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

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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 CCR5Δ32 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 8 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 of intact HIV-1 proviruses. 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 limited 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

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Editorial comment

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 loads were 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 (CTL) epitopes (restricted by autologous HLA class I alleles) that match the clade B consensus sequence and CTL epitope 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), 91US-056 (CCR5-tropic) (NIH AIDS Reagent Program, ARP-2099), and NL4-3 with a BaL-derived env (CCR5-tropic) viruses for 4 hours at 37 °C. Viral replication was monitored by p24 ELISA (PerkinElmer) in culture supernatants at days 3, 5, and 7.

HLA 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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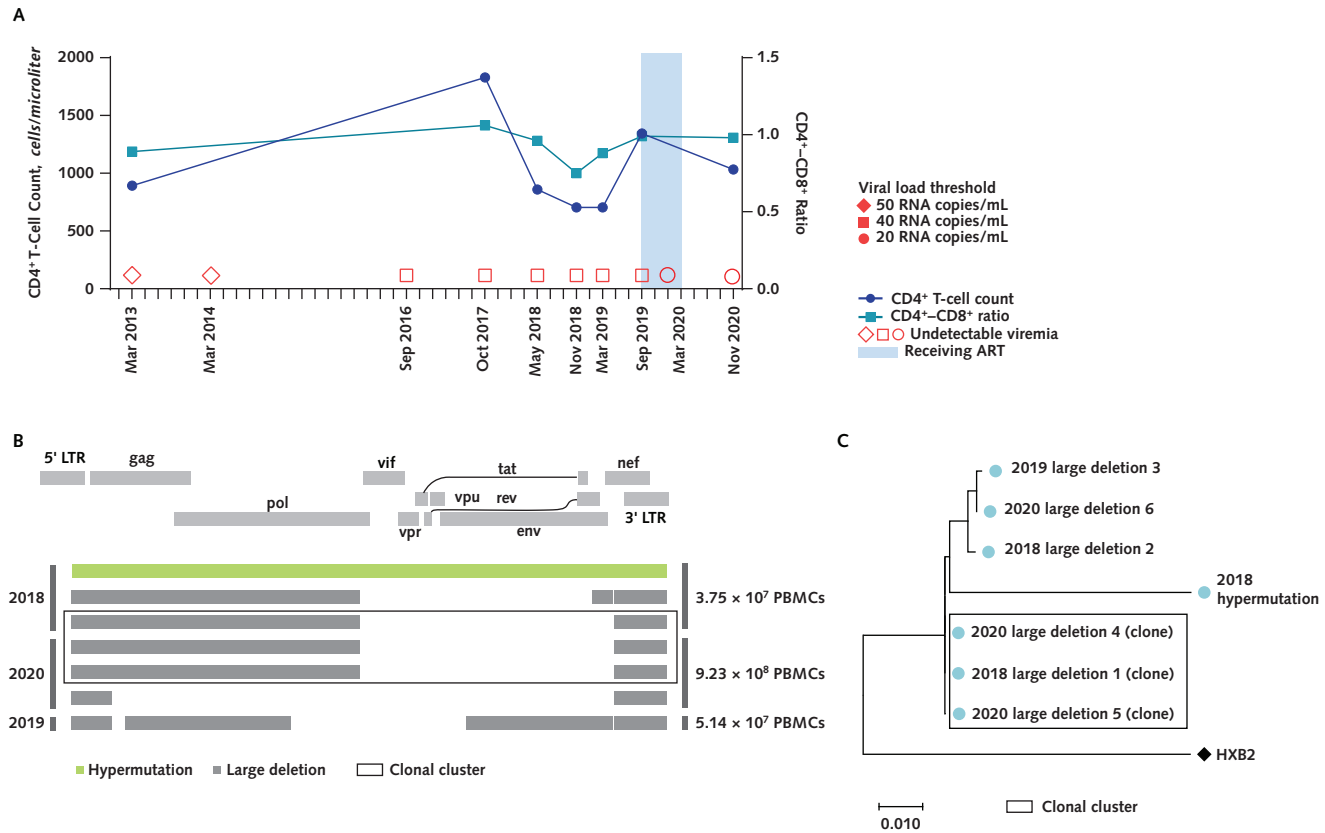
Figure. Clinical and virologic characteristics of the Esperanza patient.



ART = antiretroviral therapy; LTR = long terminal repeat; PBMC = peripheral blood mononuclear cell. **A.** Longitudinal $CD4^+$ T-cell counts (cells/microliter), $CD4^+$–$CD8^+$ 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.

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 $CD45^+$ leukocytes) from the placenta were also analyzed (**Table**; **Appendix Figure 1**, *A*, available at Annals.org). 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*). 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

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

Assay	Cells, *n*	Cell Type	Intact Proviruses, *n*	Defective Proviruses, *n*	Replication-Competent Proviruses, *n*
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 mononuclear cells from placenta	0	0	–
Viral outgrowth assay	150 million	Resting $CD4^+$ T cells	–	–	0

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, DQA1*03, DQB1*03:01, DQB1*03:01, DRB1*04:01, DRB1*04:08, DRB4*01, and DRB4*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 CCR5Δ32-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.

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Appendix Table 1. Additional HIV-1 Reservoir Profiling Assays Performed*

Assay	Cells, *n*	Sample Type	HIV-1 Detected
Total DNA	0.118 million	$CD4^+$	None
Integrated DNA	0.118 million	$CD4^+$	None
2-LTR	0.118 million	$CD4^+$	None
Cell-associated unspliced RNA	3.4 million	$CD4^+$	None
Cell-associated multiple-spliced RNA	3.4 million	$CD4^+$	None
SCA Aptima HIV-1 Quant Dx Assay	NA	4.5 mL plasma	None

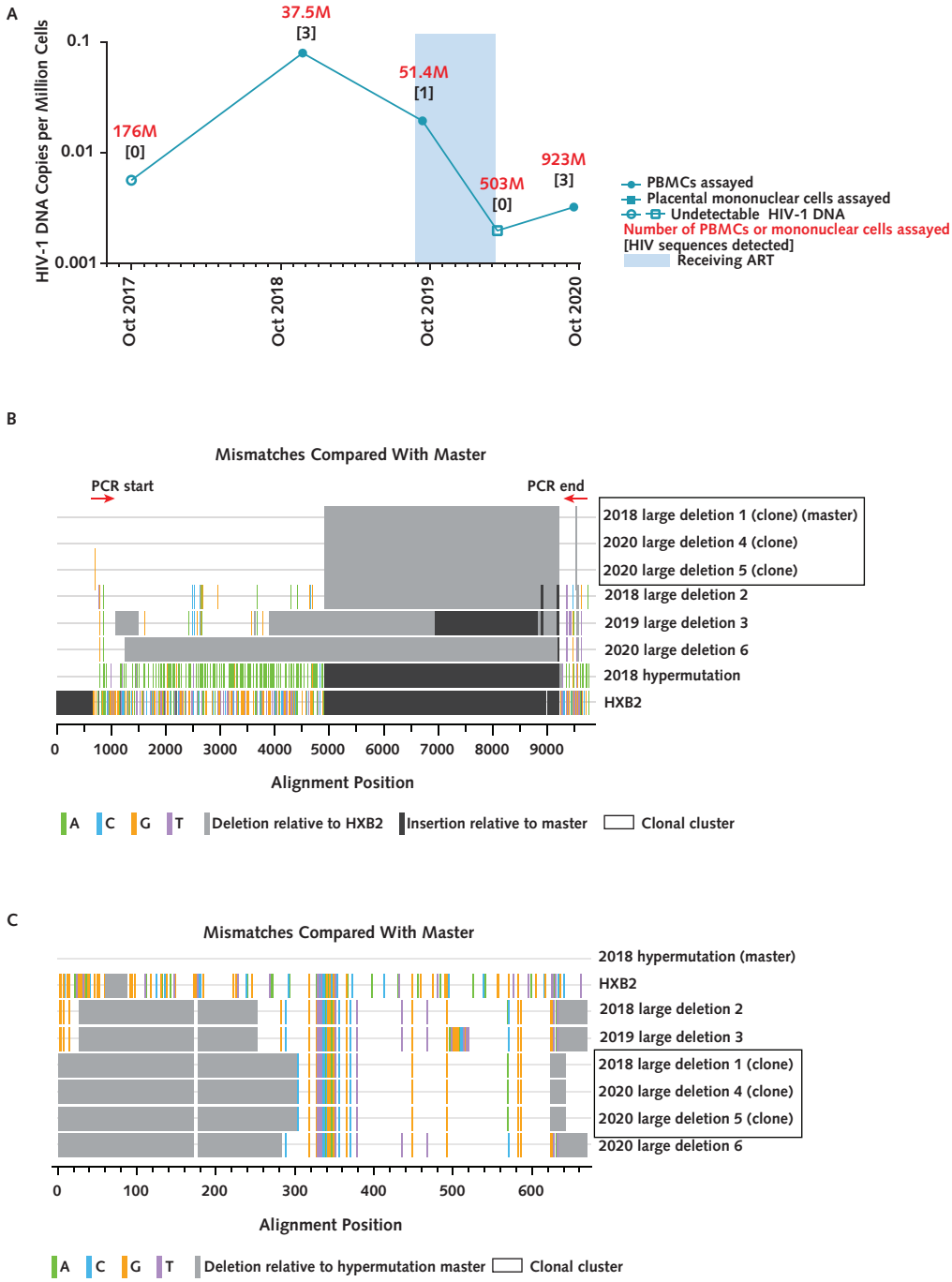
LTR = long terminal repeat; NA = not applicable; SCA = single-copy assay.
* HIV-1 DNA forms (total, integrated, and 2-LTR circles) and cell-associated HIV RNA forms (unspliced and multiple-spliced) were evaluated in the indicated number of sorted peripheral $CD4^+$ T cells by quantitative polymerase chain reaction. Ultrasensitive HIV-1 plasma viral load was evaluated by replicate testing (total plasma volume, 4.5 mL) using the Aptima HIV-1 Quant Dx Assay (Hologic). In all cases, no copies of HIV nucleic acids were detected.

Appendix Table 2. Antigen-Specific Antibody Western Blot Tests Performed*

Sample Date	Western Blot Result
March 2013	gp160/120, p24
May 2013	gp160/120, p24
September 2013	gp160/120, p24
March 2014	gp160/120, p24
September 2016	gp160/120, p24
April 2017	gp160
October 2017	gp160/120, p24
May 2018	gp160, p24
September 2019	gp160/120, p24

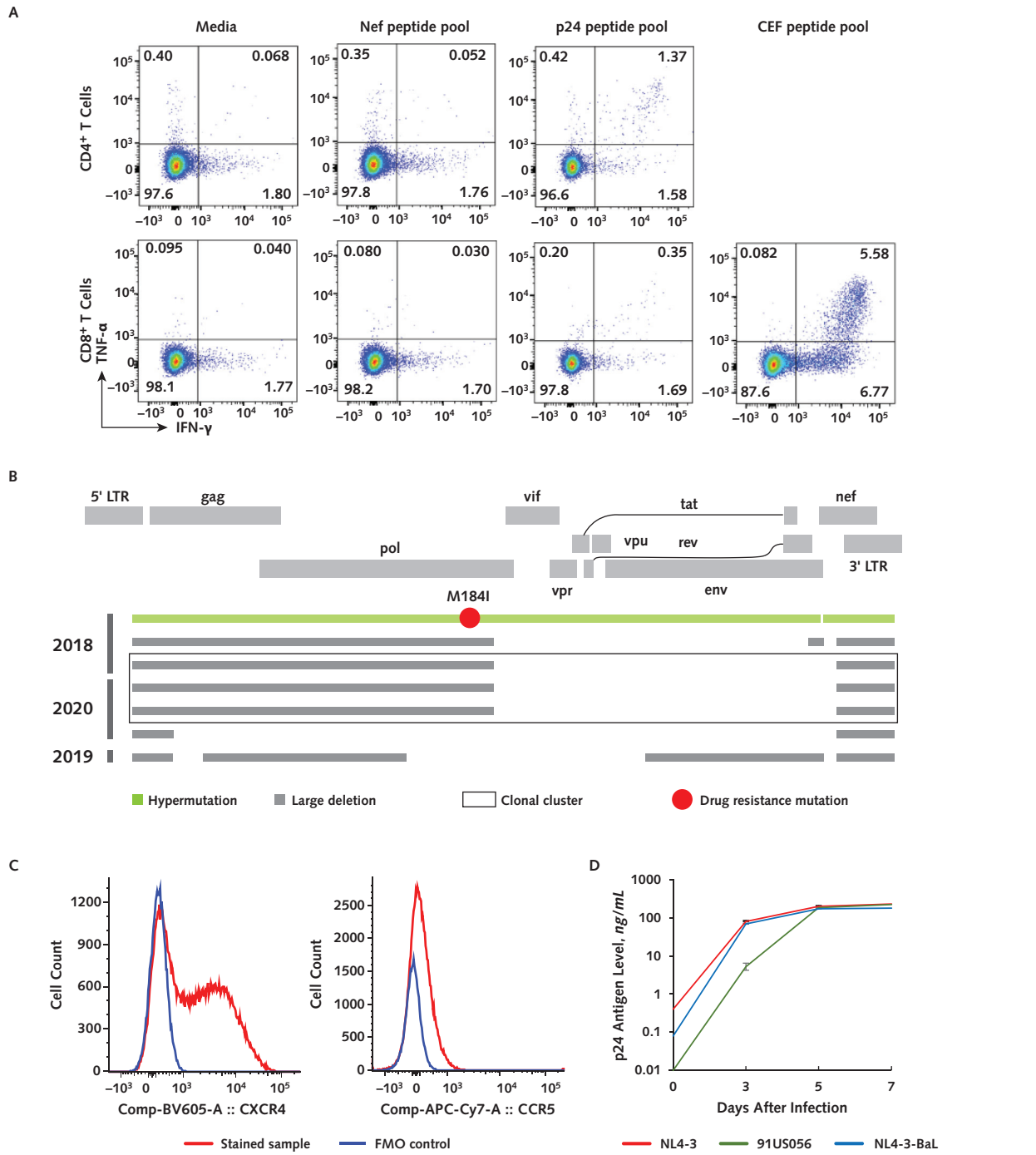
* The table shows the profile of HIV-1 antigen-specific antibody responses detectable by commercially available Western blot tests in plasma samples obtained at the time of HIV-1 diagnosis and during follow-up. All tests performed during the patient's follow-up (except for the sample obtained in April 2017) showed a positive result for HIV-1 according to the Centers for Disease Control and Prevention criteria for interpretation of HIV-1 Western blot tests. However, full band profile was not achieved, and the same 2 bands were always present during the 8-year follow-up.

Appendix Figure 1. Detailed clinical and virologic characteristics of the Esperanza patient.



ART = antiretroviral therapy; M = million; PBMC = peripheral blood mononuclear cell; PCR = polymerase chain reaction. **A.** Total HIV-1 proviral DNA levels in PBMCs or placental mononuclear cells, determined by near-full-length individual proviral sequencing and expressed in HIV DNA copies per million cells. A total of 1.188 billion PBMCs and 503 million placental mononuclear cells collected between 2017 and 2020 were analyzed. The numbers of PBMCs or placental mononuclear cells assayed for each time point are shown in red. The numbers of HIV-1 proviral sequences detected in each sample are shown in brackets. **B.** Highlighter plot reflecting variations in HIV-1 DNA sequences isolated from this patient. The clonal cluster detected in 2018 and 2020 is highlighted by the box. Base-pair mismatches relative to the clonal cluster are indicated by thin colored bars. Large deletions in the HIV-1 proviral sequences relative to HXB2 are indicated by light gray bars, and large insertions in the HIV-1 proviral sequences and HXB2 relative to the clonal cluster are indicated by dark gray bars. **C.** Highlighter plot reflecting variations in HIV-1 nef sequences isolated from this patient. Base-pair mismatches relative to the hypermutated sequence isolated from this patient are indicated by thin colored bars. The clonal cluster is highlighted by the box. The hypermutated sequence covers the entire nef region, providing evidence that this patient was not infected with a nef-deletion founder virus.

Appendix Figure 2. Immunologic characteristics of the Esperanza patient.



FMO = Fluorescence Minus One; IFN = interferon; LTR = long terminal repeat; TNF = tumor necrosis factor. **A.** HIV-1 Nef- and p24-specific memory $CD4^+$ and $CD8^+$ T-cell responses. Percentages of IFN-γ-producing and TNF-α-producing $CD4^+$ and $CD8^+$ T cells were shown in responses to HIV-1 (Nef or p24) peptide pools. Cells cultured in media alone and cells stimulated with CEF peptide pools were used as negative and positive controls, respectively. **B.** Drug resistance mutations identified in this patient's HIV-1 proviral sequences. The Stanford HIV Drug Resistance Database was used to identify mutations in the pol sequence associated with resistance to the major HIV-1 antiretroviral drugs: nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, integrase strand transfer inhibitors, and protease inhibitors. One mutation, M184I, which is associated with resistance to nucleoside reverse transcriptase inhibitors, was identified in the hypermutated sequence detected in peripheral blood mononuclear cells collected in 2018. This mutation is due to an A-to-G nucleotide APOBEC-3G/3F-induced hypermutation. This finding is not unexpected on the basis of previous work indicating that this and other mutations are enriched in hypermutated sequences. **C.** Histograms showing proportions of $CXCR4^+$ and $CCR5^+$ cells in activated $CD4^+$ T cells from the Esperanza patient. **D.** Growth kinetics of indicated viruses after infection of activated $CD4^+$ T cells from the Esperanza patient. Activated $CD4^+$ T cells were infected with the CXCR4-tropic NL4-3 (*red line*), the primary CCR5-tropic strain 91US056 (*green line*), and NL4-3 expressing an R5-tropic (BaL-derived) envelope sequence (*blue line*). HIV-1 replication was assessed by p24 antigen levels in culture supernatants at the indicated time points. Data are presented as means; error bars indicate SEs.

Appendix Figure 3. HLA class I–associated epitopes detected in each unique HIV provirus sequence from the Esperanza patient.

Protein	HLA Class I	Clade B Wild-Type Epitope	Autologous Sequence	Frequency
Gag	A02	VLAEAMSQV	VLAEAMSQV	3/4
			VLAKAISQV	1/4^
Gag	A0201	FLGKIWPSYK	FLGKIWPSSR	4/4
Gag	A0201	RSLYNTVATLY	RSLYNTVATLY	4/4
Gag	B15	YVDRFFKTL	YVDRFYKVL	4/4
Gag	B1501	VKVIEEKAF	VKVIEEKAF	4/4
Gag	B1501	GLNKIVRMY	GLNKIVRMY	4/4
Gag	B4402	AEQASQDVKNW	AEQASQDVKNW	4/4
Gag	B4402	RDYVDRFYKTL	RDYVDRFYKVL	4/4
Gag	Cw05	AEQASQEVKNWM	AEQASQEVKNWM	4/4
gp160	A02	KLTPLCVTL	KLTPLCVTL	1/1
gp160	A02	SLLNATAIAV	SLLNAITIAV	2/2
gp160	B15	SFNCGGEFF	SFSCGGEFF	1/2
			SFNCREKFF	1/2^
Nef	A02	AAVDLSHFL	AAVDLSFFL	3/3
Nef	A02	VLEWRFDSRL	VLVWKFDSIL	5/5
Nef	A02	LTFGWCFKLV	LTFGWCFKLV	4/5
			LTFG*CFKLV	1/5^
Nef	A0201	PLTFGWCYKL	PLTFGWCFKL	4/5
			PLTFG*CFKL	1/5^
Nef	B15	WRFDSRLAF	WKFDSILAS	3/3
Nef	B1501	TQGYFPDWQNY	TQGYFPDWQNY	5/5
Nef	B4402	QEILDLWVY	KDIPDLWVH	4/5
			--ILDL*VY	1/5^
Nef	Cw03	AALDLSHFL	AAVDLSFFL	3/3
Pol	A02	LVGPTPVNI	LVGPTPVNI	4/4
Pol	A02	YTAFTIPSI	YTAFTIPSI	4/4
Pol	A0201	VIYQYMDDL	VVYQYMDDL	4/4
Pol	A0201	ALVEICTEM	ALVEICTEM	4/4
Pol	A0201	KLVSQGIRKV	KLVSNGIRKV	3/4
			KLVSNKIRKV	1/4^
Pol	A0201,Cw0303	ILKEPVHGV	ILKEPVHGV	4/4
Pol	B1501	LVGKLNWASQIY	LVGKLNWASQIY	3/4
			LVRKLN*ASQIY	1/4^
Pol	Cw05	HTDNGSNF	HTDNGSNF	3/4
			HTGNGSNF	1/4^
Rev	B4402	EELLKTVRL	EELLKTVRL	1/1

Wild type
Uncharacterized mutation
* Stop codon due to APOBEC3 mutation
^ Mutation only in hypermutated sequence
-- Deletion

Optimal epitopes and escape variants associated with this patient's HLA class I alleles were obtained from the Los Alamos National Laboratory HIV Immunology Database. Cytotoxic T-lymphocyte (CTL) epitopes identified in this patient with sequences identical to the clade B wild-type consensus sequence are highlighted by blue boxes, and uncharacterized mutations in the CTL epitopes relative to the consensus sequence are highlighted by pink boxes. The red characters indicate the mutated amino acids in the autologous sequences compared with the wild-type epitope sequences. None of the mutations in this patient's CTL epitopes relative to the wild-type consensus sequence are consistent with previously described CTL-driven escape mutations.