

Does HIV-1 tropism change in patients during virological suppressive therapy?

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Background

Drug toxicities are limiting factors for the lifelong therapy of HIV infection. The CCR 5 receptor antagonist Maraviroc showed a favourable long term toxicity profile so far. Thus switching from a virological successful but not well tolerated regimen to Maraviroc might be a future option.

However, since testing of viral tropism is mandatory before the use, current tropism assays require a plasma HIV viral load of >500 c/ml. Thus it is difficult to determine viral tropism with standard assays in successfully treated patients in clinical practise.

This drawback may be overcome by analyzing historical plasma samples which have been frozen before the introduction of HAART. These samples which are available in many large centers may be used to define viral tropism if it can be excluded that viral tropism changes during virological successful therapy.

Patients and Methods

Seven male patients were selected from previous clinical study maintaining an undetectable viral load throughout their treatment period. Viral load and CD4 counts were monitored at 3 monthly intervals.

These patients received 3TC, d4T, SQV and NFV in standard dosing for a mean of 92 weeks (range 72-180). A predefined STI was performed and samples were collected at a mean of 34 days (28-59) after cessation of ART at a mean viral load of 43.967 c/ml (860- 145.000).

Historical plasma and PBMC samples were obtained and frozen at a mean of 7.4 days (0-19) before starting initial antiretroviral therapy. The mean pre-treatment HIV-1 viral load was 36.433 copies/ml (range: 1.500-133.500) and the mean CD4 T-cell count was 509/μl (356-650) (details in Table 1). PBMC and plasma were cryopreserved at -80°C after cryopreservation.

HIV co-receptor tropism was determined in PBMC and plasma **phenotypically** using the Trofile™ assay performed by Monogram Biosciences, San Francisco¹. Trofile is a recombinant test which is based on amplification of the HIV envelope (env) gene by PCR and its subsequent insertion into an env expression vector. A susceptible cell line is transfected with the env expression vector and another vector carrying the HIV genome and a luciferase reporter gene. Detection is facilitated by infecting cells carrying either the CCR5 or CXCR4 receptor, respectively and quantification of emitted light in case of expression of the luciferase reporter gene.

The **genotypic** test was accomplished by amplifying and sequencing the V3 loop of the HIV env gene from plasma or peripheral blood mononuclear cells, respectively. Nested PCR was performed using Env 31 and Env 8 as outer primers. Env7 and ED33 were used as inner and sequencing primers. Primer sequences were previously described³. For sequence analysis and prediction of HIV co-receptor usage the web-based bioinformatic tool geno2pheno_[coreceptor] was chosen² (<http://coreceptor.bioinf.mpi-inf.mpg.de/index.php>). The false positive rate (FPR), indicating the probability of classifying an R5 virus falsely as X4, was adjusted to 10 %.

Results

Genotypic results from plasma revealed R5 tropic viruses in 4/4 pre-treatment samples and in all 7/7 post-treatment samples (Table 1). Consequently R5 using viruses were detected in 3/4 pre-treatment samples and 7/7 post-treatment samples by using the standard phenotypic assay. However, in one patient a dolutropic D/M population was detected phenotypically in pre-treatment samples with a shift to R5 usage after ART, whereas genotyping failed to detect the dolutropic population in this patient before therapy.

Results cont.

Table 1

Patient	Duration of ART (weeks)	CD4-Cell count/ μl	blood collection before/post ART (days)	Viral load [copies/ml]	Results of coreceptor use		
					Phenotypic assay (Trofile)	Genotypic assay (geno2pheno _[coreceptor]) [†]	Proviral
Patient 1	72	422	-4	89.500			R5
			Baseline (BL)	133.500	X4/R5	R5	
			+14 from BL	1.000	X4/R5	R5	R5
			31 post ART	75.000	R5	R5	
Patient 2	88	558	-17	3000			R5
			28 post ART	145000	R5	R5	R5
Patient 3	88	356	-9	144000			R5
			Baseline	83000			R5
Patient 4	72	584	28 post ART	25000	R5	R5	R5
			-19	10000			R5
Patient 5	72	456	36 post ART	26500	R5	R5	R5
			-1	1500	R5	R5	R5
Patient 6	72	538	28 post ART	3010	R5	R5	R5
			-6	24000	R5	R5	R5
			28 post ART	860	R5	R5	R5
Patient 7	180	650	74 post ART	18400	R5	R5	R5
			Baseline	73500	R5	R5	R5
			59 post ART	32400	R5	R5	R5

* 10 % false positive rate (FPR) = probability of classifying an R5 virus falsely as X4.

R5: CCR5-using variant

X4/R5: CXCR4- and/or CCR5-using variant

Discussion

The genotypic test in PBMC and plasma showed no changes of tropism during virological successful therapy in our patients. No shift from R5 to X4 usage was detected during suppressive therapy by standard phenotype testing which remains the gold standard at the moment. Moreover, no differences in co-receptor usage was detected using either cryopreserved plasma in comparison to PBMC, both materials can be used as sources for the determination of viral tropism before therapy.

Thus historical samples might be used to screen selected aviremic patients before a potential switch to maraviroc. However clinical trials with larger sample sizes are necessary to confirm our findings.

References

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