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

Silvere D. Zaongo[1,2], Yue Wang[3], Ping Ma[4,5], Fang-Zhou Song[2], Yao-Kai Chen[1]

[1]Division of Infectious Diseases, Chongqing Public Health Medical Center, Chongqing 400036, China;
[2]College of Basic Medicine, Chongqing Medical University, Chongqing 400016, China;
[3]Institute for Medical Device Standardization Administration; National Institutes for Food and Drug Control, Beijing 100050, China;
[4]Department of Infectious Diseases, Tianjin Second People Hospital, Tianjin 300192, China;
[5]School 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.[1] Nevertheless, HIV management through ART requires lifelong drug treatment,[2] which may potentially be cost-effective,[3] but can often be toxic to vital organs such as the kidneys,[4] the liver,[5] 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: www.cmj.org

DOI: 10.1097/CM9.0000000000001797

Silvere D. Zaongo and Yue Wang contributed equally to this work.

Correspondence to: Dr. Yao-Kai Chen, Division of Infectious Diseases, Chongqing Public Health Medical Center, 109 Baoyu Road, Geleshan Town, Shapingba District, Chongqing 400036, China
E-Mail: yaokaichen@hotmail.com

Chinese Medical Journal 2021;134(23)
Received: 22-06-2021 **Edited by:** Peng Lyu

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

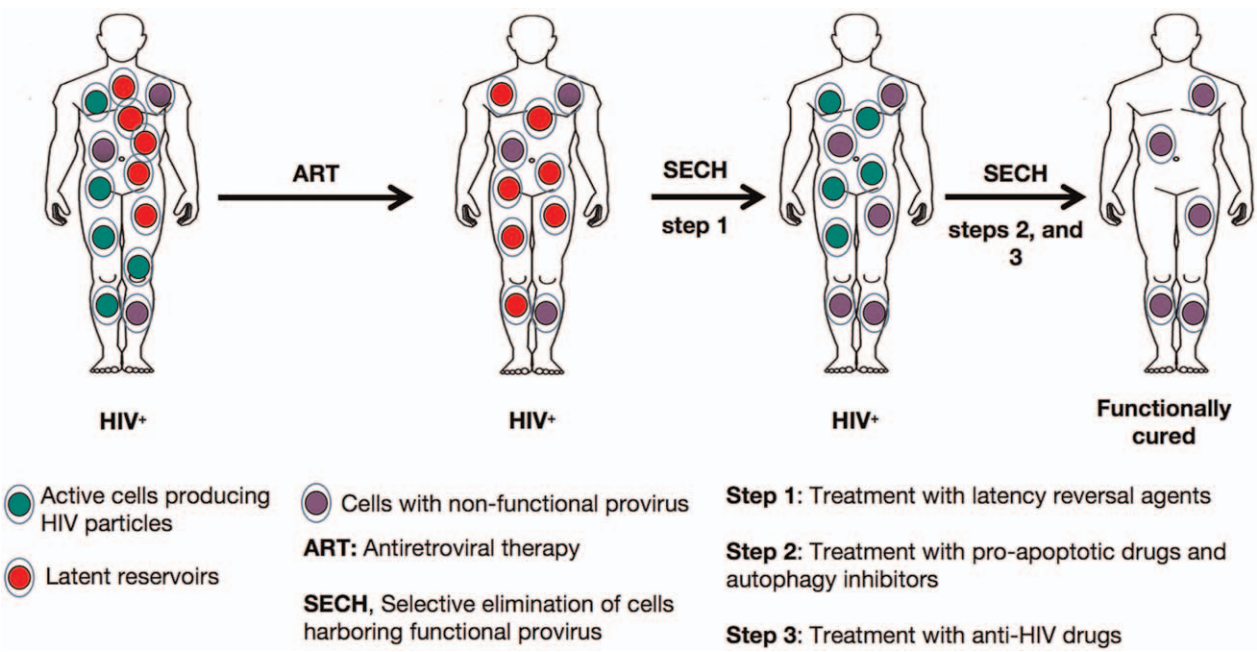

Figure 1: Principle of inducing total HIV remission with a therapeutic cocktail. Via oral or injectable administration, the patient receives specific drugs causing (i) latent reservoir reversal, (ii) elimination of HIV-infected cells harboring replication competent provirus, and (iii) new infection inhibition. 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.

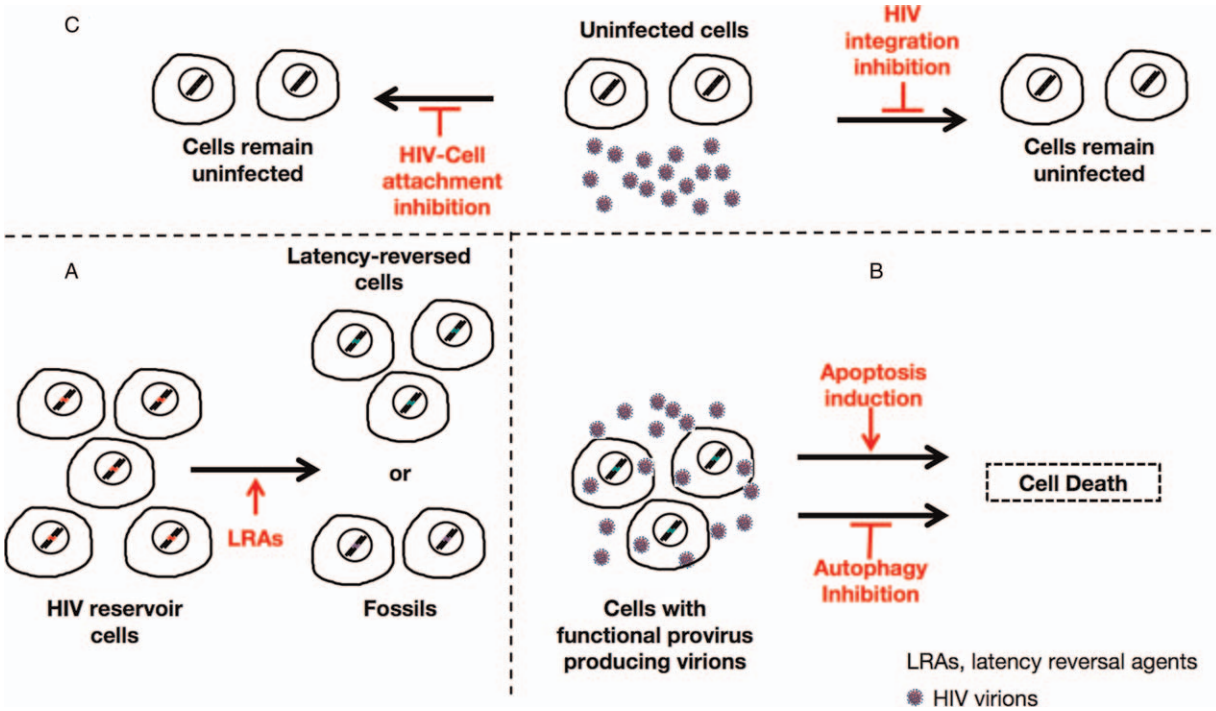

Figure 2: Overview of the key steps through which a therapeutic drug cocktail would engage. Using LRAs, cells with latent reservoirs are converted to latency-reversed cells actively producing HIV particles. On the other hand, cells harboring non-functional provirus remain inactive (fossils) and are not 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.

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,[19] *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),[20] *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),[21] *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.

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		[31]
	TCR/Co-receptor activators	Maraviroc		[32]
	TLR agonists	TLR2, 3, 7, 8, 9 agonists		[33]
Epigenetic modifiers	HDAC inhibitors	Vorinistat, panobinostat, AR-42, MS-275, chidamide	HDAC1, 2, 3	[34-38]
	Histone methyltransferase inhibitors	Chaetocin, AZ505	Suv39H1, SMYD2	[39,40]
Intracellular signaling modulators	PKC agonists	Ingenol EK-16A, gnidimacrin, bryostatin, SUW133, PEP005/Inge-nol-3-angelate, Prostratin, Bryostatin-1, IDB	NF-kappaB	[41-43]
	AMPK activators	Dibutyryl-cAMP		[44]
	JAK/STAT agonists	Benzotriazole, benzazole	STAT3	[45,46]
	IAP agonists	Debio1143	NF-kappaB (non-canonical)	[47]
Transcriptional elongation regulators	BET inhibitors	JQ1, MMQO, UMB-136, RVX-208, PFI-1, OTX015	TAT/pTEFB	[48,49]
	CDK9 activators	Chalcone, Amt-87	pTEFB	[50,51]
Unclassified	Anti-oxidant	Auranofin	NF-kappaB	[52,53]
	AKT modulators	Disulfiram, 57704		[54,55]
	S1P1 agonist	SEW2871	NF-kappaB	[56]
	Protein phosphatase 1	SMAPP1	pTEFb	[57]
	SMAC mimetics	SBI-0637142	NF-kappaB	[58]

* 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 immunodeficiency virus; IAP: Inhibitor of apoptosis; IDB: 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; SMAPP1: 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.

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.[27] 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.[28] For example, it has been reported that the enhancer of the HIV-1 long terminal repeat (LTR) binds many transcriptional activator proteins.[29] 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.[30] 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].

Autophagy, HIV, and autophagy inhibitors

Autophagy is defined as the catabolic mechanism by which intracellular components are delivered to the lysosome for degradation.[59] Of note, macroautophagy is characterized by the formation of autophagosomes which enfold intracellular components and fuse with lysosomes to allow their degradation.[59] On the other hand, microautophagy is described as the process which captures target materials through the invagination of membranes of the endo-lysosomal compartment.[60] Several publications have extensively highlighted the implications of autophagy in (i) maintaining homeostasis,[61,62] (ii) contributing to the innate immune response through multiple mechanisms,[63,64] and (iii) participating in the survival and function of B- and T cells, and lymphoid progenitors.[63]

Macroautophagy-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 autophagosome formation.[65] Then, the phagophore (autophagy machinery required for assembling the autophagosomal membrane precursor) is recruited via PI3P signaling.[66] 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.[62] 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 autophagosome fusion 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-I (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 alternates autophagy activation[79] and inhibition[73,74,80] to avoid its antiviral and immune properties,[73,76-78] 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.[17] 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).[104] It then binds with apoptotic protease activating factor-1 and adenosine triphosphate, which subsequently binds to pro-caspase-9 to create the apoptosome. The latter cleaves pro-caspase to its active form of caspase 9, which in turn cleaves and

Table 2: List of autophagy inhibitors.

Type of inhibitors	Target	Examples	References
Proximal inhibitors*	PI3K	3-Methyladenine	[82,83]
		Wortmannin	[84]
		LY294002	[85]
		PT210	[81,86]
		GSK-2126458	[87]
	VPS34	Spautin-1	[88,89]
		SAR405	[21]
		Compound 31	[90]
		VPS34-IN1	[91]
		PIK-III	[92]
	ULK	Compound 6	[93]
		MRT68921	[94]
		SBI-0206965	[95]
Late inhibitors†	Proteases	Pepstatin A	[96]
		E64d	[96]
	V-ATPase	Bafilomycin A1	[97]
	Lysosomes	Clomipramine	[98]
		Lucanthone	[99]
		Chloroquine	[99]
		Hydroxychloroquine	[99]
		Lys05	[100]
		ARN5187	[101]
		Compound 30	[102]

* Proximal inhibitors target proteins or pathways involved in the initial steps of the core autophagy machinery. † Late inhibitors act on the later stages of the autophagy process, that is, the degradation of autophagosome content by lysosomes. ATP: Adenosine triphosphate; V-ATPase: Vacuolar-type ATPase; VPS34: Class III PI 3-kinase.

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

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]
Dolutegravir	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.

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 inhibitor of receptor binding,[113] inhibitors of gp120 binding to cellular CD4+ antigen,[114] inhibitors of gp120 binding to the chemokine receptor,[115] prevention of gp41/gp120 conformational change,[116] blocking exposure of the fusion domain,[117] and the blocking of the fusion event (gp41 bundle formation).[118] It has previously been demonstrated that piperazine derivatives are potent inhibitors of HIV-1 attachment, and interfere with the interaction of viral gp120 with the host cell receptor, CD4+.[119] In addition, HIV attachment inhibitor BMS-626529, the active component of the prodrug BMS-663068, has shown excellent results *in vitro*[120] and *in vivo*,[17] with proven safety when administered to individuals.[121] Furthermore, chloroquine and hydroxychloroquine (CQ/HCQ), old therapeutic molecules used to treat malaria, should be investigated in this regard. Indeed, chloroquine has potential broad-spectrum antiviral activity via increasing endosomal pH, which is required for virus/cell fusion, as well as via interference with the glycosylation of cellular receptors.[122-124] In addition to the fact that CQ/HCQ is safe and displays excellent autophagy inhibition properties, CQ/HCQ may represent a viable therapeutic option should further compelling evidence of its anti-HIV-cell attachment activity be demonstrated.

In addition to HIV attachment inhibitors, a therapeutic cocktail aiming to treat HIV infection should contain an HIV integrase inhibitor (INI). INIs have emerged as a high-value therapeutic option. 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*[22], 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.[125]

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.[138] 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[139,140] may influence the response to LRAs and that only 12% of HIV-1 infections globally are because of subtype B.[141] 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.[142]

Further investigations are required to clarify this critical point. Indeed, the success of the approach SECH is closely associated with the ability of LRAs to induce latency reversal in reservoir cells.

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.[148] 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.[148] 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, Bansi-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.0167654.

4. Venter WDF, Fabian J, Feldman C. An overview of tenofovir and renal disease for the HIV-treating clinician. S Afr J HIV Med 2018;19:2–4. doi: 10.4102/sajhivmed.v19i1.817.
5. Taramasso L, Lorenzini P, Di Biagio A, Lichtner M, Marchetti G, Rossotti R, *et al*. Incidence and risk factors for liver enzyme elevation among naive HIV-1-infected patients receiving ART in the ICONA cohort. J Antimicrob Chemother 2019;74:3295–3304. doi: 10.1093/jac/dkz353.
6. Treisman GJ, Soudry O. Neuropsychiatric effects of HIV antiviral medications. Drug Saf 2016;39:945–957. doi: 10.1007/s40264-016-0440-y.
7. Garg H, Joshi A, Mukherjee D. Cardiovascular complications of HIV infection and treatment. Cardiovasc Hematol Agents Med Chem 2013;11:58–66. doi: 10.2174/1871525711311010010.
8. Ho YC, Shan L, Hosmane NN, Wang J, Laskey SB, Rosenbloom DIS, *et al*. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. Cell 2013;155:540–551. doi: 10.1016/j.cell.2013.09.020.
9. Siliciano RF, Greene WC. HIV latency. Cold Spring Harb Perspect Med 2011;1:1–4. doi: 10.1101/cshperspect.a007096.
10. Hütter G, Nowak D, Mossner M, Ganepola S, Müssig A, Allers K, *et al*. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. N Engl J Med 2009;360:692–698. doi: 10.1056/NEJMoa0802905.
11. Gupta RK, Abdul-Jawad S, McCoy LE, Mok HP, Peppa D, Salgado M, *et al*. HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. Nature 2019;568:244–248. doi: 10.1038/s41586-019-1027-4.
12. Barouch DH, Deeks SG. Immunologic strategies for HIV-1 remission and eradication. Science 2014;345:169–174. doi: 10.1126/science.1255512.
13. Margolis DM, Garcia JV, Hazuda DJ, Haynes BF. Latency reversal and viral clearance to cure HIV-1. Science 2016;353:5–7. doi: 10.1126/science.aaf6517.
14. Søgaard OS, Graversen ME, Leth S, Olesen R, Brinkmann CR, Nissen SK, *et al*. The depsipeptide romidepsin reverses HIV-1 latency in vivo. PLoS Pathog 2015;11:7–10. doi: 10.1371/journal.ppat.1005142.
15. Elliott JH, McMahon JH, Chang CC, Lee SA, Hartogensis W, Bumpus N, *et al*. Short-term administration of disulfiram for reversal of latent HIV infection: a phase 2 dose-escalation study. Lancet HIV 2015;2:e520–e529. doi: 10.1016/s2352-3018(15)00226-x.
16. Vansant G, Bruggemans A, Janssens J, Debyser Z. Block-and-lock strategies to cure HIV infection. Viruses 2020;12:5–10. doi: 10.3390/v12010084.
17. Li M, Liu W, Bauch T, Graviss EA, Arduino RC, Kimata JT, *et al*. Clearance of HIV infection by selective elimination of host cells capable of producing HIV. Nat Commun 2020;11:6–10. doi: 10.1038/s41467-020-17753-w.
18. de Castro S, Camarasa MJ. Polypharmacology in HIV inhibition: can a drug with simultaneous action against two relevant targets be an alternative to combination therapy? Eur J Med Chem 2018;150:206–227. doi: 10.1016/j.ejmech.2018.03.007.
19. Jiang G, Mendes EA, Kaiser P, Sankaran-Walters S, Tang Y, Weber MG, *et al*. Reactivation of HIV latency by a newly modified Ingenol derivative via protein kinase Cδ-NF-κB signaling. AIDS 2014;28:1555–1566. doi: 10.1097/qad.0000000000000289.
20. Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, *et al*. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res 2008;68:3421–3428. doi: 10.1158/0008-5472.can-07-5836.
21. Ronan B, Flamand O, Vescovi L, Dureuil C, Durand L, Fassy F, *et al*. A highly potent and selective Vps34 inhibitor alters vesicle trafficking and autophagy. Nat Chem Biol 2014;10:1013–1019. doi: 10.1038/nchembio.1681.
22. Trivedi J, Mahajan D, Jaffe RJ, Acharya A, Mitra D, Byrareddy SN. Recent advances in the development of integrase inhibitors for HIV treatment. Curr HIV/AIDS Rep 2020;17:63–75. doi: 10.1007/s11904-019-00480-3.
23. Nettles RE, Schürmann D, Zhu L, Stonier M, Huang SP, Chang I, *et al*. Pharmacodynamics, safety, and pharmacokinetics of BMS-663068, an oral HIV-1 attachment inhibitor in HIV-1-infected subjects. J Infect Dis 2012;206:1002–1011. doi: 10.1093/infdis/jis432.
24. Zhu J, Gaiha GD, John SP, Pertel T, Chin CR, Gao G, *et al*. Reactivation of latent HIV-1 by inhibition of BRD4. Cell Rep 2012;2:807–816. doi: 10.1016/j.celrep.2012.09.008.
25. Ganor Y, Real F, Sennepin A, Dutertre CA, Prevedel L, Xu L, *et al*. HIV-1 reservoirs in urethral macrophages of patients under suppressive antiretroviral therapy. Nat Microbiol 2019;4:633–644. doi: 10.1038/s41564-018-0335-z.
26. Rullo EV, Cannon L, Pinzone MR, Ceccarelli M, Nunnari G, O'Doherty U. Genetic evidence that naive T cells can contribute significantly to the human immunodeficiency virus intact reservoir: time to re-evaluate their role. Clin Infect Dis 2019;69:2236–2237. doi: 10.1093/cid/ciz378.
27. Kim Y, Anderson JL, Lewin SR. Getting the "Kill" into "Shock and Kill": strategies to eliminate latent HIV. Cell Host Microbe 2018;23:14–26. doi: 10.1016/j.chom.2017.12.004.
28. Sadowski I, Lourenco P, Malcolm T. Factors controlling chromatin organization and nucleosome positioning for establishment and maintenance of HIV latency. Curr HIV Res 2008;6:286–295. doi: 10.2174/157016208785132563.
29. Sharma AL, Hokello J, Sonti S, Zicari S, Sun L, Alqatawni A, *et al*. CBF-1 promotes the establishment and maintenance of HIV latency by recruiting polycomb repressive complexes, PRC1 and PRC2, at HIV LTR. Viruses 2020;12:6–14. doi: 10.3390/v12091040.
30. De Crignis E, Mahmoudi T. The multifaceted contributions of chromatin to HIV-1 integration, transcription, and latency. Int Rev Cell Mol Biol 2017;328:197–252. doi: 10.1016/bs.ircmb.2016.08.006.
31. Hokello J, Sharma AL, Dimri M, Tyagi M. Insights into the HIV latency and the role of cytokines. Pathogens 2019;8:8–9. doi: 10.3390/pathogens8030137.
32. Lopez-Huertas MR, Jimenez-Tormo L, Madrid-Elena N, Gutiérrez C, Vivancos MJ, Luna L, *et al*. Maraviroc reactivates HIV with potency similar to that of other latency reversing drugs without inducing toxicity in CD8 T cells. Biochem Pharmacol 2020;182:3–8. doi: 10.1016/j.bcp.2020.114231.
33. Macedo AB, Novis CL, Bosque A. Targeting cellular and tissue HIV reservoirs with toll-like receptor agonists. Front Immunol 2019;10:5–9. doi: 10.3389/fimmu.2019.02450.
34. Sung JA, Sholtis K, Kirchherr J, Kuruc JD, Gay CL, Nordstrom JL, *et al*. Vorinostat renders the replication-competent latent reservoir of human immunodeficiency virus (HIV) vulnerable to clearance by CD8 T cells. EBioMedicine 2017;23:52–58. doi: 10.1016/j.ebiom.2017.07.019.
35. Archin NM, Kirchherr JL, Sung JA, Clutton G, Sholtis K, Xu Y, *et al*. Interval dosing with the HDAC inhibitor vorinostat effectively reverses HIV latency. J Clin Investig 2017;127:3126–3135. doi: 10.1172/jci92684.
36. Brinkmann CR, Højen JF, Rasmussen TA, Kjær AS, Olesen R, Denton PW, *et al*. Treatment of HIV-infected individuals with the histone deacetylase inhibitor panobinostat results in increased numbers of regulatory T cells and limits ex vivo lipopolysaccharide-induced inflammatory responses. mSphere 2018;3:e00616–e00617. doi: 10.1128/mSphere.00616-17.
37. Li JH, Ma J, Kang W, Wang CF, Bai F, Zhao K, *et al*. The histone deacetylase inhibitor chidamide induces intermittent viraemia in HIV-infected patients on suppressive antiretroviral therapy. HIV Med 2020;21:747–757. doi: 10.1111/hiv.13027.
38. Savarino A, Mai A, Norelli S, El Daker S, Valente S, Rotili D, *et al*. "Shock and kill" effects of class I-selective histone deacetylase inhibitors in combination with the glutathione synthesis inhibitor buthionine sulfoximine in cell line models for HIV-1 quiescence. Retrovirology 2009;6:2–5. doi: 10.1186/1742-4690-6-52.
39. Kobayashi Y, Gélinas C, Dougherty JP. Histone deacetylase inhibitors containing a benzamide functional group and a pyridyl cap are preferentially effective human immunodeficiency virus-1 latency-reversing agents in primary resting CD4+ T cells. J Gen Virol 2017;98:799–809. doi: 10.1099/jgv.0.000716.
40. Samer S, Arif MS, Giron LB, Zukurov JPL, Hunter J, Santillo BT, *et al*. Nicotinamide activates latent HIV-1 ex vivo in ART suppressed individuals, revealing higher potency than the association of two methyltransferase inhibitors, chaetocin and BIX01294. Braz J Infect Dis 2020;24:150–159. doi: 10.1016/j.bjid.2020.01.005.
41. Sloane JL, Benner NL, Keenan KN, Zang X, Soliman MSA, Wu X, *et al*. Prodrugs of PKC modulators show enhanced HIV latency

reversal and an expanded therapeutic window. Proc Natl Acad Sci USA 2020;117:10688–10698. doi: 10.1073/pnas.1919408117.
42. French AJ, Natesampillai S, Krogman A, Correia C, Peterson KL, Alto A, *et al*. ReactivatinglatentHIVwithPKCagonistsinduces resistance to apoptosis and is associated with phosphorylation and activation of BCL2. PLoS Pathog 2020;16:3–9. doi: 10.1371/journal.ppat.1008906.
43. Spivak AM, Planelles V. Novel latency reversal agents for HIV-1 cure. Annu Rev Med 2018;69:421–436. doi: 10.1146/annurev-med-052716-031710.
44. Lim H, Kim KC, Son J, Shin Y, Yoon CH, Kang C, *et al*. Synergistic reactivation of latent HIV-1 provirus by PKA activator dibutyryl-cAMP in combination with an HDAC inhibitor. Virus Res 2017;227:1–5. doi: 10.1016/j.virusres.2016.09.015.
45. Sorensen ES, Macedo AB, Resop RS, Howard JN, Nell R, Sarabia I, *et al*. Structure-activity relationship analysis of benzotriazine analogues as HIV-1 latency-reversing agents. Antimicrob Agents Chemother 2020;64:e00888–e00920. doi: 10.1128/aac.00888-20.
46. Graci JD, Michaels D, Chen G, Lester GMS, Nodder S, Weetall M, *et al*. Identification of benzazole compounds that induce HIV-1 transcription. PLoS One 2017;12:7–9. doi: 10.1371/journal.pone.0179100.
47. Bobardt M, Kuo J, Chatterji U, Chanda S, Little SJ, Wiedemann N, *et al*. The inhibitor apoptosis protein antagonist Debio 1143 is an attractive HIV-1 latency reversal candidate. PLoS One 2019;14:6–7. doi: 10.1371/journal.pone.0211746.
48. Lu P, Shen Y, Yang H, Wang Y, Jiang Z, Yang X, *et al*. BET inhibitors RVX-208 and PFI-1 reactivate HIV-1 from latency. Sci Rep 2017;7:2–3. doi: 10.1038/s41598-017-16816-1.
49. Salahong T, Schwartz C, Sungthong R. Are BET inhibitors yet promising latency-reversing agents for HIV-1 reactivation in AIDS therapy? Viruses 2021;13:10–12. doi: 10.3390/v13061026.
50. Wu J, Ao MT, Shao R, Wang HR, Yu D, Fang MJ, *et al*. A chalcone derivative reactivates latent HIV-1 transcription through activating P-TEFb and promoting Tat-SEC interaction on viral promoter. Sci Rep 2017;7:2–6. doi: 10.1038/s41598-017-10728-w.
51. Marinov R, Markova N, Krumova S, Yotovska K, Zaharieva MM, Genova-Kalou P. Antiviral properties of chalcones and their synthetic derivatives: a mini review. Pharmacia 2020;67:325–337. doi: 10.3897/pharmacia.67.e53842.
52. Lewis MG, DaFonseca S, Chomont N, Palamara AT, Tardugno M, Mai A, *et al*. Gold drug auranofin restricts the viral reservoir in the monkey AIDS model and induces containment of viral load following ART suspension. AIDS 2011;25:1347–1356. doi: 10.1097/QAD.0b013e328347bd77.
53. Lu Y, Bohn-Wippert K, Pazerunas PJ, Moy JM, Singh H, Dar RD. Screening for gene expression fluctuations reveals latency-promoting agents of HIV. Proc Natl Acad Sci USA 2021;118:3–5. doi: 10.1073/pnas.2012191118.
54. Kula A, Delacourt N, Bouchat S, Darcis G, Avettand-Fenoel V, Verdikt R, *et al*. Heterogeneous HIV-1 reactivation patterns of disulfiram and combined disulfiram+romidepsin treatments. J Acquir Immune Defic Syndr 2019;80:605–613. doi: 10.1097/qai.0000000000001958.
55. Doyon G, Sobolewski MD, Huber K, McMahon D, Mellors JW, Sluis-Cremer N. Discovery of a small molecule agonist of phosphatidylinositol 3-kinase p110a that reactivates latent HIV-1. PLoS One 2014;9:4–7. doi: 10.1371/journal.pone.0084964.
56. Duquenne C, Gimenez S, Guigues A, Viala B, Boulouis C, Mettling C, *et al*. Reversing HIV latency via sphingosine-1-phosphate receptor 1 signaling. AIDS 2017;31:2443–2454. doi: 10.1097/qad.0000000000001649.
57. 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;12:2–4. doi: 10.1186/s12977-015-0190-4.
58. Pache L, Dutra MS, Spivak AM, Marlett JM, Murry JP, Hwang Y, *et al*. BIRC2/cIAP1 is a negative regulator of HIV-1 transcription and can be targeted by smac mimetics to promote reversal of viral latency. Cell Host Microbe 2015;18:345–353. doi: 10.1016/j.chom.2015.08.009.
59. Mathiassen SG, De Zio D, Cecconi F. Autophagy and the cell cycle: a complex landscape. Front Oncol 2017;7:2–3. doi: 10.3389/fonc.2017.00051.
60. Goto-Yamada S, Oikawa K, Bizan J, Shigenobu S, Yamaguchi K, Mano S, *et al*. Sucrose starvation induces microautophagy in plant root cells. Front Plant Sci 2019;10:1604. doi: 10.3389/fpls.2019.01604.
61. Mizushima N. Autophagy in protein and organelle turnover. Cold Spring Harb Symp Quant Biol 2011;76:397–402. doi: 10.1101/sqb.2011.76.011023.
62. Antonioli M, Di Rienzo M, Piacentini M, Fimia GM. Emerging mechanisms in initiating and terminating autophagy. Trends Biochem Sci 2017;42:28–41. doi: 10.1016/j.tibs.2016.09.008.
63. Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. Nat Rev Immunol 2013;13:722–737. doi: 10.1038/nri3532.
64. Liu T, Tang Q, Liu K, Xie W, Liu X, Wang H, *et al*. TRIM11 suppresses AIM2 inflammasome by degrading AIM2 via p62-dependent selective autophagy. Cell Rep 2016;16:1988–2002. doi: 10.1016/j.celrep.2016.07.019.
65. Levine B, Liu R, Dong X, Zhong Q. Beclin orthologs: integrative hubs of cell signaling, membrane trafficking, and physiology. Trends Cell Biol 2015;25:533–544. doi: 10.1016/j.tcb.2015.05.004.
66. Lamb CA, Nühlen S, Judith D, Frith D, Snijders AP, Behrends C, *et al*. TBC1D14 regulates autophagy via the TRAPP complex and ATG9 traffic. EMBO J 2016;35:281–301. doi: 10.15252/embj.201592695.
67. Lamb CA, Yoshimori T, Tooze SA. The autophagosome: origins unknown, biogenesis complex. Nat Rev Mol Cell Biol 2013;14:759–774. doi: 10.1038/nrm3696.
68. Shen HM, Mizushima N. At the end of the autophagic road: an emerging understanding of lysosomal functions in autophagy. Trends Biochem Sci 2014;39:61–71. doi: 10.1016/j.tibs.2013.12.001.
69. Khaminets A, Behl C, Dikic I. Ubiquitin-dependent and independent signals in selective autophagy. Trends Cell Biol 2016;26:6–16. doi: 10.1016/j.tcb.2015.08.010.
70. Oh JE, Lee HK. Pattern recognition receptors and autophagy. Front Immunol 2014;5:300. doi: 10.3389/fimmu.2014.00300.
71. Criollo A, Niso-Santano M, Malik SA, Michaud M, Morselli E, Mariño G, *et al*. Inhibition of autophagy by TAB2 and TAB3. EMBO J 2011;30:4908–4920. doi: 10.1038/emboj.2011.413.
72. Shi CS, Kehrl JH. TRAF6 and A20 regulate lysine 63-linked ubiquitination of Beclin-1 to control TLR4-induced autophagy. Sci Signal 2010;3:ra42. doi: 10.1126/scisignal.2000751.
73. Campbell GR, Rawat P, Bruckman RS, Spector SA. Human immunodeficiency virus type 1 Nef inhibits autophagy through transcription factor EB sequestration. PLoS Pathog 2015;11:e1005018. doi: 10.1371/journal.ppat.1005018.
74. Mandell MA, Jain A, Arko-Mensah J, Chauhan S, Kimura T, Dinkins C, *et al*. TRIM proteins regulate autophagy and can target autophagic substrates by direct recognition. Dev Cell 2014;30:394–409. doi: 10.1016/j.devcel.2014.06.013.
75. Cabrera-Rodríguez R, Pérez-Yanes S, Estévez-Herrera J, Márquez-Arce D, Cabrera C, Espert L, *et al*. The interplay of HIV and autophagy in early infection. Front Microbiol 2021;12:661446. doi: 10.3389/fmicb.2021.661446.
76. Nardacci R, Ciccosanti F, Marsella C, Ippolito G, Piacentini M, Fimia GM. Role of autophagy in HIV infection and pathogenesis. J Intern Med 2017;281:422–432. doi: 10.1111/joim.12596.
77. Kyei GB, Dinkins C, Davis AS, Roberts E, Singh SB, Dong C, *et al*. Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. J Cell Biol 2009;186:255–268. doi: 10.1083/jcb.200903070.
78. Shoji-Kawata S, Sumpter R, Leveno M, Campbell GR, Zou Z, Kinch L, *et al*. Identification of a candidate therapeutic autophagy-inducing peptide. Nature 2013;494:201–206. doi: 10.1038/nature11866.
79. Chauhan S, Mandell MA, Deretic V. IRGM governs the core autophagy machinery to conduct antimicrobial defense. Mol Cell 2015;58:507–521. doi: 10.1016/j.molcel.2015.03.020.
80. Borel S, Robert-Hebmann V, Alfaisal J, Jain A, Faure M, Espert L, *et al*. HIV-1 viral infectivity factor interacts with microtubule-associated protein light chain 3 and inhibits autophagy. AIDS 2015;29:275–286. doi: 10.1097/qad.0000000000000554.
81. Pasquier B. Autophagy inhibitors. Cell Mol Life Sci 2016;73:985–1001. doi: 10.1007/s00018-015-2104-y.

82. Wu YT, Tan HL, Shui G, Bauvy C, Huang Q, Wenk MR, *et al*. Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition on class I and III phosphoinositide 3-kinase. J Biol Chem 2010;285:10850–10861. doi: 10.1074/jbc.M109.080796.
83. Zhang A, Song Y, Zhang Z, Jiang S, Chang S, Cai Z, *et al*. Effects of autophagy inhibitor 3-methyladenine on ischemic stroke: a protocol for systematic review and meta-analysis. Medicine (Baltimore) 2021;100:e23873. doi: 10.1097/md.0000000000023873.
84. Uluer ET, Sonmez PK, Akogullari D, Onal M, Tanriover G, Inan S. Do Wortmannin and Thalidomide induce apoptosis by autophagy inhibition in 4T1 breast cancer cells in vitro and in vivo? Am J Transl Res 2021;13:6236–6247.
85. Brosinsky P, Bornbaum J, Warga B, Schulz L, Schlüter KD, Ghigo A, *et al*. PI3K as mediator of apoptosis and contractile dysfunction in TGFβ(1)-stimulated cardiomyocytes. Biology (Basel) 2021;10:670. doi: 10.3390/biology10070670.
86. Miller S, Tavshanjian B, Oleksy A, Perisic O, Houseman BT, Shokat KM, *et al*. Shaping development of autophagy inhibitors with the structure of the lipid kinase Vps34. Science 2010;327:1638–1642. doi: 10.1126/science.1184429.
87. Basu D, Salgado CM, Bauer B, Khakoo Y, Patel JR, Hoehl RM, *et al*. The dual PI3K/mToR inhibitor omipalisib/GSK2126458 inhibits clonogenic growth in oncogenically-transformed cells from neurocutaneous melanocytosis. Cancer Genom Proteom 2018;15:239–248. doi: 10.21873/cgp.20082.
88. Liu J, Xia H, Kim M, Xu L, Li Y, Zhang L, *et al*. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. Cell 2011;147:223–234. doi: 10.1016/j.cell.2011.08.037.
89. Schott CR, Ludwig L, Mutsaers AJ, Foster RA, Wood GA. The autophagy inhibitor spautin-1, either alone or combined with doxorubicin, decreases cell survival and colony formation in canine appendicular osteosarcoma cells. PLoS One 2018;13:e0206427. doi: 10.1371/journal.pone.0206427.
90. Pasquier B, El-Ahmad Y, Filoche-Romme B, Dureuil C, Fassy F, Abecassis PY, *et al*. Discovery of (2S)-8-[(3R)-3-methylmorpholin-4-yl]-1-(3-methyl-2-oxo-butyl)-2-(trifluoromethyl)-3,4-dihydro-2H-pyrimido[1,2-a]pyrimidin-6-one: a novel potent and selective inhibitor of Vps34 for the treatment of solid tumors. J Med Chem 2015;58:376–400. doi: 10.1021/jm5013352.
91. Bago R, Malik N, Munson MJ, Prescott AR, Davies P, Sommer E, *et al*. Characterization of VPS34-IN1, a selective inhibitor of Vps34, reveals that the phosphatidylinositol 3-phosphate-binding SGK3 protein kinase is a downstream target of class III phosphoinositide 3-kinase. Biochem J 2014;463:413–427. doi: 10.1042/bj20140889.
92. Dowdle WE, Nyfeler B, Nagel J, Elling RA, Liu S, Triantafellow E, *et al*. Selective VPS34 inhibitor blocks autophagy and uncovers a role for NCOA4 in ferritin degradation and iron homeostasis in vivo. Nat Cell Biol 2014;16:1069–1079. doi: 10.1038/ncb3053.
93. Lazarus MB, Novotny CJ, Shokat KM. Structure of the human autophagy initiating kinase ULK1 in complex with potent inhibitors. ACS Chem Biol 2015;10:257–261. doi: 10.1021/cb500835z.
94. Petherick KJ, Conway OJL, Mpamhanga C, Osborne SA, Kamal A, Saxty B, *et al*. Pharmacological inhibition of ULK1 kinase blocks mammalian target of rapamycin (mTOR)-dependent autophagy. J Biol Chem 2015;290:11376–11383. doi: 10.1074/jbc.C114.627778.
95. Egan DF, Chun MGH, Vamos M, Zou H, Rong J, Miller CJ, *et al*. Small molecule inhibition of the autophagy kinase ULK1 and identification of ULK1 substrates. Mol Cell 2015;59:285–297. doi: 10.1016/j.molcel.2015.05.031.
96. Müller S, Dennemärker J, Reinheckel T. Specific functions of lysosomal proteases in endocytic and autophagic pathways. Biochim Biophys Acta 2012;1824:34–43. doi: 10.1016/j.bbapap.2011.07.003.
97. Wang R, Wang J, Hassan A, Lee CH, Xie XS, Li X. Molecular basis of V-ATPase inhibition by bafilomycin A1. Nat Commun 2021;12:1782. doi: 10.1038/s41467-021-22111-5.
98. Cavaliere F, Fornarelli A, Bertan F, Russo R, Marsal-Cots A, Morrone LA, *et al*. The tricyclic antidepressant clomipramine inhibits neuronal autophagic flux. Sci Rep 2019;9:4881. doi: 10.1038/s41598-019-40887-x.
99. Sehgal AR, Konig H, Johnson DE, Tang D, Amaravadi RK, Boyiadzis M, *et al*. You eat what you are: autophagy inhibition as a therapeutic strategy in leukemia. Leukemia 2015;29:517–525. doi: 10.1038/leu.2014.349.
100. Ondrej M, Cechakova L, Fabrik I, Klimentova J, Tichy A. Lys05 - a promising autophagy inhibitor in the radiosensitization battle: Phosphoproteomic perspective. Cancer Genom Proteom 2020;17:369–382. doi: 10.21873/cgp.20196.
101. De Mei C, Ercolani L, Parodi C, Veronesi M, Vecchio CL, Bottegoni G, *et al*. Dual inhibition of REV-ERBβ and autophagy as a novel pharmacological approach to induce cytotoxicity in cancer cells. Oncogene 2014;34:2597–2608. doi: 10.1038/onc.2014.203.
102. Torrente E, Parodi C, Ercolani L, De Mei C, Ferrari A, Scarpelli R, *et al*. Synthesis and in vitro anticancer activity of the first class of dual inhibitors of REV-ERBβ and autophagy. J Med Chem 2015;58:5900–5915. doi: 10.1021/acs.jmedchem.5b00511.
103. Alberts B, Johnson A, Lewis J, Morgan D, Raff M, Roberts K, *et al*. Molecular Biology of the Cell (6th ed). 2015;Garland Science, 2.
104. Uren RT, Iyer S, Kluck RM. Pore formation by dimeric Bak and Bax: an unusual pore? Philos Trans R Soc Lond B Biol Sci 2017;372:20160218. doi: 10.1098/rstb.2016.0218.
105. Shalini S, Dorstyn L, Dawar S, Kumar S. Old, new and emerging functions of caspases. Cell Death Differ 2015;22. 526-239. doi: 10.1038/cdd.2014.216.
106. Goeddel DV. Connection map for tumor necrosis factor pathway. Sci STKE 2007;382:tw132. doi: 10.1126/stke.3822007tw132.
107. Masum AA, Yokoi K, Hisamatsu Y, Naito K, Shashni B, Aoki S. Design and synthesis of a luminescent iridium complex-peptide hybrid (IPH) that detects cancer cells and induces their apoptosis. Bioorg Med Chem 2018;26:4804–4816. doi: 10.1016/j.bmc.2018.08.016.
108. Raducka-Jaszul O, Bogusławska DM, Jędruchniewicz N, Sikorski AF. Role of extrinsic apoptotic signaling pathway during definitive erythropoiesis in normal patients and in patients with β-Thalassemia. Int J Mol Sci 2020;21:3325. doi: 10.3390/ijms21093325.
109. Wajant H. The Fas signaling pathway: more than a paradigm. Science 2002;296:1635–1636. doi: 10.1126/science.1071553.
110. Wajant H. Connection map for Fas signaling pathway. Sci STKE 2007;380:tr1. doi: 10.1126/stke.3802007tr1.
111. Murphy KM, Ranganathan V, Farnsworth ML, Kavallaris M, Lock RB. Bcl-2 inhibits Bax translocation from cytosol to mitochondria during drug-induced apoptosis of human tumor cells. Cell Death Differ 2000;7:102–111. doi: 10.1038/sj.cdd.4400597.
112. Matsuda K, Kobayakawa T, Tsuchiya K, Hattori SI, Nomura W, Gatanaga H, *et al*. Benzolactam-related compounds promote apoptosis of HIV-infected human cells via protein kinase C-induced HIV latency reversal. J Biol Chem 2019;294:116–129. doi: 10.1074/jbc.RA118.005798.
113. Falkenhagen A, Singh J, Asad S, Leontyev D, Read S, Zúñiga-Pflücker JC, *et al*. Control of HIV infection in vivo using gene therapy with a secreted entry inhibitor. Mol Ther Nucleic Acids 2017;9:132–144. doi: 10.1016/j.omtn.2017.08.017.
114. Tsou LK, Chen CH, Dutschman GE, Cheng YC, Hamilton AD. Blocking HIV-1 entry by a gp120 surface binding inhibitor. Bioorg Med Chem Lett 2012;22:3358–3361. doi: 10.1016/j.bmcl.2012.02.079.
115. Giraudy I, Ovejero CA, Affranchino JL, González SA. In vitro inhibitory effect of maraviroc on the association of the simian immunodeficiency virus envelope glycoprotein with CCR5. Virus Genes 2021;57:106–110. doi: 10.1007/s11262-020-01816-7.
116. Pacheco B, Alsahafi N, Debbeche O, Prévost J, Ding S, Chapleau JP, *et al*. Residues in the gp41 ectodomain regulate HIV-1 envelope glycoprotein conformational transitions induced by gp120-directed inhibitors. J Virol 2017;91:e02219–e02316. doi: 10.1128/jvi.02219-16.
117. Madani N, Princiotto AM, Zhao C, Jahanbakhshsefidi F, Mertens M, Herschhorn A, *et al*. Activation and inactivation of primary human immunodeficiency virus envelope glycoprotein trimers by CD4-mimetic compounds. J Virol 2017;91. e01880–e01816. doi: 10.1128/jvi.01880-16.
118. Joshi VR, Newman RM, Pack ML, Power KA, Munro JB, Okawa K, *et al*. Gp41-targeted antibodies restore infectivity of a fusion-deficient HIV-1 envelope glycoprotein. PLoS Pathog 2020;16:e1008577. doi: 10.1371/journal.ppat.1008577.

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 piperazine 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, Lataillade M, Hanna GJ, *et al*. Activity of the HIV-1 attachment inhibitor BMS-626529, the active component of the prodrug 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/aac.00513-13.
121. Lalezari JP, Latiff GH, Brinson C, Echevarría J, Treviño-Pérez S, Bogner JR, *et al*. Safety and efficacy of the HIV-1 attachment inhibitor prodrug BMS-663068 in treatment-experienced individuals: 24 week results of AI438011, a phase 2b, randomised controlled trial. Lancet HIV 2015;2:e427–e437. doi: 10.1016/s2352-3018(15)00177-0.
122. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? Lancet Infect Dis 2003;3:722–727. doi: 10.1016/s1473-3099(03)00806-5.
123. Yan Y, Zou Z, Sun Y, Li X, Xu KF, Wei Y, *et al*. Anti-malaria drug chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal model. Cell Res 2013;23:300–302. doi: 10.1038/cr.2012.165.
124. 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.
125. Overton ET, Richmond G, Rizzardini G, Jaeger H, Orrell C, Nagimova F, *et al*. Long-acting cabotegravir and rilpivirine dosed every 2 months in adults with HIV-1 infection (ATLAS-2M), 48-week results: a randomised, multicentre, open-label, phase 3b, non-inferiority study. Lancet 2021;396:1994–2005. doi: 10.1016/s0140-6736(20)32666-0.
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 CC, Smith GHR, de Vente J, Hagins DP, Eron JJ, *et al*. Cabotegravir plus rilpivirine, once a day, after induction with cabotegravir plus nucleoside reverse transcriptase inhibitors in antiretroviral-naive adults with HIV-1 infection (LATTE): a randomised, phase 2b, dose-ranging trial. Lancet Infect Dis 2015;15:1145–1155. doi: 10.1016/s1473-3099(15)00152-8.
128. Elliot E, Amara A, Jackson A, Moyle G, Else L, Khoo S, *et al*. Dolutegravir and elvitegravir plasma concentrations following cessation of drug intake. J Antimicrob Chemother 2016;71:1031–1036. doi: 10.1093/jac/dkv425.
129. Molina JM, Clotet B, van Lunzen J, Lazzarin A, Cavassini M, Henry K, *et al*. Once-daily dolutegravir versus darunavir plus ritonavir for treatment-naive adults with HIV-1 infection (FLAMINGO): 96 week results from a randomised, open-label, phase 3b study. Lancet HIV 2015;2:e127–e136. doi: 10.1016/s2352-3018(15)00027-2.
130. Sax PE, DeJesus E, Crofoot G, Ward D, Benson P, Dretler R, *et al*. Bictegravir versus dolutegravir, each with emtricitabine and tenofovir alafenamide, for initial treatment of HIV-1 infection: a randomised, double-blind, phase 2 trial. Lancet HIV 2017;4:e154–e160. doi: 10.1016/s2352-3018(17)30016-4.
131. Tsiang M, Jones GS, Goldsmith J, Mulato A, Hansen D, Kan E, *et al*. Antiviral activity of bictegravir (GS-9883), a novel potent HIV-1 integrase strand transfer inhibitor with an improved resistance profile. Antimicrob Agents Chemother 2016;60:7086–7097. doi: 10.1128/aac.01474-16.
132. Natukunda E, Gaur AH, Kosalaraksa P, Batra J, Rakhmanina N, Porter D, *et al*. Safety, efficacy, and pharmacokinetics of single-tablet elvitegravir, cobicistat, emtricitabine, and tenofovir alafenamide in virologically suppressed, HIV-infected children: a single-arm, open-label trial. Lancet Child Adolesc Health 2017;1:27–34. doi: 10.1016/s2352-4642(17)30009-3.
133. Shimura K, Kodama E, Sakagami Y, Matsuzaki Y, Watanabe W, Yamataka K, *et al*. Broad antiretroviral activity and resistance profile of the novel human immunodeficiency virus integrase inhibitor elvitegravir (JTK-303/GS-9137). J Virol 2008;82:764–774. doi: 10.1128/jvi.01534-07.
134. Quashie PK, Mesplède T, Han YS, Oliveira M, Singhroy DN, Fujiwara T, *et al*. Characterization of the R263K mutation in HIV-1 integrase that confers low-level resistance to the second-generation integrase strand transfer inhibitor dolutegravir. J Virol 2012;86:2696–2705. doi: 10.1128/jvi.06591-11.
135. Hoesley CJ, Chen BA, Anderson PL, Dezzutti CS, Strizki J, Sprinkle C, *et al*. Phase 1 safety and pharmacokinetics study of MK-2048/Vicriviroc (MK-4176)/MK-2048A intravaginal rings. Clin Infect Dis 2019;68:1136–1143. doi: 10.1093/cid/ciy653.
136. Summa V, Petrocchi A, Bonelli F, Crescenzi B, Donghi M, Ferrara M, *et al*. Discovery of raltegravir, a potent, selective orally bioavailable HIV-integrase inhibitor for the treatment of HIV-AIDS infection. J Med Chem 2008;51:5843–5855. doi: 10.1021/jm800245z.
137. Rockstroh JK, Lennox JL, Dejesus E, Saag MS, Lazzarin A, Wan H, *et al*. Long-term treatment with raltegravir or efavirenz combined with tenofovir/emtricitabine for treatment-naive human immunodeficiency virus-1-infected patients: 156-week results from STARTMRK. Clin Infect Dis 2011;53:807–816. doi: 10.1093/cid/cir510.
138. Sarabia I, Bosque A. HIV-1 latency and latency reversal: does subtype matter? Viruses 2019;11:1104. doi: 10.3390/v11121104.
139. van der Sluis RM, Derking R, Breidel S, Speijer D, Berkhout 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/vir.0.059642-0.
140. Verma A, Rajagopalan P, Lotke R, Varghese R, Selvam D, Kundu TK, *et al*. Functional incompatibility between the generic NF-κB motif and a subtype-specific Sp1III element drives the formation of the HIV-1 subtype C viral promoter. J Virol 2016;90:7046–7065. doi: 10.1128/jvi.00308-16.
141. Spach DH. Epidemiology of HIV, 2021. Available from: https://www.hiv.uw.edu/go/screening-diagnosis/epidemiology/core-concept/all#global-hiv-epidemiology. [August 13, 2021].
142. Hashemi P, Barreto K, Bernhard W, Lomness A, Honson N, Pfeifer TA, *et al*. Compounds producing an effective combinatorial regimen for disruption of HIV-1 latency. EMBO Mol Med 2018;10:160–174. doi: 10.15252/emmm.201708193.
143. Cummins NW, Sainski AM, Dai H, Natesampillai S, Pang YP, Bren GD, *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.03179-15.
144. 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.
145. Descours B, Petitjean G, López-Zaragoza JL, Bruel T, Raffel R, Psomas C, *et al*. CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. Nature 2017;543:564–567. doi: 10.1038/nature21710.
146. Fukazawa Y, Lum R, Okoye AA, Park H, Matsuda K, Bae JY, *et al*. B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers. Nat Med 2015;21:132–139. doi: 10.1038/nm.3781.
147. Huot N, Jacquelin B, Garcia-Tellez T, Rascle 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.
148. Kwaa AKR, Talana CAG, Blankson JN. Interferon alpha enhances NK cell function and the suppressive capacity of HIV-specific CD8 (+) T cells. J Virol 2019;93. e01541-e01518. doi: 10.1128/jvi.01541-18.
149. Zaongo SD, Liu Y, Harypursat V, 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/CM9.0000000000001797