Reanalysis of Coreceptor Tropism in HIV-1–Infected Adults Using a Phenotypic Assay with Enhanced Sensitivity

Timothy J. Wilkin,[1] Mathew Bidwell Goetz,[3] Robert Leduc,[5] Gail Skowron,[6] Zhaohui Su,[9] Ellen S. Chan,[8] Jayyant Heera,[10] Doug Chapman,[2] John Spritzler,[8] Jacqueline D. Reeves,[4,7] Roy M. Gulick,[1] and Eoin Coakley[4]

[1]Division of Infectious Disease, Weill Cornell Medical College, New York, New York; [2]Pfizer, New York, New York; [3]Veterans Administration Greater Los Angeles Healthcare System and Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, California; [4]Monogram Biosciences, San Francisco, California; [5]Division of Biostatistics, University of Minnesota, Minneapolis, Minnesota; [6]Division of Infectious Diseases, Roger Williams Medical Center, Providence, Rhode Island; [7]Boston University School of Medicine; [8]Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts; [9]Outcome Sciences, Cambridge, Massachusetts; and [10]Pfizer Global Research and Development, New London, Connecticut

The enhanced-sensitivity Trofile assay (TF-ES; Monogram Biosciences) was used to retest coreceptor tropism samples from 4 different cohorts of HIV-1–infected patients. Nine percent to 26% of patients with CCR5-tropic virus by the original Trofile assay had CXCR4-using virus by TF-ES. Lower CD4 cell counts were associated with CXCR4-using virus in all cohorts.

In persons not receiving combination antiretroviral therapy, viral coreceptor tropism has been associated with HIV-1 disease progression [1]. More recently, assessment of coreceptor tropism is necessary prior to initiation of CCR5 antagonist therapy. The Trofile coreceptor tropism assay (Monogram Biosciences) phenotypically characterizes a viral envelope population as using CCR5 or CXCR4 exclusively (R5 or X4, respectively) or having dual-tropic or mixed infections (DM virus) [2]. The original Trofile (TF) assay was validated to detect X4 variants composing 10% and 5% of a population with 100% and 85% sensitivity, respectively, by use of mixtures of plasmids carrying different HIV-1 envelopes [3]. This assay was modified to detect as few as .3% of X4 variants with 100% sensitivity [4–5] and is here termed Trofile–Enhanced Sensitivity (TF-ES). This report provides an analysis of available cohorts in which coreceptor tropism assessments by TF assay were retested using TF-ES. This was done to provide a more accurate understanding of the epidemiology of coreceptor tropism across a range of HIV-1–infected populations.

METHODS

We evaluated 4 cohorts in which coreceptor tropism was originally assessed using TF and retested using TF-ES. First, Community Programs for Clinical Research on AIDS (CPCRA) study 060 was an observational, noninterventional cohort study of antiretroviral therapy (ART)-naive HIV-1–infected patients, aged ⩾13 years, with a $CD4^+$ cell count ⩾ 450 cells/μL and plasma HIV-1 RNA level >1000 copies/mL [6]. Baseline coreceptor tropism was assessed to evaluate its relationship to HIV-1 progression. Second, New Works Concept Sheet (NWCS) 261R was an immunologic substudy of AIDS Clinical Trials Group (ACTG) Protocol 384, an open-label, phase 3 trial comparing 6 different initial ART regimens in HIV-1-infected adults [7]. Patients included in NWCS 261R were a nonrandom subset chosen to provide a range of baseline $CD4^+$ cell counts. NWCS 261R assessed relationships among coreceptor tropism at study entry, plasma HIV-1 RNA, $CD4^+$ cell count, replication capacity, $CD4^+$ and $CD8^+$ activation, and $CD4^+$ changes after ARTinitiation [8]. Third, MERIT (maraviroc versus efavirenz in treatment-naive patients) was a phase 3 trial of maraviroc, a CCR5 antagonist, in ART-naive adults [9]. Only samples from MERIT subjects that were randomized to twice-daily maraviroc or efavirenz were retested. Subjects who were not randomized or were randomized to once-daily maraviroc were not included. Fourth, ACTG A5211 was a phase 2 trial of vicriviroc, an investigational CCR5 antagonist, in highly treatment-experienced

Received 22 October 2010; accepted 9 December 2010.

Correspondence: Timothy J. Wilkin, MD, MPH, Division of Infectious Disease, Weill-Cornell Medical College, New York, NY 10011(tiw2001@med.cornell.edu).

Clinical Infectious Diseases 2011;52(7):925–928

1058-4838/2011/527-0001$37.00
DOI: 10.1093/cid/cir072

Table 1. Reclassification of Coreceptor Tropism in 4 Cohorts of HIV-1–Infected Subjects

Variable	CPCRA (*N*=314)	NWCS 261R (*N*=230)	MERIT (*N*=1451)	ACTG A5211 (*N*=391)
Study population	Early-stage HIV-1 infection	Chronic HIV-1 infection, prior to ART initiation	Chronic HIV-1 infection, prior to ART initiation	Highly treatment-experienced, initiating a new ART regimen
Median $CD4^+$ (cells/μL)	629	350	238	104
N with R5 by original TF	282	173	1,237	197
N (%) with TF-ES result	278 (98%)	171 (99%)	720 (58%)	190 (96%)
N (%) reclassified as DM	24 (9%)	21 (12%)	106 (15%)	49 (26%)
Coreceptor tropism prevalence (TF vs. TF-ES)				
R5 virus	90% vs. 82%	75% vs. 66%	85% vs. 73%	50% vs. 37%
DM virus	10% vs. 18%	24% vs. 33%	15% vs. 27%	46% vs. 59%
X4 virus	0% vs. 0%	1% vs. 1%	.3% vs. .3%	4% vs. 4%

NOTE. Samples sizes from each cohort are the number of participants with an available Trofile result. TF, Trofile; TF-ES, enhanced-sensitivity Trofile; R5, CCR5-using HIV-1; DM, dual-tropic or mixed HIV-1 infections; X4, CXCR4-using HIV-1 only; CPCRA, Community Programs for Clinical Research on AIDS; NWCS, New Works Concept Sheet; MERIT, Maraviroc vs. Efavirenz in Treatment-Naive Patients; ACTG, AIDS Clinical Trials Group.

patients [10–11]. Coreceptor tropism was assessed in MERIT and ACTG A5211 to determine eligibility for those trials. All subjects screening for ACTG A5211 were included.

The stored DNA test vectors defined as R5 by TF were retested using TF-ES. Samples with DM/X4 tropism were not retested. TF-ES results were only available on a subset of participants. We used the observed proportion of subjects with virus reclassified from R5 virus to DM virus to revise the overall prevalence of R5 and DM virus for the cohort. We assumed that subjects with R5 virus without TF-ES results were missing at random and that all patients with DM or X4 virus by TF would have the same result by TF-ES.

Investigators from each study team analyzed factors associated with DM/X4 virus using logistic regressions (with equal weights for all subjects). We evaluated demographic and laboratory variables common to all 4 cohorts. All variables with $P < .15$ in the univariate analyses were included in the multivariable analysis. The statistical analyses for each cohort were performed independently, and no patient-level data were shared between groups. Statistical tests were 2-sided exploratory analyses, and .05 was used for the nominal level of significance (without adjustments for multiple testing).

Written informed consents were obtained from study participants for participation in these 4 studies. Human experimentation guidelines of the U.S. Department of Health and Human Services were followed in the conduct of this research.

RESULTS

Table 1 shows the number of subjects with R5 virus by TF in each of the 4 cohorts, the proportion retested with TF-ES, and the proportion reclassified to DM virus. The table also shows the revised estimate of coreceptor tropism in these 4 cohorts and the proportion with DM or X4 according to $CD4^+$cell count. The prevalence of DM or X4 virus is now 18% for early-stage HIV-1 infection (CPCRA cohort), 34% and 27% just prior to ART initiation (NWCS 261R and MERIT, respectively), and 63% in a highly treatment-experienced population (ACTG A5211). Of note, stored DNA test vectors from 32 subjects in the CPCRA cohort with DM virus by TF were retested, and all were reported as DM virus by TF-ES.

We examined the univariate relationships of $CD4^+$T cell count, plasma HIV-1 RNA, age, sex, and race/ethnicity to the detection of DM/X4 virus by TF-ES. Higher $CD4^+$ cell counts were associated with a significantly lower prevalence of DM/X4 virus in all 4 cohorts with similar odds ratios. The odds of detecting DM/X4 virus were .7–.8 times lower for every 100 cells/μL increase in $CD4^+$cell counts and were similar in all 4 cohorts. This remained significant in the multivariable analyses. When controlling for $CD4^+$ cell counts, plasma HIV-1 RNA levels were not associated with DM/X4 virus except for the CPCRA cohort, where higher levels were associated with DM/X4 virus. Age and sex were not significantly related to coreceptor tropism in any of the cohorts. In the MERIT cohort, non-Hispanic blacks had a lower prevalence of DM/X4 virus compared with non-Hispanic whites. This was related to a high prevalence of HIV-1 subtype C among black subjects enrolled in South Africa, of which 96% had samples testing R5 by TF.

DISCUSSION

HIV-1 coreceptor tropism is a valuable tool for determining eligibility for CCR5 antagonist therapy and is predictive of HIV-

1 disease progression. In this study, we found that TF-ES detects substantially more DM virus than the original TF assay. This is most apparent in the treatment-experienced population having lower $CD4^+$ cell counts, where 26% of the population classified as R5 by TF was reclassified as DM. Use of the revised assay has led to improved observed outcomes in patients receiving CCR5 antagonists. Reanalysis of the MERIT and ACTG A5211 trials to include only those subjects having R5 virus by TF-ES revealed improved virologic outcomes to CCR5 antagonists in both studies and changed the overall interpretation of the MERIT study [9, 11]. In the revised MERIT analysis, maraviroc was found to be noninferior to efavirenz for initial treatment of HIV-1 infection.

Comparing the prevalence of DM virus across the 4 cohorts demonstrates the evolving pattern of coreceptor tropism over the course of progressive HIV-1 infection. Other studies have used deepsequencing of viral isolates from subjects with primary HIV-1 infection to isolate the single or few transmitted viruses in a given patient. These transmitted viruses rarely use CXCR4 as predicted by a phenotypic assay (54 of 55 transmitted viruses were R5) [12]. Our study suggests that CXCR4-using HIV-1 emerges early during the course of infection as part of a mixed viral population and the prevalence increases in the face of ongoing viral replication and $CD4^+$ cell decline particularly in highly treatment-experienced subjects. CCR5 antagonists do not demonstrate significant virologic activity in patients with DM virus [13]. Taken together this suggests that CCR5 antagonists are more likely to be useful earlier in HIV-1 infection and highlights that coreceptor tropism testing should be conducted just prior to initiating CCR5 antagonists.

This study has several limitations. Samples having DM virus by TF in ACTG A5211, NWCS 261R, and MERIT were not retested using TF-ES. It is possible that some of these samples could have been reclassified as R5, although none of the 32 CPCRA DM samples were reclassified as R5. We did not combine patient-level data across the 4 studies, and demographic data available for analysis were limited. All of these studies had entry criteria that limit the generalizability of these results.

In summary, this study shows that the prevalence of CXCR4-using HIV-1 varies from 18% in early-stage HIV-1 infection to 63% in a highly treatment-experienced population with advanced HIV-1 infection. This is appreciably higher than previous estimates. $CD4^+$ cell count was the only consistent predictor of coreceptor tropism.

Acknowledgments

We would like to acknowledge the patient volunteers, DAIDS/NIAID/NIH, other ACTG 384 and A5211 team members, CPCRA/INSIGHT co-investigators, the Pfizer/MERIT study staff, and clinical research sites participating in the CPCRA/INSIGHT Long-Term Monitoring Study, ACTG 384, MERIT, and ACTG A5211.

Financial Support. The CPCRA and INSIGHT networks are funded by the National Institutes of Health (U01 AI-42170, U01 AI-46362, U01 AI-68641). The ACTG is funded by the National Institutes of Health (U01 AI-68636 and AI-68634 [Statistical and Data Analysis Center]). Other grants that funded coauthors include K23 AI55038 (to T.J.W.), K24 AI51966 (to R.M.G.), AI-69419 (Cornell Clinical Trials Unit), RR024996 (Cornell CTSC), and AI-46381 (Miriam Hospital Clinical Research Site). Monogram Biosciences received an SBIR grant from the National Institutes of Health (R44AI050321).

Potential conflicts of interest. T.J.W. has served as a paid consultant to Pfizer and Quest Diagnostics. M.B.G. receives grant support from Monogram. G.S. has received honoraria from ViiV Healthcare and Boehringer-Ingelheim and has served as a paid consultant to Tobira Pharmaceuticals. Z.S. has served as a paid consultant to Pfizer and Quest Diagnostics. J.H. and D.C. are employed by and have stock in Pfizer, Inc. J.D.R. and E.C.are employed by Monogram Biosciences. R.M.G. served as an adhoc consultant to ViiV Pharmaceuticals. R.L., E.S.C., and J.S. have no conflicts to report.

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