HIV-1 Coreceptor Use in Triple-Class Treatment–Experienced Patients: Baseline Prevalence, Correlates, and Relationship to Enfuvirtide Response

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Objective. We wished to assess, in heavily treatment-experienced patients, the prevalence of and baseline characteristics associated with HIV-1 coreceptor use and their relationship to responses to enfuvirtide treatment.

Methods. Samples were obtained from participants in phase 3 studies of enfuvirtide. Multiple logistic regression and analysis of covariance were performed on data for baseline coreceptor use, virological and immunological response, and changes in coreceptor use during treatment.

Results. Baseline envelopes were phenotyped for 724 patients; 50% harbored R5 strains, 48% harbored dual/mixed (D/M) strains, and 2% harbored X4 strains. D/M strains were associated with significantly lower CD4+ cell counts but comparable viral loads, compared with R5 strains (P = .0005). Virological and immunological responses to enfuvirtide-based treatment showed no correlation with baseline coreceptor use. Changes in virus tropism from D/M to R5 strains during treatment were common, particularly in patients who received enfuvirtide (27%, vs. 14% who received no enfuvirtide; P < .05).

Conclusion. At baseline, D/M strains were associated with lower CD4+ cell counts but similar viral loads, compared with R5 strains, and were common across CD4+ cell count strata. The comparable virological and immunological responses and bias toward shifts from D/M to R5 strains in patients who received enfuvirtide support its use in triple-class treatment–experienced patients and its study as a therapeutic partner for coreceptor-binding inhibitors.

HIV-1 infection of host cells proceeds through several steps, beginning with adhesion to the cell and binding of the viral envelope to the cell surface receptor CD4 (reviewed in [1]). Envelope interaction with CD4 results in the exposure of a binding site for 1 of 2 clinically relevant coreceptors, CCR5 and CXCR4 [2]. After binding, rearrangements of the viral transmembrane protein gp41 bring the virus and target cell membranes into close proximity, leading to membrane fusion and subsequent virus entry (reviewed in [3]). Substantial efforts, driven particularly by the need for antiretroviral agents with improved toxicity profiles and activity against multidrug-resistant viruses, have been made to develop drugs that target the entry process. The first of these to be approved for clinical use was enfuvirtide, which acts at the stage of membrane fusion [4]. Additional compounds that target receptor or coreceptor binding have demonstrated proof-of-concept in clinical studies; however, at present, their future role in the management of HIV infection remains uncertain.

CCR5-using (R5) strains are generally dominant immediately after HIV transmission, whereas variants capable of using CXCR4 (dual/mixed [D/M] or X4 strains) may emerge later during the course of infection [5, 6]. Because of differential coreceptor expression, R5 strains mainly infect memory CD4+ cells, whereas X4 variants target naïve and resting CD4+ cells [7, 8]. The appearance of CXCR4-using strains (as inferred from syncytia induction in MT-2 cells) was observed in ~50% of untreated patients who had progression to AIDS.
during the pre–highly active antiretroviral therapy (HAART) era and was associated with a rapid decrease in CD4+ cell counts and poor clinical prognosis [9–11]. In 2 studies, CXCR4 coreceptor use, as predicted by envelope V3 loop genotype, was also associated with significantly lower CD4+ cell count responses to antiretroviral therapy [12, 13]. Other recent studies have characterized correlates of coreceptor use, as defined by the assay that we used, in large cohorts of mostly treatment-naive patients [14, 15]. However, such data have previously been unavailable for a large cohort of triple-class treatment–experienced patients.

Enfuvirtide has shown potent anti–HIV-1 activity in vitro and in vivo [16–20]; it acts via interaction with the HIV-1 envelope after CD4 and, possibly, coreceptor binding [4, 21]. Using the PhenoSense Entry assay, we have previously characterized envelopes from patients participating in phase 3 studies of enfuvirtide [22–25]. However, those studies defined coreceptor use on the basis of reporting of enfuvirtide IC50 results on U87 cell lines expressing CD4 and either the CCR5 or the CXCR4 coreceptor. More recently, an assay that directly measured coreceptor use was developed and used in several large studies [14, 15, 26]. Taking into consideration the need for data on virus coreceptor use in heavily treatment–experienced patients and the potential to improve and elaborate on our earlier analyses, we obtained updated determinations of coreceptor use for samples from the phase 3 studies of enfuvirtide. Here we present, for a large cohort of triple-class treatment–experienced patients, analyses of the baseline prevalence and correlates of coreceptor use and of the relationship between coreceptor use and virological and immunological responses to enfuvirtide–based treatment regimens.

PATIENTS AND METHODS

Study population. The analyzed population included all patients in 2 phase 3 studies of enfuvirtide who were randomized to receive enfuvirtide plus an optimized background regimen (n = 661) and 100 of 334 patients randomized to receive the optimized background regimen alone; methods used in those studies have been presented elsewhere [27, 28]. Enfuvirtide susceptibility was tested at baseline and again for patients who met protocol-defined criteria for virological failure through 48 weeks; the 100 patients receiving the optimized background regimen alone had all met virological-failure criteria through 48 weeks. Because the sensitivity threshold of the assay was 1000 copies/mL, there were only 18 patients tested for virus tropism during treatment who did not meet virological-failure criteria; they were therefore excluded from study.

Coreceptor use and susceptibility testing. Testing of coreceptor use and susceptibility to enfuvirtide was performed using the PhenoSense Entry assay (Monogram Biosciences) [15, 22]. In brief, the assay began with extraction of viral RNA from patient plasma samples, followed by cDNA synthesis. Env-specific primers were then used to amplify a 2.5-kb fragment including the entire open reading frame of the HIV-1 envelope; amplicons were subcloned, and representative expression vectors/envelope sequence libraries were prepared in Escherichia coli. Pseudotyped virus stocks were produced in human embryonic kidney–cell cultures by cotransfection of the expression libraries and an Env-deficient HIV-1 vector carrying a luciferase reporter gene. Virus stocks were harvested after 48 h, and pseudotyped virus populations were assessed for susceptibility to enfuvirtide and their ability to infect U87 cells expressing CD4 and the CCR5 or CXCR4 coreceptor [22]. In our previous reports [23–25], coreceptor use was determined by the reporting of an IC50 to enfuvirtide for either the CCR5- or CXCR4-expressing cell line. In the present study, data were reanalyzed for all envelope populations previously defined as CCR5 or CXCR4 tropic with reporter light units above background levels on both cell lines, and coreceptor use was determined on the basis of signal ablation by entry inhibitors specific to CCR5 (proprietary) or CXCR4 (AMD3100).

Normalized IC50 values for enfuvirtide were derived by multiplying the sample IC50 by the ratio of a standard reference value (determined by Monogram Biosciences from multiple assay runs) to the reference value obtained concurrently with the patient sample; standard IC50 values for enfuvirtide were 0.0286 µg/mL for CCR5 (JRCSF) and 0.0083 µg/mL for CXCR4 (HXB2). This methodology represents a departure from that used in earlier reports [23–29] and was adopted to facilitate comparisons across multiple cell lines while adjusting for interassay variability. Similarly, in the present study, IC50 values for D/M strains were averages from the 2 cell lines, whereas we had previously used the maximum IC50 from either cell line.

The total number of drugs to which viruses had predicted genotypic susceptibility and the baseline phenotypic sensitivity score were determined on the basis of results from the PhenoSense GT and PT tests, respectively, using cutoff values in place in 2001, when the study was initiated. Cessation of antiretroviral drugs for at least 7 days constituted an interruption of treatment; missing data were imputed as noninterruptions.

Statistical methods. Data were summarized by baseline coreceptor-use stratum (R5, D/M, or X4); strata were compared using the χ2 test for the dichotomous variables sex, prior lopinavir/ritonavir use, prior/current diagnosis of AIDS, and baseline treatment interruption. Continuous variables—including baseline age, viral RNA load, CD4+ cell count, phenotypic sensitivity score, the total number of antiretroviral drugs (excluding enfuvirtide) with predicted genotypic sensitivity, number of antiretrovirals used, duration of prior therapy, and IC50 to enfuvirtide—were compared using the Wilcoxon rank sum test. Two-sided P values were reported for all analyses. Mean R5 and mean D/M and X4 virological and immunological re-
Table 1. Patient characteristics at baseline, by HIV-1 coreceptor-use stratum.

<table>
<thead>
<tr>
<th>Characteristic at baseline</th>
<th>Model(s)</th>
<th>R5 (n = 361)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>D/M (n = 347)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>X4 (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, no. (%)</td>
<td>1</td>
<td>318 (88)</td>
<td>321 (93)</td>
<td>14 (88)</td>
</tr>
<tr>
<td>Age, median (IQR), years</td>
<td>1</td>
<td>41 (37–47)</td>
<td>41 (37–47)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma viral RNA, median(IQR), log&lt;sub&gt;10&lt;/sub&gt; copies/mL</td>
<td>1, 2, 3</td>
<td>5.19 (4.66–5.58)</td>
<td>5.16 (4.81–5.45)</td>
<td>NS</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; cell count, median (IQR), cells/mm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1, 2, 3</td>
<td>108 (23.0–218) [359]</td>
<td>70.0 (14.0–177.0) [346]</td>
<td>.0005</td>
</tr>
<tr>
<td>Antiretrovirals previously used, median (IQR), no.</td>
<td>1</td>
<td>11.0 (10.0–13.0)</td>
<td>12.0 (10.0–13.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Prior lopinavir/ritonavir use, no. (%)</td>
<td>1, 2, 3</td>
<td>303 (83.9)</td>
<td>267 (76.9)</td>
<td>.019</td>
</tr>
<tr>
<td>Enfuvirtide IC&lt;sub&gt;50&lt;/sub&gt;, geometric mean (IQR), µg/mL</td>
<td>1</td>
<td>0.047 (0.025–0.086) [360]</td>
<td>0.030 (0.016–0.055) &lt; .0001</td>
<td>0.055 (0.033–0.088) NS .0078</td>
</tr>
<tr>
<td>Phenotypic sensitivity score, mean (IQR)</td>
<td>2, 3</td>
<td>1.5 (0.0–2.5) [352]</td>
<td>1.5 (0.0–2.0) [341]</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline treatment interruption, no. (%)</td>
<td>1</td>
<td>59 (16.3)</td>
<td>60 (17.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Total susceptible agents predicted by genotype, mean (IQR)</td>
<td>1</td>
<td>4.8 (2.0–6.0) [357]</td>
<td>4.3 (2.0–6.0) [342]</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of prior therapy, median (IQR), months</td>
<td>1, 2, 3</td>
<td>89.2 (70.3–113.0)</td>
<td>79.4 (63.0–103.0)</td>
<td>.0009</td>
</tr>
</tbody>
</table>

NOTE. P values for Wilcoxon rank sum or χ² test (see Patients and Methods). Models are described in Patients and Methods. D/M, dual/mixed strain; IQR, interquartile range; NS, not significant (P > .10).

<sup>a</sup> Brackets indicate differing total nos. of patients in a group.
Figure 1. Composition of CD4⁺ cell count or HIV-1 RNA load strata, by baseline HIV-1 coreceptor use. Bars denote percentages of patients, by coreceptor stratum in each category, with the total no. of patients indicated in parentheses. A, Baseline CD4⁺ cell count strata \((n = 721)\). B, Baseline HIV-1 RNA load quartiles \((n = 724)\). Gray bars, R5 virus; black bars, dual/mixed virus; white bars, X4 virus.

responses were adjusted for baseline log₁₀ viral RNA load, CD4⁺ cell count, phenotypic sensitivity score, number of antiretroviral drugs previously received, and prior or current lopinavir/ritonavir use (factors previously associated with response in the T-20 versus Optimized Regimen Only (TORO) studies [27, 28]). For patients infected with R5 or D/M strains, logistic regression was used to assess the independent significance of associations with baseline coreceptor use (model 1). Independent predictors of baseline R5 versus D/M coreceptor use \((P < .05)\) were then included, along with the same factors used in the adjusted means analyses (listed above), in regression analyses of associations with virological suppression at 48 weeks (only patients receiving the enfuvirtide and optimized background regimen; model 2) and associations with changes from D/M to R5 during treatment (all patients with D/M strains at baseline but R5 strains at virological failure; model 3). The models in which each factor was included are listed (as numbered above) in table 1; additional factors were use of lopinavir/ritonavir in the background regimen in models 2 and 3, baseline R5 use in model 2, and treatment arm in model 3. An additional regression analysis of tropism switching was performed that included only treatment arm and week-48 CD4⁺ cell count increase, CD4⁺ cell count, viral RNA load decrease, and viral RNA load. In all analyses, factors with a univariate significance of \(P < .15\) were included in the multivariable model. Because of the exploratory, post hoc nature of the present study, findings with significance levels between \(P = .1\) and \(P = .05\) (i.e., a 90%-95% likelihood of being nonchance findings) were reported. All statistical analyses were conducted using SAS software (version 8.2; SAS Institute). As described elsewhere [27, 28], virological failure was defined as a decrease from baseline in viral RNA loads of \(<0.5 \text{ log}_10 \text{ copies/mL}\) by week 6 or \(<1.0 \text{ log}_10 \text{ copies/mL}\) by week 12 or a rebound of \(>1.0 \text{ log}\) at any time following a decrease of \(>2.0 \text{ log}_10 \text{ copies/mL}\).

RESULTS

Baseline study population. Baseline tropism data were obtained for 627 (95%) of 661 patients receiving the enfuvirtide and optimized background regimen and for 97 (97%) of 100
patients receiving the optimized background only; viral RNA loads of the 37 patients for whom viral coreceptor-use testing was unsuccessful (median, 4.9 log_{10} copies/mL) were close to those for the analysis population (median, 5.2 log_{10} copies/mL). Baseline characteristics for all patients without available results (n = 271; 37 plus the 234 patients receiving the optimized background regimen who were not selected for testing) were broadly similar (median viral RNA load, 5.1 log_{10} copies/mL; median CD4+ cell count, 98 cells/mm^3) to those of the analysis population.

Baseline prevalence and characteristics, by coreceptor-use stratum. The overall prevalences of R5, D/M, and X4 strains were 50% (361/724), 48% (347/724), and 2% (16/724), respectively (table 1). To assess the relationship between baseline factors and coreceptor use, for most patients, we first examined CD4+ cell counts and plasma viral RNA loads for the R5 and D/M populations (table 1). Baseline CD4+ cell counts were significantly lower for patients harboring D/M strains than for those harboring R5 strains (median, 70 and 108 cells/mm^3, respectively; P < .001); the prevalence of D/M strains decreased from 54% for patients with CD4+ cell counts < 25 cells/mm^3 to 38% for those with CD4+ cell counts > 300 cells/mm^3 (figure 1A). Median plasma viral RNA loads were similar for the 2 groups (both had 5.2 log_{10} copies/mL; P > .15); however, patients harboring R5 strains were disproportionately distributed in the lowest and highest viral RNA quartiles (figure 1B).

Intriguingly, patients harboring X4 strains had significantly lower plasma viral RNA loads (median, 4.6 log_{10} copies/mL) than patients harboring either R5 or D/M strains (for both comparisons, P < .003); they exhibited CD4+ cell counts similar to those of patients harboring R5 strains and somewhat higher than those of patients harboring D/M strains (median, 143 cells/mm^3; P = .079 vs. D/M). Patients harboring X4 strains were also significantly older than other patients (P = .018 vs. R5; P = .032 vs. D/M) and showed a trend toward a longer duration of treatment than patients harboring D/M strains (P = .088).

Associations with baseline coreceptor use were further evaluated through regression analysis (table 2). Baseline patient characteristics independently predictive of increased odds of harboring R5 versus D/M strains included higher CD4+ cell count (P = .002), prior/current diagnosis of AIDS (P = .0031), longer duration of antiretroviral therapy (P = .0043), and, marginally, a higher total number of drugs with predicted activity (P = .088); a higher viral IC_{50} to enfuvirtide (P < .0001) was also strongly predictive of harboring R5 rather than D/M strains.

Analyses of response to treatment with enfuvirtide. We first examined the relationship between baseline coreceptor use and virological and immunological response to enfuvirtide-based

### Table 2. Predictors of harboring R5 strains at baseline (vs. dual/mixed strains only; n = 708), by univariate and multivariate analyses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Baseline value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ cell count</td>
<td>1.002 (1.001–1.003)</td>
<td>.0009</td>
</tr>
<tr>
<td>(\log_{10}) IC_{50} to enfuvirtide</td>
<td>3.160 (2.154–4.635)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Prior or current diagnosis of AIDS</td>
<td>1.565 (1.075–2.280)</td>
<td>.020</td>
</tr>
<tr>
<td>Sex</td>
<td>0.599 (0.359–0.999)</td>
<td>.049</td>
</tr>
<tr>
<td>Susceptible agents predicted by genotype</td>
<td>1.046 (1.002–1.093)</td>
<td>.043</td>
</tr>
<tr>
<td>Duration of antiretroviral therapy</td>
<td>1.008 (1.003–1.013)</td>
<td>.0001</td>
</tr>
</tbody>
</table>

**NOTE.** Odds ratios (ORs) are given per \(\log_{10}\) unit for IC_{50}, per cell/mm^3 for CD4+ cell count, and agent for the no. of antiretrovirals previously used. CI, confidence interval.

* Male sex was the reference value.

### Table 3. Least-squares mean adjusted change in HIV-1 RNA load and CD4+ cell count from baseline to week 48 for 627 patients, by baseline coreceptor-use strata.

<table>
<thead>
<tr>
<th>Change in value from baseline to week 48</th>
<th>D/M or X4</th>
<th>R5 only</th>
<th>Difference (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 RNA load,^{a} log_{10} copies/mL</td>
<td>−1.43</td>
<td>−1.54</td>
<td>−0.11 (−0.32 to 0.09)</td>
<td>.28</td>
</tr>
<tr>
<td>CD4+ cell count,^{a} cells/mm^3</td>
<td>96</td>
<td>98</td>
<td>2 (−18 to 21)</td>
<td>.85</td>
</tr>
</tbody>
</table>

**NOTE.** Values were adjusted by least-squares means method for baseline factors: log_{10} HIV-1 RNA load, CD4+ cells/mm^3, no. of antiretrovirals previously used, baseline phenotypic sensitivity score, and prior lopinavir/ritonavir use. In a separate analysis of patients harboring X4 strains at baseline, RNA response was equivalent to that in the overall population, but the increase in CD4+ cell count was ∼20% lower (data not shown). D/M, dual/mixed strain.

* Last observation carried forward.
therapy by comparing adjusted mean changes in viral RNA load and CD4+ cell counts. As shown in table 3, no significant differences were found for week-48 changes in the viral RNA load (P = .28) or the CD4+ cell count increase (P = .86) between patients harboring D/M + X4 versus R5 strains at baseline. To confirm that the virological response was similar at earlier time points, the proportion of patients with viral RNA loads of <400 copies/mL was plotted by study week (figure 2). No difference between the baseline tropism groups was seen by intent-to-treat or as-treated analysis; similar results were obtained for patients with viral RNA loads of <50 copies/mL (data not shown). The independent significance of baseline coreceptor use in relation to virological suppression at week 48 was modeled using multivariate regression of viral RNA loads <400 copies/mL; neither coreceptor use nor the baseline IC50 to enfuvirtide was a significant predictor of viral suppression at week 48 (P = .67 and P = .21, respectively).

Changes in coreceptor use during treatment. Paired samples obtained at baseline and at the time when protocol-defined criteria for virological failure were met were available for 279 patients receiving the enfuvirtide and optimized background regimen and for 94 patients receiving the optimized background regimen alone. Changes in predicted coreceptor use during treatment were observed for 56 patients (20%) receiving the enfuvirtide and optimized background regimen and 12 patients (13%) receiving the optimized background regimen alone (P = .11) (table 4). The highest incidence of switching was between D/M and R5; this occurred significantly more frequently for patients harboring baseline D/M strains receiving the enfuvirtide and optimized background regimen than those receiving the optimized background regimen alone (27% [40/147] vs. 14% [6/42] patients, respectively; P = .043). To examine the relationship between these observations and enfuvirtide-based therapy, we performed regression analyses of the D/M-to-R5 tropism switch, including variables that had been previously associated with virological response in the TORO studies (see Patients and Methods) and factors that we identified as being independent predictors of baseline coreceptor use. Of the 10 factors in the initial analysis, only baseline CD4+ cell count and treatment arm were carried forward into the multivariate analysis; the significances of their independent associations with response were then P = .13 and P = .10, respectively. In regression analysis of the D/M-to-R5 tropism

Table 4. Summary of coreceptor use at baseline and change on treatment.

<table>
<thead>
<tr>
<th>Coreceptor tropism at baseline and at virological failure</th>
<th>ENF + OB (n = 279)</th>
<th>OB (n = 94)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/M</td>
<td>D/M</td>
<td>101 (69)</td>
<td>34 (81)</td>
</tr>
<tr>
<td></td>
<td>R5</td>
<td>40 (27)</td>
<td>6 (14)</td>
</tr>
<tr>
<td></td>
<td>X4</td>
<td>6 (2)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>R5</td>
<td>D/M</td>
<td>8 (6)</td>
<td>3 (6)</td>
</tr>
<tr>
<td></td>
<td>R5</td>
<td>120 (94)</td>
<td>45 (94)</td>
</tr>
<tr>
<td>X4</td>
<td>D/M</td>
<td>2 (50)</td>
<td>1 (25)</td>
</tr>
<tr>
<td></td>
<td>X4</td>
<td>2 (50)</td>
<td>3 (75)</td>
</tr>
</tbody>
</table>

NOTE: Data are no. (%). D/M, dual/mixed strain; ENF, enfuvirtide; OB, optimized background.

* χ² test.
switch for during-treatment variables (including changes in CD4+ cell count and viral RNA load from baseline and week 48 CD4+ cell count and viral RNA load), week-48 viral RNA load and treatment arm were carried forward into multivariable analysis; the significance of their independent predictive value for the D/M-to-R5 tropism switch was $P = .10$ and $P = .087$, respectively. Interestingly, the few patients who were receiving only the optimized background regimen and who had a D/M-to-R5 tropism switch had much higher median increases in CD4+ cell count at the time of virological failure than other patients who were receiving the optimized background regimen alone and who harbored D/M strains at baseline (72 vs. 2 cells/mm³, respectively); however, this relationship was not seen for patients receiving enfuvirtide (corresponding increases of 44 and 50 cells/mm³).

**DISCUSSION**

We have characterized the prevalence and correlates of coreceptor use in a large cohort of multiple HAART regimen–experienced patients. At study entry, patients generally had high viral RNA loads (median, $>5 \log_{10}$ copies/mL), had low CD4+ cell counts (median, <100 cells/mm³), and had been treated previously with a median of 12 antiretrovirals; 80% had previously had an AIDS-defining illness diagnosed. The baseline prevalence of harboring D/M or X4 strains was 50%, which is strikingly consistent with reports of the emergence of the syncytium-inducing phenotype in patients who had progression to AIDS during the pre-HAART era [9, 30, 31]. A significant association between lower baseline CD4+ cell counts and harboring D/M strains was observed; however, D/M strains were common even in patients with CD4+ cells counts of >200 cells/mm³. Importantly, we found no difference in response to enfuvirtide-based treatment between patients harboring R5 strains and those harboring D/M and X4 strains.

**Clinical correlates of baseline tropism.** The correlation that we observed between D/M strains and lower CD4+ cell counts is generally consistent with previous reports; however, in contrast to the findings of a recent report from Brumme et al. [14], we observed a substantial prevalence of D/M strains even at CD4+ counts of >200 cells/mm³ [15, 32]. This difference could reflect the emergence of CXCR4 use at CD4+ cell count nadirs below those observed at baseline; however, a direct impact of antiretroviral treatment on coreceptor use cannot be ruled out. In addition, our observations differ from those of recent studies, in that we found the median viral RNA loads associated with R5 and D/M strains to be equivalent. This likely reflects the role of D/M strains as a surrogate marker of disease progression (and, hence, a higher viral RNA load) in studies that examine patients who are more widely distributed across the disease spectrum [9, 33, 34]. When we examined our results in more detail, R5 strains were particularly common in patients with viral RNA loads in the lowest quartile, which is consistent with this hypothesis, but they were also unexpectedly overrepresented in patients with viral RNA loads in the highest quartile. The latter finding could reflect such factors as higher rates of infectivity or the overall in vivo fitness of strains that had successfully outcompeted D/M strains [35, 36]. Given that patients harboring R5 strains also had the highest rate of prior/current AIDS diagnoses, they could also have been experiencing increased immune activation and, thus, harboring a larger number of CCR5-expressing activated memory cells capable of contributing to the R5 viral load [37, 38]. Immune activation data were not collected as part of the present study, but they (along with host coreceptor genotype data) could prove to be of value in future studies of coreceptor binding inhibitors.

The prevalence of X4 strains in the present study was low (2%); however, it was still higher than that in other recent cohort studies [14, 15, 26]. Interestingly, X4 strains were associated with significantly lower viral RNA loads than were D/M or R5 strains and with a trend toward higher CD4+ cell counts relative to patients harboring D/M strains. Although we are unaware of previously published cohort data for patients with exclusively CXCR4-using strains for comparison, our findings are similar to those reported for HIV-infected individuals homozygous for CCR5Δ32 (who lack cell-surface expression of CCR5). The few such patients with clinical data available have generally had moderate viral RNA loads and, after a rapid initial loss, stable CD4+ cell counts in the range of what we observed for X4 strains [39]. Taken together, these data point to the capacity to use both coreceptors, rather than the emergence of X4 use per se, in the accelerated CD4+ cell count decreases and disease progression associated with the emergence of the syncytium-inducing phenotype.

**Coreceptor use and response to enfuvirtide-based treatment.** The virological response through 48 weeks of enfuvirtide-based treatment was similar, regardless of baseline coreceptor use. Importantly, the immunological response was also independent of baseline tropism. A correlation between genotypically predicted CXCR4 use and lower short- and long-term increases in CD4+ cell count during antiretroviral therapy has recently been reported; however, in a follow-up to the initial report from Brumme et al., the correlation did not extend to phenotypically predicted coreceptor use [12–14]. Of note, moderately greater increases in CD4+ cell count (controlling for viral RNA load) have been reported for patients receiving the enfuvirtide and optimized background regimen, compared with the optimized background regimen alone, in phase 3 studies of enfuvirtide [40]. Together, these data raise the possibility that the increases could have been due to an inferior CD4+ cell response for a subset of patients with D/M strains in the optimized background arm.

As in previous analyses, we did not find a significant cor-
relation between the baseline IC₅₀ and virological response [23, 29]. However, in contrast to our previous work, a significant association was observed between baseline D/M strains and a lower IC₅₀ to enfuvirtide. Factors contributing to this finding could include the substantial changes in tropism between the analyses (previously 62% for R₅, 35% for D/M, and 4% for X₄), the addition in the present study of data for 97 additional patients who received the optimized background regimen alone, and a modification of data-handling rules that resulted in lower IC₅₀ values for D/M strains. One caveat regarding the interpretation of both sets of analyses is that they compare results across separate CCR5- and CXCR4-expressing cell lines, which could introduce bias caused by different functional coreceptor expression levels. Nonetheless, this finding is consistent with those of earlier in vitro studies, which found greater susceptibility for D/M and X₄ strains and pointed to differences in coreceptor affinity and fusion kinetics as explanations [41, 42].

In the present study, we observed a higher frequency of shifts from D/M to R₅ strains in patients receiving the enfuvirtide and optimized background regimen than in patients receiving the optimized background regimen alone. This could reflect either a greater activity of enfuvirtide against the X₄, rather than the R₅, components of D/M strains or a nonspecific effect, as has been reported for other highly active therapies [43, 44]. A recently published model of tropism switching that was based on the turnover rates of different CD₄⁺ cell types also suggested that increases in CD₄⁺ cell count may be sufficient to drive a reversion to R₅ strains [45]. Interestingly, we observed a much higher median CD₄⁺ cell count for patients with shifts from D/M to R₅ strains than for those without a switch in patients receiving the optimized background regimen alone; however, this was not true for patients receiving enfuvirtide. On the basis of the aforementioned model, this observation would be consistent with an enfuvirtide-specific effect. However, we also note that baseline strains associated with subsequent D/M-to-R₅ tropism switches had lower median CXCR4 reporter signals than did other D/M strains (data not shown), which suggests that the relative prevalence of CXCR4-using viruses in those samples was low. Thus, the switches that we observed could simply reflect a failure to detect relatively small CXCR4-using populations immediately after viral rebound. Further studies of coreceptor switch and immunological response during enfuvirtide-based treatment will be necessary to determine the durability and potential clinical significance of these findings.

In conclusion, our results highlight important issues concerning the use of entry inhibitors in heavily treatment-experienced patients. From an epidemiological standpoint, they extend reports from the pre-HAART era to show that, like untreated patients, ~50% of patients who have had failure of multiple treatment regimens develop viral populations capable of CXCR4-mediated entry. Virus from these patients may therefore be at least partially refractory to suppression by CCR5-binding inhibitors. In contrast, we found no correlation between baseline coreceptor use and either virological or immunological responses to enfuvirtide treatment, and we observed a bias toward the detection of R₅ strains at the time of viral rebound. Taken together with previous reports of in vitro synergy with other entry inhibitors [46–49], our findings support the notion of combining enfuvirtide with these agents in clinical studies and with other treatment regimens in triple-class treatment–experienced patients.

References


