HIV enters cells by a complex process that involves sequential attachment to the CD4 T lymphocyte (CD4) receptor followed by binding to either the CCR5 or CXCR4 molecules and fusion of the viral and cellular membranes.\(^1\) CCR5 co-receptor antagonists prevent HIV entry into target cells by binding to the CCR5 receptors.\(^2\) Phenotypic and genotypic assays have been developed that can determine or predict the co-receptor tropism (i.e., use of CCR5, CXCR4, or both as either dual-tropic virus or a mixed population of viruses referred to for purposes of assay results as dual/mixed [D/M]) of the patient’s dominant virus population. An older generation assay (Trofile,\(^*\) Monogram Biosciences, Inc., South San Francisco, CA) was used to screen patients who were participating in clinical trials that led to the approval of maraviroc (MVC), the only CCR5 antagonist currently available. The assay has been improved and is now available with enhanced sensitivity. In addition, genotypic assays to predict co-receptor usage are commercially available.

During acute/recent infection, the vast majority of patients harbor a CCR5-utilizing virus (R5 virus), which suggests that the R5 variant is preferentially transmitted; however, up to 19% of individuals with acute/recent infection can harbor CXCR4-tropic virus.\(^3-5\) Viruses in many untreated patients eventually exhibit a shift in co-receptor tropism from CCR5 usage to either CXCR4 usage or D/M tropism. This shift is temporally associated with a more rapid decline in CD4 counts,\(^6,7\) but whether this tropism shift is a cause or a consequence of progressive immunodeficiency remains undetermined.\(^1\) Antiretroviral-treated patients with extensive drug resistance or persistently high-level viremia are more likely to harbor CXCR4- or D/M-tropic variants than untreated patients with comparable CD4 counts.\(^8,9\) The prevalence of CXCR4- or D/M-tropic variants increases to more than 50% in treated patients who have CD4 counts <100 cells/mm\(^3.\)\(^8,10\) Since CXCR4-tropic viruses may be present at initial presentation or a patient may shift to CXCR4-tropicism over the course of infection, co-receptor tropism should always be assessed prior to the use of CCR5 antagonists for treatment. Once a patient has ever been documented with detectable CXCR4- or D/M-tropic virus, it is assumed that such viruses will always be present. CCR5 co-receptor antagonists will no longer be active for that patient and should not be used.

### Phenotypic Assays

Phenotypic assays characterize the co-receptor usage of plasma-derived virus. These assays involve the generation of laboratory viruses that express patient-derived envelope proteins (i.e., gp120 and gp41). These pseudoviruses, which are replication-defective, are used to infect target cell lines that express either CCR5 or CXCR4.\(^11,12\) Using the Trofile\(^*\) assay, the co-receptor tropism of the patient-derived virus is confirmed by testing the susceptibility of the virus to specific CCR5 or CXCR4 inhibitors \textit{in vitro}. This assay takes about 2 weeks to perform and requires a plasma HIV RNA level \(\geq\)1,000 copies/mL.
The performance characteristics of these assays have evolved. Most, if not all, patients enrolled in premarketing clinical trials of MVC and other CCR5 antagonists were screened with an earlier, less sensitive version of the Trofile® assay.12 This earlier assay failed to routinely detect low levels of CXCR4 utilizing variants. As a consequence, some patients enrolled in these clinical trials harbored low levels of such variants at baseline, which were below the assay limit of detection, and these patients exhibited rapid virologic failure after initiation of a CCR5 antagonist.13 The assay has been improved and is now able to detect lower levels of CXCR4-utilizing viruses. In vitro, the assay can detect CXCR4-utilizing clones with 100% sensitivity when those clones represent 0.3% or more of the virus population.14 Although this more sensitive assay has had limited use in prospective clinical trials, it is now the only assay that is commercially available. For unclear reasons, a minority of samples cannot be successfully phenotyped with either generation of the Trofile® assay.

In patients with an undetectable viral load or detectable plasma HIV RNA <1,000 copies/mL, phenotypic co-receptor usage can be determined using proviral DNA obtained from peripheral blood mononuclear cells (e.g., Trofile® DNA, Monogram Sciences); however, the clinical utility of this assay remains to be determined.15

Genotypic Assays

Genotypic determination of HIV-1 co-receptor usage is based on sequencing of the V3-coding region of HIV-1 env, the principal determinant of co-receptor usage. A variety of algorithms and bioinformatics programs can be used to predict co-receptor usage from the V3 sequence.16 When compared to the phenotypic assay, genotypic methods show high specificity (~90%) but only modest sensitivity (~50% to 75%) for the presence of a CXCR4-utilizing virus. Studies in which V3 genotyping was performed on samples from patients screened for clinical trials of MVC suggest that genotyping performed as well as phenotyping in predicting the response to MVC.17-19 An important caveat is that the majority of patients who received MVC were first shown to have R5 virus by a phenotypic assay (Trofile®). Consequently, the opportunity to assess treatment response to MVC in patients whose virus was considered R5 by genotype but D/M or X4 by phenotype was limited to a relatively small number of patients. Other studies have also demonstrated relatively high concordance between genotypic- and phenotypic-assessed tropism;20,21 however, there is variability between different genotypic platforms.22

Given these performance characteristics, genotypic tropism assays may not be sufficiently robust to completely rule out the presence of an X4 or D/M variant;23 therefore, the Panel preferentially recommends phenotypic testing. Based on accessibility, capacity, logistics, and cost, European guidelines currently include genotypic testing as an equivalent option to phenotypic testing when determining co-receptor usage among patients with HIV RNA >1,000 copies/mL and preferentially for those with HIV RNA ≤1,000 copies/mL.24

HIV-1 proviral DNA genotypic tropism testing is available for patients with HIV RNA <1,000 copies/mL. These assays evaluate the HIV-1 proviral DNA integrated within infected cells for CXCR4-utilizing viral strains.25 As discussed above, caution is advised when using such assays, as their detection limit, concordance with plasma HIV RNA tropism, and clinical utility are not yet fully determined.

Use of Assays to Determine Co-receptor Usage in Clinical Practice

An assay for HIV-1 co-receptor usage should be performed whenever the use of a CCR5 antagonist is being considered (AI). This is true even in the setting of prior tropism testing showing CCR5 usage, as viral evolution may occur over the course of infection. In addition, because virologic failure may occur due to a shift from CCR5-using to CXCR4-using virus, testing for co-receptor usage is recommended in patients who exhibit virologic failure on a CCR5 antagonist (BIII). Virologic failure may also be caused by resistance of a CCR5-using virus to a CCR5 antagonist, but such resistance is uncommon. Compared to genotypic testing, phenotypic testing has more evidence supporting its utility. Therefore, a phenotypic test for co-receptor usage is generally preferred (AI). However, because phenotypic testing is more expensive, requires more time to
perform, and may have logistic challenges, a genotypic test to predict HIV-1 co-receptor usage should be considered as an alternative test (BII).

As with HIV resistance testing, the results of all prior tropism tests should be obtained. If CXCR4-utilizing or D/M-tropic viruses have ever been detected previously, then repeat testing is not necessary and a CCR5 co-receptor antagonist should not be used.

If a CCR5 co-receptor antagonist is being considered in a patient with an undetectable HIV RNA (e.g., in cases of regimen simplification or a toxicity-related switch), a proviral DNA tropism assay can be utilized (BII). If CXCR4-utilizing or D/M-tropic viruses are detected, then the CCR5 co-receptor antagonist should not be used.

References


