

## Review Article

# HIV-1 Tropism Test Evaluation: Assessment and Clinical Implications

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CCR5 and CXCR4 chemokines receptors are critical coreceptors for the binding of HIV to specific host cells. Guidelines recommend its assessment in case of virological failure or before prescription of CCR5 inhibitors. Strategies to assess viral tropism may be divided into phenotypic and genotypic assays; registrative trials of CCR5 inhibitors used phenotypic assay, but recently genotypic ones have been used in clinical practice. The presence of CXCR4 is increasing in naïve patients, with both acute and chronic HIV-1 infections; this coreceptor usage is associated with CD4 depletion. The assessment of viral tropism should be considered in every stage of HIV-1 infection.

## 1. Introduction

HIV enters target cells by interacting with specific surface receptors. While CD4 represents the main molecule with which the HIV envelope is able to interact, several cofactors have been identified. Among those, CCR5 (as discussed by Alkhatib et al. [1]) and CXCR4 (as discussed by Feng et al. [2]) chemokines receptors are critical coreceptors for the binding of HIV to specific host cells. Most HIV-1 quasiespecies infect cells expressing CCR5 and are thus defined as CCR5- or R5-tropic, while a smaller proportion of viruses bind to CXCR4 and are defined as CXCR4- or X4-tropic. Moreover, some patients harbour mixed viral populations, and some quasiespecies are able to use both coreceptors: those patients are defined as carrying dual/mixed viruses (DM-tropic) (as discussed by Berger et al. [3]).

Maraviroc, a CCR5 inhibitor, is the only antiretroviral agent targeting coreceptor binding currently licensed for use in HIV infection. Its use is limited to patients with a determined CCR5 tropism (as discussed elsewhere [4, 5]). Several techniques were developed to assess HIV tropism, and treatment guidelines from different countries are not uniform in defining which methods should be employed in clinical practice.

## 2. Methods

Strategies to assess viral tropism may be divided into two broad categories: phenotypic and genotypic assays. Phenotypic assays determine HIV tropism by culturing host infected cells or by engineering a recombinant virus derived from the virus population of the subject (as discussed by Raymond et al. [6]). Genotypic tests are based on amplification and subsequent sequence analysis of HIV V3 segment by means of prespecified algorithms (as discussed by Obermeier et al. [7]).

Among phenotypic assays, Trofile test has been the most widely used (as discussed by Whitcomb et al. [8]), and it has been recently replaced by an equivalent with enhanced sensitivity (Trofile ES or ESTA) (as discussed by Trinh et al. [9]). This strategy utilizes patient-derived env gene amplified by PCR and inserted into an expression vector. The vector is then cotransfected with a replication-defective proviral vector containing a luciferase reporter gene to give rise to a pseudovirus, which is subsequently used to infect cells expressing CCR5 or CXCR4. To increase sensitivity and specificity of this test, CCR5 and CXCR4 inhibitors are added to test the susceptibility of the virus to these molecules. This test requires a plasma HIV-RNA of more than 1000 cps/mL

and has recently been improved, being currently able to detect with 100% sensitivity CXCR4-tropic clones representing 0.3% or more of the viral population.

Genotypic assays are based on amplification sequencing of env gene, from both viral RNA and DNA. The sequence obtained is analyzed by a bioinformatic tool which applies an algorithm in order to determine HIV tropism. Several algorithms are currently available, the most widely used being Geno2Pheno (as discussed by Lengauer et al. [10]). This algorithm uses nucleotide sequences compared to a set of sequences corresponding to R5-, DM-, or X4-tropic viruses. The result is given as false positive rate (FPR), that is, the probability of classifying an R5 virus falsely as an X4.

### 3. Guidelines

American Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents (2013) (as discussed elsewhere [11]) recommend the use of tropism testing whenever a CCR5 inhibitor is considered for use (AI) or in case of virologic failure in patients treated with CCR5 inhibitor (BIII). European guidelines on the clinical management of HIV-1 tropism testing (as discussed by Vandekerckhove et al. [12]) suggest the assessment of viral tropism in case of virological failure or poor tolerability to current treatment if a CCR5 inhibitor is considered (AII); moreover, tropism testing is suggested in patients with virological failure or with HAART-related side-effects to give optional insight into future therapeutic choices (BII and CIII, resp.). Finally, European guidelines recommend tropism testing before starting treatment in naïve patients in whom toxicity to first-line treatment is expected (CIII), while testing in newly diagnosed patients currently has no recommendation due to insufficient data.

American guidelines differ from European guidelines in recommending a phenotypic tropism test (AI), considering a genotypic assay as an alternative (BII). European guidelines suggest the use of either phenotypic or genotypic test (BII) when plasma viremia is over 1000 cps/mL, while in patients with a plasma HIV-RNA under 1000 cps/mL genotypic test on viral RNA (CIII) or DNA (especially if under 50 cps/mL, CIII) is recommended. Values of Geno2Pheno false positive rate under which a virus should be considered X4-tropic are to be set at 10% after triplicate PCR amplification and sequencing in patients with >1000 cps/mL (CII) or <1000 cps/mL (BIII) and at 20% when only one sequence is generated (BIII); FPR threshold of 10% should also be employed when HIV-DNA is used after triplicate PCR and sequencing (BIII).

### 4. Acute Patients

Reports on determination of coreceptor usage are also studied during primary HIV-1 infection (PHI, defined according to Fiebig staging) and early HIV-1 infection (defined as diagnosed within 6 months of the presumed infection date).

During viral transmission, there is a bottleneck selection by which viral variants using CCR5 as coreceptor are strongly selected (as discussed by Zhu et al. [13]).

Gottlieb studies HIV-1 envelope genes among 38 patients at a very early stage of infection, before seroconversion. He reports that at least 46% of patients had replicating virus derived from a single viral variant; moreover, nearly all variants are predicted to be R5 viruses.

In their recent paper, Hedskog et al. [14] assess whether X4-tropic viruses are already present during viral transmission by ultradeep pyrosequencing (UDPS) of the V3 loop. He demonstrates that patients diagnosed during PHI as infected by R5-tropic viruses who switch over time to X4 viruses do not have any minor variant using X4 at the onset of infection. Indeed, the switch from R5 to X4 over time seems to be a viral evolution process from a unique variant.

Although R5 viruses (irrespective of the assay used) are the most frequent type found among early infected patients (as discussed elsewhere [13, 15]), there is multiple evidence of transmission of CXCR4-tropic viruses.

The evidence that X4 viruses can be transmitted has emerged during the last few years thanks to the use of either genotypic assays or the Trofile assay (OTA, Monogram), which is a recombinant phenotypic method aimed at assessing on lymphocytes the coreceptor used by the virus. The currently used genotypic test Geno2Pheno could slightly overestimate the frequency of X4 viruses.

As well described in the review of Verhofstede et al. [16] the prevalence of X4 or dual mixed tropic (DM) viruses among different cohorts of recent infection varies from 2% to 19% (as discussed elsewhere [17–22]).

In terms of clinical outcome, several works confirmed the worst clinical outcome of patients with X4 viruses.

Firstly, X4 viruses transmission seems not to be associated with transmission of drug resistance, as reported by de Mendoza et al. [21].

In a cohort of PHI patients, Chalmet et al. [23] reported that patients infected by X4 viruses had lower CD4 T cell counts, but no association with viral load was found.

Raymond et al. [24] found that X4 viruses in PHI patients were not associated with CD4 count and viral load, but these patients had a faster decline of CD4 T cells.

Hamlyn et al. [25] measured T cell activation in 120 PHI patients and reported that patients with X4 viruses had a higher level of immune-activation.

Conversely, Nozza et al. [26] reported that PHI patients with X4 viruses treated with cART had a better CD4 increase over time in comparison to patients carrying R5 viruses.

These contrasting results could be interpreted as the confirmation that X4 viruses are responsible for an accelerated disease progression but that early cART can restore the impairment to the immune-system, not precluding a good clinical outcome.

Anyway, the clinical impact of acquiring an X4 virus has to be further investigated.

### 5. Naïve Patients

The average of CCR5 viruses in naïve HIV-infected patients (pts) changes according to HIV history. In advanced naïve HIV-infected patients with low CD4 cell count about 40% of

patients are non-R5 (as discussed by Charpentier et al. [27]), while in HIV patients with CD4 >350 cells around 14% are non-R5 (as discussed elsewhere [28]).

Several studies correlate the usage of coreceptor to the immunovirological progression (as discussed by Moyle et al. [29]), suggesting an earlier beginning of ART in X4 HIV virus, but the impact of the CCR5 coreceptor usage on the clinical outcome after the beginning of therapy is not so clear.

Some studies as MERIT and ANRS 130 APOLLO do not show any correlation between HIV coreceptor usage and immunovirological profile. In MERIT (as discussed by McGovern et al. [30]) retrospective study, CCR5 patients have a better immunological response in maraviroc arm of treatment, while no other differences were found among arms. In ANRS 130 APOLLO study (as discussed elsewhere [27]) advance naïve patients were randomized to receive first ART regimen intensification with enfuvirtide or not. At week 24, there are no differences between patients with CCR5 or CXCR4 coreceptor neither in CD4 cell count nor in viraemia. Patients with stable R5 tropism during 24 weeks (34% of patients) display a better response to ART (tropism measured as Geno2Pheno on HIV-RNA and HIV-DNA).

While in some Italian studies HIV coreceptor usage is associated with a worse CD4 cell count trend before ART initiation, Santoro et al.'s study [31] analyses the relationship between HIV-1 coreceptor and immunovirologic parameter both in naïve and experienced patients. Decrease of CD4 cells was higher in patients harbouring X4 virus than R5 virus. This correlation is much more evident if FPR is set at 2% instead of 10%. No correlation is found between coreceptor usage and viraemia. Similar results are found in one Italian cohort (as discussed by Nozza et al. [28]) of 223 primary and chronic HIV-infected patients naïve to ART with CD4 count  $\geq 350$  cells/mL and HIV-RNA >500 copies/mL enrolled in the ICONA Foundation. In this cohort, non-R5 viral tropism at BL, determined by Geno2Pheno (FPR < 10%), is associated with an increased depletion of CD4 cells count at multivariable analysis during follow-up.

In ArTE study (as discussed by Seclén et al. [32]) the X4 correlates both with a more rapid decrease of CD4 and higher viraemia at baseline and with a poor response to ART in terms of viral load at w48. In the multivariate analysis CCR5 coreceptor usage is the independent predictor of virologic control during ART, while no significant relationship is found with CD4 cells count. No correlation with ART regimen is found. Similar results are described by Moyle in 2005 (as discussed by Moyle et al. [29]).

Finally Waters et al. [33] do not find any correlation between coreceptor usage and viral load, but there is a direct correlation with the occurrence of AIDS-defining events: X4 or dual mix HIV predicts a lower increase of CD4 at month 12 after adjustment for baseline characteristics, and the multivariate analysis shows augmented risk of adverse events (RR 2.56).

Once again the clinical impact of X4 virus is not clear and needs further investigation.

## 6. Experienced Patients

X4 HIV coreceptor usage has significant clinical implications, because it has been associated with higher viral load and lower CD4 T cell count (as discussed by Moyle et al. [29]). These viruses appear to emerge later in progression of HIV disease (as discussed by Berger et al. [34]) and several studies have demonstrated a higher prevalence of X4 variants in patients exposed to antiretroviral drugs than in drug-naïve individuals (as discussed by Johnston et al. [35] and Delobel et al. [36]).

The presence of X4 virus is associated with AIDS. As discussed by Delobel et al. [37], in a nonrandomised selection of individuals, approximately two-thirds of the men who develop AIDS have X4-tropic virus. Men who develop X4 viruses have a significant acceleration in the decline of CD3<sup>+</sup> T lymphocytes, which starts approximately one year after the first appearance of these viruses. These data are consistent with a causal relationship between emergence of non-R5 virus and onset of decreases in the T cell count.

Clinical relevance of tropism testing increases with introduction of CCR5 inhibitors as an antiretroviral treatment option. The MOTIVATE-1 and -2 studies compare maraviroc along with optimized background therapy (OBT) versus placebo along with OBT in treatment-experienced patients screened as having R5 HIV determined originally by Monogram Trofile (as discussed by Gulick et al. [4]). A retrospective analysis examines the performance of Geno2Pheno to predict coreceptor usage (as discussed by McGovern et al. [38]). Compared with Trofile, V3 genotyping has a specificity of 92.6% and a sensitivity of 67.4% for detecting non-R5 virus and virological responses are similar in considered treatment-experienced population.

There are few data from longitudinal studies on viral tropism in patients fully responding to HAART; as discussed by Parisi et al. [39] there is a correlation between viral tropism and combined HIV-proviral-DNA, residual viraemia, and drugs used in treatment. The tropism of archived virus was stable during the effective treatment, with 15–18% of subjects switching over time, despite a viraemia <50 copies/mL. R5 tropism and its stability are related to achieving and maintaining viraemia <2.5 copies/mL. A higher stability of viral tropism (88%) during ARV was reported in a small cohort by Seclén et al. [40], with 12% switching in either direction. From a clinical point of view, the performance of tropism test on HIV-DNA is suggested before prescription of maraviroc in this setting.

## 7. Closing Remarks

Current guidelines recommend the performance of tropism test in experienced patients, in particular for evaluating therapy with CCR5 inhibitors.

Different studies demonstrated that the presence of X4-tropic virus is associated with CD4 decrease and clinical progression; the percentage of this coreceptor usage is different in the clinical setting, as summarized in Table 1.

In addition, viral tropism is changeable in every patient.

TABLE 1: Percentage of CCR5 tropic virus in different stages of HIV-1 infection.

Authors, journal, year of publication	Methods	Percentage of patients with CCR5 tropic virus (median)	Total number of patients
<i>Acute and early HIV infection</i>			
Eshleman et al. [17], AIDS Res Hum Retroviruses 2007	Phenotypic (replication-defective retroviral vector assay)	98	195
Huang et al. [18], AIDS Res Hum Retroviruses 2009	Trofile	96	150
Frange et al. [19], J. Clin. Microbiol. 2010	Geno2Pheno <sub>FPR 10%</sub> Phenotypic (recombinant virus entry assay)	88%	131
Frange et al. [20], J. Antimicrob. Chemother. 2009	Genotypic (5 genotypic rules to predict tropism)	84%	390
De Mendoza et al. [21], J. Antimicrob. Chemother. 2007	Genotypic (wetcat prediction algorithm)	83%	203
Rieder et al. [22], Clin. Infect. Dis. 2011	Genotypic with 3 prediction tools (web-PSSM, wetcat, Geno2Pheno) Phenotypic (MT2 and GHOST cell culture assays)	90%, 81%, 86%	145
Chalmet et al. [23], J. Infect. Dis. 2012	Geno2Pheno	81–88%	539
Raymond et al. [24], AIDS 2010	Recombinant virus phenotypic entry assay (Toulouse tropism test assay)	93.6%	133
Hamlyn et al. [25], AIDS 2012	Geno2Pheno	88%	
	Geno2Pheno <sub>FPR 5.75%</sub>	91.7%	120
Nozza et al. [26], J. Acquir. Immune Defic. Syndr. 2014	Geno2Pheno <sub>FPR 10%</sub> plasma, <sub>FPR 20%</sub> PBMC	87.3%	
	PSSMx4r5	87%	66
	(ds) kernel	92.4%	
Charpentier et al. [27], J. Antimicrob. Chemother. 2013	Geno2Pheno <sub>FPR 10%</sub> on HIV RNA Geno2Pheno <sub>FPR 10%</sub> on HIV DNA	60% 54%	170
Nozza et al. [28], J. Antimicrob. Chemother. 2012	Geno2Pheno <sub>FPR 10%</sub>	86%	223
Simon et al. [42], AIDS 2010	Geno2Pheno <sub>FPR 20%</sub>	62%	50
Moyle et al. [29], J. Infect. Dis. 2005	ViroLogic PhenoSense assay	81%	402
Santoro et al. [31], Clin. Microbiol. Infect 2012	Geno2Pheno <sub>FPR 2%</sub> Geno2Pheno <sub>FPR 10%</sub>	4.9% 25%	532
<i>Experienced HIV patients</i>			
Gulick et al. [4], N Engl J Med 2008	Trofile	70%	1049
Caseiro et al. [43], J. Infect. 2012	Trofile	84%	857
Recordon-Pinson et al. [44], Antimicrob. Agents Chemother. 2010	Trofile Geno2Pheno <sub>FPR 10%</sub>	71.4% 77%	236
Svicher et al. [45], New Microbiol. 2010	Trofile Geno2Pheno <sub>FPR 10%</sub>	76.5% 70%	406

In naïve patients, the presence of X4-tropic virus is associated with CD4 depletion; its determination in this setting could be useful to individualize a fragile HIV-positive population who could have benefited by early starting of ART.

In successfully treated naïve subjects, the tropism of archived virus was revealed to be stable but with a substantial rate (15–18%) of subjects switching from one type to the other over time, suggesting that archived virus is dynamic.

As discussed by Paquet et al. [41], the fraction of non-R5 tropic viruses has decreased over time within 62,397 resistant viruses reported from 2003 to 2012; however, CXCR4 usage was more prevalent among multiclass resistant samples, which may be due to the more advanced disease stage of treatment-experienced patients.

In conclusion, determination of viral tropism could be useful in the prescription of CCR5 inhibitors and for better

knowledge of HIV-positive patients and their prognosis in terms of CD4 depletion.

## Conflict of Interests

The authors declare that they have no conflict of interests.

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