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PHYLOGENY AND RESISTANCE PROFILES OF HIV-1 POL SEQUENCES FROM RECTAL BIOPSIES AND BLOOD

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Keywords:	HIV resistance, anatomical site, viral RNA, proviral DNA



patient	HIV RNA cp/mL	CD4 cell count	current treatment	no previous NRTI	no previous NNRTI	no previous PI
1	96.000	112 x 10(6)/L	ddI, ABC,LPV/RTV	4	2	2
2	202.000	66 x 10(6)/L	AZT,3TC,LPV/RTV,APV	4	2	3
3	1.640	26 x 10(6)/L	AZT,3TC,LPV/RTV,APV	3	2	3
4	8.083	608 x 10(6)/L	ABC,EFV,LPV/RTV	3	0	2
5	11.600	250 x 10(6)/L	AZT,3TC,ABC	3	1	4
6	34.000	602 x 10(6)/L	3TC,ABC,EFV	3	0	4

Table I. Patient characteristics.

The CD4 cell counts, viral load and treatment regimen at time of biopsy and concurrent plasma and PBMC sampling is given. Number of previous NRTI's, NNRTI's and PI's is listed.

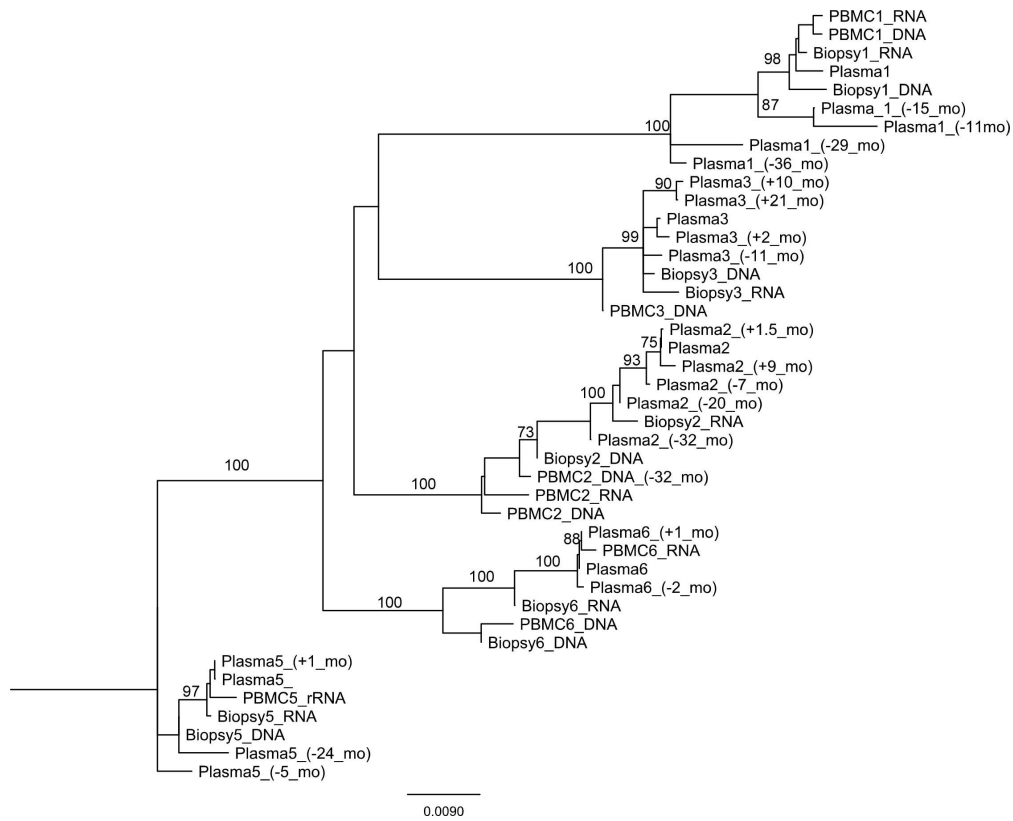
ABC; Abacavir, AZT; Zidovudine, APV; Amprenavir, ddI; Didanosine, EFV; Efavirenz, 3TC; Lamivudine, LPV/RTV; Lopinavir/Ritonavir.

297x209mm (300 x 300 DPI)

PR mutations		L10P/I/R/V	K20M/R	D30N	L33F	M36I	M46I/L	I47V	F53L	I54V/M/L	D60E	L63P	A71V/T	G73S/A	V77I	V82F/A/T/S	I84V	L90M	I93L		
PT1	PLASMA (-38 months)	I				I						P		V	GS	VI		IV	M	L	
	PLASMA (-29 months)	I				MI				V		P						IV	M	L	
	PLASMA (-15 months)	I				I						P						V	M	L	
	PLASMA (-11 months)	I								V		P							V	M	L
	PLASMA-RNA	I				I						P					VII		V	M	L
	PBMC-RNA	I				I					V	P					I		V	M	L
PBMC-DNA	I				I					V	P					VII		V	M	L	
BIOPSI-RNA	I				I					V	P					VII		V	M	L	
BIOPSI-DNA	I				I					V	P							V	M	L	
PT2	PBMC-DNA (-32 months)	LI	R			I			L	V		P	V	T		A				M	
	PLASMA (-32 months)	LI							FL												M
	PLASMA (-20 months)	LI	KR						L	V		P	V	T		A				M	
	PLASMA (-7 months)	I	R			F	I	I	IV	L	V	P	V	T		A				M	
	PLASMA-RNA	I				F	I	I	IV	L	V	P	V	T		A				M	
	PBMC-RNA	I							L	V		P	V	T		A				LM	
	PBMC-DNA	I							FL			P	X	GT						M	
	PBMC-RNA	I							L	V		P	V	T		A				M	
	BIOPSI-RNA	I							L	V		P	V	T		A				LM	
	BIOPSI-DNA	LI				F	MI			V	L	V	P	V	GT		A			M	
PT3	PLASMA (+1.5 month)	I	R			F	I		V	L	V	P	V	T		A				M	
	PLASMA (+9 months)	I	R			LF	I		V	L	V	P	V	T		A				M	
PT3	PLASMA (-1 months)	I				I	I		V			P				F	V			M	
	PLASMA (-4 months)	I	KM			F	I	I		V		P	AV			F	IV			M	
	PLASMA-RNA	I								V		P								M	
	PBMC-RNA	IF	R	DN			I	MI		V		P				VF	IV			M	
	BIOPSI-RNA	I	KR				I			V		P				F	V			M	
	BIOPSI-DNA	I	R				I			V		P				F	V			M	
	PLASMA (+2 months)	I	M				I	I		V		P				F	V			M	
	PLASMA (+10 months)	I				LF	I	I		V		P				F	V			M	
	PLASMA (+21 months)	I				LF	I	I		V		P				F	V			M	
	PLASMA (-13 months)	I	I				I			V		P	V			A				M	
PT4	PLASMA-RNA	F	I						V		P	V			A					M	
	PBMC-RNA	I							V		P	V			A					M	
	PBMC-DNA	F	I						V		P	V			A					M	
	BIOPSI-RNA	F	I						V		P	V			A					M	
PT5	PLASMA (+5 months)	F	I						V		P	V			A					M	
	PLASMA (-24 months)											P	T							M	
	PLASMA (-6 months)											P	T							M	
	PLASMA-RNA											P								M	
PT6	PBMC-RNA											P								LM	
	PBMC-DNA											P								LM	
	BIOPSI-RNA	I										P	AT							LM	
	BIOPSI-DNA	I										P	AT							LM	
PT6	PLASMA (+1 month)											P				S				M	
	PLASMA-RNA											P				S				M	
	PBMC-RNA											P				S				M	
	PBMC-DNA											P				S				M	
	BIOPSI-RNA											P				S				M	
	BIOPSI-DNA											P				S				M	
PT6	PLASMA (+1 month)											P				GS				M	
	PLASMA (+1 month)											P				S				M	

Table II. Mutations at positions conferring resistance against protease inhibitors. The samples collected at time of rectal biopsies shades light gray. Samples that could not be amplified are shades dark gray.

297x209mm (300 x 300 DPI)



184x148mm (309 x 309 DPI)

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2 1 **PHYLOGENY AND RESISTANCE PROFILES OF HIV-1 POL SEQUENCES FROM**
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4 **RECTAL BIOPSIES AND BLOOD**
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25 Short title: HIV-1 sequences in rectal biopsies and blood

1
2 26 **ABSTRACT**
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6
7 28 The phylogeny and resistance profiles of human immunodeficiency virus type 1 (HIV-
8
9 29 1) protease (PR) and reverse transcriptase (RT) sequences were compared among
10
11 30 six patients with HIV-1 who had received numerous treatments. RNA and DNA
12
13 31 fractions were obtained from concurrent blood and rectal biopsy samples.
14
15
16 32 Phylogenetic trees and resistance profiles showed that the rectal mucosa and the
17
18 33 peripheral blood mononuclear cells (PBMCs) harboured different HIV-1 strains. The
19
20 34 resistance-associated mutations found in each strain corresponded to the treatment
21
22 35 history of the patients. The resistance mutations acquired during earlier treatment
23
24 36 regimens were detected in the sequences obtained from the rectal samples and in
25
26 37 the PBMCs in several of the patients. Also, differences in the resistance profiles were
27
28 38 observed between anatomical sites and between RNA and DNA fractions. Thus, a
29
30 39 single sample probably will not be representative of the HIV-1 archived in different
31
32 40 sites. Both the resistance profile and phylogeny of HIV-1 often differed in sequences
33
34 41 obtained from RNA and DNA from the same site. These findings suggest that
35
36 42 additional information regarding the antiviral resistance profile of the patient might be
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38 43 obtained by testing different anatomical sites.
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54 48 Keywords: HIV resistance, anatomical site, proviral DNA, viral RNA
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INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) exists in chronically infected patients as a diverse population of related genetic variants. It persists as a reservoir, capable of rebounding, either in the absence of drug treatment or by evolution of resistant escape mutants. From this reservoir, integrated proviral DNA of the HIV-1 genome can add to the replicating pool of virus upon cellular activation. Several anatomical sites are potential reservoirs for HIV-1, and several studies have shown diverse virus evolution at the different sites (Blankson et al., 2002; Finzi et al., 1997; Kemal et al., 2003; Pantaleo et al., 1991; Tirado et al., 2004).

The intestinal mucosa contains most of the body's lymphoid tissue (Kotler, 2005); thus, it is potentially a vast site for persistent HIV-1 infection. The rectal mucosa is part of the intestinal compartment. Several studies have shown that detectable levels of proviral DNA and HIV RNA were present in rectal biopsies of HIV-1 infected patients, even when the plasma HIV-RNA levels were below the limit of detection (Anton et al., 2003; Monno et al., 2003). Therefore, rectal mucosa might serve as a reservoir of HIV-1 and harbour resistance mutations from earlier time periods. Furthermore, due to the pharmacokinetics of antiretroviral drugs suboptimal concentrations might be obtained at the rectal mucosa allowing development of resistant HIV-1 variants.

The aim of the present study was to determine whether the rectal mucosal was a reservoir that harboured variants of HIV-1 not found in the plasma. We compared the RNA and DNA resistance profiles and phylogeny of HIV-1 obtained from three different anatomical sites: rectal biopsies, peripheral blood mononuclear cells (PBMCs), and plasma from patients that underwent highly active antiretroviral treatments (HAART).

1
2 75 **MATERIALS AND METHODS**
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6
7 77 **Patients**
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9 78 Intestinal tissue and blood samples were collected from six patients with HIV
10
11 79 infections in November, 2001. All patients had received HAART for several years,
12
13 80 and showed virological failure. The patients exhibited a substantial number of
14
15 81 resistance mutations, based on examinations of plasma HIV RNA circulating in the
16
17 82 blood. The patient characteristics are given in Table I. The study was approved by
18
19 83 the Danish National Committee on Biomedical Research Ethics (no. 2001 0197).
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21 84 Written informed consent was obtained from all patients.
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29 86 In all six patients, HIV RNA was obtained from plasma and had been tested
30
31 87 previously for HIV-1 resistance as part of a routine follow-up analysis with the
32
33 88 Viroseq, HIV-1 Genotyping System version 2, (Abbott diagnostics, Foster City, USA).
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39 90 **Rectal biopsies**
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41 91 Two rectal mucosa biopsies were obtained from each patient. Biopsies were kept at
42
43 92 – 80 °C until nucleic acid extraction was performed. Concurrent with the biopsy,
44
45 93 blood was collected in tubes containing EDTA, separated into plasma and PBMC
46
47 94 fractions, and stored at -80 °C.
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56 97 **Extraction of viral DNA and RNA from rectal tissue and PBMCs**
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58 98 PBMCs were isolated from 500 µl blood by centrifugation at 16,000 x g for 3 min.

59 99 The supernatant was then removed, and 1 ml of PBS was added to wash the cells.

60 100 The sample was vortexed briefly and cells were collected after a second

1
2 101 centrifugation.

3
4 102

5
6
7 103 PBMC and rectal biopsies were homogenised in buffer with a micro-homogeniser.

8
9 104 Then, DNA and RNA were isolated with the Qiagen combined RNA/DNA kit (Qiagen,

10
11 105 Hilden, Germany), according to the manufacturer's instructions. Additional washing

12
13 106 steps were added to avoid contamination of RNA fragments with DNA.

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16 107

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18
19 108 **Amplification of the *pol* gene**

20
21 109 The protease region (PR) and part of the reverse transcriptase (RT) region (strain

22
23 110 HXB2, position 2136-3462) of the HIV-1 polymerase gene (*pol*) were separately

24
25 111 amplified from the DNA and RNA extracted from rectal biopsies and PBMCs. Nested

26
27 112 PCR procedures were performed, and positive and negative controls were included

28
29 113 in each set up. To prevent contamination between tests, RNA and DNA from

30
31 114 different fractions of the same patient were amplified on separate runs. To assess

32
33 115 whether RNA preparations were contaminated with HIV DNA, nested PCR assays

34
35 116 were performed without a previous reverse transcriptase step. Additionally,

36
37 117 phylogenetic analyses were performed to control for PCR contamination and sample

38
39 118 mix-ups between patients.

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47 120 **cDNA synthesis**

48
49 121 cDNA was synthesised from RNA fractions with MuLV reverse transcriptase (Applied

50
51 122 Biosystems, Foster City, USA). The conditions were as follows: the mixture was

52
53 123 heated to 65 °C for 2 min and cooled to 42 °C before adding MuLV reverse

54
55 124 transcriptase; then it was incubated at 42 °C for 60 min.

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126 **PCR amplification of the PR-encoding sequences.**

1
2 127 The PR gene was amplified with nested PCR primers: 5'Prot1 (5' AGG CTA ATT
3
4 128 TTT TAG GAA GAT CTG GCC TTC C 3'), which corresponded to positions 2078-
5
6 129 2109 of HXB2; 5'Prot2 (5' TCA GAG CAG ACC AGA GCC AAC AGC CCC ACC AG
7
8 130 3'), which corresponded to positions 2136-2167 of HXB2; 3'Prot1 (3' GCA AAT ACT
9
10 131 GGA GTA TTG TAT GGA TTT TCA GG 3'), which corresponded to positions 2734-
11
12 132 2703 of HXB2; and 3'Prot 2 (5' ATT GCT TTT ATT TTT TCT TCT GTC AAT GGC
13
14 133 3'), which corresponded to positions 2650-2621 of HXB2. AmpliTaq DNA polymerase
15
16 134 (Applied Biosystems, Foster City, USA) was used for both PCR runs. The cycling
17
18 135 conditions for the first PCR run were as follows: 35 cycles that consisted of 30 s at
19
20 136 95 °C, 30 s at 55 °C, and 30 s at 72 °C. The cycling conditions for the second PCR
21
22 137 were as follows: 35 cycles that consisted of 30 s at 95 °C, 30 sec at 59 °C, and 1 min
23
24 138 30 s at 72 °C.
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140 **PCR amplification of the RT encoding sequences.**

141 A nucleotide fragment of 1008 base pairs that corresponded to positions 2454-3462 of
142 HXB2 was amplified with a nested PCR procedure, as described by Nijhuis et al
143 (Nijhuis et al., 1995).
144

145

145 **Population based sequencing.** The primers used to sequence the PR gene were

146 5'Prot2 and Pr 2 (5' CTT TTG GGC CAT CCA TTC 3'), which corresponded to
147 positions 2610-2593 of HXB2. The primers used to sequence the RT gene were
148 RT-19; B-RT (5' GAT GGA AAG GAT CAC C 3'), which corresponded to positions
149 3004-3019 of HXB2; and K-RT (5' CAG GAT GGA GTT CAT AAC 3'), which
150 corresponded to positions 3258-3241 of HXB2.
151

151

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1
2 152 **Viral RNA extraction and sequencing of the *pol* gene from plasma samples**

3
4 153 Viral RNA was extracted from plasma and sequenced with the Viroseq, HIV-1
5
6 154 Genotyping System, version 2 (Abbott, Wiesbaden, Germany). A 1.8-kilobase (kb)
7
8 155 amplicon was generated by reverse transcription-PCR amplification and sequenced.
9
10 156 This provided sequence data for codons 1-99 of the PR gene and 1-320 of the RT
11
12 157 gene. The sequences were assembled and analysed with the HIV-1 Genotyping
13
14 158 System software version 2 (Abbott, Wiesbaden, Germany).
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16
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21 160 **Sequence alignment, phylogeny, and interpretation of drug resistance**

22
23 161 Mutations at resistance conferring positions in the HIV RT and PR regions were
24
25 162 scored according to the mutation list from the Stanford HIV RT and PR sequence
26
27 163 database (<http://hivdb.stanford.edu/>). The scores for resistance mutations in the
28
29 164 rectal RNA and DNA fractions were compared to the scores for mutations found in
30
31 165 the plasma viral RNA and PBMC RNA and DNA fractions. Comparisons were also
32
33 166 made among the available sequences from earlier and later time-points during the
34
35 167 infection of each patient. The scores for resistance mutations in all fragments and
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37 168 time-points were compared with the treatment history of each patient.
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45 170 The sequences were also compared by phylogenetic analyses. Fragments from each
46
47 171 of the PR and RT sequences were merged before the sequences were aligned. This
48
49 172 was performed with the CLUSTAL-W (Thompson et al., 1994) programme from
50
51 173 Geneious software (ver.Pro.4.75),(Biomatters, Auckland, New Zealand) with the
52
53 174 default settings. The Kimura two-parameter model (Kimura, 1980) in Geneious was
54
55 175 used to calculate the pairwise nucleotide distances. The phylogenetic trees were
56
57 176 constructed with the neighbour-joining algorithm, (Felsenstein, 1991) integrated in
58
59 177 the Geneious package. The trees were bootstrapped with 100 replications to test for
60

1
2 178 the significance of the branches of the tree with Geneious Tree Builder.

3
4 179

5
6 180 The overall amino acid divergence of the subtype B *pol* gene was calculated using
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8 181 the HXB2 strain (acc. nr K03455) as reference. The calculations were performed both
9
10 182 with and without drug resistance mutations. To analyse the intra-patient divergence
11
12 183 in drug resistance mutations, the amino acids at resistance-conferring codons in the
13
14 184 PR and RT genes were compared among samples from different tissues. For
15
16 185 example, plasma RNA sequences collected at the time of biopsies were compared to
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18 186 PBMC and rectal biopsy sequences that had been collected concurrently. The
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20 187 percentage of discordance in both genes was calculated by comparing RNA from
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22 188 plasma with RNA and DNA from other compartments for each patient.
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30 190 **RESULTS**

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34
35 192 The treatment history, viral load, and CD4-positive cell counts for the patients are
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37 193 shown in Table I. Plasma, PBMCs, and rectal biopsies were obtained from all six
38
39 194 patients. The resistance-conferring mutations for the PR and RT regions are shown
40
41 195 in Tables II and III, respectively. Furthermore, Tables II and III present a total of 20
42
43 196 *pol* sequences that were obtained from plasma samples collected at time points prior
44
45 197 to the biopsy date. It was not possible to amplify material from all the samples for
46
47 198 sequencing. Patient 4 was infected with subtype C, while all the other patients were
48
49 199 infected with subtype B HIV-1.
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55
56 201 The resistance profiles of samples obtained concurrently from the different
57
58 202 compartments showed different levels of variation (Tables II, III). The resistance
59
60 203 profiles of all included samples for the PR gene are shown in Table II. The resistance

1
2 204 profiles corresponded well to the treatment histories for the individual patients (Table
3
4 205 I -III). Samples from patients 1, 5, and 6 that were taken at the time of biopsy (light
5
6 206 gray shading) showed that the resistance profiles found in the plasma were not very
7
8
9 207 different from those found in PBMC and biopsy samples

10
11 208 The resistance profiles from the various compartments also showed a high degree of
12
13 209 concordance in the RT region. However, some variations between compartments
14
15 210 were noted in the RT gene (Table III).
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20
21 212 The RNA fractions of HIV sequences collected concurrently from each patient
22
23 213 showed variable levels of variation; for example, the samples from patient 2 showed
24
25 214 moderate divergence and the samples from patient 5 showed full concordance
26
27
28 215 (Table II, III). A similar level of variation was observed in the DNA fractions from
29
30 216 patients 1, 3, and 6 (Table II). The patients that harboured subtype B of HIV showed
31
32 217 an average of 6-14% overall divergence compared to HXB2 when drug resistance
33
34 218 mutations were included, and 3-9% when drug resistance mutations were excluded.
35
36
37 219 At the resistance conferring positions, the level of discordance for each patient was
38
39 220 calculated between the profiles in plasma RNA and the other samples .This
40
41 221 discordance ranged from 0 to 51% in the PR region and 3 to 37% for the RT region.
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44 222

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46
47 223 A phylogenetic tree was constructed that included the *pol* sequences from all
48
49 224 available samples collected from the five patients that were infected with subtype B
50
51 225 of HIV (Fig. 1). The samples from each patient formed a monophylogenetic cluster
52
53 226 with 100% bootstrap support; this indicated that contamination among patients was
54
55 227 successfully avoided in the PCR amplifications.
56
57

58 228
59
60 229 In addition, sequences were retrieved from time points before and after the time of

1
2 230 biopsy. The samples from the time of biopsy were scattered throughout the
3
4 231 phylogenetic tree. The sequences did not show any particular placement in the
5
6 232 phylogenetic tree; however, the plasma sample obtained at the same time as the
7
8 233 biopsy tended to group with samples taken a few months before and after the date of
9
10 234 sampling. This was especially true for patients 1 and 6. Three PBMC RNA samples
11
12 235 were located at the tips of the clusters for patients 1, 5, and 6; but, the PBMC RNA
13
14 236 from patient 2 was placed closer to the root of the tree. For PBMC DNA, the samples
15
16 237 from three patients (2, 3, and 6) represented phylogenetically older isolates; but this
17
18 238 was not the case for patient 1, and no samples were available for patient 5. Likewise,
19
20 239 there was no clear pattern with regard to the phylogenetic position of the rectal
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22 240 biopsy samples (DNA and RNA; Figure 1).
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30 242 **DISCUSSION**

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32 243
33
34 244 In this study, the PR and RT sequences of HIV-1 RNA and DNA from blood were
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36 245 compared to those of the rectal mucosal biopsies from six patients infected with
37
38 246 HIV-1 that underwent numerous treatments who had received numerous
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40 247 antiretroviral regimens.
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47 249 All of the patients had HIV-1 *pol* RNA and DNA sequences in the plasma, PBMC,
48
49 250 and rectal fractions, with a substantial number of mutations associated with
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51 251 resistance to antiretroviral drugs. These mutations corresponded to the treatment
52
53 252 history of the patients. Several patients showed traces of previous antiretroviral
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55 253 treatment regimens in the form of resistance mutations or reversions of resistance
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57 254 mutations. This was probably due to the fact that they had experienced various failed
58
59 255 regimens during long periods of high viral load. In this type of patient, the viral
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1
2 256 population typically consists of a potentially wide range of viruses with different
3
4 257 resistance profiles. For example, patient 1 harboured a virus population in the rectal
5
6 258 biopsy DNA that had a G190A mutation in the RT gene. This mutation was
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8
9 259 associated with resistance to non-nucleoside reverse transcriptase inhibitors
10
11 260 (NNRTIs; Table III), and the patient had previously received nevirapine (NVP) and
12
13 261 efavirenz (EFV) treatments, while the current antiretroviral treatment did not include a
14
15 262 NNRTI (Table I). The samples were collected some years ago, prior to the
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17
18 263 introduction of second generation NNRTIs, PIs, and the new drug classes that target
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20 264 CCR5 and the integrase. This is reflected both in the treatments given and in the
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22 265 failure to obtain virological suppression.
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28 267 In the phylogenetic tree (Figure 1), the *pol* sequences derived from the RNA and
29
30 268 DNA isolated from the blood and rectal biopsy of each patient were dispersed among
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32 269 plasma HIV RNA sequences derived from different time points of the infection. This
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34 270 suggested that the sequences either represented archived virus or the dominant
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36 271 population of the recently infected cells.
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41
42 273 The comparison between the rectal biopsy samples and the blood samples from the
43
44 274 six patients did not reveal a clear pattern in the distribution of mutations. The results
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46 275 showed that a single site can harbour different HIV-1 proviral DNA copies, and the
47
48 276 one selected during PCR probably varies.
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54 278 The RNA fractions found in the plasma, PBMCs, and biopsies represented
55
56 279 replicating viruses. In most of the patients, the RNA fractions from different sites
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58 280 showed diverse resistance profiles (Table II, III) and were often separated in the
59
60 281 phylogenetic tree (Fig 1). This suggested that there was continuous replication of

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2 282 viruses that had been archived at different time points. This result was in line with the
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4 283 finding that hidden resistant viruses can be rapidly recruited when a patient is
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7 284 reverted to a previous drug regimen (Deeks et al., 2001).
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11 286 Earlier studies have analysed the genotypes and resistance profiles of HIV-1 in blood
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14 287 compared to a different anatomical site, like the rectal mucosa. For example, Di
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16 288 Stefano et al.(Di Stefano et al., 2001) studied rectal mucosa samples from 14 HIV-1
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18 289 positive patients and found a high level of discordance compared to blood samples
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21 290 in the resistance positions of the RT (74.6%) and PR (88.8%) genes. The median
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23 291 percentage discordance at the amino acid level was 8% in the RT and 5% in the PR.
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26 292 In contrast, Poles et al.(Poles et al., 2001) compared HIV-1 RNAs isolated from the
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28 293 rectal mucosa, PBMCs, and plasma from 7 HIV-1 positive patients. They found a
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30 294 high level of concordance among the RT and PR resistance positions. In this study,
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32
33 295 the median percentage discordance was 11.2% in the PR gene and 11.7% in the RT
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35 296 gene at resistance positions in samples from different sites and time points. At the
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37 297 amino acid level, the percent discordances were 4.8% and 2.6% for the PR and RT
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40 298 proteins, respectively (Poles et al.,2001). Those results were similar to the results
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42 299 found in the current study. The level of discordance at resistance positions in the PR
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45 300 and RT genes between anatomical sites is probably due to the duration of infection,
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47 301 the number of changes in the treatment regimen, and the level of viral load during
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49 302 phases of failing antiretroviral treatment.
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54 304 For patients with an undetectable viral load, the genotyping of proviral DNA, either
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56 305 from PBMCs or from lymphoid tissue, might be informative. In the current study,
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59 306 resistance mutations acquired during earlier treatment regimens were detected in
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307 samples from rectal site and the PBMCs in several of the patients. This suggested

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2 308 that archived viruses were present in both compartments. Also, differences in the
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4 309 resistance profiles were observed between anatomical sites and between RNA and
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6 310 DNA fractions from the same site. Thus, a single sample probably will not be
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8 311 representative of the HIV-1 archived in different anatomical sites. Moreover, both the
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10 312 resistance profile and phylogeny of HIV-1 often differed in sequences obtained from
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12 313 RNA and DNA from the same site. This suggested that HIV-1 from different time-
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14 314 points of the infection were present in the same compartment.
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