Published in final edited form as:
Curr Opin HIV AIDS. 2009 November ; 4(6): 481–487. doi:10.1097/COH.0b013e328331b929.

Tropism Testing in the Clinical Management of HIV-1 Infection

Nina H. Lin[1,2] and **Daniel R. Kuritzkes**[2,3]

[1]Infectious Diseases Unit, Massachusetts General Hospital, Boston, MA

[2]Harvard Medical School, Boston, MA

[3]Section of Retroviral Therapeutics, Brigham and Women's Hospital, Boston, MA

Abstract

Purpose of review—A variety of methods are available to determine HIV-1 co-receptor usage, commonly referred to as viral tropism. This article reviews recent data on phenotypic and genotypic assays of HIV-1 tropism.

Recent findings—Tropism assays are used to determine co-receptor usage of HIV-1 in patients who may be candidates for treatment with CCR5 antagonists. Phenotypic assays are used most often in the clinical trials of CCR5 antagonists, and are considered the “gold standard” for comparison to other methods of tropism testing. Enhancements have allowed detection of a lower threshold of minor CXCR4-using species. When compared to phenotypic assays, genotypic methods have poor sensitivity but good specificity at detecting CXCR4-using HIV-1. Preliminary results from a recent comparative study suggest that some genotypic methods may perform as well as phenotypic tests in predicting virologic response to CCR5 antagonists. Several studies show that tropism testing may provide useful prognostic information regarding the risk of disease progression.

Summary—Understanding the characteristic of different tropism assays is important for their clinical use. Although phenotypic testing currently is favored, genotypic assays may be a suitable alternative in appropriate settings.

Keywords

CCR5; CXCR4; viral tropism; phenotypic assay; genotypic assay

I. Introduction

Discovery of the two main coreceptors for HIV-1, CCR5 and CXCR4, has lead to a better understanding of the interaction of the viral envelope with host cell and the development of novel therapeutic agents that inhibit viral entry. Viral tropism assays play an important role in identifying patients who are candidates for treatment with entry inhibitors and in the ongoing development of coreceptor inhibitors as a novel class of antiretroviral agents. In addition, epidemiological data on co-receptor usage are important for elucidating the relationship between coreceptor usage and disease progression. Here we provide an overview of the major

Address correspondence to: Daniel R. Kuritzkes, MD Section of Retroviral Therapeutics Brigham and Women's Hospital 65 Landsdowne St, Rm 449 Cambridge, MA 02139 Tel: +1 617 768 8371 Fax: +1 617 768 8738 dkuritzkes@partners.org.

Disclosure: Dr. Kuritzkes has served as a consultant to Monogram Biosciences, Pfizer, Schering-Plough, Tobira and Progenics. Dr. Lin has no potential conflicts of interest to disclose.

methods and tools used to determine HIV co-receptor usage and discuss the utility of tropism testing in the clinical setting.

Viral entry and tropism

HIV-1 entry into susceptible cells is initiated by attachment of the surface subunit of the envelope glycoprotein (gp120) to CD4 molecules on the cell surface. Subsequently, binding of gp120 to a specific C-C or C-X-C chemokine receptor triggers conformational changes in the transmembrane subunit of envelope (gp41) that lead to membrane fusion and entry of the viral core particle into the cell. Although a number of chemokine receptors can be utilized by HIV-1 for entry *in vitro*, the two most significant co-receptors *in vivo* are CCR5 and CXCR4 (reviewed in [1]).

Soon after infection, most patients harbor HIV-1 that easily infects macrophages and primary lymphocytes, but not immortalized T-cell lines (M-tropic virus). Later in infection more cytopathic variants can be isolated that have acquired the ability to replicate in immortalized T cell lines, such as H9 or MT-2 cells, but have lost the ability to infect macrophages (T-tropic viruses) [2]. Because viral replication in these T-cell lines causes cytopathic changes and syncytium formation, such isolates were termed syncytium-inducing (SI) viruses. In comparison, M-tropic viruses do not replicate on T-cell lines, and hence are termed non-SI viruses (NSI). When the role of CCR5 and CXCR4 in HIV-1 entry was discovered, it became apparent that viruses with SI phenotype preferentially use CXCR4 coreceptors, which are expressed on MT-2 cells, whereas NSI strains use CCR5 coreceptors, which are absent from this particular cell line. Viruses that preferentially use CCR5 as co-receptor are termed R5 viruses; those that use CXCR4 are termed X4 viruses. Because it is difficult to distinguish mixtures of R5 and X4 viruses from dual-tropic strains with currently available assays, viruses that are capable of using both CCR5 and CXCR4 coreceptors are often referred to as dual/mixed-tropic (D/M) viruses.

II. Tools for determining viral tropism

Currently there are several methods to determine HIV-1 tropism, but it is unclear which will prove most appropriate for routine clinical use. Although clinical trials of chemokine receptor antagonists have relied on phenotypic assays, such assays are laborious, time-consuming and expensive. More rapid and less costly options include genotypic methods that predict co-receptor usage based on the *env* gene sequence. Each of these approaches has advantages and limitations, and both share the common challenge of detecting low levels of CXCR4-using viruses present as minority variants in the viral quasispecies. Understanding the limitations of each of these assays is important for making the best clinical use of their results.

A. Phenotypic methods

Phenotypic testing to determine viral tropism has depended on two general approaches: detection of syncytium formation by HIV-1 on MT-2 cells or detection of viral infection of non-lymphoid cell lines that stably express CD4 and CCR5 or CXCR4.

1. MT-2 cell assay—Early assays to characterize the tropism of HIV-1 isolates depended on the differential ability of T- and M-tropic viruses to infect cells of an human T-cell leukemia virus type I (HTLV-1)-transformed lymphoblastoid cell line (MT-2 cells) [2,3]. The MT-2 assay requires first stimulating peripheral blood mononuclear cells (PBMC) from HIV-1 infected patients to increase viral replication. Subsequently, supernatant from the culture is used to infect MT-2 cells; alternatively, the stimulated PBMC can be co-cultured directly with MT-2 cells. Cultures are examined at regular intervals for cytopathic changes and syncytium formation, which generally are observed within 14 days when CXCR4-using variants are

present. Cells or supernatant are inoculated onto phytohemagglutinin (PHA)-stimulated PBMC from seronegative donors and monitored for p24 antigen production as a control to insure the presence of infectious virus in the sample. The use of stimulated patient PBMC as a source of infectious virus has advantages and disadvantages. On the one hand, the resulting virus stock may represent both circulating and archived populations of HIV; on the other hand, PBMC-derived virus may not accurately reflect the full diversity of the plasma virus population, and may have been altered by selection even during brief *in vitro* passage.

The presence of SI virus is correlated with accelerated CD4 count decline, increased plasma HIV-1 RNA levels, disease progression and more frequent opportunistic infections [4,5]. Although SI viruses are found in all stages of HIV-1 infection, they are most commonly detected in patients with advanced disease. Before the advent of potent antiretroviral therapy, SI virus could be detected in approximately 25% of infected persons within 5 years of seroconversion [6]. Although it became clear that the formation of syncytia in MT-2 cells indicates CXCR4-usage, the correlation between SI phenotype and coreceptor usage has proved to be more complex as new ways of testing for viral tropism have been introduced and larger cohorts of patients are studied. For instance, the SI phenotype can be observed with either pure X4 or D/M viruses, but studies suggest distinct clinical correlations associated with pure X4 and D/M viruses [7-9].

2. Assays of co-receptor usage—Once it became clear that HIV-1 tropism was related to co-receptor usage, several reporter cell lines were developed that express various combinations of HIV-1 receptors on the cell surface. These cell lines are able to support replication of primary HIV-1 isolates, infectious molecular clones or pseudotyped viruses. Virus replication can be detected by p24 antigen production or by expression of a reporter gene. Most phenotypic assays now use human glioma cell lines (e.g., U87, U373 or NP-2 cells) that stably express CD4 and either CCR5 or CXCR4. Advantages of these cell lines include their ability to easily support HIV replication, the high level of coreceptor expression and the adherent property of the cells, which makes them easier to work with in cell culture. An important caveat is that the high levels of co-receptor expression exceed those found on primary lymphocytes and monocytes *in vivo*. Some studies suggest that co-receptor usage may be affected by the level of co-receptor expression, whereas others do not [10,11]. Another frequently used indicator cell line is the GHOST cell line, which is derived from human osteosarcoma cells and carries a GFP gene under the control of HIV-1 Tat, in addition to expressing CD4 and CCR5 or CXCR4 [12]. A disadvantage of these cells is that they naturally express low levels of CXCR4, which can produce misleading results [13].

3. Phenotypic assays with recombinant or pseudotyped viruses—Phenotypic assays developed by clinical reference laboratories utilize recombinant or pseudotyped viruses constructed with envelope sequences amplified from patient plasma by RT-PCR. The resulting *env* amplicons preserve the diversity of the plasma virus population, and avoid any selection imposed by *in vitro* passage. Reliable representation of viral diversity depends largely on the sensitivity and accuracy of the RT-PCR reactions, which is partly a function of the plasma virus load. Typically, the virus load should be above 1,000 copies/ml in order to obtain a reliable result.

The most widely used phenotypic test in clinical trials of CCR5 antagonists is the Trofile assay (Monogram Biosciences, Inc., South San Francisco, CA; now Laboratory Corporation of America [LabCorp]). The *env* gene is amplified from plasma HIV-1 RNA and cloned into an expression vector, which is then cotransfected together with an *env*-deficient HIV genomic vector carrying a luciferase reporter gene into human embryonic kidney cells (HEK293). The resulting pseudotyped HIV particles are harvested and inoculated onto U87:CXCR4 and U87:CCR5 cells. Samples that produce a signal (measured as relative light units [RLU] in a

luciferase assay) only on CCR5-expressing cells are considered R5, and those that produce a signal only on CXCR4-expressing cells are considered X4; those that produce a signal on both cell types are considered dual- or mixed tropic (D/M). Signal ablation with specific coreceptor inhibitors is used for each coreceptor to confirm the assigned tropism. Viruses with an SI phenotype by MT-2 assay produce high RLU when inoculated onto CXCR4-expressing U87 cells. The original Trofile assay reliably detected X4 variants present at 10% of the viral population, but only detected variants present at 5% of the population in 85% of assays [14].

Several other laboratories have developed phenotypic assays to determine HIV-1 tropism, all based on the same principles as the Trofile assay. As of this writing, however, none of these alternative assays is commercially available.

4. Detection of minority species by phenotypic assays—An important limitation of the original Trofile assay was the challenge of detecting low abundance (minority) CXCR4-using variants. Clinical trials of CCR5 antagonists that employed the original Trofile assay to identify patients with exclusively R5 virus have found that 5-10% of subjects judged to have R5 virus at screening had D/M virus at entry [15,16**]. The apparent change in coreceptor usage most likely is explained by small shifts in minority CXCR4-using variants above and below the limit of assay sensitivity [17*]. Patients with initially undetected D/M viruses who subsequently received a CCR5 antagonist showed a blunted virologic response and high rates of early virologic failure [16**,18,19**]. However, assays using replicating viruses did not substantially increase the ability to detect presence of CXCR4-using virus [17*]. Enhancements to the Trofile assay have improved its sensitivity for detecting minority CXCR4-using variants. The current assay is validated to detect X4 variants present at 0.3% of the population with 100% sensitivity [20,21]. However, actual sensitivity in clinical samples may vary as a function of virus load and infectivity of the virus being assayed.

The enhanced Trofile assay has been applied retrospectively to retest screening samples from subjects who enrolled in two clinical trials of CCR5 antagonists, and the data reanalyzed based on the resulting reclassification of eligible subjects. The MERIT study compared the efficacy of maraviroc to that of efavirenz (both given with a fixed-dose combination of zidovudine plus lamivudine) as initial antiretroviral therapy. The primary analysis of that trial showed that the maraviroc arm failed to achieve the pre-specified threshold for non-inferiority to efavirenz. Many of the virologic failures in the maraviroc arm, however, occurred in subjects with R5 virus at screening but D/M virus at study entry [22]. Retesting of the screening samples with the enhanced Trofile assay identified most of such patients as having D/M virus at screening. A post-hoc analysis that included only those subjects found to have R5 virus by the enhanced Trofile assay showed that the maraviroc arm now met criteria for non-inferiority to efavirenz [23]. Similarly, the enhanced Trofile assay reclassified as 25 of 114 (22%) subjects enrolled into AIDS Clinical Trials Group (ACTG) protocol A5211, a phase 2b trial of vicriviroc in treatment-experienced patients, as D/M from the assignment of R5 virus by the original Trofile assay [24]. Nevertheless, the enhanced Trofile assay identified D/M virus in the screening samples from only 12 of 23 (52%) vicriviroc recipients in whom CXCR4-using virus subsequently emerged, suggesting room for further improvements in assay sensitivity [24].

B. Genotypic methods for determining HIV-1 tropism

Genetic approaches to viral tropism are based on the principle that the V3 loop is the major determinant of coreceptor usage. Bioinformatic algorithms to determine coreceptor usage are based on known genotype and phenotype pairs and have been shown to perform relatively well in clonal samples [25]. Genotyping has several advantages over phenotyping, including lower cost and shorter turn-around time. Sequencing avoids certain confounders inherent to phenotypic assays, such as lack of standardization among laboratory performing the assays,

difference in activity of different target cells and subjective interpretation of cytopathic changes and need to use stimulated or pseudotyped viruses. In addition, some authors argue that genotyping may be able to differentiate levels of CXCR4 utilization by scoring V3 sequences on a continuous scale. Fluctuations in this continuous index may detect and predict shifts in phenotype even before the emergence of biological markers [26]. However, genotypic methods fail to achieve high sensitivity and this might be explained in two ways. Firstly, several studies show that regions outside of the V3 loop are important determinants of coreceptor usage [27, 28]. Secondly, the detection of minority species in bulk sequencing is limited even when sequences are expanded into numerous possible permutations.

1. The 11/25 rule—The simplest genotypic approaches for predicting coreceptor usage include the: 1) presence of positively charged amino acids at positions 11 and 25, often referred as the "11/25 rule" and 2) total charge of V3 loop equal to or greater than +5 [29,30]. The 11/25 rule is fairly sensitive for distinguishing NSI and SI viruses, suggesting that the amino acid changes at these positions contribute to the viral phenotype on MT-2 cells. With clonal HIV sequences the 11/25 rule has >90% sensitivity and specificity for predicting the SI phenotype [25]. However, this rule has <60% sensitivity in terms for predicting CXCR4 usage, underscoring the point that the SI phenotype and coreceptor-usage are not synonymous [31]. The sensitivity and specificity of the 11/25 rule are further reduced when applied to population-based V3 loop sequences. Using direct PCR and population-based sequencing the 11/25 rule has a reported specificity of 93.1% but sensitivity of 32.7% for predicting X4 virus when compared to the phenotype assigned by the Trofile assay [32]. The sensitivity of genotypic predictions of coreceptor usage may be improved by integrating CD4 count and other clinical factors into the evaluation [33*].

Despite their overall low sensitivity, these rules-based approaches are good predictors of the risk of clinical progression in the absence of antiretroviral therapy (ART) [29,30]. Similarly, two studies of treatment-experienced cohorts showed that presence of CXCR4-using virus, as determined by the 11/25 rule, was associated with significantly a lower CD4 count and poorer immunological response to ART [34,35]. In a prospective cohort study of patients initiating ART, those whose virus was X4 by the 11/25 genotype had earlier mortality after starting therapy despite achieving viral suppression to <500 copies/ml (RR 1.70, p=0.027) compared to patients without the 11/25 genotype. This multivariate analyses controlled for viral load, CD4 cell count and time spent on ART in the first year of follow up [34].

2. Bioinformatics approaches—A number of bioinformatic approaches for interpreting V3 amino acid sequences has been developed to predict HIV coreceptor usage, including decision trees, support vector machines (SVM), neural networks (NN) and position-specific scoring matrices (PSSM). The SVM generates a categorical score of R5 or X4; the SVM-genomiac uses an amino acid dataset aligned to a standard, whereas SVM-geno2pheno uses an unaligned nucleotide dataset as input [36]. Neural network algorithms use a large population-based sequence data set from samples with known SI phenotype and/or coreceptor usage to make correlations between complex patterns of sequence variability with their corresponding phenotypes [37]. PSSM is a method for predicting coreceptor usage using a continuous scoring system that takes into account the fact that there exists a continuum of HIV genetic sequences required for shifts in tropism and is interpreted as the propensity to use CXCR4 [25].

A number of studies have examined concordance between phenotypic and genotypic methods for determining co-receptor usage. One study showed relatively poor sensitivity of genotypic approaches as compared to phenotype—the SVM geno2pheno method performed the best but had a sensitivity of only 50%; SVM genomiac performed the worst, with a sensitivity of 22% [38*]. By contrast, a subsequent study found sensitivities of 88% and 77% for the SVM

geno2pheno and PSSM methods, respectively [39*]. The varying levels of concordance between genotype and phenotype observed in these studies could be explained by differences in the phenotypic assay used as the comparator, the characteristics of the cohort, the proportion of non-subtype B infections in the cohort, and the efficiency of population sequencing.

In general, these algorithms perform less well at predicting co-receptor usage of non-subtype B viruses, which is not surprising considering that most of the algorithms were developed from datasets based mostly on subtype B virus [40*]. Performance improves when using algorithms developed specifically for the subtype in question [26], but the number of non-B viruses with genotypic and phenotypic tropism data remains relatively limited.

Preliminary results of a recent study suggest that despite the poor sensitivity of genotypic, they perform reasonably well at predicting virologic response to treatment with CCR5 antagonists. The V3 loop sequence was determined in triplicate on screening samples from 1,230 patients who enrolled in either MOTIVATE-1 [19**], a phase 3 study of maraviroc in treatment-experienced patients with R5 HIV-1 infection, or a sister trial that enrolled treatment-experienced patients with CXCR4-using virus [41**]. The sequence data were interpreted with the aid of either geno2pheno or PSSM [42]. Compared to the original Trofile assay, the two genotypic approaches had sensitivities of 63% and 56%, respectively, and specificities of 91% and 90%. However, the median log-reduction in plasma HIV-1 RNA at week 8 for patients who received maraviroc was similar for patients characterized as having R5 virus by Trofile or genotype; likewise, similar (though smaller) reductions in plasma HIV-1 RNA were observed for maraviroc-treated subjects judged by either assay to have X4 or D/M viruses. One caveat in interpreting results of this study is that the number subjects with discordant results was relatively small, so power to detect differences in outcome based on screening method was low.

3. Heteroduplex tracking assays—Another genotype-based approach for determining coreceptor tropism is the DNA heteroduplex tracking assay (HTA). In this method, PCR-amplified V3 sequences from patient samples are hybridized to known V3 sequences of known R5 or X4 viruses and subjected to electrophoresis. Differences in the V3 sequences between the probe and target sequences result in altered electrophoretic mobility due to varying degrees of mismatch in the hybridized DNA molecules. The assay can also estimate the proportion of CCR5- and CXCR4-using viruses in a population, but sensitivity is highly dependent on the probes used to separate the viral species. One study that tested 50 viral isolates previously characterized by various phenotypic assays showed concordance of 98% between the HTA and phenotypic assays, with a reported sensitivity and specificity for identifying CXCR4-using viruses of 94% and 100%, respectively [43]. Patients with X4 viruses identified by HTA had blunted CD4 cell increases in response to ART.

A commercialized version of the HTA, previously available as the SensiTrop assay from Pathway Diagnostics, was found to have low sensitivity (42%) but high specificity (92.5%) for detecting CXCR4-using viruses in a study of 100 patient samples previously tested by the Trofile assay [44]. An improved version of this assay, SensiTrop II, combined HTA and V3 loop sequence analysis. A comparison of the SensiTrop II and original Trofile assays in samples from 252 treatment-naïve patients found concordance in 79% of results. A low overall proportion of D/M viruses in the sample and absence of virologic outcome data limit the interpretation of these results. It is notable, however, that a higher proportion of inconclusive results is obtained with the Trofile assay (13%) as compared to the SensiTrop II assay (4%). The SensiTrop II assay has now been replaced by the CE-HDA assay (Quest Diagnostics), which combines V3 loop sequence analysis with heteroduplex analysis performed by capillary electrophoresis, rather than agarose gel electrophoresis [45].

IV: Clinical use of tropism testing

The principal clinical role of tropism testing is to exclude the presence of detectable D/M or X4 viruses in patients being considered for treatment with a CCR5 antagonist. At this time, phenotypic testing with the enhanced Trofile assay remains the gold standard, but genotypic assays may play a role as well, particularly as more evidence emerges from large clinical studies validating genotypic methods against clinical outcome. Given the high specificity of the genotypic tests, in some settings it may be reasonable to perform a genotype first. If the result suggests presence of a CXCR4-using virus, no further testing is needed; if the result suggests R5 virus, a confirmatory phenotypic test may be necessary.

Tropism testing may also have a role as a prognostic test. Previous studies had clearly established an association between presence of SI virus and disease progression [4]. A number of studies using contemporary tropism assays show a similar association between presence of D/M or X4 virus and disease progression [43,46*,47*,48**]. Periodic monitoring of co-receptor usage in patients not receiving ART could be useful for making therapeutic decisions, but additional studies are needed to validate viral tropism as a biomarker for initiating and switching therapy.

Conclusion

Since the identification of CCR5 and CXCR4 coreceptors, much progress has been made in the development of tropism assays and understanding the complex relationship between viral tropism and HIV disease. With the advent of chemokine receptor antagonists, there is additional impetus to decipher the complex determinants of coreceptor usage, and the mechanisms driving evolution of a CXCR4-using virus. Tropism assays are an important tool in these investigations, and in the clinical management of HIV-1 infection.

Acknowledgments

Grant support: NIH grants K24 RR016482, R37 AI055357, and U01 AI068636 (to D.R.K); and fellowship support from the Harvard Institute for Global Health, Bristol-Myers Squibb, and the American Society for Tropical Medicine and Hygiene (to N.H.L.).

References and recommended reading

1. Tsibris AMN, Kuritzkes DR. Chemokine antagonists as therapeutics: Focus on HIV-1. Ann Rev Med 2007;58:13.1–15.
2. Schuitemaker H, Koot M, Kootstra NA, et al. Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus population. J Virol Mar;1992 66(3):1354–60. [PubMed: 1738194]
3. Japour AJ, Fiscus SA, Arduino JM, et al. Standardized microtiter assay for determination of syncytium-inducing phenotypes of clinical human immunodeficiency virus type 1 isolates. J Clin Microbiol Sep; 1994 32(9):2291–4. [PubMed: 7814561]
4. Koot M, Keet IP, Vos AH, et al. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS. Ann Intern Med May 1;1993 118(9):681–8. [PubMed: 8096374]
5. Bozzette SA, McCutchan JA, Spector SA, et al. A cross-sectional comparison of persons with syncytium- and non-syncytium-inducing human immunodeficiency virus. J Infect Dis Dec;1993 168 (6):1374–9. [PubMed: 7902382]
6. de Roda Husman AM, Koot M, Cornelissen M, et al. Association between CCR5 genotype and the clinical course of HIV-1 infection. Ann Intern Med Nov 15;1997 127(11):882–90. [PubMed: 9382366]

7. Hunt PW, Harrigan PR, Huang W, et al. Prevalence of CXCR4 tropism among antiretroviral-treated HIV-1-infected patients with detectable viremia. J Infect Dis Oct 1;2006 194(7):926–30. [PubMed: 16960780]
8. Melby T, Despirito M, Demasi R, et al. HIV-1 coreceptor use in triple-class treatment-experienced patients: baseline prevalence, correlates, and relationship to enfuvirtide response. J Infect Dis Jul 15;2006 194(2):238–46. [PubMed: 16779731]
9. Wilkin TJ, Su Z, Kuritzkes DR, et al. HIV type 1 chemokine coreceptor use among antiretroviral-experienced patients screened for a clinical trial of a CCR5 inhibitor: AIDS Clinical Trial Group A5211. Clin Infect Dis Feb 15;2007 44(4):591–5. [PubMed: 17243065]
10. Reeves JD, Gallo SA, Ahmad N, et al. Sensitivity of HIV-1 to entry inhibitors correlates with envelope/coreceptor affinity, receptor density, and fusion kinetics. Proc Natl Acad Sci U S A Dec 10;2002 99(25):16249–54. [PubMed: 12444251]
11. Princen K, Hatse S, Vermeire K, et al. The antiviral activity of the CXCR4 antagonist AMD3100 is independent of the cytokine-induced CXCR4/HIV coreceptor expression level. AIDS Res Hum Retroviruses Dec;2003 19(12):1135–9. [PubMed: 14709250]
12. Weber J, Piontkivska H, Quinones-Mateu ME. HIV type 1 tropism and inhibitors of viral entry: clinical implications. AIDS Rev Apr-Jun;2006 8(2):60–77. [PubMed: 16848274]
13. Coakley E, Petropoulos CJ, Whitcomb JM. Assessing chemokine co-receptor usage in HIV. Curr Opin Infect Dis Feb;2005 18(1):9–15. [PubMed: 15647694]
14. Whitcomb JM, Huang W, Fransen S, et al. Development and characterization of a novel single-cycle recombinant-virus assay to determine human immunodeficiency virus type 1 coreceptor tropism. Antimicrob Agents Chemother Feb;2007 51(2):566–75. [PubMed: 17116663]
15. Wilkin TJ, Su Z, Kuritzkes DR, et al. HIV type 1 chemokine coreceptor use among antiretroviral-experienced patients screened for a clinical trial of a CCR5 inhibitor: AIDS Clinical Trial Group A5211. Clin Infect Dis Feb 15;2007 44(4):591–5. [PubMed: 17243065]
**16. Fätkenheuer G, Nelson M, Lazzarin A, et al. Subgroup analyses of maraviroc in previously treated R5 HIV-1 infection. N Engl J Med Oct 2;2008 359(14):1442–55. Subanalyses of the phase 3 maraviroc trials. Emergence of CXCR4-using virus was associated with virologic failure of maraviroc in one-third of csaes; 8% of subjects with R5 virus at screening with the Trofile assay had CXCR4-using virus at entry. [PubMed: 18832245]
*17. Hosoya N, Su Z, Wilkin T, et al. Assessing HIV-1 tropism: A comparison of assays using replication-competent virus versus plasma-derived pseudotyped virions. J Clin Microbiol. epublished ahead of print 2009 Jun 3. The study compares the Trofile assay with two phenotypic assays that use replication-competent viruses instead pseudotyped viruses. Good concordance was found between the different assays, but the use of replicating virus did not substantially improve the detection of CXCR4-using viruses,
18. Gulick RM, Su Z, Flexner C, et al. Phase 2 study of the safety and efficacy of vicriviroc, a CCR5 inhibitor, in HIV-1-Infected, treatment-experienced patients: AIDS clinical trials group 5211. J Infect Dis Jul 15;2007 196(2):304–12. [PubMed: 17570119]
**19. Gulick RM, Lalezari J, Goodrich J, et al. Maraviroc for previously treated patients with R5 HIV-1 infection. N Engl J Med Oct 2;2008 359(14):1429–41. Reports results of the phase 3 trials of maraviroc. Trial eligibility was based on tropism testing with the Trofile assay. [PubMed: 18832244]
20. Trinh L, Han D, Huang W, et al. Technical validation of an enhanced sensitivity Trofile HIV co-receptor tropism assay for selecting patients for therapy with entry inhibitors targeting CCR5. Antiviral Ther 2008;13(Suppl 3):A128.
21. Reeves JD, Coakley E, Petropoulos CJ, et al. An enhanced sensitivity Trofile HIV coreceptor tropism assay for selecting patients for therapy with entry inhibitors targeting CCR5. J Viral Entry. 2009 In press.
22. Saag, M.; Ive, P.; Heera, J., et al. A Multicenter, Randomized, Double-Blind, Comparative Trial of a Novel CCR5 Antagonist, Maraviroc Versus Efavirenz, both in Combination with Combivir (Zidovudine/Lamivudine), for the Treatment of Antiretroviral-Naive Subjects Infected with R5 HIV 1: Week 48 Results of the MERIT Study. 4th IAS. Sydney, Australia: 2007.

23. Saag, M.; Heera, J.; Goodrich, J., et al. Reanalysis of the MERIT Study with the Enhanced Trofile Assay (MERIT-ES) 48th ICAAC/IDSA. Washington D.C.: 2008.

24. Su Z, Gulick RM, Krambrink A, et al. Response to Vicriviroc in Treatment-Experienced Subjects Using an Enhanced Sensitivty Co-receptor Tropism Assay: Reanalysis of AIDS CLinical Trials Group A5211. J Infect Dis. 2009 In press.

25. Jensen MA, van 't Wout AB. Predicting HIV-1 coreceptor usage with sequence analysis. AIDS Rev Apr-Jun;2003 5(2):104–12. [PubMed: 12876899]

26. Jensen MA, Coetzer M, van 't Wout AB, et al. A reliable phenotype predictor for human immunodeficiency virus type 1 subtype C based on envelope V3 sequences. J Virol May;2006 80 (10):4698–704. [PubMed: 16641263]

27. Suphaphiphat P, Essex M, Lee TH. Mutations in the V3 stem versus the V3 crown and C4 region have different effects on the binding and fusion steps of human immunodeficiency virus type 1 gp120 interaction with the CCR5 coreceptor. Virology Mar 30;2007 360(1):182–90. [PubMed: 17101166]

28. Pastore C, Nedellec R, Ramos A, et al. Human immunodeficiency virus type 1 coreceptor switching: V1/V2 gain-of-fitness mutations compensate for V3 loss-of-fitness mutations. J Virol Jan;2006 80 (2):750–8. [PubMed: 16378977]

29. Fouchier RA, Groenink M, Kootstra NA, et al. Phenotype-associated sequence variation in the third variable domain of the human immunodeficiency virus type 1 gp120 molecule. J Virol May;1992 66 (5):3183–7. [PubMed: 1560543]

30. De Jong JJ, De Ronde A, Keulen W, et al. Minimal requirements for the human immunodeficiency virus type 1 V3 domain to support the syncytium-inducing phenotype: analysis by single amino acid substitution. J Virol Nov;1992 66(11):6777–80. [PubMed: 1404617]

31. Resch W, Hoffman N, Swanstrom R. Improved success of phenotype prediction of the human immunodeficiency virus type 1 from envelope variable loop 3 sequence using neural networks. Virology Sep 15;2001 288(1):51–62. [PubMed: 11543657]

32. Brumme ZL, Goodrich J, Mayer HB, et al. Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naive individuals. J Infect Dis Aug 1;2005 192(3):466–74. [PubMed: 15995960]

*33. Low AJ, Marchant D, Brumme CJ, et al. CD4-dependent characteristics of coreceptor use and HIV type 1 V3 sequence in a large population of therapy-naive individuals. AIDS Res Hum Retroviruses Feb;2008 24(2):219–28. The study identifies factors related to viral tropism which are CD4-dependent. The readout from the Trofile assay appeared to be CD4 dependent only with CCR5 but not with CXCR4 cells. Additionally, the ability to predict CXCR4 use by genotype varied with CD4 cell count. [PubMed: 18240966]

34. Brumme ZL, Dong WW, Yip B, et al. Clinical and immunological impact of HIV envelope V3 sequence variation after starting initial triple antiretroviral therapy. AIDS Mar 5;2004 18(4):F1–9. [PubMed: 15090786]

35. Delobel P, Sandres-Saune K, Cazabat M, et al. R5 to X4 switch of the predominant HIV-1 population in cellular reservoirs during effective highly active antiretroviral therapy. J AIDS (1999) Apr 1;2005 38(4):382–92.

36. Pillai S, Good B, Richman D, et al. A new perspective on V3 phenotype prediction. AIDS Res Hum Retroviruses Feb;2003 19(2):145–9. [PubMed: 12643277]

37. Resch W, Hoffman N, Swanstrom R. Improved success of phenotype prediction of the human immunodeficiency virus type 1 from envelope variable loop 3 sequence using neural networks. Virology Sep 15;2001 288(1):51–62. [PubMed: 11543657]

*38. Low AJ, Dong W, Chan D, et al. Current V3 genotyping algorithms are inadequate for predicting X4 co-receptor usage in clinical isolates. AIDS Sep 12;2007 21(14):F17–24. The study compares six different genotypic methods in a predominantly subtype B cohort and shows generally low sensitivities among the methods. The authors offer ways to improve sensitivity by integrating CD4 count and changing the cut-off. The study found that the sensitivity of the genotypic methods improved with greater infectivity on CXCR4 cells. [PubMed: 17721088]

*39. Raymond S, Delobel P, Mavigner M, et al. Correlation between genotypic predictions based on V3 sequences and phenotypic determination of HIV-1 tropism. AIDS Sep 12;2008 22(14):F11–6. This study compared tropism testing by a recombinant virus-based phenotypic method with various

genotypic methods. The data show high concordance between genotypic and phenotypic assessments of coreceptor usage. [PubMed: 18753930]

*40. Garrido C, Roulet V, Chueca N, et al. Evaluation of eight different bioinformatics tools to predict viral tropism in different human immunodeficiency virus type 1 subtypes. J Clin Microbiol Mar; 2008 46(3):887–91. This study compared coreceptor determination by eight bioinformatics approaches to interpreting V3 genotypes. It examines the largest cohort of non-subtype B samples and confirms findings from smaller studies that genotypic methods perform poorly with non-subtype B viruses. [PubMed: 18199789]

**41. Saag M, Goodrich J, Fätkenheuer G, et al. A double-blind, placebo-controlled trial of maraviroc in treatment-experienced patients infected with non-R5 HIV-1. J Infect Dis June 1;2009 199(11): 1638–47. This study found no virologic or immunologic benefit of maraviroc in patients infected with D/M or X4 HIV-1. These findings provide support for the concept that viral tropism should be determined prior to initiating treatment with a CCR5 antagonist. [PubMed: 19432546]

42. Harrigan PR, McGovern R, Dong W, et al. Screening for HIV tropism using population-based V3 genotype analylsis: a retrospective virological outcome analysis using stored plasma screening samples for MOTIVATE-1. Antiviral Ther 2009;14(Suppl 1):A17.

43. Weiser B, Philpott S, Klimkait T, et al. HIV-1 coreceptor usage and CXCR4-specific viral load predict clinical disease progression during combination antiretroviral therapy. AIDS Feb 19;2008 22(4):469–79. [PubMed: 18301059]

44. Tressler, R.; Valdez, H.; Van der Ryst, E., et al. Comparison of Results from the SensiTrop™ vs Trofile™ Assays on 100 Samples from the Maraviroc Expanded Access Program; 15th Conference on Retrovirus and Opportunistic Infections; Boston, Massachusetts. 2008.

45. Baumann R, Hamdan H, Schwab D, et al. Performance of an HIV-1 coreceptor tropism assay utilizing replicate V3 loop sequencing and heteroduplex analysis with capillary electrophoresis. Antiviral Ther 2009;14(Suppl 1):A132.

*46. Waters L, Mandalia S, Randell P, et al. The impact of HIV tropism on decreases in CD4 cell count, clinical progression, and subsequent response to a first antiretroviral therapy regimen. Clin Infect Dis May 15;2008 46(10):1617–23. This study of a large treatment-naïve cohort shows that the presence of D/M or X4 virus is associated with increased risk of clinical progression and greater decrease in CD4 cell count. Immunological and virological responses to ART were similar, regardless of viral tropism. [PubMed: 18419499]

*47. Goetz MB, Leduc R, Kostman JR, et al. Relationship between HIV coreceptor tropism and disease progression in persons with untreated chronic HIV infection. J AIDS Mar 1;2009 50(3):259–66. This study assessed the relationship of coreceptor usage with the rate of disease progression. The cohort in the study included treatment-naïve patients with relatively high CD4 counts.

**48. Shepherd JC, Jacobson LP, Qiao W, et al. Emergence and persistence of CXCR4-tropic HIV-1 in a population of men from the multicenter AIDS cohort study. J Infect Dis Oct 15;2008 198(8):1104–12. This study examined longitudinal change in coreceptor usage in a cohort of male seroconverters. CXCR4-using virus was often detected when CD4 cell count was well above the range for the onset of AIDS. The authors propose the use of tropism testing for prognostic purposes in managing HIV infection. [PubMed: 18783316]