

HIV-1 genotype and phenotype correlate with virological response to abacavir, amprenavir and efavirenz in treatment-experienced patients

Judith Falloon, Mounir Ait-Khaled^a, Deborah A. Thomas^b,
Carol L. Brosgart^c, Joseph J. Eron Jr^d, Judith Feinberg^e
Timothy P. Flanigan^f, Scott M. Hammer^g, Peter W. Kraus^h,
Robert Murphyⁱ, Ramon Torres^j, Henry Masur and the CNA2007 Study
Team^{*}

Objective: To assess the safety and efficacy of three new drugs in patients with antiretroviral failure and to correlate retrospectively baseline factors with virological response.

Design and setting: Open-label, 48-week, single-arm, multi-center phase II trial conducted at nine US university or government clinics and private practices.

Patients and interventions: Patients with HIV-1 RNA ≥ 500 copies/ml despite ≥ 20 weeks of treatment with at least one protease inhibitor received abacavir 300 mg twice a day, amprenavir 1200 mg twice a day and efavirenz 600 mg once a day. Other antiretrovirals were prohibited until week 16 except for substitutions for possible abacavir hypersensitivity.

Main outcome measures: HIV RNA at weeks 16 and 48.

Results: A total of 101 highly treatment-experienced patients enrolled; 60 were naive to non-nucleoside analog reverse transcriptase inhibitors (NNRTI). HIV RNA < 400 copies/ml was attained in 25 out of 101 (25%) patients at 16 weeks (35% of NNRTI-naive and 10% of -experienced patients) and 23 (23%) patients at 48 weeks (33% of naive and 7% of experienced patients). CD4 cells increased by a median of 15×10^6 and 43×10^6 cells/l at weeks 16 and 48, respectively. Drug-related rash occurred in 50 out of 99 (51%) of patients, and 17 out of 99 (17%) permanently discontinued one or more drugs as a result. Lower baseline viral load, fewer NNRTI-related mutations, absence of decreased abacavir (≥ 4 -fold) and efavirenz (≥ 10 -fold) susceptibility, and greater number of drugs to which virus was susceptible were associated with virological response at week 16.

Conclusions: Abacavir, amprenavir and efavirenz durably reduced HIV RNA and increased CD4 cell counts in a subset of treatment-experienced adults. Baseline viral load and some genotypic and phenotypic markers of resistance correlated with HIV RNA response.

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From the National Institutes of Health, Bethesda, Maryland, USA, ^aGlaxo Wellcome, Stevenage and Greenford, UK, ^bGlaxo Wellcome Inc., Research Triangle Park, North Carolina, the ^cEast Bay AIDS Center, Berkeley, California, the ^dUniversity of North Carolina at Chapel Hill, North Carolina, the ^eUniversity of Cincinnati, Cincinnati, Ohio, ^fThe Miriam Hospital, Providence, Rhode Island, ^gBeth Israel Deaconess Medical Center, Boston, Massachusetts, the ^hKraus Medical Partners, Los Angeles, California, ⁱNorthwestern University, Chicago, Illinois, and ^jSt Vincent's Hospital, New York, USA. *See Appendix.

Requests for reprints to: J. Falloon, Laboratory of Immunoregulation, NIAID, NIH, Building 10 Room 11C103, 10 Center Drive, Bethesda MD 20892-1880, USA.

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Introduction

Despite the durable antiviral responses and clinical benefit seen in many HIV-1-infected patients treated with combination antiretroviral regimens, practitioners are confronted by an increasing number of patients whose regimens fail to control viral replication adequately [1,2]. Selecting treatment regimens for these patients is now one of the most important issues in HIV disease management. Although the use of resistance testing in the setting of antiretroviral failure is recommended, it is not clear how to use genotype or phenotype to select a regimen for treatment-experienced patients [3,4].

We conducted an open-label, single-arm study of three (at the time) investigational agents, abacavir, amprenavir, and efavirenz, in patients with regimen failure. These drugs represent all three major classes of antiretroviral agents: abacavir is a nucleoside analog reverse transcriptase inhibitor (NRTI), amprenavir a protease inhibitor (PI), and efavirenz a non-nucleoside analog reverse transcriptase inhibitor (NNRTI). Thus, the study complied with recommendations to use three new drugs in patients with suspected drug failure [3]. The objectives of this study were to assess the safety and antiretroviral effect of abacavir, amprenavir and efavirenz in an antiretroviral-experienced population that included both NNRTI-naïve and -experienced patients. Because we anticipated cross-resistance in patients previously exposed to drugs in the same classes, we also planned to examine the correlation between baseline genotype and phenotype and viral load outcome to define a population in whom this regimen should prove most useful.

Methods

Entry criteria and procedures

CNA2007 was a phase II, open-label, single-arm, multicenter study with a planned enrollment of 100 adults (aged ≥ 13 years) with HIV RNA ≥ 500 copies/ml despite at least 20 weeks of a PI-containing combination regimen and at least 12 weeks without change in PI. Enrollment of at least 30 patients with HIV RNA $< 40\,000$ copies/ml and at least 60 patients without (fewer than 7 days) prior NNRTI exposure was mandated to ensure representation of these groups. Hemoglobin ≥ 10.0 g/dl for men and ≥ 9.0 g/dl for women, neutrophils $\geq 1 \times 10^9/l$, platelets $\geq 75 \times 10^9/l$, transaminases ≤ 5 times the upper limit of normal, serum pancreatic amylase ≤ 1.5 times the upper limit of normal, and estimated creatinine clearance > 50 ml/min were required for entry. There was no CD4 cell count restriction. Pregnant women were excluded and immunomodulators prohibited. Drugs

metabolized via cytochrome P450 were prohibited or to be used with caution. Institutional review boards at the nine sites reviewed the protocol, and all subjects provided written informed consent. Drug histories were obtained by patient report. Patients began abacavir 300 mg twice daily, amprenavir 1200 mg twice daily, and efavirenz 600 mg daily without a washout period. They had clinical and laboratory assessments at weeks 2, 4, 8, and every 8 weeks thereafter. Testing, performed at a central site, included HIV RNA, CD4 cell counts, standard hematologic and chemistry panels, amylase, and non-fasting triglyceride and cholesterol levels.

Subjects who developed rash together with systemic symptoms were to have all study medications interrupted but could be re-challenged with efavirenz and amprenavir with other NRTI substituted for abacavir. After week 16, all subjects were permitted to add other antiretroviral drugs provided they remained on at least two study drugs. Genotype and phenotype were not available for use in regimen changes. Withdrawal was mandated for a confirmed HIV RNA increase $> 1 \log_{10}$ copies/ml above baseline.

Laboratory methods

Lymphocyte subsets were analyzed by flow cytometry. HIV-1 HIV RNA was measured by the Roche Amplicor HIV-1 monitor test (Roche, Branchburg, New Jersey, USA; level of detection 400 copies/ml). For genotyping, plasma population sequencing was conducted using reverse transcription-PCR and automated sequencing of reverse transcriptase (RT), gag cleavage sites (p7/p1 and p1/p6), and protease coding regions of the *pol* gene. HIV-1 RNA was isolated from plasma, and complementary DNA of the RT and protease were made enzymatically and amplified by PCR. PCR products were population sequenced using a PRISM Big dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and resolved on an ABI377 sequencer. RT and protease mutations as catalogued by Schinazi *et al.* were analyzed [5]. Phenotyping was conducted at Virco (Mechelen, Belgium) [6]. The 50% inhibitory concentration (IC₅₀) was calculated for recombinant and wild-type reference strains and the ratio of IC₅₀ reported as fold change in susceptibility. Low HIV RNA (no PCR product) or poor growth of recombinant virus resulted in missing data.

Data analysis and statistics

Study populations

The safety population included all subjects exposed to at least one dose of drugs (Table 1). Adverse events were graded according to a modified AIDS Clinical Trials Group scale and were considered to be treatment-emergent if absent at baseline or if ≥ 1 grade increase occurred. The denominator for the intention-

Table 1. Baseline characteristics of study populations.

Characteristic	ITT population			Per protocol (virology) subpopulation (n = 74)
	Total (n = 101)	NNRTI-naïve (n = 60)	NNRTI-experienced (n = 41)	
Age (years) [median (range)]	40 (22–58)	42 (22–54)	38 (29–58)	41 (26–58)
Plasma HIV-1 RNA (log ₁₀ copies/ml) [median (range)]	5.1 (3.4–6.6)	4.7 (3.4–6.3)	5.3 (4.2–6.6)	5.0 (3.4–6.6)
CD4 cell count (× 10 ⁶ /l) [median (range)]	162 (9–782)	195 (9–782)	106 (9–607)	169 (9–782)
Patients who had received 3–4 prior PI (%)	62	52	78	61
Patients who had received 4–5 prior NRTI (%)	74	74	73	73
NNRTI naïve patients (%)	59	100	0	58

ITT, Intention-to-treat; NRTI, nucleoside analog reverse transcriptase inhibitor; NNRTI, non-nucleoside analog reverse transcriptase inhibitor; PI, protease inhibitor.

to-treat (ITT) analysis was all enrolled subjects. For analyses of treatment success, missing values, discontinuations of all study drugs, new confirmed Centers for Disease Control and Prevention Class C events, and deaths were carried forward as treatment failures. Subjects who discontinued one study drug or added antiretroviral agents after 16 weeks as permitted by the protocol could have been counted a success. Those patients receiving only the assigned combination at the time of analysis formed the per protocol subpopulation; this population at week 16 was used for the virology analysis (virology subpopulation).

Analysis of predictors of response

Correlation between baseline resistance, virological and immunological parameters and week 16 virological response was performed on the virology subpopulation using univariate and multivariate analyses. Virological success, defined as week 16 HIV RNA < 400 copies/ml, was the outcome variable. For genotype analysis, predictor variables included HIV RNA, CD4 cell count, each specific resistance-associated mutation, and number of mutations associated with each drug class. The following mutations were considered resistance-associated mutations: for RT, M41L, A62V, K65R, D67N, T69D, 69 insertions, K70R, L74V, V75M/I, F77L, Y115F, F116Y, Q151M, M184V/I, L210W, T215Y/F, and K219Q/E and the NNRTI mutations A98G, L100I, K101E/I/Q, K103N, V106A, V108I, V179D/E, Y181C/I, and G190A; for protease, L10I/V/F/R, D30N, V32I, F33V/F, M36I, M46I/L, G48V, I54V/L, L63P, A71I/V/T, G73S, V77I, V82A/F/T, I84V, N88S and L90M. For phenotype analysis, predictor variables included HIV RNA, CD4 cell count, four- and 10-fold reduced susceptibility to efavirenz, amprenavir and abacavir, and the cumulative number of active drugs (defined by phenotype). Predictors of virological response were assessed with logistic regression models (*P* values from likelihood ratio tests) or a decision tree-based procedure (based on the χ^2 test) as appropriate.

Results

Study population

The study opened in December 1997 and enrolled 101 subjects, 17% female and 21% non-white. Baseline characteristics are presented in Table 1. Eighty-seven percent of subjects had > 2 years of prior antiretroviral therapy. NNRTI-experienced patients (59% of the total) had higher median baseline viral loads, more advanced HIV disease, and more PI exposure at baseline.

Subject accountability

Ninety-nine patients received study drugs and thus formed the safety population (Fig. 1); 82 were on study at week 16 and 54 at week 48. For the analysis of correlates of response, baseline reverse transcriptase and cleavage sites/protease genotypes were obtained for 73 out of 74 and 95 out of 101 patients in the virology subpopulation and the ITT population, respectively. Baseline phenotypic resistance data were obtained for 57 and 69 patients in the virology subpopulation and the ITT population, respectively.

Plasma HIV RNA and CD4 cell response to therapy

The median decrease in HIV RNA from baseline was 0.33 (n = 83) and 0.85 (n = 51) log₁₀ copies/ml at weeks 16 and 48, respectively. The majority of patients had some antiviral response: 61% (62/101) of patients experienced >0.5 log₁₀ decrease in HIV RNA (a pre-specified secondary endpoint) between baseline and week 2; however, the proportion with this response diminished to 35% at week 16; it was then approximately stable (32%) at week 48. The proportion with HIV RNA < 400 copies/ml was smaller: 25 out of 101 (25%) at week 16 (Fig. 2). Patients were permitted to change drugs because of toxicity prior to week 16; thus, eight (35%) out of 23 patients with HIV RNA < 400 copies at week 16 had substituted other NRTI for abacavir.

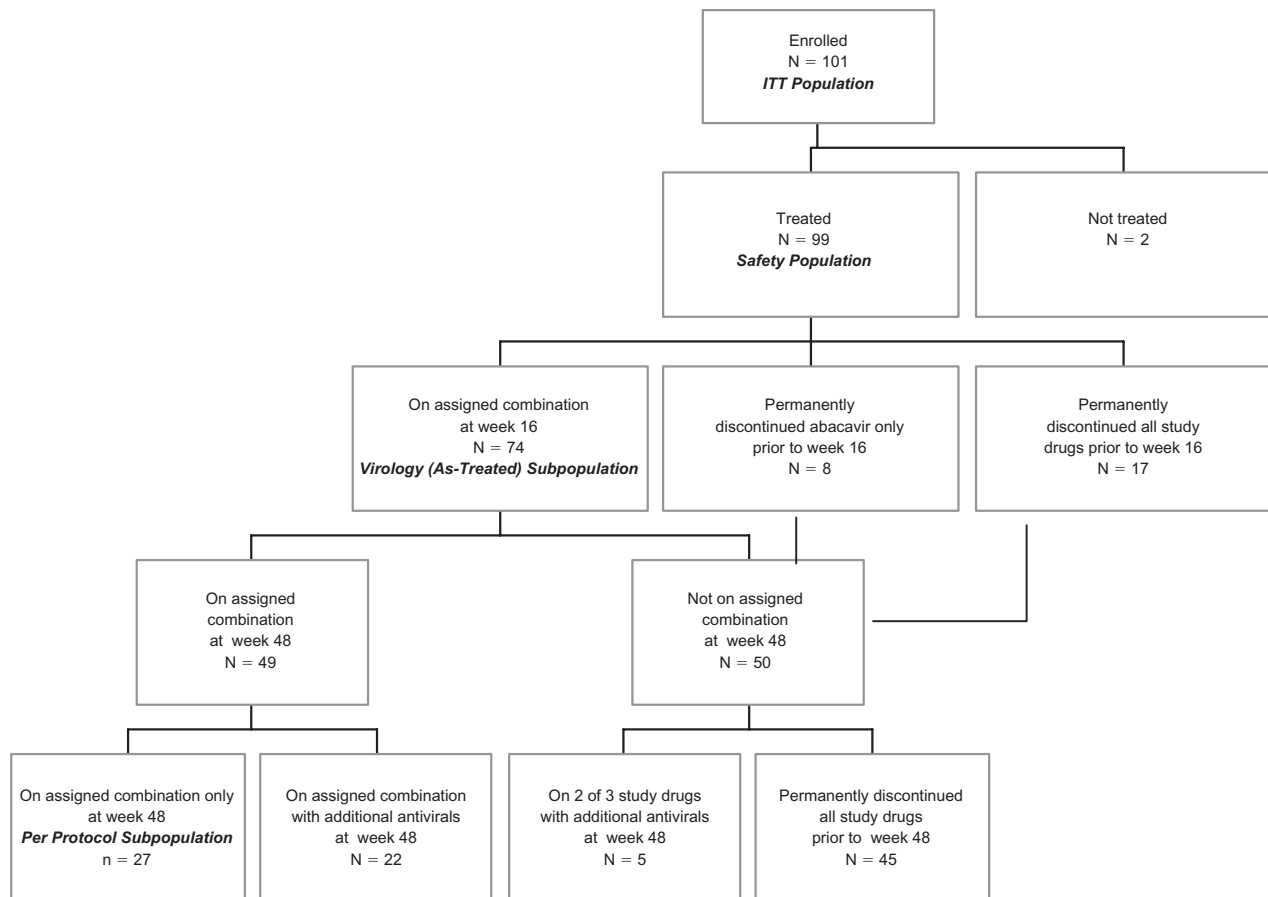


Fig 1. Subject accountability and definition of study populations.

Subjects were allowed to add other antiviral agents for reasons other than toxicity after week 16, and by week 48, 27 (50%) of the 54 subjects on study had added to the study regimen. The 23 subjects (23%) who had HIV RNA < 400 copies/ml at week 48 represent 20 of the 25 who achieved this viral load at week 16 plus three others; eight patients had added other drugs to the regimen.

The antiviral activity of the regimen was different in the different treatment strata. Of NNRTI-experienced patients with pre-entry HIV RNA >40 000 copies/ml, only two out of 35 (6%) had HIV RNA < 400 copies/ml at week 16 compared to 12 out of 28 (43%) NNRTI-naïve patients with HIV RNA < 40 000 copies/ml ($P < 0.001$, Fisher's exact test). Those who were NNRTI-naïve were more likely to have remained exclusively on all three study drugs and to have HIV RNA < 400 copies/ml at week 48 (14/22 if NNRTI-naïve vs. 1/5 if NNRTI-experienced).

In a week 16 per protocol analysis (virology subpopulation), 32% (24/74) of patients had HIV RNA < 400 copies/ml: 47% (20/43) of the NNRTI-naïve and 13% (4/31) of the NNRTI-experienced patients.

In the week 48 per protocol analysis, 56% (15/27) of patients had HIV RNA < 400 copies/ml.

The median increase in CD4 cells was $15 \times 10^6/l$ ($n = 82$) at week 16 and $43 \times 10^6/l$ ($n = 50$) at week 48.

Safety

All study drugs were stopped because of adverse events in 12 out of 99 (12%) and 21 out of 99 (21%) patients prior to week 16 and week 48, respectively. Eight others stopped abacavir only. No patient stopped efavirenz or amprenavir alone prior to week 16. Rash was the most common cause of study drug discontinuation (17/99, 17%). Signs and symptoms possibly attributable to abacavir hypersensitivity occurred in 14 out of 99 (14%) patients. Common (> 20% of subjects) drug-related adverse events grades 1–4 (99 subjects assessed) and treatment emergent grade 3/4 laboratory abnormalities (92 subjects assessed) through study week 72, the time of study closure, were as follows: rash, 50 (51%); nausea, 41 (41%); diarrhea, 27 (27%); sleep disorders, 27 (27%); dizziness, 25 (25%); fatigue, 23 (23%); hypertriglyceridemia (non-fasting specimen), 18 (20%); neutropenia, eight (9%); hyperamylasemia, four

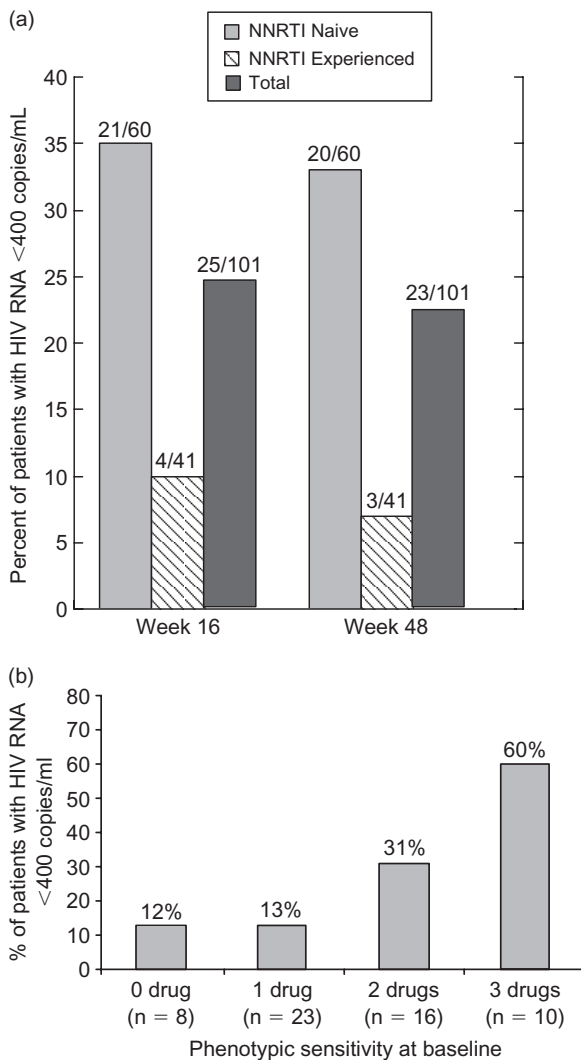


Fig 2. Virological outcome according to baseline factors. (a) Proportion of subjects with plasma HIV RNA < 400 copies/ml at 16 and 48 weeks according to prior exposure to NNRTI. The data are from an ITT population ($n = 101$) with missing values and discontinuations of all study drugs considered as failing to achieve HIV RNA < 400 copies/ml; subjects who added or changed antiretroviral agents as permitted by the protocol could be included in those < 400 copies/ml. (b) Proportion of subjects who had plasma HIV RNA < 400 copies/ml at 16 weeks according to baseline phenotypic susceptibility (< 4-fold resistance) to 1, 2, or 3 study drugs; $P = 0.0042$ for the slope of the relationship between week 16 virological response and baseline drug sensitivity. Data are from patients ($n = 57$) in the virology subpopulation for whom baseline phenotype was available for all three study drugs.

(4%); leukopenia, three (3%); hypercholesterolemia, two (2%); elevated alkaline phosphatase, one (1%); elevated aspartate aminotransferase, one (1%). No rash was serious (grade 3 or 4). Minor changes in median triglyceride values occurred (median increase of

0.050 mmol/l at week 16). Median increases in cholesterol were observed with a maximum change of 1.25 mmol/l at week 48, but medians remained within the normal range. Two patients died, one of lung cancer and one of lymphoma.

Analysis of predictors of response

The baseline characteristics of the virology subpopulation were similar to those of the ITT study population (Table 1). Genotypes and phenotypes in the virology subpopulation were similar to those in the ITT population (data not shown).

Baseline genotype

Baseline genotypic resistance was common in the 95 subjects for whom complete data were obtained: 75% had four or more RT mutations and 81% had four or more protease mutations. The median (range) number of RT and protease mutations at baseline was 5 (0–10) and 6 (0–9), respectively. The number of NNRTI-associated mutations in viruses from NNRTI-experienced patients ranged from 0 (in six subjects) to five. Eight NNRTI-naive patients had viruses with one to three NNRTI-associated mutations. The most common NNRTI-associated mutations were T215F/Y (85%), M41L (67%), M184V (66%), D67N (53%) and T69D (34%). L74V and Y115F were present in eight and two isolates, respectively. Mutations associated with multiple NNRTI resistance were detected in 7% (7/95): Q151M complex in three patients and 69 insertion in four. In protease, the most common substitution was at position 10 (74%); other common mutations were L90M (68%), A71V or T (66%), M46L or I (54%), V82A/F/T (42%), I54V (41%) and I84V (28%); I50V was not detected. Cleavage site mutations A to V at residue P2 of p7/p1 and L to F, P or V at residue P1 of p1/p6 were observed in 44% and 15% of isolates, respectively. NNRTI-associated mutations observed were Y181C (28%), K103N (24%), and A98G (11%).

Baseline phenotype

Phenotype was obtained for isolates from 63 to 69 subjects depending on the drug considered. Phenotypic sensitivity (≤ 4 -fold) to amprenavir was found in 55%; abacavir in 45%, and efavirenz in 59%. Less than 10-fold decrease in sensitivity was found in 94% for abacavir, 86% for amprenavir and 71% for efavirenz. Fifty-seven percent of NNRTI-experienced patients had ≥ 10 -fold efavirenz resistance. Most isolates had susceptibility to other PI reduced at least fourfold: indinavir 81%, nelfinavir 86%, ritonavir 84% and saquinavir 78%. Stepwise multiple regression identified a correlation between reduced susceptibility to amprenavir and the presence of mutations at I84V, which was enhanced when linked with L101/V/F/R, and V32I ($P < 0.02$) but not V82A/F/T, I54V or L90M. Reduced susceptibility (≥ 4 -fold) to NNRTI was also

common: zidovudine, 87%; lamivudine, 88%; stavudine, 57%; didanosine, 38%. Seventy-six percent (22/29) of isolates with reduced susceptibility to nevirapine were resistant (≥ 10 fold) to efavirenz.

Relationship between genotype and response

Complete genotypic data were available for 73 out of 74 patients in the virology subpopulation. In a univariate analysis, higher baseline HIV RNA, lower baseline CD4 cell count, and greater number of NRTI and NNRTI mutations were associated with lack of virological response (Table 2). Neither the number of protease mutations nor any specific protease mutation predicted response. After adjusting for all other variables, the number of NNRTI mutations remained the only predictor of response. The loss of significance of baseline HIV RNA is explained by the high correlation between number of NNRTI mutations and baseline viral load. Decision tree analysis using combined NNRTI and NRTI mutations found K103N, Y181C and T69D to be significant predictors of lack of virological response (Fig. 3).

Relationship between phenotype and response

Phenotypic data for all study drugs were available for 57 patients from the virology subpopulation. In a univariate analysis, susceptibility (< 4 -fold reduction) to efavirenz and to abacavir as well as lower baseline HIV RNA, but not higher CD4 cell count or susceptibility to amprenavir, was associated with virological response (Table 2). In the multivariate analysis using a logistic model with *P* values calculated for each term after correction for all other terms, only lower HIV RNA and susceptibility to abacavir predicted response (Table 2). The loss of significance of susceptibility to efavirenz (< 4 -fold reduction) in the multivariate model can be explained by the high correlation of this covariate with other variables. Using a 10-fold cutoff for susceptibility, HIV RNA and susceptibility to efavirenz were associated with virological response in both univariate and multivariate analyses. Abacavir susceptibility was not significant in this model, but only three out of 57 isolates had > 10 -fold resistance to abacavir. In a multivariate model using a fourfold cutoff for abacavir and a 10-fold cutoff for efavirenz, both of

Table 2. Logistic regression models of probability of week 16 HIV RNA < 400 copies/ml.

Baseline factor	Univariate analysis	Multivariate analysis ^b	
	<i>P</i> (χ^2) ^a	Odds ratio (95% CI)	<i>P</i> (χ^2) ^a
Genotypic model (n = 73)			
HIV-1 RNA (copies/ml)	0.0007	0.71 (0.19–2.38)	0.58
CD4 cell count ($\times 10^6/l$)	0.0138	1.00 (1.00–1.01)	0.24
Total number of PI-associated mutations	0.466	n.i.	
Total number of NRTI-associated mutations	0.0024	0.84 (0.58–1.16)	0.29
Total number of NNRTI-associated mutations	0.0001	0.14 (0.03–0.41)	0.0001
Phenotypic models (n = 57)			
Analysis using 4-fold cutoff to define baseline viral susceptibility			
HIV-1 RNA (copies/ml)	0.0272	0.23 (0.04–0.92)	0.037
CD4 cell count ($\times 10^6/l$)	0.0949	n.i.	
< 4 FR for efavirenz	0.0167	3.19 (0.77–16.56)	0.11
< 4 FR for abacavir	0.0249	4.99 (1.25–24.33)	0.022
< 4 FR for amprenavir	0.5042	n.i.	
Analysis using 10-fold cutoff to define baseline viral susceptibility			
HIV-1 RNA (copies/ml)	0.0272	0.26 (0.07–0.84)	0.023
CD4 cell count ($\times 10^6/l$)	0.0949	n.i.	
< 10 FR for efavirenz	0.0051	11.99 (1.97–235.2)	0.004
< 10 FR for abacavir	0.7818	n.i.	
< 10 FR for amprenavir	0.9362	n.i.	
Analysis using 4-fold cutoff for abacavir and 10-fold cutoff for efavirenz			
HIV-1 RNA (\log_{10} copies/ml)	0.0272	0.19 (0.03–0.72)	0.012
CD4 cell count ($\times 10^6/l$)	0.0949	n.i.	
< 4 FR for abacavir	0.0249	5.11 (1.22–26.92)	0.025
< 10 FR for efavirenz	0.0051	9.82 (1.58–192.6)	0.011
< 4 FR for amprenavir	0.5042	n.i.	
Analysis using number of drugs to which baseline virus susceptible (< 4 FR)			
HIV-1 RNA, copies/ml	0.0272	0.22 (0.04–0.83)	0.024
CD4 cell count ($\times 10^6/l$)	0.0949	n.i.	
Number of active drugs	0.0048	2.81 (1.36–6.65)	0.0042

^a*P* values from likelihood ratio tests based on a logistic model. ^bMultivariate analysis uses Type 3 logistic regression models. n.i., Not included in the model; NRTI, nucleoside analog reverse transcriptase inhibitor; NNRTI, non-nucleoside analog reverse transcriptase inhibitor; PI, protease inhibitor; CI, confidence interval; FR, fold resistance of baseline virus to specified drug.

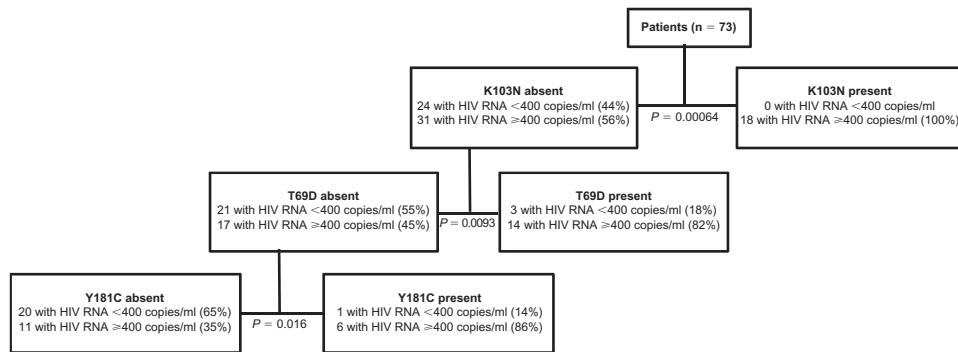


Fig. 3. Virological response decision tree based on logistic regression approach and χ^2 test for all NNRTI- and NRTI-related mutations at baseline. Data from patients remaining on all study drugs through week 16 were included. Virological response is assessed as < 400 copies/ml (responder) or \geq 400 copies/ml (non-responder). This tree had a misclassification rate of 21% (15/73).

these variables remained significantly associated with virological response. In univariate and multivariate analyses using the number of active drugs to which the baseline isolate was susceptible at the fourfold cutoff, lower HIV RNA and greater number of active drugs but not higher CD4 cell counts were significantly associated with virological response. When the isolate was sensitive to none or to one drug, 13% of patients had week 16 HIV RNA < 400 copies/ml as compared to 31% and 60% when susceptible to two or three drugs, respectively (Fig. 2).

Discussion

In this phase II salvage study, an abacavir, amprenavir and efavirenz regimen resulted in substantial (< 400 copies/ml) and durable (48 week) decreases in viral load in 23% of highly antiretroviral-experienced patients, an outcome consistent with responses in other salvage studies [1,7–11]. Although most patients initially had at least a 0.5 \log_{10} decline in viral load, these early responses were often brief. In contrast, most of the 25% who had HIV RNA < 400 copies/ml at week 16 maintained this response to week 48. We had anticipated that many patients would not have optimal responses despite the use of three drugs to which the patients had not previously been exposed, and thus we planned to look at baseline factors that might predict virological response. We chose to examine factors that predicted a viral load of < 400 copies/ml at week 16 because we assumed that this response represented a more clinically relevant outcome than lesser responses at earlier time-points. In addition, patients were not permitted to add or change drugs until week 16 except for substitutions for suspected abacavir intolerance.

Our analysis showed that subgroups of the study

population who were more likely to have an optimal antiviral response could be predicted by baseline characteristics. Patients who were NNRTI-naïve by history and those with lower baseline viral loads had a substantially better outcome than those who were NNRTI-experienced or who had higher baseline viral loads; however, in our study population, NNRTI experience and baseline viral load were highly correlated. Thus, we used multivariate analysis to examine the independent contribution of phenotypic and genotypic resistance to each study drug. For genotype, variables assessed included number of NNRTI-, NRTI- and PI-associated mutations and, separately, individual mutations. For phenotype, susceptibility to each study drug and, separately, phenotypic susceptibility to one, two or three study drugs were examined.

In our study, phenotype predicted virological response: antiretroviral response improved in association with phenotypic sensitivity to an increasing number of drugs in the regimen. When susceptibility is defined as a lower than fourfold change compared to wild-type virus, susceptibility to abacavir was the only predictor of virological success independent of baseline HIV RNA. Multivariate statistical analyses on a small data set can be complicated by interactions between variables included in the model; in this case, susceptibility to efavirenz was highly correlated with baseline HIV RNA. When using < 10-fold change in susceptibility, a cut-off more appropriate for assessment of efavirenz resistance [12], susceptibility to efavirenz was a strong independent predictor of success. In a model that used less than fourfold change for abacavir and < 10-fold change for efavirenz, susceptibility to abacavir and to efavirenz as well as baseline viral load were significantly associated with virological response. This demonstrates the effect of breakpoint on the utility of susceptibility testing; a lack of validated breakpoints is one limitation

of phenotyping. Amprenavir susceptibility was not associated with virological response although the improved response in those with baseline virus susceptible to all three drugs suggests that it did contribute to outcome.

Genotypic analysis showed that the total number of NNRTI- and NRTI- but not PI-associated mutations correlated with virological response. In a multivariate model, the total number of NNRTI-associated mutations remained the only predictor of response. In a separate analysis, individual mutations associated with lack of response included K103N and Y181C, both sites of NNRTI resistance, and T69D, which is not a specific marker for abacavir resistance but is associated with multiple NRTI-associated mutations and thus NRTI cross-resistance [4,13]. Sixty-five percent (20/31) of those whose virus lacked all three of the genotypic predictors (K103N, T69D, and Y181C) achieved viral loads < 400 copies/ml at week 16. No single or double combination of key PI mutations predicted response. These data confirm the importance of viral susceptibility to efavirenz and abacavir to successful outcome. The heterogeneity of baseline viruses, the complexity of mutational patterns and their interactions, particularly in the protease region, and the use of a three-drug regimen probably contributed to difficulties in selecting specific genotypes predictive of success [14].

We evaluated retrospectively baseline correlates of response to a study-specified regimen administered in a prospective clinical trial. Retrospective analysis has previously shown genotypic or phenotypic susceptibility to NRTI and PI to correlate with outcome [7,11,15–21], and the prospective use of resistance testing in the design of a salvage regimen has been shown to improve antiviral response [8,22,23]. Although using resistance testing to design salvage regimens is recommended [3,4], the utility of resistance testing and the superiority of genotype or phenotype testing is probably highly dependent on the treatment experience of the patient population and on how well each test reflects susceptibility to particular antiretroviral drugs. By multivariate analysis, in addition to baseline viral load, both genotype and phenotype provided information predictive of outcome: abacavir phenotype, efavirenz phenotype, number of active drugs, and number of NNRTI mutations.

Because efavirenz, a cytochrome P450 inducer, decreases concentrations of amprenavir (a substrate) this study may have been compromised by the use of the standard dose of amprenavir. Efavirenz decreases amprenavir peak and trough concentrations by 33% and 43%, respectively [24]. Lower plasma concentrations of PI have been associated with poorer virological response [25]. This might explain the lack of strong

statistical association between phenotypic or genotypic susceptibility to amprenavir and outcome despite the preservation of *in vitro* susceptibility to amprenavir in 55% of viruses and the improved virological response in patients with viruses sensitive to all study drugs. Concomitant ritonavir or nelfinavir has been shown to abrogate the effect of efavirenz on plasma concentrations of amprenavir [26]. It is reasonable to include one of these or to increase amprenavir dosing in an amprenavir–efavirenz regimen.

The adverse events seen in this study, although frequent, were consistent with known side-effects of the drugs [27–31]. Except for rash as part of possible abacavir hypersensitivity syndrome, they were rarely treatment limiting. Although rash occurred in half of the patients, no rash was assessed as serious. Treatment can be continued despite non-serious rash in the absence of systemic symptoms, a strategy that was successful in 42 of our patients. In our study, 14% of patients stopped abacavir for possible hypersensitivity compared to an expected 4% [31,32]. It is unlikely that amprenavir or efavirenz potentiated abacavir hypersensitivity because studies of abacavir–amprenavir and abacavir–efavirenz have shown low frequencies of hypersensitivity [32,33]. The frequency of rash combined with gastrointestinal side-effects from the combination regimen may have led to over-diagnosis of abacavir hypersensitivity. There is no definitive way to substantiate this diagnosis, and investigators had to make individualized clinical decisions. Differences in the proportion with hypersensitivity at different study sites suggest differing standards for the diagnosis.

The long-term virological or clinical benefit of resistance testing as compared to expert care alone in highly treatment-experienced patients remains to be established. This study supports the idea that, in a heavily pretreated population, resistance assays can provide useful information about the likelihood of response. Optimizing drug selection could minimize cost, inconvenience, toxicity, and drug resistance by avoiding the use of inactive drugs and of regimens in which only a single drug is active. Although resistance testing is expensive, costs might be recouped by avoiding the use of inactive drugs. Clearly, cross-resistance makes lack of prior use of a drug an inadequate criterion for inclusion in a regimen. In complex populations similar to the one in our study, resistance testing is probably useful for the selection of a multi-drug salvage regimen.

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Appendix

The CNA2007 Study Team

D. J. Manion, DuPont Pharmaceuticals Co., Wilmington DE; M. Rogers, Glaxo Wellcome Inc., Research Triangle Park NC; J. Wolfram, G. E. Amphlett, A. Rakik and M. Tisdale, Glaxo Wellcome, Stevenage and Greenford, UK.