Title

Circulating CD30+CD4+ T Cells Increase Before Human Immunodeficiency Virus Rebound After Analytical Antiretroviral Treatment Interruption

Journal

The Journal of Infectious Diseases, 221(7)

ISSN

0022-1899

Authors

Prator, Cecilia A
Thanh, Cassandra
Kumar, Shreya
et al.

Publication Date

2020-03-16

DOI

10.1093/infdis/jiz572

Peer reviewed

The Journal of Infectious Diseases

MAJOR ARTICLE

Circulating CD30$^+$CD4$^+$ T Cells Increase Before Human Immunodeficiency Virus Rebound After Analytical Antiretroviral Treatment Interruption

Cecilia A. Prator,[1] Cassandra Thanh,[1] Shreya Kumar,[1] Tony Pan,[1] Michael J. Peluso,[1,2] Ronald Bosch,[3] Norman Jones,[4] Jeffrey M. Milush,[4] Sonia Bakkour,[5] Mars Stone,[5] Michael P. Busch,[5] Steven G. Deeks,[6] Peter W. Hunt,[1] and Timothy J. Henrich[1]

[1]Division of Experimental Medicine, University of California San Francisco, San Francisco, California, USA, [2]Division of Infectious Diseases, University of California San Francisco, San Francisco, California, USA, [3]Center for Biostatistics in AIDS Research, Boston, Massachusetts, USA, [4]Core Immunology Laboratory, Division of HIV, Infectious Diseases and Global Medicine, University of California San Francisco, San Francisco, California, USA, [5]Vitalant Research Institute, San Francisco, California, USA, [6]Division of HIV, Infectious Diseases and Global Medicine, University of California San Francisco, San Francisco, California, USA

Background. Identification of nonviral markers of human immunodeficiency virus (HIV) infection that increase before viral rebound during analytical treatment interruption (ATI) may affect HIV persistence research. We previously showed that HIV ribonucleic acid (RNA) is enriched in CD30$^+$CD4$^+$ T cells in many individuals. Here, we studied CD30$^+$CD4$^+$ T-cell dynamics before ATI, during ATI (before detectable plasma RNA), and after HIV rebound.

Methods. Peripheral blood mononuclear cells from 23 participants collected longitudinally from 5 Adult AIDS Clinical Trials Group studies incorporating ATI were included in this study. Flow cytometric characterization of expression of CD30 and markers of T-cell activation and exhaustion were performed along with HIV-1 RNA and deoxyribonucleic acid quantification and measurement of soluble plasma CD30 and CD30 ligand.

Results. The percentage of CD4$^+$ T cells expressing CD30 significantly increased from pre-ATI to postinterruption time points before detectible viremia (1.65 mean relative increase, P = .005). Seventy-seven percent of participants experienced an increase in CD30$^+$ cells before viral rebound. In contrast, there were no significant differences between pre-ATI and postinterruption pre-rebound time points in percentages of lymphocytes expressing CD69, CD38/HLA-DR, or PD-1 until after HIV recrudescence.

Conclusions. CD30 may be a surrogate marker of early replication or viral transcriptional activity before detection by routine peripheral blood sampling.

Keywords. analytical treatment interruption; CD30; HIV biomarkers; HIV-1 persistence; monitored antiretroviral pause.

Despite advances in antiretroviral therapy (ART), human immunodeficiency virus (HIV)-1 persists in tissue and blood cellular reservoirs in treated individuals. Latently infected CD4$^+$ T cells that harbor integrated HIV-1 deoxyribonucleic acid (DNA) comprise a large proportion of this long-lived reservoir and appear similar to uninfected cells, especially when in a quiescent state [1–3]. Investigations of HIV persistence may require analytical treatment interruption (ATI) to determine effects of interventions, such as the use of HIV-specific monoclonal antibodies, immune modulation, or strategies that lead to loss of detectable HIV in blood and tissues, such as stem cell transplantation or very early ART initiation [4–9]. Although useful for determination of viral rebound, these studies are logistically complicated and are accompanied by various risks [8, 10]. Certain factors, such as pre-ATI cell-associated HIV-1 ribonucleic acid (RNA), DNA, and various immune phenotypes before ART initiation or ATI, have been associated with decreased time to rebound [11–13], but there is a lack of information regarding more specific biomarkers that can be used in real time during treatment interruption to monitor and, ideally, anticipate viral rebound. Identifying impending rebound may also allow for prospectively guided tissue sampling or a noninvasive procedure (such as whole-body positron emission tomography imaging) to gain important insights in the anatomic origins of HIV recrudescence. As a result, identification of such nonviral, but specific, markers of impending HIV recrudescence that increase before detectable plasma HIV-1 RNA by routine clinical assays would have a potentially profound impact on the design of HIV curative studies and provide novel insights in the pathogenesis of HIV persistence.

One potential marker, CD30, is a member of the tumor necrosis factor receptor (TNFR) superfamily and has been implicated in the activation, proliferation, and death of selected cell populations [14–16]. Only a very small percentage of hematopoietic cells in healthy individuals express CD30, but it is upregulated in the setting of various hematologic malignancies

Received 6 August 2019; editorial decision 28 October 2019; accepted 30 October 2019; published online November 2, 2019.

Correspondence: Timothy J. Henrich, MD, 1001 Potrero Avenue, Building 3, Room 605, San Francisco, CA 94110, USA (timothy.henrich@ucsf.edu).

The Journal of Infectious Diseases® 2020;221:1146–55

DOI: 10.1093/infdis/jiz572

such as Hodgkin lymphoma. In addition, infection with various viral pathogens increases surface CD30 expression on lymphocytes, and increased CD30 expression has previously been associated with HIV clinical progression before the introduction of ART [15–21]. We recently demonstrated that HIV RNA is variably enriched in $CD30^+CD4^+$ T cells in participants on suppressive ART [22]. We also observed in a single case that targeting CD30 using a cytotoxic antibody-drug conjugate was associated with reduction cell-associated HIV RNA and DNA, an effect we recapitulated ex vivo using cells from antiretroviral-treated people with HIV [22, 23]. These data collectively indicate that HIV infection of a cell is associated with CD30 expression through mechanisms that remain undefined.

In this study, we investigated surface expression of CD30 and the CD30 ligand, CD153, on $CD4^+$ T cells before ATI, during ATI (before detectable plasma RNA), and after HIV rebound in 5 AIDS Clinical Trial Group (ACTG) trials involving planned treatment interruptions. Cell surface expression and soluble levels of CD30 in plasma were explored in relation to T-cell activation markers and measures of HIV reservoirs before and during ATI and after HIV rebound. Overall, we observed that $CD4^+$ T-cell expression of CD30 significantly increases after interruption and before detectable viremia in a majority of individuals.

MATERIALS AND METHODS

Study Population

The cohort assembled for this study included 23 participants collected longitudinally from 5 ACTG studies incorporating ATI (A5187, A371, A5197, A5024, A5068) [24–28]. Both peripheral blood mononuclear cells (PBMCs) and plasma were curated from time points collected before ATI (baseline, Time point 1), during ATI (before detectable plasma RNA, Time point 2), and after HIV rebound (Time point 3). Clinical history obtained included ATI start dates, viral load, date of plasma or PBMC sample collection, $CD4^+$ T-cell counts, race/ethnicity, and age. All participants provided informed consent to participate in the ACTG studies and subsequent analyses. With exception of samples from the A5068 trial, samples were obtained from individuals who were not receiving vaccination or other immune-modifying intervention as part of the parent trials (ie, control arms from the vaccine/therapy studies) to minimize potential treatment bias. Samples were chosen based on sufficient sample remaining from the 3 time points as defined above.

Antibody Staining and Flow Cytometry

Peripheral blood mononuclear cells (1×10^7) from 23 participants were analyzed by 2 multiparameter flow cytometric panels that included the following: (1) CD30 expression on $CD4^+$ T cells with markers to exclude $CD14^+$, $CD16^+$, and $CD19^+$ cells and (2) markers of T-cell activation (CD69, CD38/HLA-DR) and immune checkpoint (PD-1) and immune subsets (T_N, T_{CM}, T_{TM}/T_{EM}, T_{EMRA}). Full panels and antibodies used for staining are described in Supplementary Table 1. The first panel was used to exclude potential contamination of non $CD4^+$ lymphocytes such as B cells and $CD14/CD16^+$ myeloid cells. Samples stained with all but 1 fluorescent antibody (FMO) were used in addition to unstained controls to adequately gate on the low-frequency cell populations while accounting for spectral overlap, even after rigorous compensation. Cells were analyzed on a BD LSR II (BD Biosciences) and data were analyzed in FlowJo V10 (TreeStar). Single-stained beads (Life Technologies) were used for compensation. Sample gating for CD30 expression on $CD4^+$ T cells is shown in Supplementary Figures 1 and 2.

Enzyme-Linked Immunosorbent Assay

Both soluble (s)CD30 and CD30L levels were measured in plasma samples collected from 22 participants using either a Human sCD30 Platinum ELISA Kit (Invitrogen) or a Human CD30L/CD153 ELISA Kit (LifeSpan Biosciences), respectively. Protocols were followed according to the manufacturer's instructions. Plasma specimens were measured in duplicate, and values were averaged to obtain adjusted sCD30 or CD30L values for each specimen. Assays were repeated twice to ensure reproducible results.

Quantification of Cell-Associated Human Immunodeficiency Virus-1 Ribonucleic Acid and Deoxyribonucleic Acid

Human immunodeficiency virus-1 DNA and RNA were purified from PBMC aliquots saved from the 23 patient samples used for flow cytometry using a QIAGEN AllPrep DNA/RNA Mini Kit, following the manufacturer's standard protocol, with an additional DNAse treatment (QIAGEN). Quantitative polymerase chain reaction was performed as previously described [22].

Statistical Methods

Non-parametric analyses and linear mixed models were used to determine paired significant differences between baseline, on-ATI, and post-HIV rebound time points using Prism (GraphPad Software) and STATA 15 (StataCorp, College Station, TX). Continuous variables were log-transformed to satisfy model assumptions, and results were back-transformed to derive relative changes from baseline.

RESULTS

Study Population, Design, and Analytical Treatment Interruption

We examined CD30 expression in the context of ATI in 23 ART-suppressed people with HIV who underwent ATI in 5 previous ACTG studies: A5187, A371, A5197, A5024, and A5068 [24–28]. These trials involved treatment interruption as part of longitudinal cohorts of antiretroviral-treated individuals or in vaccine alone and combination vaccine immunotherapy studies [24–29]. A371 and A5187 involved acute or early-treated individuals [24], whereas the remaining involved participants

treated during established infection. The duration of ART before ATI was at least 6 months in all studies, with certain trials requiring longer periods of continuous suppression (eg, at least 2 years of ART in the A5195 study) [28]. Sampling was performed every 2–4 weeks during ATI in the parent ACTG trials.

Table 1 shows participant demographics, ATI details, and median $CD4^+$ T-cell counts and CD4/CD8 T-cell ratios before, during, and after ATI. Median age was 43, 87% of participants were male, and a majority of individuals were on an nonnucleoside reverse-transcriptase inhibitor-based regimen before ART cessation. In general, these values were similar to the parent study arms from which samples were able to be obtained. For example, the median ages of participants were 41 years (A5187), 34 years (A371), 42 years (A5197), and 43 years (A5068); no demographic data were provided for A5024. These studies comprised a majority of white (70%, 69%, 66%, and 57%) and male participants (100%, 95%, 94%, respectively; no data were provided for the A5024 or A5068 trials). Median CD4 counts were 934, 535, 853, and 730 cells/μL, respectively [24, 25, 27, 28]. In this study, median $CD4^+$ T-cell counts, percentages, and $CD4^+/CD8^+$ T-cell ratios were stable after treatment interruption, but they decreased after viral recrudescence. The last sample time point with no detectable HIV-1 RNA from routine sampling with available PBMCs was used in this study, which was a median of 25 days after initiation of ATI and 10 days before first detectable viral load.

Lymphocyte Phenotype and Surface Markers During Analytical Treatment Interruption

Twenty of the 23 participants had samples from all 3 time points, and paired data were analyzed using non-parametric tests with Dunn's corrections for multiple comparisons, which are shown in Figure 1. In addition, data from all time points and participants were included in mixed linear effects models (Table 2). The percentage of $CD4^+$ T cells expressing CD30 significantly increased from pre-ATI (Time point 1) to on-ATI (Time point 2) samples before detectible viremia (Time point 3) in both non-parametric analyses (median = 0.06, interquartile range [IQR] = 0.03–0.11 and median = 0.10, IQR = 0.04–0.19, respectively; $P = .029$), and a mean 1.65-fold relative increase from Time point 1 in the $\%CD30^+CD4^+$ T cells was also observed at Time point 2 (95% confidence interval [CI], 1.16–2.34) and Time point 3 (1.50 fold relative increase; 95% CI, 1.05–2.12) by mixed-effects regression analysis. Overall, 77% of participants experienced an increase in $CD30^+$ cells from Time point 1 to Time point 2. Median fluorescent intensity of CD30 expression on $CD4^+$ T cells also increased significantly from Time point 1 to Time point 2 in paired analyses (Figure 1) and approached significance in linear effects regression analyses (Table 2). Up to 512 individual $CD30^+CD4^+$ T cells were analyzed (mean 83 for all participants, including pre-ATI time points; 95% CI, 62–103).

In contrast to CD30 expression, there were no significant differences in percentages of $CD4^+$ T cells expressing CD69, CD38/HLA-DR, PD-1, and CD153 or in percentages of central or effector/transitional memory $CD4^+$ T-cell subsets (all $P > .05$) in either paired (Figure 1) or mixed-effects (Table 2) analyses between Time point 1 and Time point 2. However, the percentage of CD38/HLA-DR^+ $CD4^+$ T cells significantly increased from Time point 1 to Time point 3 (after rebound) in paired analyses (median = 1.59% [IQR, 1.06–2.59] and median = 2.43% [IQR, 1.47–3.64], respectively). Although not significant, there was a trend toward increasing PD-1^+ $CD4^+$

Table 1. Participant Demographics and Treatment/ATI Parameters

N (%)	Pre-ATI	During ATI	After HIV Rebound
Male	20 (87%)	-	-
Race Ethnicity			
White Non-Hispanic	17 (74%)	-	-
Hispanic	4 (17%)	-	-
Asian/Pacific Islander	1 (4%)	-	-
Black Non-Hispanic	1 (4%)	-	-
Pre-ATI ART			
PI	5 (21.7%)	-	-
NNRTI	17 (73.9%)	-	-
NRTI only	1 (4.3%)		-
Median (IQR)			
Age	43 (40–49)	-	-
Duration (days) from ATI to first sampling[a]	25 (13–27)	-	-
Duration (days) from ATI to viral rebound	35 (27–42)	-	-
CD4 cell count	798 (682–1020)	768 (618–953)	679 (440.3–810)
CD4%	37 (31–48)	36 (31–46)	30.5 (26.3–40.5)
CD4/CD8 ratio	1.3 (0.8–1.7)	1.3 (0.7–1.7)	0.9 (0.6–1.1)

Abbreviations: ART, antiretroviral therapy; ATI, analytical treatment interruption; HIV, human immunodeficiency virus; IQR, interquartile range; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

[a]Sampling frequency = 2–4 weeks.

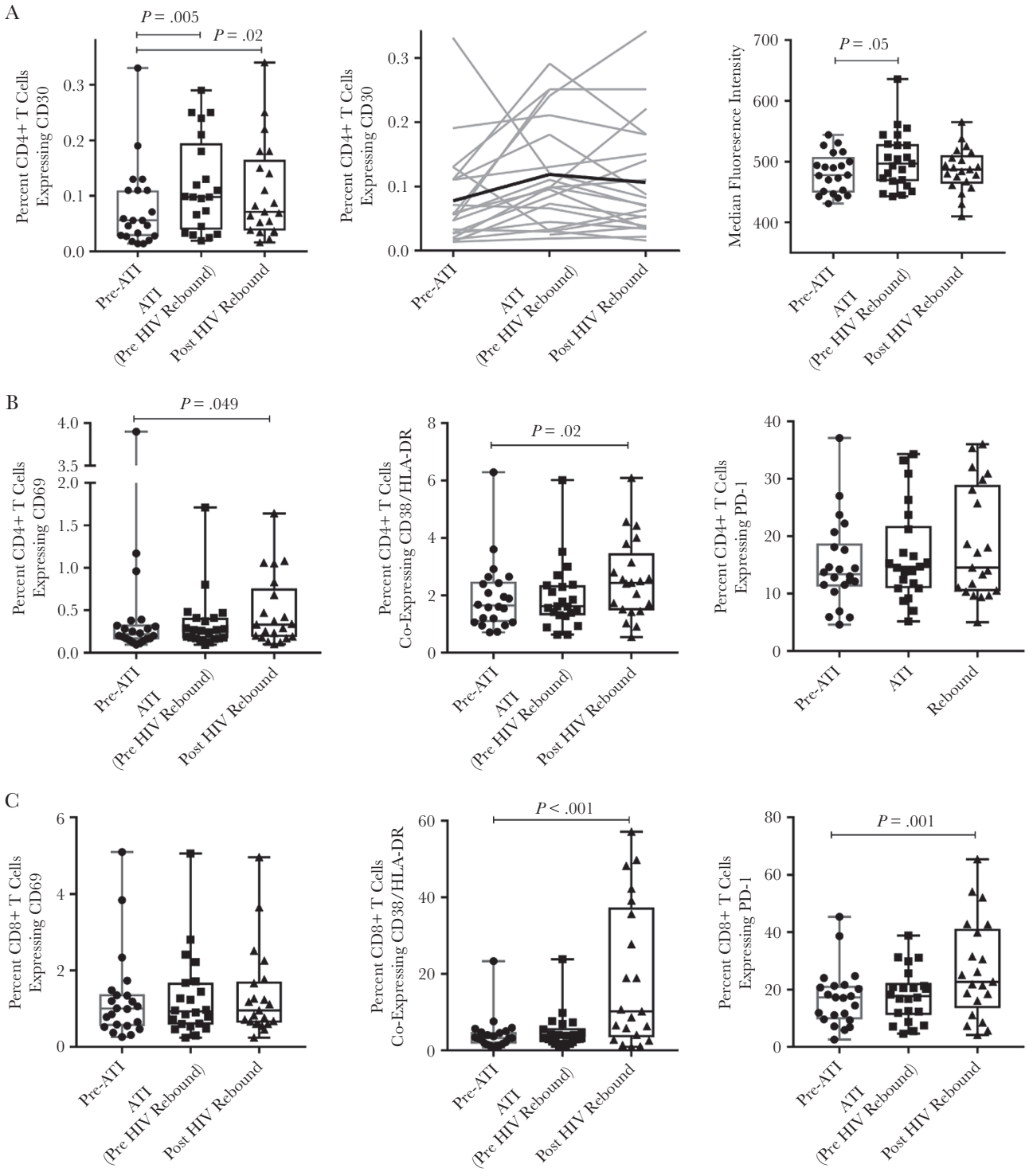

Figure 1. Changes in expression of CD30 and markers of T-cell activation and exhaustion on $CD4^+$ and $CD8^+$ T cells before analytical treatment interruption (ATI) (Time point 1), during ATI (Time point 2), and after human immunodeficiency virus (HIV) rebound (Time point 3). The percentage of $CD30^+CD4^+$ T cells (box plots with all data points and matched participant line plot) and CD30 median fluorescent intensity (MFI) on $CD4^+$ lymphocytes are shown in A. The percentage of $CD4^+$ and $CD8^+$ T cells expressing CD69, CD38/HLA-DR, and PD1 at each time point are shown in B and C, respectively. Box plots show median, interquartile range, and minimum/maximum values. Mean absolute change is shown in bold in the line plot. *P* values are shown for significant relative changes in cell percentages or MFI between Time point 1 versus Time points 2 and from mixed linear effects analyses.

T cells from Time point 1 to Time point 3 (median = 13.35 [IQR, 11.50–17.98]; $P = .053$). In linear fixed-effects models, the percentage of $CD4^+$ T cells expressing CD69 and CD38/HLA-DR and $CD8^+$ T cells expressing CD153, CD38/HLA-DR, and PD-1 increased significantly from Time point 1 to Time point 3 (all $P < .05$) (Table 2). As we have previously

Table 2. Changes From Baseline in the Percentage of T-Cell Subsets Expressing CD30 or Markers of T-Cell Activation and Exhaustion

	Time Point 1 to Time Point 2 [Pre-ATI to ATI (Pre-HIV Rebound)]				Time Point 1 to Time Point 3[b] (Pre-ATI to Post-HIV Rebound)			
Marker	Absolute Change[a]	Relative Change[b]	RC 95% CI[b]	*P*[b]	Absolute Change[a]	Relative Change[b]	RC 95% CI[b]	*P*[b]
%$CD4^+CD30^+$ T Cells	0.04%	1.65	1.16–2.34	.005	0.03%	1.50	1.05–2.12	.02
$CD4^+CD30^+$ MFI	21.10	1.04	0.99–1.09	.05	5.33	1.01	0.97–1.05	.66
%$CD4^+CD153^+$ T Cells	0.01%	0.98	0.74–1.29	.89	<–0.01%	0.88	0.67–1.17	.38
%$CD4^+$ $CD69^+$ T Cells	–0.11%	1.02	0.78–1.34	.88	0.02%	1.32	1.00–1.74	.049
%$CD4^+CD38$/HLA-DR^+ T Cells	–0.02%	1.01	0.80–1.29	.93	0.59%	1.33	1.04–1.69	.02
%$CD4^+$ $T_{TM}/T_{EM}{}^+$ T Cells	1.57%	1.06	0.93–1.20	.38	0.55%	1.05	0.93–1.19	.44
%$CD4^+$PD-1^+ T Cells	1.67%	1.07	0.94–1.23	.32	3.59%	1.20	1.04–1.37	.38
%$CD8^+CD30^+$ T Cells	–0.32%	0.73	0.30–1.76	.48	–1.00%	0.74	0.29–1.89	.53
%$CD8^+CD153^+$ T Cells	<0.01	0.64	0.35–1.18	.15	–0.07	0.47	0.24–0.91	.03
%$CD8^+CD69^+$ T Cells	0.01%	0.99	0.81–1.20	.89	0.09%	1.00	0.83–1.23	.95
%$CD8^+CD38$/HLA-DR^+ T Cells	0.61%	1.15	0.70–1.91	.58	14.77%	3.17	1.90–5.30	<.01
Cell-associated HIV-1 RNA (copies/106 $CD4^+$ T cells)	17 468	2.68	0.63–11.41	.18	267 912	18.89	4.44–80.32	<.01
Cell-Associated HIV-1 DNA (copies/106 $CD4^+$ T cells)	–6	0.63	0.33–1.18	.15	22	1.84	0.98–3.46	.06

Abbreviations: ATI, analytical treatment interruption; CI, confidence interval; DNA, deoxyribonucleic acid; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; MFI, mean fluorescence intensity; RC, relative change; RNA, ribonucleic acid.

[a]Calculated from all values for each time point (difference from pre-ATI baseline).

[b]As determined by mixed-linear effects modeling.

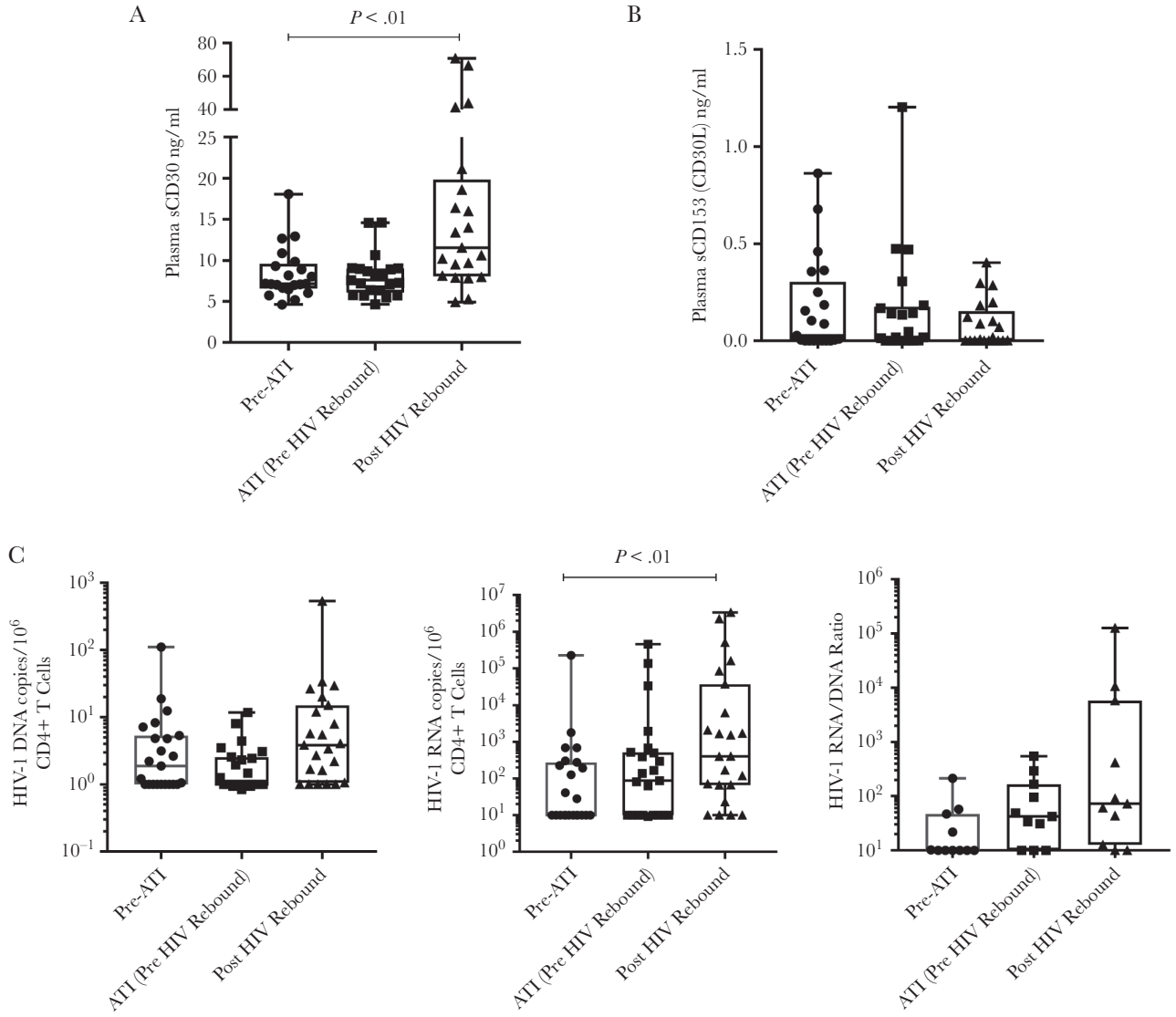

Figure 2. Plasma soluble (s)CD30 and sCD153 (sCD30L) at baseline, during analytical treatment interruption (ATI) and after human immunodeficiency virus (HIV) rebound are shown in A and B, respectively. $CD4^+$ T cell-associated HIV-1 deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) levels and RNA/DNA ratios for each timepoint are shown in C. Box plots show median, interquartile range, and minimum/maximum values. *P* values are shown for significant relative changes in cell percentages or median fluorescent intensity between baseline (pre-ATI) versus ATI and postrebound timepoints from mixed linear effects regression analyses.

reported, a majority of $CD30^+CD4^+$ T cells (59%) were of effector memory phenotype ($CCR7^-CD45RA^-$), but there were no significant changes in $CD30^+CD4^+$ memory subtypes over time. Given the relatively low frequency of $CD30^+CD4^+$ cells, even in the setting of HIV infection that we have previously reported [22], these subset analyses were limited. Of note, there were no significant changes in the percentages of CD30 expressing $CD14^+/CD16^+$ myeloid cells between any time points (all $P > .37$).

Soluble Plasma CD30 and CD153 During Analytical Treatment Interruption

Soluble plasma levels of CD30 (sCD30) and sCD153 (CD30L) were measured using sensitive commercial enzyme-linked immunosorbent assays. Unlike cell-surface CD30 expression, soluble CD30 only increased after viral recrudescence (Time point 3), as shown in Figure 2. Soluble CD153 was low overall and did not change in plasma over any time point (Figure 2).

Baseline CD30 Expression and Time to Viral Rebound

Survival analysis including log-rank, Mantel-Cox tests to determine whether baseline CD30 expression levels predicts time to viral rebound was performed. The hazard ratio (HR) between time to rebound since stopping ART between participants with baseline $CD30^+CD4^+$ T-cell percentages greater than or less than 0.05 was not significant (HR = 1.74; 95% CI, 0.71–4.28). However, the median time to rebound in the >0.05% baseline $CD30^+CD4^+$ T-cell group was 39 days compared with 33.5 days for the <0.05% group.

Cell-Associated Human Immunodeficiency Virus-1 Deoxyribonucleic Acid and Ribonucleic Acid During Analytical Treatment Interruption

To determine the relationship between $CD30^+CD4^+$ T cells and the HIV reservoir, we compared the total PBMC-associated HIV-1 RNA and DNA between all time points. Adjusting levels for the percentage of $CD4^+$ T cells, no significant change was observed in HIV DNA levels between between all time points (Figure 2). In contrast, significant changes in HIV RNA levels and RNA/DNA ratios from Time point 1 to Time point 3 were observed in paired analyses (Figure 2), and HIV RNA levels increased significantly in the linear effects model from Time point 1 to Time point 3 (Table 2). There were no significant correlations between HIV-1 RNA or HIV-1 DNA and the frequency of CD30-expressing cells (all R^2 values <0.01).

DISCUSSION

Overall, we observed that, unlike traditional markers of lymphocyte activation and exhaustion, CD30 expression on $CD4^+$ T cells significantly increased before HIV-1 rebound in participants in ACTG clinical trials involving ATI. Although correlations between pre-ATI cell-associated nucleic acid measures HIV and various immunological parameters have been identified with the time to viral rebound [11–13], to our knowledge, we report the first specific, nonviral $CD4^+$ T-cell marker that anticipates impending HIV-1 rebound during ATI. Although T-cell exhaustion markers, such as PD-1, Lag-3, and Tim-3 measured before ART initiation have been shown to predict time of HIV rebound during ATI [13], we did not identify increases in generalized markers of lymphocyte activation or exhaustion before detectable HIV-1 during ATI compared with baseline levels on ART. Frequencies in activated or PD-1-expressing lymphocytes increased only after viral recrudescence. In addition, changes in CD30 expression in this study appear to be limited to $CD4^+$ lymphocytes because the expression of CD30 on $CD8^+$ T cells or mononuclear cells expressing CD14 and/or CD16 did not differ significantly between any time points.

CD30 signaling occurs predominately through TNFR-associated factors (eg, TRAF2 and TRAF5) and mediates DNA transcription via NF-κB, MAPK8/JNK, and through antiapoptotic signals from various TNFRs [30–32]. However, CD30 is largely restricted in healthy resting cells [15–17, 33, 34]. Although its functions are still largely undefined, CD30 has been implicated in the activation, proliferation, and death of selected cell populations [14–16, 32], and stimulation of this receptor has been shown to activate signaling pathways that regulate immune responses to infection [16, 32, 35]. Furthermore, viral infections have been shown to markedly increase CD30 expression on T and B lymphocytes [15–17, 19, 34]. For example, Epstein-Barr virus (EBV), human T-lymphotropic virus (HTLV)1, and HTLV2 infections have been shown to dramatically increase CD30 expression, and parapoxvirus infections can lead to CD30-positive coetaneous infiltrates in humans [16, 36]. CD30 can be cleaved by metalloproteases relatively rapidly after surface expression, and higher levels of the unbound, soluble 85-kDa proteolytic fragment (sCD30) is readily detected in patients with EBV infection, hepatitis B and C, and in primary/acute HIV-1 infection despite being very low or undetectable in healthy individuals [14, 37–41]. In contrast, decreased CD30 has been associated with initiation of ART [42]. However, CD30 is rarely expressed on nonmalignant, uninfected cells [19, 32], even in the setting of various generalized immune-stimulating or -activating processes.

The above studies suggest that viral replication or de novo infection are closely linked with CD30 expression on lymphocytes. Furthermore, we recently reported that CD30-expressing $CD4^+$ T cells are enriched in HIV-1 RNA (ie, viral transcriptionally active) and that in vivo anti-CD30 cancer therapy may transiently decrease cell-associated HIV-1 RNA and DNA levels [22, 23]. Altogether, these data suggest that $CD4^+$ T cells upregulate CD30 in the setting of active HIV replication or increased transcriptional activity. It is possible that the increased CD30 expression on $CD4^+$ T cells during ATI in this study is either a direct effect of HIV within $CD4^+$ cells or a close bystander effect of ongoing viral transcriptional activity. The frequency of $CD4^+$ T cells expressing CD30 is somewhat higher than would be expected from viral nucleic acid quantification alone, and there was no significant correlation observed between the percentage of blood-derived $CD4^+$ T cells expressing CD30 and cell-associated HIV-1 RNA levels. As a result, there may be a combination of direct and indirect HIV effects on CD30 expression. It is also possible that $CD4^+$ T cells that were observed before viral rebound may originate from transient relocalization of a particular subset from lymphoid tissues where viral recrudescence is likely to first occur. Given the limited number of cells that could be obtained from this study, we were unable to investigate more specific markers of tissue residence or cellular homing, but further study is certainly warranted. The lack of correlation between cell-associated RNA and the frequency between $CD30^+CD4^+$ T cells across all time points may also be due to previously observed dichotmy between the number of individual HIV transcrtiptionally active cells and total RNA from bulk cellular extracts [43, 44]. This disconnect may be a result of a small number of cells that express high levels of HIV RNA. Direct tissue studies will be a critical component of future ATI studies examining HIV reservoir and persistence to determine the potential for productive infection and to correlate expression of markers such as CD30 and direct measures of HIV recrudescence.

CD30 or other markers may play an important role in the design of future ATI studies. Many concerns have been raised about the safety of interrupting ART and the risks of viral rebound, which at times may be associated with acute retroviral syndromes [10, 45]. Measuring markers that are specific for HIV activity that are evident before generalized immune activation

may help inform on timing of ART re-initiation, trigger timely tissue sampling or noninvasive HIV-specific whole-body imaging techniques during ATI to determine specific anatomical foci of viral recrudescence, and provide valuable information as to the impact of various curative interventions on the overall state of the viral reservoir. One caveat is that CD30 expression is very low overall, even it is significantly increased in paired analysis and mixed linear-effects modeling between baseline and ATI time points. The low expression introduces challenges if CD30 expression on circulating cells is used for prospective clinical decision making in ATI studies.

CONCLUSIONS

This study has several other limitations. For example, sample size was relatively small, because longitudinal samples during ATI studies are difficult to obtain. Furthermore, the sampling frequency of viral load monitoring (every 2–4 weeks) is suboptimal for time-to-rebound analyses, and we were limited by availability of remaining samples from individuals. The lack of earlier timepoints during the early ATI period precludes formal sensitivity/specificity analyses for predicting rebound, and it should be incorporated into future studies involving ART cessation. Despite these limitations, however, we had access to samples from 5 longitudinal clinical trials within the ACTG, and we observed significant increases in $CD30^{+}CD4^{+}$ T-cell expression during ATI. Furthermore, the finding that low-level viremia may be detected up to 1 month before clinically evident HIV rebound is also important and dictates the need for direct tissue sampling during ATI studies.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank the participants, staff, and principal investigators of the AIDS Clinical Trial Group (ACTG) studies A371 (Paul Volberding and Elizabeth Connick), A5024 (J. Michael Kilby and Ronald Mitsuyasu), A5068 (Jeffrey Jacobson, Ian Frank, Michael Saag, and Joseph Eron), A5187 (Daniel Barouch, Eric Rosenberg, and Daniel Kuritzkes), and A5197 (Robert Schooley, Michael Lederman, and Diane Havlir).

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Financial support. This research was funded by the National Institute of Allergy (Grants AI068634, AI068636, AI10670, AI141003, and MH116716) and the amfAR Institute for HIV Cure Research (amfAR 109301) and in part by a Center for AIDS Research grant (P30 AI027763).

Potential conflicts of interest. T. J. H. has consulted for Merck and receives grant support from Gilead Biosciences. S. G. D. receives grant support from Gilead, Merck, and ViiV. He has consulted from AbbVie and is a member of the scientific advisory boards for BryoLogyx and Enochian Biosciences. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Duong YT, Qiu M, De AK, et al. Detection of recent HIV-1 infection using a new limiting-antigen avidity assay: potential for HIV-1 incidence estimates and avidity maturation studies. PLoS One **2012**; 7:e33328.
2. Keating SM, Hanson D, Lebedeva M, et al. Lower-sensitivity and avidity modifications of the vitros anti-HIV 1 + 2 assay for detection of recent HIV infections and incidence estimation. J Clin Microbiol **2012**; 50:3968–76.
3. Kassanjee R, Pilcher CD, Keating SM, et al.; Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA). Independent assessment of candidate HIV incidence assays on specimens in the CEPHIA repository. AIDS **2014**; 28:2439–49.
4. Chun TW, Murray D, Justement JS, et al. Broadly neutralizing antibodies suppress HIV in the persistent viral reservoir. Proc Natl Acad Sci U S A **2014**; 111:13151–6.
5. Bar KJ, Sneller MC, Harrison LJ, et al. Effect of HIV antibody VRC01 on viral rebound after treatment interruption. N Engl J Med **2016**; 375:2037–50.
6. Gaudinski MR, Coates EE, Houser KV, et al.; VRC 606 Study Team. Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: a Phase 1 open-label clinical trial in healthy adults. PLoS Med **2018**; 15:e1002493.
7. Henrich TJ, Hanhauser E, Marty FM, et al. Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases. Ann Intern Med **2014**; 161:319–27.
8. Henrich TJ, Hatano H, Bacon O, et al. HIV-1 persistence following extremely early initiation of antiretroviral therapy (ART) during acute HIV-1 infection: An observational study. PLoS Med **2017**; 14:e1002417.
9. Sáez-Cirión A, Bacchus C, Hocqueloux L, et al.; ANRS VISCONTI Study Group. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. PLoS Pathog **2013**; 9:e1003211.
10. Li JZ, Smith DM, Mellors JW. The need for treatment interruption studies and biomarker identification in the search for an HIV cure. AIDS **2015**; 29:1429–32.

11. Li JZ, Etemad B, Ahmed H, et al. The size of the expressed HIV reservoir predicts timing of viral rebound after treatment interruption. AIDS **2016**; 30:343–53.
12. Williams JP, Hurst J, Stöhr W, et al.; SPARTACTrial Investigators. HIV-1 DNA predicts disease progression and post-treatment virological control. Elife **2014**; 3:e03821.
13. Hurst J, Hoffmann M, Pace M, et al. Immunological biomarkers predict HIV-1 viral rebound after treatment interruption. Nat Commun **2015**; 6:8495.
14. Biswas P, Cozzi-Lepri A, Delfanti F, et al. Significant link between sCD30 changes and HIV viremia in patients treated with HAART. J Med Virol **2006**; 78:1513–9.
15. Biswas P, Mantelli B, Delfanti F, Ferrarini M, Poli G, Lazzarin A. CD30 ligation differentially affects CXCR4-dependent HIV-1 replication and soluble CD30 secretion in non-Hodgkin cell lines and in gamma delta T lymphocytes. Eur J Immunol **2003**; 33:3136–45.
16. Falini B, Pileri S, Pizzolo G, et al. CD30 (Ki-1) molecule: a new cytokine receptor of the tumor necrosis factor receptor superfamily as a tool for diagnosis and immunotherapy. Blood **1995**; 85:1–14.
17. Agrawal B, Reddish M, Longenecker BM. CD30 expression on human CD8+ T cells isolated from peripheral blood lymphocytes of normal donors. J Immunol **1996**; 157:3229–34.
18. Chiarle R, Podda A, Prolla G, Podack ER, Thorbecke GJ, Inghirami G. CD30 overexpression enhances negative selection in the thymus and mediates programmed cell death via a Bcl-2-sensitive pathway. J Immunol **1999**; 163:194–205.
19. Dürkop H, Foss HD, Eitelbach F, et al. Expression of the CD30 antigen in non-lymphoid tissues and cells. J Pathol **2000**; 190:613–8.
20. Higuchi M, Matsuda T, Mori N, et al. Elevated expression of CD30 in adult T-cell leukemia cell lines: possible role in constitutive NF-kappaB activation. Retrovirology **2005**; 2:29.
21. Makino M, Yashiki S, Fujiyoshi T, Baba M, Sonoda S. An expression of anaplastic large cell lymphoma-associated antigens on HTLV-I-infected CD4+ T cells. Ann Hematol **1998**; 76:31–5.
22. Hogan LE, Vasquez J, Hobbs KS, et al. Increased HIV-1 transcriptional activity and infectious burden in peripheral blood and gut-associated CD4+ T cells expressing CD30. PLoS Pathog **2018**; 14:e1006856.
23. Wang CC, Thanh C, Gibson EA, et al. Transient loss of detectable HIV-1 RNA following brentuximab vedotin anti-CD30 therapy for Hodgkin lymphoma. Blood Adv **2018**; 2:3479–82.
24. Rosenberg ES, Graham BS, Chan ES, et al.; AIDS Clinical Trials Group A5187 Team. Safety and immunogenicity of therapeutic DNA vaccination in individuals treated with antiretroviral therapy during acute/early HIV-1 infection. PLoS One **2010**; 5:e10555.
25. Volberding P, Demeter L, Bosch RJ, et al.; ACTG 371 Team. Antiretroviral therapy in acute and recent HIV infection: a prospective multicenter stratified trial of intentionally interrupted treatment. AIDS **2009**; 23:1987–95.
26. Kilby JM, Bucy RP, Mildvan D, et al.; Adult AIDS Clinical Trials Group A5024 Protocol Team. A randomized, partially blinded phase 2 trial of antiretroviral therapy, HIV-specific immunizations, and interleukin-2 cycles to promote efficient control of viral replication (ACTG A5024). J Infect Dis **2006**; 194:1672–6.
27. Jacobson JM, Pat Bucy R, Spritzler J, et al.; National Institute of Allergy and Infectious Diseases-AIDS Clinical Trials Group 5068 Protocol Team. Evidence that intermittent structured treatment interruption, but not immunization with ALVAC-HIV vCP1452, promotes host control of HIV replication: the results of AIDS Clinical Trials Group 5068. J Infect Dis **2006**; 194:623–32.
28. Schooley RT, Spritzler J, Wang H, et al.; AIDS Clinical Trials Group 5197 Study Team. AIDS clinical trials group 5197: a placebo-controlled trial of immunization of HIV-1-infected persons with a replication-deficient adenovirus type 5 vaccine expressing the HIV-1 core protein. J Infect Dis **2010**; 202:705–16.
29. Skiest DJ, Su Z, Havlir DV, et al.; AIDS Clinical Trials Group 5170 Study Team. Interruption of antiretroviral treatment in HIV-infected patients with preserved immune function is associated with a low rate of clinical progression: a prospective study by AIDS Clinical Trials Group 5170. J Infect Dis **2007**; 195:1426–36.
30. Tsitsikov EN, Wright DA, Geha RS. CD30 induction of human immunodeficiency virus gene transcription is mediated by TRAF2. Proc Natl Acad Sci U S A **1997**; 94:1390–5.
31. Kennedy MK, Willis CR, Armitage RJ. Deciphering CD30 ligand biology and its role in humoral immunity. Immunology **2006**; 118:143–52.
32. Gilfillan MC, Noel PJ, Podack ER, Reiner SL, Thompson CB. Expression of the costimulatory receptor CD30 is regulated by both CD28 and cytokines. J Immunol **1998**; 160:2180–7.
33. Chiarle R, Podda A, Prolla G, Gong J, Thorbecke GJ, Inghirami G. CD30 in normal and neoplastic cells. Clin Immunol **1999**; 90:157–64.
34. Biswas P, Smith CA, Goletti D, Hardy EC, Jackson RW, Fauci AS. Cross-linking of CD30 induces HIV expression in chronically infected T cells. Immunity **1995**; 2:587–96.
35. Senter PD, Sievers EL. The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. Nat Biotechnol **2012**; 30:631–7.

36. Rose C, Starostik P, Bröcker EB. Infection with parapoxvirus induces CD30-positive cutaneous infiltrates in humans. J Cutan Pathol **1999**; 26:520–2.
37. Fattovich G, Vinante F, Giustina G, et al. Serum levels of soluble CD30 in chronic hepatitis B virus infection. Clin Exp Immunol **1996**; 103:105–10.
38. Monsalve-De Castillo F, Romero TA, Estevez J, et al. Concentrations of cytokines, soluble interleukin-2 receptor, and soluble CD30 in sera of patients with hepatitis B virus infection during acute and convalescent phases. Clin Diagn Lab Immunol **2002**; 9:1372–5.
39. Pizzolo G, Vinante F, Morosato L, et al. High serum level of the soluble form of CD30 molecule in the early phase of HIV-1 infection as an independent predictor of progression to AIDS. AIDS **1994**; 8:741–5.
40. Pizzolo G, Vinante F, Nadali G, et al. High serum level of soluble CD30 in acute primary HIV-1 infection. Clin Exp Immunol **1997**; 108:251–3.
41. Oflazoglu E, Grewal IS, Gerber H. Targeting CD30/CD30L in oncology and autoimmune and inflammatory diseases. Adv Exp Med Biol **2009**; 647:174–85.
42. Sadeghi M, Süsal C, Daniel V, et al. Short communication: decreasing soluble CD30 and increasing IFN-gamma plasma levels are indicators of effective highly active antiretroviral therapy. AIDS Res Hum Retroviruses **2007**; 23:886–90.
43. Yucha RW, Hobbs KS, Hanhauser E, et al. High-throughput characterization of HIV-1 reservoir reactivation using a single-cell-in-droplet PCR assay. EBioMedicine **2017**; 20:217–29.
44. Wiegand A, Spindler J, Hong FF, et al. Single-cell analysis of HIV-1 transcriptional activity reveals expression of proviruses in expanded clones during ART. Proc Natl Acad Sci U S A **2017**; 114:E3659–68.
45. Julg B, Dee L, Ananworanich J, et al. Recommendations for analytical antiretroviral treatment interruptions in HIV research trials-report of a consensus meeting. Lancet HIV **2019**; 6:e259–68.