

## Frequently Asked Questions regarding Trofile™

### Physician-Specific Questions and Answers

#### ***What is Trofile™?***

Trofile™ is a diagnostic test performed to determine the viral tropism of HIV-1. It provides an accurate assessment of whether a patient's virus uses the CCR5, CXCR4, or both co-receptors to enter CD4+ T-cells.

#### ***How do you determine a patient's viral tropism?***

Monogram Biosciences has developed Trofile™, the only commercially available diagnostic assay that can determine whether an individual patient's HIV infection is R5, X4 or D/M HIV.

#### ***What are the benefits of Trofile™?***

Trofile™ identifies the tropism of an individual patient's virus. As CCR5-antagonists are (approved exclusively) for patients with R5 HIV, understanding a patient's viral tropism can help determine what treatment options are available.

#### ***Is Trofile™ the only available tropism assay?***

Trofile™ is currently the only commercially available, CLIA- and clinically validated tropism assay. Trofile has been used in all clinical trials of CCR5 antagonists.

#### ***When should Trofile™ be used?***

Trofile™ can be ordered at any time but is recommended prior to initiation of CCR5 antagonist therapy.

#### ***How does the Trofile™ assay work?***

Trofile™ is a patient selection co-receptor tropism assay that determines which co-receptor a patient's HIV strain uses for viral entry into CD4+ cells – CCR5, CXCR4, or both.

Using a small blood sample, Trofile™ amplifies a patient's HIV genome to make HIV particles specific to that individual patient. These HIV particles are used to infect CCR5- and CXCR4-expressing cell lines. Once the virus infects the cell and undergoes a single round of replication, a reporter gene expresses its indicator gene (Luciferase), which gives a visible signal. This identifies the patient's viral tropism. Viral load must be at least 1000 copies/mL to determine viral tropism.

#### ***What is the collection and testing process?***

Physicians collect and send patient samples directly to us, which we then test with the specified diagnostics at our CLIA-certified facility.

#### ***Are you prepared to handle the volume of testing/logistics?***

We process tens of thousands of samples each year and have conducted more than 23,000 Trofile™ assays since the CLIA validation three years ago. Those samples have originated from all over the world.

### ***How is the Trofile™ report interpreted?***

The Trofile™ test results arrive in a compact, easy-to-understand report. The report will clearly indicate whether a patient has R5 or X4 virus. If the patient is infected with dual-tropic virus, or with a combination of R5, X4, or dual-tropic strains, the readout will indicate D/M (dual/mixed tropic). The report will also specify whether activity of a CCR5 antagonist is anticipated or not based on the presence of an R5 tropic virus.

### ***How much will the tropism assay cost?***

Trofile™ is the only commercially available assay to identify a patient's tropism and has been used in the pivotal trials leading to maraviroc's approval.

Trofile™ holds high clinical value to physicians who need to understand a patient's tropism prior to initiation of maraviroc. Since only about half of treatment experienced patients are CCR5 tropic, it will be important to understand the tropism status of a patient prior to initiation of therapy with maraviroc.

### ***Will the tropism assay be covered by payors?***

Monogram Biosciences is working directly with providers and payors to provide reimbursement as quickly as possible. However, because this technology is so new, many insurance plans are evaluating the assay and have yet to set policy. The company will work with each patient regarding coverage and reimbursement.

### ***What is maraviroc?***

Maraviroc is a CCR5 antagonist that targets the CCR5 co-receptor on the surface of the CD4+ cell. Maraviroc represents the first in a new oral class of HIV medications to become available in more than 10 years, has been approved by the FDA for use in combination with other HIV medications in treatment-experienced adult patients with CCR5-tropic HIV.

### ***What makes maraviroc different from other HIV medications?***

Maraviroc is the first orally available entry inhibitor on the market. Other antiretroviral HIV drugs tend to target the virus once it has entered healthy cells, disrupting viral functions. Entry inhibitors seek to stop HIV from entering CD4 cells in the first place, thus preventing the cascade of effects caused by HIV breach, replication and spread throughout the body.

### ***What is tropism and how does it relate to HIV infection?***

HIV targets CD4+ (or T-helper) immune cells. Once inside, the virus reprograms the cell to begin replicating HIV, spreading copies of the virus throughout the body and depleting the supply of healthy CD4 cells necessary to fight infection.

HIV tropism refers to which cell surface gateway or *chemokine co-receptor* that a particular strain of HIV uses to enter healthy CD4+ cells. Depending on which co-receptor a particular virus uses to enter cells, a patient's HIV infection can be categorized as one of the following:

- R5 HIV (CCR5-tropic) enters CD4+ cells exclusively via the CCR5 co-receptor
- X4 HIV (CXCR4-tropic) enters exclusively via the CXCR4 co-receptor
- D/M HIV (dual- or mixed- tropic) enters via either or both co-receptors

### ***How reliable is the assay?***

The Trofile™ assay has been shown to be accurate, precise, sensitive, reproducible, and robust in the measurement of HIV-1 co-receptor tropism. To date, in over 23,000 clinical samples received for ongoing clinical trials of co-receptor antagonists in North America and Europe, 94% have been successfully tested. Trofile has been used to identify tropism in all clinical trials of CCR5 antagonists.

***What percentage of X4-tropic virus would need to be present in order to be detectable?***

The Trofile™ assay is able to detect X4 HIV 100% of the time when it comprises at least 10% of the total viral population, and 85% of the time when X4 virus comprises at least 5% of the total viral population.

***Is an assay mandatory prior to therapy with a CCR5 antagonist?***

Maraviroc is indicated for treatment-experienced patients infected with CCR5-tropic HIV-1. An assay is needed to determine a patient's viral tropism.

***What happens if a CCR5 antagonist is administered to a patient with D/M or X4 virus?***

CCR5 antagonists show potent *in vitro* and *in vivo* antiviral activity against a broad range of viruses that use CCR5 to enter CD4+ cells, but do not show antiviral activity in CXCR4-using strains. A clinical trial exploring the use of maraviroc in patients who had D/M virus showed that the addition of maraviroc to optimized background therapy (OBT) did not reduce viral load further than OBT alone. However, there were no deleterious effects on viral load and, contrary to what was seen when X4 virus emerged during the natural course of disease, patients treated with maraviroc experienced a greater increase in CD4+ cell count at time of virologic failure than patients receiving OBT alone.

***Will a patient have to take the tropism test during treatment?***

Clinicians are using Trofile™ to select patients most likely to respond to CCR5 antagonists prior to beginning treatment. In the clinical trial setting, Trofile™ was used to monitor whether any patients experienced a shift in viral tropism. Many clinicians have expressed interest in monitoring and identifying tropism changes which can help in ongoing treatment decisions. The assay requires a viral load >1000 copies/mL; however, patients on therapy may not meet this requirement.

***What role did Monogram play in maraviroc development?***

Monogram's co-receptor tropism assay, Trofile™, was used to select patients for the maraviroc clinical trials. Trofile™ identified which candidates were infected with CCR5-tropic HIV and thus eligible to participate. Monogram's advanced genotypic and phenotypic resistance tests, PhenoSense GT™, also provided the necessary data to help clinicians optimize background therapies for patients enrolled in maraviroc trials. Finally, Monogram's Trofile™ assay was used to monitor whether any patients experienced a shift in viral tropism while using maraviroc.

***Why use Trofile™ to select patients for maraviroc?***

As a CCR5 antagonist, maraviroc targets CCR5-tropic versions of the HIV virus. According to the information Pfizer submitted to the FDA Advisory Panel meeting, clinical trial results in treatment-experienced patients validate Monogram's Trofile™ assay as an effective and appropriate means to identify patients with CCR5-tropic HIV-1, and who are therefore likely to respond to maraviroc.

***What other drugs are in development in this class of HIV medications? Is Monogram involved in those clinical development programs?***

Monogram's testing services have been involved in the clinical trial programs for all CCR5 antagonists currently in development, including Schering-Plough's vicriviroc. Trofile™, Monogram's co-receptor tropism assay, has been used for patient selection and monitoring; and our PhenoSense GT™ test has been used for optimizing a patient's background therapies.