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.[1,2] 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.[3–11] 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, we set out to study whether our previous findings on reduced TAK-779 sensitivity at low $CD4^+$ T-cell levels[8,11] 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 IC_{90} values ($r = -0.64$, $P = 0.007$, Fig. 1A). Similar results were obtained when correlating MVC IC_{50} values and $CD4^+$ T-cell counts (data not shown). It has been suggested that phenotypic resistance assays should include determination of IC_{90} because 10%–15% residual replication of resistant mutants have been detected at drug concentrations several magnitudes higher than the IC_{50} 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), IC_{90} values may better detect the presence of virus variants in clinical samples with reduced sensitivity to MVC compared with IC_{50}.

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 IC_{90} 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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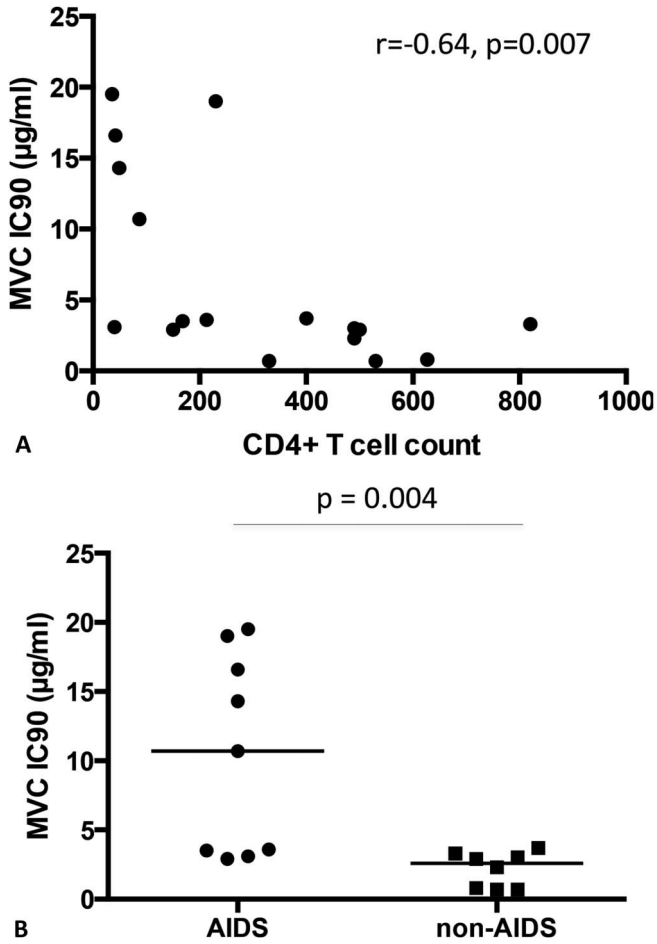

FIGURE 1. R5 HIV-1 AIDS isolates display reduced baseline sensitivity to MVC inhibition. A, $CD4^+$ T-cell counts correlate with R5 virus baseline sensitivity to MVC inhibition (r = 0.64, $P = 0.007$). B, Non-AIDS R5 isolates were more sensitive to inhibition by MVC (lower IC_{90}) than patients with R5 HIV-1 AIDS ($P = 0.004$). Figures display 1 representative experiment of 3 performed.

alternative explanation of our findings could be an increased replicative capacity by R5 HIV-1 isolates from severely immunosuppressed individuals,[8,10] and the reduced basal sensitivity to MVC may therefore not be exclusively specific to the compound.

Several Env mutations, mainly within, but also outside of the V3 region of the *env* gene, have been linked to CCR5 antagonist resistance.[15–18,21,27,29–32] However, these mutations have been Env context-dependent and there are no universal genotypic markers to distinguish resistant R5 isolates from sensitive strains.[29,33] Several single or combined mutations within the Gp120 V3 region have also been linked to MVC resistance in vitro and in vivo.[21,32,34] In the 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.[32,34] 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,[32,34] 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). Two isolates (13 and 23) that consistently had among the highest MVC IC_{90} values displayed the single amino acid polymorphisms 4L and 19S, respectively (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/A763). The 4L and 19S polymorphisms are rare, occurring only in 1%–2% of V3 sequences from individuals in various disease stages.[35] 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.[36] 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.[37] 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.[38] 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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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).[1] 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.[2] 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 nonprivate 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 staff orders a test, blood receipt time was obtained from the timestamp generated on the receipt of the specimen by the laboratory, discharge time was obtained from the timestamp generated when a patient 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 $\geq$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 Stata version 13 (StataCorp 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

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