BlueCross BlueShield of Alabama

Name of Blue Advantage Policy:

Laboratory Testing for HIV Tropism

Policy #: 322
Category: Laboratory

Latest Review Date: January 2019
Policy Grade: Effective January 31, 2019: Active Policy but no longer scheduled for regular literature reviews and updates

Background:

Blue Advantage *medical policy does not conflict with Local Coverage Determinations (LCDs), Local Medical Review Policies (LMRPs) or National Coverage Determinations (NCDs) or with coverage provisions in Medicare manuals, instructions or operational policy letters. In order to be covered by Blue Advantage the service shall be reasonable and necessary under Title XVIII of the Social Security Act, Section 1862(a)(1)(A). The service is considered reasonable and necessary if it is determined that the service is:*

1. *Safe and effective;*
2. *Not experimental or investigational*;*
3. *Appropriate, including duration and frequency that is considered appropriate for the service, in terms of whether it is:*
   - *Furnished in accordance with accepted standards of medical practice for the diagnosis or treatment of the patient's condition or to improve the function of a malformed body member;*
   - *Furnished in a setting appropriate to the patient's medical needs and condition;*
   - *Ordered and furnished by qualified personnel;*
   - *One that meets, but does not exceed, the patient's medical need; and*
   - *At least as beneficial as an existing and available medically appropriate alternative.*

Routine costs of qualifying clinical trial services with dates of service on or after September 19, 2000 which meet the requirements of the Clinical Trials NCD are considered reasonable and necessary by Medicare. Providers should bill* ***Original Medicare* *for covered services that are related to* ***clinical trials*** *that meet Medicare requirements (Refer to Medicare National Coverage Determinations Manual, Chapter 1, Section 310 and Medicare Claims Processing Manual Chapter 32, Sections 69.0-69.11).*

Description of Procedure or Service:

HIV tropism testing can determine the predominant co-receptor protein used by the human immunodeficiency virus (HIV) to infect target cells. Tropism testing can help select patients for treatment with HIV co-receptor antagonists, such as Maraviroc, which block specific co-receptor proteins.

HIV

The human immunodeficiency virus (HIV-1), which causes acquired immunodeficiency syndrome, uses co-receptor proteins (either CCR5 or CXCR4) on the surface of target cells to enter and infect the cells. The most commonly transmitted strains of HIV-1 bind to CCR5 and are said to have "tropism" for CCR5-expressing cells. Dual or mixed (D/M) tropic viruses can bind to either receptor type. It is estimated that around 85% of treatment-naive patients harbor CCR5-tropic virus only, around 15% harbor D/M virus, and less than 1% are infected with CXCR4-tropic virus alone. CXCR4-tropic virus is associated with immunosuppression and later stages of disease. Co-receptor antagonists have been designed to interfere with the interaction between HIV-1 and its co-receptors.

HIV Coreceptor Antagonists

Maraviroc (Selzentry™, Pfizer) is the first co-receptor antagonist to be approved by the U.S. Food and Drug Administration (FDA). Maraviroc is a selective, slowly reversible, small-molecule antagonist of the interaction between human cell surface CCR5 and HIV-1 gp120, necessary for HIV-1 cell infection. Blocking this interaction prevents CCR5-tropic HIV-1 entry into cells. However, CXCR4-tropic HIV-1 entry is not prevented. According to the drug's original label, Maraviroc, in combination with other antiretroviral agents, is indicated for adult patients who are infected with only CCR5-tropic detectable HIV-1, who have evidence of viral replication and HIV-1 strains resistant to multiple antiretroviral agents.

The currently-approved maraviroc label indicates that maraviroc is indicated for combination antiretroviral treatment for adults infected with only CCR5-tropic HIV-1, without discussion of the presence of viral replication. The FDA-approved full prescribing information states "Tropism testing must be conducted on a current sample with a highly sensitive tropism assay that has demonstrated the ability to identify patients appropriate for use of SELZENTRY." This is because efficacy was not demonstrated in a phase II study of maraviroc in patients with dual/mixed or CXCR4-tropic HIV-1. Due to potential adverse effects (hepatic and cardiotoxicity), maraviroc should only be used in indicated patients.

Other HIV coreceptor antagonists are in the drug development pipeline. Cenicriviroc (Tobira Therapeutics) is a small-molecule antagonist of both CCR5 and CCR2, a receptor involved in a number of inflammatory diseases that is currently being investigated for treatment of CCR5-tropic HIV. In January 2015, cenicriviroc was granted fast track designation by FDA for the treatment of nonalcoholic steatohepatitis in patients with liver fibrosis, but the drug does not yet have FDA approval.

HIV Tropism Testing

HIV tropism testing is available by either phenotypic or genotypic methods. Tropism testing with a phenotypic assay, a cellular-based assay that functionally determines tropism, is

available with the enhanced sensitivity TrofileTM assay (Monogram Biosciences, South San Francisco, CA) assay (ESTA). This phenotypic assay uses virus stocks pseudotyped with envelope sequences derived from patient plasma to infect cell lines engineered to express CCR5 or CXCR4 HIV-2 coreceptors. Genotypic tropism testing is based on sequencing the third variable (V3) loop of the HIV glycoprotein 120 gene, because the V3 loop interacts with the HIV coreceptor, and mutations in V3 are associated with measurable changes in HIV tropism. Tropism assignment is derived from the sequence data using a bio-informatic algorithm such as geno2pheno (G2P). In the U.S., the only commercially available genotypic HIV coreceptor tropism assay is available from Quest Diagnostics, which uses triplicate population sequencing with reflex to ultra-deep sequencing if only CCR5-tropic virus is detected. Quest Diagnostics also offers a proviral DNA tropism test (Trofile DNA) which sequences the tropism of HIV-1 DNA that has integrated into the host genome of infected T-lymphocytes via triplicate population sequencing, without the use of ultra-deep sequencing.

Policy:

Effective for dates of service on or after May 1, 2015:

Blue Advantage will treat **HIV tropism testing with either the phenotypic assay or V3 population (via Sanger or V3 deep sequencing method) genotyping** as a **covered benefit** for selecting patients for treatment with HIV co-receptor antagonists, such as maraviroc, when there is an immediate plan to prescribe a co-receptor antagonist.

Blue Advantage will treat **HIV tropism testing** without immediate plans to prescribe HIV co-receptor antagonists such as maraviroc as a **non-covered benefit**.

Blue Advantage will treat **repeat HIV tropism testing** during co-receptor antagonist treatment or after failure with co-receptor antagonists as a **non-covered benefit** and as **investigational**.

Blue Advantage will treat **HIV tropism testing** to predict disease progression (irrespective of co-receptor antagonist treatment) as a **non-covered benefit** and as **investigational**.

Refer also to Blue Advantage medical policy *#264 HIV Genotyping and Phenotyping* for additional information.

Effective for dates of service on or after March 1, 2014 and prior to May 1, 2015:

Blue Advantage will treat **HIV tropism testing with either the phenotypic assay or V3 population (via Sanger or V3 deep sequencing method) genotyping** as a **covered benefit** for selecting patients for treatment with HIV co-receptor antagonists such as maraviroc when there is an immediate plan to prescribe a co-receptor antagonist. Patients indicated for testing:

- Have evidence of viral replication, and
- Have failed multiple antiretroviral treatment regimens, or
- Are treatment naïve

Blue Advantage will treat **HIV tropism testing** without immediate plans to prescribe HIV co-receptor antagonists such as maraviroc as a **non-covered benefit**.

Blue Advantage will treat **repeat HIV tropism testing** during co-receptor antagonist treatment or after failure with co-receptor antagonists as a **non-covered benefit** and as **investigational**.

Blue Advantage will treat **HIV tropism testing** to predict disease progression (irrespective of co-receptor antagonist treatment) as a **non-covered benefit** and as **investigational**.

Blue Advantage does not approve or deny procedures, services, testing, or equipment for our members. Our decisions concern coverage only. The decision of whether or not to have a certain test, treatment or procedure is one made between the physician and his/her patient. Blue Advantage administers benefits based on the members' contract and medical policies. Physicians should always exercise their best medical judgment in providing the care they feel is most appropriate for their patients. Needed care should not be delayed or refused because of a coverage determination.

Key Points:

This policy was updated with the literature available through October 4, 2018.

Evidence reviews assess whether a medical test is clinically useful. In order to determine if a test is clinically useful, the test must provide information to make a clinical management decision that improves the net health outcome. The balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The formulation of the clinical context and purpose of the test is the first step in assessing a medical test. The test must be technically reliable, clinically valid, and clinically useful for that purpose.

HIV Tropism Testing to Identify Candidates for HIV Coreceptor Antagonist Therapy

Clinical Context and Test Purpose

The purpose of HIV tropism testing in patients who have HIV infection is to inform a decision whether the patient might be a candidate for treatment with HIV coreceptor antagonist therapy.

The question addressed in this evidence review is: Does assessment of HIV tropism, to identify HIV-infected patients who are candidates for HIV coreceptor therapy, result in an improved health outcome compared with HIV coreceptor therapy without HIV tropism testing?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant populations of interest are treatment-naive and treatment-experienced HIV-infected patients.

Interventions
The interventions of interest are HIV tropism testing using the Trofile assay, the enhanced sensitivity Trofile assay (ESTA), V3 sequencing, or V3 deep sequencing.

Comparators
The comparator of interest is no HIV tropism testing.

Outcomes
The potential beneficial outcomes of primary interest would be identification of HIV-infected patients who might benefit from treatment with HIV coreceptor antagonist therapy.

The potential harmful outcomes are those resulting from a false test result. False-positive or false-negative test results can lead to the initiation of unnecessary treatment and adverse events from that treatment or undertreatment.

Timing
HIV tropism testing is conducted before starting HIV coreceptor antagonist therapy.

Setting
Ordering and interpreting HIV tropism testing should be done by physicians specializing in infectious diseases. Most patients are likely to be tested in an outpatient setting. Referral for genetic counseling is important for the explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

Technically Reliable
The technical reliability of different HIV tropism testing and comparison in performance of these testing techniques are discussed in this section.

Tropism Testing using the Trofile Assay or Enhanced Sensitivity Trofile Assay
For the clinical studies of patients with treatment failure, Whitcomb et al (2007) determined tropism at enrollment and again at baseline using the original phenotypic Trofile assay for 2560 potential enrollees; 56% were CCR5- tropic only and were eligible for the clinical trials. Most other patients had dual/mixed HIV infection; CXCR4-infection alone is rare. Of the patients enrolled, 90% had CCR5-tropic virus at baseline, 4% had dual-mixed tropic virus, and 5% had nontypable virus infection. The original phenotypic Trofile assay had a turnaround time of 14 to 18 days, failed to work in 3% to 7% of patients, and required at least 1000 copies/mL of HIV RNA. The assay was 100% effective in detecting model CXCR4-tropic or dual/mixed HIV present in a 10% mixture, and 83% effective at a 5% mixture. Validation studies also indicated 100% accuracy of results for 38 samples with known tropism, and 100% reproducibility including repeat assays using multiple operators, instrumentation, reagent lots, and conducted over a 14- day period. No false positive results were obtained on samples that were HIV-negative but positive for either hepatitis B or C virus.

An enhanced sensitivity Trofile assay (ESTA) has replaced the original Trofile. The ESTA can detect CXCR4-tropic virus present at levels less than 0.3% of the total virus population, and at that level of virus or higher, the assay is stated to be 100% sensitive. Total viral concentration of at least 1000 copies/mL is required. However, ESTA remains limited by long turnaround time and the relatively high minimum level of viremia required, making it not useful in patients in virologic failure with low viremia. Additionally, a small proportion of samples cannot be successfully phenotyped with either generation of the Trofile assay.

The Maraviroc versus Efavirenz Regimens as Initial Therapy trial (MERIT) study (2010) of treatment-naïve patients was retrospectively reanalyzed using ESTA; approximately 15% of the subjects originally identified as CCR5-tropic had dual/mixed- or CXCR4-tropic virus by ESTA. Removing these from the analysis resulted, as already noted, in similar responses in both trial arms, indicating that maraviroc in a combination regimen is at least as good as another well-accepted combination regimen for treatment-naïve patients.

Wilkin et al (2011) used ESTA to reanalyze samples from four large cohort studies that had originally been evaluated for HIV tropism with the original Trofile assay. Nine percent to 26% of patients with CCR5-tropic virus by the original Trofile assay had CXCR4-using virus by ESTA.

V3 Population Genotyping to Determine Tropism

The Trofile assay is a cell-based, functional (phenotypic) assay. Genotypic assays are based on the sequencing of the patient-derived HIV-1 gp120 V3 domain, which determines the protein amino acid sequence for the major determinant of co-receptor binding. This sequencing method results in a V3 sequence that represents the average or dominant viral population sequence for each patient. The patient-derived HIV V3 sequence is used to predict HIV-1 tropism using web-based bioinformatic interpretation tools developed from prior data. These are most often the support vector machine-based geno2pheno coreceptor (G2P; available online at: coreceptor.bioinf.mpi-inf.mpg.de/index.php) and position-specific scoring matrices (PSSM; available online at; indra.mullins.microbiol.washington.edu/webpssm/).

Genotyping can be conducted on either viral RNA samples (plasma) or on proviral DNA (peripheral blood mononuclear cells), the latter allowing tropism determination in the context of undetectable viremia. Other potential advantages of genotypic assays are reduced cost, shorter turnaround time, and fewer sample failures.

Early genotyping studies with comparisons with original Trofile assay results reached contradictory conclusions regarding the adequacy of genotyping for predicting CXCR4 coreceptor usage. Some of the variability in genotype-phenotype assay correlation may have been due to the lower sensitivity of the original Trofile assay, and some variability may have accrued from inclusion of samples containing HIV subtypes other than B (the dominant form in Europe, the Americas, Japan, Thailand, and Australia). Ultimately, the best indication against which tropism assay results should be compared is the virologic outcome of patients who receive CCR5-antagonist medication.

Comparison of different tropism assay techniques with reference to virologic outcome of patients is discussed in the Clinical Validity section below.

Newer bioinformatics algorithms continue to be developed, some of which incorporate clinical variables such as HIV-1 viral load and nadir CD4+ count, into their prediction modeling. Some studies, such as one reported by Ceresola et al (2015) in a cohort of 67 subjects with HIV, suggest that the G2P algorithm may be more likely to overestimate the frequency of CCX4-tropic viruses compared with other methods.

Table 1 summarizes studies that evaluated the results of V3 sequencing using enhanced sensitivity Trofile assay (ESTA) as the reference standard; treatment outcomes were not considered in these analyses. All studies sequenced HIV V3 RNA from plasma (standard assay); two additionally sequenced HIV V3 DNA from whole blood, which targets proviral DNA (useful for patients with low plasma levels of virus). In general, the sensitivity results indicate that V3 genotyping detects somewhat fewer CXCR4-tropic viral samples than does ESTA; the specificity results indicate that the FPR is not high, i.e., few CCR5-tropic samples are identified as CXCR4-tropic. Assay concordance is relatively high. Where reported, genotyping results for proviral DNA appear very similar to those for RNA in paired samples from the same patient population (see also, "Tropism Testing in Patients with Undetectable Viral Load" section).

Overall and based largely on the studies of tropism assays with reference to maraviroc treatment outcome (see Clinical Validity section), the evidence suggests that HIV V3 genotyping classifies patients as well as Trofile assays. Genotyping has additional advantages of shorter turnaround time, ability to generate results for patients who cannot be assayed by Trofile, and more access to assay providers

Table 1: Performance of HIV V3 Genotyping with Reference to Enhanced Sensitivity Trofile Assay (ETSA)

Study	N	Patients	RT-PCR Replicates	V3 Genotyping Algorithm	V3 Genotyping vs ESTA		
					Sensitivity	**Specificity**	**Concordance**
Prosperi (2010)	55	Patients failing antiretroviral treatment	1x	G2P clonal, FPR=5.75% G2P clonal, FPR=10% G2P clonal, FPR=5.75% G2P clonal, FPR=10%	RNA: 55% 55% DNA: 68% 67%	RNA: 96% 79% DNA: 86% 71%	RNA: 83% 71% DNA: 82% 71%
Svicher (2010)	365	63% treatment-experienced patients	1x	G2P clonal, FPR=5% G2P clonal, FPR=10%	49% 55%	96% 89%	81% 78%
Sanchez (2010)	119	Naïve and treatment-experienced	1x (?)	G2P clonal, FPR=5% G2P clonal,	37% 57%	93% 84%	79% 77%

		patients		FPR=10%			
Strang (2009) (Abs)	79	Patients evaluated for maraviroc therapy	?	G2P, FPR range, 1%-20%	NR	NR	Range, 70%-94%
Pou (2009) (Abs)	79	Banked samples, pre-ART	3x	G2P	RNA: 40% DNA: 36%	RNA: 100% DNA: 100%	RNA: 78% DNA: 77%

Abs: abstract; ART: antiretroviral therapy; Conc: concordance; ESTA: enhanced sensitivity Trofile assay; G2P: geno2pheno coreceptor system; FPR: false-positive rate (used as cutoff value); NR: not reported; RT-PCR: reverse-transcriptase polymerase chain reaction; Sens: sensitivity; Spec: specificity.

Tropism Testing by Deep Sequencing

Because of concern that standard V3 sequencing methods used for tropism testing, might miss clinically significant minor HIV variants, so-called "deep sequencing," (i.e., V3 sequencing using next-generation sequencing methods) has been investigated for tropism testing. While standard sequencing essentially determines a population average V3 loop sequence, deep sequencing allows simultaneous sequencing and quantifying of thousands of individual V3 variants within a viral population. From this, the proportion of non-R5 variants in a given sample can be calculated using bioinformatic interpretation tools similar to those for standard V3 genotyping. Similar to the standard V3 sequencing methods, the FPR for tropism prediction must be prespecified. Retrospective analyses have used G2P and a FPR of 3.5% or less. The proportion of the viral population that can be detected as non-CCR5 for maraviroc treatment to remain effective has been established as 2% or less. Other studies have also reported high concordance between deep sequencing and current tropism assays and between different sequencing platforms. Gibson et al reported high concordance between tropism prediction for samples sequenced with deep sequencing and those sequenced with population-based sequencing.

Tropism Testing in Patients with Undetectable Viral Load

The original studies of genotypic tropism tests, such as those shown in Table 1, were conducted on RNA samples from viremic patients. However, there has been interest in the use of maraviroc as part of a simplification strategy in patients already on antiretroviral therapy with undetectable plasma HIV RNA levels. Another potential indication is as intensification strategy in patients with prolonged suppression of HIV levels but with impaired CD4 gains. A 2012 study by Svicher et al demonstrated the feasibility of determining viral tropism using sequencing of proviral DNA with prediction of tropism with the geno2pheno algorithm in peripheral blood mononuclear cells from 53 subjects with HIV, most of whom had undetectable (94.3%) or low (3.7%) viral loads. Additional studies, outlined in Table 2, have demonstrated high rates of concordance between tropism predicted by proviral DNA or RNA sequencing.

Table 2. Performance of HIV Proviral DNA Genotyping

Study	Study Population	DNA Sequence Success Rate	V3 Genotyping Algorithm	Comparison	Concordance	Sens	Spec
Prosperi et al (2010)	55 patients failing antiretroviral treatment	NR		Proviral DNA vs RNA (ref) (n=29)	87.5% (k=0.74; 95% CI 0.53 to 0.95; p<0.001)	NR	NR
Svicher et al (2014)	253 patients with plasma HIV-1 RNA <50 copies/mL	· 93.5% for VL >100 copies HIV DNA/10^6PBMCs · 60% for VL<100 copies HIV DNA/10^6 PBMCs	G2P clonal, FPR=5.75%	Proviral DNA (whole blood or PBMCs) vs RNA (ref) (n=143)	96.5%	NR	NR
Brown et al (2014)	42 patients with plasma HIV-1 RNA ≥1000 copies/mL	97.6%	G2P clonal, FPR=10%	Proviral DNA (whole blood) vs RNA (ref)	93% (k=0.85)	100%	89%
				Proviral DNA (PBMCs) vs RNA (ref)	95% (k=0.90)	100%	93%
				Proviral DNA (whole blood or PBMCs) vs RNA (ref)	98% (k=0.95)	100%	96%

CI: confidence interval; ESTA: enhanced sensitivity Trofile assay; FPR: false-positive rate; G2P; geno2pheno; NR: not reported; PBMC: peripheral blood mononuclear cell; ref: reference group; Sens: sensitivity (defined as concordant ´4 results between test and reference methods/reference method CCX4); Spec: specificity (defined as concordant CCR5 results between test and reference methods/reference method CCR5); VL: viral load.

Section Summary: Technically Reliable

The evidence comparing HIV V3 population genotyping with original Trofile and ESTA using maraviroc response as the reference for all assays, strongly suggests that genotyping is equivalent to the Trofile assays in selecting patients likely to respond to maraviroc, the outcomes of interest. Studies evaluating genotyping and using paired ESTA results for reference suggest that genotyping might be somewhat less sensitive for detecting CXCR4-tropic samples; however, these studies were smaller, and most did not test in triplicate. V3 ultra-deep sequencing methods appear to have greater sensitivity in identifying CXCR4-tropic viruses, and therefore are likely to identify additional patients with HIV tropism who are negative on standard sequencing. Based largely on the maraviroc response results, HIV V3 population genotyping may be considered medically necessary for patients considering immediate maraviroc treatment.

Clinically Valid

HIV Coreceptor Antagonist Therapy in Treatment-Experienced Patients

The Maraviroc versus Optimized Therapy in Viremic Antiretroviral Treatment-Experienced Patients (MOTIVATE) I and II trials assessed the efficacy of maraviroc in patients previously treated or resistant to three antiretroviral drug classes and with HIV-1 RNA levels exceeding 5,000 copies/mL. MOTIVATE-I was conducted in Canada and the United States, and MOTIVATE-II in Australia, Europe, and the United States, using identical study designs. A total of 1,075 patients were randomized to three trial arms, and 1,049 received at least one dose of study drug: placebo (n=209), maraviroc once daily (n=414), or maraviroc twice daily (n=426). Selected subjects had only CCR5-tropic HIV-1 infections, as determined by the original Trofile assay for HIV tropism (see 'Tropism Testing,' following). At 48-weeks follow-up in an intention-to-treat analysis, 16% in the placebo group and 45% in both maraviroc-treated groups had HIV-1 RNA levels less than 50 copies/mL. The mean increase in CD-4 count from baseline was 60 in the placebo group compared to 120 in the maraviroc groups. Based on the early trial results and review by the FDA Antiviral Drugs Advisory Committee, the FDA concluded that, compared to placebo, maraviroc significantly reduced HIV RNA copy number, and significantly increased CD4 cells, both validated markers of improved health outcomes. At nearly two years of follow-up (96 weeks), 81% to 87% of maraviroc-treated patients maintained these responses with no new or unexpected events impacting safety. At five years of follow up, 46 deaths were reported, with ongoing low rates of hepatic failure, malignancy, and myocardial infarction.

In contrast, in a 2009 trial of 167 patients infected with dual- or mixed-tropic HIV-1, randomized to receive optimal therapy plus maraviroc or placebo, there was no difference in outcomes between treatment groups, indicating maraviroc treatment failure in patients harboring assay-detectable CXCR4-tropic HIV-1 populations.

HIV Coreceptor Antagonist Therapy in Treatment-Naïve Patients

The MERIT study (discussed above) was a randomized, double-blind, multicenter study in subjects infected with CCR5-tropic HIV-1 according to the original Trofile assay. Patients had plasma HIV-1 RNA levels of at least 2,000 copies/mL and did not have: 1) prior antiretroviral therapy for longer than 14 days, 2) an active or recent opportunistic infection or primary HIV-1 infection, or 3) resistance to zidovudine, lamivudine, or efavirenz. Subjects were randomized to two doses of either maraviroc or efavirenz, each in combination with zidovudine/lamivudine. In a pre-planned interim analysis, the lower dose of maraviroc failed to meet pre-specified efficacy criteria and was discontinued. Patients were stratified by screening HIV-1 RNA levels and by geographic region. The median CD4 cell counts and mean HIV-1 RNA at baseline were similar for both treatment groups.

At 96 weeks, after re-analysis using results from an enhanced sensitivity Trofile assay (see 'Tropism Testing,' section next), virologic response rates in both treatment arms were approximately equal, and there were fewer discontinuations due to adverse events in the maraviroc arm.

Although most newly infected patients harbor CCR5-tropic HIV virus alone, a study of 150 individuals from two recent seroconverter cohorts documented 4% infection with detectable

CXCR4-tropic virus (either mixed or, rarely, CXCR4-only), indicating that tropism analysis is necessary, even for the recently infected.

Comparison of HIV Tropism Testing Methods to Identify Candidates for HIV Coreceptor Antagonist Therapy

Table 3 summarizes the results of studies comparing V3 genotyping results with virologic outcomes after maraviroc treatment. Because most studies use G2P for interpretation, only these results are presented. Where reported, results of original Trofile and enhanced-sensitivity Trofile assay results are also shown. Only the study reported by Gonzalez-Serna was prospective; for the others, V3 genotyping was conducted retrospectively on banked samples. McGovern (2010) likely includes data reported by Harrigan (2009). Results depend on the false positive rate (FPR) cutoff value chosen for the G2P algorithm. If the result provided by G2P for a specific V3 sequence is higher than the chosen cutoff, the prediction of HIV-1 coreceptor tropism is CXCR4. Because the G2P distributions for CCR5- and CXCR4- tropic viruses overlap, no cutoff value allows perfect classification. Using a higher cutoff value is considered a conservative choice because predictions of CXCR4-tropism are more likely to be true predictions; the trade-off is that some true CXCR4-tropic HIV infections will be falsely identified as CCR5- tropic. For example, a cutoff value of 5.75% was optimized retrospectively for the MOTIVATE trial data, but for routine clinical practice, the European guidelines on HIV-1 tropism testing recommend a cutoff of 10% for sequencing of samples in triplicate, or a cutoff of 20% when only a single sequence is generated.

The results in Table 3 indicate that, depending on the G2P cutoff value chosen, V3 sequencing results can be generated that are very similar in their ability to predict response to maraviroc to both the original Trofile and the enhanced sensitivity Trofile assays. The Gonzalez-Serna study reports somewhat different results, with lower sensitivity and higher specificity for maraviroc response using similar G2P cutoff values. This study prospectively enrolled patients attending the infectious disease service of a university hospital, as opposed to the other retrospective studies of carefully selected clinical trial participants, but was also much smaller. Sequencing in this study was not done in triplicate as it was in the other studies.

Table 3. Performance of HIV V3 Genotyping, Trofile, and ESTA Assays with Reference to Maraviroc Treatment Outcomes

Characteristics	McGovern (2012)	Harrigan (2009) (Abs)	Gonzalez-Serna (2011)	McGovern (2010)
Sample size	705	623	73	1164
Patients	Drug-naïve patients from MERIT trial	Treatment experienced patients from MOTIVATE and 1029 studies	Patients with persistent viral load and on treatment hiatus	Treatment experienced patients from MOTIVATE and 1029 studies
RT-PCR replicates	3x	3x	1x	3x
Virologic response definition	<50 copies/mL at week 48	<50 copies/mL or reduction ≥2 log at week 8	<50 copies/mL or reduction ≥1 log on day 8	<50 copies/mL or reduction ≥2 log at week 8
V3 genotyping algorithm	G2P, FPR=5.75%	G2P, FPR=5%	G2P clonal, FPR=5% FPR=10%	G2P, FPR=5%

V3 genotyping vs virologic response to MVC	Sens=94% Spec=13%	Sens=85% Spec=36%	Sens=58% Spec=89% Sens=68% Spec=83%	Sens=89% Spec=24%
Original Trofile vs virologic response to MVC	NR	Sens=90% Spec=31%	NR	Sens=92% Spec=20%
ESTA vs virologic response to MVC	Sens=91% Spec=22%	NR	NR	NR

RT-PCR: reverse-transcriptase polymerase chain reaction; ESTA: enhanced sensitivity Trofile assay; MVC: maraviroc; G2P: geno2pheno co-receptor system; FPR: false-positive rate (used as cutoff value); Sens: sensitivity; Spec: specificity; MERIT: (Maraviroc versus Efavirenz Regimens as Initial Therapy trial; MOTIVATE: Maraviroc Plus Optimized Therapy in Viremic Antiretroviral Treatment-Experienced Patients trials; NR: not reported.

The sample data in Table 4 suggest that deep sequencing performs similarly to ESTA and the original Trofile assay at predicting response to maraviroc treatment. Moreover, as noted by Swenson et al (2011), the group of patients with 2% to 20% non-CCR5 virus according to deep sequencing had minority non-CCR5 variants that were not reliably detected by the original Trofile assay, but this group of patients had poor response to maraviroc, with 27% of the patients achieving virologic suppression at week 48, similar to the non-CCR5 group as a whole (26%) and to patients with greater than 20% non-R5 virus (25%). Both ultradeep sequencing methods demonstrated improved sensitivity in identifying maraviroc responders compared with standard sequencing. These results suggest that detection of minority non-CCR5 variants by deep sequencing may be important for predicting response.

Table 4. Performance of HIV V3 Deep Sequencing, Trofile, and ESTA Assays with Reference to Maraviroc Treatment Outcomes

Characteristics	Gonzalez-Serna 2011	Swenson 2011	Swenson 2011	Kagan 2012
Sample size	27	859	851	327
Patients	Patients with persistent viral load and on treatment hiatus	Drug-naïve patients from MERIT trial	Treatment-experienced patients from MOTIVATE and A4001029 studies	Treatment-experienced patients from MOTIVATE and A4001029 studies who received maraviroc
RT-PCR replicates	3□	3□	3□	3□
Virologic response definition	<50 copies/mL or reduction ≥1 log on day 8	<50 copies/mL at week 48	<50 copies/mL at week 48	<50 copies/mL or >2 log decline at week 8
V3 genotyping algorithm	G2P clonal, FPR≤3.5%	G2P clonal, FPR≤3.5%	PSSMx4/R5 FPR ≥ -4.75 [~90% concordance with G2P]	G2P, FPR≤5.75% PSSMX4R5, FPR ≥ - 4.75
V3 genotyping vs virologic response to MVC	Sens=83% Spec=22%	Sens=93% Spec=15%	Sens=83% Spec=36%	PPV[a] =65% NPV=61%
Original Trofile vs virologic response to MVC	NR	NR	Sens=93% Spec=17%	NR
ESTA vs virologic response to MVC	NR	Sens=90% Spec=21%	NR	PPV=66% NPV=59%

conc: concordance; ESTA: enhanced sensitivity Trofile assay; G2P: geno2pheno coreceptor system; FPR: false-positive rate (used as cutoff value); MVC: maraviroc; NPV: negative predictive value; NR: not reported; PPV: positive predictive value; RT-PCR: reverse-transcriptase polymerase chain reaction; Sens: sensitivity; Spec: specificity. [a] PPV refers to the proportion of CCR5 subjects who achieved virologic response at eight wk. NPV refers to the proportion of non-CCR5 subjects who failed to have a virologic response at eight wk.

A 2014 prospective, phase 3 trial by Heera et al, which randomized treatment-naïve patients with HIV to genotypic or phenotypic (Trofile) testing showed no significant differences in treatment response. Previously-presented results of European cohort studies have shown maraviroc virologic extended response rates of 69% to 82% in those patients in which HIV variants were genotypically classified CCR5-tropic.

Garcia et al (2014) reported in abstract form the results of the PROTEST study, which evaluated the initiation of maraviroc plus two nucleoside reverse-transcriptase inhibitors in aviremic subjects based on genotypic tropism testing of proviral DNA, rather than viral RNA. The study included 74 maraviroc naïve HIV-1 patients with viral load less than 50/mL on stable antiretroviral therapy, requiring medication change due to toxicity, and CCR5 HIV by proviral DNA genotypic tropism testing. Of the included subjects, 62 (84%) maintained a viral load less than 50/mL through 48 weeks of therapy. A remaining 12 (16%) discontinued treatment: two (3%) withdrew informed consent; two (3%) died to non-study-related causes; five (7%) developed protocol-defined virologic failure; and one each (1% each) had a shift to CCX4 between the screening and baseline visits, was lost to follow up), or developed an antiretroviral therapy-related adverse event.

Nozza et al (2016) conducted a multicenter, randomized, open-label, noninferiority trial among treatment-experienced subjects with HIV-1 RNA of 500 or more copies per milliliter. One hundred fifty-five participating patients were randomized (1:1) to undergo coreceptor tropism testing by the G2P algorithm (false-positive rate >10%) or the Trofile assay before starting a new antiretroviral regimen. Only patients with an R5 tropic virus were enrolled and received treatment with maraviroc plus optimized background therapy. The primary end point was the 48-week proportion of patients with treatment success (defined as HIV RNA <50 copies/mL). In the Trofile arm, 87% of patients achieved treatment success at 48 weeks, and in the G2P arm, 89% achieved treatment success at 48 weeks; these results suggest noninferiority.

The purpose of gaps tables (see Tables 5 and 6) is to display notable gaps identified in each study. This information is synthesized as a summary of the body of evidence following each table and provides the conclusions on the sufficiency of the evidence supporting the position statement.

Table 5. Relevance Gaps

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Follow-Up[e]
MOTIVATE 1 & 2 (2008)	4. Only 10% of participants were women and <20% nonwhite				
MERIT (2010)					
OSCAR (2016)	4. Small number of participants included (n=155)				

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Not clearly defined; 2. Version used unclear; 3. Delivery not similar intensity as comparator; 4.Not the intervention of interest.
[c] Comparator key: 1. Not clearly defined; 2. Not standard or optimal; 3. Delivery not similar intensity as intervention; 4. Not delivered effectively.
[d] Outcomes key: 1. Key health outcomes not addressed; 2. Physiologic measures, not validated surrogates; 3. No CONSORT reporting of harms; 4. Not establish and validated measurements; 5. Clinical significant difference not prespecified; 6. Clinical significant difference not supported.
[e] Follow-Up key: 1. Not sufficient duration for benefit; 2. Not sufficient duration for harms.

Table 6. Study Design and Conduct Gaps

Study	Allocation[a]	Blinding[b]	Selective Reporting[c]	Follow-Up[d]	Power[e]	Statistical[f]
MOTIVATE 1 & 2 (2008)	3. Allocation concealment unclear	1. Unclear if administrator also blinded				
MERIT (2010)						
OSCAR (2016)		2. Unclear if investigators blinded to treatment				

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Allocation key: 1. Participants not randomly allocated; 2. Allocation not concealed; 3. Allocation concealment unclear; 4. Inadequate control for selection bias.
[b] Blinding key: 1. Not blinded to treatment assignment; 2. Not blinded outcome assessment; 3. Outcome assessed by treating physician.
[c] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[d] Follow-Up key: 1. High loss to follow-up or missing data; 2. Inadequate handling of missing data; 3. High number of crossovers; 4. Inadequate handling of crossovers; 5. Inappropriate exclusions; 6. Not intent to treat analysis (per protocol for noninferiority trials).
[e] Power key: 1. Power calculations not reported; 2. Power not calculated for primary outcome; 3. Power not based on clinically important difference.
[f] Statistical key: 1. Intervention is not appropriate for outcome type: (a) continuous; (b) binary; (c) time to event; 2. Intervention is not appropriate for multiple observations per patient; 3. Confidence intervals and/or p values not reported; 4. Comparative treatment effects not calculated.

Clinically Useful

Among patients who are undergoing HIV tropism testing to determine if they are suitable for maraviroc treatment, there is no direct evidence that HIV tropism testing results in improved health outcome in terms of overall or disease-specific survival. However, there is evidence that selection of candidates for HIV coreceptor antagonist therapy using HIV tropism tests results in a high rate of treatment success, demonstrated as increased virologic suppression. Plasma viral load is the single best predictor of progression to AIDS and death. Successful virologic suppression leads to longer overall survival and disease-specific survival among HIV-infected patients.

Section Summary: HIV Tropism Testing to Identify Candidates for HIV Coreceptor Antagonist Therapy

Evidence from randomized controlled trials (RCTs) and observational studies has suggested high sensitivity of the Trofile assay, the ESTA test, V3 sequencing, and V3 deep sequencing in identifying treatment-naive and treatment-experienced HIV-infected candidates for HIV coreceptor antagonist therapy, with treatment outcome as the reference. Studies have also suggested a moderate (>70%) level of concordance between different HIV tropism testing techniques.

HIV Tropism Testing for Treatment Monitoring and at Virologic Failure

Clinical Context and Test Purpose

The purpose of HIV tropism testing in patients with HIV infection receiving treatment with HIV coreceptor antagonist or who have failed coreceptor antagonist therapy is to monitor or detect possible tropism switching.

The question addressed in this evidence review is: Does assessment of HIV tropism among HIV-infected patients undergoing maraviroc therapy, or patients who have experienced virologic failure while on maraviroc therapy, result in an improved health outcome compared with no testing to identify HIV tropism switching?

The following PICOTS were used to select literature to inform this review.

Patients

The relevant populations of interest are 1 of 2 patient populations: (1) HIV-infected patients undergoing treatment with HIV coreceptor antagonists; or (2) patients who have failed coreceptor antagonist therapy.

Interventions

The interventions of interest are HIV tropism testing using the Trofile assay, ESTA, V3 sequencing, or V3 deep sequencing.

Comparators

The comparator of interest is no HIV tropism testing.

Outcomes

The potential beneficial outcomes of primary interest would be identification of HIV-infected patients who might benefit from changes in antiretroviral therapy regimen.

The potential harmful outcomes are those resulting from a false test result. False-positive or false-negative test results can lead to the initiation of unnecessary treatment and adverse events from that treatment.

Timing
HIV tropism testing should be conducted before starting HIV coreceptor antagonist therapy.

Setting
Ordering and interpreting of HIV tropism testing should be done by physicians specializing in infectious diseases. Most patients are likely to be tested in an outpatient setting. Referral for genetic counseling is important for the explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

Technically Reliable
Evidence on the technical reliability of different HIV tropism testing techniques has been discussed in the section on identifying candidates for HIV coreceptor antagonist therapy.

Clinically Valid
Viral strains transmitted in vivo are usually CCR5-tropic. Over time and more often after antiretroviral treatment, detectable CXCR4-tropic virus emerges in about half of patients and is associated with rapid CD4 cell depletion and clinical disease progression. However, patients whose infection remains predominately CCR5-tropic can also experience disease progression. . HIV-1 viral load is a strong prognostic indicator of HIV disease progression, and suppression of viral load is a critical goal of antiretroviral therapy. Viral rebound (virologic failure) is typically followed by a reduction in CD4 cell count (immunologic failure), and if not adequately addressed by changes in treatment, by HIV-related events (clinical progression). Thus, success of any antiretroviral treatment regimen is monitored by measuring HIV-1 RNA level and CD4 cell count; significant changes direct patient management.

The prominent reason for individual treatment failure in the clinical studies was outgrowth of a minor CXCR4-tropic virus population not detected at screening. However, treatment failure with CCR5-tropic virus alone also occurred, indicating that resistance to CCR5 antagonists occurs independent of tropism. In vitro studies have provided extensive information on resistance; mechanisms may involve the ability of HIV to bind the CCR5 inhibitor-receptor complex. Resistance to CCR5 antagonists has been associated with increased affinity for CCR5, changes in the gp 120 V3 loop, and with other gp 120 (or other envelope) changes.

A concern regarding treatment with CCR5 coreceptor antagonists is that small, undetectable populations of CXCR4-tropic virus would be enriched and would accelerate disease progression. However, in a randomized, placebo-controlled Phase II study of maraviroc treatment of patients with dual or mixed (D/M)-tropic infections, there was no evidence that this was the case. The association of CXCR4 tropism (defined with the original Trofile assay) with clinical progression has been shown to be independent of CD4 cell count and HIV-1 RNA level (adjusted hazard ratio=3.82, 95% CI, 1.69 to 8.60, p=0.001 compared with patients with CCR5-tropic infection only).

Fatkenheuer et al performed a post hoc analysis of the virologic response according to tropism at baseline and at treatment failure using pooled data from the MOTIVATE I and II trials. Virologic failure occurred in 53% of placebo-treated patients and in 22% to 23% in the maraviroc treatment arms. However, of the 133 treatment failures in the maraviroc groups, 76 (57%) had CXCR4 or D/M tropism compared with only 6 of 95 (6%) in the placebo group, raising concerns that maraviroc treatment could lead to emergence of CXCR4-tropic subpopulations and more rapid development of clinical progression. This was not the case, as the CXCR4 maraviroc treatment failures were not associated with declines in CD4 cell counts nor with disease progression.

Raymond et al (2015) conducted a multicenter study to characterize virologic failure in patients treated with maraviroc (n=27). Patients had been screened for HIV tropism using population-based V3 genotyping before maraviroc initiation. Authors determined HIV tropism and resistance of R5 viruses to maraviroc at baseline and at virologic failure retrospectively using an ultra-sensitive recombinant virus assay. Among the 27 patients experiencing virologic failure, 12 harbored CXCR4-using viruses, and 15 had R5 viruses at failure. Four of the 12 harboring CXCR4 viruses were infected with D/M-tropic viruses, according to the recombinant virus assay before maraviroc initiation.

The most common mechanism of maraviroc treatment failure is emergence of a CXCR4-tropic viral population. However, this is not necessarily correlated with rapid clinical progression.

Clinically Useful

For HIV-infected patients who are receiving maraviroc treatment, there is no direct evidence that HIV tropism testing-both during treatment monitoring and at virologic failure-results in improved health outcomes. The lack of evidence that HIV tropism testing might predict treatment failure among patients who are on maraviroc therapy, therefore, suggests that HIV tropism testing in this population might not result in improved health outcomes. Treatment failure is detected by increased viral load and decreased CD4 cell count, indicating that maraviroc treatment can be discontinued.

Section Summary: HIV Tropism Testing for Treatment Monitoring and Therapy Failure

The evidence for the use of HIV tropism testing for treatment monitoring and virologic failure in patients receiving maraviroc treatment includes post hoc analysis of data from RCTs and observational studies. While the emergence of the CXCR4-tropic viral population is the most common mechanism of maraviroc treatment failure, treatment failure is also common among patients with CCR5-tropic viruses. There is no evidence that tropism testing for treatment monitoring might predict treatment failure.

HIV Tropism Testing for HIV Prognosis

Clinical Context and Test Purpose

The purpose of HIV tropism testing in patients who have HIV infection is to identify patients who might experience rapid disease progression, such as the short-term risk of AIDS and death.

The question addressed in this evidence review is: Does assessment of HIV tropism to predict disease progression among HIV-infected patients result in an improved health outcome compared with CD4 count or viral load testing?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant population of interest is HIV-infected patients.

Interventions
The interventions of interest are HIV tropism testing using the Trofile assay, ESTA, V3 sequencing, or V3 deep sequencing.

Comparators
The comparator of interest is no HIV tropism testing.
Outcomes
The potential beneficial outcomes of primary interest would be identification of HIV-infected patients who might benefit from changes in antiretroviral therapy regimen.

The potential harmful outcomes are those resulting from a false test result. False-positive or false-negative test results can lead to the initiation of unnecessary treatment and adverse events from that treatment or undertreatment.

Timing
HIV tropism testing should be conducted before starting HIV coreceptor antagonist therapy.

Setting
Ordering and interpreting of HIV tropism testing should be done by physicians specializing in infectious diseases. Most patients are likely to be tested in an outpatient setting. Referral for genetic counseling is important for the explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

Technically Reliable
Evidence on the technical reliability of different HIV tropism testing techniques has been discussed in the section on identifying candidates for HIV co-receptor antagonist therapy.

Clinically Valid
Aside from the specific situation of maraviroc treatment failure, CXCR4-tropic virus infection has been associated with more rapid disease progression, compared with CCR5 infection, in several studies (e.g., see Wilkin et al [2011], Almeida et al [2014], and Visseaux et al [2014]). However, other studies have demonstrated no independent association between the HIV tropism and HIV-related outcomes, including short term risk of AIDS and death and hepatic fibrosis in HIV/hepatitis C virus-co-infected patients.

Castagna et al (2016) conducted a longitudinal cohort study of HIV-1-treated adults to determine the rate of HIV tropism switch among subjects using antiretroviral therapy both in presence of

persistently detectable (PD) or undetectable (PU) viral load and to evaluate the association between tropism switch and disease progression. Over a median follow-up period of 22.6 months (range, 19.8-28.1 months), 124 PD and 71 PU patients showed similar rates of switch to a non-R5 virus (PD=6.9/100 person-years; 95% CI, 3.7 to 11.2/100 person-years; PU=8.0/100 person-years; 95% CI, 3.4 to 14.5/100 person-years). Switch to non-R5 virus was predicted by nadir CD4-positive count before the start of the follow-up period. Twenty-two (18%) PD and 4 (6%) PU subjects experienced disease progression (p=0.02). The risk of disease progression was independently associated with disease progression (adjusted hazard ratio, 4.06; 95% CI, 1.20 to 13.80).

Casadella et al (2017) conducted a nested case-control study within the EuroSIDA cohort to investigate whether plasma HIV-1 tropism testing could identify subjects at higher risk for clinical progression and death in routine clinical management. Cases (N=100) were subjects with AIDS or who had died from any cause, with a plasma sample of HIV-1 RNA greater than 1000 copies/mL available for tropism testing 3 to 12 months prior to the event. At least one matched (for age, HIV-1 RNA, and HCV status) control per case was selected (N=166). Baseline tropism was not associated with the risk of clinical progression or death (OR=0.66; 95% CI, 0.33 to 1.33). Female gender (OR=2.13; 95% CI, 1.04 to 4.36), being on antiretroviral therapy (OR=2.12; 95% CI, 1.15 to 4.41), baseline CD4 count (OR=0.90; 95% CI, 0.80 to 1.00), per 100 cells/mm3 higher and calendar year of sample (OR=0.84; 95% CI, 0.77 to 0.91) per more recent year were independently associated with disease progression.

Clinically Useful

Currently, there is no direct evidence that HIV tropism testing for assessment of disease progression among HIV-infected patients results in improvement of health outcomes. More studies are required comparing HIV tropism testing with other tests (CD4, viral load) for predicting disease progression.

Section Summary: HIV Tropism Testing for HIV Prognosis

The evidence for the use of tropism testing for HIV prognosis includes nested case-control and cohort studies. While some studies demonstrated an association between the HIV tropism and HIV-related outcomes, the findings have been inconsistent. Viral load and CD4 count remain independently associated with disease progression among HIV-infected patients across studies.

Summary of Evidence

For individuals who have HIV infection who are being considered for HIV coreceptor antagonist therapy who receive HIV tropism testing, the evidence includes RCTs. Relevant outcomes are overall survival, disease-specific survival, morbid events, quality of life, hospitalizations, medication use, and treatment-related morbidity. RCTs on treatment-naive and treatment-experienced HIV-infected patients have provided evidence that selection of candidates for HIV coreceptor antagonist therapy using HIV tropism testing results in higher rates of treatment success compared with HIV coreceptor antagonist therapy without HIV tropism testing. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals with HIV infection receiving HIV coreceptor antagonist therapy or who have failed coreceptor antagonist therapy who receive HIV tropism testing, the evidence includes post hoc analysis of RCTs and observational studies. Relevant outcomes are overall survival, disease-specific survival, morbid events, quality of life, hospitalizations, medication use, and treatment-related mortality and morbidity. Current evidence does not indicate improved outcomes with additional tropism monitoring during treatment. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals with HIV infection who are undergoing tests to predict disease progression who receive HIV tropism testing, the evidence includes observational studies. Relevant outcomes are overall survival, disease-specific survival, morbid events, quality of life, hospitalizations, and medication use. Current evidence is inconsistent in as relates to whether HIV tropism testing independently predicts disease progression among HIV-infected patients. The evidence is insufficient to determine the effects of the technology on health outcomes.

Practice Guidelines and Position Statements

HIV Medicine Association of the Infectious Disease society of North America

The HIV Medicine Association of the Infectious Disease Society of North America released updated guidelines on the on the management of persons infected with HIV in 2013. These guidelines state that tropism testing should be performed if the use of a CCR5 antagonist is being considered (strong recommendation, high quality evidence). The guidelines also state that "routine tropism testing is not recommended prior to initiation of other regimens because of cost and lack of demonstrated benefit." The guidelines do not specify the preferred method of tropism testing.

European Consensus Group

The European Consensus Group on clinical management of tropism testing states that tropism testing is indicated for patients who fail treatment or have unacceptable toxicity and a CCR5 inhibitor is being considered. In the absence of evidence, the group provides no guidance regarding tropism testing for newly diagnosed patients whose immediate treatment plan does not include a CCR5 inhibitor. In the absence of adequate data, the group could provide no guidance regarding the question of testing treatment-naïve patients prior to the start of a regimen not including a CCR5 inhibitor, in anticipation of need for a fast change to a CCR5 inhibitor due to the toxicity of the initial treatment regimen. For patients with a plasma HIV RNA load >1,000 copies/mL, tropism testing can be done by Trofile ESTA or by population genotypic analysis of the V3 loop, indicating for both a moderate level of evidence based on well-designed, nonrandomized trials or cohort studies with long-term clinical outcomes. For patients with a plasma HIV RNA load <1,000 copies/mL, genotyping is the preferred method.

Department of Health and Human Services

The Health and Human Services Panel on Antiretroviral Guidelines for Adults and Adolescents published federally approved HIV/AIDS medical practice guidelines in 2014, which make the following recommendations on coreceptor tropism assays:

- Recommendations with "A" (strong) rating:

    - A coreceptor tropism assay should be performed whenever the use of a CCR5 coreceptor antagonist is being considered (level of evidence: I [data from randomized controlled trials]).
    - A phenotypic tropism assay is preferred to determine HIV-1 coreceptor usage (level of evidence: I).
- Recommendations with "B" (moderate) rating:
    - Coreceptor tropism testing is also recommended for patients who exhibit virologic failure on a CCR5 antagonist (level of evidence: III [expert opinion]).
    - A genotypic tropism assay should be considered as an alternative to predict HIV-1 coreceptor usage (level of evidence: II [data from well-designed nonrandomized trials or observational studies with long-term clinical outcomes]).

U.S. Preventive Services Task Force Recommendations
Not applicable.

Key Words:
Maraviroc (Selzentry™, Pfizer), Trofile™ (Monogram Biosciences, South San Francisco, CA) assay, SensiTrop assay, HIV-1 Coreceptor Tropism, Tropism testing, Genotypic tropism testing, tropism assay, V3 genotyping, HIV V3, ESTA, antiretroviral drug resistance testing

Approved by Governing Bodies:
The FDA-approved full prescribing information for maraviroc (Selzentry™, Pfizer) states that "Tropism testing must be conducted with a highly sensitive and specific tropism assay that has demonstrated the ability to identify patients appropriate for [maraviroc] use."

Currently-available HIV tropism tests are performed as laboratory developed tests (LDTs). Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; LDTs must meet the general regulatory standards of the Clinical Laboratories Improvement Act (CLIA). HIV tropism tests are is available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the FDA has chosen not to require any regulatory review of this test.

Benefit Application:
Coverage is subject to member's specific benefits. Group specific policy will supersede this policy when applicable.

Current Coding:
CPT Codes:

87999	Unlisted microbiology procedure

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This medical policy is not an authorization, certification, explanation of benefits, or a contract. Eligibility and benefits are determined on a case-by-case basis according to the terms of the member's plan in effect as of the date services are rendered. All medical policies are based on (i) research of current medical literature and (ii) review of common medical practices in the treatment and diagnosis of disease as of the date hereof. Physicians and other providers are solely responsible for all aspects of medical care and treatment, including the type, quality, and levels of care and treatment.

This policy is intended to be used for adjudication of claims (including pre-admission certification, pre-determinations, and pre-procedure review) in Blue Cross and Blue Shield's administration of plan contracts.