

# Clinical utility of HIV-1 genotyping and expert advice: the Havana trial

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**Objective:** To determine whether HIV-1 genotyping and expert advice add additional short-term virologic benefit in guiding antiretroviral changes in HIV+ drug-experienced patients.

**Design:** A two factorial (genotyping and expert advice), randomized, open label, multi-center trial. The patients were stratified according to the number of treatment failures.

**Patients and methods:** HIV-1 infected patients on stable antiretroviral therapy who presented virological failure were included into the study. Genotypic testing was performed by using TrueGene HIV Genotyping kit and the results were interpreted by a software package (RetroGram<sup>®</sup>, version 1.0). An expert advisory committee suggested the new therapeutic approach based on clinical information alone or on clinical information plus HIV-1 genotyping results. Plasma HIV-1 RNA load, CD4+ cell count and adverse events were recorded at baseline and every 12 weeks.

**Results:** A total of 326 patients were included. The baseline CD4+ cell count and plasma HIV-1 RNA were  $387 (\pm 224) \times 10^6$  cells/l and  $4 (\pm 1) \log_{10}$  respectively. The proportion of patients with plasma HIV-1 RNA < 400 copies/ml at 24 weeks differed between genotyping and no genotyping arms (48.5 and 36.2%,  $P < 0.05$ ). Factors associated with a higher probability of plasma HIV-1 RNA < 400 copies/ml were HIV-1 genotyping [odds ratio (OR), 1.7; 95% confidence interval (CI), 1.1–2.8;  $P = 0.016$ ] and the expert advice in patients failing to a second-line antiretroviral therapy (OR, 3.2; 95% CI, 1.2–8.3;  $P = 0.016$ ).

**Conclusions:** HIV-1 genotyping interpreted by a software package improves the virological outcome when it is added to the clinical information as a basis for decisions on changing antiretroviral therapy. The expert advice also showed virologic benefit in the second failure group.

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**HIV-1, genotypic resistance, expert advice, viral load, antiretroviral therapy, virological failure, resistance mutations**

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## Introduction

Virologic failure of antiretroviral (ARV) medications during therapy for HIV infection has many potential causes. A result and a possible cause of this failure is the development of mutations associated with reduced viral susceptibility to ARV medications. The relationship between resistance-related mutations and virologic outcome have been tentatively explored by the use of HIV-1 genotypic and phenotypic assays. Resistance to all available ARV medications and cross resistance between some agents have been demonstrated and may limit options [1–20].

There is retrospective evidence for a link between genetic mutations and virologic failure *in vivo* [4–11]. In addition, there have been a number of studies that have prospectively demonstrated the virologic benefits of HIV-1 genotyping in clinical settings [12–15]. These studies provided preliminary proof of concept, and in addition they have extended the acceptance of HIV genotyping in clinical settings and have helped stimulate the publication of guidelines recommending the use or consideration of resistance testing [16–20]. However, many questions regarding the clinical utility of HIV resistance testing remain to be answered.

There are some data demonstrating that expert advice may contribute to improve virologic outcomes with the clinical use of HIV-1 genotyping [12], however there is a lack of information on the role of expert advice in standard of care practice. Previous studies have shown that, in the setting of treating HIV, the experience of the clinician can have a direct impact on the clinical and cost outcomes of therapy [21–23]. Given the complexity of the clinical use of genotypic resistance testing [24–27], this is a likely case where expert interpretation may be of greater impact. Previous studies did not attempt to differentiate the benefits of resistance testing and expert advice [12,13]. This study investigates the influence that HIV-1 genotyping with software interpretation, and expert advice, each have on virologic outcomes in ARV-experienced patients.

## Patients and methods

### Patients

HIV-1-infected patients were screened and enrolled from 13 hospitals in 10 cities in Spain. The study protocol was approved by the institutional review boards or ethic committees from all participating study sites. Written, informed consent was obtained from all study participants prior to randomization.

The inclusion criteria were: to have plasma HIV-1

RNA  $\geq 1000$  copies/ml and to be on stable ARV therapy combination for more than 6 months. Patients were excluded if they had substantial ARV-related adverse events history, poor adherence or active drug abuse was reported by the treating physician.

### Trial design

This randomized, open label, multi-centre trial evaluated the utility of the genotyping testing and expert advice in guiding changes of ARV therapy in individuals with virologic failure.

The primary endpoint was the proportion of patients with plasma HIV-1 RNA load (pVL)  $< 400$  copies/ml at 24 weeks. The secondary endpoint was the change in pVL at 12 and 24 weeks from baseline.

Consecutive eligible patients were enrolled. This was a factorial study with two randomizations: genotyping (G+) versus no genotyping (G-) and expert advice (EA+) versus no expert advice (EA-), stratified by whether the patient had one (first failure group), two (second failure group) or three or more (third failure group) treatment failures.

This double randomization led to the definition of four treatment groups:

- Group 1: genotyping without expert advice (G+/EA-)
- Group 2: genotyping and expert advice (G+/EA+)
- Group 3: no genotyping and no expert advice (G-/EA-)
- Group 4: no genotyping with expert advice (G-/EA+).

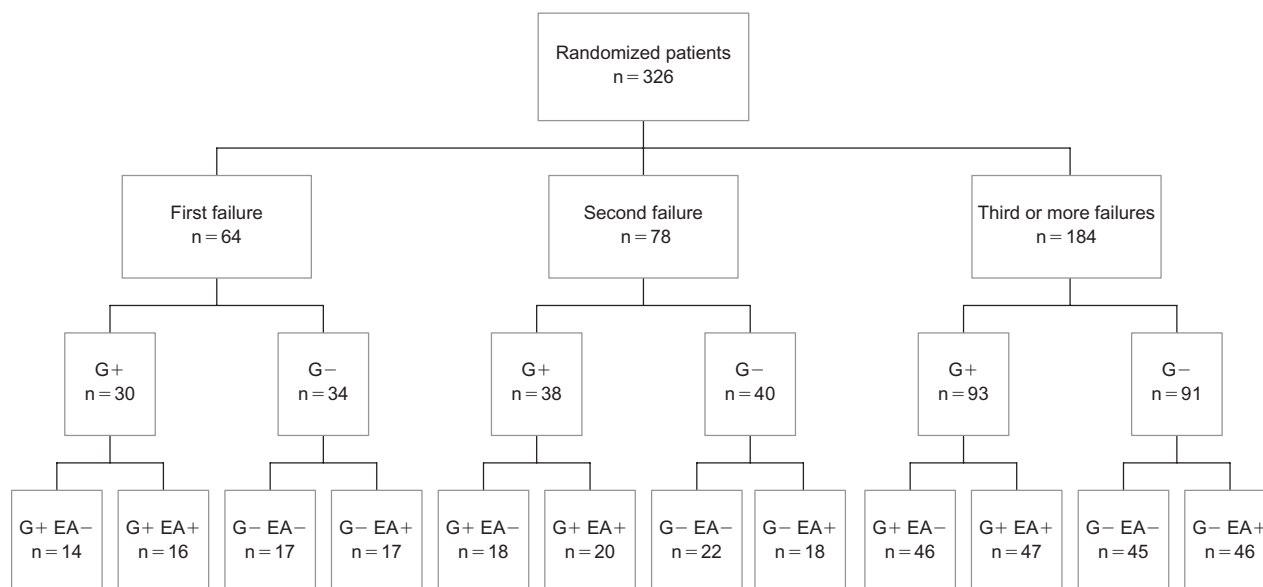
Patients were randomly assigned in a 1:1 ratio to the two levels of each factor. Randomization was carried out by the co-ordinating centre (Fig. 1).

### Genotyping groups (groups 1 and 2)

The genotyping test results were interpreted by a software (RetroGram<sup>®</sup>, version 1.0; Virology Networks, Utrecht, The Netherlands) which reported the description of all the reverse transcriptase and protease substitutions found and a ranking of the available drugs within each group depending on the pattern of mutation. This report was sent to the physician in less than 4 weeks after the randomization date. In addition to the genotyping results and interpretations provided, changes in therapy were managed according to the best clinical judgement and based on most recent published guidelines [28].

### Expert advice groups (groups 2 and 4)

The expert advisory committee was made up of four clinicians and two virologists all with more than 10



**Fig. 1.** Havana trial design with the total number of patients randomized. G+, genotype; G-, non-genotype; EA+, expert advice; EA-, non-expert advice.

years experience in the area of HIV clinical care or HIV-specific virology in a HIV Unit taking care of more than 2000 patients. This committee had information about the past pharmacological history, CD4 cell count and pVL evolution with previous treatment approaches, drug adverse events and adherence capability of the patient. All recommendations for therapy took into account the information provided (including the software interpretations of the genotyping test results when the patient was assigned to a G+ treatment group), panel member experience and published guidelines. The expert advisory committee decision was sent to the physician in less than 4 weeks after the randomization date.

### Control Group (group 3)

Patients assigned to group 3 were managed according to the best clinical judgement and based on most recent published guidelines [28].

### Study monitoring and enrolment

At baseline, pVL and CD4+ cell counts were taken along with demographic, previous pVL and CD4+ cell count, drug adverse event history, a subjective adherence assessment by the treating provider, concomitant medications and previous ARV history. Every 12 weeks the patients were asked to return for a pVL, CD4+ cell count, routine hematological and biochemical parameters and an assessment of adverse events. Physicians were allowed to change therapy if no virologic success was achieved (decrease from baseline  $< 0.5 \log_{10}$ ) at 12 weeks of follow-up.

### Software interpretation of genotypic results

The RetroGram<sup>®</sup> software accepts a list of substitutions in the protease and reverse transcriptase genes with respect to the NL4-3 reference strain. The application contains approximately 200 rules relating substitutions on the HIV-genome to reported effects on drug response. The rules are based on scientific literature on HIV-resistance and references associated with individual rules are available for display to the user. Based on the rules, the drugs are reported to the physician at one of the five suitability levels: (A) can be used; (B) consider if no class A drug is available; (C) consider use if no class A or B drugs are available; (D) consider if no class A, B or C drugs are available; and (U) unranked, insufficient data available. The drugs are then ranked within each group depending on the pattern of substitutions, with the drugs least likely to be associated with clinical resistance appearing at the top of each of the three lists.

### Laboratory measurements

Plasma HIV-1 RNA load and genotyping resistance testing were obtained from the same baseline plasma sample.

Viral RNA was extracted using the QIAamp viral RNA mini Kit (Quiagen, Barcelona, Spain) following the manufacturer's instructions. Plasma HIV RNA load was measured by the standard method used in each participating centre [either Amplicor<sup>®</sup> (Roche Diagnostic Systems, Barcelona, Spain) or NASBA<sup>®</sup> (Organon-Tecknika, Barcelona, Spain)].

Genotypic resistance testing was performed in the coordinating centre. All genotyping samples were sequenced on the TRUGENE 'HIV-1 Genotyping Kit and the OpenGene' (Visible Genetics, Madrid, Spain) automated DNA sequencing system. Briefly, the entire protease gene (codons 1–99) and codons 37–248 of the reverse transcriptase gene of HIV-1 were sequenced. Viral RNA was isolated from a plasma sample and converted to 1.3 kb cDNA. The cDNA was then amplified by polymerase chain reaction (PCR) in a single tube reverse transcriptase-PCR reaction. These reaction products were then added, without purification, to a set of sequencing reaction tubes. Sequencing were carried out using CLIP<sup>®</sup>, a DNA sequencing technique for direct sequencing of small quantities of amplified templates. Reaction tubes were prepared containing two oligonucleotide primers, each labelled with a different fluorescent dye. When hybridized to the cDNA, the primers were oriented to allow chain extension towards each other (one primer on the sense strand and one on the anti-sense strand). The reaction tubes also contained all reagents necessary for chain-extension, along with one chain-terminating ddNTP per tube. The reaction was initiated with the addition of the sample and a thermostable DNA polymerase. The vast majority of extension products ended with a chain-terminating nucleotide, although a minority of the reaction products were extended far enough to serve as a template for hybridization of the opposite primer. The reaction proceeded through 30 cycles. Upon completion of the cycling program a STOP solution (formamide + dye) was added to each reaction tube. The reaction products were then loaded onto the MicroCel<sup>®</sup> (Visible Genetics) cassette and electrophoresed. The products were then detected by a laser based detection system and analyzed using supplied software (GeneObjects<sup>®</sup>; Visible Genetics). The sequence was compared to a known, reference HIV-1 sequence (LAV-1) for determination of mutational patterns.

The classification of primary mutations for the statistical analysis was based on that made by Hirsch *et al.* [27].

### Antiretroviral treatments

During the trial there were six nucleoside reverse transcriptase inhibitors (NRTI), (didanosine, lamivudine, stavudine, abacavir, zalcitabine and zidovudine), two non-nucleoside reverse transcriptase inhibitors (NNRTI) (nevirapine and efavirenz), four protease inhibitors (PI) (indinavir, ritonavir, nelfinavir and saquinavir) and one miscellaneous drug (hydroxyurea) available for routine use in Spain. At the time of study enrolment ritonavir was only available in a liquid formulation.

Selection of the ARV medications used was ultimately decided by the practising physician with the additional

information provided by the study (expert recommendations and HIV-1 genotyping results).

### Statistical analysis

The number of patients enrolled provide 80% power to detect a treatment difference of 50% in percentage of patients with pVL < 400 copies/ml for the two factorial comparisons, using a 0.05 level test.

Primary analysis included all randomized patients (intention to treat analysis, ITT) and the last observation carried forward (LOCF) method was used to impute the information at week 24 for missing values. Per protocol (PP) analysis was performed as a secondary analysis and included all randomized patients with at least one measurement with the initial ARV approach.

The proportion of patients with pVL < 400 copies/ml, genotypic data, drug-related adverse events and ARV drugs used were tested using  $\chi^2$  analysis. All demographic data were tested using one way analysis of variance (ANOVA).

A repeated-measures ANOVA analysis was performed to compare the independent groups of patients when each of whom were subjected to repeated measurements of the same response variable (pVL and CD4 cell count) at three periodic visits (baseline, 3 and 6 months). Scheffé's test for multiple comparison was used for comparing each pair of group means. When statistically significant differences were detected among groups, a two-way ANOVA analysis was performed to test the visit effects at each group of treatment. Plasma HIV-1 RNA load values at 12 and 24 weeks that were below the limit of detection for each assay were transformed to 399 copies/ml before the analysis. Normality and variance homogeneity were assumed in the previous analysis. The significance level was fixed to 0.05.

A logistic regression was performed to identify factors associated with pVL < 400 copies/ml at 24 weeks follow-up. Associations of categorical independent variables with pVL < 400 copies/ml were assessed with  $\chi^2$  tests when variables or an interaction reached statistical significance. Failure group, HIV-1 genotyping, expert advice and all-possible two-way interactions between these variables were eligible for entry into a model. Using the method of maximum likelihood, estimated coefficients and their standard errors were calculated. After initial assessment of the performance of the model, variables and interactions were eliminated from the model, one at time, based on likelihood ratio tests eliminating non-significant ( $P > 0.10$ ) variables and interactions.

The statistical software used was the SAS system release

6.12 for Windows (SAS Institute Inc., Cary, North Carolina, USA).

## Results

Enrolment took place between March 1999 and February 2000. A total of 326 patients were randomized into the study (Fig. 1). A lower than expected number of patients were included in first and second failure groups due to the fact that genotyping became an useful tool for managing HIV-infected patients during the recruitment period, and the relative low rate of virologic failure in first and second groups.

Baseline characteristics were similar across all randomization groups (Table 1). Patients in the third failure group had a significantly higher pVL at baseline than those in the first or second failure groups [ $3.9 (\pm 0.8) \log_{10}$ ,  $3.9 (\pm 0.7) \log_{10}$  and  $4.2 (\pm 0.8) \log_{10}$ , respectively;  $P < 0.05$ ]. In addition, the third failure group patients were on ARV treatment and on PI-containing regimens for a significantly longer time [ $2.4 (\pm 1.7)$ ,  $3.5 (\pm 1.7)$  and  $5.4 (\pm 2.0)$  years on ARV therapy;  $P < 0.0001$ ; and  $1.1 (\pm 1.1)$ ,  $1.8 (\pm 1.1)$  and  $2.4 (\pm 0.8)$  years on PI-containing regimens,  $P < 0.0001$  for the first, second and third failure groups].

Patients in the G+ arms, had a mean of  $1.8 (\pm 1.4)$  primary mutations in the retrotranscriptase (RT) gene

and  $0.9 (\pm 1.0)$  primary mutations in the protease (P) gene per patient. There was a significant difference in the mean number of primary mutations between failure groups (mean RT primary mutations:  $0.9 (\pm 0.9)$ ,  $1.5 (\pm 1.2)$  and  $2.2 (\pm 1.5)$  in the first, second and third failure groups,  $P < 0.0001$ ; mean P primary mutations:  $0.4 (\pm 0.5)$ ,  $0.5 (\pm 0.6)$  and  $1.3 (\pm 1.1)$  in the first, second and third failure groups,  $P < 0.0001$ ). Overall, patients had a high prevalence of 190A (6.0%), 44D (7.5%), Y181C (11.9%), 118I (14.2); K103N (15.7%), K70R (17.2%), T215Y (34.3%) and M184V (55.2%) in the RT gene and D30N (5.2%), M84V (10.4%), 46I (17.9%), V82A (20.9%), and L90M (29.9%) in the P gene.

Expert advice treatment recommendations were followed by 81% of the patients randomized to EA+ arms. Prescribed therapies included a mean of  $4 (\pm 0.9)$  ARV drugs across all study subjects. Of note, the number of drugs prescribed varied between failure groups [ $3.4 (\pm 0.6)$ ,  $3.8 (\pm 0.7)$  and  $4.4 (\pm 0.9)$  in the first, second and third groups respectively,  $P < 0.0001$ ]. However, no differences were observed between genotype or expert advice randomized arms (G+,  $4 (\pm 0.9)$  versus G-,  $4 (\pm 0.8)$ ; EA+,  $4.1 (\pm 0.9)$  versus EA-,  $3.9 (\pm 0.9)$ ]. Moreover, the proportion of patients receiving efavirenz and a double PI-containing regimen including ritonavir differed between failure groups: (first, 21.8%; second, 35.9%; third, 51.3%;  $P < 0.0001$  for the double PI-containing regimen and first, 18.7%; second, 26.9%; third, 37.5%;  $P = 0.013$  for efavirenz) and between expert advice arms (EA+,

**Table 1.** Baseline characteristics in all the patients evaluated according to the genotype (G+/G-) and expert advice (EA+/EA-) randomizations.

	Genotype		Expert advice		All patients
	G-/G+	P	EA-/EA+	P	
Patients (n)	165/161		162/164		326
CDC stage (%)		NS		NS	
A	34.4/31.3		35.6/30.1		32.8
B	31.9/32.5		27.6/36.8		32.2
C	33.7/36.2		36.8/33.1		35.0
Mean age (years)	36.6/37.6	NS	37.5/36.6	NS	37.2
(SD)	(7.2)/(7.8)		(8.2)/(6.6)		(7.7)
Mean pVL ( $\log_{10}$ )	4.0/4.1	NS	4.1/4.0	NS	4
(SD)	(0.8)/(0.8)		(0.8)/(0.8)		(1)
Mean CD4+ ( $\times 10^6$ cells/l)	401/372	NS	376/398	NS	387
(SD)	(225)/(223)		(226)/(223)		(224)
Mean time on ARV therapy (years)	4.4/4.3	NS	4.4/4.3	NS	4.3
(SD)	(2.3)/(2.2)		(2.2)/(2.3)		(2.2)
Mean time on PI therapy (years)	2/2	NS	2/2	NS	2
(SD)	(1.1)/(1.1)		(1.1)/(1.1)		(1.1)
Pharmacological history (%)		NS		NS	
Only NRTI combinations	11.6/7.4		9.2/9.8		9.5
NRTIs+PI (first ARV regimen)	17.2/10.4		12.2/15.3		13.8
First PI regimen (with previous NRTIs)	22.5/17.4		23.3/17.1		20.2
$\geq 2$ PI-containing regimens	53.2/59.5		58.1/54.6		56.4
NVP exposure(%)	19.2/26	NS	19.8/25.4	NS	22.6

G+, genotyping group; G-, non-genotyping group; EA+, expert advice group; EA-, non-expert advice group; CDC, Centers for Disease Control and Prevention; pVL, plasma viral load; ARV, antiretroviral therapy; PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NVP, nevirapine; NS, not significant.

53.3% EA-, 30.6%  $P < 0.0001$  for the double PI-containing regimen and EA+, 23.9% versus EA-, 38.6%;  $P = 0.0041$  for efavirenz).

A sub-analysis within the third failure group showed differences in the mean number of prescribed drugs between expert advice arms [EA+, 4.5 ( $\pm 0.9$ ) and EA-, 4.2 ( $\pm 1$ );  $P = 0.038$ ] but not between the genotyping arms [G+, 4.35 ( $\pm 1$ ) versus G-, 4.41 ( $\pm 0.1$ )] and in the proportion of patients receiving a double PI-containing regimen including ritonavir between the expert advice arms (EA+, 64.1% versus EA-, 39.1%;  $P = 0.0007$ ).

At 24 weeks the proportion of patients who showed pVL  $< 400$  copies/ml differed between ARV stratification groups in the ITT analysis ( $P < 0.0001$ ). Likewise, differences were found in the percentage of patients with pVL  $< 400$  copies/ml between genotyping arms ( $P < 0.05$ ) but not between expert advice arms (Fig. 2).

A multivariate analysis including genotyping, expert advice, stratification group as independent variables, and all possible two-way interactions between them stated that the factors associated with a higher probability of pVL  $< 400$  copies/ml at 24 weeks follow-up were HIV-1 genotyping and the expert advice in patients failing to a second line ARV therapy; whereas patients in the third failure group had a higher probability of virologic failure (Table 2).

A significant pVL decrease from baseline was observed between failure groups and the two genotype arms in the combined 12 and 24 week analysis ( $P < 0.05$ ) but not between expert advice arms (Fig. 3).

Further exploratory analysis in the third failure group showed that there was no significant difference in the proportion of patients with plasma HIV-1 RNA  $< 400$  copies/ml at 24 weeks among patients randomized to receive genotype or not and those randomized to receive expert advice or not (G+, 37.9% versus G-, 24.7%; EA+, 29.3% versus EA-, 33.7%). However, in this third failure group significant differ-

ences were observed in the mean decrease of pVL in the combined at 12 and 24 week analysis between genotype randomized arms [G+ mean decrease of  $-0.84 (\pm 0.9)$  and  $-0.7 (\pm 0.9)$  at 12 and 24 weeks versus G- mean decrease of  $-0.7 (\pm 0.7)$  and  $-0.5 (\pm 0.7)$ ,  $P = 0.039$ ] but not between EA randomized arms [EA+ mean decrease of  $-0.80 (\pm 0.8)$  and  $-0.66 (\pm 0.8)$  at 12 and 24 weeks versus EA- mean decrease of  $-0.80 (\pm 0.8)$  and  $-0.66 (\pm 0.8)$ ]. No significant differences were observed in the first and second failure groups.

In addition, we also evaluated the main endpoint of the Havana trial by a PP analysis. At 24 weeks significant differences were found in the proportion of patients with pVL  $< 400$  copies/ml between stratification groups ( $P < 0.0001$ ), genotyping arms ( $P < 0.05$ ) and expert advice arms ( $P < 0.05$ ) (Fig. 2).

A multivariate analysis including genotyping, expert advice and failure group as independent variables, showed that genotyping [odds ratio (OR), 1.92; 95% confidence interval (CI), 1.16–3.17;  $P = 0.011$ ] and expert advice (expert advice: OR, 2.13; 95% CI, 1.3–3.53;  $P = 0.003$ ) were associated with a higher probability of pVL  $< 400$  copies/ml at 24 weeks whereas the third failure group was negatively associated with pVL  $< 400$  copies/ml at 24 weeks (OR, 0.30; 95% CI, 0.16–0.59;  $P = 0.0004$ ).

The proportions of patients that dropped out from the study between baseline and week 12 were 12.8% due to drug adverse events that required a change in the initial approach and 1.8% because of loss to follow-up. The distribution of those 48 patients among failure groups and the two randomization arms was similar.

Twenty eight (8.5%) patients were lost to follow-up following the 12 week endpoint, twenty two (6.7%) experienced a drug-related adverse event, ten (3%) changed therapy because of virologic failure and two (0.6%) died. No differences were observed in the proportion of patients that discontinued the initial approach between the 12 and 24 weeks of follow-up among failure groups or genotype or expert advice randomized arms.

**Table 2.** Factors associated with plasma HIV-1 RNA  $< 400$  copies/ml at 24 weeks in a multivariate analysis.

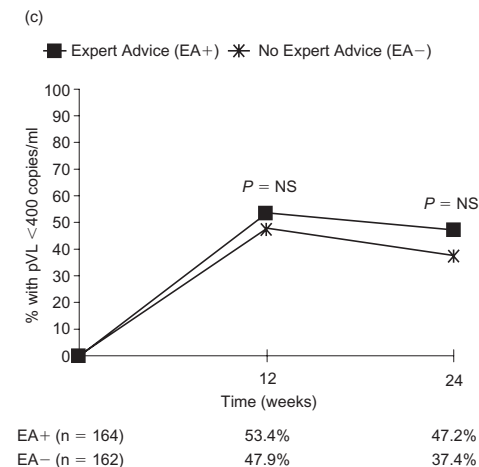
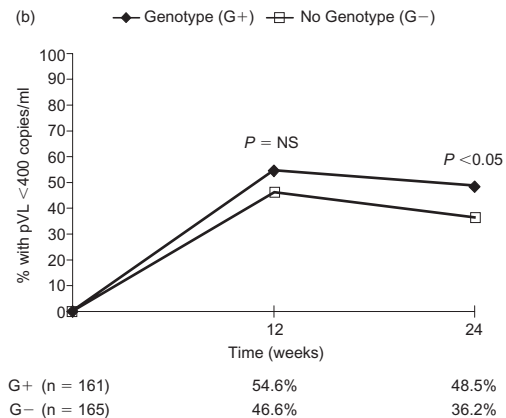
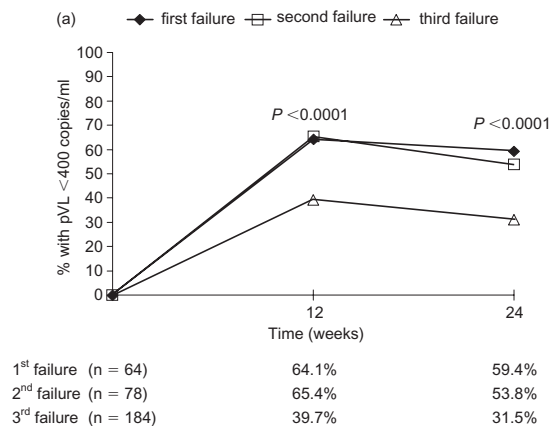
	OR	95% CI	P
All patients (n = 326)			
Genotype	1.7	1.1–2.8	0.016
Second failure	0.4	0.2–1.0	0.057
Third failure	0.3	0.1–0.5	0.0001
EA * second failure <sup>a</sup>	3.2	1.2–8.3	0.016

<sup>a</sup>Interaction between expert advice and second failure group. OR, odds ratio; CI, confidence interval; EA, expert advice.

## Discussion

In the present study, we have found a significantly better virologic outcome (percentage of patients with pVL  $< 400$  copies/ml and decrease in pVL from baseline) at 6 months follow-up, in those patients randomized to guide the change of therapy according to the genotypic-resistance testing results than in those who received a change in therapy based on standard of care.

1) Intention to treat analyses



2) Per protocol analyses

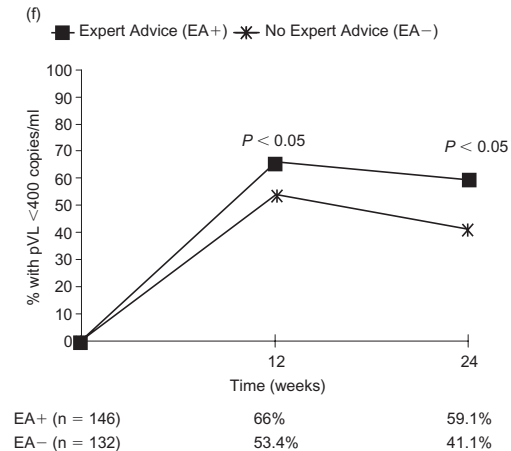
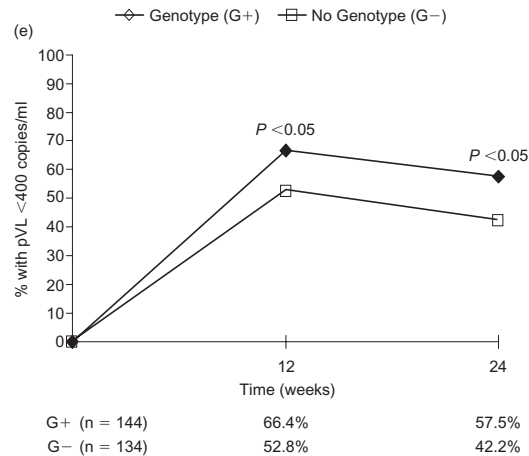
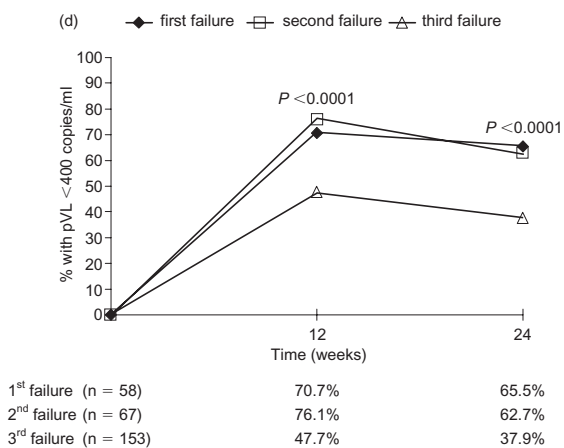
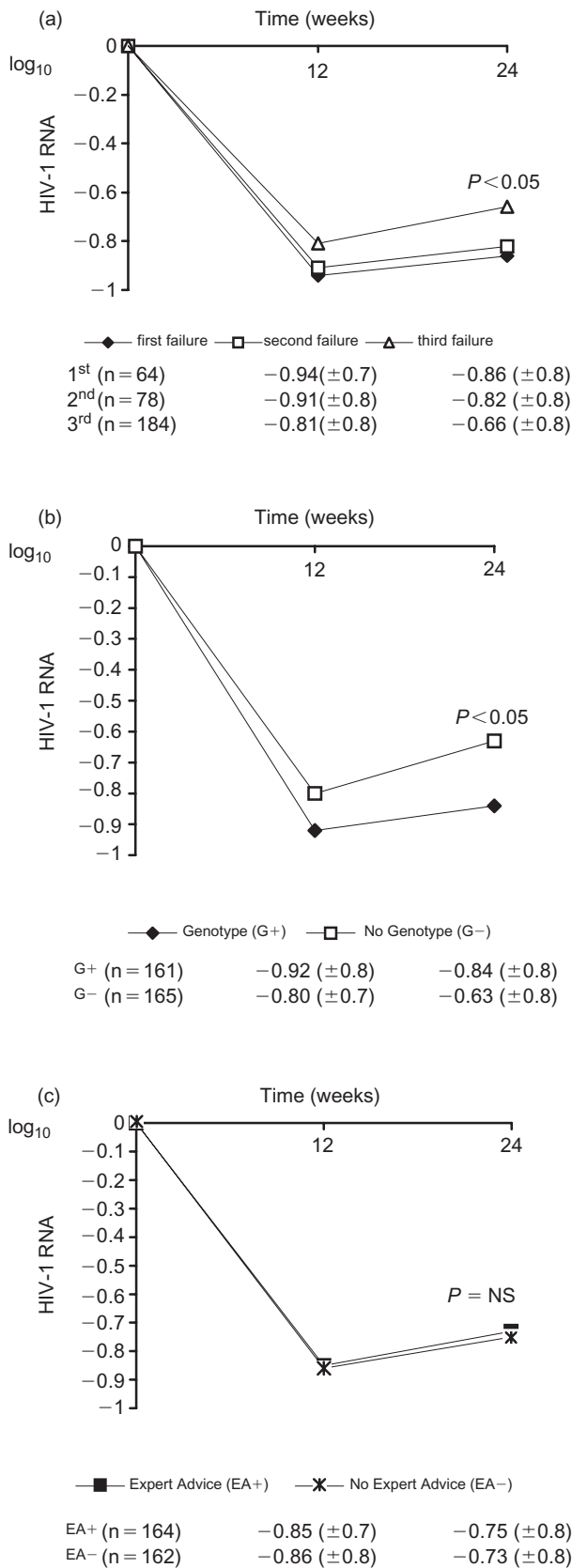


Fig. 2. Proportion of patients with plasma HIV-1 RNA below 400 copies/ml by intention to treat (1) and per protocol (2) analyses according to failure groups (figures a and d); genotype (figures b and e); and expert advice (figures c and f)



**Fig. 3.** Mean decrease of plasma HIV-1 RNA from baseline by intention to treat analysis according to (a) failure groups, (b) genotyping groups, and (c) expert advice groups.

In addition, in a multivariate analysis, we observed in the second failure group a beneficial effect of the expert advice recommendations for achieving undetectable pVL. Finally, in the third failure group, significant differences were found in the mean reduction of pVL in those randomized to a genotype arm (ITT).

When we analysed the results without considering those lost to follow-up during the first 12 weeks, (PP analysis) the outcome supports HIV-1 genotyping and expert advice recommendations as being of virologic benefit at both 12 and 24 weeks of follow-up.

As has been shown previously in both retrospective and some prospective trials, we also found that genotyping was superior to no genotyping in virologic outcomes at 24 weeks [12–14]. However, not all the studies aimed to show the benefit of resistance testing have been successful and some of them have not demonstrated any improvement in the virologic outcome when using either genotype or phenotype [15,29].

An important feature of this study was the demonstration by PP analysis and in the second failure group, by ITT, of virologic benefit from the use of expert advice recommendations for changes in ARV therapy. Previous studies relating to HIV therapy had shown differences in the treatment approaches provided by experienced HIV providers, cost savings and the potential for improved outcomes [12,21–23]. However, this is the first trial to show a direct virologic benefit from the incorporation of expert advice into the clinical care of HIV infected patients. The randomization of patients to EA+ and EA– arms in the Havana study, as well as G+ and G–, allowed us, in the PP analysis, to determine that both genotypic testing and expert advice can contribute individually to improved virologic outcomes. As it is hard to determine whether these effects were additive or synergistic, it may be prudent at this time to consider both these interventions as independently beneficial to patient management.

It is difficult to establish if the influence of the expert committee advice in the proportion of patients with HIV-1 RNA < 400 copies/ml in the Havana trial was due to their higher knowledge on resistance issues or to their excellence in the overall management of HIV-1 infection. However, the lack of significance of the interaction between genotype and expert advice in the multivariate analysis and the current limitations in the ARV drug arsenal to overcome resistance once it is acquired (lack of drugs with alternative resistance patterns or drug families with alternative sites of action) suggests that the impact of the expert committee recommendations on our study results could be primarily due to their overall experience in the management of HIV infection.

The complexity of HIV disease and antiretroviral treatment, the rapidly updating guidelines to HIV patient management and the specialized nature of HIV therapy are all potential causes for the benefit of expert advice. The challenges of incorporating resistance-testing data into routine patient management may magnify these needs.

All patients receiving genotypic analysis had result interpretations provided by a specialized software program. Such software does not propose a specific therapeutic strategy to follow, but establishes a ranking of suitability of the different drugs based on the existing pattern of resistance mutations. The individualized information for each drug, allows the clinician to select the most suitable treatment considering other clinical variables (adverse effects, toxicity, adherence, etc), that are as relevant as drug resistance for the tailoring of salvage therapies.

Our results provide evidence that even where expert advice is not available, genotyping with automated interpretation is superior to no testing. As no patients received genotyping without software interpretation, the relative contribution of automated interpretation cannot be determined.

Although Gart[12], Viradapt[13] and the Havana studies have all demonstrated the utility of genotyping for the design of salvage therapy in the short-term; inherent differences between the three studies make the comparison of their respective results difficult.

The Gart study incorporated expert advice into the interpretation of genotyping results but it was impossible to separate out the effects that genotyping and expert advice had independently, as all patients received both interventions. Another aspect that makes comparisons of the results difficult is the different follow-up periods (8 weeks in the Gart study and 24 weeks in the Havana) [12].

As compared to the Viradapt trial [13] the Havana study had a greater proportion of patients below limit of detection, whereas the Viradapt trial had a greater difference between the two arms at 6 months (Viradapt: 32% genotyping versus 14% standard of care, difference 18% Havana: 48.5% genotyping, 36.2 % non-genotyping, difference 12.3%). The differences seen may be due to sample sizes, differences in treatments available and differences in genotype interpretation or statistical analysis [13]. In addition, the results from the genotyping arm in the Havana trial may be influenced by the software interpretation of the genotype results and by the interpretation by the expert committee to a greater or lesser degree.

The difference in the time periods when the Gart,

Viradapt and Havana studies were developed is relevant. This is mainly due to the progressive knowledge acquired by clinicians on drug resistance management and the prognostic factors of virologic response not related only to the intrinsic potency of antiretrovirals (adherence, therapeutic drug monitoring, etc) [14,29].

The appropriate use of HIV genotypic resistance testing in clinical practice will require better understanding of its utility in different populations. The Gart, Viradapt and Narval studies failed to address this issue [12,13,15]. However, in the present study we found significant improvement of the virologic outcome in the reduction of pVL when therapeutic choice was guided by genotyping in the most drug-experienced group (three or more combination therapy failures).

Although further work is required to better define this issue, it may not be appropriate now to assume genotyping is of reduced benefit in highly drug experienced patients [16].

The importance of expert interpretation of resistance assay results highlighted in this trial gives a particular sense of urgency to increased access to experts as well as training of clinicians in the area of HIV-1 resistance. As the widespread use of resistance testing in general and specifically genotyping becomes more common, there may be a patient benefit to education efforts directed towards the training of the HIV-treating clinicians to become familiar and comfortable with the area of interpretation of genotypes.

In summary, the Havana trial has demonstrated that both genotyping interpreted by a software and expert advice may improve short-term virologic outcome in HIV-infected patients. The relative utility of genotypic testing in different drug experienced population needs further defining. Our findings provide further support for the widespread use of resistance testing in pre-treated patients to achieve optimal care.

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## Appendix

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