HIV-1 tropism testing and clinical management of CCR5 antagonists: Quebec review and recommendations

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HIV-1 tropism assays play a crucial role in determining the response to CCR5 receptor antagonists. Initially, phenotypic tests were used, but limited access to these tests prompted the development of alternative strategies. Recently, genotyping tropism has been validated using a Canadian technology in clinical trials investigating the use of maraviroc in both experienced and treatment-naive patients. The present guidelines review the evidence supporting the use of genotypic assays and provide recommendations regarding tropism testing in daily clinical management.

Key Words: *CCR5; HIV; Maraviroc; Tropism*

Les tests de détermination du tropisme du VIH-1 et la prise en charge clinique des antagonistes du CCR5 : une analyse au Québec et des recommandations

Les tests de détermination du tropisme du VIH-1 jouent un rôle capital dans la détermination de la réponse aux antagonistes des récepteurs du CCR5. Au début, on utilisait des tests phénotypiques, mais leur accès limité a suscité l'élaboration d'autres stratégies. Récemment, le génotypage du tropisme a été validé à l'aide d'une technologie canadienne, dans le cadre d'essais cliniques faisant appel au maraviroc tant chez des patients déjà en traitement que chez des patients naïfs au traitement. Les présentes lignes directrices passent en revue les données probantes en appui à l'utilisation de tests génotypiques et contiennent des recommandations au sujet des tests de détermination du tropisme dans la prise en charge clinique quotidienne.

The discovery of the C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4) chemokine receptors as coreceptors for HIV binding and viral entry into the host cell has significantly improved our knowledge of HIV pathogenesis, including primary HIV infection and the course of disease progression. The concurrent identification of viral populations with different cell tropisms paved the way for the development of a new class of antiretrovirals: CCR5 coreceptor antagonists. The determination of HIV viral tropism is even more relevant because this class of molecules is effective only against viruses that use the CCR5 coreceptor, also known as R5 viruses.

The purpose of the present review, which is targeted to health care professionals, is to describe the different methods assessing HIV-1 coreceptor tropism and to define the pertinence of such tests in a clinical setting. Given the constant evolution of HIV tropism assays, future assay developments will likely lead to modifications of the recommendations provided in the present article.

METHODS

A literature review of published scientific articles and abstracts presented at international conferences before February 20, 2013, was performed using the following key words: "maraviroc", "vicriviroc" and "CCR5". The literature was screened by an HIV expert panel. Five face-to-face meetings occurred between members of the panel. Articles were analyzed based on their relevance to the use of phenotypic and genotypic tropism tests. Group members also reviewed articles regarding the emergence of drug resistance in the past two years. Each recommendation was agreed on by consensus among clinicians and virologists. The resulting document was then approved by the Advisory Committee on the Clinical Management of People Living with HIV.

Each recommendation is identified, according to a classification code that was adapted from the Panel on Antiretroviral Guidelines for Adults and Adolescents (1), using a letter (A to C) followed by a Roman numeral (I to III) separated by a hyphen. The letter corresponds to the strength of the recommendation, evaluated by the experts of the Advisory Committee, whereas the Roman numeral refers to the grounds for the recommendation (Table 1).

STATE OF KNOWLEDGE AND BACKGROUND

Viral entry into the cell involves several inter-related steps

The attachment and entry of HIV-1 to $CD4^+$ lymphocytes involve several steps that are all potential targets for antiretroviral therapy (2). The glycoproteins that form the HIV-1 envelope, namely glycoprotein (gp) 120 and gp41, exist as trimeric complexes on the surface of the virion and serve as mediators, both for the attachment of the virus to the target cell as well as for the fusion of the viral and cell membranes (3). For the HIV-1 membrane to fuse with the target cell, gp120 must first bind to the CD4 receptor. This binding results in a conformational change of the gp120 protein, which exposes a high-affinity binding site for a chemokine receptor on the surface of the cell (CCR5 or CXCR4). This site is a conserved region of gp120 on the bridging sheet between two domains of the protein, near the V3 loop. The binding of gp120 to the coreceptor induces other conformational changes resulting in the activation of gp41. The fusion peptide, located at the N-terminal region of gp41, is exposed and penetrates the membrane of the target cell. This structure, composed of three helices, folds back on itself to form a six-helix bundle, thereby resulting

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in the apposition of the viral and cellular membranes that enables virus entry into the host cell (4-11). Several coreceptors involved in HIV entry into the cell have been identified in vitro; nonetheless, only CCR5 and CXCR4 have a clinical significance in vivo.

Viral tropism may vary during disease progression

The viral strains isolated at the onset of HIV infection easily infect macrophages and primary lymphocytes, but are unable to infect immortalized T cell lines. These strains are known as M-tropic. At a later infection stage, it is possible to isolate more cytopathic variants that have acquired the ability to replicate in cell lines, such as H9 and MT-2, but can no longer replicate in macrophages. Such viruses are termed T-tropic. Because viral replication in such cell lines causes cytopathic effects and the formation of syncitium, these viral strains are called syncytium inducing (SI). In comparison, M-tropic viral strains that do not form syncytium are termed non-SI (NSI). Following the discovery of CCR5 and CXCR4 chemokine receptors and their role in HIV cell entry, it was observed that SI viruses preferentially used the CXCR4 coreceptor whereas NSI used the CCR5 coreceptor. Viruses using the CXCR4 coreceptor to enter the cell are referred to as X4 viruses whereas those using the CCR5 coreceptor are referred to as R5 viruses. The term 'R5/X4 dual-tropic' describes a single virus using both coreceptors, whereas mixed tropic defines a population of viruses, some of which use CCR5 and others that use CXCR4. Thus, in a tropism assay, a population of viruses could exhibit dual and/or mixed tropism, termed D/M-tropic (12). Non-R5 tropism determined by genotype has been associated with a greater mean CD4 decrease in antiretroviral-naive patients (13). Both antiretroviral-naive and -experienced patients infected with X4 viruses are more likely to have advanced HIV-1 disease (CD4 cell count <200 cells/mm^3) (14). In patients receiving first-line antiretroviral therapy (atazanavir/ritonavir or nevirapine plus tenofovir/emtricitabine), viral tropism was observed to be independently associated with virological response, but not CD4 cell count recovery at week 24 (15). In nontreated patients, the time to clinical progression did not differ between patients infected with R5 or X4 viruses (16). Therefore, it may not be useful to determine coreceptor tropism at the time of diagnosis to decide when to start antiretroviral therapy. Throughout the course of antiretroviral treatment, viral tropism can evolve and patients can experience a tropism switch (17). Although most individuals are infected by R5 viruses, the error-prone reverse transcriptase coupled with the immune selective pressures quickly lead to the emergence of viral quasispecies, among which some may have a different cell tropism (18). Recent data suggest that there may be differences in tropism distribution among HIV-1 subtypes (19). The overall prevalence of X4 viruses is only approximately 16%, and is significantly higher in antiretroviral-experienced than antiretroviral-naive patients (20,21). However, several studies have confirmed that, at an advanced stage of the disease, X4 viral strains or strains that can use both coreceptors are found in approximately 50% of HIV-infected individuals (22-33). Newer and more sensitive assays to characterize viral tropism have shown that X4 or D/M-tropic strains can be present early in the course of HIV infection, but at a very low level (12,34,35). The factors leading to a transition from R5 to X4 viral population during the course of disease have yet to be determined. Moreover, we do not know whether this transition is responsible for a more aggressive form of the illness or if it simply reflects the availability of certain target cells (36). In this respect, the determination of viral tropism is not recommended for predicting the natural evolution of the disease (C-III) (16).

Nevertheless, determination of viral tropism is essential for predicting the response to treatment with CCR5 antagonists. These molecules, which bind to the CCR5 coreceptor and block the interaction with gp120, are efficacious only in individuals infected with R5 viruses. Indeed, data from phase II/III clinical trials in which the CCR5 antagonists maraviroc (MVC) or vicriviroc were used have demonstrated that the detection of D/M-tropic strains at study onset was associated with a poorer response to treatment (12,37). The double-blind Maraviroc versus Efavirenz in Treatment-Naive Patients (MERIT) study compared MVC with efavirenz in 720 antiretroviral-naive patients. The underestimation of minority X4 or D/M-tropic viral strains before MVC treatment led to excessive virological failures in this arm compared with the efavirenz arm. At week 48, MVC did not meet the noninferiority criterion of <50 copies/mL (65.3% for MVC versus 69.3% for efavirenz) at a threshold of –10%. Following a post hoc analysis that excluded 15% of patients with non-R5 screening virus by current, more sensitive tropism assays, the final conclusions of the study were modified and noninferiority was demonstrated (38,39).

TABLE 1
Recommendation classification code

Recommendation strength	
A	Strong recommendation
B	Moderate recommendation
C	Optional recommendation
Recommendation grounds	
I	At least one randomized placebo-controlled clinical trial
II	Clinical trials without placebo-control, case-control studies or prospective surveys
III	Expert opinion

Adapted from reference 1

It is important to note that it is possible for HIV to develop resistance to MVC in the absence of a tropism change; thus, a tropism assay performed after treatment failure may not necessarily be adequate to indicate that CCR5 antagonists will be active in a given patient (40). Currently, MVC is the only CCR5 antagonist that has been approved by Health Canada for clinical use (41-44). Other CCR5 antagonists are under development. It is essential to be able to adequately determine viral tropism before treatment with CCR5 antagonists to ensure the best possible use of our therapeutic arsenal.

TROPISM ASSAYS

Both phenotypic and genotypic assays to assess HIV-1 tropism are available. Each testing method has its distinct advantages and inconveniences. Clinical trials investigating coreceptor antagonists have relied on phenotypic assays, which are costly, complex and time consuming. Moreover, they typically require a minimum viral load of 1000 copies/mL, which excludes patients with recent virological failure who have a low viral load as well as patients for whom a change in therapy could be considered for tolerability reasons despite their undetectable viral load (12). It is possible to determine coreceptor usage from HIV DNA when the viral load is below the limit of detection, although the clinical utility of this test has yet to be established (45,46).

Genotypic assays are faster and less costly. The challenge that both phenotypic and genotypic assays share is their ability to detect minority viral populations that are clinically significant.

Phenotypic assays

The first generation of phenotypic tests were based on the detection of syncytium formation by HIV-1 on MT-2 cells. More recent phenotypic tests detect viral infection of cell lines that specifically express the CD4 receptor, and the CCR5 or CXCR4 coreceptors.

MT-2 assay: The MT-2 assay evaluates the capacity of HIV-1 isolates to induce (or not) the formation of syncytium in an in vitro model. The correlation between SI phenotype and X4 coreceptor usage is not perfect and this test is not commonly used in clinical practice.

Phenotypic assays of coreceptor usage: The purpose of phenotypic assays using the coreceptor is to evaluate viral infection in cell lines that express either CCR5 or CXCR4 coreceptor exclusively. To this end, a recombinant virus that expresses the HIV envelope amplified from a patient's plasma is produced, and its capacity to infect cells expressing the CD4 receptor as well as the CCR5 or the CXCR4 coreceptor is observed using reporter genes that express a luminescent signal, such as luciferase (12).

The phenotypic test that is most commonly used in practice, and that was the benchmark test in CCR5 antagonist clinical trials, is the Enhanced Sensitivity Trofile Assay (ESTA, Monogram Biosciences Inc, USA). It is considered to be the gold standard phenotypic assay for HIV tropism determination (47). The ESTA uses the type of pseudovirus described above and U87:CXCR4 and U87:CCR5 cells. The samples producing a luminescent signal only on CCR5 cells are characterized as R5, those that produce a signal only on CXCR4 cells are characterized as X4, and those that produce a luminescent signal on both cell lines are D/M-tropic. Moreover, the test is validated by using coreceptor antagonists specific to each cell type to block HIV entry. The original Trofile assay reliably detected X4 variants present in mixed virus populations of R5 and X4 variants only if they represented at least 10% of the total viral population (12). The capacity of the assay to detect minority X4 populations is clinically significant. Four cohorts involving CCR5 antagonists using this test to identify R5 viruses have demonstrated that 9% to 26% of the samples initially labelled as R5 were, in fact, D/M-tropic (48). The patients for whom these minority populations were poorly identified did not respond as well to treatment and experienced more virological failures (38). Thus, the ESTA is a more sensitive version of the original Trofile test. This test has been shown to detect minority populations present at levels of 0.3% of the viral population in in vitro studies (49). Nevertheless, its sensitivity on clinical samples varies according to the viral load and other factors (12,47,50).

Genotypic assays

Genotypic assays for determining HIV-1 tropism are based on the sequencing of the V3 loop, which contains the key determinants of viral tropism. It is known that several V3 loop residues are important for coreceptor usage. In fact, the presence of positively charged amino acids in positions 11 and 25, commonly known as the 11/25 rule, associated with an electrical charge of the V3 loop greater than +5, enables the prediction of CXCR4 tropism with 90% sensitivity in in vitro clones (51-53). The performance of this rule in clinical samples is expected to be lower. Nonetheless, this rule has a lower than 60% sensitivity for predicting CXCR4 usage, because other amino acids in the V3 loop also play a role in tropism (36,54-66). Furthermore, isolates using the same coreceptor can have a highly variable V3 sequence (4,67-75). Although the V3 loop sequence of gp120 is the main determinant for viral tropism, it appears that other regions of this glycoprotein can intervene in the binding to the coreceptor, such as the V1/V2/V4/V5, C1, C2, C3 and C4 regions (76-78). Thus, it has been reported that V1/V2 mutations, in the absence of a change in V3, frequently increased the capacity of the virus to use CCR5, but were, however, unable to confer CXCR4 use. Finally, certain changes in gp41 can also influence tropism; nonetheless, the incorporation of these mutations in the interpretation models did not improve their predictive value (79-84).

The interpretation of these sequences relies on bioinformatic algorithms based on several variables: the known genotype/phenotype associations, the electrical charge of the various amino acids and the clinical variables (nadir CD4 and viral load) in particular. Examples of these bioinformatic tools include geno2pheno [coreceptor] (http://coreceptor.bioinf.mpi-inf.mpg.de/) and the position-specific scoring matrices (http://indra.mullins.microbiol.washington.edu/webpssm/). Moreover, it has been demonstrated that the interpretation parameter of geno2pheno [coreceptor], called False Positive Rate (FPR) (which determines the probability of falsely classifying an R5 virus as X4), should be set at a value between 5% and 10% to avoid false classification of X4 strains as R5 (85); more sensitive methodologies may be more valuable when population sequencing yields a FPR >20% to improve the accuracy of identifying X4 variants (86).

Phenotypic and genotypic assays were compared, along with their clinical outcomes, to validate their use. Thus, the availability of frozen plasma collected as part of MVC clinical trials allowed two retrospective evaluations of subjects included in the MERIT, MOTIVATE and 1029 trials (85,87,88). These studies demonstrated that genotypic assays (on plasma viral RNA, triplicate sequencing) predicted the virological results in a similar way that the Trofile test did in antiretroviral-experienced patients (MOTIVATE trial [87]) and corroborated the ESTA test in treatment-naive patients (MERIT trial [85,88]). The results from these retrospective studies, as well as other prospective studies (89-91), have led several experts to state that genotypic tests developed according to certain parameters (described below) are adequate for predicting tropism during routine clinical testing (92). Duplicate PCR amplification and bulk sequencing have been shown to improve the sensitivity and specificity of population genotyping (93). In addition, genotypic testing in triplicate can improve X4 tropism prediction in individual cases (94). The performance of genotypic assays in 10 European laboratories was evaluated in a pairwise comparison (95). The concordance rates between the Trofile assay and genotypic assays were 89% for B subtypes and 79% for non-B subtypes. In a more recent retrospective analysis of Study A4001078, 91.7% concordance (FPR 5.75%) was observed between ESTA and population-based genotyping (96).

A genotypic assay based on ultra-deep sequencing (UDS) of the V3 loop was used in an attempt to detect minority X4 populations with a higher sensitivity (97,98). This technology allows for the sequencing, from a patient sample, of thousands of different viral clones based on a sophisticated system of DNA capture on microscopic beads. The test is able to identify the selection of X4/R5 variants earlier in experienced patients being treated with a CCR5 antagonist (MOTIVATE study [97]), but not in treatment failure. In this study, the UDS tests and original Trofile test produced concordant results. More recently, the UDS assay demonstrated concordance with the ESTA assay for screening tropism assessments (96,99) and for predicting short-term virological response as a function of tropism status in treatment-experienced patients in the MOTIVATE and A4001029 trials (100). The UDS assay was also able to distinguish between responders and nonresponders of MRV in a reanalysis of antiretroviral-naive patients in the MERIT trial (99). This analytical method is now used clinically at several sites. The majority of published studies of UDS tropism have used the 454 Life Sciences platform (454 Life Sciences/Roche, USA); however, three other platforms have also demonstrated similar concordance with virological response in comparison with the ESTA assay; namely, Illumina (Illumina Inc, USA), PacBio RS (Pacific Biosciences, USA) and Ion Torrent (Ion Torrent/Life Technologies, USA) (101). All of these platforms detected non-R5 variants at similar levels. Several studies are underway to determine the threshold of the minority variants that predict virological failure.

Proviral DNA

To date, assays for determining HIV-1 tropism have been performed on circulating viral strains (plasma RNA), which implies the existence of a replicating virus. In the event that we would want to use a CCR5 antagonist in a patient whose viral load has been completely suppressed (eg, treatment change as a result of poor tolerability), some suggest using proviral DNA integrated in peripheral blood mononuclear cells to determine whether the patient has been a carrier of X4 strains in the past (102-106). Numerous studies have compared the genotype of the proviral DNA with the genotype of plasma RNA before the start of treatment. The concordance of the results varied from 74% to 95.2% (102,106-108). A recent study used quantitative deep sequencing to demonstrate concordance between the genotypic tropism test on the pretreatment plasma RNA and the proviral DNA in patients who initiated antiretroviral treatment without a CCR5 antagonist and who had an undetectable viremia for at least two years (109). These results indicated that there was no evidence of X4 virus evolution in patients' sustained undetectable viremia. The concordance in R5 tropism between HIV-RNA (ESTA) and HIV-DNA provirus at the start of MRV therapy ranged between 71% and 85% (106,110,111). Differences between the circulating quasispecies and the proviral compartment could be observed in some patients, as part

of a comparison between the UDS technology and the original Trofile test (112). Therefore, these differences imply that the threshold used to classify a virus with an X4 tropism must be better defined when a technology as sensitive as the UDS is used. Although tropism tests on proviral DNA can be useful for identifying carriers of X4 strains, the correlation with response to treatment based on a CCR5 antagonist has never been demonstrated.

RECOMMENDATIONS

Based on the existing data, the HIV expert panel has issued the following recommendations:

1. A tropism assay must be performed before treatment initiation with a CCR5 receptor antagonist. This test should be performed four to six weeks before treatment initiation (A-I).
2. A tropism assay can be performed in case of treatment failure with a CCR5 receptor antagonist (B-II).
3. Determining the viral tropism is not recommended to predict the natural evolution of disease (C-III).
4. A V3 loop genotypic test performed on the plasma viral RNA should be used to assess viral tropism, as a result of its availability and its greater technical ease (B-II).
5. Use of a phenotypic assay should be possible in the event that repeated amplification for genotyping cannot be performed (A-I for the use of phenotypic assay in general; C-III for this subpopulation).
6. A tropism assay can be performed on proviral DNA when the viral loads are <400 copies/mL and in the presence of limited therapeutic options. Nevertheless, the use of a CCR5 receptor antagonist based on this test has yet to be validated by clinical trials in patients with suppressed viral load or who are experiencing therapeutic failure (C-III).

CONCLUSION

The viral tropism assay is an essential tool for ensuring the adequate use of CCR5 receptor antagonists. This new class of molecules has been used successfully in clinical trials involving patients experiencing treatment failures as well as treatment-naive patients. Reports on the use of these molecules to replace a treatment that has been interrupted as a result of intolerance in patients with a controlled viral load are only anecdotal, but trials are currently being conducted with genotype testing on proviral DNA to validate this clinical indication. Access to genotype testing for HIV-1 tropism is essential for the management of patients who will be prescribed a CCR5 receptor antagonist and this access must be supported. The laboratory techniques used to assess HIV-1 tropism are constantly evolving and the validation of genotypic assays as well as new technologies such as UDS must continue.

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