Comparison of Trofile® and ViroTect^{Tropism} Assays in Treatment-Experienced Subjects

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Abstract

Background: CCR5 and CXCR4 HIV viral populations can coexist during HIV infection and coexistence is a dynamic process. There is increasing interest in understanding the sensitivity of tests of HIV-1 coreceptor tropism in light of the development of new drugs that inhibit HIV replication by CCR5 receptor antagonism.

Methods: Coreceptor tropism was determined independently using the standard Trofile® (Monogram Biosciences) and ViroTect^{Tropism} (Invirion Diagnostics) assays on plasma and whole blood specimens obtained from 288 HIV-1-infected treatment-experienced individuals in the US. The Trofile® assay determines tropism by infection of coreceptor-expressing cell lines with reporter viruses expressing patient-derived gp160. The ViroTect^{Tropism} assay uses flow cytometry to combine detection of HIV replication in patients' cells by *in situ* hybridization with simultaneous immuno-phenotyping.

Results: Among the 288 samples examined, 36 (13%) inconclusive results were reported by Trofile® and 18 (6%) by ViroTect^{Tropism}. The number of inconclusive results using the Trofile® assay was related to low HIV RNA load (≤10,000 copies) and CD4 cell count (>200 cells). Exclusive CCR5 viruses were detected in 145 (50%) and 126 (44%) by Trofile® and ViroTect^{Tropism}, respectively, while 107 (37%) and 144 (50%) mixed CCR5/CXCR4 viruses were detected by Trofile® and ViroTect^{Tropism}, respectively. Seventy-five (32%) subjects with detectable CCR5/CXCR4 by ViroTect^{Tropism} were reported as exclusively CCR5 by Trofile® (95% CI: 26.0-37.9). Conversely, 48 (20%) had CCR5/CXCR4 detected by Trofile® but were reported as exclusively CCR5 by ViroTect^{Tropism} (95% CI: 15.3-25.6).

Conclusions: Tropism determinations obtained from different diagnostic tropism assays can vary, and both viral load and CD4 count may affect the performance and sensitivity of the tests. In addition, ViroTect^{Tropism} assay may be more likely than standard Trofile® assay to detect CXCR4 viruses in this patient population. Longitudinal clinical studies are needed to elucidate the relation of HIV-1 tropism and long-term clinical outcome.

Background/Introduction

- Vicriviroc is a novel CCR5 antagonist in clinical development for patients with CCR5-tropic HIV.
- Current guidelines dictate that tropism testing be performed to assure that CCR5 antagonists are prescribed only for individuals infected with R5-only infection.
- The first commercially available tropism assay was the Trofile® assay (Monogram Biosciences). This assay has a reported sensitivity to detect minor populations of X4-tropic viruses present in plasma at 5% with about 85% certainty.[1]
- New assays, including the enhanced Trofile® assay (Trofile® ES) and the ViroTect^{Tropism} assay (Invirion Diagnostics), have been or are being developed to assess tropism more satisfactorily.
- These new assays utilize different techniques to determine viral tropism; thus, the differences between the assays and their performance in clinical samples must be evaluated.
- We conducted an independent evaluation comparing the performance of the phenotypic Trofile® and the immunophenotypic ViroTect^{Tropism} assays in a population of treatment-experienced patients.

Study Objectives

- To compare the results of tropism determinations by Trofile® and ViroTect^{Tropism} assays using blood screening samples from individuals in a Phase III trial.

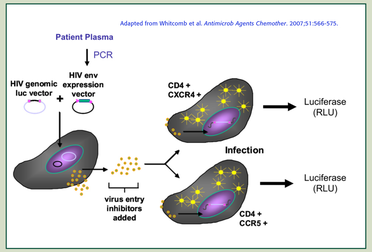
Study Subjects and Methods

- Tropism was determined at screening using aliquots of a single blood specimen from each of the 300 individuals enrolled in Study P04889.
- Study P04889 is designed to evaluate the safety and efficacy of vicriviroc in treating antiretroviral-experienced patients with R5-tropic HIV-1.
  - Key inclusion criteria:
    - HIV-1 RNA >1000 copies/mL
    - Documented resistance to at least 2 classes of antiretroviral treatment (ART)

Assay Methodologies

Trofile® is a phenotypic assay based on infection of coreceptor-expressing cell lines with reporter viruses expressing patient-derived gp160 (**see Figure 1**).

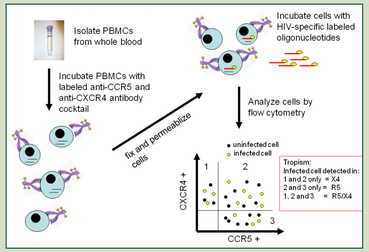
Figure 1: Trofile® HIV Coreceptor Tropism Assay (Monogram Biosciences)



- Method:
  - Plasma samples collected in EDTA are aliquoted, frozen, and shipped to Monogram Biosciences.
  - HIV gp160 sequences are amplified by PCR from plasma-derived RNA, gel purified, and cloned into an expression vector.
  - Pooled envelope clones are cotransfected with a full-length HIV expression vector lacking a functional gp160 gene and containing a luciferase reporter gene to replication-defective pseudovirus particles containing patient-derived gp160.
  - The pseudoviruses are separately incubated with U87-CCR5 and U87-CXCR4 CD4+ target cells for 72 hours and viral infection is determined by measurement of luciferase activity in cell lysates.
- Note: all assays were performed using the standard Trofile® assay, as the enhanced Trofile® (Trofile® ES) was not available at the time of study initiation.

The ViroTect^{Tropism} assay uses *in stiu* hybridization combined with cell surface CCR5 and CXCR4 receptor staining to identify the coreceptor expression pattern of PBMCs productively infected with HIV.

Figure 2: ViroTect^{Tropism} Coreceptor Tropism Assay (Invirion Diagnostics)



- Method:
  - Whole blood is collected in 5 mL Cytochex BCT tubes (Streck Laboratories, Omaha, NE) and shipped at ambient temperature within 24-48 hours to Invirion Diagnostics.
  - 100 µL of whole blood are placed in a 1.5 mL microcentrifuge tube and cells are separated.
  - Cells are incubated with a cocktail of fluorescently labeled antibodies to CD4, CCR5, and CXCR4, 30 min at 4°C, followed by washing.
  - Cells are fixed and permeabilized by addition of CellPerm buffer followed by incubation with a cocktail of end labeled HIV-specific oligonucleotides (43°C, 0.5-2 hours).
  - Cells are washed to remove unbound probe and analyzed by flow cytometry to determine the coreceptor expression pattern of HIV-infected cells.
  - Lymphocytes are gated using forward scatter and side scatter dot plots. This gate is also used to assess sample quality prior to analysis.

Tropism was determined as described in **Table 1**.

Table 1: Infected Cell Coreceptor Expression

	Tropism Call
CCR5+ / CXCR4– (Quadrants 2 and 3)	R5
CCR5– / CXCR4+ (Quadrants 1 and 2)	X4
CCR5+ / CXCR4+ (Quadrants 1, 2 and 3)	R5/X4

Quanititative cut-offs are established using gates established in each quadrant.

- Statistical methods
  - The marginal distribution of a single assay is the relative frequency of tropism determination by that assay. We evaluated the homogeneity of the marginal distributions of the two assays to compare the results of tropism determinations by the Trofile® and ViroTect^{Tropism} assays.

Results

Table 2: Summary of Screening Characteristics (N=288)

Characteristic	
Median HIV-1 RNA, $\log_{10}$ copies/mL (range)	4.21 (2.60-5.88)
Median CD4+ cell count, cells/mm^3 (range)	261 (1-2233)

98% (282/288) samples were HIV clade B.

The different characteristics of the two assays are described in **Table 3**.

Table 3: Comparison of the Trofile® and ViroTect^{Tropism} Assays

Feature	Trofile®	ViroTect^{Tropism}
Method of tropism determination	Infection-based phenotype	Cell-based immunophenotype
Sample required	Frozen plasma	Fresh whole blood
Sample handling	Plasma collected, aliquoted and frozen on site, shipped to Monogram for analysis	Collection tubes shipped within 48 hrs at ambient temp to Invirion for processing and analysis
Reported assay sensitivity for detection of minor X4 populations	5-10% X4 virus	0.5% X4 virus
Stated turnaround time for data reporting	14-18 days	1-2 days

Tropism results of all 300 samples are shown in **Table 4**.

Table 4: Comparison of Tropism Results with the Trofile® and ViroTect^{Tropism} Assays on Paired Samples

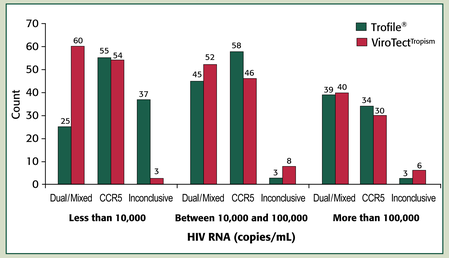
		ViroTect^{Tropism}			
		Dual/Mixed	CCR5	Inconclusive	Total (MD)
Trofile®	Dual/Mixed	53	48	9	110 (37%)
	CCR5	76	63	8	147 (49%)
	Inconclusive	23	19	1	43 (14%)
	Total (MD)	152 (51%)	130 (43%)	18 (6%)	300

(MD = Marginal distribution)

Tropism Determinations with Trofile® and ViroTect^{Tropism} Assays:

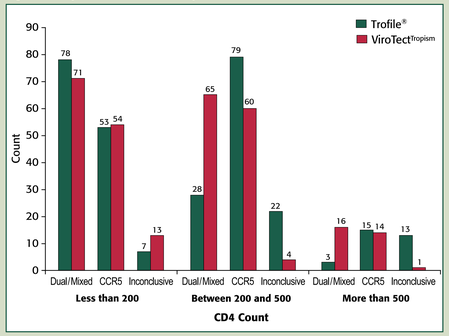
- The test of marginal homogeneity indicated that the marginal distributions of Trofile® and ViroTect^{Tropism} were statistically different ($p = 0.0002$).
- Of 300 samples tested, 240 had reportable results for both assays. Among these samples, 116 (48%) samples had concordant tropism results and 124 (52%) were discordant.
  - Among discordant samples, the ViroTect^{Tropism} assay reported a greater proportion of samples as D/M (76/124 [61%]), compared with 48/124 (39%) D/M as determined by the Trofile® assay.
- The number of inconclusive results was 43/300 (14%) for the Trofile® assay and 18/300 (6%) for the ViroTect^{Tropism} assay. Only 1 sample gave an inconclusive result in both assays.
  - Reasons for inconclusive results included inadequate sample quality and assay performance issues.
- Subset analyses based on viral load and CD4 count were performed to assess whether there was any association between these parameters and assay discordance or performance.

Figure 3: Tropism Determinations by Viral Load



- The number of discordant D/M results detected by the ViroTect^{Tropism} was highest in samples with HIV RNA <10,000 copies/mL.
  - Trofile® had a greater number of inconclusive results with HIV RNA <10,000 copies/mL.

Figure 4: Tropism Determinations by CD4 Count



- More D/M results were detected by the ViroTect^{Tropism} assay than by Trofile® in samples from subjects with CD4 counts >200 cells/mm^3.
- CD4 count <200 cells/mm^3 was associated with more inconclusive results from ViroTect^{Tropism}.
- CD4 count >200 cells/mm^3 was associated with more inconclusive results from Trofile®.

Conclusions

- **Comparison of the standard Trofile® assay and ViroTect^{Tropism} showed concordance between the two assays in 48% of samples yielding results.**
- **In this treatment-experienced population, the ViroTect^{Tropism} assay detected D/M viruses in 51% of samples, compared with Trofile®, which reported 37% of samples with D/M virus.**
- **Inconclusive results emerged from 14% of samples tested by Trofile®, compared with 6% tested by ViroTect^{Tropism}.**
- **Viral load, CD4 count and sample quality may influence the performance of either tropism test.**
- **Clinical outcome studies are needed to determine the predictive value of different tropism assays with respect to long-term benefit of CCR5 antagonists.**

References

1. Whitcomb JM, Huang W, Fransen S, et al. Development and characterization of a novel single-cycle recombinant-virus assay to determine human immunodeficiency virus type 1 coreceptor tropism. *Antimicrob Agents Chemother*. 2007;51,566-575.

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