

Validation of an Enhanced Sensitivity Trofile™ HIV-1 Co-receptor Tropism Assay

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BACKGROUND

- Several HIV entry inhibitors which block infection via CCR5 are in clinical development and maraviroc, a CCR5 antagonist, has been approved for use in treatment-experienced patients with CCR5-tropic (R5) virus.
- Trofile™ is a clinically validated HIV co-receptor tropism assay (Monogram Biosciences) (**Figure 1**) for selecting patients for appropriate treatment with CCR5 antagonists. Trofile determines whether a patient's viral population is CCR5 (R5), CXCR4 (X4) or dual (R5/X4)/mixed (D/M)-tropic and has demonstrated utility in clinical trials of CCR5 antagonists including maraviroc and vicriviroc.
- In mixed envelope (*env*) populations, the original Trofile assay was validated to detect minor R5 and X4 variants at 10 and 5% of the population with 100 and 85% sensitivity, respectively (**Figure 2**) (levels below 5% were not tested) (1).
- Low level CXCR4-using variants below the detection limit of the original Trofile assay can sometimes be identified by clonal analysis of patient *Env* populations and may be selected following therapy with CCR5 antagonists.
- As the detection of CXCR4-using virus below the original Trofile assay sensitivity limit may further optimize patient selection (2,3), we validated an enhanced sensitivity version of Trofile that allows an average ~30-fold improved detection of X4 variants in *env* clone mixtures (100% sensitivity at detecting 0.3% X4 *Env*s) and earlier detection of minor CXCR4-using subpopulations in longitudinal samples from PR/RT inhibitor experienced patients (4,5,6,7).

METHODS

- Experiments were performed to validate the performance of enhanced Trofile for patient management applications in compliance with CAP and CLIA regulations. The co-receptor tropism of a panel of well characterized viral isolates and patient-derived HIV-1 envelopes (*Env*s) were evaluated to assess assay accuracy. Precision and reproducibility were assessed by replicate testing. Minor variant sensitivity was determined using mixtures of paired R5 and X4 *env* clones derived from 4 patients. R5 and X4 pairs were selected to exhibit similar infectivity of CCR5 and CXCR4 expressing cells, respectively.

Figure 1: Trofile HIV Co-receptor Tropism Assay

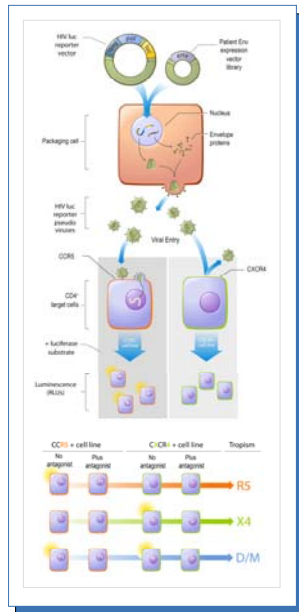


Figure 2: Validation Performance of Original and Enhanced Trofile Assays

Assay Attribute	Original Trofile	Enhanced Trofile
Accuracy	Accurately determined tropism of 58 viruses representing multiple subtypes	Accurately determined tropism of 16 viruses of multiple subtypes and 30 clonally analyzed patient samples
Precision	100%	100%
Reproducibility	100%	99%
Sensitivity	≥ 1,000 copies/mL (95% analytical success)	Equivalent to original Trofile
Sensitivity	100% at 10% mixture	100% at 0.3% mixture

• The performance characteristics of original and enhanced Trofile assays are comparable, apart from sensitivity to detect minor variants

Figure 3: Determining Assay Sensitivity for Detection of Low Level X4 and R5 Variants

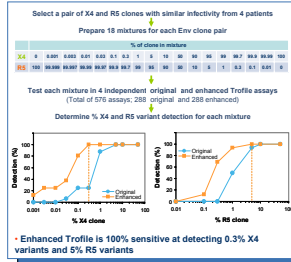


Figure 4: Detection of Low Level X4 and R5 Variants is Env Specific

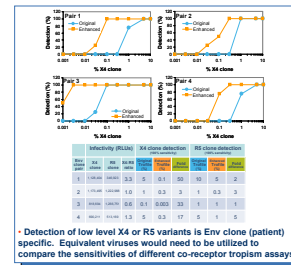
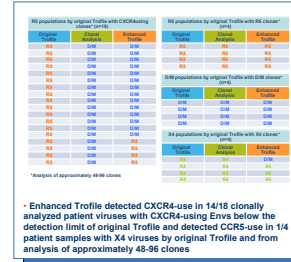


Figure 5: Detection of CXCR4-using Variants in Patient Samples Below the Detection Limit of Original Trofile



RESULTS

- **Figure 2.** Enhanced Trofile accurately determined the coreceptor tropism of a panel of 46 well characterized *Env*s representing multiple subtypes and clonally analyzed patient *Env* populations. Intra-assay precision (100%) and inter-assay reproducibility (99%) were demonstrated from concordant results for 135/135 and 228/230 pair-wise comparisons of R5, X4 and DM *Env*s clones and from repeat testing of 46 patient *Env* populations, respectively. Assay validation performance characteristics were equivalent between the original and enhanced Trofile assays, with the exception that enhanced Trofile is validated to detect 0.3% X4 *Env*s with 100% sensitivity, compared to 10% minor variant with 100% sensitivity for original Trofile.
- **Figure 3.** Across 288 assays to evaluate minor variant sensitivity, enhanced Trofile detected X4 clones in 100% of assays when present at 0.3% and in 81% of assays at 0.1%. R5 clone detection was also improved compared to original Trofile, with 5% R5 clones detected in 100% of assays and 1% in 94% of assays.
- **Figure 4.** The lower limit of X4 and R5 variant detection was *env* clone pair (patient) dependent and ranged from 100% sensitivity to detect 0.003-0.3% X4 clones and 0.3-5% R5 clones by enhanced Trofile.
- **Figure 5.** Enhanced Trofile detected CXCR4-use in 14/18 clonally analyzed patient samples with CXCR4-using variants below the level of detection of the original Trofile assay and detected CCR5-use in 1/4 clonally analyzed X4 patient samples.

SUMMARY & CONCLUSIONS

- Enhanced Trofile has improved sensitivity to detect low levels of CXCR4-using variants in *env* clone mixtures and patient *env* populations compared to the original assay, while assay accuracy, precision and reproducibility are maintained.
- Enhanced Trofile has increased utility for selecting patients for CCR5 antagonist therapy and replaced the original Trofile assay for patient selection in June 2008.

ACKNOWLEDGEMENTS

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