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Research article

HIV-1 proviral DNA in purified peripheral blood CD34^{+} stem and progenitor cells in individuals with long-term HAART; paving the way to HIV gene therapy

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ABSTRACT

Human immunodeficiency virus (HIV)-1 infection is an important public health problem worldwide. After primary HIV-1 infection, transcribed HIV-1 DNA is integrated into the host genome, serving as a reservoir of the virus and hindering a definite cure. Although highly active antiretroviral therapy suppresses active viral replication, resulting in undetectable levels of HIV RNA in the blood, a viral rebound can be detected after a few weeks of treatment interruption. This supports the concept that there is a stable HIV-1 reservoir in people living with HIV-1. Recently, a few individuals with HIV infection were reported to be probably cured by hematopoietic stem transplantation (HSCT). The underlying mechanism for this success involved transfusion of uninfected hematopoietic stem and progenitor cells (HSPCs) from CCR5-mutated donors who were naturally resistant to HIV infection. Thus, gene editing technology to provide HIV-resistant HSPC has promise in the treatment of HIV infections by HSCT. In this study, we aimed to find HIV-infected individuals likely to achieve a definite cure via gene editing HSCT. We screened for total HIV proviral DNA by Alu PCR in peripheral blood mononuclear cells (PBMCs) of 20 HIV-infected individuals with prolonged viral suppression. We assessed the amount of intact proviral DNA via a modified intact proviral DNA assay (IPDA) in purified peripheral CD34^{+} HSPCs. PBMCs from all 20 individuals were positive for the *gag* gene in Alu PCR, and peripheral CD34^{+} HSPCs were IPDA-negative for six individuals. Our results suggested that these six HIV-infected individuals could be candidates for further studies into the ability of gene editing HSCT to lead to a definite HIV cure.

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Key points

1. HIV-1 proviral DNA negative in $CD34^+$ cells
2. The potential of using HIV-1 proviral DNA negative $CD34^+$ stem cells for gene therapy to achieve a definite cure for HIV infection

1. Introduction

Human immunodeficiency virus (HIV) establishes a persistent infection after primary infection by integrating its genomic DNA into the host genome. This makes a definite cure rarely possible [1]. Despite tremendous advances in the optimization of highly active anti-retroviral therapy (HAART) leading to the prolonged suppression of viral replication, HAART does not completely eliminate the virus from the human body, nor can it completely solve the persistent inflammation caused by HIV infection [2]. Importantly, HAART discontinuation leads to viral replication rebound from various cell subsets containing replication-competent viruses [3,4]. This represents the main obstacle in achieving a definite cure [5]. The HIV reservoir has a complex and heterogeneous nature, where each of the subsets that compose the viral reservoir contributes differently to viral persistence [6,7]. Central memory T cells are one of the main populations contributing to the total reservoir size [8], effector memory cells support HIV transcription [9] and contain higher proportions of intact viral regions [10–12], and memory stem cells and resident memory T cells are potentially long-lived niches for HIV [3]. Unfortunately, our knowledge of the reservoir's establishment, maintenance, and composition remains incomplete, and markers to target persistent HIV-infected cells remain elusive [13,14]. Hematopoietic stem cells and progenitor cells (HSPCs) are long-lived cell types that are also infected in vivo. These cells are capable of propagating integrated provirus to $CD4^+$ and $CD4^-$ progeny [15–17]. Infection of HSPCs by HIV-1 in vivo remains controversial. Some studies found no HIV DNA in bone marrow HSPCs [18] and purified $CD34^+$ HSPCs [19]. This discrepancy may be, in part, due to the use of different methods of detecting the proviral DNA and the heterogenous nature of $CD34^+$ HSPC harboring HIV DNA [11]. A novel approach to detecting latent HIV reservoirs includes the intact proviral DNA assay (IPDA), which detects intact viral genomes capable of transcribing infective virus rather than defective viral genomes [20]. This technique would definitely indicate whether intact viruses can be produced from HSPCs. Because hematopoietic stem transplantation (HSCT) has proved to be a treatment modality that can lead to a definite cure in HIV-infected individuals [21], donor HSPCs that are resistant to HIV infection are mandatory in this treatment process. The link between genetic variation and HIV resistance has been conceptually and practically proven [22]. However, finding HSPC donors can be difficult; thus, gene editing technology has begun to play a role in this setting [23]. If stem cells free of HIV proviral DNA of HIV-infected individuals can be genetically edited to be resistant to HIV infection and transferred back after their bone marrow cells have been eradicated, these HIV-resistant stem cells could proliferate and confer HIV resistance to these individuals, and a definite cure would be expected. Currently, autologous hematopoietic stem cell transplantation (HSCT) can provide definite cures for many diseases, such as

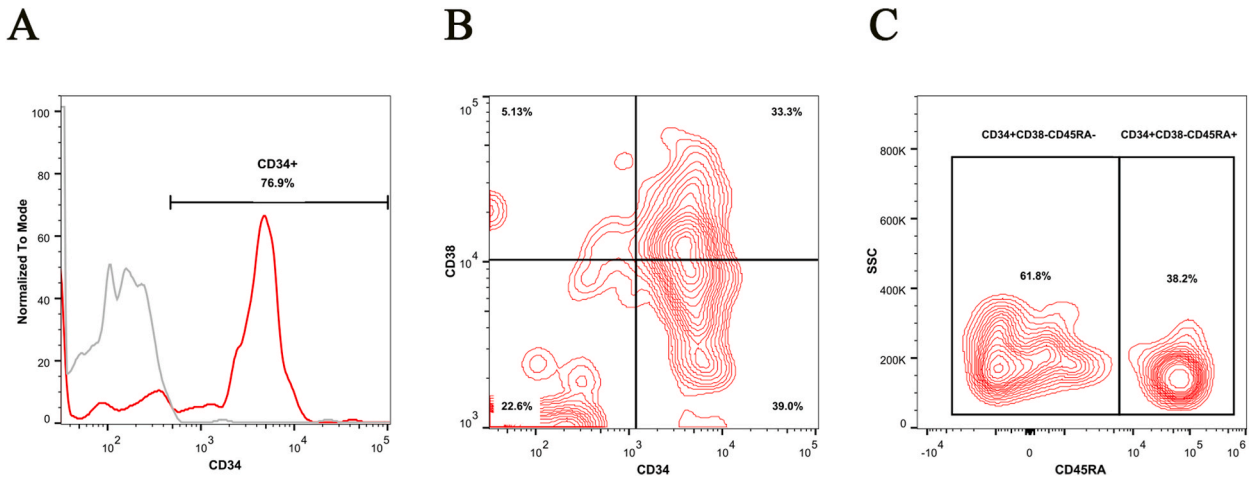

Fig. 1. Flow cytometry analysis of hematopoietic stem cell subpopulations. Following isolation of $CD34^+$ cells from PBMC fraction using a CD34 micro-bead kit, cells were stained with antibodies specific to surface markers and analyzed by flow cytometry. A) Red histogram depicting the expression of $CD34^+$ within the isolated cell population. B) Subsequently, gated cells were further analyzed on $CD34^+$ and $CD38^-$parameters within the $CD34^+$ subpopulation. C) Representative expression profile of three surface markers (CD34, CD38, and CD45A) in the $CD34^+CD38^-CD45A$ + subpopulation. This subpopulation represents primitive hematopoietic stem cells isolated from patient samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

solid tumors [24], storage disease [25], and hematological disorders [26]. This process needs healthy HSPCs from the individuals themselves to be transferred back after they have undergone intensive chemotherapy to destroy abnormal cells. The healthy HSPCs then proliferate, resulting in new hematopoietic progenitor cells to replace the abnormal cells. Autologous stem cell transplantation may then provide a method for the treatment of HIV infection. In this study, we aimed to find virally suppressed HIV-infected individuals who have been on long-term HAART and who have no proviral DNA in their HPSCs.

2. Participants and methods

2.1. Participants

Twenty HIV-infected individuals who had been on HAART with viral suppression (viral load <40 copies/mL) were recruited. Informed consent was obtained. The Faculty of Medicine Ramathibodi Hospital (MURA2020/531) and the Faculty of Medicine Siriraj Hospital (Si 700/2020), Mahidol University Institutional Review Board approved the study protocol regarding conformation with the principles of the Declaration of Helsinki.

2.2. $CD34^+$ HSPC purification and culture

The $CD34^+$ cells from the individuals were isolated immediately after blood collection using the CD34 MicroBead kit UltraPure (Miltenyi Biotec, Germany #cat no. 130-100-453) following the manufacturer's protocol. Briefly, peripheral blood mononuclear cells (PBMCs) were isolated through density gradient centrifugation. The $CD34^+$ cells were then magnetically labeled with CD34 MicroBeads UltraPure and the cell suspension was loaded onto a MACS® Column placed in the magnetic field of a MACS Separator. The magnetically labeled $CD34^+$ cells were retained within the column, while the unlabeled cells ran through, effectively depleting the cell fraction of $CD34^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD34^+$ cells were eluted as the positively selected cell fraction. The purity of $CD34^+$ cells after sorting is depicted in Fig. 1, with $CD34^+$ cells constituting 76.9% of the total cell population, as determined by flow cytometry.

2.3. Alu-gag PCR

According to the manufacturer's protocol, total DNA from PBMCs was extracted using the DNeasy Blood & Tissue Kit (Qiagen). Integration sites were sequenced as described previously [27,28]; the following primers were used: Alu forward, 5′-CCACTGCTTAAAGCCTCAAT-3′; and HIV-1 gag reverse, 5′-GCAAGCAGAGAGTTAGAA AG-3′. The PCR solution contained 1 × Ex Taq Buffer, 0.2 mM dNTP mixture, 1 U/μL Ex Taq DNA polymerase (TaKaRa Bio Inc, China), 0.3 μM Alu forward primer, 0.3 μM gag reverse primer, and 200 ng of DNA for every 25 μl of PCR solution. The thermocycler (T100 Thermal cycler, Bio-Rad) was programmed to perform a 3-min hot start at 94 °C, followed by 35 steps of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and final extension 72 °C for 5 min. For HIV-1 DNA integration detection, a second-round Alu-gag nested PCR was

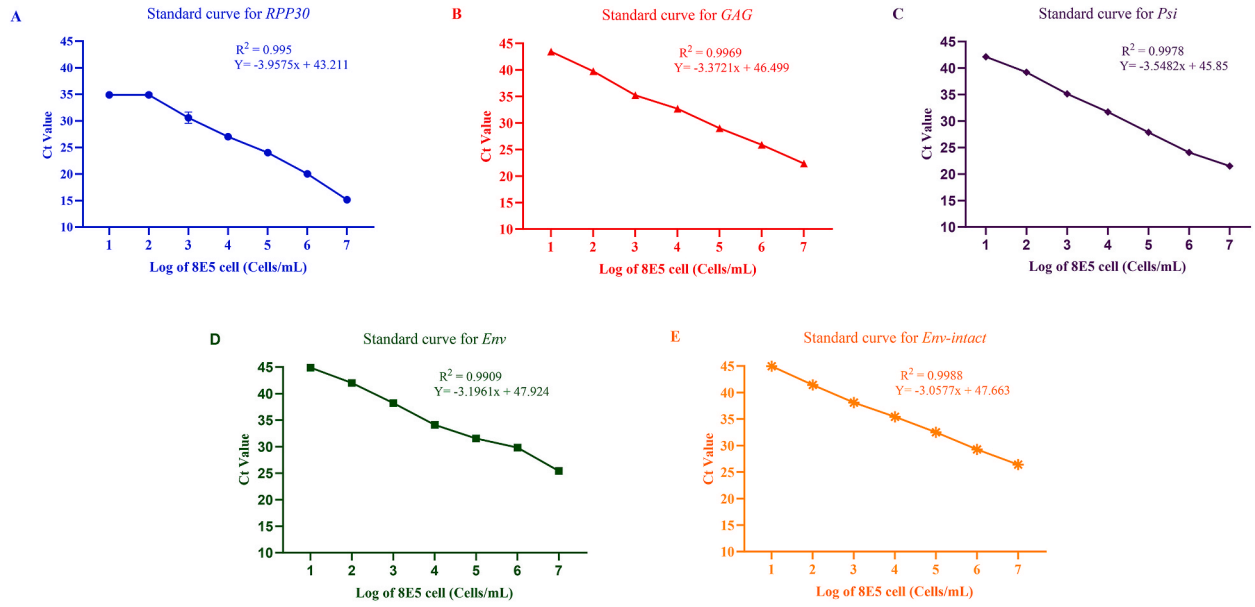

Fig. 2. Standard curves of number of cells and Ct of qRT-PCR for each primer. Inversed linear relationship between number of cells and Ct is noted for A) *RPP30*, B) *GAG*, C) *Psi*, D) Env and E) *Env-intact* primers.

performed using 5 μL of the product from the first-round PCR step. The primers' sequences were R forward, 5′-AAAATCTCTACCAGTGGCGC-3′; and U5 reverse, 5′-GAAAAAATCGGTTGCGGCC-3′. The PCR solution contained 1 × Ex Taq Buffer, 0.2 mM dNTP mixture, 1 U/μL Ex Taq DNA polymerase (TaKaRa Bio Inc, China), 0.3 μM Alu forward primer, 0.3 μM gag reverse primer, and 200 ng of DNA for every 25 μl of PCR solution. The thermocycler (T100 Thermal cycler, Bio-Rad) was programmed to perform a 3-min hot start at 94 °C, followed by 35 steps of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and final extension 72 °C for 5 min. For gel electrophoresis, 4 μL of PCR product were loaded into 2.5% agarose gel containing 0.2% red gel and electrophoresis was performed under 100 V for 35 min.

2.4. Intact proviral DNA assay

According to the manufacturer's protocol, total DNA from the cultured $CD34^+$ cells was extracted using the DNeasy Blood & Tissue Kit (Qiagen). An intact proviral DNA assay (IPDA) targeting *RPP30, GAG*, *Psi*, *Env,* and Env-intact genes [20] were performed on Rotor-Gene Q R09151 (Qiagen, Hilden, Germany) using the oasig™PLEX Lyophilised OneStep 2X RT-qPCR Master Mix (PrimerDesign). The reaction conditions for the qRT-PCR, sequences of the specific primers, and conditions are shown in Supplementary Table 1. A cycle threshold (Ct) of more than 42 was considered to indicate the sample being negative for proviral DNA [29]. We used 8E5 as a positive control and the standard curves of number of cells and Ct of each reaction for each gene are shown in Fig. 2A–E. At least 10^4 $CD34^+$ cells were used for the assay as at least 1 in 10^4 cells harbored an HIV genome is necessary to detect HIV provirus in $CD34^+$ cells [30].

2.5. Statistics

Statistical analysis was performed using STATA version 16 (STATA Corp, College Station, TX, USA). Data are presented as the mean ± SD if they were normally distributed or as the median (interquartile range [IQR]) if they were not. Comparisons of continuous data between proviral DNA positive and negative groups were performed by Student's t-test or Mann–Whitney *U* test if they were normally distributed or not normally distributed, respectively. A comparison of categorical data was performed using the chi-square test. A *p*-value less than 0.05 was considered to indicate statistical significance.

3. Results

Twenty virally suppressed HIV-infected individuals from the infectious disease clinics at Ramathibodi Hospital were recruited from 2019 to 2022. All individuals reported good compliance to antiretroviral drugs and appeared healthy. According to Thai guidelines, individuals who achieve viral suppression should have viral load and CD4 count monitoring once a year. In this cohort, 15 were male, and five were female. The median age of the individuals was 28.6 years old (IQR 21.4, 48.4). The median duration of antiretroviral therapy was 7.39 years (IQR 4.9, 11.6), and the median duration of viral suppression was 4.8 years (IQR 3.5, 8.5). The median CD4 count was 504 cells/μL (IQR 366.5, 719). Five individuals acquired HIV via mother-to-child transmission. Individual characteristics,

Table 1
Individual characteristics.

Individual ID	Congenital/ acquired	Age (years)	Sex	Duration of ARV (years)	Current ARV	Duration of suppression (years)	Proviral DNA (IPDA)
1	Congenital	15.56	M	8.76	AZT+3 TC + LPV/r	6.21	Positive
2	Congenital	16.48	M	11.54	AZT+3 TC + LPV/r	10.15	Positive
3	Congenital	17.58	F	7.32	TDF + FTC + EFV	6.29	Positive
4	Congenital	21.12	M	5.17	TDF + FTC + EFV	4.93	Positive
5	Congenital	21.56	M	16.43	TDF + FTC + EFV	15.81	Positive
6	Acquired	17.76	M	3.72	TDF + FTC + EFV	2.45	**Negative**
7	Acquired	25.58	F	14.69	TDF + FTC + EFV	4.56	**Negative**
8	Acquired	25.59	M	0.26	TDF/FTC/EFV	0.02	Positive
9	Acquired	26.98	M	4.51	TDF/FTC/EFV	4.00	Positive
10	Acquired	27.99	M	6.07	TDF/FTC + RPV	4.67	Positive
11	Acquired	29.30	M	7.45	TDF/FTC + RPV	NA	**Negative**
12	Acquired	31.69	M	2.81	TDF/FTC + RPV	2.33	**Negative**
13	Acquired	36.10	M	2.98	TDF/FTC/EFV	2.31	Positive
14	Acquired	42.44	M	4.98	TDF + AZT/3 TC + LPV/r	3.29	Positive
15	Acquired	46.32	M	8.61	TAF/FTC/BIC	7.91	Positive
16	Acquired	54.75	F	17.30	TDF/FTC/EFV	16.89	**Negative**
17	Acquired	58.42	F	13.67	TDF/FTC/EFV	NA	Positive
18	Acquired	58.48	F	11.86	TDF/FTC + NVP	11.29	Positive
19	Acquired	66.13	M	5.55	TAF/FTC/DTG	4.67	Positive
20	Acquired	76.96	M	9.21	3 TC + DTG	8.64	**Negative**

ID: identification number; ARV: antiretroviral drugs; NA: not available; TDF: tenofovir disoproxil fumarate; TAF: tenofovir alafenamide; FTC: emtricitabine; EFV: efavirenz; RPV: rilpivirine; LPV/r: lopinavir/ritonavir; AZT: zidovudine; 3 TC: lamivudine; IPDA: intact proviral DNA assay.

including current antiretroviral therapy, are summarized in Table 1.

We used Alu PCR as a screening assay to detect HIV-1 proviral DNA because it is widely accepted and easy to perform. We found the *gag* gene of HIV in all individuals (Fig. 3). This suggested that an HIV reservoir still existed in all individuals, even if their viral load was undetectable (<40 copies/mL). Next, we assessed the presence of intact HIV-1 proviral DNA from peripheral $CD34^+$ HSPCs using a novel IPDA assay. The number of cells and DNA concentration used for the assay is summarized in Supplementary Table 2. Proviral DNA was not detected by IPDA in six individuals (Table 2). The results of IPDA are summarized in Supplementary Table 3 and proviral frequencies across different individuals are demonstrated in Fig. 4. There were no differences in age, duration of viral suppression, or duration of HAART between those who were positive and those negative for proviral DNA in peripheral $CD34^+$ HSPCs. None of the individuals who had acquired HIV via mother-to-child transmission route were negative for proviral DNA in these cells. Of note, individual ID5, who acquired HIV via mother-to-child transmission, had quite a high Ct (>40), indicating the presence of a very small amount of proviral DNA. This individual had been virally suppressed for 15 years, which is longer than the viral suppression periods of the other individuals who acquired HIV via mother-to-child transmission.

4. Discussion

Our study demonstrated that some individuals who had been treated with HAART and had undergone viral suppression still had a HIV genome detected in their peripheral blood, as evidenced by the positive HIV DNA detected by the Alu PCR. However, six individuals had peripheral $CD34^+$ HSPCs negative for proviral DNA. This highlighted the potential of using these cells as stem cells for gene therapy to achieve a definite cure for HIV infection.

Since the HIV-1 pandemic in the 1980s, the successful implementation of HAART has improved the quality of life of individuals living with HIV infection (PLWH) worldwide, in that their immune response is allowed to recover to a near-normal state. However, HAART cannot eliminate HIV-1 proviruses or reservoirs, especially in $CD4^+$ T cells. HIV-1 viremia rebound can occur following treatment interruption. HIV-1 reservoirs are a major hindrance to curing HIV-1. Most HIV-1 reservoir studies have focused on reservoirs within the $CD4^+$ T cell compartment [11,31–33]. HSPCs are capable of self-renewal and expansion but can be infected and turn into viral reservoirs [15,16] even in HAART-treated individuals.

A recent env-sequencing study showed that HIV-1 DNA could be detected in $CD34^+$ HSPCs from the bone marrow of PLWH [34], although this may not have been the intact proviral DNA. However, there are conflicting data on HIV DNA detection in HPSCs. Some studies found no HIV DNA in bone marrow HSPCs [18] or purified $CD34^+$ HSPCs [19]. In this study, we aimed to identify HIV-1-infected individuals who could be good candidates for autologous hematopoietic stem cell transplantation. The quantitative viral outgrowth assay (QVOA) is considered the reference assay for quantifying replication-competent HIV-1 reservoirs; however, this assay requires large amounts of blood and 7–10 days of culturing time. Recently, results of IPDA have been proven to correlate well with QVOA results [20,35]. Our study showed that the PBMCs of 20 subjects were all positive in the Alu PCR assay. However, 70% of the enrolled individuals also had proviral DNA in their peripheral $CD34^+$ HSPCs assayed by IPDA. This supports the previous observation that HIV-1 can infect $CD34^+$ cells because these cells also harbor CXCR4 [36]. The sample size was relatively small in this study, but the preliminary results show that age, duration of HAART, and duration of viral suppression or CD4 count at the time of investigation were not associated with negative proviral DNA. Proviral DNA was detected in isolated $CD34^+$ cells, even in the two individuals in this cohort (ID2 and ID5) who had undergone more than ten years of viral suppression. The two individuals were started on HAART at a very young age, both at around five years old. This supports the previous finding that intact proviral DNA can persist in children who receive HAART, even in early life [37]. Interestingly, none of the individuals who acquired HIV via mother-to-child

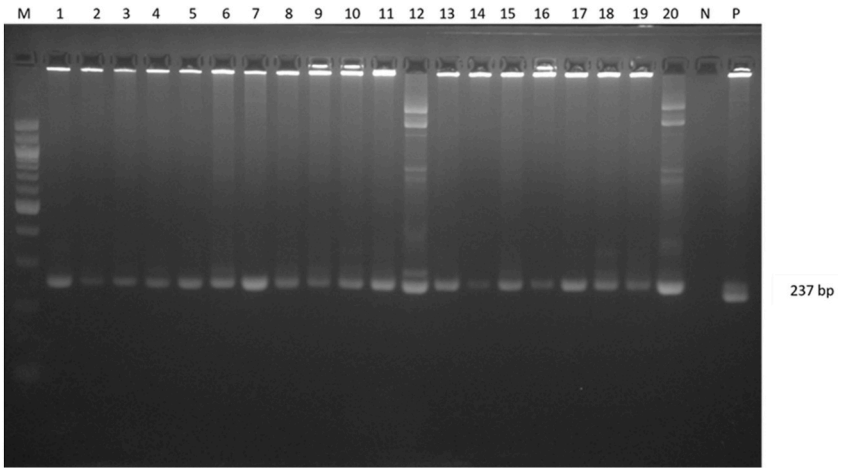

Fig. 3. Alu PCR results for peripheral blood mononuclear cells isolated from 20 individuals. Four microliters of PCR product were loaded into 2.5% agarose gel containing 0.2% red gel and electrophoresis was performed under 100 V for 35 min. DW represent negative control and 8E5 represent positive control, respectively. The full, non-adjusted image is provided in Supplementary Fig. 1.

Table 2
Comparison of individuals with positive and negative proviral DNA in peripheral CD34$^+$ cells.

	Proviral DNA positive (n = 15)	Proviral DNA negative (n = 6)	*p*-value
Age: years (mean ± sd)	34.3 ± 17.2	39.3+/22.2	0.591
Sex			0.573
Male	11	4	
Female	3	2	
Mode of acquisition			0.091
Mother to child	5	0	
Postnatal	9	6	
Duration of HAART: years (mean ± sd)	7.7 ± 4.4	9.2 ± 5.8	0.534
Duration of viral suppression: years (mean+/sd)	6.3 ± 4.2	7.0 ± 6.1	0.782
Number of CD4 count: cells/uL (median, IQR)	573 (302, 832)	467 (369, 512)	0.509

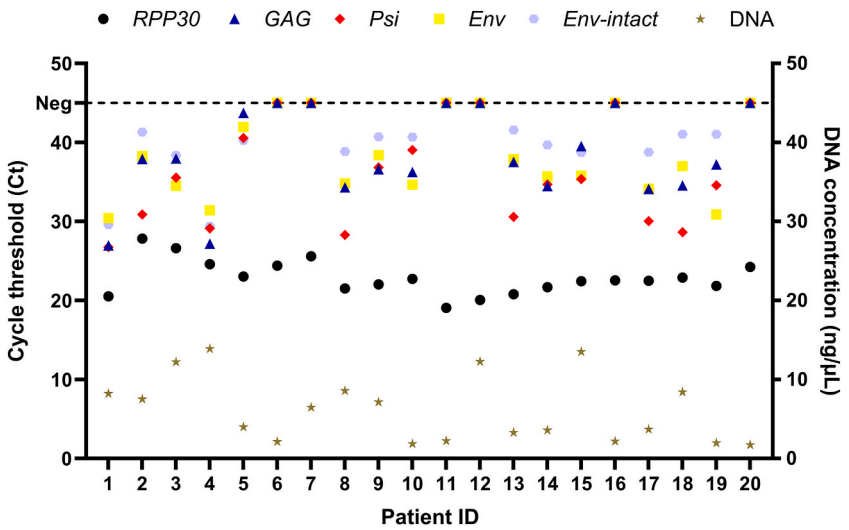

Fig. 4. Proviral frequencies across different individuals. The relationship of cycle threshold (Ct) of Env, Intact Env (Env-intact), Gag, RPP30, Psi and DNA concentration (ng/mL) of peripheral CD34$^+$ cells of all individuals is demonstrated. Ct ≥ 42 is considered to be negative.

transmission had CD34$^+$ HSPCs negative for proviral DNA. These individuals were not started on HAART once HIV infection was diagnosed because HIV treatment guidelines at the time recommended starting individuals on anti-retroviral drugs when their CD4 count starts to decline. This might lead to the establishment of a long-lived viral reservoir [38,39]. It is interesting to further explore whether after the implementation of new guidelines suggesting starting individuals on HAART early, would lead to more proportion of individuals having reduced HIV reservoir and subsequently non-infected CD34$^+$ HSPCs. The early treatment of neonates with HIV infection has been shown to reduce the size of their reservoir [38].

There are many possible reasons why some individuals were negative for proviral DNA in their CD34$^+$ HSPCs. The initiation of very early antiretroviral therapy may play a role, though this could not be proved in this study. Many studies have shown that early antiretroviral treatment in HIV-infected individuals can reduce the size of the reservoir [40–48]. The type of virus predominant during the initial infection may also influence whether CD34$^+$ HSPCs are infected because HIV enters these cells through CXCR4 molecules [36]. Therefore, individuals with predominantly X4 virus may have a higher probability of having infected CD34$^+$ HPSCs. Coinfection with other viruses may also influence infectivity of HIV in HSPC. HSPCs latently infected with cytomegalovirus may have be more vulnerable to HIV-1 infection [49].

Allogeneic stem cell transplantation has been proven to achieve functional cures in at least six individuals [50–55]. These individuals who received uninfected HSPCs from CCR5-mutated donors stem cells that were naturally resistant to HIV infection had a hematologic malignancy requiring allogeneic HSCT. However, this procedure needs preconditioning chemotherapy and immunosuppressive drugs for prophylaxis against graft versus host disease (GvHD) [56], rendering individuals vulnerable to opportunistic infections [57]. Therefore, treating HIV-infected individuals with allogeneic HSCT without other indications may pose an increased risk of the side effects from stem cell transplant procedure in stable individuals who have been on HAART. In contrast, in autologous HSCT, individuals use their own stem cells; therefore, they have a very low risk of GvHD, and hence GvHD prophylaxis is not needed [58]. Currently, the morbidity and mortality rates of autologous HSCT are quite acceptable and individuals do not need long-term anti-GvHD prophylaxis [59,60]. Because implementation of HAART has led to individuals infected with HIV living as normally as those who are not infected, HAART is currently still the first-line therapy for HIV infection. Autologous stem cell transplant with gene editing of stem cells could be considered as an alternative treatment especially for individuals tending to be non-compliant to the

medications, those who cannot tolerate the medications or those who have co-occurring conditions requiring HSCT such as lymphoma. In addition, to use peripheral blood as stem cells, a larger amount of peripheral $CD34^+$ cells would be needed, however, this could be accomplished by leukapheresis. Another issue that has to be addressed in this point is that HIV reactivation does not occur after cells being stimulated with growth factors during leukapheresis.

Our results suggest that the six HIV-infected individuals who had negative HIV proviral DNA in $CD34^+$ HSPC, though proviral DNA may still persist in other cells, could be ideal candidates for autologous HSCT aimed at achieving an HIV cure. Because the $CD34^+$ HSPCs from these individuals were free of HIV proviral DNA, they could be genetically edited to make them HIV-resistant [23]. After the gene editing process, these cells could be transfused into individuals through autologous stem cell transfusion. Many gene editing tools available at the moment [61–64] provide the possibility that a definite cure for HIV infection can be achieved in the near future.

There are several aspects that have to be further addressed. First, the propagation of HIV proviral DNA-negative $CD34^+$ HSPCs is needed to prove that HIV proviral DNA is still absent after their differentiation to lymphoid precursor cells. This would require a larger amount of peripheral blood, or the use of bone marrow cells, to accomplish. Second, follow-up is needed to ensure that, after a certain period of time, these cells are still negative for HIV proviral DNA. Lastly, factors determining HIV proviral DNA negativity in these cells should be explored.

In conclusion, HIV-infected individuals on HAART were demonstrated to have $CD34^+$ HSPCs negative for HIV proviral DNA. This suggests that, after gene editing of these cells, a definite cure for HIV infection using autologous HSCT is possible.

Data availability

The data used to support the findings of this study are included within the articles.

CRediT authorship contribution statement

Boonrat Tassaneetrithep: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Angsana Phuphuakrat:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Ekawat Pasomsub:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Kanit Bhukhai:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Wasinee Wongkummool:** Visualization, Methodology, Investigation, Conceptualization. **Thongkoon Priengprom:** Methodology, Investigation. **Wannisa Khamaikawin:** Methodology, Investigation, Conceptualization. **Sujittra Chaisavaneeyakorn:** Visualization, Investigation, Conceptualization. **Usanarat Anurathapan:** Writing – review & editing, Visualization, Investigation, Conceptualization. **Nopporn Apiwattanakul:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Suradej Hongeng:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26613.

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