HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance

Robert W. Shafer[a], Soo-Yon Rhee[a], Deenan Pillay[b], Veronica Miller[c], Paul Sandstrom[d], Jonathan M. Schapiro[a], Daniel R. Kuritzkes[e] and Diane Bennett[f]

Objectives: Monitoring regional levels of transmitted HIV-1 resistance informs treatment guidelines and provides feedback on the success of HIV-1 prevention efforts. Surveillance programs for estimating the frequency of transmitted resistance are being developed in both industrialized and resource-poor countries. However, such programs will not produce comparable estimates unless a standardized list of drug-resistance mutations is used to define transmitted resistance.

Methods: In this paper, we outline considerations for developing a list of drug-resistance mutations for epidemiologic estimates of transmitted resistance. First, the mutations should cause or contribute to drug resistance and should develop in persons receiving antiretroviral therapy. Second, the mutations should not occur as polymorphisms in the absence of therapy. Third, the mutation list should be applicable to all group M subtypes. Fourth, the mutation list should be simple, unambiguous, and parsimonious.

Results: Applying these considerations, we developed a list of 31 protease inhibitor-resistance mutations at 14 protease positions, 31 nucleoside reverse transcriptase inhibitor-resistance mutations at 15 reverse transcriptase positions, and 18 non-nucleoside reverse transcriptase inhibitor-resistance mutations at 10 reverse transcriptase positions.

Conclusions: This list, which should be updated regularly using the same or similar criteria, can be used for genotypic surveillance of transmitted HIV-1 drug resistance.

AIDS 2007, **21**:215–223

Keywords: HIV-1, Antiretroviral drug resistance, protease, reverse transcriptase, mutations, epidemiology, surveillance

Introduction

HIV-1 drug resistance can be acquired (developing in a person receiving antiretroviral treatment) or transmitted (occurring because a virus with drug-resistance mutations was transmitted to a drug-naive person; Fig. 1). Although both acquired and transmitted HIV-1 drug resistance are public health concerns, transmitted resistance has the potential to more rapidly reverse the effectiveness of first-line antiretroviral therapy at the population level. Persons with transmitted drug resistance begin antiretroviral therapy with a lower genetic barrier to resistance, a higher risk of virologic failure, and a higher risk of developing resistance even to those drugs in their regimen that were originally fully active [5,10–12]. Surveillance of transmitted resistance can supply information to support public

From the [a]Division of Infectious Diseases, Stanford University, Stanford, California, USA, the [b]Centre for Virology, Division of Infection and Immunity, University College London and Centre for Infections, Health Protection Agency, London, UK, the [c]Forum for Collaborative HIV Research, The George Washington University, Washington, DC, USA, the [d]Centre for Infectious Disease Prevention and Control, Public Health Agency of Canada, Ottawa, Ontario, Canada, the [e]Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA, and the [f]World Health Organization, Geneva, Switzerland.

Correspondence to Robert W. Shafer, Division of Infectious Diseases, Room S-169, Stanford University, Stanford, CA, 94305, USA.

E-mail: rshafer@stanford.edu

Received: 11 February 2006; revised: 6 July 2006; accepted: 14 September 2006.

ISSN 0269-9370 © 2007 Lippincott Williams & Wilkins

Primary or transmitted drug resistance: Drug resistance in previously untreated persons. Because drug resistance seldom occurs without drug exposure, primary drug resistance implies that a virus with drug resistance mutations was transmitted either directly, or through one or more intermediates, from a person with acquired drug resistance. Previously untreated persons include drug-naïve persons with laboratory evidence for recent infection (e.g. within the preceding 6 to 18 months depending on the particular study); newly diagnosed with infection of uncertain duration; and previously diagnosed with infection of uncertain duration. Primary HIV-1 infection should be distinguished from primary drug resistance. In the first case, 'primary' is used to describe persons who have recently been infected. In the second case, 'primary' is used to describe persons with transmitted resistance.

Acquired or secondary drug resistance: Drug resistance developing in a person who has received antiretroviral therapy. Acquired drug resistance results from the generation of genetic variation in the population of viruses within a person followed by the selection of drug-resistant variants during therapy.

Mutation: Because HIV-1 is highly variable, there is no standard wild-type strain. Therefore for drug-resistance studies, mutations are defined as amino acid differences from one of several wild-type reference sequences. The most commonly used reference sequences are of the laboratory viruses HXB2 and NL43 and a consensus reference sequence comprising the most common amino acid at each position in wild-type subtype B viruses (subtype B consensus). These sequences are nearly identical, differing at only a few amino acids not involved in drug resistance. The use of subtype B sequences as reference sequences is based on historical precedence.

Polymorphism: Polymorphisms are mutations occurring frequently in viruses not exposed to selective drug pressure. A nonpolymorphic mutation is one that does not occur in the absence of therapy. No frequency cut-off has been proposed to distinguish polymorphic from nonpolymorphic positions.

Electrophoretic mixture: The presence of more than one fluorescent peak at the same position in a dideoxynucleoside sequence indicates that two populations of viruses with different nucleic acids at the same position are each present in large enough proportions (> 10–20%) to be detected by sequencing.

T215 revertants: The nucleoside reverse transcriptase (RT) inhibitor-resistance mutations, T215Y/F evolve from T by acquiring two nucleotide mutations: ACT/C (T) → TAT/C (Y) or TTT/C (F). Patients primarily infected with strains containing T215Y/F often develop viruses with partial reversion mutations at this position [1–3]. T215C/D result from the back mutation of TAT/C (Y) to TGT/C (C) or GAT/C (D). T215I/V result from the back mutation of TTT/C (F) to ATT/C (I) or GTT/C (V). T215S (TCT/C) may result from back mutation of either TAT/C (Y) or TTT/C (F). T215E (GAA/G) requires two changes from T215Y (TAT/C) or one change from the common revertant T215D (GAT/C). Although most of the revertant mutations do not reduce nucleoside susceptibility (data for T215I/V are not available), they have been associated with an increased risk of virologic failure to a new treatment regimen [4]. The T215 revertants are also among the most common mutations observed in viruses from previously untreated persons, occurring in about 3% of such patients [5–9].

Fig. 1. Definitions of terms relevant to the epidemiology of drug resistance.

health bodies in designing education and prevention programs to minimize the development and transmission of drug-resistant viruses, and to support the rational use of antiretroviral drugs by treatment programs, clinicians and policy makers [WHO HIV Drug Resistance Surveillance Programme (V. 07-08-05), unpublished].

In Europe, North America, and Brazil, prevalence studies generally performed in specialist clinical centers have reported prevalences of drug resistance ranging from 5 to 15% in newly diagnosed persons and 10 to 25% in acutely infected persons (reviewed in [12,13]). However, it has been difficult to compare national, regional, and local estimates of transmitted resistance because of differences in study design and because estimates have been based on different lists of resistance mutations [13]. As antiretroviral treatment and surveillance programs are now beginning in many resource-limited countries, it is particularly important to develop a standard list of mutations to characterize the epidemiology of transmitted resistance [WHO HIV Drug Resistance Surveillance Programme (V. 07-08-05), unpublished].

This paper summarizes the points to be considered when selecting mutations for epidemiologic studies. Mutations should be commonly recognized as causing or contributing to resistance, should be nonpolymorphic in untreated persons, and should be applicable to all HIV-1 subtypes. Furthermore, the list should be relatively short: inclusion of a large number of mutations, particularly rare mutations, would unnecessarily complicate surveillance efforts and would decrease the uniform acceptance and widespread adoption of a standard list. In this review of the considerations for choosing drug-resistance mutations for the epidemiology of transmitted HIV drug resistance, we use publicly available data on the associations between genotype and antiretroviral treatment from the Stanford HIV Drug Resistance Database. We apply our criteria to propose a set of candidate mutations and then apply this set of mutations to five published studies of primary

HIV-1 infection for which sequences have been submitted to GenBank.

Criterion 1: association with drug resistance

Several types of data can be used to assess the role of a mutation in causing drug resistance: (1) correlations between a mutation and treatment: does drug therapy select for the mutation? (2). Correlations between a mutation and decreased in-vitro drug susceptibility; and (3) correlations between a mutation and a diminished in-vivo virologic response to a new antiretroviral regimen. These data often require expert interpretation. Therefore, for the purposes of this manuscript, we defined drug-resistance mutations primarily as those that are included in three or more of the following five expert panel lists of HIV-1 drug resistance mutations: (1) IAS-USA [14]; (2) Los Alamos HIV Sequence Database [15]; (3) HIVdb drug resistance interpretation algorithm (version 4.1.9) [16]; (4) ANRS drug resistance interpretation algorithm (version 2005.07) [17]; and (5) Rega Institute drug resistance interpretation algorithm (version 6.4.1) [18].

However, because none of the above mutation lists was designed for epidemiologic purposes, we have extended our criterion to include one category of mutations that is not present in three of the above lists. This category includes the T215 revertant mutations (T215C/D/E/I/S/V), which evolve from the nucleoside reverse transcriptase inhibitor (NRTI)-resistance mutations T215F/Y when viruses with T215F/Y are transmitted to a new patient in which case reversion to wild-type must evolve by back mutation rather than from a reservoir of drug-susceptible viruses [1–3,6]. Although most of the T215 revertants do not directly reduce drug susceptibility, they have been associated with an increased risk of virologic failure either because they are surrogates for the presence of T215F/Y or because they lower the genetic barrier to a major resistance mutation from two to one nucleotide [4,6]. Among the possible amino acids at this position (other than wild-type T and drug-resistant F/Y), the revertant mutations were selected for inclusion based on their frequency in published papers, their frequency in unambiguous form in published sequences (e.g. not part of a complex mixture of amino acids), their association with previous but discontinued drug therapy, and their nucleotide distance from the mutant forms F and Y.

Criterion 2: nonpolymorphic

Polymorphic drug-resistance mutations cannot be used for surveillance because they would lead to falsely high estimates of transmitted resistance. However, identifying mutations that are nonpolymorphic in the absence of antiretroviral drug therapy is confounded by three problems. First, nonpolymorphic treatment-associated mutations may appear polymorphic if the analysis includes a large proportion of untreated persons who were primarily infected with resistant viruses – a frequent occurrence in areas where antiretroviral drugs have been available for many years. Second, publicly available sequence data have been generated by many research groups using different methods of sequencing with varying levels of accuracy. Third, the high mutation rate of HIV-1 ensures that, in each patient, every mutation is created thousands of time each day. Genetic drift within a patient and the occasional disproportionate amplification of a rare variant prior to sequencing may lead to the detection in untreated persons of a mutation that usually occurs only in treated persons.

In the following paragraphs, we describe methods for identifying nonpolymorphic drug-resistance mutations using publicly available sequence data from treatment-naive persons infected with viruses belonging to different subtypes. For this analysis only a single isolate per person was used.

Influence of transmitted resistance on estimates of polymorphism rates

Two approaches were used to minimize the influence of transmitted resistance on estimating the polymorphism frequency in the absence of selective drug pressure. First, we excluded isolates from studies of untreated persons with primary HIV-1 infection in regions where transmitted resistance is common. Second, we excluded isolates from untreated persons with two or more drug-resistance mutations at residues that are already known either to be nonpolymorphic or to have low polymorphism rates. This second exclusion criterion is predicated on the strong likelihood that the presence of two or more drug-resistance mutations at nonpolymorphic positions in untreated persons is unlikely to reflect natural variation in protease and reverse transcriptase (RT) but is instead most consistent with previous selective drug pressure.

Effect of sequencing method and sequence quality on estimates of polymorphism rates

Two approaches were taken to minimize the effect that heterogeneous sequencing methods and outright sequencing errors could have on estimates of polymorphism rates: (1) published sequences performed using oligonucleotide arrays were excluded because these sequences have a higher error rate than HIV-1 sequences determined using dideoxynucleoside sequencing [19]; (2) A sequence quality score – defined as the total number of stop codons, highly ambiguous nucleotides (B, D, H, V, N) and highly unusual mutations (frequency $< 0.05\%$ in pooled viruses of all Group M subtypes from treated and untreated persons) – was assigned to all sequences in the database. Protease sequences with a sequence quality score of four or higher and RT sequences (positions

1–240) with a sequence quality score of six or higher were excluded from the dataset.

Distinguishing nonpolymorphic from rarely polymorphic drug-resistance mutations

In human genetics, a polymorphism is arbitrarily defined as a mutation that occurs in at least 1% of a population. However, because of the large number of HIV-1 positions associated with drug resistance, using a cut-off of 1% could lead to inflated estimates of transmitted resistance. As a more conservative criterion, we propose that the list of resistance mutations used for epidemiology should ideally exclude mutations occurring in untreated persons at a frequency of $> 0.5\%$. However, we cannot yet apply this criterion uniformly to all subtypes, because the numbers of isolates currently available from non-B subtypes are relatively small and may not be representative. The appearance of a mutation once or twice among the sequences from a non-B subtype with fewer than 200 sequences available could lead to that mutation's unjustified exclusion from the list. Therefore, we excluded from the list any mutation in more than 1% of untreated isolates from any one non-B subtype in our dataset, and have provisionally included those occurring > 0.5 to 1% until a larger dataset for each of the non-B subtypes become available.

An epidemiologic list may lead to overestimates of prevalence of resistance if the list includes mutations that occur at a low level without exposure to drug pressure. For example, nearly one-half of the drug-resistance surveillance mutations derived here occurred at a level of 0.1% or higher in one or more subtypes (see Tables 1–3 below). The presence of these mutations in untreated persons probably reflects a combination of two phenomena: transmitted resistance and natural occurrence. There is no definitive approach for distinguishing between these two possibilities because, even in recently infected persons, the genotype and treatment history of the source of infection are rarely known.

Criterion 3: applicability to all HIV-1 subtypes

Most data on the genetic mechanisms of HIV-1 drug resistance come from studies of subtype B viruses, the predominant subtype in the North American and Europe. Nonetheless several preliminary observations have been made about drug-resistance in non-B viruses. With few exceptions, nearly all of the differences between subtypes in reported viruses from untreated persons occur at positions that are also polymorphic in subtype B. Indeed, the vast majority of in-vitro and in-vivo studies suggest that the currently available protease and RT inhibitors are as active against non-B viruses as they are against subtype B viruses [20–25].

The mutations that cause drug resistance in subtype B viruses appear to be the main mutations that cause drug resistance in non-B viruses [26]. However, the spectrum of mutations at some drug-resistance positions differs among subtypes. For example, the RT mutation V106M, which rarely occurs in subtype B isolates after non nucleoside reverse transcriptase inhibitor (NNRTI) exposure, is one of the most common mutations responsible for NNRTI resistance in subtype C isolates [27,28]. The protease mutation V82M, which rarely occurs in subtype B isolates after protease inhibitor exposure, is a common cause of protease inhibitor resistance in subtype G isolates [29]. Failure to consider these exceptions would lead to falsely low estimates of transmitted resistance for persons infected with viruses belonging to these subtypes. Conversely, including mutations that are polymorphic in any subtype in a mutation list could lead to falsely high estimates of drug resistance in epidemiologic studies where non-B subtypes are common or where their prevalence is increasing.

These preliminary observations support the concept that a single mutation list applicable to epidemiology involving all subtypes can be created. Many surveys involve more than one subtype, and maintaining different lists for different subtypes would be impractical. Further, as sequencing of viral isolates from around the world increases an increasing number of new infections appear to be inter-subtype recombinants. We therefore propose a single list excluding mutations found to be polymorphic among sequences from any subtype, and including the drug-resistance mutations associated with treatment in any subtype.

Criterion 4: parsimonious and unambiguous

HIV-1 drug resistance incidence and prevalence surveys will often be performed by epidemiologists who do not have expert knowledge of HIV-1 drug resistance. Therefore, the simplest and shortest possible list of drug-resistance mutations that reliably assesses the prevalence of resistance should be used for epidemiologic studies of transmitted drug resistance. The process of developing the list should be transparent and reproducible. Although the list does not require all clinically relevant mutations, the majority of the mutations should be meaningful to clinicians at the sites of epidemiologic studies.

To simplify the list, we excluded mutations occurring at positions that are known to be highly polymorphic. Specifically, the protease mutations L10F/R/Y and A71I are each in three or more expert lists and are non-polymorphic in untreated persons. However, because these mutations occur at positions for which the most common drug-resistance mutations (L10I/V and A71V/T) are

Table 1. HIV-1 protease mutations meeting the proposed criteria for protease inhibitor resistance surveillance: prevalence in untreated persons according to subtype and prevalence in treated persons.

		Percentage polymorphic in untreated persons								
AA	Mut	A ($n=686$)	01 ($n=684$)	02 ($n=811$)	B ($n=3704$)	C ($n=1025$)	D ($n=355$)	F ($n=180$)	G ($n=270$)	Percentage with Rx[a] ($n=4154$)
L24	I	0	0	0.1	0	0	0	0	0	7.8
D30	N	0	0	0	0	0.1	0	0	0	13
V32	I	0	0	0.1	0	0	0	0	0	4.4
M46	I	0.4	0.3	0	0.2	0.2	0	0.6	0	21
I47	A	0	0	0	0	0	0	0	0	0.3
	V	0	0	0	0.1	0	0.3	0	0.7	2.3
G48	V	0	0	0	0	0	0	0	0	5.4
I50	V	0	0	0	0	0	0	0	0	2.3
	L	0	0	0	0	0	0	0	0	2.7
F53	L	0	0	0	0.1	0.1	0	0	0.4	7.0
I54	V	0	0	0	0	0	0	0	0	39
	L	0	0	0	0	0	0	0	0	3.1
	M	0	0	0	0	0	0	0	0	1.2
	A	0	0	0	0	0	0	0	0	1.2
	T	0.2	0	0	0	0	0	0	0	1.6
	S	0	0	0	0	0	0	0	0	0.2
G73	C	0	0	0	0	0	0	0	0	1.1
	S	0	0	0	0.1	0	0	0.6	0	9.1
	T	0	0	0	0	0	0	0	0	1.9
	A	0	0	0	0	0	0	0	0	0.5
V82	A	0	0	0	0	0.1	0	0	0	32
	F	0	0	0	0	0	0	0	0	2.4
	T	0	0	0	0	0	0	0	0	13
	S	0	0	0	0	0	0	0	0	3.6
	M	0	0	0	0	0	0	0	0	2.4
I84	V	0	0	0	0	0	0	0	0	13
	A	0	0	0	0	0	0	0	0	0.2
	C	0	0	0	0	0	0	0	0	1.2
N88	D	0	0.3	0	0.1	0	0	0	0	7.6
	S	0	0	0.1	0	0	0	0	0.7	6.2
L90	M	0	0.4	0	0.2	0.1	0	0	0.7	43

AA, amino acid position preceded by the one-letter code for the consensus B amino acid; Mut, drug-resistance mutation. The header for columns 3–10 contains the subtype followed by the number of protease inhibitor-naive persons with sequences in the Stanford HIV Drug Resistance Database. 01 and 02 indicate circulating recombinant forms 01 (AE) and 02 (AG).
[a]Percentage with Rx shows the mutation prevalence in persons receiving a protease inhibitor in the subtype with the highest prevalence for that mutation. Mutations for which the prevalence in untreated persons is $>0.5\%$ in a subtype are underlined.

polymorphic, the inclusion of these mutations would complicate the performance of resistance analyses in epidemiologic settings.

Protease inhibitor-resistance surveillance mutations

Table 1 lists 31 mutations at 14 protease positions likely to be appropriate for genotypic resistance epidemiology. Commonly recognized drug-resistance mutations excluded from the list because of occurrence above the cut-off in at least one subtype with no evidence of exposure to drug pressure included L10I/V, K20R/M/I, L33F, M36I/L/V, M46L, L63P, A71V/T, and V77I. Three of these exclusions are based on data from non-B isolates: K20I is the consensus amino acid for CRF01_AG and subtype G, M46L has been reported in 1.5% (4/264) of subtype G sequences and L33F has been reported in 1.0% (7/686) of subtype A and 1.2% (8/684) of CRF01_AE sequences.

Drug-resistance mutations that occur with a frequency of >0.5 to 1.0% in datasets with relatively small numbers of non-B subtypes have been provisionally included: M46I (0.6%, 1/180 of subtype F sequences), I47V (0.7%, 2/270 of subtype G sequences), G73S (0.6%, 1/180 of subtype F sequences), N88S (0.7%, 2/270 of subtype G sequences), and L90M (0.7%, 2/270 of subtype G sequences). When a larger number of subtype F and G sequences become available for analysis, the frequency of these mutations can be estimated more precisely. In the interim, they are included, but the possibility that these mutations could be sufficiently polymorphic to bias estimates of transmitted resistance upward should be recognized.

Nucleoside reverse transcriptase inhibitor-resistance surveillance mutations

Table 2 lists 31 NRTI-resistance mutations at 15 RT positions likely to be appropriate for genotypic resistance epidemiology. Commonly recognized

Table 2. HIV-1 reverse transcriptase mutations meeting the proposed criteria for nucleoside reverse transcriptase inhibitor resistance surveillance: prevalence in untreated persons according to subtype and prevalence in treated persons.

		Percentage polymorphic in untreated persons								
AA	Mut	A ($n=499$)	01 ($n=635$)	02 ($n=484$)	B ($n=2240$)	C ($n=915$)	D ($n=192$)	F ($n=137$)	G ($n=145$)	Percentage with Rx[a] ($n=4035$)
M41	L	0	0.2	0.2	0.5	0	1.0	0	0	40
K65	R	0	0	0	0.1	0.1	0	0	0	4.6
D67	N	0	0	0	0	0	0	0	0	46
	G	0	0	0	0	0	0	0	0	1.7
	Del	0	0	0	0	0	0	0	0	0.2
T69	D	0	0	0	0.1	0	0	0	0	6.5
	ins	0	0	0	0	0	0	0	0	0.5
K70	R	0	0.3	0	0.3	0	0	0	0.7	30
L74	V	0	0	0	0	0.1	0	0	0.7	3.3
V75	A	0	0	0	0	0	0	0	0	1.5
	M	0	0.6	0	0	0	0	0	0	1.8
	T	0	0	0	0	0	0	0	0	3.4
	S	0	0	0	0	0	0	0	0	0.2
F77	L	0	0.3	0	0.1	0	0	0	0	2.4
Y115	F	0	0	0	0	0	0	0	0	1.2
F116	Y	0	0.2	0	0	0	0	0	0	2.4
Q151	M	0	0	0	0	0	0	0	0	4.2
M184	V	0.2	0.2	0.4	0.2	0.2	0	0	0	66
	I	0	0.2	0	0.1	0	0.5	0	0.7	1.8
L210	W	0	0	0	0	0	0	0	0	23
T215	Y	0	0	0	0	0.1	0	0	0	37
	F	0	0	0	0	0	0	0	0	22
	C	0	0	0	0.3	0.1	0	0	0	1.5
	D	0	0	0	0.4	0	0	0	0	0.9
	E	0	0	0	0.1	0	0	0	0	3.6
	S	0	0	0	0.5	0.1	0	0	0	1.8
	I	0.4	0	0	0	0	0	0	0	3.5
	V	0	0	0	0	0	0	0	0	1.5
K219	Q	0.2	0.5	0.4	0.1	0	1.0	0	0	31
	E	0	0	0	0	0	0	0	0	7.1
	R	0	0	0.2	0.2	0.1	0	0	0	1.8

AA, amino acid position preceded by the one-letter code for the consensus B amino acid; Mut, drug-resistance mutation. The header for columns 3–10 contains the subtype followed by the number of reverse transcriptase inhibitor-naive persons with sequences in the Stanford HIV Drug Resistance Database. 01 and 02 indicate circulating recombinant forms 01 (AE) and 02 (AG).
[a]Percentage with Rx shows the mutation prevalence in persons receiving nucleoside reverse transcriptase inhibitor in the subtype with the highest prevalence for that mutation. Mutations for which the prevalence in untreated persons is $>0.5\%$ in a subtype are underlined.

drug-resistance mutations provisionally excluded from the list because of occurrence at $>1\%$ in one or more subtype sets in the database with no evidence of exposure to drug pressure included E44D, reported in 1.6, 1.5 and 0.7% of persons with subtype D, F, and G sequences, respectively; A62V, reported in 17% of persons with subtype A sequences; T69S, reported in 0.6, 1.0, and 1.1% of persons with subtype B, C, and D sequences, respectively; T69N, reported in 0.7% of persons with subtype B and F sequences; V75I, reported in 2.6% (5/189) persons with subtype D sequences; and V118I, reported in $>1\%$ of persons with subtype B, C, D, F, and G sequences.

Drug-resistance mutations that occur with a frequency of >0.5 to 1.0% among a relatively small set of one non-B subtype in the database have been provisionally included: M41L (1.0%, 2/192 in subtype D); K70R (0.7%, 1/145 in subtype G); L74V (0.7%, 1/145 in subtype G), V75M (0.6%, 4/635 in CRF01_AE), and K219Q (1.0%, 2/192 in subtype D). When a larger number of subtype D, F, and G sequences become available for analysis, their frequency can be estimated more precisely. The possibility that these mutations could be sufficiently polymorphic to bias estimates of transmitted resistance upward should be recognized.

Non-nucleoside reverse transcriptase-resistance surveillance mutations

Table 3 lists 18 NNRTI-resistance mutations at 10 RT positions likely to be appropriate for genotypic resistance epidemiology. Commonly recognized drug-resistance mutations provisionally excluded from the list because of occurrence at $>1\%$ in one or more subtypes with no evidence of exposure to drug pressure include: V108I, reported in 0.6 to 1.1% of sequences from untreated persons with subtypes, A, CRF02, D, and G; V179D, reported in $>1.0\%$ in of persons with subtypes B, F, and CRF01_AE sequences; V179E, reported in 4.3% of

Table 3. HIV-1 reverse transcriptase mutations meeting the proposed criteria for non-nucleoside reverse transcriptase inhibitor resistance surveillance: prevalence in untreated persons according to subtype and prevalence in treated persons.

		Percentage polymorphic in untreated persons								
AA	Mut	A ($n=499$)	01 ($n=635$)	02 ($n=484$)	B ($n=2244$)	C ($n=915$)	D ($n=192$)	F ($n=137$)	G ($n=145$)	Percentage with Rx[a] ($n=2568$)
L100	I	0	0	0	0	0	0	0	0	7.7
K101	E	0	0	0	0.3	0	0	0	0	16
K103	N	0	0	0.4	0.2	0.2	0	0	0	61
	S	0	0	0	0	0.1	0	0	0	0.7
V106	A	0	0	0.2	0	0	0	0	0	2.6
	M	0	0	0	0	0	0	0	0	15
Y181	C	0.2	0.2	0	0	0	0	0	0	33
	I	0.2	0	0	0	0	0	0	0	3.6
Y188	L	0	0	0	0	0	0	0	0	8.8
	H	0	0	0	0.1	0	0	0	0	1.5
	C	0	0	0	0	0	0	0	0	6.8
G190	A	0	0	0	0	0	0.5	0	0	36
	S	0	0	0	0	0	0	0	0	16
	E	0	0	0	0	0.1	0	0	0	1.8
	Q	0	0	0	0	0	0	0	0	0.3
P225	H	0	0	0	0	0	0	0	0	10
M230	L	0	0	0	0	0	0	0.7	0	2.3
P236	L	0	0	0.2	0.1	0	0	0	0	1.0

AA, amino acid position preceded by the one-letter code for the consensus B amino acid; Mut, drug-resistance mutation. The header for columns 3–10 contains the subtype followed by the number of reverse transcriptase inhibitor-naive persons with sequences in the Stanford HIV Drug Resistance Database. 01 and 02 indicate circulating recombinant forms 01 (AE) and 02 (AG).
[a]Percentage with Rx shows the mutation prevalence in persons receiving non-nucleoside reverse transcriptase inhibitor in the subtype with the highest prevalence for that mutation. Mutations for which the prevalence in untreated persons is $>0.5\%$ in a subtype are underlined.

persons with subtype G sequences; and F227L, reported in 1.9% of persons with subtype F sequences.

Drug-resistance mutations that occur with a frequency of >0.5 to 1.0% among a relatively small set of one non-B subtype in the database have been provisionally included: M230L (0.7%, 1/137 in subtype F).

Application to published studies of primary HIV-1 infection

There have been five studies of primary HIV-1 infection for which RT and/or protease sequence data have been submitted to GenBank [30–34] (Table 4). Twenty-three of the 32 NRTI-resistance mutations and nine of the 18 NNRTI-resistance mutations on our list were present in the 656 RT sequences in these studies; and nine of the 31 protease-resistance mutations on our list were present in the 407 protease sequences in these studies.

For four of the 15 estimates of drug class resistance in these studies, there was complete agreement between the results described in the published study and those obtained using the mutation list we have derived. For 10 of the estimates, the results obtained using our mutation list differed from the results reported by the authors by a median of 1.2% (range, 0.9–3.6%). For one study, protease sequences were not available and no comparison was possible.

Although none of the five studies enumerated a specific list of drug resistance surveillance mutations, the analysis of the sequences from these studies in conjunction with tables in the primary publication containing the mutations of drug-resistant isolates made it possible to identify the likely sources for differences between the published analyses and our results. In one study, the difference arose from the authors' inclusion of the NRTI mutation V118I and the NNRTI mutation V179D, mutations that are not on our list as they occur at a frequency of more than 1% in the absence of therapy.

In the remaining studies, differences arose from mutations that are in our mutation list but did not appear to be counted in the primary study, including the RT mutations F77L (present as a mixture in two persons), K103N (three persons), T215D/E/S (three persons), K219Q (three persons), P236L, and M41L+L74V+ M184V+L210W+ T215Y and the protease mutations L24I (present as a mixture in two persons), F53L, I54T, G73A, and I54V+V82F+ L90M. Some of these differences appear to reflect differences in how mutations were classified and others to reflect differences in how the final results were tabulated.

Conclusion

In this paper, we propose criteria for selecting mutations for drug resistance surveillance and develop a provisional list of mutations based on these criteria. Widespread application of the list proposed here, or of a similar list based on standard criteria, will facilitate inter-study comparisons and will support ongoing representative surveillance to evaluate trends over time.

Table 4. Prevalence of HIV-1 genotypic resistance in five studies of primary HIV-1 infection using a standard list of drug-resistance mutations.

					Prevalence of resistance reported by authors			Prevalence of resistance using standard list		
Reference	Location	Years	*N*	Criteria for genotypic resistance	NRTI	NNRTI	PI	NRTI	NNRTI	PI
Balotta [30]	Italy	1994–1997	38	Not stated	21.1	2.6	0	21.1	5.2	0
Simon [31]	New York City, USA	1995–2001	154	Not stated. M46I, V82A, and L90M considered primary PI-resistance mutations	13.0	4.5	3.2	11.7	2.6	3.9
Romano [32]	Tuscany, Italy	1996–2000	116	Not stated. M46I and L90M considered key PI-resistance mutations	13.0	0.9	0.9	13.8	0.9	4.3
Toni [33]	Abidjan, Cote d'Ivoire	1997–2000	99	ANRS consensus method [17]	0	0	0	0	1.0	1.0
Chaix [34]	France	1999–2000	249	IAS USA 2002 [14] excluding L63P and possibly other mutations	8.4	4.1	5.6	12.0	5.2	NA

NA, not available; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor. Sequences were available from a subset of virus isolates from two additional studies of primary HIV-1 infection [35,36]. It was not possible, however, to correlate the prevalence of drug-resistance mutations in this subset with the prevalence reported in the published studies.

Although there is no gold standard for the identification of transmitted resistance, continued studies of virus sequences of different subtypes from untreated and treated chronically infected persons are required to avoid the exclusion of mutations that are selected by therapy in one or more subtypes and to avoid the inclusion of mutations that are nonpolymorphic in one or more subtypes. Continued studies of virus sequences of different subtypes from untreated acutely infected persons facilitates the identification of mutations important for surveillance such as the T215 revertants that evolve from known drug-resistant variants in the absence of selective drug pressure and competing wild-type variants.

A definitive, unambiguous list of genotypic mutations for surveillance systems and other epidemiologic studies is also likely to change over time with the introduction of new drugs, the identification of new drug-resistance mutations, and the availability of more non-B sequences for analysis. Active efforts are required to base the analyses on sufficient numbers of sequences from all countries in the world and all recognized subtypes.

References

1. Goudsmit J, de Ronde A, Ho DD, Perelson AS. **Human immunodeficiency virus fitness in vivo: calculations based on a single zidovudine resistance mutation at codon 215 of reverse transcriptase.** *J Virol* 1996; **70**:5662–5664.
2. Yerly S, Rakik A, De Loes SK, Hirschel B, Descamps D, Brun-Vezinet F, Perrin L. **Switch to unusual amino acids at codon 215 of the human immunodeficiency virus type 1 reverse transcriptase gene in seroconvertors infected with zidovudine-resistant variants.** *J Virol* 1998; **72**:3520–3523.
3. de Ronde A, van Dooren M, van Der Hoek L, Bouwhuis D, de Rooij E, van Gemen B, *et al*. **Establishment of new transmissible and drug-sensitive human immunodeficiency virus type 1 wild types due to transmission of nucleoside analogue-resistant virus.** *J Virol* 2001; **75**:595–602.
4. Violin M, Cozzi-Lepri A, Velleca R, Vincenti A, D'Elia S, Chiodo F, *et al*. **Risk of failure in patients with 215 HIV-1 revertants starting their first thymidine analog-containing highly active antiretroviral therapy.** *AIDS* 2004; **18**:227–235.
5. Little SJ, Holte S, Routy JP, Daar ES, Markowitz M, Collier AC, *et al*. **Antiretroviral-drug resistance among patients recently infected with HIV.** *N Engl J Med* 2002; **347**:385–394.
6. Garcia-Lerma JG, Nidtha S, Blumoff K, Weinstock H, Heneine W. **Increased ability for selection of zidovudine resistance in a distinct class of wild-type HIV-1 from drug-naive persons.** *Proc Natl Acad Sci U S A* 2001; **98**:13907–13912.
7. Cane P, Chrystie I, Dunn D, Evans B, Geretti AM, Green H, *et al*. **Time trends in primary resistance to HIV drugs in the United Kingdom: multicentre observational study.** *BMJ* 2005; **331**:1368.
8. Wensing AM, van de Vijver DA, Angarano G, Asjo B, Balotta C, Boeri E, *et al*. **Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: Implications for clinical management.** *J Infect Dis* 2005; **192**:958–966.
9. Oette M, Kaiser R, Daumer M, Petch R, Fatkenheuer G, Carls H, *et al*. **Primary HIV drug resistance and efficacy of first-line antiretroviral therapy guided by resistance testing.** *J Acquir Immune Defic Syndr* 2006; **41**:573–581.
10. Grant RM, Hecht FM, Warmerdam M, Liu L, Liegler T, Petropoulos CJ, *et al*. **Time trends in primary HIV-1 drug resistance among recently infected persons.** *JAMA* 2002; **288**:181–188.
11. Markowitz M, Mohri H, Mehandru S, Shet A, Berry L, Kalyanaraman R, *et al*. **Infection with multidrug resistant, dual-tropic HIV-1 and rapid progression to AIDS: a case report.** *Lancet* 2005; **365**:1031–1038.

12. Daar ES, Richman DD. **Confronting the emergence of drug-resistant HIV type 1: impact of antiretroviral therapy on individual and population resistance.** *AIDS Res Hum Retroviruses* 2005; **21**:343–357.
13. Pillay D. **Current patterns in the epidemiology of primary HIV drug resistance in North America and Europe.** *Antivir Ther* 2004; **9**:695–702.
14. Johnson VA, Brun-Vezinet F, Clotet B, Conway B, Kuritzkes DR, Pillay D, *et al*. **Update of the Drug Resistance Mutations in HIV-1: 2005.** *Top HIV Med* 2005; **13**:51–57.
15. Clark S, Calef C, Mellors JW. **Mutations in retroviral genes associated with resistance.** In: Leitner T, Foley B, Hahn B, Marx P, McCutchan F, Mellors J, *et al*. editors. *HIV sequence compendium*. Los Alamos, NM: Theoretical Biology and Biophysics Group, Los Alamos National Laboratory; 2005. pp. 83–128.
16. Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. **Human immunodeficiency virus reverse transcriptase and protease sequence database.** *Nucleic Acids Res* 2003; **31**:298–303.
17. Meynard JL, Vray M, Morand-Joubert L, Race E, Descamps D, Peytavin G, *et al*. **Phenotypic or genotypic resistance testing for choosing antiretroviral therapy after treatment failure: a randomized trial.** *AIDS* 2002; **16**:727–736.
18. Van Laethem K, De Luca A, Antinori A, Cingolani A, Perno CF, Vandamme A-M. **A genotypic drug resistance interpretation algorithm that significantly predicts therapy response in HIV-1-infected patients.** *Antivir Ther* 2002; **7**:123–129.
19. Vahey M, Nau ME, Barrick S, Cooley JD, Sawyer R, Sleeker AA, *et al*. **Performance of the Affymetrix GeneChip HIV PRT 440 platform for antiretroviral drug resistance genotyping of human immunodeficiency virus type 1 clades and viral isolates with length polymorphisms.** *J Clin Microbiol* 1999; **37**:2533–2537.
20. Weidle PJ, Malamba S, Mwebaze R, Sozi C, Rukundo G, Downing R, *et al*. **Assessment of a pilot antiretroviral drug therapy programme in Uganda: patients' response, survival, and drug resistance.** *Lancet* 2002; **360**:34–40.
21. Pillay D, Walker AS, Gibb DM, de Rossi A, Kaye S, Ait-Khaled M, *et al*. **Impact of human immunodeficiency virus type 1 subtypes on virologic response and emergence of drug resistance among children in the Paediatric European Network for Treatment of AIDS (PENTA) 5 trial.** *J Infect Dis* 2002; **186**:617–625.
22. Kebba A, Atwine D, Mwebaze R, Kityo C, Nakityo R, Peter M. **Therapeutic responses to AZT + 3TC + EFV in advanced antiretroviral naive HIV type 1-infected Ugandan patients.** *AIDS Res Hum Retroviruses* 2002; **18**:1181–1187.
23. Landman R, Schiemann R, Thiam S, Vray M, Canestri A, Mboup S, *et al*. **Once-a-day highly active antiretroviral therapy in treatment-naive HIV-1-infected adults in Senegal.** *AIDS* 2003; **17**:1017–1022.
24. Parkin NT, Schapiro JM. **Antiretroviral drug resistance in non-subtype B HIV-1, HIV-2 and SIV.** *Antivir Ther* 2004; **9**:3–12.
25. Laurent C, Kouanfack C, Koulla-Shiro S, Nkoue N, Bourgeois A, Calmy A, *et al*. **Effectiveness and safety of a generic fixed-dose combination of nevirapine, stavudine, and lamivudine in HIV-1-infected adults in Cameroon: open-label multicentre trial.** *Lancet* 2004; **364**:29–34.
26. Kantor R, Katzenstein DA, Efron B, Carvalho AP, Wynhoven B, Cane P, *et al*. **Impact of HIV-1 subtype and antiretroviral therapy on protease and reverse transcriptase genotype: results of a global collaboration.** *PLoS Med* 2005; **2**:e112.
27. Brenner B, Turner D, Oliveira M, Moisi D, Detorio M, Carobene M, *et al*. **A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to non-nucleoside reverse transcriptase inhibitors.** *AIDS* 2003; **17**:F1–F5.
28. Grossman Z, Istomin V, Averbuch D, Lorber M, Risenberg K, Levi I, *et al*. **Genetic variation at NNRTI resistance-associated positions in patients infected with HIV-1 subtype C.** *AIDS* 2004; **18**:909–915.
29. Camacho R, Godinho A, Gomes P, Abecasis A, Vandamme A-M, Palma C, *et al*. **Different substitutions under drug pressure at protease codon 82 in HIV-1 subtype G compared to subtype B infected individuals including a novel I82M resistance mutations.** *Antiviral Therapy* 2005; **10**:S151.
30. Balotta C, Berlusconi A, Pan A, Violin M, Riva C, Colombo MC, *et al*. **Prevalence of transmitted nucleoside analogue-resistant HIV-1 strains and pre-existing mutations in pol reverse transcriptase and protease region: outcome after treatment in recently infected individuals.** *Antiviral Therapy* 2000; **5**:7–14.
31. Simon V, Vanderhoeven J, Hurley A, Ramratnam B, Louie M, Dawson K, *et al*. **Evolving patterns of HIV-1 resistance to antiretroviral agents in newly infected individuals.** *AIDS* 2002; **16**:1511–1519.
32. Romano L, Venturi G, Ferruzzi R, Riccio ML, Corsi P, Leoncini F, *et al*. **Detection of genotypically drug-resistant HIV-1 variants and non-B subtypes in recently infected antiretroviral-naive adults in Italy.** *AIDS* 2000; **14**:2204–2206.
33. Toni T, Masquelier B, Bonard D, Faure M, Huet C, Caumont A, *et al*. **Primary HIV-1 drug resistance in Abidjan (Cote d'Ivoire): a genotypic and phenotypic study.** *AIDS* 2002; **16**:488–491.
34. Chaix ML, Descamps D, Harzic M, Schneider V, Deveau C, Tamalet C, *et al*. **Stable prevalence of genotypic drug resistance mutations but increase in non-B virus among patients with primary HIV-1 infection in France.** *AIDS* 2003; **17**:2635–2643.
35. Little SJ, Daar ES, D'Aquila RT, Keiser PH, Connick E, Whitcomb JM, *et al*. **Reduced antiretroviral drug susceptibility among patients with primary HIV infection.** *JAMA* 1999; **282**:1142–1149.
36. Weinstock HS, Zaidi I, Heneine W, Bennett D, Garcia-Lerma JG, Douglas JM Jr, *et al*. **The epidemiology of antiretroviral drug resistance among drug-naive HIV-1-infected persons in 10 US cities.** *J Infect Dis* 2004; **189**:2174–2180.