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In vitro Phenotypic Susceptibility of HIV-2 Clinical Isolates to Protease Inhibitors

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

Delphine Desbois¹, Bénédicte Roquebert¹, Gilles Peytavin², Florence Damond¹, Gilles Collin¹, Antoine Bénard⁵, Pauline Campa⁴, Sophie Matheron³, Geneviève Chêne⁵, Françoise Brun-Vézinet¹ and Diane Descamps^{1*} for the French ANRS HIV-2 Cohort (ANRS CO 05 VIH-2).

1-Laboratoire de Virologie, Service de Microbiologie, Hôpital Bichat-Claude Bernard, Paris, France

2-Laboratoire de Toxicologie, Service de Pharmacie, Hôpital Bichat-Claude Bernard, Paris, France

3-Service de Maladies Infectieuses et Tropicales, Hôpital Bichat-Claude Bernard, Paris, France

4-Service de Maladies Infectieuses et Tropicales, Hôpital Saint-Antoine, Paris, France

5-ISPED Université Victor Segalen Bordeaux 2 INSERM U593, Bordeaux, France

*Corresponding author:

21 Dr Diane Descamps
22 Laboratoire de Virologie
23 Hôpital Bichat Claude Bernard
24 46 rue Henri Huchard
25 75018 Paris, France
26 Tel: +33 1 40 25 61 54;
27 Fax: +33 1 40 25 67 69;
28 E-mail: diane.descamps@bch.aphp.fr
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31 **Abstract**

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

36

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

38 resistance mutations

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41 Few data are available on HIV-2 susceptibility to antiretroviral agents. In France, as
42 recommended by the national expert group on the treatment of HIV infection, HIV-2-infected
43 patients receive highly active antiretroviral therapy (HAART) regimens as HIV-1-infected
44 individuals except non nucleoside reverse transcriptase inhibitors or fusion inhibitors classes
45 (17) (12). However, a recent study showed that CD4 cell recovery was poor in antiretroviral-
46 naive HIV-2 infected patients starting treatment with HAART (8). Thus it appears crucial to
47 determine HIV-2 susceptibility to the current PIs available, in order to define the optimal
48 regimen to be recommended.

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

63 The protease sequences of wild-type HIV-2 strains, when compared to HIV-1 clade B consensus
64 sequence, contained several amino acids associated with HIV-1 PI resistance, such as 10I/V
65 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/regacev/links/regacev_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

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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 IC₅₀ and IC₉₀ 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 μ M