

Drug-resistance genotyping in HIV-1 therapy: the VIRAD APT randomised controlled trial

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Summary

Background Growing evidence has linked HIV-1 resistance mutations and drug failure. The use of genotypic-resistance analysis to assist therapeutic decision-making in patients failing therapy has not been investigated. We assessed the virological and immunological impact of genotypic-resistance testing.

Methods We did a prospective, open, randomised, controlled study of HIV-1-infected patients in whom combination therapy was not successful. We randomly assigned patients standard care (control, n=43) or treatment according to the resistance mutations in protease and reverse-transcriptase genes (genotypic group, n=65). The major endpoint was the change in HIV-1 RNA viral load. Analysis was by intention to treat.

Findings 108 patients were enrolled. All patients were similar for risk factors, age, sex, previous treatment, CD4-cell count (214/ μ L [SD14]) and log HIV-1 RNA viral load at baseline (4.7 copies/mL [0.1]). At month 3, the mean change in HIV-1 RNA was -1.04 log (0.14) in the study group compared with -0.46 log (0.17) in the control group (mean difference 0.58 log [95% CI 0.14–1.02], $p=0.01$). At month 6, changes were -1.15 (0.15) log copies/mL, and -0.67 (0.19) log copies/mL in the genotypic group and the control group, respectively (mean difference 0.48 log [0.01–0.97], $p=0.05$). Difference in the drop in viral load combined at 3 months and 6 months was significant ($p=0.015$). At month 3, HIV-1 RNA was lower than detection level (200 copies/mL) in 29% (19/65) of patients in the genotypic group versus 14% (6/43) in the control group ($p=0.017$). At month 6, the values were 32% (21/65) and 14% (6/43) ($p=0.067$) for the genotypic group and the control group, respectively. Therapy was generally well tolerated, with ten patients (six in the genotypic group, four in the control group) requiring toxic-effect-related drug modification.

Interpretation We found genotypic-resistance testing to have a significant benefit on the virological response when choosing a therapeutic alternative. Further study of the use of genotypic-resistance testing in assisting clinical decision-making is warranted.

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Introduction

Drug resistance is the inevitable consequence of incomplete suppression of HIV-1 replication. The rapid turnover of HIV-1 RNA and its genetic variability have led to the production of many HIV-1 variants with decreased drug susceptibility.^{1,2} There are currently ten drugs available to treat HIV-1-infected patients in France, including four protease inhibitors, and an additional three drugs available through the expanded access programme. Despite this selection, many patients do not achieve or maintain complete viral suppression. Virological response rates to initial therapy with a protease inhibitor and two nucleoside reverse-transcriptase inhibitors range from 60% to 90% and success is less likely in advanced stages of the disease.^{3,4} Many factors (drug, host, viral) contribute to drug failure. However, drug-resistance mutations in the HIV-1 reverse-transcriptase and protease genes lead to lower sensitivity to antiretroviral agents and are an important cause of drug failure. An increasing number of retrospective studies link the appearance of such mutations with a rebound in the viral load.⁵⁻⁷ Information on patterns of resistance to and cross-resistance between antiretroviral agents is increasingly available and may be important for decisions on how to combine drugs to achieve an optimum antiviral effect.⁸

Improved genotypic assays to assess resistance mutations are becoming available, but the clinical use of these results has not yet been investigated.² We assessed the clinical benefit of HIV-1 resistance testing in a drug-experienced population in a prospective, randomised, open, controlled pilot study.

Patients and methods

Patients

We did the study in three hospitals in southern France. The study protocol was approved by the institutional ethics committee of our hospital. Written informed consent was obtained from all study participants.

Entry criteria were a plasma HIV-1 RNA of more than 10 000 copies/mL despite at least 6 months' treatment with nucleoside analogues and at least 3 months' treatment with a protease inhibitor. Eligibility criteria also included age older than 18 years and a Karnofsky score higher than 50. Criteria for exclusion were haemoglobin concentration of less than 6 mmol/L, absolute neutrophil count less than $0.8 \times 10^9/L$, creatinine concentration of more than 200 μ mol/L, and liver aminotransferase values at more than five times the normal upper limit. Patients with foreseeable non-compliance were excluded from the study.

Trial design

We enrolled consecutive patients meeting the criteria who agreed to participate. Randomisation was done by permutation table in blocks of six, with a two/three ratio in the control group (n=43) and treatment group (n=65), respectively. Each patient was assigned to a study group according to his or her study-entry number, which was kept in an opaque sealed envelope.

We changed the therapeutic regimen of patients in the control group based on our current optimum care, according to the published guidelines.⁸⁻¹⁰ Knowledge of the results of the genotypic assay were not made available to the physician. For patients in the genotypic group, the physician waited for the genotypic results before changing therapy, so that treatment was adapted with this additional knowledge available. Patients who failed their second or third regimens were classified as difficult patients and were discussed by two or three study physicians before a decision was made on the next treatment. Discussions took into account data available based on study group only. We assessed clinical status and adverse effects at each visit. We measured viral load, CD4-cell counts, and did genotypic-resistance assays about every 3 months in all patients.

For the analysis, results were aligned every 3 months. Treatment was modified after 3 months on a new regimen if HIV-1 RNA remained more than 10 000 copies/mL or less than 0.5 log lower than baseline. We did the analysis by comparing standard of care in the control group with adapted treatment in the genotypic group. We measured plasma HIV-1 RNA about 3 months after additional changes.

Laboratory measurements

Complete sequencing of the major part of the reverse-transcriptase gene (nucleotides 25–230) and the entire protease gene was done on plasma HIV-1 RNA. HIV-1 RNA was extracted from patients' plasma samples and amplified with RT-PCR. Because of the lack of widely available registered kits at the start of the study, we used multiple procedures to identify the genetic sequences during the course of the trial. From the beginning of the study until January, 1998, several PCR-extraction and DNA-sequencing technologies (ACT Gene Laboratory, Baudry, France) were used, following standard operating procedures.¹¹ RNA extractions (QIAGEN, Düsseldorf, Germany, or Boehringer Mannheim, Mannheim, Germany) were used, followed by RT-PCR and nested-PCR with *pol* gene oligonucleotides separately producing two fragments, an 800 bp reverse-transcriptase amplicon, and a 350 bp protease amplicon. The amplicons were sequenced (ALF Express, Pharmacia, Biotech, Upsala, Sweden and ABI 377 Foster City, CA, USA, respectively), with single-stranded or double-stranded data. From February, 1998, until July, 1998, we used a prereleased version of a single-step RNA-extraction and RT-PCR assay (TruGene HIV-1 assay, Visible Genetics, Toronto, Ontario, Canada), whereas DNA sequencing was done on DNA sequencers. From August, 1998 until the end of the study the approved TruGene assay was used.

Plasma RNA extraction (500 mL) was done after centrifugation. The pellet was treated with RNAzol (Biotecs Laboratories, Houston, TX, USA), followed by chloroform, isopropanol, and ethanol steps. Extracted RNA was stored at -80°C until use. The viral RNA was retrotranscribed in cDNA and subsequently amplified by a single-tube RT-PCR with an HIV-1 assay high-resolution genotyping kit (TruGene, Visible Genetics) which gave a 1.3 kb amplicon that covered the whole protease gene and the major part of the reverse-transcriptase gene of HIV-1. Bidirectional DNA sequencing of the amplification products was performed with a sensitive sequencing method (CLIP, Visible Genetics, Toronto, Ontario, Canada). Each sequencing reaction was loaded on an automated DNA sampler (MicroGene Clipper, Visible Genetics). For each sample sequenced, the resulting assays were base called with GeneObjects (Visible Genetics), aligned, and assembled together with GeneLibrarian (Visible Genetics). The sequence for each sample was compared to a database of known drug-resistance mutations to find out which mutations were present in the HIV-1 RNA. All the initial samples were tested with the TruGene kits, which seemed to be more sensitive than the inhouse PCRs in identification of mutations. HIV-1 RNA was measured by PCR (Amplicor, Roche, Basel, Switzerland). Classification of the mutations into primary, secondary, and polymorphism, associated or not associated with decreased drug sensitivity was established according to the consensus statement on antiretroviral drug-resistance testing.¹²

Mutations and codons	Drug not suggested
Nucleoside analogues	
V75T	Stavudine
T215F or K70R±(M41L, D67N, L210W, K219E)	Zidovudine
M184V	Lamivudine
M184V+T215F±(R211K or L214F or G333D/E)	Zidovudine and lamivudine
M184V+(K65R or L74V)	Abacavir
L74V±(K65R, M184V)	Didanosine
T69D±(K65R, L74V, M184V)	Zalcitabine
Q151M±(A62V, V75I, F77L, F116Y)	Multinucleoside resistance
Non-nucleoside analogues	
K103N or V106A or Y181I or Y188C/1 or G190A/S or P236L	Nevirapine, delavirdine, or efavirenz
Protease inhibitors	
G48V and L90M	Saquinavir
G48V or L90M and any two of the following mutations (L10I/R/V, I54V, A71V/T, G73S, I84V)	Saquinavir and partial resistance to ritonavir or indinavir
M461I/L or V82A/F/T/V+any two of the following mutations (L10I, K20M/R, L24/I, V32I, I54V/L, A71V/T, G73S, I84V, L90M)	Ritonavir, indinavir
D30N or any three of the following mutations (M36I, M46I/L, A71V/T, V77I, I84V, N88D, L90M)	Nelfinavir

Table 1: Resistance mutations

Treatment

During the study, new drugs became available (nelfinavir, nevirapine, efavirenz, and abacavir) and were incorporated in the therapeutic regimen.

Decisions about therapeutic changes in the genotypic group were guided by correlations linking specific mutations to decreased activity of specific drugs, which increased over time (table 1). When specific mutations were found, we no longer selected corresponding drugs for treatment. If no resistance mutations were found, the choice of antiretroviral therapy was determined by the physician's best clinical judgement.

Statistical analysis

The number of patients required to obtain statistical power was difficult to calculate because of the lack of previous studies. Therefore, we chose empirically the number of 100 patients—40 patients in the control group and 60 patients in the genotypic group. The number of patients in the two groups did not balance because we had initially planned further randomisation of patients in the genotypic group into two subgroups for assessment of treatment modification based on the appearance of genotypic mutations on treatment before viral load rose. Since the limit of the genotyping technique was 1000 copies/mL, viral load lower than the level of detection (200 copies/mL plasma RNA) could not be amplified to be sequenced. Subgroup randomisation was, therefore, abandoned.

We included in the analysis all patients who had observations after the start of the study. Our primary endpoint was the variation of HIV-1 RNA from baseline to month 3 and month 6 (log

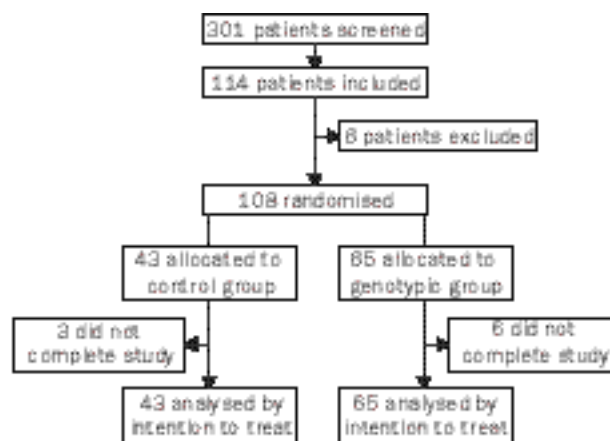


Figure 1: Trial profile

transformed). We did the analysis with last observation carried forward. Secondary efficacy variables were CD4-cell count and the proportion of patients with plasma HIV-1 RNA lower than the limit of detection (200 copies/mL). Difference in the proportion of patients with viral load less than 200 copies/mL was analysed by intention to treat (dropout equalled failure).

The plasma HIV-1 RNA was log transformed. We compared mean change by ANOVA repeated-measures analysis (data for months 3 and 6 combined). We did post-hoc analysis of drug use and mutations. All p values reported are two-sided and all CI are 95%. Data were analysed by Statview (version 5.0) software.

Results

From March, 1997, until March, 1998, 114 patients were screened, of whom 108 were randomised (figure 1). 41 patients in the control group completed 3-month follow-up after treatment adaptation, and 40 completed 6-month follow-up. In the genotypic group, 62 patients completed 3 months of follow-up after treatment adaptation, and 59 completed 6 months of follow-up. 103 (95.4%) of 108 patients could be assessed at month 3, and 99 (91.6%) of 108 at month 6 (four patients were lost to follow-up, four patients died, and one patient did not complete 24 weeks of study). We ended the study, originally planned to last 1 year, after 6 months because interim analysis showed a significant virological advantage in the genotypic group.

Baseline characteristics were similar in the two groups (table 2). The overall prevalence of primary mutations for the reverse-transcriptase gene was 90%. All patients had at least one secondary mutation. 7% of patients never treated with a non-nucleoside reverse-transcriptase inhibitor presented with primary mutations to these drugs. We found one strain with the 151 mutation and one strain with 69S insertion mutation conferring multidrug resistance. Mutations at position D67N (44.6 vs 23.3%), T69D (21.5 vs 4.6%), and T215Y/F (69 vs 49%) on the reverse-transcriptase gene were more common at baseline in the genotypic group than in the control group. The overall prevalence of primary mutations in the protease gene was 48%. All patients had at least one secondary mutation in the protease gene, which led to a mean of 6.2 (SD 2.9) mutations per patient.

All patients were antiretroviral experienced, with exposure to a mean of 3.9 (0.9) nucleoside analogues for a mean duration of 39.5 months (26) and to a mean of 1.8 protease inhibitors for a mean duration of 11.6 months (5.4). Treatment at baseline was similar in the two groups. The proportion of patients with first-line failure (previous treatment with one protease inhibitor), second-line failure, and third-line failure (previous treatment with two or more protease inhibitors) were, in the control and genotypic groups, respectively, 32.1% compared with 46.2% and 66.1% compared with 53.9% ($p=0.17$). The most common combinations of drugs at study entry were: zidovudine, lamivudine, and indinavir; stavudine, lamivudine, and indinavir; and stavudine, lamivudine, and saquinavir (table 2).

At month 3, the mean change in HIV-1 RNA was -1.04 log (0.14) in the genotypic group and -0.46 log (0.17) in the control group (mean difference 0.58 log [95% CI 0.14–1.02], $p=0.01$). This decrease persisted at month 6, with a change of -1.15 log copies/mL (0.15) in the genotypic group and -0.67 log copies/mL (0.19) in the control group (mean difference 0.48 [0.01–0.97], $p=0.05$; figure 2). The difference in the decrease in viral load combined at 3 months and 6 months was significant ($p=0.015$). At month 3, HIV-1 RNA was lower

Characteristic	Control group (n=43)	Genotypic group (n=65)	p
Mean (SD) age (years)	40.1 (7.5)	39.4 (8.2)	0.43
Sex (M/F)	34/9	47/18	0.64
Risk factor for HIV-1			0.48
Injecting drug user	18	30	
Homosexual/bisexual	17	18	
Heterosexual	7	16	
Other	1	1	
Plasma HIV-1 RNA			0.82
Median copies/mL (range)	141 395 (5300–1 500 000)	152 002 (2300–1 500 000)	
Mean log (SD)	4.8 (0.5)	4.7 (0.6)	0.45
Mean CD4 cell count $\times 10^6$ (SD)	201.7 (22.0)	220.8 (18.0)	0.49
CDC stage			
A	5	16	
B	16	14	
C	22	35	0.11
Previous RT inhibitor			
Zidovudine	43 (100%)	64 (99%)	
Didanosine	28 (65%)	43 (66%)	
Zalcitabine	20 (47%)	29 (45%)	
Stavudine	36 (84%)	52 (79%)	
Lamivudine	42 (98%)	63 (97%)	
Nevirapine	1 (2%)	2 (3%)	0.72
Previous protease inhibitor			
Indinavir	32 (74%)	42 (65%)	
Ritonavir	28 (65%)	42 (65%)	
Saquinavir	19 (44%)	22 (34%)	
Nelfinavir	1 (2%)	3 (4%)	0.54
Primary mutations in the RT gene			
K70R	10 (23%)	18 (28%)	0.60
L74V	2 (5%)	0	0.30
V75T	2 (5%)	3 (5%)	0.99
K103N	2 (5%)	1 (2%)	0.34
Q151M	1 (2%)	0	0.21
M184V	22 (51%)	36 (55%)	0.67
T215Y/F	21 (49%)	45 (69%)	0.05
Primary mutations in the protease gene			
M46I/L	7 (17%)	9 (4%)	0.69
G48V	1 (2%)	4 (6%)	0.37
V82A/F/T/V	12 (29%)	24 (37%)	0.37
L90M	10 (24%)	14 (15%)	0.78

CDC=Centers for Disease Control and Prevention; RT=reverse transcriptase.

Table 2: Baseline characteristics

than the detection level (200 copies/mL) in 19 (29%) of 65 patients in the genotypic group and six (14%) of 43 in the control group ($p=0.017$). At month 6, the values were 21 (32%) of 65 and six (14%) of 43 ($p=0.067$, figure 3).

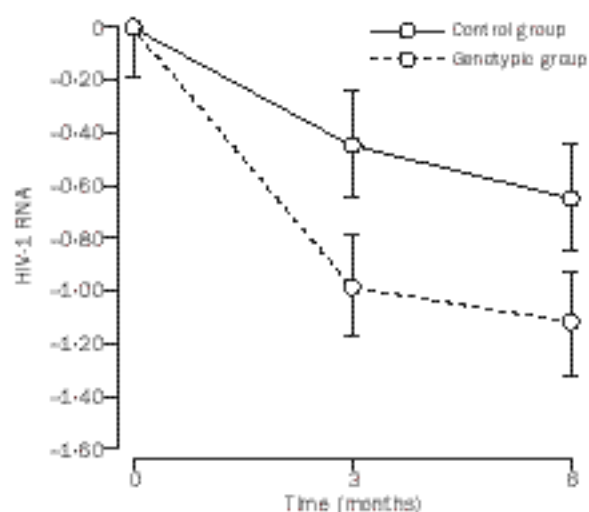


Figure 2: Mean change in plasma HIV-1 RNA from baseline to months 3 and 6

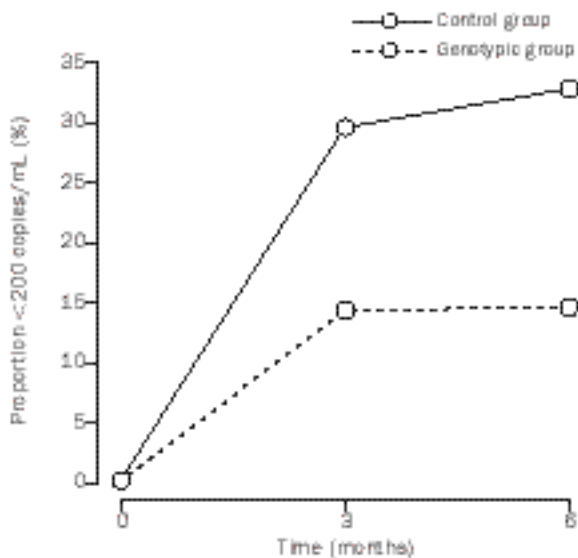


Figure 3: Proportion of patients with plasma HIV-1 RNA lower than limit of detection (200 copies/mL) at months 3 and 6

The proportion of patients who showed no decrease in viral load after treatment adaptation was significantly higher in the control group than in the genotypic group (6 vs 32%, $p=0.01$). Some primary mutations were significantly associated with worse progression, irrespective of study group. Those mutations include the K70R and T215Y/F ($p=0.03$) positions of the reverse-transcriptase gene, and the M46I/L and V82A/F/T/V ($p=0.04$) positions of the protease gene. Patients more heavily pretreated (second-line and third-line regimen failure) also had a lesser virological response ($p=0.02$).

The mean change from baseline of CD4-cell count did not differ significantly in the two groups. The mean increase in CD4 T lymphocytes at months 3 and 6 was, respectively, 36 cells/ μ L (19) and 21 cells/ μ L (18) in the genotypic group and 18 cells/ μ L (20) and 33 cells/ μ L (21) in the control group.

Time to treatment modification from screening was 1.7 months (0.7) in the control group and 2.3 months (1.3) in the genotypic group because of laboratory turn-around time. Patients were kept on their initial treatment while awaiting changes. HIV-1 RNA was measured 3.1 months (0.8) and 3.2 months (1.1) after treatment change in the control and genotypic groups, respectively. 25 patients in the control group and 27 patients in the genotypic group were classified as difficult patients and were discussed by physicians.

Treatment at baseline was similar in the two groups. After changes in therapy, the preferred regimens used in the control group were nelfinavir in combination with stavudine and didanosine or stavudine and lamivudine (30%), and ritonavir and saquinavir with stavudine and

lamivudine or stavudine and didanosine (37%). Published guidelines suggesting changes to at least two new drugs, were not followed in eight (19%) of 43 of patients in control group, compared with 18 (28%) of 65 patients in the genotypic group. Treatment regimens in the genotypic group were more diverse, with more combinations of reverse-transcriptase inhibitors and protease inhibitors being used, which resulted in more individualised treatment patterns. Typically, patients in the control group were more frequently switched to new protease inhibitor and nucleoside reverse-transcriptase-inhibitor combinations (table 3). A large proportion of the patients received saquinavir-ritonavir combinations with one or two nucleoside reverse-transcriptase inhibitors (45%).

Comparison of viral-load evolution for patients changed to the ritonavir-saquinavir combination in the two groups shows that the genotypic-assisted decision to give this regimen resulted in a greater mean decrease in HIV-1 RNA than in the control group (-1.25 vs -0.65 log RNA copies/mL, $p=0.018$). Use of drugs in newly available classes (abacavir, efavirenz, nevirapine) was equally distributed in the two groups.

Ten patients experienced one or more drug-related side-effects that lead to treatment modification. Six patients in the genotypic-group had one or more adverse events: increase in liver aminotransferases attributed to stavudine, nevirapine, or nelfinavir in three, stavudine-related pancreatitis in two, polyneuritis on stavudine and zalcitabine in one, rash on didanosine in one, neutropenia attributed to didanosine in one, ritonavir-related diarrhoea in one. Four patients in the control group experienced a side-effect: nevirapine-related rash in one, stavudine-related polyneuritis in one, and indinavir-related hepatitis in two. Treatment was modified according to group.

Four deaths occurred during this study period, two in the control group (cirrhosis and cerebral toxoplasmosis) and two in the genotypic arm (pulmonary Kaposi's sarcoma and gram-negative septic shock). Six new AIDS-defining disorders were reported, four in the control group (cerebral toxoplasmosis, cytomegalovirus colitis and microsporidiosis, pneumocystosis, lymphoma and oesophageal candidosis) and two in the genotypic group (disseminated *Micobacterium avium* complex infection, cytomegalovirus colitis).

Discussion

Genotypic-resistance testing was beneficial for decisions about changes to treatment. The decrease in HIV-1 RNA, and the percentage of patients with HIV-1 RNA lower than the detection level was better in the genotypic group than in the control group.

Several retrospective studies have shown that the presence of drug-resistance mutations at baseline was predictive of the virological responses. The probability of virological response to the combination of saquinavir and ritonavir in patients pretreated with a protease inhibitor decreased with the number of primary mutations in the protease gene.⁵ In a multivariate analysis, the best predictive model of virological response was the combined knowledge of genotype and treatment history. In another series, the number of primary mutations in the protease gene (G48V, V82A/F/T, I84V, L90M) was correlated with the response to nelfinavir.⁶ One retrospective study showed baseline genotype or phenotype to be significant predictors of virological response in patients treated with the combination of saquinavir and ritonavir.⁶

Treatment combination	Control group (n=43)	Genotypic group (n=65)	p
2 NRTI, 1 protease inhibitor	20/43 (47%)	18/65 (28%)	0.05
2 NRTI, 2 protease inhibitors	13/43 (31%)	22/65 (31%)	0.69
1 NRTI, 2 protease inhibitors	2/43 (5%)	6/65 (9%)	0.36
1 NRTI, 1 NNRTI, 1 protease inhibitor	5/43 (12%)	12/65 (18%)	0.33
2 NRTI, 1 NNRTI	1/43 (2%)	3/65 (5%)	0.52
Other combinations (2 protease inhibitors, 3 or 4 NRTI; 2 protease inhibitors, 1 NNRTI; 2 or 3 NRTI, 1 NNRTI)	2/43 (5%)	4/65 (6%)	0.73

NRTI=nucleoside reverse-transcriptase inhibitor; NNRTI=non-nucleoside reverse-transcriptase inhibitor.

Table 3: Treatment regimens in control and genotypic groups

The difference between the two groups in our study in the evolution of viral load seemed to be because of the proper use of the drugs available and not because of different drugs. Published guidelines, which suggest changing to at least two new drugs, were not followed in 19% of the patients in the control group compared with 27% in the genotypic group. This difference was owing to the lack of another option in the control group. In the genotypic group the difference was owing to HIV-1 RNA still being sensitive to the one or two drugs in the previous regimen. For patients changed in the two groups to zidovudine and zalcitabine combinations, a greater decrease in viral load was obtained if the decision to use this regimen was based on genotypic results and not standard of care.

In some patients, no mutation could explain the drug failure. 11% of the reverse-transcriptase genes and 52% of the protease genes at baseline contained no primary mutations. This finding could be because of other causes, such as non-compliance, lack of absorption, poor drug metabolism, release of virions from sanctuaries, or, possibly, clinically significant minor quasispecies.^{13,14} The previously described wild-type virus escape, in which wild-type virus rebounds because of incomplete suppression and increased CD4 target-cell availability, may also have had a role.¹⁵

Another explanation for the lack of mutations could be technological limitations. Only the major quasispecies are detected by genotyping. Even with the use of genotyping, many of our patients did not achieve complete viral suppression. This effect may be due to the drug-experienced and advanced-disease status of our population, and to the present limitations of genotypic assays.

Interpretation of the mutation pattern is difficult, and clear clinical guidelines for the interpretation of resistance mutations are urgently required. Some mutational patterns are well characterised for drugs such as zidovudine, lamivudine, and the non-nucleoside reverse-transcriptase inhibitors. Other drugs, such as stavudine or didanosine and zalcitabine, have less well-defined resistance mutations. The field of resistance mutations is rapidly evolving and new mutations have been described that explain failure of drugs such as stavudine, abacavir, or nelfinavir. The clinical relevance of new mutations and the mutational patterns of new drugs needs to be investigated. The interpretation of genotypic resistance must also take into account that some mutations may be found only in archival HIV-1 DNA, and that mutations arising under combination therapy may be different than those arising under monotherapy, for which most knowledge about resistance exists. Finally cross-resistance, drug re-sensitisation because of mutation interactions, and mutation-related loss of viral replicative capacity further increase the complexity of use of genotypic-resistance testing.

The groups did not differ for changes in CD4-cell count, since modest increases were seen in both. This similarity may be explained by several factors. In the two groups HIV-1 RNA decreased and, although this change was significantly greater in the genotypic group, it may not have been large enough to be associated with a difference in short-term changes in CD4-cell count. The drug-experienced nature of the population may have contributed to this finding. In addition, since these patients were receiving combination therapy at baseline, CD4-cell count

may have continued to rise despite loss of viral suppression.¹⁶

In conclusion, this pilot study shows promising results for use of genotypic-resistance assays to manage patients. Genotypic technology is applicable to clinical practice, but further studies are needed to find the optimum indications for genotyping in the general HIV-1-positive population or in other situations. The long-term effects of this strategy must also be assessed, as well as other important factors to improve therapy in drug-experienced patients.

Contributors

J Durant and P Clevenbergh were responsible for the protocol design, study coordination, statistical analysis, and the writing of the paper. P Halfon was responsible for the virological analysis and interpretation of genotypic results. S Porsin was responsible for the data management and analysis, P Del Giudice, P Simonet, and N Montagne were responsible for the study at the Frejus and Cannes sites. C Boucher and J Schapiro were responsible for critical reviewing of the data, data analysis, and writing the paper. P Dellamonica is the head of the infectious diseases department and was the primary investigator of the study.

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