Ongoing Clinical Trials of Human Immunodeficiency Virus Latency-Reversing and Immunomodulatory Agents

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In chronic human immunodeficiency virus (HIV-1) infection, long-lived latently infected cells are the major barrier to virus eradication and functional cure. Several therapeutic strategies to perturb, eliminate, and/or control this reservoir are now being pursued in the clinic. These strategies include latency reversal agents (LRAs) designed to reactivate HIV-1 ribonucleic acid transcription and virus production and a variety of immune-modifying drugs designed to reverse latency, block homeostatic proliferation, and replenish the viral reservoir, eliminate virus-producing cells, and/or control HIV replication after cessation of antiretroviral therapy. This review provides a summary of ongoing clinical trials of HIV LRAs and immunomodulatory molecules, and it highlights challenges in the comparison and interpretation of the expected trial results.

Keywords. anti-HIV agents; clinical trial; HIV-1; latency-reversing agents; viral reservoir.

Long-lived infected cells harboring human immunodeficiency virus (HIV) deoxyribonucleic acid (DNA) account for persistent viral reservoirs in blood and tissues during long-term effective antiretroviral therapy (ART). A cure for HIV may require complete eradication of these reservoirs (a “sterilizing cure”), or a cure may be achieved by the effective immune control of a persistent, but limited reservoir of infected cells (variably referred to as a “functional cure” or “remission”). Regardless of the strategy, the efficient targeting and reduction of the viral reservoir is a critical first step.

One strategy termed “shock and kill” is aimed at decreasing the numbers of latently infected cells after the activation of HIV transcription in order for host cells to produce HIV-1 proteins (“shock”); this will presumably allow the cells to be cleared by virus-associated cell death or by a host response (“kill”). This procedure would be performed under the cover of ART to prevent additional rounds of viral replication. Activation of HIV-1 production would ideally take place in the setting of minimal immune activation to avoid increasing inflammation and immune exhaustion.

A wide variety of compounds are under investigation as candidate latency-reversing agents (LRAs) for the shock step. These include the following: (1) histone deacetylases inhibitors (HDACis) and histone methyltransferases inhibitors, which play a major role in maintaining HIV latency through chromatin conformation-mediated repression of the viral long terminal repeat (LTR) [1–3]; (2) protein kinase C (PKC) agonists, which activate transcription factor nuclear factor-κB (NF-κB), leading to increased transcription from a wide variety of promoters including the HIV LTR, which contains 2 NF-κB binding sites [4–6]; (3) bromodomain extraterminal motif inhibitors, which enhance transcription factor positive transcriptional elongation factor b (P-TEFb) interaction with the HIV-1 LTR, thus activating HIV transcription [7, 8]; and (4) cytokine, chemokine, and Toll-like receptor (TLR) agonists, which stimulate immune signaling pathways in T cells, leading to transcriptional modifications and NF-κB activation [9, 10].

Early-phase clinical trials have demonstrated that these agents can be used to enhance HIV transcription. Published studies using the HDACis vorinostat and panobinostat as well as disulfiram all showed increased levels of cell-associated unspliced HIV ribonucleic acid (CA-RNA) or residual viremia [11–14] after LRA exposure. However, none of the studies demonstrated a significant reduction in the frequency of infected cells as measured by HIV DNA or quantitative viral outgrowth assay (QVOA). Since the publication of these studies, a substantial number of clinical trials have been initiated with a broadening range of LRA classes, dosing intervals, and combination strategies. This review will focus on ongoing phase I/II LRA clinical trials, testing single agents or combination interventions, as well as immunomodulatory molecules.

HISTONE DEACETYLASES INHIBITORS

Hydroxamic Acid Group

The major class of HDACi is the hydroxamic acid group, 3 compounds of which have US Food and Drug Administration
(FDA) approval for cancer therapy. Vorinostat (Zolinza, Merck) is a pan-HDACi given by the oral route for the treatment of relapsed or refractory cutaneous T-cell lymphoma [15]. In humans, vorinostat has the potential to increase histone acetylation and upregulate cellular transcription of CA-RNA in CD4+ cells [11,16], but vorinostat failed to demonstrate a reduction of the viral reservoir, measured by HIV-1 DNA quantification, and/or QVOA [16–18]. It of interest to note that a repeated dosing study of 5 participants, all of whom showed CA-RNA increases after a single dose of vorinostat, suggests a decline in the induction of HIV transcription after multiple doses [17].

Panobinostat (Farydak, Novartis) was approved in 2015 for the treatment of refractory or relapsed myeloma. Panobinostat was tested over 8 weeks as a single LRA in 15 ART-treated patients and increased plasma HIV RNA levels and CA-RNA in total CD4+ cells, but there was no cohort-wide reduction in the total or integrated HIV DNA levels, and viral rebound occurred in all patients undergoing an analytical treatment interruption (ATI) [12].

Ongoing clinical trials of hydroxamate-based HDACis are listed in Table 1. Vorinostat is currently being tested in 4 clinical trials. It is being evaluated as a single LRA in a repeated administration design over 8 weekly cycles in 30 participants (Table-trial: 1–1). Three phase I/II clinical trials include vorinostat as part of a combination strategy. RIVER is a randomized controlled open-label trial in 52 participants with primary HIV infection. Participants will be randomized 1:1 to receive either ART or ART with therapeutic vaccination followed by 10 doses of vorinostat (1–2). Vaccination will combine a ChAdV-HIVconsv prime (chimpanzee adenovirus 63-HIVconsv chimeric gene, nonreplicating vector) and MVA-HIVconsv (modified vaccine ankara-HIVconsv gene, nonreplicating vector) boost, both designed to elicit broad cytotoxic responses towards the 14 most conserved regions of the viral proteome (“consv” construct). Safety and immunogenicity of this vaccine strategy were demonstrated in the phase I trial HIV-CORE002, with broad T responses and inhibition of HIV-1 replication in vitro in autologous CD4+ cells [19–21]. The VOR VAX study will evaluate the AGS-004 dendritic cell therapy followed with 10 doses of vorinostat, with the primary outcome the changes in quantity of infectious units per million resting CD4+ T cells by the viral outgrowth assay (QVOA) (1–3). AGS-004 is an immunotherapy method that loads the participant’s dendritic cells with autologous viral RNAs encoding HIV antigens. In a recent phase IIb study of AGS-004 including an ATI, HIV-specific effector memory CD8+ cells were induced in the treatment arm, although there was no significant impact on plasma viremia or the frequency of cells harboring HIV-integrated DNA [22]. The SEARCH 019 study is enrolling participants who initiated ART during acute infection and randomizing them to either continued ART or ART with a combination of vorinostat, hydroxychloroquine, and maraviroc (VHM) (1–4). It has been hypothesized that hydroxychloroquine could potentiate the activity of vorinostat through inhibition of autophagy, and this combination has been evaluated for safety in the oncology setting [23]. The first results from 14 SEARCH 019 participants (9 VHM + ART, 5 ART) were presented at the 21st International AIDS Conference 2016. Vorinostat, hydroxychloroquine, and maraviroc induced low-level plasma viremia. There were no changes in total HIV-1 DNA in peripheral blood mononuclear cells (PBMCs) and no difference in viral rebound off treatment between both arms, underlining the difficulty in reducing HIV reservoirs even in participants who started ART early in the course of infection [24].

Another registered trial (NCT01193842) is currently testing vorinostat along with chemotherapy in HIV-infected patients treated for B-cell lymphoma with a primary focus on its antitumor effects. Secondary outcomes include changes in plasma HIV viral load and CD4+ cell counts.

Panobinostat is being tested in the randomized ACTIVATE trial. This study will evaluate panobinostat alone or in combination with immune stimulation by subcutaneous polyethylene glycol-interferon (IFN)-α2a in patients suppressed on ART for more than 2 years. Due to toxicity concerns, this trial is planned as a dose-escalation study with the final dose of panobinostat lower than that used for myeloma treatment (1–5). Interferon-α2a enhances antiviral immune responses; it upregulates anti-HIV cellular restriction factors and interferes with the viral replication cycle and hence may act as a killing agent. When used for the treatment of HCV, IFN-α decreased CA-DNA levels in CD4+ T cells [25, 26]. Entinostat and givinostat, 2 class 1 HDACis, exhibit potent latency reversal activity ex vivo [27, 28], but these inhibitors have not been tested in clinical trials so far.

**Benzamides**

Chidamide (Epidaza, Chipscreen Biosciences) is the only member of the benzamide group of HDACi molecules that is being evaluated in HIV-infected individuals (1–6). Chidamide was approved for the treatment of peripheral T-cell lymphoma in China in 2015, and it is currently under study for breast cancer and nonsmall cell lung cancer therapy. There is no published preclinical data available in PubMed on the effect of this compound on HIV-infected cells.

**Short-Chain Fatty Acids**

Valproic acid (VPA) has been used for decades for the treatment of epilepsy and is a weak HDACi [29]. The first trial of VPA as a HDACi in 4 HIV-infected patients in 2005 showed significant reduction of the viral reservoir measured by QVOA at week 18 [30]. However, further trials in larger numbers of participants did not confirm the reduction of the reservoir [31, 32], and VPA is not currently being investigated in active LRA trials.

Sodium butyrate has been known for years to increase transcription of the HIV-1 LTR in cell lines [33, 34] and ex vivo [35]. It also displayed latency reversing activity towards the feline
<table>
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<td>1</td>
<td>NCT01319383</td>
<td>Vorinostat</td>
<td>ChAdVV3.HIVconsv + MVA.HIVconsv</td>
<td>400 mg/d x 28 d starting W32</td>
<td>—</td>
<td>ART &gt; 18 mo, VL &lt; 50 cp/mL for 24 wks, CD4 &gt; 300 µL</td>
<td>30</td>
<td>1</td>
<td>Step I - VOR single group</td>
<td>15 wks</td>
<td>N</td>
<td>CA-RNA, CA-DNA, SCA-VL, QVOA</td>
<td>Recruiting</td>
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<td>2</td>
<td>NCT02338074</td>
<td>Vorinostat</td>
<td>ChAdVV3.HIVconsv + MVA.HIVconsv</td>
<td>10 x 400 mg/d x 28 d starting W32</td>
<td>5 x 10^10vp W24 Boost 2 x 10^9vp W32</td>
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<td>VOR VAX</td>
<td>Vorinostat</td>
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<td>4 injections x 2 cycles</td>
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<td>N</td>
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<td>NCT02475915</td>
<td>VOR VAX</td>
<td>SEARCH 018</td>
<td>400 mg/d x 14d of W0, W4, W8</td>
<td>200 mg x 150-600 mg/d x 10 wks</td>
<td>ART &gt; 42 wks (started during PHI) VL &lt; 50 cp/mL for 2 cycles CD4 &gt; 450 µL</td>
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<td>2</td>
<td>ART only/ART only</td>
<td>Phase I (I) randomized open label</td>
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<td>Y</td>
<td>Rebound features, CA-RNA, CA-DNA, 2-LTR</td>
<td>Ongoing</td>
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<tr>
<td>5</td>
<td>NCT02471430</td>
<td>Vorinostat</td>
<td>SCA-VL, QVOA</td>
<td>5, 10, 15 mg Q0, D1, D2</td>
<td>180 µg SC on W0</td>
<td>ART &gt; 2 yr, VL &lt; 50 cp/mL for 2 yrs CD4 &gt; 400 µL</td>
<td>8</td>
<td>2</td>
<td>—</td>
<td>Phase II (VL) randomized open label</td>
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<td>N</td>
<td>CA-RNA, CA-DNA, QVOA, 2-LTR, VL</td>
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<tr>
<td>6</td>
<td>NCT02513901</td>
<td>Chidamide</td>
<td>CHARTER</td>
<td>—</td>
<td>10 mg x 2 wk or 30 mg x 2/ w for 4 wks</td>
<td>ART &gt; 18 mo, RNA &lt; 50 for &gt;1 yr CD4 &gt; 350/µL</td>
<td>12</td>
<td>2</td>
<td>—</td>
<td>Phase I (I) single site open label</td>
<td>10 wks</td>
<td>N</td>
<td>CA-RNA, CA-DNA, VL</td>
<td>Recruiting</td>
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<tr>
<td>7</td>
<td>NCT02092116</td>
<td>Romidepsin</td>
<td>REDUC</td>
<td>5 mg/m² x 4 wk weekly for 3 wks</td>
<td>1.2 mg x 0.33 x 10^5 IU x 6 over 12 wks</td>
<td>ART &gt; 1 yr, RNA &lt; 50 cp/mL for &gt;1 yr CD4 &gt; 500 µL</td>
<td>26</td>
<td>2</td>
<td>RMD safety/ RMD + Vacc-4x and ATI</td>
<td>Phase II (II) single group open label</td>
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<td>Rebound features, CA-RNA, CA-DNA, QVOA, VL, anti-HIV responses</td>
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<td>8</td>
<td>NCT01933594</td>
<td>Romidepsin</td>
<td>—</td>
<td>0.5 or 2 or 5 mg/m²</td>
<td>—</td>
<td>ART (EFV, RAL or DTG) VL &lt; 50 cp/mL for 1 yr, SCA-VL &gt; 0.4 cp/mL at screening, CD4 &gt; 300 µL</td>
<td>45</td>
<td>6</td>
<td>RMD or placebo + x 3 dosing</td>
<td>Phase I (II) multiclasser placebo-controlled randomized double-blind, dose-finding</td>
<td>4 wks</td>
<td>N</td>
<td>CA-RNA, SCA-VL</td>
<td>Recruiting</td>
</tr>
<tr>
<td>9</td>
<td>NCT02616874</td>
<td>Romidepsin</td>
<td>MVA.HIVconsv vaccine</td>
<td>5 mg/m², W0, W4, W5</td>
<td>10^8 pfu: W0 + W9</td>
<td>Subjects from ChAdMVA-HIVconsv_BCN01 study + BCN01-RI extension study. ART with RAL or DTG, VL &lt; 50 cp/mL for 3 yrs, CD4 &gt; 600 µL</td>
<td>24</td>
<td>1</td>
<td>—</td>
<td>Phase I single group open label</td>
<td>52 wks</td>
<td>Y</td>
<td>Rebound features, CA-RNA, CA-DNA, SCA-VL, VL</td>
<td>Pre-enrollment</td>
</tr>
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<td>10</td>
<td>RV 438</td>
<td>Romidepsin</td>
<td>3BNC117 Ab</td>
<td>5 mg/m² W0, W1, W2, W3</td>
<td>30 mg/kg: W0, W12, W24, W27</td>
<td>ART &gt; 2 yrs started during PHI VL &lt; 50 cp/mL, CD4 &gt; 400 µL</td>
<td>42</td>
<td>4</td>
<td>RMD + 3BNC117/ RMD only/ 3BNC117 only/no intervention</td>
<td>Phase II (II) randomized open label</td>
<td>78 wks</td>
<td>Y</td>
<td>Control &lt;50 cp/mL, rebound features, reservoir markers</td>
<td>Pre-enrollment</td>
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<td>11</td>
<td>NCT02269005</td>
<td>Bryostatin</td>
<td>BRYOLAT</td>
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<td>10 or 20 µg/m²</td>
<td>—</td>
<td>ART &gt; 2 yrs, VL &lt; LOD, CD4 &gt; 350/µL</td>
<td>12</td>
<td>3</td>
<td>—</td>
<td>Phase I randomized placebo-controlled double-blind dose-finding</td>
<td>4 wks</td>
<td>N</td>
<td>CA-RNA, VL, 2-LTR</td>
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<tr>
<td>12</td>
<td>NCT02531295</td>
<td>Euphorbia kansui</td>
<td>Euphorbia kansui</td>
<td>—</td>
<td>—</td>
<td>ART &gt; 3 yrs, VL &lt; 40 cp/mL for 3 yrs, CD4 &gt; 350 µL</td>
<td>9</td>
<td>3</td>
<td>—</td>
<td>Phase I single group open label</td>
<td>4 wks</td>
<td>N</td>
<td>CA-RNA, VL, T-cell activation</td>
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<td>13</td>
<td>ANRS VR04470</td>
<td>rhIL7</td>
<td>HIV-Lipo5 DC therapy</td>
<td>20 µg/kg: W0 W1 W2 or W4 W8 W12 W16</td>
<td>30 x 10^6 DCs: W4 W6 W12 W16</td>
<td>ART &gt; 12 mo, VL &lt; 50 &gt; 60 mo, CD4 &gt; 500 µL</td>
<td>80</td>
<td>4</td>
<td>RMD only/ DC vaccine/ DC + IL7/IL7 before DC/IL7</td>
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<td>N</td>
<td>CA-DNA in blood and rectal biopsies</td>
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<tr>
<td>14</td>
<td>—</td>
<td>GS-9620</td>
<td>—</td>
<td>—</td>
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<tr>
<td>15</td>
<td>NCT02443935</td>
<td>GMN1703</td>
<td>TEACH</td>
<td>60 mg x 2 wk for 4 wks</td>
<td>—</td>
<td>ART &gt; 1 yr, VL &lt; 50 cp/mL CD4 &gt; 350 µL</td>
<td>16</td>
<td>1</td>
<td>—</td>
<td>Phase II (II) open label 2</td>
<td>12 wks</td>
<td>N</td>
<td>CA-RNA, CA-DNA, VL</td>
<td>Ongoing</td>
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<td>16</td>
<td>NCT02071095</td>
<td>Poly-ICLC</td>
<td>—</td>
<td>1.4 mg: D1, D2</td>
<td>—</td>
<td>ART &gt; 48 wks, VL &lt; 50 cp/mL for 48 wks, baseline CA-RNA &gt; 10 cp/µg RNA, CD4 &gt; 500 µL</td>
<td>15</td>
<td>2</td>
<td>—</td>
<td>Phase II (II) placebo-controlled randomized double blind</td>
<td>48 wks</td>
<td>N</td>
<td>CA-DNA, CA-DNA, VL</td>
<td>Ongoing</td>
</tr>
</tbody>
</table>

Abbreviations: Ab, antibody; ART, antiretroviral therapy; ATI, analytical treatment interruption; CA-DNA, cell-associated HIV-1 DNA; CA-RNA, cell-associated unspliced HIV-1 RNA; DC, dendritic cell; DNA, deoxyribonucleic acid; DTG, dolutegravir; EFV, efavirenz; GM-CSF, granulocyte-macrophage colony-stimulating factor; HIV, human immunodeficiency virus; IFN, interferon; IL, interleukin; IV, intravenous; peg, pegylated; pfu, plaque-forming units; PHI, primary HIV infection; RAL, raltegravir; QVOA, viral outgrowth assay; rh, recombinant human; RMD, romidepsin; RNA, ribonucleic acid; SCA-VL, single-copy assay ultrasensitive viral load; VL, plasma viral load; VOR, vorinostat; vp, viral particle; 2-LTR, 2-long terminal repeat circles.
immunodeficiency virus [36], but it has not been tested in humans.

Cyclic Tetrapeptides
Romidepsin (Istodax, Celgene) was granted FDA approval in 2009 for the treatment of refractory T-cell lymphoma. In simian immunodeficiency virus (SIV)-infected ART-treated macaques, romidepsin infusions increased plasma viremia, but viral rebound kinetics were similar in romidepsin-treated and untreated animals after ART discontinuation [37]. In the REDUC study (1–7), 6 ART-treated participants received 3 weekly infusions of romidepsin at the reduced dosage of 5 mg/m² (oncology dosage is 14 mg/m²). Romidepsin infusions led to peaks in CA-RNA in resting CD4⁺ cells approximately 30 minutes after completion of each infusion, and to plasma HIV-1 RNA viral load blips above 20 copies/mL in 5 of 6 participants, peak viremia being highest after the second infusion. However, there were no overall changes in the numbers of latently infected cells after treatment as quantified by cell-associated HIV DNA and QVOA [38].

A multicenter, randomized, double-blind controlled dose-escalation study is currently underway through the AIDS Clinical Trials Group ([ACTG] A5315) to evaluate the safety and latency reversal activity of single and multiple doses of romidepsin (0.5, 2, 5 mg/m²) (1–8). The primary outcome will be the induction of HIV-1 transcription by CA-RNA in CD4⁺ T cells at 24 hours and ultrasensitive plasma viral load at 48 hours.

Two ongoing trials are testing a combination strategy of 3 weekly infusions of 5 mg/m² romidepsin with anti-HIV therapeutic vaccines. The second arm (phase II) of the REDUC study (1–7) combines romidepsin with the Vacc-4x anti-HIV vaccine to improve anti-HIV cytotoxic responses and enhance reservoir clearance. Preliminary results were presented at the 2016 Conference on Retroviruses and Opportunistic Infections (CROI) [39]: there was a provocative trend suggesting that the magnitude of romidepsin-mediated latency reversal (as defined by viremia) was lower in the vaccinated subjects than in the unvaccinated subjects, suggesting that vaccine-mediated enhancement of immune function was efficiently clearing virus-producing cells. However, the time to viral rebound over 50 cp/mL in a monitored ATI was generally rapid, at a median of 14 days. The BCN02-Romi trial (1–9), starting in 2016, is recruiting participants from the BCN01 phase I study of ChAdV63-HIVconsv and MVA-HIVconsv in 0–8 weeks and 0–24 weeks prime-boost schedules in HIV-infected volunteers (NCT01712425). Participants will receive 2 booster injections of MVA-HIVconsv, before and after treatment with romidepsin.

The RV438 trial will test a combination of romidepsin and 3BNC117 antibody in participants who initiated ART during acute infection (1–10). 3BNC117 is a broadly neutralizing monoclonal antibody targeting HIV CD4 binding site and is reported to significantly reduce viremia in HIV-infected individuals [40–42]. The study design includes an antiretroviral pause after 2 cycles of romidepsin + 3BNC117, with the primary endpoint the time to detectable viremia over 50 cp/mL.

NUCLEAR FACTOR-κBp SIGNALING

Protein Kinase C Agonists
Bryostatin 1, derived from the marine organism Bugula neritina, is a strong PKC activator. Protein kinase C signaling pathways are involved in many cellular functions, and bryostatin has originally been studied as an anticancer agent. It is currently investigated as a memory enhancer in severe Alzheimer’s disease for its impact on synapse formation and function, and it is also investigated as a potent activator of NF-κB in HIV latency reversal studies. Bryostatin displayed latency reversal activity in vitro and ex vivo, both by itself and in combination with other LRAs [43–46]. The BRYOLAT study was a phase 1, randomized, double-blind, placebo-controlled dose-finding study of bryostatin in HIV-1 infected participants on suppressive ART (1–11). Due to concerns regarding the toxicity of this drug class, a very conservative dose escalation study was performed. Four participants were included in each treatment arm (10 µg/m² and 20 µg/m²) and received a single dose of bryostatin. There were no changes in plasma viral load or in CA-RNA levels compared with the placebo group. Of note, plasma concentrations of bryostatin were very low at all time points, and there was no modification of PKC activity in CD4⁺ cells during the study [47]. Future studies will need to explore either higher doses or combinations of low-dose bryostatin and other LRAs.

Ingenols are bioactive compounds extracted from Euphorbia pechpegae and have latency reversal activity in HIV-infected cell lines and ex vivo CD4⁺ T cells [5, 48, 49], moreover displaying synergy with other PKC agonists and latency-reversing drugs [46, 50, 51]. A phase 1 clinical trial of oral Euphorbia kansui (as a tea) is planned and will address the safety of ingenols in vivo and focus on plasma viral load and CA-RNA quantification over 31 days, after daily intake of 1 g kansui extract powder for 1 to 3 days (1–12).

Cytokines, Chemokines, and Toll-Like Receptor Agonists
Interleukin (IL)-7 plays a major role in homeostasis and survival of T cells. The IL-7 receptor belongs to the common γ-chain cytokine receptor family (IL-2, IL-4, IL-7, IL-9, IL-15, IL-21), essential for the establishment and function of immune cells. Conflicting data is available concerning the latency-reversing ability of IL-7 in infected resting CD4⁺ T cells [52–54]. A trial of IL-7 combined with raltegravir and maraviroc intensification was recently reported to transiently increase CA-DNA levels in CD4⁺ cells, with no change in HIV-1 DNA in PBMCs or rectal tissue over 18 months [55]. The VRI04-DALIA2 trial (1–13) will investigate the effects of IL-7 combined with a boost of anti-HIV responses with the dendritic cell-based therapeutic vaccine LIPO-5-DC. This therapeutic vaccine was shown to
be well tolerated and immunogenic, and the induction of poly-functional T cells was inversely correlated with viral load after an ATI [56, 57].

Toll-like receptors are activator immune receptors involved in non-specific pathogen recognition. During infection, TLR signaling promotes inflammation, priming of innate responses, and intake and processing of non-self-antigens by antigen-presenting cells. The TLR agonists may act both as enhancers of anti-HIV responses and as indirect LRAs in the setting of T-cell activation by a proinflammatory environment. In addition, TLR agonists were also reported to reactivate HIV transcription in T cells [58], even in the absence of T-cell activation and proliferation, through NF-kB/NFAT/AP-1 pathways [59]. GS-9620, an agonist of TLR7 originally developed for the treatment of chronic viral hepatitis, led to virus production after ex vivo treatment of PBMCs from HIV-1-infected ART-treated donors [60] and elicited recurrent plasma viral load blips after administration in ART-treated rhesus macaques [61]. A phase 1 trial of GS-9620 is now ongoing (1–14). Alternatively, the TEACH study in Denmark is testing a course of 8 injections of the TLR9 agonist MGN1703 over 4 weeks (1–15), after a decrease in HIV-1 DNA was observed in participants of a trial testing the TLR9 agonist CpG7909 as an adjuvant for anti-pneumococcal vaccination [62]. MGN1703 was recently reported to moderately enhance HIV CA-RNA transcription and increase activation of natural killer (NK) cells and T cells ex vivo along with inhibition of HIV production by autologous CD4+ T cells [63]. Lastly, a randomized, placebo-controlled, double-blind trial is underway investigating the potential of a 2-day course of a TLR3 agonist (Poly-ICLC) to reactivate HIV-1 production and as a vaccine adjuvant (1–16). Short-term TLR3 activation has been associated with upregulation of transcription factors, increased histone acetylation, and elevated levels of HIV transactivation [64].

Disulfiram
Disulfiram has been used for over 60 years in the treatment of alcohol dependence. Disulfiram was shown to increase HIV production in the BCL-2 cellular model of HIV latency, likely via activation of Akt signaling and downstream enhancement of NF-kB [65, 66]. In a phase I trial of 14 days of disulfiram in HIV-infected participants, there was no change in plasma HIV RNA (using an ultrasensitive viral load assay) and the frequency of latently infected cells (by QVOA) through day 84 [13]. A further phase II dose-escalating trial recently investigated 3-day courses of 500 mg, 1000 mg, or 2000 mg/day disulfiram and reported an increase in CA-RNA by approximately 2-fold, present as early as 2 hours after administration and sustained for 30 days with a modest but significant increase in plasma HIV RNA in participants who received 2000 mg/day [14].

Immune-Based Therapies
In addition to IL-7 and TLR agonists, other immune-based therapies are being evaluated as part of HIV cure trials for their potential to boost HIV-specific immune responses; such drugs could be effective in combination strategies with LRAs, and ongoing trials in HIV-1 infection are reviewed in Table 2.

“Immune checkpoints” are the set of complex regulatory pathways that restrain immune responses in order to maintain self-tolerance and prevent the harmful effects of overreactivity. Programmed-death receptor 1 (PD-1) signaling promotes energy and apoptosis in T cells. Inhibition of this negative signal with anti-PD-1 or anti-PD-1 ligand (PD-L1) monoclonal antibodies may boost T-cell responses and is under investigation both in a variety of solid and hematopoietic malignancies as well as in HIV infection [67, 68]. In HIV-infected participants, the administration of anti-PD-1 immunotherapy is being evaluated in participants with advanced solid tumors or lymphoma, in 2 trials running through 2018 and 2020, respectively. Pembrolizumab (MK3475) monotherapy will be given every 3 weeks for up to 2 years (2–1). Alternatively, a dose-defining study for nivolumab (BMS-936558) given every other week combines anti-PD-1 treatment with the other immune checkpoint inhibitor ipilimumab (an anti-CTLA-4 monoclonal antibody) for the first 4 cycles of treatment, before maintenance with nivolumab only (2–2). A trial of anti-PD-L1 monoclonal antibody BMS-936559 (ACTG A5326) was originally scheduled to enroll 56 participants in a phase 1 dose-escalation study, but it was halted after enrolling 8 participants (6 drug, 2 placebo) at the lowest dose of 0.3 mg/kg due to the concern for ocular toxicity in animal studies (2–3). In a presentation at CROI 2016 [69], 2 of the 6 participants who received 0.3 mg/kg had increased HIV-specific T-cell responses and reversal of CD8+ T-cell exhaustion. One individual was diagnosed with pituitary insufficiency (hypophysitis) several months after completion of the trial, which is a well known complication of nivolumab in the treatment of malignancy [70].

Other strategies aiming at enhancing T-cell responses involve chemokines agonists and stimulation with IFN-α as well as the reduction of baseline inflammation. Interleukin-15 superagonist ALT-803 (IL-15N72D + IL-15RαSu/Fc fusion protein) is a modified version of IL-15 with enhanced potency to boost NK and T-cell responses, and it has a potential role in activating HIV transcription and boosting cytotoxic T-cell activity [71, 72]. ALT-803 will be investigated in a phase 1 dose-escalating study of weekly injections over 4 weeks, during which the size of the inducible reservoir will be estimated by quantification of early intracellular multisliced HIV RNA after ex vivo stimulation of CD4+ cells (Tat/Rev-induced limiting dilution assay [TILDA]) (2–4).

Interferon-α is a proinflammatory cytokine supporting antiviral immune responses. It is approved for the treatment of viral hepatitis C. Polyethylene glycol-IFN-α2a was reported to decrease integrated HIV-1 DNA levels in PBMCs and delay rebound after ATI in a small cohort [25]. In addition to combination studies with HDACi (1–5), it is now under
<table>
<thead>
<tr>
<th>No.</th>
<th>Clinical Trials</th>
<th>Agent 1</th>
<th>Agent 2</th>
<th>Dosing 1</th>
<th>Dosing 2</th>
<th>Inclusion Criteria</th>
<th>N</th>
<th>Arms</th>
<th>Design</th>
<th>Follow-up</th>
<th>HIV Outcome</th>
<th>Status</th>
<th>Completion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NCT02595866 CITN-12</td>
<td>Pembrolizumab</td>
<td>—</td>
<td>200 mg every 21 d</td>
<td>—</td>
<td>Advanced malignancy, ART &gt; 4 wks, VL &lt; 200 cp/mL CD4 &gt; 100 µL</td>
<td>36</td>
<td>1 arm</td>
<td>Phase I single group open label</td>
<td>104 wks</td>
<td>CA-RNA, SCA- VL, TILDA, integrated HIV-1 DNA</td>
<td>Pre-enrollment</td>
<td>2018</td>
</tr>
<tr>
<td>2</td>
<td>NCT02408861 AMC095</td>
<td>Ipilimumab</td>
<td>Nivolumab</td>
<td>3 mg/kg D1, D15, D29, D43 or D1, D22, D43, D64 Maintenance every 14 d</td>
<td>1 mg/kg D1, D22, D43, D64 with nivolumab</td>
<td>Advanced solid tumor malignancy, VL &lt; 75 cp/mL with or w/o cART, CD4 &gt; 200 µL</td>
<td>42</td>
<td>2 arms nivolumab 2 arms nivolumab + ipilimumab</td>
<td>Phase I single group open label, dose-finding</td>
<td>156 wks</td>
<td>VL, QVOA</td>
<td>Recruiting</td>
<td>2020</td>
</tr>
<tr>
<td>3</td>
<td>NCT02028403 BMS-936559</td>
<td>—</td>
<td>—</td>
<td>Single dose: 0.3 mg/kg; 1 mg/kg; 3 mg/kg; 10 mg/kg</td>
<td>—</td>
<td>ART, VL below limit for 2 yrs, SCA &gt; 0.4 cp/mL in last 3 mo, CD4 &gt; 350 µL</td>
<td>56</td>
<td>4 arms: 4 doses</td>
<td>Phase I multicenter randomized double-blind placebo-controlled, dose-escalation</td>
<td>48 wks</td>
<td>CA-RNA, CA-DNA, 2-LTR, SCA-VL, anti-gag CD8 responses</td>
<td>Completed</td>
<td>2015</td>
</tr>
<tr>
<td>4</td>
<td>NCT02191098 ALT-803</td>
<td>—</td>
<td>—</td>
<td>Weekly injection</td>
<td>—</td>
<td>ART &gt; 3 yrs, VL &lt; 50 cp/mL for 2 yrs, CD4 &gt; 500 µL</td>
<td>10</td>
<td>1 arm</td>
<td>Phase I open label single arm, dose-escalation</td>
<td>4 wks</td>
<td>TILDA</td>
<td>Pre-enrollment</td>
<td>2016</td>
</tr>
<tr>
<td>5</td>
<td>NCT01935089 peg-IFNα2b</td>
<td>—</td>
<td>—</td>
<td>1 µg/kg per wk for 20 wks</td>
<td>—</td>
<td>ART &lt; 1 yr, VL &lt; 60 cp/mL for 1 yr, CD4 &gt; 450 µL</td>
<td>25</td>
<td>1 arm</td>
<td>Phase II single group open label</td>
<td>24 wks</td>
<td>CA-DNA</td>
<td>Ongoing</td>
<td>2016</td>
</tr>
<tr>
<td>6</td>
<td>NCT01295515 peg-IFNα2b</td>
<td>—</td>
<td>—</td>
<td>Weekly injection (HCV dose) for 4 wks</td>
<td>—</td>
<td>ART, VL &lt; 50 cp/mL for 1 yr, CA-RNA &gt; 5 cp/10^6 PBMC, CD4 &gt; 300 µL</td>
<td>65</td>
<td>1 arm</td>
<td>Phase II/II single group open label</td>
<td>52 wks</td>
<td>VL, CA-RNA</td>
<td>Recruiting</td>
<td>2020</td>
</tr>
<tr>
<td>7</td>
<td>NCT02475655 Ruxolitinib</td>
<td>—</td>
<td>—</td>
<td>10 mg x 2/d for 5 wks</td>
<td>—</td>
<td>ART &gt; 2 y, VL &lt; 50 &gt; 1 yr CD4 &gt; 350</td>
<td>60</td>
<td>2 arms: ART + ruxolitinib/ ART only</td>
<td>Phase II multicenter randomized open label</td>
<td>12 wks</td>
<td>SCA-VL, CA-RNA, CA-DNA, integrated HIV-1 DNA, 2-LTR</td>
<td>Pre-enrollment</td>
<td>2016</td>
</tr>
<tr>
<td>8</td>
<td>NCT02440789 Sirolimus</td>
<td>—</td>
<td>—</td>
<td>0.025 or 0.05 mg/kg per day for 20 wks</td>
<td>—</td>
<td>ART &gt; 2 yrs, VL below limit for 2 yrs, CD4 &gt; 400/µL</td>
<td>30</td>
<td>2 arms: 2 doses depending on ART regimen</td>
<td>Phase II/II multicenter single group open label</td>
<td>44 wks</td>
<td>CA-RNA, CA-DNA, SCA-VL, anti-gag CD8 responses, VL</td>
<td>Recruiting</td>
<td>2017</td>
</tr>
<tr>
<td>9</td>
<td>NCT02429869 Everolimus</td>
<td>—</td>
<td>—</td>
<td>Target trough level 3-8 ng/mL for 6 mo</td>
<td>—</td>
<td>Liver/kidney transplant, ART, VL &lt; 50 cp/mL for 2 yrs, CD4 &gt; 350 µL</td>
<td>10</td>
<td>1 arm</td>
<td>Phase IV single group open label</td>
<td>52 wks</td>
<td>CA-DNA</td>
<td>Pre-enrollment</td>
<td>2017</td>
</tr>
</tbody>
</table>

Abbreviations: ART, antiretroviral therapy; CA-DNA, cell-associated HIV-1 DNA; CA-RNA, cell-associated unspliced HIV-1 RNA; DNA, deoxyribonucleic acid; HIV, human immunodeficiency virus; IFN, interferon; PBMC, peripheral blood mononuclear cells; peg, pegylated; QVOA, viral outgrowth assay; RNA, ribonucleic acid; SCA-VL, single-copy assay ultrasensitive viral load; TILDA, Tat/Rev-induced limiting dilution assay; VL, plasma viral load; 2-LTR, 2-long terminal repeat circles.
investigation as a single agent in 2 new trials, evaluating the effects of low dosing (1 µg/kg per week) on HIV-1 DNA quantification in CD4+ cells (2–5) and of full dose (180 µg/week) on viremia and CA-RNA (2–6).

Chronic inflammation within disease tissue upregulates a number of immunosuppressive pathways. These complex signaling environments generally prevent optimal T-cell responses. Reversing these pathways to enhance adaptive immunity is a central theme in ongoing cancer research [73] and increasingly HIV cure research [74, 75]. Ruxolitinib is a specific inhibitor of Janus kinases 1 and 2 approved for the treatment of myelofibrosis. It inhibits the JAK/STAT cascade downstream of cytokine signaling, thus reducing immune overactivity, inflammation, and potentially immune exhaustion. The impact of ruxolitinib on HIV-1 reservoir will be investigated for the first time in a multicenter randomized trial of 5 weeks of daily treatment (2–7). Data collection will include soluble and cellular markers of inflammation and activation in addition to viral load and reservoir quantification. Another class of anti-inflammatory candidates for the immunomodulation of anti-HIV responses are mammalian target of rapamycin (mTOR) inhibitors, widely used to prevent organ transplant rejection. These drugs reduce T-cell activation/proliferation, reduce CCR5 expression [76], and enhance formation of memory responses (in nonhuman primates) [77]. Sirolimus has been linked to lower HIV DNA levels posttransplant [78] and is being evaluated in a phase I/II dose-defining trial in HIV-infected participants without other immunosuppressive drugs (ACTG A5337, 2–8). Everolimus is a derivative of sirolimus, and its effects on the viral reservoir will also be studied in a phase IV study of cell-associated HIV-1 DNA changes after kidney or liver transplantation (2–9).

Challenges and Future Directions
The existence of elite controllers (ECs) and posttreatment controllers (PTCs) of HIV infection suggests that the goal of sustained HIV remission is achievable. Both ECs and PTCs have relatively small viral reservoirs compared with HIV progressors [79–83], and ECs combine these characteristics with robust anti-HIV immune responses [84, 85]. A combination of individual and/or viral factors are most likely to participate in the control of viral replication, and model predictions of viral dynamics indeed suggest that the right combination of reservoir reduction and immune boosting may lead to sustained virologic remission in infected individuals [86]. As summarized above, there are a variety of pathways and strategies that are undergoing early-phase clinical trials. One challenge to the interpretation of these trials is the lack of a well validated biomarker that reliably predicts a delayed viral rebound timing. The heterogeneity of primary outcome measures amongst the studies highlights the need for reliable surrogate markers of clinical outcomes such as sustained HIV remission [87, 88].

In addition to the LRAs described here, new latency-reversing candidates are being evaluated, both from new chemical classes (e.g., triazoles, Galcetin 9) and from amongst well known drugs marketed for other indications (DNA methylation inhibitor 5-aza-2’-deoxycytidin) [89–91]. These agents may become available for in vivo trials after completion of preclinical phases.

The LRA trials have been complicated by concerns surrounding safety, acceptability, and transmission risk in participants who are doing well on ART and will almost certainly receive no clinical benefit from early-phase studies. The design of such trials must balance the potential side effects and risks with the potential scientific gain and long-term benefits. Many LRA candidates were initially developed as antineoplastic agents and have significant dose-limiting toxicities. Trials may require dosing or administration frequency different from oncology care standards given the lower tolerance for toxicities in the nononcology population. This is the reason behind the low starting dose used for several LRA clinical trials, including studies with panobinostat (1-5) and bryostatin (BRYOLAT, 1–10). In addition, the ACTG A5326 study of anti-PD-L1 was discontinued after enrolling the lowest dose cohort due to concerns about ocular toxicity seen in animal models and occurrence of hypophysitis in 1 participant [69].

Although many compounds have been identified as potent LRAs both in vitro and in clinical trials, these findings have not been accompanied by reductions in the size of the reservoir [11, 12, 38]. One potential cause is the limited potency of the currently studied compounds and dosing strategies: these clinical trials involved only single or relatively few doses of LRAs. The use of repeated dosing has yielded inconsistent results: the induction of replication-competent HIV-1 virus from latent CD4+ cells was strongest after the first dose in a vorinostat study [17], whereas repeated dosing has been associated with potentially increased activity of other LRAs [12, 38, 61]. Optimized dosing and inter-dose intervals may further improve latency reversing activity. The development of compounds with greater specificity may enhance the potential of these drugs in reversing HIV-1 latency, particularly if given in combination. For instance, ex vivo studies have shown that PKC agonists may synergize with both bromodomain inhibitors and HDACis [92, 93], and 5-aza-2‘-deoxycytidin can potentiate the effect of HDACi with a sequential dosing schedule [91]. Another possible reason for the absence of detectable reservoir reduction is the inefficient clearance of reactivated cells. This was demonstrated in ex vivo studies performed by Shan et al [84] showing that cytotoxic T lymphocytes (CTLs) from ART-treated participants did not efficiently eliminate HIV-expressing cells, in contrast to those from HIV ECs. However, this activity could be stimulated in a dose-dependent manner with pre-exposure of the CTLs to HIV antigens, akin to the effect of a therapeutic vaccine [84]. These results suggest that immune-stimulating agents may be
needed in order to realize the potential of LRAs. Furthermore, experiences with the Boston bone marrow transplant patients and Mississippi child show that solely depleting the reservoir may be inadequate to prevent viral rebound despite a $> 3 \log_{10}$ reduction in the reservoir size [94–97]. A number of therapeutic vaccines are under investigation (some in combination with HDACis), along with novel immunomodulatory molecules such as TLR agonists, or by a blockade of inhibitory pathways (anti-PD-1/PD-L1 antibodies). In addition, a variety of trials are underway or in development that are aimed at restoring effective antiviral responses by decreasing chronic inflammation and its deleterious effects to the immune system, as well as targeting the mTOR and JAK/STAT pathways.

CONCLUSIONS

The shock and kill strategy for viral reservoirs reduction in ART-treated HIV-infected participants relies on the development of potent and safe LRAs combined with strong anti-HIV cytotoxic immune responses. Building on results from former clinical trials of LRAs and recent ex vivo findings, current trials are testing an increasing number of compounds and immunomodulatory strategies, including combinations and repeated administration over longer timeframes, from which valuable data shall be expected over the coming years.

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