

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

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

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

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6 Collin<sup>1</sup>, Antoine Bénard<sup>5</sup>, Pauline Campa<sup>4</sup>, Sophie Matheron<sup>3</sup>, Geneviève Chêne<sup>5</sup>,

7 Françoise Brun-Vézinet<sup>1</sup> and Diane Descamps<sup>1\*</sup> for the French ANRS HIV-2 Cohort

8 (ANRS CO 05 VIH-2).

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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-naive 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

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

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

93 Crystallographic studies with HIV-1 showed that darunavir interacts directly with the main  
94 chains of aspartic acid residues (Asp-29 and Asp-30), whereas other PIs interact with side  
95 chains in the S2 subsite of the HIV-1 enzyme (6) (7). Moreover, it has been reported in HIV-1  
96 that the binding affinity of darunavir for wild-type protease was > 100-fold higher than other PIs  
97 due to a slower dissociation rate of this molecule from the protease active site (5). In the same  
98 way, crystallography structure studies of the HIV-2 protease and binding affinity experiments  
99 might help us to understand the difference observed in the natural susceptibility of HIV-2 strains  
100 for these two drugs as well as phenotypic resistance in HIV-2 mutated strains. The IC<sub>50</sub> and  
101 IC<sub>90</sub> values of atazanavir, indinavir, nelfinavir and tipranavir for the HIV-2 isolates were higher  
102 than those observed for HIV-1 raising the hypothesis of a lower activity of these PIs against  
103 HIV-2. However these values were lower than their respective trough plasma concentrations.  
104 This might be explained by the fact that HIV-2 wild-type isolates harbored several amino acids  
105 associated with PI resistance in HIV-1.

106 PI treatment-associated amino acid changes in the HIV-2 protease gene occurred at positions  
107 known to confer PI resistance in HIV-1 and were not associated with the use of a particular PI  
108 without any order of accumulation. They altered the phenotypic susceptibility of the isolates to  
109 all the PIs tested here. These results are in keeping with data published elsewhere (2) (3) (9)  
110 (11) (13) (15) . Mutagenesis experiments coupled with phenotypic susceptibility testing might  
111 help to determine the impact of each substitutions in PI resistance. Saquinavir, lopinavir and  
112 darunavir appear to be the best choices for first-line therapy of HIV-2 infection, while  
113 amprenavir should not be used. Atazanavir and tipranavir might be used with care (17). Our  
114 results suggest that treatment guidelines for HIV-1-infected patients should not be directly  
115 extrapolated to HIV-2-infected patients. Virological efficacy data in vivo might help us to  
116 evaluate the place of PIs in HIV-2 antiretroviral strategy.

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122 Infection, February 25-28, 2007, Los Angeles, CA, USA.

123

124 **Drugs and sources**

125 Amprenavir was provided by GlaxoSmithKline (Marly-le-Roi, France), atazanavir by

126 Bristol-Myers Squibb (Rueil-Malmaison, France), darunavir by Tibotec (Mechelen,

127 Belgium), indinavir by Merck Sharp & Dohme-Chibret (West Point, PA, USA), lopinavir

128 by Abbott (Rungis, France), nelfinavir and saquinavir by Roche (Neuilly sur Seine,

129 France) and tipranavir by Boehringer-Ingelheim (Ridgefield, CT, USA).

130



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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 <sub>50</sub>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>
<b>BRU (HIV-1 reference isolate)</b>	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
<b>HIV-2 Isolates</b>																
<b>ROD (HIV-2 reference isolate)</b>	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
<b>Patient 1 (subtype H)</b>																
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 <sub>50</sub> between T1/T0	<b>12</b>	<b>30</b>	<b>16</b>	<b>32</b>	<b>225</b>	<b>126</b>	<b>621</b>	<b>193</b>	<b>57</b>	<b>17</b>	<b>236</b>	<b>79</b>	<b>252</b>	<b>114</b>	<b>19</b>	<b>20</b>
<b>Patient 2 (subtype A)</b>																
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 <sub>50</sub> between T1/T0	<b>8</b>	<b>3</b>	<b>4</b>	<b>11</b>	<b>71</b>	<b>222</b>	/	/	<b>1</b>	<b>5</b>	<b>463</b>	<b>450</b>	/	/	<b>24</b>	<b>12</b>
<b>Patient 3 (subtype A)</b>																
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 <sub>50</sub> between T1/T0	<b>5</b>	<b>9</b>	<b>11</b>	<b>6</b>	<b>18</b>	<b>45</b>	<b>304</b>	<b>206</b>	<b>12</b>	<b>21</b>	<b>68</b>	<b>65</b>	/	/	<b>15</b>	<b>5</b>
<b>Patient 4 (subtype A)</b>																
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 <sub>50</sub> between T1/T0	<b>22</b>	<b>9</b>	<b>2</b>	<b>6</b>	<b>216</b>	<b>88</b>	<b>15</b>	<b>28</b>	<b>75</b>	<b>42</b>	<b>24</b>	<b>37</b>	<b>45</b>	<b>33</b>	<b>12</b>	<b>9</b>
<b>Patient 5 (subtype A)</b>																
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 <sub>50</sub> between T1/T0	<b>36</b>	<b>60</b>	<b>0.8</b>	<b>6</b>	<b>124</b>	<b>105</b>	<b>400</b>	<b>143</b>	<b>5</b>	<b>10</b>	/	/	<b>1287</b>	<b>2221</b>	<b>8</b>	<b>4</b>
Fold increase in IC <sub>50</sub> between T2/T1	<b>35</b>	<b>60</b>	<b>9</b>	<b>14</b>	<b>254</b>	<b>351</b>	<b>705</b>	<b>252</b>	<b>7</b>	<b>14</b>	<b>66</b>	<b>193</b>	<b>4421</b>	<b>7635</b>	<b>13</b>	<b>7</b>
<b>Patient 6 (subtype B)</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 <sub>50</sub> between T1/T0	<b>24</b>	<b>24</b>	<b>38</b>	<b>51</b>	<b>246</b>	<b>327</b>	<b>31</b>	<b>50</b>	<b>4</b>	<b>20</b>	<b>67</b>	<b>107</b>	<b>0.6</b>	<b>0.8</b>	<b>11</b>	<b>10</b>
Fold increase in IC <sub>50</sub> between T2/T1	<b>30</b>	<b>30</b>	<b>3</b>	<b>4</b>	<b>324</b>	<b>429</b>	<b>100</b>	<b>161</b>	<b>3</b>	<b>12</b>	/	/	<b>0.5</b>	<b>0.6</b>	<b>10</b>	<b>7</b>
<b>Patient 7 (subtype B)</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
<b>Patient 8 (subtype B)</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
<b>Patient 9 (subtype A)</b>																
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
<b>Median IC<sub>50</sub> and IC<sub>90</sub> value at T0 (+/- SD)</b>	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. IC<sub>50</sub> and IC<sub>90</sub> values are measured in μM