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Impact of Chemotherapy for HIV-1 Related Lymphoma on Residual Viremia and Cellular HIV-1 DNA in Patients on Suppressve Antiretroviral Therapy

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Abstract

The first cure of HIV-1 infection was achieved through complex, multimodal therapy including myeloablative chemotherapy, total body irradiation, anti-thymocyte globulin, and allogeneic stem cell transplantation with a CCR5 delta32 homozygous donor. The contributions of each component of this therapy to HIV-1 eradication are unclear. To assess the impact of cytotoxic chemotherapy alone on HIV-1 persistence, we longitudinally evaluated low-level plasma viremia and HIV-1 DNA in PBMC from patients in the ACTG A5001/ALLRT cohort on suppressive antiretroviral therapy (ART) who underwent chemotherapy for HIV-1 related lymphoma without interrupting ART. Plasma HIV-1 RNA, total HIV-1 DNA and 2-LTR circles (2-LTRs) in PBMC were measured using sensitive qPCR assays. In the 9 patients who received moderately intensive chemotherapy for HIV-1 related lymphoma with uninterrupted ART, low-level plasma HIV-1 DNA did not change significantly, with median pre-chemotherapy HIV-1 DNA of 355 copies/10⁶ T cells versus 228 copies/10⁶ T cells post-chemotherapy. 2-LTRs were detectable in 2 of 9 patients pre-chemotherapy and in 3 of 9 patients post-chemotherapy. In summary, moderately intensive chemotherapy for HIV-1 related lymphoma in the context of continuous ART did not have a prolonged impact on HIV-1 persistence.

Clinical Trials Registration Unique Identifier: NCT00001137

Introduction

Effective antiretroviral therapy (ART) reduces plasma HIV-1 RNA to levels that are undetectable by FDA-approved assays, but low-level viremia and HIV-1 DNA in PBMC persist [1,2]. The persistence of replication-competent HIV-1 in long-lived memory CD4+ T cells despite prolonged ART administration is a major obstacle to curing HIV-1 infection [3–5]. Nevertheless, in one HIV-infected individual, allogeneic hematopoietic stem cell transplantation (ASCT) with a CCR5 delta32/delta32 donor resulted in the first definitive cure of HIV-1 infection [6]. This cure has generated enthusiasm for further investigation of potentially curative interventions for HIV-1, including allogeneic stem cell transplantation [7,8] and autologous transplantation with genetically modified CD4+ T cells [9] or stem cells [10,11]. Along these lines, the National Heart, Lung and Blood Institute (NHLBI) recently identified the possible role of hematopoietic stem cells in curative approaches for HIV-1 infection as an essential question that needs to be addressed [12]. Although there is considerable interest in stem cell-mediated interventions to achieve a cure of HIV-1, the question remains as to which components of the Berlin patient’s cancer therapy were necessary to achieve a cure. Components of ASCT that may have contributed to the eradication of HIV-1 reservoirs include chemotherapy, total body irradiation, immunosuppressive drugs, allogeneic transplantation with CCR5 delta32/delta32 donor cells and graft versus host disease. We have shown previously that myeloablative chemotherapy followed by autologous hematopoietic stem cell transplantation is not sufficient to eliminate low-level HIV-1 RNA in plasma or HIV-1 DNA in PBMC in patients on...
ART with <50 cps/mL of HIV-1 RNA in plasma, but changes
from before myeloablative therapy to after autologous transplant
were not compared in this prior study [13], thus the impact
chemotherapy alone is undefined. Intensive chemotherapy is
known to cause significant depletion of circulating CD4+ T cells
[14–16], which could reduce levels of plasma viremia or HIV-1
DNA in PBMC in the context of uninterrupted ART by killing
HIV-infected cells. To investigate this possibility, we measured
HIV-1 levels in plasma and PBMC samples, before and after
chemotherapy, in 9 patients who underwent moderately intensive
chemotherapy for HIV-1 related lymphoma and who remained on
continuous ART throughout the sampling period.

Methods
NWGS 334 was a retrospective study of HIV-1-infected patients
in the ACTG A5001: AIDS Clinical Trials Group Longitudinal
Linked Randomized Trials (ALLRT) cohort who received
moderately intensive chemotherapy for the treatment of HIV-1
related lymphoma, who continued on ART pre- and post-
chemotherapy, and who maintained suppressed plasma HIV-1
RNA<50 copies/mL (Roche Amplicor HIV Monitor assay
versions 1.0/1.5; Branchburg, NJ). The ALLRT parent study is
registered at ClinicalTrials.gov under the unique identifier
NCT00001137, and the rationale, design, and baseline charac-
teristics of the overall cohort have been previously described [17].
The University of Pittsburgh Institutional Review Board approved
the parent study (ALLRT), which allowed participants to
contribute samples for future use in ACTG-approved research.
Patients gave written informed consent for the information to be
obtained from their clinic records as part of the ALLRT study, and
this information was kept confidential at each ALLRT site. Patient
information was anonymized and de-identified prior to analysis.

Stored plasma samples were evaluated for HIV-1 RNA using
two-step real-time quantitative PCR assays with two different
primer/probe sets targeting HIV-1 gag or integrate sequences using
previously described assay conditions with single-copy sensitivity
(limit of detection<1 copy/mL of plasma) [13,18]. Levels of total
HIV-1 DNA (limit of quantification = 5 copies/sample) and 2-long
terminal repeat circles (2-LTRs; limit of quantification = 7.5 copy-
ies/sample) in PBMC were assayed as described previously [13]
and were run in parallel with positive and negative controls from
the Virology Quality Assurance Laboratory (Rush University).
HIV-1 DNA quantitative PCR (qPCR) data were normalized per
10^6 CD4+ T cells using qPCR for the CCR5 gene [19] and the
percent CD4+ T cells. The percent CD4+ T cells was available
from the A5001/ALLRT database (N=8) or was determined by
standard flow cytometry in the Pitt Virology Support Laboratory
(N = 1).

Statistical analysis using McNemar’s Test was applied to
determine if the proportion of patients with plasma viremia at
undetectable levels pre-chemotherapy was significantly different
from the proportion of patients with undetectable levels post-
chemotherapy. A non-parametric sign test was used to determine
if there was a significant difference between CD4+ and CD8+ cell
counts pre- and post-chemotherapy, and between HIV-1 DNA
copies per 10^6 CD4+ T cells pre- and post-chemotherapy.

Results
A total of 40 patients in the A5001/ALLRT cohort were
diagnosed with HIV-1 related lymphoma, 18 had plasma HIV-1
RNA<50 cps/mL pre- and post-chemotherapy with uninterrupted
ART, and of these 18 had plasma and PBMC samples
available pre- and post-chemotherapy for further analysis. To
confirm the efficiency of qPCR amplification for HIV-1 RNA,
plasma samples from prior to the initiation of ART in these 10
patients were tested and results were compared to the FDA-
approved Roche Amplicor assay. HIV-1 RNA in pre-ART
samples from 9 of 10 subjects amplified efficiently by qPCR,
and longitudinal samples from these 9 patients were studied. The
relevant clinic characteristics of the study patients are shown in
Table 1. All patients were males diagnosed with HIV-1 related
Hodgkin’s (HL; N = 3) or Non-Hodgkin’s lymphoma (NHL; N = 6).

All 9 patients received moderately intensive chemotherapy for
lymphoma consisting of doxorubicin, bleomycin, vinblastine and
dacarbazine (ABVD) in 4 patients, cyclophosphamide, doxorubi-
cin, vincristine, prednisone (CHOP) in 3 patients, and CHOP with
rituximab in 2 patients (Table 1). The number of days between
pre- and post-chemotherapy sampling varied, with a median of
285 days between samples (interquartile range: 225 to 685 days).
Median CD4+ cell count pre-chemotherapy was 296 cells/μL (median
%CD4+ = 20.0) and was 315 cells/μL (median %CD4+ = 20.0)
post-chemotherapy; median CD8+ cell count pre-chemotherapy was
803 cells/μL (median %CD8+ = 50.0%) and 693 cells/μL (median
%CD8+ = 49.0%) post-chemotherapy. Differences in the CD4+
and CD8+ cell counts between pre- and post-chemotherapy time
points were not statistically significant.

All subjects received ART throughout their chemotherapy and
post-chemotherapy follow-up. Low-level plasma HIV-1 RNA
(Figure 1A), as determined by qPCR with single-copy sensitivity
[13], did not show a consistent pattern of change from pre- to post-
chemotherapy time points. Plasma viremia decreased in 5 patients
(median decrease = 8 copies/mL) increased in 3 (median increa-
se = 3 copies/mL), and remained below the LOD in one patient
(Figure 1A). The median HIV-1 plasma RNA pre-chemotherapy
was 1 copy/mL, and the median post-chemotherapy was 4 copies/
M. A total of 4 patients had undetectable plasma HIV-1 RNA
pre-chemotherapy and 3 had undetectable plasma HIV-1
RNA post-chemotherapy. There was no significant difference in
the proportion of patients with undetectable plasma HIV-1 RNA
before versus after chemotherapy (p = 0.6, McNemar’s test).

Levels of total HIV-1 DNA and 2-LTRs in PBMC (Figures 1B
and 1C) were evaluated for changes between pre- and post-
chemotherapy time points. Total HIV-1 DNA was detectable in
all 9 patients pre- and post-chemotherapy and showed no
consistent pattern of change. The median total HIV-1 DNA level
pre-chemotherapy was 355 (interquartile range: 70.0 to 469)
copies/10^6 CD4+ T cells versus 228 (interquartile range: 106 to
452) copies/10^6 CD4+ cells post-chemotherapy (p = 1.0, sign test).
Total HIV-1 DNA levels per 10^6 CD4+ cells decreased in 4
patients and increased in 5 patients between pre- and post-
chemotherapy time points. 2-LTRs were detectable in only 4 of
9 subjects at either time point, with 2-LTRs increasing in 1 patient
post-chemotherapy, becoming detectable (from below the limit of
quantification) in 2 subjects, and becoming undetectable in 1
subject.

Discussion
In this small initial study (N = 9), we found no significant
differences between pre- and post-chemotherapy levels of HIV-1
RNA in plasma and HIV-1 DNA in PBMC from patients receiving
chemotherapy for HIV-1 related lymphoma. The absence of a durable
effect on plasma viremia following chemotherapy suggests that there was not a reduction in the number of infected cells that can produce virus. Although much of the HIV-1 DNA that persists despite ART has deletions or is
hypermutated [20–22], the lack of a reduction in HIV-1 DNA levels is also consistent with chemotherapy not causing a sustained reduction in infected cell number.

One reason why chemotherapy would not impact HIV-1 persistence is that some subpopulations of CD4+ T cells may be resistant to chemotherapeutic agents. In this regard, Turtle et al. have described a population of CD8+ T cells in peripheral blood that survive intensive chemotherapy [23], and Casorati et al. have described a population of bone marrow resident CD4+ T cells that survive conditioning chemotherapy for autologous transplantation [24]. Importantly, the latent reservoir of HIV-1 resides within the resting memory CD4+ T cell population [3], and while chemotherapy significantly depletes CD4+ T cells in the periphery [8,24], resting memory CD4+ T cells may be more resistant to cytotoxic chemotherapy because of their quiescent state [15]. In addition, CD4+ T cells that survive cytoreductive chemotherapy and harbor HIV-1 DNA are likely to proliferate in response to chemotherapy-induced lymphopenia through IL-7 mediated homoestatic proliferation [25,26]. Hence, although chemotherapy may kill some HIV-infected cells, those that survive could repopulate HIV-1 reservoirs through cell proliferation in response to lymphopenia.

Limitations of our study are the small sample size and long interval (median 285 days) between pre- and post-chemotherapy samples. As a consequence, transient reductions in low-level viremia and HIV-infected CD4+ T cells from chemotherapy could have been missed, as could have restoration of viremia and HIV-infected cells through proliferation of surviving CD4+ T cells. Nevertheless, the findings from this current study are similar to those found in a cross-sectional study of 10 patients on suppressive ART, where HIV-1 RNA in plasma and HIV-1 DNA in PBMC remained detectable following myeloablative chemotherapy and autologous stem cell transplantation [13]. The current study adds to this prior post-transplant cross-sectional study by comparing pre- and post-chemotherapy levels of HIV-1 persistence.

The failure of either moderately intensive or myeloablative chemotherapy to have a sustained effect on HIV-1 persistence points to the importance of allogeneic transplantation with a CCR5 delta32 homozygous donor in achieving the first definitive cure of HIV-1 infection [6]. For HIV-1 to infect target cells, CD4 and one of two major coreceptors, either CCR5 or CXCR4, must be expressed on the cell surface [27,28]. A 32 base pair deletion (CCR5 delta32) provides resistance to CCR5 tropic HIV-1 [29,30], and is present in 2–5% of persons from Europe, the Middle East and the Indian subcontinent [31]. Complete replacement of the Berlin patient’s immune system by allogeneic cells with the CCR5 delta32 mutation was likely critical in eradicating HIV-1 reservoirs. Recently, the elimination of HIV-1 DNA from PBMC in 2 patients who received ART throughout reduced-intensity allogeneic transplantation with CCR5 wild-type donors has been reported [7], confirming the importance of allogeneic transplantation in eliminating evidence of HIV-1 persistence in blood. The recent report of viral rebound in both of these patients 3–8 months after cessation of ART indicates that HIV-1 reservoirs were not eliminated by allogeneic transplantation and that the CCR5 wild-type donor cells supported HIV-1 replication in the absence of ART [32].

In summary, this study provides evidence that chemotherapy alone does not have a sustained impact on HIV-1 persistence in patients on ART and that future therapeutic interventions to reduce or eliminate HIV-1 reservoirs will need to have greater specificity for HIV-infected cells.

### Table 1. Characteristics of the 9 patients studied from the ALLRT cohort.

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<th>Patient ID Number</th>
<th>Sex</th>
<th>Race/Ethnicity</th>
<th>Age at Lymphoma Diagnosis</th>
<th>Lymphoma Diagnosis</th>
<th>Chemotherapy Administered</th>
<th>CD4+ T cell Counts (cells/mm³)</th>
<th>Time from Pre- to Post-Chemotherapy Sampling (Days)</th>
<th>Pre-Chemo</th>
<th>Post-Chemo</th>
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Acknowledgments

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Author Contributions

Conceived and designed the experiments: ARC SK DKM RTM MFP JWM. Performed the experiments: ARC. Analyzed the data: ARC SK JWM. Contributed reagents/materials/analysis tools: ARC SK DKM RTM MFP JWM. Wrote the paper: ARC JWM.

References


