HIV enters cells by a complex process that involves sequential attachment to the CD4 receptor followed by binding to either the CCR5 or CXCR4 molecules and fusion of the viral and cellular membranes. CCR5 co-receptor antagonists prevent HIV entry into target cells by binding to the CCR5 receptors. Phenotypic and, to a lesser degree, genotypic assays have been developed that can determine or predict the co-receptor tropism (i.e., CCR5, CXCR4, or both) 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, a genotypic assay to predict co-receptor usage is now 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. Viruses in many untreated patients eventually exhibit a shift in co-receptor tropism from CCR5 usage to either CXCR4 or both CCR5 and CXCR4 tropism (i.e., dual- or mixed-tropic; D/M-tropic). This shift is temporally associated with a more rapid decline in CD4 T-cell counts, but whether this tropism shift is a cause or a consequence of progressive immunodeficiency remains undetermined. Antiretroviral (ARV)-treated patients with extensive drug resistance are more likely to harbor X4- or D/M-tropic variants than untreated patients with comparable CD4 counts. The prevalence of X4- or D/M-tropic variants increases to more than 50% in treated patients who have CD4 counts <100 cells/mm³.

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. 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 in vitro. This assay takes about 2 weeks to perform and requires a plasma HIV RNA level ≥1,000 copies/mL.

The performance characteristics of these assays have evolved. Most, if not all, patients enrolled in pre-marketing clinical trials of MVC and other CCR5 antagonists were screened with an earlier, less sensitive version of the Trofile assay. 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 CXCR4-utilizing virus at baseline that were below the assay limit of detection and exhibited rapid virologic failure after initiation of a CCR5 antagonist. The assay has been revised 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. Although this more sensitive assay has had limited use in prospective clinical trials, it is now the only one that is commercially available. For unclear
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. When compared to the phenotypic assay, genotypic methods show high specificity (~90%) but only modest sensitivity (~50%-70%) for the presence of a CXCR4-utilizing virus. Given these performance characteristics, these assays may not be sufficiently robust to completely rule out the presence of an X4 or D/M variant.12

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.13-15 On the basis of these data, accessibility, and cost, European guidelines currently favor genotypic testing to determine co-receptor usage.16 An important caveat to these results 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.

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). 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 also may 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 usefulness. Therefore, a phenotypic test for co-receptor usage is generally preferred (AI). However, because phenotypic testing is more expensive and requires more time to perform, a genotypic test to predict HIV-1 co-receptor usage should be considered as an alternative test (BII).

A tropism assay may potentially be used in clinical practice for prognostic purposes or to assess tropism before starting ART if future use of a CCR5 antagonist is anticipated (e.g., a regimen change for toxicity). Currently, sufficient data do not exist to support these uses.

References


