Selective elimination of host cells harboring replication-competent human immunodeficiency virus reservoirs: a promising therapeutic strategy for HIV cure

Silvere D. Zaongo¹,², Yue Wang³, Ping Ma⁴,⁵, Fang-Zhou Song², Yao-Kai Chen¹

¹Division of Infectious Diseases, Chongqing Public Health Medical Center, Chongqing 400036, China;
²College of Basic Medicine, Chongqing Medical University, Chongqing 400016, China;
³Institute for Medical Device Standardization Administration; National Institutes for Food and Drug Control, Beijing 100050, China;
⁴Department of Infectious Diseases, Tianjin Second People Hospital, Tianjin 300192, China;
⁵School of Medicine, Nankai University, Tianjin 300071, China.

Abstract

Many seminal advances have been made in human immunodeficiency virus (HIV)/AIDS research over the past four decades. Treatment strategies, such as gene therapy and immunotherapy, are yielding promising results to effectively control HIV infection. Despite this, a cure for HIV/AIDS is not envisioned in the near future. A recently published academic study has raised awareness regarding a promising alternative therapeutic option for HIV/AIDS, referred to as “selective elimination of host cells capable of producing HIV” (SECH). Similar to the “shock and kill strategy,” the SECH approach requires the simultaneous administration of drugs targeting key mechanisms in specific cells to efficiently eliminate HIV replication-competent cellular reservoirs. Herein, we comprehensively review the specific mechanisms targeted by the SECH strategy. Briefly, the suggested cocktail of drugs should contain (i) latency reversal agents to promote the latency reversal process in replication-competent reservoir cells, (ii) pro-apoptotic and anti-autophagy drugs to induce death of infected cells through various pathways, and finally (iii) drugs that eliminate new cycles of infection by prevention of HIV attachment to host cells, and by HIV integrase inhibitor drugs. Finally, we discuss three major challenges that are likely to restrict the application of the SECH strategy in HIV/AIDS patients.

Keywords: HIV; SECH; Latency reversal; Autophagy; Apoptosis; Cell infection inhibition

Introduction

Forty years after its official emergence, the human immunodeficiency virus (HIV) pandemic remains a major public health burden globally. Initially, HIV infection was considered to be inevitably lethal as there was no effective treatment for HIV disease available at the time, and thus most HIV infections unavoidably resulted in death. After the discovery and widespread implementation of antiretroviral therapy (ART), HIV infection has subsequently evolved into the status of a chronic infectious disease, with affected patients on appropriate therapeutic drugs expected to live a relatively conventional lifespan.[3] Nevertheless, HIV management through ART requires lifelong drug treatment,[5] which may potentially be cost-effective[3] but can often be toxic to vital organs such as the kidneys,[4] the liver,[3] the central and peripheral nervous system,[6] and the heart.[7] In addition, even if the use of ART results in the successful suppression of HIV viral load, an interruption or cessation of ART treatment without elimination of dormant HIV provirus from the genomes of infected cells inevitably leads to HIV viral rebound.[8] Indeed, HIV gene integration into the host genome is the major challenge to curing HIV infection, as this results in the establishment of HIV reservoirs within infected patients.[9]

The persistence of non-functional provirus, referred to as “fossils” in the cells of the Berlin patient and the London patient, has also been reported.[10,11] “Fossils” confirm that only reservoirs harboring functional provirus are likely to be of concern. Both patients mentioned above received allogeneic bone marrow transplants from a naturally mutated CCR5 gene (CCR5 delta 32) donor. The strategy consisted of replacing their immune cells (using whole-body irradiation and chemotherapy) with those of the donor that are capable of blocking HIV replication. This is a painful, expensive, and complicated exercise and is restricted by the limited size of the donors’

Access this article online

Quick Response Code:  
Website:  
DOI: 10.1097/CM9.0000000000001797
immune cell population. Besides bone marrow transplant (which is not a viable option on any kind of scale), other strategies have been tested and two options are particularly interesting. The first is the “shock and kill” concept,[12] where the “shock” consists of using drugs to reactivate functional latent provirus concealed in reservoir cells. These latent reservoirs thereafter display viral antigens, which in turn trigger appropriate immune responses against latently infected cells.[13] These reactivated cells can thus potentially be targeted and killed by the body immune system or by anti-HIV drugs. However, one of the major disadvantages of the “shock and kill” strategy is the fact that no trial has as yet demonstrated changes in the size of the latent reservoir[14,15] as it may not be realistic to rely solely on the immune system and anti-HIV drugs to eliminate all HIV-infected cells in an HIV-infected person. The second is the “block and lock” approach, which aims to permanently silence all provirus, even after treatment interruption. Using this strategy, several mechanisms acting on different factors of HIV transcription could be targeted such as trans-activator of transcription (TAT) inhibition by didehydro-cortistatin A and Janus Kinase/Signal transducer and activator of transcription inhibitors, facilitates chromatin transcription inhibition by Curaxin CBL0100, and mechanistic target of rapamycin (mTOR) inhibition, to list a few. However, the “block and lock” strategy does not seem to be an ultimately viable means to cure HIV infection, as it is challenging to permanently and irretrievably silence all provirus.[16]

In this article, we review the concept of selective elimination of host cells capable of producing HIV (SECH), which has been recently demonstrated in vitro and in vivo.[17] Compared with the “shock and kill” and the “block and lock” strategies, the SECH technique includes pro-apoptotic agents and autophagy inhibitors to provide greater benefits in terms of eliminating HIV-infected cells, and thus could provide permanent remission from HIV infection. We believe that the SECH approach could help to develop effective future interventions to cure HIV infection via the use of a therapeutic cocktail of drugs. This cocktail, based on our review of contemporary literature, should contain drugs promoting (i) the latency reversal process, (ii) autophagy inhibition, (iii) apoptosis activation in infected cells, and (iv) inhibition of new infections. Herein, each of these mechanisms is comprehensively reviewed. We also discuss major challenges to the practical utilization of the SECH strategy.

Concept of SECH

It has been postulated that the most successful therapeutic approach to efficiently inhibit HIV-1 replication would be a cocktail of inhibiting agents, which block infection at several points, including potential escape pathways.[18] Using the SECH strategy, we also reinforce the premise that the most promising therapeutic approach to cure HIV will likely be a cocktail of drugs (administered via the oral or the parenteral route) [Figure 1] exerting their combined widespread influence on key viral mechanisms.

In a recent academic publication, the concept of SECH was proffered and discussed, providing constructive information regarding how HIV infection could be cured in the future.[17] The SECH concept involves the elimination of

![Figure 1: Principle of inducing total HIV remission with a therapeutic cocktail. Via oral or injectable administration, the patient receives specific drugs causing (i) latent reservoir reversal, (ii) elimination of HIV-infected cells harboring replication competent provirus, and (iii) new infection inhibition. Latent reservoirs (red) are converted into active cells producing HIV particles (green) or remain cells harboring non-functional provirus (purple). Progressively, infected cells with functional provirus are eliminated over time through the conventional action of the immune system and other drugs (pro-apoptotic and anti-autophagy), and eventually, only the non-functional provirus-infected cells remain at the end of treatment. ART: Antiretroviral therapy; HIV: Human immunodeficiency virus; SECH: Selective elimination of host cells capable of producing HIV.](image-url)
host cells harboring HIV-1 provirus through viral reactivation, induction of apoptosis, inhibition of autophagy, and blocking of new infections. Indeed, Li et al. [17] advised that SECH treatments could contain (i) a latency reversal agent (LRA) acting as non-tumorigenic protein kinase c (PKC)-ε activator, viz., ingenol-3,20-dibenzoate (IDB, at 2.5 mg/kg b.w.), (ii) an apoptosis inducer which inhibits B-cell lymphoma 2 (BCL-2) and B-cell lymphoma-extra-large (BCL-XL), viz., ABT-263 (50 mg/kg b.w.), and (iii) an autophagy inhibitor that prevents autophagy initiation by suppressing Class III PI 3-kinase (VPS34), viz., SAR405 (50 mg/kg b.w.). This therapeutic cocktail was formulated in a solvent mixture of 10% ethanol, 30% polyethylene glycol 400, and 60% Phosal 50 PG for administration to HIV-infected mice via oral gavage. An integrase strand transfer inhibitor (raltegravir) [22] and an attachment inhibitor (BMS-663068, 20 mg/kg b.w.) [23] were also included as a daily ART regimen. After 40 cycles of treatment (once every 2 days equating to one cycle) followed by 2 months of ART treatment withdrawal, >50% (8 out of 15) of treated mice were functionally cured. Furthermore, the above study group included Thienotriazolodiazepine (JQ1) (an inhibitor of the Bromodomain and extra-terminal motif family of bromodomains that can promote the reactivation of HIV-1 [24]) in the SECH cocktail. The results revealed that 10 of 13 (77%) mice showed no virus rebound when JQ1 was included in the SECH treatment regimen. Most importantly, no HIV-1 production was detected in the spleen or bone marrow cells from these newly HIV-1-negative mice. In vitro experiments with HIV-positive patients’ infected cells revealed that SECH treatments killed infected T cells but not uninfected T cells. These findings indicate that HIV infection may be curable using a combination of specific drugs [Figure 2] targeting specific cellular mechanisms.

**Mechanisms of the SECH strategy**

**HIV reservoir latency reversal and LRAs**

HIV infection can currently be effectively controlled with modern ART, and ART use is also capable of blocking the transmission of HIV from one person to another. ART drugs target different specific steps of the HIV viral replication cycle, such as reverse transcription, viral entry, integration, and viral budding in all infected host cells, except for those cells that are latently infected [9]. Although other cell types contribute to the HIV reservoir, Cluster of differentiation 4 (CD4)+ T cells are believed to be one of the main latent reservoirs [25,26]. To eliminate these reservoirs, several strategies resulting from core concepts were developed. Among them, there is the well-recognized “shock and kill” strategy [12]. The “shock” in this strategy consists of using drugs called LRAs to reactivate latent HIV concealed within immune cells. These LRAs can efficiently promote viral protein expression through several distinct mechanisms, such as relieving repressive epigenetic modifications or supplying host transcription factors and other cellular factors necessary for viral gene expression. The latent reservoir then may express viral antigens, which in turn trigger immune responses against latently infected cells [13]. These reactivated cells can thus be identified and neutralized by the body immune system.

![Figure 2: Overview of the key steps through which a therapeutic drug cocktail would engage. Using LRAs, cells with latent reservoirs are converted to latency-reversed cells actively producing HIV particles. On the other hand, cells harboring non-functional provirus remain inactive (fossils) and are not of concern (A). Thus, latency-reversed cells together with other infected cells exhibiting HIV replication are eliminated using pro-apoptotic drugs and autophagy inhibitors (B). To avoid a new cycle of infection, the therapeutic cocktail should contain an HIV-cell attachment inhibitor and an HIV integration inhibitor (C). The aftermath of this process is the elimination of HIV reservoirs, interruption of new infections, and progressive clearance of HIV particles from the body, resulting in total remission in the patient. HIV: Human immunodeficiency virus; LRAs: Latency reversal agent.](https://www.cmj.org)
Both (SECH and “shock and kill”) strategies involve the reactivation of latently infected cells by using LRAs. LRAs are small molecules that induce the expression of HIV-1 in latently infected cells. Thus, latent reservoirs display viral antigens, which in turn trigger their elimination by virus-mediated cytopathogenesis or immune-mediated removal by natural killer (NK) or Cluster of differentiation 8 (CD8)+ T cells. The latent HIV genome responds to multiple signaling pathways downstream of the T-cell receptor in addition to a variety of cytokines and innate immune stimuli. For example, it has been reported that the enhancer of the HIV-1 long terminal repeat (LTR) binds many transcriptional activator proteins. In addition, it has been revealed that the viral trans-activator TAT, which promotes transcriptional elongation from the core promoter, recruits the Positive transcription elongation factor complex, phosphorylates the RNA polymerase II C-terminal repeat domain, and inhibits the pausing factors Negative elongation factor and DRB Sensitivity Inducing Factor. Regarding the multitude of potential targets for positive regulation, it is obvious that a large variety of chemical interventions would be capable of producing elevated expression from the LTR. According to the “shock” concept, five classes of LRAs have been reported [Table 1].

### Table 1: Classes of HIV-1 provirus latency reversing agents.

| Class                          | Type                                | Examples of LRAs                                      | Target                                      | References |
|-------------------------------|-------------------------------------|-------------------------------------------------------|---------------------------------------------|------------|
| Cytokines/receptor agonists   | IL                                  | IL-2, IL-7, IL-15                                      | HDAC1, 2, 3                                 | [31]       |
| TCR/Co-receptor activators    | Maraviroc                           |                                                       |                                             |            |
| TLR agonists                  | TLR2, 3, 7, 8, 9 agonists            |                                                       |                                             |            |
| Epigenetic modifiers          | HDAC inhibitors                     | Vorinostat, panobinostat, AR-42, MS-275, chidamide    | HDAC1, 2, 3                                 | [34-38]    |
| Histone methyltransferase     | Chaetocin, AZ505                     |                                                       | Suv39H1, SMYD2                              | [39,40]    |
| inhibitors                    |                                     |                                                       |                                             |            |
| Intracellular signaling       | PKC agonists                         | Ingenol EK-16A, g nidimacrin, bryostatin, SUW133,    | NF-kappaB                                   | [41-43]    |
| modulators                    |                                     | PEI005/Inge-nol-3-angelate, Prostratin,               |                                             |            |
|                               |                                     |                                                       |                                             |            |
|                                    | AMPK activators                      | Dibutyril-cAMP                                         | STAT3                                       | [44]       |
|                               |                                     |                                                       |                                             |            |
|                                    | JAK/STATAT agonists                  | Benzotrazole, benzoazene                               |                                             | [45,46]    |
|                               |                                     |                                                       |                                             |            |
|                                    | IAP agonists                         | Debo1143                                              | NF-kappaB (non-canonical)                   | [47]       |
|                               |                                     |                                                       |                                             |            |
|                                    | BET inhibitors                        | Q1, MMQO, UMB-136, RVX-208, PFI-1, OTX015             | TAT/pTEFB                                   | [48,49]    |
|                               |                                     |                                                       |                                             |            |
| Transcriptional elongation     | CDK9 activators                      | Chalcone, Amt-87                                       | pTEFB                                       | [50,51]    |
| regulators                    | Anti-oxidant                         | Auranofin                                              |                                             | [52,53]    |
|                               |                                     |                                                       |                                             | [54,55]    |
|                                    | AKT modulators                       | Disulfiram, 57704                                     |                                             | [56]       |
|                               |                                     |                                                       |                                             |            |
|                                    | S1P1 agonist                         | SEW2871                                               |                                             | [57]       |
|                               |                                     |                                                       |                                             |            |
|                                    | Protein phosphatase 1                | SMAPPI                                                |                                             | [58]       |
|                               |                                     |                                                       |                                             |            |
|                                    | SMAC mimetics                        | SBI-0637142                                           |                                             | [59]       |

*HIV LTR-associated transcription factor stimulated by the LRA. AKT: Protein kinase B; AMBK: AMP-activated protein kinase; BET: Bromodomain and extra-terminal motif; CDK9: Cyclin-dependent kinase 9; HDAC: Histone deacetylase; HIV: Human immuno-deficiency virus; IAP: Inhibitor of apoptosis; IDR: Ingenol-3,20-dibenzoate; IL: Interleukins; JAK/STAT: Janus Kinase/Signal transducer and activator of transcription; JQ1: Thienotriazolodiazepine; LRA: Latency reversal agent; LTR: Long terminal repeat; MMQO: 8-methoxy-6-32 methylquinolin-4-ol; NF-kappaB: Nuclear factor kappa light chain enhancer of activated B cells; PPKC: Protein kinase C; pTEFb: The positive transcription elongation factor; S1P1: Sphingosine-1-phosphate receptor 1; SMAC: Second mitochondria-derived activator of caspases; SMAPPI: Small molecule activator of protein phosphatase 1; SMYD2: SET (Suppressor of variegation, Enhancer of Zeste, Trithorax) and MYND (myeloid-nervy-DEAF 1) domain containing 2; STAT3: Signal transducer and activator of transcription 3; Suv39H1: Suppressor of variegation 3-9 homolog 1; TCR: T-cell receptor; TLR: Toll-like receptor.

**Autophagy, HIV, and autophagy inhibitors**

Autophagy is defined as the catabolic mechanism by which intracellular components are delivered to the lysosome for degradation. Of note, macroautophagy is characterized by the formation of autophagosomes which engulf intracellular components and fuse with lysosomes to allow their degradation. On the other hand, microautophagy is described as the process which captures target materials through the invagination of membranes of the endo-lysosomal compartment. Several publications have extensively highlighted the implications of autophagy in (i) maintaining homeostasis, (ii) contributing to the innate immune response through multiple mechanisms, and (iii) participating in the survival and function of B- and T cells, and lymphoid progenitors.
Macrouptosis-related genes (ATGs) were first identified in yeasts by Yoshinori Ohsumi, who was awarded the 2016 Nobel Prize in Physiology or Medicine. Thus far, macroautophagy is the best characterized form of autophagy and is hereafter referred to as autophagy. Depending on the ATG proteins, different steps of autophagy may be noted. By triggering Class III PI3K VPS34 to generate phosphatidylinositol 3-phosphate (PI3P), Beclin-1 (the ortholog of the yeast ATG6) can launch autophagosomal formation.[67] Then, the phagophore (autophagosome machinery required for assembling the autophagosomal membrane precursor) is recruited via PI3P signaling.[68] Most importantly, Beclin-1 operates in tandem with ATG14 and VPS15 proteins while its activity is balanced by several positive and negative regulators, such as Ambra-1 and BCL-2, respectively.[69] It has been demonstrated that several ATGs, including ATG9 and LC3 (the ortholog of the yeast ATG8), control the expansion and closure of the nascent autophagosome.[67] Finally, formed autophagosomes fuse to the lysosome is monitored by a different Beclin-1 complex with UV radiation resistance-associated gene protein (UVRAG) replacing ATG14 which acts in tandem with RAB proteins (ex RAB7), SNARE proteins (ex Syntaxin 17), and the HOPS-tethering complex.[68] LC3 implication in the selection of the cargo to be degraded has been pointed out in the literature. Essentially, it interacts with a series of autophagy receptor proteins (p62, NBR1, NDP52, and OPTINEURIN), which bind ubiquitinated or glycosylated proteins.[69]

The process is induced by different upstream signals, mostly from Beclin-1 complex or indirectly through the upstream kinase Unc-51 like autophagy activating kinase 1 (the ortholog of yeast ATG1), depending on the stress stimulus.[62] During infections, autophagy is triggered by several immune-related signaling pathways activated by inflammatory cytokines and pattern recognition receptors (PRRs).[70] In the same context, kinase TAK1[71] and the E3 ubiquitin ligase TRAF6[72] have been shown to be the signal transduction proteins that mediate autophagy induction by inflammatory cytokines and PRRs, including toll-like proteins, nucleotide oligomerization-domain-like proteins, C-type lectin receptors, RIG-1 (retinoic acid-inducible gene I)-like proteins, and cGAS/STING.

From recently published investigations into SECH, it appears that inhibition of autophagy plays a major role in eliminating HIV-1 infected cells. Therefore, it is of fundamental importance to further explain the complex relationship between HIV and autophagy.

The complexity of the relationship between HIV and autophagy is well documented.[73-75] For instance, it is well known that HIV, to execute early replication steps, depends on autophagy. However, HIV also develops multiple strategies to avoid the recognition and degradation of newly synthesized viral particles.[76] Furthermore, it appears that ATG7, gamma-aminobutyric acid receptor-associated protein-like 2, ATG12, and autophagy-related 16-like 2 are required for productive HIV infection.[76] Another study demonstrated that the autophagosome may provide membrane support for viral replication, as HIV group-specific antigen precursor was found to interact with the autophagosome protein LC3.[77] Moreover, researchers have shown that negative factor (Nef) interacts with Beclin-1 to inhibit autophagosome maturation,[77,78] this step is under the monitoring of the UVRAG-containing Beclin-1 complex. Curiously, the interaction between Nef and Beclin-1 mimics the function of glioma pathogenesis-related protein 2, a host autophagy inhibitor that sequesters Beclin-1 on the Golgi apparatus. In 2015, the Nef/Beclin-1 interaction was shown to be responsible for inhibition of autophagy at the transcriptional level, by preventing the nuclear translocation of the pro-autophagic transcription factor EB in an mTOR-dependent manner.[73] This said we believe that HIV not only alters autophagy activation[79] and inhibition[73,74,80] to avoid its antiviral and immune properties,[75,76-78] but also to avoid cell stress,[62,76] which could eventually lead to the cell death. As long as the cell remains viable, HIV replication continues. The inhibition of such a process has been proven to be damaging for cells in general,[61] and cells infected with active provirus become highly sensitive, with a nearly zero survival rate, as demonstrated recently.[71] This is the reasoning behind why autophagy inhibitors should be included in HIV treatment protocols upon diligent investigation of reasonable dosage formulations and assessment of appropriate information regarding their safety.

With regards to the potential role of autophagy in many diseases, several studies have been conducted to develop therapeutic agents that inhibit autophagy. However, to date, the autophagy process remains difficult to measure and quantify. For instance, an accumulation of autophagosomes does not necessarily demonstrate an increase in autophagy itself but may simply imply that autophagy is blocked at a late stage.[81] Contemporary literature has reported several potential inhibitors of autophagy. In [Table 2], we present a list of the best-characterized autophagy inhibitors based on their target.

**Apoptosis in the latency-reversed HIV reservoir**

Apoptosis is a process that inevitably leads to the death of the cell,[103] and relies on two well-understood activation mechanisms, the intrinsic and the extrinsic pathways. While the intrinsic pathway is activated by intracellular signals generated when cells are stressed and depend on the release of proteins from the intermembrane space of mitochondria, the extrinsic pathway, on the other hand, is activated by extracellular ligands binding to cell-surface death receptors, which leads to the formation of the death-inducing signaling complex.[103] The intrinsic pathway is also referred to as the mitochondrial pathway. Indeed, during apoptosis, cytochrome c is released from mitochondria through the actions of the proteins BCL-2-associated X protein (BAX) and BCL-2 homologous antagonist/killer (BAK). It then binds with apoptotic protease activating factor-1 and adenosine triphosphate, which subsequently binds to procaspase-9 to create the apoptosome. The latter cleaves pro-caspase to its active form of caspase 9, which in turn cleaves and
activates pro-caspase into the effector caspase 3, which proteolytically degrades a host of intracellular proteins to carry out the cell death program. The mitochondrial pathway can also be initiated when mitochondria release a second mitochondria-derived activator of caspases (SMACs) into the cytosol. SMACs bind to the proteins that inhibit apoptosis (IAPs), thereby deactivating the IAPs to allow apoptosis to proceed. The degradation of the cell is, thence, carried out by a group of cysteine proteases called caspases that are normally suppressed by IAPs.[105] It has been reported that the extrinsic pathway is induced by the tumor necrosis factor (TNF) path[106-108] and FAS path,[109,110] both involving receptors of the TNF receptor family.[109] After the activation of this pathway, a balance among proapoptotic BAX,[111] BH3 interacting-domain death agonist (BID), BAK, or BCL-2-associated agonist of cell death (BAD), and anti-apoptotic (BCL-XL and BCL-2) members of the BCL-2 family are established. Proapoptotic proteins render the mitochondrial membrane permeable for the release of caspase activators such as cytochrome c and SMAC, which promote apoptosis[109,110] as described above.

From the results of Li et al[17], we know that promotion of apoptosis leads to clearance of the latency-reversed HIV reservoir. For instance, treatment with IDB did not change the expression of anti-apoptotic BCL-2 but increased the expression of anti-apoptotic BCL-XL and Mcl-1 in CD4+ T cells with or without HIV-1 infection. However, BCL-XL expression in HIV-1-infected cells was greater than that in uninfected controls. It becomes obvious that HIV in infected cells, through overexpression of anti-apoptotic BCL-XL, for example, tends to keep infected cells viable to promote viral particle production. Consequently, using an inhibitor of BCL-XL may trigger cell death and terminate the HIV life cycle. This has been demonstrated as well, as administration of ABT-263 (an inhibitor of BCL-2 and BCL-XL) increased IDB-mediated cell death in latently infected T cells. Matsuda et al[112], demonstrated that Benzolactam-related compounds exhibit latency-reversing activity, which was followed by the enhanced release of HIV particles in ACH-2 and J-Lat cells latently infected with HIV. One of these compounds, referred to as BL-V8-310, displayed activity that was superior to the activity of another highly active PKC activator, pro-stratin. These observations were confirmed with peripheral blood cells from HIV-infected patients. Furthermore, it was observed that Benzolactam-related compounds up-regulate the expression of caspase 3 and enhance apoptosis, specifically in latently HIV-infected cells. This implies that instead of two drugs with distinct mechanisms of action (1 LRA plus 1 apoptosis inducer), BL-V8-310 alone may be enough to induce apoptosis. BL-V8-310 tested alone was, however, more toxic compared with its combination regimen with JQ1, which further enhanced HIV latency-reversing activity.

Thus, induction of apoptosis in cells harboring HIV reservoirs is essential to cure HIV, as apoptosis promotes reservoir decay, and ultimately there will be no more host cells available capable of replicating the virus. The
remaining challenge is to preclude infection of naive cells by HIV particles present in the bloodstream.

**Blocking the occurrence of new infections**

This last and as-important step should be carried out rigorously to avoid potential new infections and eventual establishment of new HIV reservoirs. Since it is known that HIV needs, first, to attach to target cells before initiating its penetration, molecules especially adept at blocking attachment are required at this stage. Several options exist, such as using soluble CD4 antigen as a competitive attachment inhibitor of receptor binding, or using soluble CD4 antigen as a competitive attachment inhibitor of receptor binding.

Inhibitors of gp120 are required at this stage. Several options for penetrating the host cell exist, especially adept at blocking HIV entry, including molecules that specifically target HIV-1 attachment, and interference with the virus/cell fusion, as well as via interference with the glycosylation of cellular receptors.

In addition to HIV attachment inhibitors, a therapeutic cocktail aiming to treat HIV infection should contain an HIV integrase inhibitor (INI). INIs are divided into two categories known as (i) IN strand transfer inhibitors (INSTIs) that bind to the catalytic core domain of the enzyme, IN, to block the binding of IN to dsDNA, and (ii) IN binding inhibitors that bind to the allosteric pocket of IN, and thus disrupts the conformational changes required for the strand transfer reaction. Currently, all USFDA-approved INIs belong to the group of INSTIs. A list of USFDA-approved INIs, with at least in vivo and in vitro test results available, as reported by Trivedi et al, is provided in Table 3. Of note, raltegravir, dolutegravir, and cabotegravir, to list a few, display excellent results in the treatment of HIV-positive individuals. For example, one injection of cabotegravir combined with rilpivirine has been observed to maintain viral loads at undetectable levels for 2 months.

### Challenges of the SECH strategy

#### Challenges from latency and LRAs

The impact of HIV clades on latency establishment and latency reversal remains to be clarified. Sarabia and Bosque have reported that HIV subtypes may play a crucial role in HIV latency. Indeed, HIV-1 LTR, the site of integration, IN variants, the Nef, viral infectivity factor, viral protein r, and viral particle unit of some HIV subtypes could explain how latency occurs and the potential mechanisms required for its reversal. Given that several in vitro tests on subtype B demonstrated that LRAs are effective, it is believed that the effect should be the same for other subtypes. This topic, however, requires further study, considering that factors such as sequence differences in the LTR may influence the response to LRAs and that only 12% of HIV-1 infections globally are because of subtype B. In addition, differential responsiveness of proviruses integrated at various chromosomal locations represents another major challenge that treatment with only one LRA does not overcome. Therefore, there has been a recent trend toward the development of combinations of reagents that affect multiple pathways to produce broader and synergistic transcriptional responses.

---

### Table 3: List of approved Integrase inhibitors with reported in vivo and in vitro testing.

| Integrase inhibitor | **In vitro efficacy (nmol/L)** | **In vivo dosage** | References |
|---------------------|-------------------------------|-------------------|------------|
| Cabotegravir        | 3                             | 400 mg (or 200 mg split injection once a month) | [126,127] |
| Doltegravir         | 0.51–2                        | 50 mg per day     | [128,129] |
| Bictegravir         | 1.5–2.4                       | Available in clinics as single-tablet fixed-dose combination of bictegravir 50 mg, emtricitabine 200 mg, and tenofovir alafenamide 25 mg (Biktarvy) | [130,131] |
| Elvitegravir         | 0.7–1.5                       | Available in clinics as single-tablet fixed-dose combination of elvitegravir 150 mg, cobistat 150 mg, emtricitabine 200 mg, and tenofovir alafenamide 25 mg (Genvoya) | [132,133] |
| MK-2048             | 1.5–2.6                       | 30 mg once daily  | [134,135] |
| Raltegravir         | 2–7                           | 400 mg twice per day | [136,137] |

INI: Integrase inhibitor.
Challenges from pro-apoptotic drugs

Pro-apoptotic drugs currently do not discriminate between HIV-infected and non-HIV-infected cells, and this is a functionally critical component of the SECH strategy. Therefore, Kim et al.[27] proposed to first administer pro-apoptotic drugs to sensitize latently infected cells to apoptosis, followed by administration of LRAs to reactivate latently infected cells to promote the production of pro-apoptotic viral products. This may result in the selective elimination of HIV-infected cells only. This method has already been demonstrated with the pro-apoptotic drug, Venetoclax,[143] which when combined with LRAs led to the selective apoptosis and clearance of HIV-infected cells. Also, potential interactions between pro-apoptotic drugs and LRAs should be considered and studied, especially regarding their synergy, antagonism, and toxicity. The effects of pro-apoptotic drugs on non-T-cell reservoirs should be contemplated as well. It has already been established that some cells, like macrophages, may be particularly resistant to apoptosis, and this information provides adequate justification for the aforementioned concern. Additionally, a better understanding of such drugs on actively dividing and non-dividing cells (to understand how effectively various HIV-infected cell types will be cleared) is warranted. Furthermore, it will be critical to assess the penetration and cellular consequences of these drugs when introduced into particular sites such as the central nervous system and gut-associated lymphoid tissue.[144] Nanoparticles coated with specific antibodies targeting CD4+ antigen or a latency marker such as CD32a[145] may be necessary to enhance pro-apoptotic drug penetration into the aforementioned issues.

Challenges from immune system effector cells

After the application of LRAs, the immune system, via CD8+ T cells and NK cells, can eliminate the HIV reservoir. Actually, through their cytotoxic properties, CD8+ T cells play a critical role in killing HIV-infected cells, which in turn facilitates the control of HIV infection. However, CD8+ T cells are (i) slower to respond to viral infections, (ii) susceptible to viral escape strategies, and (iii) generally excluded from B-cell follicles in lymph nodes[146] which may become subsequent hotspots for productive HIV infection. These limitations demonstrate the necessity to rely on other effector cells, such as NK cells. NK cells may complete CD8+ T cells activity and greatly enhance the immune system ability to clear latency-reversed cells. Indeed, NK cells are (i) rapid in responding to viral infection, without a need for clonal expansion, (ii) present in lymph nodes, where they can control viral replication,[147] and (iii) able to destroy infected cells that evade CD8+ T-cell-mediated elimination.[148] However, being cognizant of the fact that no trial has as yet demonstrated changes in the size of the latent reservoir after treatment with LRAs (in “shock and kill” strategies),[14,15] we believe that strategies aimed at improving the suppressive capacity of CD8+ T cells and NK cell function should be an integral part of the SECH strategy. Various cytokines have been shown to be effective at augmenting NK cell function, including some interleukins (IL-15, 18, and 21) and type 1 interferons.[14] We envisage that perhaps, upon future investigation, these cytokines (or perhaps others) may also be useful in enhancing CD8+ T cell suppressive capacity. For now, it is known that IFN-α treatment simultaneously enhances (i) cytokine secretion, polyfunctionality, degranulation, cytotoxic potential, and the suppressive capacity of NK cells, and (ii) the suppressive capacity of CD8+ T cells.[146] Moreover, supplementation with IFN-γ is likely to trigger P-selectin glycoprotein ligand-1 expression, and thus enhance the recruitment of effector cells, inhibit virion infectivity, and suppress HIV replication, as explored in detail in a recent review by our group.[149]

Conclusion

In summary, considering the knowledge gleaned from the recent literature, we believe that the proposed SECH strategy for HIV cure is dependent upon specific therapeutics administered simultaneously and acting specifically on each of the following distinct processes: (1) the activation of latent reservoirs, (2) the inhibition of autophagy, (3) the induction of apoptosis, (4) the inhibition of HIV attachment, and (5) the inhibition of HIV integration. However, several challenges remain. Indeed, intention-to-treat protocols, reservoir size-based treatment duration, category of HIV-positive individuals (infants, children, adults, elite controllers, and immunological non-responders), and robust study concerning drug–drug interactions are critically important areas to address and overcome going forward. Ultimately, well-designed and executed clinical investigations are warranted in the future to explore the feasibility, safety, and the efficacy of such an approach to possible HIV cure in humans.

Funding

This work was funded by the Medical Research Project of Chongqing Municipal Science and Technology Bureau and Health Commission (No. 2020GDRC004) and the Key Medical Research Project of Chongqing Municipal Science and Technology Bureau and Health Commission (No. 2019ZDXM012).

Conflicts of interest

None.

References

1. Antela A, Rivero A, Llibre JM, Moreno S. RET Group. Redefining therapeutic success in HIV patients: an expert view. J Antimicrob Chemother 2021;dkab168. doi: 10.1093/jac/dkab168.
2. Pace M, Frater J. A cure for HIV: is it in sight? Expert Rev Anti Infect Ther 2014;12:783–791. doi: 10.1586/14787210.2014. 910112.
3. Phillips AN, Cambiano V, Nakagawa F, Banssi-Matharu L, Sow PS, Ehrenkranz P, et al. Cost effectiveness of potential ART adherence monitoring interventions in Sub-Saharan Africa. PLoS One 2016;11:8–14. doi: 10.1371/journal.pone.0167634.
reversal and an expanded therapeutic window. Proc Natl Acad Sci USA 2020;117:10686–10698. doi: 10.1073/pnas.1919408117.

40. Frech T, Altena E, Natesampillai S, Krogaan A, Correia C, Peterson KL, Alto A, et al. Reactivating latent HIV with PKA activator dibutyryl-cAMP in combination with an HDAC inhibitor. Virus Res 2017;227:1–5. doi: 10.1016/j.virusres.2016.09.015.

41. Sorensen ET, Mavraganis GB, Grivas A, Howard JN, Nell R, Sarabia I, et al. Structure-activity relationship analysis of benzotriazole analogues as HIV-1 latency-reversing agents. Antimicrob Agents Chemother 2020;64:e00888–e00920. doi: 10.1128/Aac.6400888.

42. French AJ, Natesampillai S, Krogman A, Correia C, Peterson KL, Alto A, et al. Reactivating latent HIV with PKA activator dibutyryl-cAMP in combination with an HDAC inhibitor. Virus Res 2017;227:1–5. doi: 10.1016/j.virusres.2016.09.015.

43. Spivak AM, Altena E, Natesampillai S, Krogaan A, Correia C, Peterson KL, Alto A, et al. Reactivating latent HIV with PKA activator dibutyryl-cAMP in combination with an HDAC inhibitor. Virus Res 2017;227:1–5. doi: 10.1016/j.virusres.2016.09.015.

44. Lim H, Kim KC, Son J, Shin Y, Yoon CH, Kang C, et al. Reversing HIV latency via sphingosine-1-phosphate receptor 1 signaling. AIDS 2017;31:2443–2450. doi: 10.1097/QAD.0000000000001649.

45. Tyagi M, Iordanskiy S, Ammosova T, Kumari N, Smith K, Breuer D, et al. Reactivation of latent HIV-1 provirus via targeting protein phosphatase-1. Retrovirology 2015;12:1–4. doi: 10.1186/s12977-015-0190-4.

46. Yu J, Boon RT, Yang H, Wang Y, Jiang Z, Yang X, et al. BET inhibitors RXV-208 and PFI-1 reactivate HIV-1 from latency. Sci Rep 2015;5:8098–8105. doi: 10.1038/srep08098.

47. Shi CS, Kehrl JH. TRAF6 and A20 regulate lysine 63-linked ubiquitination of benzamide compounds that induce HIV-1 transcription. PLoS One 2014;9:4–7. doi: 10.1371/journal.pone.0119910.

48. Bobardt M, Kuo J, Chatterji U, Chandra S, Little SJ, Wiedemann N, et al. The inhibitor apoptosis protein antagonist Debrid 1433 is an attractive HIV-1 latency reversal candidate. PLoS One 2019;14:6–7. doi: 10.1371/journal.pone.0217146.

49. Lu P, Shen Y, Yang H, Wang Y, Jiang Z, Yang X, et al. BET inhibitors RXV-208 and PFI-1 reactivate HIV-1 from latency. Sci Rep 2017;7:2–3. doi: 10.1038/s41598-017-1681-6.

50. Wu J, Ao MT, Wang G, Yang X, Jiang Z, Xu D, et al. Pattern recognition receptors and autophagy. Cell Mol Life Sci 2016;73:985–996. doi: 10.1007/s00018-015-1204-y.
119. Wang T, Kadow JF, Zhang Z, Yin Z, Gao Q, Wu D, et al. Inhibitors of HIV-1 attachment. Part 4: a study of the effect of piperazone substitution patterns on antiviral potency in the context of indole-based derivatives. Bioorg Med Chem Lett 2009;19:5140–5145. doi: 10.1016/j.bmcl.2009.07.076.

120. Li Z, Zhou N, Sun Y, Ray N, Laatiaillde M, Hanna GJ, et al. Activity of the HIV-1 attachment inhibitor BMS-626329, the active component of the produg BMS-663068, against CD4-independent viruses and HIV-1 envelopes resistant to other entry inhibitors. Antimicrob Agents Chemother 2013;57:4172–4180. doi: 10.1128/acc.00513-13.

121. Lalazar JF, Latif GH, Brimson C, Echevarria J, Treviño-Pérez S, Bogner J, et al. Safety and efficacy of the HIV-1 attachment produg BMS-663068 in treatment-experienced individuals: 2 week results of A438011, a phase 2b, randomized controlled trial. Lancet HIV 2015;2:e427–e437. doi: 10.1016/j.lancet.hiv.2015.09.017.

122. Savarino A, Boelaert JR, Cassone A, Majori G, Caquina R, Allocco M, et al. Antimalarial drug chloroquine is highly effective in treating influenza A H5N1 virus infection in an animal model. Cell Res 2013;23:300–302. doi: 10.1038/cr.2012.165.

123. Devaux CA, Rolain JM, Colson P, Raoult D. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? Int J Antimicrob Agents 2020;55:105938. doi: 10.1016/j.ijantimicag.2020.105938.

124. Overton ET, Richmond G, Rizzardi G, Jaeger H, Orrell C, Nagmur C, et al. Long-acting cabotegravir and rilpivirine dosing for HIV patients with a BCL-2 antagonist before HIV reactivation reduces HIV reservoir size. J Gen Virol 2014;95:968–979. doi: 10.1099/jgv.0.05964-0.

125. Verma A, Rajagopalan P, Lotke R, Varghese R, Selvam D, Kundu TK, et al. Functional incompatibility between the genetic NF-κB motif and a subtype-specific SpI1l1l1 element drives the formation of the HIV-1 subtype C viral promoter. J Virol 2016;90:7046–7065. doi: 10.1128/jvi.00308-16.

126. Markowitz M, Frank I, Grant RM, Mayer KH, Elion R, Goldstein D, et al. Safety and tolerability of long-acting cabotegravir injections in HIV-uninfected men (ECLAIR: a multicentre, double-blind, randomised, placebo-controlled, phase 2a trial. Lancet HIV 2017;4:e331–e340. doi: 10.1016/s2352-3018(17)30068-1.

127. Margolis DA, Brinson CL, Smith GHR, de Vente J, Hagins DP, Elliot E, Amara A, Jackson A, Moyle G, Else L, Khoo S, et al. Once-daily dolutegravir versus darunavir plus tenofovir/emtricitabine for treatment-naive human immunodeficiency virus-1 infected patients: 156-week results from STARTMRK. Clin Infect Dis 2011;53:830–816. doi: 10.1093/cid/cir510.

128. Carabia J, Bosque A. HIV-1 latency and latency reversal: does subtype matter? Viruses 2019;11:1104. doi: 10.3390/v111112104.

129. van der Sluis RM, Derking R, Breidel S, Speijer D, Berkout B, Jeeninga RE. Interplay between viral Tat protein and c-Jun transcription factor in controlling LTR promoter activity in different human immunodeficiency virus type I subtypes. J Gen Virol 2014;95:968–979. doi: 10.1099/jgv.0.05964-0.

130. Cummings NW, Sainski AM, Dai H, Natesampillai S, Pang YP, Brennan CD, et al. Prime, shock, and kill: priming CD4 T cells from HIV patients with a BCL-2 antagonist before HIV reactivation reduces HIV reservoir size. J Virol 2016;90:4032–4048. doi: 10.1128/jvi.00308-16.

131. Eisele E, Siliciano RF. Redefining the viral reservoirs that prevent HIV-1 eradication. Immunity 2012;37:377–388. doi: 10.1016/j.immuni.2012.08.010.

132. Descours B, Petitjean G, López-Zaragoza JL, Brueil T, Raffel F, Psomas C, et al. CD52 is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. Nature 2017;543:564–567. doi: 10.1038/nature21710.

133. Fukazawa Y, Lum R, Okoye AA, Park H, Matsuda K, Bae JY, et al. The cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers. Nat Med 2015;21:132–139. doi: 10.1038/nm.3781.

134. Huot N, Jacquelin B, Garcia-Telles T, Rasche P, Ploquin MJ, Madec Y, et al. Natural killer cells migrate into and control simian immunodeficiency virus replication in lymph node follicles in African green monkeys. Nat Med 2017;23:1277–1286. doi: 10.1038/nm.4421.

135. Kwa AKR, Talana CAG, Blankson JN. Interferon alpha enhances NK-cell activity, Goldstein the suppressor capacity of HIV-specific CD8 (+) T cells. J Virol 2019;93,e01541-e01518. doi: 10.1128/jvi.01541-18.

136. Zaongo SD, Liu Y, Harpurvurt S, Song F, Xia H, Ma P, et al. P-selectin glycoprotein ligand 1: a potential HIV-1 therapeutic target. Front Immunol 2021;12; doi: 10.3389/fimmu.2021.710121.

How to cite this article: Zaongo SD, Wang Y, Ma P, Song FZ, Chen YK. Selective elimination of host cells harboring replication-competent human immunodeficiency virus reservoirs: a promising therapeutic strategy for HIV cure. Chin Med J 2021;134:2776–2787. doi: 10.1097/CM9.000000000001797.