The Evolution of Coreceptor Tropism in HIV-infected Patients Interrupting Suppressive Antiretroviral Therapy

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CCR5 antagonists may provide a well-tolerated switch option for patients experiencing tolerability or toxicity of their antiretroviral regimen. We analyzed stored samples from patients undergoing planned treatment interruptions for reasons other than virological failure, in order to analyze tropism evolution during fully suppressive antiretroviral therapy (ART). Two of 37 patients showed evidence of switching. Tropism switching after suppressive ART was uncommon in this cohort. Pretreatment human immunodeficiency virus (HIV) RNA tropism testing may help guide the switch to CCR5 antagonists in patients with undetectable HIV RNA.

Drug-related adverse events are an important cause of poor adherence to and treatment failure of and a precipitating factor for switching antiretroviral therapy (ART) [1]. Timely switch of antiretroviral agents, where alternative options are available, is an important strategy for managing drug-related adverse events.

When human immunodeficiency virus (HIV) enters host cells, 1 of 2 cell surface coreceptors are utilized during the binding process: CC chemokine receptor 5 (CCR5) or CXC chemokine receptor 4 (CXCR4). CCR5 antagonists, in combination with other antitrovirals, may provide a well-tolerated switch option in patients experiencing tolerability or toxicity issues. Maraviroc, the only currently licensed CCR5 antagonist, was associated with low levels of toxicity in studies of treatment-naive [2] and treatment-experienced [3] patients; CXCR4 antagonists are in much earlier stages of development. HIV can be classified by coreceptor use: R5-tropic (uses only the CCR5 receptor to enter cells), X4-tropic (uses only CXCR4), and dual/mixed (able to use both coreceptor types). Use of maraviroc requires the presence of R5-tropic virus, and until recently, the only recommended method for tropism determination was by phenotypic assay (Trofile; Monogram Biosciences). Phenotyping requires HIV RNA and therefore cannot be performed in patients with suppressed viremia. Genotypic algorithms based on samples of peripheral blood mononuclear cells (PBMCs) can be performed in subjects with undetectable HIV RNA, and recent consensus guidelines now recommend phenotypic and genotypic methods for tropism determination [4, 5].

We hypothesized that a change in HIV tropism during periods of suppressive ART would be uncommon and that a phenotypic tropism test on a stored sample prior to treatment initiation could provide a reliable guide to tropism during suppressive therapy. A previous study comparing tropism before treatment (ascertained by 2 genotypic algorithms, Geno2pheno and PSSM, on plasma and PBMC samples) and after 48 weeks of suppressive ART (genotypic algorithms on PBMC samples only) showed that 1 of 34 patients experienced tropism change (from R5-tropic to dual/mixed) [6]. To study the evolution of phenotypic tropism during ART, we selected patients who had interrupted therapy with of a suppressed viral load in order...
to compare Trofile results on stored plasma samples collected before and after highly active ART (HAART).

METHODS

At our center, data including demographics, disease characteristics, treatment history, and surrogate markers (including CD4 cell count and HIV RNA level) are entered on a prospectively collected database. We used this database to identify patients who achieved viral suppression after receiving at least 12 weeks of continuous ART (excluding any CCR5-containing regimens) and then had an interruption of therapy for reasons other than virological failure. Viral suppression was defined as an HIV RNA level of <50 copies/mL, although viral blips (defined as a single viral load of up to 500 copies/mL preceded and followed by a viral load of <50 copies/mL) were permitted. All patients had stored plasma samples frozen at −80°C available from within 3 months of initiating their regimen and within 3 months of stopping their drugs. These frozen samples were tested by the Trofile ES, and tropisms before and after suppressive therapy were compared.

The following information was collected: age, sex, year of diagnosis, treatment history (including line of therapy, history of prior virological failure, and history of mono/dual nucleoside reverse transcriptase inhibitor therapy), and viral load before, during, and after interruption of ART. Viral loads in samples yielding successful and failed Trofile results were compared using Mann-Whitney nonparametric testing.

RESULTS

Thirty-seven patients (33 male and 4 female) with stored plasma samples available from within 3 months of starting and 3 months of interrupting suppressive ART were identified. All had been receiving therapy for at least 12 weeks with a mean duration of 862 d (range, 120–2,708 d). Reasons for interrupting ART included pregnancy (in which case ART had been initiated for prevention of mother-to-child transmission), participation in a clinical trial, and patient choice (predominantly due to adverse events).

Of the 74 samples, 19 did not amplify (assay failure rate, 26%), leaving 55 patients with paired amplifiable samples. Failed assays were equally divided across the pre-ART and post-ART samples. Most sample volumes were less than the 3 mL usually required for Trofile testing (mean volume, 1 mL). There were no differences in storage time between the samples that did amplify and those that did not; the median time from sample collection for samples measured 82 months (range, 3–123 months) and 68 months (range, 3–123 months) for samples that did and did not amplify, respectively. However, the difference in viral load between amplifiable and nonamplifiable samples was significant at

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with R5-tropic virus (n = 18)</th>
<th>Patients with dual/mixed or X4-tropic virus (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, no. (%)</td>
<td>13 (72)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>Median age, years</td>
<td>36</td>
<td>38</td>
</tr>
<tr>
<td>Median CD4 cell count, cells/μL</td>
<td>283</td>
<td>161</td>
</tr>
<tr>
<td>Median nadir CD4 cell count, cells/μL</td>
<td>245</td>
<td>84</td>
</tr>
<tr>
<td>Median duration of HIV infection, months</td>
<td>76.3</td>
<td>109.6</td>
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<tr>
<td>Median no. of lines of therapy (range)</td>
<td>1 (1–7)</td>
<td>5 (1–11)</td>
</tr>
<tr>
<td>Median no. of virological failures (range)</td>
<td>0 (0–4)</td>
<td>2 (0–6)</td>
</tr>
</tbody>
</table>

69,729 (4.84 log_{10}) copies/mL compared with 12,412 (4.09 log_{10}) copies/mL, respectively (P = .001). In addition, all successful Trofile assays were performed on samples with a viral load of >1,000 copies/mL, whereas 2 of 19 failed Trofile tests were performed on samples with a viral load of <1,000 copies/mL (viral load of failed test samples, 626 and 639 copies/mL, respectively).

The median line of therapy at the time of the study was second line (range, 1–11 lines of therapy), and the median number of previous virological failures was 5 (range, 0–6 virological failures). Baseline characteristics of patients with R5-tropic virus or dual/mixed (DM) or X4-tropic virus at the baseline test (before suppressive ART) are described in Table 1.

Of the 26 individuals whose samples were analyzed, 24 retained the same viral tropism after treatment interruption: 17 of 18 with R5-tropic virus at baseline rebounded with R5-tropic virus and 7 of 8 with DM or X4-tropic baseline HIV rebounded with the same strain. One patient with baseline R5-tropic virus rebounded with DM or X4-tropic virus, and 1 patient with a baseline DM or X4-tropic viral population rebounded with R5-tropic virus. Both patients who experienced tropism change were highly treatment experienced (seventh and eighth line of therapy and 2 and 4 prior virological failures, respectively). Most patients maintained a viral load of <50 copies/mL during therapy and preceding interruption (including the 2 patients with tropism switch); 5 individuals experienced blips (defined as a viral load of 50–500 copies/mL preceded and followed by a viral load of <50 copies/mL).

DISCUSSION

The need to determine tropism limits the use of CCR5 antagonists in patients receiving suppressive ART. Despite marked improvements over recent years in terms of ART tolerability,
dosing, and pill burden, adverse events remain the main reason for switching ART [1] and are associated with poor adherence [7]. Switching away from the offending agent may limit toxicity while maintaining efficacy [8]. Maraviroc was noninferior to efavirenz when the Maraviroc versus Efavirenz in Treatment-Naïve Patients (MERIT) Study was reanalyzed using the more sensitive version of the Trofile assay (Trofile ES) [9] and performed similarly to efavirenz among patients with a baseline viral load of <100,000 copies/mL in the original analysis [2]. Data for other CCR5 antagonists in this setting are lacking. Maraviroc was associated with fewer reports of dizziness and abnormal dreams, although the overall rates of adverse events and category C events were similar [2]. Finally, maraviroc was associated with a significantly higher increase in CD4 cell count than that for efavirenz at 48 weeks [9], and it may confer a CD4 cell count benefit even in patients with DM or X4-tropic virus [10]. The long-term clinical benefit, if any, is unclear, but a recent analysis demonstrated that longer duration with a CD4 cell count of >500 cells/μL correlated with lower mortality rates [11].

The need to determine tropism may limit the utility of CCR5 antagonists as a switch option in individuals with suppressed viremia. However, our data confirm the findings of previous studies showing that tropism change is uncommon during periods of viral suppression, although it is very difficult to make comparisons between studies. An alternative to phenotypic testing is the use of genotypic algorithms to predict coreceptor use. These genotypic tests can be performed on proviral DNA and therefore do not depend on detectable HIV RNA, and a retrospective analysis of the Maraviroc versus Optimized Therapy in Viremic Antiretroviral Treatment-Experienced Patients Study (maraviroc vs placebo with optimized background therapy in treatment-experienced individuals) showed that geno2pheno, a genotypic algorithm, predicted virological response to maraviroc as reliably as a phenotypic method (Trofile) [12]. In addition, a post hoc analysis of the MERIT Study, a comparison of maraviroc and efavirenz in treatment-naive subjects, demonstrated that genopheno predicted virological response to maraviroc as well as the enhanced sensitivity Trofile assay [13]. Limitations of this study include its retrospective design, the small number of subjects, and the relatively high assay failure rate; several stored samples were of suboptimal volume, which may explain this.

Both of the patients who demonstrated a change in viral tropism after suppressive HAART were highly treatment experienced. We conclude that Trofile testing of stored samples, in combination with clinical history, can reliably predict tropism in patients receiving suppressive HAART.

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References