Phenotypic Drug Susceptibility Testing Predicts Long-Term Virologic Suppression Better than Treatment History in Patients with Human Immunodeficiency Virus Infection

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To assess the value of phenotypic drug susceptibility testing as a predictor of antiretroviral treatment response in human immunodeficiency virus (HIV)–infected people, drug susceptibility testing was performed retrospectively on plasma samples collected at baseline in a cohort of 86 antiretroviral-experienced, HIV-infected people experiencing treatment failure and initiating a new antiretroviral treatment regimen. Two separate criteria for reduced drug susceptibility were evaluated. In multivariate analyses, phenotypic susceptibility was an independent predictor of time to treatment failure (adjusted hazards ratio [HR], 0.70; 95% confidence interval [CI], 0.55–0.90; and adjusted HR, 0.76; 95% CI, 0.61–0.95, with reduced drug susceptibility cutoffs defined as 4.0-fold and 2.5-fold higher than reference virus IC_{50} values, respectively). Previous protease inhibitor experience was also a significant independent predictor. Notably, drug susceptibility predicted on the basis of treatment history alone was not predictive of time to treatment failure. In this cohort, phenotypic testing results enhanced the ability to predict sustained long-term suppression of virus load.

Recent advances in potent combination antiretroviral therapy for human immunodeficiency virus (HIV) infection have led to a remarkable decline in HIV-associated mortality and morbidity [1–5]. Despite these positive trends, however, the management of HIV-infected patients remains highly complex. One of the greatest challenges is the management of patients who experience treatment failure despite treatment with aggressive combination regimens. Several mechanisms for failure have been postulated, including decreased adherence to complex medical regimens, suboptimal pharmacodynamics, and the emergence of viral drug resistance [6–12]. The relative role of each of these factors in predicting the success of salvage therapy is currently unclear, which makes the selection of subsequent therapeutic regimens for treatment-experienced HIV-infected patients particularly difficult.

Viral drug resistance testing has been proposed as a useful adjuvant to clinical and virus load assessments when selecting salvage regimens [11–19]. Several retrospective studies have demonstrated an association between viral drug resistance testing results and subsequent virologic response to therapy [13–15, 20]. In addition, 3 prospective, randomized, intervention-based trials have indicated that drug selection made on the basis of HIV type 1 (HIV-1) drug resistance testing is associated with improved short-term virologic response [16–18]. However, previous studies have not examined the additional benefit of phenotypic testing results when they are added to a detailed drug-specific history for treatment regimen selection in a routine clinical setting. The aim of this study was to determine the associations between sustained plasma viral suppression and either baseline viral phenotypic drug susceptibility testing results, new drug regimen selection based on antiretroviral treatment history alone, or other clinical variables in a well-characterized cohort of antiretroviral drug–experienced HIV-infected patients starting a new treatment regimen in a clinical practice setting.

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Subjects and Methods

Study population. Subjects were HIV-positive patients followed in an ongoing prospective cohort study designed to investigate associations among clinical characteristics, virologic parameters, and clinical outcomes before and during antiretroviral therapy. The cohort participants were drawn from the population of patients routinely followed in the HIV outpatient clinic at the University of Alabama at Birmingham School of Medicine. All charts were screened at the time of routine primary care clinic visits. Inclusion in the prospective cohort study required a plasma virus load measurement ⩾5000 HIV-1 RNA copies/mL and plans by the primary care provider either to initiate or to change antiretroviral therapy. Once identified, patients were included in the study if they agreed to participate and were able to complete either a paper or computerized questionnaire. Enrollment in the prospective cohort began on 1 March 1997 and is still ongoing.

The retrospective analyses presented here are based on data obtained through 1 January 1999 in the subset of HIV-infected patients with ⩾3 months of antiretroviral experience. Analyses were further limited to those patients with available baseline plasma virus load measurements and viral resistance results, available stored plasma samples taken within 1 month of antiretroviral therapy change, and ⩾1 follow-up virus load measurement ⩾8 weeks after enrollment. This resulted in a final patient cohort of 86 subjects.

Demographic and clinical history data. Data collected at enrollment included sociodemographic information, complete medical history (including medications), and laboratory values. Study personnel obtained historical information from the clinic's existing computerized clinical database, chart extraction, and personal interview. Follow-up history and medication changes were collected at 3–4-month intervals by interview. Interim clinical and laboratory data were verified and collected on a continuous basis through a real-time computerized clinical database system. Historical exposure to a specific drug or drug class was defined as >2 consecutive weeks of treatment at any time before enrollment. A random selection of 5% of study charts was reviewed monthly for quality control.

Laboratory data. Baseline plasma HIV-1 RNA level and $CD4^+$ T lymphocyte count were assessed on the day of enrollment and at subsequent visits. Plasma HIV-1 RNA levels were measured with the standard Amplicor HIV-1 Monitor assay (Roche Diagnostic Systems; quantification limit of 400 HIV-1 RNA copies/mL). Resistance assays were performed on banked frozen (−70°C) specimens of plasma collected from each subject at enrollment. HIV-1 drug susceptibility was determined by testing patient plasma samples, using the PhenoSense HIV-1 assay (ViroLogic) [21]. This assay involves the assembly of recombinant HIV-1 test vectors, which are created by inserting polymerase chain reaction–amplified reverse transcriptase (RT) and protease sequences (derived from the patient) into a modified HIV-1 genome that carries a luciferase indicator gene and is restricted to a single round of viral replication [21]. Susceptibility of recombinant viruses derived from the patient isolates were compared with the susceptibility of a drug-sensitive reference virus derived from an infectious molecular clone of HIV-1 (NL4-3). Patient viruses with IC_{50} values that differed by ⩾2.5-fold from the reference virus IC_{50} run in parallel were considered to have altered drug susceptibility (confidence interval [CI] >95%) [21, 22]. The clinical significance of small differences in drug susceptibility (e.g., ⩾2.5 fold or ⩾4-fold increases or decreases in IC_{50}) have not been clearly defined. In the present study, 2 separate criteria for reduced drug susceptibility were used in the analyses of virologic outcomes: virus IC_{50} values ⩾2.5-fold or ⩾4-fold higher than reference virus IC_{50} values.

Statistical analysis. All analyses were based on the new treatment regimen initiated at time of enrollment into this cohort, regardless of whether patients discontinued treatment or had treatment regimen changes during follow-up. The primary outcome was time to treatment failure, defined by any single plasma virus load measure that was not at least 0.5 $\log_{10}$ below the baseline plasma value after ⩾8 weeks of follow-up. Follow-up for people who did not meet the definition of failure was censored at the time of the last available virus load measurement.

Two variables reflecting possible viral drug susceptibility to the new treatment regimen were analyzed in this study: predicted susceptibility by history, defined as the number of antiretroviral drugs in the new treatment regimen to which the person had not been previously exposed; and results of viral phenotypic drug susceptibility testing, defined as the number of antiretroviral drugs in the new regimen to which a person's plasma virus population was sensitive by phenotypic testing. Both phenotypic sensitivity cutoffs were examined, but always in separate analyses.

Kaplan-Meier analyses were used to estimate failure rates over time. Comparisons of failure rates between groups stratified by the number of drugs to which a patient's virus was susceptible, either by phenotypic drug testing or predicted by history, were performed by the log-rank test [23]. Relative hazards for treatment failure associated with clinical factors and phenotypic susceptibility test results were determined from Cox proportional hazards regression models involving either single or multiple independent variables [24]. The following variables were coded as dichotomous: previous protease inhibitor experience, previous nonnucleoside RT inhibitor experience, protease inhibitor–inclusive new treatment regimen, and nonnucleoside RT inhibitor–inclusive new treatment regimen. All others were analyzed as continuous variables.

Multivariable Cox proportional hazards models were used to determine the factors most predictive of time to treatment failure. Through stepwise regression, a simplified clinical model most predictive of failure was derived on the basis of all clinical information that would routinely be available to a physician at the time of a regimen change (i.e., all variables were included except for the phenotypic drug susceptibility variables). Variables with $P < .10$ on univariate analysis were included in the stepwise process; variables with $P < .05$ were considered to be independently predictive of time to treatment failure. To assess the additional predictive value of each of the 2 phenotypic susceptibility variables when added to the model, each was next included in the multivariate analysis and analyzed separately. Multivariate models controlling for all clinical parameters routinely available to the provider at the time of a regimen change were also developed by including these variables into forced Cox proportional hazards models.

The baseline $CD4^+$ T lymphocyte count was not included in multivariate models because of lack of significance in the univariate analysis and missing data for some subjects. All P values presented are 2-sided; all CIs are 95%. The assumption of proportionality of

Table 1. Baseline characteristics of human immunodeficiency virus (HIV)–infected study participants at the time of new treatment regimen initiation.

Characteristic	Cohort (n = 86)
Mean age, y (SD)	39 (7.6)
Male sex, %	87
White race, %	64
Homosexual HIV risk factor, %	75
Injection drug use HIV risk factor, %	7
Previous AIDS-defining diagnosis, %[a]	85
Median plasma HIV-1 RNA level, copies/mL (range)	63,321 (5000–1,983,088)
Median $CD4^+$ T lymphocyte count, cells/mm^3 (range) (n = 80)	142 (1–720)
Median no. of previous antiretroviral regimens (range)	5 (1–15)
Previous protease inhibitor experience, %	73
Previous nonnucleoside reverse-transcriptase inhibitor experience, %	13

[a] Previous AIDS-defining diagnosis includes all patients with a history of Centers for Disease Control and Prevention class C opportunistic infection or a previous $CD4^+$ T lymphocyte cell count ≤200 cells/mm^3.

the hazards for all covariates was tested before modeling. All statistical analyses were done by SAS version 6.12 (SAS Institute).

Results

Study population. A total of 86 subjects were included in this analysis; median follow-up was 454 days (range, 73–630 days). The demographics of the cohort were similar to the clinic demographics and are presented in table 1. The majority of patients had advanced HIV disease; 85% of patients had a history of an AIDS-defining event. At entry, median $CD4^+$ T lymphocyte count was 142 cells/mm^3; median plasma HIV RNA level was 63,321 copies/mL. The study population was heavily antiretroviral experienced (the median number of antiretroviral regimens before entry was 5), with a median previous treatment duration of 31 months (range, 0.5–92.3 months). Most patients had previous protease inhibitor experience (73%); 26% had received >1 protease inhibitor, and 9% had received dual protease inhibitor drug regimens. Only 13% had previous nonnucleoside RT inhibitor experience.

The new treatment regimen initiated at enrollment was selected by each patient's primary provider. The median duration of this new regimen was 158 days (range, 6–625 days). The median number of total drugs in the new treatment regimen initiated at enrollment was 3 (range, 2–5 drugs). The distribution of the number of drugs in the new treatment regimen to which an individual patient's isolate was predicted to be sensitive on the basis of treatment history (i.e., the predicted drug susceptibility by history) is presented in figure 1. Most of the patients in the cohort (64%) received new treatment regimens that included 2–3 drugs to which the patient had not been previously exposed. Most regimens included a protease inhibitor (94%); a proportion of patients received dual or triple protease inhibitor therapy (33% and 5%, respectively). Nonnucleoside RT inhibitor–inclusive regimens were started in 31% of the study population.

As shown in figure 2, the number of drugs to which a patient's virus was sensitive, as determined by phenotypic susceptibility testing, ranged from 0 to 4. By use of a drug sensitivity cutoff of a <4.0-fold increase in IC_{50}, we found that the majority of patients (65%) received regimens that included either 2 or 3 drugs to which their virus was susceptible. A similar distribution was observed when we used the drug sensitivity cutoff of a <2.5-fold increase in IC_{50} (figure 2).

Time to treatment failure. The definition of treatment failure was met in 70% of the study cohort during follow-up. The median time to failure was 182 days. Kaplan-Meier curves depicting the proportion of the cohort achieving sustained plasma HIV RNA suppression over time are presented in figure 3 for each of the 3 variables analyzed: predicted drug susceptibility by history (figure 3*A*); a phenotypic drug sensitivity cutoff of a <4.0-fold increase in IC_{50} (figure 3*B*); and a phenotypic drug sensitivity cutoff of a <2.5-fold increase in IC_{50} (figure 3*C*). In the Kaplan-Meier analysis of drug susceptibility as predicted by treatment history (figure 3*A*), the time to treatment failure was not significantly different among the 3 patient strata defined by the number of drugs in the treatment regimen to which the virus was predicted to be sensitive: 0–1 drug, 2 drugs, and 3–5 drugs (P = .28; log rank test). The median times to treatment failure among the 3 patient strata were 198, 182, and 231 days, respectively.

In contrast, in the Kaplan-Meier analyses of drug susceptibility as determined by phenotypic testing (figure 3*B,* 3*C*), the time to treatment failure was significantly different among the strata by use of either sensitivity cutoff: a <4.0-fold increase in IC_{50} (P = .01; log rank test) or a <2.5-fold increase in IC_{50} (P = .005; log rank test). The median time to treatment failure for the 0–1, 2, and 3–4 drug strata was 107, 182, and 242 days, respectively, for the <4.0-fold cutoff analysis and 112, 182, and 245 days, respectively, for the <2.5-fold cutoff analysis. Thus,

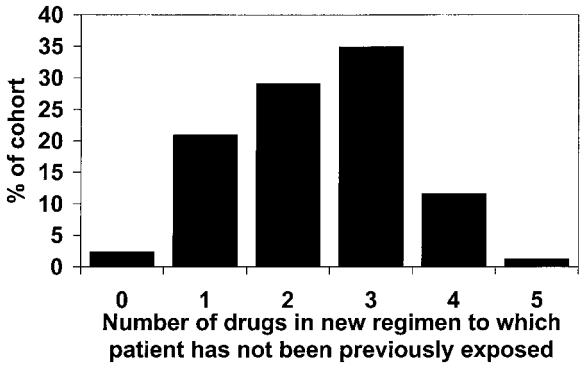

Figure 1. Distribution of drug susceptibility of study cohort human immunodeficiency viral isolates, as predicted by previous treatment history.

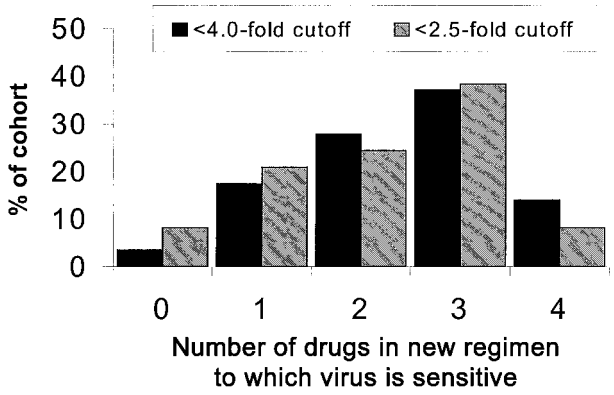

Figure 2. Distribution of phenotypic drug sensitivity of study cohort human immunodeficiency virus (HIV) isolates to drugs in new treatment regimen. Sensitivity was based on phenotypic drug susceptibility assay cutoffs of either a <4.0-fold increase in IC_{50} or a <2.5-fold increase in IC_{50} in the PhenoSense HIV assay [21].

having more sensitive drugs in the new treatment regimen delayed the time to treatment failure. Notably, there was no statistically significant difference in the proportion of patients in each stratum receiving either a protease inhibitor–inclusive or a nonnucleoside analogue–inclusive new regimen (data not shown).

Factors predicting time to treatment failure. Univariate analyses indicated that several factors were significantly associated with time to treatment failure (table 2). Patient baseline laboratory characteristics (plasma virus load and $CD4^+$ T lymphocyte count) were not significantly associated with failure rate by univariate analyses. Among variables indicating history of antiretroviral use, the number of previous antiretroviral regimens and previous protease inhibitor experience were statistically significantly associated with time to treatment failure. None of the variables characterizing the new treatment regimens was predictive of time to treatment failure. It is of particular note that predicted drug susceptibility by history was not significantly associated with time to treatment failure. In contrast, the number of drugs in the new treatment regimen to which a patient's virus was sensitive by phenotypic testing was significantly associated with time to failure; the inclusion of more drugs in the new treatment regimen to which a person's virus was sensitive was associated with a longer time to failure. This association was found by use of either sensitivity cutoff (table 2).

Through stepwise multivariate regression analysis that included only clinical variables with P values <.10 on univariate analyses, previous protease inhibitor experience alone was identified as the strongest predictor of the time to treatment failure (adjusted hazards ratio [HR], 2.75; 95% CI, 1.43–5.31). To assess the additional predictive value of the phenotypic testing results, the phenotypic susceptibility variables were added to the stepwise process in separate models including clinical factors. Inclusion of the <4.0-fold sensitivity cutoff variable revealed that past protease inhibitor experience and the phenotypic susceptibility variable were independent predictors of time to treatment failure (adjusted HR, 2.65; 95% CI, 1.37–5.12; and adjusted HR, 0.70; 95% CI, 0.55–0.90, respectively). Inclusion of the <2.5-fold sensitivity cutoff variable yielded similar results (previous protease inhibitor experience adjusted HR, 2.44; 95% CI, 1.25–4.77; and phenotypic susceptibility variable adjusted HR, 0.76; 95% CI, 0.61–0.95).

Multivariate Cox proportional hazards models adjusted for baseline clinical data, treatment history, and new regimen characteristics are presented in table 3. The first model included only information that would be routinely available to a clinician at the time of selection of a new regimen (table 3, model 1). In this model, previous protease inhibitor experience remained significantly associated with time to treatment failure. The phenotypic susceptibility testing variables were added independently to the clinical factors in the second and third models presented (table 3, models 2 and 3). Again, previous protease inhibitor experience remained significantly associated with time to treatment failure, and in each model, the phenotypic susceptibility variable remained an independent predictor of time to treatment failure.

Others have reported an association between treatment failure and the duration of past therapy with protease inhibitors [14]. To address this issue, both univariate and multivariate analyses assessing duration of past protease inhibitor therapy as a continuous variable were performed in the subset of 63

Table 2. Results of univariate Cox proportional hazards analysis of predictors of time to treatment failure in 86 human immunodeficiency virus (HIV)–infected patients.

Factor	Failure,[a] HR (95% CI)	P
Plasma HIV-1 RNA, $\log_{10}$ copies/mL	1.32 (0.90–1.94)	.15
$CD4^+$ T lymphocyte count, cells/mL[b]	1.00 (0.99–1.00)	.14
No. of previous antiretroviral regimens	1.09 (1.01–1.17)	.02
Duration of previous antiretroviral therapy, y	1.11 (0.99–1.24)	.09
Previous protease inhibitor experience	2.75 (1.43–5.31)	.003
Previous nonnucleoside RT experience	1.60 (0.83–3.10)	.16
Total no. of drugs in new treatment regimen	1.32 (0.86–2.02)	.20
Protease inhibitor–inclusive new treatment regimen	2.45 (0.76–7.94)	.13
Nonnucleoside RT inhibitor–inclusive treatment regimen	1.44 (0.85–2.45)	.18
Predicted drug susceptibility by history[c]	0.93 (0.74–1.17)	.52
Phenotypic drug sensitivity (<4.0-fold increase in IC_{50} cutoff)[d]	0.67 (0.52–0.87)	.003
Phenotypic drug sensitivity (<2.5-fold increase in IC_{50} cutoff)[d]	0.70 (0.56–0.88)	.002

NOTE. CI, confidence interval; HR, hazards ratio; RT, reverse transcriptase.

[a] Failure to maintain 0.5 $\log_{10}$ reduction in plasma HIV RNA levels from baseline. HR for time to failure was determined by Cox proportional hazards univariate analysis.

[b] Baseline $CD4^+$ T lymphocyte count was available for only 78 patients.

[c] Predicted drug susceptibility by history was defined as the number of antiretroviral drugs in the new treatment regimen to which the individual had not been previously exposed.

[d] Viral drug phenotypic susceptibility was defined by the PhenoSense HIV assay [21].

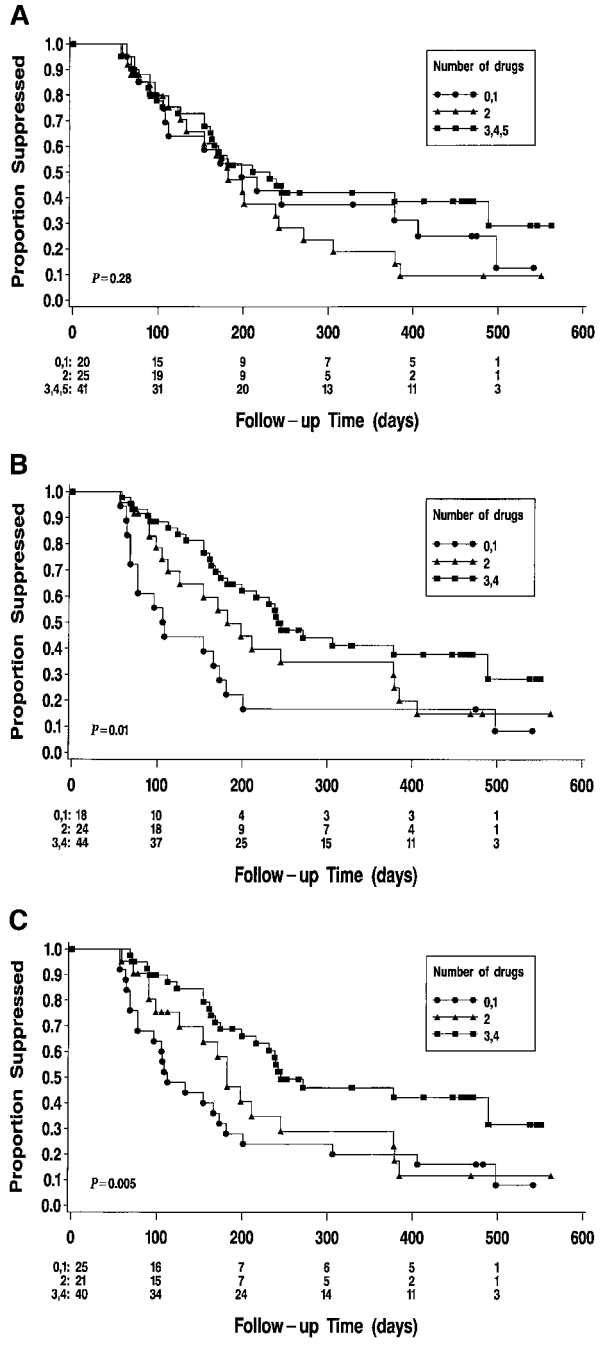

Figure 3. Kaplan-Meier curves showing proportion of the cohort achieving sustained suppression of plasma human immunodeficiency virus RNA over time, stratified by each susceptibility variable. *A,* Predicted susceptibility by treatment history. Strata are defined by the number of drugs in the treatment regimen to which the virus was predicted to be sensitive: 0–1, 2, or 3–5 drugs. *B,* Phenotypic drug sensitivity cutoff of <4.0-fold increase in IC_{50}. Strata are defined by the number of drugs in the regimen to which the virus was sensitive in the assay: 0–1, 2, or 3–4 drugs. *C,* Phenotypic drug sensitivity cutoff of <2.5-fold increase in IC_{50}. Numbers below the figures represent the number of patients in each stratum who had follow-up and who did not experience treatment failure at that time point.

patients in our cohort who had previous protease inhibitor experience. Duration of previous protease inhibitor treatment was not statistically significantly associated with time to treatment failure (unadjusted HR, 1.46; $P = .27$).

Discussion

Among patients starting a new regimen in the setting of antiretroviral failure, phenotypic drug susceptibility assay results significantly enhanced the ability to predict the success of a treatment regimen. Multivariate models that included clinical and treatment history plus phenotypic drug susceptibility results (by use of either a 2.5-fold or a 4.0-fold sensitivity cutoff) demonstrated that the phenotypic drug susceptibility result was a strong predictor of time to treatment failure. Specifically, having more drugs in the new treatment regimen to which a person's viral isolate was sensitive by phenotypic testing was associated with a significant delay in time to treatment failure. In these multivariate models, previous protease inhibitor experience was also an independent predictor of time to treatment failure. In contrast, other baseline virologic and clinical factors, including other prior treatment history and characteristics of the new treatment regimen, were not as predictive. Overall, our results suggest that viral resistance testing can significantly improve the ability to select subsequent therapy for antiretroviral-experienced patients beyond knowledge of treatment history or other clinical parameters.

Previous standards of practice have suggested discontinuation of all agents within a patient's failing regimen and replacing them with drugs that the patient has not received previously [19, 25, 26]. In our study, the number of agents in the new treatment regimen to which the patient had not been exposed previously was not a significant predictor of time to treatment failure in either univariate or multivariate analyses. Indeed, the use of treatment history may be misleading in 2 important ways: (1) drugs not used in previous regimens may not be effective because of the development of unanticipated viral cross-resistance established with the previous use of another related agent; and, conversely, (2) some drugs used in previous regimens may be assumed to be inactive when, in fact, no viral resistance has developed to that agent [11, 12, 27–29]. Thus, the emerging guidelines for use of antiretroviral therapy recommend the routine use of resistance testing in the setting of multiple regimen failure, to tailor an individualized regimen that is more reflective of the actual biologic circumstances in vivo [11, 12, 19, 25, 26].

Our primary outcome measure was time to treatment failure. We selected our failure end-point definition on the basis of controlled clinical outcomes studies demonstrating that the ability to sustain a 0.5 $\log_{10}$ decrease from baseline virus load was associated with improved clinical outcome [30–33]. We used both Cox proportional hazards models and Kaplan-Meier plots to allow the flexibility of assessing multiple covariates in this cohort with variable durations of follow-up. The analysis was

Table 3. Results of multivariate Cox proportional hazards analysis to determine predictors of time to treatment failure in 86 human immunodeficiency virus (HIV)–infected patients.

	Cox proportional hazards model[a]					
	Model 1		Model 2		Model 3	
Factor	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Plasma HIV-1 RNA, $\log_{10}$ copies/mL	1.16 (0.74–1.84)	.52	1.15 (0.74–1.80)	.54	1.14 (0.73–1.77)	.58
No. of previous antiretroviral regimens	1.00 (0.90–1.10)	.92	0.98 (0.89–1.08)	.69	0.98 (0.88–1.09)	.69
Duration of previous antiretroviral therapy, y	1.12 (0.98–1.29)	.10	1.06 (0.91–1.23)	.45	1.04 (0.89–1.21)	.64
Previous protease inhibitor experience	2.57 (1.23–5.38)	.01	2.24 (1.05–4.76)	.04	2.22 (1.04–4.75)	.04
Previous nonnucleoside RT inhibitor experience	1.53 (0.75–3.09)	.24	1.50 (0.75–3.01)	.25	1.48 (0.72–3.02)	.29
Total no. of drugs in new treatment regimen	1.03 (0.60–1.77)	.91	1.38 (0.79–2.41)	.27	1.12 (0.66–1.89)	.68
Nonnucleoside RT inhibitor–inclusive treatment regimen	1.11 (0.62–2.03)	.71	1.06 (0.58–1.93)	.86	1.20 (0.66–2.20)	.54
Predicted drug susceptibility by history[b]	0.94 (0.71–1.25)	.68	1.19 (0.86–1.63)	.30	1.0 (0.71–1.40)	.47
Phenotypic drug sensitivity (<4.0-fold increase in IC_{50} cutoff)[c]	—	—	0.58 (0.41–0.83)	.003	—	—
Phenotypic drug sensitivity (<2.5-fold increase in IC_{50} cutoff)[c]	—	—	—	—	0.70 (0.49–0.99)	.04

NOTE. CI, confidence interval; HR, hazards ratio; RT, reverse transcriptase.

[a] HR for time to failure was determined by Cox proportional hazards multivariate analysis.

[b] Predicted drug susceptibility by history was defined as the number of antiretroviral drugs in the new treatment regimen to which the individual had not been previously exposed.

[c] Viral drug phenotypic susceptibility was defined by the PhenoSense HIV assay [21].

done on the basis of the first salvage regimen initiated at the time of enrollment to this study (at the time of phenotypic drug testing). Any changes in therapy after this date were not accounted for, creating a parallel to the intention-to-treat type of analysis used in randomized, controlled trials. Because all subjects enrolled in our ongoing prospective cohort study had plasma stored that was then used to perform resistance testing for this retrospective study, we avoided the selection bias inherent in evaluation of only patients who had resistance testing available to them in the analysis.

Previous studies have shown an association between results of HIV drug resistance testing and short-term virologic outcomes [13–18, 20]. However, most of the retrospective studies reported to date have focused on viral resistance to either a specific drug or a specific combination regimen, in contrast to our clinical practice-based study, where a variety of treatment regimens were employed. Zolopa et al. [14] demonstrated that genotypic testing results were the strongest predictors of treatment failure in their retrospective cohort study. They examined virologic response to a single salvage regimen, saquinavir and ritonavir, in subjects who had failed to respond to previous protease inhibitor–containing antiretroviral regimens. Their multivariate regression models based on clinical parameters (e.g., baseline plasma HIV RNA levels) and treatment history by class of agent alone were not as predictive of virologic response as those including genotypic testing results. They concluded that genotypic testing in HIV-1 infection provided information that could not otherwise be derived from standard clinical assessment of people in whom antiretroviral therapy is failing virologically. Their results mirrored our demonstration that phenotypic testing was a better predictor of long-term virologic response than treatment history alone in our antiretroviral-experienced, HIV-infected patient cohort.

Few studies have investigated the clinical role of phenotypic resistance testing, and with the exception of the recently presented interim results of the VIRA 3001 study [18], these are generally retrospective studies that have again focused on specific drugs or drug classes in select populations over short times. Deeks et al. [15] examined the virologic response to a new salvage regimen in HIV-infected patients experiencing therapeutic failure during indinavir therapy. Their study, like ours, indicated that the number of drugs to which a person's virus was sensitive by phenotypic testing was associated with virologic success. However, their study population was small ($n = 18$), and follow-up was limited (24 weeks), whereas our study included 86 subjects with a median follow-up of 65 weeks.

Our study has several limitations. The cohort studied was limited in number and included only patients enrolled in one university-associated HIV outpatient center in the southeastern United States. Although our clinic population is diverse in its drug experience and clinical history, the results of this study may not apply to all clinical settings. Because of the nature of the study, we were unable to randomize patients, but we did attempt to control for confounding variables by assessing the impact of several covariates on the results. Treatment regimens were chosen without knowledge of the phenotypic testing results or direction from the study investigators; however, the clinicians in our center are highly experienced caregivers to the HIV population, which may have impacted their selection of drug regimens. Although this study demonstrated that phenotypic drug testing was associated with improved virologic outcomes, it did not address

the role of phenotypic testing in facilitating better selection of new treatment regimens by clinicians.

In summary, our results indicate that HIV phenotypic susceptibility testing results are strongly predictive of time to treatment failure in antiretroviral-experienced, HIV-infected patients. Patients with drug-susceptible virus at the time of treatment initiation experienced a longer time to treatment failure. Thus phenotypic testing results appear to add important information that physicians can use when making management decisions in the clinical setting of therapeutic failure. The continuous collection of other measures in this cohort will enable us to further study the impact of resistance testing on clinical outcomes, health care utilization, cost, and other patient-based measures, such as quality of life. Ongoing and planned prospective, randomized, controlled, longer-term clinical trials will evaluate the direct impact of phenotypic resistance testing on the selection of optimal antiretroviral treatment regimens.

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References

1. Centers for Disease Control and Prevention. Update: trends in AIDS incidence—United States, 1996. JAMA **1997**;278:1485–6.
2. Hammer SM, Squires KE, Hughes MD, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. N Engl J Med **1997**;337:725–33.
3. Gulick RM, Mellors JW, Havlir D, et al. Treatment with indinavir, zidovudine and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. N Engl J Med **1997**;337:734–9.
4. Gulick RM, Mellors JW, Havlir D, et al. 3-Year suppression of HIV viremia with indinavir, zidovudine, and lamivudine. Ann Intern Med **2000**;133:35–9.
5. Palella FJ, Delaney KM, Moorman AC, et al., for the HIV Outpatient Study Investigators. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med **1998**;338:853–60.
6. Deeks SG, Hecht FM, Swanson M, et al. HIV RNA and CD4 cell count response to protease inhibitor therapy in an urban AIDS clinic: response to both initial and salvage therapy. AIDS **1999**;13:F35–43.
7. Fätkenheuer G, Theisen A, Rockstroh J, et al. Virological treatment failure of protease inhibitor therapy in an unselected cohort of HIV-infected patients. AIDS **1997**;11:F113–6.
8. Lorenzi P, Yerly S, Abderrakim K, et al. Toxicity, efficacy, plasma drug concentrations and protease mutations in patients with advanced HIV infection treated with ritonavir plus saquinavir. AIDS **1997**;11:F95–9.
9. Lorenzi P, Opravil M, Hirschel B, et al. Impact of drug resistance mutations on virologic response to salvage therapy. AIDS **1999**;13:F17–21.
10. Shafer RW, Winters MA, Palmer S, Merigan TC. Multiple concurrent reverse transcriptase and protease mutations and multidrug resistance of HIV-1 isolates from heavily treated patients. Ann Intern Med **1998**;128:906–11.
11. Hirsch MS, Conway B, D'Aquila RT, et al. Antiretroviral drug resistance testing in adults with HIV infection: implications for clinical management. International AIDS Society—USA Panel. JAMA **1998**;279:1984–91.
12. Hirsch MS, Brun-Vézinet F, D'Aquila RT, et al. Antiretroviral drug resistance testing in adult HIV-1 infection: recommendations of an International AIDS Society—USA Panel. JAMA **2000**;283:2417–26.
13. Harrigan PR, Hertogs K, Verbiest W, et al. Baseline HIV drug resistance profile predicts response to ritonavir-saquinavir protease inhibitor therapy in a community setting. AIDS **1999**;13:1863–71.
14. Zolopa AR, Shafer RW, Warford A, et al. HIV-1 genotypic resistance patterns predict response to saquinavir-ritonavir therapy in patients in whom previous protease inhibitor therapy had failed. Ann Intern Med **1999**;131:813–21.
15. Deeks SG, Hellmann NS, Grant RM, et al. Novel four-drug salvage treatment regimens after failure of a human immunodeficiency virus type 1 protease inhibitor–containing regimen: antiviral activity and correlation of baseline phenotypic drug susceptibility with virologic outcome. J Infect Dis **1999**;179:1375–81.
16. Baxter JD, Mayers DL, Wentworth DN, et al. A randomized study of antiretroviral management based on plasma genotypic antiretroviral resistance testing in patients failing therapy. AIDS **2000**;14:F83–93.
17. Durant J, Clevenbergh P, Halfon P, et al. Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial. Lancet **1999**;353:2195–9.
18. Cohen C, Hunt S, Sension M, et al. Phenotypic resistance testing significantly improves response to therapy: a randomised trial (VIRA 3001) [abstract 237]. In: Program and abstracts of the 7th Conference on Retroviruses and Opportunistic Infections, San Francisco, 30 January–2 February 2000. Alexandria, VA: Foundation for Retrovirology and Human Health, **2000**.
19. Panel on Clinical Practices for Treatment of HIV Infection convened by the Department of Health and Human Services (DHHS) and the Henry J. Kaiser Family Foundation (online). Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents; available at http://www.hivatis.org.
20. Miller V, Phillips A, Rottman C, et al. Dual resistance to zidovudine and lamivudine in patients treated with zidovudine-lamivudine combination therapy: association with therapy failure. J Infect Dis **1998**;177:1521–32.
21. Petropoulos CJ, Parkin NT, Limoli KL, et al. A novel phenotypic drug susceptibility assay for human immunodeficiency virus type 1. Antimicrob Agents Chemother **2000**;44:920–8.
22. Hellmann N, Johnson P, ViroLogic Clinical Reference Laboratory, Petropoulos C, and ViroLogic Research Laboratory. Validation of the performance characteristics of a novel, rapid phenotypic drug susceptibility assay, PhenoSense HIV [abstract 51]. Antiviral Ther**1999**;4(Suppl 1):34–5.
23. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assn **1958**;53:457–81.
24. Cox DR. Regression models and life-tables. J R Stat Soc 1972;B34:187–220.
25. Panel on Clinical Practices for Treatment of HIV Infection convened by the Department of Health and Human Services (DHHS) and the Henry J. Kaiser Family Foundation. Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. Ann Intern Med **1998**;128:1079–100.
26. Carpenter CC, Cooper DA, Fischl MA, et al. Antiretroviral therapy in adults: updated recommendations of the International AIDS Society—USA Panel. JAMA **2000**;283:381–90.

27. Havlir DV, Hellmann NS, Petropoulos CJ, et al. Drug susceptibility in HIV infection after viral rebound in patients receiving indinavir-containing regimens. JAMA **2000**; 283:229–34.
28. D'Aquila RT, Johnson VA, Welles SL, et al. Zidovudine resistance and HIV-1 disease progression during antiretroviral therapy. Ann Intern Med **1995**; 122:401–8.
29. Young B, Johnson S, Bahktiari M, et al. Resistance mutations in protease and reverse transcriptase genes of human immunodeficiency virus type 1 isolates from patients with combination antiretroviral therapy failure. J Infect Dis **1998**; 178:1497–501.
30. Marschner IC, Collier AC, Coombs RW, et al. Use of changes in plasma levels of human immunodeficiency virus type 1 RNA to assess the clinical benefit of antiretroviral therapy. J Infect Dis **1998**; 177:40–7.
31. Marschner IC, Betensky RA, DeGruttola V, Hammer SM, Kuritzkes DR. Clinical trials using HIV-1 RNA-based primary endpoints: statistical analysis and potential biases. J Acquir Immune Defic Syndr Hum Retrovirol **1999**; 20:220–7.
32. Phillips AN, Eron J, Bartlett J, et al. Correspondence between the effect of zidovudine plus lamivudine on plasma HIV level/CD4 lymphocyte count and the incidence of clinical disease in infected individuals. AIDS **1997**; 11:169–75.
33. Deeks SG, Barbour JD, Martin JN, Swanson MS, Grant RM. Sustained $CD4^+$ T cell response after virologic failure of protease inhibitor–based regimens in patients with human immunodeficiency virus infection. J Infect Dis **2000**; 181:946–53.