Phenotypic evaluation of resistance to tipranavir (TPV) in non B subtypes possibly resistant according to genotype interpretation rules

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BACKGROUND

A higher prevalence of mutations involved in resistance to antiretroviral drugs (ARV) in B subtypes has been described in naive non B subtypes[1]. Several resistance algorithms have been developped to predict resistance to the protease inhibitor (PI) TPV, most of them based on data from clinical studies including a majority of subtype B infected patients, and possibly leading to discrepant interpretations [2][3][4]. The prevalence of some mutations involved in resistance to TPV in B subtypes is known to be high in naïve non-B subtypes, leading to frequent genotypic possible resistance[5].

OBJECTIVES

The number of patients infected with a non B subtype of HIV-1 and under antiretroviral therapy is constantly increasing in France. Such patients experiencing a treatment failure and who could benefit of a salvage therapy including TPV are not uncommon. In order to find an alternative way of testing susceptibility to TPV in PIs experienced patients with a non B subtype, this preliminary study was designed to evaluate the feasability of a phenotypic measurement of the susceptibility to TPV with PHENOSCRIPT® and to compare genotypic and phenotypic data obtained from PIs naive patients considered as possibly resistant to TPV by genotypic evaluation.

MATERIAL AND METHODS

Between january 2006 and march 2007, 1586 samples were received at Laboratoire Pasteur Cerba for HIV resistance genotyping. Genotyping and subtyping were performed by RT-PCR and sequencing using the Bayer/Siemens Trugene HIV reagents and software according to the manufacturer's recommandations.
Final mutation profile interpretation was done according to the ANRS 2005 (upgraded in 2006) algorithm including the rules available at that time for TPV[2].
A patient was then considered as resistant to TPV if at least 8 mutations among L10V, I13V, K20M/R/V, L33F, E35G, M36I, K43T, M46L, I47V, I54A/M/V, Q58E, H69K, T74P, V82L/T, N83D and I84V were present, and possibly resistant if only 4 to 7 of these mutations were present.
32 successive patients infected by a non B subtype, PI naive and considered as possibly resistant to TPV according to the 2005/2006 algorithm were included in the study.

Patients Statistics:
Origin:
Africa: 21 France: 9 Middle-east (Koweit, Lebanon): 2

Subtype repartition:

HIV-1 subtype A:	4
HIV-1 subtype C:	2
HIV-1 subtype F:	1
HIV-1 subtype G:	1
HIV-1 subtype K/J:	2
HIV-1 subtype CRF-01_AE:	10
HIV-1 subtype CRF-02_AG:	9
HIV-1 subtype CRF-15_01B:	3

Mean viral load: 4.56 log (3.07 – 5.94)

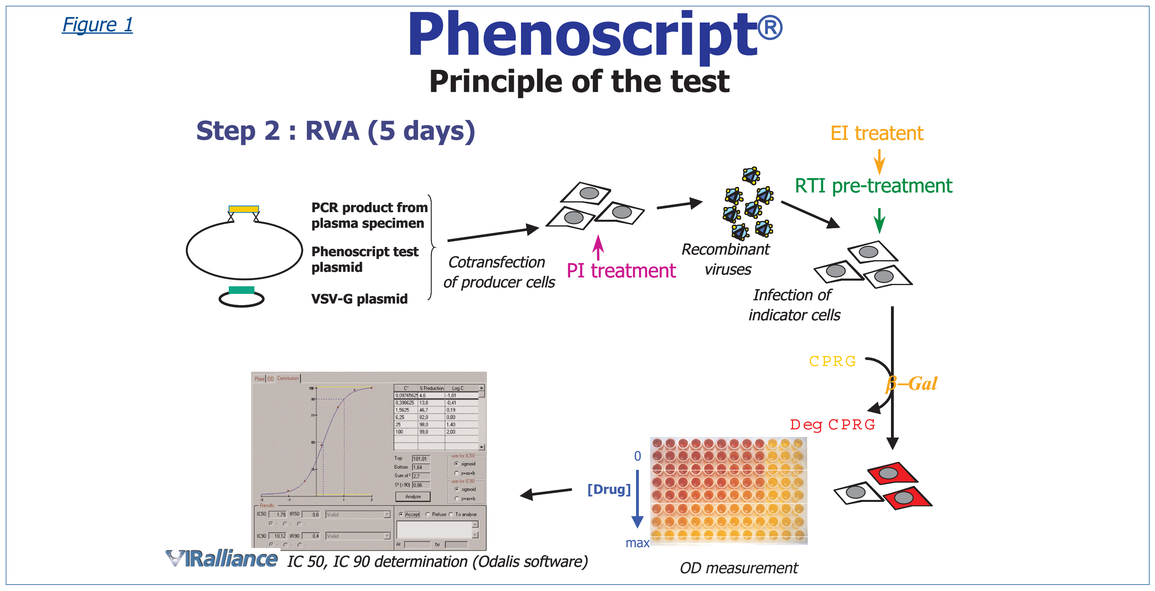
Mean CD4 count: 205 (41-472)

Previous history of ARV therapy:

PI naive:	32 (100%)
ARV naive:	19 (59.4%)
NRTI experienced:	13 (40.6%)
NNRTI experienced:	7 (21.9%)

PHENOSCRIPT®
RNA was extracted from 1 ml EDTA plasma after ultra-centrifugation using Qiagen reagents.
Phenotyping was performed using the Recombinant Virus Assay (RVA) PHENOSCRIPT® technology *(figure 1)*, with the calculation of a fold change index 50% (FC50) by comparison of the specimen IC50 and the control wild-type pNL-43 IC50.
Interpretation was done according to TPV package insert values with a low clinical cut-off for TPV efficiency below 3.0 (likely) and a high clinical cut-off for resistance above 10.0 (unlikely), TPV being possibly active with a FC50 result between these two cut-off values.

Figure 1



RESULTS

- List of the mutations of interest and PHENOSCRIPT® results according to subtype are presented in *table 1*
- Mutation frequency for all samples and those for which a phenotype could be obtained are presented in *table 2*.
- When re-interpreted with the 2008 Stanford HIVdb algorithm version, all profiles demonstrated susceptibility to TPV.
- PHENOSCRIPT® success rate was 65% with 5 PCR failures and 6 RVA failures. Failure could not be linked to a particular profile.
- Eighteen out of 21 samples (86%) considered as possibly resistant to TPV according to genotype showed no decrease in susceptibility to TPV using PHENOSCRIPT®, with a mean FC50 of 1.35.
- Three samples (14%) showed a low decrease in susceptibility to TPV, with a mean FC50 of 4.4.
- The small number of samples tested did not allow an analysis of the FC50 repartition according to subtype and mutation profile.

Table 2

	L10V	I13V	I15V	G16	K20I/R/M/V	E35D/G	M36I	R41K	D60E	I62V	L63P/V
All samples	16	31	7	12	27	15	31	29	3	2	6
	50,0%	96,9%	21,9%	37,5%	28,0%	46,9%	96,9%	90,6%	9,4%	6,3%	18,8%
Samples with PHENOSCRIPT result	12	21	5	7	19	9	21	21	1	2	6
	57,1%	100,0%	23,8%	33,3%	90,5%	42,9%	100,0%	100,0%	4,8%	9,5%	28,6%

Table 1

PATIENT	SUBTYPE	L10	I13	I15	G16	K20	E35	M36	R41	D60	I62	L63	H69	V77	L89	PHENOSCRIPT FC50	PHENOSCRIPT INTERPRETATION
1	CRF-02	V	V	V		I		I	K				K		M	3.5	Possibly
2	CRF-01		V			R	D	I	K				K			1.9	Likely
3	CRF-02		V			R		I	K			P	K		I	< 0.1	Likely
4	CRF-02	V	V			I		I	K			P	K		M	2.7	Likely
5	CRF-02	V	V	V	E	I		I	K			P	K		M	1.9	Likely
6	CRF-01	V	V		E/G		D	I	K				K		M	2.4	Likely
7	A		V			R	D	I	K				K		M	ND	NA
8	A		V			R	D	I	K				K		M	1.6	Likely
9	CRF-02		V			R		I	K				K		M	2.4	Likely
10	F	V	V	V	E	R	D	I			V	V			I	6.6	Possibly
11	CRF-02	V	V		E	I		I	K				K		M	ND	NA
12	CRF-01	V	V			I		I	K				K		M	2.8	Likely
13	CRF-01		V		E	R	D	I	K				K		M	ND	NA
14	CRF-01	V	V		E		D	I	K				K		M	ND	NA
15	K/J	I	V			R				E			K		I/M	ND	NA
16	C	V				R		I	K				K		M	ND	NA
17	CRF-01	V	V		E		D	I	K				K		M	ND	NA
18	K/J		V		E	R		I	K	E	V		K			0.4	Likely
19	CRF-02	V	V			I		I	K				K		M	3.2	Possibly
20	G		V			I	G	I	K	E			K	I	M	ND	NA
21	CRF-01	V	V		E		D	I	K				K		M	0.9	Likely
22	C		V	V		R		I					K		M	ND	NA
23	CRF-01		V			M		I	K			P	K		M	0.7	Likely
24	CRF-01		V			R	D	I	K				K	I	M	1.6	Likely
25	A		V	V		R	D	I	K				K		M	ND	NA
26	CRF-01	V	V			I		I	K				K		M	1.2	Likely
27	A	I	V	V	E	R		I	K				K		M	0.4	Likely
28	CRF-15	V	V	V	E	I	D	I	K				K		M	0.1	Likely
29	CRF-15		V			R	D	I	K				K		M	1	Likely
30	CRF-02	V	V			I		I	K			P	K		M	1.3	Likely
31	CRF-15		V			R	D	I	K				K		M	1	Likely
32	CRF-02		V		E	R	D	I	K				K		I	ND	NA

DISCUSSION

Discordance between TPV possible resistance genotype interpretation performed with the initial (2005/2006) algorithm[2] and phenotypic susceptibility obtained with PHENOSCRIPT® is consistent with an excess of sensitivity of rules defined mostly on subtype B to predict susceptibility or resistance in non B subtypes.
A comparable study with genotypic and phenotypic results on 57 naïve B and non B subtypes demonstrated a 100% susceptibility to TPV[6].
The ANRS 2007 interpretation rules[3] defined on a population of more than 80% of B subtypes are not valid for testing resistance to TPV in non B subtypes.
New algorithms developped with a scoring of the different mutations as the 2008 Standford HIVdb algorithm or the one proposed at EACS 2007[7] may demonstrate a better efficacy as predictors of response to TPV for both B and non B subtypes. However, 3 samples out of 21 (14%) showed a low decrease in susceptibility to TPV, with a mean FC50 of 4.4. The small number of samples tested did not allow an analysis of the FC50 repartition according to subtype and mutation profile.

Therefore further studies are needed:
- to confirm and explore this possible primary decrease in TPV susceptibility for these particular patients,
- to test a larger number of unselected PI naïve non B subtypes to evaluate the frequency of such possible decrease in susceptibility to TPV of some non B subtypes, as in comparable studies performed with other PIs with the PHENOSCRIPT® technology[8] or other phenotype assays[9], or with TPV[6].

As TPV is not used in first line ARV therapy but only in salvage therapy, determining its possible efficacy in PIs experienced patients will be more frequently necessary in clinical practice as a constantly increasing number of non B patients are now under ARV therapy including PIs. When necessary, testing for phenotypic suceptibility to TPV with technologies such as PHENOSCRIPT® may be of some help.

CONCLUSION

This small study demonstrates that in the absence of subtype-specific genotypic interpretation rules, testing for TPV resistance with phenotypic tests as PHENOSCRIPT® can be an helpfull alternative when needed for PIs experienced patients with non-B subtypes.

More *in vitro* and clinical studies are needed with ARV naive non B subtypes infected patients to achieve a comparable efficacy in the definition of subtype adapted rules for predicting response or resistance for B and non B subtypes, and to evaluate the role of polymorphism mutations in a possible decrease of sensitivity to current and new PIs, as well as in possible different ways and kinetics of acquisition of resistance to PIs under therapy.

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