Reduced Baseline Sensitivity to Maraviroc Inhibition Among R5 HIV-1 Isolates From Individuals With Severe Immunodeficiency

To the Editors:

The recognition that the chemokine receptors CCR5 and CXCR4 act as essential receptors for the entry of human immunodeficiency virus type 1 (HIV-1) into CD4+ target cells has provided the basis for new treatment strategies. Although HIV-1 with CCR5 restricted phenotypes (R5) predominate during asymptomatic infection, viruses with the ability to use CXCR4 (R5X4 or X4) emerge in 13%–76% of individuals during disease progression.1–3 A growing bulk of evidence has also revealed that individuals with low CD4+ T-cell counts at late-stage disease, where a switch to CXCR4 tropism has not occurred, can harbor R5 viruses that are distinct from R5 viruses isolated at earlier disease stages.4–11 Importantly, R5 virus isolates from individuals with low CD4+ T-cell counts have been found less sensitive to in vitro inhibition by natural CCR5 ligands and the CCR5 antagonist TAK-779.3–8,11 Through the use of CXCR4/CCR5 chimeric receptors, we previously showed that this correlated with an altered use of CCR5, including a decreased dependency on the native N-terminus of CCR5 for target cell entry.6,11

Maraviroc (MVC) interacts with CCR5 and is currently the only CCR5 antagonist approved for the treatment of patients infected with R5 viruses.12,13 Before the initiation of therapy, it is recommended to perform tropism testing, in order to exclude the presence of naturally resistant R5X4 or X4 virus variants. However, also R5 viruses can display resistance to CCR5 antagonists, including isolates from treatment-naive individuals.14,15 Furthermore, alterations in baseline sensitivity to CCR5 antagonists in vitro may be of relevance for the clinical utilization of MVC.

As cross-resistance between different CCR5 antagonists is highly unpredictable,16–21 and MVC is the only approved compound for clinical use, we set out to study whether our previous findings on reduced TAK-779 sensitivity at low CD4+ T-cell levels8,11 also applied to MVC. Primary R5 isolates derived from plasma of 17 HIV-1–infected patients with varying CD4+ T-cell counts at the time of virus isolation were evaluated for their ability to infect phytohaemagglutinin-stimulated peripheral blood mononuclear cells in the presence of increasing MVC concentrations (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/A763). All isolates could be completely inhibited by MVC, ie, no isolate could be defined as MVC resistant. However, although the MVC inhibitory concentrations varied considerably between the virus isolates, we found an inverse correlation between CD4+ T-cell counts at the time of virus isolation and MVC IC90 values (r = −0.64, P = 0.007, Fig. 1A). Similar results were obtained when correlating MVC IC50 values and CD4+ T-cell counts (data not shown). It has been suggested that phenotypic resistance assays should include determination of IC90 because 10%–15% residual replication of resistant mutants have been detected at drug concentrations several magnitudes higher than the IC50 value.22 Moreover, because the presence of virus variants with reduced sensitivity within heterogeneous virus isolates likely impact the upper part of the response curve (see Figure S1, Supplemental Digital Content, http://links.lww.com/QAI/A763), IC90 values may better detect the presence of virus variants in clinical samples with reduced sensitivity to MVC compared with IC50.

Our studies also showed that reduced baseline sensitivity to MVC was a common finding for R5 isolates from individuals with AIDS, whereas isolates from individuals without AIDS generally were highly sensitive to MVC, P = 0.004 (Fig. 1B). These findings suggest that reduced baseline sensitivity to in vitro inhibition by MVC is a common feature also for R5 isolates from patients in late stage disease. These results are also in line with a previous study showing that late-stage macrophage-tropic R5 Env pseudoviruses displayed reduced sensitivity to MVC.23

The clinical relevance of shifts in R5 virus sensitivity to MVC in vitro is unclear. Reduced levels of MVC in cerebrospinal fluid reflect a relatively poor penetration of the compound to the central nervous system, where modest reductions in viral sensitivity to MVC may result in insufficient viral suppression.24 Furthermore, in vitro selection studies have shown that parental viruses of 2 MVC resistant clones had 3–100 times higher baseline MVC IC90 values than 3 isolates that did not develop resistance under the same conditions.21 Thus, at least in vitro, reduced baseline sensitivity to CCR5 antagonists may favor the development of fully resistant R5 viruses.

In a previous study, we dissected the mode of CCR5 use of the R5 isolates analyzed in this study.11 Interestingly, by combining results from our previous study with MVC sensitivity results obtained here, we found that R5 isolates with a reduced viral dependency on the CCR5 N-terminus were less sensitive to MVC inhibition (data not shown). In support of this observation, macrophage-tropic isolates less dependent on the CCR5 N-terminus have been reported to display reduced MVC sensitivity.25 In contrast, noncompetitive and high-grade resistance has been attributed to an enhanced ability of the virus to use the N-terminus of drug-bound CCR5 receptors.15,26 However, exceptions from this emerging paradigm exist, underscoring the complexity of the mechanisms involved in CCR5 antagonist resistance.15,26–28 An

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In the recent MOTIVATE studies, analysis of HIV-1 V3 sequences collected before treatment initiation showed that 4L, 11R, and 19S polymorphisms were the only V3 polymorphisms that were associated with virologic failure. Whether these polymorphisms are related to alterations in susceptibility to MVC in vitro has not been investigated. To determine whether any of the R5 isolates displayed polymorphisms previously related to virologic failure during MVC treatment, the env gp120 V1–V3 region of the analyzed R5 isolates was amplified, cloned, and sequenced (see Supplemental Digital Content, http://links.lww.com/QAI/A763). The 4L and 19S polymorphisms were rare, occurring only in 1%–2% of V3 sequences from individuals in various disease stages. In our data set, these polymorphisms were found in 2 of the 3 least MVC sensitive isolates and in 2 of 9 individuals with severe immunodeficiency, suggesting that they are more common late in the disease. However, further studies on the role of the 4L and 19S polymorphisms as predictors for virologic failure at MVC treatment are needed.

In conclusion, we believe that decreased R5 HIV-1 baseline sensitivity to CCR5 antagonists displayed by isolates from individuals with severe immunodeficiency maybe clinically relevant. In line with this have low CD4+ T-cell counts previously been shown to be an independent risk factor for treatment failure in antiretroviral regimens including MVC. Recent results from the MODERN study also showed that an inferior treatment outcome among individuals receiving ritonavir-boosted darunavir combined with MVC, as compared with tenofovir/emtricitabine, was specifically pronounced in patients with low CD4 T-cell count and high viral load. We believe that our in vitro observation that non-AIDS R5 isolates generally were highly sensitive to MVC provides theoretical support for in vivo studies, suggesting a benefit of earlier initiation of CCR5 antagonist treatment rather than later. Not only because the risk of the development of CXCR4 using virus variants increases but also due to the emergence of HIV-1 R5 viruses with reduced baseline sensitivity to MVC during severe immunodeficiency.

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REFERENCES

1. Esbjörnsson J, Mansson F, Martinez-Arias W, et al. Frequent CXCR4 tropism of HIV-1 subtype A and CRF02_AG during late-stage disease-indication of an evolving epidemic in West Africa. Retrovirology. 2010;7:23.

2. Fenyo EM, Esbjörnsson J, Medstrand P, et al. Human immunodeficiency virus type 1 biological variation and coreceptor use: from concept to clinical significance. J Intern Med. 2011;270:520–531.

3. Jansson M, Popovic M, Karlsson A, et al. Sensitivity to inhibition by beta-chemokines correlates with biological phenotypes of primary HIV-1 isolates. Proc Natl Acad Sci U S A. 1996;93:15382–15387.

4. Jansson M, Backstrom E, Bjornad A, et al. Coreceptor usage and RANTES sensitivity of non-syncytium-inducing HIV-1 isolates obtained from patients with AIDS. J Hum Virol. 1999;2:325–338.

5. Koning FA, Kwa D, Boerse-Nunnink B, et al. Decreasing sensitivity to RANTES (regulated on activation, normally T cell-expressed and secreted) neutralization of CC chemokine receptor 5-using, non-syncytium-inducing virus variants in the course of human immunodeficiency virus type 1 infection. J Infect Dis. 2003;188:864–872.

6. Karlsson I, Antonsson L, Shi Y, et al. Coevolution of RANTES sensitivity and mode of CCR5 receptor use by human immunodeficiency virus type 1 of the R5 phenotype. J Virol. 2004;78:11807–11815.

7. Gray L, Sterjojvski J, Churchill M, et al. Uncoupling coreceptor usage of human immunodeficiency virus type 1 (HIV-1) from macrophage tropism reveals biological properties of CCR5-restricted HIV-1 isolates from patients with acquired immunodeficiency syndrome. Virology. 2005;337:384–398.

8. Repits J, Oberg M, Esbjörnsson J, et al. Selection of human immunodeficiency virus type 1 R5 variants with augmented replicative capacity and reduced sensitivity to entry inhibitors during severe immunodeficiency. J Gen Virol. 2005;86:2859–2869.

9. Borggren M, Repits J, Kuylenstierna C, et al. Evolution of DC-SIGN use revealed by fitness studies of R5 HIV-1 variants emerging during AIDS progression. Retrovirology. 2008;5:28.

10. Repits J, Sterjojvski J, Badia-Martinez D, et al. Primary HIV-1 R5 isolates from end-stage disease display enhanced viral fitness in parallel with increased gp120 net charge. Virology. 2008;379:125–134.

11. Karlsson U, Antonsson L, Repits J, et al. Mode of coreceptor use by R5 HIV-1 correlates with disease stage: a study of paired plasma and cerebrospinal fluid isolates. AIDS Res Hum Retroviruses. 2009;25:1297–1305.

12. Gulick RM, Lalezari J, Goodrich J, et al. Maraviroc for previously treated patients with R5 HIV-1 infection. N Engl J Med. 2008;359:1429–1441.

13. Tan Q, Zhu Y, Li J, et al. Structure of the CCR5 chemokine receptor-HIV entry inhibitor maraviroc complex. Science. 2013;341:1378–1390.

14. Kritisos KM, Amrine-Madsen H, Irbeck DM, et al. Virologic failure in therapy-naive subjects on aplaviroc plus lopinavir-ritonavir: detection of aplaviroc resistance requires clonal analysis of envelope. Antimicrob Agents Chemother. 2009;53:1124–1131.

15. Tilton JC, Amrine-Madsen H, Miamidian JL, et al. HIV-1 type I from a patient with baseline resistance to CCR5-antagonists uses drug-bound receptor for entry. AIDS Res Hum Retroviruses. 2010;26:13–24.

16. Armand-Ugon M, Moncunill G, Gonzalez E, et al. Different selection patterns of resistance and cross-resistance to HIV-1 agents targeting CCR5. J Acquir Immune Defic Syndr. 2010;55:417–424.

17. Baba M, Miyake H, Wang X, et al. Isolation and characterization of human immunodeficiency virus type 1 resistant to the small-molecule CCR5 antagonist TAK-652. Antimicrob Agents Chemother. 2007;51:707–714.

18. Marozsan AJ, Kuhmann SE, Morgan T, et al. Generation and properties of a human immunodeficiency virus type 1 isolate resistant to the small molecule CCR5 inhibitor, SCH-417690 (SCH-D). Virology. 2005;338:182–199.

19. Pugach P, Ketas TJ, Michael E, et al. Neutralizing antibody and anti-retroviral drug sensitivities of HIV-1 isolates resistant to small molecule CCR5 inhibitors. Virology. 2008;377:401–407.

20. Trikola A, Kuhmann SE, Strizki JM, et al. HIV-1 escape from a small molecule, CCR5-specific entry inhibitor does not involve CXCR4 use. Proc Natl Acad Sci U S A. 2002;99:395–400.

21. Westby M, Smith-Burchnell C, Mori J, et al. Reduced maximal inhibition in phenotypic susceptibility assays indicates that viral strains resistant to the CCR5 antagonist maraviroc utilize inhibitor-bound receptor for entry. J Virol. 2009;83:2270–2273.

22. Geipel A, Seiz PL, Niekamp H, et al. Entecavir allows an unexpectedly high residual replication of HIV-1 infected patients. J Acquir Immune Defic Syndr. 2008;51:2504–2507.

23. Dorr P, Westby M, Dobbs S, et al. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. Antimicrob Agents Chemother. 2007;51:4721–4727.

24. Yilmaz A, Watson V, Else L, et al. Cerebrospinal fluid maraviroc concentrations in HIV-1 infected patients. AIDS. 2009;23:2537–2540.

25. Sterjojvski J, Roche M, Churchill MJ, et al. An altered and more efficient mechanism of CCR5 engagement contributes to macrophage tropism of CCR5-using HIV-1 envelopes. Virology. 2010;404:269–278.

26. Roche M, Jakobsen MR, Sterjojvski J, et al. HIV-1 escape from the CCR5 antagonist maraviroc associated with an altered and less-efficient mechanism of gp120-CCR5 engagement that attenuates macrophage tropism. J Virol. 2011;85:4330–4342.

27. Ogert RA, Hou Y, Ba L, et al. Clinical resistance to vicriviroc through adaptive V3 loop mutations in HIV-1 subtype D gp120 that alter interactions with the N-terminus and ECL2 of CCR5. Virology. 2010;400:145–155.

28. Berro R, Sanders RW, Lu M, et al. Two HIV-1 variants resistant to small molecule CCR5 inhibitors differ in how they use CCR5 for entry. PLoS Pathog. 2009;5:e1000548.

29. Moore JP, Kuirzikes DR. A piece of dance: how HIV-1 escapes small molecule CCR5 inhibitors. Curt Opin HIV AIDS. 2009;4:118–124.

30. Kuhmann SE, Pugach P, Kunsten KN, et al. Genetic and phenotypic analyses of human immunodeficiency virus type 1 escape from a small-molecule CCR5 inhibitor. J Virol. 2006;80:2980–2987.

31. Anastassopoulou CG, Ketas TJ, Klasse PJ, et al. Resistance to CCR5 inhibitors caused by sequence changes in the fusion peptide of HIV-1 gp41. Proc Natl Acad Sci U S A. 2009;106:5318–5323.

32. Lewis M, Mori J, Simpson P. Changes in V3 loop sequence associated with failure of maraviroc treatment in patients enrolled in the MOTIVATE 1 and 2 trials. Program and abstracts of the 15th Conference on Retroviruses and Opportunistic Infections; February 3–6, 2008; Boston, Massachusetts. Abstract 871.

33. Ogert RA, Wojcik L, Buontempo C, et al. Mapping resistance to the CCR5 co-receptor antagonist vicriviroc using heterologous chimeric HIV-1 envelope genes reveals key determinants in the C2-V5 domain of gp120. Virology. 2006;337:387–399.

34. Lewis M, Simpson P, Delonge C. Genotypic analysis of the HIV-1 gp120 V3 loop for treatment-experienced patients enrolled into the MOTIVATE studies and who received maraviroc + optimized background therapy. 17th CROI Conference on the N-terminus and ECL2 of CCR5. Retroviruses and Opportunistic Infections; February 16–19, 2010; San Francisco CA.

35. Seclen E, Gonzalez Mdel M, Lapaz M, et al. Primary resistance to maraviroc in a large set of R5-V3 viral sequences from HIV-1-infected patients. J Acquir Immune Defic Syndr. 2010;53:259–267.

36. Schapiro JM, Boucher CA, Kuirzikes DR, et al. Baseline CD4(+)* T-cell counts and weighted background susceptibility scores strongly predict response to maraviroc regimens in treatment-experienced patients. Antivir Ther. 2011;16:395–404.
Hepatitis C Virus Antibody Testing: Result Availability at Time of Discharge for Emergency Department Patients

To the Editors:

The Centers for Disease Control and Prevention recommend targeted hepatitis C virus (HCV) screening in health care settings including emergency departments (EDs). In April 2014, we integrated triage nurse HCV screening and adjunctive physician diagnostic HCV testing into ED clinical operations, using a laboratory-based testing protocol and native staffing to offer, perform, and disclose results. Because of concerns regarding the potential impact of HCV screening on ED throughput, our protocol did not require patients to wait for the results of their HCV tests before discharge. An accurate understanding of ED length of stay in relation to HCV test turnaround times, however, is needed to better inform screening policies and procedures.

We performed a retrospective cohort study to determine the proportion of ED patients tested for HCV whose test results were available before discharge in an attempt to quantify the impact of our policy of not holding patients in the ED pending their HCV test result. We compared prospectively collected timestamped laboratory data with timestamped hospital admission and discharge times. We used logistic regression to determine factors associated with HCV test result availability before patient discharge. The study received hospital institutional review board approval with a waiver of written informed consent.

Highland Hospital is an urban teaching hospital and trauma center with an accredited emergency medicine residency program in Oakland, CA. The annual ED census is 90,000 patients, 45% are Black, 44% are women, and 85% have public insurance. Patients presenting for care are triaged in a non-private centralized area and designated for treatment in either the main ED (70%) or the Fast Track (FT) (30%). All blood is sent by tube system and processed immediately by the laboratory. Anti-HCV-antibody tests are performed on the Abbott Architect (Abbott Laboratories, Abbott Park, IL) with a laboratory median turnaround time of 70 minutes. The median laboratory turnaround time for complete blood count (CBC) testing is 22 minutes.

Data routinely collected during an ED visit, including demographic information and timestamped laboratory and discharge data, were exported to spreadsheets (Microsoft Excel 2007; Microsoft Corporation, Redmond, WA). Patient-specific laboratory data, including reason for HCV-antibody testing, results of HCV testing, and whether a CBC test was performed (a surrogate for other blood testing), were captured from the laboratory electronic medical record (Novius, Siemens Corporation) and linked to the spreadsheet by means of patient account numbers. Patient identifying information was then removed and each visit was assigned a unique study number.

The primary outcome is the proportion of HCV-tested ED patients whose tests results were available before discharge. Order time was obtained from the timestamp generated when a patient leaves the department for admission or leaves the department for admission or discharge, and result availability time was obtained from the timestamp generated when the laboratory uploads the result electronically to the electronic medical record. We dichotomized HCV tests as being received in the laboratory either < or ≥30 minutes from the time the test was ordered.

Visit level data are presented and descriptive analyses were performed for all variables. Continuous data are reported as medians with interquartile ranges (IQRs) and categorical data are reported as numbers and percentages. We excluded patients with missing discharge or admission timestamp data and those who eloped or left against medical advice. Bivariate analyses were performed to explore the relationships between various visit characteristics and having the HCV-antibody result available before discharge. We then specified logistic regression models to explore relationships between variables believed to plausibly affect result availability, using HCV test results available before discharge as the dependent variable. All statistical analyses were performed using Statat version 13 (StatCorp LP, College Station, TX). This study is supported by a grant from Gilead Sciences. The funding agency had no role in study design, results interpretation, or manuscript preparation.

From April 2014 through March 2015, the medical center recorded 83,721 visits to the ED and 3360 HCV-antibody tests were performed of which 363 (10.8%) were anti-HCV-antibody positive. The mean age of HCV-tested patients was 47.9 years (SD = 13.2), 1844 (55%) were men, 1617 (48%) were Black, 161 (5%) were homeless, 2414 (72%) received care in the ED, 2885 (86%) were discharged home, and 1620 (48%) also had a CBC test performed. Patients in the main ED were more likely to test HCV-antibody positive than FT patients [ED prevalence 11.6% (280/2414) vs. FT prevalence 8.8% (83/940), P = 0.02].

Hepatitis C virus test results were available in the electronic medical record before discharge for 1797 of the 3360 (53%) HCV-tested patients. Of the 1563 patients tested for HCV whose test results were available before discharge, the result was positive for 252 (15.3%). Of the 1215 patients whose HCV test results were not available before discharge, 201 (16.5%) patients received the result before discharge. The proportion of patients who eloped or left against medical advice was not significantly different for patients whose HCV results were available before discharge compared to those who did not receive the result before discharge.