In Vitro Phenotypic Susceptibility of Human Immunodeficiency Virus Type 2 Clinical Isolates to Protease Inhibitors.

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Running title: Phenotypic susceptibility of HIV-2 to protease inhibitors

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Abstract

We determine phenotypic susceptibility of HIV-2 isolates to amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, saquinavir and tipranavir. Saquinavir, lopinavir and darunavir are potent on wild-type HIV-2 isolates and should be preferred as first-line options for HIV-2-infected patients. Other protease inhibitors are less active on HIV-2 than on HIV-1.

Key words: HIV-2, phenotypic susceptibility, protease inhibitors, phenotypic resistance, resistance mutations

Few data are available on HIV-2 susceptibility to antiretroviral agents. In France, as recommended by the national expert group on the treatment of HIV infection, HIV-2-infected patients receive highly active antiretroviral therapy (HAART) regimens as HIV-1-infected individuals except non nucleoside reverse transcriptase inhibitors or fusion inhibitors classes (17) (12). However, a recent study showed that CD4 cell recovery was poor in antiretroviral-naive HIV-2 infected patients starting treatment with HAART (8). Thus it appears crucial to determine HIV-2 susceptibility to the current PIs available, in order to define the optimal regimen to be recommended.

We selected nine PI-naive HIV-2-infected patients from the French HIV-2 ANRS cohort. Six of these patients subsequently received HAART regimens including a PI (indinavir, nelfinavir, saquinavir and/or ritonavir) for a median of 13 months (range 2-36), and had plasma viruses harboring mutations in the protease gene. PBMC co-culture isolates were available for these patients before (T0, n=6) and during (T1 (n=6), T2 (n=2)) PI exposure. Protease gene sequences of plasma and PBMC isolates were determined as previously described (3). Amino acid changes were compared with those associated with drug resistance in HIV-1 (International AIDS Society-USA (IAS-USA). We determine the *in vitro* phenotypic susceptibility of clinical HIV-2 isolates and HIV-2 ROD and HIV-1 BRU reference strains to amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, saquinavir and tipranavir using the ANRS PBMC assay (4). Phenotypic inhibitory quotients (PIQs) were calculated for each clinical HIV-2 isolate, as the ratio between the trough plasma PI concentration and the IC50. The PIQs were not adjusted for protein binding. The Table shows the phenotypic results and protease gene sequences of the HIV-2 clinical isolates, and the HIV-2 and HIV-1 reference isolates.

The protease sequences of wild-type HIV-2 strains, when compared to HIV-1 clade B consensus sequence, contained several amino acids associated with HIV-1 PI resistance, such as 10I/V 16E, 20V, 32I, 33V, 35G, 36I, 43T, 46I, 47V, 58E, 62V, 69K, 71V, 73A, 82I and 93L. Other

differences were observed at positions involved in but not associated with HIV-1 resistance, such as 13A, 34A, 60K, 63E, 76M, 77T, 85F and 89I. Relative to HIV-1 reference strain, the median IC50 values of the HIV-2 wild-type isolates were 31-fold higher for amprenavir, 8-fold higher for atazanavir, 7-fold higher for tipranavir, and 3-fold higher for indinavir and nelfinavir. Darunavir, lopinavir and saquinavir IC50 and IC90 values were similar for HIV-1 and the wild-type HIV-2 isolates (table). Viruses isolated from the six PI–experienced patients at T1 and T2 harboured the I82F, I84V and L90M substitutions, alone or in combination with minor HIV-1 PI mutations such as V10I, V33I, I54M, I64V, V71I and I89V. Compared to the corresponding wild-type isolates, the eight mutants showed 4 to >10-fold increases in the IC50 and/or IC90 values of all tested PIs, at both T1 and T2. The PIQs values of amprenavir, atazanavir, indinavir, nelfinavir and tipranavir were respectively 33-fold, 8-fold, 3-fold, 3-fold and 7-fold lower for HIV-2 wild-type strains than for HIV-1. Darunavir, lopinavir and saquinavir PIQ values were similar.

PIs are designed to fit the active site of the HIV-1 protease and are sensitive to structural changes in the viral protein. It has been reported that the therapeutic outcome of HIV-2-infected patients might be influenced by the choice of PIs (1) (15). For amprenavir our data are in keeping with those reported elsewhere, showing significantly lesser activity against HIV-2 wild-type strains than against HIV-1 (9) (14) (16). These phenotypic results could be explained by the natural presence in HIV-2 protease of amino acids associated with resistance to HIV-1, which might influence the binding affinity of the PIs for HIV-2 protease (2) (3) (9) (10) (11). In our study, all the HIV-2 wild-type strains protease sequences naturally presented the amino acids 32I and 47V associated with resistance in HIV-1 infection to amprenavir according to IAS USA list and to different genotypic resistance interpretation algorithms (www.hivfrenchresistance.org,www.hivdb.stanford.edu,www.kuleuven.be/rega/cev/links/rega_algorithm). We found that clinical HIV-2 isolates and HIV-1 reference strains had similar phenotypic susceptibility to saquinavir, lopinavir and darunavir. As amprenavir and darunavir are structurally close, we expected darunavir to be relatively ineffective in HIV-2.

Crystallographic studies with HIV-1 showed that darunavir interacts directly with the main chains of aspartic acid residues (Asp-29 and Asp-30), whereas other PIs interact with side chains in the S2 subsite of the HIV-1 enzyme (6) (7). Moreover, it has been reported in HIV-1 that the binding affinity of darunavir for wild-type protease was > 100-fold higher than other PIs due to a slower dissociation rate of this molecule from the protease active site (5). In the same way, crystallography structure studies of the HIV-2 protease and binding affinity experiments might help us to understand the difference observed in the natural susceptibility of HIV-2 strains for these two drugs as well as phenotypic resistance in HIV-2 mutated strains. The IC50 and IC90 values of atazanavir, indinavir, nelfinavir and tipranavir for the HIV-2 isolates were higher than those observed for HIV-1 raising the hypothesis of a lower activity of these PIs against HIV-2. However these values were lower than their respective trough plasma concentrations. This might be explained by the fact that HIV-2 wild-type isolates harbored several amino acids associated with PI resistance in HIV-1.

PI treatment-associated amino acid changes in the HIV-2 protease gene occurred at positions known to confer PI resistance in HIV-1 and were not associated with the use of a particular PI without any order of accumulation. They altered the phenotypic susceptibility of the isolates to all the PIs tested here. These results are in keeping with data published elsewhere (2) (3) (9) (11) (13) (15) . Mutagenesis experiments coupled with phenotypic susceptibility testing might help to determine the impact of each substitutions in PI resistance. Saquinavir, lopinavir and darunavir appear to be the best choices for first-line therapy of HIV-2 infection, while amprenavir should not be used. Atazanavir and tipranavir might be used with care (17). Our results suggest that treatment guidelines for HIV-1-infected patients should not be directly extrapolated to HIV-2-infected patients. Virological efficacy data in vivo might help us to evaluate the place of PIs in HIV-2 antiretroviral strategy.

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Drugs and sources

Amprenavir was provided by GlaxoSmithKline (Marly-le-Roi, France), atazanavir by Bristol-Myers Squibb (Rueil-Malmaison, France), darunavir by Tibotec (Mechelen, Belgium), indinavir by Merck Sharp & Dohme-Chibret (West Point, PA, USA), lopinavir by Abbott (Rungis, France), nelfinavir and saquinavir by Roche (Neuilly sur Seine, France) and tipranavir by Boehringer-Ingelheim (Ridgefield, CT, USA).

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194 **Legend of the table**

195 Table

196 Title : Phenotypic susceptibilities to PIs and protease mutations compared to HIV-2

197 subtypes A and B consensus sequences of the 9 wild-type isolates

Phenotypic susceptibilities to PIs and protease mutations compared to HIV-2 subtypes A and B consensus sequences before (T0) and after (T1. T2) PIs initiating treatment of the 9 HIV-2 isolates.

ISOLATES	Amprenavir		Atazanavir		Darunavir		Indinavir		Lopinavir		Nelfinavir		Saquinavir		Tipranavir	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
BRU (HIV-1 reference isolate)	0.02	0.20	0.007	0.03	0.004	0.009	0.005	0.04	0.03	0.10	0.002	0.10	0.01	0.04	0.05	0.40
HIV-2 Isolates																
ROD (HIV-2 reference isolate)	0.60	4.60	0.03	0.10	0.005	0.02	0.03	0.30	0.02	0.07	0.05	0.90	0.01	0.09	0.40	3.50
Patient 1 (subtype H)																
T0: 10I-40P-41Y-60H-63N-70T-73G-89L-92E	0.90	2.70	0.10	0.50	0.004	0.04	0.02	0.50	0.06	0.50	0.04	0.30	0.03	0.30	0.40	0.80
T1: 10I-34E*-40P-41Y-60H-63N-70T-73G-82F*-89L-92E	10.0	82.0	1.90	15.0	0.90	4.70	12.0	99.0	3.20	8.70	9.00	24.0	7.30	31.0	7.30	16.0
Fold increase in IC_{50} between T1/T0	**12**	**30**	**16**	**32**	**225**	**126**	**621**	**193**	**57**	**17**	**236**	**79**	**252**	**114**	**19**	**20**
Patient 2 (subtype A)																
T0: 14H-17D-43T-68N/D	0.60	3.80	0.07	0.20	0.009	0.03	<0.01	<0.01	0.03	0.08	0.03	0.30	<0.006	<0.006	0.30	2.20
T1: 5L/F*-14Y/H*-17G/D*-43T-54I/M*-62V/A*-70R/K*-71I*	4.40	13.0	0.30	2.10	0.60	7.30	18.0	2794	0.04	0.40	14.0	110	>12.5	>12.5	6.70	26.0
Fold increase in IC_{50} between T1/T0	**8**	**3**	**4**	**11**	**71**	**222**	/	/	**1**	**5**	**463**	**450**	/	/	**24**	**12**
Patient 3 (subtype A)																
T0: 14H-60K/N-65E	0.90	6.80	0.04	0.30	0.01	0.03	0.03	0.40	0.04	0.20	0.06	0.80	0.005	0.05	0.30	2.50
T1: 54M*-65E-71I*-74N*-90M*	4.00	60.0	0.40	1.80	0.20	1.40	10.0	82.0	0.40	3.30	3.80	53.0	>12.5	>12.5	4.70	14.0
Fold increase in IC_{50} between T1/T0	**5**	**9**	**11**	**6**	**18**	**45**	**304**	**206**	**12**	**21**	**68**	**65**	/	/	**15**	**5**
Patient 4 (subtype A)																
T0: 10I-17D-40D-43I-46V-66V/A-70R/K	0.40	3.50	0.10	0.30	0.004	0.03	0.004	0.10	0.02	0.20	0.05	0.40	0.002	0.02	0.30	2.70
T1: 10I-17D-40D-43I-45K/R*-46V-54M*-64I/V*-69K/R*-71V/I*-90M*	9.50	31.0	0.20	1.80	0.80	3.00	0.20	3.10	1.70	8.50	1.20	15.0	0.09	0.70	4.10	23.0
Fold increase in IC_{50} between T1/T0	**22**	**9**	**2**	**6**	**216**	**88**	**15**	**28**	**75**	**42**	**24**	**37**	**45**	**33**	**12**	**9**
Patient 5 (subtype A)																
T0: 14H-40D-70K-72R/K-91T/S	0.30	1.60	0.04	0.20	0.004	0.02	0.02	0.40	0.02	0.08	0.20	0.40	0.005	0.02	0.40	4.20
T1: 10I*-40D-43I*-70K-82F*-84V*-85L*-89V*-90M*-91T/L*-98N/K*	12.0	96.0	0.04	1.30	0.50	2.40	6.40	51.0	0.10	0.80	>17.6	>17.6	6.40	51.0	2.80	16.0
T2: 10V/I*-40D-43I*-56V*-70K-82F*-84V*-89V*-90M*	12.0	95.0	0.40	3.00	1.00	8.10	11.0	90.0	0.10	1.10	10.0	81.0	22.0	180	4.80	29.0
Fold increase in IC_{50} between T1/T0	**36**	**60**	**0.8**	**6**	**124**	**105**	**400**	**143**	**5**	**10**	/	/	**1287**	**2221**	**8**	**4**
Fold increase in IC_{50} between T2/T1	**35**	**60**	**9**	**14**	**254**	**351**	**705**	**252**	**7**	**14**	**66**	**193**	**4421**	**7635**	**13**	**7**
Patient 6 (subtype B)																
T0: 14Y-61N-99L	0.60	4.60	0.06	0.30	0.002	0.01	0.10	0.60	0.04	0.07	0.20	1.00	14.0	82.0	0.40	1.70
T1: 14Y-19P*-33I*-61N-71I*-75M*-84V*-90M*	14.0	110	2.20	17.0	0.50	3.90	3.80	30.0	0.20	1.50	14.0	107	8.20	65.0	4.80	17.0
T2: 14Y-19P*-61N-64V*-71I*-90M*-95I*	17.0	140	0.20	1.40	0.70	5.10	12.0	95.0	0.10	0.9	>17.6	>17.6	6.70	52.0	4.00	12.0
Fold increase in IC_{50} between T1/T0	**24**	**24**	**38**	**51**	**246**	**327**	**31**	**50**	**4**	**20**	**67**	**107**	**0.6**	**0.8**	**11**	**10**
Fold increase in IC_{50} between T2/T1	**30**	**30**	**3**	**4**	**324**	**429**	**100**	**161**	**3**	**12**	/	/	**0.5**	**0.6**	**10**	**7**
Patient 7 (subtype B)																
T0: 12T-14Y-19P-40N-41D-61N-62I-96S-99L	0.40	2.40	0.20	0.40	0.004	0.02	0.004	0.02	0.03	0.10	0.06	0.20	0.007	0.02	0.40	3.00
Patient 8 (subtype B)																
T0: 12Q-14R-17G/D-19P-61N-62I-92A	0.40	2.80	0.03	0.30	0.01	0.05	0.40	2.60	0.02	0.20	0.04	0.80	0.06	0.30	0.30	2.60
Patient 9 (subtype A)																
T0: 41D	0.80	3.30	0.03	0.30	0.10	0.50	0.006	0.20	0.04	0.10	0.20	2.10	0.02	0.20	0.30	0.7
Median IC_{50} and IC_{90} value at T0 (+/- SD)	0.60 (+/- 0.20)	3.30 (+/- 1.50)	0.06 (+/- 0.05)	0.30 (+/- 0.08)	0.004 (+/- 0.04)	0.03 (+/- 0.16)	0.02 (+/- 0.10)	0.40 (+/- 0.80)	0.03 (+/- 0.01)	0.14 (+/- 0.10)	0.06 (+/- 0.08)	0.40 (+/- 0.60)	0.008 (+/- 4.60)	0.05 (+/- 27.0)	0.30 (+/- 0.05)	2.50 (+/- 1.10)

*Substitutions selected between T0 and T1/T2. IC50 and IC90 values are measured in µM