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Antiretroviral drug susceptibility among drug-naive adults with recent HIV infection in Rakai, Uganda

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Abstract

Objective—To analyze antiretroviral drug susceptibility in HIV from recently infected adults in Rakai, Uganda, prior to the availability of antiretroviral drug treatment.

Methods—Samples obtained at the time of HIV seroconversion (1998–2003) were analyzed using the GeneSeq HIV and PhenoSense HIV assays (Monogram Biosciences, Inc., South San Francisco, California, USA).

Results—Test results were obtained for 104 samples (subtypes: 26A, 1C, 66D, 9A/D, 1C/D, 1 intersubtype recombinant). Mutations used for genotypic surveillance of transmitted antiretroviral drug resistance were identified in six samples: three had nucleoside reverse transcriptase inhibitor (NRTI) surveillance mutations (two had M41L, one had K219R), and three had protease inhibitor

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Authors' contributions: all authors contributed substantially to the study. All authors contributed to preparation of the manuscript. In addition, individual contributions are listed below.

S.H.E designed the resistance substudy, analyzed the data, and prepared the manuscript.

O.L. assisted with the design of the resistance substudy, selected samples for testing.

N.P. provided expertise in the interpretation and presentation of resistance test results.

W.H. provided expertise in the interpretation and presentation of resistance test results.

C.C. identified mutations associated with antiretroviral hypersusceptibility.

A.P. analyzed data related to HIV-replication capacity.

D.S., Co-Principal Investigator of the Rakai Health Science Program (RHSP), contributed to the design and monitoring of the study that identified recent seroconverters, and contributed to the creation of data sets.

S.J.R. supervised laboratory activities in Uganda, and assisted with the design of resistance substudies for the RHSP.

N.K., Co-Principal Investigator of the RHSP, which identified recent seroconverters, oversaw study conduct, data collection and the creation of data sets.

T.C.Q. assisted with the design of the resistance substudy.

R.G., Co-Principal Investigator of the RHSP, contributed to the design of the study, which identified recent seroconverters, and monitored study conduct, data collection, and the creation of data sets.

M.W., Co-Principal Investigator of the RHSP, contributed to design of the study that identified recent seroconverters, and monitored study conduct, data collection and the creation of data sets.

Conflict of interest: W.H., C.C., and A.P. are employees and stockholders of Monogram Biosciences, Inc., (provider of the GeneSeq HIV and PhenoSense HIV assays). N.P. was an employee and stockholder of Monogram Biosciences, Inc. at the time that the study was performed.

surveillance mutations (I47V, F53L, N88D); none had nonnucleoside reverse transcriptase inhibitor (NNRTI) surveillance mutations. Other resistance-associated mutations were identified in some samples. However, none of the samples had a sufficient number of mutations to predict reduced antiretroviral drug susceptibility. Ten (9.6%) of the samples had reduced phenotypic susceptibility to at least one drug (one had partial susceptibility to didanosine, one had nevirapine resistance, and eight had resistance or partial susceptibility to at least one protease inhibitor). Fifty-three (51%) of the samples had hypersusceptibility to at least one drug (seven had zidovudine hypersusceptibility, 28 had NNRTI hypersusceptibility, 34 had protease inhibitor hypersusceptibility). Delavirdine hyper-susceptibility was more frequent in subtype A than D. In subtype D, efavirenz hypersusceptibility was associated with substitutions at codon 11 in HIV-reverse transcriptase.

Conclusion—Phenotyping detected reduced antiretroviral drug susceptibility and hypersusceptibility in HIV from some antiretroviral-naive Ugandan adults that was not predicted by genotyping. Phenotyping may complement genotyping for analysis of antiretroviral drug susceptibility in populations with nonsubtype B HIV infection.

Keywords

antiretroviral drug; hypersusceptibility; phenotype; resistance; subtype; Uganda

Introduction

With the advent of the President's Emergency Plan for AIDS Relief program (PEPFAR) and other programs, antiretroviral drugs are becoming increasingly available for treatment of HIV infection in sub-Saharan Africa and other resource-limited settings. In Uganda, antiretroviral drugs first became widely available under PEPFAR funding in June 2004. In Rakai District, Uganda, approximately 5000 people with HIV infection have been screened under the Rakai Health Sciences Program (RHSP) to identify those eligible for antiretroviral therapy, using the criteria of CD4 cell count less than 250 cells/μl and/or World Health Organization stage 3 or 4 HIV disease. On the basis of surveillance data from 2006–2007, approximately 26% of those who agreed to counseling and HIV testing were eligible for antiretroviral treatment. Approximately, 1500 people followed by the RHSP were receiving antiretroviral treatment as of October 2008; these constitute approximately 76% of those eligible for antiretroviral treatment.

The purpose of this study was to obtain information about the antiretroviral drug susceptibility of HIV isolates from Rakai, collected prior to the widespread availability of antiretroviral treatment. In Uganda, most HIV infections are caused by HIV subtypes A and D, with some subtype C and intersubtype recombinant infections [1–3]. In 2000, subtype A and subtype D accounted for approximately 30 and 5%, respectively, of new HIV infections around the world [4]. In a previous study of antiretroviral drug-naive women from Kampala, Uganda, we found no major antiretroviral resistance mutations among 207 women (120 subtype A and 87 subtype D samples) [5]. However, genotypic studies may not provide a full picture of antiretroviral drug susceptibility. Nonsubtype B HIV frequently contains amino acid polymorphisms in HIV protease and reverse transcriptase at positions associated with drug resistance in subtype B, and different antiretroviral resistance mutations may emerge in different HIV subtype backgrounds (reviewed in [4,6]).

In this report, we analyzed antiretroviral drug susceptibility in HIV isolates collected at the time of HIV seroconversion from individuals in Rakai, Uganda using both genotypic and phenotypic assays.

Methods

Source of samples used for analysis

Serum samples were collected annually between 1998 and 2003 in a longitudinal study of HIV infection in Rakai, Uganda. For individuals with incident HIV infection, clinical and plasma samples were collected for laboratory assessment including HIV viral load and CD4 cell counts. For individuals who were initially seronegative, but became seropositive after approximately 1 year of follow-up, HIV infection was confirmed by two enzyme immunoassays with western blot confirmation. We identified 145 samples from participants collected at the time of seroconversion who had subtype A, subtype D, or intersubtype recombinant HIV, based on previous HIV subtyping using a multiple hybridization assay [6], and had sufficient plasma for analysis.

HIV resistance testing

The GeneSeq HIV and PhenoSense HIV assays were performed at Monogram Biosciences, Inc. (South San Francisco, California; ordered as the PhenoSenseGT assay package) [7]. For both assays, the HIV *pol* region is amplified from a test sample and the amplified DNA is cloned into a test vector. In the GeneSeq HIV assay, vector pools are sequenced to determine the HIV genotype. In the PhenoSense HIV assay, recombinant virus generated from the vector pools is used to infect cells in the presence of varying concentrations of a drug. The amount of drug needed to inhibit viral replication of the test vector by 50% (IC_{50}) is then compared with the IC_{50} of a reference strain; this ratio (IC_{50} test vector/IC_{50} reference) is referred to as the fold change in IC_{50}. The fold change in IC_{50} is compared with defined clinical cutoff values (either a sole cutoff value, or if available, lower and upper cutoff values), to predict drug susceptibility. The lower clinical cutoff indicates the fold change IC_{50} that provides the best discrimination of reduced clinical response; the upper clinical cutoff indicates the fold change IC_{50} above which a clinical response is unlikely [8]. Samples are characterized as susceptible (fold change IC_{50} >0.4 and <lower or sole cutoff value), partial susceptibility (fold change IC_{50} >lower and <upper cutoff value), resistant (fold change IC_{50} >upper cutoff value), or hypersusceptible (fold change IC_{50} <0.4).

HIV subtyping

Pol region subtype was determined based on phylogenetic analysis of protease and reverse transcriptase sequences. Nucleotide sequences were compared with a set of reference sequences representing HIV-1 group M subtypes and circulating recombinant forms (CRFs) [9], using the BLAST local similarity search algorithm [10]. Each subtype was represented by at least two reference sequences.

Informed consent

The Rakai study was approved by the institutional review boards at each of the participating institutions (Uganda Virus Research Institute, Walter Reed Army Institute of Research, and Johns Hopkins and Columbia Universities) and participants provided written informed consent. Human experimentation guidelines of the Department of Health and Human Services were followed in the conduct of this research.

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Results

We identified 145 samples from individuals in the Rakai cohort collected at the time of HIV seroconversion (see Methods). Previous analysis of HIV subtype in the *gag*, *pol*, *vpu*, *env*, and gp41 regions using a multiregion hybridization assay [6] identified these samples as subtype

A (*n* = 37), subtype D (*n* = 61) or intersubtype recombinant (*n* = 47). HIV resistance testing (GeneSeq HIV and PhenoSense HIV assays) was successful for 104 (71.7%) of the 145 samples. Failure to obtain results for the remaining 41 samples most likely reflected the low volume of plasma available for testing (0.2 ml versus 3 ml typically requested). In these low-volume samples, low viral load also contributed to assay failure; the 41 samples that failed testing had significantly lower viral loads than the 104 samples with test results (*P* = 0.003, Table 1). There were no significant differences in the age, gender, year of seroconversion/sample collection, or CD4 cell count of individuals with vs. without resistance test results (Table 1). Individuals who had samples collected closer to their last study visit with a negative HIV test result were more likely to have a resistance test result; however, this association was not statistically significant (*P* = 0.063, Table 1).

For each of the 104 samples with resistance test results, we determined the *pol* region subtype. The subtypes were: A (*n* = 26, includes subtypes A and A1), C (1), D (*n* = 66), A/D (*n* = 9), C/D (*n* = 1), and complex intersubtype recombinant (*n* = 1). Genotypic testing (GeneSeq HIV assay) was performed to identify mutations associated with reduced susceptibility to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors. Seven of the 104 samples (6.7%) had a mutation associated with NRTI resistance (one mutation in each sample: M41L (*n* = 2), E44D (*n* = 3), V118V/I (*n* = 1), K219K/R (*n* = 1)), and one sample had a mutation associated with NNRTI resistance (E138A). However, none of these mutations was sufficient to predict reduced NRTI or NNRTI susceptibility in the GeneSeq HIV assay. The mutations M41L (detected in two subtype D samples) and K219K/R (detected in one subtype A1 sample) were the only mutations identified that are among those used for genotypic surveillance of transmitted NRTI drug resistance [11]. We did not detect any of the mutations used for genotypic surveillance of transmitted NNRTI drug resistance [11]. All of the 104 samples had at least one mutation detected in HIV protease associated with reduced protease inhibitor susceptibility (e.g. K20R, M36I). However, none of the 104 samples had a mutation pattern predictive of reduced protease inhibitor susceptibility, and only three samples had a mutation used for genotypic surveillance of transmitted protease inhibitor drug resistance [11]: one subtype A sample had I47V, one subtype D sample had F53L, and one subtype A1 sample had N88D. It should be noted that the presence of single mutations from the list used for genotypic surveillance of transmitted drug resistance is not expected to always be reflected by phenotypic changes [11].

The PhenoSense HIV assay measures the susceptibility of HIV in the test sample to a panel of antiretroviral drugs. Ten (9.6%) of the 104 samples had evidence of reduced susceptibility to one or more antiretroviral drugs in the PhenoSense HIV assay (two subtype A and eight subtype D samples, Table 2), including one individual with partial susceptibility to didanosine (ddI), one individual with resistance to nevirapine (NVP), and eight individuals with resistance and/or partial susceptibility to one or more of the protease inhibitors. Some of the samples with discordant resistance results (GeneSeq HIV = susceptible, susceptible, PhenoSense HIV = resistant or partially susceptible) had genotypic polymorphisms identified; complex interactions of these polymorphisms may have resulted in reduced drug susceptibility to NVP [12] and some of the protease inhibitors. We found no genotypic explanation for reduced phenotypic susceptibility to ddI, saquinavir/ritonavir, or tipranavir/ritonavir (see Table 2).

In the PhenoSense HIV assay, hypersusceptibility is defined as a fold change IC_{50} less than 0.4 (indicating that replication of the test virus is inhibited by 50% at a drug level 2.5-fold or less compared with the reference strain). Hypersusceptibility to one or more antiretroviral drugs was detected in 53 (51.0%) of the 104 samples, including 19 (73.1%) of 26 samples with subtype A, 28 (42.4%) of 66 samples with subtype D, four (44.4%) of nine samples with A–D recombinant strains, and two (66.6%) of the three samples with other strains (one each: C, C–D, and complex; *P* value for A vs. D = 0.012 [19/26 vs. 28/66]; *P* value for A vs. non-A =

0.013 [19/26 vs. 33/78], z-test). Seven (6.7%) of the 104 individuals had hypersusceptibility to the NRTI, zidovudine; 28 (26.9%) of the 104 individuals had hypersusceptibility to one or more of the NNRTIs; 34 (32.7%) of the 104 individuals had hypersusceptibility to one or more of the protease inhibitors. The proportion of individuals who had hypersusceptibility to the NNRTI, delavirdine (DLV), was significantly higher for subtype A than D (P <0.001, Table 3). In a previous study, subtype A samples were found to have a lower replication capacity than subtype D samples [13]. However, in our data set, there was no significant difference between the median replication capacity of samples with vs. without DLV hypersusceptibility for subtype A (median: 28.23 vs. 27.49, P = 0.526.) or subtype D (median: 37.99 vs. 71.96 P = 0.276).

We next analyzed the Rakai sequence set to see whether we could identify mutations associated with hypersusceptibility to any of the antiretroviral drugs. In subtype D, we found that EFV hypersusceptibility was associated with amino acid substitutions at codon 11 in HIV reverse transcriptase. Among the 66 subtype D samples, five of 12 samples with EFV hypersusceptibility had substitutions at codon 11 (3Q, 1T, and 1H); in contrast, only two of 54 samples without EFV hypersusceptibility had a substitution at codon 11 (1Q, 1N; P value = 0.001, Fisher Exact test). As this association was observed within a single subtype (D), it should not be influenced by subtype-based difference in compatibility with the resistance test vector. The association of EFV hypersusceptibility with codon 11 substitutions was also observed in a separate data set of 182 subtype D samples previously submitted to Monogram Biosciences for testing (unpublished data).

Discussion

Our results show that HIV genotyping may not provide a full picture of antiretroviral drug susceptibility in nonsubtype B HIV strains. In our sample set, none of the samples had genotypic evidence of antiretroviral drug resistance, even though 10 (9.6%) of the samples had reduced susceptibility to one or more antiretroviral drug in a phenotypic assay. In interpreting these results, one should note that the reduction in susceptibility was relatively low in all cases (IC_{50} near the assay cutoff). In cases where lower and upper clinical cutoffs were available (reduced susceptibility to ddI, amprenavir, saquinavir, and tripranavir) the fold change IC_{50} of the test sample was below the upper cutoff for the drug, indicating partial drug susceptibility. It is also important to recognize that the cutoffs used in the PhenoSense assay were derived from clinical outcome data from antiretroviral treatment studies in which most of the participants were likely to have subtype B HIV. It is not known whether different clinical cutoffs for drug susceptibility are needed to predict treatment response in individuals with nonsubtype B infection. Currently, there is little data to suggest that different HIV subtypes have inherently different susceptibilities to antiretroviral drugs. A few other studies have evaluated the phenotypic susceptibility of nonsubtype B isolates from antiretroviral-naive individuals; relatively few subtype A or D isolates were included in those studies [14–17]. In most cases, nonsubtype B isolates were fully susceptible to NRTIs and NNRTIs. One report described a CRF01_AE isolate with reduced NNRTI susceptibility (associated with I135T) and two CRF02_AG isolates with reduced susceptibility to abacavir (associated with D123N plus I135V) [18]. In our study, one sample had reduced susceptibility to ddI and one sample had reduced susceptibility to NVP; both samples were subtype D. Nonsubtype B HIV frequently contains amino acid polymorphisms in HIV protease that are associated with protease inhibitor resistance in subtype B [4,19], and several studies have identified nonsubtype B isolates with reduced susceptibility to protease inhibitors (e.g. [17,18,20–23]). All of the samples analyzed in this report had polymorphisms in HIV protease that are typical of polymorphisms previously described in HIV isolates from Uganda [24], and eight (7.7%) of the 104 samples had reduced protease inhibitor susceptibility (two subtype A and six subtype D samples). The polymorphisms in those samples explained the reduced susceptibility to some

protease inhibitors, but did not explain the reduced susceptibility to saquinavir/ritonavir, or tipranavir/ritonavir.

Our study also found that 53 (51.0%) of the 104 samples from Rakai had hypersusceptibility to one or more antiretroviral drug; this included hypersusceptibility to ZDV and NNRTIs, in addition to protease inhibitors. Two previous studies identified nonsubtype B isolates with antiretroviral hypersusceptibility [21,25]. In one study, CRF02_AG isolates were more susceptible to nelfinavir and ritonavir than other subtypes (associated with K70R in protease) [21]; in the other study, subtype C isolates were hypersusceptible to lopinavir (associated with I93L) [25]. We are not aware of previous reports of hypersusceptibility to NRTIs or NNRTIs in nonsubtype B HIV. We recognize the possibility that hypersusceptibility of some nonsubtype B test samples may reflect incompatibility of the test sample with the subtype B PhenoSense resistance test vector; this incompatibility is not seen with subtype C test samples [13]. In our study, the proportion of samples that exhibited DLV hypersusceptibility was greater for subtype A than D. However, we found no association between DLV susceptibility and viral replication capacity in the absence of the drug. This makes it less likely that the observed difference between subtype A and D DLV hypersusceptibility was due to incompatibility of subtype A samples with the resistance test vector.

The reduced antiretroviral susceptibility and antiretroviral hypersusceptibility that we observed in HIV from some individuals from Rakai are likely to represent natural variations in antiretroviral susceptibility in these strains. However, it is possible that some individuals acquired HIV strains with reduced antiretroviral susceptibility from individuals who were exposed to antiretroviral drugs in other regions of Uganda or other countries, where antiretroviral were more widely available at the time of sample collection. We also note that single-dose NVP was introduced in Rakai for prevention of mother-to-child transmission (pMTCT) in 2000. Samples tested in this study were collected between 1998 and 2003. Use of single-dose NVP for pMTCT is associated with emergence of resistance in some women [26]. However, the resistant strains typically fade to low levels after delivery, and may only be detectable using tests designed to detect minority variants [27]. In this study, none of the 58 women tested had genotypic evidence of NVP resistance; virus from the one woman who did have HIV with reduced phenotypic susceptibility to NVP did not have any of the known NVP resistance mutations. With the rollout of antiretroviral drug treatment programs in Rakai and elsewhere in Uganda, it will be important to monitor antiretroviral drug resistance, both at treatment failure, and in the population over time. The findings in this report suggest that phenotypic resistance testing may complement HIV genotyping in this setting.

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Table 1

Characteristics of study participants.

		Resistance test results			Resistance		
	Available samples	**Yes**	**No**	***P* value**	**Yes**	**No**	***P* value**
	***N* = 145**	***N* = 104**	***N* = 41**	**—**	***N* = 10**	***N* = 94**	**—**
Median Age (years)	27.7	27.9	26.5	0.265[c]	31.6	27.8	0.766[c]
Male (%)	44.8%	44.2%	46.3%	0.854[b]	30%	45.7%	0.272[b]
Year of HIV seroconversion	2000	2000	2000	0.634[c]	2000	2000	0.583[c]
Median time since last negative HIV result (months)	13.4	13.3	14.4	0.063[c]	12.3	13.4	0.041[c]
Median CD4 cell count (per 100 cells)[a]	544	516	562	0.496[c]	545	512	0.559[c]
Median $\log_{10}$ HIV RNA copies/ml (per log HIV RNA)[a]	4.69	4.89	4.35	0.003[c]	4.71	4.93	0.391[c]
Subtype (all)[d]							0.716[b]
A		26			2 (7.8%)	24 (92.3%)	
C		1			0 (0.0%)	1 (100.0%)	
D		66			8 (12.1%)	58 (87.9%)	
R		11			0 (0.0%)	11 (100.0%)	

[a] Viral load results were available for 143/145 individuals; CD4 cell count results were available for 126/145 individuals.

[b] Fisher's exact test. The *P* value in the table (0.716) is for the overall association between subtype and resistance. The *P* values for the association of subtype and resistance are $P = 0.72$ for A vs. D, $P = 1$ for A vs. non-A (D, C, and recombinant combined), and $P = 0.32$ for D vs. non-D (A, C, and recombinant combined).

[c] Mann–Whitney rank sum test.

[d] Results are from phylogenetic subtyping of the HIV *pol* region. R: Recombinant, includes A/D ($n = 9$), C/D ($n = 1$), and complex intersubtype recombinant ($n = 1$).

Table 2

PhenoSense HIV and GeneSeq HIV results for samples with reduced antiretroviral drug susceptibility.

A. Reduced susceptibility to reverse transcriptase inhibitors.

		ABC	ddI	FTC	3TC	d4T	TFV	ZDV	DLV	EFV	NVP	
Cutoff	**ST**	**4.5–6.5**	**1.3–2.2**	**3.5**	**3.5**	**1.7**	**1.4–4**	**1.9**	**6.2**	**3**	**4.5**	**RT mutations**
E235	D	1.12	(1.40)	0.68	0.93	1.11	0.99	0.59	0.64	0.30	0.45	V8V/I, V35K, K49R, V60I, S68G, Q102K, **V118V/I**, I159V, C162S, D177E, T200I, Q207E, R211K, V245T, D250E, R277K, K281K/R, L282C, T286A, V293I
H306	D	0.79	0.60	0.67	0.76	0.57	0.82	0.71	3.20	1.33	5.22	K13R, V35T, K49R, V60I, Q102K, K104R, D121Y, K122E, E138Q, D177E, I180V, T200R, Q207G, R211K, V245K, E248D, D250E, I257I/L, A272S, R277K, K281R, L282C, T286A

B. Reduced susceptibility to protease inhibitors.

		ATV	DRV	AMP	IDV	LPV	NFV	RTV	SQV	TPV	
Unboosted cutoff		**2.2**		**2**	**2.1**		**3.6**	**2.5**	**1.7**		
Boosted cutoff	**ST**	**5.2**	**10–90**	**4–11**	**10**	**9–55**			**2.3–12**	**2–8**	**Protease mutations**
A316	A1	1.34	1.40	1.41	1.05	0.74	1.30	1.89	1.38	(2.22)	**I13V**, K14R, E35D, **M36I**, R41K, R57K, **H69K**, **L89M**
H287	A1	1.59	1.70	(4.09)	1.03	1.98	1.21	2.29	1.23	(2.68)	T4T/N, **I13V**, **L33F**, **M36I**, R41K, R57K, E65D, H69K, L89M
E054	D	1.35	1.69	1.38	1.79	0.86	1.32	1.09	(2.20)[a]	1.57	**I13I/V**, I15V, R41K, **F53F/L**, I64V, H69Y

B. Reduced susceptibility to protease inhibitors.

		ATV	DRV	AMP	IDV	LPV	NFV	RTV	SQV	TPV	
Unboosted cutoff		**2.2**		**2**	**2.1**		**3.6**	**2.5**	**1.7**		
Boosted cutoff	**ST**	**5.2**	**10–90**	**4–11**	**10**	**9–55**			**2.3–12**	**2–8**	**Protease mutations**
J450	D	1.55	1.26	0.92	1.58	0.99	1.76	1.15	(1.73)[a]	(2.43)	**I13V**, **G16E**, **L33I**, **M36I**, R41K, I64V, I72I/V
K122	D	1.61	1.44	1.81	2.03	1.37	1.91	1.93	(1.87)[a]	(2.54)	**I13V**, **M36I**, R41K, D60E, L63E, I64V
F392	D	2.24[a]	1.64	1.61	2.07	2.50	3.06	1.82	(2.25)[a]	(3.27)	**I13V**, **K20K/R**, **M36I**, R41K, I64V, K70R
C252	D	2.31[a]	2.05	1.34	2.00	2.26	3.02	2.57	(2.20)[a]	(3.42)	**L10V**, **I13V**, **G16E**, **M36I**, R41K, **L63Q**, I64V, V75V/I
D084	D	2.39[a]	2.14	1.95	2.23[a]	2.34	3.92	3.29	(2.44)	(2.81)	**L10V**, **I13V**, **K20R**, **M36I**, R41K, **L63T**, I64V, E65D, I72V

Test results are shown for samples with evidence of reduced drug susceptibility to NRTIs and NNRTIs (Table 2A) and to protease inhibitors (Table 2B). (2A) Reverse transcriptase inhibitors [ABC, abacavir; ddI, didanosine; FTC, emtricitibine; 3TC, lamivudine; d4T, stavudine; TFV, tenofovir; ZDV, zidovudine; DLV, delavirdine; EFV, efavirenz; NVP, nevirapine]. (2B) Protease inhibitors [ATV, atazanavir; DRV, darunavir; AMP, amprenavir; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; RTV, ritonavir; SQV, saquinavir; TPV, tipranavir]. The cutoff value for each drug is shown (Cutoff); if upper and lower cutoffs are available for a drug, both are shown (e.g. 4.5–6.5). In Table 2B, cutoffs are shown for unboosted protease inhibitors (Unboosted cutoff) and also for protease inhibitors coformulated with ritonavir (Boosted cutoff). ST: subtype. Fold change IC_{50} values that indicate reduced drug susceptibility are shaded; parentheses indicate partial drug sensitivity (fold change IC_{50} between the upper and lower cutoff). None of the samples had genotypic evidence of reduced drug susceptibility. Differences from the NL4-3 reference sequence are shown for reverse transcriptase (Table 2A, RT Mutations) and protease (Table 2B, Protease Mutations); mutations associated with reduced drug susceptibility are shown in bold.

[a]The values shown (fold-change IC_{50}) are above the lower cutoff for the unboosted drug, but are below the cutoff for the ritonavir-boosted (boosted/r) drug.

Table 3

Hypersusceptibility of subtype A vs. subtype D strains.

				NNRTI			Protease inhibitor								
Subtype	**#Tested**	**Any ARV**	**NRTI ZDV**	**DLV**	**EFV**	**NVP**	**ATV**	**DRV/r**	**AMP**	**IDV**	**LPV/r**	**NFV**	**RTV**	**SQV**	**TPV/r**
A/A1	*n* = 26	19	1	9	9	8	0	0	9	3	6	1	4	0	0
	%	73.1	3.8	34.6	34.6	30.8	0.0	0.0	34.6	11.5	23.1	3.8	15.4	0.0	0.0
D	*n* = 66	28	6	2	12	9	5	5	12	7	12	5	8	6	2
	%	42.4	9.1	3.0	18.2	13.6	7.6	7.6	18.2	10.6	18.2	7.6	12.1	9.1	3.0
A vs. D*	*P* value	**0.01**	0.67	<**0.001**	0.10	0.07	0.32	0.32	0.10	1.00	0.57	0.67	0.74	0.18	1.00

Test results are shown for samples with evidence of hypersusceptibility to antiretroviral drugs. NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non nucleoside reverse transcriptase inhibitor. Antiretroviral drug abbreviations are defined in the legend for Table 2. In the PhenoSense HIV assay, hypersusceptibility is defined as a fold change IC_{50} <0.4 (indicating that replication of the test sample is inhibited by 50% at a drug level 40% of that needed for comparable inhibition of a reference strain). The table shows the number and percentage of samples with *pol* region HIV subtypes A/A1 or D with hypersusceptibility to each antiretroviral drug. *P* values are shown for comparisons between subtype A and D isolates (Fishers Exact test).

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