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A broad drug arsenal to attack a strenuous latent HIV reservoir

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HIV cure is impeded by the persistence of a strenuous reservoir of latent but replication competent infected cells, which remain unsusceptible to c-ART and unrecognized by the immune system for elimination. Ongoing progress in understanding the molecular mechanisms that control HIV transcription and latency has led to the development of strategies to either permanently inactivate the latent HIV infected reservoir of cells or to stimulate the virus to emerge out of latency, coupled to either induction of death in the infected reactivated cell or its clearance by the immune system. This review focuses on the currently explored and non-exclusive pharmacological strategies and their molecular targets that 1. stimulate reversal of HIV latency in infected cells by targeting distinct steps in the HIV-1 gene expression cycle, 2. exploit mechanisms that promote cell death and apoptosis to render the infected cell harboring reactivated virus more susceptible to death and/or elimination by the immune system, and 3. permanently inactivate any remaining latently infected cells such that c-ART can be safely discontinued.

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Introduction

Millions worldwide are infected with HIV and depend on daily antiretrovirals for survival. Combination antiretroviral therapy (cART) suppresses HIV replication and halts disease progression. However, a small reservoir of replication-competent virus lingers in long-lived resting memory CD4+T cells, which, because the virus is in a latent state, are not targeted by cART [1]. Persistence of these cells leads to inevitable rebound of viral replication once cART is interrupted and constitutes a roadblock to cure. Viable HIV cure dictates either elimination of the latent reservoir or its permanent containment such that cART can be safely discontinued. Ongoing progress in molecular understanding of HIV latency has led to development of pharmacological strategies that target the latent HIV infected cell reservoir (Figure 1). While 'block and lock' [2•] relies on permanent suppression of latent virus, other approaches aim to reverse HIV-1 latency in infected cells via latency reversal agents (LRAs) [3] such that either cell death is induced, or HIV infected cells are 'seen' and eliminated by an immune response. This review focuses on the arsenal of pharmacological agents and mechanisms they exploit to target the reservoir for latency reversal, permanent inactivation, and/or cell death. Other important strategies not discussed include the breadth of interventions to boost HIV-specific immunity for viral elimination [4–7].

Pipeline of latency reversal agents (LRAs)

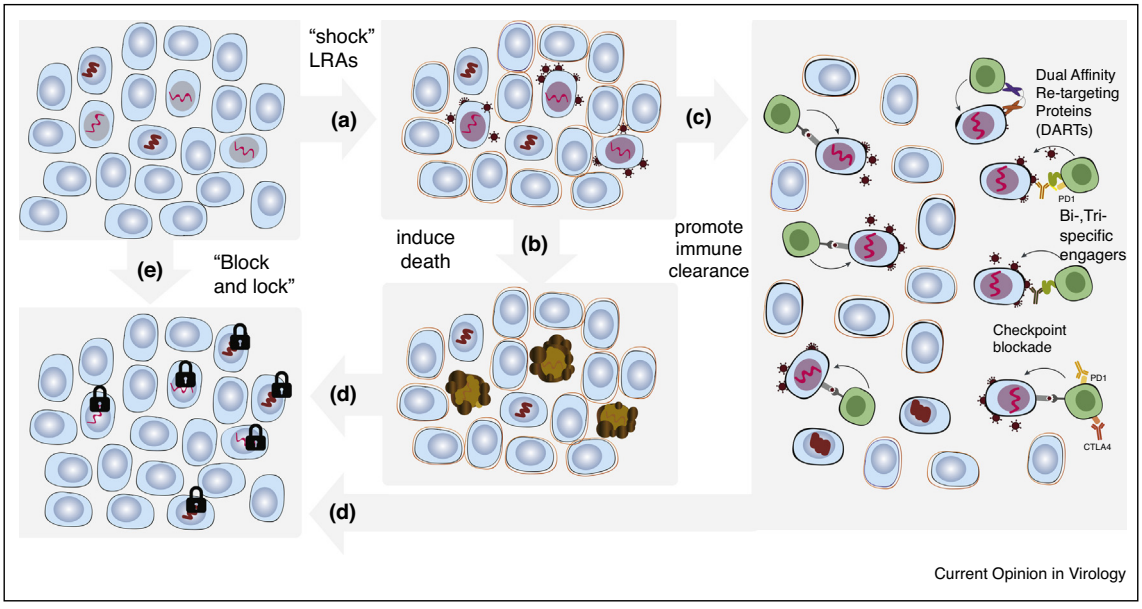
Following integration, transcription at the proviral promoter or 5′ long terminal repeat (5′LTR) is controlled by the host transcription machinery and influenced by surrounding chromatin landscape [8]. Regardless of genomic position, 5′LTR latent structure is defined by Nucleosome-0 (Nuc0) connected by a stretch of accessible DNA (HSS1) to the strictly positioned repressive Nuc1 downstream of the transcription start site (TSS), which is remodeled upon activation (Figure 2a) [8,12,15]. HIV-1 transcription is initiated by engagement of inducible sequence-specific transcription factors (TFs) and associated cofactors at the 5′LTR, controlling accessibility to RNA Polymerase II (Pol II) and permissiveness to transcription (Figure 2). Under basal conditions transcription is initiated but Pol II pauses, producing short transcripts [9,12,15]. The HIV transactivator Tat, a major determinant of reactivation from latency, when expressed, recruits the positive transcription elongation factor (PTEFb) to the nascent TAR RNA, releases Pol II pausing, activating transcription elongation [8,12,15]. HIV-1 expression is also restricted post-transcriptionally via previously underappreciated mechanisms that can also be explored pharmacologically to modulate latency [10,11].

De-repressors: pharmacological interventions that counter repressive chromatin

Targeting PTMs

A broad category of LRAs affect post-translational modifications (PTMs) of N-terminal histone tails, modulating

Figure 1



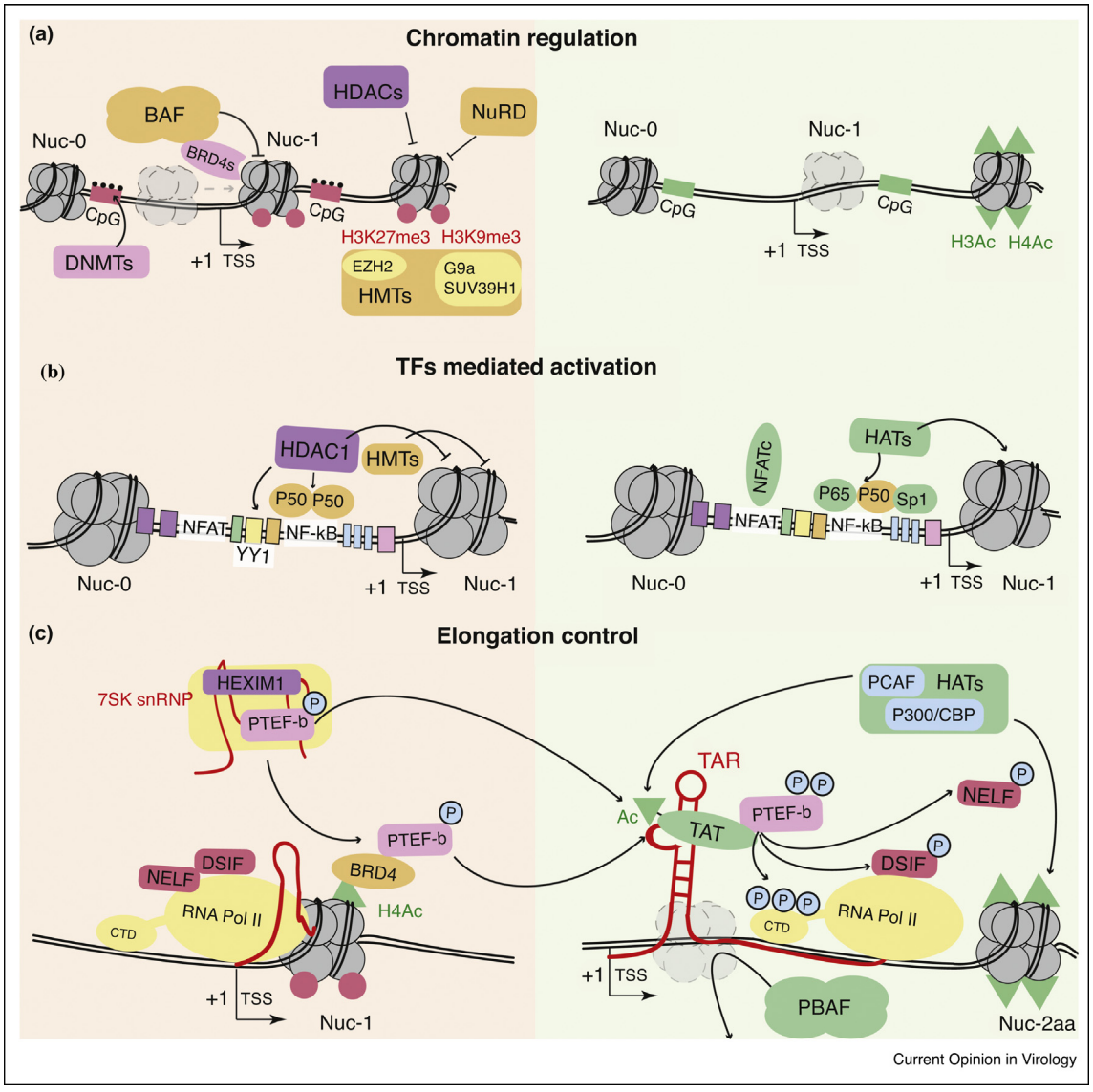
Pharmacological strategies to target the latent HIV-1 reservoir. **(a)** The inducible fraction of the HIV-1 latent reservoir is 'shocked' with LRAs to induce expression of the provirus. **(b)** Cells expressing viral particles die due to the associated cytotoxicity and via pharmacological interventions that sensitize HIV reactivated cells toward cell death. **(c)** Reactivated cells are also recognized and killed by the immune system which can be strengthened and boosted via a number of strategies including small molecule checkpoint inhibitors that enhance T cell function, bi/tri-specific T cell engagers (BI/TRIES) and dual-affinity re-targeting proteins (DARTs). **(d)** In case of inefficient activation and insufficient clearance of latently infected cells, a deeper state of latency is pharmacologically promoted in the remaining fraction of the reservoir ('block and lock'). **(e)** Efficient 'block and lock' strategies, capable of driving the whole reservoir into a deep latency state, could also, in principle, be used alone without the need of additional interventions.

the strength of DNA-nucleosomal core interaction and can serve as marks for recruitment of protein complexes that regulate chromatin structure [12,13]. The best-characterized modification, histone acetylation is deposited by histone acetyltransferases (HATs) and removed by histone deacetylases (HDACs), which are associated with the latent HIV-1 promoter and can be targeted with HDAC inhibitors (HDACis) for derepression [13]. The repressed HIV-1 promoter is also characterized by latency-associated H3K27me3, deposited by polycomb group repressive complex 2 (PRC2) histone methyltransferase (HMT) EZH2, and serves as a mark to recruit other repressors including HDACs, PRC1 and DNA methyltransferases (DNMTs) [14–16]. As well, heterochromatin associated HMTs G9a and Suv39H1-deposited H3K9di/tri-methyl marks [14–16] occupy the latent LTR. A previously underappreciated modification, H4K3 Crotonylation, found to be associated with latency reversal, can be enhanced by sodium crotonate as substrate [17].

HDACis Romidepsin, Panobinostat, Vorinostat, and Valproic acid have been extensively studied for their latency reversal potential [18–21]. The metabolite acetate, highly concentrated in the gut and blood, inhibits HDAC activity and boosted HIV replication [22]. Clinical trials and *in vitro* data have confirmed their sufficient clinical tolerance and effectiveness as LRAs that mechanistically enhance transcriptional noise and synergize with signal-dependent HIV-1 activation [23,8], inducing viral RNA and protein [24]. But clinically, no significant reservoir depletion with HDACis has been observed [18–22]. A multitude of HDACis, targeting all or specific HDAC classes have been developed (Table 1). Class I appear to play a prominent role in latency with Class I HDACis inducing stronger HIV-1 derepression [25,26•]. A recent comparison of HDACis pointed to benzamide moiety and pyridyl cap group molecules, such as Chidamide to be most active with least associated cytoxicity [27].

HMTis. The potential of HMTIs as LRAs has more recently emerged. Inhibition of SUV39H1 with Chaetocin, or targeting G9a with the quinazoline BIX-01294 and more recently UNC-0638, a BIX-01294 derivative with better toxicity profile, reversed latency in CD4+T cells of suppressed patients [14–16,28•]. H4K20 monomethylation, deposited by SMYD2, was linked to HIV-1 latency and its inhibition by AZ391 led to increased cell associated HIV-1 RNA in c-ART treated patient CD4+T cells [29]. Wide spectrum EZH2 HMTis including

Figure 2



Distinct steps in control of the HIV-1 LTR transcription cycle represented in the latent and active states, simplified overview. **(a)** The chromatin architecture of the HIV-1 promoter consists of three strictly positioned nucleosomes (Nuc-0, Nuc-1 and Nuc-2) separated by nucleosome free regions. In the repressed state (left panel), the BAF complex is recruited to the LTR tethered by BRD4S and mediates the positioning of the repressive Nuc-1, downstream of the transcriptional start site (TSS). The latent HIV-1 promoter is also characterized by the presence of repressive cofactors, including HDACs, HMTs and the NuRD complex. **(b)** Upon signal-dependent activation, sequence-specific TFs bind their consensus sites at the HIV-1 5′ LTR and mediate the recruitment of RNA Pol II, required for transcription initiation, and HATs, rendering the chromatin more permissive to transcription. **(c)** In basal conditions RNA Pol II processivity is restricted by the activity of negative elongation factors NELF and DSIF which promote the early dissociation of RNA Pol II from the DNA template, and inhibit the production of full length viral RNAs. Additionally, the availability of P-TEFb is restricted by sequestration within the 7SK snRNP complex and by BRD4-dependent chromatin recruitment. Productive infection requires sufficient expression of the viral transactivator Tat that dramatically potentiates transcription elongation. Tat binds TAR, a hairpin loop RNA structure of the nascent transcript, and recruits P-TEFb to the 5′ LTR. Within P-TEFb, the kinase activity of CDK9 promotes phosphorylation of NELF, DSIF and the RNA Pol II CTD, hence increasing RNA Pol II processivity. Tat PTMs modulates its association with cellular cofactors including HATs and PBAF, remodeling chromatin and enhancing transcription.

DZNep reactivated latent HIV in cell lines, although with substantial toxicity, while recently, specific EZH2 inhibitors EPZ-6438, GSK-343 more effectively reversed latency in resting CD4+T cells from infected individuals [14–16,28•].

DNMTis. HIV 5′LTR CpG methylation promotes binding of methyl–CpG-binding protein (MBD2) and recruitment of the repressive NuRD complex [8]. While the importance of this mechanism *in vivo* has been questioned [8,12,15], sequential treatment with DNMTis and

Table 1

Pharmacologic interventions to target the latent HIV-1 reservoir

Class	Subclass	Function/Target	Compounds	Experimental system	References (Fully listed in reference list)
Chromatin modulators	**Histone methyl transferases inhibitors (HMTis)**	ACSS2 agonist	Sodium crotonate (Na-Cro)	A, F	Jiang *et al.*, 2018
		HMT (SMYD2 inhibitor)	AZ391	A, F	Boehm *et al.*, 2017
		HMT (G9a inhibitor)	BIX-01294	A	Imai *et al.*, 2010
			UNC-0638	D, F	Nguyen *et al.*, 2017
		Polycomb (L3MBTL1 inhibitor)	UNC-926	A, F	Boehm *et al.*, 2017
		Polycomb (SUV39H1 inhibitor)	Chaetocin	A	Bernhard *et al.*, 2011
		Polycomb (EZH1/EZH2 inhibitor)	UNC-1999	D	Kobayashi *et al.*, 2017
		Polycomb (EZH2 inhibitor)	3-deazaneplanocin A (DZNep)	A	Friedman *et al.*, 2011
			EPZ-6438; GSK-343	A, F	Nguyen *et al.*, 2017
			CG05; CG06	A	Choi *et al.*, 2010
			Thiophenyl benzamide (TPB)	A, F	Huang *et al.*, 2018
	Histone deacetylases inhibitors (HDACis)		Chidamide	A, F, G (NCT02902185, NCT02513901)	Yang *et al.*, 2018
		HDAC Class I	Entinostat	A, F	Wightman *et al.*, 2013
			Largazoles (SDL148; JMF1080; SDL256)	A, D	Albert *et al.*, 2017
			Mocetinostat	C	Zaikos *et al.*, 2018
			Romidepsin	D; G (NCT02092116, NCT01933594, NCT02850016, NCT03041012, NCT03619278, NCT02616874, NCT01933594)	Wei *et al.*, 2014
			Pimelic diphenylamide 106, Pyroxamide	D	Kobayashi *et al.*, 2017
		HDAC3/6	Apicidin	A	Lin *et al.*, 2011
		HDAC3	BRD3308	A, F	Barton *et al.*, 2014
		HDAC3/6/8	Droxinostat		
		(pan)HDAC	Belinostat	A	Matalon *et al.*, 2011
			Givinostat	A	Matalon *et al.*, 2010
			KD5170, Pracinostat (SB939)	D	Kobayashi *et al.*, 2017
			M344	A	Ying *et al.*, 2012
			Metacept-1; Metacept-2; Oxamflatin	A	Shehu-Xhilaga *et al.*, 2009
			Panobinostat	F, G (NCT02471430, NCT01680094)	Bullen *et al.*, 2014

Table 1 (*Continued*)

Class	Subclass	Function/Target	Compounds	Experimental system	References (Fully listed in reference list)
Chromatin modulators			Psammaplin A	A, E	Richard K *et al.*, 2018
			Scrpitaid	A	Ying *et al.*, 2010
			Sodium butyrate (Na-But)	A	Reuse *et al.*, 2009
		(pan)HDAC	ST7612AA1	E	Badia *et al.*, 2015
			Trichostatin A (TSA); Trapoxin A (TPX)	A	Van Lint *et al.*, 1996
			Valproic Acid (VPA)	G (NCT03525730, NCT00614458)	Lehrman *et al.*, 2005
	Histone deacetylases inhibitors (HDACis)		Vorinostat (SAHA)	D, F, G (NCT02336074, NCT03198559, NCT03803605, NCT03212989, NCT03382834, NCT02475915, NCT02707900, NCT01319383)	Contreras *et al.*, 2009
		SIRT2 inhibitor	AGK2	D	Kobayashi *et al.*, 2017
		HDACI/II	acetate	E	Bolduc *et al.*, 2017
	BRG-1-associated factors complex inhibitors (BAFis)	BAF complex	CAPE; MGD-486; Pyrimethamine	D, F, G (NCT03525730)	Stoszko *et al.*, 2016
		ARID1A subunit of BAF	Macrolactams	D, F	Marian *et al.*, 2018
	DNA methyltransferases inhibitors (DNMTis)		Decitabine (5-aza-2′-deoxycytidine)	A, D	Kauder *et al.*, 2009
			Zebularine	A, F	Blazkova *et al.*, 2009
Activators of Transcription		CD122/CD132	ALT-803 (IL-15 superagonist complex)	D, E (NCT02191098)	Jones *et al.*, 2016
		CD122/CD132	IL-2, Il-6, TNFα	F, G (NCT03382834)	Tae-Wook Chun *et al.*, 1998
		CD127/CD132	CYT107 (recombinant IL-7)	F, G (NCT01019551)	Wang *et al.*, J 2005; Katlama *et al.*, 2016
		CD28	αCD28	A, D	Tong-Starkesen *et al.*, 1989; Spina *et al.*, 2013
	Extracellular stimulators	Surface glycans	rGal9 (recombinant Gal9)	A, F	Abdel-Mohsen *et al.*, 2016
			Phytohemagglutinin (PHA)	A, D	Spina *et al.*, 2013
		EGFR inhibitor	AG555	A	Calvanese *et al.*, 2013
		TCR agonist	αCD3	A, D	Spina *et al.*, 2013
		S1P1	S1P1 agonists	C,	Duquenne *et al.*, 2017
		αPD1 antibodies	Pembrolizumab	E, G (NCT02595866, NCT03239899)	Fromentin *et al.*, 2019

Table 1 (*Continued*)

Class	Subclass	Function/Target	Compounds	Experimental system	References (Fully listed in reference list)
Activators of transcription			12-deoxyphorbol 13-phenylacetate (DPP)	A	Bocklandt *et al.*, 2003
			3-(2-Naphthoyl)ingenol	A	Liu *et al.*, 2018
			Aplysiatoxin; Debromoaplysiatoxin	A, E	Richard *et al.*, 2018
			Bryologs	A, D	Marsden *et al.*, 2018
			Bryostatin-1	G (NCT02269605)	Gutiérrez *et al.*, 2016
			C3-esterified ingenol derivatives	A, F	Spivak *et al.*, 2018
			EK-16A	A, F	Wang *et al.*, 2017
			Euphoria Kansui extract	D, E, G (NCT02531295)	Cary *et al.*, 2016
			Gnidimacrin	A, E	Huang *et al.*, 2011; Lai *et al.*, 2015
	Protein kinase C agonists (PKC agonists)		IDB (ingenol 3, 20-dibenzoate)	F	Spivak *et al.*, 2015
			Ingenol-B (ingenol-3-hexanoate)	D, F	Jiang *et al.*, 2014; Pandeló José *et al.*, 2014
			LMC03; LMC07	F	Hamer *et al.*, 2003
			Namushen-1; Namushen-2	A	Tietjen *et al.*, 2018
			PEP005 (ingenol-3- angelate)	A, F,	Jiang *et al.*, 2015
			Phorbol 12-myristate 13-acetate (PMA)	A, D	Folks *et al.*, 1988; Spina *et al.*, 2013
			Prostratin	A, E	Gulatosky *et al.*, 1997; Kulkosky *et al.*, 2001
			Sesterterpenoids	A	Wang *et al.*, 2016
			SJ23B	A	Bedoya *et al.*, 2009
		TLR1/2	Pam2CSK4, Pam3CSK4; Imiquimod	D, E	Novis *et al.*, 2013, Macedo *et al.*, 2018
		TLR2	HKLM	A	Alvarez-Carbonell *et al.*, 2017
			PIM6	D	Rodriguez *et al.*, 2013
		TLR3	Poly-ICLC	A, G (NCT02071095)	Alvarez-Carbonell *et al.*, 2017
	Toll-like receptor agonists	TLR2/7	CL413	D, E	Macedo *et al.*, 2018
		TLR5	Flagellin	A	Thibault *et al.*, 2009
		TLR7/8	R-848	A, C (productive infection)	Schlaepfer *et al.*, 2006
			vesatolimod (GS- 9620)	E, G (NCT03060447, NCT02858401)	Tsai *et al.*, 2017
		TLR7	GS-986	H	Lim *et al.*, 2018
		TLR8	3M-002	A, F	Schlaepfer and Speck, J, 2011; Rochat *et al.*, 2017

Table 1 (*Continued*)

Class	Subclass	Function/Target	Compounds	Experimental system	References (Fully listed in reference list)
Activators of transcription	**Toll-like receptor agonists**	TLR9	CPG-7909	G (NCT00562939)	Winckelmann *et al.*, 2013
			MGN1703	A, E, G (NCT02443935)	Offersen *et al.*, 2016
			CpG oligonucleotides: ODN-2006; ODN-2040	A	Scheller *et al.*, 2004
		NF-κB - CCR5	Maraviroc	D, G (NCT02486510, NCT02475915, NCT00935480, NCT00808002)	López-Huertas *et al.*, 2017; Madrid-Elena *et al.*, 2018
		NF-κB	Juglone (5HN)	A, D	Yang *et al.*, 2009
		NF-κB and MSK1 activation	Cocaine	A	Sahu *et al.*, 2015
		NF-κB activation	As2O3 (Aresenic trioxide; FDA-approved drug)	A	Wang *et al.*, 2013
		STAT5 sumoylation inhibitors	Benzotriazoles (HODHBt, HBt, HOBt, HOAt)	F	Bosque *et al.*, 2017
		NFAT activator	AV6	D	Micheva-Viteva *et al.*, 2011
		RUNX1 inhibitor	Ro5-3335	E	Klase *et al.*, 2014
		SRC agonist	MCB-613	A, B	Nikolai *et al.*, 2017 (8th HIV Persistence during Therapy Workshop)
	Activators of transcription factors	HSF-1 stimulators	Resveratrol; Triacetyl resveratrol	A	Pan *et al.*, 2016; Zeng *et al.*, 2017
		PTEN dysregulation	Disulfiram	D, G (NCT03198559; NCT01286259; NCT01944371, NCT01571466)	Elliott *et al.*, HIV 2015; Spivak *et al.*, 2014
		PKA agonist	Bucladesine (dibutyryl-cAMP)	A	Lin *et al.*, 2018
		PI3K agonist	Oxoglaucine (57704)	A, E	Doyon *et al.*, 2014
		Heme oxygenase-1 agonist	Heme arginate	A	Shankaran *et al.*, 2011
		GSK3 inhibitors	SB-216763; Tideglusib	F	Gramatica *et al.*, 2017 (8th HIV Persistence during Therapy Workshop)
		GADD34 / PP1 inhibitor	Salubrinal	A, F	Pan *et al.*, 2016
		Calcineurin agonist	Ionomycin	A, D	Siekevitz *et al.*, 1988; Spina *et al.*, 2013
		BTK inhibitor	Terreic acid	A	Calvanese *et al.*, 2013
		Sp1	Hydroxyurea	A	Oguariri *et al.*, 2007
			Debio 1143	B, F	Bobardt, Kuo, Gallay, 2019
	Inhibitors of apoptosis (IAPs)	BIRC2	Birinapant; SBI-0637142; LCL-161	F	Pache *et al.*, 2015

Table 1 (*Continued*)

Class	Subclass	Function/Target	Compounds	Experimental system	References (Fully listed in reference list)
Transcription elongation control		KAT5 inhibitor	MG-149	D, F	Li *et al.*, 2018
			8-methoxy-6- methylquinolin-4-ol (MMQO)	D, E	Abner *et al.*, 2018; Gallastegui *et al.*, 2012
			Apabetalone (RVX-208), PFI-1	A, F	Lu *et al.*, 2017
	Inhibition of BET	BET inhibitors	I-BET; I-BET-151; MS-417	A, D	Nilsson *et al.*, 2016
			JQ1	D, F	Banerjee *et al.*, 2012
			OTX-015	A, F	Lu *et al.*, 2016
			UMB-136	D, F	Huang *et al.*, 2017
			Hexamethylene bisacetamide (HMBA)	C, F	Vlach and Pitha, 1993; Klichko *et al.*, 2005
		HEXIM	HMBA	A C, D	Contreras *et al.*, 2009; Spina *et al.*, Pathogens 2013
	7SK snRNP	7SK RNA	Gliotoxin	D, F	Stoszko *et al.*, 8th HIV Persistence during therapy workshop, Miami 2017
	Tat	TAR-LTR	TatR5M4	A, D, F	Geng *et al.*, 2016
			EXO-Tat	A, D, F	Tang *et al.*, 2018
	Immune checkpoint inhibitors		Durvalumab (anti-PD1)	G	NCT03094286
			Cemiplimab (anti-PD1)	G	NCT03787095
			Nivolumab (anti-PD1)	G	NCT02408861
			BMS-936559 (anti-PD1)	G	NCT02028403
			Pembrolizumab	F, G case study, $N = 1$	Fromentin *et al.*, 2019, NCT02595866
			Ipilimumab (anti- CTLA-4)	E (case study, $N = 1$); G (NCT02408861, NCT03407105)	Wightman *et al.*, 2015
Post transcriptional control		SF3B1 inhibitor	sudemycin D6	A, D	Kyei *et al.*, 2018
		SR protein family: SRp20/ SRSF3	Digoxin	C, E	Wong *et al.*, 2013
			Cardiac glycoside/aglycones	A, C, E	Wong *et al.*, 2018
		SR protein family: SF-2	DHA-type compound 9 (1C8)	A	Cheung *et al.*, 2016
			Clomifene	A	Prado *et al.* 2016
		Rev-RRE formation	8-Azaguanine, 2-(2-[5-Nitro-2-thienyl]vinyl)quinolone	C, E	Wong *et al.*, 2013
		CRM1 inhibitors	LMB, ratjadone A	A	Fleta-Soriano *et al.*, 2014
			PKF050-638	A	Daelemans *et al.*, 2002
		CBC inhibitor	ABX464	D, G (NCT02735863, NCT02990325)	Vautrin *et al.*, 2019; Steens *et al.*, 2017
Miscellaneous		Deoxyhypusyl hydroxylase	Deferiprone	C, G (NCT02191657)	Saxena *et al.*, 2016

Table 1 (*Continued*)

Class	Subclass	Function/Target	Compounds	Experimental system	References (Fully listed in reference list)
Miscellaneous		Adenosine reuptake inhibitor	Dilazep	A	Calvanese *et al.*, 2013
		Proteasome inhibitors	Carfilzomib (CFZ)	A, F	Pan *et al.*, JBC 2016
			MG-132 (ONX-0914/PR-957); Velcade; CLBL	A, D	Miller *et al.*, 2013
			PR-957 (ONX-0914/MG-132)	A, F	Li *et al.*, 2018
			Abyssomicin-2	D, F	Leon *et al.*, 2015
			HHODC	A, E	Kapewangolo *et al.*, 2017
		Unknown	Piceatannol	A	Elbezanti *et al.*, 2017 (oral presentation); Zeng *et al.*, JAFC 2017
			PH01; PH02; PH03; PH04; PH05	A, F	Hashemi *et al.*, 2018
			Quinolin-8-ol derivatives	A, D	Xing *et al.*, J 2011
			Radicicol; Pochonin B; Pochonin C	D	Mejia *et al.*, J 2014
Block and Lock approaches		Kinase inhibitors	Danusertib, PF-3758309	D	Vargas *et al.*, 2018
		mTOR inhibitors	Torin1, pp242 and rapamycin (Sirolimus)	F, G (NCT02440789)	Besnard *et al.*, 2017
		Tat inhibitor	Didehydro-cortistatin A (dCA)	A, B, F	Mosseau *et al.*, 2015; Kessing *et al.*, 2017
			Triptolide wilfordii	A, G (NCT02219672)	Wan and Chen, 2014
		JAK-STAT inhibitors	Tofacitinib and ruxolitinib	D, F	Gavegnano *et al.*, 2017
		LEDGF/p75 inhibitors	LEDGINs	D	Vranckx *et al.*, 2016
		FACT complex, elongation	curaxin 100 (CBL0100)	D, E	Jean *et al.*, 2017
		Inhibition of NFKB activation, through Hsp90 inhibition	GV1001	A	Kim *et al.*, 2016
		calcineurin inhibitor	cyclosporin A	A, D	Chan *et al.*, 2013
		CDK9 inhibitors	F07#13	B	Van Duyne *et al.*, 2013
			FIT-039	A	Okamoto *et al.*, 2015
			Panel of inhibitors	A	Nemeth *et al.*, 2011
			2-fluorophenyl (12 d), flavopiridol analogue	A	Ali *et al.*, 2009
		PKC	Benzolactam derivative, BL-V8-310	A, E	Matsuda *et al.*, 2019
Induction of cell death		BET inhibitor	Apabetalone	A, F	Xuan-xuan Zhang *et al.*, 2019
		Bcl-2 agonists	Venetoclax, Navitoclax	F	Campbell *et al.*, 2015, CROI, conference
		PI3K/Akt inhibitors	Edelfosine, Perifosine, Miltefosine	A	Lucas *et al.*, 2010
			Lancemaside A, Compound K, Arctigenin	A	Kim *et al.*, 2016
Induction of cell death			Birinapant, GDC- 0152, Embelin	F	Campbell *et al.*, 2018, Hattori, 2018
		SMAC mimetics	AZD5582; AT406; BV6; SM164; GDC0152	A, F	Sampey *et al.*, 2018
			SM-AEG40730, SM-LCL161	A, C	Ashok Kumar *et al.*, 2019
		RIG-I	Acitretin	F	Li *et al.*, 2016; Garcia-Vidal *et al.* [90]

Table 1 (*Continued*)

Class	Subclass	Function/Target	Compounds	Experimental system	References (Fully listed in reference list)
Promote cell killing		Bispecific T-cell engaging (BiTE) antibodies	B12; VRC01; CD4(1+2)L17b	C	Brozy *et al.*, 2018
			MGD014	G	NCT03570918
		Dual-affinity re- targeting (DART)	HIVxCD3	A, D, F	Sung *et al.*, 2015
			HIVxCD3	D, E	Sloan *et al.*, 2015

Model systems:
A – cell lines.
B – mouse models.
C – *ex vivo* infected PBMCs.
D – *ex vivo* infected primary CD4+ T cells.
E – PBMCs from aviremic participants.
F – CD4+ T cells from aviremic participants.
G – aviremic participants (*in vivo*).

HDACis synergized to reactivate HIV-1 in cART treated patient cells [30].

Targeting chromatin structure

A major determinant of HIV latency, chromatin, is restructured by the activity of ATP-dependent remodelers. The CHD3 containing NuRD remodeller and related CHD1 repress HIV-1 [8,9]. The INI-1 containing ATP-dependent BAF remodeller is associated with the 5′LTR and represses HIV-1 by actively positioning Nuc-1 [8]. Interestingly, BRD4S, a short isoform of the bromodomain protein BRD4, tethers BAF to the 5′LTR, silencing HIV-1 [31•]. Such enforced chromatin structure represents a mechanical block for HIV-1 transcription, subject to pharmacological intervention for reversal [8,31•,32,33•,34].

BAF inhibitors (BAFis). Small molecule BAFis re-activated latent HIV-1 in a spectrum of *in vitro* latency models and in c-ART suppressed HIV-1 infected patient CD4+T cells [32]. BAFis CAPE and Pyrimethamine enhance transcriptional noise [34]. When combined with PKC agonists showed significantly increased potency than single treatments, pointing, similar to HDACis, to their potential in combinatorial LRA approaches. Recently, a screen of almost 350 000 compounds led to identification of ARID1A targeting macrolactam scaffold BAFis, which reversed HIV-1 latency in primary CD4+T cells with limited cytoxicity, representing promising LRAs for clinical investigation [33•].

BET inhibitors (BETis), in addition to enhancing HIV-1 transcription elongation (Section 'Enhancing HIV-1 transcriptional elongation'), act as derepressors of HIV-1 transcription in a Tat independent manner [8]. BETis inhibited 5′LTR-bound BRD2, and BRD4S, inducing LTR chromatin derepression in a BAF-dependent manner [8,31•]. Small molecule BETis are under development with differing potency and specificity to circumvent clinical limitations of JQ1 (Table 1).

Inducing HIV-1 transcription activation

The 5′LTR contains a plethora of consensus sequences for TFs whose binding leads to HIV-1 5′LTR recruitment of Pol II and basal TFs [8,12,15] (Figure 2b). NF-κB/p65, arguably the strongest activator of HIV-1 transcription initiation, and molecular effectors that facilitate its binding such as those in the protein kinase C (PKC), TLR, and TNFα signaling pathways, are high potential pharmacological targets for latency reversal [9,35]. AP-1, STAT5 and NFAT are also among important HIV-1 transcription activators [8,9,36].

Targeting NFkB

In latent HIV-1 infected resting CD4+T cells, p65 is sequestered in the cytoplasm while the 5′LTR is repressed by p50 homodimers. Upon canonical NFκB

activation, p65 translocates to the nucleus, binds 5′LTR as a p65/p50 heterodimer and recruits Pol II, HATs, as well as PTEFb, leading to initiation and elongation of HIV transcription [8,9,35]. While an attractive pathway for LRA-based interventions, NFkB signaling is a master regulator of immune and other functions and its pharmacological modulation exposes risks of serious side effects [35]. Interestingly, small molecule mimetics of mitochondria-derived activator of caspases (SMAC mimetics) (Section 'Inhibitors of IAPs'), activated non-canonical NF-κB and binding of RELB/p52 heterodimers to the 5′LTR resulting in latency reversal (Table 1) without causing T cell activation, pointing to non-canonical NFκB as an interesting avenue for further exploration [27,37,38].

PKC agonists. A spectrum of drugs targeting the PKC pathway, including Prostratin, Bryostatin-1 and Ingenols activate NFAT, NFκB and AP-1 binding to the 5′LTR (Table 1), leading to strong proviral transcription initiation [18,39–45]. While PKCα and PKCθ stimulation targets HIV-1 [46], most currently available PKC agonists target many PKC isoforms resulting in pleiotropic and consequent toxic effects, highlighting need for novel more specific PKC agonists [18,45,46].

Maraviroc, a CCR5 antagonist HIV entry blocker was shown to also reverse latency via NFkB activation [47,48]. Maraviroc induced NFκB phosphorylation and HIV transcription as shown by increased cell associated HIV-1 RNA in patient CD4+ T cells [48]. Maraviroc is attractive for inclusion in pharmacological LRA strategies because of its mechanistic versatility as an LRA and antiviral.

TLR agonists have gained much attention due to their multifactorial effects on the HIV-1 reservoir [49••,50–54]. At least ten TLRs are described that function as first line of pathogen recognition and induce innate and adaptive immune defenses. Dual TLR agonists such as CL413 showed potent HIV-1 reactivation via complementary targeting of TLR2 and TLR7, leading to NFkB activation concomitant with TNFα production [49••]. MGN1703, a TLR9 agonist induced HIV plasma RNA in 6 of 15 study participants concomitant with increased activation of NK and CD8+T cells, although no reduction in latent reservoir was observed [50]. The TLR7 agonists GS-986 and GS-9620, suggested to also enhance anti-HIV immune effector function, reversed latency in patient cells [51]. These TLR7 agonists also increased plasma HIV-1 RNA concominant with decreased HIV-1 DNA in the infected rhesus model, where two of nine animals have remained aviremic [52]. Because of this functional versatility, TLR agonists show much promise in reservoir elimination strategies.

Other TFs

LRAs can reduce or enhance HIV-1 5′LTR binding of repressive/activating TFs [8,12,15]. Resveratrol promotes histone acetylation and activation of HSF1, an HIV-1 transcription activator [55]. Benzotriazoles were recently shown to stabilize the active form of STAT5 and reactivate HIV-1 [36].

Enhancing HIV-1 transcriptional elongation

Inefficient transcription elongation via promoter-proximal Pol II 5′LTR pausing is a major rate-limiting step in latency reversal [56••] (Figure 2c), which is released by Tat; when expressed at sufficient levels, Tat orchestrates a strong positive transcriptional feedback loop [8]. Tat binds TAR and recruits PTEFb, whose CDK9 component phosphorylates the Pol II C-terminal domain (CTD) as well as NELF and DSIF (which promote Pol II dissociation when unphosphorylated), enhancing Pol II processivity. In latent cells, PTEFb is predominantly sequestered within the 7SKsnRNP complex, a ribonucleoprotein scaffold in which PTEFb activity is inhibited [8]. Tat also competes for PTEFb with BRD4, which binds and sequesters PTEFb [9]. To enhance transcription elongation, in addition to PTEFb, Tat recruits a number of other interactors, including chromatin modifiers, whose binding is regulated by deposition and removal of PTMs and these can also be exploited pharmacologically [8,57–59].

BETis. Inhibition of BRD4 releases PTEFb, increasing its availability for binding Tat. BETis activate latent HIV in a spectrum of latency models and after treatment of cells from HIV infected patients [60–63] (Table 1). Interestingly, inhibition of the lysine acetyltransferase KAT5 reduced 5′LTR histone H4 acetylation and impaired BRD4 recruitment, similar to BETis, resulting in increased PTEFb pool for Tat reactivation of latent HIV-1 [64]. Thus BETis are promising LRAs that act via a dual mechanism, relieving BRD4S-BAF-mediated LTR repression as well as increasing availability of PTEFb for Tat.

Compounds disrupting 7SK snRNP. In resting CD4+T cells the majority of PTEFb is sequestered in an inactive form within the 7SK snRNP complex [8]. Inhibition of the HEXIM subunit of 7SK snRNP by HMBA enhanced PTEFb activity and latency reversal [9,63,65]. We recently found Gliotoxin, a small molecule secreted by *Aspergillus fumigatus* reversed latency in HIV infected patient CD4+T cells by disrupting 7SK snRNP causing PTEFb release and transcription elongation at the HIV LTR (submitted).

Tat has remarkable specificity for the HIV 5′LTR and can penetrate cell membranes. In an attenuated form [66], or exosomally delivered [67], Tat activated HIV-1 in CD4 +T cells obtained from c-ART suppressed infected individuals and significantly increased the potency of other LRAs. The potential of Tat as a therapeutic vaccine

candidate has also been explored [68] and may play a role in efforts toward reservoir depletion.

Immune checkpoint (IC) blockers. PD-1 has been suggested to confer persistence of HIV-1 latency during c-ART, likely via inhibition of signaling pathways that lead to PTEFb activity [69,70]. IC blockers reversed latency in cells obtained from suppressed patients [71], although another study found less robust effects [72]. Further investigation will determine effectiveness of IC blockers as LRAs and/or in alleviating CD8+T cell exhaustion.

Targeting post transcriptional regulation

Viral proteins were shown to be produced in a small fraction of LRA-reactivated cells which transcribed viral RNA [73••]. This points to the presence of post-transcriptional blocks in viral reactivation [56••], where HIV-1 RNA is subjected to splicing and polyadenylation and RNA surveillance proteins influence viral RNA metabolism. Lack of polyadenylated mRNA compromises transcript stability, export and HIV-1 protein production while block in splicing decreases HIV-1 expression [10,11,56••,74–77]. The significant contribution of post-transcriptional and transcription elongation blocks to efficient HIV latency reversal have only recently come to light. Although these regulatory mechanisms have not been extensively explored in the context of HIV reactivation, effective latency reversal may require interventions that improve viral RNA stability, splicing, export and translation in order to boost viral protein production.

Pipeline of block and lock agents

On the flip side of reversing latency as a stepping stone to viral elimination, 'block and lock' [2•] is a functional cure strategy to permanently shut down viral expression, eliminating the need for continued antiviral therapy.

Tat inhibition

The HIV-1 Tat inhibitor Didehydro-Cortistatin A (dCA) binds Tat and effectively disrupts Tat/TAR axis [78], restricting HIV-1 transcription and replication. dCA treatment was shown to restrict PBAF recruitment while enhancing BAF 5'LTR occupancy and Nuc-1 mediated repression [79]. Consistently, *ex vivo* dCA treatment of CD4+T cells from HIV-1 infected individuals both improved c-ART suppression of infection and led to strengthened 5′LTR chromatin and epigenetic repression, restricting viral reactivation in latently infected cells and leading to a delayed viral rebound after c-ART interruption [2•].

Targeting host factors to reinforce latency

In line with block and lock, compounds targeting host factors DDX3, DDX5, Matrin3, Mov10, splicing factors, UPF proteins, involved in HIV-1 post-transcriptional processing, including inhibitors of mTOR, cardiotonic steroids, SR proteins, inhibit HIV-1 latency reversal and lead to a block in translation [74–81]. Inhibition of HIV-1 Rev and Rev response element (RRE) association on the viral RNA or the cellular factor CRM1 can block nuclear export of unspliced viral mRNA [82]. ABX464-mediated inhibition of the cap binding complex increased viral splicing, halting production of unspliced RNA required for viral assembly [83]. LEDGINs, molecules that inhibit HIV-1 integrase-LEDGF interaction were described to shift preferential sites of HIV-1 integration out of active transcription units, and retarget HIV into regions refractory to reactivation [84]. Block and lock strategies, similarly to LRAs, can in principle work most effectively in combination; dCA, LEDGINs, compounds that strengthen proviral epigenetic repression, and ultimately modulators of splicing and viral export, may act synergistically to induce a deeper state of latency to delay or permanently suppress viral rebound.

Inducing cell death

An attractive approach to eliminate HIV-1 emerging out of latency is to pharmacologically target danger sensing, stress and apoptotic pathways in order to induce cell death in LRA-reactivated HIV expressing cells [85]. This would bypass necessity for an anti-HIV immune response to eliminate reactivated cells. To this end, coupling LRA-induced HIV activation with inhibitors of inhibitors of apoptosis (IAPs), stimulation of danger sensing pathways, and indirect triggering of stress by blocking the cell's physiological processes have drawn much attention as a way to eliminate latently infected cells.

Inhibitors of IAPs

SMAC mimetics (SMs), molecules which target cell survival factors XIAP and cIAP1/BIRC2 have shown much promise as both LRAs that act through noncanonical NFkB activation as well as compounds that induce apoptosis in HIV-1 infected cells through proteasomal degradation of IAPs. SMs SBI-0637142 and LCL161 down-regulated BIRC2/IAP, leading to proviral transcription [37]. Debio 1143 targets BIRC2 for degradation inducing non-canonical NFκB with subsequent HIV-1 latency reversal in resting CD4+T cell from aviremic participants [86]. SMs birinapant [38], GDC-0152, and embelin induced apoptosis selectively in HIV-1 infected (but not uninfected) central memory CD4+Tcells, leading to their elimination [87••]. Benzolactam related compound BL-V8-310 induced apoptosis in HIV infected cells reactivated in a PKC induced manner [44]. Interestingly, *in vitro* treatment with the pro-apoptotic drug Venetoclax, which blocks Bcl-2, followed by anti-CD3/CD28 stimulation resulted in fast decay of productively infected primary T cells *in vitro* and reduction of the latent reservoir *in vitro* [88].

Stimulation of TLRs and RIG-I-like receptors (RLRs)

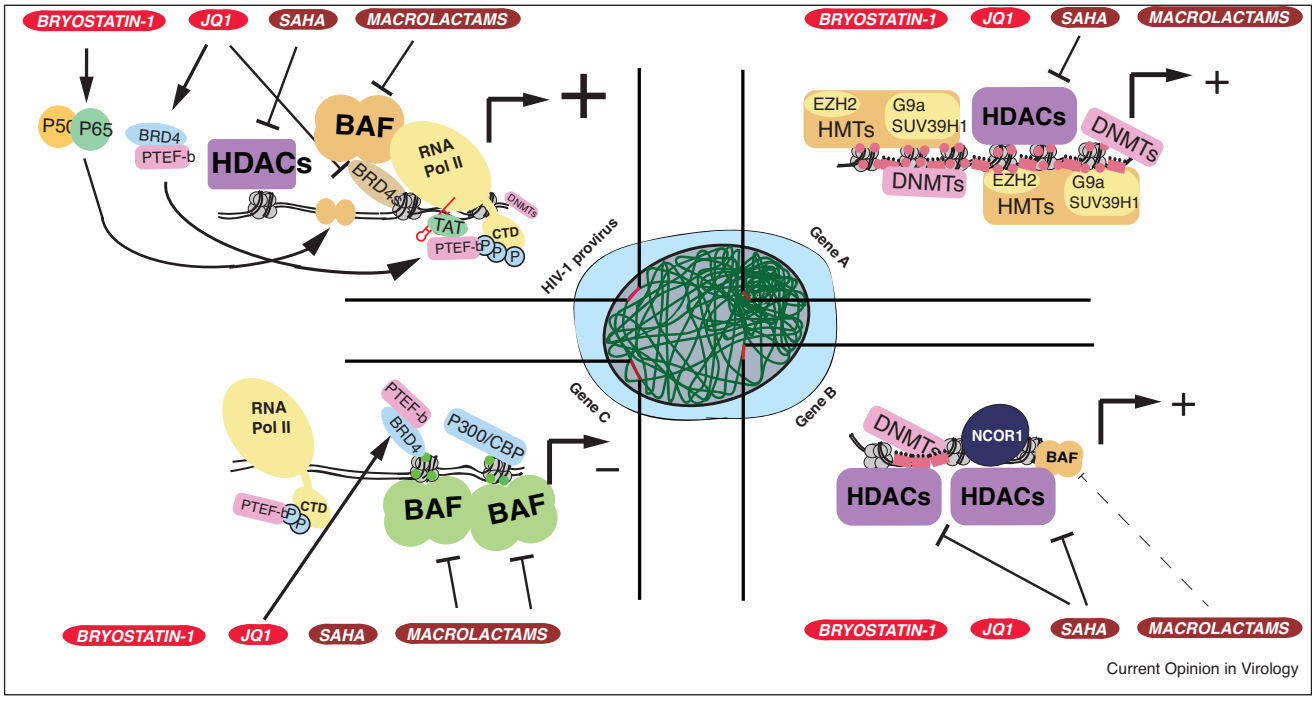
When latent HIV is reactivated, TLRs, RLRs and their molecular effectors, act as sensors that trigger NFkB,

MAP kinase and interferon signaling and initiate an innate immune response. Subsequent to detection of viral RNA, RIG-1 induces apoptosis. Interestingly, retinoic acid (RA) induces expression of RIG-I and p300, which in turn stimulates HIV-1. Acitretin a derivative of RA reversed HIV-1 latency and induced apoptosis in infected cells [89,90]. When combined with Vorinostat even higher depletion of proviral DNA was observed. A later study however challenged these findings showing only weak latency reversal and cell death, pointing to need for further evaluation. TLRs may also play multiple roles, as LRAs, and mediators of HIV-infected cell death [51,54]. A remaining question is whether and which TLRs become activated by HIV-1 transcripts and proteins upon latency reversal.

Combination, synergism and scalable therapy

Current LRAs reactivate only 5% of latently infected cells [91••], of which only an approximated 2–10% produce viral protein in addition to expressing viral RNA [73••]. Administration of certain LRA combinations in intervals, rather than at once [19,30], stimulated higher proviral expression, while sequential treatment rounds yielded new infecious particles. These observations point to a limitation in potency of current LRAs as well as transcriptional stochasticity of a diverse and strenuous latent reservoir. The heterogeneous nature of molecular mechanisms controlling HIV latency predicts that a combination of compounds targeting distinct regulatory pathways will be most effective to activate the reservoir. Synergistic effects of LRAs have been shown ex vivo [8,9,30,32,33•,40,43,63]. While ongoing and future clinical trials will shed more light on which mechanisms of latency should be targeted in concert for most roboust reversal, mechanistic and preclinical observations point to combinations that include derepressors (eg. Vorinostat, BAFis), activators of NFkB (eg. dual TLR agonists or SMAC mimetics) and activators of transcription elongation (eg. BETis, Gliotoxin) to have high potential. The use of LRAs in combination allows for lower concentrations of each molecule to induce HIV activation. Hence, combinatorial approaches emerge not only as a way to improve the activation efficacy of individual LRAs, but also as a way to govern a level of specificity towards HIV-1 latency reversal, limiting the pleiotropic and toxic effects of each intervention (Figure 3).

Figure 3



Combinatorial targeting to obtain synergism and selectivity for the HIV promoter to achieve HIV latency reversal with minimal associated pleiotropic effects and cytotoxicity. Combinatorial use of different classes of LRAs (e.g. bryostatin-1, JQ1, Vorinostat and macrolactam scaffold BAFis shown here) may confer specificity for transcriptional reactivation at the latent HIV-1 promoter relative to endogenous genes. The HIV-1 promoter is targeted by the activity of each LRA, which together strongly synergize to re-activate HIV-1 transcription. Gene A, is highly repressed and targeted only by Vorinostat for re-activation, with limited effect. Gene B, predominantly repressed by NCOR1, histone hypoacetylation and DNA methylation, and partially by the repressive BAF is moderately re-activated by the combination of LRAs. Gene C is an actively transcribed gene, dependent on p300, BAF and BRD4 and undergoes partial repression as result of the combination LRAs.

In contrast to antivirals, which target HIV, pharmacological interventions to eliminate the HIV reservoir (Table 1), with the exception of Tat and Tat and Rev-RRE inhibitors, all target host molecular effectors, harbor inherent pleiotropic effects and are subject to variability in response. In this context, pharmacogenetics to investigate the patient-specific response to distinct molecules may identify robust treatments, which synergize at sufficient magnitudes to overrule individual variability, paving the way for scalable therapy options. Importantly, the complex nature of the latent reservoir points to the likelihood of future combinations of nonexclusive pipelines of interventions. For example, potent latency reversal and cell death promoting combination regimens could be used, in presence of c-ART, to activate and eliminate a more reactivatable fraction of the reservoir. Here promoting clearance of latent cells via apoptosis and immune boosting strategies could be used concomitantly to improve reservoir elimination. Upon clearance of this more labile latent reservoir, 'block and lock' regimens may be employed to lock the remaining reservoir in a permanently repressed state. A strengthened immune system would then control the latent virus in case of escape from the blocked state, in combination allowing cessation of c-ART.

Conflict of interest statement

Nothing declared.

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