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Depletion of latent HIV-1 infection in vivo: a proof-of-concept study

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Contributors

G Lehrman, I B Hogue, and D M Margolis isolated resting CD4 T cells and did outgrowth assays; S Palmer, A Wiegand, J W Mellors, and J M Coffin were responsible for the one-copy HIV-RNA assays; C Jennings and A L Landay did CD8-depleted cell cultures, flow cytometry, and lymphocyte proliferation assays; R W Coombs did seminal HIV RNA assays; R J Bosch did statistical analyses; and D M Margolis designed the protocol, and oversaw its implementation. D M Margolis wrote the report with input from all authors.

Conflict of interest statement

We declare that we have no conflict of interest.

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Summary

Background—Persistent infection in resting CD4⁺ T cells prevents eradication of HIV-1. Since the chromatin remodeling enzyme histone deacetylase 1 (HDAC1) maintains latency of integrated HIV, we tested the ability of the HDAC inhibitor valproic acid to deplete persistent, latent infection in resting CD4⁺ T cells.

Procedures—We did a proof-of-concept study in four volunteers infected with HIV and on highly-active antiretroviral therapy (HAART). After intensifying the effect of HAART with subcutaneous enfuvirtide 90 ug twice daily for 4–6 weeks to prevent the spread of HIV, we added oral valproic acid 500–750 mg twice daily to their treatment regimen for 3 months. We quantified latent infection of resting CD4⁺ T cells before and after augmented treatment by limiting-dilution culture of resting CD4⁺ T cells after ex-vivo activation.

Findings—The frequency of resting cell infection was stable before addition of enfuvirtide and valproic acid, but declined thereafter. This decline was significant in three of four patients (mean reduction 75%, range 68% to 84%). Patients had slight reactions to enfuvirtide at the injection site, but otherwise tolerated treatment well.

Interpretation—Combination therapy with an HDAC inhibitor and intensified HAART safely accelerates clearance of HIV from resting CD4⁺ T cells in vivo, suggesting a new and practical approach to eliminate HIV infection in this persistent reservoir. This finding, though not definitive, suggests that new approaches will allow the cure of HIV in the future.

Introduction

Antiretroviral therapy suppresses plasma HIV RNA concentrations below the limits of detection and restores immune function. However, latent replication-competent provirus in resting CD4⁺ T lymphocytes [1-3] and persistent viral replication [4,5] prevent eradication of HIV infection. Nevertheless, since lifelong suppression of HIV infection with antiretroviral therapy is difficult, eradication should be the therapeutic aim. Approaches to deplete latent infection within resting CD4⁺ T cells are, therefore, needed. Results of a long-term study, [6] which followed-up patients for up to 7 years, confirm the stability of latent infection. Although a modest increase in the decay of the number of recoverable cells that contain replication-competent HIV has been reported after intensified antiretroviral therapy, [7] the rate of decay of plasma HIV RNA [8] or integrated HIV DNA decreases with time [9,10]. As such, a proportion of the pool of latently infected cells is probably highly stable and unlikely to be affected by potent inhibition of viral replication.

Intensive antiretroviral therapy in combination with T-cell activation does not eradicate HIV infection [11-14].

Activation can induce viral replication and increase the number of susceptible uninfected target cells beyond the threshold that can be contained by antiretroviral therapy [15]. The discovery of reagents that selectively induce the expression of quiescent proviral genomes, but that have limited activating effects, might allow outgrowth of latent HIV and avoid the pitfalls of global T-cell activation [16-19]. Histone deacetylation is important for quiescence of HIV gene expression in infected resting CD4⁺ T lymphocytes. Histone deacetylase 1 (HDAC1) mediates chromatin remodeling, repression of viral gene expression, and virion production [20-23]. Blockade of HDAC activity ex vivo results in HIV outgrowth from the resting T cells of aviraemic patients [23,24]. Valproic acid, an anticonvulsant that inhibits HDAC, induces HIV expression ex vivo from the resting CD4⁺ T cells of aviraemic patients treated with highly-active antiretroviral therapy (HAART) as efficiently as activation with mitogen, but without

upregulation of cell-surface markers of activation, or increased susceptibility to de-novo HIV infection [25]. Our aim, therefore, was to test the ability of valproic acid to deplete HIV infection of resting CD4+ T cells in vivo.

Methods

Patients

Between July, 2002, and February, 2005, we did a proof-of-concept study to which we enrolled HIV infected volunteers with long-term suppression of viraemia (<50 copies/mL for 2 years) who were taking HAART. Inclusion criteria were age 18 years or older, presence of documented HIV infection, ability to comply with protocol requirements and self-administer enfuvirtide for up to 18 weeks. Exclusion criteria were poor adherence to antiretroviral therapy, presence of contraindications to valproate therapy, receipt of interferon, other immunomodulators, or other experimental treatments. All patients provided written informed consent, and the study was approved by the Dallas Veterans' Administration Medical Center institutional review board.

Procedures

We intensified the effect of HAART by concomitant subcutaneous administration of the fusion inhibitor enfuvirtide 90 mcg twice daily after two rounds of leukopheresis. Once patients had adhered to HAART and enfuvirtide for 4–6 weeks, oral valproic acid 500–750 mg twice daily was initiated and continued for 3 months. We measured total and free serum concentrations of valproic acid, and did routine clinical haematology and blood chemistry monitoring once a week for a month, then monthly, and once again 1 month after protocol therapy was discontinued. We adjusted the dose of valproic acid to maintain plasma concentrations of 50–100 mg/L. We obtained plasma, peripheral blood mononuclear cells (PBMCs), and semen (when available) for analysis at scheduled intervals. Leucopheresis was done twice before the initiation of enfuvirtide and after 16–18 weeks' treatment with HAART, valproic acid, and enfuvirtide. We obtained leucocytes by continuous-flow leucopheresis. The leucocytes were washed and separated from contaminating red cells by Ficoll gradient centrifugation. We isolated resting CD4 T cells as described previously.^{2,24} FACS analysis confirmed greater than 99.9% purity of CD4 cells with less than 0.5% of cells exhibiting activation markers. We obtained 200–1200 million resting CD4 cells. After negative selection, we cultured the cells for 2 days in the presence of an integrase inhibitor (L-708906, 10 mmol/L; provided by D Hazuda, Merck Research Laboratory, West Point, PA, USA) and a selected reverse transcriptase inhibitor.²⁶ We used efavirenz (15 nmol/L; provided by Bristol Meyers-Squibb, Wallingford, CT, USA) if the patient has not been treated with nonnucleoside reverse transcriptase inhibitors; alternatively we used abacavir (4 mmol/L, provided by Glaxo SmithKline, Research Triangle Park, NC, USA). For the recovery and quantification of replication competent HIV, we cultured 140–263 million resting cells in replicate dilution series of 5, 2.5, 1.25, and 0.625 million cells per well for every experimental condition [24]. The serial dilution range was designed for optimum efficiency as per the statistical analysis of Macken [27,28]. We induced the cells of patients to express HIV by exposure to 2 mg/L PHA-L (Remel, Lanexa, KS, USA), 6×10^6 allogeneic irradiated PBMCs, and 100 U/mL interleukin 2. As a control for low-level contamination of cultures with activated cells, we exposed parallel cultures to 100 U/mL interleukin 2 for 72 h. Cultures were subsequently fed and maintained as previously described [24]. We scored cultures as positive if p24 was detectable 21 days after cell harvest, and rising p24 concentrations were confirmed on day 25. Virus was detected in culture supernatant by p24 antigen capture ELISA (Coulter, Miami, FL, USA).

We measured plasma HIV RNA concentrations by Roche Amplicor (Roche Molecular Systems, Branchburg, NJ, USA) with an assay detection limit of 50 copies per mL, and by a

quantitative real-time, RTPCR assay capable of detecting and quantifying HIV RNA concentrations of 1 copy per mL [29]. We confirmed the ability of the primers and probe used to amplify and detect the viral RNA in every patient by testing of a sample of banked plasma taken before initiation of fully suppressive HAART. Since such a sample was not available for patient 3, we tested primers and probes on viral RNA recovered from the outgrowth assay of that patient's resting T cells.

For real-time PCR assays of integrated HIV DNA, cells were snap-frozen after resting cell isolation as above. We thawed and resuspended the dry cell pellets in batches in 200 uL calcium-free and magnesium-free Dulbecco's phosphate buffered saline (Mediatech, Herndon, VA, USA). We extracted DNA with the QIAmp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and eluted in 50–100 uL of 10 mmol/L Tris, pH 8.0. To isolate total genomic DNA, we electrophoresed up to 2 mcg per sample on a 0.5% Seakem Gold agarose gel (Cambrex Bio Science, Rockland, ME, USA) in TAE buffer (40 mmol/L Trisacetate, 1 mmol/L EDTA, pH 8.3; Invitrogen Life Technologies, Carlsbad, CA, USA) at 60 V for 2.5 h. We excised the band at 23 kbp with a Gene Capsule cutter (Geno Technology, St Louis, MO, USA) and extracted it with a modified QIAEX II protocol (Qiagen, Valencia, CA, USA), shearing the DNA. Briefly, we dissolved gel slices in 600 uL of Buffer QX1 at 50°C for about 15 min. We added 400 uL water and 10 uL QIAEx beads and bound DNA for 20 min at 50°C, mixing beads by inverting tube every 2 min. Beads were pelleted by microcentrifugation at full speed for 30 s. If more than 1 ug DNA was loaded per lane, we subsequently bound the supernatant to a fresh aliquot of 10 uL beads. We washed the beads once with 500 uL Buffer QX1, twice with 500 uL Buffer PE, and dried at 50°C for 10–15 min. We eluted bound DNA with 10 mmol/L Tris, pH 8.0, and we pooled multiple loadings or sequential extractions for a total volume of 60 uL. We did real-time PCR on an ABI Prism 7700 Sequence Detector in MicroAmp optical 96-well reaction plates with optical caps, using Taqman universal PCR Master Mix and beta actin control reagents from Applied Biosystems (Foster City, CA, USA). We bought NL4-3 gag p24 primers (panel) from Integrated DNA Technologies (Coralville, IA, USA). We ran duplicate 50 uL reactions containing 20 uL of sample DNA, 900 nmol/L each PCR primer, and 200 nmol/L probe per reaction with standard curve of 10^6 to 5 copies of linearised pNL4-3 HIV DNA diluted in 4 mg/L human genomic DNA. Cycle conditions were: 50°C for 2 min, 95°C for 10 min, 45 cycles of 95°C for 15 s, 60°C for 1 min. The amount of DNA assayed was quantified by beta actin real-time PCR with 5 uL of eluate and 2.5 uL of each primer and probe in 25 uL reactions in duplicate. Results are normalised to 500 ng high molecular weight DNA (80 000 resting CD4+ cell equivalents). We did CD8-depleted PBMC outgrowth assays [30], seminal HIV RNA assays [31], immunostaining, flow cytometric analyses, and lymphocyte proliferation assays [32] as previously described.

Statistical analysis

We estimated the number of resting CD4+ T cells in infected units per billion (10^9 , IUPB) by a maximum likelihood method [28]. A parametric, model-based analysis was applied to the preprotocol and postprotocol IUPB data, to quantitatively estimate the effect of enfuvirtide and valproic acid. This approach provides an estimate (95% CI) of the proportional reduction in IUPB after treatment with enfuvirtide and valproic acid, using a generalised linear model with a binary response—i.e., a positive or a negative culture [33].

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The four patients enrolled tolerated the treatment regimen and adhered well to therapy, according to self-report, refill history, and enfuvirtide vial count (Table 1). All had minor reactions at the injection site related to administration of enfuvirtide, as commonly reported [34]. Patient 4, with a distant history of antiretroviral-related anaemia and baseline HAART that included zidovudine, developed grade 1 anaemia during the last 4 weeks of treatment with valproic acid. Anaemia resolved when valproic acid was discontinued. Total CD4 and CD8 counts and proportions were stable. With one exception, plasma HIV RNA remained undetectable (<50 copies per mL). Patient 3 had a self-limited upper respiratory tract infection 3–4 weeks after initiation of enfuvirtide, but before initiation of valproic acid. Plasma HIV-1 RNA concentrations at that time were 75 copies per mL, but were less than 50 copies at all other time points.

Immune activation might have arisen if HIV expression had been induced by valproic acid, and viraemia not contained by HAART and enfuvirtide. We noted no significant changes, however, in concentrations of expression of cell-surface markers of activation at entry (HAART alone), week 4–6 (HAART and enfuvirtide), or week 16–18 (HAART, enfuvirtide, and valproic acid). We detected no significant change in the proportion of resting or activated, memory or naive CD4+ or CD8+ T-cell populations. Lymphoproliferative assay responses to HIV p24 antigen were low at baseline and remained unchanged (data not shown).

To assess extent of viraemia, we used a sensitive assay able to detect a single copy of HIV RNA per mL of plasma (Table 2) [29]. Plasma viraemia was undetectable in patient 2. There was no evidence of further suppression of low-level viraemia in patients 1 or 3, nor was there evidence of increased or re-emergent plasma viraemia after addition of valproic acid. Plasma viraemia was initially quantifiable at 1 copy per mL, and then declined to less than 1 copy per mL after addition of enfuvirtide to HAART in patient 4, a decline that might not be significant. Cultures of more than 37 million PBMCs from patient 4 yielded HIV after ex-vivo CD8 depletion and activation at weeks 0–6 (Table 2), but none was detected at the end of the study. CD8-depleted cultures did not grow HIV in patients 1 and 2. HIV was also not recovered from CD8-depleted cultures of PBMCs from patient 3, despite low-level plasma viraemia and detectable viraemia at week 5. We noted a correlation between plasma viraemia and seminal HIV RNA in patients 3 and 4 (semen samples not available for patients 1 and 2). Seminal HIV RNA was not detected at entry, week 6, or week 18 in patient 4 at a limit of detection of less than 200–700 copies per mL. 1025 copies per mL of HIV RNA was detected in patient 3 1 week after low-level plasma viraemia (75 copies per mL) was detected. We did not detect HIV RNA in the semen of this patient at weeks 0 or 18, at a limit of detection of less than 500 copies per mL. We detected integrated HIV DNA genomes much more frequently than replication competent HIV. During the abbreviated study period, we did not note a substantial decline of integrated HIV DNA. Integrated HIV genomes were detected at week 0 and week 18 in a mean of 275 (SD 88) cells per million resting CD4 cells in patient 1, 375 (288) cells per million in patient 2, 1250 (1013) cells per million in patient 3, and 213 (13) cells per million in patient 4. The frequency of infection in resting CD4+ Tcells on standard HAART was stable (Table 3). The greatest decline noted was 28%. Pooled IUPB from resting cell outgrowth assays before addition of enfuvirtide and valproic acid to the treatment regimen was higher than after (Table 4). Since we did not recover any virus in outgrowth assays of patient 2 at week 18, maximum IUPB and minimum decline is modelled on an estimate of virus recovery in one macroculture. The decline in resting CD4+ T-cell infection was substantially more rapid than predicted by the half-life estimates previously reported. Resting cell IUPB declined by at least 29% in all patients. If the IUPB changes after protocol therapy were from the same distribution as the changes in patients on standard HAART, the likelihood that the largest decreases were observed only after treatment with enfuvirtide and valproic acid is less than 0.016 (Wilcoxon rank-sum

two-sided test). We compared the IUPB measured at time points before the addition of enfuvirtide and valproic acid to the IUPB measured after 16–18 weeks of enfuvirtide and valproic acid therapy. In view of the previous estimates of the half life of latently infected resting CD4+ T cells, we tested the null hypothesis that a 50% depletion of IUPB would not be observed after 16–18 weeks of protocol therapy. A decline of more than 50% (95% CI 40–80) was measured in three of four patients after protocol therapy. If the single time point determined is reflective of the decrease in infected resting cell half-life, the half-life of infected resting CD4+ T cells decreased to 2–3 months in the three responders.

Discussion

Our findings show that 16–18 weeks' treatment with a standard clinical dose of valproic acid, in the setting of intensified HAART, produces a substantial decline in the frequency of replication-competent HIV in circulating resting CD4+ T cells. We measured replication-competent HIV in resting CD4+ T cells under stringent conditions: our volunteers had been successfully treated with HAART for at least 2 years; we used an additional incubation step with reverse transcriptase and integrase inhibitors to reduce to a minimum the likelihood of recovery of replication-competent HIV from recently infected cells; and we did multiple assessments in every patient before initiating treatment with enfuvirtide and valproic acid, none of which showed a consistent fall in IUPB. Depletion of HIV recovered from circulating resting CD4+ cells after treatment, however, indicated a pronounced shortening of the half-life of the latent reservoir.

An examination of the response of individual patients is revealing. Patient 1 had a single episode of transient viraemia, possibly associated with an intercurrent infection, before protocol therapy, and also had low-level viraemia that was unaffected by the addition of enfuvirtide. Resting cell IUPB did not decline much in this patient after therapy with enfuvirtide and valproic acid. Low-level viraemia continued while on intensified HAART. Perhaps these clinical characteristics explain the lack of depletion of resting cell infection seen in this individual. Patient 2 had durable and complete suppression of detectable viraemia, without known episodes of viraemia, without recoverable HIV in CD8-depleted cultures, and without detectable viraemia in the single-copy assay. IUPB of resting cells had fallen greatly by the end of the study. Patient 3 had two episodes of transient viraemia during a state of immune activation before protocol therapy, and one while on study treatment. Low-level viraemia was detected throughout the study, despite the addition of enfuvirtide to HAART. After treatment with enfuvirtide and valproic acid, resting cell IUPB declined. It is noteworthy that resting cell IUPB declined in this patient during therapy with intensified HAART and valproic acid despite continued viraemia. Finally, the IUPB of patient 4's resting CD4+ T cells measured before protocol therapy was stable. Low-level viraemia was detected before the addition of enfuvirtide, decreased after enfuvirtide therapy, and was less than 1 copy per mL 1 month after enfuvirtide was discontinued. However, this apparent decline might be within the range of experimental variation of the one-copy assay.

Although seminal HIV RNA was not detected, HIV was initially recovered from CD8-depleted PBMCs. Consistent with the suppression of low-level replication by intensified HAART, CD8-depleted cultures were negative at the end of study. After 12 weeks of exposure to valproic acid and intensified HAART a decline of resting cell IUPB was noted.

Our pilot study is limited and leaves many questions unanswered. First, we did not note any immune activation in our patients. This finding is probably due to a combination of factors. HIV expressed in resting cells might have been too rare to induce detectable immune activation, a response of HIV-specific T cells present in these durably suppressed patients might have been too rare to detect, or immune responses could have been blunted by effective containment

of new rounds of HIV replication by HAART and enfuvirtide. Second, one patient, whose background HAART included zidovudine, developed anaemia. Valproic acid inhibits glucuronidation of zidovudine and increases its bioavailability [35], theoretically increasing the risk of zidovudine-induced anaemia. Whether this adverse effect is infrequently seen in clinical practice, or is under-reported [36] is unclear. In our ongoing studies, we plan to avoid the coadministration of valproic acid and zidovudine. Third, treatment with valproic acid and enfuvirtide seems to have reduced latent HIV infection to an unprecedented extent. We postulate that HDAC inhibition is essential to achieve this effect. However, this study is too small to separate the effects of entry and HDAC inhibition. Furthermore, two of our patients had persistent, low-level viraemia despite the addition of enfuvirtide, an antiretroviral with a new mechanism to which they had not previously been exposed. The source and importance of this viraemia is unclear, and an important topic for future study. Nevertheless, we noted depletion of resting cell infection in one individual despite continuous low level viraemia. Finally, we have studied a small number of patients, with a single course of therapy of an arbitrary duration. The depletion of resting cell infection we noted seems much greater than that reported after intensification [7], and at least as great as that reported after long-term therapy with interleukin 2 [11]. However, further studies are needed to confirm, expand, and deepen our observations.

Our findings suggest that eradication of established HIV infection might be achieved in a staged approach. Patients should perhaps first be treated with standard antiretroviral regimens at an early stage of infection. For those in whom viral replication is suppressed, latent viral infection should then be tackled with HDAC inhibitors, intensified therapy, or both. Alternatively, or additionally, therapeutic vaccination might augment the antiviral immune response.

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References

1. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, Quinn TC, Chadwick K, Margolick J, Brookmeyer, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997;278:1295–1300. [PubMed: 9360927]
2. Chun TW, Carruth L, Finzi D, Shen X, DiGiuseppe JA, Taylor H, Hermankova M, Chadwick K, Margolick J, Quinn TC, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 1997;387:183–188. [PubMed: 9144289]
3. Wong JK, Hezareh M, Gunthard HF, Havlir DV, Ignacio CC, Spina CA, Richman DD. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 1997;278:1291–1295. [PubMed: 9360926]
4. Dornadula G, Zhang H, VanUitert B, Stern J, Livornese L Jr, Ingerman MJ, Witek J, Kedaris RJ, Natkin J, DeSimone J, et al. Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. *JAMA* 1999;282:1627–1632. [PubMed: 10553788]
5. Gunthard HF, Frost SD, Leigh-Brown AJ, Ignacio CC, Kee K, Perelson AS, Spina CA, Havlir DV, Hezareh M, Looney DJ, et al. Evolution of envelope sequences of human immunodeficiency virus type 1 in cellular reservoirs in the setting of potent antiviral therapy. *J. Virol* 1999;73:9404–9412. [PubMed: 10516049]

6. Zhang L, Ramratnam B, Tenner-Racz K, He Y, Vesanen M, Lewin S, Talal A, Racz P, Perelson AS, Korber BT, et al. Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. *N. Engl. J. Med* 1999;340:1605–1613. [PubMed: 10341272]
7. Fischer M, Gunthard HF, Opravil M, Joos B, Huber W, Bisset LR, Ott P, Boni J, Weber R, Cone RW. Residual HIV-RNA levels persist for up to 2.5 years in peripheral blood mononuclear cells of patients on potent antiretroviral therapy. *AIDS Res. Hum. Retroviruses* 2000;16:1135–1140. [PubMed: 10954888]
8. Lewin SR, Vesanen M, Kostrikis L, Hurley A, Duran M, Zhang L, Ho DD, Markowitz M. Use of real-time PCR and molecular beacons to detect virus replication in human immunodeficiency virus type 1-infected individuals on prolonged effective antiretroviral therapy. *J. Virol* 1999;73:6099–6103. [PubMed: 10364365]
9. Ramratnam B, Mittler JE, Zhang L, Boden D, Hurley A, Fang F, Macken CA, Perelson AS, Markowitz M, Ho DD. The decay of the latent reservoir of replication-competent HIV-1 is inversely correlated with the extent of residual viral replication during prolonged anti-retroviral therapy. *Nat. Med* 2000;6:82–85. [PubMed: 10613829]
10. Sharkey ME, Teo I, Greenough T, Sharova N, Luzuriaga K, Sullivan JL, Bucy RP, Kostrikis LG, Haase A, Veyard C, et al. Persistence of episomal HIV-1 infection intermediates in patients on highly active anti-retroviral therapy. *Nat. Med* 2000;6:76–81. [PubMed: 10613828]
11. Havlir DV, Strain MC, Clerici M, Ignacio C, Trabattini D, Ferrante P, Wong JK. Productive infection maintains a dynamic steady state of residual viremia in human immunodeficiency virus type 1-infected persons treated with suppressive antiretroviral therapy for five years. *J Virol* 2003;77:11212–9. [PubMed: 14512569]
12. Pierson T, McArthur J, Siliciano RF. Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. *Ann. Rev. Immunol* 2000;18:665–708. [PubMed: 10837072]
13. Pomerantz RJ. Reservoirs, sanctuaries, and residual disease: the hiding spots of HIV-1. *HIV Clin. Trials* 2003;4:137–143. [PubMed: 12671782]
14. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, Lloyd AL, Nowak MA, Fauci AS. Presence of an inducible HIV latent reservoir during highly active antiretroviral therapy. *Proc. Natl Acad. Sci. USA* 1997;94:13193–13197. [PubMed: 9371822]
15. Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. *Proc. Natl Acad. Sci. USA* 1998;95:8869–8873. [PubMed: 9671771]
16. Finzi D, Blankson S, Siliciano JD, et al. Latent infection of CD4 + T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat. Med* 1999;5:512–517. [PubMed: 10229227]
17. Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, Kovacs C, Gange SJ, Siliciano RF. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat Med* 2003;9:727–728. [PubMed: 12754504]
18. Ramratnam B, Ribeiro R, He T, Chung C, Simon V, Vanderhoeven J, Hurley A, Zhang L, Perelson AS, Ho DD, et al. Intensification of antiretroviral therapy accelerates the decay of the HIV-1 latent reservoir and decreases, but does not eliminate, ongoing virus replication. *J. Acquir. Immune Defic. Syndr* 2004;35:33–37. [PubMed: 14707789]
19. Di Mascio M, Dornadula G, Zhang H, Sullivan J, Xu Y, Kulkosky J, Pomerantz RJ, Perelson AS. In a subset of subjects on highly active antiretroviral therapy, human immunodeficiency virus type 1 RNA in plasma decays from 50 to <5 copies per milliliter, with a half-life of 6 months. *J. Virol* 2003;77:2271–2275. [PubMed: 12525664]
20. Strain MC, Gunthard HF, Havlir DV, Ignacio CC, Smith DM, Leigh-Brown AJ, Macaranas TR, Lam RY, Daly OA, Fischer M, et al. Heterogeneous clearance rates of long-lived lymphocytes infected with HIV: intrinsic stability predicts lifelong persistence. *Proc. Natl Acad. Sci. USA* 2003;100:4819–4824. [PubMed: 12684537]
21. Strain MC, Little SJ, Daar ES, Havlir DV, Gunthard HF, Lam RY, Daly OA, Nguyen J, Ignacio CC, Spina CA, et al. Effect of treatment during primary infection on establishment and clearance of cellular reservoirs of HIV-1. *J. Infect Dis* 2005;191:1410–8. [PubMed: 15809898]2005

22. Chun TW, Engel D, Mizell SB, Hallahan CW, Fischette M, Park S, Davey RT Jr, Dybul M, Kovacs JA, Metcalf JA, et al. Effect of interleukin-2 on the pool of latently infected, resting CD4+ T cells in HIV-1-infected patients receiving highly active anti-retroviral therapy. *Nat. Med* 1999;5:651–655. [PubMed: 10371503]
23. Prins JM, Jurriaans S, van Praag RM, Blaak H, van Rij R, Schellekens PT, ten Berge IJ, Yong SL, Fox CH, Roos MT, et al. Immuno-activation with anti-CD3 and recombinant human IL-2 In HIV-1-infected patients on potent antiretroviral therapy. *AIDS* 1999;13:2405–2410. [PubMed: 10597782]
24. van Praag RM, Prins JM, Roos MT, Schellekens PT, Ten Berge IJ, Yong SL, Schuitemaker H, Eerenberg AJ, Jurriaans S, de Wolf F, et al. OKT3 and IL-2 treatment for purging of the latent HIV-1 reservoir in vivo results in selective long-lasting CD4+ T cell depletion. *J. Clin. Immunol* 2001;21:218–226. [PubMed: 11403229]
25. Dybul M, Hidalgo B, Chun TW, Belson M, Migueles SA, Justement JS, Herpin B, Perry C, Hallahan CW, Davey RT, et al. Pilot study of the effects of intermittent interleukin-2 on human immunodeficiency virus (HIV)-specific immune responses in patients treated during recently acquired HIV infection. *J. Infect. Dis* 2002;185:61–68. [PubMed: 11756982]
26. Kulkosky J, Nunnari G, Otero M, Calarota S, Dornadula G, Zhang H, Malin A, Sullivan J, Xu Y, DeSimone J, et al. Intensification and stimulation therapy for human immunodeficiency virus type 1 reservoirs in infected persons receiving virally suppressive highly active antiretroviral therapy. *J. Infect. Dis* 2002;186:1403–1411. [PubMed: 12404155]
27. Stellbrink HJ, van Lunzen J, Westby M, O'Sullivan E, Schneider C, Adam A, Weitner L, Kuhlmann B, Hoffmann C, Fenske S, et al. Effects of interleukin-2 plus highly active antiretroviral therapy on HIV-1 replication and proviral DNA (COSMIC trial). *AIDS* 2002;16:1479–1487. [PubMed: 12131185]
28. Fraser C, Ferguson NM, Ghani AC, Prins JM, Lange JM, Goudsmit J, Anderson RM, de Wolf F, et al. Reduction of the HIV-1-infected T-cell reservoir by immune activation treatment is dose-dependent and restricted by the potency of antiretroviral drugs. *AIDS* 2000;14:659–669. [PubMed: 10807189]
29. Brooks DG, Hamer DH, Arlen PA, Gao L, Bristol G, Kitchen CM, Berger EA, Zack JA, et al. Molecular characterization, reactivation, and depletion of latent HIV. *Immunity* 2003;19:413–423. [PubMed: 14499116]
30. Demonte D, Quivy V, Colette Y, Van Lint C. Administration of HDAC inhibitors to reactivate HIV-1 expression in latent cellular reservoirs: implications for the development of therapeutic strategies. *Biochem. Pharmacol* 2004;68:1231–1238. [PubMed: 15313421]
31. Lehrman G, Ylisastigui L, Bosch RJ, Margolis DM. Interleukin-7 induces human immunodeficiency virus type 1 outgrowth from peripheral memory resting CD4+ T cells. *J. Acquir. Immune Defic. Syndr* 2004;36:1103–1104. [PubMed: 15247565]
32. Wang FX, Xu Y, Sullivan J, Souder E, Argyris EG, Acheampong EA, Fisher J, Sierra M, Thomson MM, Najera R, et al. IL-7 is a potent and proviral strain-specific inducer of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART. *J. Clin. Invest* 2005;115:128–137. [PubMed: 15630452]
33. Margolis DM, Somasundaran M, Green MR. Human transcription factor YY1 represses HIV-1 transcription and virion production. *J. Virol* 1994;68:905–910. [PubMed: 8289393]
34. Romero F, Gabriel M, Margolis DM. Repression of HIV-1 through the novel cooperation of the human factors YY1 and LSF. *J. Virol* 1997;71:9375–9382. [PubMed: 9371597]
35. Coull J, Romero F, Sun JM, Volker JM, Galvin KM, Davie JR, Shi Y, Hansen U, Margolis DM. The human factors YY1 and LSF repress the human immunodeficiency virus type-1 long terminal repeat via recruitment of histone deacetylase 1. *J. Virol* 2000;74:6790–6799. [PubMed: 10888618]
36. He G, Margolis DM. Counter-regulation of chromatin acetylation and histone deacetylase occupancy at the integrated promoter of human immunodeficiency virus type 1 by the HIV-1 activator tat and the HIV-1 repressor YY1. *Mol. Cell Biol* 2002;22:2965–2973. [PubMed: 11940654]
37. Coull JJ, He G, Melander C, Rucker V, Dervan PB, Margolis DM. Targeted derepression of the human immunodeficiency type 1 long terminal repeat by pyrrole-imidazole polyamides. *J. Virol* 2002;76:12349–12354. [PubMed: 12414976]

38. Ylisastigui L, Coull JJ, Rucker V, Brodie SJ, Bosch RJ, Sodora D, Dervan P, Margolis DM. Polyamides reveal a role for repression in viral latency within HIV-infected donors' resting CD4+ T cells. *J. Infect. Dis* 2004;190:1429–1437. [PubMed: 15378435]
39. Ylisastigui L, Archin N, Lehrman G, Bosch RJ, Margolis DM. Coaxing human immunodeficiency virus type 1 from resting CD4+ T cells: can the reservoir of HIV be purged? *AIDS* 2004;18:1101–1108. [PubMed: 15166525]
40. Clotet B, Raffi F, Cooper D, Delfraissy JF, Lazzarin A, Moyle G, Rockstroh J, Soriano V, Schapiro J. Clinical management of treatment-experienced, HIV-infected patients with the fusion inhibitor enfuvirtide: consensus recommendations. *AIDS* 2004;18:1137–1146. [PubMed: 15166529]
41. Palmer S, Wiegand AP, Maldarelli F, Bazmi H, Mican JM, Polis M, Dewar RL, Planta A, Liu S, Metcalf JA, et al. New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. *J. Clin. Microbiol* 2003;41:4531–4536. [PubMed: 14532178]
42. Lertora JJ, Rege AB, Greenspan DL, Akula S, George WJ, Hyslop NE Jr, Agrawal KC. Pharmacokinetic interaction between zidovudine and valproic acid in patients infected with human immunodeficiency virus. *Clin. Pharmacol. Ther* 1994;56:272–278. [PubMed: 7924122]
43. Antoniou T, Gough K, Yoong D, Arbess G. Severe anemia secondary to a probable drug interaction between zidovudine and valproic acid. *Clin. Infect. Dis* 2004;38:e38–40. [PubMed: 14986271]
44. Hazuda DJ, Felock P, Witmer M, Wolfe A, Stillmock K, Grobler JA, Espeseth A, Gabryelski L, Schleif W, Blau C, et al. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science* 2000;287:646–650. [PubMed: 10649997]
45. Macken C. Design and analysis of serial limiting dilution assays with small sample sizes. *J. Immunol. Methods* 1999;222:13–29. [PubMed: 10022369]
46. Myers LE, McQuay LJ, Hollinger FB. Dilution assay statistics. *J. Clin. Microbiol* 1994;32:732–739. [PubMed: 8195386]
47. McCullagh, P.; Nelder, JA. *Generalized Linear Models*. 2nd ed.. Chapman and Hall; New York, NY: 1989.
48. Jennings, C.; Brambilla, D.; Huang, D.; Bremer, J. An improved culture method for stimulating the production of HIV from PBMCs; 10th Conference on Retroviruses and Opportunistic Infections; Boston, MA. 2003. abstr. 672
49. Coombs RW, Speck CE, Hughes JP, Lee W, Sampoleo R, Ross SO, Dragavon J, Peterson G, Hooton TM, Collier AC, et al. Association between culturable human immunodeficiency virus type 1 (HIV-1) in semen and short-term variation of HIV-1 RNA levels in semen and blood: evidence for compartmentalization of HIV-1 between semen and blood. *J. Infect. Dis* 1998;177:320–330. [PubMed: 9466517]
50. Al-Harthi L, Siegel J, Spritzler J, Pottage J, Agnoli M, Landay A. Maximum suppression of HIV replication leads to the restoration of HIV-specific responses in early HIV disease. *AIDS* 2000;14:761. [PubMed: 10839583]

Figure 1.

Effect of T-20 and VPA on resting CD4 cell IUPB: Pooled estimates of the number of cells (◆) containing replication-competent HIV per billion resting CD4+ cells (IUPB) on prolonged HAART, and after 16-18 weeks of continued HAART with VPA and T-20 as assayed by limiting-dilution culture is shown. Also displayed is the predicted decay of IUPB (half-life of 44.2 months; Siliciano 2003), and accelerated decay (half-life of 10.3 months; Ramratnam 2004) reported following intensified antiretroviral therapy. For illustrative purposes only, an estimate of half-life following T-20 and VPA therapy is displayed.

Table 1

Clinical characteristics prior to and during study

	Year HIV+	HAART	Duration <50 copies/ml	CD4/μl (%)	VPA μg/ml	
					total	free
Pt. 1	1995	TDF/ABC/D4T/APV/r	38 mo [*]	1285 (43%)	65.1-83.1	5.8-6.7
Pt. 2	1999	DDI/FTC/Efv	>43 mo [†]	558 (26%)	52.4-89.6	3.0-9.8
Pt. 3	1985	TDF/ABC/3TC/Efv/Nfv	>24 mo ^{†*}	350 (18%)	56.7-81.0	3.9-6.8
Pt. 4	1995	ZDV/3TC/Nvp	>75 mo [†]	372 (35%)	33.9-74.8	2.2-8.7

ZDV: zidovudine, 3TC: lamivudine, Nvp: nevirapine, DDI: didanosine, FTC: emtricitabine, Efv: efavirenz, TDF: tenofovir, ABC: abacavir, Nfv: nelfinavir, D4T: stavudine, APV/r: amprenavir with low-dose ritonavir

[†] Viremia was suppressed prior to the first visit of these patients to our clinic.

* Patient 1: single measurement of 71 copies/ml HIV-1 RNA at week -96. Patient 3 single measurements of 636 copies/ml at week -12, and 98 copies/ml at week -72.

Table 2
Plasma HIV-1 RNA single-copy assay, CD8-depleted cultures, and seminal HIV DNA

Week	Patient 1		Patient 2		Patient 3		Patient 4	
	1-copy assay	CD8-culture	1-copy assay	CD8-culture	1-copy assay	CD8-culture	1-copy assay	CD8-culture
0	2	Neg.	<1	Neg.	n.d.*	Neg.	2*	Pos.
4	<1	Neg.	<1		n.d.		1	
5	1							
6	1		<1	Neg.	n.d. [†]	Neg.	<1*	Pos.
7			<1		1		1	
8	1		<1		7		<1	
10			<1		6		<1	
14	<1		<1		9		<1	
16	2	Neg.						
18			n.d.	Neg.	2*	Neg.	<1* [#]	Neg.
22	<1		n.d.		7		<1	

HAART alone (unshaded); HAART and T-20 (light gray); HAART, T-20 and VPA (gray)

n.d.: not determined due to real-time instrument failure

* Seminal HIV RNA not detected

[†] Roche Ultrasensitive plasma HIV-1 RNA = 75 copies/ml during at week 5, <50 copies/ml at week 6; seminal HIV RNA 1025 copies/ml at week 6

[#] single-copy assay internal standard below acceptable limit

Table 3

Infected cells per billion (IUPB) resting CD4+ T cells

Patient	IUPB on HAART	IUPB on HAART	Interval (months)
1	57	41	1
2	42	72	3
3	290	240	2
4	140	130	15
5	20	23	1

IUPB measurements from resting cell outgrowth assays on HAART prior to protocol therapy. Patient 5 was stably aviremic as per the entry criteria but did not receive T20 or VPA.

Table 4

Decline in infected cells per billion (IUPB) resting CD4+ T cells following addition of T20&VPA to HAART

Patient	Pooled IUPB on HAART	HAART & T20/HAART&20&VPA therapy (wks)	IUPM after T20&VPA	Observed reduction in IUPM
1	49	4/12	35	29%
2	57	6/12	<9	>84%
3	250	4/12	81	68%
4	136	6/12	37	73%

IUPM measurements from resting cell outgrowth assays on HAART prior to and following 16-18 weeks of T-20 and VPA exposure. Percent reduction in IUPM after protocol therapy is shown. Maximal IUPM (†) and minimal percent reduction for patient 2 is estimate assuming one culture had been positive, as virus was not recovered after protocol therapy.