

Rationale and Uses of a Public HIV Drug-Resistance Database

Robert W. Shafer

Division of Infectious Diseases, Department of Medicine, Stanford University, Stanford, California

Knowledge regarding the drug resistance of human immunodeficiency virus (HIV) is critical for surveillance of drug resistance, development of antiretroviral drugs, and management of infections with drug-resistant viruses. Such knowledge is derived from studies that correlate genetic variation in the targets of therapy with the antiretroviral treatments received by persons from whom the variant was obtained (genotype-treatment), with drug-susceptibility data on genetic variants (genotype-phenotype), and with virological and clinical response to a new treatment regimen (genotype-outcome). An HIV drug-resistance database is required to represent, store, and analyze the diverse forms of data underlying our knowledge of drug resistance and to make these data available to the broad community of researchers studying drug resistance in HIV and clinicians using HIV drug-resistance tests. Such genotype-treatment, genotype-phenotype, and genotype-outcome correlations are contained in the Stanford HIV RT and Protease Sequence Database and have specific usefulness.

Publicly available databases have revolutionized how biological research is done and have led to an exponential increase in discovery brought on through secondary analyses of publicly available data. As medical research is increasingly turning to studies of sequence variation in genes of medical importance, a need has arisen for databases that link sequence data to other forms of data, such as the evolutionary history of genetic variants, functional information about genetic variants, and the clinical implications of genetic variation (genotype-outcome correlations).

The Stanford HIV RT and Protease Sequence Database (HIVRT&PrDB; also called the "HIV Drug Resistance Database") was formed in 1998 with HIV reverse transcriptase (RT) and protease sequences from persons with well-characterized antiretroviral treatment histories (genotype-treatment correlations) [1]. In 2001, correlations between genotype and in vitro susceptibility test results (genotype-phenotype correlations) were added. In 2004, correlations between genotype and the

virological response to a new treatment regimen (genotype-outcome correlations) were added. The correlations in the database have been obtained from nearly 600 published studies (<http://hivdb.stanford.edu/cgi-bin/Reference.cgi>). This review summarizes the genotype-treatment, genotype-phenotype, and genotype-outcome correlations in the HIVRT&PrDB and provides specific examples of their utility.

GENOTYPE-TREATMENT CORRELATIONS

Sequences of HIV-1 isolates from patients who experienced failure of antiretroviral therapy indicate which virus mutations are most significant in vivo, enabling the virus to escape from the inhibitory effects of particular drugs. As of May 2005, the HIVRT&PrDB contained protease and RT sequences from >10,000 persons with well-characterized antiretroviral treatment histories (table 1). The Genotype-Treatment section of the Web site has 10 pages with customizable queries for viewing or downloading data linking protease and RT mutations and sequences to specific drug treatments (<http://hivdb.stanford.edu/pages/genotype-rx.html>). Examples of such queries and the type of data returned are shown in table 2. Two recently published studies based on these data show how genotype-treatment correlations can inform the development of surveillance studies of HIV drug resistance.

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Reprints or correspondence: Dr. Robert W. Shafer, Div. of Infectious Diseases and Geographic Medicine, Rm. S-169, Stanford University, Stanford, CA 94305 (rshafer@stanford.edu).

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Table 1. Genotype-treatment correlations: number of persons with sequenced HIV-1 isolates in Stanford HIV RT and Protease Sequence Database, according to HIV-1 subtype, gene, and treatment (May 2005).

Subtype, treatment status	Protease	RT
B		
Untreated	3369	2077
Treated	3354	4977
Non-B		
Untreated	3738	2612
Treated	793	1797
Total	11,254	11,463

NOTE. Data are no. of persons with sequenced isolates. For the protease and RT categories, treatment status refers to protease and RT inhibitors, respectively. For example, protease genes from persons who were protease inhibitor naive were considered to be untreated regardless of whether the persons from whom the sequences were obtained had received RT inhibitors. The non-B subtype category includes isolates belonging to HIV-1 subtypes A, B, C, D, F, G, CRF01_AE, and CRF01_AG. Several hundred additional circulating recombinant forms and non-circulating recombinant form recombinants are not included in the totals.

Correlation of protease and RT mutations with antiretroviral therapy in HIV-1 subtype B isolates. HIV-1 mutations that emerge during in vitro passage experiments, reduce in vitro drug susceptibility in site-directed mutants, or occur in large numbers of clinical isolates are widely recognized to be associated with drug resistance [2, 3]. These established mutations, however, have 2 limitations to their usefulness in surveillance of drug resistance. First, most are identified during preclinical and early clinical drug development. In contrast, mutations conferring drug resistance that are uncommon, that emerge after prolonged therapy, or that act primarily in combination with other mutations often remain unrecognized. Second, several of the established mutations are polymorphic and occur commonly in untreated viruses.

To identify mutations resulting from selective drug pressure, we compared the prevalence of protease and RT mutations in HIV-1 subtype B sequences from persons with and without previous treatment with protease inhibitors (PIs), nucleoside RT inhibitors (NRTIs), and nonnucleoside RT inhibitors (NNRTIs). Treatment-selected mutations in protease isolates from 5867 persons and in RT isolates from 6247 persons were also categorized as to whether they were polymorphic (prevalence of >0.5%) in untreated persons and whether they were considered to be established mutations conferring drug resistance [4]. New methods were introduced to minimize misclassification result-

ing from transmitted resistance, population stratification, sequencing artifacts, and multiple hypotheses testing.

Of 74 PI-selected mutations, 60 (36 established and 24 additional mutations) at 34 positions were nonpolymorphic. Of 47 NRTI-selected mutations, 43 (21 established and 22 additional mutations) at 24 positions were nonpolymorphic. Of 37 NNRTI-selected mutations, 26 (15 established and 11 additional mutations) at 15 positions were nonpolymorphic. The additional treatment-selected mutations were less frequent than established mutations and were more likely to occur in combination with established drug-resistance mutations than to occur alone. Nonpolymorphic treatment-selected mutations are likely to be more sensitive (because of an increased number of mutations) and more specific (because of the exclusion of polymorphic treatment-selected mutations) indicators of transmitted resistance than are polymorphic treatment-selected mutations.

Correlation of protease and RT mutations with antiretroviral therapy in non-B subtypes. Because HIV-1 subtype B predominates in North America and western Europe, antiretroviral drugs used to treat HIV infection were developed using biophysical, biochemical, and in vitro studies of subtype B isolates, and most data on the genetic mechanisms of drug resistance in HIV-1 were generated through the study of subtype B viruses. However, subtype B accounts for only ~10% of the global HIV pandemic. An increasing number of in vitro and in vivo studies suggest that the currently available PIs and RT inhibitors are as active against non-subtype B viruses as they are against subtype B viruses [5–10]. However, fewer data are available on the genetic mechanisms of drug resistance in non-subtype B viruses. Identifying drug-resistance mutations in non-B subtypes is necessary for monitoring the evolution and transmission of drug resistance, for determining initial treatment strategies for persons infected with non-subtype B viruses, and for interpreting genetic resistance in patients who experience failure of antiretroviral treatment regimens.

To assess the impact of HIV-1 subtype and antiretroviral treatment on the distribution of mutations in protease and RT, researchers from 15 institutions collaborated to collect and analyze genotypes and treatment histories from persons infected with non-B subtype viruses [11]. The analysis showed that mutations at each of the established subtype B drug-resistance positions occurred in ≥ 1 non-subtype B isolate and that mutations at 80% of the known subtype B drug-resistance positions were significantly associated with antiretroviral treatment in ≥ 1 non-B subtype. Conversely, there was no compelling evidence for positions in protease or RT being uniquely associated with therapy in non-subtype B isolates.

However, 2 simplifications, likely to become unnecessary in future analyses as sufficient data become available, were made to increase the statistical power of the analysis. First, no distinction was made between different substitutions at the same

Table 2. Genotype-treatment correlations: examples of possible queries and significance of their results.

Query, criteria (examples)	Result	No. ^a
Treatment		
No treatment; HIV-1 subtype B	Natural variability of HIV-1 subtype B	3440
No treatment; HIV-1 subtype C	Natural variability of HIV-1 subtype C	733
NFV; 1 PI; HIV-1 subtype B	Mutations in HIV-1 subtype B isolates from persons receiving NFV	785
NFV; 1 PI; HIV-1 subtype C	Mutations in HIV-1 subtype C isolates from persons receiving NFV	72
RTV/SQV; all HIV-1 subtypes	Mutations in isolates from all persons receiving RTV + SQV (whether or not other PIs were also received)	921
≥3 PIs; LPV; all HIV-1 subtypes	Mutations from persons receiving LPV and ≥3 PIs	264
2 NRTIs; AZT/3TC; ≥1 NNRTI; CRF01_AE	Mutations in CRF01_AE viruses from persons receiving AZT/3TC and an NNRTI but no other NRTIs	23
≥5 NRTIs; ≥2 NNRTIs; all HIV-1 subtypes	Mutations in isolates from persons receiving ≥5 NRTIs and ≥2 NNRTIs	316
Mutation		
V82I (no mutations at other drug-resistance positions)	Mutation at a drug-resistance position that occurs in viruses from PI-naive persons, particularly in HIV-1 subtype G isolates	311
V82A (no restrictions on other positions)	Nearly always occurs in persons receiving ≥1 PI; 94% of sequences with V82A have additional mutations conferring PI-resistance mutations	1319
K103N/R/S	Position summary page shows that N (<i>n</i> = 1058) and S (<i>n</i> = 25) but not R (<i>n</i> = 198) are significantly associated with NNRTI therapy	1281
L33F/I/V	The position summary page shows that F (<i>n</i> = 183) and I (<i>n</i> = 99) but not V (<i>n</i> = 108) are significantly associated with PI therapy	390
M41L; M184V; L210W; T215Y	A very common pattern that almost never occurs with mutations at position 65; K65R occurs only with T215Y/F in sequences containing an electrophoretic mixture at position 65 or 215	964

NOTE. Criteria represent options that can be selected by the user. There are 2 types of treatment queries: (1) standard queries, which return a tabular summary of all isolates meeting the user-defined criteria with 1 row per isolate; complete sequences meeting the user-defined query can be retrieved from the standard query pages; and (2) profile queries, which return a composite summary of mutations at each position. AZT, zidovudine; LPV, lopinavir; NFV, nelfinavir; NNRTI, nonnucleoside RT inhibitor; NRTI, nucleoside RT inhibitor; PI, protease inhibitor; RTV, ritonavir; SQV, saquinavir; 3TC, lamivudine.

^a No. of results meeting the query criteria as of May 2005.

position; all differences from consensus B were considered to be mutations. Second, viruses were classified only by the classes of drugs to which they were exposed rather than by individual drugs or drug regimens. Therefore, further research is required to identify differences in the spectrum of mutations at individual positions and differences in the patterns of mutations associated with particular drugs. Indeed, 3 such differences have been reported and are shown in figure 1, through the use of the Profile query pages in HIVRT&PrDB [12–15, 17].

GENOTYPE-PHENOTYPE CORRELATIONS

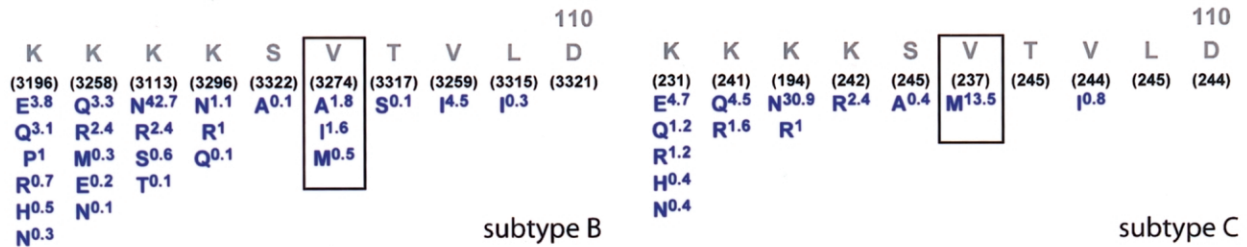
The impact of specific mutations and combinations of mutations on drug susceptibility makes it possible to determine the extent of cross-resistance among different drugs of the same class. The HIVRT&PrDB contains the only publicly available data linking genotype and phenotype for clinical isolates (table 3). The Genotype-Phenotype section has 7 pages with customizable queries for viewing or downloading data linking mutations or combinations of mutations to changes in drug susceptibility (<http://hivdb.stanford.edu/pages/genotype-phenotype.html>). Two recently published studies based on genotype-phenotype correlations in the HIVRT&PrDB are described below.

Precision and sensitivity of current phenotypic assays.

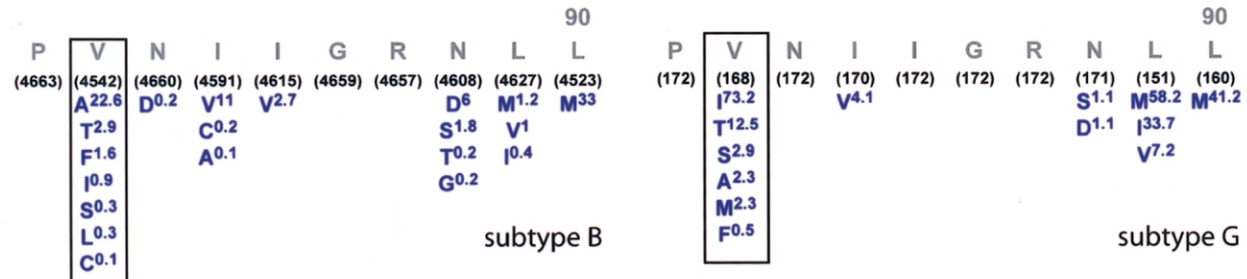
Although 2 susceptibility assays (Antivirogram [Virco] and PhenoSense [Monogram Biosciences]) have been widely used in clinical management for >5 years, the precision and sensitivity of these assays could not be compared until the HIVRT&PrDB had accumulated sufficient drug-susceptibility data. On the basis of routine queries of the database, we previously observed that the PhenoSense assay appeared to be more sensitive at detecting resistance to each of the NRTIs with the exception of zidovudine, lamivudine, and emtricitabine. For example, a stavudine-susceptibility query on isolates with the RT mutations M41L, L210W, and T215Y returns 74 PhenoSense and 53 Antivirogram results (figure 2). Susceptibilities of isolates with these mutations (*dark blue dots*) were, in all cases, greater than those of wild-type isolates (*light blue dots*) for the PhenoSense but not the Antivirogram assay.

On the basis of these initial observations, we used isolates sharing common patterns of drug-resistance mutations to query each assay. We then compared the proportion of these isolates with levels of resistance higher than those of wild-type isolates for various antiretroviral drugs [18]. Our analysis showed that the SD and median absolute deviance of the fold

A Treatment with non-nucleoside RT inhibitors, position 106



B Treatment with protease inhibitors, position 82



C Treatment with nelfinavir, positions 30 and 90

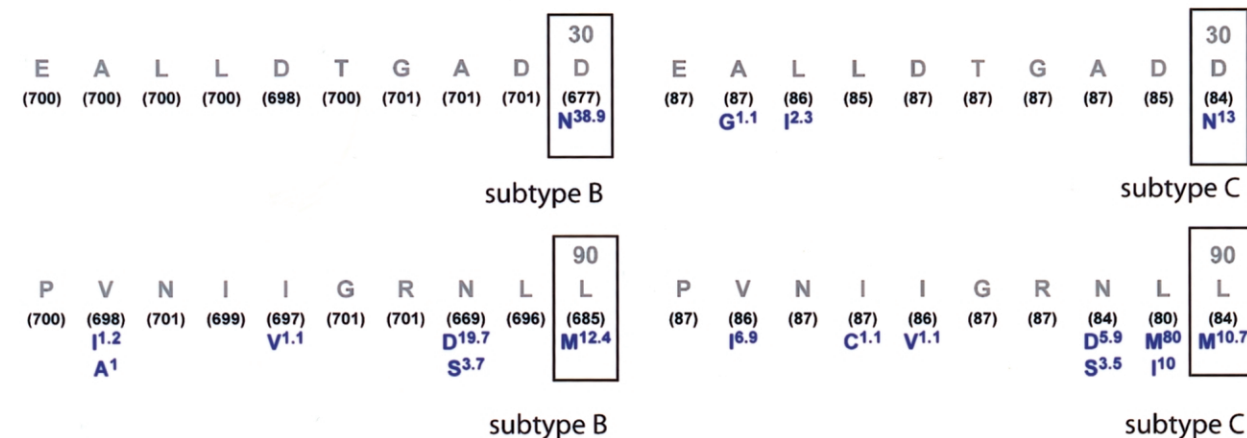


Figure 1. Examples of 3 published intersubtype differences in the genetic mechanisms of HIV-1 drug resistance, as demonstrated by Profile Queries on the Stanford HIV RT and Protease Sequence Database (<http://hivdb.stanford.edu/pages/genotype-rx.html>). *A*, Association of the RT mutation V106M with nonnucleoside RT inhibitor therapy in subtype C viruses [12, 13]. *B*, Association of the protease mutation V82T and V82M with protease inhibitor therapy in subtype G viruses [14]. *C*, Decrease in the D30N:L90M ratio during nelfinavir therapy in subtype C compared with subtype B [15, 16]. For each section, the top line contains the consensus B reference amino acids. The number in parenthesis under the reference amino acid contains the number of persons in the database (meeting the query's criteria) with sequences encompassing that position. The entries beneath the number in parenthesis indicate reported differences from the reference amino acid. Superscripts indicate the proportion of persons in whom the superscripted amino acid is found.

resistance of the log-transformed NRTI-susceptibility results were significantly lower for the PhenoSense than for the Antivirogram assay, indicating that the PhenoSense assay is more precise. The PhenoSense assay was also significantly more likely than the Antivirogram assay to detect resistance to abacavir, didanosine, and stavudine in isolates with the common drug-resistance mutations M41L, M184V, and T215Y (\pm L210W),

when published wild-type cutoffs were used for each assay. A receiver operator characteristic analysis showed that, regardless of the cutoff used to distinguish wild-type isolates from those with reduced susceptibility, the PhenoSense assay was significantly more sensitive than the Antivirogram assay in detecting decreased abacavir, didanosine, and stavudine susceptibility [18].

Panel of multidrug-resistant viruses. Antiretroviral drugs

Table 3. Genotype-phenotype correlations: number of isolates and susceptibility test results in Stanford HIV RT and Protease Sequence Database, according to gene and test method (May 2005).

Gene, test method	Isolates, no.	Test results, no. ^a
Protease		
PhenoSense	860	4811
Antivirogram	424	2189
Other	646	2809
Total	1930	9809
RT		
PhenoSense	695	5476
Antivirogram	477	3387
Other	996	5573
Total	2168	14,436

NOTE. The PhenoSense assay is performed by Monogram Biosciences; the Antivirogram assay is performed by Virco. Other assays include plaque-reduction assays, assays using peripheral blood mononuclear cells, and in-house pharmaceutical assays.

^a The number of test results is greater than the number of isolates because each susceptibility result on a different drug is considered to be a different test result.

are usually designed for and tested against wild-type HIV-1 laboratory isolates that were originally isolated in the mid-1980s. New compounds that demonstrate *in vitro* activity against these laboratory isolates are then tested on a range of drug-resistant clinical isolates. However, because no standard set of clinical isolates has been identified for testing, it is difficult to determine the activity of a new compound relative to that of other approved drugs and experimental compounds. Moreover, it is often not known whether the isolates used in many of these studies accurately reflect a clinically relevant spectrum of HIV-1 drug resistance.

Although many mutations are responsible for drug resistance, these mutations occur in common patterns. Indeed, there are a limited number of genetic pathways to resistance to mul-

iple NRTIs. We developed a panel of 12 recombinant molecular infectious clones containing each of the known patterns of RT mutations responsible for resistance to multiple NRTIs [19]. The specific combinations of mutations and their associated drug susceptibilities are shown in table 4. Eight clones have reduced susceptibility to each of the 7 approved NRTIs. The remaining 4 clones have reduced susceptibility to 3–6 inhibitors. Testing the activity of new antiretroviral compounds against this panel of drug-resistant clones will help to determine their relative activities and may increase the likelihood that new drugs will be efficacious against clinically relevant drug-resistant viruses.

Genotype-outcome correlations and patient management.

Many retrospective and prospective studies have demonstrated that preexisting drug resistance is an independent predictor of success of a treatment regimen. However, although genotypic resistance testing is recommended to assist with selection of antiretroviral drugs, there are no guidelines on how such tests should be used. Because HIV-1 develops drug resistance through a variety of genetic mechanisms, the development of resistance segregates patients into many strata and makes it impossible for any one study to identify optimal treatments for all, or even most, subsets of patients. Although comprehensive and specific guidelines exist for initial HIV treatment, published guidelines for treating persons with drug-resistant virus are vague and often contradictory. Treatment of such persons is often a process of trial and error, which increases a patient's risk of treatment failure, drug toxicity, and increased drug resistance.

This astounding incongruity between the guidelines for treating drug-susceptible versus drug-resistant HIV-1 has occurred because the standard drug-development paradigm does not generate results robust enough to support guidelines for treating patients with drug-resistant virus. The problem of HIV drug resistance, like immunological escape from vaccine-induced immunity, cannot be solved by any single study or clinical trial but only by the effective synthesis of data from multiple studies and clinical trials.

Nearly all publications to date on genotype and clinical out-

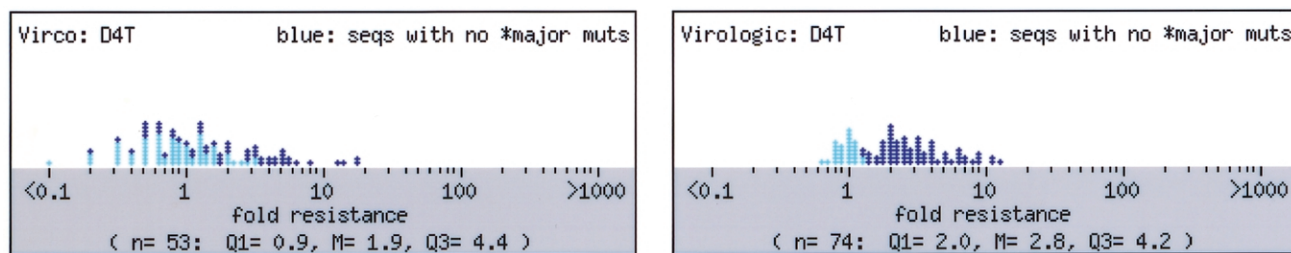


Figure 2. Stanford HIV RT and Protease Sequence Database query for results concerning stavudine (D4T) susceptibility on isolates containing the RT mutations M41L, L210W, and T215Y, using the PhenoSense (Monogram Biosciences) and Antivirogram (Virco) assays. When this query is performed on the Web (http://hivdb.stanford.edu/cgi-bin/RT_Phenotype.cgi), these summary figures are followed by a table containing a line-by-line listing of each result and its associated publication.

Table 4. Nucleoside RT inhibitor–resistance mutations and drug-susceptibility results of the 12 infectious molecular RT clones.

Clone	GenBank no.	NIH no.	Amino acid at position																	Fold decrease in susceptibility ^a					
			41 ^b	67 ^b	70 ^b	210 ^b	215 ^b	219 ^b	184	69	65	75 ^c	77 ^c	116 ^c	151 ^c	74	115	44	118	AZT	d4T	TDF	ABC	ddl	3TC
1	AY351774	6463-13	L	N	...	W	Y	...	V		24	4.1	1.5	7.4	2.1	»	
2	AY351750	7303-3	L	N	...	W	Y	D	D		»	6.7	5.9	8.4	2.3	7.5	
3	AY351769	4755-5	L	N	...	W	Y	...	V	D	D		61	3.9	2.3	7.7	2.4	»	
4	AY351719	7324-4	...	N	R	...	F	E	464	2.3	5.2	3.8	1.5	3.6	
5	AY351744	7295-1	...	N	R	...	F	Q	V	N	9.9	1.9	1.1	6.1	1.9	»	
6	AY351717	7324-1	L	N	R	...	F	E	...	N	923	2.6	8.1	4.2	1.7	4.1	
7	AY351729	52534-2	L	W	Y	...	V	Ins	V	719	9	4.2	22	3.3	»	
8	AY351767	1617-1	G	V	K	...	I	L	Y	M	...	Y	...	261	11	2.4	»	23	»	
9	AY351770	35764-2	I	L	Y	M	55	11	1.9	7.1	11	5.4	
10	AY351736	56252-1	R	R	I	L	Y	M	»	20	11	»	28	89	
11	AY829262	71361-1	R	0.7	1.9	3.8	3.6	3.0	10	
12	AY829263	8415-2	V	...	R	0.3	1.2	1.1	9.1	3.0	»	

NOTE. The complete sequences, list of mutations, and list of drug-susceptibility results for each clone can be found on the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program Web site (<http://www.aidsreagent.org>) or in the Stanford Drug Resistance Database (<http://hivdb.stanford.edu>). Ellipses denote positions at which there was no mutation. “Ins” denotes the presence of a double-amino acid (SS) insertion after a T69S substitution at position 69. ABC, abacavir; AZT, zidovudine; ddl, didanosine; d4T, stavudine; TDF, tenofovir; 3TC, lamivudine.

^a Determined by the PhenoSense assay (Monogram Biosciences). Results in bold type exceed the PhenoSense assay clinical cutoff. » indicates that the reduction in drug susceptibility was greater than the upper limit of assay detection (300-fold for 3TC and ~1,000 fold for AZT). Although susceptibilities to emtricitabine—the most recently approved nucleoside RT inhibitor—were not determined, the resistance profile is considered to be identical to that of 3TC.

^b Thymidine analog mutation.

^c 151-associated mutation.

come describe the results of a single analysis on a single data set. The underlying raw data have not been shared, in part because, until now, there had been no resource for making such data publicly available. The HIVRT&PrDB has begun to catalog these studies (http://hivdb.stanford.edu/pages/geno_clinical_review.html) and, as described below, has created a template for making the underlying data—specifically, the temporal relationship between antiretroviral drug treatments, genotypes, and plasma HIV-1 RNA levels and CD4⁺ T cell counts—available. Access to these data will allow other researchers to validate the primary analysis, perform one or more alternative analyses, and conduct meta-analyses on pooled data.

AIDS CLINICAL TRIALS GROUP (ACTG) DATA ANALYSIS CONCEPT SHEET 224 (DACS 224)

DACS 224 is an ongoing collaboration between the ACTG virology leadership, the Statistical Data Analysis Center at the Harvard School of Public Health, the chairs of completed ACTG trials, and the Stanford HIVRT&PrDB. The collaboration is designed to pool genotypic-clinical outcome data from published ACTG trials and to make such data available on the Web in a variety of formats. The first 2 studies in this collaboration, ACTG 320 and ACTG 364, were pivotal trials of salvage therapy in which baseline genotypic data had been collected [20, 21]. ACTG 320 compared salvage therapy with zidovudine plus lamivudine versus zidovudine plus lamivudine plus didanosine in patients previously treated with NRTIs other than lamivudine. ACTG 364 compared nelfinavir, efavirenz, and nelfinavir plus efavirenz in combination with 2 NRTIs in patients who had previously been treated with NRTIs for ≥ 5 years.

The data from these 2 clinical trials are available in HIVRT&PrDB in a variety of formats. Genotypes can be downloaded in a FASTA or spreadsheet format. Treatments, HIV-1 RNA levels, and CD4⁺ T cell counts can be viewed using HTML or can be downloaded as tab-delimited text files or Excel spreadsheets. Finally, a combined data set containing genotypes, treatments, HIV-1 RNA levels, and CD4⁺ T cell counts can be downloaded as a Microsoft Access database. Each subject's data (genotype, treatment, HIV-1 RNA level, CD4⁺ T cell count) can also be viewed in an integrated graphical format enabling a visual check for data consistency that is not possible when the genotype, treatment, HIV-1 RNA level, and CD4⁺ T cell count are in separate files. Data redaction includes using relative (rather than absolute) dates and rounding HIV-1 RNA levels to 0.1 log and CD4⁺ T cell counts to the nearest multiple of 10. XML schemas representing genotypic data and treatment history facilitate the interchange of data between the database, graphical format, input forms, and the various downloadable formats.

CONCLUSION

Data are the most important commodity in science, and data management is of critical importance. An HIV drug-resistance database that provides unfettered access to the types of data described above must be publicly available to the broadest number of users to promote discovery in the most efficient manner. Proprietary databases that deny access to the majority of researchers are not only inefficient but also counterproductive: the company or small group of researchers with a stake in such a database will typically act to thwart the nonproprietary dissemination of data, to maintain the perceived commercial or research value of their monopoly. Conclusions drawn from a proprietary database may also be unduly influenced by the interests of those who submit and retrieve the data. Although pharmaceutical and diagnostic companies may contribute to a drug-resistance database, the group that maintains the database should not have a potential for bias in favor of particular drugs or diagnostic approaches.

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