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Review

Clinical pharmacology in HIV cure research - what impact have we seen?

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Abstract

Introduction

Combined antiretroviral therapy (cART) has transformed an inexorably fatal disease into a chronic pathology, shifting the focus of research from the control of viral replication to the possibility of HIV cure.

Areas covered

The present review assesses the principal pharmacological strategies that have been tested for an HIV cure starting from the *in vitro* proof of concept and the potential rationale of their *in vivo* applicability. We evaluated the possible pharmacological procedures employed during the early-stage HIV infection and the possibility of cART-free remission. We then analysed the shock and kill approach from the single compounds *in vitro* mechanism of action, to the *in vivo* application of single or combined actions. Finally, we briefly considered the novel immunological branch through the discovery and development of broadly neutralizing antibodies in regard of the current and future *in vivo* therapeutic strategies aiming to verify the clinical applicability of these compounds.

Expert opinion

Despite an incredible effort in HIV research cure, the likelihood of completely eradicating HIV is unreachable within our current knowledge. A better understanding of the mechanism of viral latency and the fully characterization of HIV reservoir are crucial for the discovery of new therapeutic targets and novel pharmacological entities.

Keywords

HIV, eradication, shock and kill, pharmacology.

1.0 Introduction

Combined antiretroviral therapy (cART), with the introduction of protease inhibitors in middle 90', has led to a dramatic change in the life expectancy of HIV infected patients by transforming an inexorably progressive disease into a chronic one. cART is able to control the human immunodeficiency virus type-1(HIV)'s replication by interfering with different steps of the HIV life cycle, including cell entry, reverse transcription, integration, and assembling of the virion. Several problems are still present in the treatment of HIV infection and in particular if on the one-hand cART is able to inhibit the virus replication reaching undetectable levels of HIV-RNA in plasma, on the other the persistence of the virus in the so-called reservoir prevents the possibility of its eradication [1]. The HIV reservoir is composed by quiescent cells that can undergo clonal expansion and either maintain or increase the size of the reservoir without producing life-long persisting virus. Moreover, the quantification of the reservoir and the measurement of its size are still subject of debate and different techniques to quantify HIV reservoir are applied in different studies [2]. At the moment, no standardized assay is available to assess the magnitude of viral reservoir and this limits the comparability of different studies. For example, PCR based methods are likely to overestimate the size of viral reservoir whereas culture-based methods are likely to underestimate it, leading to potential misclassification of patients as cured or not. Thus, the real efficacy of HIV eradication strategies is actually hardly to account [2]. One of the major challenges with which scientist has to face up is the difficulty in the identification of specific markers able to specifically target the cells which composed the HIV reservoir. Descours B et al suggested that $CD32a^+$ cells could play an important role as HIV reservoir because those cells were highly enriched in inducible replication-competent proviruses. These authors suggested that $CD32a^+$ lymphocytes, representing the elusive HIV-1 reservoir, may lead to insights that will facilitate the specific targeting and eventually its elimination [3]. Unfortunately, these findings have been

reconsidered according to the work of Abdel-Mohsen *et al*. that examined T cells from treated HIV patients. They found that $CD32^+$ HIV-infected T cells had an activated phenotype and HIV RNA, indicating active HIV transcription. In contrast, the majority of HIV DNA resided in $CD32^-$ cells. Their results suggest that targeting $CD32^+$ cells is unlikely to hit the HIV latent reservoir [4]. Moreover, a recent work by Bertagnolli et al showed how there is no evidence that CD32 expression indicates the presence of latent HIV-1, and found that a not negligible fraction of the HIV latent reservoir is in $CD3^+CD4^+CD32^-$ T cells. Moreover, the authors suggested that the findings by Descours et al could be driven by the use of an ultrasensitive p24 ELISA assay that may account for the apparent enrichment observed in culture experiments [5]. Indeed, if cART is interrupted, the HIV production by these cells is observed within 2 to 4 weeks. Thus, in the absence of cART, cells that harbour quiescent replication-competent virus can rekindle HIV replication and transmission [1]. Recently an alternative and broader definition of viral reservoir has been suggested: all infected cells containing all forms of HIV persistence that can participate in HIV pathogenesis [6]. This broader definition includes defective proviruses that could theoretically play a role in HIV pathogenesis through the production of viral proteins and the subsequent induction of chronic immune activation [7]. In other words, proviruses that are not fully replication-competent, but that are capable of transcribing viral mRNAs and/or translating viral proteins, may constitute an additional dimension to persistence studies [8]. Moreover, a chronic state of immune activation combined with a persistent pro-inflammatory state leads to a premature aging and to an increased frequency of non-AIDS related illness [9]. In the context of a possible virological control with a deferred progression of HIV infection due to cART, the focus in HIV research is now posed on the possibility of HIV control in the absence of cART and, in the end, on the possibility of HIV complete eradication from the body. The greatest challenge in the prospect to achieve a cure of HIV infection is to eliminate all replication-competent virus in the reservoir or, if this could not be achieved, to obtain a lifelong remission without the use of cART. Several strategies have been addressed both in animals and human models but nowadays all have failed to achieve HIV cure [10]. In fact, the only patient who have been cured from HIV infection was the so-called “Berlin patient”. Timothy Brown received a hematopoietic stem cell transplant from a donor whose cells were resistant to HIV infection due

to the delta32 mutation in the HIV coreceptor CC chemokine receptor 5 (CCR5). Mr. Brown, who has not been receiving cART for more than 10 years, has no evidence of replication-competent HIV [11]. Although the transplantation approach is intriguing, it has to be considered just a proof of concept for possible future eradication strategies due to the high mortality risk for the patient and also to the large cost.

In this review we are going to examine all the possible pharmacological approaches that have been tested, the one which still are ongoing in trial and also the future perspectives regarding HIV cure.

2.0 Very early treatment and ART free remission

Several studies have assessed the possibility of reducing the viral reservoir and consequently to pose the foundation for a possible HIV cure by using cART in the early phase of infection. These studies start from the observation that HIV reservoir begins to increase dramatically as soon as HIV-RNA becomes detectable in plasma and it persists to increase in the following weeks [12]. One of the strategies tested was treatment intensification during the early stage of HIV infection: the addition of maraviroc to a cART regimen results in faster reduction of 2-long terminal repeat (LTR) newly infected cells and recovery of CD4 T-cell counts, and a modest reduction in total reservoir size after 48 weeks of treatment. However, CCR5 blockade also induced a slower decrease in plasma viremia and immune activation [13]. As shown by Crowell and colleagues, the presence of detectable colonic HIV RNA at the time of cART initiation during acute HIV infection, resulted associated with higher levels of proviral DNA suggesting that the seeding of HIV in the gut may have long-lasting effects on the size of persistent viral reservoirs and may represent an important therapeutic target in eradication strategies [14]. Due to its rapid infiltration by HIV during the early phase of infection, the colon may be a target to reduce the rapid instauration of the reservoir. A possible intervention designed to prevent colonic infiltration could be the use of anti-α4β7 monoclonal antibody administration, to block the interaction between the HIV envelope and gut-homing integrins. This kind of intervention has to be conducted before peripheral blood HIV RNA reaches peak levels. In animal model, anti-α4β7 has been able to prevent mucosal infection by simian immunodeficiency virus and in the early phase of infection it is able to protect both blood and gut-associated lymphoid tissue CD4 T cells from

infection [15]. Another potential intervention tested during SIV acute infection in rhesus macaques is the caspase inhibitor Q-VD-OPH which demonstrated first of all to reduce T cell death, secondly to preserve of $CD4^+/CD8^+$ T cell ratio in lymphoid organs and in the gut and also to maintain memory $CD4^+$ T cells with increased specific $CD4^+$ T cell response with the production of cytotoxic molecules [16]. If it is true that very early cART start, within 2 weeks from HIV infection, could significantly reduce the size of viral reservoir, making almost impossible its quantification, unfortunately, it does not correspond to a significant clinical improvement for the patient [17]. In fact, infection rebounds when treatment is interrupted by reactivation of virus production from this reservoir [18]. This finding was also confirmed in subjects treated in an extremely early phase of HIV infection, i.e. Fieibig I stage, leading to an almost complete undetectability of HIV-DNA in blood and tissue sampled. However, it is possible that the very small size of the latently infected cells could lead to a delayed rebound of HIV viremia [19, 20]. Lymphoid tissues are the principal sites of production and persistence before initiating cART and if cART is interrupted a rebound of the plasmatic HIV-RNA is observed shortly thereafter. One possible explanation about the persistent replication of HIV at tissue level, albeit an undetectable HIV-RNA in plasma, could be a low penetration of antiretrovirals in lymphoid tissues despite therapeutic concentrations obtained in blood [21]. Sequence analysis revealed a great number of rebounding viruses representing recrudescent viremia from multiple sources. These findings suggest that the HIV reactivation arises from many latently infected cells at multiple sites. Unfortunately, the large pool of cells and sites that are able to rekindle recrudescent infection highlights the challenges in eradicating HIV [22]. The first data showing a possible role in HIV "functional" cure of early antiretroviral treatment came from the VISCONTI study in which 14 patients, who initiated cART within 3 months of their estimated date of infection, remained on durable treatment for 1–8 years controlling HIV replication after cART interruption [23, 24]. The so-called "post-treatment controllers" have maintained their plasma HIV RNA levels to <50 copies per mL, with the exception of a few viral blips with a good median CD4 cell count after cART interruption. Apart from the VISCONTI cohort, amid patients there have also been other anecdotal reports of post treatment controllers [25]. The patient's subsets with primary HIV infection are the ideal candidates for intervention strategies with therapeutic vaccines. Such

interventions could lead to an interruption of cART with a control of HIV replication hopefully through the employment of a "functional cure" ,since unfortunately they cannot cause an HIV eradication [26]. The eradication strategy of a very early cART treatment has not worked even in children, who are in many ways the best population to apply it [27, 28]. To achieve sustained virological control of HIV-1, without the need of continuous cART, and to clear virus-expressing cells, it is likely that immune-based therapies will be needed, nevertheless therapeutic HIV vaccine trials in chronic HIV infection have obtained very few clinical benefits. The subset of subjects with a primary HIV infection is a specific target population allowing to study the role of HIV therapeutic vaccines in individuals who have experienced an early cART start. It was observed that this approach has led to few or no viral escape, less CD4 depletion and more preserved memory T cells.

In conclusion if on the one hand it is unlikely that future improvement in cART strategies could led to a potential HIV "functional" cure on the other hand more studies are required for the characterization of immune responses following treatment initiation at different stages of primary HIV infection, since responses to therapeutic vaccines may vary depending on the pre-existing HIV-specific immune responses [26].

3.0 Shock and Kill strategies

The most explored strategy in tackling HIV-1 reservoirs — "shock and kill" — describes the broadly explored pharmacological way of kicking the latent provirus, with subsequent killing of the virus-producing cells by the immune system [29]. One strategy to eliminate latency is to activate virus production using latency reversing agents (LRAs) with the goal of triggering cell death through virus-induced cytolysis or immune-mediated clearance. However, multiple studies have demonstrated that activation of viral transcription alone is insufficient to induce cell death and some LRAs may counteract cell death by promoting cell survival. Some have advocated approaches that activate latent reservoir cells, thereby allowing them to be destroyed. Clinical trials of such latency-reversing classes of drugs like histone deacetylase inhibitors (HDACi) suggest that they can induce some expression of latent HIV although not its clearance [30]. Other

drug classes, such as activators of protein kinase C or toll-like receptors, may be more effective [31]. Given potentially large HIV reservoirs , the extent to which these agents could provide adequate awakening of latent virus is unclear [32]. Their potential role should not just be considered alone but combined together with an immune-based therapy to kill the re-activated cells.

2.2.2 ***The "shock"***

LRAs which are currently being investigated, include HDACi inhibitors, toll-like receptor agonists, activators of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB), disulfiram, immune checkpoint inhibitors, and agents that affect the STAT5 signaling pathway and mTOR signalling (*Table 1*) (*Figure 1*). However, studies to date indicate that, compared with maximal T-cell activation, few LRAs worked effectively well ex vivo with cells from HIV-infected patients. It has been well recognized that epigenetic regulation of histone tails at the HIV LTR is critical for the establishment of latent reservoirs. Recent studies showed that HDACi or histone methyltransferase can reactivate HIV and some of these inhibitors have been investigated as LRAs in human clinical trials *(Table 2)*[33]. First of all, it is worth mentioning the valproic acid, a well-known drug used commonly against seizures or migraine, which emerged as a potential LRA thank to a study supervised by Margolis and published on Lancet in 2005. This work gained such huge resonance because scientists noticed that three out of four patients, under a HAART regimen and successfully suppressed, underwent a significant decrease in the frequency of latently infected CD4+ T-cells when enfuvirtide and valproic acid were added for 12 weeks [34]. Although this work presented many weak points such as the fact that it was not controlled, the impossibility to quantify the effect of the single drug, or again that the number of CD4+ T cell is significantly fluctuant even in stable patients , it raised great enthusiasm about the subject [35]. However successive research failed to confirm such promising results, defining the usefulness of valproic acid addition of limited value [36- 38]. Next, vorinostat administration has led to an increase in cell-associated HIV RNA within circulating resting cd4+ t cells in patients under cART [30, 39]. However, there is not an agreement about the dose regimen to apply in order to obtain a complete depletion of the reservoirs [40, 41]. Another potent member of these series is panobinostat,

which demonstrated to be able to reverse latency with a high level both of cell-associated unspliced HIV RNA and plasma viremia other than to increase the amount of sytemic histone acetilation both in vitro and in BLT (bone marrow, liver, thymus) mice [42, 43]. Despite these promising results, panobinostat has not proved to obtain a drop in the levels of latent-infected CD4+ T cells suggesting that a combination therapy may be necessary [42, 43]. Finally, romidespin has to be mentioned within the HADC pool of compounds. It has shown to be able to induce HIV expression ex vivo at concentrations that can be achieved clinically, indicating that the drug may reactivate latent HIV in patients on suppressive cART and also resulted much more potent than its above-mentioned counterparts [44]. Romidepsin has also displayed significant viral reactivation in vivo in long-term suppressed HIV-1 individuals on antiretroviral therapy after different cycles of infusions. In fact, Søgaard *et al*. observed clear increases in lymphocyte H3 acetylation, HIV-1 transcription, and plasma HIV-1 RNA. Importantly, this reversal of HIV-1 latency could be measured using standard clinical assays for detection of plasma HIV-1 RNA. Furthermore, romidepsin did not alter the proportion of HIV-specific T cells nor inhibit T cell cytokine production which is critically important for future trials combining HDACi with interventions [45]. Interestingly, a work by Winckelmann *et al*. shed light on the source of the romidespin-induced viremia. They found out that, in spite of the intermingling of the cell-associated HIV-1 RNA with DNA sequences indicates transcription from a diverse range of proviruses, the expansions of identical viral plasma sequences with few defects show that the romidepsin-induced viremia arises from intact proviruses with highly similar or identical genetic backgrounds. This evidence may suggest that a specific subset of transcriptionally activated proviruses contributed to the majority of viremia [46].

Several bromodomain inhibitors have been investigated in vitro as possible compounds able to reactivate HIV-1 latent proviruses [47]. Bromodomain and extraterminal (BET) protein family is a highly-conserved class of transcriptional regulators that are characterized by the presence of tandem bromodomains, conserved domains that recognize and bind acetyl-lysine residues, and a so-called extraterminal (ET) domains. BRD4 represents the best-studied BET family member, which contains a domain, namely the

positive transcription elongation factor b (P-TEFb)-interacting domain, that binds the HIV cofactor P-TEFb [48]. Such bond prevents the reaction between HIV Tat and P-TEFb, which is crucial to trigger HIV transcription. Thus, BRD4 represents a potent HIV activation inhibitor [49]. In the light of these experimental observations, the bromodomain inhibitor JQ1, a cell-permeable compound with potent anti-cancer activity, which prevents the sequestration of P-TEFb by BRD4 hence promoting Tat-transactivation, has been recruited amid the strategies to disrupt HIV latency [48-50]. Li *et al*. demonstrated in vitro that the binding of JQ1 with BRD4 enhances HIV transcription [49]. It seems, indeed, that JQ1 is able to dissociate BRD4 from the HIV promoter thus allowing the Tat transcriptional activity [49]. Although Li *et al* claimed that JQ1 activity is TAT-dependent, a study by Boehm *et al*. showed that even after the removal of Tat from the equation, cells treated with JQ1 were able to start transcription [48]. They also demonstrated a role, even if of minor relevance than BRD4, of BRD2 in the activation of HIV transcription: knockdown of BRD2 resulted in a robust activation of the HIV LTR, and this effect was only slightly enhanced in response to JQ1 [49].

Among LRA, disulfiram has been initially tested in HIV infected patients on stable cART to study the ability to induce plasma viremia since in vitro studies have demonstrated the capacity of this compound of inducing HIV transcription in a T cell model [51]. Despite the mechanism of action is not completely understood, It appears to be mediated by the depletion of the phosphatase and tensin homolog and the activation of protein kinase B [52]. Even though a rapid increase in the HIV viremia soon after the disulfiram administration, which resulted well tolerated, no effect on the size of HIV reservoir was observed in a pilot study by Spivak *et al* [53]. In a phase 2 study escalating doses of disulfiram were tested showing an increase in the cell-associated unspliced HIV-RNA at all doses [54]. The illustrated findings, combined with the good tolerability profile of this drug, suggest the possibility of use of disulfiram as LRA alone or combined with other molecules [55].

Other key molecular mechanisms involved in HIV-1 latency are sequestration of transcription initiation/elongation factors and blockade of the assembly of the active elongation factor, P-TEFb. The NF-

κB pathway appears to be one of the most relevant pathways concerning HIV-1 reactivation. Protein kinase C agonists (PKCA) activate PKC isoforms which then phosphorylate IκB and consequently activate NF-κB. Several PKC agonists have been considered for purging the reservoirs of latent HIV-1[56]. First of all, prostratin is a unique phorbol esterthat it induces potent T cell activation signals and is not tumorigenic. Besides the ability of prostratin to induce T-cell activation through PKC, without tumor promoting ability, another unique property is that, despite being able to reactivate latent HIV-1, it exerts an inhibitory effect on active HIV-1 replication through downregulation of CD4 [57]. Furthermore, bryostatin-1 has appeared to be an effective reversing agent. Bryostatins represent an important group of pharmaceutically promising substances. These compounds are produced by commensal microorganisms naturally occurring in marine invertebrates, mainly in bryozoans. The most frequently investigated substance is bryostatin-1, which is a highly oxygenated macrolide with a polyacetate backbone [58]. Its mechanism of action involves a bound to the diacylglycerol-binding region within the C-1 regulatory domain of PKC. Bryostatin-1 has shown to reactivate latent HIV-1 in vitro in monocytoid and lymphoid cell line models of latency and was approximately 1,000-fold more potent than prostratin [57]. However, since economic and environmental factors have severely limited its availability, De Cristopher et al. reported the activity of seven bryostatin-1 analogues, dubbed “bryologs”, specifically designed to obtain molecules easier to operate with. These compounds incorporate a tetrahydropyranyl B-ring formed through a versatile prins macrocyclization. They demonstrated that these simplified bryostatin analogues, which share the functional activity of the clinical candidate prostratin, are up to four orders of magnitude more potent [59].

Although research on latency reversing agents has collected a good amount of promising results, none of these compounds has provided an effective reduction in HIV reservoir size. Thus, scientists have been proposing combinations of different agents in order to obtain a maximal synergistic effect able to cause a significant contraction to the viral latent pool. First of all, it is worth mentioning the work of Reuse *et al*. who tested the combined effect of prostatin (PKC agonists) and several HADCis [60]. They demonstrated that the combination of prostratin plus valproic acid or suberoylanilide hydroxamic acid disrupted more

effectively than each compound alone the HIV-1 latency both in several latently-infected cell lines and in $CD8^+$-depleted PBMCs isolated from HIV-1-infected patients receiving HAART and with undetectable viral load [60]. Interestingly, they also found that HADCis can degrade cytoplasmic IκBα which enhances the PKC agonistic activity hence boosting their combined synergism [60]. Moreover, they proved that this combination is effective on all the viral subtypes belonging to the HIV group M except for subtype G [60]. Secondly, Darcis *et al*. later considered the combination of PKC agonists and P-TEFb releasing compounds like JQ1 and the results obtained were quite promising. Initially they assessed the capability of these drugs of reaching a synergistic effect if administered together, so they measured the HIV-1 production and transcription both in U1 and in a J-Lat strain. They discovered that the combination PKC agonists plus iBET/hexamethylene bisacetamide strongly enhanced both production and transcription in such *in vitro* post-integration latency cellular models of T-lymphoid and myeloid lineages [61]. Furthermore, the combined effect of these molecules was tested ex vivo on primary cells isolated from aviremic patients on cART. Cultures of Isolated PBMC depleted of CD8+ T cells and cultures of resting CD4+ T cells expressed HIV-1 RNA levels to a degree comparable to that obtained after anti-CD3+anti-CD28 antibodies stimulation in presence of JQ1/Ing-B+bryostatin-1 [61]. The mechanism of action underlying the synergism was also partially uncovered: it seems, in fact, that on the one hand JQ1 increased bryostatin-1-induced NF-κB DNA-binding activity and on the other hand that JQ1 and bryostatin-1 caused a higher activation of P-TEFb than the compounds alone [61]. Finally, the activity of PKC agonists plus JQ1 on cell surface activation markers was investigated. They noted that first, JQ1 did not cause the activation of resting CD4+ T cells and CD4+ cells - analyzed from the CD8-depleted pool -and second, that the combination of PKC agonists and JQ1 led to the activation of cell markers such as CD69 and HLA-DR and to a downregulation of CD38 and CD25 compared with the PKC agonists effect alone. Consequently, it is reasonable to say that the combination causes a minor T cell activation which is fundamental towards a clinical prospective, since compounds which trigger a non-specific or robust immune activation are inappropriate for in vivo applications. Moreover, the combination PKC agonist plus JQ1 led to the downregulation of CD4 receptor, which could be beneficial for the blockade of de novo HIV infections [61].

Toll–like receptor molecules (TLRs) are a class of antigen-presenting cells trans membrane proteins located on the cell surface or on the membrane of endocytic vesicles and other intra-cellular organelles. They are capable of recognizing a wide range infectious disease pathogen-associated molecular patterns (PAMP). TLRs are divided in two main groups: i) surface TLRs such as TLR-1, TLR-2, TLR-4, TLR-5, TLR-6, and TLR-11 mainly capable of identifying microbial membrane components such as lipids, lipoproteins, and flagella and ii) TLR-3, TLR-7, TLR-8, and TLR-9 which are expressed in intracellular vesicles of the endoplasmic reticulum, endosomes, and lysosomes and recognize both viral and microbial nucleic acids [62]. TLRs activation by their ligands implicates the expression of the adaptor protein My88 and the nuclear factor NF-κB. In particular TLR-7 is well known to be involved in the recognition of single-stranded RNA fragments located in endosomes and its activation is a common feature of viral infections.(59). In fact, TLR-7 agonists have already been studied as potential HIV inhibition targets [63, 64]. Moreover, as already mentioned by Sloan at Croi 2015 and confirmed by Tsai et al., TLR-7 agonists also have (especially GS-9620) a high potential as HIV inducers in HIV siero-converted patients on HAART. Indeed, Tsai found that GS-9620 can induce extracellular RNA ex vivo on the PBMCs of 27 out 36 of the patients tested, independently of their basal HIV-RNA levels and is capable of stimulating the production of INF-α, which plays a key role in HIV induction [65]. Besides, it was discovered that GS-9620 can upregulate the expression of CD69 and consequently activate CD4 and CD8 both in the presence and absence of HIV antigens, even though in the first case the level is much higher [65]. Furthermore, it was noticed that the molecule was able to enhance the CD8 cytolytic effect and that this same effect is strictly connected with the activation of IFN-α (62). Finally, Tsai et al. proved that immune effector cells, like monocyte and dendritic cells, due to the upregulation of CD80 as well as CD69 consequent to the exposure to GS-9620, more effectively recognize and kill HIV-infected target cells in the presence of effector-competent bNAbs [65]. Also, maraviroc have been investigated among LRAs, alone or combined with PKC-agonists, in fact this CCR5 antagonist can activate NF-κB and, subsequently, induce latent HIV-1-transcription in resting $CD4^+$ T-cells from HIV-1-infected individuals on suppressive antiretroviral therapy [66, 67].

3.2 **The "kill"**

As to the "kill" part of the strategy, various studies have shown that neither the virus nor the immune system is effective in clearing infected cells after latency reversal; in one in vitro model, infected resting CD4+ T cells survived despite viral cytopathic effects [68]. Further, because most of the virus has mutated to escape immune responses, escape variants dominate in the latent reservoir of people with chronic infection [69]. Unfortunately, therapeutic vaccines to augment immune responses have resulted in transient expansion of T cells that do not recognize escaped HIV epitopes [70]. Several trials of vaccines to increase the strength of HIV-specific immune response have been completed in the last years, and overall results show that vaccination is safe and immunogenic, but useless in eliminating HIV. Moreover, a great number of shock-and-kill studies have combined LRAs with approaches such as therapeutic vaccines, interferon, and broadly neutralizing antibodies to enhance immune response. In one study of 20 individuals on antiretroviral therapy who had a viral load below 50 HIV RNA copies/mL for more than 3 years, the combination of the HDACi romidepsin and the HIV peptide vaccine resulted in no change in integrated DNA or infectious virus. If on one side a statistically significant decline in total HIV DNA was observed on the other the effect was clinically insignificant, because viral rebound after cessation of antiretroviral therapy was always observed within 2 to 4 weeks [71]. In a recent study, the use of a different HIV vaccine in combination with romidepsin was associated with viral rebound within 4 weeks of interruption of antiretroviral drugs in 8 participants; 5 other participants exhibited sustained lower level viremia during the interruption [72]. Lim *et al*. treated SIV-infected rhesus macaques on antiretroviral therapy with up to 19 doses of the TLR-7 agonists GS-986 or GS-9620. By the third dose, all macaques experienced a transient SIV plasma viremia within 48 hours after dosing. The dose regimen was also associated with activation of lymphocytes (T, NK, and B cells) and a reduction in SIV DNA in cells from the peripheral blood, lymph nodes, and gastrointestinal tract. When antiretroviral therapy ceased, 2 out of 13 treated macaques did not show rebound of virus and remained virus-free and disease-free for more than 2 years [73]. In the same study, the depletion of CD8 cells did not induced a rebound of the SIV RNA in the 2 aviremic macaques. Moreover,

the transfer of cells from aviremic animals to uninfected ones did not produce de novo infection. These findings suggested a possible role of TLR-7 agonist in reducing the viral reservoir in a subset of macaque infected with SIV.

In recent years the possible use of compounds employed in cancer therapy, with the ability of interfering with apoptotic pathways, was postulated for potential HIV curative intervention (*Table 3*) [29]. Among these bcl-2 antagonists, venetoclax, which causes selective killing of HIV-infected cells resulting in a decreased of HIV DNA-containing cells, has been tested in vitro as a potential strategy to reduce reservoir size [74]. Another class of compounds is the phosphoinositide 3-kinase (PI3K)/AKT inhibitors, i.e. elefosine, perifosine and milefosine, which have the ability of reducing the HIV-1 production from long-living virus-infected macrophages. The potential application of these compounds could be the block of the establishment of the HIV reservoir by interfering with the cytoprotective effect of HIV-1 infection, which extends the lifespan of infected macrophages by the PI3k/AKT pathway interference [75]. Another approach potentially able to revert HIV latency could be the block of Baculoviral IAP Repeat Containing 2 (BIRC2) by small molecules named Smac mimetics. In fact, ubiquitin ligase BIRC2 which is a repressor of the NF-κB pathway, is a potent negative regulator of LTR-dependent HIV-1 transcription [29]. Successful in vitro strategies have combined the HDACi panobinostat with Smac mimetics with a synergistic effect in the reversal of HIV latency [76]. Finally, retinoic acid inducible gene 1 (RIG-1) inducers, i.e. acitrecin, which have been tested as potential compounds able to revert and selectively kill the HIV latently infected cells, alone or combined with vorinostat, failed in reverting latently HIV infected cells and in selectively killing of HIV positive cells [77].

4.0 Immune therapy

A detailed understanding of how the host immune system shapes the size and distribution of the viral reservoir should lead to the development of a new generation of immune-based therapeutics, which might eventually contribute to a curative intervention [78]. CTLA-4+/ programmed death-1(PD1)- CD4+ T cells

with features of T regulatory cells are critical for viral persistence in lymphoid tissues and should be targeted as part of HIV cure strategies, as well as CD8 depletion in SIV-infected macaques on ART , suggesting that CD8 cells play an ongoing role in viral control even during suppressive ART [79, 80]. Finally, recent studies using IL-21 and interferon alpha have modulated viral rebound and improved immune control in infected animals after ART interruption [81].

Immunosuppressive pathways or immune checkpoints are known to be inhibitory receptors expressed on the surface of several immune cell subsets and have the function of maintaining self-tolerance and controlling the physiological course of immune response in peripheral tissues, in order to minimize collateral tissue damage [82]. Such Immunosuppressive pathways lead to 'exhausted' T cells, which show inferior effector function, sustained expression of immune checkpoint molecules such as PD1, poor recall responses and an uncommon transcriptional state [83]. Many pathogens and malignancies use these pathways as immune escape strategy, such as HIV. Indeed, several clinical trials involving checkpoint inhibitors as pharmacological targets have been starting on cancer patients [82]. PD1, Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3 (Tim-3) and Lymphocyte-activation gene 3 (LAG-3) are the most commonly upregulated proteins, expressed both on CD4+ and CD8+ T cells. Their expression is majorly observed in HIV-untreated infection, nevertheless even patients on cART, albeit lower, present an elevated level of immune checkpoint proteins in comparison with controls [84]. It was proved that the administration of PD-1 antibody to SIV-infected macaques resulted in a rapid expansion of CD8+ T cells with a reduction in the interferon signaling pathways and reduction in intestinal microbial translocation [85, 86]. Moreover, a single study in a patient with metastatic melanoma and HIV treated with anti-CTLA4 (ipilimumab) and anti-PD1 (nivolumab) antibodies showed an increase in cell-associated HIV-1 RNA [87, 88]. So far, the only trial assessing an immune checkpoint blocker in individuals with HIV infection without malignancy was a phase II dose-escalation study with an anti-PDL1 antibody (BMS-936559), which was prematurely interrupted for concomitant retinal toxicity in a simultaneous study in macaques. Moreover, one patient, among the six tested, developed hypophysitis

after several months from the discontinuation of the compound, thus raising concern about the safety of this class of compounds [89]. Furthermore, a recent study released on June 2018 by Porichis et al describes the relationship between restored CD4+ T cells and NK cells [90]. Their *ex vivo* analysis shows that the inhibition of two main immunosuppressive pathways contributing to HIV-specific T cell exhaustion, PD-1 and IL-10, increases NK function through reinvigorated HIV-specific CD4 T cell help both in patients on cART or untreated. These results suggest that reversion of CD4 T cell exhaustion in HIV infection can provide a more efficient viral control by boosting the innate immunity response. This model considers cytokines as important mediators of CD4 T cell help to NK cells; as a matter of fact, IL-2 secreted by CD4+ T cells can stimulate NK and elicit secretion of cytokines such as IL-12 by APCs, which in turn is a potent activator of NK cell function. Their results suggested that the reversal of adaptive immunity exhaustion can improve the innate immune response and immune checkpoint modulation could be used as adjuvant therapy in HIV infection [90].

It has been shown the importance of other interleukins, i.e. IL-15, in enhancing the survival and also the effector function of HIV specific CD8+ specific lymphocytes which are fundamental for the control of HIV replication [91]. Recent data have shown how the use of IL-15 superagonist ALT-803 is able to invert the trafficking of SIV specific CD8+ cells from peripheral blood to lymph nodes, and in particular inside B cells follicles [92]. Moreover, in rhesus macaques IL-15 superagonist ALT-803 has the ability to increase both NK cells and CD8 + cells with an impact in the reduction of SIV viremia, albeit transiently [93].

5.0 Non pharmacological strategies

5.1 Broadly neutralizing antibodies

Along with the discovery of numerous HIV-1–specific broadly neutralizing antibodies (bNAbs)[94-96], it has become evident that viral co-evolution is likely required to induce potent bNAbs during the natural course of infection by mature B lymphocytes [97, 98]. One of the bNAbs' possible mechanisms of action against HIV infected cells may be the increase of the host immunity or FcγR-dependent mechanism by forming an immune complex for eliciting CD8+ T cell responses [99, 100]. Previous studies have shown that bNAbs

administered at the time of ART discontinuation can provide direct antiviral effects, but whether bNAbs can effectively target the viral reservoir during ART suppression remains to be determined. Recent work has shown that bNAbs can clear cell-free virus and infected cells that express HIV [101]. Proviral DNA was reduced in macaques treated with these antibodies [102, 103]. However, the extent of the antibodies ability to clear latently infected cells is not well understood. The first bNAbs studied in the VRC01 phase I study showed that a single infusion was associated with various degrees of viral suppression in viremic patients ranging from a sustained suppression of HIV replication to no inhibition of viral replication [104]. The emergence of resistant virus strains has arisen as the major problem with this therapeutic approach leading to the recrudescence of viral replication in plasma after a median time of about five weeks after cART interruption, albeit therapeutic levels of VRC01 were detected[105]. Similar findings have been observed in a analogous investigation by using 3BNC117 bNAbs [106]. The next step in the development of such types of bNAbs has been the increase of the potency of the autoantibodies combined with a broader coverage of the envelope escaping variants. Engineering strategy has led to the extension of the half-life of this bNAbs by modifying the Fc region causing a block of the endosomal degradation. Moreover, this new technology has permitted the co-formulation of different bNabs in a single compound accounting for a greater breadth of coverage when compared to first generation bNAbs [107, 108]. in vitro and ex-vivo data have shown how bispecific antibodies directed against GP-120 HIV surface protein, using either the scFv B12 or VRC01, are able to inhibits HIV replication not only in PBMCs but also in macrophages co-cultured with autologous CD8+ T-cells. The combination of these antibody constructs with latency reversible agents could potentially be an option to taking into account as future approach for HIV-cure since has shown an efficient killing of GP-120 expressing cells and the inhibition of HIV replication both in PBMCs as well as in macrophages [109]. An in vivo study, in humanized mice, has shown that an injection of a bispecific bNAns, BiIA-SG, can confer protection against the infection by several different HIV strains when administered before the challenges with live HIV-1 viruses. Moreover, BiIA-SG delayed HIV infection when compared to cART. A single injection of adeno-associated virus–transferred BiIA-SG gene resulted in prolonged in vivo

expression of BiIA-SG leading to HIV replication control and subsequently to the elimination of infected cells in humanized mice [110].

Several human studies are planned in order to evaluate the effects of passively infused antibodies on the HIV reservoir. Immune checkpoint blockers such as anti-programmed cell death-1 antibody could improve function and persistence of effector T cells [78]. Targeted cytotoxic therapy using immunotoxin is another potential path [111]. One of the possible bNAbs routs of administration assessed was the delivery by an adeno associated virus in rhesus monkeys with the achievement of a sustained viral remission by using two different bNAbs [112]. Despite the maintenance of viral replication control of a period covering approximately 20 months, viral replication rebounded in rhesus monkeys showing how this kind of intervention could potentially be accounted for a "functional cure" approach but not for strategies aiming the eradication of the viral reservoir [113]. ConverselyBorducchi et al. have assessed the impact of the V3 glycan-dependent bNAb PGT121 combined with the TLR-7 agonist GS-9620 in ART-suppressed SHIV-infected rhesus monkeys. The Authors showed that in rhesus monkeys infected with SHIV, that initiated cART during acute infection, a combination of PGT121 and GS-9620 during cART suppression substantially delayed and controlled viral rebound following cART discontinuation. Such data suggest that bNAb administration together with innate immune stimulation during effective cART may effectively target the viral reservoir [114] . Two trials are now planned. The first one will be a controlled test of a conserved gag-pol peptide-stimulated dendritic cell vaccine in 28 acutely infected participants (Fiebig stage I–III) from the RV254 clade AE cohort in Thailand. In this trial an analytical treatment interruption (ATI) will determine the effect of the vaccine on viral rebound and control [115]. The other one will test passive administration of a combination of two bNAbs (PGT121 + PGDM1400) in 30 participants from the clade C FRESH cohort of early-treated individuals (Fiebig stage I–III) in South Africa in a placebo controlled, randomised study [116].

One of the last drugs developed for HIV treatment is an anti-CD4 (IgG4 humanized) monoclonal antibody, ibalizumab [117]. Ibalizumab was developed initially for HIV prevention strategies and actually is licensed for the treatment of drug resistance viruses as a component of salvage strategies [118]. Although

ibalizumab showed in a phase 3 study to be effective against multi-drug resistance viruses, the development of escape mutants has been observed [119].

Another humanized monoclonal antibody is PRO 140, which binds to CCR5 inhibiting R5 tropic viruses without any role in blocking CXCR4-using viruses [120]. This compound was tested in a phase II study administered intravenously in which a single intravenous infusion has been demonstrated to have potent, long-lasting antiretroviral activity with a favourable safety profile [121]. Nowadays, there are ongoing phase IIb and III trials in patients with virologic failure and as monotherapy maintenance strategy in patients with viral suppression [122].

5.2 Gene and cell therapy

As it has previously mentioned, the anecdotal case of the "Berlin patient" highlights the possibility of HIV eradication by allopoietic bone marrow cells transplantation with implanted cells intrinsically resistant to HIV infection [11]. Nevertheless, this case was not further reproduced; a recent work by Colonna et al. in cART-suppressed non-human primates infected by SIV showed how allopoietic hematopoietic cell transplantation is insufficient for HIV eradication despite high-level donor chimerism and graft versus host disease due to the persistence of HIV in multiple sites including the brain [123]. Nowadays, several strategies based on gene and cell therapy have been tested in order to evaluate HIV cure approaches from in vitro studies to clinical trial [124]. These include engineering HIV-specific immunity in T-cells, gene editing approaches to render all blood cells in the body HIV-resistant starting from the proof of concept of the "Berlin patient", and a combination of both these strategies. As for pharmacological intervention, it is unlikely that a single intervention could lead to complete HIV eradication. Conversely, a combination of multiple intervention based on cell and gene therapy are more likely to give some results.

6.0 Conclusion

In this review, we assessed several pharmacological strategies, which have been tested as HIV curative interventions. As things stand, no one of these approaches have been demonstrated, in vivo, capable of leading to viral eradication despite strong in vitro and ex vivo data, i.e. shock and kill. A deep understanding

of HIV viral latency mechanisms combined with a better knowledge and characterization of HIV reservoir are crucial for the development of future pharmacological strategies.

7.0 Expert opinion

Despite an incredible effort has been made to decode and interpret the mechanisms underlying HIV viral latency and the establishment and perpetuation of viral reservoir, no effective strategy has been demonstrated capable to completely eradicate HIV infection.

One of the potential targets of pharmacological curative research is primary HIV infection. In fact, it represents a great opportunity for potential curative intervention due to the possibility of reducing the size of viral reservoir with very early pharmacological treatment. It is now clear that cART alone, even if started in the first phase of infection, *i.e.* Fiebig I, is not able to eradicate the virus, but only in few cases it potentially led to a post-treatment control of viral replication after structured treatment interruption. Future studies need to focus on the combination of multiple interventions both pharmacological, *i.e.* cART, and immunological, *i.e.* vaccines and bNAbs, with the aim to hit different targets of the viral replicative cycle and latency mechanisms.

In regards to "shock and kill", which is the most widely investigated among curative intervention strategies, the great challenge is the possibility to apply in vitro and ex vivo discovery firstly to animal model and secondly to clinical practice. This gap is unfortunately not negligible and the great majority of research in the field had the limit of the difficult applicability in vivo. In fact, when we face up with new interventions we have to take into account important ethical, social and behavioural research questions in parallel [125, 126]. The great majority of these strategies poses significant health risks with realistically modest benefit to trial participants. Moreover, the proof of concept of HIV cure and remission is the structured treatment interruption. If on the one hand we could limit the potential problems related to viral rebound for the subject with a close monitor and a rapid resumption of cART, on the other hand we pose the subject in a condition of medicalization that would not be necessary if the patient was still on cART. Furthermore, the effect of a potential resettlement of the viral reservoir during cART interruption is unclear [127].

Eventually, immunological approaches alone are unlikely to be effective in HIV cure. Regarding immune therapy, check-points inhibitors seem to be promising. Unfortunately, toxicity concern has arisen from the first trial on HIV-positive patients without cancer, thus delaying it involvement as therapeutic strategy in HIV positive individuals. Options accounting for the use of bNAbs may have the most clinical applicable with good safety data in vivo. One limitation of this therapeutic intervention is the route of administration and the appearance of viral resistant strains. bNAbs could likely be an option for cART-free remission interventions, whether they could not be effective as curative action.

Since every single strategy alone have failed to reach the ambitious goal of curing HIV, the next step will be the development of multiple steps and target interventions, thus considering the combination of pharmacological and immune agents, i.e. LRA, inflammatory pathway blockage, therapeutic vaccines and other interventions. The objective is at least to induce a cART free remission and whether possible a complete viral eradication.

After the description of the VISCONTI cohort [25] and the explanation of the “shock and kill” [29] line of research, the field of the HIV cure has been dramatically developed. Together with excellent data and sporadic pitfalls, alternate waves of hope and discomfort are still present in the most prominent research initiatives.

The “Berlin patient” remains a great success, yet anecdotal. In parallel, the “Mississippi” and “Milan” babies and the “Boston” patients [18, 27, 128] revealed that the reality is harsher that scientific promises, although well built. As clinicians and researchers who care for HIV-positive patients, we do not know if these strategies will ever be feasible, but we surely realize that it is worth trying to find a solution. In the next 5 years, a series of issues need to be elucidated, as a substantial step before adding them into the clinical practice, such as (i) identifying biomarkers that do rationally have an impact on the inflammation profile and (ii) ways of measuring the viral reservoir. This couple of activities aim at assessing both sides of the same medal, *i.e.* the reaction by the immune system and the ways in which HIV replicates or hides. The challenge will be to measure them in the most effective and accurate way.

Other than these obstacles, some approaches look in a better shape than others and will be more extensively developed in the next 5 years. These tools will include (i) several check-point inhibitors developed in cancer research, the most promising being those which block the axis PD-1/PDL-1 and CTLA-4 and (ii) multiple broadly neutralizing antibodies, some of which already concluded clinical trials.

It is not going to be a *déjà vu* and the best way to use these new resources will be as multi-target combination therapies, which, as expected, will need to take into consideration not only the power of the combination but the complex burden of side effects.

8. Article highlights

- In the early phases of HIV infection the establishment of a reservoir composed by quiescent and long lasting cells represents the major obstacle to HIV eradication.
- Intervention strategies in acute HIV infection could lead to a significant reduction in the reservoir's size but inexorably HIV-RNA rebounds in plasma after cART interruption even if the reservoir result unquantifiable.
- Shock and kill strategies have the aim of revert the latency of quiescent cells composing the HIV reservoir and subsequently kill the same cell. Several models have demonstrated how this kind of intervention can be effective in vitro and in animal model, but at the moment no strategy has been demonstrated to be effective in reducing the reservoir size in clinical studies.
- Immunological strategies accounting for the use of check-point inhibitors developed in cancer research seems to be promising. To date, the major concern regards the safety profile for this class of compounds.
- Broadly neutralizing antibodies represent not only an option among strategies for long term control of HIV viremia and HIV prevention but they could be also part of combined strategies for targeting HIV reservoir as a part of kill interventions.

- Since every single strategy has failed in HIV eradication, the future interventions will have to focus on multitarget combination therapies.

Figure legend

Figure 1. Pharmacological classes of drugs with specific compounds, stage of development and potential application in the current strategies of HIV eradication.

[†]Clinical trial

[χ]Animal model

*In vitro/ex vivo

List of abbreviations: HDACi (Histone Deacetylase Inhibitors), TLR7 (TOOL-LIKE RECEPTOR 7 AGONISTS), BETi (BROMODOMAIN EXTRATERMINAL INHIBITORS), PD-1 (Programmed cell Death protein-1), Bcl-2 (B-cell lymphoma 2), PI3K/AKT (Phosphatidylinositol-4,5-bisphosphate 3-kinase/Protein kinase B), Smax (Second Mitochondria-derived Activator of Caspases), XIAP (X-linked inhibitor of apoptosis protein), RIG-1 (retinoic acid-inducible gene I).

Table legend

Table 1: Class of drug tested as a part of shock strategies, rationale and mechanism of action and relative stage of development.

List of abbreviations: HDACi (Histone Deacetylase Inhibitors), PKCA (PROTEIN KINASE AGONISTS), NF-kb (nuclear factor kappa-light-chain-enhancer of activated B cells), TLR7 (TOOL-LIKE RECEPTOR 7 AGONISTS), BETi (BROMODOMAIN EXTRATERMINAL INHIBITORS), p-TEFb (positive transcription elongation), Bromodomain-containing protein 4 (BRD4), PKB (Protein kinase B), AKT (Protein kinase B).

Table 2: List of histone deacetylase inhibitors, their use outside HIV infection and potential effect as latency reverting agents.

List of abbreviations: HDACi (Histone deacetylase inhibitors), HIV-1 CA-US-RNA (Human Immunodeficiency Virus-1 Cell Associated-Unspliced-RNA), FDA (Food and Drug Administration).

Table 3: Class of drug tested as a part of kill strategies, rationale and mechanism of action and relative stage of development.

List of abbreviations: Bcl-2 (B-cell lymphoma 2), PI3K/AKT (Phosphatidylinositol-4,5-bisphosphate 3-kinase/Protein kinase B), FOXO1 (Forkhead box protein O1), Smax (Second Mitochondria-derived Activator of Caspases), XIAP (X-linked inhibitor of apoptosis protein), RIG-1 (retinoic acid-inducible gene I), DexD/H (DexD/Helicase)

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Pharmacological family	Mode of action	Agents tested	Phase of development
HDACi	Inhibition of histone deacetylation→chromatin relaxion→transcription induction [129, 133]	- Valproic acid [34, 130]	- Proof-of-concept and multicenter crossover study
		- Vorinostat [30]	- Phase I-II
		- Panobinostat [132]	- Phase I-II
		- Romidepsin [71]	- Phase Ib/IIa
PKCA	Activation of PKC isoforms → iκB phosphorylation → NF-κB activation → P-TEFb elongation factor activation [55]	- Prostratin [58]	- *ex vivo*
		- Bryostatin-1 [131]	- *in vivo*
TLR7 AGONISTS	Recognition of single-stranded RNAs → expression of My-88 → cytokine production [63]	- Vesatolimod (GS-9620) [64.65.73]	- animal model - Ongoing Phase I trial
DISULFIRAM	Not clear involvement in PTEN depletion → phosphorylation of PKB → Akt pathway activation [51]	- Disulfiram [53, 54]	- Phase II
BETi	Inhibition of BRD4-P-TEFb binding → P-TEFb-Tat binding → transcription [49]	- JQ1 [48, 131]	- in *vivo*

Table 1: Class of drug tested as a part of shock strategies, rationale and mechanism of action and relative stage of development.

List of abbreviations: HDACi (Histone Deacetylase Inhibitors), PKCA (PROTEIN KINASE AGONISTS), NF-kb (nuclear factor kappa-light-chain-enhancer of activated B cells), TLR7 (TOOL-LIKE RECEPTOR 7 AGONISTS),

BETi (BROMODOMAIN EXTRATERMINAL INHIBITORS), p-TEFb (positive transcription elongation), Bromodomain-containing protein 4 (BRD4), PKB (Protein kinase B), AKT (Protein kinase B).

HDACi	OTHER USES	Increase of HIV-1 CA-US-RNA	Increase of plasmatic HIV-1 RNA	HIV-1 DNA reduction
VALPROIC ACID (31)	FDA-approved against seizures, migraine, bipolarism	YES	NO	NO
VORINOSTAT (34)	FDA-approved against cutaneous T-cell Lymphoma (122)	YES	NO	NO
PANOBINOSTAT (121)	FDA-approved against Multiple Mieloma (122)	YES	YES	NO
ROMIDESPIN(42)	FDA-approved against cutaneous T-cell Lymphoma and peripheral T-cell Lymphoma(122)	YES	YES	NO

Table 2: List of histone deacetylase inhibitors, their use outside HIV infection and potential effect as latency reverting agents.

List of abbreviations: HDACi (Histone deacetylase inhibitors), HIV-1 CA-US-RNA (Human Immunodeficiency Virus-1 Cell Associated-Unspliced-RNA), FDA (Food and Drug Administration).

Pharmacological family	Mode of action	Stage of development
Bcl-2 antagonist	Mimicking of BH3 with higher bindgin affinity for Bcl-2 binding → blockade of anti-apoptotic effect [29]	venetoclax and navitoclax (in vitro) [74, 132, 134]
PI3K/Akt inhibitors	Inhibition of Akt activation → activation of FOXO-1 transcrition factor → expression of pro-apoptotic molecules [29]	elefosine, perifosine a milefosine (in vitro) [75]
Smac mimetics/XIAP inhibitors	Competition for the binding with XIAP → activation of cascape 3, 7 and 9 [29]	Birinapant, AEG40730 –SM- (*in vitro*) [136] GDC-0152, embelin (*in vitro*) [137]
RIG-1 inducers	Rig-I is a DexD/H box RNA helicase that sense viral Rna → apoptosis induction [29]	Retinoi acid, retinoic acid-like, Acicretin (*in vitro*) [135]

Table 3: Class of drug tested as a part of kill strategies, rationale and mechanism of action and relative stage of development.

List of abbreviations: Bcl-2 (B-cell lymphoma 2), PI3K/AKT (Phosphatidylinositol-4,5-bisphosphate 3-kinase/Protein kinase B), FOXO1 (Forkhead box protein O1), Smax (Second Mitochondria-derived Activator of Caspases), XIAP (X-linked inhibitor of apoptosis protein), RIG-1 (retinoic acid-inducible gene I), DexD/H (DexD/Helicase)

Figure 1. Pharmacological classes of drugs with specific compounds, stage of development and potential application in the current strategies of HIV eradication.

[†]Clinical trial

[χ]Animal model

*In vitro/ex vivo

List of abbreviations: HDACi (Histone Deacetylase Inhibitors), TLR7 (TOOL-LIKE RECEPTOR 7 AGONISTS), BETi (BROMODOMAIN EXTRATERMINAL INHIBITORS), PD-1 (Programmed cell Death protein-1), Bcl-2 (B-cell lymphoma 2), PI3K/AKT (Phosphatidylinositol-4,5-bisphosphate 3-kinase/Protein kinase B), Smax (Second Mitochondria-derived Activator of Caspases), XIAP (X-linked inhibitor of apoptosis protein), RIG-1 (retinoic acid-inducible gene I).