Update on Clinical and Methodological Recommendations for Genotypic Determination of HIV tropism to Guide the Usage of CCR5 Antagonists

Eva Poveda[1], Roger Paredes[2], Santiago Moreno[3], José Alcamí[4], Juan Córdoba[5], Rafael Delgado[6], Félix Gutiérrez[7], Josep M. Llibre[8], Miguel García Deltoro[9], José Hernández-Quero[10], Federico Pulido[11], José Antonio Iribarren[12] and Federico García[13]

[1]Infectious Diseases Department, Hospital Carlos III, Madrid, Spain; [2]HIV Unit and IrsiCaixa, Hospital Germans Trias i Pujol, Badalona, Spain; [3]Service of Infectious Diseases, Hospital Ramón y Cajal, Madrid, Spain; [4]AIDS Immunopathogenesis Unit, National Center of Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain; [5]Service of Microbiology, Hospital La Fe, Valencia, Spain; [6]Laboratory of Molecular Microbiology, Instituto de Investigación Hospital 12 de Octubre (i+12), Madrid, Spain; [7]Infectious Diseases Unit, Hospital Universitario de Elche, Miguel Hernández University, Alicante, Spain; [8]Hospital Universitari Germans Trias i Pujol, Fundació Lluita contra la SIDA, Badalona, Spain; [9]Infectious Diseases Unit, Consorcio Hospital General Universitario, Valencia, Spain; [10]Infectious Diseases Unit, Hospital Universitario San Cecilio, Granada, Spain; [11]HIV Unit, Instituto de Investigación Hospital 12 de Octubre, Madrid, Spain; [12]Infectious Diseases Unit, Hospital Donostia, San Sebastián, Spain; [13]Service of Microbiology, Hospital Universitario San Cecilio, Granada, Spain

Abstract

The genotypic determination of HIV tropism to guide the use of maraviroc, the first CCR5 antagonist with specific antiviral activity against CCR5 (R5)-tropic HIV variants, has been widespread in the last two years. Retrospective analyses from maraviroc clinical trials (MOTIVATE and MERIT) demonstrated that specific genotypic tools and the phenotypic assay Trofile™ are comparable in predicting virologic response to maraviroc. Moreover, recent studies performed in cohorts of patients outside clinical trials have reported overall rates of virologic response to maraviroc up to 82% in patients harboring HIV R5-tropic variants according to genotypic tools. Specific technical requirements as well as recommendations for proper HIV tropism determination in the clinical setting have been improving, according to new data reported in several studies related with this issue. This review updates clinical and methodological recommendations for genotypic determination of HIV tropism to guide therapeutic decisions using CCR5 antagonists, considering the most recently reported data. **(AIDS Rev. 2012;14:208-17)**

Corresponding author: Eva Poveda, evapoveda@hotmail.com

Key words

HIV tropism. CCR5 antagonists. V3 genotyping. Maraviroc.

Introduction

During the HIV entry process, CD4-gp120 interaction induces conformational changes in the viral envelope that expose a chemokine receptor binding site and consequently allow the CD4-gp120 complex to interact with a chemokine coreceptor, typically CCR5 or CXCR4. The CD4-gp120 complex binds to either coreceptor through interactions mainly with the V3 region of gp120, though other HIV gp120 regions, such as V1/V2, C4, and the bridging sheet, are also involved[1]. The use of CCR5 or CXCR4 coreceptors by HIV is mainly determined by the amino acid sequence of the V3 region of gp120. Accordingly, HIV isolates are classified as either R5 tropic, X4 tropic, or dual/mixed tropic, depending on their coreceptor use[2]. The term "dual/mixed" refers to isolates that may contain true dual-tropic particles that can use either or both chemokine coreceptors or mixtures of viruses that exclusively use CCR5 and others that use CXCR4.

Correspondence to:
Eva Poveda
Departamento de Enfermedades Infecciosas
Hospital Carlos III
Sinesio Delgado, 10
28029 Madrid, España
E-mail: evapoveda@hotmail.com

The CCR5 antagonists represent the second class of HIV entry inhibitors approved for the treatment of HIV infection that exclusively inhibit replication of R5-tropic HIV variants. Maraviroc (Selzentri®) is so far the only CCR5 antagonist approved for treatment of HIV infection[3]. It is an allosteric inhibitor of the CCR5 chemokine coreceptor, orally bioavailable, and binds to the transmembrane coreceptor cavity within the 2, 3, 6, and 7 helix[4]. Following binding, CCR5 coreceptor conformational changes occur, especially in the second extracellular loop (ECL2) region, which ultimately inhibits the interaction of the ECL2 with the V3 region of gp120, and consequently the HIV entry process.

Due to its mechanism of action, the antiviral activity of CCR5 antagonists is limited to R5-tropic variants, and the presence of detectable X4 or R5/X4 dual-tropic viruses has been associated with therapeutic failure using CCR5 antagonists[5-7]. Therefore, determination of HIV coreceptor usage is required before recommending treatment with this drug family. Several phenotypic and genotypic assays have been developed to determine HIV tropism in clinical samples[8,9].

The Trofile™ phenotypic assay (Monogram Biosciences, USA), which is based on recombinant virus technology, has been extensively utilized to provide tropism information in clinical trials, showing good correlation with virologic outcomes[10]. However, this method displays logistical (specimens must be shipped to the reference laboratory in the USA) and technical (> 15% of specimens are non-reportable) limitations that make it far from convenient as a diagnostic test in clinical practice. Genotypic assays, based on analysis of the V3 region, represent a more feasible alternative to phenotypic assays since they are more rapid, cheaper, and more widely available among laboratories specializing in HIV diagnosis[11]. The use of specific genotypic tools such as geno2pheno and PSSM have demonstrated, in retrospective analyses of maraviroc trials (MOTIVATE/A4001029 and MERIT)[12-14] and different studies performed in cohorts of HIV-infected patients outside clinical trials[15-18], their ability to predict virologic responses to a CCR5 antagonist-based therapy, even though their sensitivity to detect X4 variants is lower compared with Trofile™ [19-23].

Specific technical requirements and recommendations for a proper HIV tropism determination in the clinical setting have been recorded in several guidelines for the treatment of HIV-infected patients. Indeed, a consensus document on HIV-tropism determination proposed by a Spanish panel of experts in 2010[24] and the European guidelines published in 2011[25] specifically record the main clinical and methodological recommendations for genotypic determination of HIV coreceptor usage in the clinical setting. During the last two years, new relevant data have emerged from several studies related with this issue that require consideration for a more reliable determination of HIV tropism. In this context, the Spanish panel of experts met again to analyze and discuss the new published data and include it in a new document by consensus. This review updates clinical and methodological recommendations for genotypic determination of HIV tropism to guide therapeutic decisions using CCR5 antagonists, considering the most relevant data recently published.

HIV tropism determination in the clinic: the widespread of genotypic methods

The phenotypic assay Trofile™ has been extensively used to provide tropism information in the pivotal maraviroc clinical trials, and accordingly it has been widely used to date. The MOTIVATE/A4001029 and MERIT trials demonstrated the ability of Trofile™ to identify responders and nonresponders to maraviroc-based therapy[12-14], but also revealed its limitations for the detection of minority X4-tropic variants associated with virologic failure to maraviroc[5-7]. Consequently, Monogram Biosciences developed an enhanced sensitivity tropism assay (ESTA), which is 10- to 100-fold more sensitive for detecting X4 minor populations[26]. In June 2008, ESTA replaced the original Trofile™ assay used in the pivotal clinical trials. Initially, the current version of Trofile™ to determine HIV tropism was retrospectively validated in the MERIT trial. ESTA reclassified as dual/mixed nearly 15% of viruses from samples originally scored as having R5 at baseline by the original Trofile™. However, a detailed analysis of the results showed that even though the higher sensitivity of the new version to detect minority X4 variants, ESTA seems not improve the ability of the assay to discriminate between responders and nonresponders to maraviroc, since nearly 43% of patients reclassified as dual/mixed had reached HIV RNA < 50 copies/ml at week 48, even harboring detected X4 variants[5,27]. More recently, ESTA was also retrospectively validated in treatment-experienced patients in the MOTIVATE/A4001029 trials to identify responders and nonresponders to maraviroc[14].

In addition to Trofile™, other phenotypic methods have been designed for HIV tropism determination, mainly based on cloning or recombination of polymerase chain reaction (PCR)-amplified sequences encompassing partial regions of the gp160 envelope. The

Table 1. Genotypic rules and algorithms for determining viral tropism

Methodology	Principle
Rules and algorithms	
– 11/25 rule[40]	R or K at position 11 and/or 25 is associated with an X4-tropic phenotype
– 11/24/25 rule[41]	R or K at positions 11, 24, or 25 is associated with an X4-tropic phenotype
– Net charge[42]	K+R – (D+E) ≥ 5 is associated with an X4-tropic phenotype
– Wetcat[46] (http://genomiac2.ucsd.edu:8080/wetcat/v3.html)	
– Geno2pheno[47] coreceptor (http://coreceptor.bioinf.mpi-inf.mpg.de/index.php)	HIV tropism predictions are inferred from genotypic/phenotypic paired dataset employing statistical methods. These algorithms for HIV tropism interpretation are freely available on websites
– WebPSSM[48] (http://indra.mullins.microbiol.washington.edu/webpssm)	At this time, geno2pheno is one of the most accepted and widely used algorithms for genotypic determination of viral tropism
– Fortinbras PSSM (http://fortinbras.us/cgibin/fssm/fssm.pl)	
Deep sequencing	This technology allows investigating whether a higher sensitivity for the detection of X4-tropic minority variants might improve the ability to identify responders and nonresponders to maraviroc-based therapy. The best cutoff for the detection of X4 variants is still controversial
	It is a sophisticated and expensive method that is only available in a few research facilities. However, these limitations are being resolved with the advent of new cheaper generations (454 junior) of this technology and new useful tools for the interpretation of results (geno2pheno-454)

R: arginine; K: lysine; D: aspartic acid; E: glutamic acid.

sensitivity and specificity of some of these assays to detect X4 variants have been validated using Trofile™ and/or ESTA as gold-standard[28-38].

Genotypic assays represent a more feasible alternative to phenotypic assays since they are more rapid, cheaper and more broadly available among laboratories specialized in HIV diagnosis. Since the early 1990s, several rules and algorithms have been developed to predict HIV-coreceptor usage based on V3 sequences. Many of them are now freely available via publicly accessible websites[5]. Table 1 summarizes the main methodological characteristics of genotypic rules and algorithms for determining viral tropism[39-49].

The validation of genotypic tropism prediction methods do rather than a perfect concordance with the Trofile™ (or ESTA) assay and evidence a similar ability to correctly identify patients who will benefit from the use of maraviroc. In this context, recent studies have evaluated the reliability of genotypic tropism prediction tools to guide the therapeutic use of CCR5 antagonists[12-18].

A retrospective analysis of the MOTIVATE trials[12] has demonstrated that specific genotypic tools and the Trofile™ assay are comparable in predicting virologic response to maraviroc, although the sensitivity to detect X4 variants of the genotypic algorithms used, geno2pheno (FPR 5%) and PSSM, was 63 and 59%, respectively, compared with Trofile™. Likewise, a reanalysis of the MERIT trial demonstrated the ability of geno2pheno-5.75% to identify responders and nonresponders to maraviroc similarly to ESTA, even when the sensitivity to detect X4 variants was 55% compared with ESTA[14]. More recently, a retrospective analysis of MOTIVATE/A4001029 in treatment-experienced patients demonstrated the feasibility of geno2pheno-5.75% and geno2pheno-10% to identify responders and nonresponders to maraviroc similarly to ESTA[14].

Table 2. Rates of virologic response to maraviroc based on genotypic determination of HIV tropism outside clinical trials

Study	Patient population	Clinical setting	Type of sample (plasma vs. proviral DNA)	% of patients reaching or maintaining < 50 copies/ml after starting maraviroc-based therapy
Obermeier, et al.[15]	n = 160	Salvage therapy (HIV RNA > 50 copies/ml) and patients with HIV RNA < 50 copies/ml	Both*	69% at week 96 for pooled groups of patients
Seclén, et al.[16]	n = 62	Salvage therapy (HIV RNA > 50 copies/ml) and immune recovery or toxicity/intolerance switches (HIV RNA < 50 copies/ml)	Both	83% at week 96 in salvage therapies. 92% at week 96 in patients with baseline HIV RNA < 50 copies/ml
Chueca, et al.[17]	n = 54	Simplification (dual regimen with maraviroc + darunavir/r; HIV RNA < 50 copies/ml)	DNA	87% at week 24
Bellecave, et al.[18]	n = 71	Patients with suppressed plasma viremia (HIV RNA < 50 copies/ml)	DNA	88% at week 36 82% at week 48

*Tropism information was inferred either from phenotypic assays in plasma or genotypic assays in plasma and proviral DNA.

New reports have shown results from studies outside clinical trials performed in different European cohorts in whom the virologic response to maraviroc has been evaluated based on a genotypic determination of viral tropism. Overall, the results obtained have shown rates of virologic response to maraviroc up to 82% in those patients in which HIV variants were classified genotypically as R5-tropic viruses[15-18] (Table 2).

Moreover, the contribution of baseline $CD4^+$ counts and the drugs administered along with maraviroc for achieving viral suppression has been highlighted by recent studies[50]. Valdez, et al. showed that a weighted, optimized background treatment susceptibility score, rather than low-level X4 viruses at baseline, was the strongest predictor of virologic response at 48 weeks in the MOTIVATE trials[51]. Therefore, the activity of the accompanying drugs is of paramount importance to enable maraviroc to benefit patients with a low proportion of X4 variants. Therefore, in the contemporary therapeutic context, with potent drugs available to be given along with maraviroc, the presence of X4 variants most likely might have only a minor impact on virologic outcomes.

In view of these data, different guidelines for HIV infection management, such as the Spanish (http://www.gesida.seimc.org)[52], British (http://www.bhiva.org/Tropism.aspx)[53], and European (http://www.europehivresistance.org)[54] guidelines, specifically include within their recommendations the use of genotypic methods to guide the clinical use of CCR5 antagonists. Moreover, as previously mentioned, Spanish[24] and European[25] recommendations have been published to guide the use of CCR5 antagonists in clinical practice, based on the genotypic determination of viral tropism.

In this context, the use of genotypic methods for HIV tropism determination has rapidly spread over the last two years in Europe, replacing the initial phenotypic assay. In the USA, the experience with genotypic methods is more limited because there are fewer logistical barriers to obtain HIV tropism determination by Trofile™ since the reference laboratory (Monogram Bioscience) is based in the USA.

Update on clinical recommendations for genotypic determination of HIV tropism

Current HIV treatment guidelines recommend HIV tropism testing whenever the use of a CCR5 inhibitor is being considered. Overall, the European guidelines are more disposed to use genotypic assays than the USA's since the experience with this methodology in the USA is more limited. The following are the main recommendations for genotypic determination of HIV tropism, considering specific clinical settings (Table 3).

Table 3. Clinical recommendations for determining HIV tropism in the clinical setting

Patient population	Recommendation grading	Specific recommendation
Drug-naive HIV-infected patients candidates for antiretroviral therapy initiation	BIII	When maraviroc is considered as a therapeutic option (presence of primary resistance or toxicity/intolerance to first-line regimen or pharmacokinetic interactions), perform HIV tropism test closest to antiretroviral therapy initiation (1-3 months before)
Antiretroviral-experienced patients	AIII	Tropism test must be done in each treatment failure and results seen simultaneously to genotypic resistance tests
Antiretroviral-experienced patients under suppressive antiretroviral therapy	CIII	In the context of a switch to maraviroc for any reason. It is recommended to perform HIV tropism test from proviral DNA or from viremic stored plasma samples right before the initiation of beginning a suppressive antiretroviral therapy

Strength of recommendation. A: strong recommendation for the statement; B: moderate recommendation for the statement; C: optional recommendation for the statement. Quality of evidence for recommendation. I: one or more randomized trials; II: one or more well-designed, nonrandomized trials; III: expert opinion.

Drug-naive HIV-infected patients (BIII)

To date, there is no data to extend the recommendation for HIV tropism determination in patients who are going to start antiretroviral therapy. However, the U.S. Department of Health and Human Services (DHHS)[55] and European AIDS Clinical Society (EACS)[56] antiretroviral guidelines consider the use of maraviroc as an acceptable or alternative regimen for antiretroviral-naive patients. Therefore, maraviroc use might be considered in drug-naive patients in special clinical situations, such as the presence of primary resistance, or in case of toxicity/intolerance to drugs included in first-line therapy. In these situations, it is advisable to determine HIV tropism closest to antiretroviral therapy initiation to avoid potential tropism evolution between tropism determination and the time of starting maraviroc therapy. Viral tropism evolves in the course of HIV infection, and switches in viral tropism from R5 to X4 might occur before HAART initiation[5,6].

Antiretroviral-experienced patients (AIII)

Assessment of HIV tropism is recommended in all patients who experience virologic failure. Viral tropism information should be available together with each drug resistance test to facilitate the design of an optimal rescue therapy.

Antiretroviral-experienced patients under suppressive antiretroviral therapy (CIII)

In those patients under suppressive antiretroviral therapy in which a switch to maraviroc is being planned for any reason (intolerance/toxicity, drug-drug interactions, and simplification or intensification strategies), HIV tropism could be performed genotypically from peripheral blood mononuclear cells (PBMC). Although there is as yet scarce data regarding the clinical validation of this therapeutic strategy, the results reported to date support the use of this tool to guide the use of maraviroc in this scenario[57-59]. There are ongoing prospective clinical trials to validate the determination of HIV tropism from proviral DNA to guide the use of CCR5 antagonists in HIV patients under suppressive anti-HIV therapy.

As an alternative, HIV tropism might be also determined from viremic plasma samples stored (–80ºC) right before initiation of the last suppressive antiretroviral therapy, as long as full HIV-1 plasma suppression (< 50 copies/ml) has been maintained. Several studies have demonstrated a relatively good correlation (~82%) between RNA and DNA tropism estimations using genotypic methods[57-59]. Moreover, the rate of HIV tropism switches over time under suppressive HAART has been estimated. Overall, viral tropism switches from R5 to X4 are rare and ranged from 6.1 to 14.9%, depending on the time of follow-up considered and the type of sample analyzed (RNA vs. DNA)[57,60-62].

Technical and methodological recommendations for proper V3 genotyping in the clinical setting

The specific methodological recommendations for determining HIV tropism in the clinical setting have been improving, according to new data reported in several studies related with this issue (Table 4). In this context, there are recently published data of particular relevance for V3 genotyping that must to be taken into consideration.

Table 4. Technical and methodological recommendations for determining HIV tropism in the clinic

Topic	Specific recommendation	Grade	Comments
Report	R5 tropism/X4 tropism	AIII	Genotypic assays based on bulk sequencing cannot distinguish between dual/mixed tropic variants. When geno2pheno is used for HIV tropism interpretation, it is recommended to include the percentage of FPR in the report as a comment. Moreover, when V3 genotyping is performed from non-B subtype samples, it should be indicated
	In parallel together with the resistance test for RT, protease, integrase and fusion inhibitors	AIII	CCR5 antagonist might be considered similarly to other drugs in rescue therapies
Interpretation	Geno2pheno FPR 10% and/or (http://coreceptor.bioinf.mpi-inf.mpg.de/)	AII	In case that V3 genotyping is performed using one single RT-PCR
	PSSM X4R5/SiNSi (http://indra.mullins.microbiol.washington.edu/webpswsm/)		
	Geno2pheno FPR: 5.75%	AII	In case that V3 genotyping is performed using three RT-PCR
Plasma volume	≥ 500 µl	AII	Increase the sensitivity to detect X4-tropic variants
Proviral DNA	When HIV RNA viral load is ≤ 500 copies/ml or RNA amplification from plasma samples is not possible	BII	There are several data outside clinical trials in cohorts of patients in which maraviroc therapy was initiated based on HIV tropism determination using geno2pheno with a FPR of 10 or 20%
	Interpretation: geno2pheno 10-20%	BII	
Sequence analysis	It is indicated to expand the V3 sequence in the case of nucleotide mixtures in all possible permutations	AIII	Increase the sensitivity to detect X4-tropic variants
	If the V3 sequence has ≥ 8 nucleotide mixtures, do not consider it for subsequent analysis	AIII	A heterogeneous V3 sequence might cause errors during interpretation
Non-B subtypes	To advise in the HIV tropism report regarding the poor correlation between genotypic and phenotypic methods for HIV tropism determination in non-B subtypes compared with B	AIII	The overall correlation between genotypic and phenotypic methods for the detection of X4-tropic variants is lower in non-B subtype samples than in B. Moreover, the higher genetic variability among V3 sequences from non-B subtypes might lead to inaccuracies in the HIV tropism predictions. To date there is scarce data regarding the feasibility of genotypic and phenotypic tools to predict clinical response to maraviroc in non-B subtypes patients
	Interpretation: as for B subtypes, except for subtype C variants for which there is a specific matrix ("matrix C") in PSSM website		

Strength of recommendation. A: strong recommendation for the statement; B: moderate recommendation for the statement; C: optional recommendation for the statement.
Quality of evidence for recommendation. I: one or more randomized trials; II: one or more well-designed, nonrandomized trials; III: expert opinion.

Choosing the best algorithm for viral tropism interpretation (AII)

Although there are several rules and algorithms available for viral tropism interpretation[39-49], geno2pheno[47] and PSSM[48] are considered the most appropriate for use in the clinical setting. Both have demonstrated to be comparable with Trofile™ to identify responders and nonresponders to maraviroc[12-18]. For each algorithm, it is possible to obtain different rates of sensitivity to detect X4 variants depending on the false-positive rate (FPR) used in the case of geno2pheno or the matrix

selected for interpretation in the case of PSSM (R5X4 or SINSI). An increase in sensitivity for the detection of X4 variants is accompanied by a loss in specificity.

Seclén, et al. have recently reported a high concordance (88%) between PSSM and geno2pheno in the genotypic interpretation of HIV-1 tropism in clinical samples[63]. However, at this time, geno2pheno is one of the most accepted and widely used algorithms for genotypic determination of viral tropism[47]. The main disadvantage of this algorithm was that the server does not allow batch predictions of V3 sequences; therefore, V3 sequences should be introduced independently. However, the latest version of geno2pheno allows analyzing up to 50 V3 sequences simultaneously. Although this option does not automatically give the HIV tropism interpretation (R5 tropic or X4 tropic), it generates a FPR for each V3 sequence that can be interpreted subsequently. Geno2pheno also gives the opportunity to introduce additional clinical data (HIV RNA levels, $CD4^+$ counts, and the presence of the Δ32 deletion in the CCR5 gene) to improve the accuracy of predictions. However, this model is only based in V3 sequences from antiretroviral-naive patients and to date it has not been validated. Therefore, the use of these clinical parameters is not recommended for HIV tropism interpretation in clinical practice. As mentioned before, geno2pheno permits the selection in each prediction of the degree of sensitivity to detect X4 variants choosing different FPR. A higher FPR indicates a more sensitive prediction for the detection of X4 variants, but a lower specificity for the detection of R5 viruses. Considering the data published to date, the recommendations are to use a FPR of 5.75 or 10% based on the number of reverse transcriptase polymerase chain reaction (RT-PCR) assays performed for V3 genotyping.

Single versus triplicate (AII)

The number of RT-PCR assays needed for V3 genotyping has been a cause of debate. In a reanalysis of the MOTIVATE and MERIT trials, in which V3 genotyping was clinically validated to discriminate between responders and nonresponders to maraviroc, the number of RT-PCR assays performed was three. The performance of three RT-PCR has demonstrated to increase the sensitivity to detect X4 variants from 4 to 8% compared with the performance of a single PCR using geno2pheno with a FPR of 5.75%. More recently, Swenson, et al. have presented new data of this reanalysis using geno2pheno with a FPR of 10%, and the rates of virologic response to maraviroc are comparable either using a single or three RT-PCR[64]. Therefore, whether V3 genotyping is realized using one single PCR, the recommendation is to use geno2pheno with a FPR of 10%.

Non-B subtypes (AIII)

The overall sensitivity of genotypic methods for the detection of X4 variants is lower in non-B HIV-1 subtypes than for B subtypes. For example, using geno2pheno with a 20% FPR, the sensitivity to detect X4 variants was 94% for B subtypes and 63% for non-B ones, considering as gold standard the phenotypic assay HIV-1 Phenoscript Env™ (VIRalliance, France). Similarly, in the same set of samples, the sensitivity to detect X4 variants using PSSMr5x4 was 89% for clade B and 58% for non-B subtypes[65]. The feasibility of genotypic tools was also evaluated for specific HIV-1 subtypes (CRF02_AG, G and C)[65-67]. Subtypes CRF02_AG and G are the most prevalent in Spain (47%)[68] and several other European countries[69]. The sensitivity of geno2pheno (FPR 10%) and PSSMr5x4 to detect X4 variants in specimens from patients infected with CRF02_AG and G was 71%[65]. In patients infected with subtype C, the most prevalent worldwide[70], it is recommended to use a specific matrix of PSSM for samples from HIV-1 subtype C-infected patients, showing a sensitivity of 93%[67].

The algorithms for HIV tropism interpretation currently in use are based on paired genotypic/phenotypic databases constituted by 100 to 1,100 V3 sequences with paired phenotypic data[45]. From these, the number of V3 sequences with paired phenotypic data from non-B subtypes is very limited and might explain the overall poor performance of genotypic methods in non-B subtypes. Moreover, the higher genetic variability among V3 sequences from non-B subtypes might lead to inaccuracies in HIV tropism prediction in non-B subtypes. To date, there is scarce data regarding the feasibility of genotypic tools to predict clinical response to maraviroc in patients infected with non-B subtypes. A recent report from a small cohort of HIV-infected patients has shown that the clinical response to maraviroc was comparable between B and non-B subtypes using geno2pheno (FPR 20%)[71].

New data in proviral DNA (BII)

Genotypic determination of HIV tropism from proviral DNA is indicated in patients with HIV RNA ≤ 500 copies/ml or in those in which RNA amplification from plasma samples is not successful. The biological specimen

should be complete blood from which DNA can be extracted directly or from PBMC obtained by conventional methods. In this case, the use of geno2pheno using a FPR of 10 or 20% is recommended. New data from retrospective analyses of the MOTIVATE/A4001029 trials in antiretroviral-experienced patients have demonstrated that HIV tropism determination from proviral DNA is a good predictor of virologic response to maraviroc, comparable to Trofile™ and ESTA[72]. There are several data outside clinical trials in cohorts of patients in which maraviroc therapy was initiated based on HIV tropism determination using geno2pheno with a FPR of 10 or 20%. In these studies, the rates of virologic response to maraviroc were up to 82%[16-18] (Table 2). At this time, a prospective validation of the use of proviral DNA for HIV tropism determination to guide the use of CCR5 antagonists in clinical practice is ongoing in Europe.

HIV tropism report (AIII)

The HIV tropism report needs to be clear and easily understood. It is recommended to use terms such as "R5-tropic" or "X4-tropic" instead of terms like "CCR5-antagonists like maraviroc are likely to be or not effective". When geno2pheno is used for HIV tropism interpretation, it is recommended to include the percentage of FPR in the report as a comment. This data might be helpful for the clinicians because it gives additional information regarding the probability of the sample to be R5-tropic. Considering a FPR of 10% for interpretation, when the FPR value is ≥ 10, there is a high probability of R5 tropism, and conversely, when the FPR value is closer to zero, there is a high probability of X4 tropism[47]. Moreover, for V3 sequences from non-B subtype, it is recommended to include in the report a note highlighting the poor correlation between genotypic and phenotypic assays for HIV tropism determination in these samples.

New technologies: massive pyrosequencing by 454

Virologic failure to CCR5 antagonist-based therapies is mainly characterized by the selection of X4 viruses that preexist as a minority population before antiretroviral therapy initiation, below the detection level of the assay. Indeed, phylogenetic analysis demonstrated that X4-tropic variants detected in patients failing CCR5-antagonist-based therapy are identical to X4 variants present as minor populations before treatment initiation. The relevance of the presence of minority X4 viruses in the virologic efficacy of CCR5 antagonists was reported in the MERIT trial, which evaluated the safety and efficacy of maraviroc vs. efavirenz, each in combination with zidovudine and lamivudine, in drug-naive HIV-1 patients. In this study, maraviroc only showed non-inferior efficacy to efavirenz in terms of virologic efficacy (68.5 vs. 69.3%, respectively)[13], when at baseline, only those subjects with R5 viruses identified with ESTA, more sensitive for the detection of X4 variants, were considered.

The use of deep-sequencing technology has allowed investigation of whether improvements in prediction of X4 variants can be achieved by searching a larger number of genomes in comparison with the use of conventional ("bulk") sequencing with sensitivity for the detection of minority variants in the range of 10-20%. Therefore, deep sequencing provides a unique opportunity to enhance the sensitivity for identification of minority variants, including those from X4-tropic viruses.

Currently, 454 (454 Life Sciences/Roche Diagnostics) is the best-adapted platform of massive sequencing for determining viral tropism[73]. This technology has recently demonstrated to be comparable to Trofile™ and ESTA to predict virologic response to maraviroc in naive and antiretroviral-experienced patients[74]. Moreover, the Max Plank Institute has developed a new tool for viral tropism interpretation for V3 sequences derived from 454, named geno2pheno-454, which is freely available at http://g2p-454.bioinf.mpi-inf.mpg.de/index.php. This tool generates data regarding the number of unique V3 sequences and the total percentage of X4 viruses in the viral population using different FPR. At this time, the best cutoffs for the detection of X4 variants that could more accurately predict responders and nonresponders to CCR5 antagonist-based therapy using 454 are still controversial. In a reanalysis of the maraviroc clinical trials (MOTIVATE/A4001029 and MERIT), 454 technology demonstrated to be comparable to ESTA in the prediction of virologic response to maraviroc, considering X4 if ≥ 2% of the V3 sequences analyzed have a FPR ≤ 3.5%[74,75]. Moreover, the proportion of X4 variants in plasma samples was inversely associated with the rate of virologic response to maraviroc in antiretroviral-experienced patients. In addition, similar to other antiretroviral regimens, the virologic response to CCR5 antagonists might be also influenced by other parameters such as baseline $CD4^+$ counts[50] or the number of active drugs in the therapeutic regimen[51].

The accuracy of 454 technology to identify responders and nonresponders to maraviroc from proviral DNA (PBMC samples) has recently been assessed in 181 patients enrolled in the MOTIVATE/A4001029 trials. Here,

the prediction of virologic response using 454 was poorer when V3 sequences were obtained from PBMC than from plasma[72]. The poorer performance in viremic PBMC samples may be due to higher variability in this compartment. Indeed, a recent phylogenetic analysis performed by Pou, et al. demonstrated a high degree of virus compartmentalization in plasma and PBMC using V3 sequences from either plasma or PBMC, which might explain the results obtained[76].

Interpretation of the large amount of sequencing data generated by each sample remains challenging. Moreover, deep sequencing is a sophisticated and very expensive method that is only available in a few research facilities. However, these technical and economic limitations are being resolved with the advent of new cheaper generations of this technology, such as 454 Junior (www.454.com), and the development of new tools such as geno2pheno-454 that facilitate the interpretation of a large amount of data generated by this technology.

Conclusions

Current HIV treatment guidelines recommend HIV tropism testing whenever the use of a CCR5 inhibitor is being considered. The use of genotypic methods for HIV tropism determination has been widespread in the last two years, especially in Europe, replacing the phenotypic assays. Indeed, different guidelines for HIV-infection management specifically include within their recommendations the use of genotypic methods to guide the clinical use of CCR5 antagonists. The specific methodological recommendations for determining HIV tropism in the clinical setting have been improved according to the new data reported. At this time, geno2pheno is one of the most accepted and widely used algorithms for interpretation of the genotypic determination of HIV tropism. When V3 genotyping is performed using a single PCR, it is recommended to use geno2pheno with a FPR of 10%. In patients under suppressive antiretroviral therapy in which a switch to maraviroc is planned for any reason (intolerance/toxicity, drug-drug interactions, and simplification or intensification strategies), HIV tropism could be performed from proviral DNA. The data reported to date, although scarce, support the use of this tool to guide the use of maraviroc in this scenario. A prospective validation of the use of proviral DNA for HIV tropism determination to guide the use of CCR5 antagonists in clinical practice is currently ongoing. The clinical and methodological recommendations updated in this review may be useful for the proper performance of genotypic HIV tropism determination in the clinical setting.

Acknowledgments

This work was funded in part by grants from Fundación Investigación y Educación en SIDA (FIES), Red de Investigación en SIDA (RIS, ISCIII-RETIC-RD06/0040), Fondo de Investigación Sanitaria-FIS (CP08/00214; CP08/0752; and CP07/0207), and Junta de Andalucia-SAS (PI08/0408).

References

1. Comier E, Dragic T. The crown and stem of the V3 loop play distinct roles in HIV type 1 envelope glycoprotein interactions with the CCR5 coreceptor. J Virol. 2002;76:8953-7.
2. Berger E, Doms R, Fenyö E, et al. A new classification for HIV-1. Nature. 1998;391:240.
3. Anonymous. FDA approves maraviroc tablets. AIDS Patient Care STDs. 2007;21:702.
4. Dorr P, Westby M, Dobbs S, et al. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-HIV type 1 activity. Antimicrob Agents Chemother. 2005;49:4721-32.
5. Cooper D, Heera J, Goodrich J, et al. Maraviroc versus efavirenz, both in combination with zidovudine-lamivudine for the treatment of antiretroviral-naive subjects with CCR5-tropic HIV-1 infection. J Infect Dis. 2010;201:803-13.
6. Fätkenheuer G, Nelson M, Lazzarin A, et al. Subgroup analyses of maraviroc in previously treated R5 HIV-1 infection. N Engl J Med. 2008;359:1442-55.
7. 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. 2009;199:1638-47.
8. Poveda E, Briz V, Quinones-Mateu M, Soriano V. HIV tropism: diagnostic tools and implications for disease progression and treatment with entry inhibitors. AIDS. 2006;20:1359-67.
9. Perez-Olmeda M, Poveda E. Methods for determining viral tropism: genotype and phenotype tests. Enferm Infecc Microbiol Clin. 2008;(Suppl 11):40-8.
10. Whitcomb J, 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. 2007;51:566-75.
11. Jensen M, Van't Wout A. Predicting HIV-1 coreceptor usage with sequence analysis. AIDS Rev. 2003;19:145-9.
12. McGovern R, Thielen A, Mo T, et al. Population-based V3 genotypic tropism assay: a retrospective analysis using screening samples from the A4001029 and MOTIVATE studies. AIDS. 2010;24:2517-25.
13. McGovern R, Dong W, Zhong X, et al. Population-based sequencing of the V3-loop is comparable to the enhanced sensitivity trofile assay in predicting virologic response to maraviroc of treatment-naive patients in the MERIT trial. 17th CROI 2010, San Francisco [Abstract 92].
14. Brumme C, Wilkin T, Su Z, et al. Relative performance of ESTA, Trofile, 454 Deep sequencing, and "reflex" testing for HIV tropism in the MOTIVATE screening population of therapy-experienced patients. 18th CROI 2011, Boston [Abstract 666].
15. Obermeier M, Carganico A, Cordes C, et al. Week 96 update on the Berlin maraviroc cohort. 10th European Meeting on HIV and Hepatitis treatment strategies and antiviral drug resistance 2012, Barcelona, Spain. [Abstract P_24].
16. Seclén E, Soriano V, González M, et al. Clinical validation of genotypic HIV tropism assessment to guide the therapeutic use of CCR5 antagonists. 52nd ICAAC 2012, San Francisco.
17. Chueca N, Recio E, Macías J, et al. Proviral DNA tropism using genotypic tools can identify responders to a maraviroc/ boosted darunavir dual therapy regimen. 10th European Meeting on HIV and Hepatitis treatment strategies and antiviral drug resistance 2012. Barcelona, Spain. [Abstract P-67].
18. Bellecave P, Paredes R, Anta L, et al. Determination of HIV-1 tropism from proviral HIV-1 DNA in patients with suppressed plasma HIV-1 RNA using population based- and deep-sequencing: impact of X4-HIV variants on virologic response to maraviroc. International Workshop on HIV and hepatitis virus drug resistance and curative strategies 2012. Sitges, Spain. [Abstract A53].
19. Low A, Dong W, Chan D, et al. Current V3 genotyping algorithms are inadequate for predicting X4 co-receptor usage in clinical isolates. AIDS. 2007;21:F17-24.
20. Chueca N, Garrido C, Alvarez M, et al. Improvement in the determination of HIV-1 tropism using the V3 gene sequence and a combination of bioinformatic tools. J Med Virol. 2009;81:763-7.
21. Poveda E, Seclén E, González M, et al. Design and validation of new genotypic tools for easy and reliable estimation of HIV tropism before using CCR5 antagonists. J Antimicrob Chemother. 2009;63:1006-10.
22. Sánchez V, Masiá M, Robledano C, Padilla JM, Gutiérrez F. Performance of genotypic algorithms for predicting HIV-1 tropism measured against the enhanced-sensitivity Trofile coreceptor tropism assay. J Clin Microbiol. 2010;48:4135-9.

23. Sanchez V, Masiá M, Robledano C, et al. A highly sensitive and specific model for predicting HIV-1 tropism in treatment-experienced patients combining V3 loop sequences interpretation and clinical parameters. J Acquir Immune Defic Syndr. 2011;56:51-8.
24. Poveda E, Alcamí J, Paredes R, et al. Genotypic determination of HIV tropism – clinical and methodological recommendations to guide the therapeutic use of CCR5 antagonists. AIDS Rev. 2011;12:135-48.
25. Vandekerckhove L, Wensing AM, Kaiser R, et al. European guidelines on the clinical management of HIV-1 tropism testing. Lancet Infect Dis. 2011;11:394-407.
26. Trinh L, Han D, Huang W, et al. Technical validation of an enhanced sensitivity Trofile HIV coreceptor tropism assay for selecting patients for therapy with entry inhibitors targeting CCR5. Antiviral Ther. 2008;13(Suppl 3):A128.
27. Sax P. Report from the 2008 joint ICAAC/IDSA meeting. Maraviroc vs. efavirenz: a reanalysis of MERIT. AIDS Clin Care. 2008;98:104.
28. Brumme C, Wilkin T, Su Z, et al. Relative performance of ESTA, Trofile, 454 Deep sequencing, and "reflex" testing for HIV tropism in the MOTIVATE screening population of therapy-experienced patients. 18th CROI 2011, Boston. [Abstract 666].
29. Hamy F, Vidal V, Hubert S, Klimkait T. Hybridization-based assay and replicative phenotyping as diagnostic platform for determination of coreceptor tropism. 5th European HIV Drug Resistance Workshop 2007. Cascais, Portugal. [Abstract 60]
30. Van Baelen K, Vandenbrouke I, Rondelez E, Van Eygen V, Vermeiren H, Stuyver L. HIV-1 coreceptor usage determination in clinical isolates using clonal and population-based genotypic and phenotypic assays. J Virol Methods. 2007;146:61-73.
31. Poveda E, Rodés B, Labernardière JL, et al. Evolution of genotypic and phenotypic resistance to Enfuvirtide in HIV-infected patients experiencing prolonged virologic failure. J Med Virol. 2004;74:21-8.
32. Alcami J, Sánchez-Palomino S, García J, Gonzalez N. Novel HIV-based recombinant viral clones and use thereof in analytical methods, PCT/ES2005/000250 _ (WO 2005108588, EP175241 A1, P200401116).
33. González N, Pérez-Olmeda M, Gacía-Pérez J, A sensitive phenotypic assay for the determination of human immunodeficiency virus type 1 tropism. J Antimicrob Chemother. 2010;65:2493-501.
34. Raymond S, Delobel P, Mavigner M, et al. Development and performance of a new recombinant virus phenotypic entry assay to determine HIV-1 coreceptor usage. J Clin Virol. 2010;47:126-30.
35. Saliou A, Delobel P, Dubois M, et al. Concordance between two phenotypic assays and ultradeep pyrosequencing for determining HIV-1 tropism. Antimicrob Agents Chemother. 2011;55:2831-6.
36. Gonzalez-Serna A, Leal M, Genebat M, et al. TROCAI (tropism coreceptor assay information): a new phenotypic tropism test and its correlation with Trofile enhanced sensitivity and genotypic approaches. J Clin Microbiol. 2010;48:4453-8.
37. Ruiz-Mateos E, González-Serna A, Genebat M, et al. Virological response after a short-term CCR5 antagonist exposure in HIV-infected patients: frequency of subjects with virological response and associated factors. Antimicrob Agents Chemother. 2011;55:4664-9.
38. Li W, Webb E, Nary L, Robins T. SensiTrop QT: A novel molecular diagnostic assay for the detection and quantification of HIV co-receptor tropism. 15th CROI 2008, Boston. [Abstract 919].
39. Isaka Y, Sato A, Miki S, et al. Small amino acid changes in the V3 loop of human immunodeficiency virus type 2 determines the coreceptor usage for CXCR4 and CCR5. Virology. 1999;264:237-43.
40. de Jong J, Goudsmit J, Keulen W, et al. HIV-1 clones chimeric for the envelope V3 domain differ in syncytium formation and replication capacity. J Virol. 1992;66:757-65.
41. Cardozo T, Kimura T, Philpott S, Weiser B, Burguer H, Zolla-Pazner S. Structural basis for coreceptor selectivity by the HIV type 1 V3 loop. AIDS Res Hum Retroviruses. 2007;23:415-26.
42. Briggs D, Tuttle D, Sleasman J, Goodenow M. Envelope V3 amino acid sequence predicts HIV-1 phenotype (co-receptor usage and tropism for macrophages). AIDS. 2000;14:2937-9.
43. Delobel P, Nugeyre M, Cazabat M, et al. Population-based sequencing of the V3 region of env for predicting the coreceptor usage of human immunodeficiency virus type 1 quasispecies. J Clin Microbiol. 2007;45:1572-80.
44. Hoffman NG, Seillier-Moiseiwitsch F, Anh J, Walker JM, Swanstrom R. Variability in the human immunodeficiency virus type 1 gp120 Env protein linked to phenotype-associated changes in the V3 loop. J Virol. 2002;76:3852-64.
45. Sierra S, Kaiser R, Thielen A, Lengauer T. Genotypic coreceptor analysis. Eur J Med Res. 2007;12:453-62.
46. Pillai S, Good B, Richman D, Corbeil J. A new perspective on V3 phenotype prediction. AIDS Res Hum Retroviruses. 2003;19:145-9.
47. Sing T, Low A, Beerenwinkel N, et al. Predicting HIV coreceptor usage on the basis of genetic and clinical covariates. Antivir Ther. 2007;12:1097-106.
48. Jensen M, Li F, van't Wout A, et al. Improved coreceptor usage prediction and genotypic monitoring of R5-to-X4 transition by motif analysis of human immunodeficiency virus type 1 env V3 loop sequences. J Virol. 2003;77:13376-88.
49. Jensen M, Coetzer M, van't Wout A, Morris L, Mullins J. A reliable phenotype predictor for human immunodeficiency virus type 1 subtype C based on envelope V3 sequences. J Virol. 2006;80:4698-704.
50. Schapiro J, Boucher C, Kuritzkes D, et al. Baseline CD4+ T cell counts and weighted background susceptibility scores strongly predict response to maraviroc regimens in treatment-experienced patients. Antivir Ther. 2011;16:395-404.
51. Valdez H, Lewis M, Delogne C, Simpson P. Weighted OBT susceptibility score (Wobtss) is a stronger predictor of virologic response at 48 weeks than baseline tropism result in MOTIVATE 1 and 2. Program and abstract of the 48th Annual ICAAC/IDSA 2008. 46th Annual Meeting, Washington, DC. [Abstract H-1221].
52. Recomendaciones de Gesida/Plan Nacional sobre el SIDA respecto al tratamiento antiretroviral en adultos infectados por el virus de la inmunodeficiencia humana (actualización enero 2012). http://www.gesida.seimc.org.
53. Geretti A, Mackie N. Determining HIV-1 tropism in routine clinical practice. http://www.bhiva.org/documents/Guidelines/Tropism/HIV-1Tropism.doc
54. European tropism guidelines. http://www.europehivresistance.org.
55. Panel on Antiretroviral Guidelines for Adults and Adolescents 2012. Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. Department of Health and Human Services. http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf.
56. European AIDS Clinical Society 2011. Guidelines-clinical management and treatment of HIV infected adults in Europe. http://www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/1_treatment_of_hiv_infected_adults.pdf.
57. Seclén E, del Mar González M, De Mendoza C, Soriano V, Poveda E. Dynamics of HIV tropism under suppressive antiretroviral therapy: implications for tropism testing in subjects with undetectable viremia. J Antimicrob Chemother. 2010;65:1493-6.
58. Obermeier M, Walter H, Korn K, et al. Multicenter comparison of genotypic tropism testing: results from viral RNA and proviral DNA. 9th European Workshop on HIV & Hepatitis Treatment Strategies & Antiviral Drug Resistance 2011, Paphos, Cyprus. [Abstract O_21].
59. Verhofstede C, Brudney D, Reynaerts J, et al. Concordance between HIV-1 genotypic coreceptor tropism predictions based on plasma RNA and proviral DNA. HIV Med. 2011;12:544-52.
60. De Luca A, Meini G, Rossetti B, et al. HIV-1 co-receptor tropismo evolution in naive patients undergoing successful ART: concordance of DNA vs. RNA and triplicate vs singlicate sequencing. 10th European Meeting on HIV & Hepatitis treatment strategies & antiviral drug resistance 2012. Barcelona, Spain. [Abstract O_17].
61. Chueca N, Alvarez M, Peña A, et al. Long term follow up of longitudinal plasma and proviral DNA coreceptor usage in HIV-1 patients under HAART. 10th European Meeting on HIV & Hepatitis treatment strategies & antiviral drug resistance 2012. Barcelona, Spain. [Abstract P_28].
62. Mortier V, Staelens D, Schauvliege M, et al. Correlates and prevalence of co-receptor switch in ART naive HIV patients. 10th European Meeting on HIV & Hepatitis treatment strategies & antiviral drug resistance 2012. Barcelona, Spain. [Abstract O_16].
63. Seclén E, Soriano V, González M, Gómez S, Thielen A, Poveda E. High concordance between PSSM and geno2pheno algorithms for genotypic interpretation of HIV-1 tropism- V3 length as the major cause of disagreement. J Clin Microbiol. 2011;49:3380-2.
64. Swenson L, Knapp D, Harrigan R. Calibration and accuracy of the geno2pheno co-receptor algorithm for predicting HIV tropism for single and triplicate measurements of V3 genotype. J Int AIDS Soc. 2010;13(Suppl 4):O8.
65. Seclén E, Poveda E, González M, et al. High sensitivity to detect X4 variants using specific genotypic tools in antiretroviral-experienced HIV patients suitable to CCR5 antagonists therapy. J Antimicrob Chemother. 2010;65:1486-92.
66. Raymond S, Delobel P, Mavigner M, et al. Genotypic prediction of human immunodeficiency virus type 1 CRF02-AG tropism. J Clin Microbiol. 2009;47:2292-4.
67. Raymond S, Delobel P, Mavigner M, et al. Prediction of HIV type 1 subtype C tropism by genotypic algorithms built from subtype B viruses. J Acquir Immune Defic Syndr. 2010;53:167-75.
68. Treviño A, Soriano V, Rodríguez C, et al. Changing rate of non-B subtypes and coinfection with hepatitis B/C viruses in newly diagnosed HIV type 1 individuals in Spain. AIDS Res Hum Retroviruses. 2011;27:633-8.
69. Leoz M, Chaix M, Delaugerre C, et al. Circulation of multiple patterns of unique recombinant forms B/CRF02_AG in France precursor signs of the emergence of an upcoming CRFB/02. AIDS. 2011;25:1371-7.
70. Buonaguro L, Tornesello M, Buonaguro F. HIV-1 subtype distribution in the worldwide epidemic: pathogenetic and therapeutic implications. J Virol. 2007;81:10209-19.
71. Sierra S, Thielen A, Reuter S, et al. Tropism determination and clinical outcome of 61 patients under maraviroc treatment. 8th European HIV Drug Resistance Workshop 2010, Sorrento, Italy. [Abstract 20].
72. Swenson L, McGovern R, James I, et al. Genotypic analysis of cellular HIV V3 DNA to predict virologic response to maraviroc: performance of population-based and 454 deep V3 sequencing. 18th CROI 2011, Boston [Abstract 668].
73. Margulies M, Egholm M, Altman W, Attiya S, Bader J, Bemben L, Genome sequencing in microfabricated high-density picolitre reactors. Nature. 2005;437:376-80.
74. Swenson L, Dong W, Mo T, et al. Deep sequencing to infer HIV-1 coreceptor usage: application to three clinical trials of maraviroc in treatment-experienced pateints. J Infect Dis. 2011;203:237-45.
75. Swenson L, Mo T, Dong W, et al. Deep V3 sequencing for HIV type 1 tropism in treatment-naïve patients: a reanalysis of the MERIT trial of maraviroc. Clin Infect Dis. 2011;53:732-42.
76. Pou C, Codoñer F, Thielen A, et al. Plasma and PBMC viruses provide equivalent genetic information for genotypic tropism testing: analysis using quantitative deep HIV-1 sequencing. 18th CROI 2011, Boston [Abstract 669].