Decreases in Inflammatory and Coagulation Biomarkers Levels in HIV-Infected Patients Switching from Enfuvirtide to Raltegravir: ANRS 138 Substudy

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Stored plasma specimens from 164 participants in the ANRS 138 trial were analyzed to determine interleukin 6 (IL-6), high-sensitivity C-reactive protein (hsCRP), and D-dimer levels at baseline and weeks 24 and 48. These virologically suppressed, treatment-experienced patients were randomly assigned to undergo an immediate switch (IS) or a deferred switch (DS; at week 24) from an enfuvirtide-based antiretroviral therapy (ART) regimen to a raltegravir-based regimen. At week 24, a significant decrease from baseline was observed in the IS arm, compared with the DS arm, for IL-6 level (−30% vs +10%; *P* < .002), hsCRP level (−46% vs +15%; *P* < .0001), and D-dimer level (−40% vs +6%; *P* < .0001). At week 48, there was a reproducible decrease in levels of all biomarkers in the DS arm.

**Keywords.** HIV; enfuvirtide; raltegravir; IL-6; CRP; D-dimer; switch; biomarkers.

Despite major benefits associated with the use of antiretroviral therapy (ART), human immunodeficiency virus (HIV)-infected patients still experience high rates of non–AIDS-defining events, particularly cardiovascular diseases and cancers that have been associated with high levels of inflammatory and coagulation biomarkers [1–3]. Levels of these biomarkers remain elevated during ART, and new treatment strategies to reduce biomarker levels are a focus of increased interest [4–5]. Also, the effect of antiretroviral drugs on these biomarkers remains unclear [6–9]. We studied the potential effect of raltegravir, an HIV integrase inhibitor, on these biomarkers, using stored specimens obtained from individuals enrolled in the ANRS 138 EASIER trial [10].

**PATIENTS AND METHODS**

**Trial Population**

The ANRS 138 EASIER study was a 48-week randomized trial that compared treatment-experienced HIV-infected patients who underwent either an immediate switch (IS) or a deferred switch (DS; at week 24) from an enfuvirtide-based ART regimen to a raltegravir-based ART regimen [10]. Eligible patients were integrase inhibitor naive, had a history of triple-class failure or intolerance, and had achieved virologic suppression (defined as a plasma HIV RNA level of <400 copies/mL) with an enfuvirtide-based regimen.

**Biomarkers Substudy**

The primary objective was to compare the effect of switching from enfuvirtide to raltegravir on biomarker levels after 24 weeks. Secondary objectives were to compare changes in biomarker levels after 48 weeks between arms and to study the correlation between baseline biomarker levels and the presence of enfuvirtide-induced injection-site reactions. This substudy included all subjects with available stored plasma specimens obtained at baseline and at weeks 24 and 48 who provided written inform consent.

**Biomarker Assays**

Plasma specimens were collected in ethylenediaminetetraacetic acid tubes and shipped frozen to the ANRS central repository in Lyon. Plasma samples were stored at −80°C without prior thawing, until analysis. Levels of 2 inflammatory markers, interleukin 6 (IL-6) and high-sensitivity C-reactive protein (hsCRP), and of 1 coagulation marker, D-dimer, were measured. IL-6 levels were measured with the Quantikine HS human IL-6 immunoassay (an enzyme-linked immunosorbent assay solid-phase test; R&D Systems, Minneapolis, MN). The Tina-quant CRP kit, third generation (Cobas, Roche/Hitachi, 892 • JID 2013:208 (15 September) • BRIEF REPORT
Indianapolis, IN), was used to measure the hsCRP level on a MODULAR P instrument. D-dimer levels were measured by STA Liatest D-DI immunoturbidimetry (Diagnostica Stago, Asnière sur Seine, Ile de France, France). The lower limits of detection for hsCRP, IL-6, and D-dimer were 0.10 μg/mL, 1.5 pg/mL, and 0.210 μg/mL, respectively. Samples from all subjects were analyzed by investigators who were blinded to the treatment group to which each subject had been randomly assigned.

Enfuvirtide-Related Injection-Site Reactions
Patients were asked to answer questions related to enfuvirtide-induced injection-site reactions at baseline. Four questions addressed the presence or absence of erythema, pain, skin induration, and nodules at the injection sites. The severity of discomfort was graded from 1 (no discomfort) to 4 (very disturbing). The absence of a reaction was assigned a grade of 0.

Statistical Methods
The primary objective was to compare changes in IL-6, hsCRP, and D-dimer plasma levels from baseline to week 24 between the IS and DS arms. Analyses were performed using intent-to-treat principles. Because of the skewed distribution of the data, biomarker levels were log10 transformed before analysis. Within regimen, 1-sample t tests were used to assess median changes from baseline, whereas comparisons between regimen components used 2-sample t tests with no adjustment for baseline factors.

Biomarker levels below assay detection limits (15%, 34%, and 54%) of specimens tested for IL-6 at baseline, week 24, and week 48, respectively; 0%, 1%, and 2% tested for hsCRP at baseline, week 24, and week 48, respectively; and 24%, 46%, and 67% tested for D-dimer at baseline, week 24, and week 48 (week 48) were set to the lower level of detection. Similar analyses were performed to compare changes from baseline to week 48 between arms. Comparisons were made with a 2-sided α level of 0.05. Statistical analyses were performed with SAS software, version 9.2 (SAS Institute, Cary, NC).

RESULTS
Among the 170 patients randomized in the trial, 164 (96.5%) had plasma available to measure biomarkers at baseline, 157 (92%) had plasma available at week 24, and 147 (86.5%) had plasma available at week 48. The most relevant baseline characteristics of patients are described in Table 1, and values were well balanced across treatment arms. These patients were extensively treatment experienced and mainly male, with a median age of 48 years. Most patients (54%) had a prior AIDS-defining event (54%), and the median nadir CD4+ T-cell count was <50 cells/mm3. All patients had <400 copies/mL of plasma HIV-RNA, and 86% had <50 copies/mL of plasma HIV RNA. Most patients were receiving at baseline a complex regimen including enfuvirtide, a ritonavir-boosted protease inhibitor (PI; darunavir or tipranavir in 76%), and 2 nucleoside reverse-transcriptase inhibitors (NRTIs; mainly tenofovir disoproxil fumarate or abacavir plus lamivudine or emtricitabine). During the 48-week follow-up period, no patient died, but 5 developed a coronary event, and 1 developed Hodgkin lymphoma.

Baseline Biomarker Levels
Median baseline levels and interquartile ranges of the 3 biomarkers in the IS and DS arms are shown in Table 2. Although levels were similar in both arms, they were high, compared with levels reported in previous studies [1, 3, 5].

Randomized Comparison of Biomarker Levels Over Follow-up in the IS and DS Arms
At week 24, all patients maintained a plasma HIV RNA level of <400 copies/mL, and 86% and 87% in the IS and DS arms, respectively, had a plasma HIV RNA level of <50 copies/mL (P = .84). Median levels of the biomarkers during the follow-up period, by treatment arm, are shown in Table 2 (crude and log10-transformed levels) and Supplementary Figure 1A–C (log10-transformed levels). Changes from baseline in IL-6, hsCRP, and D-dimer levels were all significantly greater in the IS arm as compared to the DS arm (P <.0001, P <.0003, and P <.0001, respectively; Table 2). Indeed, a significant decrease in mean percentage fold-change from baseline was seen in the IS arm, with changes in IL-6, hsCRP, and D-dimer levels of −30% (95% confidence interval [CI], −42% to −17%), −46% (95% CI, −60% to −29%), and −40% (95% CI, −47% to −32%), respectively, whereas in the DS arm mean percentage fold-changes from baseline were not significant, with findings of +10% (95% CI, −11% to +38%) for the IL-6 level, +15% (95% CI, −11% to +47%) for the hsCRP level, and +6% (95% CI, −9% to +24%) for the D-dimer level.

At week 48, all patients maintained a plasma HIV RNA level of <400 copies/mL, and 88% in both arms also had a plasma HIV RNA level of <50 copies/mL (P = .93). Changes from baseline in IL-6, hsCRP, and D-dimer levels were no longer different between the arms. Indeed, significant changes in mean percentage fold-change from week 24 to week 48 were seen in the DS arm for IL-6, hsCRP, and D-dimer levels, with values of −47% (95% CI, −57% to −35%), −71% (95% CI, −79% to −61%), and −50% (95% CI, −58% to −41%), respectively, which were very similar to what was seen in the IS arm during the first 24 weeks after the switch. From week 24 to week 48, mean percentage fold-changes in the IS arm were −13% (95% CI, −30% to +8%), −31% (95% CI, −52% to −0%), and +11% (95% CI, −3% to +27%) for IL-6, hsCRP, and D-dimer levels, respectively. Yet, median levels of all 3 biomarkers at week 48 were similar in both study arms (Table 2).
Injection-site Reactions and Biomarkers

Questionnaires were available at baseline for 125 patients (76%) enrolled in the biomarker study, 74% of whom complained of erythema (grade 3 in 30%), 78% of pain (grade 3 in 30%), 83% of skin indurations (grade 3 in 34%), and 90% of subcutaneous nodules (grade 3 in 45%). For each biomarker, median levels at baseline were measured according to the presence or absence of each symptom and according to symptom severity. No significant trend in median biomarker levels could be seen for any of these injection-site reactions, regardless of their severity grade (data not shown).

DISCUSSION

This study clearly demonstrated a significant decrease in IL-6, hsCRP, and D-dimer plasma levels following the switch from an enfuvirtide-based regimen to a raltegravir-based regimen. Indeed, following the switch to raltegravir, mean fold-change
decreases from baseline in IL-6, hsCRP, and D-dimer levels were 30%, 46%, and 40%, respectively, at week 24. These decreases in biomarker levels following the switch from enfuvirtide to raltegravir were further confirmed by similar decreases in levels of all 3 biomarkers in the DS arm at week 48, 24 weeks after the switch to raltegravir. At week 48, there was no difference between arms in plasma levels of the 3 biomarkers, suggesting that there was no further benefit of the switch to raltegravir beyond 24 weeks. However, because of the high proportion of patients with biomarker levels below the lower limit of detection at week 48, it is also possible that further decreases in biomarker levels could have been masked by the detection limits of the assays.

A number of studies have shown both in the general population and in HIV-infected individuals that high levels of hsCRP, IL-6, and D-dimers are associated with increased cardiovascular risk and overall mortality [1-3]. Also, a reduction of 37% in the hsCRP level and of 50% in the low-density lipoprotein cholesterol level in the rosuvastatin arm of the JUPITER study after a median follow-up of 1.9 years in HIV-uninfected patients with a high baseline hsCRP level was associated with a 46% reduction of severe cardiovascular disease or death [11]. The range of decrease in biomarker levels observed in our study is thus quite significant, although no change in lipid levels was reported in the ANRS 138 EASIER trial [10].

Data are limited regarding the effect of specific antiretroviral drugs on these inflammatory and coagulation biomarkers. McComsey et al recently reported a 1.5-fold increase in hsCRP levels following initiation of abacavir/lamivudine and efavirenz treatment, compared with no change after initiation of tenofovir/emtricitabine or atazanavir/ritonavir treatment, in antiretroviral-naive patients and a similar decrease in IL-6 levels, from 22% to 40% [8]. Even fewer data are available for raltegravir. No significant changes in the hsCRP or IL-6 levels were seen after 48 weeks in patients initiating therapy with abacavir/lamivudine and raltegravir [12]. Another study did not find any change in hsCRP levels 24 weeks after a switch from a PI or nonnucleoside reverse-transcriptase inhibitor (NNRTI) to raltegravir, but only 37 patients completed follow-up [13]. However, a recent substudy of the SPIRAL trial, in which patients with well-suppressed viremia during receipt of a boosted PI-based regimen were randomly assigned to switch to raltegravir while maintaining the same NRTIs or to continue their baseline regimen, reported significant decreases of 40%, 46%, and 8% in hsCRP, IL-6, and D-dimer levels in the raltegravir arm after 48 weeks, again unrelated to lipid changes [14].

It is tempting to speculate that the effect of the switch to raltegravir could be related to better control of HIV replication in these patients. However, in our study we failed to demonstrate a higher proportion of patients with a plasma viral load of <50 copies/mL over time in the raltegravir arm. Also, a number of intensification studies with raltegravir in patients with suppressed viral replication in plasma have failed to demonstrate a benefit of this strategy in further reducing viral replication in plasma, even by use of ultrasensitive methods [15].

Because of the frequent occurrence of inflammatory subcutaneous nodules at the injection site with enfuvirtide, it was tempting to hypothesize that reduction in biomarker levels was merely due to the discontinuation of enfuvirtide. To address this issue, we assessed the relationships between self-reported

### Table 2. Levels of Interleukin 6 (IL-6), High-Sensitivity C-Reactive Protein (hsCRP), and D-dimer Levels at Baseline, Week 24, and Week 48 Among Patients Enrolled in the Biomarker Substudy of the ANRS 138 EASIER Trial

<table>
<thead>
<tr>
<th>Variable</th>
<th>IL-6 Level, pg/mL</th>
<th>hsCRP Level, µg/mL</th>
<th>D-dimer Level, µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IS Arm (n = 83)</td>
<td>DS Arm (n = 81)</td>
<td>IS Arm (n = 83)</td>
</tr>
<tr>
<td>Baseline value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>3.5 (2.2–6.4)</td>
<td>3.6 (2.1–5.8)</td>
<td>4.6 (2.5–11.1)</td>
</tr>
<tr>
<td>Log10 transformed</td>
<td>0.544</td>
<td>0.556</td>
<td>0.633</td>
</tr>
<tr>
<td>Week 24 value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.7 (1.5–3.6)</td>
<td>4.1 (2.1–7.8)</td>
<td>2.1 (1.2–5.7)</td>
</tr>
<tr>
<td>Log10 transformed</td>
<td>0.230</td>
<td>0.607</td>
<td>0.322</td>
</tr>
<tr>
<td>Change from baseline</td>
<td>–0.166</td>
<td>0.080a</td>
<td>–0.301</td>
</tr>
<tr>
<td>Week 48 value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.5 (1.5–2.5)</td>
<td>1.9 (1.5–2.8)</td>
<td>1.6 (0.8–3.2)</td>
</tr>
<tr>
<td>Log10 transformed</td>
<td>0.176</td>
<td>0.279</td>
<td>0.204</td>
</tr>
<tr>
<td>Change from baseline</td>
<td>–0.177</td>
<td>–0.239</td>
<td>–0.448</td>
</tr>
</tbody>
</table>

Data are median value or median value (interquartile range). Patients were randomly assigned to undergo an immediate switch (IS) or a deferred switch (DS; at week 24) from an enfuvirtide-based ART regimen to a raltegravir-based regimen.

a P < .0001, by the Wilcoxon test, for the difference between treatment groups in the change in median log10-transformed value from baseline to week 24.
b P = .0003, by the Wilcoxon test, for the difference between treatment groups in the change in median log10-transformed value from baseline to week 24.
c P < .0001, by the Wilcoxon test, for the difference between treatment groups in the change in median log10-transformed value from baseline to week 24.
injection-site reactions and biomarker levels but have found no clear association. Although this lack of association could be explained by a lack of power and does not exclude such a relationship, these results suggest that the potential activity of raltegravir on these biomarkers should be further explored.

Indeed, one of the limitations of our findings is that we currently have no mechanistic explanation of how raltegravir could decrease biomarker levels. Stored specimens from randomized studies of raltegravir-based regimens should be tested to provide more information. Other limitations of our study are its relative small size and short follow-up period. We also could not assess the potential clinical relevance of these findings, since the number of non–AIDS-defined clinical events was too small.

In summary, we have found that a switch from enfuvirtide to raltegravir in multitreatment-experienced patients with suppressed viral replication was associated with a significant decrease in inflammatory and coagulation biomarkers. Whether these changes are due to the initiation of raltegravir, discontinuation of enfuvirtide, or any other factor, and whether these changes are associated with clinical benefit, will require further studies with longer follow-up periods.

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Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not co-edited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.
Notes

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Potential conflicts of interest. J. M. M. has participated on Merck advisory boards. All other authors report no potential conflicts.

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.

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