A randomized study of antiretroviral management based on plasma genotypic antiretroviral resistance testing in patients failing therapy

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Objective: To determine the short-term effects of using genotypic antiretroviral resistance testing (GART) with expert advice in the management of patients failing on a protease inhibitor and two nucleoside reverse transcriptase inhibitors.

Design: Prospective randomized controlled trial.

Setting: Multicenter community-based clinical trials network.

Patients: One-hundred and fifty-three HIV-infected adults with a threefold or greater rise in plasma HIV-1 RNA on at least 16 weeks of combination antiretroviral therapy.

Interventions: Randomization was either to a GART group, where genotype interpretation and suggested regimens were provided to clinicians, or to a no-GART group, where treatment choices were made without such input.

Main outcomes measures: Plasma HIV-1 RNA levels and CD4 cell counts were measured at 4, 8, and 12 weeks following randomization. The primary endpoint was change in HIV-1 RNA levels from baseline to the average of the 4 and 8 week levels.

Results: The average baseline CD4 cell count was 230×10^6 cells/l and the median HIV-1 RNA was 28 085 copies/ml. At entry, 82 patients were failing on regimens containing indinavir, 51 on nelfinavir, 11 on ritonavir, and nine on saquinavir. HIV-1

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Disclosure: Some years ago, one of the study investigators (Thomas C. Merigan, M.D.) filed patents on the use of drug target gene sequencing testing to direct HIV therapy because his group observed immunologic and virologic deterioration following the development of drug resistance mutation during monotherapy. Several diagnostic firms are paying royalties to Stanford on these patents, which are distributed according to NIH guidelines. Although he was the senior virologist and involved in drug selection advice, he has not been directly involved in data management or statistics of this study.

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RNA, averaged at 4 and 8 weeks, decreased by 1.19 $\log_{10}$ for the 78 GART patients and -0.61 $\log_{10}$ for the 75 no-GART patients (treatment difference: −0.53 log, 95% confidence interval, −0.77 to −0.29; $P = 0.00001$). Overall, the best virologic responses occurred in patients who received three or more drugs to which their HIV-1 appeared to be susceptible.

Conclusion: In patients failing triple drug therapy, GART with expert advice was superior to no-GART as measured by short-term viral load responses.

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Introduction

The development of HIV-1 resistance to antiretroviral drugs is considered to be a major contributing factor to the loss of plasma HIV-1 RNA suppression (virologic failure) in patients taking highly active antiretroviral therapy (HAART) [1]. Resistance to nucleoside reverse transcriptase inhibitors (NRTI) has been associated with a more rapid decline in CD4 cell counts and an increased risk of disease progression and death [2–5]; resistance to protease inhibitors (PI) has been associated with a poor short-term virologic response [6–9]. HIV-1 resistance mutations have been characterized for all of the commercially available antiretroviral agents and are associated with reduced *in vitro* phenotypic sensitivity to these compounds [10]. The application of molecular diagnostic technologies has resulted in rapid assays for HIV drug resistance which have been used to assess the frequency and clinical impact of drug resistant HIV-1 in different populations [11–18].

Although drug resistance mutations identified at the time of treatment failure have been associated with a poor response to subsequent antiretroviral drug combinations, there is limited evidence that treatment guided by knowledge of resistance mutations can result in a more effective salvage regimen than careful consideration of the patient's treatment history [19]. We conducted a short-term, randomized trial to assess the utility of plasma genotypic antiretroviral resistance testing (GART) along with expert advice in the management of patients failing on a PI-containing antiretroviral regimen.

Materials and methods

Study population

Patients were enrolled from 14 units of the Terry Beirn Community Programs for Clinical Research on AIDS (CPCRA) and the Walter Reed Army Medical Center. The CPCRA is a U.S. clinical trials group sponsored by the National Institute of Allergy and Infectious Diseases (NIAID). Following local institutional review board approval at study sites, patients gave written informed consent for participation in the study.

Patients with HIV-1 infection who were at least 13 years of age were eligible if they were failing virologically on a combination antiretroviral regimen containing a single PI (indinavir, nelfinavir, saquinavir, or ritonavir) and two NRTI. For virologic failure, four conditions had to be met: (i) patient taking a current triple drug regimen for at least 16 weeks; (ii) a locally determined screening HIV-1 RNA level $> 20\,000$ copies/ml by the Roche Amplicor HIV-1 assay or $> 10\,000$ copies/ml by the Chiron branched chain (bDNA) assay within 6 weeks prior to a required baseline visit; (iii) documentation that the screening HIV-1 RNA level was threefold greater than the nadir HIV-1 RNA level while on the triple drug regimen, or that the nadir was < 500 copies/ml; and (iv) a centrally determined HIV-1 RNA level > 5000 copies/ml by the Chiron 2.0 bDNA assay using plasma collected at the baseline visit. This definition for virologic failure was used because a threefold rise in the HIV-1 RNA level represents a significant change beyond the expected variability of these assays [20]. In addition, because the Roche Amplicor PCR assay has been shown to be twofold higher on average than the Chiron 2.0 bDNA assay in use at the time of the study, different minimum HIV-1 RNA levels were used for eligibility [21]. Patients were also required to have at least 12 months of cumulative antiretroviral therapy and a screening CD4 cell count between 50 and 500×10^6 cells/l. Patients were excluded if they used an antiretroviral drug other than those in the qualifying regimen in the 16 weeks prior to the baseline visit or if they had access to previous genotypic or phenotypic test results.

Study procedures

Patients were screened for eligibility at participating sites through chart reviews and patient interviews. Patients meeting eligibility criteria had a baseline visit

while taking their qualifying regimens; at this visit, plasma for HIV-1 RNA and GART was obtained, and CD4 cell count determined. HIV-1 RNA testing was performed by a single laboratory using the Chiron 2.0 bDNA assay. GART was performed by one of three laboratories, the Coriell Institute Laboratory, Stanford Center for AIDS Research Virology Laboratory, and SRA Technologies, Inc., using reverse transcription–PCR of patient plasma derived viral RNA [PCR product encompassed the entire protease gene and the first 250 amino acids of the reverse transcriptase (RT) gene] and standard dideoxyterminator sequencing (ABI 377 sequencers) as described by Winters *et al.* [22]. A mixture of mutant and wild-type nucleotides at a particular position was called when the sequencing analysis software revealed that the peak height of the minor peak was at least 30% of the total signal in both sequencing directions. Software was used to deduce the amino acid sequence from the nucleotide sequence and mixtures of wild-type and mutant amino acids were considered to be mutant for resistance interpretation. Erroneous results due to laboratory contamination were ruled out by creating a phylogenetic tree of each laboratory's HIV-1 sequence results [23].

GART results were transmitted to the Statistical Center at the University of Minnesota. Three protocol virologists (J.D.B., D.L.M., T.C.M.) independently reviewed the mutations identified, the patient's treatment history, and possible treatment contraindications as reported by the site clinician; characterized the virus as sensitive, possibly resistant, or resistant to each antiretroviral drug; and suggested up to four treatment regimens. The presumed drug susceptibility of individuals' viral quasispecies to specific antiretroviral agents was determined by evaluation of patterns of genotypic mutations: sensitive (absence of resistance mutations associated with a particular drug); possibly resistant (presence of 'minor' or 'secondary' associated resistance mutations or possible cross-resistance for a particular drug); and resistant ('major' mutations or patterns of mutations associated with phenotypic resistance to a specific agent). A written algorithm was used by the study virologists for interpretation of resistance mutations (see Appendix). The factors considered by the protocol virologists in selecting salvage regimens for each patient included: (i) interpretation of the genotypic resistance mutations present, (ii) potential cross-resistance between antiretroviral agents, (iii) prior drug exposure, (iv) available drug options, (v) limited available data from clinical studies of response to salvage treatments in patients failing with drug resistance, and (vi) knowledge of pharmacologic and synergistic interactions which may be used to optimize drug activity of antiretroviral agents when used in combination. The rationale for including treatment suggestions with the genotype result was to assist study clinicians in making treatment decisions based on the information provided by this new assay. Study clinicians were not required by the protocol to follow these treatment suggestions.

A virologists' teleconference was conducted to reach consensus on the contents of the GART report for each patient. The GART report was prepared by the virologists without knowledge of the patient's clinical site or the group to which the patient would be randomized; and it contained the genotypic mutations identified, an interpretation with drug susceptibilities, and a set of treatment suggestions. On average, teleconferences occurred 21 days (range, 8–41 days) following the baseline visit. The protocol stipulated that there would be no contact between study sites and the virologists concerning the interpretation of the report and the treatment suggestions.

After the GART report was prepared, the Statistical Center notified sites that the patient could be randomized. Prior to randomization, the site clinician was asked to specify a proposed treatment regimen that he or she would prescribe if the patient were randomized to the no-GART group. Patients were randomly assigned in a 1 : 1 ratio to the GART group or the no-GART group; randomization was stratified according to CD4 cell count ($50–199 \times 10^6$ cells/l or $200–500 \times 10^6$ cells/l) and PI in the failing regimen (indinavir, nelfinavir, ritonavir, or saquinavir) (eight strata total). Randomization schedules for each stratum were prepared using permuted blocks. At the randomization visit, the clinician was informed of the patient's randomization assignment and plasma HIV-1 RNA level and CD4 cell count were measured.

Participating clinicians were provided regular updates of current consensus treatment guidelines, which included expert recommendations for changing therapy based on prior treatment history [24–26]. The protocol permitted only licensed antiretroviral drugs (including hydroxyurea) to be prescribed after randomization in both treatment groups. For patients randomly assigned to the no-GART group, the treatment regimen proposed prior to randomization was prescribed by the site clinician. For patients assigned to the GART group, the GART report was transmitted by the Statistical Center to the clinical site immediately following randomization and was available to the site clinician for use in determining the patient's treatment regimen. Thus, the 'GART intervention' investigated in this study consisted of the GART report with its three components: the mutations identified, the interpretation of drug susceptibilities, and the treatment suggestions. The study was not designed to evaluate the three components separately.

Patients were seen at 4, 8, and 12 weeks following randomization. At each follow-up visit, changes in antiretroviral treatment were recorded, adherence to

treatment was assessed (by number of missed doses), and blood was obtained for CD4 cell counts and plasma HIV-1 RNA levels by the Chiron 2.0 bDNA assay.

The primary endpoint of the study was change in plasma HIV-1 RNA ($\log_{10}$) from baseline (the geometric mean of measurements from the baseline and randomization visits) to the average (geometric mean) of the measurements determined at 4 and 8 weeks post-randomization. The rationale for the primary endpoint was that these measurements would reflect time on the regimen prescribed following randomization, as patients were asked to remain on those medications (barring toxicity) until the 8-week visit, at which time a change was allowed if the 4-week viral load measurement was deemed suboptimal by the treating clinician. Power was estimated at 0.90 to detect a 0.26 $\log_{10}$ difference between treatment groups if 160 patients were randomized (80 per group). Secondary endpoints included change in plasma HIV-1 RNA through 12 weeks, and change in CD4 cell count through the average of 4 and 8 weeks and through 12 weeks.

Statistical analysis

Unless otherwise specified, analyses were performed according to intention-to-treat. Plasma HIV-1 RNA values were $\log_{10}$ transformed before analysis, imputing 499 copies/ml for values below the level of detection of the assay (500 copies/ml). For the analyses of plasma HIV-1 RNA and CD4 cell count, analysis of variance was used with strata corresponding to the PI in the qualifying baseline regimen and with the baseline value as a covariate. For both plasma HIV-1 RNA and CD4 cell count averages, a single follow-up measurement was used, if available, when both measurements were not available. To compare percentages between treatment groups, the Mantel–Haenszel procedure was used to combine binary data over strata.

Table 1. Patient baseline characteristics.

	GART	No-GART	Total
	(n = 78)	(n = 75)	(n = 153)
Mean age (years)	41.1	40.7	40.9
Non-White race (%)	64.1	60.0	62.1
Female (%)	12.8	12.0	12.4
Previous opportunistic event (%)	51.3	45.3	48.4
Baseline protease inhibitor (%)			
Indinavir	53.8	53.3	53.6
Nelfinavir	33.3	33.3	33.3
Ritonavir	6.4	8.0	7.2
Saquinavir	6.4	5.3	5.9
Baseline nucleoside analog combination (%)			
Zidovudine and lamivudine	44.9	44.0	44.4
Stavudine and lamivudine	41.0	38.7	39.9
Other combination	14.1	17.3	15.7
Mean time on baseline regimen (months)	12.6	13.1	12.8
Failing on first protease (%)	52.6	44.0	48.4
Mean CD4 cell count[a] ($\times 10^6$ cells/l)	230.5	228.6	229.6
(range)	(40–561)	(47–505)	(40–561)
Mean $\log_{10}$ HIV RNA[a]	4.47	4.37	4.42
(SD)	(0.46)	(0.44)	(0.45)
Any major reverse transcriptase mutation (%)	93.6	93.3	93.5
65R, 69D, or 74V	11.5	10.7	11.1
75T	1.3	0.0	0.7
151M	2.6	2.7	2.6
184V	84.6	78.7	81.7
215F/Y	55.1	68.0	61.4
103N, 106A, 181C/I, or 188C	2.6	5.3	3.9
Any major protease mutation (%)	73.1	77.3	75.2
30N	14.1	13.3	13.7
46I/L, 82A/F/T, or 84V	50.5	52.0	51.0
48V or 90M	26.9	38.7	32.7

[a]Mean of measurements from baseline and randomization visits.

Results

Baseline characteristics

Between July 1997 and December 1998, 153 patients were randomized, 78 to the GART group and 75 to the no-GART group. The baseline characteristics of patients in the two treatment groups were similar (Table 1). The mean CD4 cell count was 230×10^6 cells/l (range, $40–561 \times 10^6$ cells/l); the median plasma HIV RNA was 28 085 copies/ml (range, 3318–292 100 copies/ml). Overall mean values for the baseline and randomization visits were similar, suggesting that the patient's clinical status remained stable between these study visits; the baseline visit mean plasma HIV-1 RNA was 4.45 $\log_{10}$ and mean CD4 cell count was 230.7×10^6 cells/l while the randomization visit mean plasma HIV-1 RNA was 4.39 $\log_{10}$ and mean CD4 cell count was 229.1×10^6 cells/l. Forty-four percent of patients in the no-GART group were failing on their first PI compared with 52.6% in the GART group, but this difference was not statistically significant ($P = 0.27$ after adjustment for stratum). Ten percent of patients had prior exposure to a non-NRTI (NNRTI). The frequency of major RT and protease resistance mutations identified by GART analysis was similar for both groups. Overall, 73% had evidence of at least one major RT mutation and at least one major protease resistance mutation (GART group 72%, no-GART group 75%). Twenty percent had at least one major RT mutation without a major protease mutation, 2% had at least one major protease mutation without a major RT mutation, and 5% had no major mutations. Twenty-seven percent of patients had viral strains resistant to all three drugs in the qualifying regimen,

and 34% had viral strains resistant to one NRTI and the PI in the qualifying regimen.

Treatment regimens proposed by site clinicians and study virologists

Prior to randomization, site clinicians proposed modification of the PI in the qualifying regimen for 98% of patients and modification of the entire triple drug regimen for 61% of patients. For most patients, three or fewer (52%) or four (39%) drugs were proposed (Table 2). The most common regimens proposed by the site clinicians consisted of one or two PI plus one or more NRTI (47%) or one or two PI plus an NNRTI plus one or more NRTI (44%), with or without hydroxyurea. In contrast, study virologists recommended four (38%) or five or more (56%) drugs for most patients. For 88% of patients the study virologists recommended one or two PI plus an NNRTI plus one or more NRTI, with or without hydroxyurea. The site clinicians' proposed regimens agreed exactly with one of the virologists' treatment suggestions for only four patients (3%).

Treatment regimens prescribed following randomization

For 65 of the 78 patients in the GART group (83%), review of the GART report led to a change in the site clinicians' planned treatment regimen. One of the treatment suggestions was prescribed for 42 of the 78 patients in the GART group (54%). An equivalent regimen (an NNRTI substitution for two patients and ritonavir instead of indinavir for one patient) was used for another three patients (4%). For the remaining 33 patients (42%), the site clinician prescribed something different from all of the virologists' treatment suggestions. Reasons for not following one of the treatment recommendations included too many drugs (seven patients), patient choice/refusal (eight patients), saving NNRTI for future therapy (five patients), concerns about potential drug toxicity (five patients), started part of regimen and might add remainder at a later date (five patients), and other (three patients). Eleven of the 33 patients not using a recommendation were prescribed a regimen that contained all but one of the drugs in one of the treatment recommendations. Fifteen of the remaining 22 patients who did not follow one of the recommendations were prescribed fewer drugs than in any of the recommendations.

The regimens ultimately used for the two groups differed in the number of drugs (74% of patients assigned GART were prescribed four or more drugs versus 45% of patients assigned no-GART; $P = 0.001$); the use of an NNRTI (77% versus 55%; $P = 0.003$); and the use of hydroxyurea (42% versus 12%; $P = 0.001$) as shown in the last two columns of Table 2.

Patients in the GART group were prescribed more drugs with susceptibilities considered sensitive or only possibly resistant (referred to as active drugs). Hydroxyurea was counted as one active drug and was usually prescribed with didanosine. Eighty-six percent of pa-

Table 2. Antiretroviral regimens proposed by site clinicians, suggested by virologists, and prescribed after randomization in the GART and no-GART groups.

	Regimens proposed prior to randomization (%)		Regimens prescribed after randomization (%)	
	Site clinician (n = 153)	Virologists[a] (n = 153)	GART (n = 78)	No-GART (n =75)
Number of drugs				
Three or fewer	52.3	5.9	25.6	54.7
Four	39.2	37.9	44.9	38.7
Five or more	8.5	56.2	29.5	6.7
Number of new drugs[b]				
Two or fewer	41.2	26.3	46.2	44.0
Three	42.5	32.2	21.8	41.3
Four or more	16.3	41.4	32.1	14.7
Specific combinations[c]				
Nucleoside analog(s), non-nucleoside analog, and protease inhibitor(s)	43.8	87.6	62.8	48.0
One protease inhibitor	31.4	31.4	26.9	38.7
Two protease inhibitors	12.4	56.2	35.9	9.3
Nucleoside analog(s) and protease inhibitor(s)	47.1	5.2	21.8	44.0
One protease inhibitor	26.1	2.6	10.3	26.7
Two protease inhibitors	20.9	2.6	11.5	17.3
Nucleoside analog(s) and non-nucleoside analog	3.3	6.5	9.0	2.7
Other regimens	5.9	0.7	6.4	5.3
Specific classes				
Nucleoside analog(s)	94.1	99.3	93.6	94.7
Protease inhibitor(s)	96.7	93.5	89.7	97.3
Non-nucleoside analog	52.3	94.8	76.9	54.7
Hydroxyurea	15.7	62.7	42.3	12.0

[a]From first suggested treatment regimen. [b]Drugs to which the patient was naive; includes hydroxyurea. [c]With or without hydroxyurea.

tients assigned to the GART group were prescribed a regimen with at least three active drugs compared with 44% of patients assigned to the no-GART group ($P < 0.001$). Irrespective of the total number of drugs prescribed, proportionately more patients in the GART group received three or more active drugs. For instance, among patients prescribed a three-drug regimen, 67% of patients in the GART group and 30% of patients in the no-GART group received three active drugs. For patients given a four-drug regimen, 91% of patients assigned GART and 62% of patients assigned no-GART received at least three active drugs. Among those given five drugs, 100% of patients assigned GART and 60% assigned no-GART received at least three active drugs.

Follow-up and treatment changes

Of the 153 patients randomized, 4, 8, and 12 week HIV-1 RNA measurements are available for 150 (98%), 148 (97%), and 148 (97%) respectively. One patient, in the GART group, had missing HIV-1 RNA levels at both 4 and 8 weeks. This patient is excluded from the primary endpoint analysis. Eighteen patients in the GART group and 15 patients in the no-GART group discontinued at least one drug in their antiretroviral regimen prior to the 12-week visit. Discontinuation due to drug toxicity occurred in 11 patients in the GART group and eight patients in the no-GART group. One patient in each treatment group died before to the 12-week visit.

HIV-1 RNA levels

Following randomization and change in therapy, viral load levels declined in both treatment groups (Table 3). For the primary endpoint based on the average of 4 and 8 week levels, the changes in viral load averaged $-1.19\ \log_{10}$ in the GART group and $-0.61\ \log_{10}$ in the no-GART group. The average treatment difference for the primary endpoint favored the GART group by $-0.53\ \log_{10}$ [95% confidence interval (CI), -0.77 to -0.29; $P = 0.00001$]. The average differences between treatment groups were similar throughout follow-up; 4, 8, and 12 week treatment differences were -0.46 ($P = 0.0003$), -0.58 ($P = 0.001$), and -0.44 ($P = 0.003$) respectively ($P = 0.22$ for treatment group by follow-up visit interaction). The percentage of patients with HIV-1 RNA values < 500 copies/ml (the lower level of detection of the assay) at 4, 8, and 12 weeks was 45% versus 23% ($P = 0.004$), 55% versus 25% ($P = 0.0001$), and 34% versus 22% ($P = 0.10$), for the GART versus no-GART groups respectively.

CD4 cell count

For the GART group, CD4 cell count increased 23×10^6 cells/l from baseline to the average of 4 and 8 weeks; for the no-GART group, the corresponding increase was 22×10^6 cells/l ($P = 0.97$ for treatment difference). Through 12 weeks, the average increases

Table 3. HIV-RNA changes by treatment group.

	GART				No-GART				
	n	Median HIV RNA (copies/ml)	Mean change from baseline ($\log_{10}$) (SE)	< 500 copies (%)	n	Median HIV RNA (copies/ml)	Mean change from baseline ($\log_{10}$) (SE)	< 500 copies (%)	*P* for treatment difference in mean change[a]
Baseline	78	28 785		0.0	75	25 860		0.0	
Week 4	77	569	−1.26 (0.09)	45.5	73	3112	−0.75 (0.09)	23.3	0.0003
Week 8	75	499	−1.12 (0.11)	54.7	73	8598	−0.52 (0.09)	24.7	0.0001
Week 12	76	1543	−0.94 (0.11)	34.2	72	10 197	−0.47 (0.09)	22.2	0.003
Week 4 & 8	77	811	−1.19 (0.09)	36.4	75	8286	−0.61 (0.09)	18.7	0.00001

[a]Adjusted for baseline HIV-RNA and qualifying regimen.

from baseline were 25×10^6 cells/l and 18×10^6 cells/l for the GART and no-GART groups ($P = 0.67$).

Subgroup analyses according to pre-randomization characteristics

The effect of GART on change in viral load was consistent across baseline subgroups as defined by the qualifying PI, CD4 cell counts, plasma HIV-1 RNA levels, the number of prior PI received, and genotypic profile (Fig. 1). Among the subgroups considered, the favorable effect of GART compared with no-GART ranged from -0.3 to -0.8 $\log_{10}$. Although power is low for detecting subgroup differences in the treatment effect, for nearly every subgroup (which included by definition fewer patients than in the overall results), the virologic response was significantly greater in the GART compared with the no-GART group.

Although a significantly greater number of patients in the GART group compared with the no-GART group used an NNRTI, in and of itself, greater use of this class of drugs in the GART group did not explain the overall viral load difference between treatment groups. Among the 80 patients (38 GART and 42 no-GART) for whom the site clinicians pre-specified that they would prescribe an NNRTI (93% of whom had not previously used an NNRTI), the viral load change was -1.38 $\log_{10}$ copies/ml for patients subsequently assigned GART and -0.63 $\log_{10}$ copies/ml for patients assigned no-GART ($P = 0.0002$). For this subgroup (which is based on a pre-randomization characteristic and for which the treatment comparison is protected by randomization), patients in the no-GART group were given an NNRTI according to protocol (in the absence of the consensus virology report, the clinician prescribed the pre-specified regimen), and 95% of the GART group received an NNRTI. Thus, even though nearly all patients in both treatment groups received an NNRTI, the reduction in viral load through the average of 4 and 8 weeks was 0.71 $\log_{10}$ greater for the 38 patients in the GART group. For 82% of these 38 patients, the consensus report resulted in the clinicians changing the planned regimen. As a consequence, 87% of the 38 patients in the GART group and only 50% of the 42 patients in the no-GART group were prescribed three or more active drugs.

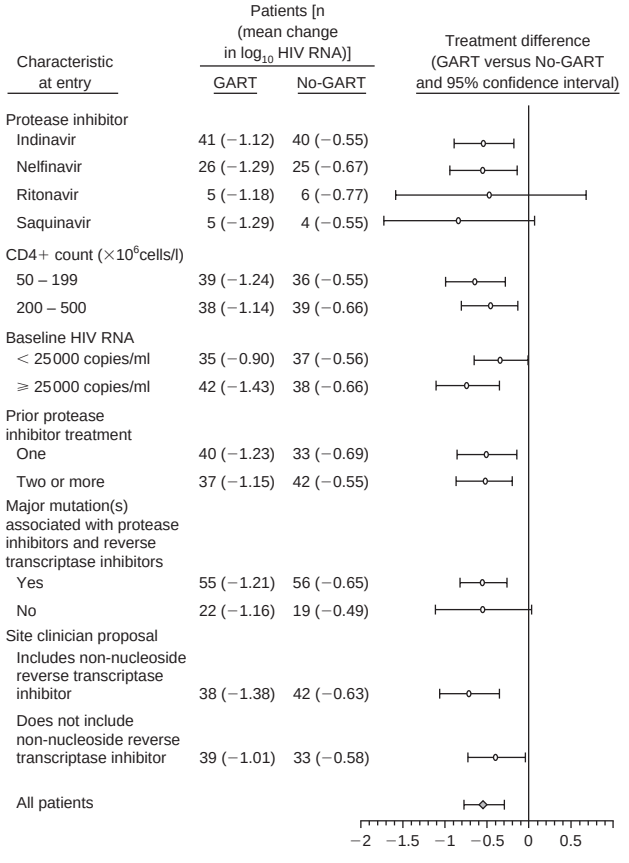

Fig. 1. Treatment differences by selected baseline-defined subgroups. The change in HIV RNA level through the average of 4 and 8 weeks for each treatment group and the 95% confidence interval for the treatment difference are shown for *a priori* defined subgroups.

Subgroup analyses according to post-randomization characteristics

Analyses were carried out to assess which aspects of the treatment regimen prescribed after randomization were associated with reductions in HIV-1 RNA. The viral load response (average of 4 and 8 weeks) in both treatment groups was associated with the number of active drugs prescribed. For both groups combined, the $\log_{10}$ HIV-1 RNA change was -0.10 for those given one or fewer active drugs, -0.59 for those given two active drugs, -1.04 for those given three active drugs; and -1.25 for those given four or more active drugs (Fig. 2). Based on a linear regression analysis including patients in both treatment groups with predictors corresponding to baseline HIV-1 RNA, number of active drugs prescribed, and number of inactive drugs prescribed (i.e. a drug for which the patient is considered to have resistant virus), each additional active drug prescribed was associated with a -0.37 $\log_{10}$ viral load change (95% CI, -0.51 to -0.22; $P = 0.0001$), and each additional inactive drug was associated with a -0.17 $\log_{10}$ viral load change (95% CI, -0.34 to $+0.01$; $P = 0.06$).

As noted previously, the number of active drugs prescribed was significantly greater in the GART group compared with the no-GART group; this difference explains part, but not all of the treatment difference between groups. In an analysis that adjusted for the number of active drugs and inactive drugs prescribed, the estimated $\log_{10}$ plasma HIV-1 RNA treatment difference for the primary endpoint was reduced from -0.53 ($P = 0.0001$) to -0.33 ($P = 0.01$). In other

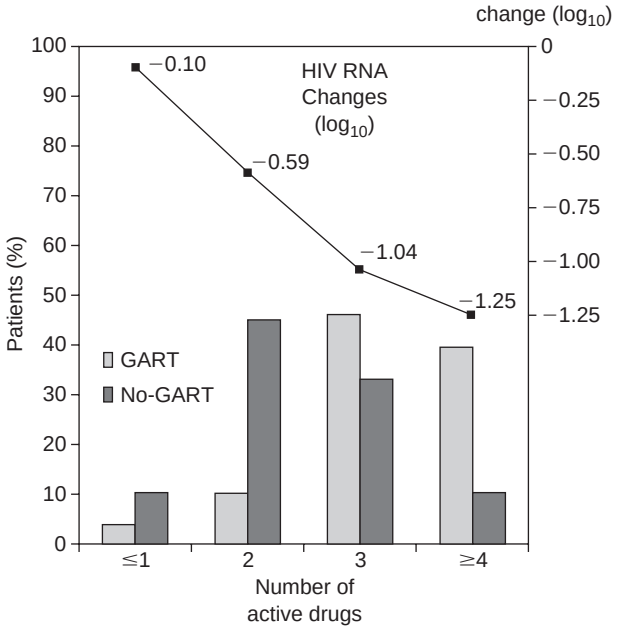

Fig. 2. HIV RNA changes by number of active drugs prescribed. The upper line represents change in HIV RNA through the average of 4 and 8 weeks for both treatment groups combined according to the number of active drugs prescribed in the salvage regimen. The bars represent the percentage of patients in each treatment group that received the specified number of active drugs.

analyses adjusting for the total number of drugs used, classes of drugs used, and specific drug regimens, further reduction of the estimated treatment difference was not observed.

In another subgroup analysis, the participating clinical units were classified into three groups: (i) the four units in which $> 80\%$ of patients assigned to the GART group were prescribed one of the virologists' suggested regimens ($n = 50$); (ii) the three units in which 60–80% of GART patients were prescribed one of the suggested regimens ($n = 46$); and (iii) the eight units in which $< 60\%$ were prescribed one of the suggested regimens ($n = 57$). While this subgroup was defined based on a post-randomization characteristic, by classifying on clinical unit instead of patients, the randomized comparison within subgroup was maintained. Within these three groups of units the viral load response of patients assigned the GART and no-GART group were compared. The plasma HIV-1 RNA treatment difference (GART versus no-GART) was -0.81 $\log_{10}$ (95% CI, -1.26 to -0.36; $P = 0.001$) for the first group of units, -0.72 (95% CI, -1.11 to -0.33; $P = 0.001$) for the second group of units, and -0.28 (95% CI, -0.71 to 0.15; $P = 0.21$) for the third group of units. For each 10% increase in unit adherence to one of the virologists' suggestions for patients assigned GART, the treatment difference favoring GART increased by -0.05 $\log_{10}$ (P corresponding to the test for interaction between the treatment difference and percentage using one of the virologists' recommendations is 0.19).

Discussion

Drug resistant HIV-1 is a growing clinical problem. With the expanded use of antiretroviral drugs, many patients are currently experiencing virologic failure on combination drug regimens. Potential reasons for virologic failure include interruption or discontinuation of medications, failure to absorb the antiretroviral drugs, accelerated drug metabolism, and development of drug resistance. The development of cross-resistance within each class of antiretroviral drugs can significantly limit the number of effective drugs available to manage patients failing on these combination drug regimens. The reported response rates to salvage therapy in patients failing on antiretroviral drug regimens containing a PI have been quite variable but generally disappointing [27–30]. Methods to optimize therapy with available antiretroviral drugs as well as newer antiretroviral agents are needed to improve responses in patients failing HAART.

The results of our study indicate that management based on GART with expert advice results in a greater reduction in viral load over a 12-week follow-up period than management without GART. The impact of GART on short-term virologic response was similar for patients failing on their first PI and for those who previously had taken other PI treatment. More generally, the beneficial effect of using GART on short-term virologic response was evident across a number of subgroups based on pre-randomization characteristics. Both treatment groups experienced a modest rise in CD4 cell counts without a significant difference observed between groups. It is possible that longer follow-up would be required to observe a difference in CD4 cell responses or that the greater use of hydroxyurea in the GART group could have blunted the CD4 cell response.

Post hoc analyses, based on factors defined post-randomization, indicate that the treatment difference can be attributed in part to the greater number of active drugs that patients in the GART group received as compared with the no-GART group. Other factors such as the total number of drugs used, classes of drugs used, and specific drug regimens did not appear to offer additional explanation for the treatment difference observed. It is possible that there were additional effects such as drug synergy or favorable pharmacokinetic interactions with the combinations of agents used in the GART group. Irrespective of whether three, four,

or five or more drugs were prescribed, patients in the GART group were more likely to receive three active drugs. The better response in the GART group suggests that, similar to initial therapy in HIV-infected patients, effective salvage therapy may require at least three drugs to which a patient's virus remains susceptible.

Further evidence for the need for multiple active drugs in a salvage regimen is provided by the large subgroup of patients for whom site clinicians indicated, prior to randomization, that they planned to use an NNRTI based on treatment history. In this subgroup, for whom the addition of the NNRTI represented a new active drug, the viral load reduction was more than double in the GART (-1.38 $\log_{10}$ copies/ml) compared with the No-GART group (-0.63 $\log_{10}$ copies/ml), reflecting more optimal choices of antiretroviral agents (either number of active drugs or specific drugs) that were prescribed in combination with the NNRTI.

The results of this study are consistent with another recently conducted trial (VIRADAPT Study) [19]. In that randomized trial, 108 patients failing on combination drug regimens that included a PI were randomized to GART versus best clinical judgment. The VIRADAPT trial allowed the physicians to obtain more than one GART test, if needed. After 3 months, patients receiving GART had a -1.04 log copies/ml change in plasma HIV-1 RNA compared with -0.46 log copies/ml in the standard of care arm ($P = 0.01$). Twenty-nine percent of patients in the GART treatment group had plasma HIV-1 RNA values < 200 copies/ml compared with 14% of patients in the standard of care arm ($P = 0.02$). The beneficial effect persisted through 6 months. In the CPCRA GART Study, comparable improvements in short-term viral load reduction were found for the GART compared with the no-GART group. Despite the improved management of antiretroviral medications with GART in both trials, less than half of patients in either trial had undetectable plasma virus by conventional assays after 3 months of therapy.

The components of the intervention for the GART group included both resistance testing with expert interpretation and suggested treatment regimens. Given this study design, we cannot determine the relative contributions of the resistance assay and the expert advice. However, the expert advice was based primarily on the HIV-1 genotype and resulted in the GART group receiving more active drugs per number of drugs prescribed, suggesting that the genotype result impacted patient outcome. There was a trend (although it was not significant) that the treatment effect was greatest among units that more often followed suggested treatment regimens. This suggests that the overall treatment effect was due to both the GART result and the expert advice.

One limitation of this study is the short follow-up period. In both treatment groups, the average viral load began to increase after 4 weeks of follow-up. The longer-term durability of viral load suppression and clinical outcome in patients whose salvage regimens are based on GART remains to be demonstrated. Ongoing studies in the USA and Europe, examining the utility of HIV resistance testing, should provide more definitive data in the future. Another limitation of the study is that the majority of patients enrolled were naive to NNRTI. These findings may not be generalizable to patients failing on NNRTI-containing regimens or for whom no NNRTI treatment option is available.

Given the number of drugs available for use and their cross-resistance patterns, changes in therapy must be carefully considered. The optimum time to make a change in antiretroviral therapy when virologic failure occurs has not yet been determined. It is not clear whether early modification of failing drug regimens based on GART will lead to early exhaustion of remaining antiretroviral options or will maximize the efficiency of these choices over the longer term for individual HIV-infected patients. The results of this short-term study indicate that GART with expert advice can be helpful in choosing an effective regimen for patients failing on combination antiretroviral drug regimens.

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Appendix

Algorithm for interpretation of resistance mutations

Reverse Transcriptase Mutation	Expected Drug Resistance
75T	stavudine
215F/Y ± (41L, 67N, 70R, 210W, 219E/Q)	zidovudine
74V or 65R or 69D	didanosine, zalcitabine
184V	lamivudine possible: didanosine, zalcitabine
215F/Y + [74V or 65R or 69D]	zidovudine, didanosine, zalcitabine
151M ± (62V, 75I, 77L, 116Y)	zidovudine, didanosine, zalcitabine, stavudine possible lamivudine
103N or 106A or 181C/I or 188C	non-nucleoside reverse transcriptase inhibitors
Protease Mutation	**Expected Drug Resistance**
90M or 48V ± (63P, 71T/V)	saquinavir possible nelfinavir
30N ± (36I/L, 46I/L, 71T/V, 77I, 88D)	nelfinavir
90M + 48V	saquinavir possible: ritonavir, indinavir, nelfinavir
≥ Any 3 of the following: 10I/R/V, 20M/R, 24I, 36I/L, 46I/L, 48V, 54V, 63P, 64V, 71T/V, 82A/F/T, 84V, 90M (either 46I/L, 82A/F/T or 84V must be present)	indinavir, ritonavir possible: nelfinavir, saquinavir