

HIV Tropism testing now available in Switzerland

August 2010

How to request the HIV Tropism Test prior to prescribing Celsentri (Maraviroc):

The Swiss HIV cohort project #553, - Cross validation of "Genosorting" against the "Trofile" test ESTA (Monogram) - has now been completed.

The diagnostic version of the test "XTrack" is now available through the Institute of Medical Microbiology, Basel.

Until reimbursement through the Analysenliste via BAG is in place, the test cost will be carried by IMM, Basel and ViiV Healthcare Switzerland.

Material to be provided by treating physician:

- 1x 8mL tube or 2x 4mL tubes of EDTA plasma
- Viral load should be ≥ 500 c/mL
- for lower VL, please provide 1x 8mL CPT tube, and we will perform the determination from proviral DNA
- shipment overnight (e.g. Luna Express) to

Institut für Medizinische Mikrobiologie
attn. Prof. Th. Klimkait
Petersplatz 10
4003 Basel

Time to result: 5-10 days

the 5-day procedure is performed 1x per week on Mondays

(More time needed if a repetition due to low viral load is necessary)

Contact:

at IMM, Basel:

Prof. Dr. Thomas Klimkait, Tel. 061 267 3272

at ViiV Healthcare, Switzerland:

(to be named)

VALIDATION PROTOCOL

BACKGROUND: Coreceptor testing

Prior to the use of CCR5-antagonists in HIV-1 therapy the Health Authorities require a determination of the HIV tropism in the respective patient. The requirement for the currently highly complex US-based phenotyping test poses an unacceptable hurdle for an entire drug class in Switzerland and therefore asks for research on possible alternative diagnostic methods. We aimed at validating a new rapid diagnostic tool, called XTrack[©], that omits cell culture and utilizes key features of the V3 region for an interpretation that goes beyond simple sequence-based genotyping.

Scientific Basis of XTrack[©]

XTrack[©] is based on a new methodology for co-receptor tropism testing. It has been developed at the IMM Basel and InPheno, Basel, and was validated against the current standard test, Trofile ESTA, by direct assay comparison on prospective clinical samples.

In contrast to phenotypic or genotypic methods for tropism testing, e.g. Trofile or G2P, XTrack[©] focuses on V3 loop features at the DNA level and combines genotypic with structural information. XTrack[©] is based on the hybridization of a fluorescently labelled single-stranded V3 loop reference sequence to a V3 loop cDNA fragment derived from patients' plasma samples. The hybrids, run in a specific semi-denaturing capillary electrophoresis, yield a certain running behavior that discriminates imperfectly matched DNA (hetero-duplex) from a perfect homoduplex, allowing to discriminate R5 from X4 viruses and from X4/R5 mixed virus populations or from dualtropic virus (Figure 1).

Sequence panels of clinical samples with known tropism were used to yield the optimal set of diagnostic probes. A newly developed algorithm then assigns the coreceptor usage of HIV-1 in the clinical sample. It identifies the tropism of the main viral population in every sample but also reveals the tropism of emerging viral minorities. Virus samples of the various HIV-1 subtypes were assessed in the study.

XTrack[©] will provide a fast method for assigning co-receptor tropism and is able to provide a result within 2-4 days.

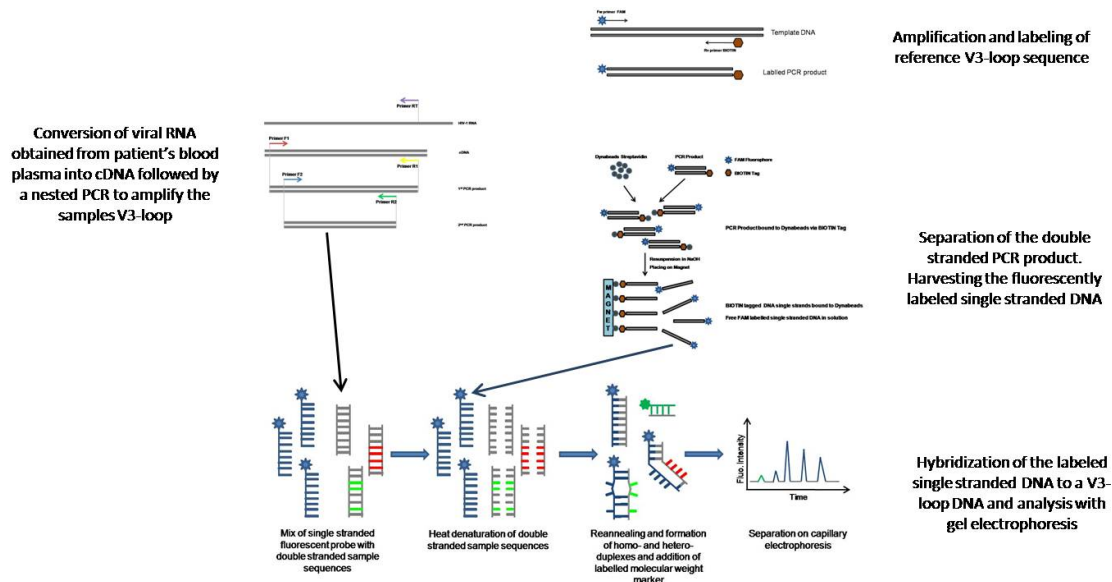


Figure 1: Overview of the principle of XTrack[©]

Study outline

Prospective collection of 100 Swiss samples was from the clinical context of therapy-experienced patients, of whom plasma was submitted for testing prior to prescribing Celsentri. For analysis either Trofile was used or, on a parallel plasma sample, the new test system XTrack[®]. An independent site (University Hospital Basel) collected the results from both systems for comparison.

As required numbers as initially defined for this study could not be recruited in due time, a collaboration with the University of Cologne, Germany (Dr. R. Kaiser) was initiated and 50 samples acquired through this link. Moreover, Dr. Kaiser also provided Trofile results as well as G2P data for the samples from Germany.

At completion of the study all samples were compared, for which positive answers were available for all three systems and relevant statistics (sensitivity and specificity) established. The data serve directly as basis for submission to the Swiss federal authorities at BAG for application for equivalence with the currently accepted Trofile test and for reimbursement through the Swiss Health system (Analysenliste). Further, a subsequent analysis with InPheno's replicative phenotyping method, PhenX-R, will provide the functional confirmation or assignment for discordant samples.

The validated XTrack[®] assay, which considers sequence- as well as structure determinants, is thought to provide a very rapid and accurate new method for a reliable prediction of the HIV-1 coreceptor use.

If the study can be concluded successfully the new assay is expected to have utility for clinical diagnostics.

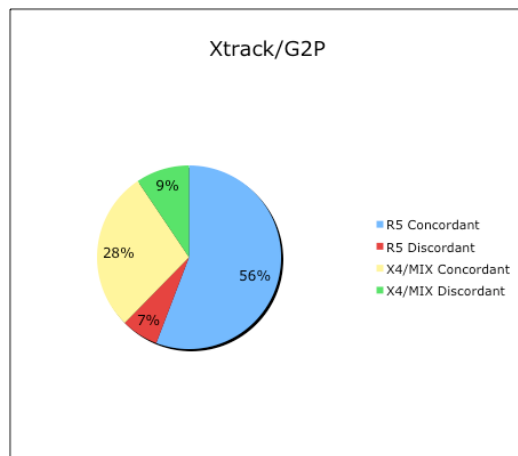
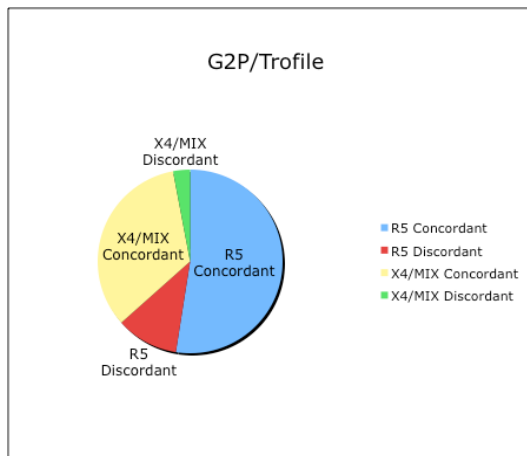
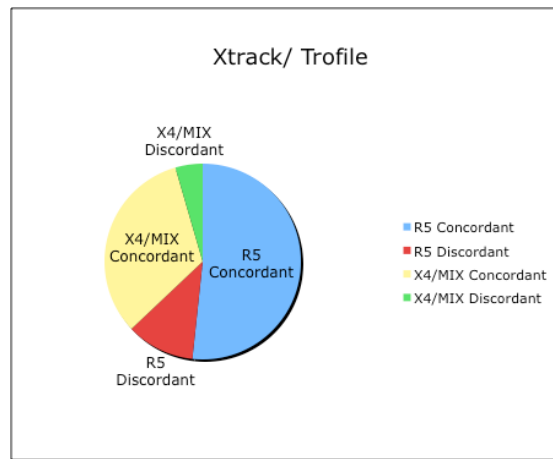
Test Setup Facts:

XTrack[®] uses a small 140bp PCR fragment allowing amplification of patient material with viral loads to below 300 copies/mL. As the system works at the nucleotide level the analysis does not use parameters such as amino acid charge or peptidic structure of the V3-loop. While it is possible to detect with high sensitivity (<5%) mixed virus populations the system is currently not validated for the assessment of dual-tropic viruses.

For identification of optimal probe candidates, HIV-1 V3 loop nucleotide sequences from 635 patient's viruses were obtained either through the Los Alamos database or from characterized clinical samples. For all these sequences tropism and HIV subtype were known.

With the help of the alignment program ClustalW 655 sequences were aligned in a multiple alignment process comparing each sequence of the dataset to every one of the other sequences; their respective relatedness was determined and sequences grouped based on their nucleotide sequence homology. This new method to determine the optimal probe candidates aimed at using a correlation between the degree of hybridization of probe and sample and the resulting binding enthalpy (ΔH) and hypothesizing that a tight hybridization corresponds to a higher ΔH value than a less perfect fit. X4 sequences are in general more distant and variable than R5 sequences, will result in a poorer hybridization with an R5 probe resulting in a significantly lower ΔH value than R5/R5 hybrids. The resulting values were used as reference and an in silico optimization carried out using the online tool "RNA fold". All available samples were subjected to hybridization in parallel and the three best chosen as probes to represent the entirety of known HIV sequences with the highest probability to discriminate between CXCR5- and CXCR4 tropism.

The resulting tropism prediction was then separately compared wither with the prediction by Geno2Pheno (G2P) or with the available results from phenotypic determination by Trofile. Results are depicted for each pair of comparison in a separate pie-chart and summarized in the table below:



Summary of the Data:

Sensitivity of the Xtrack^c assay for the correct determination of CCR5 tropism (vs. Trofile, enhanced format ESTA, taken as reference) was 92 % with a positive predictive value (determination agreeing / disagreeing) for CCR5 tropism of 82 %.

<i>Xtrack/</i> <i>G2P</i>	<i>Xtrack/</i> <i>Trofile</i>	<i>G2P/</i> <i>Trofile</i>	
48	44	46	<i>R5 Concordant</i>
6	10	9	<i>R5 Discordant</i>
26	29	30	<i>X4/MIX Concordant</i>
7	4	2	<i>X4/MIX Discordant</i>
87	87	87	<i>Sum</i>
87.3	91.7	95.8	<i>Sensitivity R5</i>
81.3	74.4	76.9	<i>Specificity X4</i>
88.9	81.5	83.6	<i>PPV R5</i>
78.8	87.9	93.8	<i>NPV X4</i>

For CXCR4 determination the agreement between both tests is lower as, due to the greater heterogeneity of CXCR4-tropic HIV strains, their unequivocal determination is more difficult. Of note the absolute number of CXCR4-discordant samples was too low to establish useful statistics!

Against the Geno2Pheno assay, which has recently been published to possess high equivalence with Trofile [PR Harrigan et al.; Screening for HIV tropism using population-based V3 genotypic analysis: a retrospective virological outcome analysis using stored plasma screening samples from MOTIVATE-1. IAS 2009, Cape Town, South Africa] the Sensitivity for CCR5 is 87% with a positive predictive value of 89%.

In order to maximize performance and for providing additional validation data, the IMM, Basel performs simultaneously, on a scientific basis, and free of charge, also sequencing of all current diagnostic samples and, if no unambiguous tropism results can be obtained, also a replicative phenotype for verification.

Reimbursement Plan for Patient:

The results of this evaluation of XTrack^c have been submitted to the Swiss BAG in order to apply for acceptance & reimbursement.

In the meantime an agreement has been reached with ViiV Healthcare, Switzerland, to provide the HIV tropism test result free of charge for the patient. This offer is valid until the end of 2010 with possible extension beyond the year-end if necessary.

Testing can be requested through the IMM, Basel

Please request the standard diagnostic order form of the laboratory at Tel: 061-267 3264 (and soon directly via internet: www.zid.ch)

Until available on the Analysenliste, until the end of 2010, all tests will be directly paid by ViiV Healthcare, Switzerland, and billing will be handled directly by the IMM, Basel.

For further information or questions, please do not hesitate to contact us directly.
(contact: Thomas Klimkait, Tel. 061 267 3272)

Basel, August 19, 2010

Prof. Thomas Klimkait

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