

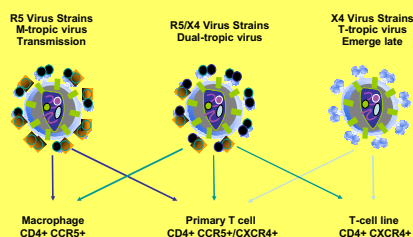
# Performance of genotypic coreceptor measurement using geno2pheno<sub>[coreceptor]</sub> in B- and non-B HIV subtypes in a large cohort of therapy-experienced patients in Germany

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## Background:

Coreceptor usage determination is mandatory for prescription of the CCR5-antagonist Maraviroc (MVC). Recently, the clinically validated standard Trofile assay has been replaced by an enhanced version (Trofile-ES) capable of detecting also CXCR4-using minor variants to levels below 0.3%. For the fast and inexpensive genotypic approaches which can also be used in case of low viral loads below 1000 cp/ml or below 50 cp/ml using proviral DNA from PBMCs, no prospective clinical validation is available yet. In this work, we compare results of both Trofile assays with predictions from geno2pheno<sub>[coreceptor]</sub> (g2p) in a large German cohort of treatment-experienced patients and analyze the effects of different subtypes.



## Materials & Methods:

HIV coreceptor usage was determined with the Trofile test (Trofile® or Trofile-ES® (ESTA) by Monogram), which was used in the maraviroc approval studies. In addition, the V3-loop of gp120 was sequenced using standard bulk-sequencing techniques and tropism as well as subtype inferred from genotype with g2p and the REGA-subtyping tool. Subtypes were grouped into B and non-B. Fisher's exact test was used to assess statistical significance.

## Methods to determine HIV-1 coreceptor tropism

### Trofile® by Monogram

- Used in approval studies
- clinically validated phenotypic assay

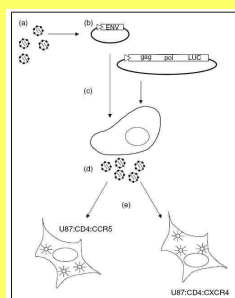
### Trofile-ES® (ESTA)

- „enhanced-sensitivity“
- Minority detection improved from 10% to 0.3%

### Sequence analyses

- RT-PCR from plasma viral RNA and Sanger sequencing
- Interpretation by geno2pheno<sub>[coreceptor]</sub>
- Subtyping by geno2pheno<sub>[coreceptor]</sub>

## Trofile® test



- Viral RNA is extracted from patient sample
- viral gp120 is cloned into an env-expression-vector
- 293 cells are co-transfected with the env-expression-vector and a HIV-genomic vector
- HIV pseudotype particles are harvested
- Reporter cell lines are infected

## Results:

### Subtyping

	Geno2pheno <sub>[coreceptor]</sub>	Rega subtyping tool
No result	2 (0.5%)	367 (49.7%)
Subtype B	642 (86.7%)	314 (84.6%)*
Non-B	92 (12.4%)	57 (15.4%)*

\* Of successful predictions

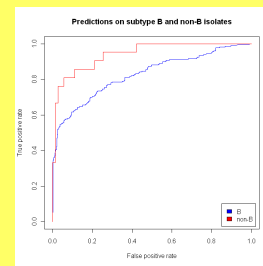
Overall concordance between both subtyping systems: 96.2%

Short sequence length was the most frequent reason for unsuccessful subtyping.

B isolates were significantly more often D/M than non-B isolates

B:  
60.3% sensitivity  
90.3% specificity

Non-B:  
85.7% sensitivity  
87.3% specificity



Subtyping can be used as additional information to support the confidence the coreceptor determination by geno2pheno<sub>[coreceptor]</sub>.

## Tropism testing comparisons

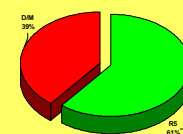
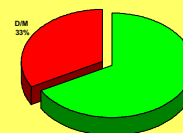
738 genotype-phenotype pairs  
619 standard Trofile®  
119 ESTA

### standard Trofile®

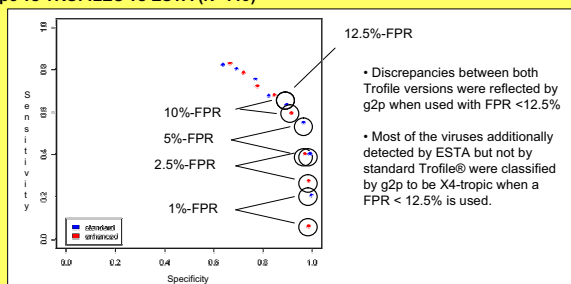
414 (66.9%) R5  
205 (33.1%) D/M

### ESTA

72 (60.5%) R5  
47 (39.5%) D/M



## Genotype vs TROFILE® vs ESTA (N=119)



## Conclusions:

This analysis shows that with the enhanced assay more samples were detected to be D/M-tropic. In the context of the most recently presented clinical data comparing responders and non-responders to MVC-use in patients harboring minority CXCR4-variants, the question arises whether more people suitable for MVC will be screened out when using the enhanced assay.

In contrast to other studies, these results show very good agreements between Trofile and geno2pheno<sub>[coreceptor]</sub> for non-B isolates suggesting to use the subtype-predictions as an additional confidence measure.