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A Possible Sterilizing Cure of HIV-1 Infection Without Stem Cell Transplantation

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Abstract

Background: A sterilizing cure of HIV-1 infection has been reported in 2 persons living with HIV-1 who underwent allogeneic hematopoietic stem cell transplantations from donors who were homozygous for the CCR5 32 gene polymorphism. However, this has been considered elusive during natural infection.

Objective: To evaluate persistent HIV-1 reservoir cells in an elite controller with undetectable HIV-1 viremia for more than 7 years in the absence of antiretroviral therapy.

Design: Detailed investigation of virologic and immunologic characteristics.

Setting: Tertiary care centers in Buenos Aires, Argentina, and Boston, Massachusetts.

Patient: A patient with HIV-1 infection and durable drug-free suppression of HIV-1 replication.

Measurements: Analysis of genome-intact and replication-competent HIV-1 using near-full-length individual proviral sequencing and viral outgrowth assays, respectively; analysis of HIV-1 plasma RNA by ultrasensitive HIV-1 viral load testing.

Results: No genome-intact HIV-1 proviruses were detected in analysis of a total of 1.188 billion peripheral blood mononuclear cells and 503 million mononuclear cells from placental tissues. Seven defective proviruses, some of them derived from clonally expanded cells, were detected. A viral outgrowth assay failed to retrieve replication-competent HIV-1 from 150 million resting CD4 T cells. No HIV-1 RNA was detected in 4.5 mL of plasma.

Limitations: Absence of evidence for intact HIV-1 proviruses in large numbers of cells is not evidence of absence HIV-1 infection. A sterilizing cure of HIV-1 can never be empirically proved.

Conclusion: Genome-intact and replication-competent HIV-1 were not detected in an elite controller despite analysis of massive numbers of cells from blood and tissues, suggesting that this patient may have naturally achieved a sterilizing cure of HIV-1 infection. These observations raise the possibility that a sterilizing cure may be an extremely rare but possible outcome of HIV-1 infection.

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Although antiretroviral therapy (ART) can effectively suppress viral replication, HIV-1 is one of the few infectious diseases for which a sterilizing cure during natural disease is currently considered elusive. Indeed, HIV-1 is known to establish a population of latently infected CD4⁺ T cells that harbor chromosomally integrated proviral DNA that displays little or no transcriptional activity (1). These cells persist throughout the lifespan, are not susceptible to ART, and can effectively fuel rebound viremia when ART is stopped. Attempted elimination of these cells through pharmacologic or immunologic interventions has been unsuccessful in the past, except in 2 reported patients with leukemia who underwent allogeneic hematopoietic stem cell transplants that resulted in what are widely considered to be sterilizing cures (2, 3). In a small subgroup of persons living with HIV-1 who are frequently termed “elite controllers” or “natural suppressors,” HIV-1 plasma viremia remains durably undetectable by commercial polymerase chain reaction (PCR) assays in the absence of ART. However, genome-intact proviral DNA and replication-competent viruses can readily be isolated in these persons by using in vitro laboratory assays, indicating that drug-free viral control in these persons results from host-dependent inhibition of viral replication and does not reflect elimination of all virally infected cells (4, 5). Similarly, a small proportion of persons living with HIV-1 have sustained viral control after stopping ART; such “posttreatment controllers” are also known to harbor persistent reservoirs of replication-competent HIV-1, indicating that this clinical phenotype is not associated with viral eradication (6). In this article, we describe a person who may have achieved complete clearance of all replication-competent HIV-1 proviruses during natural infection.

Methods

Peripheral Blood Mononuclear Cell and Placental Samples

Peripheral blood from the person described in this study was collected in October 2017, January 2018, and August 2019; leukapheresis was performed in September 2020. Peripheral blood mononuclear cells (PBMCs) were isolated and cryopreserved according to standard procedures. Placental tissues were collected in March 2020, after vaginal delivery of a healthy baby. Placenta mononuclear cells were isolated and cryopreserved as previously described, with minor modifications (7, 8). The proportion of CD45 leukocytes in placental mononuclear cells was determined by flow cytometry.

Full-Length Individual Proviral Sequencing

DNA was extracted from PBMCs and placental mononuclear cells by using commercial kits (DNeasy Blood & Tissue Kit [QIAGEN]). Total HIV-1 DNA and cell numbers were quantified with Droplet Digital PCR (ddPCR [Bio-Rad]), using primers and probes

that have been described previously (9). DNA diluted to single-genome levels based on Poisson distribution statistics and ddPCR results was subjected to single-genome near-full-length HIV-1 amplification, as previously described (9). Individual amplification products were sequenced on the Illumina MiSeq platform. Resulting short reads were de novo assembled and aligned to HXB2. Intact and defective proviral sequences were distinguished using an automated pipeline written in Python code (<https://github.com/BWH-Lichterfeld-Lab/Intactness-Pipeline>). The presence or absence of APOBEC-3G/3F-associated hypermutations was determined using the Los Alamos HIV Sequence Database Hypermut 2.0 program. Viral sequences were considered clonal if they had completely identical sequences.

Quantitative Viral Outgrowth Assay

CD4⁺ memory cells were isolated from PBMCs by using the EasySep Human CD4 Positive Selection Kit II (STEMCELL Technologies). Large-scale quantitative viral outgrowth measurements on cells from the patient were performed by a similar standard method (10), with a p24 enzyme-linked immunosorbent assay (ELISA) used to detect viral outgrowth.

Analysis of Cell-Associated HIV-1 RNA and DNA

Cell-associated HIV-1 DNA (total, integrated, and 2-LTR HIV-1 DNA) and unspliced and multiple-spliced HIV-1 RNA were quantified by quantitative real-time PCR as previously described (11).

Plasma HIV-1 Viral Load

Plasma viral load was determined using commercial assays with limits of detection of 50, 40, and 20 HIV-1 RNA copies/mL, depending on the assay. One sample obtained in 2017 was subjected to ultrasensitive HIV-1 viral load quantification by repetitive sampling of 4.5 mL of plasma using the Aptima HIV-1 quantification assay (Hologic) on the Panther system; the estimated limit of detection was 0.4 copies/mL.

Intracellular Cytokine Staining Assay

Peripheral blood mononuclear cells were stimulated for 14 days with HIV-1 peptide pools (individual peptide concentration, 1 µg/mL) spanning the clade B consensus sequence of nef or p24 or a control peptide pool, as described previously (12). Afterward, cells were restimulated with the designated peptide pool (at 2 µg/mL) in the presence of anti-CD28 and anti-CD49d antibodies (1 µg/mL; BD Biosciences), monensin (GolgiStop, 0.7 µL/mL; BD Biosciences), and brefeldin A (10 µg/mL; BD Biosciences). After surface staining with CD3, CD4, and CD8 antibodies, intracellular cytokine staining was performed according to standard protocols. Flow cytometry data acquisition was performed on a BD FACSAria Fusion Flow Cytometer using the BD FACSDiva v8.0.1 software (BD Biosciences). Acquired data were analyzed using FlowJo v10.

Sequence Analysis

The proportions of optimal cytotoxic T-lymphocyte epitopes (restricted by autologous HLA class I alleles) that match the clade B consensus sequence and cytotoxic T-lymphocyte

escape variants restricted by selected HLA class I alleles and supertypes described in the Los Alamos National Laboratory HIV Immunology Database (www.hiv.lanl.gov/content/index) were determined.

In Vitro Infection Assays

Peripheral blood mononuclear cells were stimulated with an anti-CD3/CD8 bispecific antibody (0.5 µg/µL; NIH AIDS Reagent Program, 12277). After 5 days in culture, the expression levels of CXCR4 and CCR5 were detected by flow cytometry. CD4⁺ T cells were infected with replication-competent NL4-3 (CXCR4-tropic), 91US056 (CCR5-tropic) (NIH AIDS Reagent Program, ARP-2099), and NL4-3 with a BaL-derived env (CCR5-tropic) virus for 4 hours at 37 °C. Viral replication was monitored by p24 ELISA (PerkinElmer) of culture supernatants at days 3, 5, and 7.

HLA/CCR5 Genotyping

HLA typing was performed using a targeted next-generation sequencing method, as described previously (13).

Western Blots

The HIV-specific antibody profile was evaluated in plasma using the Western Blot HIV Blot 2.2 kit (MP Diagnostics).

Detection of Antiretroviral Drugs in Plasma

Qualitative testing of 18 antiretroviral drugs (etravirine, elvitegravir, efavirenz, amprenavir, atazanavir, darunavir, lopinavir, maraviroc, raltegravir, rilpivirine, ritonavir, dolutegravir, tenofovir, lamivudine, emtricitabine, abacavir, zidovudine, and nevirapine) was performed by the Clinical Pharmacology and Analytical Chemistry Laboratory of the University of North Carolina at Chapel Hill.

Institutional Review Board Approval

The study participant gave written informed consent to participate in accordance with the Declaration of Helsinki. The study was approved by the institutional review boards of Massachusetts General Hospital, Brigham and Women's Hospital, and Fundación Huésped.

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Results

We report a 30-year-old woman who was first diagnosed with HIV-1 in March 2013 through a requested serologic test; her last negative HIV-1 test result was in 2011. The patient's partner, who was living with HIV-1, had a plasma viral load of 186 000 copies/mL in February 2013 and died of AIDS in July 2017.

During the patient's 8 years of follow-up, results from a total of 10 commercial viral load tests were below detection thresholds (Figure, A), and there were no clinical or laboratory signs of HIV-1-associated disease. No ART was started until 2019, when she became pregnant and began treatment with tenofovir, emtricitabine, and raltegravir for 6 months (September 2019 to March 2020) during the second and third trimesters. After delivering a healthy (HIV-1-negative) baby, she stopped ART. After this, the patient's HIV-1 viral loads remained undetectable by commercial PCR assays. She had negative results on serologic tests for hepatitis C virus and hepatitis B virus and no history of other sexually transmitted infections. Her baby received 4 weeks of zidovudine treatment and was not breastfed; HIV-1 plasma RNA was negative at ages 6 and 62 days, and an HIV-1 ELISA showed a negative result at age 17 months.

To evaluate persistent HIV-1 reservoir cells in this patient, we used near-full-length individual proviral sequencing for single-genome amplification of HIV-1 DNA (9, 14). A total of 1.188 billion PBMCs, collected in 2017 to 2019 (265 million) and in 2020 (923 million), were subjected to this analysis, and 503 million mononuclear cells (32% of which were CD4⁺ leukocytes) from the placenta were also analyzed (Table). In total, only 7 defective proviral HIV-1 DNA species were detected (all from PBMCs; none from the placenta): 1 near-full-length sequence with APOBEC-3G/3F-induced lethal hypermutations, and 6 sequences with large deletions, of which 3 were clonal (Figure, B and C; Appendix Figure 1, B, available at [Annals.org](#)). These HIV-1 DNA products clearly indicate that this person was infected with HIV-1 in the past and that active cycles of viral replication had occurred at one point. A total of 150 million resting CD4⁺ T cells were subsequently analyzed using a viral outgrowth assay, without retrieving a single replication-competent viral particle (Table). Ultrasensitive analysis of HIV-1 RNA from 4.5 mL of plasma failed to detect any viral RNA copies (Appendix Table 1, available at [Annals.org](#)).

Immunologic assays in this person showed HIV-1-specific memory CD4⁺ and CD8⁺ T-cell responses against HIV-1 p24 (Appendix Figure 2, A, available at [Annals.org](#)) in the background of the HLA class I alleles A*02:01, A*31:01, B*15:01, B*44:02, Cw*03:03, and Cw*05:01 and the class II alleles DPA1*01:03, DPA1*01:03, DPB1*02:01, DPB1*04:01, DQA1*03:03, DQA1*03:03, DQB1*03:01, DQB1*03:01, DRB1*04:01, DRB1*04:08, DRB4*01:01, and DRB4*01:01. The patient's CCR5 gene was homozygous for the wild-type allele. Her HIV-1 Western blots consistently showed an incomplete pattern consisting of gp160/120 and p24 bands (Appendix Table 2, available at [Annals.org](#)), suggesting an incomplete seroconversion. There was no evidence of ART-related or HLA class I-associated viral escape mutations in the detected proviral sequences (Appendix Figure 2, B; Appendix Figure 3, available at [Annals.org](#)), and results of plasma testing for 18 commonly used antiretroviral agents were negative in 2019. Activated CD4⁺ T cells from this patient expressed clearly detectable levels of CCR5 and CXCR4 (Appendix Figure 2, C) and were able to effectively support HIV-1 replication in in vitro infection assays with R5- and X4-tropic viral isolates (Appendix Figure 2, D).

Discussion

The person described in this article displays the clinical phenotype of an HIV-1 elite controller or posttreatment controller, defined by durably undetectable HIV-1 plasma viremia in the absence of ART. What distinguishes her from all other described elite controllers and posttreatment controllers is the absence of detectable intact HIV-1 proviruses and replication-competent HIV-1 viral particles in large numbers of cells (>1.5 billion in total). This has previously been described only in a 67-year-old woman with 28 years of drug-free HIV-1 control in whom no intact proviral sequence was detected despite analysis of more than 1.5 billion PBMCs (5). Notably, the person described here resembles the “Berlin patient,” a patient with HIV-1 who underwent a transplant with CCR5-32-encoding hematopoietic stem cells, enabling cell-intrinsic resistance to HIV-1 infection. In the Berlin patient, no replication-competent HIV-1 proviruses were detected in 1.4 billion CD4+ T cells leading to the conclusion that he had achieved a sterilizing cure of HIV-1 infection (15).

Does this imply that our patient has developed a sterilizing cure during natural infection? We believe this is likely, but it cannot be proved. Although this might sound unsatisfying, it reflects an intrinsic limitation of scientific research: Scientific concepts can never be proved through empirical data collection; they can only be *disproved*. In the context of HIV-1 research, this means that it will be impossible to empirically prove that anybody has achieved a sterilizing cure. All that can reasonably be done is to show that someone is *not* cured, by isolating intact proviruses and/or replication-competent HIV-1 from patient-derived material, as we and others have done in almost all prior analyzed patients (4–6). In contrast, in the person described here, we failed to detect any intact or replication-competent proviruses, despite what we consider a serious and comprehensive effort to detect them using massive numbers of cells and multiple complementary virologic assays. Therefore, we currently cannot reject the hypothesis that this patient has achieved a sterilizing cure.

The mechanisms that enable such a remarkable disease outcome are difficult to ascertain. Innate immune cells, HIV-1-specific T-cell or B-cell responses, or cell-intrinsic restriction of viral replication steps leading to abortive HIV-1 infection may all have contributed, although it is noteworthy that we detected 1 near-full-length hypermutated provirus. Such hypermutated sequences result from APOBEC-3G/3F-mediated immune effects and imply that productive viral replication cycles must have occurred at one point in our patient (16). In addition, proviral sequences with large deletions differed at multiple base pair residues (Appendix Figure 1, B), further supporting the notion that multiple rounds of productive infection have occurred in the past and that the proviral landscape does not result from abortive infection of the founder virus. Notably, the near-full-length hypermutated sequence did not show evidence of nef deletions (Appendix Figure 1, C), which have previously been associated with drug-free HIV-1 control (17). Therefore, infection with an attenuated viral strain is unlikely.

Collectively, our results raise the possibility that a sterilizing cure of HIV-1 infection, defined by the absence of detectable intact HIV-1 proviruses, is an extremely rare but possible clinical outcome. The person described here is originally from the city of

Esperanza, Argentina, and in line with her wishes, we propose to refer to her as the “Esperanza patient” to send a message of hope for finding a cure for HIV-1 infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Ruelas DS, Greene WC. An integrated overview of HIV-1 latency. *Cell*. 2013;155:519–29. doi:10.1016/j.cell.2013.09.044 [PubMed: 24243012]
2. Gupta RK, Abdul-Jawad S, McCoy LE, et al. HIV-1 remission following CCR5 32/ 32 haematopoietic stem-cell transplantation. *Nature*. 2019;568:244–8. doi:10.1038/s41586-019-1027-4 [PubMed: 30836379]
3. Hütter G, Nowak D, Mossner M, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med*. 2009;360:692–8. doi:10.1056/NEJMoa0802905 [PubMed: 19213682]
4. Blankson JN, Bailey JR, Thayil S, et al. Isolation and characterization of replication-competent human immunodeficiency virus type 1 from a subset of elite suppressors. *J Virol*. 2007;81:2508–18. [PubMed: 17151109]
5. Jiang C, Lian X, Gao C, et al. Distinct viral reservoirs in individuals with spontaneous control of HIV-1. *Nature*. 2020;585:261–7. doi:10.1038/s41586-020-2651-8 [PubMed: 32848246]
6. Sáez-Cirión A, Bacchus C, Hocqueloux L, et al. ; ANRS VISCONTI Study Group. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. *PLoS Pathog*. 2013;9:e1003211. doi:10.1371/journal.ppat.1003211 [PubMed: 23516360]
7. Rasheed FN, Bulmer JN, Morrison L, et al. Isolation of maternal mononuclear cells from placentas for use in in vitro functional assays. *J Immunol Methods*. 1992;146:185–93. [PubMed: 1538142]
8. Xu Y, Plazyo O, Romero R, et al. Isolation of leukocytes from the human maternal–fetal interface. *J Vis Exp*. 2015:e52863. doi:10.3791/52863 [PubMed: 26067211]
9. Lee GQ, Orlova-Fink N, Einkauf K, et al. Clonal expansion of genome-intact HIV-1 in functionally polarized Th1 CD4+ T cells. *J Clin Invest*. 2017;127:2689–96. doi:10.1172/JCI93289 [PubMed: 28628034]
10. Laird GM, Rosenbloom DI, Lai J, et al. Measuring the frequency of latent HIV-1 in resting CD4+ T cells using a limiting dilution coculture assay. *Methods Mol Biol*. 2016;1354:239–53. doi:10.1007/978-1-4939-3046-3_16 [PubMed: 26714716]

11. Ghiglione Y, Polo ML, Urioste A, et al. Hepatitis C virus (HCV) clearance after treatment with direct-acting antivirals in human immunodeficiency virus (HIV)–HCV coinfection modulates systemic immune activation and HIV transcription on antiretroviral therapy. *Open Forum Infect Dis.* 2020;7:ofaa115. doi:10.1093/ofid/ofaa115
12. Salido J, Ruiz MJ, Trifone C, et al. Phenotype, polyfunctionality, and antiviral activity of in vitro stimulated CD8+ T-cells from HIV+ subjects who initiated cART at different time-points after acute infection. *Front Immunol.* 2018;9:2443. doi:10.3389/fimmu.2018.02443 [PubMed: 30405632]
13. Digitale JC, Callaway PC, Martin M, et al. HLA alleles B*53:01 and C*06:02 are associated with higher risk of *P. falciparum* parasitemia in a cohort in Uganda. *Front Immunol.* 2021;12:650028. doi:10.3389/fimmu.2021.650028 [PubMed: 33815410]
14. Einkauf KB, Lee GQ, Gao C, et al. Intact HIV-1 proviruses accumulate at distinct chromosomal positions during prolonged antiretroviral therapy. *J Clin Invest.* 2019;129:988–98. doi:10.1172/JCI124291 [PubMed: 30688658]
15. Yukl SA, Boritz E, Busch M, et al. Challenges in detecting HIV persistence during potentially curative interventions: a study of the Berlin patient. *PLoS Pathog.* 2013;9:e1003347. doi:10.1371/journal.ppat.1003347 [PubMed: 23671416]
16. Esnault C, Heidmann O, Delebecque F, et al. APOBEC3G cytidine deaminase inhibits retrotransposition of endogenous retroviruses. *Nature.* 2005;433:430–3. [PubMed: 15674295]
17. Kirchhoff F, Greenough TC, Brettler DB, et al. Brief report: absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection. *N Engl J Med.* 1995;332:228–32. [PubMed: 7808489]

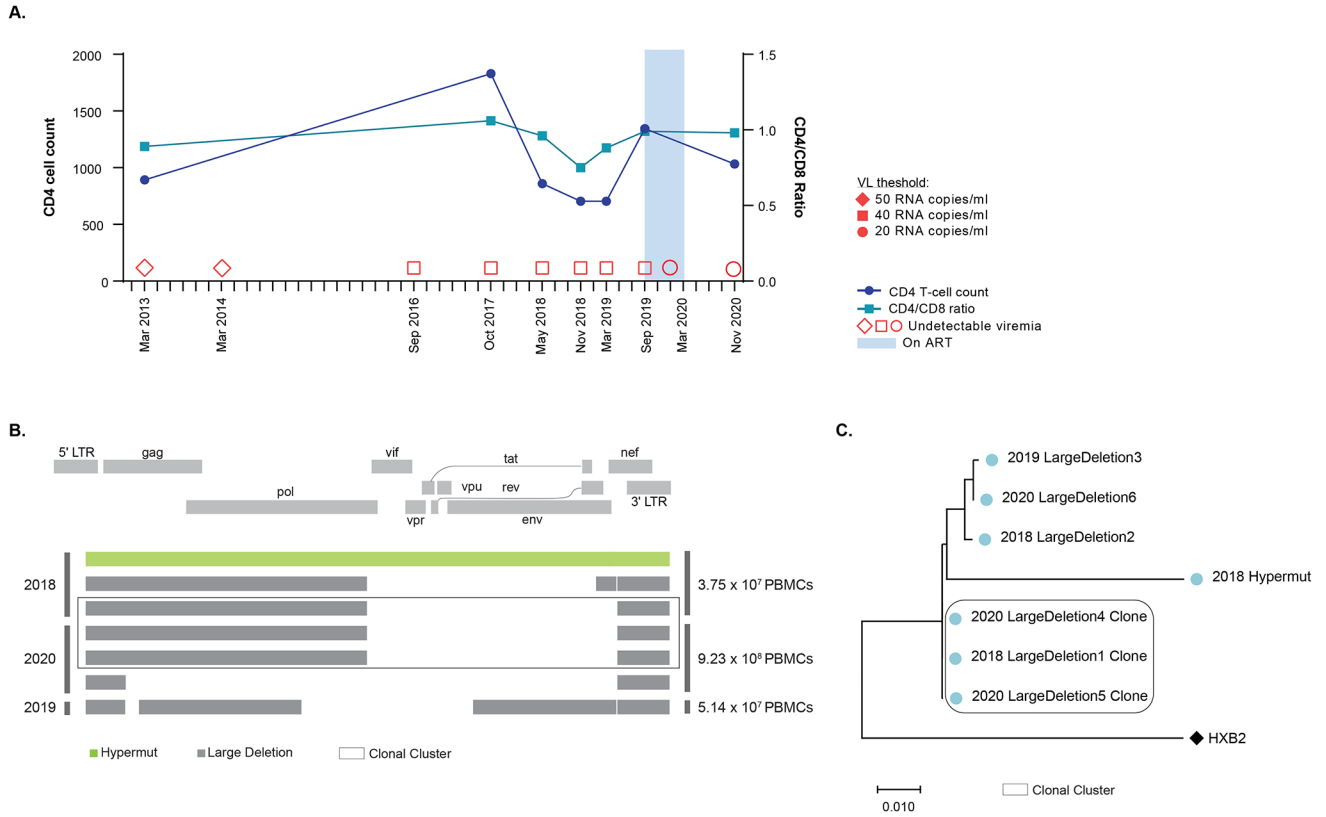


Figure. Clinical and virologic characteristics of the Esperanza patient. ART = antiretroviral therapy; LTR = long terminal repeat; PBMC = peripheral blood mononuclear cell. **A.** Longitudinal CD4⁺ T-cell counts (cells/mL), CD4–CD8 ratios, and HIV-1 viral loads in the Esperanza patient. The recorded diagnosis date of HIV-1 infection is shown as the first date on the *x*-axis. The detection threshold for each viral load test is represented by a diamond (50 RNA copies/mL), a square (40 RNA copies/mL), or a circle (20 RNA copies/mL). **B.** Virogram indicating proviral HIV-1 DNA sequences isolated from a total of 1.188 billion PBMCs in the Esperanza patient. Sequences with hypermutations and large deletions are indicated by different colors; sequence-identical (clonal) sequences are boxed. **C.** Linear maximum-likelihood phylogenetic tree of HIV-1 proviral sequences detected in the Esperanza patient, relative to HXB2. The clonal cluster of proviral sequences with a large deletion was detected in PBMCs collected in 2018 and 2020 and is highlighted by the box.

Table.

HIV-1 Reservoir Profiling Assays Performed on Cells From the Esperanza Patient

Assay	Cells, <i>n</i>	Cell Type	Intact Proviruses, <i>n</i>	Defective Proviruses, <i>n</i>	Replication-Competent Proviruses, <i>n</i>
Near-full-length individual proviral sequencing assay	1.188 billion	Peripheral blood mononuclear cells	0	7	–
Near-full-length individual proviral sequencing assay	503 million	Isolated cells from placenta	0	0	–
Viral outgrowth assay	150 million	Resting CD4 ⁺ T cells	–	–	0

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