitates the I $\kappa$ B interaction with dimers for the activation of the signaling pathways [31].

Two NF- $\kappa$ B pathways exist: canonical and noncanonical NF- $\kappa$ B signaling, which generally cooperate with each other. However, distinct features, such as required stimuli, protein synthesis and other functions, distinguish between the canonical and noncanonical signaling pathways [42]. In canonical NF- $\kappa$ B (cNF- $\kappa$ B) signaling, once phosphorylation of I $\kappa$ B kinase occurs, ubiquitination and proteasomal degradation of I $\kappa$ B $\alpha$  releases p65/p50 from the cytoplasm for nuclear translocation, leading to p65/p50 binding to gene promoter for transcription [28]. Noncanonical NF- $\kappa$ B (ncNF- $\kappa$ B) signaling responds to a subset of tumor necrosis factor receptor (TNFR) superfamily members that activates NF- $\kappa$ B-inducing kinase (NIK), which further phosphorylates IKK $\alpha$ , leading to the phosphorylation of p100 followed by its cleavage into p52 and nuclear translocation of p52/RelB heterodimer to drive its target gene transcription [43]. Vpr, a vital gene for HIV virulence, is associated with NF- $\kappa$ B signaling by which Vpr hijacks both NF- $\kappa$ B pathways via I $\kappa$ B kinase (IKK) $\alpha/\beta$  enhancement for productive viral infection [44].

NF- $\kappa$ B exhibits crosstalk with other signaling molecules resulting in atypical signaling [41,45]. A well-known example is the positive upregulation of p100 in ncNF- $\kappa$ B during the induction of cNF- $\kappa$ B signaling [46]. Unlike canonical or noncanonical NF- $\kappa$ B signaling whose activation occurs in membrane-bound receptors, atypical NF- $\kappa$ B signaling is triggered by genotoxic stress (DNA damage) after the activation of nuclear factor  $\kappa$ B essential modulator (NEMO or IKK $\gamma$ ). NEMO initially translocates into nucleus, where it is sumoylated (sumoylation) and subsequently ubiquitinated (ubiquitination) via PTM. These sequential PTMs are regulated by the ataxia telangiectasia mutated (ATM) checkpoint kinase. Then, ubiquitinated NEMO/ATM complex returns to the cytosol and phosphorylates IKK $\beta$  for the subsequent activation of atypical NF- $\kappa$ B [41,43,47]. This is achieved via the phosphorylation of I $\kappa$ B, followed by its protein degradation and RelA/p50 nuclear translocation to activate its target gene transcription.

## 5. Targeting canonical NF- $\kappa$ B in HIV cure through PKC agonists

The cNF- $\kappa$ B pathway is well established in HIV transcription where the degradation of I $\kappa$ B $\alpha$  triggers the cNF- $\kappa$ B signaling cascade for translocation of p65/p50 to the nucleus, leading to the initiation of HIV transcription (Fig. 1b). In contrast, low expression of cNF- $\kappa$ B or impaired translocation of cNF- $\kappa$ B into nucleus is associated with HIV latency [31]. When I $\kappa$ Bs were knocked down, latent HIV was reactivated [48,49], indicating that this pathway can be modulated to achieve latency disruption. By mimicking the physiologic ligand diacylglycerol, protein kinase C agonist binds to the regulatory domains of PKC isoforms, targets PKC/cNF- $\kappa$ B pathway to degrade I $\kappa$ Bs, and activates HIV transcription from latency [31]. PKC agonist includes ingenol derivatives such as ingenol mebutate (PEP005) and ingenol B, phorbol esters such as phorbol 12-myristate 13-acetate (PMA), prostratin, bryostatin-1 and others [50] (Fig. 2 and Table 1), which have been tested in BLT humanized mice model and ART-suppressed HIV-positive individuals *in vivo* and shows some promise in executing the “shock and kill” strategy.

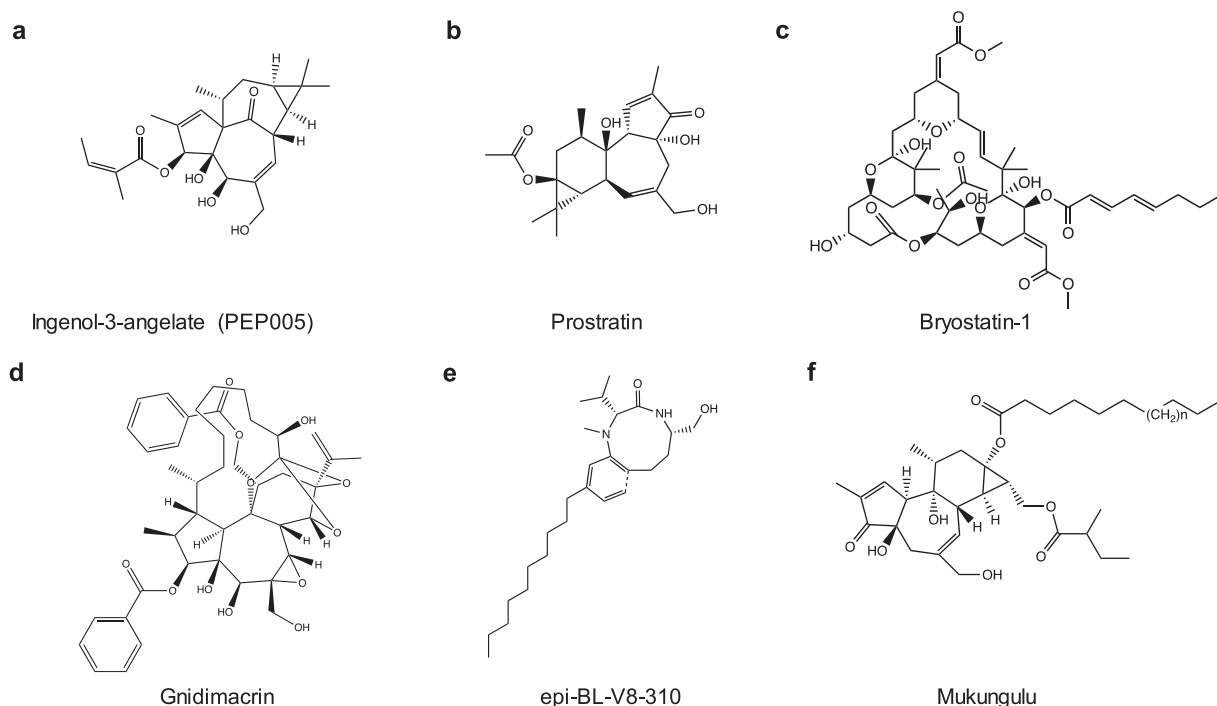
Prostratin is an active ingredient from plant derivatives such as *Homalanthus nutans* which was used by Samoan healers for non-HIV related conditions such as hepatitis. One issue with plant derivatives is the ability to quantify the active ingredient accurately as content

varies between sections of the plant [51]. Wender and colleagues report a practical synthesis of prostratin, which can yield milligrams to grams of the active ingredient as a resolution [52]. Soon after, Beans and colleagues synthesized prostratin analogs that elicited latent HIV with 100-fold potency [53]. The practical synthesis proposed by Wender and colleagues provides insight into extending similar syntheses to other PKCa that are low yield such as bryostatin-1. Bryostatin-1 has been tested in a clinical trial. While it was safe in low dosage, it failed to reactivate latent HIV *in vivo*, indicating that higher doses may be needed [54,55]. To investigate this issue, Marsden and colleagues have generated bryologs, which have been successful in latency reversal in a J-Lat cell line model of HIV latency, CD4+ T cells isolated from ART-suppressed HIV-infected individuals, and BLT-humanized mice [56].

Ingenol mebutate, a compound derived from *Euphorbia peplus* sap, is a PKC agonist and is highly potent when used singly or combined with other known LRAs, such as JQ1, a small molecule of BET bromodomain inhibitor, the reactivation of latent HIV *in vitro* and *ex vivo* resulted in a synergy compared to single treatment [57]. Jiang and colleagues conducted a study to examine cutaneous administration of PICATO, an FDA-approved gel, *in vivo*. PICATO utilizes ingenol mebutate (PEP005) as its active ingredient where short dosing of PICATO effectively clears actinic keratosis, a pre-malignant and common skin lesion often diagnosed in older HIV-infected individuals [58]. The administration of ingenol mebutate in ART-suppressed HIV-infected individuals cleared actinic keratosis and reversed transcriptional blocks of latent HIV without adverse effects [22]. Skin inflammation at administration site and site-associated mild pain were observed, however, actinic keratosis was resolved, and symptoms subsided [22]. Throughout treatment, a decrease in blocks to stages of HIV transcription was observed in both peripheral CD4+ T cells and skin biopsies. The reactivation of latent HIV by PICATO did not cause systemic immune activation, signifying that ingenol mebutate, the active ingredient, is able to reactivate latent HIV *in vivo*. However, many compounds of PKCa are toxic *in vivo*. To this end, Cao et al., recently formulated hybrid nanocarriers for ingenol mebutate to not only maintain the efficacy of ingenol mebutate in latency reversal but also reduce its side effect *in vivo* [59].

In addition, *Euphorbia Kansui* (EK), which contains many novel ingenol derivatives [29,60] is undergoing a safety trial in the form of a tea for the reactivation of latent HIV [61]. EK is a traditional Chinese medicine used for thousands of years as treatments for fluid retention and cancer. A plethora of individual compounds have been screened from EK extracts for latency reversal by which nanomolar concentrations of the compounds were able to induce HIV *via* PKC/cNF- $\kappa$ B signaling *in vitro* and *ex vivo* [60,62]. These studies may ensure an alternative direction to apply PKCa to the cure of HIV in future.

Recent studies started to uncover the preference of PKCa to different subset of CD4+ T cells for latency reversal. PKC agonists are effective in inducing NF- $\kappa$ B phosphorylation in the naïve (T<sub>N</sub>) and central memory CD4+ T cells (T<sub>CM</sub>) cells (T<sub>N</sub>>T<sub>CM</sub>>transitional memory CD4+ T cells> effector memory CD4+ T cells (T<sub>EM</sub>)) while they prefer to activate P-TEFb in the T<sub>EM</sub> cells [63]. HDAC inhibitor (HDACi), such as panobinostat and romidepsin, had a limited effect on latency reversal. Instead, ingenol mebutate (PEP005) induced HIV transcription from latency in all tested samples [63]. Ingenol mebutate displayed activity to a broad range of memory CD4+ T cell subsets compared with bryostatin-1 which preferred to disrupt latent HIV in the T<sub>EM</sub> cells [27]. When in combination with HDAC inhibitor, ingenol mebutate significantly enhanced latency reversal in T<sub>CM</sub> cells [63]. Similarly, the combination of romidepsin and ingenol was able to increase HIV RNA+ cells in most of the CD4+ T cell subsets. Interestingly, only the combination of panobinostat and bryostatin-1 was able to significantly increase HIV RNA+ cells in the population of memory stem cells [64]. These studies indicate that the cellular HIV reservoir is heterogeneous and responds differentially to different



**Fig. 2.** Pharmacological compounds for the activation of canonical NF- $\kappa$ B. Chemical structures of PKC agonists were shown. These PKC agonists have been tested in HIV cure studies. While latency reversal efficacy has been shown *in vitro* and *ex vivo*, *in vivo* evidence of latency disruption is lacking.

**Table 1**  
Targeting canonical and noncanonical NF- $\kappa$ B signaling pathways through pharmacological compounds.

Pathway Target	Pharmacological compounds	Model system	References
<b>Canonical NF-<math>\kappa</math>B</b>	Bryostatin-1 and analogs (bryologs)	A, B, E, F, H (NCT02269605)	DeChristopher et al., 2012; Gutierrez et al., 2016; Marsden et al., 2017; Marsden et al., 2018
	<b>Phorbol esters</b>		
	• Prostratin and analogs	B, D, E	Beans et al., 2013; Marsden et al., 2018
	• 4 $\beta$ -dPE A	A, B, C, D, E	De la Torre-Tarazona et al., 2020
	Gnidimacrin	A, D	Huang Li et al., 2011; Lai W, et al., 2015
	Ingenol-3-hexanoate (IngB)	A, B, C, E	José et al., 2014; Jiang et al., 2014
	Ingenol-3-angelate (PEP005)	A, C, E, H	Jiang et al., 2015; Jiang et al., 2019
	Ingenol-3,20-dibenzoate (IDB) and other synthesized ingenol derivatives	A, E	Spivak et al., 2015; Spivak AM et al., 2018
	Benzolactam derivatives	A, B, E	Matsuda et al., 2019
	Mukungulu	A, H	Tietjen et al., 2018
Kansui and its derivatives	A, D, E, H (NCT02531295)	Cary, Fujinaga, and Peterlin, 2016; Lee et al., 2019; Yang et al., 2019	
<b>Noncanonical NF-<math>\kappa</math>B</b>	<b>SMAC mimetics (SMACm)</b>		
	• Debio 1143/AT-406	A, C, D, E, F	Bobardt et al., 2019; Bobardt et al., 2020
	• AZD5582	E, F, G	Pache et al., 2015; Sampey et al., 2018; Nixon et al., 2020
	• SBI-0.637,142	A, C	Pache et al., 2015;
	• LCL161	C	Pache et al., 2015;
	• BV6	A, C	Hattori et al., 2018; Campbell et al., 2018; Sampey et al., 2018
	• SM164	A, C	
	• Birinapant	A, E	
	• GDC-0152		
	• Embelin		
<b>Experimental model system:</b>			
A – cell line of HIV latency, <i>in vitro</i>			
B – PBMCs isolated from healthy blood donors, <i>ex vivo</i>			
C – Primary CD4+ T cells isolated from healthy blood donors, <i>ex vivo</i>			
D – PBMCs isolated from HIV-positive patients, <i>ex vivo</i>			
E – Primary CD4+ T cells isolated from HIV-positive patients, <i>ex vivo</i>			
F – BLT (bone marrow, liver, thymus) humanized mouse model, <i>in vivo</i>			
G – ART-suppressed SIV-infected rhesus macaques, <i>in vivo</i>			
H – ART-suppressed HIV-positive individuals, <i>in vivo</i>			

classes of LRAs, and combination therapy is required in order to achieve optimal latency reversal.

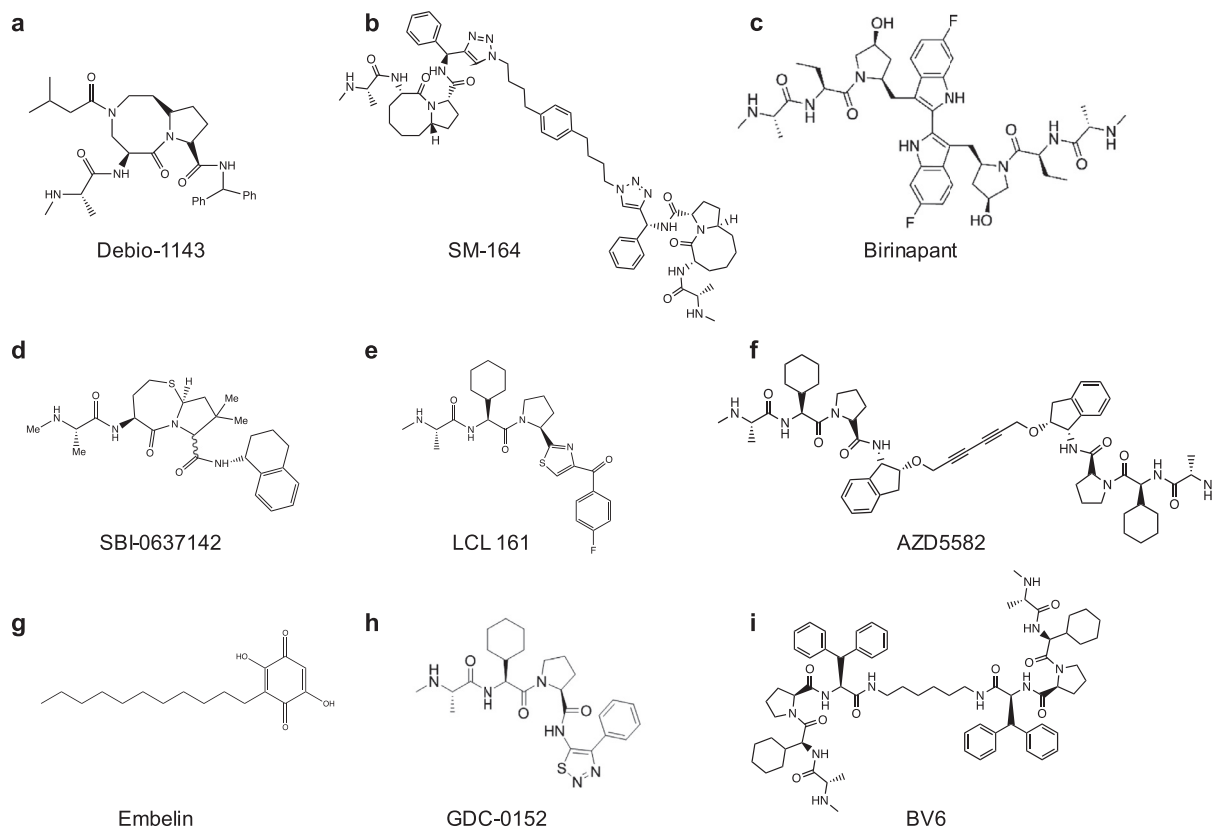
## 6. Targeting canonical NF- $\kappa$ B in HIV cure through PEBP1/Raf1 signaling

Although much is known upon the downstream NF- $\kappa$ B signaling, less of the upstream NF- $\kappa$ B signaling has been investigated in the regulation of HIV transcription. We recently discovered that phosphatidylethanolamine binding protein 1 (PEBP1), a Raf1 kinase inhibitor protein (RKIP), is implicated in the induction of HIV latency through a genome-wide library screening [65] (Fig. 1b). Raf1 is involved in IKK regulation via the Ras/Raf/MEK/ERK signaling pathway where phosphorylation of Raf1 leads to IKK activation in the NF- $\kappa$ B signaling pathway, thus releasing the sequestration of p65 in the cytosol and allowing nuclear translocation [66,67]. PEBP1 knockout in C11 cell model of HIV latency demonstrated an enhanced MAPK/IKK activation and latency reversal, indicating that PEBP1 inhibits upstream phosphorylation of Raf1 kinase, which thwarts the activation of the NF- $\kappa$ B signaling cascade, thereby establishing HIV latency. Yang and colleagues' working model proposes PEBP1 as a suppressor of HIV transcription where PEBP1 induction inactivates NF- $\kappa$ B signaling by dephosphorylation of Raf1 or by protein complex formation with Raf1 or IKK, thereby establishing HIV latency [65,68,69]. Interestingly, PEBP1 inducer, such as epigallocatechin-3-gallate (EGCG) or dihydroartemisinin (DHA), inhibited  $\alpha$ -CD3/CD28 reversal of latent HIV in resting CD4+ T cells isolated from ART-suppressed HIV-infected individuals [65]. An interaction between PEBP1 and Nef has been reported, however, it is unclear whether this is related to PEBP1 induction of latency [70]. Considering that PEBP1 is an antagonist of NF- $\kappa$ B signaling to establish HIV latency, upstream NF- $\kappa$ B signaling may be of interest for curative strategies of HIV.

## 7. Understanding the importance of noncanonical NF- $\kappa$ B in HIV cure

Given that NF- $\kappa$ B is a master regulator of inflammatory and immune response, it is important to explore other approaches to prevent global T cell activation or hypercytokinemia that PKCa application may trigger, due to the broad specificity induced by PKCa. NcNF- $\kappa$ B signaling juxtaposes cNF- $\kappa$ B signaling by acting as a slow, persistent and stimulus-selective mechanism. Noncanonical NF- $\kappa$ B is favored for its stimulus-selective mechanism where fewer genes are induced compared to cNF- $\kappa$ B stimulated by PKCa. This reinforces the activation of the noncanonical signaling pathway to ensure minimization of off-target effects [25,42], unique from cNF- $\kappa$ B signaling which permits a broad inflammatory response through tumor necrosis factor receptors [43] (Fig. 1c).

Pache et al. found that an ubiquitin ligase, Baculoviral IAP repeat-containing protein 2 (BIRC2)/cIAP1 (inhibitor of apoptosis protein-1), acts as a repressor of the ncNF- $\kappa$ B pathway to negatively regulate HIV transcription [42]. This study uncovered a family of IAP inhibitors (IAPi) or mimetics of the second mitochondrial-derived activator of caspases (SMACm) as a new class of LRAs. IAPi/SMACm modulates tumor activity and is known to induce ncNF- $\kappa$ B through cIAP1/cIAP2 degradation [71]. Similarly, Bobardt and colleagues found that another SMACm, Debio 1143, (Fig. 3) reverses latent HIV through the degradation of BIRC2/cIAP1 to permit cleavage of p100 into p52 for activation of ncNF- $\kappa$ B signaling in latently infected cell models of HIV latency, in ART-suppressed BLT humanized mice and ART-suppressed patient resting CD4+ T cells [24] (Table 1). When a proteasome inhibitor MG132 was included during Debio 1143 treatment, latency reversal was inhibited [24]. Inhibition of BIRC2 ultimately allows the degradation of a complex comprised of BIRC2, BIRC3, TRAF2 and TRAF3, protecting NIK and activating ncNF- $\kappa$ B. Interestingly, there



**Fig. 3.** Pharmacological compounds for the activation of noncanonical NF- $\kappa$ B. Chemical structures of SMACm were shown. Many monovalents and divalents of SMACm have been tested in HIV cure studies, which displayed potency and safety *in vitro*, *ex vivo* and *in vivo*.



was no observed increase in cytokine induction when Debio 1143 was administered in nanomolar concentrations to PBMCs and BLT humanized mice, highlighting its desirable potency as a potential LRA. The efficacy of IAPi is currently in clinical trials with cancer patients, which will provide future information about its safety *in vivo* [72,73]. IAPi exhibited synergism with HDACis such as vorinostat *in vitro*, which favors the use of IAPi in conjugation with other LRAs to exploit diverse mechanisms for elimination of latent HIV reservoirs. The structure-activity relationship analysis indicates that bivalent molecule of IAPi is more potent than monovalent compound where its potency may be enhanced by the two binding motifs of BIR domains in cIAP protein [74] (Fig. 3).

Another IAPi/SMACm that has garnered interest as a tool to disrupt latency *via*  $\text{ncNF-}\kappa\text{B}$  signaling is AZD5582. *In vitro*, AZD5582 degrades BIRC2/cIAP1 for activation of  $\text{NF-}\kappa\text{B}$ , similar to other IAPi such as birinapant and Debio 1143. Further, Nixon and colleagues revealed AZD5582 therapy in the ART-suppressed SIV-infected animal model of AIDS resulted in the induction of SIV RNA from latency in lymph node tissues of rhesus macaques. Remarkably, latent HIV in the resting CD4+ T cells was reactivated in the ART-suppressed HIV-infected BLT humanized mouse model, signifying the presence of latency reversal as a result of AZD5582. Additionally, the safety of AZD5582 was tested where both models showed that AZD5582 was efficacious and did not increase cytokine production. The ART-suppressed SIV-infected rhesus macaques tolerated AZD5582 intravenous injections over an 8-week period where 2 of the 12 rhesus macaques exhibited adverse reactions. In ART-suppressed HIV-infected mice, levels of alanine aminotransferase and aspartate aminotransferase were mildly elevated. Together, these studies support that IAPi/SMACm could be further developed to directly activate  $\text{ncNF-}\kappa\text{B}$  signaling as a new tool to disrupt HIV latency [25].

## 8. Outstanding questions

$\text{NF-}\kappa\text{B}$  is a desirable target due to its biological function as a regulator of HIV transcription and other signaling pathways [40]. However, concerns of toxicity using PKCa to disrupt latency remains. Discovery of effective and safe PKCa is still urgently needed and an ongoing safety trial of *Euphorbia Kansui* may provide us some guidelines of use. PEBP1, an inhibitor of Raf1 kinase in the upstream  $\text{cNF-}\kappa\text{B}$  signaling, is a new inducer of HIV latency, which could be developed for cure of HIV [65].

Noncanonical  $\text{NF-}\kappa\text{B}$  is implicated in the establishment of HIV latency, however, the underlying molecular mechanisms of latency and latency disruption are not fully understood. However, it may be a more favorable pathway as it is a slow and persistent process that does not lead to global T cell activation. Tolerability of IAPi for the induction of  $\text{ncNF-}\kappa\text{B}$  in both ART-suppressed HIV-infected BLT humanized mice and SIV-infected rhesus macaques provides some insight for curative HIV strategies *in vivo* [25]. However, a recent study applying AZD5582 in the SHIV model of HIV latency failed to induce latency reversal nor reservoir reduction in combination with clearance tool of bispecific HIVxCD3 DART molecules [75], indicating that latency reversal by IAPi alone may be not potent enough to present the viral antigens for immune clearance and our understanding of underlying *in vivo* mechanism of latency reversal is not complete.

An optimal combination strategy with other LRAs may lead to a more robust latency disruption than single LRA. It is quite possible that optimization of current IAPi is needed in order to find potent and safe IAPi combination strategy in the disruption of latency in patients. In addition, how  $\text{ncNF-}\kappa\text{B}$  signaling is regulated in HIV transcription and latency is not clear. P100 cleavage into p52 is one of the essential steps in activation of  $\text{ncNF-}\kappa\text{B}$  signaling. Many proteins, such as NIK/IKK and  $\beta$ -TrCP ubiquitination complex, are involved in this cleavage process, indicating that fine-tuning this step is essential for its biological functions. Lastly, the induction of apoptosis is a

suitable aim as programmed cell death prevents viruses from propagation, thereby eradicating HIV reservoir. It has been reported that the induction of  $\text{ncNF-}\kappa\text{B}$  signaling causes cell death in memory CD4+ T cells where latent HIV is harbored [76]. Whether this is specific for cells with reactivated HIV is a very interesting question, which is intriguing for the field. Therefore, much work remains in the understanding of  $\text{NF-}\kappa\text{B}$  signaling subpathways and developing effective and safe IAPi for latency reversal.

## Search strategy and selection criteria

Data search and selection occurred through searches from PubMed with the following search terms: "HIV reservoir", "HIV latency", "HIV deep latency", "shock and kill", "block and lock", "canonical  $\text{NF-}\kappa\text{B}$ " and "noncanonical  $\text{NF-}\kappa\text{B}$ ". Selection of articles particularly emphasized articles between 2015 and 2020 with several distinguished papers as early as the 1990s.

## Contributors

GJ generated the initial concepts of this review article. LMW and GJ wrote the article and approved the final version of the manuscript.

## Declaration of Competing Interest

The authors declare no conflicts of interests.

## Acknowledgments

We apologize for not being able to cite all the excellent studies and reviews due to space limitations. GJ is supported by Qura Therapeutics funding, University of North Carolina at Chapel Hill Center for AIDS Research (P30AI50410), and NIAID/CARE (1UM1AI126619). The funders have no roles in experimental design, data collection and analysis, interpretation of the data, or writing of this paper.

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