Intensification of Antiretroviral Therapy With Raltegravir or Addition of Hyperimmune Bovine Colostrum in HIV-Infected Patients With Suboptimal CD4⁺ T-Cell Response: A Randomized Controlled Trial

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Background. Despite virally suppressive combination antiretroviral therapy (cART), some HIV-infected patients exhibit suboptimal CD4⁺ T-cell recovery. This study aimed to determine the effect of intensification of cART with raltegravir or addition of hyperimmune bovine colostrum (HIBC) on CD4⁺ T-cell count in such patients.

Methods. We randomized 75 patients to 4 treatment groups to receive raltegravir, HIBC, placebo, or both raltegravir and HIBC in a factorial, double-blind study. The primary endpoint was time-weighted mean change in CD4⁺ T-cell count from baseline to week 24. T-cell activation (CD38⁺ and HLA-DR⁺), plasma markers of microbial translocation (lipopolysaccharide, 16S rDNA), monocyte activation (soluble (s) CD14), and HIV-RNA (lowest level of detection 4 copies/mL) were monitored. Analysis was performed using linear regression methods.

Results. Compared with placebo, the addition of neither raltegravir nor HIBC to cART for 24 weeks resulted in a significant change in CD4⁺ T-cell count (mean difference, 95% confidence interval [CI]: 3.09 cells/μL, 2.14; 14.27; 20.45, P = .724 and 9.43 cells/μL, 2.78; 26.68, P = .279, respectively, intention to treat). There was no significant interaction between HIBC and raltegravir (P = .275). No correlation was found between CD4⁺ T-cell count and plasma lipopolysaccharide, 16S rDNA, sCD14, or HIV-RNA.

Conclusion. The determinants of poor CD4⁺ T-cell recovery following cART require further investigation.

Clinical Trials Registration. ClinicalTrials.gov identifier: NCT00772590, Australia New Zealand Clinical Trials Registry: ACTRN12609000575235.

Combination antiretroviral therapy (cART) in human immunodeficiency virus (HIV)-infected individuals is associated with control of viral replication and immune recovery. In the majority of patients, this response is associated with increases in CD4⁺ T cells in peripheral blood [1]. However, the extent of CD4⁺ T-cell recovery following cART is highly variable [2, 3]. Despite achieving reliable and sustained suppression of viral replication, up to 30% of patients exhibit suboptimal increase in CD4⁺ T-cell count [2, 4, 5]. Disconcertingly, a greater risk of non-AIDS-related complications is consistently observed in patients with lower CD4⁺ T-cell counts on suppressive therapy [6, 7].
The factors that have been consistently associated with limited CD4+ T-cell recovery in patients on cART include advanced age [2, 8, 9], viral coinfection [10, 11], and a lower nadir CD4+ T-cell count [9, 12]. Persistent immune activation is thought to play an important role in poor immunological response to cART [13–15]. Two potential mechanisms have been proposed as play an important role in poor immunological response to cART. First, HIV-associated damage to intestinal mucosal integrity may result in increased microbial translocation into the circulation from the gastrointestinal lumen, though this observation is not consistently demonstrated [16–19]. Second, persistent low-level HIV replication, even in the setting of suppressive cART, may result in persistent immune activation [20, 21]. The observation of higher levels of proviral DNA in memory and naive CD4+ T cells of patients with poor CD4+ T-cell recovery on suppressive cART supports the hypothesis that HIV antigen–driven CD4+ T-cell activation may play a key role in continuous loss of CD4+ T-cell loss in these individuals [22].

Currently, no consensus has been reached on the most efficacious management for suboptimal immunological response to cART. Raltegravir is a potent HIV-1 integrase inhibitor, and it blocks viral replication through a mechanism different from more commonly used antiretroviral agents like protease inhibitors, nonnucleoside reverse transcriptase inhibitors, and reverse transcriptase inhibitors [23, 24]. Therefore, it provides an opportunity to investigate the hypothesis that intensification of cART might decrease ongoing virus replication [25], and, as a result, improve CD4+ T-cell response. Hyperimmune bovine colostrum (HIBC) containing antilipopolysaccharide immunoglobulin offers protection against infection with a wide number of enteric pathogens, mainly through passive immunization in the mucosa of the gut and suppression of gut-associated inflammation with promotion of mucosal repair and regeneration [26, 27]. The activity of HIBC is thought to occur through luminal binding and neutralizing of toxin [28]. Bovine colostrum was shown to reduce acute nonsteroidal anti-inflammatory drug-induced increase in intestinal permeability [29] and to lower lipopolysaccharide (LPS) levels in surgical patients [30, 31], suggesting it may have an effect on microbial translocation. Therefore, HIBC may reduce translocation of bacterial products, diminishing immune activation and resulting in improved CD4+ T-cell recovery. The CORAL study aimed to determine the effect of cART intensification with raltegravir or addition of HIBC on CD4+ T-cell count in HIV-infected patients with suboptimal CD4+ T-cell response despite prolonged viral-suppressive cART.

METHODS

Study Participants

Patients were recruited from 20 Australian sites between March and September 2009. Eligibility criteria at randomization were

- HIV-1 infection, age ≥18 years, cART exposure ≥12 months (without change for a minimum of 6 months), 2 documented consecutive plasma HIV-RNA measurements <50 copies/mL in the preceding 9 months, CD4+ T-cell count <350 cells/μL for the preceding 6 months, and a CD4+ T-cell count rise of <50 cells/μL in the preceding 12 months. Exclusion criteria at randomization included use of immune-modulating medications within preceding 60 days, current cART containing an integrase inhibitor, allergy to cow’s milk, pregnancy or breastfeeding, or a known cause of impaired CD4+ T-cell gain, for example, splenomegaly or a current cART regimen containing both tenofovir and didanosine.

The study received ethics approval from the Human Research Ethics Committee at the University of New South Wales and at each participating site. All participants provided written informed consent.

Study Design and Interventions

This was a randomized factorial design, double-blind, placebo-controlled, multicenter study. Eligible patients were randomized to 1 of 4 treatment groups: group I, HIBC + raltegravir; group II, HIBC + raltegravir placebo; group III, HIBC placebo + raltegravir; and group IV, HIBC placebo + raltegravir placebo. Randomization was stratified by site and screening CD4+ T-cell count (≤200, >200 cells/μL).

The active substance in HIBC is freeze-dried bovine colostrum powder. It contains approximately 40% bovine immunoglobulins (predominantly immunoglobulin [Ig] G and IgA with small amounts of IgM and IgE) that have high binding activity against the LPS and cell wall antigens of Gram-negative bacteria [28, 32]. HIBC contains bovine transforming growth factor β and insulin-like growth factor. These cytokines are bioactive in humans and are expected to assist in quelling immune activation [27] and reducing intestinal permeability to microbial products [29]. HIBC tablets contain an excipient that includes milk-derived casein and calcium carbonate to buffer stomach acid. A small amount of bovine IgG makes its way into the bloodstream with an estimated half-life of 15 hours observed in mice [33]. Immunoglobulins are excreted intact or in the form of immunoglobulin fragments in stool [34]. A dose of three 600-mg tablets was taken twice daily. The dosage of raltegravir was 400 mg (1 tablet) twice daily.

Clinical Assessments and Laboratory Testing

Assessments performed during the screening visit and weeks 0 (randomization visit), 4, 8, 12, 24, 36, and 48 included measurement of CD4+ T-cell count, T-cell immune activation markers (CD4+ and CD8+ T-cell expression of CD38+, and HLA-DR+), plasma microbial translocation markers (LPS and 16S rDNA), soluble CD14 (sCD14; marker of monocyte activation), plasma HIV-RNA, routine safety biochemistry and hematology, assessment for adherence (estimated patient self-report), and adverse events. All adverse events were graded for...
CD4⁺ and CD8⁺ T cells coexpressing CD38⁺ and HLA-DR⁺ were enumerated in whole blood within 72 hours of collection by flow cytometry using a standardized protocol on either a FACS Canto II or FACS aria cell sorter. Each subject had flow measurements starting from week 0 performed in 1 lab. All assays were undertaken in 1 of 3 core laboratories, which took part in an ongoing quality assurance exercise to standardize analysis and reporting methods. To improve the consistency of gating of activation markers, a bulk data analysis was performed by 1 experienced flow cytometrist blinded to treatment allocation.

Microbial translocation markers were measured from plasma samples that had been stored at −70°C following blood collection. Plasma LPS was measured using a commercial chromogenic limulus amebocyte lysate assay (Lonza). The sCD14 in plasma was measured using the Quantikine Human sCD14 kit (R&D Systems). Details of both methods are described elsewhere [19]. Plasma 16S rDNA levels were assessed by real-time quantitative polymerase chain reaction on DNA extracted from plasma (DNeasy Kit, Qiagen).

Plasma HIV-1 RNA levels were measured using the Amplicor HIV-1 Monitor assay (version 1.5; Roche) or Abbott RealTime ultrasensitive assays with lower limits of detection at 50 and 40 copies/mL, respectively. In addition, levels of HIV-RNA were measured at 1 central laboratory using Amplicor HIV-1 Monitor assay test (MWP, version 1.5), with the Ultra Boosted specimen preparation protocol with a lower limit of detection of 4 copies/mL.

**Statistical Considerations**

A comparison of HIBC or raltegravir versus placebo with an expected mean CD4⁺ T-cell change of 75 cells/µL (SD: 100 cells/µL) for participants receiving HIBC or raltegravir and 0 (zero) cells/µL (SD: 80 cells/µL) in the placebo arm would require 32 participants on HIBC and 32 participants on raltegravir to give a 90% power. An additional 10% (±) of patients would provide for modest losses to follow-up over 24 weeks. This resulted in a recruitment objective of 72 participants (18 participants in each of 4 study arms). These sample sizes assumed no interaction between HIBC and raltegravir.

The primary analysis was conducted when all participants had completed at least 24 weeks or had permanently discontinued from the study. The primary efficacy endpoint was the time-weighted area under the curve for mean change in CD4⁺ T-cell count from baseline to week 24 for main effects: HIBC (treatment groups I + II) versus placebo (treatment groups III + IV), and raltegravir (treatment groups I + III) versus placebo (treatment groups II + IV)—intention-to-treat (ITT) population. The ITT population included all randomized participants who received at least 1 dose of study medication and had 1 follow-up visit. For the mean change analyses, ITT analysis was conducted by carrying forward the last observed value if the week 24 result was missing. For binary outcomes with missing data, participants were imputed to be failures in the ITT analysis. For secondary outcomes, data were analyzed on both the ITT and per protocol (PP) populations. The PP population was all participants included in the ITT population censored when or if randomized therapy was stopped.

Comparison of mean change from baseline to study week of interest of outcomes for main effects was assessed by ANOVA; differences in proportions were calculated using exact methods. Linear regression was used to test for interaction between raltegravir and HIBC on continuous outcomes and exact linear regression was used for binary outcomes. Comparison of proportions was assessed by χ² tests. We examined the relationship between CD4⁺ T cell count, microbial translocation, immune activation, and HIV plasma viremia at baseline and at week 24 using Spearman’s rho.

In all analyses, 2-sided α was considered statistically significant at .05. SAS software version 9.2 was used for analysis of all continuous outcomes; Stata software version 10.1 was used for analysis of all binary outcomes.

**RESULTS**

**Study Participants and Baseline Characteristics**

Of 100 patients screened, 75 were randomized (Figure 1). Two participants withdrew consent, (1 before commencing study drugs and the other shortly after commencing drugs) and their data were not included in the primary or secondary analyses. The ITT population comprised 73 participants (3 of these completed 24 weeks on study but were off study medications).

Primary analysis results showed no benefit of study drugs to the patients; therefore, all participants were advised to discontinue study drugs immediately. At this point, 70 participants (64 on randomized therapy) had completed week 36, and 43 participants (38 on randomized therapy) had completed week 48.

Baseline demographic and clinical characteristics were similar across randomized arms (Table 1). The mean (SD) estimated duration of infection was 11.6 (7.5) years, and mean age was 53.1 (10.2) years. At baseline, 41 (57%) of participants had a detectable HIV-RNA level by the ultrasensitive assay used in this study (lowest level of detection: 4 copies/mL).

**Effect of Addition of HIBC or Raltegravir to cART on CD4⁺ T-Cell Count**

The addition of HIBC or raltegravir resulted in no significant difference in time-weighted area under the curve for change from baseline through week 24 in CD4⁺ T-cell count compared
with placebo (mean difference, 95% confidence interval [CI]: 9.43 cells/\( \mu \)L, -7.81 to 26.68, \( P = .279 \); and 3.09 cells/\( \mu \)L, -14.27 to 20.45, \( P = .724 \), respectively)—ITT (Table 2). There was no significant interaction between HIBC and raltegravir (\( P = .275 \)). A similar result was obtained in a PP population analysis in which participants were censored when randomized therapy was stopped (data not shown).

There were no significant differences in absolute changes in CD4\(^+\) T-cell count or CD4\(^+\) T-cell percentage at any time point between HIBC or raltegravir and placebo; neither were there any significant differences in proportions of patients with CD4\(^+\) T cells >350 cells/\( \mu \)L at weeks 4, 8, and 24 (data not shown).

For the 38 participants that completed week 48 on randomized therapy, there were no significant differences observed in proportion of participants with CD4\(^+\) T cells >350 cells/\( \mu \)L, time weighted area under the curve for change from baseline in CD4\(^+\) T-cell count, plasma microbial translocation markers, T-cell immune activation markers, or plasma HIV-RNA between participants who received HIBC or raltegravir and those who received placebo.

Effect of HIBC or Raltegravir on Microbial Translocation, Immune Activation, and Plasma HIV-RNA

Compared with placebo, we observed no significant differences in changes from baseline to week 24 in plasma levels of LPS, 16S rDNA and sCD14, proportion of CD4\(^+\) and CD8\(^+\) T cells expressing CD38\(^+\) and HLA-DR\(^+\), and plasma HIV-RNA in participants who received either HIBC or raltegravir (Table 3).

Associations Between CD4\(^+\) T-Cell Count, Microbial Translocation Markers, T-Cell Activation, and Plasma HIV-RNA

LPS and 16S rDNA were significantly correlated (\( r = 0.37, P < .001 \); and \( r = 0.36, P < .001 \), respectively) at baseline and week 24, respectively. There was a significant negative correlation between LPS and sCD14, both at baseline and week 24 and between the proportion of CD8\(^+\) T cells expressing CD38\(^+\) and HLA-DR\(^+\) and CD4\(^+\) T-cell counts (Table 4). There was no correlation between CD4\(^+\) T-cell count and microbial translocation markers or plasma HIV-RNA; neither was there any association between microbial translocation markers, T-cell activation, or plasma HIV-RNA levels.

Safety and Tolerability of HIBC and Raltegravir

Two participants stopped study medications due to grade 2 adverse symptoms thought to be related to study drugs. The following 8 serious adverse events were reported: myocardial infarction; unstable angina pectoralis; and hospitalization for cellulitis (event occurred twice in the same participant), possible influenza A, a methamphetamine-induced psychotic episode, and investigation of chest pain (event occurred in two different

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**Figure 1.** CORAL study participant disposition. Abbreviations: HIBC, hyperimmune bovine colostrum; RAL, raltegravir.
participants). All serious adverse events resolved, none were related to the study drugs, and all participants continued study medications. Adherence to study drugs was approximately 100% for all 4 treatment arms.

**DISCUSSION**

Addition of HIBC or raltegravir intensification of a suppressive cART regimen did not result in an increase in CD4$^+$ T-cell count or demonstrable changes in plasma microbial translocation markers, plasma HIV-RNA levels, or T-cell activation. Neither plasma HIV-RNA nor markers of microbial translocation were associated with CD4$^+$ T-cell count. Markers of T-cell activation were inversely correlated with CD4$^+$ T-cell recovery during cART. However, no correlation was found between immune activation and microbial translocation or plasma HIV viremia.

An earlier study investigating the use of bovine colostrum in patients with HIV-associated diarrhea showed a CD4$^+$ T-cell count.

### Table 1. Patient Characteristics at Baseline

<table>
<thead>
<tr>
<th></th>
<th>HIBC + RAL (n = 19)</th>
<th>HIBC + Placebo (n = 19)</th>
<th>Placebo + RAL (n = 18)</th>
<th>Placebo + Placebo (n = 17)</th>
<th>All (N = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male), no.</td>
<td>17</td>
<td>18</td>
<td>18</td>
<td>16</td>
<td>69</td>
</tr>
<tr>
<td>Age (y), mean (SD)</td>
<td>52 (11)</td>
<td>56 (9)</td>
<td>50 (10)</td>
<td>55 (10)</td>
<td>53 (10)</td>
</tr>
<tr>
<td>Duration of infection (y), mean (SD)</td>
<td>11.5 (6.9)</td>
<td>12.0 (8.2)</td>
<td>11.4 (7.2)</td>
<td>11.6 (8.1)</td>
<td>11.6 (7.5)</td>
</tr>
<tr>
<td>CDC category C, no. (%)</td>
<td>8 (42)</td>
<td>8 (42)</td>
<td>7 (39)</td>
<td>7 (41)</td>
<td>30 (41)</td>
</tr>
<tr>
<td>Duration from start of ART (y), median (IQR)</td>
<td>6 (3–7)</td>
<td>4 (2–8)</td>
<td>5 (3–7)</td>
<td>4 (2–6)</td>
<td>5 (2–7)</td>
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<tr>
<td>Current ART, no. (%)</td>
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- PI                  | 4 (21)             | 7 (37)                  | 6 (33)                 | 6 (35)                    | 23 (32)     |
- NRTI                | 19 (100)           | 19 (100)                | 18 (100)               | 17 (100)                  | 73 (100)    |
- NNRTI               | 15 (79)            | 13 (68)                 | 12 (67)                | 12 (71)                   | 52 (71)     |

**Ultraspesitive plasma viral load**

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<tr>
<td>Nadir CD4$^+$ T-cell count (cells/μL)</td>
<td>83 (69)</td>
<td>100 (73)</td>
<td>68 (52)</td>
<td>62 (48)</td>
<td>79 (60)</td>
</tr>
<tr>
<td>CD4$^+$ T-cell count (cells/μL)</td>
<td>230 (68)</td>
<td>208 (73)</td>
<td>201 (63)</td>
<td>194 (56)</td>
<td>209 (66)</td>
</tr>
<tr>
<td>CD4$^+$ T-cell %</td>
<td>17.3 (6.6)</td>
<td>15.7 (4.9)</td>
<td>14.4 (4.6)</td>
<td>14.5 (4.9)</td>
<td>15.5 (6.0)</td>
</tr>
<tr>
<td>CD8$^+$ T-cell count (cells/μL)</td>
<td>811 (496)</td>
<td>773 (306)</td>
<td>887 (465)</td>
<td>807 (428)</td>
<td>819 (422)</td>
</tr>
<tr>
<td>CD8$^+$ T-cell %</td>
<td>50.1 (16.3)</td>
<td>55.7 (10.8)</td>
<td>57.3 (11.4)</td>
<td>54.0 (12.7)</td>
<td>54.3 (13.0)</td>
</tr>
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</table>

**Proportion with CD4$^+$ T-cell count ≥200, no (%)**

- 12 (63)             | 10 (53)             | 8 (44)                 | 9 (53)                 | 39 (53)                   |

**T-cell activation markers (%), mean (SD)**

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<tr>
<td>CD4 CD38$^+$ HLA-DR$^+$</td>
<td>0.7 (1.2)</td>
<td>0.8 (0.9)</td>
<td>1.5 (2.4)</td>
<td>0.4 (0.4)</td>
<td>0.9 (1.4)</td>
</tr>
<tr>
<td>CD8 CD38$^+$ HLA-DR$^+$</td>
<td>1.7 (0.9)</td>
<td>3.3 (2.9)</td>
<td>3.3 (2.8)</td>
<td>2.2 (1.6)</td>
<td>2.6 (2.3)</td>
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**Microbial translocation markers, mean (SD)**

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<tr>
<td>LPS (pg/mL)</td>
<td>79.6 (48.0)</td>
<td>74.6 (31.7)</td>
<td>75.0 (22.7)</td>
<td>85.0 (25.3)</td>
<td>78.4 (33.3)</td>
</tr>
<tr>
<td>16S rDNA (copies/mL)</td>
<td>6933 (8140)</td>
<td>4562 (2117)</td>
<td>7113 (6616)</td>
<td>6035 (3253)</td>
<td>6151 (5606)</td>
</tr>
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</table>

**Monocyte activation, mean (SD)**

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<tr>
<td>sCD14 (× 10$^6$ pg/mL)</td>
<td>2.1 (0.7)</td>
<td>2.2 (0.7)</td>
<td>2.0 (0.6)</td>
<td>1.9 (0.5)</td>
<td>2.1 (0.6)</td>
</tr>
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</table>

**Table 2. Time-Weighted Area Under the Curve for Change From Baseline Through Week 24 in CD4$^+$ T-Cell Count (cells/μL)—Intention to Treat**

<table>
<thead>
<tr>
<th>Main effects</th>
<th>No.</th>
<th>Mean</th>
<th>HIBC$^*$ 95% CI</th>
<th>N value</th>
<th>No.</th>
<th>Mean</th>
<th>Raltegravir$^*$ 95% CI</th>
<th>N value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>35</td>
<td>15.09</td>
<td>1.31, 28.87</td>
<td>...</td>
<td>36</td>
<td>11.74</td>
<td>−4.9, 23.98</td>
<td>...</td>
</tr>
<tr>
<td>Active</td>
<td>38</td>
<td>5.65</td>
<td>−5.43, 16.73</td>
<td>...</td>
<td>37</td>
<td>6.85</td>
<td>−4.07, 21.38</td>
<td>...</td>
</tr>
<tr>
<td>Difference</td>
<td>...</td>
<td>9.43</td>
<td>−7.81, 26.68</td>
<td>.279</td>
<td>...</td>
<td>3.09</td>
<td>−14.27, 20.45</td>
<td>.724</td>
</tr>
</tbody>
</table>

**Abbreviations:** CI, confidence interval; HIBC, hyperimmune bovine colostrum.

* P value for interaction between HIBC and raltegravir was .275.
A causal relationship between biomarkers of microbial translocation or immune activation and CD4+ T-cell count during cART is not established. Similar to previous reports, we found an association between higher CD8+ T-cell activation (measured by CD38+ and HLA-DR+ expression), and lower CD4+ T-cell counts [14, 37]. Increased susceptibility of activated T cells to apoptosis and turnover have been described as possible mechanisms [13, 14]. We also found a positive correlation between plasma levels of LPS and 16S rDNA as reported in earlier studies [17, 19]. However, unlike previous reports, we did not find an association between 16S rDNA and CD8+ T-cell activation or CD4+ T-cell count [17], nor an association between plasma HIV-RNA and CD8+ T-cell activation or CD4+ T-cell count [21].

Interestingly, we found plasma LPS levels were negatively correlated with sCD14 levels. LPS can be cleared by multiple mechanisms and has a complex relationship with sCD14. LPS activates the release of sCD14 [38] but then LPS is cleared via high-density lipoprotein [39], formation of lipopolysaccharide binding protein (LBP)–LPS complexes [39], scavenging by endotoxin core antibody (EndoCAb) [40], and by sCD14 itself [41]. LBP, EndoCAb, and high-density lipoprotein were not measured in this study. The interactions between each of these factors are important for the clearance of LPS, and may be different in the presence and absence of cART. Although plasma levels of LPS and sCD14 have been shown to decrease with initiation of cART [16, 42], there are no extensive data sets available describing the relationship between sCD14 and LPS in cART-treated individuals. In a recent report, a positive correlation was observed between LPS and sCD14 in the absence of opportunistic infection, and a significant negative correlation in the presence of opportunistic infections in ART-naïve patients. However, there was no correlation seen in cART-treated individuals [42].

In this study, we also examined the effect of raltegravin intensification on CD4+ T-cell count recovery. Our findings are consistent with those from a small single-arm study [43] that found no significant increase in CD4+ T-cell counts following 4 weeks of raltegravin intensification [43]. Similarly, in a retrospective study of 9 patients with poor CD4+ T-cell recovery despite suppressive cART, no significant change in CD4+ T-cell counts was observed after 25 weeks of intensification with maraviroc [44]. By contrast, in a recently published trial of 53 patients randomized to receive raltegravin or matched placebo, a trend toward increased CD4+ T-cell counts was observed during 12 weeks of raltegravin intensification. However, participants in this study had CD4+ T-cell counts >200/µL, and increased CD4+ T-cell counts were not associated with an effect of raltegravin on T-cell activation [45].

Our results are also consistent with those from other studies showing no effect of raltegravin intensification on low-level viremia and immune activation [43, 45–47]. Of particular note is
a study in which 30 patients receiving suppressive ART with CD4+ T-cell count <350 cells/µL for at least 1 year were randomized to receive raltegravir intensification or placebo [47]: raltegravir had no effect on plasma HIV-RNA level or immune activation as measured by the proportion of CD8+ T cells expressing CD38 and HLA-DR+. These findings together suggest that raltegravir intensification does not further suppress low-level HIV replication and that residual viremia in these fully suppressed patients does not arise from ongoing cycles of HIV-1 replication [47]. In this study, we were unable to draw any conclusions about the role of low-level HIV-RNA on CD4+ T-cell recovery.

In a recent study of raltegravir intensification, a significant transient increase in 2-long terminal repeat (2-LTR) circles was observed at weeks 2 and 4 [48]. Further, increased immune activation at baseline was associated with 2-LTR circle detection, and this normalized after raltegravir intensification. The authors interpreted these data to imply that active HIV replication persists in some HIV-infected patients despite suppressive cART and that viral replication drives immune activation. Similar to results from our study, there was no significant change in residual low-level viremia. We were unable to measure rapid changes in 2-LTR circles and therefore could not analyze our data according to similar parameters as they did with respect to residual immune activation.

Patients in our study were older than those of previous cohorts of ART-treated patients, possibly affecting CD4+ T-cell recovery [14, 15, 47]. An inverse correlation between age and the magnitude of CD4+ T-cell recovery is consistently reported [8, 9], possibly arising from decreased thymic function and other regenerative mechanisms with older age. Moreover, the number of circulating naive CD4+ T cells observed in HIV-infected patients is positively correlated to thymic mass and function after both short-term [49] and long-term ART [50].

Very low levels of CD4+ and CD8+ expression of CD38+ and HLA-DR+ were observed in our study compared with other cohorts of ART-treated patients with poor CD4+ T-cell gain [14, 15, 47]. We can only speculate that persistent T-cell activation is unlikely to be the major cause of the low CD4+ T-cell recovery in this group of patients.

There is no consensus on the definition of poor immunological response to cART with regard to the duration of evaluation of the CD4+ T-cell response, the threshold of CD4+ T-cell gain from pretherapy levels, or the extent of CD4+ T-cell recovery to a specific threshold. Therefore, findings from this study may not be generalizable. Another limitation of this study is that we did not investigate the effect of the addition of HIBC or raltegravir at the level of different anatomical sites, such as the gut and central nervous system.

In conclusion, in this patient group, the addition of HIBC or raltegravir intensification of suppressive cART regimens was not associated with an increase in CD4+ T-cell count or changes in immune activation, plasma HIV viremia, or microbial translocation. The absence of CD4+ T-cell increases does not exclude the possibility that either microbial translocation or low-level plasma viremia plays a role in poor CD4+ T-cell recovery, but makes it unlikely in this group with a relatively long duration since the start of ART. The determinants of CD4+ T-cell

Table 4. Correlation of CD4+ T-Cell Counts, Microbial Translocation Markers, Immune Activation, and HIV-RNA Levelsa

<table>
<thead>
<tr>
<th></th>
<th>CD4+ T cell</th>
<th>sCD14</th>
<th>LPS</th>
<th>CD4+ CD8+ HLA-DR+</th>
<th>CD8+ CD8+ HLA-DR+</th>
<th>16S rDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week 0</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ T-cell count (cells/µL)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>sCD14 (x 106 pg/mL)</td>
<td>−0.01, .918</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>LPS (pg/mL)</td>
<td>0.03, .828</td>
<td>−0.25, .038</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>CD4+ CD8+ HLA-DR+</td>
<td>0.04, .736</td>
<td>0.11, 0.348</td>
<td>0.08, .518</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>CD8+ CD8+ HLA-DR+</td>
<td>−0.25, .037</td>
<td>0.14, .268</td>
<td>0.01, .927</td>
<td>0.52, &lt;.001</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>16S rDNA (copies/mL)</td>
<td>−0.00, 1.000</td>
<td>−0.19, .123</td>
<td>0.37, &lt;.001</td>
<td>−0.08, .512</td>
<td>−0.03, .782</td>
<td>...</td>
</tr>
<tr>
<td>HIV-RNA (log10 copies/mL)</td>
<td>0.02, .866</td>
<td>−0.06, .607</td>
<td>0.09, .445</td>
<td>−0.20, .102</td>
<td>0.04, .726</td>
<td>0.03, .809</td>
</tr>
<tr>
<td><strong>Week 24</strong></td>
<td></td>
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</tr>
<tr>
<td>CD4+ T-cell count (cells/µL)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>sCD14 (x 106 pg/mL)</td>
<td>−0.02, .876</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>LPS (pg/mL)</td>
<td>0.03, .780</td>
<td>−0.25, .034</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>CD4+ CD8+ HLA-DR+</td>
<td>0.03, .798</td>
<td>0.12, .318</td>
<td>0.07, .588</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>CD8+ CD8+ HLA-DR+</td>
<td>−0.26, .032</td>
<td>0.14, .243</td>
<td>−0.00, .991</td>
<td>0.54, &lt;.001</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>16S rDNA (copies/mL)</td>
<td>−0.00, .983</td>
<td>−0.18, .133</td>
<td>0.36, &lt;.001</td>
<td>−0.06, .643</td>
<td>−0.01, .923</td>
<td>...</td>
</tr>
<tr>
<td>HIV-RNA (log10 copies/mL)</td>
<td>0.02, .846</td>
<td>−0.07, .561</td>
<td>0.10, .429</td>
<td>−0.21, .086</td>
<td>0.03, .826</td>
<td>0.02, .869</td>
</tr>
</tbody>
</table>

Abbreviations: HIV, human immunodeficiency virus; LPS, lipopolysaccharide; sCD14, soluble CD14.

* Ultrasensitive assay with lowest level of detection of 4 copies/mL used for measurement of HIV-RNA. All data are expressed as Spearman’s correlation coefficient, P value.
recovery on cART require more investigation. Further research is required to delineate causal relationships between biomarkers of microbial translocation and CD4+ T-cell count responses to cART.

**Notes**

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**References**


