A randomized trial assessing the impact of phenotypic resistance testing on antiretroviral therapy

Calvin J. Cohen[a], Susan Hunt[b], Michael Sension[c], Charles Farthing[d], Marcus Conant[e], Susan Jacobson[f], Jeffrey Nadler[g], Werner Verbiest[h], Kurt Hertogs[h], Michael Ames[i], Alex R. Rinehart[j], Neil M. Graham[j] and the VIRA3001 Study Team*

Objective: To compare the effect of treatment decisions guided by phenotypic resistance testing (PRT) or standard of care (SOC) on short-term virological response.

Design: A prospective, randomized, controlled clinical trial conducted in 25 university and private practice centers in the United States.

Participants: A total of 272 subjects who failed to achieve or maintain virological suppression (HIV-1-RNA plasma level $>$ 2000 copies/ml) with previous exposure to two or more nucleoside reverse transcriptase inhibitors and one protease inhibitor.

Interventions: Randomization was to antiretroviral therapy guided by PRT or SOC.

Main outcome measures: The percentage of subjects with HIV-1-RNA plasma levels less than 400 copies/ml at week 16 (primary); change from baseline in HIV-1-RNA plasma levels and number of 'active' (less than fourfold resistance) antiretroviral agents used (secondary).

Results: At week 16, using intent-to-treat (ITT) analysis, a greater proportion of subjects had HIV-1-RNA levels less than 400 copies/ml in the PRT than in the SOC arm ($P = 0.036$, ITT observed; $P = 0.079$, ITT missing equals failure). An ITT observed analysis showed that subjects in the PRT arm had a significantly greater median reduction in HIV-1-RNA levels from baseline than the SOC arm ($P = 0.005$ for 400 copies/ml; $P = 0.049$ for 50 copies/ml assay detection limit). Significantly more subjects in the PRT arm were treated with two or more 'active' antiretroviral agents than in the SOC arm ($P = 0.003$).

Conclusion: Antiretroviral treatment guided prospectively by PRT led to the increased use of 'active' antiretroviral agents and was associated with a significantly better virological response.

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From the [a]Community Research Initiative of New England, Boston, MA 02215, USA; [b]University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA; [c]North Broward Hospital District, Fort Lauderdale, FL 33312, USA; [d]AIDS Healthcare Foundation, Los Angeles, CA 90027-6069, USA; [e]Dermatology/HIV Consultations, San Francisco, CA 94117, USA; [f]East Bay AIDS Center, Clinical Faculty, Department of Family and Community Medicine, Berkeley, CA 94705, USA; [g]University of South Florida, Tampa, FL 33602, USA; [h]Tibotec Virco NV, B-2800 Mechelen, Belgium; [i]GlaxoSmithKline, Research Triangle Park, NC 27709, USA; and [j]Tibotec Virco USA, Durham, NC 27713, USA, formally at Glaxo Wellcome, Inc.

*For members of the VIRA3001 Study Team see Appendix.

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Correspondence to: Neil Graham, MD, Vice President, Medical Department, Tibotec-Virco USA, 2505 Meridian Parkway, Suite 350, Durham, NC 27713, USA.

Tel: +1 919 361 9010 (Ext 3245); fax: +1 919 361 9020; e-mail: neil.graham@tibotec-virco.com

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Introduction

The goals of treating HIV infection with antiretroviral drugs are to suppress HIV-1-RNA plasma levels (viral load) to undetectable levels for as long as possible, to restore or preserve immunological function, to improve quality of life, and to reduce HIV-related morbidity and mortality [1]. Current guidelines established by the Department of Health and Human Services call for the initiation and maintenance of antiretroviral therapy with one of the following types of regimens composed of three antiretroviral agents: two nucleoside reverse transcriptase inhibitors (NRTI) plus a protease inhibitor (PI); two NRTI plus a non-nucleoside reverse transcriptase inhibitor (NNRTI), or three NRTI [1]. Highly active antiretroviral therapy (HAART) regimens allow as many as 60–90% of antiretroviral-naive subjects in clinical trials and up to 44% of patients in inner-city clinic settings to achieve the maximal suppression of HIV-1-RNA levels [2,3]. Typically, a lower HIV-1-RNA nadir correlates with a longer time before drug failure occurs [4].

However, when antiretroviral drugs are given in combinations that only partly suppress HIV-1 replication, or when viral rebound occurs after initial successful suppression, viral mutations that confer drug resistance are selected and resistant viral strains can predominate within weeks, leading to poor clinical response and, ultimately, treatment failure [5–7]. In recent years, the prevention, characterization, and clinical management of resistance to HAART have received increasing attention. Testing for the resistance of HIV to antiretroviral drugs is now considered a rational adjunct to guide HAART [1,8,9]. Resistance is commonly measured in one of two ways. A sequencing (genotypic) analysis of the viral genome can identify point mutations that are known to be associated with resistance. As there are over 200 different mutations that are known to affect resistance and as these mutations can interact in complex ways, the interpretation of genotypic information can be highly complex and challenging [10].

Phenotypic resistance testing (PRT) utilizes an in vitro-based assay system, in which the 50% inhibitory concentration (IC_{50}) to each antiretroviral agent is determined by culturing the recombinant viral strain in the presence of increasing concentrations of each drug. The IC_{50} for the recombinant virus is then compared with that of a genetically wild-type reference virus, to give the relative fold change in susceptibility of the subject's virus to each drug. PRT is a direct measure of HIV-1 drug susceptibility, and takes into account the net effect of resistance mutations and their interactions. Although these results can be used by most clinicians to help treatment decisions, expert advice is still generally recommended [8].

Testing a subject's virus for susceptibility to antiretroviral drugs and using this information to help make treatment decisions could reasonably be expected to help select active drugs, avoid drugs to which the subject's virus is resistant, and improve clinical outcome. Results from two published prospective trials of genotypic resistance testing VIRADAPT [11] and genotypic antiretroviral resistance testing (GART) [12] demonstrated an improvement in virological response when therapy decisions were guided by genotypic test results. Before VIRA3001, studies suggesting the potential clinical benefit of therapy guided by PRT were retrospective [13–15]. The VIRA3001 trial was an open-label, multicenter, randomized, controlled 16 week study designed to assess the impact on virological outcome of prospective PRT. The primary objective of the trial was to determine whether treatment guided by PRT results leads to greater viral load suppression than treatment guided by the standard of care (SOC), i.e. using treatment history, subject records and following published treatment recommendations, without PRT.

Methods

Study population

Eligible male and female subjects were 13 years of age or older, and had documented HIV-1 infection; HIV-1-RNA plasma levels of 2000 copies/ml or more; antiretroviral-experience; and were experiencing virological failure on antiretroviral treatment consisting of at least two NRTI and only one PI, taken for at least one month before screening. Subjects were excluded if they had a history of alcohol or drug use that was judged likely to interfere with therapy, had previous PRT, had participated in an antiretroviral drug trial within 30 days of selection or during the trial, had a life expectancy of less than 6 months, or had diseases that could interfere with assessments (e.g. lymphoma requiring ongoing chemotherapy, Kaposi's sarcoma requiring systemic therapy, active or life-threatening opportunistic infections, severe peripheral neuropathy, or cytomegalovirus retinitis). The protocol was amended to allow subjects with previous NNRTI therapy in May 1999. Four out of 272 randomly selected subjects had had previous genotypic testing. The study protocol was approved by Institutional Review Boards at all participating study sites, and all subjects provided written informed consent.

Study design

This open-label, randomized study was conducted over a 16 week treatment period at 25 study sites in the United States from January 1998 to September 1999, with a maximum of 30 randomly selected subjects per site. The primary objective of the study

was to compare the virological outcome of antiretroviral regimens chosen with (PRT arm) or without (SOC arm) PRT. Investigators had access to published treatment guidelines and their subjects' treatment histories (but not study-provided external 'expert opinion') to assist treatment decision-making for subjects in both arms. At some sites treatment decisions were made by the investigator in collaboration with the subject's treating physician. The use of IL-2, granulocyte colony-stimulating factor, or granulocyte/macrophage colony-stimulating factor during the study was prohibited.

The endpoints for the study related to virological and immunological response. These included the proportion of subjects with HIV-1-RNA levels below 400 copies/ml at 16 weeks (primary endpoint). Secondary endpoints were absolute and average area under the curve minus baseline (AAUCMB) changes in HIV-1 RNA from baseline to week 16 and the degree and duration of immunological change (CD4 cell count), as assessed by absolute and AAUCMB changes from baseline to week 16. The proportion of subjects with HIV-1-RNA levels below 50 copies/ml at 16 weeks was also determined by re-analysing all week 16 samples with HIV-1-RNA levels below 5000 copies/ml using the Roche Amplicor Ultrasensitive assay (Roche Molecular Systems, Pleasanton, CA, USA). Baseline phenotypic resistance profiles in both groups were also evaluated.

Fig. 1 summarizes the study design. Study candidates were screened (informed consent, HIV-1-RNA plasma levels, and CD4 cell counts) 5 weeks before the initiation of new treatment regimens (week −5). A plasma sample was obtained at this time and submitted to Virco NV, Mechelen, Belgium, for PRT using the Antivirogram phenotypic assay. At week −4, eligible subjects were randomly assigned either to the PRT or SOC arms, demographic and medical history information was collected, and a physical examination and assessment of HIV clinical events were performed. Randomization was performed centrally, and occurred in blocks of size four (two subjects per treatment arm) independently at each site. Arm assignments were not blinded to either the investigator or subject. At week −2, before seeing the results of resistance testing, investigators completed a Choice of Therapy Survey, describing which treatment regimen they would prescribe at the time of change to a new regimen (irrespective of randomization). PRT results were then provided for subjects randomized to the PRT arm at week −1. At baseline, investigators were permitted to recommend a change in any treatment regimen component for subjects randomly assigned to either arm. Antiretroviral agents, which were tested for phenotypic susceptibility and were available for baseline regimen choices, were zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, nevirapine, delavirdine, efavirenz, indinavir, ritonavir, saquinavir, nelfinavir, and amprenavir. All baseline treatment regimens were limited to either three or four antiretroviral drugs. A subtherapeutic, pharmacological enhancing dose of ritonavir (< 400 mg twice a day) was not considered as an antiretroviral drug. Plasma HIV-1-RNA levels (Amplicor HIV-1 Monitor, 400 copies/ml detection limit), CD4 cell count, and concomitant medication use were assessed at baseline, and at weeks 2, 4, 8, 12, and 16. Although the study was not powered for more sensitive virological cut-offs, an exploratory analysis was performed only on subjects with HIV-1-RNA plasma values of less than 5000 copies/ml (< 400 copies/ml detection limit) at week 16 using the Roche Amplicor Ultrasensitive assay (< 50 copies/ml limit of detection). Subjects were permitted to change baseline antiretroviral therapy during the 16 week period for reasons of intolerance or toxicity only. Baseline PRT results for subjects randomly assigned to the SOC arm were released to the investigator when the subject completed all 16 weeks of the study or failed to achieve virological suppression. All subjects were tested for phenotypic resistance upon completion of 16 weeks or after confirmed failed virological suppression. A lack of virological suppression was defined as the failure to achieve a 0.5 $\log_{10}$ or greater decrease below baseline at 8 weeks, a 0.5 $\log_{10}$ or greater increase from the lowest viral measurement achieved during the study, or the return of virus levels above baseline levels.

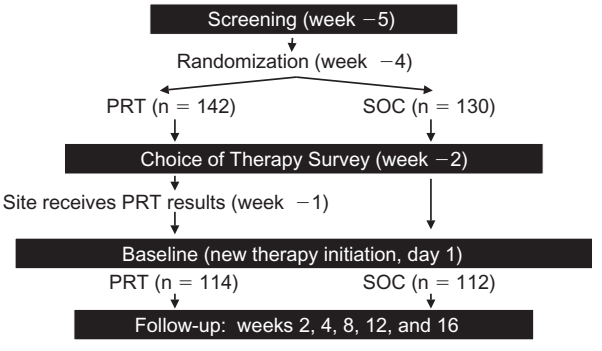

Fig. 1. Study design. Events at week −2 and week −1 did not require a subject visit. PRT, Phenotypic resistance testing; SOC, standard of care.

Phenotypic resistance testing

The Antivirogram assay was performed according to the standardized methodology [16,17]. Amplification of subject virus sequences included HIV-1 *gag* (p7/p1 and p1/p6 cleavage sites), protease, and reverse transcriptase (codons 1–400) sequences, covering all the known resistance mutations. A CD4 T cell line, MT-4, was transfected via electroporation with this part of the HIV-1 genome, together with an HIV-DNA construct from which this part of the genome was deleted. Upon

intracellular recombination, progeny HIV-1 was produced within 5–10 days, and the newly formed chimeric viruses were analysed for phenotypic sensitivity to the 14 antiretroviral drugs in an automated, cellular-based assay. From the comparison of the subject's virus strain IC_{50} with the IC_{50} of the wild-type HIV-1 laboratory strain, a report showing the relative changes in susceptibilities (as fold changes in resistance) for each of the antiretroviral drugs was generated.

Sample size and statistical analysis

The percentage of subjects expected to be virological failures on the basis of the 400 HIV-1-RNA copies/ml of plasma detection limit during the first 16 weeks of the evaluation period was estimated to be 60% in the SOC arm and 40% in the PRT arm, on the basis of previous study findings [18–20]. To detect this difference with 80% power and a 5% two-sided significance level, 134 subjects were deemed necessary per treatment arm (after adjustment for a 20% dropout rate between random selection and 16 weeks of the study). A total study population of 268 subjects was thus planned. The recruitment of subjects was to continue until 134 subjects were randomly assigned to each treatment arm. The primary endpoint was virological response achieved at 16 weeks. In the analysis this was based on the percentage of subjects who achieved HIV-1-RNA values of less than 400 copies/ml of plasma in the two arms. Subjects who were exposed to antiretroviral drugs at baseline were included in the intent-to-treat (ITT) populations for efficacy analysis. In the ITT, missing equals failure (ITT, M = F) analysis, subjects were considered failures if they permanently discontinued the study for any reason, or had missing data, or failed to demonstrate virological suppression. In the ITT observed (ITTO) analysis, the change from baseline and AAUCMB were computed without imputing missing values; subjects who withdrew because of failure to demonstrate virological suppression were included in the denominator as 'failures' in the calculations of the percentage of subjects with HIV-1-RNA levels of less than 400 copies/ml of plasma.

The proportion of subjects with HIV-1-RNA levels below the limit of assay quantitation, as measured by quantitative HIV-1 RNA polymerase chain reaction, were compared across treatment arms using the Fisher's exact test. The median change from baseline to each study visit was reported for $\log_{10}$ HIV-1 RNA and CD4 cell count. The Wilcoxon rank sum test was used to compare the change from baseline and AAUCMB for $\log_{10}$ HIV-1 RNA and CD4 cell count between the study arms. Hodges–Lehmann estimators were used to compute 95% confidence intervals (CI) for the difference between study arms. A difference between the two treatment arms was considered statistically significant if the P value was less than 0.05. Additional post-hoc analyses examined the exploratory analysis carried out on subjects with HIV-1-RNA plasma values of less than 5000 copies/ml (< 400 copies/ml detection limit) at week 16 using the Roche Amplicor Ultrasensitive assay (< 50 copies/ml limit of detection), the effect of the PI initiated before study entry on virological outcome, and the effect of adding a NNRTI to the baseline regimen on virological outcome. The proportion of subjects receiving two or more 'active' antiretroviral agents, defined as agents to which the subject's virus exhibited less than a fourfold increase in resistance when compared with the standard wild-type reference virus, was also included in post-hoc analyses.

Results

Subject characteristics

In total, 142 and 130 subjects were randomly assigned to the PRT and SOC arms, respectively. Twenty-eight subjects (20%) in the PRT arm and 18 subjects (14%) in the SOC arm were removed from the ITT population as a result of withdrawal before or at baseline or the unplanned receipt of PRT result (one SOC subject) or missing PRT result (one PRT subject). Eighty-two subjects (43 in SOC and 39 in PRT arms; 30%) withdrew before study completion for the following reasons: lack of virological suppression (36 subjects, 13%); lost to follow-up (21 subjects, 8%); protocol violation (10 subjects, 4%); consent withdrawn (eight subjects, 3%); adverse event (five subjects, 2%); clinical progression (one subject); and other (one subject). Six subjects in the PRT arm and 11 subjects in the SOC arm changed baseline therapy as a result of intolerance or toxicity during the trial. The percentage of ITT subjects with previous exposure to PI was 53% for nelfinavir, 37% for indinavir, and 10% with other PI, whereas any previous exposure to NRTI was 96% for lamivudine, 84% for zidovudine, 61% for stavudine, 32% for didanosine, and 12% for zalcitabine. Previous NRTI use for over 2 years, 1–2 years, and less than 1 year was 41, 30, and 29%, respectively. Previous PI use for over 2 years, 1–2 years, and less than 1 year was 39, 33, and 28%, respectively. Four per cent of subjects (nine out of 226) had previously received NNRTI therapy. Four subjects in the PRT arm and seven subjects in the SOC arm used a subtherapeutic, pharmacological enhancing dose of ritonavir (< 400 mg twice a day) as part of their baseline regimen.

The demographic characteristics of the 114 subjects in the PRT and 112 subjects in the SOC arms who were exposed to antiretroviral drugs at baseline were similar between the two groups (Table 1). At baseline, the median plasma HIV-1-RNA level in the PRT arm was

Table 1. Characteristics of the study subjects.

Characteristic	SOC (n = 112)	PRT (n = 114)
Age, years		
Median	38	39
Range	24–65	22–73
Sex, no. (%)		
Male	99 (88)	99 (87)
Female	13 (12)	15 (13)
Race, no. (%)		
Caucasian	69 (62)	66 (58)
Black	24 (21)	28 (25)
Hispanic	14 (13)	16 (14)
Asian	3 (3)	2 (2)
Median HIV-1-RNA level, $\log_{10}$ copies/ml		
At screening (week −5)	3.95	4.01
At baseline (day 1)	3.92	4.18
Median CD4 cell count, cells/mm^3	347	348
CDC-defined AIDS, no. (%)	38 (34)	33 (29)
Antiretroviral use as part of baseline regimen, no. (%)		
Abacavir	32 (29)	31 (27)
Adefovir	1 (< 1)	0 (0)
Amprenavir	2 (2)	12 (11)
Delavirdine	0 (0)	1 (< 1)
Didanosine	46 (41)	47 (41)
Efavirenz	38 (34)	33 (29)
Indinavir	23 (21)	14 (12)
Lamivudine	33 (30)	40 (35)
Nelfinavir	15 (13)	16 (14)
Nevirapine	25 (22)	15 (13)
Ritonavir	46 (41)	37 (32)
Saquinavir	41 (37)	41 (36)
Stavudine	59 (53)	75 (66)
Zalcitabine	0 (0)	1 (< 1)
Zidovudine	23 (21)	26 (23)
Baseline antiretroviral regimen, no. (%)[a]		
Two NRTI/one PI	24 (21)	37 (32)
Two NRTI/one NNRTI	25 (22)	26 (23)
Two NRTI/two PI	16 (14)	19 (17)
Two NRTI/one NNRTI/one PI	5 (4)	4 (4)
One NRTI/two PI	4 (4)	2 (2)
One NRTI/one NNRTI/one PI	5 (4)	2 (2)
One NRTI/one NNRTI/two PI	16 (14)	13 (11)
Other combinations	17 (15)	11 (10)

CDC, Centers for Disease Control and Prevention; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PRT, phenotypic resistance testing; SOC, standard of care.
[a]A ritonavir dose of less than 400 mg twice a day was not considered to be a PI.

slightly higher (4.18 $\log_{10}$ copies/ml) than in the SOC arm (3.92 $\log_{10}$ copies/ml), and the median CD4 cell counts were nearly identical (348 versus 347 cells/mm^3). Randomly selected subjects who were not exposed to antiretroviral therapy at baseline had median plasma HIV-1-RNA screening values of 3.80 $\log_{10}$ copies/ml (n = 27) and 4.35 $\log_{10}$ copies/ml (n = 17) for the PRT and SOC arms, respectively ($P = 0.755$).

Phenotypic susceptibility

At the screening visit, the proportion of subjects with virus that was susceptible (less than a fourfold increase in resistance) to PI was observed most frequently for amprenavir (92%) and saquinavir (84%), and a greater than 10-fold increase in resistance was observed most often (46% of subjects) for nelfinavir (Fig. 2a). In subjects who had virus with a greater than fourfold increase in resistance to indinavir or nelfinavir, susceptibility to amprenavir and saquinavir was consistently observed in the majority (Fig. 2b). Virus from most subjects demonstrated a greater than 10-fold increase in resistance to lamivudine (72%), whereas 79% of subjects remained susceptible to abacavir, 91% to didanosine, 94% to stavudine, and 70% to zidovudine (Fig. 2c). The numbers of subjects tested for phenotypic resistance to abacavir, amprenavir, and efavirenz were less than those observed for other antiretroviral agents because these antiretroviral agents were not added to the testing panel until Food and Drug Administration approval was obtained.

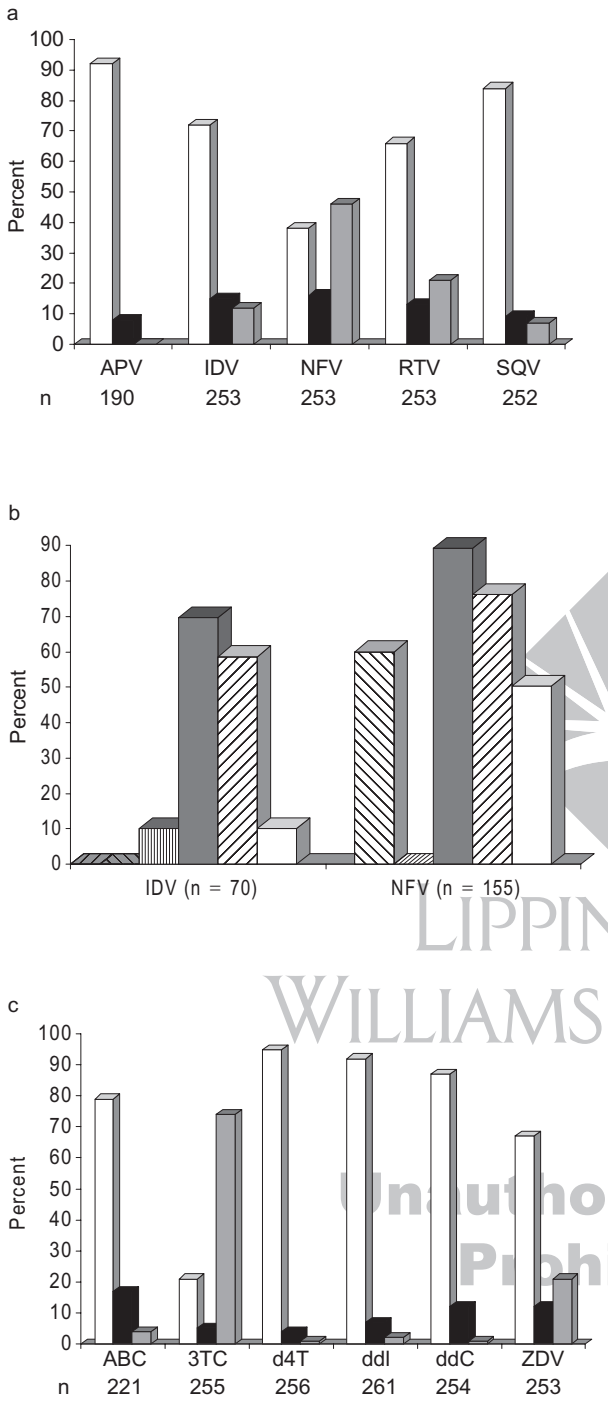

Fig. 2. (a) Phenotypic susceptibility to protease inhibitors at study entry. APV, Amprenavir; IDV, indinavir; NFV, nelfinavir; RTV, ritonavir; SQV, saquinavir. □ Less than fourfold; ■ Four- to 10-fold; ■ More than 10-fold. (b) Distribution of susceptibility to protease inhibitors with greater than fourfold resistance to indinavir and nelfinavir at study entry. ▨ Indinavir (IDV); ▥ nelfinavir (NFV); ■ amprenavir; ▨ saquinavir; □ ritonavir. (c) Phenotypic susceptibility to nucleoside reverse transcriptase inhibitors at study entry. ABC, Abacavir; ddC, zalcitabine; ddI, didanosine; d4T, stavudine; 3TC, lamivudine; ZDV, zidovudine. □ Less than fourfold; ■ Four- to 10-fold; ■ More than 10-fold.

Influence of phenotypic resistance testing results on choice of antiretroviral therapy

Changes in antiretroviral therapy at baseline (the addition or subtraction of antiretroviral agents) between the predicted (week −2 Choice of Therapy Survey) and actual baseline regimens were significantly more frequent in the PRT arm when compared with the SOC arm with respect to overall treatment (76 versus 44%), NRTI (63 versus 34%), PI (55 versus 31%), and NNRTI (46 versus 24%) ($P = 0.001$ for all comparisons). Table 2 shows how phenotype results impacted treatment choices.

At baseline, significantly more subjects in the PRT arm when compared with the SOC arm were treated with two or more 'active' (less than fourfold increase in resistance) antiretroviral agents (92 versus 77%, $P = 0.016$). Similarly, a greater percentage of subjects in the PRT arm compared with the SOC arm were treated with two or more active NRTI (61 versus 40%,

Table 2. Impact of prospective phenotype results on choices of antiretroviral agents[a].

	SOC, no. (% of 112)	PRT, no. (% of 114)
Abacavir		
Chosen and prescribed	29 (26)	16 (14)
Chosen but not prescribed	7 (6)	9 (8)
Not chosen but prescribed	3 (3)	15 (13)
Amprenavir		
Chosen and prescribed	2 (2)	3 (3)
Chosen but not prescribed	2 (2)	1 (< 1)
Not chosen but prescribed	0 (0)	9 (8)
Didanosine		
Chosen and prescribed	41 (37)	35 (31)
Chosen but not prescribed	12 (11)	23 (20)
Not chosen but prescribed	5 (4)	12 (11)
Lamivudine		
Chosen and prescribed	16 (14)	15 (13)
Chosen but not prescribed	3 (3)	10 (9)
Not chosen but prescribed	17 (15)	25 (22)
Nelfinavir		
Chosen and prescribed	9 (8)	2 (2)
Chosen but not prescribed	6 (5)	6 (5)
Not chosen but prescribed	6 (5)	14 (12)
Ritonavir		
Chosen and prescribed	41 (37)	29 (25)
Chosen but not prescribed	17 (15)	34 (30)
Not chosen but prescribed	5 (4)	8 (7)
Saquinavir		
Chosen and prescribed	39 (35)	31 (27)
Chosen but not prescribed	17 (15)	33 (29)
Not chosen but prescribed	2 (2)	10 (9)
Stavudine		
Chosen and prescribed	48 (43)	48 (42)
Chosen but not prescribed	10 (9)	14 (12)
Not chosen but prescribed	11 (10)	27 (24)
NNRTI (efavirenz or nevirapine)		
Chosen and prescribed	51 (46)	25 (22)
Chosen but not prescribed	18 (16)	32 (29)
Not chosen but prescribed	12 (11)	23 (20)

[a]Antiretroviral agents with greater than 5% difference between arms within any category are listed.

$P = 0.005$) and one or more active PI (60 versus 46%, $P = 0.002$). The median number of antiretroviral drugs administered at baseline was 3.4 in both treatment arms.

Virological outcome

In an ITT, M = F analysis, the percentage of subjects in the PRT arm with plasma HIV-1-RNA levels of 400 copies/ml or less at week 16 (46%) was greater than in the SOC arm (34%), but did not reach statistical significance [$P = 0.079$; 95% CI for the difference (PRT minus SOC) −1.0–24.4%]. In an ITTO analysis, significantly more subjects in the PRT arm had plasma HIV-1-RNA levels of 400 copies/ml or less (59 versus 43%, $P = 0.036$; 95% CI for the difference 1.9–30.9%) (Fig. 3b). The percentage of subjects with plasma HIV-1-RNA levels of 400 copies/ml or less was comparable in the two treatment arms from weeks 2 to 12 for both the ITT, M = F and ITTO analyses. In an exploratory analysis, the percentage of subjects with plasma HIV-1-RNA levels of 50 copies/ml or less at week 16 was comparable between the arms in the ITTO analysis (27 versus 28%).

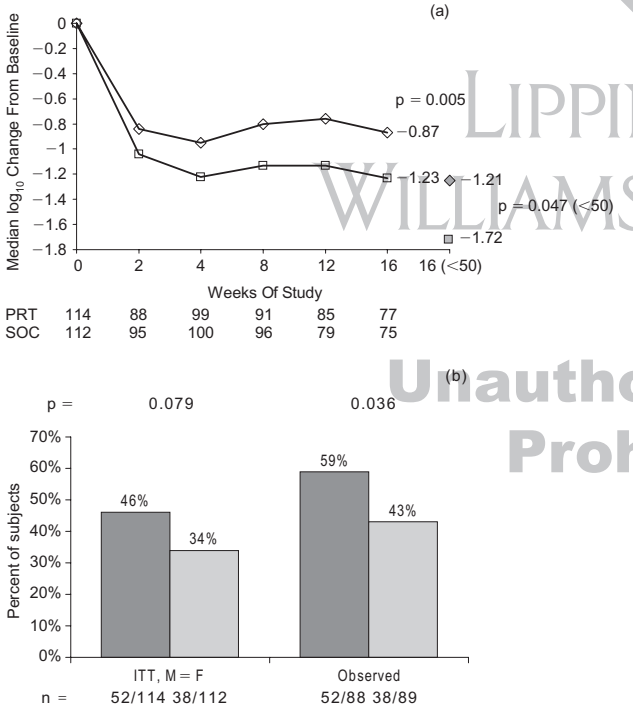

Fig. 3. (a) HIV-1-RNA response (intent-to-treat observed analysis). —◇— Standard of care (SOC; < 400 copies/ml); —□— phenotypic resistance testing (PRT, < 400 copies/ml); —◆— SOC (< 50 copies/ml); —■— PRT (< 50 copies/ml). (b) Proportion of subjects with plasma HIV-1-RNA levels of 400 copies/ml or less at week 16. ITT, M = F, Intent-to-treat, missing equals failure; ■ phenotypic resistance testing; □ standard of care.

An ITTO analysis revealed that the reduction in HIV-1-RNA plasma levels was consistently greater in the PRT arm than in the SOC arm from weeks 2 to 16 (Fig. 3a). At week 16, the decrease from baseline in plasma HIV-1-RNA levels was significantly greater in the PRT arm using assay detection limits of either 400 copies HIV-1-RNA/ml ($P = 0.005$, medians −1.23 versus −0.87 $\log_{10}$ copies/ml; 95% CI for the difference between study arms −0.72 to −0.13) or 50 copies HIV-1-RNA/ml ($P = 0.049$, medians −1.72 versus −1.21 $\log_{10}$ copies/ml; 95% CI for the difference between study arms −0.81 to −0.00). Similarly, an ITTO analysis of the $\log_{10}$ change in HIV-1-RNA levels using AAUCMB to week 16 also revealed a statistically significant difference with a less than 400 copies/ml assay detection limit ($P = 0.012$, medians −0.92 for PRT versus −0.72 for SOC; 95% CI for the difference between study arms −0.47 to −0.06).

Virological outcome by antiretroviral agent and antiretroviral class

An ITTO analysis of virological outcomes stratified by the PI initiated before study entry showed that the percentage of subjects who achieved plasma HIV-1-RNA levels of 400 copies/ml or less with previous exposure to indinavir was 55 and 28% ($P = 0.040$) in the PRT and SOC arms, respectively. The percentage of subjects who achieved plasma HIV-1-RNA levels of 400 copies/ml or less with previous exposure to nelfinavir was 63 and 50% ($P = 0.298$) in the PRT and SOC arms, respectively. Moreover, the median $\log_{10}$ change in HIV-1-RNA levels from baseline to week 16 was −1.20 and −0.34 ($P = 0.009$) in the PRT and SOC arms, respectively, for previous indinavir exposure, and −1.30 and −0.92 ($P = 0.058$) in the PRT and SOC arms, respectively, for previous nelfinavir exposure.

An additional analysis of ITTO data on the effect of adding a NNRTI to the baseline regimen demonstrated that when a NNRTI was not added, 49 and 23% ($P = 0.015$) of subjects achieved plasma HIV-1-RNA levels of 400 copies/ml or less in the PRT and SOC arms, respectively. In this subgroup of subjects not initiating NNRTI therapy, the median $\log_{10}$ change in HIV-1-RNA levels from baseline to week 16 was −1.07 and −0.26 ($P = 0.003$) in the PRT and SOC arms, respectively. When a NNRTI was added to the baseline regimen of NNRTI-naive subjects, 75 and 60% ($P = 0.230$) of subjects achieved plasma HIV-1-RNA levels of 400 copies/ml or less. The median $\log_{10}$ change in HIV-1-RNA levels from baseline to week 16 in this subgroup was −1.38 and −1.03 ($P = 0.056$) in the PRT and SOC arms, respectively.

Immunological outcome

At baseline, the median CD4 cell counts in the SOC and PRT treatment groups were similar (347 and 348

cells/μl, respectively). The median increase in CD4 cell counts from baseline to week 16 was 40 cells/μl for the SOC arm and 27 cells/μl for the PRT arm, and was not statistically different ($P = 0.772$).

Discussion

The results demonstrate that prospective PRT had a significant effect on improving virological response by several measures [absolute and AAUCMB $\log_{10}$ change in viral load from baseline and the proportion of subjects below detection (< 400 HIV-1-RNA copies/ml)]. In addition, physicians prescribed more 'active' drugs in the PRT arm, and the overall use of more active drugs was associated with a significantly better virological response both in terms of a greater reduction in HIV-1-RNA levels and the percentage of subjects attaining HIV-1-RNA plasma levels of less than 400 copies/ml.

The availability of prospective phenotypic test results had a considerable effect on the decision process. Overall, when compared with a hypothetical regimen chosen before baseline, a significantly larger number of changes were made to baseline regimens in the PRT arm compared with the SOC arm. This effect was more pronounced for certain antiretroviral agents (Table 2). For example, more abacavir and stavudine and less ritonavir and NNRTI were initiated as a result of the prospective PRT results.

Additional analyses based on antiretroviral class and individual antiretroviral agents were also conducted. In this mostly NNRTI-naive population, it was important to determine whether simply adding a NNRTI to the baseline regimen could outweigh the benefit of knowing prospectively the antiretroviral agents to which a subject might or might not respond. As expected, subjects who did not initiate a NNRTI had a dramatically improved virological response in the PRT arm compared with the SOC arm. However, in subjects initiating a NNRTI in the PRT arm, the difference in virological response compared with those in the SOC arm was not as marked.

Using a post-hoc analysis, virological response was also stratified by subjects who took either nelfinavir- or indinavir-containing regimens before study entry. A statistically significant improvement in virological response was observed in the PRT arm for those subjects on previous indinavir. However, there was less of an observed difference in virological response between arms for subjects who had previously received nelfinavir, although the trend again favored the PRT arm. This may suggest, at least among subjects with a lack of virological suppression on their first PI-containing regimen, that some initial regimens may result in less complicated resistance profiles, in which clinically guessing the next regimen may be successful.

The VIRA3001 trial was designed to emulate a clinical setting by not blinding the investigators or subjects to the randomization, by permitting subjects to change any part of their regimen at baseline, and by permitting investigators to interpret the PRT test results and 'override' them if desired. This is an important distinction between VIRA3001 and the prospective genotyping trials, in which treatment choices were recommended with the input of either a panel of experts in the GART or a rules-based algorithm in the VIRADAPT trials. The difference in HIV-1-RNA change from baseline between the intervention and control arms reached statistical significance in this trial as well as the prospective VIRADAPT and GART trials. Recent data from a large comparative trial (NARVAL) of phenotyping versus genotyping versus SOC showed no difference in the percentage of subjects (33% for phenotyping, 41% for genotyping, and 34% for SOC) with HIV-1-RNA plasma levels of less than 200 copies/ml at 12 weeks ($P = 0.249$) [21]. A major difference between this trial and the other prospective trials was the significantly greater antiretroviral experience, which may have limited treatment options. An underpowered study, as a result of incomplete enrollment, had similar limitations [22].

The question of whether the use of phenotypic or genotypic testing or both methodologies has greater clinical utility remains unresolved and is being addressed in ongoing clinical trials. PRT may offer some advantages, in that PRT results already take into account the net effect of any and all resistance mutations and their interactions. A limitation to phenotypic resistance testing has been the determination of cut-off values for resistance or susceptibility, which relate to clinical outcome. Historically, cut-off values were based on assay reproducibility and were the same for all antiretroviral agents, which for some antiretroviral agent (e.g. dideoxynucleoside analogues and NNRTI) represented an under- or over-reporting of resistance. Recent work has led to the determination of new drug-specific cut-off values [23,24]. Genotyping has a faster turnaround time, which may offset the interpretation limitations of this technology for some clinicians.

The study was not powered to detect differences in immunological response, and at 16 weeks immunological improvement was marginal and not significantly different between the arms.

It is noteworthy that overall only 54% of subjects (ITTO analysis) achieved plasma HIV-RNA levels of less than 400 copies/ml after 16 weeks of therapy. In the GART and VIRADAPT trials the percentage of

subjects achieving HIV-1-RNA levels of between less than 500 copies/ml and 200 copies/ml of plasma was 46 and 32%, respectively. Both trials enrolled more highly antiretroviral-experienced subjects; 53% of subjects in GART and 46% of subjects in VIRADAPT were enrolled after the failure of their first PI-containing regimen. Although drug resistance accounts for a large proportion of all virological failures, several other causes of virological failure exist that would not be remedied by the use of prospective PRT in treatment decision-making. These include limited intrinsic antiviral potency, inadequate adherence, defective absorption of the antiretroviral drug (i.e. as a result of malabsorption or diarrhea), pharmacokinetic interactions, inadequate activation of the antiretroviral drug (i.e. intracellular phosphorylation), and viral replication at sanctuary sites.

An additional limitation of the trial is the short follow-up period. VIRA3001 was designed as a proof of concept trial in late 1997, at which time it was believed that a 16 week study would be sufficient to demonstrate any clinical value of prospective PRT. In addition, the issues of randomly assigning subjects to potentially suboptimal care, and the increased possibility of a learning effect over time also supported a shorter trial design.

A relatively high number of subjects (17%) discontinued before baseline therapy was initiated. This occurred in the earliest stages of the trial, and may have been caused by the concern involving the 4–5 week wait period on non-suppressive therapy before regimen component changes were permitted. Despite this concern, recently presented data showed that there was no significant difference in either HIV-1-RNA levels or genotypic mutational patterns between samples taken at screening and baseline visits [25]. This suggests that partly suppressive therapy can be continued during the period between taking a sample and receiving resistance test results without deleterious consequences. Although the percentage of subjects below detection was significantly greater in the PRT arm in the ITTO analysis ($P = 0.036$), statistical significance was not achieved in the ITT, M = F analysis ($P = 0.079$). The failure to reach statistical significance in one analysis and not the other was probably caused by the slightly different rates of discontinuation with respect to consent withdrawn and those lost to follow-up. Although exploratory analyses were carried out post-hoc using the viral load assay with a less than 50 copies/ml limit of detection, we did not expect to observe a difference because the study lacked the statistical power for this endpoint as a result of increased assay variability at these low levels. Nonetheless, with 226 subjects initiating 96 different baseline antiretroviral regimens among a group of subjects who had failed only one PI, the difference in virological response observed between the arms was remarkably clear-cut using the standard assay.

Conclusion

In HIV-infected subjects not responding to their first PI-containing HAART regimen, antiretroviral treatment choices guided by PRT were associated with a significantly better virological response than treatment choices guided by SOC. In view of this, PRT treatment guidance may be an important new clinical tool for determining the most appropriate HAART regimen for individuals not responding adequately to therapy.

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Appendix

Members of the VIRA3001 Study Team: Roberto Arduino, MD (Houston, TX, USA), Carol Brosgart, MD, (Berkeley, CA, USA), Stephen Brown, MD (West Hollywood, CA, USA); Calvin Cohen, MD (Brookline, MA, USA), Ann Collier, MD (Seattle, WA, USA); Marcus Conant, MD (San Francisco, CA, USA), Steven Davis, MD (Dallas, TX, USA); Charles Farthing, MD (Los Angeles, CA, USA), Jeffrey Galpin, MD (Tarzana, CA, USA); Jeffrey Goodgame, MD (Altamonte Springs, FL, USA); Howard Grossman, MD (New York, NY, USA); W. Keith Henry, MD (St Paul, MN, USA); Susan Hunt, MD (Pittsburgh, PA, USA); Susan Jacobson, MD (Berkeley, CA, USA); Harold Kessler, MD (Chicago, IL, USA); Martin Markowitz, MD (New York, NY, USA); Douglas Mayers, MD (Detroit, MI, USA); Jeffrey Nadler, MD (Tampa, FL, USA), Michael Saag, MD (Birmingham, AL, USA); Shannon Schrader, MD (Houston, TX, USA); Michael Sension, MD (Fort Lauderdale, FL, USA), Allan Stein, DO (Coral Gables, FL, USA); Richard Stryker, MD (Beverly Hills, CA, USA); Melanie Thompson, MD (Atlanta, GA, USA); Gabriel Torres, MD (New York, NY, USA); Deborah Copeland (Glaxo Wellcome Inc., Research Triangle Park, NC).

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