

Recommendations for determining HIV-1 coreceptor usage

CCR5 antagonists proved their good efficacy in ART-naïve and ART-experienced HIV-patients harbouring CCR5-tropic virus only (Heera 2008, Hardy 2008). A rapid diagnostic should be available for all HIV-patients to enable access to all licenced drugs. For CCR5-antagonists, this can only be realized by fast and reliable methods to screen for X4-tropic viruses in human plasma.

According to the intra- and inter-laboratory validation by ten reference laboratories in Germany testing viral coreceptor usage (coordinated by the NRC for Retroviruses) simultaneously with genotypic and phenotypic assays we conclude genotypic testing by conventional sequence analysis to be equivalent to the validated and most frequently used phenotypic method (Monogram, Trofile).

For other phenotypic methods like the Eurofins, TRT and the InPheno assays, an acceptable concordance with Monogram Trofile could be shown (table 1). For newly developed tests, the same validation criteria have to be applied.

For genotypic analyses, the usage of interpretation systems is mandatory. Several interpretation systems are available via Internet

Wetcat: <http://genomiac2.ucsd.edu:8080/wetcat/tropism.html>

WebPSSM: <http://ubik.microbiol.washington.edu/computing/pssm/>

geno2pheno_[coreceptor]: <http://coreceptor.bioinf.mpi-inf.mpg.de/index.php>

Due to our experiences, a differentiated approach using the geno2pheno system provides the most reliable results in CXCR4 usage prediction.

For patients with severely limited therapeutic options we suggest the use of CCR5 blockers only, if the geno2pheno system, using a false positive rate (FPR) of 10%, does predict CCR5 usage. The other side of the patient spectrum with a large variety of antiretroviral therapy options, requires a more stringent setting with a FPR of 20%. The higher the FPR is set, the better the exclusive CCR5 usage is predicted, leading to a high safety in the administration of CCR5-coreceptor blocker (tables 2 and 3). For this highly personalized approach a close cooperation between the clinicians and virologists is essential and therefore recommended.

In conclusion, genotypic classification approaches show similar results as the comparison of phenotypic approaches (table 3).

However, in case of discordant predictions of coreceptor usage by geno2pheno when using FPR of 20% and FPR of 10%, additional phenotyping in patients with limited therapy options is recommended.

Furthermore, as genotypic predictions are based on phenotypic analyses, it is mandatory to assure a supply of phenotypic assays. Due to the differences observed between phenotypic assays, only a spectrum of different phenotypic assays can improve the genotypic predictions without being biased by a single test system.

Since there is a fast progression in the field coreceptor tropism testing, the details of this recommendation will be updated regularly.

Table 1: Agreement between Trofile and TRT (Skrabal et al. 2007)

Agreement Trofile vs. TRT 85,1 %	FNR 28,0 %
Agreement TRT vs. Trofile 85,1 %	FNR 12,5 %

Table 2: German validation study: Agreement between genotypic tropism results (using g2p classification with different false positive rates (FPR)) and phenotypic results (Trofile); N=234 samples (all laboratories).

Method	Concordant results (%)	False negative rate (FNR in %) *
G2P 20 % FPR	76.06	24.66
G2P 15 % FPR	76.49	30.14
G2P 10 % FPR	80.34	35.62
G2P 5 % FPR	83.76	43.84
G2P 2.5 % FPR	79.91	61.64
G2P 1 % FPR	76.06	75.34

* named R5-tropic by g2p but X4-tropic by Trofile

Table 3: Agreement between genotypic classification methods and Trofile results (N=103, Labor Berg).

Method	Concordant results [%]	FNR (%)
G2p 20 % FPR	77	20
G2p 15 % FPR	77	26
G2p 10 % FPR	77	32
G2p 5 % FPR	82	45
G2p 2.5 % FPR	82	58
G2p 1 % FPR	77	74
WepPSSM	82	38
WETCAT:C4.5	74	84

WETCAT: C4.5 p8 and p12	74	87
Wetcat: Part	78	55
Wetcat: SVM	77	52
Wetcat: Charge	82	58
one of the algorithms X4	67	10

References

Hardy D, Reynes J, Konourina I, Wheeler D, Moreno S, van der Ryst E, Towner W, Horban A, Mayer H, Goodrich J. Efficacy and Safety of Maraviroc plus Optimized Background Therapy in Treatment-experienced Patients Infected with CCR5-Tropic HIV-1: 48-Week Combined Analysis of the MOTIVATE Studies. 15th CROI 2008, Boston, MA, USA. Abstract 792.

Heera J, Saag M, Ive P, Whitcomb J, Lewis M, McFadyen L, Goodrich J, Mayer H, van der Ryst E, and Westby M. Virological Correlates Associated with Treatment Failure at Week 48 in the Phase 3 Study of Maraviroc in Treatment-naive Patients. 15th CROI 2008, Boston, MA, USA. Abstract 40LB.

Skrabal, K.; Low, A. J.; Dong, W.; Sing, T.; Cheung, P. K.; Mammano, F. & Harrigan, P. R. (2007), 'Determining human immunodeficiency virus coreceptor use in a clinical setting: degree of correlation between two phenotypic assays and a bioinformatic model.', *J Clin Microbiol* **45**(2), 279--284.