Response to Vicriviroc in Treatment-Experienced Subjects, as Determined by an Enhanced-Sensitivity Coreceptor Tropism Assay: Reanalysis of AIDS Clinical Trials Group A5211

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The enhanced-sensitivity Trofile assay (Monogram Biosciences) was used to retest coreceptor use at both study screening and study entry for 118 treatment-experienced subjects in AIDS Clinical Trials Group A5211 who had CCR5-tropic (R5) virus detected by the original Trofile assay at study screening. Among 90 recipients of vicriviroc, a significantly greater mean reduction in HIV-1 RNA was observed in 72 subjects with R5 virus versus 15 subjects reclassified as having dual/mixed-tropic viruses at screening: −1.11 versus −0.09 log10 copies/mL at day 14 and −1.91 versus −0.57 log10 copies/mL at week 24, respectively. Results suggest that the enhanced-sensitivity assay is a better screening tool for determining patient eligibility for CCR5 antagonist therapy.

Entry of human immunodeficiency virus (HIV) into target cells requires 3 steps: viral attachment to the CD4 receptor, viral attachment to a chemokine receptor (CCR5 or CXCR4), and viral cell membrane fusion [1]. CCR5 antagonists are designed to prevent entry of HIV into target cells by blocking a functional interaction with the CCR5 coreceptor [2]. The Trofile HIV coreceptor tropism assay (Monogram Biosciences) determines whether the viral population of a patient uses the CCR5 or CXCR4 coreceptor exclusively (R5 or X4 virus, respectively) or whether it consists of a population of dual-tropic (R5X4) or mixed-tropic viruses (ie, dual/mixed [DM] virus) [3]. This assay is useful for selecting appropriate patients to receive treatment with CCR5 antagonists.

The original tropism assay was validated to detect minority X4 variants comprising 10% and 5% of a population with 100% and 85% sensitivity, respectively, by use of mixtures of plasmids carrying different HIV type 1 (HIV-1) envelopes [3]. Low-level CXCR4-using (X4 or dual-tropic) variants below the detection limit of the original assay are better identified by an enhanced version of the assay validated to detect as little as 0.3% of X4 variants with 100% sensitivity [4–6].

Vicriviroc is a CCR5 antagonist that potently inhibits R5 HIV-1 in vitro and in vivo [7]. We previously reported that, in AIDS Clinical Trials Group (ACTG) A5211, a total of 10 vicriviroc recipients with DM virus detected at study entry had significantly reduced virologic responses, compared with 71 vicriviroc recipients with R5 virus detected at entry, as determined by the original tropism assay [8]. We recently used the enhanced-sensitivity tropism assay to retest the samples obtained at screening and study entry from individuals enrolled in ACTG A5211. The key virologic and immunologic end points of the study were reanalyzed based on tropism classifications by the enhanced assay, to determine whether the enhanced tropism assay would better identify patients who might benefit from CCR5 antagonist therapy.

Subjects and methods. The design of ACTG A5211 has been described in detail elsewhere [8]. In brief, the study was a double-blind, randomized study of vicriviroc in treatment-experienced subjects with a 48-week follow-up. Within 6 weeks

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before study entry, subjects were screened for R5 virus by use of the original Trofile assay. At study entry, eligible subjects were randomly assigned to receive either 1 of 3 doses of vicriviroc (5, 10, or 15 mg given once daily; Schering-Plough Research Institute) or placebo in addition to their failing background antiretroviral regimen that contained ritonavir (100–800 mg/day). After 14 days, subjects continued receiving their randomized, double-blind study treatment and started receiving a new optimized antiretroviral regimen that contained ritonavir.

The original tropism assay was used to determine viral coreceptor use for ACTG A5211 participants at study screening; entry; day 14; weeks 8, 24, and 48; and, if applicable, at the time of confirmation of virologic failure, which was defined as a confirmed plasma HIV-1 RNA level of <1 log_{10} copies/mL (Ultrasensitive Roche Amplicor HIV-1 Monitor assay) below the baseline level noted at or after week 16 of the study. Patient envelope expression vectors generated from samples obtained at study screening and study entry from subjects enrolled in ACTG A5211 were retested using the enhanced-sensitivity Trofile assay [6]. Written informed consent was obtained from study participants. Human experimentation guidelines of the US Department of Health and Human Services were followed in the conduct of this research.

The Wilcoxon rank-sum test and Fisher’s exact test were used to compare the characteristics of the subjects at baseline. Changes in the HIV-1 RNA level (expressed as the number of log_{10} copies/mL) were analyzed using linear regression for censored data, to account for values below the quantification limit of the assay (50 copies/mL), and were adjusted for the log_{10} HIV-1 RNA level at baseline and each of 3 stratification factors used in the randomization (ie, previous enfuvirtide use, anticipated enfuvirtide use, and a CD4 cell count of <50 cells/mm³). The proportions of subjects achieving various HIV-1 RNA thresholds were compared using Fisher’s exact test. An intent-to-treat approach was used. Analysis of changes in the CD4 cell count followed the same approach used for analysis of changes in the log_{10} HIV-1 RNA level. All tests were 2-sided. P < .05 was considered to denote statistical significance. The analyses were performed using SAS software (version 9.1; SAS Institute).

**Results.** ACTG A5211 enrolled 118 subjects who were found to have R5 virus at study screening by use of the original tropism assay [8]. Among subjects who had tropism results available from the enhanced-sensitivity assay, 89 (78%) of 114 had R5 virus and 25 (22%) of 114 were reclassified as having DM virus at study screening, and 85 (77%) of 111 subjects had R5 virus and 26 (23%) of 111 had DM virus at study entry; samples were unavailable for 4 subjects at study screening and for 7 subjects at study entry. At study entry, all 12 subjects with DM virus detected by the original assay [8] also had DM virus detected by the enhanced assay. Characteristics at baseline, including age, sex, race/ethnicity, HIV-1 RNA level at screening, CD4 cell count at screening, nadir CD4 cell count, and genotypic and phenotypic susceptibility scores at screening [9–11], were not significantly different between subjects with R5 virus at study screening and entry and those with DM virus at either study screening or entry by the enhanced assay (data not shown).

The association between coreceptor use determined by the enhanced-sensitivity assay at study screening and a change in coreceptor tropism results determined by the original assay at study entry and during the study was assessed for 90 subjects randomized to receive vicriviroc (Table 1). Fifteen vicriviroc recipients were reclassified as having DM virus at study screening, by use of the enhanced assay, and they would have been excluded from the trial if the enhanced assay had been used to determine study eligibility. Of these 15 recipients, 12 (80%) had DM/X4 viruses detected by the original assay by week 24 and would have been appropriately excluded. Of the other 3 subjects, 2 experienced virologic failure at week 16 and week 32, respectively, and the third discontinued the study at week 11 because of severe debilitation. Conversely, the original tropism assay detected DM/X4 viruses in 23 (26%) of 90 vicriviroc recipients by week 24 (Table 1). Of these 23 subjects, 12 (52%) had DM virus detected by the enhanced assay at study screening.

Eight subjects had DM virus detected at study screening, but only R5 virus was detected at study entry by use of the enhanced assay. Four of these 8 subjects were randomized to receive vicriviroc. During study follow-up, 2 of these 4 vicriviroc recipients were found to have DM virus by use of the original assay (at week 2). The other 2 recipients had R5 virus detected by the original assay during the study.

Among subjects randomized to receive vicriviroc, the mean reductions in the HIV-1 RNA level from baseline to day 14 and week 24 were significantly greater for the 72 subjects with

<table>
<thead>
<tr>
<th>Tropism detected by the enhanced assay</th>
<th>Tropism detected by the original assay between study entry and week 24a</th>
<th>Total subjects, no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>R5</td>
<td>61 (85)</td>
<td>72</td>
</tr>
<tr>
<td>DM</td>
<td>3 (20)</td>
<td>15</td>
</tr>
<tr>
<td>Missing</td>
<td>3 (100)</td>
<td>3</td>
</tr>
<tr>
<td>All</td>
<td>67</td>
<td>90</td>
</tr>
</tbody>
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a No. (%) of subjects with the specified tropism results.

b Of these 123 subjects, DM or X4 viruses were first detected at study entry for 10 subjects, at week 2 for 8, at week 8 for 2, and between week 19 and 24 for 3 subjects.
R5 virus (ie, the R5 virus group) than for the 15 subjects with DM virus (ie, DM virus group), by use of the enhanced assay at study screening: −1.11 versus −0.09 log_{10} copies/mL, respectively, at day 14 (P < .001) (Figure 1A) and −1.91 versus −0.57 log_{10} copies/mL, respectively, at week 24 (P < .001) (Figure 1B). Thirty-six subjects in the R5 virus group (55%) achieved an HIV-1 RNA level of <400 copies/mL at week 24. In contrast, only 1 subject in the DM virus group (7%) achieved an HIV-1 RNA level of <400 copies/mL (P = .001) (Figure 1C); this subject had R5 virus detected by the enhanced-sensitivity assay at study entry. Similar trends were noted in a comparison of the R5 and DM virus groups with respect to the proportions of the groups with <50 copies/mL (33% vs 7%, respectively; P = .057) at week 24 (Figure 1D) and the proportions with decreases of ≥1 log_{10} copies/mL (76% vs 29%, respectively; P = .001) (Figure 1E). Subgroup analysis showed that subjects found to have R5 virus at screening and entry by use of the enhanced tropism assay (ie, the R5_R5 group; n = 64) had greater reductions in plasma HIV-1 RNA levels at day 14 (−1.15 log_{10} copies/mL) and week 24 (−1.95 log_{10} copies/mL), compared with those found to have R5 virus at screening but DM virus at entry (ie, the R5_DM group; n = 5) (−0.66 log_{10} copies/mL at day 14 and −1.20 log_{10} copies/mL at week 24, respectively) (Figure 1A and 1B); however, these differences were not statistically significant. Four subjects (80%) in the R5_DM group achieved a viral load reduction of >1 log_{10} copies/mL at week 24, but no subjects in this group attained a viral load of <50 or <400 copies/mL (Figure 1C, 1D, and 1E).

The mean changes in the CD4 cell count at week 24 were +145 cells/mm³ for the R5 virus group and +43 cells/mm³ for the DM virus group; in the subgroup analysis, the mean changes were +140 cells/mm³ for the R5_R5 virus group and +75 cells/mm³ for the R5_DM virus group. These differences were not statistically significant in an analysis adjusted for CD4 cell count at baseline and study stratification factors (Figure 1F).

Discussion. In the present study, samples obtained from ACTG A5211 subjects who had R5 virus detected at study screening, as determined by use of the original tropism assay, were retested using an enhanced-sensitivity assay. Subjects reclassified at study screening as having DM virus by use of the enhanced assay had suboptimal virologic responses to vicriviroc, compared with those with R5 virus detected by the enhanced assay. Only one vicriviroc recipient who had DM virus detected at study screening but had R5 virus detected at study entry by the enhanced assay had a positive response to vicriviroc (achieving an HIV-1 RNA level of <400 copies/mL at week 24). Therefore, the enhanced-sensitivity tropism assay identified a subset of treatment-experienced patients who were more likely to achieve suppression of HIV-1 viremia with an antiretroviral regimen containing a CCR5 antagonist, at least through 24 weeks.

A maximum period of 6 weeks between study screening and entry visits was allowed in ACTG A5211. Differences in co-receptor use between these visits were observed for some patients by use of the original and enhanced assays. Differences in tropism calls during this time frame could denote (1) the presence of low levels of DM virus fluctuating around the limit of detection of each assay; (2) very low levels of DM virus at both screening and entry, which are only amplified at one time point because of the stochastic nature of polymerase chain reaction; or (3) a more discernable change in the relative levels of DM virus between screening and entry because of natural fluctuations in viral populations or adherence to the failing antiretroviral regimen.

Interestingly, a small number of vicriviroc recipients (n = 5) with R5 virus detected at screening and DM detected at entry by use of the enhanced assay had an intermediate reduction in viral load from baseline to day 14 and week 24, compared with subjects with R5 virus detected at screening and entry; the significance of this finding could not be ascertained because of the small sample size. However, these patients did not achieve <400 or <50 HIV RNA copies/mL at week 24. Therefore, the presence of presumably very low levels of DM virus around the limit of detection of the enhanced assay also portends reduced CCR5 antagonist efficacy.

The limitations of the present study include the fact that it represents a retrospective analysis of ACTG A5211 participants who had R5 virus detected by the original assay at study screening. Some analyses may have insufficient power because of the relatively small sample size. For example, we saw a clinically relevant but not statistically significant improvement in immunologic responses at week 24 for subjects with R5 virus, compared with those with DM virus, by use of the enhanced assay as evaluated at study screening.

Our main study findings in this treatment-experienced cohort are supported by a similar reanalysis of the Maraviroc Versus Efavirenz Regimens as Initial Therapy (MERIT) trial of the CCR5 antagonist maraviroc versus efavirenz (both in combination with coformulated zidovudine and lamivudine) in treatment-naive individuals with R5 virus detected at screening by the original tropism assay [12]. In the original study analysis, the virologic response to maraviroc did not reach a prespecified noninferiority threshold at 48 weeks. Reanalysis of MERIT samples by use of the enhanced tropism assay revealed that 15% of the study subjects had DM virus at screening. Exclusion of these subjects in a retrospective analysis showed that maraviroc would have achieved noninferiority to efavirenz in subjects with R5 virus detected at screening by the enhanced tropism assay.

In summary, the enhanced-sensitivity coreceptor tropism assay identified ACTG A5211 subjects with DM virus below the limit of detection of the original assay at study screening. These subjects experienced significantly inferior virologic responses to the CCR5 inhibitor vicriviroc. Therefore, these results, along
Figure 1. Virologic and immunologic responses (A–F), according to coreceptor use by the enhanced-sensitivity tropism assay, among subjects randomized to receive vicriviroc. Data for the 3 vicriviroc dose arms are combined. An intent-to-treat approach was used, with the last observation carried forward after virologic failure or study discontinuation. The day 14 results include 15 subjects who had DM virus at screening (the DM virus group), 72 who had R5 virus at screening (the R5 virus group), 5 who had R5 virus at screening but DM virus at entry (the R5_DM virus group), and 64 who had R5 virus at both screening and entry (the R5_R5 virus group). For the same 4 groups, at week 24 there were 14 subjects in the DM virus group, 66 in the R5 virus group, 5 in the R5_DM group, and 58 in the R5_R5 group. Of the subjects in the group that received 5 mg of vicriviroc, 7 subjects who increased their dose to 15 mg before week 24 were not included in the analysis at week 24. The 15 subjects in the DM virus group include 4 subjects who had R5 virus according to the enhanced-sensitivity assay at study entry. \( P \) values were from linear regression analysis adjusted for study stratification factors (see Subjects and Methods) and the baseline log_{10} human immunodeficiency virus type 1 (HIV-1) RNA level (for change in the HIV-1 RNA level) or CD4 cell count (for change in the CD4 cell count). Vertical bars on the graph denote the lower bounds of 95% confidence intervals.
with those from reanalysis of the MERIT trial, suggest that the enhanced tropism assay would be a better screening tool for determining the eligibility of HIV-1–infected patients to receive CCR5 antagonist therapy.

Additional members of the ACTG A5211 team and research staff. Other protocol team members: Robert Gross, Scott Hammer, Martin Hirsch, and Andrew Zolopa (co-investigators); Catherine Godfrey and Carla Pettinelli (co-medical officers); Beatrice Kallungal (clinical trials specialist); David Clifford (co-investigator and protocol neurologist); Mary Dobson (laboratory data coordinator); Antoine Simmons (laboratory technologist); Valery Hughes (field representative); Ana Martinez (protocol pharmacist); Susan Owens (data manager); and Jim Smith (community representative).

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References