A 12-22 Month Follow-Up of HIV Patients Whose Therapy Was Optimized by Using HIV Genotyping

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ABSTRACT

The purpose of this study was to determine whether treatment of HIV-1-infected patients using antiretroviral therapy (ARVT) based on HIV-1 genotyping data and expert advice would result in decreases in viral load to <50 copies/mL or by ≥0.5 $\log_{10}$ units and maintenance at those levels for ≥12 months. A prospective, longitudinal study using genotyping to optimize ARVT for HIV-1-infected patients was conducted from June 1999 to May 2001 at the Stratton VA Medical Center, HIV clinic. Seventeen patients failing ARVT were enrolled. Genotyping was performed on the reverse transcriptase and protease genes. Data were analyzed by 3 infectious disease physicians and an HIV virologist and used to select ARVT. Patients were then followed for 12 to 22 months. Genotyping revealed mutations for nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors in 88%, 53%, and 94% of patients, respectively. Thirty to 46% of patients had a viral load of <50 copies/mL within 3 months of the beginning of genotyping-directed ARVT. Genotyping was repeated for patients with a viral load of >50 copies/mL (47% of patients). Subsequent therapy changes decreased viral load by ≥0.5 $\log_{10}$ in 63% of those patients. Fifty-three percent of the patients with a viral load of ≥50 copies/mL maintained decreases in viral load of ≥0.5 $\log_{10}$ for up to 22 months. This study demonstrates that HIV therapy directed by genotyping and expert advice is highly beneficial for attaining a viral load <50 copies/mL for up to 16 months or decreases in viral load of ≥0.5 $\log_{10}$ for up to 22 months.

INTRODUCTION

Antiretroviral therapy improves longevity and quality of life of patients infected with the human immunodeficiency virus (HIV-1).[1] However, HIV-1 often develops mutations that can result

in resistance to one or more of the antiretroviral drugs.[2] HIV RNA levels are known to rebound in 50% of patients following initial viral suppression with antiretroviral agents.[3] It is estimated that 20% of newly infected and treatment-naïve patients and 50% of the HIV patients under care in the United States have resistant virus.[4,5] Until the development of resistance testing by genotyping or phenotyping, therapy for HIV-1 patients was chosen based solely on clinical practice experience, and frequently required changing of drug regimens.

Genotyping detects mutations in the reverse transcriptase and protease genes that can be an indirect measure of susceptibility to antiretroviral drugs.[6] HIV-1 genotypic resistance patterns may also be predictive of the response to specific drugs.[7,8] Although all of the indications for genotyping have not been determined, the International AIDS Society-USA Panel endorses HIV-1 resistance testing.[9] Clinical benefits have been reported in both short-term treatments (12-24 weeks)[10-13] and longer-term (12 months) studies.[14] Conversely, the CERT study[15] did not find genotyping to be helpful. All of these studies defined undetectable viral load as <200 to <500 copies/mL.[10-12] The role of repeat genotype testing in the event of failure or adverse drug effects has not been extensively studied to date. Of the 5 prospective studies, repeat genotyping was either not performed,[10-12] performed in a small number of patients,[15] or performed selectively and found not to result in a patient response different from that using standard care.[16]

We conducted a prospective, 12 to 22 month study on 17 HIV-1 drug-experienced male patients attending our HIV clinic. It should be emphasized that our study was performed in a real-life situation and describes what happens in actual clinical practice. Our group of 17 patients included only patients who were highly experienced with anti-HIV drugs and for whom the selection of future drug regimens was limited, unless genotyping could be performed and a proper interpretation of these test results could be made. Therefore, therapy was directed by genotyping and the expert advice of 3 infectious disease physicians and an HIV-1 virologist. The primary objective was to attain and maintain an undetectable viral load (<50 copies/mL) for ≥ 12 months. In patients who did not attain this goal, results of repeat genotyping, resistance patterns, changes in therapy, and adherence to therapy were evaluated.

SUBJECTS, MATERIALS, AND METHODS

Study Population

Of the 100 HIV-1-positive patients in the HIV Clinic at the Stratton VA Medical Center, 17 were chosen for the study either because they were failing therapy, as demonstrated by increasing viral load and declining CD4 cell counts, or because their viral load remained >50 copies/mL, or both. Patients with histories of variable adherence to therapy, but who thought that they would adhere to therapy while on the study were also included. Patients whose viral load was <50 copies/mL, who were non-adherent, whose virus could not be genotyped, or who were antiretroviral drug- naïve were excluded. The 17 patients were enrolled over a 9-month period as they became available. Therefore, their follow-up periods varied. All patients were receiving antiretroviral therapy (ARVT) at the time of genotyping.

Laboratory Procedure

Blood drawn into acid citrate dextrose and ethylenediaminetetraacetic acid tubes for genotyping and viral load

was centrifuged for 15 minutes at 1,000xg. Plasma was separated from blood cells within 4 hours of specimen collection and frozen at –70˚C. Levels of HIV-1 RNA were measured by the Clinical Microbiology Laboratory, Stratton VAMC, Albany, NY, using the Bayer Diagnostics (Chiron, Tarrytown, NY) HIV-1 RNA 3.0 Assay (bDNA) with a quantitative lower limit of 50 RNA copies/mL. Genotyping of the reverse transcriptase and protease genes was performed by the Virology Laboratory at GlaxoSmithKline Laboratories, Durham, NC using the TruGene HIV-1 Assay (Visible Genetics, Atlanta, Ga). T4/T8 lymphocyte counts were determined at the Buffalo VAMC using flow cytometry.

Study Design

A prospective, longitudinal study designed to optimize antiretroviral therapy of 17 HIV-1 patients by using genotyping was conducted at the Stratton VA Medical Center from June 1999 until May 2001. Therapy regimens were selected following evaluation of genotyping data, the patients' clinical progress, and using the insight of 3 infectious disease physicians and an HIV virologist. An active drug was defined as one for which the genotyping data disclosed no primary resistance mutations in the reverse transcriptase and/or protease genes and no significant polymorphisms in the protease gene. Patient response was defined as an HIV-1 viral load decrement of at least 0.5 $\log_{10}$ copies/mL. Compliance was assessed through patient recall and drug refill history by the pharmacy. Patient follow-up continued for up to 22 months. All patients gave informed consent approved by the Institutional Review Board of the Stratton VA Medical Center, Albany, NY. Patients were seen in the HIV Clinic within 2 to 4 weeks after beginning therapy. Thereafter, they were evaluated at 1 to 2 month intervals. HIV-1 viral load and T4/T8 lymphocyte studies were performed at the start of the study, 1 to 2 months later, and at 2 to 3 month intervals thereafter. If a virologic and/or clinical response was not observed in 3 months, genotyping was repeated and ARVT therapy altered as indicated.

RESULTS

The clinical and laboratory characteristics of 17 HIV-1 patients are presented in Table 1. Most patients were white, older than 30 years of age, and had previously been followed in the HIV clinic. Their mean viral load was 41,285 copies/mL and mean CD4 count was 290 cells/mL. They were known to have had HIV for from 2 to 17 years (mean 7.4 yrs) and had been treated with an average of 5.2 antiretroviral drugs for a mean of 6.4 years. Ten patients (59%) met the CDC definition for AIDS.[17]

Table 2 demonstrates drug exposure and primary and secondary HIV-1 mutations of 17 HIV-1-positive patients at the time of initial genotyping. The highest percent of nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations occurred for zidovudine, lamivudine, didanosine, and abacavir. For 3 of the 5 protease inhibitors (ritonavir, amprenavir, saquinavir) the number of patients with HIV-1 mutations exceeded the number who had received these drugs. Similar observations were noted with the NNRTIs.

Sixty-five percent of the patients were currently receiving a protease inhibitor and 41% were receiving a nonnucleoside reverse transcriptase inhibitors (NNRTI). All patients had received a regimen of NRTI, 94% had received a protease inhibitor, and 47% had received an NNRTI at some time. The population was highly drug experienced: upon enrollment in the study 16 of 17 patients had failed regimens with

Table 1. Clinical and Laboratory Characteristics of Seventeen HIV-1-Positive Patients

Characteristics	Number		
Total Patients (all male)	17		
Age in years: average (range)	44 (31-52)		
Time in study: number of patients (range of weeks)	2 (92-94) 11 (62-73) 4 (52-59)		
White	9		
African American	6		
Hispanic	1		
Native American	1		
	Mean	Median	Range
HIV-1 viral load (copies/mL)	41,285	5,928	897-338,193
CD_4 lymphocytes (cells/mm^3)	290	274	9-819
Past exposure to antiretroviral drugs (number)	5.2	6	2-7
Known HIV-1 infection (years)	7.4	8	2-17
Antiretroviral therapy (years)	6.4	7.5	1-11

at least one protease inhibitor, including 5 patients who had failed 2 protease inhibitor regimens. Eight were NNRTI experienced. Primary drug resistance mutations for at least one class of drugs were detected in all patients. While 88% and 94% of the patients showed evidence of mutations for NRTI and protease inhibitor resistance, respectively, only 53% had resistance mutations to NNRTIs.

Table 3 depicts the primary mutations for NRTI, NNRTI, and protease inhibitor drugs of 17 patients at the time of entry into the study; the most common mutations were M184V and T215Y, K103N and Y181C, and M46I and V77I, respectively. No patient had the Q151M complex or 69X insertion that confers multidrug resistance.

Sixteen (94%) HIV-1 viruses had one or more primary mutations and 16 (94%) had at least one secondary mutation in the protease gene. The HIV of 6 patients (35%) had mutations in all 3 classes, and 9 patients' viruses (53%) had mutations in 2 classes of drugs. Only 2 patients (12%) had viral mutations to just one class of drug. In spite of the presence of mutations to multiple

Table 2. Current and Previous Antiretroviral Drug Exposure and Primary and Secondary Mutation Frequency of 17 HIV-1 Study Patients at the Time of Initial Genotyping*

	Number of Patients (%)					
	On drug at time of genotyping or previously		With primary mutations to drug		With secondary mutations to drug	
NRTI						
	Number	%	Number	%	Number	%
Zidovudine	16	89	11	61	10	55
Lamivudine	17	94	13	72	0	0
Stavudine	14	78	0	0	0	0
Didanosine	7	39	2	66	8	44
Abacavir	2	11	12	66	9	50
NNRTI						
Nevirapine	5	28	9	50	4	22
Delavirdine	0	0	8	44	0	0
Efavirenz	3	17	8	44	6	33
Protease Inhibitor						
Nelfinavir	10	55	8	44	16	89
Ritonavir	1	5	6	33	15	83
Indinavir	8	44	9	50	16	89
Saquinavir	2	11	6	33	14	78
Amprenavir	0	0	3	17	8	44

*NRTI indicates nucleoside reverse transcriptase inhibitors; and NNRTI, non-nucleoside reverse transcriptase inhibitors.

classes of drugs, genotyping was still useful for selecting 3 or more active drugs for 16 patients (94%) and 2 new drugs for 11 patients (65%) of the 17 study patients. Nine patients with the M184V mutation had lamivudine selected as part of their new regimen and seven responded; 4 attained viral loads of <50 and the viral loads of 2 others decreased to 400 copies/mL. One other patient had a 2 $\log_{10}$ drop in viral load. The majority of patients who had lamivudine selected in the presence of M184V responded to the change with a decrease in their viral loads.

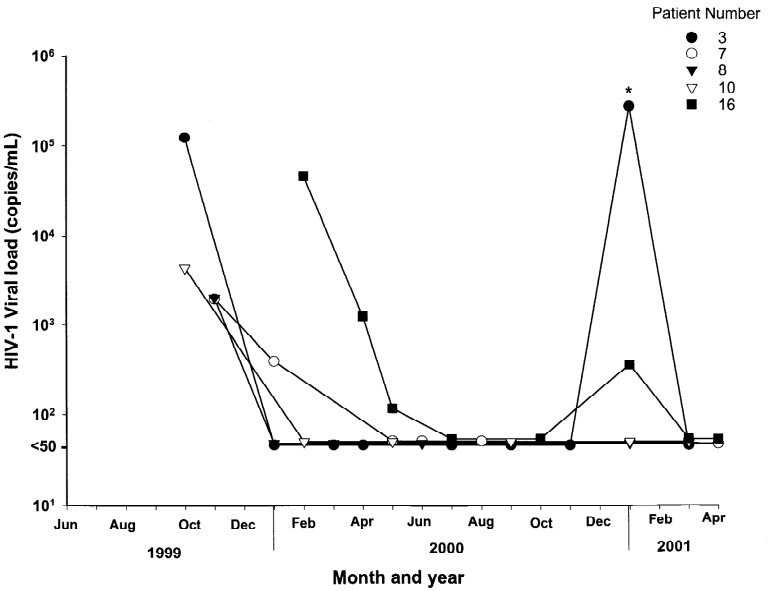
Figures 1A to D demonstrate the response of HIV-1 viral load (copies/mL) following HIV-1 genotyping and change of the ARVT over 12 to 22 months. The patients listed in Figures 1A and 1B attained viral loads of <50 and <600 copies/mL, respectively. Patients represented in Figures 1C and 1D required more than one genotyping and therapy revision. Patients represented in Figure 1D did not respond to the changes in therapy. The patients represented in Figure 1A had prompt and sustained responses; the viral load in 3 patients decreased to <50 copies/mL in one month, and in the other 2 patients in 2 to 5 months. All but one patient (#3) maintained an HIV-1 viral load of <50 copies/mL through the follow-up. Patient # 3 interrupted therapy, with a resulting rapid rise in viral load; upon resumption of the same genotyping-directed therapy, the viral load again

Table 3. Primary HIV Resistance Mutations in 17 Patient Assays at the Time of Initial Genotyping

Primary mutations in the reverse transcriptase (RT) gene	Number (%)
M184V	13 (76)
T215Y/F	8 (47)
Y181C	4 (24)
K103N	4 (24)
G190A	3 (18)
T69D	3 (18)
K70R/G	2 (12)
Primary mutations in the protease gene	
M46I/L/M	7 (41)
V771	6 (35)
L90M	4 (24)
D30N	4 (24)
L10I	4 (24)
N88D	3 (18)
V82A/F/T/V	3 (18)
I84V	2 (12)

dropped to <50 copies/mL. Patients whose viral loads are shown in Figure 1B attained and maintained their HIV-1 viral loads at 51-583 copies/mL. For 3 patients, the decreases in viral load were more gradual, reaching their lowest levels (50 to 167 copies/mL) in 1 to 5 months. Patient #2 had his regimen intensified at month 11 and experienced a subsequent decrease to 51 copies/mL in one month. Patient #9 decreased his genotyping-directed therapy dosage by 50%. This change in therapy was followed by an immediate rise in viral load from 195 to 6393 copies/mL. Resumption at the original dose was accompanied by a prompt decline in the viral load to 583 copies/mL. The patients shown in Figure 1C had partial responses. The viral loads of these patients decreased following changes in therapy made as a result of the initial genotyping data. A second genotyping was done in one week to 3 months if the viral load decline was suboptimal or if there was a rebound in the viral load. Following evaluation of the data from the second genotyping, the regimen was changed again. Two of 5 patients (#4 and #5) had excellent and sustained responses. Because of inadequate response, a third genotyping was performed in 2 patients (#1 and #11) within 2.5 months. The viral load of patient #1 did not respond to his third genotyping-directed regimen. However, his CD4 count rose from 819 to 1100. Quantification of patient #1's CD4 and CD8 cell subsets demonstrated memory and naïve cell numbers similar to those found in normal donors (data not shown), indicative of a healthy immune system. Consequently, his regimen was not changed. Patient #11 attained a viral load of <50 copies/mL on his first genotyping-directed regimen, discontinued his regimen, rebounded, and had repeat genotyping while off ARVT. This patient's third genotyping revealed additional mutations not apparent on the

Figure 1A



*Patient #3 stopped therapy demonstrating an increase in viral load; upon resumption of same therapy viral load rapidly decreased.

Figure 1B

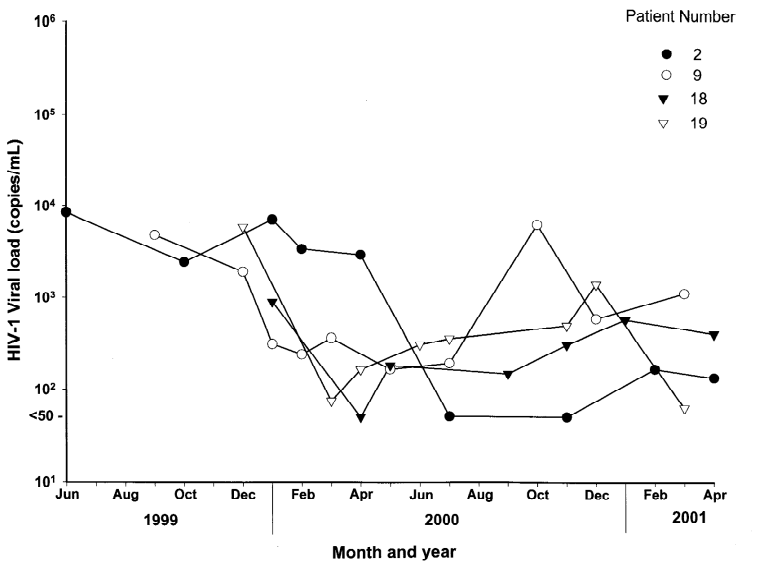


Figure 1A-D. Depicted are four groups of HIV patients and their responses to therapies chosen using genotyping data. Figure 1A includes patients whose viral loads promptly decreased to <50 upon changing therapy.* Figure 1B includes patients whose viral load decrease was gradual and was maintained at 51-583 copies/mL. Figure 1C includes patients who had repeated genotyping because of original suboptimal response to change in therapy. At the close of the study the viral load for this group ranged from <50 to 3,490 copies/mL. Figure 1D includes patients who had high numbers of viral mutations and/or did not adhere to genotyping-recommended therapy changes.

Figure 1C

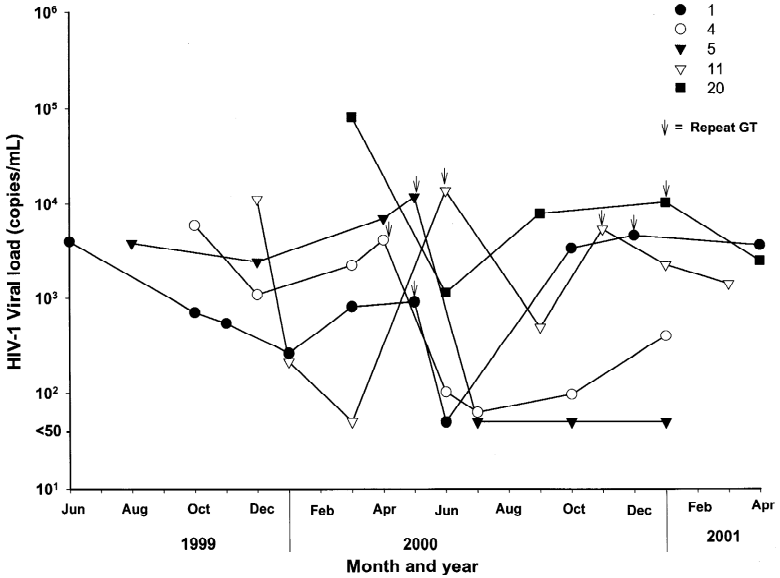
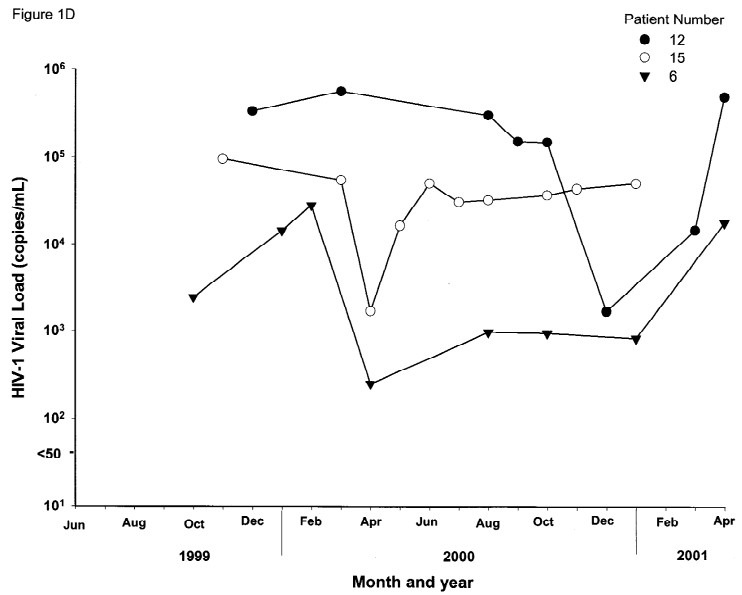


Figure 1D



second genotyping. Therapy was changed accordingly and the patient's viral load declined to <50 copies/mL (data not shown beyond study period). Patient # 4 had had HIV for >17 years and was thought to be doing well, with a viral load of □400 copies/mL sustained for >8 months. At the close of the study the viral loads for the patients in this group ranged from <50-3,590 copies/mL.

Patients whose viral loads are shown in Figure 1D responded partially or not at all. Of these 3 patients, 2 did not adhere to the regimen (#6 and #12) and 2 (#12 and #15) had a high number of key viral mutations.

Sixteen of 17 patients (94%) had a rise in CD4 cell count on genotyping-directed therapy. The mean rise in CD4 cell count at the close of the study was 107/mm^3 (median 88/mm^3; range 5-357). Patients whose viral load was <50 copies/mL had a mean rise in CD4 cells of 135/mm^3.

It should be emphasized that without the benefit of genotyping, the infectious disease physicians would have chosen a different regimen 90% of the time, and that only 54% of the time the regimen would have included 1 to 3 drugs suggested by the genotyping. Without genotyping data, 23% of the drugs selected by the infectious disease physicians would likely have been ineffective due to the presence of mutations in the HIV genome conferring resistance to antiretroviral drugs.

At the close of the study, 13 patients (76%) had decreases in HIV-1 viral load of ≥0.5 $\log_{10}$ as compared with their beginning viral loads. Six patients (35%) had HIV-1 viral loads that were <50 copies/mL. The viral loads of 10 patients (59%) dropped to <50 copies/mL on at least one occasion. Seven of these had viral loads of <50 copies/mL following changes in therapy resulting from the first genotyping analysis. The viral loads of the other 3 patients reached undetectable levels following therapy changes resulting from repeat genotyping.

DISCUSSION

This study demonstrates the importance of genotype testing, communication between infectious disease physicians and an HIV virologist, and patient adherence to the drug regimen for selecting and maintaining effective antiretroviral therapy. Furthermore, it demonstrates the importance of repeat genotype testing within 3 months of changing a drug regimen if the decrease in viral load is suboptimal after the change.

It has been noted by some investigators that the extent of drug resistance at baseline influences the treatment success or failure in the new genotyping-guided regimen for patients, and that regimens including 3 or more active drugs are needed in order to provide a higher barrier to the emergence of resistance.[2,3,7,14] Our patient population was highly drug-experienced. In contrast to the VIRADAPT study, twice as many of our patients (94%) harbored primary resistance mutations in the protease gene when they were first genotyped.[11] In our study, 35% of the patients (6 patients) had resistance in all 3 ARVT classes and 53% (9 patients) had resistance to 2 classes of drugs. Despite resistance mutations for multiple classes of drugs, we were able to select ≥3 active drugs for 94% of patients and 2 new drugs for 61% of the patients, using genotyping data and expert advice. Among the 6 patients (35%) with resistance mutations to all 3 drug classes, 2 (#3 and #7) had complete responses, 3 (#1, #11, and #15) had partial responses, and one (#12) had no response.

The International AIDS Society-USA Panel endorses the use of HIV-1 resistance testing. However, whether the benefit of testing is long-term has not been determined.[9,18] Earlier studies have demonstrated time-limited benefits utilizing genotyping testing. The VIRADAPT, GART, HAVANA, and ARGENTA studies showed short-term (12-24 weeks) virologic benefits of genotyping testing to guide antiretroviral therapy, while longer studies demonstrated an advantage at 12 months in a subgroup of patients with limited HIV

mutations.[10-14,19] The longest study (22-26 months) showed that using genotyping to select ARVT provided a modest benefit only in patients with complex treatment histories.[15] Our study of patients who had taken numerous antiretroviral drugs and whose HIV possessed many drug resistance mutations clearly demonstrates the benefits of selecting ARVT based on genotyping data and expert advice. These therapeutic benefits persisted for up to 22 months, with 30% of the compliant patients maintaining viral loads of <50 copies/mL and another 24% maintaining viral loads of <580 copies/mL.

In contrast to other prospective clinical trials where repeat genotyping was not performed,[10-12] was performed once,[16] or was performed in a small fraction of patients,[15] we found repeat genotyping to be essential in the event of failure or adverse drug effects. In our study, repeat genotyping was performed in all patients (47%) whose responses to therapy changes, made as a result of genotyping data analysis and expert advice, were suboptimal. Virologic responses following changes in therapy occurred in 63% of our patients, demonstrating the efficacy of repeat genotype testing.

Our goal was to decrease the viral load to the lowest level possible, ie < 50 copies/mL. In contrast to earlier studies, which used genotyping data for the selection of ARVT and which included viral load cutoffs of <200, <400, <500, and <1000 copies/mL as their endpoints,[10-12,14] we found that after our patients began their genotyping-directed ARVT, 41% had undetectable viral loads at 12 months and 33% at 18 months. In contrast, in one study utilizing genotyping, in which the goal was to achieve a viral load of <200 copies/mL, the viral load goal was attained in 29% of patients at 3 months, 32% at 6 months, and 30% at 12 months.[11] Seventy percent of our patients attained viral loads of <200 copies/mL in 3 months, 50% in 6 months, and 50% in 12 months. In addition to a virologic response, utilizing genotype testing and expert advice led to improved immune function, with 94% of patients having a rise in CD4 cell counts. The mean rise in CD4 cell count at the end of the study for all patients was 107 cells/mm^3. In the patients whose viral load remained at <50 copies/mL, the mean rise was 135 cells/ mm^3. Smaller CD4 cell increases were described in other studies in a 3 to 6 months follow-up.[10,11]

Previous investigators have found that utilizing resistance testing leads to more selective ARVT and that without the benefit of such testing clinicians tend to change to new drugs or new classes of drugs at frequent intervals.[16,20] Of the 17 patients in our study, for whom ARVT was selected by using genotyping data and expert advice, 9 (53%) remained on the first drug regimen selected using data from the first genotyping throughout the study period. Of the 8 patients (47%) who underwent a second genotyping, all were switched to a second drug regimen, and 7 (41%) remained on the second regimen for the remainder of the study. Using genotyping-directed regimens, we noted that 36% of the individual drugs remained the same as before genotyping. This is an important finding because if a drug retains its effectiveness, it may obviate the need to change all the drugs in a failing regimen. This is particularly important when the number of potentially useful drugs is limited.

This study has several possible limitations: it was done in a single institution and no control group was planned or included because of the small number of patients. However, it was planned as a real-life study in an HIV clinic of highly HIV drug-experienced patients. In addition, at the time this study was conduct-

ed, the mutations associated with stavudine and didanosine resistance were under-recognized, and this may have adversely affected some of our drug selections.

In conclusion, our prospective study demonstrates the benefit of selecting ARVT based on genotype testing and expert advice. This benefit persisted for up to 22 months with 30% of the compliant patients maintaining viral loads of <50 copies/mL and another 24% maintaining viral loads of <580 copies/mL.

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