HIV tropism testing can determine the predominant coreceptor protein used by HIV to infect target cells. Tropism testing can help select patients for treatment with HIV coreceptor antagonists, such as maraviroc, which block specific coreceptor proteins.

**Related Policies**

- HIV Genotyping and Phenotyping for Drug Resistance

**Policy**

**Policy**

HIV tropism testing (see Policy Guidelines for testing methods) may be considered **medically necessary** for selecting patients for treatment with HIV coreceptor antagonists such as maraviroc when there is an immediate plan to prescribe a coreceptor antagonist. Patients indicated for testing include **both** of the following:

- Have evidence of viral replication
- **One** of the following:
  - Have failed multiple antiretroviral treatment regimens
  - Are treatment naïve

HIV tropism testing without immediate plans to prescribe HIV coreceptor antagonists such as maraviroc is considered **not medically necessary**.

Repeat HIV tropism testing during coreceptor antagonist treatment or after failure with coreceptor antagonists is considered **investigational**.

HIV tropism testing to predict disease progression (irrespective of coreceptor antagonist treatment) is considered **investigational**.

**Policy Guidelines**

There are no specific CPT codes for HIV tropism testing. It was suggested that the following CPT code be used for this test along with “Trofile test for maraviroc” in the claim comments field:

- **87999**: Unlisted microbiology procedure

**Testing**

Testing should be conducted immediately before intended prescribed use of maraviroc to obtain the most accurate prediction of tropism at the start of treatment.
Either phenotypic or V3 population genotypic testing may be used to determine HIV tropism; both are not necessary.

V3 population genotypic testing may be conducted by either standard V3 sequencing via Sanger methods (amplification and population sequence analysis of patient-derived V3 region) or V3 deep sequencing methods (synonyms: ultra-deep sequencing; pyrosequencing; next-generation sequencing). In the United States, the only currently commercially available plasma HIV DNA coreceptor genotypic test (requires HIV viral load of 1000 copies/mL or more) includes step-wise testing, with an initial standard sequencing with reflex to V3 deep sequencing if standard sequencing detects only CCR5-tropic virus.

FDA has not regulated the Trofile test because it is a laboratory-developed test (LDT) conducted only at Monogram Biosciences’ Clinical Laboratory Improvement Amendments (CLIA)-licensed laboratory, and it does not meet the definition of an in vitro diagnostic multivariate index assay (IVDMIA), the only type of LDT that FDA is currently regulating. Laboratories performing LDTs not regulated by the FDA must only be certified for high-complexity testing under the CLIA of 1988.

**Benefit Application**

Benefit determinations should be based on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates (e.g., Federal Employee Program (FEP)) prohibit Plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

**Rationale**

**Background**

HIV-1, which causes AIDS, uses coreceptor 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-naïve 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. New, experimental drugs, termed coreceptor antagonists, have been designed to interfere with the interaction between HIV-1 and its coreceptors.

Maraviroc (Selzentry™, Pfizer) is the first coreceptor 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, also 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 label, maraviroc, in combination with other antiretroviral agents, is indicated for adult patients who:

- are treatment experienced, or
- are treatment naïve (approved as of November 24, 2009);
- are infected with only CCR5-tropic detectable HIV-1;
- have evidence of viral replication.

The FDA-approved full prescribing information for the drug 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.” This is because efficacy was not demonstrated in a phase II study of maraviroc in patients with D/M 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. This 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 Trofile 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 bioinformatic algorithm such as geno2pheno. In the U.S., the only commercially available genotypic HIV coreceptor tropism assay is available from Quest Diagnostics.

**Literature Review**

This literature review first discusses the evidence for the role of HIV tropism testing in clinical practice in 3 settings: in conjunction with the use of HIV coreceptor antagonist therapy, for monitoring response to therapy, and for prognosis of HIV infection. Subsequently, aspects of the technical performance of different types of HIV tropism testing are reviewed.

**The Role of HIV Tropism Testing in Clinical Practice**

The primary use of HIV tropism testing is to identify candidate patients for HIV coreceptor antagonist therapy. The approval by the U.S. Food and Drug Administration (FDA) of maraviroc is based on safety and effectiveness data from 3 studies in adult subjects infected with CCR5-tropic HIV-1: A4001027 and A4001028, in antiretroviral treatment-experienced adult patients; and A4001026 in treatment-naïve patients.

**Clinical Studies of HIV Coreceptor Antagonist Therapy in Treatment-Experienced Patients**

The Maraviroc versus Optimized Therapy in Viremic Antiretroviral Treatment-Experienced Patients (MOTIVATE) 1 and 2 trials assessed the efficacy of maraviroc in patients previously treated or resistant to 3 antiretroviral drug classes and with HIV-1 RNA levels exceeding 5000 copies/mL.(2) MOTIVATE-1 was conducted in Canada and the United States, and MOTIVATE-2 in Australia, Europe, and the United States, using identical study designs.
A total of 1075 patients were randomized to 3 trial arms, and 1049 received at least 1 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 section below). At 48-week 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 CD4 count from baseline was 60 in the placebo group compared with 120 in the maraviroc groups. Based on the early trial results and review by the FDA Antiviral Drugs Advisory Committee, FDA concluded that, compared with placebo, maraviroc significantly reduced HIV RNA copy number, and significantly increased CD4 cells, both validated markers of improved health outcomes.(3) At nearly 2 years of follow-up (96 weeks), 81% to 87% of maraviroc-treated patients maintained these responses with no new or unexpected events impacting safety.(4) At 5-year follow-up, 46 deaths were reported, with ongoing low rates of hepatic failure, malignancy, and myocardial infarction.(5)

In contrast, in a 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.(6)

Clinical Studies of HIV Coreceptor Antagonist Therapy in Treatment-Naïve Patients

The MERIT (Maraviroc versus Efavirenz in Treatment-Naïve Patients) study is 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 2000 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 2 doses of either maraviroc or efavirenz, each in combination with zidovudine/lamivudine. In a preplanned interim analysis, the lower dose of maraviroc failed to meet prespecified 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 reanalysis using results from an enhanced sensitivity Trofile assay (ESTA; see Tropism Testing section below), virologic response rates in both treatment arms were approximately equal, and there were fewer discontinuations due to adverse events in the maraviroc arm.(7)

Although most newly infected patients harbor CCR5-tropic HIV virus alone, a study of 150 individuals from 2 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.(8)

Tropism Testing for Treatment Monitoring and at Virologic Failure

Viral strains transmitted in vivo are usually CCR5-tropic.(9) 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.(10,11) 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.(12) 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%
confidence interval: 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 1
and 2 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.

There currently is no recommended management change based on a CCR5 to CXCR4
tropism switch during treatment with maraviroc. Treatment failure is detected by
increased viral load and decreased CD4 cell count, indicating that maraviroc
treatment can be discontinued. The most common mechanism of maraviroc treatment
failure is emergence of a CXCR4-tropic viral population. However, this is not necessarily
related with rapid clinical progression.

Tropism Testing for HIV Prognosis

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). But current management
recommendations are based on monitoring CD4 cell count and viral load, rather than
viral tropism.

Tropism Testing

For the clinical studies of patients with treatment failure, tropism at enrollment and again
at baseline was determined 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.(18) 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 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.(19) 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.(12)

The MERIT study 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.(7)

Wilkin et al used ESTA to reanalyze samples from 4 large cohort studies that had originally been evaluated for HIV tropism with the original Trofile assay.(17) 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 coreceptor 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: http://coreceptor.bioinf.mpi-inf.mpg.de/index.php)(20) and position-specific scoring matrices (PSSM; available online at: http://indra.mullins.microbiol.washington.edu/webpssm).(21) Newer genotypic assays have incorporated additional components of the HIV envelope genotype (e.g., gp41) and/or components of the gp 120 gene other than the V3 domain.(22) 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.(23) Other potential advantages of genotypic assays are reduced cost, shorter turnaround time, fewer sample failures.(24)

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
The best indication against which tropism assay results should be compared is the virologic outcome of patients who receive CCR5-antagonist medication. (25)

Table 1 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. (26) 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. (27)

The data in Table 1 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 ESTAs. The Gonzalez-Sema 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 2 summarizes studies that evaluated the results of V3 sequencing using ESTA as the reference standard; treatment outcomes were not considered in these analyses. All studies sequenced HIV V3 RNA from plasma (standard assay); 2 additionally sequenced HIV V3 DNA from whole blood, which targets proviral DNA (useful for patients with low plasma levels of virus). These studies are much smaller than the studies in Table 1, and largely, where it could be determined, did not test samples in triplicate. It is important to remember that the test performance characteristics reported in Table 2 cannot be compared with those reported in Table 1, as the reference standards differ. 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.

Recently 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. (28)

Overall and based largely on the studies of tropism assays with reference to maraviroc treatment outcome (Table 1), 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, Trofile, and ESTA Assays With Reference to Maraviroc Treatment Outcomes

<table>
<thead>
<tr>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>705</td>
<td>623</td>
<td>73</td>
<td>1164</td>
</tr>
<tr>
<td>Patients</td>
<td>Drug-naïve patients from MERIT trial</td>
<td>Treatment-experienced patients from MOTIVATE and 1029 studies</td>
<td>Patients with persistent viral load and on treatment hiatus</td>
<td>Treatment-experienced patients from MOTIVATE and 1029 studies</td>
</tr>
<tr>
<td>RT-PCR replicates</td>
<td>3x</td>
<td>3x</td>
<td>1x</td>
<td>3x</td>
</tr>
<tr>
<td>Virologic response definition</td>
<td>&lt;50 copies/mL at week 48</td>
<td>&lt;50 copies/mL or reduction ≥2 log at week 8</td>
<td>&lt;50 copies/mL or reduction ≥1 log on day 8</td>
<td>&lt;50 copies/mL or reduction ≥2 log at week 8</td>
</tr>
<tr>
<td>V3 genotyping algorithm</td>
<td>G2P, FPR=5.75%</td>
<td>G2P, FPR=5%</td>
<td>G2P clonal, FPR=5%</td>
<td>G2P, FPR=5%</td>
</tr>
<tr>
<td>V3 genotyping vs. virologic response to MVC</td>
<td>Sens=94% Spec =13%</td>
<td>Sens=85% Spec=36%</td>
<td>Sens=58% Spec=89% Sens=68% Spec=83%</td>
<td>Sens=89% Spec=24%</td>
</tr>
<tr>
<td>Original Trofile vs. virologic response to MVC</td>
<td>NR</td>
<td>Sens=90% Spec=31%</td>
<td>NR</td>
<td>Sens=92% Spec=20%</td>
</tr>
<tr>
<td>ESTA vs. virologic response to MVC</td>
<td>Sens=91% Spec=22%</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

RT-PCR: reverse-transcriptase polymerase chain reaction; ESTA: enhanced sensitivity Trofile assay; MVC: maraviroc; G2P: geno2pheno coreceptor 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.

### Table 2. Performance of HIV V3 Genotyping With Reference to ESTA

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Patients</th>
<th>RT-PCR Replicates</th>
<th>V3 Genotyping Algorithm</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prosperi (2010) (24)</td>
<td>55</td>
<td>Patients failing antiretroviral treatment</td>
<td>1x</td>
<td>G2P clonal, FPR=5.75 %</td>
<td>RNA:55%</td>
<td>RNA:96%</td>
<td>RNA: 83%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G2P clonal, FPR=1.00 %</td>
<td>55%</td>
<td>79%</td>
<td>71%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G2P clonal, FPR=5.75 %</td>
<td>DNA: 68%</td>
<td>DNA: 86%</td>
<td>DNA: 82%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G2P clonal, FPR=5.75 %</td>
<td>67%</td>
<td>71%</td>
<td>71%</td>
</tr>
</tbody>
</table>
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 (see Table 3). 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 (25, 37) and between different sequencing platforms. The sample data in Table 3 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, 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%). (39) Kagan et al reanalyzed samples from the...
MOTIVATE and A4001029 studies to compare ultra-deep sequencing either alone or as a reflex test following standard triplicate V3 sequencing with the ESTA test. Both ultra-deep 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>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Sample size</td>
<td>27</td>
<td>859</td>
<td>851</td>
<td>327</td>
</tr>
<tr>
<td>Patients</td>
<td>Patients with persistent viral load and on treatment hiatus</td>
<td>Drug-naive patients from MERIT trial</td>
<td>Treatment-experienced patients from MOTIVATE and A4001029 studies</td>
<td>Treatment-experienced patients from MOTIVATE and A4001029 studies who received maraviroc</td>
</tr>
<tr>
<td>RT-PCR replicates</td>
<td>3x</td>
<td>3x</td>
<td>3x</td>
<td>3x</td>
</tr>
<tr>
<td>Virologic response definition</td>
<td>&lt;50 copies/mL or reduction ≥1 log on day 8</td>
<td>&lt;50 copies/mL at week 48</td>
<td>&lt;50 copies/mL at week 48</td>
<td>&lt;50 copies/mL or &gt;2 log decline at week 8</td>
</tr>
<tr>
<td>V3 genotyping algorithm</td>
<td>G2P clonal, FPR≤3.5%</td>
<td>G2P clonal, FPR≤3.5%</td>
<td>PSSMx4/R5 FPR ≥-4.75</td>
<td>G2P, FPR ≤5.75%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[~90% concordance with G2P]</td>
<td>PSSMx4R5, FPR ≥-4.75</td>
</tr>
<tr>
<td>V3 genotyping vs. virologic response to MVC</td>
<td>Sens=83% Spec=22%</td>
<td>Sens=93% Spec=15%</td>
<td>Sens=83% Spec=36%</td>
<td>PPV a =65% NPV=61%</td>
</tr>
<tr>
<td>Original Trofile vs. virologic response to MVC</td>
<td>NR</td>
<td>NR</td>
<td>Sens=93% Spec=17%</td>
<td>NR</td>
</tr>
<tr>
<td>ESTA vs. virologic response to MVC</td>
<td>NR</td>
<td>Sens=90% Spec=21%</td>
<td>NR</td>
<td>PPV=66% NPV=59%</td>
</tr>
</tbody>
</table>

aPPV (positive predictive value) refers to the proportion of CCR5 subjects who achieved virologic response at 8 weeks. NPV (negative predictive value) refers to the proportion of non-CCR5 subjects who failed to have a virologic response at 8 weeks.

**Summary**

Based on the evidence from the clinical studies used for FDA approval, and the labeled requirement for tropism testing immediately before initiating a course of maraviroc, HIV tropism testing using the enhanced sensitivity version of the phenotypic Trofile assay is considered medically necessary for both treatment-experienced and treatment-naive patients who are being considered for immediate treatment with maraviroc.
The evidence comparing HIV V3 population genotyping to original Trofile and enhanced sensitivity Trofile assay (ESTA), using maraviroc response as the reference for all assays, strongly suggests that genotyping is equivalent to 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 may be somewhat less sensitive for detecting CXCR4-tropic samples, but 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 is considered medically necessary for patients considering immediate maraviroc treatment.

Either phenotyping or genotyping may be used to determine tropism when considering maraviroc treatment; both are not required.

Currently, patient management decisions are based on monitoring of CD4 cell counts and HIV plasma viral load. Studies would be needed to support improved outcomes with additional tropism monitoring during treatment. Pending such studies, tropism testing during treatment with coreceptor antagonists is investigational. In addition, data are not available to support the use of phenotypic tropism testing to predict prognosis, or to determine tropism in advance of a possible need for a regimen change to a coreceptor antagonist at a later date; accordingly, these indications are also investigational.

**Practice Guidelines and Position Statements**

The HIV Medicine Association of the Infectious Disease Society of North America released updated guidelines on the management of persons infected with HIV in 2013.(42) 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.

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.(27) 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 greater than 1000 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 less than 1000 copies/mL, genotyping is the preferred method. The Health and Human Services Panel on Antiretroviral Guidelines for Adults and Adolescents Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents states that tropism assays should be used whenever the use of a CCR5 inhibitor is being considered (based on strong evidence from randomized trials with clinical outcomes) and possibly for patients who exhibit virologic failure during CCR5 inhibitor treatment (optional recommendation based on expert opinion, no data cited).(12) Other uses of tropism assays, e.g., for prognostic purposes or in case use of a CCR5 inhibitor is needed later do not have sufficient data to support a recommendation.
ESTA is recognized as the more sensitive version of the original assay used in the qualifying maraviroc clinical trials. Genotyping is noted as apparently less sensitive, but based on 2 “recent studies,” possibly as accurate as phenotyping in predicting response to treatment. However, these guidelines do not yet cite all the large, published studies summarized in Table 1; rather, citing uncertainty of the evidence, they recommend phenotyping as the preferred method of tropism testing.

**Medicare National Coverage**

There is no national coverage determination (NCD). In the absence of an NCD, coverage decisions are left to the discretion of local Medicare carriers.

**References**


Documentation Required for Clinical Review

- History and physical and/or consultation notes including:
  - Description of the lab test being ordered from the prescribing physician
  - Previous treatment plan and response

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to benefit design; therefore, contract language should be reviewed before applying the terms of the Policy. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement.

**MN/NMN/IE**

The following service/procedure may be considered medically necessary in certain instances and investigational in others. Services may be medically necessary when policy criteria are met. Services are considered investigational when the policy criteria are not met or when the code describes application of a product in the position statement that is investigational.

<table>
<thead>
<tr>
<th>Type</th>
<th>Code</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>CPT®</td>
<td>See Policy Guidelines</td>
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<tr>
<td></td>
<td>87999</td>
<td>Unlisted microbiology procedure</td>
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<tr>
<td>ICD-9 Procedure</td>
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Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

<table>
<thead>
<tr>
<th>Effective Date</th>
<th>Action</th>
<th>Reason</th>
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<tbody>
<tr>
<td>12/31/2014</td>
<td>BCBSA medical policy adoption</td>
<td>Medical Policy Committee</td>
</tr>
</tbody>
</table>
Medical Policy

Definitions of Decision Determinations

Medically Necessary: A treatment, procedure or drug is medically necessary only when it has been established as safe and effective for the particular symptoms or diagnosis, is not investigational or experimental, is not being provided primarily for the convenience of the patient or the provider, and is provided at the most appropriate level to treat the condition.

Investigational/Experimental: A treatment, procedure or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

Split Evaluation: Blue Shield of California / Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a Split Evaluation, where a treatment, procedure or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements

This service (or procedure) is considered medically necessary in certain instances and investigational in others (refer to policy for details).

For instances when the indication is medically necessary, clinical evidence is required to determine medical necessity.

For instances when the indication is investigational, you may submit additional information to the Prior Authorization Department.

Within five days before the actual date of service, the Provider MUST confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should also be directed to the Prior Authorization Department. Please call 1-800-541-6652 or visit the Provider Portal www.blueshieldca.com/provider.

The materials provided to you are guidelines used by this plan to authorize, modify, or deny care for persons with similar illness or conditions. Specific care and treatment may vary depending on individual need and the benefits covered under your contract. These Policies are subject to change as new information becomes available.