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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		L10F/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 (- 36 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	
	PLASMA (- 11 months)	I								V		P					V	M	
	PLASMA-RNA	I				I						P				VII	V	M	
	PBMC-RNA	I				I				V		P				I	V	M	L
	PBMC-DNA	I				I				V		P				VII	V	M	L
PT2	BIOPSI-RNA	I				I				V		P				VII	V	M	L
	BIOPSI-DNA	I				I				V		P				V	V	M	L
	PBMC-DNA (- 32 months)	LI	R			I			L	V		P	V	T		A		M	
	PLASMA (- 32 months)	LI							FL										
	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	
	PBMC-RNA	I							L			P	V	T				LM	
PT3	PBMC-DNA	I				I			FL			P	X	QT				M	
	BIOPSI-RNA	I				I			L			P	V	T				M	
	BIOPSI-DNA	LI				MI						P		QT				LM	
	PLASMA (+ 1.5 months)	I	R		F			V	L	V		P	V	T		A		M	
	PLASMA (+ 9 months)	I	R	LF				V	L	V		P	V	T		A		M	
	PLASMA (- 11 months)	I				I	I		V			P				F	V	M	
	PLASMA (- 4 months)	I							V			P							
PT4	PLASMA-RNA	I	KM		F	I	I		V			P	AV			F	IV	M	
	PBMC-RNA	I										P							
	PBMC-DNA	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		V			P				F	V	M	
PT5	PLASMA (+ 21 months)	I			LF		I		V			P				F	V	M	
	PLASMA (- 13 months)	I	I			I			V			P	V			A		M	
	PLASMA-RNA	F	I			I			V			P	V			A		M	
	PBMC-RNA																		
	PBMC-DNA	F	I			I			V			P	V			A		M	
	BIOPSI-RNA																		
	BIOPSI-DNA	F	I			I			V			P	V			A		M	
PT6	PLASMA (+ 6 months)	F	I			I			V			P	V						
	PLASMA (- 24 months)											P	T		I			M	
	PLASMA (- 6 months)											P	T		I			M	
	PLASMA-RNA											P						LM	
	PBMC-RNA											P							
	PBMC-DNA	I										P				I		LM	
	BIOPSI-RNA	I										P	AT			I		LM	
PT9	BIOPSI-DNA																		
	PLASMA (+ 1 month)											P		S		I		M	
	PLASMA-RNA											P		S		I		M	
	PBMC-RNA											P		S		I		M	
	PBMC-DNA											P	del 4 bp			I		M	
	BIOPSI-RNA											P				I		M	
	BIOPSI-DNA											P		GS		I		M	
PT10	PLASMA (+ 1 month)											P		S		I		M	
	PLASMA (- 1 month)											P		S		I		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)

RT-mutations		W41L	E41D	D47N	R66Q	T69D	K70R	V75T/A	L76V	A80Q	L101E	K103N	V106A	V108N	V118Q	V172D	V181G	M184V	V186L	G186A	L210W	T215V/Y	K219Q/E			
PT1	PLASMA (36 months)	ML	N					RR	I	S							C				A	W	Y	E		
	PLASMA (39 months)	L	N					RR	V	S											A	W	Y	E		
	PLASMA (45 months)	L	ED	N				RG	T	I	S										A	W	Y	REGO		
	PLASMA (52 months)	L	D	S		SG		G	T	I	S										A	W	Y	R		
	PLASMA-RNA	L	D	S				G	T	I	S													YR		
	BIOPS-DNA	L	D	S				G	T	I	S													YR		
	BIOPS-RNA	L	D	S				G	T	I	S													YR		
PT2	BIOPS-DNA (32 months)	L	D	N				G	T	I	S										A	W	Y	KE		
	PLASMA (32 months)	L	ED	N				R	W				N	I									W	Y	KE	
	PLASMA (39 months)	L	ED	N				R	W				N	W	I							W	Y	E		
	PLASMA (47 months)	L	ED	N		TS		R	W				N	W	I					V		W	Y	E		
	PLASMA-RNA	L	D	N				R	W				N	I						MV			W	Y	E	
	BIOPS-DNA	ML	D	N				RR												MV			W	Y	KE	
	BIOPS-RNA	L	D	N				R	W				N	I	I					V			W	Y	KE	
PT3	BIOPS-DNA	L	D	N				R	W				N	I								W	Y	KE		
	PLASMA (47.5 months)	L	D	N				R	W				N	I						V			W	Y	KEGR	
	PLASMA (52 months)	L	D	N				R	W				N	I										Y	N	
	PLASMA (1 month)	L	D	G	G															V	L		W	Y	N	
	PLASMA (4 months)	L	D	G	G															V	L		W	Y	N	
	PLASMA-RNA	L	D	G	G															V	L		W	Y	N	
	BIOPS-DNA	L	D	V	G	G														V	L		W	Y	N	
PT4	BIOPS-RNA	L	D	G	G	G														V	L		W	Y	N	
	BIOPS-DNA	L	D	G	G	G														V	L		W	Y	N	
	PLASMA (19 months)	L	D	G	G															V	L		W	Y	N	
	PLASMA (46 months)	L	D	Y	G	G		HR												V	L		W	Y	N	
	PLASMA (47 months)	L	D	Y	G	G														V	L		W	Y	N	
	PLASMA (47 months)	L	D	N																					Y	N
	PLASMA (47 months)	L	D	N																					Y	N
PT5	PLASMA (34 months)																									
	PLASMA (4 months)																									
	PLASMA-RNA	L	D	N																						
	BIOPS-DNA																									
	BIOPS-RNA																									
	PLASMA (4 months)	L	D	N																						
	PLASMA (4 months)	L	D	N																						
PT6	PLASMA (13 months)																									
	PLASMA-RNA																									
	BIOPS-DNA	L	D	N																						
	BIOPS-RNA																									
	PLASMA (4 months)	L	D	N																						
	PLASMA (4 months)	L	D	N																						
	PLASMA (4 months)	L	D	N																						
PT7	PLASMA (13 months)																									
	PLASMA-RNA																									
	BIOPS-DNA																									
	BIOPS-RNA																									
	PLASMA (4 months)	L	D	N																						
	PLASMA (4 months)	L	D	N																						
	PLASMA (4 months)	L	D	N																						
PT8	PLASMA (13 months)																									
	PLASMA-RNA																									
	BIOPS-DNA																									
	BIOPS-RNA																									
	PLASMA (4 months)	L	D	N																						
	PLASMA (4 months)	L	D	N																						
	PLASMA (4 months)	L	D	N																						
PT9	PLASMA (13 months)																									
	PLASMA-RNA																									
	BIOPS-DNA																									
	BIOPS-RNA																									
	PLASMA (4 months)	L	D	N																						
	PLASMA (4 months)	L	D	N																						
	PLASMA (4 months)	L	D	N																						
PT10	PLASMA (13 months)																									
	PLASMA-RNA																									
	BIOPS-DNA																									
	BIOPS-RNA																									
	PLASMA (4 months)	L	D	N																						
	PLASMA (4 months)	L	D	N																						
	PLASMA (4 months)	L	D	N																						
PT11	PLASMA (13 months)																				</

184x148mm (309 x 309 DPI)

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

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Short title: HIV-1 sequences in rectal biopsies and blood

ABSTRACT

The phylogeny and resistance profiles of human immunodeficiency virus type 1 (HIV-1) protease (PR) and reverse transcriptase (RT) sequences were compared among six patients with HIV-1 who had received numerous treatments. RNA and DNA fractions were obtained from concurrent blood and rectal biopsy samples.

Phylogenetic trees and resistance profiles showed that the rectal mucosa and the peripheral blood mononuclear cells (PBMCs) harboured different HIV-1 strains. The resistance-associated mutations found in each strain corresponded to the treatment history of the patients. The resistance mutations acquired during earlier treatment regimens were detected in the sequences obtained from the rectal samples and in the PBMCs in several of the patients. Also, differences in the resistance profiles were observed between anatomical sites and between RNA and DNA fractions. Thus, a single sample probably will not be representative of the HIV-1 archived in different sites. Both the resistance profile and phylogeny of HIV-1 often differed in sequences obtained from RNA and DNA from the same site. These findings suggest that additional information regarding the antiviral resistance profile of the patient might be obtained by testing different anatomical sites.

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).

MATERIALS AND METHODS

Patients

Intestinal tissue and blood samples were collected from six patients with HIV infections in November, 2001. All patients had received HAART for several years, and showed virological failure. The patients exhibited a substantial number of resistance mutations, based on examinations of plasma HIV RNA circulating in the blood. The patient characteristics are given in Table I. The study was approved by the Danish National Committee on Biomedical Research Ethics (no. 2001 0197). Written informed consent was obtained from all patients.

In all six patients, HIV RNA was obtained from plasma and had been tested previously for HIV-1 resistance as part of a routine follow-up analysis with the Viroseq, HIV-1 Genotyping System version 2, (Abbott diagnostics, Foster City, USA).

Rectal biopsies

Two rectal mucosa biopsies were obtained from each patient. Biopsies were kept at – 80 °C until nucleic acid extraction was performed. Concurrent with the biopsy, blood was collected in tubes containing EDTA, separated into plasma and PBMC fractions, and stored at -80 °C.

Extraction of viral DNA and RNA from rectal tissue and PBMCs

PBMCs were isolated from 500 µl blood by centrifugation at 16,000 x g for 3 min. The supernatant was then removed, and 1 ml of PBS was added to wash the cells. The sample was vortexed briefly and cells were collected after a second

1
2 101 centrifugation.
3
4 102
5
6 103 PBMC and rectal biopsies were homogenised in buffer with a micro-homogeniser.
7
8 104 Then, DNA and RNA were isolated with the Qiagen combined RNA/DNA kit (Qiagen,
9
10 105 Hilden, Germany), according to the manufacturer's instructions. Additional washing
11
12 106 steps were added to avoid contamination of RNA fragments with DNA.
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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.**

The PR gene was amplified with nested PCR primers: 5'Prot1 (5' AGG CTA ATT TTT TAG GAA GAT CTG GCC TTC C 3'), which corresponded to positions 2078-2109 of HXB2; 5'Prot2 (5' TCA GAG CAG ACC AGA GCC AAC AGC CCC ACC AG 3'), which corresponded to positions 2136-2167 of HXB2; 3'Prot1 (3' GCA AAT ACT GGA GTA TTG TAT GGA TTT TCA GG 3'), which corresponded to positions 2734-2703 of HXB2; and 3'Prot 2 (5' ATT GCT TTT ATT TTT TCT TCT GTC AAT GGC 3'), which corresponded to positions 2650-2621 of HXB2. AmpliTaq DNA polymerase (Applied Biosystems, Foster City, USA) was used for both PCR runs. The cycling conditions for the first PCR run were as follows: 35 cycles that consisted of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C. The cycling conditions for the second PCR were as follows: 35 cycles that consisted of 30 s at 95 °C, 30 sec at 59 °C, and 1 min 30 s at 72 °C.

PCR amplification of the RT encoding sequences.

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

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

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

Viral RNA was extracted from plasma and sequenced with the Viroseq, HIV-1 Genotyping System, version 2 (Abbott, Wiesbaden, Germany). A 1.8-kilobase (kb) amplicon was generated by reverse transcription-PCR amplification and sequenced. This provided sequence data for codons 1-99 of the PR gene and 1-320 of the RT gene. The sequences were assembled and analysed with the HIV-1 Genotyping System software version 2 (Abbott, Wiesbaden, Germany).

Sequence alignment, phylogeny, and interpretation of drug resistance

Mutations at resistance conferring positions in the HIV RT and PR regions were scored according to the mutation list from the Stanford HIV RT and PR sequence database (<http://hivdb.stanford.edu/>). The scores for resistance mutations in the rectal RNA and DNA fractions were compared to the scores for mutations found in the plasma viral RNA and PBMC RNA and DNA fractions. Comparisons were also made among the available sequences from earlier and later time-points during the infection of each patient. The scores for resistance mutations in all fragments and time-points were compared with the treatment history of each patient.

The sequences were also compared by phylogenetic analyses. Fragments from each of the PR and RT sequences were merged before the sequences were aligned. This was performed with the CLUSTAL-W (Thompson et al., 1994) programme from Geneious software (ver.Pro.4.75),(Biomatters, Auckland, New Zealand) with the default settings. The Kimura two-parameter model (Kimura, 1980) in Geneious was used to calculate the pairwise nucleotide distances. The phylogenetic trees were constructed with the neighbour-joining algorithm, (Felsenstein, 1991) integrated in the Geneious package. The trees were bootstrapped with 100 replications to test for

the significance of the branches of the tree with Geneious Tree Builder.

The overall amino acid divergence of the subtype B *pol* gene was calculated using the HXB2 strain (acc. nr K03455) as reference. The calculations were performed both with and without drug resistance mutations. To analyse the intra-patient divergence in drug resistance mutations, the amino acids at resistance-conferring codons in the PR and RT genes were compared among samples from different tissues. For example, plasma RNA sequences collected at the time of biopsies were compared to PBMC and rectal biopsy sequences that had been collected concurrently. The percentage of discordance in both genes was calculated by comparing RNA from plasma with RNA and DNA from other compartments for each patient.

RESULTS

The treatment history, viral load, and CD4-positive cell counts for the patients are shown in Table I. Plasma, PBMCs, and rectal biopsies were obtained from all six patients. The resistance-conferring mutations for the PR and RT regions are shown in Tables II and III, respectively. Furthermore, Tables II and III present a total of 20 *pol* sequences that were obtained from plasma samples collected at time points prior to the biopsy date. It was not possible to amplify material from all the samples for sequencing. Patient 4 was infected with subtype C, while all the other patients were infected with subtype B HIV-1.

The resistance profiles of samples obtained concurrently from the different compartments showed different levels of variation (Tables II, III). The resistance 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
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6 206 gray shading) showed that the resistance profiles found in the plasma were not very
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8
9 207 different from those found in PBMC and biopsy samples
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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
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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
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23 213 showed variable levels of variation; for example, the samples from patient 2 showed
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25 214 moderate divergence and the samples from patient 5 showed full concordance
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27 215 (Table II, III). A similar level of variation was observed in the DNA fractions from
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29 216 patients 1, 3, and 6 (Table II). The patients that harboured subtype B of HIV showed
30
31 217 an average of 6-14% overall divergence compared to HXB2 when drug resistance
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33 218 mutations were included, and 3-9% when drug resistance mutations were excluded.
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35 219 At the resistance conferring positions, the level of discordance for each patient was
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37 220 calculated between the profiles in plasma RNA and the other samples .This
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39 221 discordance ranged from 0 to 51% in the PR region and 3 to 37% for the RT region.
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47 223 A phylogenetic tree was constructed that included the *pol* sequences from all
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49 224 available samples collected from the five patients that were infected with subtype B
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51 225 of HIV (Fig. 1). The samples from each patient formed a monophylogenetic cluster
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53 226 with 100% bootstrap support; this indicated that contamination among patients was
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55 227 successfully avoided in the PCR amplifications.
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60 229 In addition, sequences were retrieved from time points before and after the time of

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

242 DISCUSSION

244 In this study, the PR and RT sequences of HIV-1 RNA and DNA from blood were
245 compared to those of the rectal mucosal biopsies from six patients infected with
246 HIV-1 that underwent numerous treatments who had received numerous
247 antiretroviral regimens.

249 All of the patients had HIV-1 *pol* RNA and DNA sequences in the plasma, PBMC,
250 and rectal fractions, with a substantial number of mutations associated with
251 resistance to antiretroviral drugs. These mutations corresponded to the treatment
252 history of the patients. Several patients showed traces of previous antiretroviral
253 treatment regimens in the form of resistance mutations or reversions of resistance
254 mutations. This was probably due to the fact that they had experienced various failed
255 regimens during long periods of high viral load. In this type of patient, the viral

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2 256 population typically consists of a potentially wide range of viruses with different
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4 257 resistance profiles. For example, patient 1 harboured a virus population in the rectal
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6 258 biopsy DNA that had a G190A mutation in the RT gene. This mutation was
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9 259 associated with resistance to non-nucleoside reverse transcriptase inhibitors
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11 260 (NNRTIs; Table III), and the patient had previously received nevirapine (NVP) and
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14 261 efavirenz (EFV) treatments, while the current antiretroviral treatment did not include a
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16 262 NNRTI (Table I). The samples were collected some years ago, prior to the
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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
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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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42 273 The comparison between the rectal biopsy samples and the blood samples from the
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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
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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
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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
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60 281 phylogenetic tree (Fig 1). This suggested that there was continuous replication of

viruses that had been archived at different time points. This result was in line with the finding that hidden resistant viruses can be rapidly recruited when a patient is reverted to a previous drug regimen (Deeks et al., 2001).

Earlier studies have analysed the genotypes and resistance profiles of HIV-1 in blood compared to a different anatomical site, like the rectal mucosa. For example, Di Stefano et al.(Di Stefano et al., 2001) studied rectal mucosa samples from 14 HIV-1 positive patients and found a high level of discordance compared to blood samples in the resistance positions of the RT (74.6%) and PR (88.8%) genes. The median percentage discordance at the amino acid level was 8% in the RT and 5% in the PR. In contrast, Poles et al.(Poles et al., 2001) compared HIV-1 RNAs isolated from the rectal mucosa, PBMCs, and plasma from 7 HIV-1 positive patients. They found a high level of concordance among the RT and PR resistance positions. In this study, the median percentage discordance was 11.2% in the PR gene and 11.7% in the RT gene at resistance positions in samples from different sites and time points. At the amino acid level, the percent discordances were 4.8% and 2.6% for the PR and RT proteins, respectively (Poles et al.,2001). Those results were similar to the results found in the current study. The level of discordance at resistance positions in the PR and RT genes between anatomical sites is probably due to the duration of infection, the number of changes in the treatment regimen, and the level of viral load during phases of failing antiretroviral treatment.

For patients with an undetectable viral load, the genotyping of proviral DNA, either from PBMCs or from lymphoid tissue, might be informative. In the current study, resistance mutations acquired during earlier treatment regimens were detected in 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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9 311 representative of the HIV-1 archived in different anatomical sites. Moreover, both the
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11 312 resistance profile and phylogeny of HIV-1 often differed in sequences obtained from
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13 313 RNA and DNA from the same site. This suggested that HIV-1 from different time-
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16 314 points of the infection were present in the same compartment.
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