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HIV-1 Remission and Possible Cure in a Woman after Haplo-Cord Blood Transplant

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[§]While this manuscript was under review, the City of Hope patient who achieved HIV remission following stem cell transplant, was presented at the AIDS meeting in 2022[88].

Inclusion and Diversity

DECLARATION OF INTERESTS

The authors declare no competing interests.

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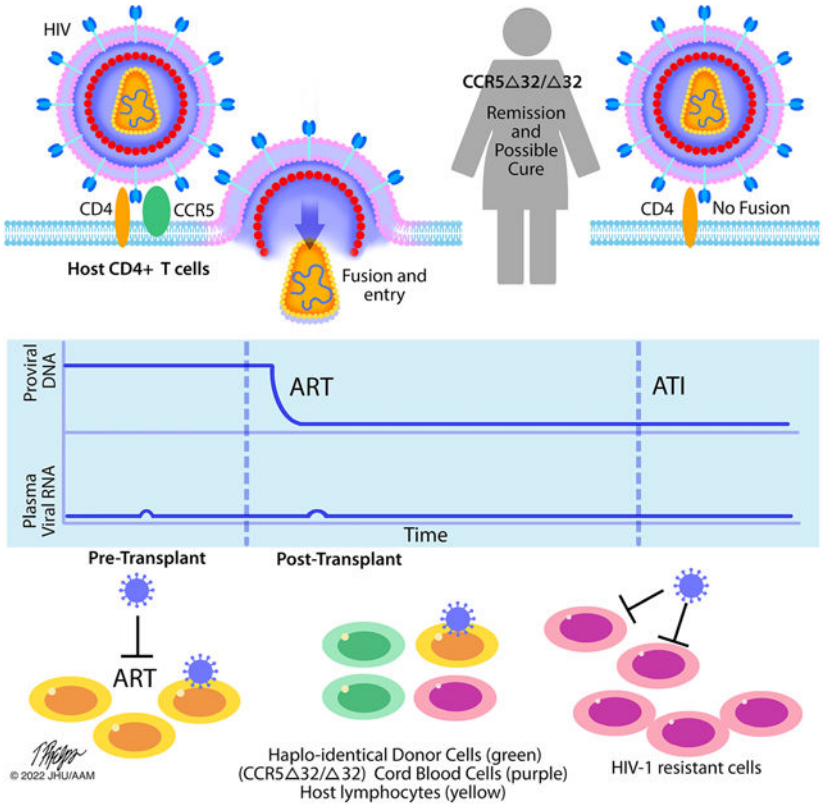
SUMMARY

Previously, two men were cured of HIV-1 through CCR5Δ32 homozygous (CCR5Δ32/Δ32) allogeneic adult stem cell transplant. We report the first remission and possible HIV-1 cure in a Mixed-race woman who received a CCR5Δ32/Δ32 haplo-cord transplant (cord blood cells combined with haploidentical stem cells from an adult) to treat acute myeloid leukemia (AML). Peripheral blood chimerism was 100% CCR5Δ32/Δ32 cord blood by Week 14 post-transplant and persisted through 4.8 years of follow-up. Immune reconstitution was associated with (1) loss of detectable replication-competent HIV-1 reservoirs, (2) loss of HIV-1-specific immune responses, (3) *in vitro* resistance to X4 and R5-laboratory variants, including pre-transplant autologous latent reservoir isolates, and (4) 18 months of HIV-1 control with aviremia, off antiretroviral therapy, starting at 37 months post-transplant. CCR5Δ32/Δ32 haplo-cord transplant achieved remission and a possible HIV-1 cure for a person of diverse ancestry, living with HIV-1, who required a stem cell transplant for acute leukemia.

Following the previous cure of two men of HIV-1 through allogenic stem cell transplantation procedures, a study reports a broader application of this approach by demonstrating the first such possible cure in a mixed-race woman using CCR5Δ32/Δ32 haplo-cord transplant to treat acute myeloid leukemia. This transplant resulted in resistance to HIV strains and no detectable virus was seen even with the removal of antiretroviral medications.

Graphical Abstract



INTRODUCTION

Worldwide, nearly 38 million people live with HIV-1 (PLWH),[1] with an estimated 1.2 million in the US.[2] Infection with HIV-1 is incurable due to the early establishment of HIV-1 latency in long-lived, resting-memory CD4+ T-cells. [3–6] These latent proviruses fuel viremia when antiretroviral therapy (ART) stops, necessitating treatment for life.[7–9] CCR5, a β-chemokine receptor gene, is the main co-receptor used by HIV-1 to infect CD4+ T-cells. [10–16] A homozygous CCR5 Δ32 frameshift mutation that deletes 32 base pairs (CCR5 Δ32/Δ32) in the coding region eliminates cell surface expression of CCR5. This change confers natural resistance to CCR5-tropic HIV-1 variants. [12–14,17,18] About 10% percent of Northern European Caucasians carry the Δ32 mutation in CCR5, however only 1% are homozygous for the mutation. [19,20] Transplantation with CCR5 Δ32/Δ32 stem cells could eradicate HIV-1 reservoirs, replacing an immune system with one resistant to HIV-1, ultimately yielding HIV-1 remission and cure for PLWH who requires transplant for underlying medical conditions.[21] Two published cases of HIV-1 cure were reported using unrelated adult CCR5Δ32/Δ32 stem cell donors.[22–25]

- **The Berlin Patient**, Timothy Brown was a Caucasian man living with HIV-1 and acute myeloid leukemia (AML). He received a 10/10 human lymphocyte antigen (HLA)-matched CCR5Δ32/Δ32 adult unrelated donor stem cell transplant. Following AML relapse, he received a 2nd transplant with the same donor. He

was deemed cured of HIV-1 and AML. He died 12 years later of AML relapse without evidence of HIV-1 rebound off ART.[23,26,27]

- **The London Patient,** Adam Castillejo, received a 9/10 HLA-matched CCR5Δ32/Δ32 adult unrelated donor stem cell transplant for his Hodgkin lymphoma. He was reported in remission after 18 months of aviremia off ART, and cured after 30 months with no rebound of HIV-1 viremia. [24,25]

In addition, the Duesseldorf patient, a Caucasian man living with HIV-1, who also received an adult CCR5 Δ32/Δ32 stem cell transplant for AML had six months of aviremia following ATI.[28] At the time of this report, there is no updated published information available to ascertain a longer duration of remission or HIV-1 cure.

Stem cell transplantion with CCR5Δ32/Δ32 is rare due to the exceedingly low prevalence of CCR5Δ32/Δ32 in the population,[19,29] and the fact that few registries screen adult stem cell donors for this mutation. However, cord blood (CB) could be a better donor stem cell for PLWH who are racially diverse.[30] CB can successfully engraft even when the grafts are only partially-matched for HLA and has less graft versus host disease.[31,32] In the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) P1107 study (NCT02140944), we posited that access to pre-screened umbilical cord blood units (CBUs) for the CCR5Δ32/Δ32 gene mutation would expand the pool of donor cells for non-Caucasians living with HIV-1.

Here, we report remission and possible HIV-1 cure in a middle-aged, Mixed race woman who developed AML four years into her diagnosis of acute HIV-1 infection and while on ART. She was transplanted with a CCR5Δ32/Δ32 homozygous CBU from the StemCyte cord blood registry, combined with CD34-selected stem cells from a haploidentical related donor with wild-type CCR5 allele. [33,34]

The participant's transplant resulted in rapid engraftment and immune replacement by CCR5Δ32/Δ32 cord blood cells, with AML in remission. HIV-1 replication was suppressed to clinically undetectable levels prior to transplant and maintained even after ART interruption. Correlative viral, immunological, and latent HIV-1 reservoir studies were consistent with the elimination of HIV-1 in peripheral blood, which were detectable pre-transplant. She remained cancer-free at 55 months post-transplant and without viremic rebound after 18 months off ART, suggesting a remission and possible HIV-1 cure. There was no detectable circulating replication-competent proviral reservoirs post-transplant while off ART. Antiretrovirals (ARVs) were detected in blood plasma at multiple points before ART interruption but none post-ART interruption, confirming ART-free HIV-1 remission and possible cure (Please see the STAR Methods section for details on the tests used).

RESULTS

IMPAACT P1107 was an observational study designed to include individuals >12 months of age through adulthood living with HIV-1 who need an allogeneic stem cell transplant for underlying illnesses, such as hematologic malignancy. The study used pre-screened CBUs for the CCR5 Δ32 allele for heterozygotes and homozygotes from the StemCyte

International Cord Blood Center Registry (StemCyte), a combination of private and public cord blood banks, as a strategy to lead to cures of the underlying condition and HIV-1 simultaneously. [31] At the time of study development in 2013, StemCyte had screened 18,000 CBUs and identified 171 CCR5Δ32/Δ32 homozygous units,[31] now it has greater than 300 homozygous cord units. The study was co-endorsed by the NIH-sponsored AIDS Clinical Trials Group (ACTG) to enroll adult participants and the Center for International Blood and Marrow Transplant Research (CIBMTR) to retrieve clinical and laboratory data collected for stem cell transplants in the US.

Two participants were enrolled in IMPAACT P1107; the first was a middle-aged man living with HIV-1 who developed Hodgkin lymphoma and underwent a haplo-cord transplant with CCR5Δ32/Δ32 CBU. He succumbed to relapsed Hodgkin lymphoma after losing CCR5Δ32/Δ32 CBU donor graft within a year following transplant. He had no reservoir assessments between transplantation and lymphoma relapse. However, we detected HIV-1 DNA (11.1 copies/ml) post-transplantation for up to 51 weeks and detectable low-level viremia at 1.3 HIV-1 RNA copies/ml while remaining undetectable by clinical assay <20 cp/ml, confirming persistence of HIV-1 producing cells. The second participant, a woman, is described below.

Case study:

A woman was diagnosed with acute HIV-1- infection four years before a diagnosis of AML. The patient self-identified as Mixed race. At the time of HIV-1 diagnosis, her plasma viral load (VL) was above the upper limit of quantification at > 1,000,000 HIV-1 RNA copies/mL (Figure 1A).

She was treated during acute HIV-1 infection with tenofovir disoproxil fumarate/ emtricitabine and raltegravir. Her treatment during acute infection is relevant, given the known impact of early ART on HIV-1 persistence.[35,36] However, our participant had a latent HIV-1 reservoir detected prior to transplant at 1.38 infectious units per million (IUPM), at levels seen in adults during chronic infection.[4,37] After five months on ART, her VL decreased to levels below limits of quantification of clinical assays (<20 HIV-1 RNA copies/mL). Throughout her HIV-1 infection course, she had several changes to her ART regimen: none for virologic failure. These included a change to a rilpivirine-containing regimen (tenofovir alafenamide/ emtricitabine/ rilpivirine), followed by a dolutegravir-containing regimen (abacavir/ lamivudine/ dolutegravir) and ultimately tenofovir alafenamide/ emtricitabine/ bictegravir. She had sustained control of plasma viremia except for one episode of transient viremia to 30 HIV-1 RNA copies/ mL when her ART was briefly held for neutropenia and before being ultimately diagnosed with AML (Figure 1A).

Four years following her diagnosis of acute HIV-1 infection, she was diagnosed with high-risk AML with monosomy 7 cytogenetic abnormality. She received standard induction chemotherapy with idarubicin and cytarabine, following which she achieved morphologic and cytogenetic remission. She also received one cycle of high-dose cytarabine consolidation. During induction chemotherapy, she had transient low-level viremia to150

HIV-1 RNA copies/mL, supporting the persistence of HIV-1-producing cells on ART (Figure 1A).

We searched the StemCyte registry and identified five CCR5Δ32/Δ32 mutated compatible CBU with a 4/8 or higher HLA match. We also identified a related haploidentical adult donor with CCR5 wild-type allele to support a haplo-cord transplant with early transient engraftment. In 2017, she underwent a haplo-cord transplant consisting of CD34-selected cells from the peripheral blood of the haploidentical adult donor, followed by the 5/8 HLA-matched CCR5 Δ32/Δ32 CBU. The cord blood graft contained 2×10^7 nucleated cells per kg and 2.2×10^5 CD34 cells per kg of the recipient's weight. The transplant conditioning regimen included fludarabine, melphalan, total body irradiation 4 Gray, and ATG. Graft-versus-host disease (GVHD) prophylaxis consisted of mycophenolate mofetil for 28 days and tacrolimus until Day 180 (Figure 1B). Before receiving the conditioning regimen, she was given a dose of rituximab for prophylaxis against post-transplant lymphoproliferative disease associated with Epstein-Barr Virus (EBV).[38–40] Her initial hospital course was uncomplicated, with neutrophil and platelet engraftment by Days 16 and 10, respectively. She was discharged home on Day +17 post-transplant. At two weeks post-transplant, 82% of her lymphoid (CD3+ cells) and 98% of myeloid (CD33+ cells) lineages were from the haploidentical adult donor. By 14 weeks, there was 100% engraftment with the CCR5Δ32/Δ32 CBU for both lineages (Figure 2). Six months post-transplant, B-cells (CD19+ cells) returned to normal levels, and by 13 months, CD4 and CD8 T-cell subsets and natural killer (NK) cells (CD 16 and CD 56) showed recovery to normal levels and have remained normal (Figure 1C).

The participant's post-transplant course was notable for asymptomatic cytomegalovirus (CMV) viremia two months post-transplant with a peak VL of 1,374 DNA copies/mL, for which she received a course of oral valganciclovir. Twenty-eight months post-transplant, she had asymptomatic EBV reactivation with a peak VL of 26,885 copies/mL, for which she received one dose of rituximab, resolving her EBV viremia. The B-cells normalized at five months post-rituximab treatment (Figure 1C).

During the first two years post-transplant, our participant had several documented episodes of mild upper respiratory tract infections with adenovirus, rhinovirus, non-SARS-CoV-2 coronavirus, and respiratory syncytial virus. She remained aviremic for HIV-1 throughout the post-transplant period while on and off ART. She did not develop acute or chronic GVHD. Bone marrow biopsy at four years post-transplant showed continued AML remission. She remains in AML remission 55 months post-transplant with 100% chimerism for the CCR5Δ32/Δ32 cord donor cells (Figure 2).

Over 55 months of follow-up post-transplant, all clinical laboratory testing showed undetectable HIV RNA levels, including undetectable HIV-1 RNA in cerebrospinal fluid, using a standard VL assay with a limit of quantification of 20 copies/ml. Notably, we detected trace low-level HIV-1 RNA by single copy assay in plasma at a single timepoint 107 weeks post-transplant, but this did not signify an imminent rebound as subsequent tests did not find HIV-1 RNA. Please see the STAR Methods section for more information on tests of HIV-1 reservoir and persistence markers.

After counseling and discussion, the study participant opted to interrupt her ART regimen at 30 months post-transplant to assess for HIV-1 remission and possible cure. The first ART interruption lasted only 13 days as the initial COVID-19 pandemic surged, raising concern about the ability to conduct the required frequent monitoring for viral rebound off ART. At 37 months post-transplant, ART was interrupted again (Figure 1A). HIV-1 RNA levels remained undetectable at < 20 copies/mL with no target detected through18 months after ATI (Figure 1A), heralding HIV-1 remission and a possible HIV-1 cure.

Timing of ART discontinuation:

We chose an optimal time to stop ART in our study participant based on the published literature on the London patient (off ART 16 months post-transplant), participant readiness to stop ART, and access to health care services during the COVID-19 pandemic, which led us to roughly three years post-transplant. This three-year post-transplant period aligns with the initial time estimate to virus eradication towards a cure with ART in seminal decay dynamic studies.[41] The caveat is that no long-lived reservoirs persisted.

Virologic markers:

Immediately before haplo-cord transplant, at approximately four years into effective ART, all assays used to quantify HIV-1 persistence, including assessment of the latent reservoir with the quantitative viral outgrowth assay (QVOA), were positive (Figure 3). HIV-1 RNA was detected at 3.30 HIV-1 RNA copies/mL of plasma (Figure 3A) confirming persistence of inducible HIV-1 reservoir cells. HIV-1 infected cells were detected at 137.4 HIV-1 DNA copies per million peripheral blood mononuclear cells (PBMC) (Figure 3B). Our PCR assays detected two long terminal repeat DNA circles (2-LTR) at 6.30 copies/10^6 million PBMC, confirming the persistence of long-lived HIV-1 reservoir-producing cells *before* transplant (Figure 3C). The size of the latent reservoir was 1.38 (95% confidence interval (CI) 0.419–4.549) infectious units per million (IUPM) CD4+ T-cells (Figure 3D).

Once the participant was off ART, we sampled 74.5 million CD4+ T-cells cumulatively from Weeks 3–53 and found them negative for viral outgrowth, with no replication-competent latent viral reservoir. The estimated cumulative size of the latent reservoir post-transplant was ≤ 0.009 IUPM, reflecting a minimum 153-fold reduction in the latent reservoir size (Figure 3D).

Post-transplant, HIV-1 DNA and plasma HIV-1 RNA with a single-copy assay [42] were essentially undetectable and remained so through 147 weeks post-transplant. However, we detected transient trace HIV-1 DNA in 2-LTR circles estimated at 1.10 copies/10^6 million PBMC on analyses of cellular equivalents of 1.23 million PBMC at a single timepoint 176 weeks post-transplant, coinciding with 15 weeks post-ATI. Repeat testing on a different aliquot of PBMC from this timepoint also showed HIV-1 DNA at 1.1 copies per million PBMC. We detected a single episode of low-level viremia at the single-copy assay quantification limit at 0.9 HIV-1 RNA copies/mL at 107 weeks post-transplant (Figure 3A). Altogether, the clinical significance is unclear, but will require further follow-up to understand the implication.

Except for those two, all timepoints tested post-ATI, including bone marrow and purified CD4+ T-cells, were negative for HIV-1 DNA and RNA. At 55 weeks post-transplant, the latent reservoir size was estimated at < 0.46 IUPM CD4+ T-cells, though only 1.5 million CD4+ T-cells were available for analysis at that timepoint.

Analyses of the proviral pool pre-transplant showed that the patient's HIV-1 was CCR5-tropic, with no detection of CXCR4-tropic HIV-1. We tested the engrafted donor cord PBMCs from 27 months post-transplant alongside normal donor PBMC for their susceptibility to infection by the two autologous latent reservoir clones recovered before transplant, along with R5-tropic (HIV-1 BAL) and X4-tropic (NL-4–3) laboratory strains. The engrafted CCR5Δ32/Δ32 CBU showed intrinsic resistance to both autologous latent reservoir clones, along with R5- and X4-tropic laboratory strains. The control donor PBMC showed normal replication kinetics for all isolates tested (Figure 4).

Measurement of antiviral drug levels provides objective adherence information that helps monitor patients' adherence to antiretroviral medications. To ensure that our participant was not taking antiretrovirals during the post-ATI period, we measured serial plasma samples collected at post-transplant intervals while on ARV and post-ATI. We found detectable levels of ARVs in plasma before the ATI but none in any samples during the 18 months off ARV.

Immunology:

The participant showed T-cell immune reconstitution, loss of markers of immune activation, and vaccine immune responses. Coincident with aviremia off ART, we monitored immune biomarkers associated with HIV-1 infection. CBU T-cells are predominantly naïve.[43,44,45] The reservoir for HIV-1 resides mainly in memory CD4+ T-cells [46–50] and (in adults) largely in central memory CD4+ T-cells.[50] We analyzed the phenotypes of the engrafted CBU for naïve (T_N), effector (T_E), central memory (T_{CM}), and effector memory (T_{EM}) T-cells. From Day 100 through 161 weeks post-transplant (just before ART interruption), the frequencies of $CD4^+$ T_N increased, and memory T-cells decreased. The T-cell change was more evident in T_{CM} than T_{EM}, with fluctuating levels of T_E (Supplemental Figure 1A). Likewise, the frequencies of CD8+ T_N increased with a coincidental decrease in CD8 T_{CM} and similarly fluctuating levels of T_{EM} and T_E. Thus, immune cell phenotype post-transplant in this recipient resembled developmental immunity[51,52] and remained stable after ATI.

A hallmark of HIV-1 infection is high levels of immune activation manifesting as increased levels of activated CD4+ and CD8+ T lymphocytes that are not fully reversed with current antiretroviral treatment strategies.[53,54,55] At approximately 100 days (Week 15) post-transplant, the first timepoint studied, levels of immune activation were high in both CD4+ (13.6%) and CD8+ (19.4%) T-cell subsets. Frequencies of immune-activated T-cells decreased to 1.54% and 3.97% at 76 weeks post-transplant. They remained low at 1.2% and 3.6 % for CD4+ and CD8+ T-cells, respectively, through Week 170 post-transplant and pre-ATI and at 52 weeks post-ATI consistent with the lack of active HIV-1 replication (Supplemental Figure 1B).

To investigate soluble biomarkers of inflammation, we assessed levels of cytokines TNFα, IL-6, IL-1β, monocyte activation marker (sCD14), and gut microbial translocation intestinal

fatty-acid-binding protein (I-FABP) post-transplantation and after ATI (Supplemental Figure 2). Post-transplant, TNF_{α}, $IL\text{-}1_{\beta}$, I-FABP, and IL-6 levels increased and peaked at 26 weeks before returning to pretransplant level by 156 weeks, consistent with known prolonged inflammation post-transplant. Interestingly there was an increase in these proinflammatory cytokine levels again pre-ATI and decreased to baseline levels by 52 weeks post-ATI (the monocyte activation marker sCD14 was initially elevated, then declined at 130 weeks post-transplant and remained low post-ATI).

To further investigate immune reconstitution, we examined the functional capacity of the engrafted CBU at various timepoints post-transplant (26, 78, 104, 148, 156, 170 weeks) and post-ATI (4, 12, 26, 52 weeks). We noted production of the intracellular cytokine's interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) in response to polyclonal staphylococcal enterotoxin B (SEB) toxin stimulation (Supplemental Figure 3). We detected no intracellular cytokine responses to antigen-specific HIV-1-Gag stimulation in the CD4+ or CD8+ T-cell subsets, indicating the absence of HIV-1-specific memory T-cells. We did not specifically assess CMV or EBV-specific T-cell responses.

Our participant lost HIV-1-specific humoral responses over time, a unique feature compared with the Berlin and London patients. When diagnosed with HIV-1, she had positive reactivity to the HIV-1 gp160, p31, p18, and indeterminate reactivity to gp40 and p24 by western blot (WB). Pre-transplant and at Day 100 post-transplant, analysis showed evidence for HIV-1-specific antibody responses to five of the 10 proteins (Figure 5). By 52 weeks post-transplant, however, there was a complete loss of HIV-1 antibody reactivity to all 10 proteins tested which remained negative through 12 months post-ATI.

Post-transplant, our participant received a series of vaccines, including pneumococcal vaccine PCV13, tetanus/diphtheria/pertussis (TDaP), haemophilus influenzae type B, and inactivated polio vaccine. She also received a pneumococcal (PPSV23) booster and measles, mumps, and rubella (MMR) vaccines. She was immunized with SARS-CoV-2 mRNA vaccines 189- and 193-weeks post-transplant (Figure 1B, Supplemental Table 1). Vaccine-induced antibody responses to tetanus, poliovirus, selected pneumococcal serotypes, measles, and rubella vaccines indicated a functional immune system. In addition, anti-spike antibodies to the novel SARS-CoV-2 mRNA vaccine were detected, supporting the presence of an intact immune system (Supplemental Table 1).

DISCUSSION

We report the first case of remission and possible HIV-1 cure in a woman of Mixed race following a CCR5 Δ32/Δ32 CBU combined with a haploidentical graft transplant to cure AML and HIV-1. Adult CCR5 Δ32/Δ32 donor stem cells were used in Timothy Brown and Adam Castillejo and the possible remission in the Dusseldorf patient,[22–25,28] making our case of CCR5 Δ32/Δ32 CBU cells with a haplo-cord transplant unique. This case also adds to the growing evidence for the critical role of intrinsic resistance of CCR5Δ32/Δ32 cells to HIV-1 in mediating HIV-1 cures, since the "Boston patients" who underwent allogeneic stem cell transplants for malignancies with wildtype CCR5 cells experienced

viremic rebound within 32 weeks of ART interruption, despite achieving undetectable HIV-1 DNA and replication-competent reservoirs.[56]

Our case study also has implications for racial health equity. In the Berlin and the Duesseldorf patients, who were Caucasian, the likelihood of finding an HLA identical adult unrelated donor match without regard for CCR5Δ32/Δ32 cells was approximately 72%.[57] The chance for the London patient, of Hispanic and Dutch ancestry, was 41–44%.[57] For our patient of Mixed ancestry, the chance was below 20%.[58,59] The use of CCR5Δ32/Δ32 CBU cord blood cells, because of less stringent match requirements, broadens the opportunity for CCR5Δ32/Δ32 therapy in PLWH who are of diverse ancestry and require a transplant for other diseases.

Differences from previous case studies:

A notable difference between our participant and the Berlin, London, and Duesseldorf patients is genetic: she is female and of Mixed race, and received a haplo-cord transplant where the cord blood cells were CCR5Δ32/Δ32 mutated.

Our participant never developed GVHD, however despite this, she experienced 18 months of HIV-1 aviremia off ART, associated with no detectable HIV-1-specific immune responses. In contrast, GVHD was considered critical to HIV-1 cure in Timothy Brown. She had normal immune responses to routine immunization, intermittent traces of HIV-1 DNA near assay detection limits, and a non-detectable replication-competent HIV-1 reservoir in nearly 75 million CD4+ T-cells off ART, all of which support a state of possible HIV-1 cure.

HIV-1 DNA:

Detection of HIV-1 DNA may indicate persistent recipient-infected cells or new infection of the transplanted cells. The complete loss of HIV-1-specific antibody responses by one-year post-transplant and throughout the post-transplant period supports resistance to new rounds of HIV-1 infection, even with ATI. The absence of HIV-1-specific cellular immune responses indicates that the trace HIV-1 infection was not associated with productive virus replication that stimulated an immune response. Alternatively, detection of HIV-1 DNA at such low-levels may represent false positives. In both the Berlin and London patients, HIV-1 DNA was detected in tissues (gut and lymph nodes) sampled while off ART. In addition, trace HIV-1 DNA and 2-LTR circles remained transiently detectable in blood samples during long-term follow-up of the Berlin and London patients who were cured of HIV-1.[25,60,61]

During ATI, in our patient we also detected trace HIV-1 DNA, including 2-LTR circles, at 15 weeks post-ART interruption post-transplant. The trace DNA was not associated with rebound viremia during the ensuing 38 weeks. Immune cells bearing 2-LTR circles may be longlived.[25] Alternatively, a hidden reservoir of cells harboring HIV-1 could have reactivated but have been unable to replicate due to the newly engrafted HIV-1-resistant immune system.[25,60] Further, HIV-1 antibody responses waned over time in the Berlin and London patients, but responses in those patients were not completely negative, as observed in our participant.

Infection-resistant CB stem cells:

In vitro studies show resistance to autologous latent reservoir clones and laboratory-adapted HIV-1 strains HIV-1_{BAL}(CCR5-tropic) and HIV-$1_{NL4–3}$ (CXCR4-tropic) in our case. The engrafted CCR5Δ32/Δ32 CB stem cells resisted infection by CXCR4-tropic HIV-1 was surprising since CB cells express high levels of CXCR4.[62] However, studies report lowered levels of CXCR4 expression with CCR5Δ32 homozygosity.[62,63] This finding is consistent with previous reports by our group [31] and others [64] of reduced susceptibility of CD4+ T-cells from adult individuals with engrafted homozygous CCR5Δ32/Δ32 CB cells to CXCR4-tropic HIV-1.

By contrast, *in vitro* engrafted adult CCR5Δ32/Δ32 cells were susceptible to CXCR4 lab strains in the London patient. Susceptibility to CXCR4 is variable since some individuals homozygous for this mutation were infected persistently with X4 strains.[65–67] It appears that CCR5Δ32/Δ32 homozygous CBU cells can exhibit reduced susceptibility to CCR5-and CXCR4-tropic HIV-1 and influence the expression of CXCR4. This finding requires further investigation, as such resistance could alter the selection of CXCR4 HIV-1 variants under CCR5Δ32/Δ32 immune pressure or susceptibility to new infection.[68]

Individuals with the CCR5Δ32/Δ32 mutation are usually healthy. There are theoretical concerns for potential increased risk with West Nile Virus [69] and possible beneficial effects such as protection against severe SARS-CoV-2 infection.[70] Our participant demonstrated normal humoral responses to routine childhood immunizations and mRNA vaccination against SARS-CoV-2, demonstrating the immune competence of nascent immune cells.

Limitations of the study:

Our study had several limitations. It lacked tissue biopsy and assessment of cell-associated HIV RNA, although we used the gold standard QVOA to detect intact, inducible, replication-competent proviruses.[71] Also, we lacked data on HIV-1-specific immune responses before the transplant; this prevented a comprehensive analysis of the effects of a CBU transplant on the loss of HIV-1-specific cellular immune responses, although the responses became undetectable over time. We also lacked specific cellular responses to other viruses such as CMV or EBV. The extent of rituximab contribution to the loss of the humoral immunity is unknown as we did not perform lymph node biopsies. The repeated courses of rituximab could have disrupted or destroyed B-cell follicles where HIV-1 persistence is commonly found.[72–74] Thus, the rituximab may be a confounding factor for interpreting HIV-1 specific immune responses. In addition, we did not analyze the size of the replication-competent reservoir just before ART cessation, so we could not model estimates of time to rebound as in the London patient.[25]

In the London patient, 24 million CD4+ T-cells were analyzed for replication-competent virus with 30 months follow up and no viral rebound and an estimated probability of remission for life (cure) of 98% with 80% donor chimerism in total HIV-1 target cells. Using a different model for the London patient,[75] the probability of remission for life was even higher at >99%, with 90% or greater donor chimerism. Furthermore, the lack of data on chimerism at tissue sites in our case precluded estimates of chimerism in various

T-cell populations in tissues. However, a previous study reported that host-derived T-cells can persist in skin and tissues despite 100% donor chimerism in the blood,[76] making it challenging to assess the probability of cure in cases without long-term follow-up.

Despite the two reported cure cases, several unsuccessful stem cell transplants using CCR5 Δ32/Δ32 donor grafts, including haplo-cord have been reported. Most patients died from relapsed disease, infectious complications or GVHD.[64,77–81] At least one patient had reported CXCR4-tropic HIV-1 rebound.[79] Others had detected proviral DNA in tissues such as ileum, spleen and lymph node post-transplant.[81] Although there is hope for HIV-1 cures with reports of these successful cases, allo-transplant remains a complex procedure with risk for both relapse and fatal complications and should be considered at this time, only in PLWH who have another potential life-threatening disease that requires a transplant.

With further evidence, particularly longer follow-up periods for participant like ours, it is possible that CCR5Δ32/Δ32 homozygous cord stem cell therapies could be prioritized for HIV-1-specific cases. Stemcyte banks carry only a limited number of CCR5Δ32 homozygous cord blood products because of the lack of ongoing screening and their rarity. Consideration for active screening of donor banks for this mutation in worldwide registries may lead to more intentional HIV-1 cures. Focused collection and screening of CB products could also broaden access. Our approach could serve as a model for future efforts using gene therapy to modify CCR5 and attenuate co-receptor expression toward HIV-1 cure.[7,82,83] Conceivably, with the current HIV-1 cure research agenda, CB-based cell therapy and CCR5-targeted gene editing efforts could be developed to serve more PLWH.

STAR METHODS

Resource Availability

Lead contact: Further information and requests for resources should be directed to and will be fulfilled by, Jingmei Hsu, MD/PhD, Jingmei.hsu@nyulangone.org

Materials availability: This study did not generate unique reagents

Experimental model and subject details

Patient recruitment: The study was conducted per the Declaration of Helsinki and approved by the Institutional Review Board of Weill Cornell Medical College. Human subjects were enrolled in the study with informed consent approved by the Institutional Review Board of Weill Cornell Medical College. Two participates were recruited (1 man and 1 women both middle aged).

Method Details

Clinical Viral load assay: No resistance mutations were detected at the onset of our participant's HIV-1 diagnosis. The lower limit for New York Presbyterian hospital-based assay was 20 copies per mL. Quantification of HIV-1 viral RNA in plasma was by reverse transcription of viral RNA followed by real-time PCR amplification and detection using dual-labeled fluorescent oligonucleotide probes on the Roche Diagnostics COBAS TaqMan

48 Instrument. CSF viral load was done by real-time PCR to quantitatively measure HIV-1 RNA (Quest Diagnostics).

Laboratory methods: Peripheral blood was collected before and following transplant—at study entry, Day 100 (week 15), week 27, 55, and approximately every six months thereafter (weeks 88, 107, 128, and 147) for immune profiling and quantitative markers of HIV-1 persistence. We collected bone marrow samples at weeks 15, 27, and 107 post-transplant to monitor AML status. We also analyzed remnant bone marrow cells for HIV-1 DNA concentrations. Quantifying the latent HIV-1 reservoir size was planned for pre-transplant and serially (weeks 15, 26, and 52) and then every six months following the transplant. However, we only collected blood samples for the latent replication-competent HIV-1 reservoir pre-transplant, at week 55 post-transplant, and again at week 2, 26 and 52 post-ATI.

Cellular immune reconstitution and activation: CD4 and CD8 T-cell immune activation markers were analyzed longitudinally using cryopreserved PBMCs. Briefly, PBMCs were thawed and rested overnight in RPMI-1640 medium (Gibco, Thermo Fisher, USA) with 10% fetal bovine serum (Sigma-Aldrich, USA). 1×10^6 cells were stained with LIVE/DEAD® Aqua, followed by staining for the cell surface markers: CD3 (SK7, BD), CD4 (L200, BD), CD8 (RPA-T8, BD), CD45RO (UCHL1, Beckman Coulter), CD27 (M-T271, BD), and the immune activation markers (HLA-DR (L243, Biolegend), and CD38 (HIT2, Biolegend)). Cells were then fixed and acquired on a flow cytometer (BD LSRFortessa, San Jose, CA) and analyzed by FlowJo V10 (Treestar, Ashland, OR). Frequencies of CD4 and CD8 T-cell maturation subsets were analyzed based on CD45RO and CD27 expression as either naïve (CD45RO-CD27+), central memory (CD45RO+CD27+), effector memory (CD45RO+CD27-), or effector (CD45RO-CD27-). We measured immune activation as frequencies of dual positive (HLA-DR+CD38+) CD4+ and CD8+ T-cells.

HIV-1 specific T-cell responses and assessment of T-cell function: Thawed PBMCs were rested overnight in RPMI-1640 medium with 10% fetal bovine serum a. The following day we stimulated 1.5×10^6 PBMCs for 6 h in the presence of 2 μg/ml Gag potential T-cell epitopes (PTE) peptides pool (AIDS Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH]. 1.5×10^6 PBMCs were simultaneously stimulated with staphylococcal enterotoxins B (SEB; List Biological Laboratories) at 1 μg/ml and another aliquot in the presence of media alone as the negative control. At the beginning of the culture, we added 1 μg/ml anti-CD28 mAb (BD Biosciences) to each condition as a costimulatory molecule and 10 μg/ml brefeldin A Sigma-Aldrich, USA) to block the protein transport process during cell activation. Following stimulation, we stained cells for surface markers (CD3, CD4, CD8). We used Fixable Blue LIVE/DEAD fixed and permeabilized to stain for intracellular IFN-γ. We acquired stained cells on a BD LSRFortessa (BD Biosciences) and performed analysis using FlowJo v10.0.8 (Tree Star) software.

HIV-1 antibody responses: We determined the presence of antibodies against HIV-1 using western blot analysis by Bio-Rad GS (Hercules, CA) and according to the manufacturer's directions.

HIV-1 persistence biomarkers: We determined HIV-1-infected cell frequencies in PBMC, purified CD4+ T-cells, and bone marrow cells using our previously published CLIA-certified ultrasensitive droplet digital polymerase chain reaction (ddPCR) assay, with detection capability to 1.25 HIV-1 DNA copies per million cells and a detection limit (concentration detected 95% of the time) of <4.09 copies/million cells.[84] Briefly, genomic DNA was isolated from PBMC cell pellets, bone marrow, and CD4+ T-cells with a DNA Blood Midi Kit (Qiagen) (STAR Methods Table). We generated droplets for ddPCR using a QX200 AutoDG, with ddPCR Supermix for probes and previously published primers and probes.[84] We performed PCR with a C1000 Touch Thermal Cycler (Bio-Rad), read with a QX200 Droplet Reader (Bio-Rad). We assessed HIV-1 proviral concentrations with QuantaSoft v1.7 (Bio-Rad). 2-LTR circles were also quantified in peripheral blood mononuclear cells using our previously published PCR methods.[85,86] We measured ongoing viremia with a single-copy viral load assay.[42,87]

HIV-1 latent reservoir size: The size of the replication-competent HIV-1 reservoir was determined using previously published methods for the QVOA.[71] Briefly, total CD4+ T-cells were purified by negative selection (Miltenyi Biotec) and placed in a limiting dilution culture where they were stimulated in the presence of phytohemagglutinin (PHA) and irradiated feeders for 48 hours to stimulate latent provirus reactivation. Following stimulation, culture media is replaced with CD8-depleted lymphoblasts for virus amplification for 14 days. We assessed the presence of p24 in the supernatant with an ultrasensitive Simoa p24 Antigen Assay (Quanterix).

HIV-1 tropism: We tested HIV-1 tropism for infection with R5 and X4-tropic variants on the proviral pool in PBMC genomic DNA using the clinically validated assay by Monogram BioSciences (South San Francisco, California, USA).

***In vitro* susceptibility of engrafted CBU cells to HIV-1:** The participant engrafted CBU cells from 107 weeks post-transplant were challenged with the two latent reservoir clones cultured from resting CD4+ T-cells immediately before the haplo-cord transplant, along with laboratory strains HIV-1_{BAL} (CCR5-tropic) and HIV-1_{NL43} (CXCR4-tropic) in a virus infectivity assay. We infected healthy donor PBMCs from an HIV-1 uninfected volunteer as controls (equal viral titers of p24 = 1.5ng with polybrene were used to infect 10^6 PHA-stimulated PBMCs in 2mL RPMI 20%FBS, pen/strep, 10U/mL IL-2). We fed additional participant and healthy PBMCs at the same concentrations to respective cultures on Day 7. We did not add exogenous normal PBMC to participant cell cultures. We measured supernatants for p24 antigen production at Days 5, 7, and 14.

Antiretroviral drug levels: We serially tested plasma samples for emtricitabine and abacavir at study entry, weeks 15, 26, 104, and 156 post-transplant, and at weeks 4, 12, 26, and 52 following ATI. We determined emtricitabine levels using a reversed-phase high-performance liquid chromatographic assay coupled to a triple quadrupole mass spectrometer

(MS/MS). After the addition of stable isotope internal standards (TFV-d_6, FTC-$^{15}N_2$, ^{13}C), we used a solid-phase extraction (SPE) procedure with Waters Oasis PRIME MCX 30mg (1cc) solid-phase extraction cartridges (Milford, MA) to clean up samples and extract the analyte of interest. We performed chromatographic separation on an XSelect HSS T3, 2.5μm, analytical column. The assay had a linear range of 5.0–5,000ng/mL and a lower limit of quantitation of 5.00ng/mL using a 20ul aliquot of human plasma. We determined abacavir levels by using protein precipitation and LC-MS/MS assay. We added labeled abacavir ($^2H_4$) internal standards to the plasma samples, followed by protein precipitation with acetonitrile. Extracts were then analyzed by hydrophilic interaction chromatography (HILIC) using a Phenomenex Luna HILIC column under isocratic conditions. We used a triple quadrupole mass spectrometer (AB Sciex 5500) equipped with TurboV Ion Spray operating in positive-ion mode.

Plasma biomarkers of inflammation and microbial translocation: We assessed the functional capacity of CBU cells by measuring intracellular cytokines following polyclonal stimulation with staphylococcal enterotoxin B (SEB) and antigen-specific stimulation with HIV-1 polyprotein Gag. We measured IL-6, TNFα, IL-1$_\beta$, and soluble CD14 (sCD14) levels using a customized magnetic bead panel (R&D Systems). Briefly, samples were thawed, mixed, and centrifuged at 16,000 x g for 4 minutes before testing. We incubated two-fold diluted samples with beads specific for various analytes, followed by biotinylated detection antibodies and streptavidin-phycoerythrin. We analyzed the beads on a Luminex Magpix instrument (Luminex Corporation, USA). We measured plasma intestinal fatty-acid-binding protein (I-FABP) levels by ELISA using an R&D systems kit as per manufacturer's recommendations.

Pre- and post-transplant biomarkers of inflammation: To investigate soluble biomarkers of inflammation, we assessed levels of cytokines TNFα, IL-1$_\beta$, IL-6, the gut microbial translocation intestinal fatty-acid-binding protein (I-FABP), and monocyte activation marker (sCD14) at pre-transplant (Day 0), post-transplantation and after ATI. Post-transplant levels of TNFα, IL-1$_\beta$, I-FABP, and IL-6 increased and peaked at 26 weeks before returning to pre-transplant level by 156 weeks, consistent with known prolonged inflammation post-transplant. Interestingly, these proinflammatory cytokine levels increased again pre-ATI and decreased to baseline levels by 52 weeks post-ATI. The monocyte activation marker sCD14 was initially elevated, declined at 130 weeks post-transplant and remained low post-ATI.

Quantification and Statistical Analysis—We analyzed column effluents by multiple reaction monitoring. We fit the calibration curve using a weighted ($1/x^2$) linear regression analysis of the analyte/IS peak area ratio versus the analyte concentration from 5.00 – 5,000 ng/mL. We reported results from 5.00–5,000 ng/mL from 10ug of human plasma. In studying inflammation, we analyzed the median fluorescence intensity data with MILLIPLEX Analyst Software V.3.5 (EMD Millipore) and calculated concentrations with an R&D standard expressed in pg/ml.

Additional Resources—IMPAACT P1107: Effects of Cord Blood Transplantation with CCR5Δ32 Donor Cells on HIV Persistence

ClinicalTrials.gov Identifier: NCT02140944 https://clinicaltrials.gov/ct2/show/NCT02140944?term=IMPAACT+p1107&draw=2&rank=1

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- First Mixed-race woman with HIV-1 remission post CCR5Δ32/Δ32 haplo-cord transplant
- 100% immune reconstitution by CCR5Δ32/Δ32 cord graft and resistance to HIV strains
- No HIV viral rebound ≥18 mos off antiretroviral therapy without graft vs host disease
- No detectable HIV-1 DNA/RNA, replication competent virus & loss of HIV Ab response

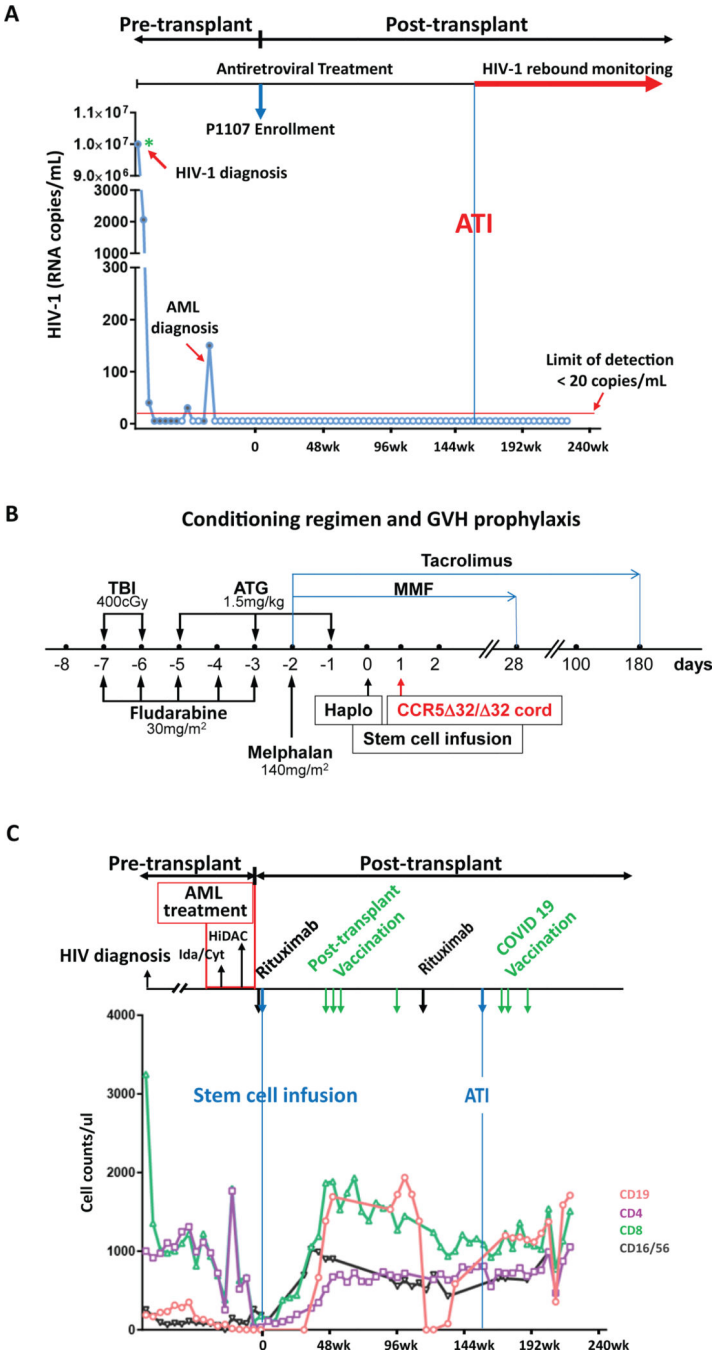

Figure 1. HIV-1 and AML Treatment Course and Rebound Monitoring

(A) HIV-1 plasma viral loads at HIV-1 diagnosis, before and after AML diagnosis, and stem cell transplant. The participant was enrolled in the P1107 trial at the time of stem cell transplant with HIV-1 control on ART. The blue line indicates the timing of antiretroviral treatment interruption (ATI) relative to the transplant. There was no viremic rebound off ART for 18 months. A clinical assay measured plasma viral load (VL) with a limit of detection of <20 copies/mL, indicated by the red line. At diagnosis, the HIV-1 VL was greater than the upper limit of quantitation (1,000,000 copies/mL), indicated by the green *.

(B) Schema of the haplo-cord stem cell transplant. The participant received an allogeneic stem cell transplant per institutional standard care. Conditioning regimen was fludarabine 30mg/m^2 daily on Days −7 to −3, melphalan 140mg/m^2 x 1 dose (Day −2) and total body irradiation at 400 CGy on Days −7 to −6. Haploidentical stem cells were infused on D0, and CCR5 Δ32/Δ32 cord stem cells were infused on D+1. Graft versus host (GVH) disease prophylaxis included: antithymocyte globulin (ATG) 1.5 mg/kg on Days −5, −3, and −1, mycophenolate mofetil (MMF) one gram three times daily on Days −2 through Day +28, and tacrolimus from Day −2 to Day 180 post-transplant.

(C) Clinical course, immune status pre-transplant, and post-transplant immune reconstitution. T-cells (CD4, CD8), B-cells (CD19), and natural killer (NK) cells (CD16/CD56) levels. Induction chemotherapy consisted of idarubicin/cytarabine (ida/cyt) and consolidation of one cycle of high-dose cytarabine chemotherapy. The first blue line shows the time of stem cell infusion after conditioning chemotherapy. Rituximab (375 mg/m2) was given one week before the conditioning regimen for prophylaxis of Epstein-Barr Virus (EBV) associated post-transplant lymphoproliferative disease. A second dose of rituximab was given for EBV viremia treatment. Rituximab resulted in transient undetectable CD19 levels before full recovery. ATI (second blue line), post-transplant immunizations (green arrows), and COVID-19 vaccinations with an mRNA vaccine (green arrows).

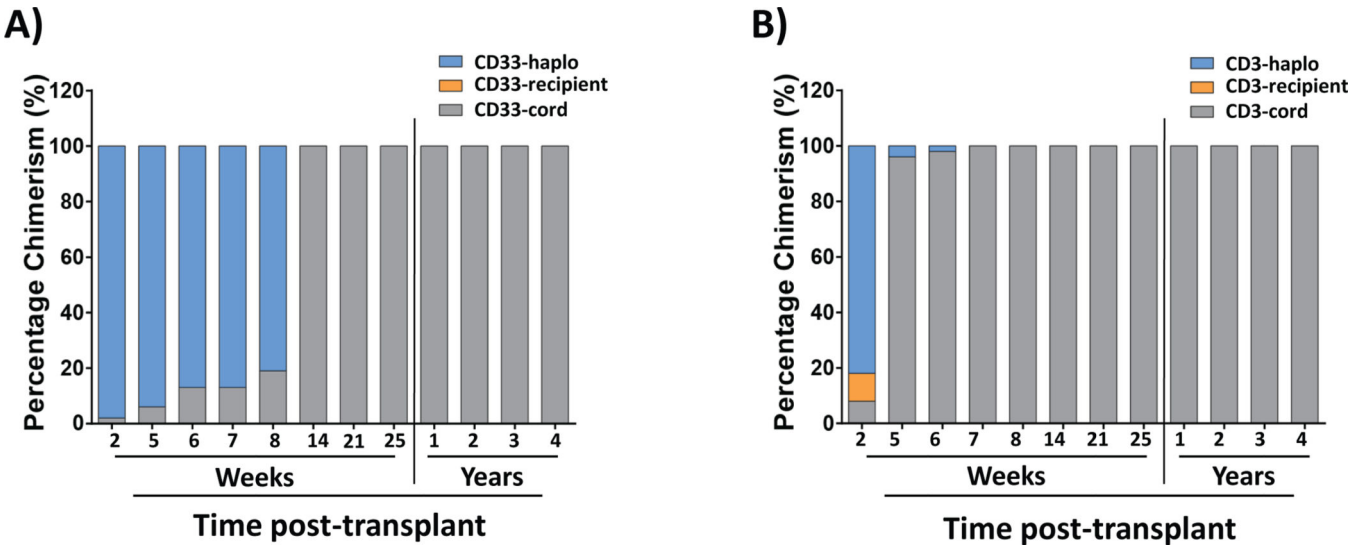

Figure 2: Chimerism Dynamics Post Haplo-cord Stem Cell Transplant Over Time.

(A) Myeloid lineage chimerism was measured by CD33;

(B) Lymphoid lineage chimerism was measured by CD3. By 14 weeks post-transplant, CD3 and CD33 chimerism were at 100% CCR5 Δ32/Δ32 CBU cells and maintained at four years post-transplant. Recipient (orange shaded), haploidentical donor (blue), and cord blood cells (gray) chimerism. The black line indicates the time change from weeks to years.

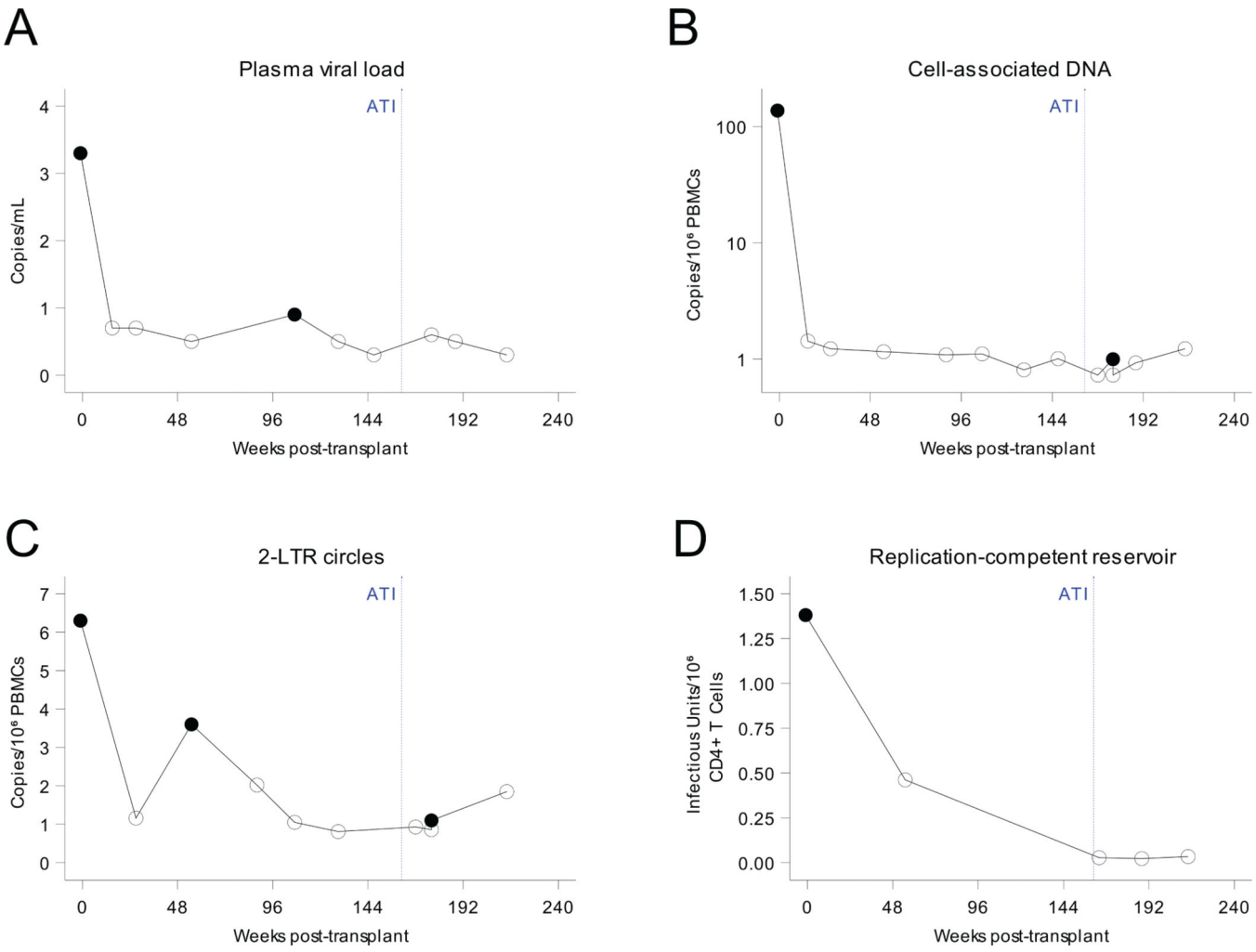

Figure 3. Biomarkers of HIV-1 Persistence Pre-and Post-transplant.

(A) Plasma viral load (HIV-1 RNA copies/mL) quantified with a single-copy viral load assay; the limit of detection of the plasma viral load assay ranged from <0.5- to <0.9 HIV-1 RNA copies/mL).

(B) Cell-associated HIV-1 DNA (copies/10^6 cells) measured with a droplet digital PCR assay that detects to 1.25 copies/ 1 ×10^6 cells analyzed with a limit of detection (concentration detected 95% of the time) of < 4.09 copies/10^6 cells;

(C) 2-LTR circles (copies/10^6 cells) assayed with a droplet digital PCR assay;

(D) Latent reservoir size determined by QVOA expressed as infectious units per million (IUPM) CD4+ T-cells. Closed and open symbols represent each biomarker's detectable and non-detectable levels. The blue dashed line indicates ATI.

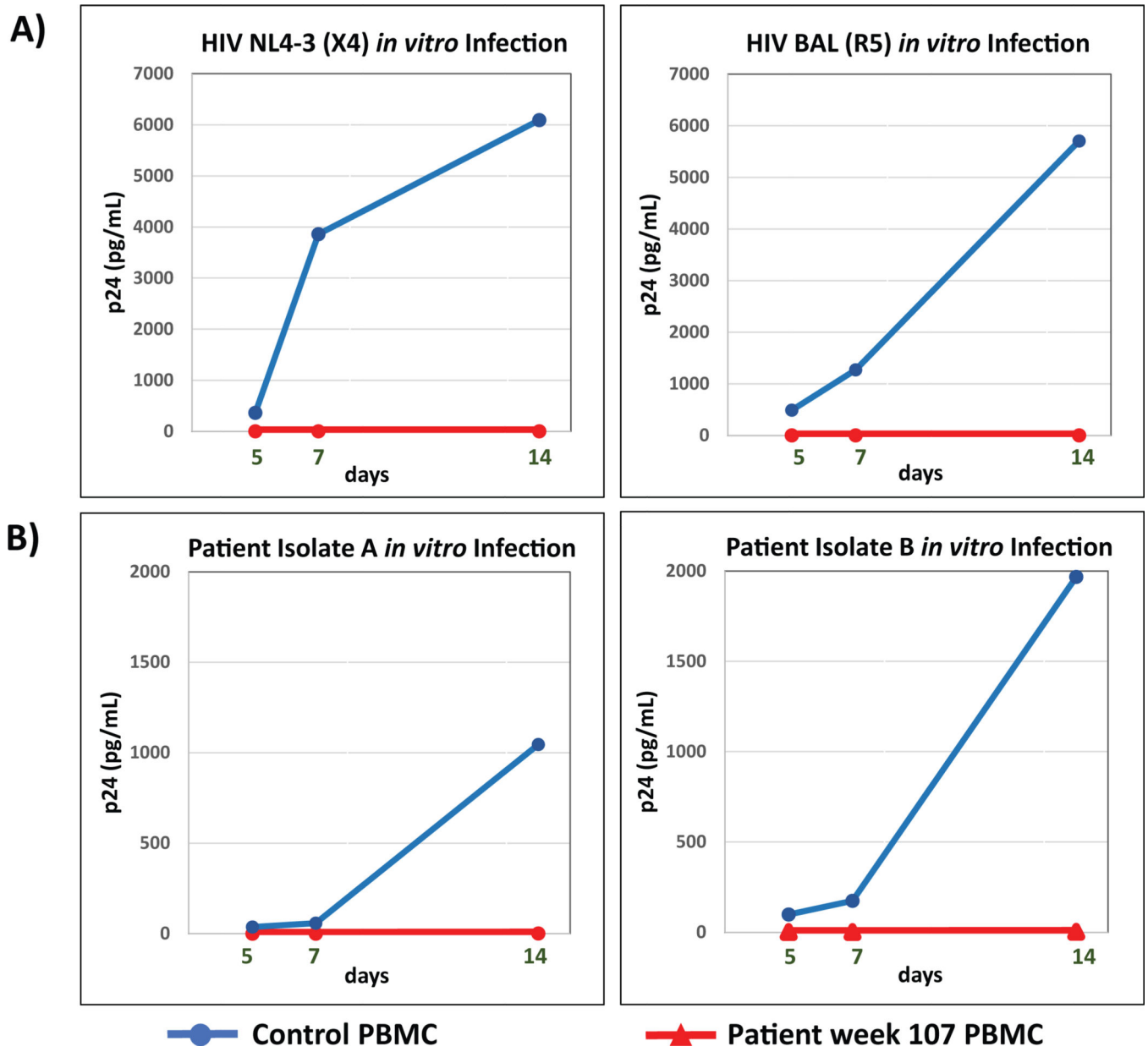

Figure 4. ***In vitro*** **HIV-1 Resistance.**

(A) *In vitro* resistance of patient PBMC post-transplant Week 107 (red line) to CCR5-tropic (HIV-1-BAL) and CXCR4- tropic (HIV-1-NL4–3) compared to replication in healthy control PBMC (blue line), measured by p24 antigen in culture supernatants.

(B) *In vitro* resistance of patient PBMC post-transplant Week 107 (red line) to the two autologous latent reservoir clones isolated pre-transplant (A & B) compared to replication in healthy control PBMC (blue line) as measured by p24 antigen in culture supernatants.

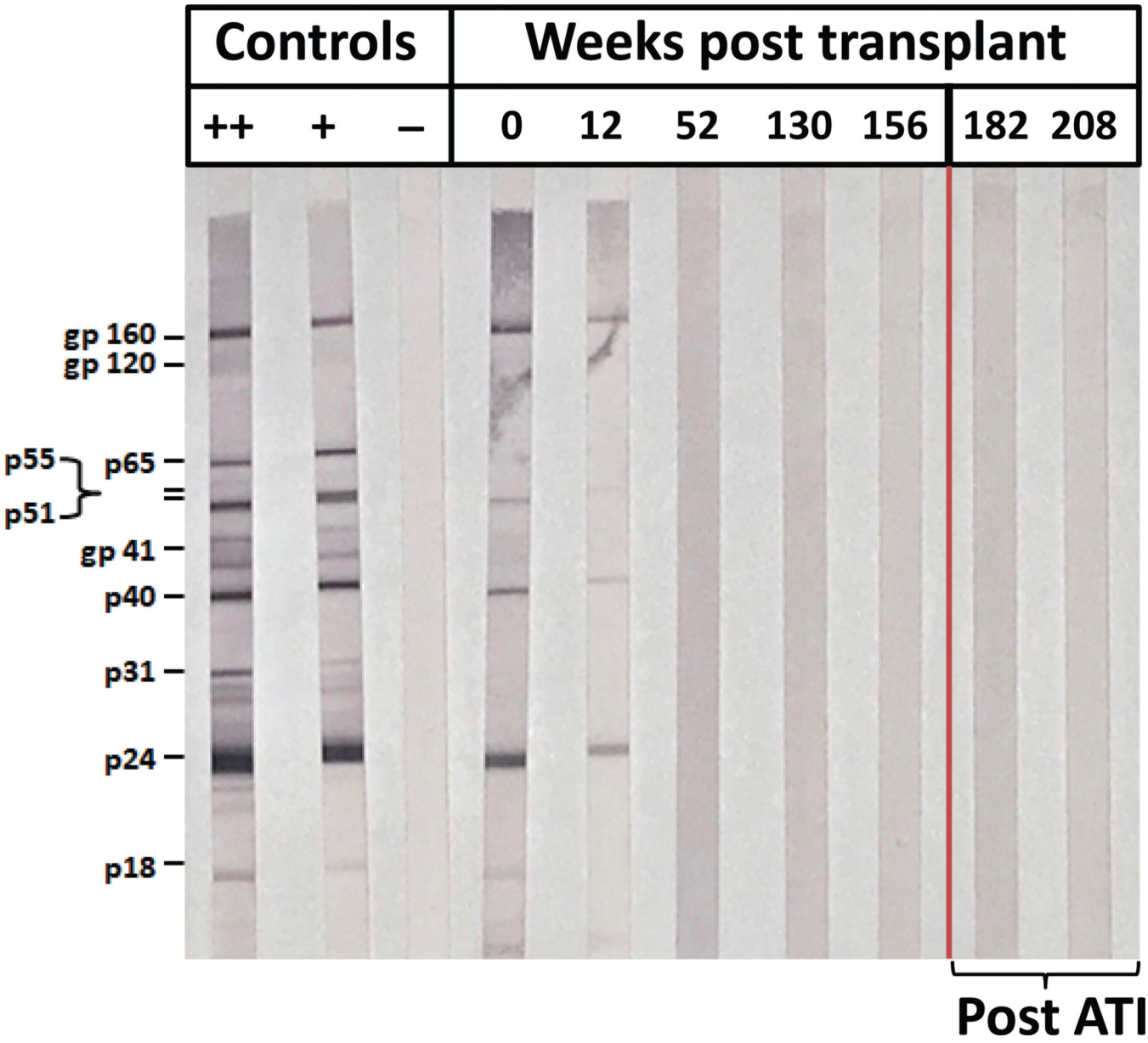

Figure 5. HIV-1 Antibody Profiles by Western Blot.

HIV-1 proteins and molecular weights are indicated. Detectable HIV-1 antibodies are shown in high (++), low (+) positive and negative controls (neg) and antibody profiles at the entry into IMPAACT P1107 (Week 0) and several timepoints following haplo-cord transplant, including post-ATI. There was a loss of HIV-1 antibody responses by 52 weeks post-transplant and maintained even after ATI (red line).

Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
CD27 APC-H7	BD	Cat#560222
CD3 BUV395	BD	Cat#564001
CD45RO ECD	Beckman Coulter	Cat#IM2712U
CD38 BV605	Biolegend	Cat#303531
HLA-DR BV711	Biolegend	Cat#307644
CD8 Alexa700	BD	Cat#561453
CD4 PerCP-Cy5.5	BD	Cat#552838
TNF-alpha APCCy7	Biolegend	Cat#502944
IFN-gamma PECy7	BD	Cat#557643
CD28 Purified	BD	Cat#555722
Chemicals, peptides, and recombinant proteins		
GAG Potential T-Cell Epitopes (PTE) peptides pool	DAIDS/NIAID	Cat#ARP-12437
Staphylococcal Enterotoxins B (SEB)	VWR	Cat#103007–764
Brefeldin A	Sigma	Cat#B7651–5MG
LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit	Thermo-Fisher	Cat# L34966
Ficoll-Plaque Plus	GE Healthcare	Cat#17–1440-03
BUFFER A (IN-HOUSE BUFFER): 1 M TRIS pH 8.0 1 M KCL 100% TWEEN ULTRA-PURE DNAse RNAse FREE WATER (10 × 500 ML BOTTLES)	 FISHER SIGMA SIGMA INVITROGEN	 Cat#AM9856 Cat#60142–500ML-F Cat#P9416–100ML Cat#10977023
dNTP's (4 × 1 ml) 100 μMole, 100 mM dNTP's (4 × 1ml) 100 μMole, 100mM	MERIDIAN BIOSCIENCE FISHER	Cat#BIO-39049 Cat#FERR0182
DTT, 100 mM	AGILENT	Cat#600107
EDTA, 0.5 M, PH 8.0	AMBION	Cat#AM9260G
MOLECULAR GRADE ETHANOL, 200 PROOF	DECON BIOSCIENCE STOCKROOM U PITT	Cat#2716 Cat#200PP0125
GLYCOGEN, 20 MG/ML	SIGMA	Cat#10901393001
GUANIDIUM HCL, 8 M	SIGMA	Cat#G9284–100 ML
GUANIDINIUM THIOCYANATE, 6 M, 50 ML	SIGMA	Cat#50983–50 ML
ISOPROPANOL 100%	SIGMA	Cat#I9516–500 ML
MgCL2, 25 mM	THERMO-FISHER	Cat#AB0359
PROTEINASE-K, 20 mg/ml	FISHER	Cat#AM2548
RANDOM HEXAMERS, 500 g/ml	FISHER	Cat#PR-C1181
RECOMBINANT RNAsin, 10000 U	FISHER	Cat#PR-N2115
Tris-HCL, 1 M PH 7.6	SIGMA	Cat#T2444–100 ML
CaCL2, 1 M	SIGMA	Cat# 21115–100 ML

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
QIAamp Blood Midi Kit	Qiagen	Cat#51104
ddPCR Supermix for Probes (No dUTP)	Bio-Rad	Cat#1863024
CD4+ T cell Isolation Kit	Miltenyi Biotec	Cat#130–096-533
SIMOA HIV p24 Assay for SR-X	Quanterix	Cat#102215
2X ROCHE MASTERMIX LC480 (10 × 5 ML)	ROCHE	Cat#4887301001
STRATAGENE AFFINITYSCRIPT REVERSE TRANSCRIPTASE, 2000 U/ l	AGILENT	Cat#600107
Oligonucleotides		
HIV-1 DNA ddPCR: Forward: TCTCTAGCAGTGGCGCCCGAACA Reverse: TCTCCTTCTAGCCTCCGCTAGTC Probe: /HEX/CAAGCCGAG/ZEN/TCCTGCGTCGAGAG/I BFQ/	IDT	N/A
HIV-1 2-LTR Circle ddPCR: Forward: AACTAGGGAACCCACTGCTTAAG Reverse: TCCACAGATCAAGGATATCTTGTC Probe: /FAM/ACACTACTT/ZEN/GAAGCACTCAAGGCAA GCTTT/IBFQ/	IDT	N/A
RPP30 ddPCR: Forward: GATTTGGACCTGCGAGCG Reverse: GCGGCTGTCTCCACAAGT Probe: /HEX/CT+G+ACCTGA+AG+GCTCT/3IBFQ/	IDT	N/A
RCAS Fw: 5’ GTC AAT AGA GAG AGG GAT GGA CAA A 3’ RCAS Rv; 5’ TCC ACA AGT GTA GCA GAG CCC 3’ RCAS Probe: [6FAM]TGGGTCGGGTGGTCGTGCC[TAM]	IDT IDT SIGMA	N/A N/A N/A
HIV INT-Fw: 5’-TTTGGAAAGGACCAGCAAA-3’ HIV INT-Rv: 5’-CCTGCCATCTGTTTTCCA-3’ HIV INT-Probe:5’- [6FAM]AAAGGTGAAGGGGCAGTAGTAATACA[TAMRA]-3’	IDT IDT SIGMA	N/A N/A N/A
Software and algorithms		
Quantstudio version 1.7	Bio-Rad	https://www.biorad.com/en-us/life-science/digital-pcr/qx200-droplet-digital-pcr-system/quantasoft-software-regulatory-edition?ID=1864011
FlowJo v10.0.8	TreeStar	https://www.flowjo.com/
LIGHTCYCLER 480 SW 1.5	ROCHE	https://lifescience.roche.com/en_us/brands/realtime-pcroverview.html#software Lightcycler Program: Step 1: 95°C for 10min Step 2: 95°C for 15sec Step 3: 60°C for 1 min Repeat 2–3 steps for 50 cycles
C1000/S1000 BioRADThermal Cyclers	BIORAD	CDNA Thermal Cycling Conditions: Step 1: 25°C for 15 min Step 2: 42°C for 40 min Step 3: 85°C for 10 min Step 4: 25°C for 30 min Step 5: 4°C hold
Other		

REAGENT or RESOURCE	SOURCE	IDENTIFIER
VQA 0, 5, 20 cp/ml HIV-1 RNA Plasma Controls	Duke Human Vaccine VQA	N/A
RCAS Internal Control for RNA Recovery	HIV Dynamics and Replication Program (HIV DRP) at NCI	Courtesy of Steve Hughes (https://home.ncifcrf.gov/hivdrp/rcas/contact.html)