European guidelines on the clinical management of HIV-1 tropism testing

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Viral tropism is the ability of viruses to enter and infect specific host cells and is based on the ability of viruses to bind to receptors on those cells. Testing for HIV tropism is recommended before prescribing a chemokine receptor blocker. In most European countries, HIV tropism is identified with tropism phenotype testing. New data support genotype analysis of the HIV third hypervariable loop (V3) for the identification of tropism. The European Consensus Group on clinical management of tropism testing was established to make recommendations to clinicians and clinical virologists. The panel recommends HIV-tropism testing for the following groups: drug-naive patients in whom toxic effects are anticipated or for whom few treatment options are available; patients who have poor tolerability to or toxic effects from current treatment or who have CNS pathology; and patients for whom therapy has failed and a change in treatment is considered. In general, an enhanced sensitivity Trofile assay and V3 population genotyping are the recommended methods. Genotypic methods are anticipated to be used more frequently in the clinical setting because of their greater accessibility, lower cost, and faster turnaround time than other methods. For the interpretation of V3 loop genotyping, clinically validated systems should be used when possible. Laboratories doing HIV tropism tests should have adequate quality assurance measures. Similarly, close collaboration between HIV clinicians and virologists is needed to ensure adequate diagnostic and treatment decisions.

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Introduction

Viral tropism is the ability of viruses to enter and infect specific host cells and is based on the ability of viruses to bind to receptors on those cells. C-C chemokine receptor type 5 (CCR5) antagonists, such as maraviroc and vicriviroc, specifically inhibit the entry into host cells and subsequent replication of CCR5-tropic HIV variants (R5 virus) by an allosteric mechanism after binding to the transmembrane CCR5 co-receptor cavity. The European Medicines Agency (EMA) has approved maraviroc for use in treatment-experienced adults in whom only CCR5-tropic virus is detected. The US Food and Drug Administration (FDA), but not the EMA, has also approved maraviroc for use in treatment-naive R5-only individuals. Hence, assessment of viral tropism is needed for clinical use of the drug. In registration trials, the original Trofile assay (Monogram Biosciences, San Francisco, CA, USA) was used for this purpose. An enhanced version of the Trofile assay with improved sensitivity for the detection of HIV variants capable of using the chemokine C-X-C-motif receptor 4 (CXCR4 receptor; X4 virus) has now replaced the original Trofile assay. Additionally, several other phenotypic and genotypic approaches for establishing tropism have been developed. As the number of tropism assessment methods increases, guidelines for their use and interpretation are needed. We review published work and summarise the consensus statement of the European Consensus Group on clinical management of tropism testing. The recommendations of the panel comprise clinical indications for tropism testing, selection of the appropriate method to establish tropism, and guidance for the adequate interpretation of results obtained with these methods.

Methods

Search strategy and selection criteria

We systematically reviewed published work in accordance with the Quality of Reporting of Meta-analyses (QUOROM) guidelines.[1] We searched PubMed for articles published in English from Jan 1, 2006, to March 31, 2010, with the terms "tropism", "CCR5-antagonist", "CCR5 antagonist", "maraviroc", or "vicriviroc". Additional articles or abstracts were identified from references in the identified articles. We systematically searched the abstract books from key conferences that were held in the same period: the Conference on Retroviruses and Opportunistic Infections, the European HIV Drug Resistance Workshop, the International HIV Drug Resistance Workshop, and the International AIDS Conference.

We included original research papers or abstracts of studies on clinical validation of tropism testing and tropism test comparisons. We included randomised controlled trials, non-randomised trials, retrospective analysis of these trials, cohort studies, or cross-sectional studies. We excluded in-vitro studies, review articles, studies with fewer than ten patients or with follow-up of less than 12 weeks, monotherapy studies, studies on CXCR4 co-receptor blockers, studies of extended analysis on small subgroups, studies on identification of tropism without a comparator tropism test or without clinical outcome data, and studies of a tropism test not available for clinical use. We assessed all titles identified by our search and excluded reviews or reports describing obviously different topics than the evaluation of tropism tests (exclusion step one). Of the remaining reports, we read the abstracts and excluded reports if they dealt with non-clinical factors, described in-vitro studies only,

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involved a small number of patients, or had short follow-up (exclusion step two). Subsequently, we retrieved full-length papers if they were not abstract-only reports. We screened these papers for clinical relevance (exclusion step three). LPRV and AMJW independently assessed all reports remaining after exclusion step two according to a set format (ie, studies on establishing tropism without a comparator test or without clinical outcome data).

Studies of tropism tests were divided into three groups. Group A studies prospectively or retrospectively evaluated virological response on highly active antiretroviral therapy in clinical trials in relation to tropism assays. Group B studies evaluated virological response on highly active antiretroviral therapy in cohorts in relation to tropism assays. Group C studies evaluated the performance of different tropism tests in plasma samples of patients independent of maraviroc treatment.

Consensus panel

There are 60 panellists from 31 European countries, from the EuropeHIVResistance Network, and from other academic groups active in diagnostic testing or tropism research. This panel comprises medical doctors with a background in infectious diseases (n=12) or clinical virology (n=21), molecular virologists (n=26), and one member of the European AIDS treatment Group. Panel members from all three disciplines volunteered for the writing committee. A full panel meeting was organised in October, 2008, followed by a writing committee meeting in March, 2009, and a final full panel meeting in November, 2009. Abstracts and papers selected according to the described method were listed on the EuropeHIVResistance Network website. Discussions within the writing committee were done in online and face-to-face meetings from October, 2008, to November, 2009.

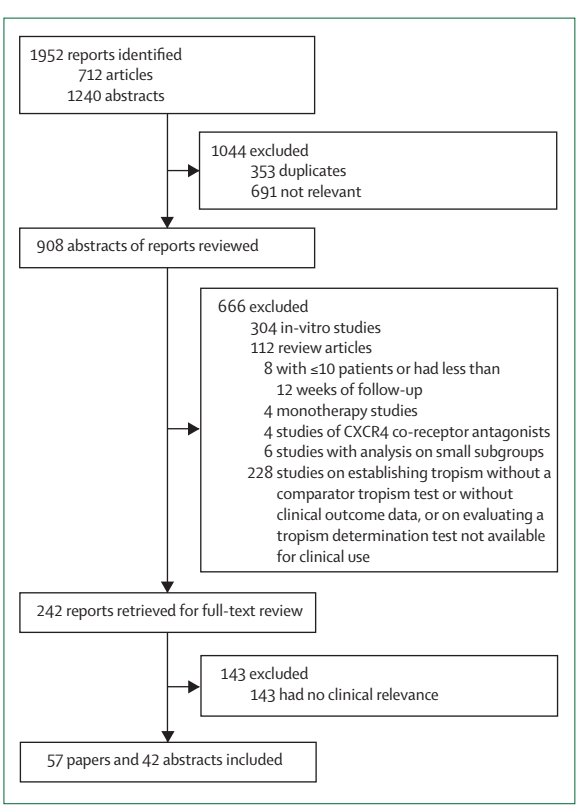

***Figure*: Study selection**

Consensus statements

Consensus statements are based on the data obtained by the systematic search. The key topics to be addressed by the recommendations were first identified at the writing committee meeting in March, 2009. A questionnaire was developed by the writing committee that presented the key concerns and circulated to the full panel for their votes and comments. 48 (80%) of the panel members responded to a first questionnaire and 60 (100%) to a more detailed questionnaire on interpretation and technical factors.

The recommendations incorporate a rating scheme as used in other international guidelines.[2] Consensus was defined as 75% of panellists agreeing with a statement. The final document was approved by all the panel members. The strength of the recommendation for every statement is indicated by A (strong), B (moderate), and C (optional) recommendation. The quality of evidence for every recommendation is indicated as: one or more prospective randomised trials with clinical outcomes or validated laboratory endpoints (I); one or more well designed, non-randomised trials or observational cohort studies with long-term clinical outcomes (II); or expert opinion (III).

Results

57 papers and 42 conference abstracts met our inclusion criteria (figure).

Virus entry into target cells and tropism testing

HIV entry into target cells is initiated by the binding of the viral envelope glycoprotein gp120 to the cellular receptor protein CD4.[3,4] In gp120, both the CD4 binding site and the conserved co-receptor binding site are partly masked by the hypervariable V1V2 loop structure. Attachment between gp120 and a CD4 molecule displaces the V1V2 loop and the third hypervariable loop (V3), creating the co-receptor binding site.[5–7] Several possible co-receptors have been identified in vitro but only the chemokine receptors CCR5 and CXCR4 have a major role in HIV-1 attachment in vivo.[8] Co-receptor tropism refers to the ability of HIV-1 to enter CD4 cells by the CCR5 receptor (R5 virus), the CXCR4 receptor (X4 virus), or both receptors (dual tropism).[9] Mixed tropism describes a mixed population of viruses with different co-receptor tropism.

The co-receptor binding site comprises the V3 loop of gp120 as the major factor for co-receptor specificity and

potentially other gp120 regions such as V1V2, C4, and the bridging sheet.[6,10] The V3 loop and both co-receptors are charged because of the presence of basic aminoacids (lysine or arginine), acidic aminoacids (aspartic acid or glutamic acid), and post-transcriptional modifications (mainly N-glycosylations, O-glycosylations, or tyrosine sulphation). Electrostatic interactions have a major role in co-receptor binding.[11–13] In general, R5 viruses are associated with HIV transmission and predominate during the early stages of infection, whereas dual and X4 viruses are associated with disease progression and emerge at later stages of infection in about half of infected individuals;[14–18] however, X4 viruses can occasionally be present in individuals with high CD4 cell count or recent infection.[15,19,20]

Tropism discordance between viral populations in cerebrospinal fluid and plasma have been reported; in general, R5-tropic virus is found in cerebrospinal fluid.[21,22] The clinical consequences of virus populations in specific body and cellular compartments with different tropism compared with the viral population in plasma is poorly understood.[23–25]

Identification of HIV-1 tropism

Viral tropism can be assessed with either genotypic or phenotypic approaches (table 1). Assessment of sensitivity and specificity of the different assays is not possible because there is no distinct gold standard.[26]

The MT-2 assay is a phenotypic assay in which patient-derived cells or established isolates are co-cultured with MT-2 cells without PCR amplification. These cells express the CXCR4 receptor but not the CCR5 co-receptor.[27] X4 and dual tropic viruses are capable of infecting MT-2 cells, resulting in formation of syncytia visible with light microscopy or production of viral antigen in culture supernatant. R5-tropic viruses are not capable of infecting the cells and do not induce syncytia.[27] A potential limitation of this assay is that it does not implement a control cell line that only expresses the CCR5 co-receptor; therefore, this assay cannot distinguish a true result, based on the presence of R5-tropic virus only, from a false negative when technical difficulties prevent infection of the MT-2 cell line. Moreover, few data are available on the reliability of the assay across different viral load ranges and CD4 cell counts.

In recombinant or pseudovirus phenotypic assays, replication-competent or pseudoviruses containing *env* genes derived from the virus population in a given patient are analysed in cell cultures. Several commercial and non-commercial recombinant virus assays that can establish viral co-receptor tropism exist. Trofile,[28] XTrack^{C}/PhenX-R (InPheno AG, Basel, Switzerland),[29] and the Toulouse Tropism Test (Université Toulouse III Paul-Sabatier, Toulouse, France)[30] are available for use in clinical practice.

Trofile is a single-cycle, recombinant virus assay.[28] The entire patient-derived *env* gene is amplified by PCR and inserted into an expression vector. This vector and a replication-defective proviral vector containing a luciferase reporter gene are co-transfected in a HEK293 cell line to produce a pseudovirus population, which is subsequently used to infect U87 cell lines expressing either the X4 or R5 receptor on their surface. If infection occurs in one or both cell lines, there is quantifiable light emission. Co-receptor antagonists are added as additional controls. The reliability of the assay depends mainly on the sensitivity and accuracy of the RTPCR reactions to indicate the diversity of the in-vivo HIV quasispecies. The assay can be used with plasma HIV RNA loads greater than 1000 copies per mL. In the original Trofile version, X4 virus variants comprising 10% of the population could be detected with 100% sensitivity when using clonal mixtures.[28] From June, 2008, the original Trofile assay has been replaced by a more sensitive version in which X4 variants that comprise 0·3% of the population could be detected with 100% sensitivity when

	Description	Disadvantages
Phenotypic assessment using whole virus		
MT-2 assay (in-house methods)	Co-culture of patient-derived peripheral blood mononuclear cells with MT-2 cells; viruses that enter cells via CXCR4 will form syncytia	Can only be used to detect viruses that enter cells via CXCR4, no control; biosafety level 3 facility needed
Phenotypic assays using recombinant viruses		
Enhanced sensitivity Trofile assay: XTrack^{C}/PhenX-R (combination of genotypic and phenotypic method)	Parts or the whole *env* gene are amplified from plasma HIV RNA to generate recombinant or pseudovirions; these virions are used to infect human cell lines expressing CD4 and either CXCR4 or CCR5	Restricted availability; special facilities and expertise are needed; can only be done at specialised centres
Genotypic sequence analysis		
Population sequence analysis (in-house methods)	Genotypic analysis of nucleotide sequence of the V3 region of *env* that strongly affects viral co-receptor usage	Complicated interpretation; use of interpretation algorithm warranted; cutoff for false-positive rate of the interpretation algorithm needs to be preset
Ultradeep 454 sequencing (in-house methods)	Can be used to detect minority HIV variants by sequencing a large number of clones within a single sample	Expensive and complicated interpretation; can be done only in specialised settings; non-viable minority variants might be classified as X4; cutoff for false-positive rate of the interpretation algorithm needs to be preset

CXCR4=chemokine C-X-C-motif receptor type 4. CCR5=C-C chemokine receptor type 5.

Table 1: **Overview of the different tests to identify HIV-1 tropism**

clonal mixtures are used.[31] The test can be done on both viral RNA and DNA; however, in Europe, this test is commercially available only for plasma RNA.

The XTrack^{C}/PhenX-R tropism assay combines a genotypic hybridisation assay (XTrack^{C}) and a phenotyping assay (PhenX-R).[29] Rapid testing is done by gene sorting based on fluorescence-labelled probes specific for R5 and X4 viruses. In cases of ambiguous results or a possible mixed or dual tropic viral population, phenotyping is done. Patient-derived *env* sequences (1·1 kb V1–V3) are ligated into a provirus without *env* and transfected into a reporter cell harbouring an HIV-dependent β-galactosidase gene. Infectivity is measured after three to four replication cycles by expression of β-galactosidase. Insufficient data exist to assess the reliability of this method for samples with low viral loads.

The Toulouse Tropism Test is a recombinant virus assay. Patient-derived *env* fragments encompassing the gp120 and the ectodomain of gp41 are amplified by PCR. Subsequently, recombinant virus particles are produced by homologous recombination of a *delta env* luciferase-containing vector (a vector without the *env* gene) and the gp140 PCR product. These particles are used to infect U87 CCR5-positive CD4 cells and U87 CXCR4-positive CD4 cells in parallel. The infection of indicator cell lines and thereby HIV tropism is assessed by measuring the luciferase activity. Co-receptor antagonists are added as additional controls.[30] Insufficient data exist to assess the reliability of this method using samples with low HIV RNA concentrations.

Tropism genotype testing is based on amplification and population sequence analysis of the patient-derived V3 region[20,32–37] Two different sequencing approaches—population-based and pyrosequencing—have been used for both viral RNA and DNA.[20,32,38–41] In clinical trials and several cohorts of patients, amplification and sequence analysis of the V3 region has been done repeatedly (ie, in triplicate),[42,43] whereas in other cohorts single testing has been done.[44–47]

A web-based bioinformatic interpretation technique is used to predict co-receptor use from the consensus sequence. Minority species that make up less than 10–20% of the viral population generally remain undetected, as with all conventional Sanger sequence methods. The test is fast compared with phenotypic assays but experience is needed for the quality assessment and editing of the highly variable viral envelope gene. Few data from cohort studies exist to assess the reliability of population sequencing on plasma samples with low viral load (<1000 RNA copies per mL) in clinical settings.[44,48]

Ultradeep 454 sequencing technology enables analysis of several thousand individual V3 sequences from a single sample.[49–51] Subsequent tropism prediction is done with similar web-based bioinformatic interpretation techniques as used for population-based testing. This pyrosequencing allows a very sensitive and quantitative analysis of sequence variability in every patient. However, this facility is only available at specific academic or commercial service units. Much computing capacity and interpretation expertise are needed for the volume of data produced. Moreover, the current costs are substantially higher than for other assays, restricting the use of this technology for current routine clinical practice. Insufficient data are available to assess the reliability of this method using samples with low HIV RNA concentrations.

For more on **WebPSSM** see http://indra.mullins.microbiol.washington.edu/webpssm

For more on **Fortinbras PSSM** see http://fortinbras.us/cgi-bin/fssm/fssm.pl

For more on the **Geno2pheno [co-receptor] system** see http://coreceptor.bioinf.mpi-inf.mpg.de/index.php

CCR5 antagonists

Several CCR5 antagonists have entered clinical evaluation: maraviroc (ViiV Healthcare) is approved for use in treatment-experienced patients by the FDA and the EMA and for the treatment of drug-naive patients by the FDA in patients with only CCR5-tropic virus.[52] Dual-tropic virus can respond in vitro to maraviroc, but the clinical relevance of this finding is unclear.[53] TBR-652 (Tobira Therapeutics) is in clinical development.[54] Aplaviroc (GlaxoSmithKline) was discontinued because of liver toxicity,[55,56] and vicriviroc (Merck) was discontinued because of poor efficacy.[57]

Interpretation systems

Several bioinformatic methods have been developed to predict viral co-receptor use on the basis of sequence data. The simplest algorithm is the so-called 11/25 charge rule, which takes into account only the charge of aminoacids at key positions 11 and 25 in the V3 loop. The technique has not been broadly assessed in clinical settings. In comparative studies, only a moderate correlation with results from the original Trofile assay was reported.[58]

The position-specific scoring matrix (PSSM) is a more advanced method that analyses complete V3 sequences. The technique calculates the likelihood that the sequence is derived from an X4 virus for every possible aminoacid at every individual position. In general, a higher total score indicates a higher likelihood that a specific sequence is derived from an X4 virus. Sequences with values below –6·96 are considered R5, whereas sequences with values above –2·88 are predicted to be X4. Intermediate scores can be interpreted using the 11/25 rule. This method ignores insertions, gaps, and aminoacid mixtures.

PSSM has been evaluated in several cohort studies and retrospective analyses of clinical trials.[59] PSSM can be accessed via WebPSSM. Recently, a modified and more sensitive PSSM method has increased the sensitivity for detecting X4 viruses,[34] which is freely available online.

Another advanced interpretation system is the geno2pheno[co-receptor] (G2P) system, which analyses complete V3 sequences. The system uses support vector machine technology trained with a set of nucleotide sequences with corresponding R5 or dual or mixed tropism or X4 phenotypes. Nucleotide sequences are used as inputs for the system and, therefore, aminoacid mixtures are considered. The clonal variant of G2P has

been investigated in several cohort studies and retrospective analyses of clinical trials. Another variant of G2P in which clinical data (the nadir of CD4 and the baseline viral load) are taken into account has not extensively been studied. Both G2P interpretation systems can be accessed online. The result of the interpretation is given as a quantitative value, the false-positive rate, which defines the probability of classifying an R5 virus falsely as X4. Varying the threshold value for the classification of false-positive rates changes the sensitivity and specificity for X4 prediction.

Originally, the developers of the G2P algorithm suggested that the preferential false-positive rate should vary depending on the clinical setting. When using a single genotypic population procedure for patients with multiple treatment options, a stringent setting with a false-positive rate of 20% was originally suggested, whereas for patients with severely restricted treatment options, a false-positive rate of 5% was proposed.[60] In the retrospective investigations of the MOTIVATE-1 and MOTIVATE-2 trials (Maraviroc Plus Optimized Therapy in Viremic Antiretroviral Treatment Experienced Patients), the 1029 trial (A4001029), and the MERIT study (Maraviroc versus Efavirenz Regimens as Initial Therapy), population sequencing was done in triplicate. In these analyses, a false-positive rate greater than 5·75% was correlated with a favourable response on a regimen containing maraviroc.[61,62] Apart from the small group of patients in the 1029 trial, the patients in these studies were included on the basis of R5-tropism results established by the original Trofile assay. The subsequent identification of the population genotypic tropism was therefore done retrospectively on a mostly R5-prescreened population of patients. Furthermore, the re-analyses were done with an automated approach for alignment and interpretation of the V3 sequence, which is not widely validated and implemented in most routine diagnostic settings. Therefore, this false-positive rate cannot be automatically translated to routine diagnostic use in clinical settings.

None of the available interpretation techniques take into account additional regions of *env*, outside the V3 loop. In one study,[63] a significant increase in the accuracy of prediction was reported when both V2 and V3 were used compared with V2 or V3 alone. The clinical relevance of including additional HIV-1 genomic regions for prediction of HIV-1 tropism is unknown.[64,65]

Interpretation based on proviral DNA instead of viral RNA

Current phenotypic assays need a plasma sample with a minimum HIV RNA concentration of 1000 copies per mL to generate a reliable result. Some patients for whom a CCR5 inhibitor is useful might, therefore, remain deprived of the drug because their viraemia is too low to investigate tropism. Since prolonged suppressive treatment seems not to result in tropism shifts,[66–68] retrospective analysis of tropism from stored plasma collected before viral suppression was achieved is sometimes used as an alternative.

Genotypic analysis is usually offered at low HIV RNA concentrations, depending on local laboratory procedures. If amplification of HIV RNA is not possible, genotypic analysis of proviral DNA is an inherently attractive strategy. Although the use of proviral DNA for viral tropism testing has not been clinically validated in large cohorts, emerging data indicate a good correlation with results derived from viral RNA.[69,70] In general, X4-predicted sequences are more commonly retrieved from proviral DNA than from RNA.[71,72] Although a low nadir CD4 T-cell count correlates well with the presence of dual mix and X4 viruses, virus populations using either co-receptor can be present in DNA in patients with a high nadir CD4 cell count and an undetectable viral load at the time of sampling.[73] The possibility of doing tropism testing on proviral DNA even during suppressed viraemia would facilitate the use of CCR5 inhibitors as part of switching, simplification, or intensification strategies.[44]

Identification of tropism across different HIV-1 subtypes

Europe has a much higher and rising prevalence of non-B subtypes than does North America. This high rate is especially true for countries with a strong historical link to Africa and for some eastern European countries in which the epidemic in some risk groups is mostly driven by non-B subtypes and circulating recombinant forms. The original Trofile assay seems to be reliable across different HIV-1 subtypes (A, B, C, D, E, G) based on a small dataset (n=38).[74] For the enhanced sensitivity Trofile assay, primers have been optimised to improve testing of a broad range of diverse HIV envelope subtypes.[75]

For population tropism genotyping, several in-house protocols have been optimised and cover most subtypes and circulating recombinant forms.[44,76,77] The techniques to identify genotypic tropism have been developed using training sets with different subtypes. The largest dataset has been used for the geno2pheno system, mainly based on 1100 genotypic–phenotypic pairs from the Los Alamos National Laboratory database (NM, USA).[78] In a separate study,[79] good correlation between genotypic tropism prediction with the G2P interpretation system and the identification of tropism by an in-house phenotypic assay (Toulouse Tropism Test) was reported for subtype C viruses. WebPSSM was originally trained with subtype B variants and then separately using a smaller set of subtype C variants.[80] For circulating recombinant form CRF02_AG, less correlation between genotypic testing using G2P or PSMM and a phenotypic assay (Toulouse Tropism Test) was reported in one study.[81] In a large cohort of treatment-experienced patients in Germany, G2P was evaluated in HIV subtype B (642 patients) and non-HIV subtype B (92 patients) and had good agreements between Trofile and geno2pheno (co-receptor) for non-HIV subtype B isolates.[82] In both MOTIVATE trials, the 1029 study, and the MERIT study,

large numbers of subtype B and C viruses were tested with the original Trofile assay, but other subtypes were only present in low numbers.[83–86] Thus, sufficient information on the accuracy of tropism tests and interpretation algorithms to predict clinical outcome is available for subtypes B and C. For other subtypes, only certain information is available on the accuracy of the identification of tropism.

Tropism assay evaluation

None of the available tropism assays have been validated in prospective, randomised, double-blind clinical trials with the performance as a primary endpoint. The inclusion of patients in prospective randomised clinical trials has been based on tropism identified with the original Trofile assay only (table 2). Retrospective analysis of the MERIT study[103] showed that the virological response was decreased in patients with a shift from R5 to X4 tropism on the original Trofile assay between screening and baseline timepoints (separated 3–4 weeks on average), suggesting limitations with the sensitivity of this assay. Stored samples from several clinical trials have subsequently been analysed retrospectively to investigate the association between baseline tropism and treatment response with other methods to establish tropism, such as the enhanced sensitivity Trofile assay, population genotypic analysis of the V3 loop, and pyrosequencing.

The enhanced version of the Trofile assay was assessed in a retrospective analysis of the MERIT trial, which studied maraviroc versus efavirenz (both along with zidovudine and lamivudine) as initial antiretroviral treatment. 106 of 721 (14·7%) patient isolates reported as R5 with the original Trofile assay were classified as having dual or mixed tropism using the enhanced version.[86,87,104] Furthermore, the enhanced sensitivity Trofile assay was more predictive of the virological responses in these antiviral-naive individuals starting maraviroc than was the original assay. Similar retrospective analysis done in treatment-experienced participants in the ACTG 5211 study of vicriviroc confirmed the increased sensitivity of the enhanced sensitivity Trofile assay for detection of X4 variants compared with the original assay.[31]

Genotypic population sequencing of the V3 loop was retrospectively studied in a pooled analysis of the 1029 study, a trial that recruited antiviral-experienced patients with a dual or mixed tropic virus, and the MOTIVATE-1 and MOTIVATE-2 studies, which included antiretroviral-experienced patients with the R5 virus.[61,88] The original Trofile assay and the triplicate population sequencing of the V3 loop were equally successful in predicting virological response at weeks 12 and 24 with either G2P or WebPSSM for predicting co-receptor tropism.[61,88] In this retrospective analysis, a G2P false-positive rate with a very low cutoff (<2) was strongly associated with only little or no response to treatment containing maraviroc. A false-positive rate in the range of 2–5·75 was predictive for loss of antiviral activity. Above this range, the false-positive rate (>5·75) was a good predictor of sustained response and this cutoff was subsequently validated as predictor for response in a second retrospective analysis with data from the MERIT trial.[61] Moreover, population V3 sequencing in triplicate and the enhanced sensitivity Trofile assay were equally predictive of virological response to maraviroc in this analysis.[43,62] A similar validation study has not been done so far for the interpretation algorithm WebPSSM.

Finally, ultradeep pyrosequencing was retrospectively analysed on the pooled dataset of both the MOTIVATE studies and the 1029 study using a false-positive rate of 3·5% and discarding minority strains present below 2%. Based on triplicate input, this sensitive method was also a better predictor of virological response at weeks 12 and 24 than was the original Trofile assay.[42,49] This promising technique had similar sensitivity as the enhanced sensitivity Trofile assay, and few patients who could benefit from maraviroc were excluded, suggesting a better specificity.

Data from several European cohorts have been used to assess the performance of different tropism tests in routine clinical practice. The inclusion of patients in most cohorts has been based on identification of tropism using either the original Trofile assay or population V3 sequencing on plasma or proviral DNA.[76,94] Other cohorts have based inclusion on either the MT-2 in combination with other methods or the enhanced sensitivity Trofile assay.[93,96] Although the number of patients included in most cohorts was small, the results were consistent in cohorts. Within every cohort, concordance between different tropism assays was high and virological outcome was comparable for phenotypic or genotypic methods (table 2).

Tropism genotyping and phenotyping have been assessed in different sets of plasma and DNA samples.[97,101] Most commonly, the original Trofile assay or the enhanced version were used as the comparator test. In initial reports, there was a poor correlation between V3 loop population genotyping to predict X4 co-receptor use compared with the original Trofile assay in clinical samples.[58,105] By use of improved interpretation algorithms, a good concordance between phenotypic and genotypic tests was reported by several groups.[41,69,100]

Recommendations for the clinical management of HIV-1 tropism: European guidelines

Clinical indications for tropism testing

Before treatment with a CCR5 antagonist is started, co-receptor tropism should be identified (recommendation level AII; table 3). Tropism testing is strongly recommended in all patients who have virological failure for whom a CCR5 antagonist is being considered as part of the subsequent regimen (AII). Tropism testing is moderately recommended in all patients for whom treatment has failed to provide insight into future treatment options (BII).

In patients who have adverse events with their current regimen or unexplained neurological dysfunction, CCR5

	OTA	ESTA	Other phenotypic tropism test	Population genotypic analysis	Ultradeep 454 genotypic analysis	FPR (pop GT)	Patients (n)	Conclusion
Clinical trials								
MERIT (maraviroc; treatment-naive patients)[42,43,86–88]	Y	Y	..	Y	..	5·75	721	Retrospective ESTA/pop GT_{G2P} resulted in improved prediction of VR compared with OTA
Study 3802 (vicriviroc; treatment-naive patients)[89]	Y	..	..	..	..	..	92	Patients with R5 results based on OTA had inferior VR compared with efavirenz
VICTOR-E1 (vicriviroc; treatment-experienced patients)[90]	Y	..	..	..	..	..	118	Retrospective ESTA had improved detection of X4 variants compared with OTA
ACTG 5211 (vicriviroc; treatment-experienced patients)[31,91,92]	Y	N	N; MT-2	..	..	..	55	Good concordance between MT-2 and OTA
MOTIVATE-1 and MOTIVATE-2 (maraviroc; treatment-experienced patients)[83,84]	Y	..	..	..	..	..	1049	Patients with R5 results based on OTA had superior VR compared with placebo
1029 study (maraviroc; treatment-experienced patients, non R5)[85]	Y	..	..	..	..	..	167	Overall, no viral response was reported in patients without R5 results based on OTA
MOTIVATE-1 and MOTIVATE-2 and 1029 study (maraviroc, treatment-experienced patients)[49,61,62,88]	Y	..	..	Y	Y	5·75	1216	Retrospective ESTA/pop $GT_{G2P,FPR\ 5.75}$/454 $GT_{G2P,\ FPR\ 3.5}$ resulted in improved prediction of VR compared with OTA
MOTIVATE-1 and MOTIVATE-2 and 1029 study and MERIT (maraviroc, treatment-naive and treatment-experienced patients)[42]	Y	..	..	..	Y	5·75	1937	Retrospective ESTA/pop $GT_{G2P,FPR\ 5.75}$/454 $GT_{G2P,\ FPR\ 3.5}$ resulted in improved prediction of VR compared with OTA
Cohort studies								
Aachen, Germany[45]	Y	..	..	Y	..	20	51	Although 20% FPR was used for inclusion, 12·5% FPR had similar predictive value as Trofile
Utrecht, Netherlands[93]	..	Y	Y	Y	..	10	17	Pop GT_{G2P}/ESTA/MT-2 equal in predicting VR
Berlin, Germany[94]	..	..	..	Y	..	10*	121	High rate of VR based on pop GT_{G2P} on RNA or DNA in treatment-experienced patients
Cologne, Germany[95]	Y	..	..	Y	..	10–20	61	OTA/pop GT_{G2P} equal in predicting VR in treatment-experienced patients
Granada II, Spain[50]	Y	..	..	Y	Y	10–20	18	OTA/pop GT/454 GT equal in predicting VR in treatment-experienced patients
Belgian Centres, Belgium[76]	Y	..	..	Y	..	10	49	OTA/pop GT_{G2P}/454 GT equal in predicting VR in treatment-experienced patients
French Centers, France (ANRS)[46]	Y	Y	..	Y	..	10†	189	Good correlation between pop GT_{G2P} and VR in treatment-experienced patients
London, UK[48]	..	..	..	Y	..	6	103	High rate of VR based on pop GT_{G2P} on RNA or DNA in treatment-experienced patients
Test comparisons without clinical data								
Madrid, Spain[32]	N	..	..	N	..	20	148	Good correlation of pop $GT_{PSSM(X4R5-8)}$ and $GT_{PSSM\ (SINSI-6.4)}$ with OTA
Barcelona, Spain[96]	..	N	MT-2	N	..	10–20	30	ESTA on pretreatment plasma and pop $GT_{DNA\ (G2P)}$ correlated well when HIV RNA <50 copies per mL
Toulouse, France[97]	..	..	TTT	N	..		103	Good correlation between TTT and pop $GT_{G2P/PSSM}$
London, UK[41]	..	N	..	N	..	1–20	106	Good correlation between ESTA and pop GT_{G2P}
Rome, Italy[69]	..	N	..	N	..	..	45	Good correlation between ESTA and pop GT with G2P clonal but not with G2P-clinical
Granada I, Spain[98]	N	..	..	N	..	..	178	Good correlation between Trofile and pop GT using a combination of several bioinformatic methods
Amsterdam, Netherlands[99]	N	N	MT-2	..	..	..	10	Good correlation between ESTA and pop GT_{G2P}
Swiss HIV cohort study, Switzerland[100]	N	..	XTrack[c]	..	..	..	110	Good correlation between OTA and XTrack[c]
Italy Tropism Study, Italy[101]	..	N	..	N	..	10	348	Poor correlation between ESTA and pop $GT_{(G2P/PSSM)}$
San Francisco (Stanford University), CA, USA[102]	..	..	MT-2	N	..	..	55	Good correlation between MT-2 and pop GT_{PSSM}

OTA=original Trofile assay. ESTA=enhanced sensitivity Trofile assay. TTT=Toulouse Tropism Test. VR=virological response. PSSM=position-specific scoring matrix. G2P=geno2pheno[co-receptor] system. FPR=false-positive rate. MERIT=Maraviroc versus Efavirenz Regimens as Initial Therapy. MOTIVATE=Maraviroc Plus Optimized Therapy in Viremic Antiretroviral Treatment Experienced Patients. VICTOR-E1=Vicriviroc (SCH 417690) in Combination Treatment With Optimized ART Regimen in Experienced Subjects. pop GT_{G2P}=population genotypic analysis using G2P. pop $GT_{G2P,FPR\ 5.75}$/454 $GT_{G2P,FPR3.5}$=population genotypic analysis using G2P with FPR of 5·75 and ultradeep 454 genotypic analysis using G2P with FPR of 3·5. pop $GT_{PSSM(X4R5-8)}$=population genotypic analysis using PSSM with the matrix X4R5 with cut-off –8 for X4 prediction. pop $GT_{PSSM\ (SINSI-6.4)}$=population genotypic analysis using PSSM with the matrix SINSI with cut-off –6·4 for X4 prediction. Y=clinical outcome, data available. N=test comparisons without clinical outcome. ANRS=French AIDS Research Agency. *For DNA, FPR was 20%. †Inclusion mainly on OTA.

Table 2: **Overview of the evaluation of the different HIV-1 tropism assays**

co-receptor antagonist-containing treatment can be of potential value (CIII). Therefore, tropism testing is strongly recommended if use of a CCR5 antagonist is considered (AII).

In newly diagnosed patients, the role of viral tropism testing as an indicator for future use of CCR5 antagonists or as a prognostic marker is not sufficiently known to warrant any recommendation. If testing is done, detection of the X4 virus indicates that future use of CCR5 antagonists is unlikely to be beneficial. If, however, R5 viruses are detected, the fact that X4 viruses might appear in the future while patients remain untreated cannot be ruled out.

	Consensus	Communication to clinicians	Comments
Clinical indications			
Tropism testing in patients who fail treatment	Undertake tropism testing if a CCR5 antagonist is considered as part of the subsequent regimen (AII) or to give optimal insight in all future therapeutic options (BII)	In patients who have virological failure, take a sample for tropism testing whenever a CCR5 antagonist is considered in the subsequent regimen; ideally, material for the tropism and resistance test should be sent to the laboratory at the same time; delay in reporting of an R5-tropism test result might either exclude the use of CCR5 antagonists or, if the failing regimen is continued, increase the accumulation of mutations	For the most appropriate sample for testing in patients with low-level viraemia, read the section on "Choice of tropism test"
Tropism testing in treated patients who have poor tolerability or toxicity of current treatment or CNS pathology	Undertake tropism testing if use of a CCR5 antagonist is considered (AII) or if CCR5 co-receptor antagonist-containing treatment can be of potential value (CIII)	In patients with poor tolerability, toxicity, or CNS pathology, take a sample to identify tropism whenever a CCR5 antagonist is considered in the subsequent regimen	For the most appropriate sample for testing in patients with low-level viraemia, read the section on "Choice of tropism test"
Tropism testing in newly diagnosed patients	The role of tropism testing is insufficiently elucidated to warrant any recommendation	In newly diagnosed patients, there is no evidence that detection of R5-tropism will be of value in the future, because tropism might change over time, especially in patients with detectable viral load	..
Tropism testing in drug-naive patients before starting treatment	Undertake tropism testing before starting treatment in treatment-naive patients in whom toxicity to first-line treatment is expected (CIII)	Identification of tropism before the start of treatment enables a prompt treatment switch to CCR5 antagonist-containing treatment in case of toxicity of first-line treatment	In the absence of adequate data, the panel is unable to provide guidance on the durability of an R5-tropism result
Choice of tropism test			
In patients with a plasma HIV RNA load of >1000 copies per mL	Tropism testing can be done by Trofile ESTA (BII) or population genotypic analysis of the V3 loop (BII)	There is sufficient evidence that an R5 result from both ESTA and population genotypic tropism testing can be used in clinical practice to guide start of CCR5 inhibitor treatment (BII); the choice of the test should be based on local assessment of capacity, logistics, cost, and desired turn-around time	Quality assurance on the performance of the test is a mandatory requirement for all laboratories involved
In treated patients with an HIV RNA load of <1000 copies per mL or suppressed viraemia (plasma HIV RNA <50 copies per mL)	The preferred tropism test is population genotypic analysis of the V3 loop (CIII); if the HIV RNA load of the sample is <50 copies per mL or below the level of viraemia that is accepted by the laboratory for reliable amplification, genotypic tropism testing can be done on proviral HIV DNA (CIII)	Discuss the most appropriate sample and volume with the laboratory	..
Technical aspects of genotypic population analysis of the V3 loop			
Choice of gene fragment for amplification	If undertaking genotypic tropism testing, the panel advises the use of the V3 loop (AII)	Clinical evidence only supports the use of V3 sequences; there are insufficient data on the addition of other *env* regions	
Number of test repeats	..	Clinical validation of genotype-based tropism testing in clinical trials has been done using triplicate PCR amplification	The additional benefit of triplicate testing is under evaluation, but is expected to be more important at lower viral load
Number of test repeats if the plasma HIV viral load is >1000 copies per mL	Undertake triplicate PCR amplification and sequencing testing and use the G2P interpretation technique (clonal model) with an FPR of 10% (CII)	..	..
Number of test repeats if the plasma HIV load <1000 copies per mL	Undertake triplicate PCR amplification and sequencing testing and use the G2P interpretation technique (clonal model) with an FPR of 10% (BIII)	..	..
Number of test repeats if the DNA is used as a source for genotyping	Undertake triplicate PCR amplification and sequencing, and use the G2P interpretation technique (clonal model) with an FPR 10% (BIII)	..	..
Number of test repeats if only one sequence can be generated	Increase the FPR up to 20% (BIII)	..	..

G2P=geno2pheno[co-receptor] system FPR=false-positive rate. ESTA=enhanced Trofile assay. CCR5=C-C chemokine receptor type 5. The strength of the recommendation for every statement is indicated by A (strong), B (moderate), and C (optional) recommendation. The quality of evidence for every recommendation is indicated as: one or more prospective randomised trials with clinical outcomes or validated laboratory endpoints (I); one or more well designed, non-randomised trials or observational cohort studies with long-term clinical outcomes (II); or expert opinion (III).

Table 3: **Summary of recommendations from the European Consensus Group on clinical management of HIV-1 tropism testing**

In antiretroviral-naive patients at high risk of toxic effects of first-line treatment (eg, liver cirrhosis, neuropsychological abnormalities), CCR5-tropism testing could be done before initiating any treatment so that, if toxic effects develop, treatment can be modified to include CCR5 antagonists without additional tests (CIII). Samples should be collected as close as possible to the time of starting treatment. The use of maraviroc in antiretroviral-naive patients is not approved by the EMA.

Choice of co-receptor tropism test

In the absence of a distinct gold standard for the identification of viral tropism, the panel assessed the different tests on the basis of the availability of clinical outcome data (table 3). An R5-tropism result from either the phenotypic enhanced sensitivity Trofile assay or V3 loop genotypic sequencing correlated with a favourable outcome in retrospective analyses of clinical trials and cohort studies. Most clinical data are based on subtype B and C viruses, whereas little information is available on other subtypes.

In patients with a plasma HIV RNA load greater than 1000 copies per mL, tropism testing can be done with the enhanced sensitivity Trofile assay (BII) or V3 loop genotypic population analysis (BII).

The choice of the test should be based on the local capacity, logistics, cost, and desired turnaround time. In general, V3 loop population sequencing is the preferred method because of its better availability and faster turnaround time (BII). If this method is used, the laboratory should have appropriate expertise in sequence analysis and use of interpretation techniques and should participate in quality control procedures to validate their accuracy.

In patients with plasma HIV RNA loads greater than 50 copies per mL but less than 1000 copies per mL, the preferred tropism test is population genotypic analysis of the V3 loop (CIII). If plasma HIV RNA load is below the level of viraemia that is accepted by the laboratory for reliable sequence results, tropism testing can be done on proviral HIV DNA (CIII). A good correlation with RNA testing and increased sensitivity for the detection of X4-tropic viruses lends support to HIV DNA tropism analysis for this indication. However, few data are available on clinical outcome after initiation of CCR5 antagonists on the basis of a proviral DNA tropism assay. In patients with suppressed viraemia (plasma HIV RNA load <50 copies per mL), a tropism test can be done on proviral HIV DNA based on the same criteria (CIII). In patients for whom treatment with a CCR5 antagonist has failed, a tropism test can be used to detect a switch of viral tropism, but cannot give information on the susceptibility of an R5 virus population to future treatment with a CCR5 antagonist.

Ultradeep 454 genotypic tropism testing is highly predictive of clinical outcome in retrospective analyses of large clinical studies. However, this promising method is expensive, needs complex analyses, and is not widely available. Given that sequence technology is developing fast, availability and quality control measure might improve rapidly, so this option might change in the near future.

The MT-2 assay has a good correlation with the original and enhanced Trofile assays, but insufficient data are available on its association with clinical and virological outcome. Moreover, this test does not have an adequate control for detection of R5. Furthermore, for the Toulouse Tropism Test and XTrack^{c}/PhenX-R analysis, insufficient clinical outcome data are available. On the basis of these arguments, the panel does not recommend the use of ultradeep 454 sequencing, MT-2, Toulouse Tropism Test, or the heteroduplex mobility assay in routine clinical settings.

Turnaround time and longevity

In people who need a change in their antiretroviral-drug regimens, the panel recommends that results of tropism tests should be available at the same time as the results of resistance tests. New regimens can therefore be started immediately, avoiding the continuation of failing treatment and associated risk of the accumulation of drug resistance mutations while the tropism test results are awaited (AII).

In the absence of adequate data, the panel is unable to provide guidance on the durability of an R5-tropism result in patients with ongoing viraemia. In patients with suppressed viraemia, preliminary data suggest a low risk for tropism change over time. In general, minimisation of the time between tropism testing and the start of the treatment is crucial to maximise future CCR5 inhibitor treatment response.

In patients with very low CD4 T-cell counts and a high risk of AIDS-defining illnesses, or in patients who are at risk for accumulating additional drug-resistance mutations, if they remain on a failing treatment, a test with a shorter turnaround time is preferred. In this case, genotypic assays using population sequencing are preferable to phenotypic assays.

Interpretation and technical aspects of population sequencing

When population genotyping is used, the panel strongly recommends sequencing the V3 loop (AII; table 3). Clinical evidence supports use of V3 sequences alone, which is a pragmatic approach for high-volume testing given the complexity reported in sequences.

The settings of interpretation algorithms should be based on current clinical evidence and periodically updated as new data emerge. The tropism genotyping report sent to clinicians should include clear advice as to whether the tropism result supports use of a CCR5 antagonist or not. Furthermore, the report should include the interpretation system used, including version number and the applied cutoff. Virologists

providing the results should have knowledge of the association between the sensitivity and specificity of tropism prediction and cutoff settings. In individual cases, the virologist can vary the cutoff according to the clinical situation.

Most data are available on the G2P interpretation system. Although a G2P cutoff (false-positive rate) of 5·75% was a good predictor of a sustained response in retrospective analyses of clinical trial data, the panel has concerns for direct translation of these data into routine clinical practice and prefers to advise a more conservative higher false-positive rate cutoff. One drawback of the current system is the presence of a predefined cutoff. The panel strongly feels that the interpretation system should be defaulted to force users to actively choose the cutoff (false-positive rate) level. In the future, the panel advises that the cutoffs as recommended in the updated European guidelines are incorporated in the pull-down menu as one of the default choices. The system would benefit from a procedure that enables computation of the three FASTA files from a triplicate procedure at once.

In general, in samples with plasma HIV RNA loads greater than 1000 copies per mL, the panel advises triplicate PCR amplification and sequencing testing and to use the G2P interpretation system with a false-positive rate of 10% (CII). In samples with plasma HIV RNA loads less than 1000 copies per mL, the panel recommends triplicate PCR and sequencing, using the G2P interpretation system with a false-positive rate of 10% (BIII). If only one sequence can be generated (HIV RNA <1000 copies per mL), the panel recommends increasing the false-positive rate up to 20% (BIII).

If proviral DNA is used as a source for V3 genotyping, the panel recommends triplicate PCR and sequencing, using the G2P interpretation system with a false-positive rate 10% (BIII). If only one sequence can be generated from a DNA sample, the panel recommends increasing the false-positive rate up to 20% (BIII). If the R5 and X4 virus are detected with triplicate genotypic analysis, the panel advises reporting the presence of mixed tropic viruses.

Conclusions

After the EMA approval of maraviroc, the first CCR5 co-receptor antagonist for the treatment of HIV-1 infection, tropism testing is needed for clinical practice. The European Consensus Group on clinical management of tropism testing provide an overview of available published work, evidence-based recommendations for the clinical use of tropism testing, and guidance on unresolved factors and developments. Current data lend support to both the use of population genotyping and the commercially available enhanced sensitivity Trofile assay for establishing co-receptor tropism. For practical reasons, genotypic population sequencing is the preferred method in Europe.

Contributors

The guidelines were an initiative of CABB, LPRV, and AMJW in collaboration with the European Society for Antiviral Resistance. CABB chaired the discussion and started with AMJW the consensus group. LPRV did the initial search of published work. LPRV and AMJW checked all full-text articles and extracted data from the full reports and conference abstracts. LPVR and AMJW conceived and coordinated the analyses and wrote the first draft of the paper. All authors were involved in the guidelines and discussion and participated in reviewing and revision of the paper. All authors have seen and approved the final version of the paper.

European Consensus Group on clinical management of HIV-1 tropism testing

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Conflicts of interest

The consensus panel is an initiative of the EuropeHIVResistance Network, which receives some funding from the European Commission. The panel did not receive funding from diagnostic or pharmaceutical companies for this initiative. CABB has served as consultant to Merck, former Schering-Plough, and ViiV Healthcare and has received payments for lectures from ViiV Healthcare. FB-V has received grants and research support from GlaxoSmithKline and Tibotec Therapeutics, has served as a consultant to Merck, Pfizer, Siemens, and Tibotec Therapeutics, and has served as a paid lecturer for Bristol-Myers Squibb, GlaxoSmithKline, and Tibotec Therapeutics. BC has served as consultant to Siemens, Boehringer-Ingelheim, GlaxoSmithKline, Gilead, Merck, and Shionogi and has served as a paid lecturer for ViiV Healthcare and Janssen. AMG has served as a consultant to Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Merck, Tibotec, Pfizer, Monogram, Roche, Virco, and ViiV Healthcare, has received grants from Gilead, Merck, Tibotec, Pfizer, Monogram, Roche, Virco, and ViiV Healthcare, has received payment for lectures from Abbott, Boehringer Ingelheim, Gilead, GlaxoSmithKline, Tibotec, Pfizer, and ViiV Healthcare, and has received travel, accommodations, and meeting expenses from Abbott, Boehringer Ingelheim, Gilead, GlaxoSmithKline, Tibotec, Pfizer, and ViiV Healthcare. TK has served as a consultant to Abbott, Pfizer, and ViiV Healthcare and has received payment for lectures from Gilead. KK has received support for travel to meetings for research from ViiV Healthcare and received payments for lectures from Abbott, Siemens, Janssen-Cilag, and Roche. ADL has served as consultant to Tibotec, Monogram Biosciences, and Gilead, has served as a paid lecturer for Abbott, GlaxoSmithKline, and Merck, and has received travel, accommodations, and meeting expenses from ViiV Healthcare and Abbott. BM has served as consultant to Abbott and ViiV Healthcare, has received grants from Pfizer and Janssen-Cilag, has received payments for lectures from Merck Sharp & Dohme, Pfizer, Gilead Sciences, and Janssen-Cilag, and has received travel, accommodations, and meeting expenses from GlaxoSmithKline, Gilead Sciences, Bristol-Myers Squibb, and Janssen-Cilag. CFP has served as a consultant to ViiV Healthcare, GlaxoSmithKline, Merck, Abbott, and Gilead, has received grants from Merck, ViiV Healthcare, and Pfizer, and has received payments for lectures from Merck. JMS has served as a consultant to Merck, Roche, GlaxoSmithKline, Tibotec, Pfizer, ViiV Healthcare, Monogram Biosciences, Siemens, and Virco, has received payments for lectures from Tibotec-Janssen Cilag, Abbott, Virology Education, and Bristol-Myers Squibb, and has received payment for development of educational presentations from Virology Education. AS has served as a consultant to Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Medivir, Abbott, Merck, and Pfizer, has received grants from Abbott and Gilead, and has received payments for lectures from Roche Diagnostics, GlaxoSmithKline, and Abbott. VS has served as consultant to ViiV Healthcare, Gilead, and Roche, has received grants from Boehringer Ingelheim, ViiV Healthcare, Gilead, Merck Sharp & Dohme, and Tibotec, has received a research grant from Gilead and Merck Sharp & Dohme, and has received payment for lectures from Gilead, Merck Sharp & Dohme, Roche, Bristol-Myers Squibb, and Tibotec. A-MV has served as a consultant to ViiV Healthcare and Janssen-Cilag, and has received payments for developments of educational presentations from Abbott. Her institution has received grants from Pfizer and travel, accommodations, and meeting expenses from Pfizer, Merck Sharp & Dohme, Gilead, Abbott, Tibotec, ViiV Healthcare, GlaxoSmithKline, and Boehringer Ingelheim. LPRV has served as a consultant to and has received travel, accommodations, and meeting expenses from GlaxoSmithKline, ViiV Healthcare, Pfizer, Gilead, Virco, Tibotec, Merck, and Bristol-Myers Squibb. HW has served as consultant has served as consultant to Abbott, GlaxoSmithKline, Pfizer, Gilead Sciences, Tibotec, and Merck Sharp & Dohme, has received grants from Aicuris, Nexigen, Pfizer, and Gilead Sciences, and has received payment for lecturers from Abbott, Tibotec, GlaxoSmithKline, Pfizer, Gilead Sciences, and Merck Sharp & Dohme. AMJW has served as a consultant to Bristol-Myers Squibb, has received grants from Merck Sharp & Dohme, European Commission, and Pfizer, has served as a paid lecturer for ViiV Healthcare and GlaxoSmithKline, and has received travel, accommodations, and meeting expenses from Bristol-Myers Squibb, Tibotec, Pfizer, and Abbott. MZ has served as a consultant to Janssen-Cilag, Gilead Sciences, Abbott Molecular, and Merck Sharp & Dohme, has received grants from Pfizer, and has received payment for lectures from Abbott, Pfizer, Abbott Molecular, and Merck Sharp & Dohme. RK, SD, FG, and CV have no conflicts of interests.

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