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Review

# Shocking HIV-1 with immunomodulatory latency reversing agents

Anna Kula-Pacurar [a,*], Anthony Rodari [b], Gilles Darcis [c], Carine Van Lint [b,*]

[a] Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland
[b] Service of Molecular Virology, Department of Molecular Virology (DBM), Université Libre de Bruxelles (ULB), Gosselies, Belgium
[c] Infectious Diseases Department, Liège University Hospital, Liège, Belgium

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ABSTRACT

The "shock-and-kill" strategy is one of the most explored HIV-1 cure approaches to eliminate latent virus. This strategy is based on HIV-1 reactivation using latency reversing agents (LRAs) to reactivate latent proviruses (the "shock" phase) and to induce subsequent elimination of the reactivated cells by immune responses or virus-induced cytopathic effects (the "kill" phase). Studies using immunomodulatory LRAs such as blockers of immune checkpoint molecules, toll-like receptor agonists, cytokines and $CD8^+$ T cell depleting antibodies showed promising potential as LRAs inducing directly or indirectly cellular pathways known to control HIV transcription. However, the precise molecular mechanisms by which these immunomodulatory LRAs reverse latency remain incompletely understood. Together with the heterogenous nature of HIV-1 latency, this lack of understanding complicates efforts to develop more efficient and safer cure strategies. Hence, deciphering those mechanisms is pivotal in designing approaches to eliminate latent HIV infection.

## 1. Introduction

Nowadays, HIV-1 infection remains a major health issue with more than 38 million people worldwide living with HIV and more than 500 000 deaths from AIDS-related morbidities. Since its introduction in 1986, the combinatory antiretroviral therapy (cART) has drastically decreased HIV-1-associated mortality by efficiently suppressing viral replication in infected individuals [1]. However, cART uptake is not curative since treatment interruption is irremediably followed by a viral rebound in the majority of cases, thereby switching HIV-1 infection from a lethal disease to a chronic one [2,3]. Following this observation, understanding of this rebound of viremia has rapidly evolved and multiple mechanisms have been identified as contributing to HIV-1 persistence *in vivo* [4]. Among these mechanisms, the transcriptional and epigenetic repression of HIV-1 gene expression in latently-infected cells is the most studied source of HIV-1 persistence *in vivo* and corresponds to HIV-1-infected cells in which the provirus is stably integrated into the host cellular genome, replication-competent but silenced due to multiple transcriptional and post-transcriptional blocks [5–7]. HIV-1 latency represents the main obstacle to reach an HIV-1 cure since the cellular reservoir is insensitive to cART and to the cytopathic effects from the host immune system. However, the state of viral latency is reversible and latently-infected cells can be reactivated to produce virus in response to many stimuli, thereby providing a persistent source of viremia once cART is interrupted. Because of cART failure to cure HIV-1 infection, several therapeutic strategies have emerged and one of them, referred to as the "shock and kill" strategy, is extensively studied [8]. This strategy aims at reactivating HIV-1 latently-infected cells by using small compounds called latency reversing agents (LRAs) (the "shock" phase), while improving the immune responses to eliminate the reactivated infected cells (the "kill" phase). Identification of potent LRAs to reverse HIV-1 latency requires a deep understanding of the molecular mechanisms underlying viral latency, a phenomenon which appears to be highly heterogenous and patient-specific [5,9]. Over the years, increasing knowledge of HIV-1 latency has led to the emergence of several classes of LRAs, that reactivate HIV-1 gene expression *in vitro* and *ex vivo*, including epigenetic drugs, protein kinase C agonists, Bromodomain and Extra-Terminal motif (BET) protein inhibitors (BETis), activators of the Akt pathway, STAT5 sumoylation inhibitors, SMAC mimetics and immunomodulators [10]. However, clinical trials using these LRAs alone exhibited moderate levels of viral reactivation *in vivo* and failed to reduce HIV-1 reservoir size in $HIV^+$ individuals, thereby highlighting the importance to improve the "shock-and-kill" strategy. In this context, combinations of distinct LRAs have demonstrated encouraging results *ex vivo*, as well as combinations of LRAs with drugs improving the host immune responses against reactivated

* Corresponding authors.
*E-mail addresses:* anna.kula-pacurar@uj.edu.pl (A. Kula-Pacurar), cvlint@ulb.ac.be (C. Van Lint).

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HIV-1-infected cells [11–14].

The present review will focus on the latency reversal potential of a specific class of LRAs, corresponding to immunomodulatory LRAs including immune checkpoint molecules (IC) blockers, toll-like receptor (TLR) agonists, cytokines and monoclonal antibodies allowing the depletion of $CD8^+$ T cells. We will present the immunomodulatory LRAs characteristics that have been reported either in *in vitro*, *ex vivo* and *in vivo* studies using non-human primates or in clinical trials enrolling $HIV^+$ individuals. We will describe the molecular mechanisms underlying immunomodulatory LRAs-mediated reversal of latency and finally, we will discuss the remaining challenges for their use in the context of HIV-1 cure strategies.

## 2. Blockers of immune checkpoint molecules as LRAs

### 2.1. Immune checkpoint molecules

Persistent antigen exposure and inflammation during chronic viral infections and cancer results in progressive loss of T-cell function termed "T-cell exhaustion" that is characterized by marked expression of immune checkpoint (IC) molecules such as PD-1 (Programmed cell death protein), CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4, CD152), TIGIT (T cell Ig and ITIM domain) and LAG-3 (Lymphocyte Activation Gene 3). IC molecules are co-inhibitory receptors that bind to their ligands to tightly control immune homeostasis that must be fine-tuned to prevent excessive immune activation while maintaining tolerance to auto-antigens [15]. Several FDA-approved monoclonal antibodies (ipilimumab against CTLA-4, nivolumab and pembrolizumab against PD-1 and atezolizumab, avelumab and durvalumab against PD-1 ligand (PDL-1)) are used in patients as immunotherapy for cancers and viral infections including HIV.

Exhausted HIV-1 and SIV-specific $CD4^+$ and $CD8^+$ T cells were shown to overexpress at their surface PD-1, CTLA-4 and TIGIT [16–18]. Targeting these pathways using monoclonal antibodies has shown immune restoration and impact on viral replication. For instance, PD-1 blockade in SIV-infected macaques has been shown to restore immune responses (by rapid expansion of virus specific $CD8^+$ T cells), to reduce plasma viral load and to prolong survival of SIV-infected macaques [19,20].

In addition to IC molecules' roles in chronic HIV infection, these exhausted receptors were also linked to HIV latency establishment and persistence. Pioneering study by Chomont et al. [21] and other subsequent studies [22,18] have identified PD-1, LAG-3 and TIGIT on the surface of latently-infected $CD4^+$ T cells. Importantly, follicular helper $CD4^+$ T cells expressing high levels of PD-1 were shown to be enriched in replication-competent virus [23]. Therefore, targeting these exhaustion markers of latent reservoir could represent a convenient way to specifically unblock HIV from latency. Indeed, SIV-infected macaques treated with anti-PD-1 antibody showed latency reversal as demonstrated by transient increases in viremia that were associated with T-cell proliferation [19]. The effects on latency reversal were also demonstrated in case report studies of HIV-1-infected oncologic patients treated for cancers with either anti-PD-1 [24,25] or anti-CTLA-4 [26] blockers or both [27]. However, other studies reported conflicting results [28,29] or only modest effects with a combined treatment blocking the PD-1/PDL-1 axis with anti-PDL-1 antibody (BMS- 936559) and anti-PD1 antibody (nivolumab) in different *ex vivo* cell cultures from ART-treated $HIV^+$ patients [30]. Recently, Sharon Lewin's group highlighted the need for combinatory approaches using different blockers of IC molecules to potently reactivate latent HIV-1 [31]. These authors evaluated combined treatments of anti-PD-1 (nivolumab), anti-CTLA-4 (ipilimumab) antibodies and antibodies against TIM-3 and TIGIT in *in vitro* latency models and demonstrated HIV-1 reactivation only in the presence of an additional T-cell activating stimulus or when all four anti-IC molecules antibodies were used in combination [31].

### 2.2. Molecular mechanisms of immune checkpoint molecules engagements in HIV latency reversal

IC molecules such as PD-1 or CTLA-4 block the T-cell receptor (TCR) primarily through CD28 signaling inactivation [32]. Activation of TCR induces several signal transduction pathways such as the ZAP70, PI3K and calcineurin pathways that downstream activate the NF-κB, AP-1 and NFAT transcription factors known to control HIV transcription (Fig. 1). Nicolas Chomont's group has demonstrated that TCR-stimulated PD-$1^+$ T cells treated with PDL-1 exhibited decreased levels of active positive transcription elongation factor b (P-TEFb), a master regulator of HIV-1 transcription, thereby providing a mechanistic insight into viral reactivation through PD-1 engagement [33]. Indeed, Jonathan Karn's group has shown that TCR stimulation releases active P-TEFb through the ERK signaling pathway, leading to HIV-1 reactivation [34]. However, a direct link between P-TEFb release and viral reactivation through PD-1 engagement remains to be confirmed. Additionally, it was reported that PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation [35] and a gene profiling study has demonstrated cellular, transcriptional and epigenetic changes associated with PD-1 pathway blockade [36]. Therefore, it remains to be determined which pathways are involved in latency reversal following IC molecules blockade.

## 3. Agonists of toll-like receptors as LRAs

### 3.1. Toll-like receptors

Toll-like receptors (TLRs) are a class of proteins belonging to the family of pattern recognition receptors (PRRs) that play a role in activating the early innate immune response through recognition of pathogen-associated molecular patterns (PAMP) and in boosting immune recognition through downstream pathways. TLRs are expressed on many different cells of the immune system including natural killer (NK) cells, macrophages, B cells, dendritic cells (DCs), T cells as well as epithelial and endothelial cells. There are 10 functional TLRs discovered so far on human cells that can be divided into two main groups depending on their cellular localization. Six of them (TLR-1, 2, 4, 5, 6 and 10) are localized on the cell surface and recognize molecules present at the surface of microbes such as bacteria and fungi. Other four TLR (TLR-3, 7, 8, and 9) are on the membrane of the intracellular endosomes and recognize nucleic acids derived from bacteria and viruses (reviewed in Ref. [37]). Discovery of immunostimulatory small molecules that can "mimic" a TLR response is an area of active research for the treatment of bacterial and viral infections and of inflammatory diseases, for cancer immunotherapy and to optimize efficiency of vaccines.

In addition to boosting anti-HIV immunity, a plethora of reports have demonstrated the ability of TLRs agonists to reactivate latent HIV-1. However, HIV reactivation depended on TLR's cell type-specific expression. Agonist of TLR1/2 (that is expressed on T cells) was shown to reactivate HIV-1 from latency in central memory $CD4^+$ T cell models of latency, while agonists of TLR5, TLR7, TLR8 and TLR9 (that are not expressed on T cells) were inactive [38]. TLR8 is expressed on monocytic and myeloid cells and stimulation with a TLR agonist led to viral reactivation in U1 and OM-10 cell lines [39]. Stimulation of TLR7 and TLR9 that are expressed on plasmacytoid dendritic cells (pDCs) were shown to reactivate HIV from latently-infected $CD4^+$ T cells either in coculture experiments or in PBMCs *ex vivo* cultures [40,41]. Collectively, TLR agonists can reactivate latent HIV-1 but this activity requires the presence of cell types that express sufficient levels of the targeted TLR.

Among the TLR agonists evaluated *in vitro* and *ex vivo* only TLR7 agonists and TLR9 agonists have progressed into *in vivo* studies as promising LRAs. TLR7 agonists GS-986 and GS-9620 have been evaluated in SIV-infected macaques on ART and a study by Lim et al. [42] has reported induced expression of SIV RNA and activation of multiple

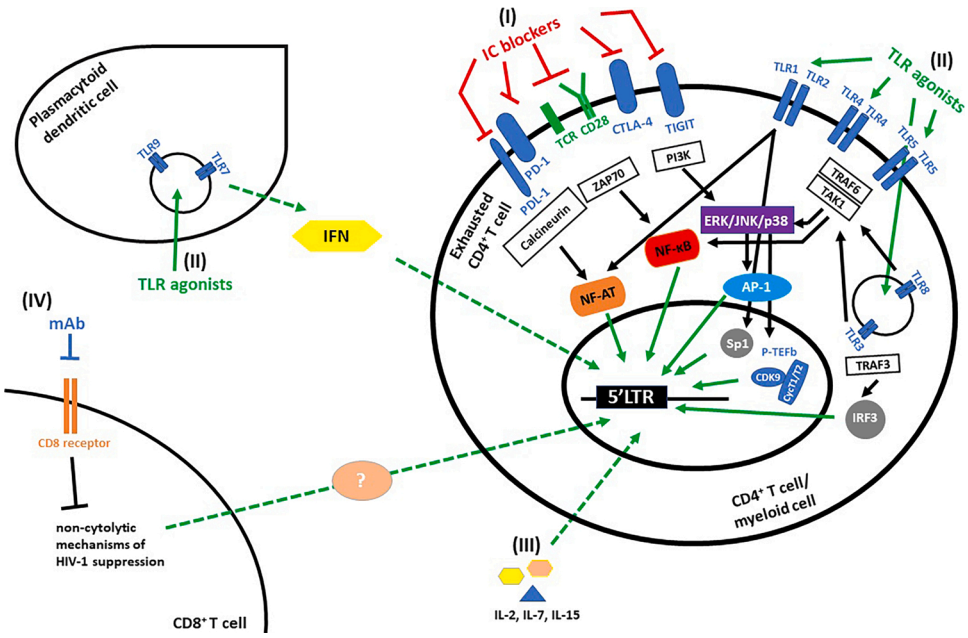


**Fig. 1.** Molecular mechanisms leading to HIV reactivation by immunomodulatory LRAs. (I) Blockade of IC molecules (expressed on the exhausted CD4$^+$ T cells) with monoclonal antibodies; (II) stimulation of TLRs present on either latently-infected CD4+ T/myeloid cells or plasmacytoid DCs; (III) administration of homeostatic cytokines and (IV) unblocking non-cytolytic suppression mechanisms by CD8$^+$ T cells depletion with monoclonal antibodies - activate HIV transcription through activation of diverse molecular pathways leading to induction of cellular transcription factors represented in the figure. Direct and indirect reactivations of the HIV-1 promoter are shown by continuous and discontinuous lines, respectively. 5'LTR, 5' long terminal repeat; AP-1, Activator protein 1, CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; CDK9, Cyclin-dependent kinase 9; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CycT1/T2, cyclin T1, cyclin T2; ERK; extracellular signal-regulated kinase; IC, immune checkpoint; IFN, interferon; IL, interleukin; IRF3, Interferon regulatory factor 3; JNK, c-Jun N-terminal kinase; mAb, monoclonal antibody; NF-AT, Nuclear factor of activated T-cells; NF-κB, Nuclear factor kappa B; PD-1, Programmed cell death protein 1; PDL-1, Programmed death-ligand 1; PI3K, Phosphoinositide 3-kinase; P-TEFb, Positive transcription elongation factor b; Sp1, specificity protein 1; TAK1, transforming growth factor-b-activated kinase 1; TIGIT, T cell immunoreceptor with Ig and ITIM domain; TLR, Toll-like receptor; TRAF, TNF receptor-associated factor; ZAP70, Zeta-chain-associated protein kinase 70.

innate and adaptive immune responses. Notably, a reduction in viral reservoir size was observed and two out of nine treated animals remained aviremic for more than 2 years after ART interruption [42]. However, these results could not be reproduced in other studies [43,44]. To increase the impact on viral reservoir, the group of Dan Barouch has evaluated a combination of the TLR7 agonist GS-9620 with the therapeutic vaccine Ad26/MVA and has reported a decrease in viral reservoir size in lymph nodes and peripheral blood, as well as a delayed viral rebound following ART discontinuation in 33 % (3 out of 9) of the animals [45]. The same group has evaluated another combination of a TLR7 agonist with PGT121, a broadly neutralizing antibody (bNAb), in Simian Human Chimeric Immunodeficiency virus (SHIV)-infected macaques and has demonstrated virus remission in 45 % (5 out of 11) of the animals [46]. Notably, adoptive transfer of PBMCs and lymph node mononuclear cells (LNMCs) from the non-rebounding animals to uninfected animals did not result in SIV infection. Another very recent study in SHIV-infected macaques using a combined treatment of the TRL7 agonist GS-986 with two bNAb (N6-LS and PGT121) has also reported delayed viral rebound after ART interruption [47].

Despite encouraging results obtained in non-human primates, TLR7 and TLR9 agonists did not impact substantially HIV reservoir size *in vivo* in human clinical trials. Increasing doses of GS-9620 resulted in no change in HIV levels except for one patient that exhibited transient increases in HIV RNA level [48]. Clinical trials using MGN1703 induced peaks in plasma HIV RNA levels in a subset of HIV$^+$ individuals, however without impact on the HIV reservoir size [49,50].

### 3.2. Molecular mechanisms of toll-like receptors agonists in HIV latency reversal

Stimulation of TLRs leads to activation of several transcription factors including NF-κB, AP-1 and interferon regulatory factors (IRFs) that induce the expression of inflammatory cytokines and type I IFNs to protect the host from microbial infection. Novis et al. [38] have provided an in-depth mechanistic insight into HIV reactivation upon TLR1/2 agonist Pam3CSK4 treatment and have reported NF-κB-, AP-1- but also NFAT- and Sp1-dependent HIV-1 reactivation (Fig. 1). Indeed, intact NF-κB/NFAT and/or Sp1 binding sites in the long terminal repeat (LTR) were required for Pam3CSK4-induced viral reactivation. Additionally, these authors reported involvement of P-TEFb upon Pam3CSK4 treatment. The same group in another study showed that a panel of TLR 2 agonists increased the levels of phosphorylation at serine 529 in the NF-κB subunit p65, a modification known to increase the transcriptional activity of NF-κB [51]. TLR3 agonist poly (I:C) was shown to reactivate HIV in microglial cells through IRF3 activation and binding to the HIV-1 promoter [52] and in monocytes through NF-κB and JNK pathways, increased HAT activities, decreased HDAC activities and epigenetic modifications on the HIV-1 promoter [53]. TLR4 stimulation by LPS was linked with NF-κB and PU.1 [54] but also with p38 mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3 (PI3) kinase pathways [55]. Interestingly, TLR8 agonist R-848 showed a bimodal mode of latency reversal in *ex vivo* PBMCs cultures by directly reactivating HIV from TLR8-expressing myeloid cells through induction of NF-κB and indirectly from neighboring latently-infected CD4$^+$ T cells through TNFalpha (also induced upon R-848 from TRL8-expressing cells) [39]. TLR7 and TLR9 agonists were shown to induce HIV from latency through an indirect mechanism that required type I IFN produced by pDCs [40,41]. Indeed, both TLR receptors are predominantly found in the endosomal compartment of pDCs and when stimulated trigger robust production of type I interferon that bridges innate and adaptive immune responses. Stimulation of PBMCs from HIV$^+$ patients on ART with TRL7 agonist GS-9620 resulted in increased INF productions, and blocking of INF receptor with specific antibodies impaired HIV latency reversal [40]. INF production following GS-9620 treatment likely leads to viral reactivation (Fig. 1), however the exact mechanism of action remains to be determined.

## 4. Cytokines as LRAs

### 4.1. HIV latency reversal by cytokines

Distinct cytokines play important roles in the modulation of T-cell

homeostasis and proliferation and thus were demonstrated to be beneficial in addition to ART in improving the impaired anti-HIV immune response [reviewed in [56]]. Moreover, a variety of cytokines such as interleukin (IL)-2, tumor necrosis factor (TNF)-α and IL-6 were shown to reactivate HIV from latently-infected resting $CD4^+$ T cells from HIV-infected individuals [57], suggesting a role of these cytokines to eliminate HIV-1 latent reservoirs. Indeed, early promising *in vivo* study with IL-2 showed a decrease in the pool of latently-infected resting $CD4^+$ T cells from patients receiving IL-2 and ART [58]. However, ART cessation in these individuals led to a rapid rebound of HIV-1 viremia, indicating that the virus was not eradicated [59]. Additionally, two large clinical trials SILCAAT and ESPRIT showed that, despite marked increases in the number of $CD4^+$ T cells, IL-2 therapy presented no clinical benefit in either study [60]. Next, a combination of IL-2 + the anti-CD3 antibody OKT3 was evaluated in a clinical study but this aggressive treatment caused excessive T-cell activation and irreversible $CD4^+$ T-cell depletion [61]. Reduction in HIV reservoir size was observed in a pilot clinical study using combined IL-2 + IFN-γ treatment but this aggressive approach did not delay the time to viral rebound after ART interruption [62]. Administration of an other homeostatic cytokine, IL-7, demonstrated viral reactivation in SCID-hu mouse model of HIV latency with minimal effects on the cell phenotype [63]. A pilot study using IL-7 showed a level of viral reactivation from resting $CD4^+$ T cells that was more potent than treatments with IL-2 or IL-2 + PHA [64]. However, additional clinical studies using IL-7 reported transient and rather modest increases in plasma HIV RNA [65,66]. Importantly, Katlama et al. [66] have reported an amplification of the HIV reservoir following IL-7 administration in a pilot study, suggesting that the homeostatic effects of IL-7 led to unwilling expansion of the pool of infected cells that may promote HIV persistence. Similarly, Vandergeeten et al. [67] have reported that IL-7 indeed promotes HIV persistence during ART by enhancing residual levels of viral production and inducing proliferation of latently-infected cells with no impact on latency reversal. Those results suggest that IL-7 does not represent a suitable therapeutic candidate for HIV eradication strategies.

More recently, latency reversing and immune boosting properties of IL-15 and IL-15 superagonists have drawn scientists' attention in the context of HIV purging strategies. Jones et al. [68] have tested several LRAs and have identified IL-2, IL-15, and two IL-15 superagonists (IL-15SA and ALT-803) as reactivating HIV from latency and priming reactivated latently-infected cells for $CD8^+$ T-cell recognition, thereby simultaneously promoting the "shock" phase and the "kill" phase. However, no effect on latency reversal in memory $CD4^+$ T cells was observed in an other study despite effects on restoring immune effector functions [69]. The potential of N-803 in HIV reactivation is being evaluated in clinical trials and preliminary data on the first 7 patients showed a transient increase in plasma HIV RNA levels while the impact on the viral reservoir has still to be determined [70].

### 4.2. Molecular mechanisms of HIV latency reversal by cytokines

Common γ-chain cytokines IL-2, IL-7 and IL-15 are central to homeostatic proliferation and survival of mature $CD4^+$ and $CD8^+$ T cells [71]. Moreover, resting $CD4^+$ T cells survive through homeostatic proliferation mediated by IL-7 and IL-15 contributing to persistence of the HIV reservoirs. Interestingly, Manganaro et al. [72] have recently reported that IL-15 contributes to viral persistence in $CD4^+$ T memory stem cells by bypassing the restriction factor SAMHD1 and inducing proliferation of this cellular reservoir. The molecular pathways of IL-2-, IL-7- and IL-15-mediated HIV latency reversal remain poorly characterized. IL-2, IL-7 and IL-15 trigger Janus family kinases (JAK1/3) pathways which then recruit signal transducers and activators of transcription (STAT) family members (STAT1, STAT3) and the PI3 kinase cascade *via* an interaction with STAT3, which are pathways known to control HIV transcription (Fig. 1). However, it remains to be determined whether these cytokine-mediated activations of latent HIV occur through a direct effect on the viral LTR promoter.

## 5. Transient $CD8^+$ T cells depletion as LRAs

### 5.1. Cytolytic and non-cytolytic activities of $CD8^+$ T cells

$CD8^+$ T cells also known as cytotoxic T lymphocytes (CTL) were shown to exhibit cytolytic activity during HIV and SIV infections. This was initially evidenced by the association with the decline in viremia during primary HIV-1 infection [73], emergence of the virus CTL escape mutants [74] and high polyfunctional responses in HIV controllers [75]. In addition to cytolytic properties of $CD8^+$ T cells, non-cytolytic mechanisms that suppress HIV replication without decreasing the number of productively-infected CD4 + T cells were reported during acute and chronic SIV infections [76,77] upon depletion of $CD8^+$ T cells using monoclonal antibodies. Importantly, a study from Guido Silvestri's laboratory [78] has reported a non-cytolytic role of $CD8^+$ T cells in suppressing HIV replication during ART. These authors have demonstrated significant increases in viral reactivation in PBMCs and lymph nodes upon $CD8^+$ T cells depletion in ART-treated, SIV-infected animals, suggesting a role of $CD8^+$ T cells depletion in latency reversal. Recently, the same group has very elegantly demonstrated in two animal models (rhesus macaques infected with SHIV and humanized mice infected with HIV) that administration of $CD8^+$ T-cell depleting antibody and immunomodulator IL-15 superagonist N-803 induced robust viral reactivation. This combined treatment led to viral reactivation in all ART-treated macaques infected with SHIV and high copy number (>1000 copies per ml) was reported in 6 out of 14 animals (42.9 %) [79].

### 5.2. Molecular mechanisms of $CD8^+$ T cells depletion in HIV latency reversal

Despite obvious impact of $CD8^+$ T cells in controlling HIV in either acute or chronic infection but also during ART, the exact mechanism of action remains incompletely understood. A favorable interpretation of the $CD8^+$ T cell-mediated decrease in HIV RNA levels was attributed to CTL killing properties of $CD8^+$ T cells. However, this was rather limited to early, pre-productive stages of the HIV-1 life cycle. Multiple studies indicate a contribution of non-cytolytic mechanisms of virus suppression by $CD8^+$ T cells [80–83]. Indeed, depletion of $CD8^+$ T cells in SIV-infected macaques did not impact the lifespan of productively-infected cells disfavoring CTL clearance mechanisms and suggesting that viral infection is primarily controlled by $CD8^+$ T cells non-cytolytic mechanisms [80,81]. However, the identity of these non-cytolytic mechanisms remains to be determined (Fig. 1). It was suggested that certain chemokines produced by $CD8^+$ T cells such as RANTES, MIP-1α and MIP-1β suppress HIV-1 through binding to CCR5 and blocking R5-tropic HIV *de novo* infection [82]. Other reports have shown an inhibitory effect on viral transcription and gene expression by unknown soluble factors [83]. A recent study from Deanna Kupla's and Guido Silvestri's groups represents a very comprehensive investigation of the antiviral role of $CD8^+$ T cells in cocultures of HIV-1-infected $CD4^+$ T cells with TCR-stimulated $CD8^+$ T cells [84]. This important study revealed that HIV is suppressed by $CD8^+$ T cell through an innate, virus-independent, immunoregulatory mechanism that is MHC-independent. These authors further demonstrated that the viral suppressive effect of $CD8^+$ T cells required the presence of certain cytokines (*i.e.* IL-4, IL-5, I-13 and sST2) but also cell-to-cell contact [84]. Moreover, this activity decreased $CD4^+$ T-cell activation and proliferation but increased $CD4^+$ T-cell survival and redirected differentiation of $CD4^+$ T cells towards a Th2 phenotype. Secretome and transcriptome analyses revealed downmodulation of multiple genes involved in inflammation, cell death, proliferation in concert with upregulation of genes involved in Th2 differentiation. However, the exact molecular pathways controlling antiviral properties of $CD8^+$ T cells have still to be

identified. Surprisingly, the authors also reported that, in the context of ART-treated HIV infections, cocultures with activated $CD8^+$ T cells led to unfavorable increases in the number of infected $CD4^+$ T cells (possibly through promotion of $CD4^+$ T-cell survival) [84]. This therefore represents a previously unrecognized obstacle to HIV eradication that paradoxically may stabilize the reservoir and contribute to HIV persistence. Additional studies are needed to fully understand the role of $CD8^+$ T cells in suppressing HIV-1 transcription under ART.

## 6. Conclusions

The advantage of immunomodulatory LRAs over classical LRAs is their double mechanism of action *i.e.,* latency reversal to facilitate the "shock" phase and immunostimulatory characteristics to facilitate the "kill" phase of the "shock-and-kill" HIV cure strategy. Preclinical and clinical studies strongly indicate that immunomodulatory LRAs such as IC molecules blockers (blockers of PD-1 and CTLA-4), TLR7 and TLR9 agonists and IL-15 superagonists have a promising potential in providing "kick" and "kill" at the same time given their ability to de-repress the blockade of HIV transcription in the reservoirs and simultaneously to restore functions in exhausted HIV-specific T cells. To date, except for a few rare cases, single interventions either with classic LRAs or immunomodulatory LRAs did not significantly impact the HIV reservoir size *in vivo*. Determinants such as the heterogeneity of the latent reservoirs and the complexity of epigenetic, transcriptional, and post-transcriptional molecular mechanisms regulating HIV-1 latency largely contribute to the limited success of the "shock-and-kill" strategy [reviewed in [5]]. Importantly, the molecular mechanisms by which immunomodulatory LRAs reverse latency are only partially determined. Studies showed that stimulation with these immunomodulatory LRAs seems to directly (for IC molecules blockers and agonists of TLRs expressed on latently-infected cells) or indirectly (other TLRs and $CD8^+$ T cells depletion) induce transcription factors known to reactivate HIV transcription such as NF-κB, AP-1 and P-TEFb but the exact mechanisms are poorly characterized. All this seriously complicates the efforts to develop more effective and safer HIV cure strategies based on specific biological functions. Safety is indeed a crucial concern. Immunotherapies that may disrupt immune homeostasis lead to unique toxicity profiles distinct from the toxicities of other LRAs therapies, depending on their mechanism of action. Interventions with cytokines such as IL-15 superagonists or $CD8^+$ T cells depletion with monoclonal antibodies led to unfavorable increases in latently-infected $CD4^+$ T cells survival [67, 84] which therefore may contribute to HIV persistence. IC molecules blockers are also of concern due to their reported immune-related adverse events which occur with the currently available antibodies [85]. Targeting TLR may lead to systemic inflammation and autoimmune side effects. These toxicities often require specific management [86]. Regarding this issue, combining different strategies based on targeted biological functions could be more effective and less toxic if doses can be reduced. Several combined treatments such as administration of immunomodulatory LRA (agonists of TLR7 or TLR9 and IL-15 superagonists) with other interventions such as $CD8^+$ T cells depletion or broadly neutralizing antibodies have shown promising results *in vivo* in non-human primates with viral reservoir size decline or even remission [42,46]. However, so far, no remission has been demonstrated in $HIV^+$ individuals following administration of diverse combined interventions with immunomodulatory LRAs. Moreover, mechanisms of HIV latency vary from one patient to the other and from one cell to the other in a single patient. We believe this heterogeneity is a major reason for which results observed in one HIV latency model or in one patient cannot be reproduced or extended to other models or patients. Deciphering the precise mechanisms of HIV latency and the mode of action of immunomodulatory LRAs taking into account the patient history diversity should allow the development of targeted individualized treatment strategies that could be more effective and safer. Although the road is still long to reach a cure, immunotherapies appear as very promising strategies that will likely occupy a growing part in the HIV cure field.

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## Declaration of Competing Interest

Authors declare no conflict of interest.

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