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HIV "shock and kill" therapy: In need of revision

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ABSTRACT

The implementation of antiretroviral therapy 23 years ago has rendered HIV infection clinically manageable. However, the disease remains incurable, since it establishes latent proviral reservoirs, which in turn can stochastically begin reproducing viral particles throughout the patient's lifetime. Viral latency itself depends in large part on the silencing environment of the infected host cell, which can be chemically manipulated. "Shock and kill" therapy intends to reverse proviral quiescence by inducing transcription with pharmaceuticals and allowing a combination of antiretroviral therapy, host immune clearance and HIV-cytolysis to remove latently infected cells, leading to a complete cure. Over 160 compounds functioning as latency-reversing agents (LRAs) have been identified to date, but none of the candidates has yet led to a promising functional cure. Furthermore, fundamental bioinformatic and clinical research from the past decade has highlighted the complexity and highly heterogeneous nature of the proviral reservoirs, shedding doubt on the "shock and kill" concept. Alternative therapies such as the HIV transcription-inhibiting "block and lock" strategy are therefore being considered. In this review we describe the variety of existing classes of LRAs, discuss their current drawbacks and highlight the potential for combinatorial "shocktail" therapies for potent proviral reactivation. We also suggest investigating LRAs with lesser-known mechanisms of action, and examine the feasibility of "block and lock" therapy.

1. Introduction

Combinatorial antiretroviral therapy (cART) against HIV has passed considerable milestones since its successful establishment in 1996. However, although the quality of life of HIV-positive patients on cART has increased remarkably, elevated risks for morbidities such as asynchronous muscle aging (Tran et al., 2018), persistent chronic immune activity and inflammation (Sereti et al., 2017), increased occurrence of various cancers (Thrift and Chiao, 2018) and decreased life-expectancy (Wandeler et al., 2016) remain as problematic aspects of the infection. One of the principal reasons for the persistence of HIV is its ability to remain latently infectious within the host, rendering the disease a permanent burden, needing constant medical monitoring and continuous ART.

Among various discussed remedies to counter the disease, several approaches are being considered to be supplemented in combination with existing cART. The life-long "block and lock" therapy aims to inhibit the emergence of the viral cycle by suppressing transcription, thereby driving the provirus into a state of deep latency (Fig. 1). In contrast, the curative "shock and kill" strategy aims to stimulate the dormant virus by regulating the host dependent pathways and to "kill" the infection, either through HIV-related cytopathic effects or via immune system-based clearance of infected cells (Hamer, 2004).

The first clinical trials that attempted to reactivate the proviral reservoir were carried out with IL-2 cytokine and antibodies against the T cell receptor CD3. However, trials with IL-2 alone did not result in any decrease of viral DNA in patient $CD4^+$ cells, nor did the therapy succeed in postponing the re-emergence of viral rebound (Stellbrink et al., 2002; Dybul et al., 2002). Activating T cells via the CD3 receptor in combination with IL-2 ended with near-catastrophic results – the three treated patients developed a cytokine storm syndrome, with one experiencing seizures and renal failure (Prins et al., 1999). A subsequent trial with more cautious dosing and treatment times proved unsuccessful (Kulkosky et al., 2002).

Following the disappointment of these early clinical trials, research continued to elaborate the conditions needed for the efficient elimination of the proviral reservoir, by developing small-molecule compounds capable of targeting the provirus more specifically. The reactivation of viral transcription has now been under investigation for two decades (Chun et al., 1998), yet a number of obstacles still obstruct the application of the "shock and kill" strategy. Here we will briefly discuss the current state of this experimental therapy and propose approaches for improving its efficacy.

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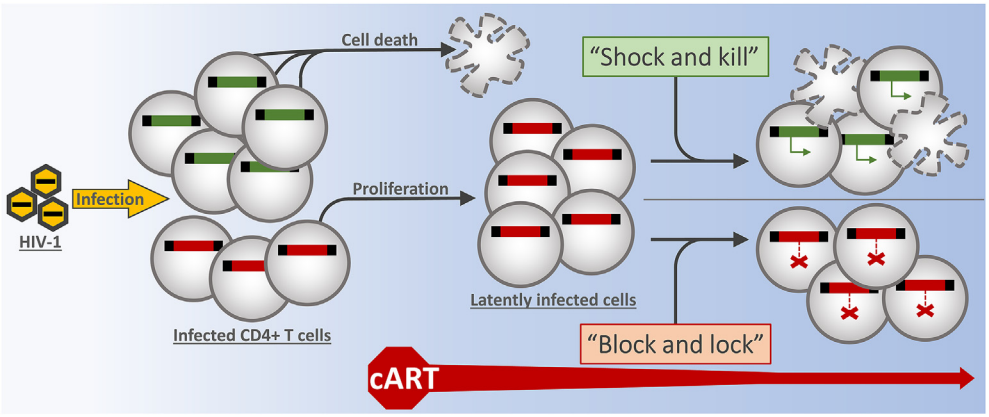

Fig. 1. Visualization of "shock and kill" and "block and lock" approaches. Following an infection event, the majority of infected CD4$^+$ T cells die, but a minor subset survives, harboring latent proviruses. The clonal expansion of the provirus is driven by the proliferation of these latently infected cells. The curative "shock and kill" strategy aims to decrease the functional HIV reservoir by chemically reactivating proviral transcription, leading to the elimination of infected cells via immune clearance and HIV-cytolysis. Alternatively, persistent "block and lock" therapy is projected to fully inhibit the proviral expression and the drive the infection into a state of deep latency. Both the of the depicted therapies are considered to function in concert with existing combinatory antiretroviral therapy (cART).

2. HIV latency and transcription

The majority of HIV-infected CD4$^+$ T cells die rapidly in response to an efficient infection, yet a significant number of latently infected cells survive. Older mathematical predictions suggested that a latent reservoir had a half-life of approximately 44 months (Finzi et al., 1999), while modern approximations of the half-life of viral DNA within a physiological setting estimate it to be around 12 years (Golob et al., 2018). Moreover, more than 99% of infected cells are predicted to accommodate clonal viral sequences in patients receiving cART, implying that the virus survives through rounds of host cell proliferation (Reeves et al., 2018), thus prolonging the elimination of these cells and viral decay to a life-long process.

Following an infection event, T cells may revert to a resting state, depriving the virus of the necessary protein elements and conditions for replication (reviewed in Mbonye and Karn, 2017). Upon regressing to the resting state, these T cells will harbor the latent proviral reservoir within various tissues (reviewed in Archin et al., 2014a,b). The establishment of proviral latency is further supported by the location of viral integration. While the bulk of provirus integrates into transcriptionally beneficial genomic loci, where host transcription factors and RNA polymerase II would be abundantly available, on rare occasions the viral genome is incorporated into sites that suppress efficient viral expression. Specifically, integration in the vicinity of a highly potent promoter or in opposing orientation of a competitive host gene, in heterochromatic or intergenic regions, or in a gene desert, will diminish the chance of viral replication (reviewed in Lusic and Siliciano, 2017). Due to the lack of active viral transcription, latently infected cells are incapable of expressing viral antigens necessary for innate and adaptive immune responses, thus rendering them imperceptible for clearance.

On a molecular level, host-dependent mechanisms play a decisive role in proviral transcription. For example, the NF-κB heterodimer is only mobilized to the nucleus following extracellular proinflammatory signals and T cell activation, while the lack of NF-κB supports the low incidence of viral expression in resting memory CD4$^+$ T cells. Repressive chromatin structure is additionally known to drastically affect viral transcription, since HIV-1 provirus is highly dependent on its own nucleosomal structure. During the integration process, HIV-1 establishes a strict nucleosomal conformation at the 5' long terminal repeat (LTR), with Nuc-0 residing at the start of the genome and Nuc-1 being localized immediately after the transcription start site (TSS) (Verdin et al., 1993). In doing so, the virus creates a ~250 bp -long nucleosome-free zone that can be accessed by chromatin modifiers and transcription factors, thus facilitating a dominant expression witnessed in active viruses. In the case of latency, access to the LTR becomes restricted by repressive transcription factors and histone deacetylates (HDAC), DNA or histone tail methylations or by sterically compact chromatin structure. For example, the suppressive class I HDACs are known to localize on the transcription factors on the NF-κB, Sp1, AP-4 and LSF motifs around the TSS (reviewed in Hakre et al., 2012). Bromodomain proteins, such as Brd4 and Brd2, have also been demonstrated to inhibit viral transcription by masking the already acetylated lysines on Nuc-0, Nuc-1 and NF-κB, while the chromatin remodeling BAF complex immobilizes Nuc-1 near the TSS (reviewed in Boehm et al., 2013a,b).

3. Not enough punch in the "shock"

Latently infected CD4$^+$ T cells are quiescent by nature and are therefore deficient in the cytosolic components and transcription factors required for efficient viral reactivation. Resting CD4$^+$ T cells are known to present inactive, sequestered or lowly abundant proteins needed for both cellular and viral activation, such as cyclin-T1, NFAT and NF-κB (Coiras et al., 2009; Chiang et al., 2012). Moreover, the chromatin state in T cells during quiescent stages of its life-cycle has been described to be exceedingly heterochromatic, further debilitating the access of effector molecules to the latent proviral loci (Wood and Bickmore, 2011). Research into "shock and kill" therapy has predominantly concentrated on developing "shocking" compounds, termed latency-reversing agents (LRAs). To date, all of them function by targeting host-dependent mechanisms (Fig. 2).

The mechanisms of action of LRAs within the host cell can be divided into six primary categories:

- Histone post-translational modification modulators: These compounds function by modulating histone tail modifications within the nucleosomes of the integrated HIV-1 genome, thus affecting proviral sensitivity for reactivation. This LRA class primarily includes histone methyltransferase (HMT) and HDAC inhibitors, but recently unique compounds targeting rare histone modifications have been described. Notable examples include vorinostat, romidepsin, DZNep, Na-Cro and MG-149.
- Non-histone chromatin modulators: This functionally broad group of agents is capable of modulating the presence and behavior of alternative elements present in the chromatin, such as transcription factors other than NF-κB, DNA methylation and various functional proteins. Notable examples include JQ1, OTX-015, BAF inhibitors, 5-aza-2′-deoxycytidine, AV6 and HODHBt.
- NF-κB stimulators: This group predominantly consists of protein kinase C (PKC) pathway agonists, which lead to the transcription factor NF-κB activation, resulting in its nuclear localization and HIV-1 reactivation. These compounds are considered the most robust

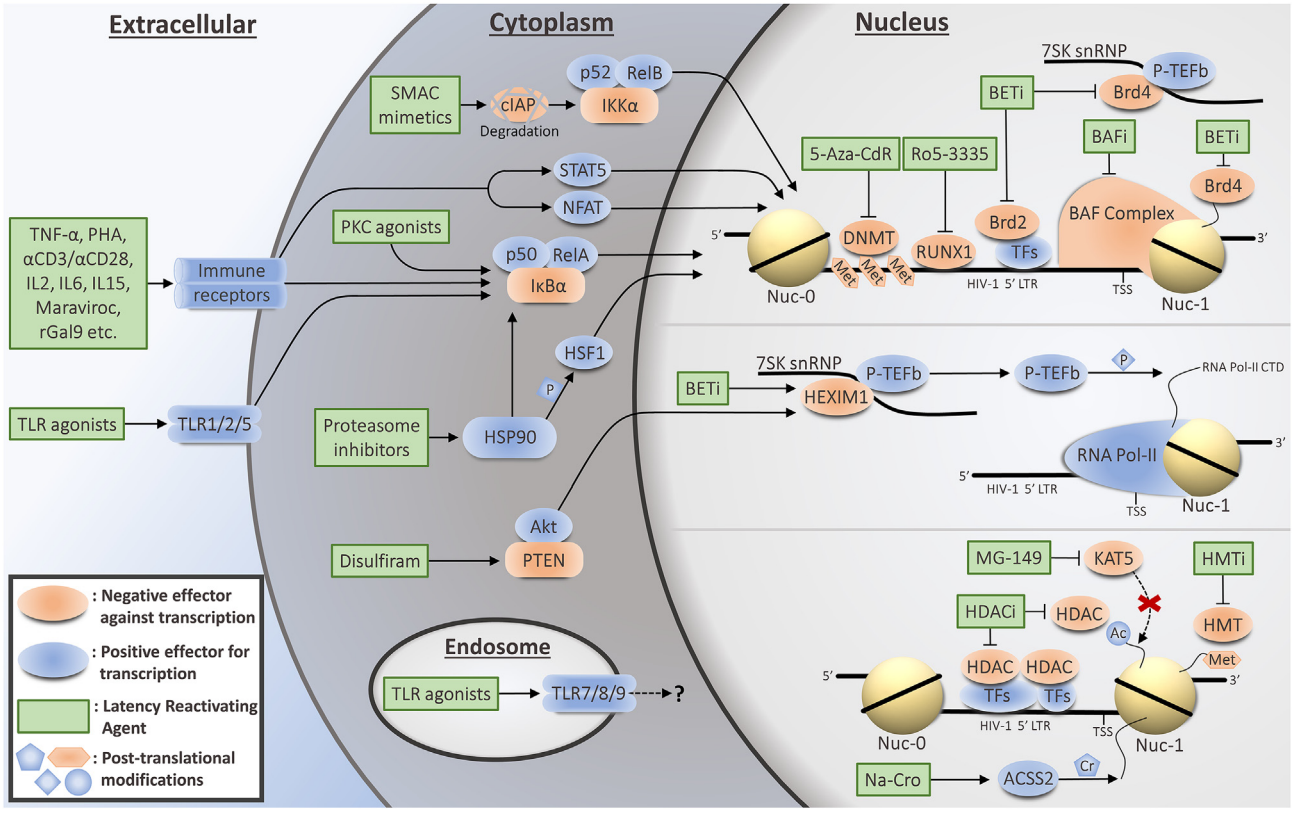

Fig. 2. Mechanisms of action of different classes of LRAs (extracellular and cytosolic). The most potent signal transduction for HIV reactivation is carried out by signaling through various immune receptors present on the infected cell surface. Among other signal transduction pathways, their stimulation canonically leads to the activation of NF-κB, STAT5 and NFAT transcription factors (Micheva-Viteva et al., 2011), which localize to the cell nucleus and participate directly in HIV reactivation. Certain membrane TLR agonists, SMAC mimetics and PKC agonists activate NF-κB as well, while the exact mechanism of action of endosomal TLR still remains to be confirmed. Within the cytosol, proteasome inhibitors induce the accumulation of HSP90, which in turn activates various transcription factors. Disulfiram reduces PTEN protein levels, allowing the activated Akt kinase to localizes to the nucleus, where it participates in releasing P-TEFb from the repressive 7SK snRNP complex. (Middle right) Intranuclearly, the release of P-TEFb complex can additionally be induced BETis. The released P-TEFb kinase assists in the regulation of RNA Pol-II dependent elongation. (Upper right) Amongst non-histone modifying mechanisms, reducing DNA methylation in the LTR or the eviction of repressive transcription factors like RUNX1 is known to result in HIV reactivation. The repressive bromodomain proteins Brd2 and Brd4 can be evicted by the use of BETis. Finally, inhibition of the BAF complex leads to its displacement from the LTR, thus allowing the release of Nuc-1. (Lower right) Proviral reactivation can also be induced by manipulating the post-translational modifications of the core histone tails via HDACis, HMTis and Na-Cro. HIV transcription can further be induced by inhibiting KAT5, which is required for H4-acetylation and Brd4 binding. TLR, Toll-like receptor; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; HSP90, Heat shock protein 90; HSF1, Heat shock factor 1; NFAT, Nuclear factor of activated T-cells; STAT5, Signal transducer and activator of transcription 5, SMACm, Second mitochondrial-derived activator of caspases mimetic; Akt, protein kinase B; PTEN, Phosphatase and tensin homolog; HEXIM1, Hexamethylene bisacetamide inducible 1; P-TEFb, Positive transcription elongation factor b; 7SK snRNP, 7SK small nuclear ribonucleoprotein complex; CTD, C-terminal domain; Brd, Bromodomain containing protein; DNMT, DNA methyltransferase; RUNX1, Runt-related transcription factor 1; BETi, Bromodomain and extraterminal domain inhibitor; BAF, BRG1-or HBRM-associated factors; 5-Aza-CdR, 5-aza-2′-deoxycytidine; TFs, transcription factors; HDAC, histone deacetylase; KAT, Lysine acetyltransferase; HMT, histone methyltransferase; Nuc, nucleosome; Pol-II, Polymerase II; KAT5, Lysine acetyltransferase 5; TSS, transcription start site; P, phosphorylation; Ac, acetylation; Cr, crotonylation; Met, methylation.

amongst LRAs, yet they are also capable of causing a life-threatening systemic inflammatory response. Notable examples include PMA, prostratin derivatives, ingenols and SMAC mimetics.

- TLR agonists: Toll-like receptor (TLR) agonists function by stimulating the TLR-based innate immune system, which can reactivate proviral transcription through a variety of pathways. These LRAs can include agonists for both the RNA-sensing or other TLR receptors. Importantly, this class of compounds is the most capable of priming the immune system to target the infected cells. Notable examples include CL413, GS-986, GS-9840, 3M-002 and MGN-1703.
- Extracellular stimulators: This category includes all compounds and biomolecules, which exert their effect typically via extracellular receptors. Due to their upstream positioning, the stimulated intracellular molecular pathways leading to viral reactivation may vary considerably. Notable examples include TNF-α, PHA, cytokines and antibodies and rGal9.
- Miscellaneous: The final category consists of uncommon compounds, which function by modulating unique cellular mechanisms or have an unknown/unconfirmed mechanism of action. Notable examples include disulfiram, BMS-936559 and Ixazomib.

The majority of LRAs were originally not described for their antiviral properties, but have rather been adopted from other scientific fields, notably from oncology. Due to the known molecular mechanisms of these compounds and the cancer model-based methodology in HIV-1 latency research, these agents have indeed proven to be invaluable in understanding proviral repression and have catalyzed progress in fundamental HIV-1 research. In Table 1 we have summarized a list of more than 160 different published LRAs, which have been classified based on the six primary categories listed above, together with the target proteins or mechanisms of action.

Clinical trials testing the feasibility of the “shock and kill” therapy have been in progress since 2012, when a single dose of the FDA-approved HDAC inhibitor (HDACi) vorinostat was demonstrated to induce

Table 1
LRAs described in literature to date. The LRAs have been categorized into six different primary classes according to their mechanisms of action. Their specific or approximated protein targets involved in viral reactivation have been listed in the second column, the third column lists the specific agents and the final column lists the references, where the specific compounds were first described or shown to function as a LRAs.

Class	Function	Compounds	References
Histone post-translational modification modulators	ACSS2 agonist	Sodium crotonate (Na-Cro)	Jiang et al. (2018)
	HMT (SMYD2 inhibitor)	AZ391	Boehm et al. (2017)
	HMT (G9a inhibitors)	BIX-01294	Imai et al. (2010)
		UNC-0638	Nguyen et al. (2017)
	KAT5 inhibitor	MG-149	Li et al. (2018)
	HDAC (Class I) inhibitors	CG05; CG06	Choi et al. (2010)
		Thiophenyl benzamide (TPB)	Huang et al. (2018)
		Chidamide	Yang et al. (2017)
		Entinostat (MS-275)	Wightman et al. (2013)
		Largazoles (SDL148; JMF1080; SDL256)	Albert et al. (2017)
		Mocetinostat	Zaikos et al. (2018)
		Romidepsin	Wei et al. (2014)
		Pimelic diphenylamide 106	Kobayashi et al. (2017)
		Pyroxamide	Macedo et al. (2018)
		Tacedinaline	Zaikos et al. (2018)
	HDAC3/6 inhibitor	Apicidin	Lin et al. (2011)
	HDAC3/6/8 inhibitor	Droxinostat	Barton et al. (2014)
	HDAC (Pan) inhibitors	4-iodoSAHA; AR-42; CBHA; MD85	Macedo et al. (2018)
		Belinostat (PXD101)	Matalon et al. (2011)
		Givinostat (ITF2357)	Matalon et al. (2010)
		KD5170, Pracinostat (SB939)	Kobayashi et al. (2017)
		M344	Ying et al. (2012)
		Metacept-1; Metacept-2; Oxamflatin	Shehu-Xhilaga et al. (2009)
		Panobinostat (LBH589)	Bullen et al. (2014)
		Psammaplin A	Richard et al. (2018)
		Scrpitaid	Ying et al. (2010)
		Sodium butyrate (Na-But)	Bohan et al. (1987)
		ST7612AA1	Badia et al. (2015)
		Suberic bishydroxamate (SBHA)	Ai et al. (2011)
		Trichostatin A (TSA); Trapoxin A (TPX)	Van Lint et al. (1996)
		Valproic Acid (VPA)	Lehrman et al. (2005)
		Vorinostat (SAHA)	Contreras et al. (2009)
	Polycomb (L3MBTL1 inhibitor)	UNC-926	Boehm et al. (2017)
	Polycomb (SUV39H1 inhibitor)	Chaetocin	Bernhard et al. (2011)
	Polycomb (EZH1/EZH2 inhibitor)	UNC-1999	Kobayashi et al. (2017)
	Polycomb (EZH2 inhibitors)	3-deazaneplanocin A (DZNep)	Friedman et al. (2011)
		EPZ-6438; GSK-343	Nguyen et al. (2017)
	SIRT2 inhibitor	AGK2	Kobayashi et al. (2017)
NF-κB stimulators	SMAC mimetics/ cIAP1 inhibitors	CAPE; MGD-486; Pyrimethamine	Stoszko et al. (2016)
		Macrolactams	Marian et al. (2018)
	Pin 1 inhibitor	Juglone (5HN)	Yang et al. (2009)
	PKC agonists	12-deoxyphorbol 13-phenylacetate (DPP)	Bocklandt et al. (2003)
		12-O-tetradecanoylphorbol-13-acetate (TPA)	Harada et al. (1986)
		3-(2-Naphthoyl)ingenol	Liu et al. (2018)
		Aplysiatoxin; Debromoaplysiatoxin	Richard et al. (2018)
		Bryologs	Marsden et al. (2018)
		Bryostatin-1	Vlach and Pitha (1992)
		C3-esterified ingenol derivatives	Spivak et al. (2018)
		EK-16A	Wang et al. (2017)
		Euphoria Kansui extract	Cary et al. (2016)
		Gnidimacrin	Huang et al. (2011); Lai et al. (2015)
		IDB (ingenol 3, 20-dibenzoate)	Spivak et al. (2015)
		Ingenol-B (ingenol-3-hexanoate)	Jiang et al. (2014); Pandeló José et al., 2014
		LMC03; LMC07	Hamer et al. (2003)
		Namushen-1; Namushen-2	Tietjen et al. (2018)
		PEP005 (ingenol-3-angelate)	Warrilow et al. (2006); Jiang et al. (2015)
		Phorbol 12-myristate 13-acetate (PMA)	Folks et al. (1988)
		Prostratin	Kulkosky et al. (2001)
		Sesterterpenoids	Wang et al. (2016)
		SJ23B	Bedoya et al. (2009)

(*continued on next page*)

Table 1 (*continued*)

Class	Function	Compounds	References
Non-histone chromatin modulators	BAF inhibitors	CAPE; MGD-486; Pyrimethamine	Stoszko et al. (2016)
	BAF (ARID1A inhibitor)	Macrolactams	Marian et al. (2018)
	Brd4 inhibitors	8-methoxy-6-methylquinolin-4-ol (MMQO)	Gallastegui et al. (2012); Abner et al. (2018)
		Apabetalone (RVX-208)	Lu et al. (2017); Zhang et al. (2018)
		BI-2536, BI-6727	Gohda et al. (2018)
		Hexamethylene bisacetamide (HMBA)	Nilsson et al. (2016)
		I-BET; I-BET-151; MS-417	Boehm et al., 2013a,b
		JQ1	Banerjee et al. (2012); Zhu et al. (2012)
		OTX-015; PFI-1	Lu et al. (2016)
		UMB-136	Huang et al. (2017)
	DNMT inhibitors	Decitabine (5-aza-2′-deoxycytidine)	Kauder et al. (2009)
		Zebularine	Blazkova et al. (2009)
	HSF-1 stimulators	Resveratrol; Triacetyl resveratrol	Pan et al., 2016a,b; Zeng et al. (2017)
	NFAT pathway activator	AV6	Micheva-Viteva et al. (2011)
	RUNX1 inhibitor	Ro5-3335	Klase et al. (2014)
	STAT5 sumoylation inhibitors	Benzotriazoles (HODHBt, HBt, HOBt, HOAt)	Bosque et al. (2017)
TLR agonists	TLR1/2 agonists	Pam3CSK4; Imiquimod	Novis et al. (2013)
	TLR2 agonists	HKLM	Alvarez-Carbonell et al. (2017)
		Pam2CSK4	Macedo et al. (2018)
		PIM6	Rodriguez et al. (2013)
	TLR2/7 agonists	CL413	Macedo et al. (2018)
	TLR5 agonist	Flagellin	Thibault et al. (2009)
	TLR7/8 agonist	R-848	Schlaepfer et al. (2006)
	TLR7-Denitric-IFNg-axis stimulators	GS-9620	Tsai et al. (2017)
		GS-986	Lim et al. (2018)
	TLR8 agonist	3M-002	Rochat et al. (2017);
	TLR9 agonists	CPG-7909	Winckelmann et al. (2013)
		MGN1703	Offersen et al. (2016)
		ODN-2006; ODN-2040	Scheller et al. (2004)
Extracellular receptor binding	CCR5	Maraviroc	López-Huertas et al., 2017; Madrid-Elena et al., 2018
	CD122/CD132	ALT-803 (IL-15 superagonist complex)	Jones et al. (2016)
		IL-2	Stellbrink et al. (2002)
	CD126	IL-6	Nakajima et al. (1989)
	CD28	αCD28	Tong-Starkesen et al. (1989)
	Cell surface glycans	rGal9 (recombinant Gal 9)	Abdel-Mohsen et al. (2016)
	EGFR inhibitor	AG555	Calvanese et al. (2013)
	Surface glycoproteins	Phytohemagglutinin (PHA)	Spina et al. (2013)
	TCR agonist	αCD3	Tong-Starkesen et al. (1989)
	TNF Receptor agonist	TNFα	Vlach and Pitha (1992)
	αCTLA4 antibody	Ipilimumab	Wightman et al. (2015)
	αPD1 antibodies	Nivolumab; Pembrolizumab	Evans et al. (2018)
Miscellaneous	Adenosine reuptake inhibitor	Dilazep	Calvanese et al. (2013)
	BTK inhibitor	Terreic acid	Calvanese et al. (2013)
	Calcineurin agonist	Ionomycin	Siekevitz et al. (1987)
	Farnesyltransferases	Farnesyltransferase inhibitors	Barnard et al., 2013 (oral presentation)
	GADD34/PP1 inhibitor	Salubrinal	Pan et al., 2016a,b
	GSK3 inhibitors	SB-216763; Tideglusib	Gramatica et al., 2017 (oral presentation)
	Heme oxygenase-1 agonist	Heme arginate	Shankaran et al. (2011)
	PI3K agonist	Oxoglaucine (57704)	Doyon et al. (2014)
	PKA agonist	Bucladesine (dibutyryl-cAMP)	Lim et al. (2017)
	Proteasome inhibitors	Carfilzomib	Pan et al., 2016a,b
		Ixazomib	Cummins et al., 2017
		MG-132 (ONX-0914); Bortezomib; CLBL	Miller et al. (2013)
		PR-957	Lin et al. (2018)
	PTEN dysregulation	Disulfiram (Antabuse)	Xing et al. (2011)
	RNR inhibitor	Hydroxyurea	Oguariri et al. (2007)
	SRC agonist	MCB-613	Nikolai and Elbezanti, 2017 (oral presentation)
	αPDL1 antibody	BMS-936559	Gay et al. (2017)
	Unknown	Abyssomicin-2	Leon et al. (2015)
		HHODC	Kapewangolo et al. (2017)
		Piceatannol	Calvanese et al. (2013)
		PH01; PH02; PH03; PH04; PH05	Hashemi et al. (2018)
		Quinolin-8-ol derivatives	Xing et al. (2012)
		Radicicol; Pochonin B; Pochonin C	Mejia et al. (2014)
		Xanax (Alprazolam)	Elbezanti et al. (2017) (oral presentation)

Reported HIV reactivating compounds listed according to mechanisms of action.

moderate production of viral RNA *in vivo* (Archin et al., 2012). Subsequent trials with longer vorinostat regimens confirmed the initial findings of an increase of HIV-1 cell-associated unspliced (CA-US) RNA (Archin et al., 2014a,b, 2017). The use of romidepsin and panobinostat, FDA-approved HDACis that are considered more potent and selective than vorinostat, did indeed lead to an increase of proviral reactivation and to detectable levels of HIV-1 RNA in plasma (Rasmussen et al., 2014; Søgaard et al., 2015). However, HDACis are known to cause

numerous side-effects at higher doses and are generally considered weak LRAs, since they primarily facilitate proviral elongation, rather than potentiating it.

Due to the host-targeting reactivity of the potent HIV-1 LRAs, considerable caution must be taken to avoid unnecessary toxicity to subjects. A dose-response clinical trial using low doses of the PKC agonist (PKCa) bryostatin-1 failed to induce any effect, either on the PKC pathway, nor on viral reactivation, probably because of the precautionary dosage used (Gutiérrez et al., 2016). As an alternative to PKCas, disulfiram is a well-tolerated drug to treat chronic alcoholism, and was found to reactivate HIV-1 transcription through PI3K/Akt pathway stimulation (Xing et al., 2011; Doyon et al., 2013). Analogously to the HDACis, disulfiram was able to induce both the production of CA-US RNA and plasma HIV-1 RNA, yet did not affect the total proviral DNA within patients (Elliott et al., 2015).

Additional clinical trials now under way include those with an HDACi (NCT02961829), an HDACi-interferon-α combination (NCT02471430), HDACi combination therapies with HIV-targeting vaccines and neutralizing antibodies (NCT03041012, NCT03619278, NCT02336074), an HDACi combination with BAF complex inhibition (NCT03525730), a PKCa (NCT02531295), TLR agonists (NCT02858401, NCT03060447), a proteasome inhibitor (NCT02946047) and a synthetic IL-15 (NCT02191098).

4. The need for unique HIV-reactivating mechanisms

The principal intention for all LRAs is to specifically reactivate proviral transcription without adversely affecting cellular homeostasis, which is where the main classes of compounds fall short. The use of the most common chromatin-modifying drugs, such as HDACis and bromodomain and extra terminal domain inhibitors (BETis), results in redundant global changes to the transcriptome, while conventional NF-κB inducing agents can often stimulate broad scale cytokine production and adverse immune activity. To that end, the development of compounds targeting poorly-studied mechanisms which target HIV-1 reactivation via unique and potentially less damaging cellular mechanisms should be prioritized. Here we will highlight various alternative transcription factors, molecular pathways or chromatin targeting compounds that could serve as more unique tools to target HIV-1.

The activation of the non-canonical NF-κB pathway via the SMAC mimetic (SMACm) class of compounds class has surfaced as an attractive mechanism to stimulate HIV-1 reactivation. This pathway results in the formation of the RelB-p52 protein NF-κB complex, which functions as a transcription factor controlled by diverse extracellular stimuli. SMACms suppress IAP family proteins, which in turn function as upstream negative regulators of non-canonical IKKα-dependent NF-κB activation. Inhibition of cIAP1 and cIAP2 proteins leads to their degradation, thereby facilitating positive regulation of the pathway (reviewed in Bai et al., 2014). SMACms have already proven being well-tolerated *in vivo* and are at the moment undergoing numerous phase I and II clinical trials against cancers and hepatitis B virus (Rathore et al., 2017). In the context of HIV-1 latency, the reactivation of this non-canonical NF-κB pathway has been demonstrated to lead to robust HIV-1 reactivation, both *in vitro* and *ex vivo*, while further exhibiting synergistic viral reactivation with HDACis (Pache et al., 2015). Crucially, the provirus reactivating SMACm AZD5582 displayed only a fraction of T cell activation and transcriptome dysregulation, when compared to other PKCas (Sampey et al., 2018, bioRxiv preprint). Following a 24-h treatment, the compound caused the dysregulation of only approximately 400 genes, as opposed to the approximately 2300 dysregulated genes caused by PKC pathway induction. Furthermore, AZD5582 failed to induce the production of the T cell activation marker CD69, even in micromolar concentrations, highlighting it as an extremely interesting candidate as a well-tolerated and potent LRA.

Likewise, novel chromatin modulators have been implicated in HIV-1 reactivation. MG-149, an inhibitor against lysine acetyltransferase 5 (KAT5), has emerged as a promising LRA, since it decreases the H4 acetylation needed for the binding of the repressive Brd4 protein on the viral promoter (Li et al., 2018). It is worth noting that selective inhibitors countering other KAT-family members had no effect on viral transcription. Importantly, the inhibition of KAT5 synergized with the BETi JQ1 in primary $CD4^+$ T cell latency models and in cells derived from patients undergoing cART. As another uncommon option for a chromatin modifying LRA, the crotonylation inducer sodium crotonate (Na-Cro) was shown to increase H3K4 acetylation and crotonylation, while decreasing H3K27 tri-methylation in the LTR, thus boosting viral reactivation. In addition, Na-Cro synergized with HDACis and PKCas *ex vivo* in $CD4^+$ T cells from patients undergoing cART, highlighting crotonylation as a potential histone modification for therapy (Jiang et al., 2018). However, it should be mentioned that histone crotonylation currently remains a poorly studied post-translational modification and the selective agents required to target this phenotype still need to be developed.

As in a normally functioning chromatin, hypermethylation of the promoter region in HIV-1 leads to transcriptional repression, while demethylation of proviral sequences by 5-aza-2′deoxycytidine (5-Aza-CdR) leads to proviral reactivation (Kauder et al., 2009). The Ubiquitin-like PHD and ring finger domain-containing protein 1 (UHRF1) protein canonically functions by recruiting DNA methyltransferase 1 (DNMT1) to DNA loci, thereby facilitating hypermethylation. The negative regulatory effect of UHRF1 towards HIV-1 has been implicated before (Victoriano and Okamoto, 2012), while more recent work has demonstrated UHRF1 to be a methylation-promoting element in HIV-1 latency, which could be shut off by the DNA methylation inhibitor 5-Aza-CdR (Bouchat et al., 2017). Importantly, the knock-down of UHRF1 resulted in the demethylation of canonically methylated nucleotides of a latent promoter, suggesting that this protein is one of the main regulators of HIV-1 DNA hypermethylation. It should be noted that UHRF1 is also considered a potential therapeutic target in oncology studies (Castillo-Aguilera et al., 2017), in which a few UHRF1-dysregulating compounds have already been developed. Hinokitiol, a naturally derived compound, has been shown to downregulate the expression of both DNMT1 and UHRF1, thus facilitating the apoptosis and cell-cycle arrest of colon cancer cells (Seo et al., 2017). Clinically, the DNTM1-UHRF1-interaction inhibitor NSC232003 might be a more tolerable LRA, due to its improved mechanistic properties and higher hypermethylation restricting ability (Myrianthopoulos et al., 2016). However, the compound might still possess unwanted affinities because of its simplistic chemical structure, and it must be further optimized before use in HIV-1 research. Furthermore, inhibition of methyltransferases has recently been proposed to lead to genomic instability (Avgustinova et al., 2018), therefore the transfer of HMTis to retroviral biology should be applied with precaution.

Another fairly under-studied element of HIV-1 latency is the role of the RUNX1-CBFβ complex, which is a heterodimeric transcription factor best known for its role as a regulator of hematopoietic stem cell differentiation. However, in the HIV-1 proviral setting, the RUNX1-CBFβ complex has been shown to interact with the *Tat* protein, thus restricting the pro-transcriptional effect of the viral protein (Cunningham et al., 2012). Both the alternative splicing of RUNX1 by long noncoding RNAs and chemical inhibition of RUNX1 by Ro5-3335, especially in combination with vorinostat, have been shown to induce proviral transcription from patient peripheral blood mononuclear cells (PBMCs) (Klase et al., 2014; Huan et al., 2018). Consequently, the use of specific RUNX1 antagonists, and possibly inhibitors against its heterodimeric partner CBFβ (Illendula et al., 2016), could prove to be a valid strategy for viral reactivation.

Finally, the histone chaperones ASF1 and HIRA have been described as negative factors of HIV-1 elongation (Gallastegui et al., 2011). Biologically, these chaperones are crucial for the histone biosynthesis pathway, in which a phosphorylated ASF1 is necessary for the delivery of core histone dimers to the chromatin and the downstream HIRA

complex deposits them onto the DNA (reviewed in Hammond et al., 2017). Recent progress in the development of numerous chemical inhibitors against ASF1 could prove to be a useful resource to rescue the provirus from latency, while further helping to elucidate the function of histone chaperones in latency establishment and maintenance (Miknis et al., 2015; Seol et al., 2015). Additionally, Tousled-like kinases (TLKs), the upstream kinases responsible for ASF1 phosphorylation, show promise for potent drug design and are considered to be conceivable targets in cancer therapy (Lee et al., 2018; Mortuza et al., 2018). Interestingly, TLKs have been shown to modulate Kaposi's sarcoma-associated herpesvirus (HSPV) and Epstein-Barr virus (EBV) latencies, highlighting the potential for TLK-specific inhibitors in virology (Dillon et al., 2013). It should be mentioned that both EBV and HSPV generally respond to similar classes of LRAs as HIV-1, so that a benefit of TLK inhibitors against all three viruses remains plausible.

5. Individual agents might not be cut out for the job

In spite of progress in developing novel anti-HIV agents, evidence against the efficacy of LRAs is slowly accumulating. Single-cell RNA sequencing of latent $CD4^+$ cells has identified two hypothetical cell classes within an infected patient: an inducible group, which responds to LRAs, and a deeply repressed class, comprised of poorly responsive cells (Rato et al., 2017). Furthermore, recent critical *in vitro* studies suggest that single LRAs will likely not be capable of targeting the entire latent proviral reservoir. Experiments utilizing a dual-label reporter virus have demonstrated that only a minor fraction of latently infected $CD4^+$ cells responded to LRAs (Battivelli et al., 2018), causing concern for the application of the “shock and kill” concept. The authors hypothesized that specific LRAs might not be able to function in distinct repressive genomic conditions, for example within enhancer regions, lamin-associated domains or on proviruses carrying unsuitable chromatin modifications.

Progress in high-throughput biology has given rise to exciting new technologies, which could improve our understanding of genome-wide latent responsiveness in reaction to an LRA, such as the impressive B-HIVE methodology (Chen et al., 2018). The “Barcoded HIV Ensembles” (B-HIVE) method consists of adding a randomized 20 nucleotide long sequence (barcode) to a recombinant HIV-1 retroviral vector, assigning each infection event a unique identification tag. This barcode can then be used to localize different integration events and correlate their expression characteristics by DNA/RNA sequencing or other similar high-throughput technologies. Utilizing B-HIVE, it has been demonstrated that the LRAs prostratin, vorinostat, MMQO, JQ1 and PHA each reactivate proviruses from distinct, unique loci. This observation affirms that no single class of compounds will be likely to provoke the total viral reservoir within a patient (Chen et al., 2017; Abner et al., 2018; Marian et al., 2018).

6. Combinatory shocks

In order to overcome the deficiencies of single LRAs, the only possibility for a thorough reactivation of all proviruses within the framework of “shock and kill” therapy might be the use of a “shocktail” – a specific combination of LRAs, which function in a highly synergistic manner in reactivating HIV, with a maximally broad effect on diverse groups of latent cells, incorporating varying types of integration loci. Crucially, a shocktail must function in resting T cells, while causing minimal side-effects for the patient (Darcis et al., 2016).

The ideal combinations for proviral reactivation will have to be considered carefully, because only compounds with markedly differing mechanisms are capable of synergizing, e.g., class I HDACis with PKCas (Zaikos et al., 2018). The HDACi/PKCa-axis supports both the loosening of the restricted chromatin state and simultaneous stimulatory signaling for T cell activation, thus facilitating a notable increase in viral RNA production. Importantly, extensive responsiveness analyses using common viral latency cell models have demonstrated that PKCas are uniformly the most potential group of LRAs (Spina et al., 2013). PKCas have also been demonstrated to be unique in their role of viral transcription throughout the different later stages of transcription, by dramatically increasing the production of completed and spliced viral transcripts (Yukl et al., 2018). On the other hand, HDAC inhibitors are among the most promiscuously synergizing group of compounds, due to their broad effect on different classes of HDAC proteins. To date, over 30 individual HDAC inhibitors have been shown to increase HIV-1 promoter activity, while keeping the activation of resting T cells at a minimal level. However, the broad mechanism of action of these compounds produces another complication – a general effect on transcription of non-target genes, potentially causing a multitude of side-effects (reviewed in (Wightman et al., 2012)). As opposed to the non-specific pan-HDACis, class-I-specific HDACis have been shown to target HIV-1 promoter with higher precision and without disrupting the activity of PKCas (Albert et al., 2017; Zaikos et al., 2018). Therefore, the use of class I HDACis should be preferred over pan-HDACis, due the lower disruption rate of the global chromatin state, which conceivably would decrease the risk of side-effects.

As an alternative combination, Toll-like receptor agonists (TLRas) deployed together with bromodomain-inhibiting compounds could prove to be an effective option (Darcis et al., 2015; Jiang et al., 2015). Agonists against TLR1, TLR2, TLR7, TLR8 and TLR9 are known to reactivate HIV-1 transcription in *ex vivo* conditions (Schlaepfer et al., 2006; Schlaepfer and Speck, 2011; Novis et al., 2013; Offersen et al., 2016; Macedo et al., 2018). While the direct stimulation of T cells via TLR1, TLR2, TLR5 and TLR9 has been shown to induce viral production in T cell based latency models (Scheller et al., 2004; Thibault et al., 2009; Rodriguez et al., 2013; Novis et al., 2013), HIV-1 reactivation via the agonists for TLR7 and TLR8 have been shown to require additional immune cell types, such as dendritic cells (Schlaepfer et al., 2006; Rochat et al., 2017). However, priming innate antiviral immunity in patient PBMCs has been demonstrated to be advantageous, through the TLR9 agonist MGN1703 as well (Offersen et al., 2016; Vibholm et al., 2017). Even though the exact key players in TLR-mediated HIV-1 reactivation remain ambiguous to date, and will vary depending on the cell type and specific TLRs being stimulated, canonical TLR-signaling is mediated via the NF-κB pathway. Concurrently, the bromodomain and extra-terminal domain (BET) family member Brd4 is known to interact with NF-κB subunit RelA at KAc-310 and enhance its global transcriptional activity, while inhibition of this interaction by BET inhibitors has been demonstrated to decrease NF-κB dependent proinflammatory signaling (Huang et al., 2009; Brown et al., 2014). Therefore, the anti-inflammatory BETis could ameliorate the excessive adverse immune response, while crucially inducing Tat-independent viral reactivation by displacing the HIV-repressive Brd4 protein from the LTR (Laird et al., 2015; Abner et al., 2018; Khoury et al., 2018).

The transcriptional responsiveness of latent proviruses has been shown to vary considerably, and to be closely associated with widely heterogeneic transcriptomic profiles of latently infected host cells (Bradley et al., 2018). More specifically, it was established that transcriptional downregulation of the HIV-1 provirus was more prevalent within proliferative cells. Therefore, directing the host cells with divergent combinations capable of pressuring the cellular transcriptome towards functionally comparable profiles might prove to be a solid rationale. A novel group of inhibitors has been developed against the BAF subtype of the SWI/SNF-complex, a structure responsible of cell proliferation by mediating nucleosomal sliding in the chromatin and a repressive factor in case of HIV. These novel compounds displayed significantly improved viral reactivation at low doses in combination with HDACis/PKCas in primary cell models with tolerable levels of toxicity (Stoszko et al., 2016; Marian et al., 2018). It should be noted that the use of a shocktail comprising of three mechanistically differing components proved highly effective in reverting proviral latency, and could therefore hypothetically be implemented to impose

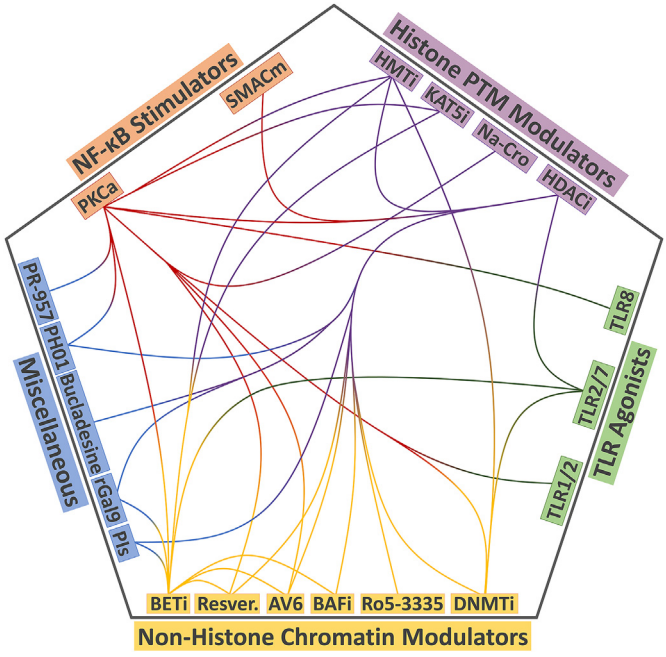

Fig. 3. Synergistic reactivation of HIV-1 between different categories of LRAs. Curved lines between the different classes of compounds highlight synergies that have been demonstrated either *in vitro/ex vivo* to date between LRAs. PTM, post-translational modification; Resver., Resveratrol; PIs, Protease inhibitors; DNMTi, DNA-methyltransferase inhibitors; BAFi, BRG1/BRM associated factor inhibitor; HMTi, histone methyltransferase inhibitors; PKCa, Protein kinase C agonists; SMACm, SMAC mimetics; Na-Cro, sodium crotonate; TLR, Toll-like receptor; BETi, Bromodomain and extraterminal domain inhibitor.

transcriptional clustering of host cells. A phase I clinical trial with the BAFi pyrimethamine and the HDACi valproic acid shocktail is currently under way (NCT03525730).

As an alternative to the simultaneous administration of two drugs in a shocktail, Bouchat et al. have demonstrated that sequential treatments via DNA methyltransferase inhibition and HDACis can reactivate HIV-1 transcription more potently, both *in vitro* and *ex vivo,* than the corresponding simultaneous treatments (Bouchat et al., 2016). It is important to note that these experiments were carried out in physiologically tolerable concentrations, utilizing clinically approved drugs. Therefore, an efficient "shock" will additionally have to take into consideration a treatment time variable for ideal results. Designing these potential shocktails could be helped along by recently developed mathematical models which determine optimal drug synergies for improved viral reactivation (Gupta and Dixit, 2018).

The variety of existing LRAs already allows for dozens of considerable combinations. In Fig. 3 we have depicted most of the known potential drug class synergies which have been demonstrated to increase viral reactivation *in vitro* and/or *ex vivo* to date.

7. Homing in for the "kill"

"Shock and kill" is a two-pronged approach, so when characterizing LRAs, we additionally must consider tackling the complications of the "kill" aspect. Clinical trials utilizing the HDACis panobinostat and romidepsin disrupt viral latency *in vivo*, but lack the capacity to reduce the number of latently infected cells (Rasmussen et al., 2014; Søgaard et al., 2015). Moreover, it has been suggested that HDACis might inhibit the functionality of cytotoxic T cells (Jones et al., 2014), while other LRAs, like disulfiram, might themselves ultimately protect the infected host cells from cell death by inducing the pro-survival Akt kinase activity (Doyon et al., 2013; Knights, 2017). In addition to evaluating the maximal viral expression as a result of shocktail regimens, combinatorial treatments must further be analyzed for their ability to decrease infected cell survivability.

Viral proteins such as *Nef, Tat* and *Vpr* are known to be capable of constraining pro-apoptotic mechanisms, thus debilitating infected cell death (reviewed in Timilsina and Gaur, 2016). This argument could be especially prominent in latently infected T cells, which are known to display atypical behavior due to an apparent exhausted state, expressing immune checkpoint receptors such as PD-1, LAG3 and TIGIT (Fromentin et al., 2016). Therefore, it has been proposed that "shock and kill" therapy should be supplemented with additional elements responsible for the removal of the cells harboring the latent reservoir, such as PI3K/Akt inhibitors, SMAC mimetics, immune checkpoint inhibitors, to name a few (Barouch and Deeks, 2014; Kim et al., 2018; Schwartz et al., 2017).

An encouraging strategy to deplete the viral reservoir lies in the induction of apoptosis, for example by inhibiting HIV-1 virion budding (Tateishi et al., 2017). Researchers have utilized the *Gag* protein inhibitor L-HIPPO to lock the virus into the infected cell, which eliminates secretion of new viral particles, leading to their accumulation and eventually to HIV-1-specific host-cell apoptosis. The induction of HIV-specific apoptosis could also be prompted by the use of proteasome inhibitors, such as the clinically approved chemotherapeutic ixazomib. In addition to processing viral proteins, the HIV-1 protease enzyme is additionally known to cleave the host procaspase-8 protein, resulting in the shorter a pro-apoptotic Casp8p41 variant (Nie et al., 2002). Casp8p41 is canonically degraded by the host proteasome mechanisms, a pathway which in turn can be switched off by ixazomib (Natesampillai et al., 2018). Excitingly, Casp8p41 is uniquely expressed in infected cells, rendering it a perfect target for the "shock and kill" therapy. This impressive proof-of-concept by study Cummins et al. further proved ixazomib to function as an NF-κB-stimulating LRA in Jurkat cell line-based latency models. Most importantly, ixazomib was demonstrated to induce preferential apoptosis in patient derived $CD4^+$ cells and decrease viral DNA load in *ex vivo* conditions. It therefore could be considered a potent candidate to diminish infected cell populations, and holds promise to be the first true dual-functioning "shock and kill" agent. Ixazomib is currently undergoing phase I clinical trials in HIV-1 patients receiving cART (NCT02946047).

Alternatively, apoptosis can be stimulated through the inhibition of the regulator protein B-cell lymphoma 2 (Bcl-2). Bcl-2 functions as an apoptosis signaling mediator and is known to play a critical role in various diseases, and is therefore considered an appealing target for therapy. It has been demonstrated that central memory $CD4^+$ cells from HIV-positive patients display elevated levels of Bcl-2, which sequesters Casp8p41, a process which in turn correlates with cell survival following an active infection event (Cummins et al., 2016). However, following CD3/CD28-based T-cell activation, the highly selective Bcl-2 inhibitor venetoclax was capable of mitigating this pro-survival effect. Importantly, in *ex vivo* conditions this treatment predominantly targeted the latently infected population and decreased drastically the proviral DNA level in eight out of eleven patients, while not affecting the viability of the total $CD4^+$ population. It should be noted that venetoclax is approved for the treatment of chronic lymphocytic leukemia and small lymphocytic lymphomas (Touzeau et al., 2018). However, because αCD3/αCD28-antibody therapy is not conceivable *in vivo* due to its toxicity, every class of LRAs should be considered beforehand to determine optimal proviral reactivation, while still retaining the functionality of venetoclax. For example, both HDACis and BETis have been demonstrated to downregulate the expression of Bcl-2 in different types of cancer, including lymphomas, and should therefore pass rigorous testing to prove their ability to preserve the "killing" mechanism achieved through Bcl-2 inhibition (Dickinson et al., 2010; Fiskus et al., 2014; Fu et al., 2015). A more feasible option could be the administration of a glycosylated recombinant IL-7 cytokine called CYT107, which has been demonstrated to induce the proliferation of circulating

$CD4^+$ and $CD8^+$ T cells in patients receiving ART, while also being well tolerated (Lévy et al., 2012). Importantly, the administration of IL-7 in combination with venetoclax has been shown to decrease the proliferation of the latent J-Lat 10.6 cell line (Cummins et al., 2017). However, the effectiveness of CYT107 in this context remains yet to be demonstrated in patient-derived cells.

Single LRA therapies have been scrutinized for the purpose of eliminating the proviral reservoir. To that end, PKCas appear to be among the most potent compounds, predominantly due to their immunomodulatory properties. Analysis of prostratin functionality on patient-derived NK cells has highlighted the ability of a PKCa to enhance NK-cell-mediated clearance of the proviral reservoir in autologous T cells (Desimio et al., 2018). Moreover, the development of highly specific agonists has shown promise for their effective use in "shock and kill". For example, various PKCas already display equally potent proviral reactivation in nanomolar or even picomolar concentrations, as does αCD3/αCD28-antibody treatment *in ex vivo* conditions (Richard et al., 2018; Spivak et al., 2018). A highly potent PKCa, gnidimacrin, which specifically targets the PKCβ isoforms, does not stimulate hypercytokinemia nor global T cell activation, yet retains potent LRA activity. More importantly, treatment of latent cells with gnidimacrin, especially in combination with HDACis, drastically reduced the viral DNA load from patient-derived PBMCs, suggesting that an immune reaction against the latent cells is triggered (Lai et al., 2015; Huang et al., 2018). Although PKC agonists are considered to provoke adverse effects due to excessive T cell activation, a selective and potent agent might achieve both potent proviral reactivation and immune system stimulation for the "killing" aspect.

To that end, TLR agonists are currently among the most intriguing immunomodulatory compounds, due to their central role in activating innate antiviral pathways. TLR1 and TLR2 are primarily expressed on cell surface of T cells, while TLR7, TLR8 and TLR9 receptors are highly expressed within endosomal compartments of plasmacytoid dendritic cells (pDCs), and their activation leads to an extensive type I interferon production. For instance, the dual TLR2/7 agonist CL413 has been shown to be a multifunctional agent by concurrently inducing NF-κB phosphorylation in infected $CD4^+$ memory cells and TNF-α production in pDCs and monocytes (Macedo et al., 2018). Similarly, the TLR7 agonist GS-9620 has been demonstrated to prime the immune system for antiviral action, by increasing the production of pDC-derived IFN-α. This interferon response resulted in the increase of $CD8^+$ T cell cytolytic activity against patient derived $CD4^+$ cells (Tsai et al., 2017). Encouraging results were obtained in studies with SIV-infected rhesus macaques, in which TLR7 agonism by either GS-986 or GS-9620 resulted in a reduction of proviral DNA in $CD4^+$ cells and a delay of viral rebound (Lim et al., 2018). Comparable observations were made with combinatory GS-9620 treatments and Ad26/MVA therapeutic vaccinations (Borducchi et al., 2016).

As another exciting example of a potential TLR7 agonist-based "shock and kill" cure, the combination of GS-9620 with the broadly neutralizing antibody PGT121 was able to considerably reduce viral rebound events in simian-human immunodeficiency virus (SHIV)-infected monkeys (Borducchi et al., 2018). The authors hypothesize that GS-9620 induced an expansive $CD4^+$ and natural killer (NK) cell activation and HIV-1 reactivation, while PGT121 stimulated the recognition of viral antigens by the immune system, resulting in the "killing" of the infected cells. Consequently, the efficacy of TLR7-dependent therapies will still have to be vigorously controlled, due to phenotypic differences between the SHIV-based primate model and an HIV-infected patient pathogenesis. For this purpose, Phase I clinical trials to test the safety and activity of GS-9620 in HIV-positive patients are currently under way (NCT02858401, NCT03060447).

Finally, LRA combinations with vaccines targeting conserved HIV-1 epitopes are also providing exciting first results. Vacc-4x, a peptide cocktail targeting four different conserved p24 capsid protein domains (Asjö et al., 2002), has passed various phase II clinical trials and has been proven to be well tolerated (Pollard et al., 2014). Sequential injections of the immunity priming Vacc-4x, followed by the HDACi romidepsin treatment proved to significantly reduce viral DNA load, but unfortunately no delay in viral rebound occurred following a cART cessation (Leth et al., 2016; Tapia et al., 2017). Another clinical trial investigating the synergy between the HDACi romidepsin and the MVA.HIVconsv viral vector vaccine, has promisingly shown to lead to an increase of viral control in patients receiving cART (NCT02616874). Analogously to natural HIV-1 viremic controllers, 5 of the 15 participants were able to delay the viral load rebound for longer than six weeks following the cessation of cART (Mothe et al., CROI, 2017, presented in conference). The researchers hypothesized that enhanced viral control was achieved by sensitizing $CD8^+$ T cells to target highly conserved vaccine-targeted viral epitopes.

Several multifunctional LRAs have been demonstrated to possess the ability to stimulate CTLs, pDCs and NK cells in an unspecific manner, however the use of HIV-specific vaccines might lead to specialized and assertive immune responses, which in the end should alleviate the viral pathogenesis. Furthermore, research into the stimulation of HIV-specific apoptotic mechanisms by LRAs may finally yield in a functional "shock and kill" compounds, as might be the case with ixazomib. It remains to be seen how well the differing methodologies perform in clinical trials.

8. Shock or block?

While "shock and kill" is envisaged to lead to an eventual cure of HIV infection, the scientific community still cannot estimate how long, expensive or toxic the therapy might end up being. Therefore, the opposite rationale to "shocking" is proposed to be the more functional alternative, namely by consistently suppressing transcription of the viral reservoir. This concept has been termed the "block and lock" method and consists of utilizing latency promoting agents (LPAs), which target either HIV or host-specific mechanisms, to drive the provirus into a state of deep latency (Gallo, 2016; Mousseau and Valente, 2016). In comparison to conventional cART, which concentrates on repressing the active viral life cycle, "block and lock" aims to entirely prevent the wide-scale production of viral RNA and protein elements.

The most promising results have been achieved with didehydrocortistatin A (dCA), an LPA which selectively interferes with interaction between viral *Tat* protein and the TAR-RNA element (Mousseau et al., 2012). Importantly, viral transcription suppression was shown to be maintained following prostratin stimulation or CD3/CD28-receptor induced T cell activation, a mechanism considered to be among the most potent physiological signals for viral reactivation (Mousseau et al., 2015). dCA-induced *Tat*-TAR-axis elimination was shown to be especially potent in combination with cART, with infected patient cells resisting prostratin treatment after 35 days of cART-dCA cocktail in *ex vivo* conditions (Kessing et al., 2017). Importantly, 25 days after cessation of cART-dCA treatment, the resurgence of viral blips (brief elevations of detectible viral load) remained considerably lower than with the cART regimen alone. These results provide an exciting proof-of-concept for directly targeting the viral *Tat* protein, and offer a convincing argument to continue developing the "block and lock" therapy (Fig. 4).

As an alternative to concentrating on targeting viral elements, recent efforts have delved into manipulating host-dependent pathways responsible for the maintenance of HIV latency. For example, mTOR complex inhibitors effectively lead to *Tat* protein degradation through autophagy, while further suppressing PMA-dependent viral reactivation in common HIV-1 cell line models, primary models and patient derived cells (Wan and Chen, 2014; Sagnier et al., 2015; Besnard et al., 2016; Zhao et al., 2016). Analogously to mTOR inhibitors, the suppression of the HSP90-NF-κB-axis with HSP90 inhibitors is known to be capable of subduing the HIV-1 reactivating effect of NF-κB-stimulatory compounds in J-Lat and ACH-2 cell lines (Anderson et al., 2014; Joshi et al., 2016;

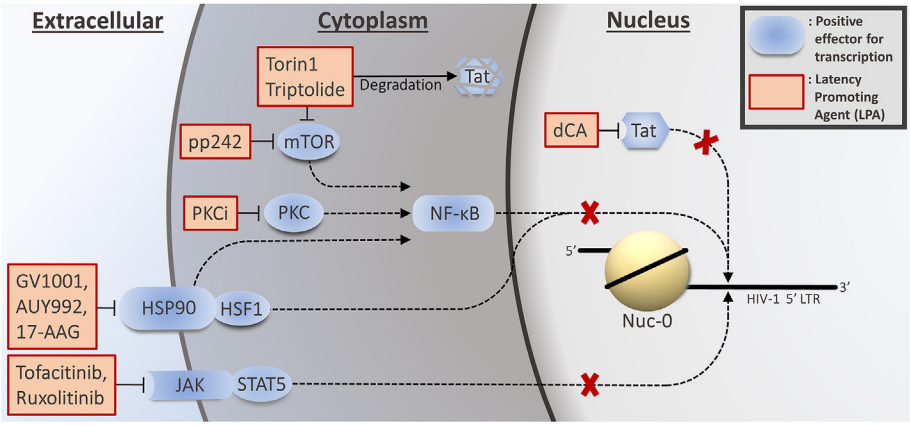

Fig. 4. Proposed mechanisms of Latency Promoting Agents (LPAs) for "block and lock" therapy. The chemical inhibition of the trans-membrane HSP90 protein leads to the suppression of both NF-κB dependent pathways and sequesters HSF1 transcription factor to the cytosol. NF-κB inhibition can also be achieved with mTOR and PKC inhibitors (Bermejo et al., 2015). The inhibition of mTOR complex by Torin 1 and Triptolide can further impede HIV-1 by inducing *Tat* degradation through autophagy. Inhibition of the JAK-STAT pathway with extracellular JAK-inhibitors limits the STAT5-dependent transcription. Intranuclearly, didehydro-Cortistatin A (dCA) binds directly into the active site of *Tat*, suppressing its binding from viral TAR RNA. Dashed arrows represent inhibited pathways. mTOR, Mammalian target of rapamycin; PKC, Protein kinase C; HSP90, Heat-shock protein 90; HSF1, Heat-shock factor 1; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; FACT, Facilitates chromatin transcription; LTR, Long terminal repeat; Nuc, nucleosome.

Kim et al., 2016). Intriguingly, HSP90 has been demonstrated to participate in the formation of PTEF-b complex (O'Keeffe et al., 2000), thus its chemical repression adds another potential layer to the viral inhibition by this drug class.

The main arguments supporting "block and lock" over the "shock and kill" hypothesis is the likelihood that "block and lock" will display less adverse side effects to patient well-being, and the possibility that "shock and kill" might never completely eradicate the proviral reservoir. Indeed, a number of the proposed LPAs are considered to be clinically well tolerated, such as the JAK-STAT inhibitors tofacitinib and ruxolitinib (Gavegnano et al., 2017). However, the proposed host-mechanism targeting "block and lock" methodologies generally impede pro-inflammatory signaling, as is the case with by inhibiting the mTOR, HSP90 or the JAK-STAT inhibitors. This presents a double-edged sword, since "block and lock" may exacerbate the already immunocompromised patient, and the therapy should therefore be carefully evaluated before clinical applications.

Foreseeably, the "block and lock" method would function in concert with existing cART to eliminate the emergence of viral rebound and sustain repression of the viral reservoir. Nevertheless, it should be noted that independent elements of the provirus may be stochastically transcribed from the deficient proviruses, which can be detrimental to patient health. HIV-1 is known to express multiple miRNA sequences, which can target crucial regulatory elements of the infected host cell, thereby altering its homeostatic pathways (Yeung et al., 2009; Schopman et al., 2012; Deeks et al., 2013; Holland et al., 2013; Fruci et al., 2017). Meanwhile, various viral proteins, like *Tat* and *Nef*, are known to be secreted from host cells and translocate to uninfected cells, thus potentially assisting in the onset of the HIV-related chronic diseases (Shelton et al., 2012; Mele et al., 2018). Perhaps then, rather than fully excluding and substituting the "shock and kill" strategy with the "block and lock" method, an alternative approach could lie in combining the proposed therapies to maximize the clinical effect against the proviral reservoir. In combination with the conventional cART, an initial "shock and kill" treatment regimen could be administered to remove the replication-competent proviral reservoir, while a consecutive continuous "block and lock" therapy represses the remaining defective, yet residually transcribable reservoir.

9. Final word

An efficient "shock and kill" therapy should ultimately be able to reactivate proviral expression in all infected T cells and target a wide distribution of integrational genomic loci. This treatment must express minimal side-effects to other cell types and should further stimulate cell death mechanisms specific to those infected cells. However, evidence is gradually showing that commonly employed LRAs might not reactivate the total HIV-1 reservoir, due to the broad integrational landscape of the provirus, condensed chromatin state of resting T cells, sequestration of necessary transcription factors and the physiological heterogeneity of the host cells. Making matters more complicated, the latent reservoir within the resting $CD4^+$ cells should ideally be reactivated without complete T cell activation. The development of highly specific LRAs must therefore continue, and additional novel methods to manipulate the latent reservoir, such as the "block and lock", should be considered. Furthermore, numerous LRAs lack the "kill" aspect, so the "shock and kill" therapy should further be studied with supplementation of immunomodulatory agents. Looking forward, the application of a combinatory shocktail regimen applying specialized LRAs, possibly in sequential schedules, or by additional stimulation with cell death-inducing compounds, should be capable of diminishing the viral reservoir for ultimate patient benefit.

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