Colinearity of Reverse Transcriptase Inhibitor Resistance Mutations Detected by Population-Based Sequencing

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Abstract: High-level resistance to multiple drugs is often detected by directly sequencing uncloned polymerase chain reaction products (population-based sequencing). It is not known, however, if this method of identifying mutations gives an accurate picture of individual viral genomes. To determine how often multidrug-resistant isolates consist of clones containing every mutation present in the population-based sequence, a mean of 2.8 molecular clones was sequenced from the plasma of 25 heavily treated persons whose population-based sequence contained multiple reverse transcriptase (RT) inhibitor resistance mutations (71 clones). The 25 population-based sequences contained a mean of 5.7 nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations and 1.2 nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance mutations. The 71 clones contained a mean of 5.3 NRTI resistance mutations and 1.0 NNRTI resistance mutations. Sequences of clones closely resembled the population-based sequence: 36 (51%) clones had each of the RT inhibitor mutations present in the population-based sequence, 25 (35%) had all but 1 RT inhibitor mutation, 4 (6%) had all but 2 RT inhibitor mutations, 3 (4%) had all but 3 RT inhibitor mutations, and 3 (4%) had all but 4 RT inhibitor mutations. Phenotypic testing of 29 clones showed that most clones were resistant to nearly all NRTIs and that those with NNRTI resistance mutations were also resistant to multiple NNRTIs. These data show that in heavily treated persons, most RT inhibitor resistance mutations are present in the same viral genomes (colinear) and that multidrug resistance often occurs within individual clones as well as within virus populations.

Key Words: HIV-1, reverse transcriptase, drug resistance, clone, genome

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HIV-1 genotypic resistance testing is commonly performed to help physicians choose antiretroviral drugs by identifying HIV-1 drug resistance mutations in the plasma virus of infected persons. Genotyping is usually performed by the direct sequencing of uncloned polymerase chain reaction (PCR) products (population-based sequencing) because it is quicker and more affordable than sequencing multiple clones. Within an infected individual, however, HIV-1 exists as a quasispecies consisting of innumerable related but genetically distinct viral variants. As a consequence, it is not known how often the mutations observed by population-based sequencing are colinear or present in the same viral genomes.

We sought to determine the frequency with which clinical HIV-1 isolates containing multiple reverse transcriptase (RT) inhibitor resistance mutations consist of clones containing all or most of the mutations in the population-based sequence rather than different subsets of the mutations in the population-based sequence. In addition, we sought to determine the drug susceptibility of those clones containing multiple RT inhibitor mutations to confirm that the clones as well as the virus population were multidrug resistant.

METHODS

HIV-1 Isolates

We selected cryopreserved plasma samples from 25 heavily treated persons who had virus isolates with multiple RT inhibitor resistance mutations detected by population-based sequencing. Each of the persons had persistent viremia despite previous treatment with 4 or more different nucleoside reverse transcriptase inhibitors (NRTIs). The median duration of NRTI treatment was 54 months (interquartile range [IQR]: 40–89 months), and the median number of months since the last treatment change was 9 months (IQR: 4–12 months). All but 1 person was receiving antiretroviral therapy at the time plasma was obtained for sequencing. Each of the selected isolates had a pattern of mutations associated with resistance to multiple NRTIs. Thirteen isolates also had 1 or more nonnucleoside reverse transcriptase inhibitor (NNRTI)–resistant mutations.

Clonal Sequencing

Plasma HIV-1 RNA was extracted, and RT-PCR was used to amplify complement DNA (cDNA) encompassing RT codons 23 through 312. Amplified RT fragments were ligated

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into an RT-deleted pNL4-3 vector (pNLPFB digested with Msc1 and PflM1[1]) and cloned in competent *Escherichia coli* to create a full-length potentially infectious molecular HIV-1 clone. One to 5 clones per isolate were selected for sequencing. Bidirectional overlapping dideoxynucleoside sequencing reactions were performed, and products were resolved electrophoretically on an ABI 377 sequencer (Applied Biosystems, Foster City, CA).

Phenotypic Susceptibility Testing

Recombinant clones with unique sequences were transfected into C8166 cells. Of 51 transfected clones, 45 (88%) were replication competent, producing syncytia and >10 ng/mL of p24 antigen (median: 275 ng, range: 10–10,000 ng). Thirty of these recombinant isolates were submitted for susceptibility testing to the currently approved RT inhibitors using the standard PhenoSense assay (ViroLogic, South San Francisco, CA).[2]

RESULTS

Clonal Sequencing

The 25 population-based sequences contained a mean of 5.7 NRTI resistance mutations, 1.2 NNRTI resistance mutations, and 11.3 mutations at non–drug-resistant positions. The 71 clones contained a mean of 5.3 NRTI resistance mutations, 1.0 NNRTI resistance mutations, and 10.2 differences at non–drug-resistant mutations. Sequences of the clones closely resembled the population-based sequences: 36 (51%) clones had each of the RT inhibitor mutations present in the population-based sequence, 25 (35%) had all but 1 RT inhibitor mutation, 4 (6%) had all but 2 RT inhibitor mutations, 3 (4%) had all but 3 RT inhibitor mutations, and 3 (4%) had all but 4 RT inhibitor mutations. Conversely, clonal sequencing detected an additional 17 drug resistance mutations not detected by population-based sequencing. Figure 1 shows a summary of the drug resistance mutations in the population-based sequence and the clonal sequences of 15 isolates for which 3 or more clones were sequenced.

The population-based sequences had electrophoretic mixtures of wild-type and mutant residues at 28 of the 158 positions with drug-resistant mutations. Positions with mixtures accounted for the majority of the mutations that were detected by population-based sequencing but not within individual clones. Of the 54 mutations that were not detected by at least 1 of the clonal sequences, 41 (76%) were at 1 of the 28 positions that contained mixtures in the population-based sequence.

Drug Susceptibility Testing

Drug susceptibility results were available for 29 of the 30 recombinant molecular infectious clones submitted for testing (Table 1). The 29 clones had reduced susceptibility to a median of 6 of the 7 approved NRTIs. Median reductions were >200-fold to lamivudine, 33-fold to zidovudine, 6-fold to abacavir, 2.3-fold to stavudine, 2.3-fold to zalcitabine, 1.8-fold to didanosine, and 1.5-fold to tenofovir. Although the reductions in susceptibilities to stavudine, zalcitabine, didanosine, and tenofovir are much lower than those to zidovudine and lamivudine, these 4 drugs begin to lose clinical activity when susceptibility is reduced by as little as 1.4- to 1.6-fold.[3–5] Although the isolates were not selected on the basis of their NNRTI resistance mutations, 12 of the 29 clones had such mutations and exhibited significantly reduced susceptibility to 1 or more NNRTIs.

DISCUSSION

Most drug resistance mutations impair virus replication.[6,7] Moreover, some mutations that confer resistance to 1 drug also hypersensitize the virus to 1 or more other drugs.[8–11] Therefore, it might be expected that the accumulation of multiple drug resistance mutations in the genome of a single virus would occur uncommonly and that multidrug resistance might instead result from the emergence of multiple virus lineages within a patient, each with resistance to different combinations of antiretroviral drugs. Indeed, several studies have shown that in less heavily treated persons or in persons undergoing treatment interruptions, viruses with "incomplete" or intermediate resistance patterns are more commonly detected than those with more complete resistance patterns.[12,13]

This study, however, shows that most individual clones from heavily treated patients contain either all or most of the mutations detected by population-based sequencing. Whether this finding is restricted to viruses from persons as heavily treated as those described in this study is not yet known. Although viruses from persons who have not been subjected to prolonged selection pressure may harbor virus clones lacking the large numbers of mutations described here, we postulate that viruses exposed to prolonged therapy may undergo extensive purifying selection. The genomic stability of such isolates may result from an interlocking of primary and compensatory drug resistance mutations that limits reversion through unfit intermediate amino acid variants.[14]

It is essential that our study not be misinterpreted to suggest that population-based sequencing provides a complete picture of the quasispecies within an individual. Several studies have shown that sequencing multiple clones often detects mutations that are not detected by population-based sequencing.[15–19] Indeed, we found 17 examples of mutations that were detected by clonal but not population-based sequencing. The main clinical implication of the insensitivity of population-based sequencing is that genotypes must be interpreted within the context of the past treatments received by the person whose virus is sequenced. Because of the increased cost associated with sequencing multiple clones, however, it is unlikely that population-based sequencing will be replaced by the sequencing of large numbers of clones. Moreover, the 2 currently US

	NRTI Mutations																		NNRTI Mutations									
	41	44	62	65	67	69	70	74	75	77	115	116	118	151	184	210	215	219	98	100	101	103	106	108	179	181	188	190
	M	E	A	K	D	T	K	L	V	F	Y	F	V	Q	M	L	T	K	A	L	K	K	V	V	V	Y	Y	G
6560	-	-	-	-	N	TN	R	-	-	-	-	-	-	-	V	-	F	Q	-	-	RS	-	-	I	-	-	-	-
1/3	V	-	-	-	N	-	R	-	-	-	-	-	-	-	V	-	F	Q	-	-	R	-	-	I	-	C	-	-
1/3	-	-	-	-	N	-	R	-	-	-	-	-	-	-	V	-	F	Q	-	-	S	-	-	I	-	-	-	-
1/3	-	-	-	-	N	-	R	-	-	-	-	-	-	-	V	-	F	Q	-	-	R	-	-	I	-	-	-	-
7324	ML	-	-	-	N	-	R	-	-	-	-	-	-	-	-	-	F	E	-	-	-	N	-	-	VI	YC	-	GA
2/3	L	-	-	-	N	N	R	-	-	-	-	-	-	-	-	-	F	E	-	-	-	-	-	-	-	-	-	-
1/3	-	-	-	-	N	-	R	-	-	-	-	-	-	-	-	-	F	E	-	-	-	-	-	-	I	C	-	-
10076	ML	-	-	-	-	-	-	-	-	-	-	-	-	-	MV	-	Y	-	-	-	-	-	-	-	-	-	-	-
3/4	L	-	-	-	-	-	-	-	-	-	-	-	-	-	V	-	Y	-	-	-	-	-	-	-	-	-	-	-
1/4	L	-	-	-	-	-	-	-	-	-	-	-	I	-	V	-	Y	-	-	-	-	-	-	-	-	-	-	-
48511	-	-	V	KR	-	-	-	-	I	L	-	-	-	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3/5	-	-	V	-	-	-	-	-	I	L	-	-	-	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1/5	-	-	-	-	-	N	-	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1/5	-	-	V	R	-	-	-	-	I	L	-	-	-	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52534	L	-	-	-	-	S_SS	-	V	-	-	-	-	VI	-	V	W	Y	-	G	-	-	-	-	-	-	-	-	C
2/4	L	-	-	-	-	S_SS	-	V	-	-	-	-	-	-	V	W	Y	-	G	-	-	-	-	-	-	-	-	C
1/4	L	-	-	-	-	S_IS	-	V	-	-	-	-	-	-	V	W	Y	-	G	-	-	-	-	-	-	-	-	C
1/4	L	-	-	-	-	S_SS	-	V	-	-	-	-	I	-	V	W	Y	-	G	-	-	-	-	-	-	-	-	C
53147	-	-	-	KR	-	-	-	V	-	-	-	-	I	-	MV	-	-	-	-	-	-	-	-	-	-	C	-	-
3/5	-	-	-	-	-	-	-	V	-	-	-	-	I	-	V	-	-	-	-	-	-	-	-	-	-	C	-	-
1/5	-	-	-	-	-	-	-	V	-	-	-	-	I	-	V	-	-	-	-	-	-	-	-	-	-	C	-	A
1/5	-	-	-	R	-	-	-	-	-	-	F	-	I	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-
56252	-	-	-	R	-	-	R	-	I	L	F	Y	-	M	-	-	-	E	-	-	-	N	-	-	-	-	-	-
3/4	-	-	-	R	-	-	R	-	I	L	F	Y	-	M	-	-	-	E	-	-	-	N	-	-	-	-	-	-
1/4	-	-	-	R	-	-	R	-	I	L	F	Y	-	M	-	-	-	-	-	-	-	N	-	-	-	-	-	-
5531	L	D	-	-	N	-	-	-	-	-	-	-	-	-	V	W	Y	Q	-	-	-	-	-	-	-	-	-	-
4/4	L	D	-	-	N	-	R	-	-	-	-	-	-	-	V	W	Y	Q	-	-	-	-	-	-	-	-	-	-
7295	ML	-	-	-	N	N	R	-	-	-	-	-	-	-	V	-	F	Q	-	-	-	-	-	-	-	-	-	-
3/4	-	-	-	-	N	N	R	-	-	-	-	-	-	-	V	-	F	Q	-	-	-	-	-	-	-	-	-	-
1/4	L	-	-	-	N	N	R	-	-	-	-	-	-	-	V	-	F	Q	-	-	-	-	-	-	-	-	-	-
7303	L	D	-	-	N	D	-	-	-	-	-	-	I	-	-	W	Y	-	S	-	-	N	-	-	-	-	-	-
3/4	L	D	-	-	N	D	-	-	-	-	-	-	I	-	-	W	Y	-	S	-	-	N	-	-	-	-	-	-
1/4	L	D	-	-	N	D	-	-	-	-	-	-	I	-	-	W	Y	-	S	-	-	N	-	-	-	D	-	-
29129	ML	-	-	-	N	-	-	-	-	-	-	-	-	-	V	LW	Y	-	-	-	-	N	-	-	-	-	-	-
2/4	L	-	-	-	N	-	-	-	-	-	-	-	-	-	V	-	Y	-	-	-	-	N	-	-	-	-	-	-
2/4	L	-	-	-	N	-	-	-	-	-	-	-	-	-	V	W	Y	-	-	-	-	N	-	-	-	-	-	-
49110	-	-	-	-	N	-	R	-	-	-	-	-	-	-	-	-	I	Q	S	-	E	-	-	-	I	-	-	A
2/3	-	-	-	-	N	-	R	-	-	-	-	-	-	-	-	-	I	Q	S	-	E	-	-	-	I	-	-	A
1/3	-	-	-	-	N	-	R	-	-	-	-	-	-	-	-	-	F	Q	S	-	-	-	-	-	I	-	-	A
50680	-	-	-	-	N	-	R	-	-	-	-	-	-	-	-	-	F	Q	-	I	-	N	-	-	I	-	-	-
4/4	-	-	-	-	N	-	R	-	-	-	-	-	-	-	-	-	F	Q	-	I	-	N	-	-	I	-	-	-
51848	L	-	V	-	E	S_SS	-	-	-	-	-	-	VI	-	MI	LW	Y	-	S	-	-	-	I	-	-	-	L	GA
2/3	L	-	V	-	E	S_SS	-	-	-	-	-	-	I	-	I	W	Y	-	S	-	-	-	I	-	-	-	L	-
1/3	L	-	V	-	-	S_SS	-	-	-	-	-	-	I	-	-	-	Y	-	S	-	-	-	I	-	I	-	L	A
7542	L	-	-	-	-	-	-	-	-	-	-	-	-	-	V	W	Y	-	S	-	-	-	-	IV	E	C	-	-
1/3	L	-	-	-	-	-	-	-	-	-	-	-	I	-	V	W	Y	-	S	-	-	-	-	I	E	C	-	-
1/3	L	-	-	-	-	-	-	-	A	-	-	-	-	-	V	W	Y	-	S	-	S	-	-	-	E	C	-	-
1/3	L	-	-	-	G	-	-	-	-	-	-	-	-	-	V	W	Y	-	S	-	-	-	-	-	E	C	-	-

FIGURE 1. Comparison of population-based versus clonal sequencing for 15 isolates for which 3 or more clones were sequenced. Columns 2 through 19 contain 18 nucleoside reverse transcriptase inhibitor (NRTI) resistance positions. Columns 20 through 29 contain 10 nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance positions; NNRTI resistance mutations at positions 225, 227, 230, 236, and 238 were not observed in this data set. The second row contains the consensus amino acid at each of the drug resistance positions. The population-based sequences are shown in bold. Clonal sequences are shown beneath each population-based sequence. Clones containing identical drug resistance mutations have been collapsed into a single line as indicated by the entry in the first column. A dash indicates that the sequence contained the consensus amino acid. A letter indicates the presence of a mutation. More than 1 letter indicates the presence of a mixture of wild-type and a mutation or a mixture of mutations.

TABLE 1. RT Inhibitor Mutations and Drug Susceptibility of 29 Recombinant Infectious Molecular HIV-1 RT Clones

Clone ID	TAMs	184	69	151-Associated	65, 74, 75	NNRTI-Associated Mutations	Other NRTI-Associated Mutations*	Fold Decrease Drug Susceptibility†									
								ABC	ddI	3TC	d4T	TDF	ddC	AZT	DLV	EFV	NVP
10076-4	41L 215Y	184V	—	—	—	—	—	3.8	1.6	>>	1.6	1.3	**1.8**	**6.3**	0.5	0.5	0.7
4676-2	41L 210W 215Y	184V	—	—	—	—	—	**5**	1.6	>>	1.6	1.2	**2.5**	**3.6**	0.4	0.4	0.6
7542-1	41L 210W 215Y	184V	—	—	—	108I 181C	43Q 118I 223Q 228R	**4.6**	1.4	>>	**2.3**	1.2	**2.2**	**5.2**	**62**	**15**	>>
7542-3	41L 210W 215Y	184V	—	—	75A	101S 181C	43Q 223Q 228R	3.4	**1.8**	>>	**2.5**	1.3	**2.3**	**11**	**60**	**15**	>>
29129-2	41L 67N 210W 215Y	184V	—	—	—	103N	—	**6**	**1.7**	>>	**2.2**	**1.6**	**2**	**30**	**9.2**	**9.6**	**26**
6463-13	41L 67N 210W 215Y	184V	—	—	—	—	39A 43E 118I 203K 208Y 223Q	**7.4**	**2.1**	>>	**4.1**	**1.5**	**3.3**	**24**	0.09	0.2	0.3
7542-7	41L 67G 210W 215Y	184V	—	—	—	181C	43Q 223Q 228R	**4.8**	**1.7**	>>	**2**	0.8	**2.2**	1.9	**15**	**3.7**	**280**
7303-3	41L 67N 210W 215Y	—	69D	—	—	98S 103N	43N 44D 118I 203K 208Y	**8.4**	**2.3**	**7.5**	**6.7**	**5.9**	**1.8**	>>	**9.2**	**5.1**	**26**
4755-5	41L 67N 210W 215Y	184V	69D	—	—	—	39A 43A 44D 118I	**7.7**	**2.4**	>>	**3.9**	**2.3**	**3.2**	**61**	0.4	0.5	0.7
819-10	41L 67N 210W 215Y	184V	69G	—	—	—	—	**6.9**	**1.8**	>>	**2**	1.2	**2.7**	**9.9**	0.3	0.2	0.5
49110-1	69N 70R 215I 219Q	—	—	—	—	98S 101E 190A	—	2.8	1.2	**4.3**	**2**	**3.5**	1.2	**177**	2.1	**76**	>>
49110-3	67N 70R 215F 219Q	—	—	—	—	98S 190A	—	3.4	1.4	**5.9**	**2.4**	**3**	1.4	**154**	0.4	**4.3**	**85**
50680-1	67N 70R 215F 219Q	—	—	—	—	100I 103N	—	0.9	0.7	0.9	1.1	1.2	0.5	**35**	>>	>>	**29**
7324-2	67N 70R 215F 219E	—	—	—	—	181C	39A 203D	2.3	1.2	2	**2.2**	**2.8**	0.9	**102**	**49**	**2.8**	>>
7324-4	67N 70R 215F 219E	—	—	—	—	—	39A 203D	3.8	1.5	**3.6**	**2.3**	**5.2**	1.4	**464**	**3**	**2.8**	**5.3**
6560-3	67N 70R 215F 219Q	184V	—	—	—	101R 108I	—	**6.5**	**1.7**	>>	1.5	1.1	**2.4**	**7.7**	0.8	1	2
7295-1	67N 70R 215F 219Q	184V	69N	—	—	—	218E	**6.1**	**1.9**	>>	**1.9**	1.1	**2.3**	**9.9**	0.7	0.5	0.5
7295-2	67N 70R 215F 219Q	184V	69N	—	—	—	208Y 218E	**5.5**	**1.8**	>>	**1.8**	0.8	**2.1**	**3.1**	0.3	0.2	0.3
7324-1	41L 67N 70R 215F 219E	—	69N	—	—	—	39A 203D	4.2	**1.7**	**4.1**	**2.6**	**8.1**	1	**923**	1.7	1.6	**3.6**
7295-3	41L 67N 70R 215F 219Q	184V	69N	—	—	—	208Y 218E	**6.8**	**1.9**	>>	**2.3**	**1.4**	**2.3**	**33**	0.5	0.3	0.5
52534-2	41L 210W 215Y	184V	69S_SS	—	74V	98G 190C	228H	**22**	**3.3**	>>	**9**	**4.2**	**4.1**	**719**	0.02	>>	>>
1617-1	70G	184V	69K	62V 77L 116Y 151M	75I	—	115F	>>	**23**	>>	**11**	**2.4**	**47**	**261**	2.3	0.9	1
35764-2	—	—	—	62V 77L 116Y 151M	75I	—	—	**7.1**	**11**	**5.4**	**11**	**1.9**	**17**	**55**	0.6	0.4	0.4
41779-2	—	—	—	116Y 151M	—	—	—	**5**	**8.1**	**3**	**7.1**	**1.4**	**10**	**9.5**	1.2	1.1	1.3
48511-11	—	—	—	62V 77L 151M	65R 75I	—	—	**11**	**22**	**64**	**20**	**4.9**	**28**	**101**	1.5	0.9	1.1
56252-1	70R	—	—	77L 116Y 151M	65R 75I	103N	115F	>>	**28**	**89**	**20**	**11**	**28**	>>	>>	>>	>>
5531-1	41L 67N 70R 210W 215Y 219Q	184V	—	—	—	—	39A 44D	**7.7**	**1.8**	>>	**2.9**	**2.5**	**2.3**	**139**	0.7	0.6	0.7
4675-1	67N 210W 215Y 219R	184V	—	—	—	—	—	**6.4**	1.6	>>	**2.2**	1	**2.6**	**4.9**	0.4	0.4	0.5
53147-24	—	—	—	—	65R	181C	39A 115F 118I 228H	**6**	**1.8**	**22**	0.9	**1.5**	**1.8**	0.4	**17**	0.5	**52**

*Includes the uncommon ABC-associated mutation Y115F and other mutations recently associated with either nucleoside RT inhibitor resistance[22] or therapy.[23] The complete sequence of each clone can be found at http://hivdb.stanford.edu/pages/mdrPanel.html.

†">>" indicates decrease in drug susceptibility greater than the upper limit of the PhenoSense assay. Bold indicates values considered by assay to indicate clinically significant drug resistance.

ABC indicates abacavir; AZT, zidovudine; ddC, zalcitabine; ddI, didanosine; d4T, stavudine; TAMs, thymidine analog mutations; 3TC, lamivudine; TDF, tenofovir.

Food and Drug Administration (FDA)–approved tests for HIV genotypic resistance testing—TruGene (Bayer Diagnostics, Emeryville, CA) and ViroSeq (Celera Diagnostics, Alameda, CA)—use population-based sequencing.

Phenotypic drug susceptibility testing is usually also performed using the population of viruses within a plasma HIV-1 sample. Our drug susceptibility results obtained testing individual virus clones complement our sequence data by showing that multidrug resistance is often a property of individual clones as well as of the population of viruses present within an isolate.

The mutations that were not detected by clonal sequencing were predominantly at positions at which the population-based sequence contained electrophoretic evidence of a mixture of wild-type and mutant residues. Therefore the presence of multiple "pure" mutations in a population-based sequence indicates mutations that are likely to be colinear, whereas the presence of mixtures indicates mutations that may or may not be colinear.

It has been proposed that most HIV-1 genomes and, possibly, most HIV-1 genes are defective because of high-level viral mutagenesis.[20,21] Nevertheless, 45 of the 51 transfected clones in this study were replication competent, showing that most RT genes, even those with multiple drug resistance mutations, are replication competent.

In conclusion, this study shows that individual clones in plasma virus samples from persons treated with multiple RT inhibitors contain most of the mutations present within the population-based sequence and may be resistant to most available RT inhibitors. The potential benefit of using a large number of NRTIs (eg, as part of a mega–highly active antiretroviral therapy [HAART] regimen) in this population is therefore likely to result from the impaired replication of viruses containing multiple RT inhibitor-resistant mutations rather than from the effects of different drugs acting on different virus subpopulations.

APPENDIX

The GenBank accession numbers of the 25 population-based RT and protease sequences are AF085089, AF088081, AF513999, AF544411, AF544428, AF514016, AF514029, AY030511, AY030546, AY030600, AY030625, AY030649, AF544507, AF514110, AY030831, AY030997, AY031121, AF544568, AF514208, AY031500, AY032222, AY032387, AF514250, AF514253, and AY351703 through AY351713. The GenBank accession numbers for the 71 RT clones are AY351714 through AY351784.

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