Phenotypic susceptibility and virological outcome in nucleoside-experienced patients receiving three or four antiretroviral drugs

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Objective: To evaluate phenotypic drug susceptibility and non-nucleoside reverse transcriptase inhibitor hypersusceptibility as predictors of the time to virological failure.

Design: In a randomized clinical trial, phenotypic susceptibility was retrospectively determined among 131 exclusively nucleoside reverse transcriptase inhibitor (NRTI)-experienced patients with baseline HIV-RNA levels greater than 2000 copies/ml. Subjects were assigned two NRTI drugs and were randomly assigned to nelfinavir, efavirenz, or both. Virological failure was defined as two HIV-RNA measurements of 2000 copies/ml or greater at or after week 16 and before treatment discontinuation.

Methods: Using biological cut-offs to define resistance, assigned NRTI and randomized drug regimens, continuous and dichotomous phenotypic susceptibility scores (PSS) were calculated for each virus. Efavirenz hypersusceptibility as a dichotomous value was defined as less than 0.4-fold resistance. Associations between virological failure and continuous and dichotomous PSS were evaluated using Kaplan–Meier curves and Cox proportional hazards regression models.

Results: A higher baseline viral load ($P < 0.02$) and lower dichotomous or continuous baseline PSS ($P = 0.004$ and $P < 0.001$, respectively) were independently associated with virological failure. In the 85 subjects who received efavirenz, efavirenz hypersusceptibility ($P = 0.042$, hazard ratio 0.43, 95% confidence interval 0.19–0.97) was independently associated with a reduced risk of virological failure.

Conclusion: Reduced phenotypic susceptibility was a significant independent risk factor for virological failure. The presence of efavirenz hypersusceptibility appeared to enhance virological responses during treatment with efavirenz in combination with NRTIs. The retrospective calculation of continuous PSS accurately identified treatment regimens containing sufficient drug activity to prevent virological failure.

AIDS 2003, **17**:821–830

Keywords: HIV RNA, hypersusceptibility, phenotype, sensitivity score, virological failure

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Received: 19 April 2002; revised: 25 October 2002; accepted: 5 November 2002.

DOI: 10.1097/01.aids.0000050869.71999.40

ISSN 0269-9370 © 2003 Lippincott Williams & Wilkins

Introduction

Antiretroviral regimens may fail to achieve or maintain suppression of HIV replication because of drug resistance, lack of adherence or suboptimal drug levels to one or more agents in a treatment regimen [1]. In the treatment of drug-experienced patients, it is increasingly recognized that resistance to one or more of the agents in the treatment regimen is often associated with extensive cross-resistance among drugs within the three currently available classes of antiretroviral drugs [1–3].

Prospective studies show improved, short-term treatment outcomes when either phenotypic or genotypic data are used to select treatment regimens, compared with outcomes when resistance assay data were not used [4–9]. In such studies, improved virological outcome was correlated with the number of drugs in the selected regimen to which the patient's virus was susceptible. A retrospective meta-analysis of multiple clinical trials and observational cohort studies has shown that drug resistance at study entry, as determined by either genotypic or phenotypic analysis, was associated with short-term virological failure in highly experienced patients [10].

The clinical utility of drug susceptibility assays for optimizing the selection of alternative treatment regimens in treatment-experienced patients critically depends on accurate drug resistance cutoffs and the interpretation of resistance assay results [11,12].

In addition to reductions in drug susceptibility, or drug resistance, recombinant phenotypic assays measure increased drug susceptibility, or hypersusceptibility. This phenomenon has been most extensively described for non-nucleoside reverse transcriptase inhibitor (NNRTI) drugs, and is frequently associated with resistance to nucleoside analog drugs [13–15]. Although the nucleoside selected mutations associated with hypersusceptibility are not fully defined, retrospective studies of short-term virological response suggest that hypersusceptibility improved virological responses to salvage regimens with NNRTI drugs [14,15].

A retrospective analysis of baseline phenotypic susceptibility and virological outcome over 144 weeks was performed among nucleoside reverse transcriptase inhibitor (NRTI)-experienced patients who were treated with three or four-drug combination regimens in a prospective randomized clinical trial. Subjects were assigned open-label dual nucleoside analog therapy based on their previous NRTI regimens, and were randomly assigned to receive one or two additional drugs: a protease inhibitor (PI), nelfinavir, a NNRTI, efavirenz, or both drugs. The phenotypic susceptibility of patient viruses to drugs in the treatment regimen was evaluated from samples stored at study entry. For virus from each tested patient, a summary measure of drug susceptibility, or phenotypic susceptibility score (PSS), was calculated for all drugs in the treatment regimen. These susceptibility scores and NNRTI hypersusceptibility were included in models of the time to virological failure.

Methods

Study design

AIDS Clinical Trials Group (ACTG) 364 enrolled 196 patients with 4 years of mono or dual NRTI treatment in ACTG 175 from 1992 to 1996 [16], followed by 48 weeks of treatment with one, two or three NRTIs in ACTG 302/303 during 1996–1997 [17–19]. None of the study subjects had previously received therapy with a PI or a NNRTI. At ACTG 364 study entry, subjects were randomly assigned to receive 48 weeks of treatment with nelfinavir, efavirenz or both (nelfinavir plus efavirenz) in addition to two NRTIs. Follow-up time for this study was extended twice, from the original 48 weeks to 96 weeks and again from 96 to 144 weeks. Each patient was assigned to receive one of three dual nucleoside analog regimens (stavudine plus didanosine, didanosine plus lamivudine, or stavudine plus lamivudine) based on the patient's previous treatment experience. A total of 156 subjects had at least one baseline HIV plasma RNA plasma value greater than 2000 copies/ml at entry into ACTG 364. Phenotypic drug susceptibility testing was performed on plasma samples from 131 of these subjects. Subjects were followed during the study for evidence of virological failure, defined as two serial determinations of HIV plasma RNA greater than or equal to 2000 c/mL after more than 16 weeks of treatment.

Plasma HIV-RNA measurements

HIV-1-RNA was measured in citrated plasma, separated within 6 h of collection and stored at −70°C, by an Ultrasensitive Roche Amplicor Monitor assay (Roche Molecular Systems, Alameda, CA, USA; lower limit of quantitation 50 copies/ml). Baseline to week 16 plasma samples from each study participant were tested retrospectively in a single assay batch, including standards containing 15 000 and 150 000 copies of HIV RNA; subsequent samples were tested in real time.

Phenotypic assay

Drug susceptibility to a panel of 15 antiretroviral drugs was measured by the PhenoSense™ HIV assay (ViroLogic, San Francisco, CA, USA). Briefly, protease and reverse transcriptase (RT) coding sequences were amplified by RT–polymerase chain reaction and cloned into a recombinant HIV vector containing a luciferase reporter gene using restriction endonucleases *Apa*I and

*Pin*AI to generate resistance test vectors. These were tranfected into 293 cells; virus was harvested and used to infect fresh 293 cells. The concentration of drug required to inhibit viral replication by 50% in a single cycle assay (IC_{50}) was determined and compared with the IC_{50} of a drug-sensitive reference strain containing protease and RT coding sequences from strain NL4-3. Assay reproducibility is within twofold or less (95% confidence intervals; CI) for all drugs except zidovudine (2.2-fold). A cutpoint for defining reduced susceptibility to didanosine and stavudine was set at 1.5-fold on the basis of recent data demonstrating that more than 99% of wild-type viruses tested with the PhenoSense assay had less than 1.5-fold reduced susceptibility to these drugs [20]. Examination of the commercially available PhenoSense cut-off values of 1.7-fold in the phenotypic susceptibility scoring reported here did not alter the association of baseline resistance measures with respect to virological outcomes.

Statistical methods and models

The PSS was calculated by summing drug susceptibility values for all drugs in the patient's treatment regimen [10]. The drug susceptibility values for each drug ranged from 0 (drug resistant) to 1 (drug sensitive). Several PSS were examined in exploratory analyses. A dichotomous PSS was calculated after assigning a value of 1 to each drug in the treatment regimen with a measured phenotypic fold-change below the individual drug cut-off, and a value of 0 for fold-changes above the cut-off. A continuous PSS was calculated after assigning a value of 1 to each drug in the treatment regimen with a measured phenotypic fold-change of 2.5 or less ($\leqslant$ 1.5 for didanosine and stavudine), 0 for drugs with a fold-change greater than 10, and a value between 0 and 1, calculated as $1 - (\text{fold-change} - 2.5)/(10-2.5)$, for drugs with a fold-change between 2.5 (1.5 for didanosine and stavudine) and 10.

Hypersusceptibility to efavirenz, defined as less than a 0.4-fold-change in susceptibility, was not incorporated into the PSS, but was used as a dichotomous variable in a separate multivariate model including other covariates significantly associated with virological failure.

Baseline $\log_{10}$ plasma HIV-RNA levels and CD4 cell counts were defined as the means of pre-entry and entry values; HIV-RNA levels below 500 or above 750 000 copies/ml were replaced by these limits, respectively, before calculating the baseline mean.

Comparisons between subjects in this baseline phenotype analysis and those not in the analysis were undertaken using the chi-square test for categorical variables and the Wilcoxon rank-sum test for continuous variables. To evaluate associations between baseline factors, including the PSS, and treatment failure (confirmed HIV-1-RNA level $\geqslant$ 2000 copies/ml at or after week 16), Kaplan–Meier curves, log-rank tests, and Cox proportional hazards regression models were used to assess the time to on-treatment virological failure. Subjects permanently discontinuing antiretroviral treatment before virological failure were censored at the time of discontinuation.

Results

Characteristics of subjects

The 131 subjects with baseline phenotypic drug susceptibility data were predominantly male, and differed from the overall ACTG 364 study population in that they had a higher median virus load (4.17 versus 3.09 $\log_{10}$ copies/ml, respectively) and lower median CD4 cell numbers (336 versus 422 cells/mm^3, respectively). These differences probably reflect the requirement for subjects to have a baseline viral load of 2000 HIV-RNA copies/ml or greater in order to be included in the phenotypic study. All subjects had extensive previous experience with NRTI (median of 5.8 years), but had received no previous treatment with NNRTI or PI antiretroviral therapies. The NRTI used in each patient's treatment regimen were assigned on the basis of the patient's previous treatment experience: 75% of subjects were assigned two NRTI that they had not previously received and 25% received one new and one previously administered NRTI. Previous nucleoside exposure, new nucleoside drug assignments and randomized treatment regimens of patients included in this analysis are shown in Table 1.

Phenotypic resistance and previous drug exposure

The baseline phenotypic drug susceptibility among the 131 subjects is displayed in Fig. 1 and Table 2. Among these PI and NNRTI-naive subjects, none had virus with reduced susceptibility as defined by an IC_{50}-fold-change greater than 2.5 to efavirenz and only five (4%) demonstrated modest reductions in susceptibility to nelfinavir.

In contrast, extensive NRTI resistance and cross-resistance was detected among this highly NRTI-experienced study population at baseline. Baseline susceptibility to zidovudine (IC_{50}-fold-change > 2.5) was reduced in 85% of study subjects, with a range of 0.4–1520-fold-change. Virus from 83% of subjects demonstrated reduced susceptibility to lamivudine, with 60% of subjects having a greater than 10-fold reduction, corresponding closely to those who had received lamivudine therapy in the preceding year. In contrast, stavudine and didanosine susceptibilities were much more tightly clustered at lower fold-change values, with more than 70% of subjects having fold-

Table 1. Baseline characteristics by whether or not in baseline phenotype analysis.

	Included in baseline phenotype analysis	
	Yes (N = 131)	No (N = 64)
Male	16 (89%)	55 (86%)
ACTG 364 treatment		
Nelfinavir + 2 RTI	46 (35%)	20 (31%)
Efavirenz + 2 RTI	45 (34%)	20 (31%)
Nelfinavir + efavirenz + 2 RTI	40 (31%)	24 (38%)
ACTG 364 assigned dual nucleoside therapy		
Stavudine + lamivudine	58 (44%)	25 (39%)
Didanosine + lamivudine	8 (6%)	2 (3%)
Didanosine + stavudine	65 (50%)	37 (58%)
HIV RNA (log_{10} copies/ml)*		
Median (25–75th)	4.17 (3.67, 4.61)	3.09 (2.80, 3.32)
CD4 cell count (cells/mm^3)**		
Median (25–75th)	336 (240, 469)	422 (282, 534)
Previous NRTI exposure > 52 weeks		
Zidovudine	131 (100%)	63 (98%)
Didanosine	74 (56%)	44 (69%)
Zalcitabine	40 (31%)	9 (14%)
Stavudine	8 (6%)	2 (3%)
Lamivudine	81 (62%)	48 (75%)
Number of 364 new drugs		
Two	33 (25%)	21 (33%)
Three	69 (53%)	31 (48%)
Four	29 (22%)	12 (19%)

ACTG, AIDS Clinical Trials Group; NRTI, nucleoside reverse transcriptase inhibitor; RTI, reverse transcriptase inhibitor.
*$P < 0.001$ comparing those subjects included versus those not included in the phenotypic analysis.
**$P = 0.026$.

change values for these two drugs of between 1.5 and 10. However, the 1.5 to 10-fold reductions in susceptibility to these drugs were correlated with significantly higher levels of cross-resistance to zidovudine (10–1000-fold). Among the subjects who had not taken didanosine or stavudine previously (naive), 91 and 63%, respectively, had drug susceptible virus at baseline, as defined by less than 2.5-fold resistance (Table 3). However, when a lower susceptibility cut-off value of 1.5-fold or less was utilized, baseline virus was fully drug susceptible in only 30 and 24% of subjects, respectively (Table 3). Similarly, among 75 didanosine-experienced subjects, only 25% had susceptible virus using a less than 1.5-fold criterion, whereas 89% were susceptible at a 2.5-fold or less cut-off . Finally, although none had been exposed to the drug, 58% of subjects demonstrated greater than 4.5-fold resistance to abacavir, consistent with the observation that zidovudine and lamivudine resistance confers decreased abacavir susceptibility (Fig. 1) [21,22].

Models of phenotypic susceptibility scores and virological failure

To determine the predictive value of summary drug susceptibility measures for on-treatment virological failure (confirmed HIV-RNA level ≥ 2000 copies/ml at or after 16 weeks), dichotomous and continuous PSS models were evaluated in Cox proportional hazards regression analysis (Table 4). In univariate and multivariate models, baseline virus load and the number of new drugs (two to four) in the treatment regimen were significant predictors of virological failure, but the CD4 cell count was not. In a model with baseline viral load, a lower dichotomous PSS (based upon the categorization of treatment drugs as sensitive or resistant using a susceptibility cut-off of 1.5-fold for stavudine and didanosine, and 2.5-fold for all other drugs) was significantly associated with virological failure ($P = 0.004$). In addition, a lower continuous PSS (based upon classification of treatment drugs by continuous susceptibility values between 0 and 1) was significantly associated with virological failure ($P < 0.001$). A Kaplan–Meier analysis of time to virological failure over the 144 weeks of the study also demonstrated longer times to virological failure among patient groups with a higher continuous PSS (Fig. 2). Considering that the PSS value and the number of drugs in the regimen are not independent (and therefore cannot be included in the same model), the overall study outcomes are substantially driven by the superior activity (and increased PSS) in the four-drug regimen [34]. However, when the analysis was restricted to the two three-drug (nelfinavir or efavirenz, plus two NRTI) arms (n = 91), the models demonstrated the anticipated trend towards an association between resistance and virological failure ($P = 0.075$ and 0.096 for dichotomous and continuous PSS, respectively, adjusting for the virus load).

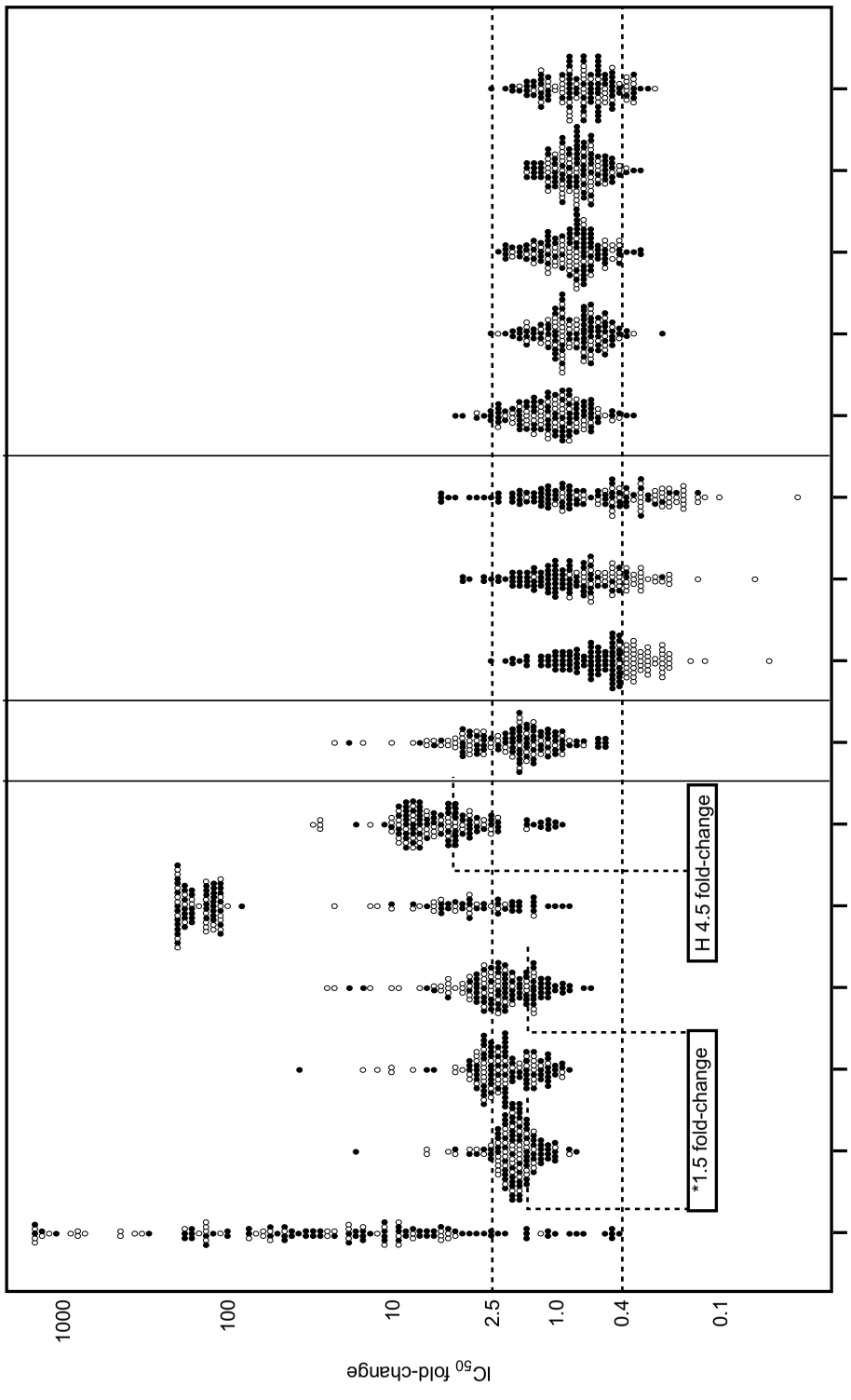

Fig. 1. Jitter plot of baseline phenotypic susceptibility in 131 subjects. Baseline IC_{50}-fold-changes to currently approved antiretroviral agents are displayed. The proportions of subjects with viral isolates susceptible (< 2.5-fold-change) to drugs within the three antiretroviral classes are featured along the horizontal axis. A less than 1.5-fold-change cut-off value was used to indicate susceptibility to didanosine and stavudine; a less than 4.5-fold-change to abacavir was deemed susceptible. Isolates with less than a 0.4-fold-change in IC_{50} were designated as hypersusceptible. ABC, Abacavir; ADV, adefovir; AMP, amprenavir; ddC, zalcitabine; ddI, didanosine; d4T, stavudine; DLV, delavirdine; EFV, efavirenz; IDV, indinavir; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir; SQV, saquinavir; 3TC, lamivudine; ZDV, zidovudine.

Table 2. Baseline concentration of drug required to inhibit viral replication by 50% in a single cycle assay (IC_{50})-fold-change by drug.

Drug	Median (25–75th percentile)	Range	⩽ 1.5 N (%)	⩽ 2.5 N (%)	> 10 N (%)	⩽ 0.4[a] N (%)
Nucleoside analog RTI						
Zidovudine	18.49 (5.62–104.71)	0.41–1520.0		19 (15)	82 (63)	0
Didanosine	1.73 (1.45–2.01)	0.76–15.50	36 (27)	118 (90)	1 (1)	0
Zalcitabine	2.09 (1.45–2.69)	0.79–35.97		90 (69)	4 (3)	0
Stavudine	2.16 (1.46–2.97)	0.63–23.53	34 (26)	84 (64)	5 (4)	0
Lamivudine	106.70 (3.60–164.78)	0.82–207.94		22 (17)	79 (60)	0
Abacavir	5.16 (3.34–7.45)	0.87–29.54		16 (12)	7 (5)	0
Nucleotide analog RTI						
Adefovir	1.66 (1.19–3.03)	0.48–22.28		89 (68)	3 (2)	0
NNRTI						
Efavirenz	0.45 (0.33–0.74)	0.05–2.36		131 (100)	0	49 (37)
Nevirapine	0.68 (0.43–1.13)	0.06–3.59		125 (95)	0	27 (21)
Delavirdine	0.53 (0.29–1.12)	0.03–4.89		123 (94)	0	49 (37)
Protease inhibitors						
Nelfinavir	1.02 (0.75–1.46)	0.33–3.93		126 (96)	0	3 (2)
Indinavir	0.77 (0.59–1.06)	0.22–2.41		131 (100)	0	5 (4)
Ritonavir	0.75 (0.59–1.06)	0.29–2.14		131 (100)	0	7 (5)
Saquinavir	0.74 (0.60–0.93)	0.28–1.54		131 (100)	0	4 (3)
Amprenavir	0.66 (0.49–0.89)	0.24–2.50		130 (99)	0	17 (13)

NNRTI, Non-nucleoside reverse transcriptase inhibitor; RTI, reverse transcriptase inhibitor.
[a]Fold-change ⩽ 0.4 (1/2.5) indicates hypersusceptibility.

Table 3. Baseline concentration of drug required to inhibit viral replication by 50% in a single cycle assay (IC_{50})-fold-change by nucleoside reverse transcriptase inhibitor previous experience.

Drug	N	Median (25–75th percentile)	Range	⩽ 1.5 N (%)	⩽ 2.5 N (%)	2.6–4.0 N (%)	4.1–10.0 N (%)	> 10 N(%)
Zidovudine								
Experienced	131	18.49 (5.32–104.71)	0.41–1520.0		19 (15)	5 (4)	25 (19)	82 (63)
Didanosine								
Experienced	75	1.79 (1.46–2.09)	0.76–15.50	19 (25)	67 (89)	5 (7)	2 (3)	1 (1)
Naive	56	1.64 (1.44–1.92)	0.83–4.54	17 (30)	51 (91)	4 (7)	1 (2)	0
Zalcitabine								
Experienced	42	2.25 (1.85–2.84)	0.92–9.62		26 (62)	14 (33)	2 (5)	0
Naive	89	2.04 (1.37–2.63)	0.79–35.97		64 (72)	18 (20)	3 (3)	4 (5)
Stavudine								
Experienced	8	1.56 (1.17–2.57)	0.92–6.76	4 (50)	6 (75)	1 (13)	1 (13)	0
Naive	123	2.16 (1.50–3.06)	0.63–23.53	30 (24)	78 (63)	28 (23)	12 (10)	5 (4)
Lamivudine								
Experienced	81	133.70 (111.56–185.65)	1.37–207.94		4 (5)	1 (1)	0	76 (94)
Naive	50	3.34 (1.79–5.15)	0.84–131.97		18 (36)	13 (26)	16 (32)	3 (6)

Table 4. Cox proportional hazards models for predicting virological failure[a].

	Model	Hazard ratio (95% CI)	*P* value
Univariate	1 $\log_{10}$ higher baseline RNA	1.61 (1.10–2.36)	0.014
	Number of 364 new drugs (1 additional new drug)	0.36 (0.24–0.53)	< 0.001
	PSS – dichotomous (1 unit higher PSS)	0.62 (0.44–0.88)	0.007
	PSS – continuous (1 unit higher PSS)	0.53 (0.37–0.75)	< 0.001
Multivariate	1 $\log_{10}$ higher baseline RNA	1.82 (1.23–2.68)	0.003
	Number of 364 new drugs (1 additional new drug)	0.33 (0.22–0.50)	< 0.001
	1 $\log_{10}$ higher baseline RNA	1.68 (1.15–2.46)	0.008
	PSS – dichotomous (1 unit higher PSS)	0.60 (0.42–0.85)	0.004
	1 $\log_{10}$ higher baseline RNA	1.59 (1.09–2.34)	0.017
	PSS – continuous (1 unit higher PSS)	0.54 (0.38–0.76)	< 0.001

CI, Confidence interval; PSS, phenotypic susceptibility scores.
[a]Virological failure: confirmed HIV-RNA values ⩾ 2000 copies/ml at or after week 16, before treatment discontinuation.

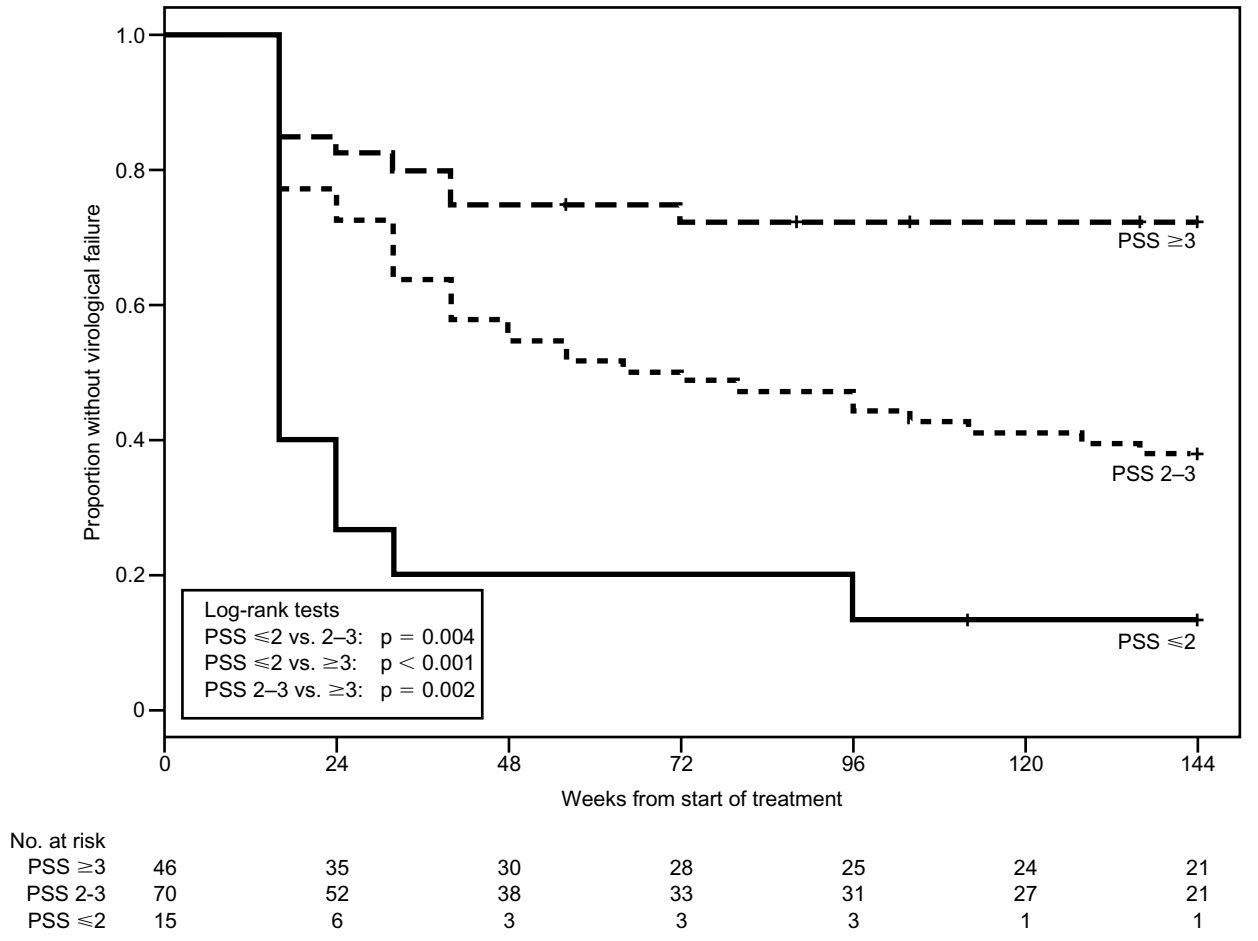

Fig. 2. Time to virological failure (Kaplan–Meier estimates) based on continuous phenotypic susceptibility scores. Virological failure was defined as a confirmed HIV-RNA level greater than 2000 copies/ml at week 16 or later, before study treatment discontinuation. PSS, Phenotypic susceptibility scores.

Efavirenz hypersusceptibility

The association between efavirenz hypersusceptibility and treatment outcomes was evaluated in a Cox proportional hazards model that was restricted to the 85 subjects who received efavirenz in the three or four-drug arms of the study. When included as a dichotomous variable (fold-change ≤ 0.4 or > 0.4), efavirenz hypersusceptibility was predictive of virological outcome in multivariate models that also contained virus load and continuous PSS variables. The model identified three covariates associated with a reduced risk of virological failure: lower baseline HIV-RNA level ($P = 0.032$), higher continuous PSS ($P = 0.003$), and efavirenz hypersusceptibility ($P = 0.042$, hazard ratio 0.43, 95% CI 0.19–0.97). Analysing this model separately in the three and four-drug efavirenz-containing arms, efavirenz hypersusceptibility showed a reduced risk of virological failure in each of these arms (hazard ratio of 0.56 and 0.23, respectively), but lacked significance based on reduced sample sizes. Efavirenz hypersusceptibility was not associated with outcomes in an analysis restricted to subjects not treated with efavirenz (nelfinavir plus two NRTI study arm: hazard ratio 0.97; $P = 0.93$).

Discussion

Several studies have demonstrated the role of baseline genotypic or phenotypic susceptibility in reducing virological failure rates during the first 16–24 weeks of treatment [4–10]. However, the long-term impact of drug resistance on virological outcomes has not been evaluated. In addition, most studies of drug resistance testing have generally focused on patients after the failure of one or more PI or NNRTI-containing regimens, a setting in which virological response to subsequent therapy was found to be heavily influenced by the presence or absence of resistance to these classes of drugs, precluding the assessment of baseline NRTI drug susceptibility on virological outcomes. The design

of the current study, which used NRTI plus efavirenz or nelfinavir, or both, to treat patients who had previously received only NRTIs, permitted the evaluation of the association between NRTI drug susceptibility and virological outcomes in the setting of sensitivity to NNRTI and PI. Because of the reported association between NRTI drug resistance and increased sensitivity to NNRTIs, the current study also provided a unique opportunity to evaluate the association between NNRTI hypersusceptibility and virological outcome.

The observation in the current study that virological failure rates during the first 24 weeks of therapy were higher among subjects treated with regimens containing (two or fewer) drugs to which their virus was sensitive at baseline (Fig. 2) is consistent with findings from previous drug-resistance testing trials. The long-term follow-up of patients in this study allowed the further differentiation of responses among patients treated with two or more active drugs beyond week 24. With continued therapy over 144 weeks, treatment failure rates were lower among patients treated with regimens in which the PSS demonstrated the equivalent of three or more sensitive drugs compared with those with fewer than three sensitive drugs. This provides evidence that susceptibility to the nucleoside analog drugs, the backbone of most antiretroviral regimens, is important in both short and long-term efficacy of drug regimens including efavirenz or nelfinavir, or both.

The activity of nucleoside drugs in experienced patients is limited by cross-resistance between different nucleoside drugs [23–28]. However, viral drug susceptibility is likely to be a continuous rather than a binary phenomenon because of partial activity against mutant virus as well as the biological variability of drug exposure, metabolism, and distribution to sites of virus replication. The analyses used in this study evaluated binary (dichotomous) and continuous measures of drug resistance. Separate analyses were performed in which dichotomous phenotypic susceptibility scores were calculated on the basis of uniform or drug-specific susceptibility cut-offs for the administered study drugs. Continuous susceptibility measures, based upon a linear range of reduced susceptibility, were calculated to model the progressive loss of drug activity as the fold-change values increase above the cut-off assigned to each drug. The continuous phenotypic scores in this study were more significant predictors of virological outcome than the dichotomous, consistent with the biology of resistance as a continuum rather than a binary event. In addition, continuous PSS calculations characterized the aggregate virological activity of nucleoside drugs, in the context of dual nucleoside combinations in this study, even when there was partial resistance to one or both drugs.

In this study of NRTI-experienced patients, efavirenz hypersusceptibility (37%) correlated with phenotypic resistance to zidovudine [29]. In the efavirenz-containing study arms, hypersusceptibility (drug susceptibility < 0.4-fold) was independently associated with a reduced risk of virological failure when included in a statistical model that also included other independent predictors of outcome (PSS and baseline viral load). In several small clinical studies, efavirenz hypersusceptibility was reported to be associated with improved virological outcomes during treatment with efavirenz regimens [13–15], although other studies have not observed a clinical impact of hypersusceptibility [30,31]. The mechanism for hypersusceptibility may be a change in the configuration of the nucleoside binding pocket with mutations in the adjacent NNRTI-binding region of the p66 subunit of RT [32,33]. Efavirenz hypersusceptibility could provide an explanation for the superior virological suppression of efavirenz in subjects with extensive nucleoside resistance [34].

Although this is the first study to show significant effects of baseline phenotype and efavirenz hypersusceptibility on long-term virological outcome, conclusions are limited by the retrospective nature of the analysis and the limited size of the study population. The extensive cross-resistance observed in this study among nucleoside drugs, especially zidovudine, stavudine, and didanosine, resulted in treatment regimens with limited potency.

This retrospective analysis explored the interpretation of drug susceptibility as a continuous measure in which the activity of all drugs in a treatment regimen, including the partial activity of nucleoside drugs, was included in a summed 'sensitivity score'. The inclusion of more active antiretroviral drugs in a salvage regimen to optimize virological response is tempered by cost, toxicity, drug intolerance and interactions, and individual patient and practitioner experience. These results provide an analytical framework for predicting the activity of a salvage regimen. However, data from additional clinical trials will be needed to translate these results into a definitive algorithm for improving long-term treatment outcomes.

The growing recognition of cross-resistance among nucleoside RT inhibitors and the results presented here suggest that long-term virological outcomes could be improved by an assessment of phenotypic susceptibility using more sensitive cut-off values that accurately characterize cross resistance among nucleoside drugs. Defining the required number of active drugs, as determined by drug-resistance test results, to sustain a virological response will require data from prospective clinical trials.

Sponsorship: This work was supported by the AIDS

Clinical Trials Group and the Stanford University VSL. Funding and drugs were provided by Bristol-Myers Squibb, GSK, and Agouron Pharmaceuticals.

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Appendix

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