

# Plasma Viremia and Cellular HIV-1 DNA Persist Despite Autologous Hematopoietic Stem Cell Transplantation for HIV-Related Lymphoma

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**Abstract:** A cure of HIV-1 has been achieved in one individual through allogeneic stem cell transplantation with a *CCR5Δ32* homozygous donor. Whether myeloablation and autologous stem cell transplantation for lymphoma in patients on suppressive antiretroviral therapy can eliminate HIV-1 reservoirs is unknown. Low-level plasma viremia and total HIV-1 DNA and 2-LTR circles in blood mononuclear cells were quantified after autologous transplantation in 10 patients on suppressive antiretroviral therapy using quantitative polymerase chain reaction assays capable of single-copy nucleic acid detection. Plasma viremia was detectable in 9 patients, whereas HIV-1 DNA was detectable in all 10 patients, indicating that HIV-1 had not been eliminated.

**Key Words:** HIV-1 pathogenesis, HIV-1 persistence, AIDS-related lymphoma

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## INTRODUCTION

Current antiretroviral therapy (ART) is effective in suppressing plasma viremia and preventing HIV-1 disease progression but is not curative because of the persistence of HIV-1 in long-lived cells, primarily CD4<sup>+</sup> memory T cells.<sup>1–3</sup> In addition, most patients on effective ART have residual low-level viremia, derived from an unknown source, that can persist for at least 7 years.<sup>1–5</sup> Allogeneic hematopoietic stem cell transplantation for relapsed acute myelogenous leukemia (AML) with a *CCR5Δ32* homozygous donor has achieved a drug-free cure of HIV-1 in one individual.<sup>6,7</sup> Although this case report has generated excitement that a cure for HIV-1 is possible, the specific components of treatment for AML that were necessary to achieve a cure of HIV-1 are undefined. In particular, whether intensive myeloablative chemotherapy followed by autologous stem cell transplantation (ASCT) in patients on suppressive ART has an impact on reservoirs of HIV-1 is unknown.

In the era of suppressive ART, cancer remains a significant cause of HIV-related mortality in both developed and developing countries.<sup>8–10</sup> ART has led to a significant decrease in AIDS-defining cancers, but the incidence and mortality of non-Hodgkin lymphoma (NHL) remain high.<sup>8,11</sup> Furthermore, the incidence of non-AIDS-defining cancers including Hodgkin lymphoma (HL) has increased in the era of ART.<sup>12,13</sup> Many studies have demonstrated the feasibility of ASCT in HIV-1 patients with both NHL and HL, but the impact of chemotherapy and ASCT on reservoirs have not been evaluated.<sup>14–17</sup> Given that intensive myeloablative chemotherapy causes depletion of CD4<sup>+</sup> T cells,<sup>18,19</sup> HIV-1-infected cells might be expected to be similarly depleted. To date, only 2 studies have examined HIV-1 DNA in peripheral blood mononuclear cells (PBMC) after ASCT.<sup>20,21</sup> However, neither study examined low-level viremia or 2-LTR circles (2-LTRs) with highly sensitive quantitative polymerase chain reaction (qPCR) assays in well-characterized patients with viremia suppressed by ART. We therefore evaluated low-level viremia, total HIV-1 DNA and 2-LTR DNA after myeloablative chemotherapy, and ASCT in patients on effective ART with plasma HIV-1 RNA suppressed to less than 50 copies per milliliter before and after transplantation.

## MATERIALS AND METHODS

We conducted a multicenter, cross-sectional study of patients with HIV-related lymphoma who underwent ASCT, had survival of at least 6 months after ASCT, and had plasma HIV-1

RNA suppressed on ART to less than 50 copies per milliliter. This study was approved by local institutional review boards, and written informed consent was obtained from all study participants.

HIV-1 RNA from plasma was initially quantified using a reverse transcriptase (RT)-qPCR assay with single-copy sensitivity targeting the *gag* region (gSCA) of the HIV-1 genome.<sup>22</sup> If HIV-1 RNA was undetectable with gSCA, an alternative assay with single-copy sensitivity (iSCA) targeting the *p31* (*integrase*) region of the HIV-1 *pol* gene was performed. Total HIV-1 DNA and 2-LTRs from PBMC were quantified using qPCR assays capable of single-copy detection.<sup>23</sup> Total HIV-1 DNA and 2-LTRs were normalized for the number of cells assayed by qPCR for the *CCR5* gene.<sup>24</sup> Complete descriptions of these assays are available in the Supplemental Digital Content (<http://links.lww.com/QAI/A410>). Pearson correlation was used to determine if there was a correlation between time from transplant and HIV-1 DNA levels.

### RESULTS

The relevant clinical characteristics of the 10 patients studied are summarized in Table 1. The median age was 51 (range: 24–60) years, and patients were either whites (N = 7) or Hispanics (N = 3) and were diagnosed with Burkitt lymphoma (N = 3), HL (N = 3), or NHL (N = 4). All patients received a myeloablative chemotherapy conditioning regimen consisting of either CBV (cyclophosphamide, carmustine, and etoposide; N = 7) or BEAM (carmustine, etoposide, cytarabine, melphan; N = 3), followed by ASCT with mobilized hematopoietic progenitor cells obtained from peripheral blood as treatment for HIV-related lymphoma.

The duration of time from ASCT to sample collection for HIV-1 qPCR assays was variable, with a median of 686 days posttransplant (range: 28–4009 days). All patients had plasma viremia suppressed to less than 50 copies per milliliter on ART before ASCT. ART was given as tolerated during conditioning chemotherapy in 6 of 9 patients and was interrupted during chemotherapy in 3 of 9 patients, and information about ART during conditioning was unavailable for

1 patient. ART regimens consisted of 2–3 nucleoside RT inhibitors and either a nonnucleoside RT inhibitor (N = 7) or a ritonavir-boosted protease inhibitor (N = 3). Additional clinical information is available in the Supplemental Digital Content (<http://links.lww.com/QAI/A410>).

As shown in Table 2, 6 of 10 patients had HIV-1 RNA detectable in plasma by gSCA, whereas in 4 patients, no HIV-1 RNA was detected. Samples from the 4 patients with undetectable HIV-1 RNA were retested by qPCR targeting *integrase* (iSCA), and 3 of these patients were found to have detectable HIV-1 RNA (Table 2). The median plasma HIV-1 RNA was 2 copies per milliliter (range of <0.2 to 26 copies per milliliter), which is consistent with levels previously reported after long-term suppressive ART in nontransplanted patients.<sup>4,5</sup> One patient (PID 7) had no HIV-1 RNA detected (<0.2 copies per milliliter) by either the gSCA or the iSCA assays performed on a total volume of 25 mL of plasma.

Total HIV-1 DNA and 2-LTRs were measured by qPCR on PBMC extracts and normalized for the number of cells assayed. Total HIV-1 DNA was detected in 9 of 10 patients using primers and probe targeting *integrase*. The patient (PID 9) with undetectable HIV-1 DNA by qPCR targeting *integrase* had 340 HIV-1 DNA copies/10<sup>6</sup> PBMC detected using qPCR targeting HIV-1 *gag*. Taken together, all 10 patients had detectable HIV-1 DNA with a median of 208 copies/10<sup>6</sup> PBMC (range: 12–415 copies/10<sup>6</sup> PBMC). Of note, the patient (PID 7) with the highest level of HIV-1 DNA (415 copies/10<sup>6</sup> PBMC) had undetectable plasma viremia by both RT-qPCR assays. There was no correlation between time post-ASCT and total HIV-1 DNA levels ( $\rho = 0.18, P = 0.625$ ). 2-LTRs were not detectable in any of the patients in this study, but 2-LTRs were readily detected in positive controls run in parallel with the patient samples.

### DISCUSSION

We found that plasma viremia and HIV-1 DNA in PBMC persist despite myeloablation and ASCT for HIV-related lymphoma and despite suppression of plasma viremia

TABLE 1. Clinical Characteristics of Patients Studied

PID	Age	Gender	Ethnicity	Lymphoma Diagnosis	Conditioning Regimen	Days Posttransplant*	CD4 <sup>+</sup> T-Cell Count Posttransplant (Cells per Microliter)	ART Regimen
1	48	M	White	BL	CBV	+637	752	EFV/TNF/FTC
2	49	M	White	NHL	CBV	+734	1015	EFV/TNF/FTC
3	39	M	Hispanic	BL	CBV	+2194	480	FPV/r/TNF/FTC
4	52	M	White	BL	CBV	+28	132	DRV/r/TNF/FTC
5	51	M	White	HL	BEAM	+210	398	EFV/TNF/FTC
6	55	M	White	HL	BEAM	+1288	422	LPV/r/TNF/FTC
7	53	M	White	NHL	CBV	+3970	697	ABC/3TC/NVP
8	24	M	Hispanic	HL	CBV	+92	210	TNF/FTC/NVP
9	60	M	Hispanic	NHL	CBV	+182	808	EFV/TNF/FTC
10	50	M	White	NHL	BEAM	+4009	920	ABC/AZT/3TC/ NVP

\*Samples obtained for current study.

BL, Burkitt lymphoma; CBV, cyclophosphamide, carmustine, etoposide; BEAM, carmustine, etoposide, cytarabine, melphalan; EFV, efavirenz; TNF, tenofovir; FTC, emtricitabine; FPV/r, ritonavir-boosted fosamprenavir; DRV/r, ritonavir-boosted darunavir; LPV/r, ritonavir-boosted lopinavir; ABC, abacavir; 3TC, lamivudine; NVP, nevirapine; AZT, zidovudine.

**TABLE 2.** Plasma HIV-1 RNA and Cellular HIV-1 DNA After ASCT

PID	gSCA HIV-1 Plasma RNA (Copies per Milliliter)	iSCA HIV-1 Plasma RNA (Copies per Milliliter)	Total PBMC Assayed by <i>CCR5</i>	Total HIV-1 DNA (Copies/10 <sup>6</sup> PBMC)	2-LTRs (Copies/ 10 <sup>6</sup> PBMC)
1	1.9	NA	1.2 × 10 <sup>6</sup>	12	<7.5
2	0.6	NA	1.3 × 10 <sup>6</sup>	48	<7.5
3	<0.6	26	7.9 × 10 <sup>5</sup>	253	<7.5
4	0.9	NA	1.0 × 10 <sup>6</sup>	186	<7.5
5	<0.3	25	9.6 × 10 <sup>5</sup>	156	<7.5
6	<0.4	2.0	5.7 × 10 <sup>5</sup>	230	<7.5
7	<0.2	<0.2	6.0 × 10 <sup>5</sup>	415	<7.5
8	7.6	NA	8.4 × 10 <sup>5</sup>	359	<7.5
9	12.0	NA	1.2 × 10 <sup>6</sup>	340*	<7.5
10	0.7	NA	3.8 × 10 <sup>5</sup>	120	<7.5

\*Patient 9 had undetectable HIV-1 DNA using a primer/probe pair targeting integrase but had detectable HIV-1 DNA using a primer/probe pair targeting *gag*. NA, not assessed.

to less than 50 copies per milliliter by ART before and after transplant. Our study is novel in that the patient group studied had undetectable plasma HIV-1 RNA by standard detection methods and that sensitive qPCR assays were required to reveal HIV-1 persistence, obviating the need for ART interruption to determine whether cure had been achieved. Simonelli et al<sup>21</sup> reported a significant decay in HIV-1 DNA 24 months after ASCT. In our study, HIV-1 DNA levels in blood did not correlate with time after ASCT, including follow-up of more than 4000 days after transplantation. The absence of detectable 2-LTRs in our patients is consistent with long-term suppressive ART and argues against ongoing viral replication in the patients studied.<sup>23</sup>

One possible explanation for persistence of HIV-1 after ASCT is the presence of HIV-1–infected resting CD4<sup>+</sup> T cells that may be resistant to the chemotherapeutic agents used for myeloablation. Chemotherapy-mediated cell death favors metabolically active cells through a variety of mechanisms. The primary reservoir of HIV-1 infection is thought to be resting memory CD4<sup>+</sup> T cells, which are metabolically quiescent. Thus, although chemotherapy reduces lymphocyte counts,<sup>18,19</sup> HIV-1–infected resting CD4<sup>+</sup> cells may not be profoundly affected. Along these lines, a recent report describes the survival of CD161<sup>hi</sup> CD8<sup>+</sup> central and effector memory T cells despite myeloablative chemotherapy in AML patients.<sup>25</sup>

Another cause of persistent HIV-1 infection may be the reinfusion of HIV-1–infected cells in the autologous transplant. If an average of 10<sup>11</sup> mononuclear cells are reinfused after conditioning chemotherapy, approximately 15% of these cells (1.5 × 10<sup>10</sup>) will be CD4<sup>+</sup> T cells. The estimated frequency of HIV-1–infected CD4<sup>+</sup> T cells in blood ranges from 1 in 100 (1.5 × 10<sup>8</sup>) to 1 in 1000 (1.5 × 10<sup>7</sup>), and approximately 1 in 1000 (1.5 × 10<sup>4</sup> to 1.5 × 10<sup>5</sup>) HIV-1–infected CD4<sup>+</sup> T cells will harbor replication-competent provirus.<sup>3</sup> Consequently, the autologous graft is likely to contain 1 × 10<sup>4</sup> to 1 × 10<sup>5</sup> cells with replication-competent proviruses that could serve as a source of persistent HIV-1.

In addition, IL-7 levels have been shown to increase in response to lymphopenia induced by chemotherapy,<sup>26,27</sup> and IL-7–induced T-cell proliferation has been reported to contribute to the size and persistence of HIV-1 reservoirs in

patients on ART.<sup>28</sup> Hence, homeostatic proliferation of CD4<sup>+</sup> T cells may have contributed to the persistence of HIV-1 after ASCT through expansion of the reinfused HIV-1–infected CD4<sup>+</sup> T cells. HIV-1–infected cells in autologous grafts could be reduced or eliminated by using purified CD34<sup>+</sup> hematopoietic progenitor cells, but this approach is not favored because of the delayed lymphocyte recovery after transplantation.<sup>29</sup>

An important distinction between autologous and allogeneic hematopoietic stem cell transplantation is the occurrence of graft versus tumor response and graft versus host response after allogeneic transplantation.<sup>30</sup> Graft versus host response may have played a key role in eliminating HIV-1–infected host cells from the one individual cured of HIV-1 infection, and transplantation of *CCR5*Δ32/Δ32 donor cells was likely to have prevented infection of the allograft. Similarly, Henrich et al<sup>31</sup> recently reported the clearance of HIV-1 DNA from PBMC in 2 HIV-1–infected patients with lymphoma who received continuous ART and allogeneic stem cell transplantation from *CCR5* wild-type donors. This report suggests that allogeneic transplantation and continuous ART to block HIV-1 infection of the allograft may have been sufficient to eliminate HIV-1 reservoirs, although proof of cure in these patients will require cessation of ART. Prior studies of allogeneic transplantation with wild-type *CCR5* donor cells without continuous ART have resulted in HIV-1 rebound after cessation of ART probably because of infection of the allograft.<sup>32</sup>

In summary, our findings show that myeloablation and autologous transplantation do not deplete HIV-1 reservoirs or cure HIV-1 infection. Genetic approaches targeting *CCR5* in autologous cells have been explored,<sup>33</sup> but whether a safe, efficient, and practical method to knock out *CCR5* in most cells targeted by HIV-1 can be achieved is not known.

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