Eur J Med Res (2007) 12: 463-472

PHENOTYPIC ASSAYS FOR THE DETERMINATION OF CORECEPTOR TROPISM IN HIV-1 INFECTED INDIVIDUALS

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Abstract
Coreceptor tropism antagonists represent a new class of antiretrovirals for the treatment of HIV infection. The knowledge of patients' viral population tropism before the initiation of and during therapy with such compounds may be critical in order to optimize treatment strategies. In this review we focus on the characteristics of phenotypic assays for the determination of HIV coreceptor tropism.

Beside traditional phenotypic assays, there are at least four phenotypic recombinant virus assays (RVA) available to predict coreceptor usage: Trofile (Monogram Biosciences), Phenoscript (VIRalliance), Xtrack^{C}/PhenX-R (inPheno) and a platform developed by Virco.

Trofile and Phenoscript represent single-cycle assays and are able to determine coreceptor tropism without cocultivation of HIV particles in cell culture. Trofile offers the most clinically validated data with currently about 25,000 analysed samples. The detection of minority variants is a limitation of all population-based assays and varies between 1 and 10%, depending on the assay used. Xtrack^{C}/PhenX-R and Virco's platform combine genotypic and phenotypic assays to analyze a patient's sample for tropism. Although all assays are validated for the assessment of coreceptor tropism in different HIV-1 subtypes, there is still a need for further evaluations. Furthermore, the establishment of cut-offs for X4 minority species will be difficult, and is affected by many factors like patient sample quality, the input volume, viral load, the detection limits and PCR variations.

Overall, RVAs confirm efficiency and accuracy thus making them suitable for the clinical management of HIV infected individuals treated with coreceptor antagonists.

Key words: HIV, coreceptor, CCR5, CXCR4, viral tropism, recombinant virus assay, phenotypic assay.

Abbreviations: bp: base pairs; eGFP: enhanced green fluorescent protein; env: envelope; NSI: non-syncytium-inducing; PBMC: peripheral blood mononoclear cells; R5-tropic: CCR5-tropic; RLU: relative light units; RVA: recombinant virus assay; SI: syncytium-inducing; X4-tropic: CXCR4-tropic.

INTRODUCTION

HIV requires one of two major chemokine coreceptors aside from the CD4 receptor in order to complete the cell internalization process into target cells [1, 2, 3]. Due to the availabilty of new antiretrovirals that target CCR5 and CXCR4 receptors, the determination of HIV tropism becomes clinically relevant [4, 5].

However, the determination of HIV-1 coreceptor usage is not yet routinely used and still restricted to a few laboratories. Several assays have been developed to determine HIV tropism. At this time, it remains to be shown which assay is the most predictive and the most suitable. In general, we distinguish between genotypic and phenotypic methods for coreceptor prediction.

In this review we discuss phenotypic tools for the determination of HIV coreceptor usage.

PHENOTYPIC TOOLS FOR THE DETERMINATION OF HIV TROPISM

As described above, HIV entry inhibitors belong to a new generation of antiretroviral drugs for the treatment of HIV infection. Accompanying their clinical development, the design of tests to evaluate viral tropism and drug susceptibility in daily routine has been improved.

At this time, beside a variety of traditional biological assays, several commercial recombinant assays have been generated for the determination of coreceptor usage of HIV-1 strains (Table 1).

Table 1. List of phenotypical assays for the determination of HIV-1 coreceptor usage in HIV-1 infected patients.

Assay	**References**
Classical	
MT-2 cells	7,8,9,10
Ghost cells	11,12,13,14
NP-2 cell lines	15
U87 cell lines	16,17
U373-MAGI cell lines	18
Recombinant virus	
Trofile™ Monogram Biosciences	19,20
Phenoscript™ HIV-1 Entry Inhibitor	21,22
PhenXR™ and XtrackC™ inPheno	23
Virco®Type HIV-1 Virco	24

Traditional Assays for Coreceptor Tropism Analysis

Since the first evaluation of viral phenotypes in cell culture back in the late 80s a variety of phenotyping assays have been developed to determine coreceptor tropism of HIV-1 (Table 1). Here we describe one representative traditional assay that was used before recombinant virus assays have been developed.

In the late 1980s the MT-2 assay served as a tool to establish the categorization of HIV strains into non-syncytium-inducing (NSI-viruses, today known as CCR5-tropic) and syncytium-inducing (SI) viruses (known as X4-tropic or R5X4 dual tropic viruses) [25, 53]. The MT-2 assay was based on the expression of CXCR4 on the cell surface of those cells. The ability of HIV to replicate in these cells associated by apparent cell fusion (syncytia) argues for the presence of X4 tropic viruses [15, 25].

One of the major disadvantages of this assay is its requirement of viral stocks from stimulated patient peripheral blood mononuclear cells (PBMC) [25]. The standard viral isolation procedure requires a co-culturing of the patient's HIV-infected PBMC with cells from a HIV-seronegative donor, stimulated with phytohemagglutinin or CD3/ CD28 antibodies in the presence of interleukin-2. The adequate collection of viral isolates is very laborious and requires highly qualified laboratories, which limits the use of this assay for clinical purposes.

Recombinant Virus Assays (RVA)

The clinical development of coreceptor tropism antagonists has defined a need for tests that are able to assess viral tropism.

Currently, there are several commercially and non-commercially available recombinant assays available that allow the determination of viral coreceptor tropism.

Apart from the inPheno (PhenX-R) method and the Virco platform, all are single-cycle recombinant virus assays (RVA) to determine HIV-1 coreceptor tropism in clinical or patient management settings. In contrast to traditional assays in which isolated viral variants were used for infection of indicator cells, these assays generate pseudoviruses which include full-length or defined parts of the envelope gene derived from the patient's virus population [19-24].

In the first step RNA, is isolated from the patient's HIV-1 plasma population and is reversely transcribed to *env* cDNA. This template is amplified by PCR using *env* specific primers. The resulting amplicons largely represent the diversity of the patient's viral plasma *env*-sequences and are then translocated into an *env*-expression vector. The expression vector generates the *env* proteins corresponding to the patient's HIV-1 population.

In addition to the construction of an *env*-expression vector, a second vector featuring a replication defective genome with a deletion in its *env* region is used. An indicator gene cassette replaces the *env* gene. While the *env*-expression vector accounts for the expression of the viral envelope, this vector generates viral particles that are not able to replicate without the envelope proteins.

Finally, in order to generate a pseudovirus, both vectors have to be cotransfected into a cell line for producing the final recombinant virus. The HIV-1-genomic vector generates virus particles and uses the patient's envelope-proteins generated by the *env*-expression vector to finish the assembly of the pseudovirus.

For the determination of coreceptor tropism, the pseudovirus is used in all assays to infect two target cell lines. These cell lines are designed to express CD4 on the cell surface along with either one of the CXCR4 or CCR5 chemokine coreceptors. The use of cell type varies with the different assay systems. Following the completion of one (single-cycle assay) to four rounds (multi-cycle assay) of viral replication, infected cells express an integrated indicator gene, which can be delivered by the pseudovirus or is a responsive cellular gene. It is quantified by bioluminescent or colorimetric signals. As result X4 tropic viruses will now preferably infect CXCR4/CD4 presenting cells, while CCR5/CD4 cells remain uninfected. Conversely, a R5-tropic virus will predominantly infect CCR5/CD4 cells instead of CXCR4/CD4 presenting cells. Mixed or dual tropic populations are considered to infect both cell lines. The bioluminescent or colorimetric signals can be used as a direct measurement and quantification of virus entry as well as for the determination of virus tropism. The verification of coreceptor tropism can be confirmed by application of specific coreceptor antagonists during the pseudovirus infection stage [19-24].

Methods and features of four recombinant viral assays are described below (see also Table 2).

Trofile™ (Monogram Biosciences, San Francisco, California, USA)

Method:

The Trofile assay [19, 20, 38] is a single-cycle recombinant virus assay developed by Monogram Biosciences. To date, this commercial assay is the most widely used and most validated RVA for the determination of HIV-1 coreceptor tropism in clinical studies.

Trofile uses a region of the *env* gene of about 2.5 kb in size to determine coreceptor tropism, which is amplified by PCR and inserted into an envelope expression vector. In this assay a HEK293 cell line (human embryonic kidney) is used for the cotransfection of the *env*-expression vector and the HIV genomic vector carrying the luciferase reporter gene. The infection process takes place in U87 cells expressing either CXCR4/CD4 or CCR5/CD4 on their surface. The quantification of emitted light by expression of the luciferase reporter gene is controlled by the presence of coreceptor-antagonists [26]. Monogram Biosciences presumes to achieve rapid and accurate results representing the patient's plasma virus population. Since the reliability depends mainly on the sensitivity and accuracy of the RT-PCR reactions to analyse the *in vivo* HIV quasispecies, this assay promises good success of coreceptor tropism analysis in patients with plasma viral loads above 1000 copies/mL. The detection limit of minority species according to the company's manual is reported as ap-

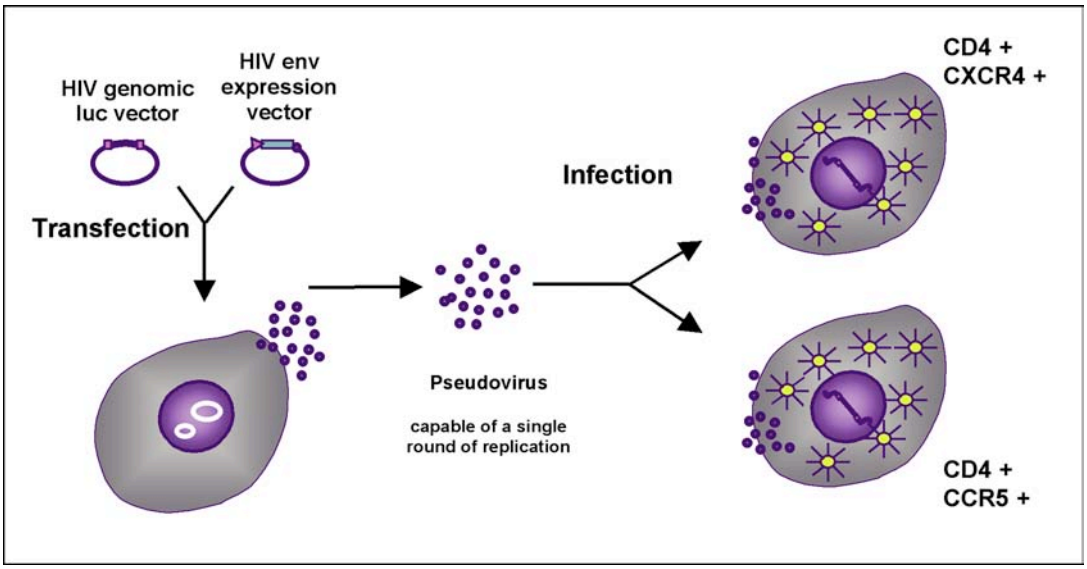

Fig. 1. Illustration of the method used in the Trofile assay. HEK-293 cells are cotransfected with an *env*-expression vector containing the *env*-sequence derived from patients' samples, and an HIV-genomic vector that harbours a luciferase reporter gene instead of the *env* region. Produced pseudovirus particles are used to infect U87 cells expressing the receptors CD4/CCR5 or CD4/CXCR4. The tropism is detected by luciferase activity of the infected cells (modified figure from Monogram Biosciences, Inc).

proximately five to ten percent (Fig.1).
Advantages and characteristics:
The Trofile assay has already been used in a number of evaluations and in the clinical development of several entry inhibitors. It shows good accuracy and adequate reproducibility. The assay can be used to assign tropisms to viral populations across diverse viral subtypes with sufficient reliability, tested for subtypes A, B, C, D, E, EA (n = 38) [27] . Furthermore, there are safety features engineered into the Trofile vector system that ensure that the recombinant viral vectors remain replication incompetent.

An additional advantage of Trofile is the fact that it includes the entire 2.5 kb *env* genes from the complex population of viruses in the patient's plasma. In contrast, previously described recombinant methods included only parts of the *env* region.

Although the V3 region is recognized as a major determinant of coreceptor specifity, the coreceptor binding site additionally includes other regions of gp120. The regions V1, V2, V4, V5 and C4 may play a role in HIV tropism [28, 29, 47, 48, 49]. Furthermore, regions outside of gp120, have been shown to determine or contribute to coreceptor tropism, as well as entry inhibitor susceptibility [30].

Since the majority of genetic determinants of the HIV-1 envelope phenotype remains largely intractable due to the extreme genetic variability of the *env* gene, it is an advantage that the entire gene is included in a coreceptor tropism assay. Such inclusions allow the elucidation of the complex molecular determinants and mechanisms of inhibition and neutralization for many chemokine receptor antagonists, which may map to other regions of the *env* gene [31].

The reported turnaround time is 16 days. The ability to detect minority quasispecies is reported to be in frequencies of as low as five to ten percent [20]. However, in reality, sensitivity to detect minor species varies with relative infectivity. Specifically, the detection of minority species with lower infectivities (relative light units, RLU) might be more difficult in a heterogeneous population dominated by many strains of higher infectivities. Conversely, the assessment may be simplified, if the minority species has a higher infectivity when compared to the other strains in the population [20].

How Trofile performs to identify low levels of particular mutants, like X4-variants especially in non-B subgroups has to be shown in the future. The failure rate of the Trofile assay is four to six percent in 25,000 clinical trial samples [www.monogrambio.com].

HIV-1 Phenoscript Env™ (VIRalliance, Paris, France)

Method:
This entry inhibitor assay from VIRalliance offers either determination of viral tropism or assessment of viral susceptibility to HIV-1 entry inhibitors. A 2,180 bp *env* sequence is used for the generation of tropism or resistance testing data while the 900 bp V1V3 region is employed for tropism-only analysis. In either case the resulting amplicon is inserted into the pNL4-3 plasmid lacking the *env* gene (gp120 and the ectodomain of gp41, positions 6480 to 8263). Replication-competent *env* recombinant viruses are produced in HEK293-T cells by homologous recombination. Infection takes place in U373MG-CD4 indicator cells, which express either CCR5/CD4 or CXCR4/CD4. This assay features a HIV-genomic vector which includes a HIV-1 LTR-*lacZ* cassette that allows the quantification of single cycle infectivity by a colorimetric assay based on the HIV-1 Tat-induced expres-

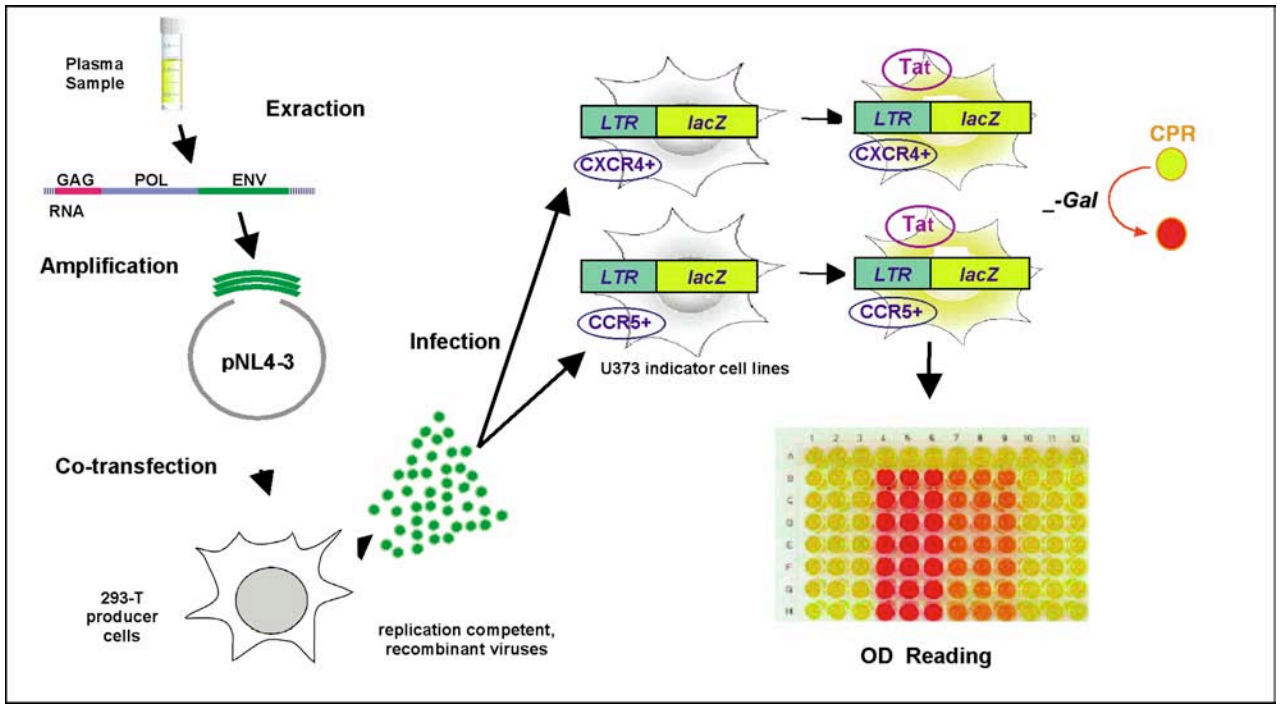

Fig. 2. Illustration of the method used by the Phenoscript Env assay. Recombinant virus particles are produced in 293-T cells after homologous recombination of the following cotransfected constructs; a linear vector pNL4-3 lacking the *env* gene and the amplified *env* region from patients' plasma samples. Two different indicator cell lines, U373MG-CD4 containing an HIV-1 *LTR-lacZ* cassette and genes encoding either CXCR4 or CCR5, are subjected to infection by recombinant virus particles. Quantification of single cycle infectivity is measured by a colorimetric assay relying on the Tat-induced expression of β-galactosidase (modified figure from VIRalliance™).

sion of β-galactosidase [22, 32, 33].
Advantages and characteristics:
VIRalliance offers an optional system for the determination of coreceptor tropism and drug susceptibility testing in one platform. The principle of this assay is similar to that of the Trofile method, although the detection is implemented by a colorimetric assay relying on a Tat-induced expression of β-galactosidase. VIRalliance promises to detect five percent of minor quasispecies from a patient's virus population with adequate reliability [34]. Reproducibility analysis on a small subset of four patients showed complete concordance in the tropism results. The assay had a 92 percent success rate in reading tropism of samples from patients with a viral load between 1,000 and 10,000 copies/mL [34]. Below 1000 copies, the success rate is dependent on the PCR success rate. The tropism assay is most successful, when PCR amplification is sufficient. The test has also been proven to be capable to evaluate the coreceptor tropism in a variety of different HIV-1 non-B subtypes (see Table 2). A panel of more than 300 plasma samples representing the eight most prevalent European non-B subtypes were tested. PCR success rate ranged between 67 pecent for subtype A (*env* V1V3) and 100 percent (gp120 gp41). The overall recombinant virus assay success rate for all non-B subtypes was 71 percent (range 36 to 95%) [34].

Xtrack^{C} and PhenX-R (InPheno AG, Basel, Switzerland)

Method:
This replicative tropism assay combines two specific assays, a probe based hybridization assay (Xtrack^{C}) and a replicative phenotyping assay (PhenX-R). Following the first process, a rapid testing is performed by genosorting to identify R5 and X4 viruses. This is realised by a set of specific fluorescence-labeled probes, one specific for R5 variants and the other for X4 variants. Both probes result in different capillary migration and peaks which can be assigned to the corresponding coreceptor. Ambiguous samples and those with suspected mix or dual-tropic virus are then assessed in the second step by replicative phenotyping [35,36]. In contrast to the described single-cycle assays, this assay replicates recombinant viruses in three to four cycles limited to total four days. This results in a higher population of for example resistant viruses and consequently an increase of susceptibility for the detection of minority populations. The addition of the respective drug classes dissects dualtropic viruses from mixed populations during the viral cycles. Patient-derived *env* sequences (1.1 kb V1-V3) are precisely ligated into position in an otherwise complete NL4-3 provirus lacking *env* and transfected into a reporter cell harbouring a Tat-responsive *lacZ* gene.

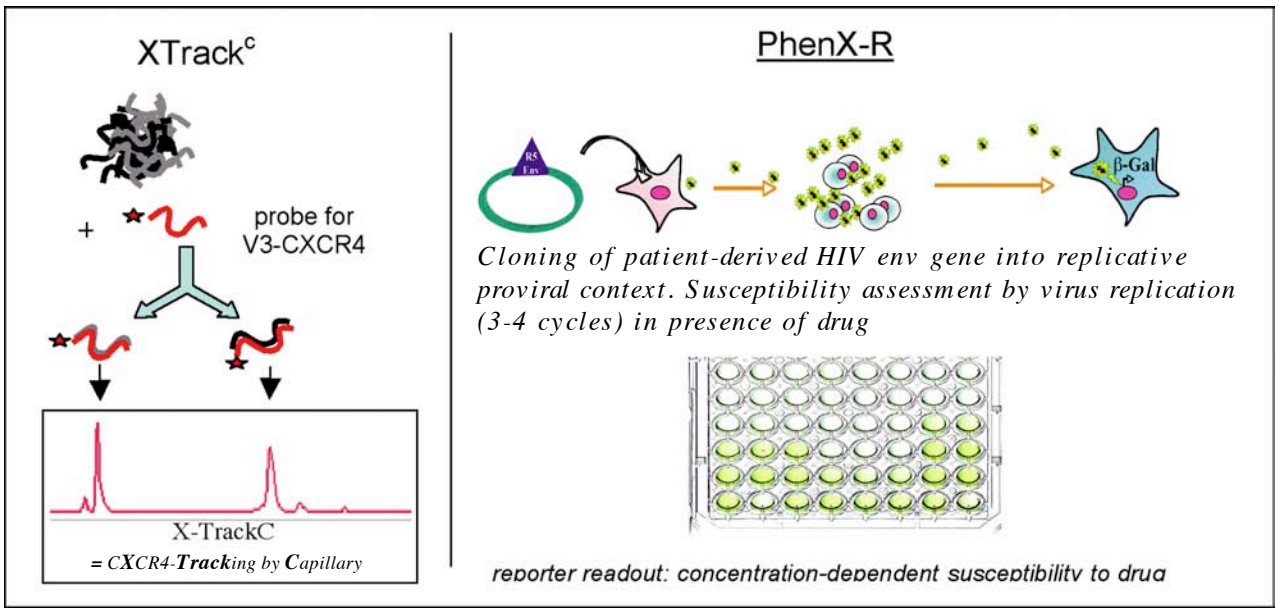

Fig. 3. Illustration of the method used by the Xtrack^C and PhenXR assays. The hybridization based genosorting method (left panel) detects and dissects individual virus populations based on sequence heterogeneity versus defined V3-DNA probes. PhenXR assesses recombinant viruses carrying patient-derived *env* (blue triangle, right panel) in parallel replicating in X4-only or X4/R5 expressing cells in the presence of drug. This format allows to amplify viral minorities and to dissect dualtropic viruses from mixed with both tropisms (modified figure from inPheno).

Advantages and characteristics:
The advantage of the Xtrack^C and the PhenX-R assays is their high sensitivity for minorities [35] and the low turnaround time of the genotypical based Xtrack^C assay of four days [36]. PhenX-R assay has a turnaround of 14 days, which is comparable to other RVA. The PhenX-R assay replicates recombinant virus populations in multi-cycle steps. This enables a higher sensitivity regarding the detection of minor quasispecies and allows to separate dualtropic viruses from virus populations with mixed tropism [35]. However, it faces the same problems as the traditional assays, where an additional replication of viral variants can push one subpopulation more forward than another less infective variant. The ability of the recombinant virus to replicate, offers the functional assessment of viral properties and fitness, but is not suitable for laboratories lacking adequate security devices.

Virco tropism platform (Virco BVBA, Mechelen, Belgium)

Method:
Virco developed a tropism testing platform which consists of four assays [6, 24, 51]. All assays are based on a one-step RT-PCR amplification of the HIV-1 gp120 region spanning the variable loop V1 through V4 (termed NH_2-V4) which has an amplicon length of 1307 bp. Each assay can be applied individually, depending on the type of results that need to be obtained: i) V3 population-based sequencing and tropism prediction assessment; ii) a clonal NH_2-V4 gp120 genotyping assay; iii) a clonal NH_2-V4 gp120 phenotyping assay; and iv) a population-based NH_2-V4 gp120 phenotyping test. For phenotyping, NH_2-V4 amplicons are cloned into an hXB2D backbone containing eGFP in *nef* and deleted for NH_2-V4 by *in vitro* recombination. The recombinant plasmid DNA, encoding a full infectious genome, is then transfected into 293T producer cells. Resulting recombinant virus particles are then used to infect U87 indicator cells (see Fig. 4). Phenotypic tropism is determined by eGFP expression analysis using an argon laser scanning microscope Currently this platform is still for research use only and no price is available yet.

Advantages and characteristics:
This new tropism testing platform offers various options depending on the needs. Here we focus only on the population based NH_2-V4 gp120 phenotyping test. In a preliminary evaluation, this assay is able to detect less than five percent of minority species in populations with a high viral load of above four log IU/mL [6, 56]. However, the success rate for minority detection of 1-2.5% ranged between 30% and 66.7%. A hundred percent success could only be confirmed for minor populations of as low as 8% from a viral load of four log IU/mL [6]. The described technology still needs further evaluation at lower viral loads between 2 log and 5 log [24]. Unlike the PhenX-R assay the NH_2-V4 gp120 phenotyping test is currently not able to distinguish between dual-tropic or mixed populations [24]. Only few data are available for the deter-

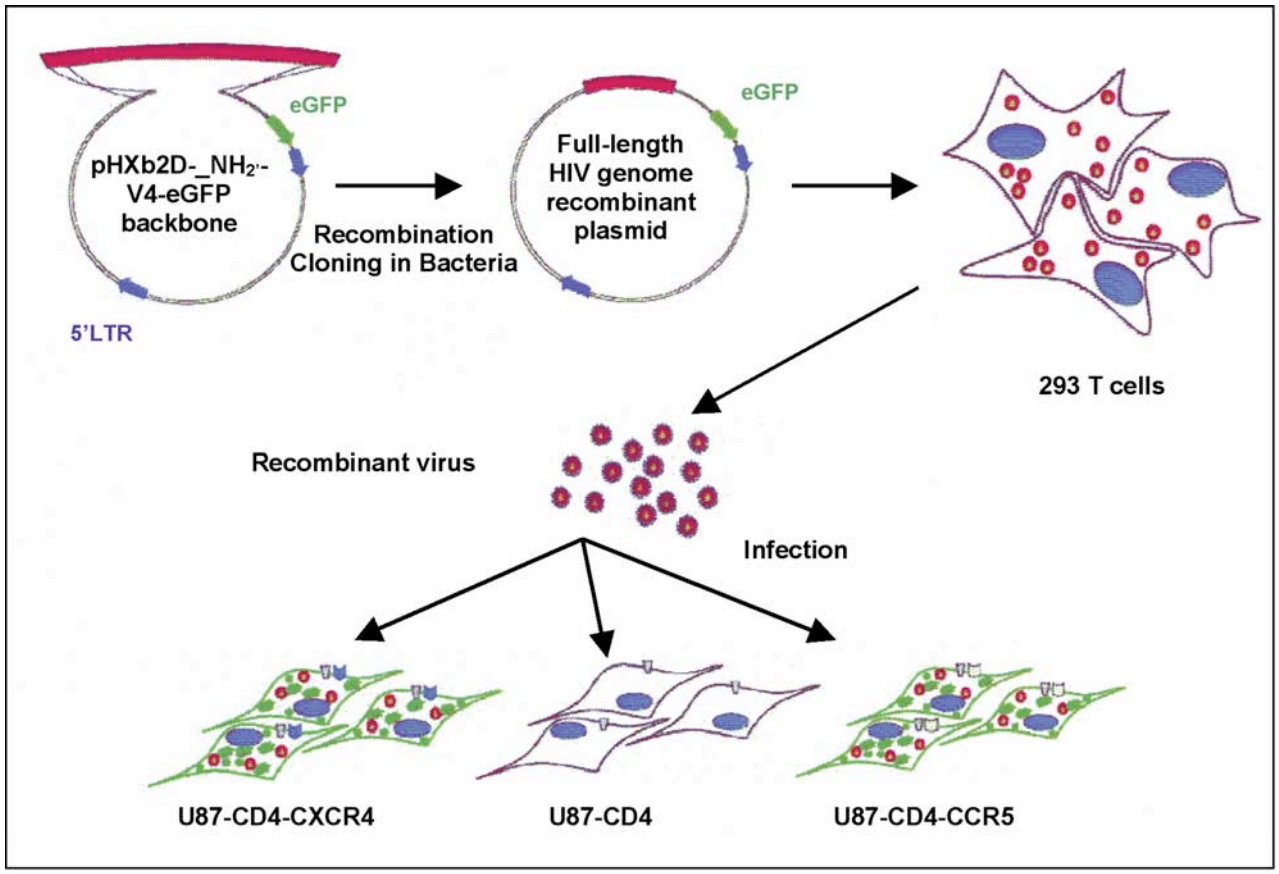

Fig. 4. Illustration of the method used by the "Virco tropism platform". *In vitro* recombination of a backbone vector, pHXB2D-ΔNH$_2$V4-eGFP, and a NH$_2$-V4 amplicon from patients' samples is followed by transformation of this construct into *E. coli cells*. Recombinant virus particles are produced in 293 T cells. The infectivity in different U87-CD4 indicator cells expressing CD4 only or CD4 in combination with CXCR4 or CCR5 is measured by the expression level of eGFP (Van Baelen et al., 2007).

mination of tropism in diverse viral subtypes.

Conclusion

Different phenotypical assays were developed and validated to determine HIV coreceptor tropism in clinical or patient management settings. The main differences lie between the traditional assays and the recombinant virus assays [20, 25].

RVAs have several distinct advantages over coreceptor assays that solely rely on virus culture on T-cells using viruses derived from stimulated patient peripheral blood mononuclear cells [19, 21, 23, 24]. As potential distortion such stimulation can be able to reactivate HIV-1 genomes integrated into host DNA, some of which may not be currently replicating in the patient [20].

Moreover, culturing patients' samples for prolonged periods as well as passaging will potentially select viruses which have adapted to such *in vitro* culture conditions that may not correctly represent the original *in vivo* HIV population [19, 20]. Stimulated patient PBMC might differ somewhat from plasma circulating viruses even in the same person. It remains to be shown if the relative coreceptor density on the surface of reporter cells (often with overexpression) versus cells in the authentic environment of a patient does not influence judgement; this possible *in vitro* artefact concerns RVAs as well as traditional assays [54].

Standard coculture techniques using CXCR4$^+$ indicator cells (e.g. MT2) can only determine whether or not the virus is capable of infecting CXCR4$^+$ expressing cells. Consequently, such assays cannot distinguish between exclucively CXCR4$^+$ using strains and those capable of using both CXCR4 and CCR5 coreceptors (i.e., dual tropic or mixed populations) [9, 53].

In contrast, RVAs (Trofile, Phenoscript, PhenX-R and the Virco-RVA) can distinguish between X4, R5 on one hand and dualtropic- or mixed viral populations on the other hand [19, 21, 23, 24]. Furthermore distinctions in dual-tropic and mixed virus populations can be assessed by PhenX-R [23]. The clinical implication of this difference will require further investigation [44].

The detection of minority quasispecies in the presence of mixed viral populations is one of the most important challenges in the correct determination of viral tropism [55]. It represents a limitation of all population-based assays [46].

Especially for research applications, the very labour-intensive and costly clonal analysis and individual *env* gene sequencing may be justified for identifying levels of particular virus strains, like X4-tropic

Table 2. Characteristics of four phenotypic assays for the determination of HIV coreceptor tropism.

Assay attribute	**Trofile**	**Phenoscript**	**PhenXR**	**NH_2-V4 gp120**
company	Monogram	VIRalliance	inPheno	Virco BVBA
method	single-cycle assay	single-cycle assay	multiplecycle assay	multiplecycle assay
considered *env* length	~2500 bp [f]	2180 bp [c] 900 bp (trop.only)	1100 bp [d]	1307 bp [e]
producer cells	HEK293	HEK293	HeLa	HEK293
reporter cells	U87	U373	SX22-HeLaR5/-X4	U87
detection	Luciferase activity	β-Gal / HIV-1 LTR *lacZ*	β-Gal/HIV LTR *lacZ*	Fluorescence intensity of eGFP
turnaround time	~ 16 days	~14 days	~14 days	~12 days
detection of minor variants	5-10% [a]	5-10% b	1% [d]	< 10% [e]
non-subtype B accuracy tested	yes	yes	yes	yes [e]
PCR success in				
subtype C	100% (n = 3) [f]	95% (n = 55) [c]	n.a.	n.a.
subtype G	n.a.	87% (n = 38) [c]	n.a.	n.a.
subtype CRF02AG	n.a.	96%(n = 45) [c]	n.a.	n.a.
subtype A	100% (n = 10) [f]	67% (n = 24) [c]	n.a.	n.a.
subtype CRF01AE	n.a.	89% (n = 19) [c]	n.a.	n.a.
subtype D	100% (n = 6) [f]	83% (n = 12) [c]	n.a.	n.a.
subtype F	n.a.	70% (n = 20) [c]	n.a.	n.a.
subtype J	n.a.	100% (n = 4) [c]	n.a.	n.a.
others	n.a.	n.a.	HIV-2 detected	n.a.
PCR success for				
VL>10.000 copies/ml	n.a.	93% [c]	n.a.	95% [g]
VL 500-10.000 copies/ml	94%	82% [c]	95%	91% [g]
VL < 500 copies/ml	77%	63% [c]	n.a.	n.a.
list price	on request	on request	on request	on request

n.a. = not available
reference index a:[52] b:[50] c:[34] d:[23] e:[24] f:[20]

variants [24].

Currently single-cycle assays are able to detect minorities of as low as five to ten percent. However, the given limits of detection may vary between X4- and R5-tropic viruses [32].

An assay with prior replication of the pseudovirus in three to four cycles is able to detect minor species with frequencies of nearly 1%. The replicative amplification of such variants in the PhenX-R system might offer a compromise, although the overrepresentation of minorities with the highest replicative fitness may have to be taken into account. Another phenotypic tropism assay currently being developed in Germany (Cologne/Erlangen) which is still under evaluation, promises to determine accurate results from minorities below one percent [45].

However, the declared values of all RVA mostly correspond to HIV-1 subtype B samples and a similar sensitivity for non-B subtypes remains to be shown.

Consequently, a universal challenge is still the insufficient data for the prediction of tropism in different HIV subtypes. Most of the phenotypical assays in use are currently validated only on a limited subset of samples. Rare HIV subtypes are often not considered. Phenoscript provides the most comprehensive data set of HIV-1 subtypes, but larger evaluations are needed for validation of the system in general [34].

With currently about 25,000 analysed samples, Trofile offers the largest clinical validation. Comparative studies demonstrated a good concordance with other systems (85.1 percent between Trofile™ and Pheno-

script™). In case of discordant results, however, due to lacking independent standards, it remains to be shown, which one of the assay is correct [38]. External quality controls are still necessary to investigate the concordance of the different tropism assays.

Since most of the cell-based HIV co-receptor tropism assays are time-consuming and costly (listprice ranges between 1000-1500 US$), there have been considerable efforts to develop methods predicting HIV-1 tropism by *env*-V3 sequence analysis. Bioinformatic methods predict coreceptor usage by correlating genotype/phenotype pairs [37, 39-42]. Such genotypic approaches might represent an attractive, faster and less expensive alternative. Virco developed an interesting strategy based on a platform for the determination of coreceptor usage which represents a link between genotypic and phenotypic analysis. It has to be shown how this strategy can be implemented into the clinical routine. Also the genosorting approach of inPhenos' Xtrack^C belongs to these genotype based systems.

In comparison to phenotypic assays most of the bioinformatic methods for coreceptor usage do not consider the three dimensional conformation of the gp120 envelope. Recently, it was shown that by incorporating the structure of the V3 loop the predictive value of such bioinformatic methods could be improved [43]. This three dimensional structure is inherently included in phenotypic assays.

In summary, the described recombinant viral assays are suitable for the clinical management of HIV infected individuals treated with coreceptor antagonists. Currently, it is still unclear which assay will show the most accurate result for phenotypic prediction of viral tropism in clinical routine. In addition, the establishment of cut-offs for X4 minority species will be difficult, and affected by many factors like patient sample quality, the input volume, viral load, the detection limits and PCR variations [56].

Nevertheless, the addition of tropism assays to other clinical tools available for the management of HIV-1 infection will assist clinicians in selecting and improving antiretroviral regimens.

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Received: August 28, 2007 / Accepted: August 30, 2007

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