Background: This study compared the predictive value for treatment failure of extended resistance detected in the current genotype resistance test (GRT) versus those from GRT history in patients with multiple combination antiretroviral therapy (cART) failures.

Methods: Patients who underwent three GRT between 1999 and 2007 were included. Extended resistance at genotypic sensitivity score (GSS) using the Rega 7.1 interpretation system compared with a non-standard definition (defined as class-wide resistance [CWR] on the basis of International AIDS Society–USA mutations) was assessed both for current and historical GRTs (a combination of mutations was detected in all three tests). The predictive role of extended resistance for treatment failure was evaluated with an adjusted Cox proportional hazard model.

Results: Overall, 177 patients were included. The historical GRT increased the number of patients with extended resistance to all three major drug classes by 25% in comparison with the current GRT. Using the GSS method, the absence of detection of any active drug in any drug class was predictive of failure with both the current and historical GRTs. Similarly, the number of active drugs in the cART regimen after the third resistance test, used as continuous variable, was also predictive of failure. Using both GSS approaches, current genotype had a higher effect than historical genotype on risk of treatment failure. Using the non-standard definition (CWR), historical resistance predicted failure better than current resistance.

Conclusions: Our results provide an epidemiological demonstration that analysis of a combined latest and historical GRT, which also considers archived mutations, might better identify the more virologically impaired patients in order to assess the best salvage treatment.

Original article

Historical resistance profile helps to predict salvage failure

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Introduction

Because of the demonstrated usefulness in clinically monitored studies [1–3], and although such usefulness was initially debated [4], genotype resistance tests (GRTs) have become the standard of care and are currently recommended to guide treatment failure [5]. In previous papers, our research group focused on cross-resistance to antiretroviral drug classes [6]. Cross-resistance can be defined as resistance to drugs to which a virus has never been exposed within a given class of antiretroviral agents. It has been found to affect all three classes of antiretroviral drugs [7,8]. By contrast with single-drug resistance, cross-resistance might result in a class-wide resistance (CWR), which could substantially reduce the clinical utility of antiretroviral drugs of the same class, seriously affecting future treatment options and even survival of HIV patients [9–14].

However, in patients undergoing multiple virological failures, the mutation patterns might continuously change. New resistance mutations are constantly developing in patients maintained on virologically ineffective combination antiretroviral therapy (cART) regimens [15]. Consequently, in patients with multiple failures, the extent of drug resistance detected at the last GRT might be underestimated, as several mutations associated with previous treatments might not be detected [16]. In case of extended resistance to ≥1 drug classes, neglecting archived mutations (which were not found at the last GRT, but detected previously) might lead to a rapid failure and even to disease progression [17].
Managing resistance related to old cART regimens remains difficult and is still unclear. Therefore, in order to estimate whether the resistance profile accounting for failure of earlier antiretroviral regimens might help to predict salvage failure, we compared the predictive value for treatment failure after salvage therapy for resistance detected in the latest genotype versus historical genotype in patients who had multiple cART failures.

Methods

Study design
The study design was a prospective observational cohort analysis of a database of all patients who underwent GRT after cART failure in the National Institute for Infectious Diseases Lazzaro Spallanzani (Rome, Italy), the largest reference centre in Rome for HIV treatment. All patients who had GRT performed in the Institute between January 1999 (the date when routine GRT at highly active antiretroviral therapy [HAART] failure was started) and June 2007 were included. The follow-up was carried out until December 2007.

Study participants
From the database, patients who underwent three GRTs, all in the course of treatment failure during the observation period, were considered in the analysis. Resistance tests executed during treatment interruption were not taken into account in order to avoid overgrowth of more wild-type-like virus and apparent disappearance of mutations. For every patient, a complete clinical history, including previous treatments, was available. The response to treatment after the third resistance test was also assessed.

Genotype resistance testing
Direct sequencing of reverse transcriptase and protease genes was performed using the ViroSeq™ HIV-1 Genotyping System (versions 1 and 2; Abbott Laboratories, Abbott Park, IL, USA) and the 3100 ABI Sequencer (Applied Biosystems, Foster City, CA, USA). Briefly, RNA was extracted from plasma samples, reverse transcribed and amplified with specific primers. The PCR products covered the whole pol region coding for all amino acids of the protease, including the first 335 amino acids of reverse transcriptase, which provide the sequence where the majority of (and most relevant) mutations conferring resistance to antiretrovirals are found.

Follow-up
Follow-up started at the third GRT. For each patient, the genotype-guided therapy in our setting was usually established by the reference physician on the basis of indications of the active drugs and of the extended interpretation of the resistance test routinely provided by the virologists, together with response of the test. The most controversial resistance tests are discussed weekly in a setting including both physicians and virologists, and the best treatment for these patients is usually decided, taking into account their behaviour and adherence characteristics. This discussion panel works within the Institute as expert advice. The subsequent clinical course, including the nature and timing of laboratory testing, was entirely under the responsibility of the treating physician.

During follow-up, time to virological failure was assessed and defined as time to the first of two consecutive detectable HIV RNA measures (>50 copies/ml) within 6 months after the third GRT. HIV RNA was determined using a branched DNA method and the limit of detection of 50 copies/ml was the standard limit in our centre during the whole period of the study. The specimen were regularly shipped in EDTA tubes and this method did not change during the study period.

Definition of current and historical genotype resistance tests
After the third resistance test, we compared the predictive value for virological failure of resistance to the three main classes of antiretroviral drugs (nucleoside reverse transcriptase inhibitors [NRTIs], non-NRTIs [NNRTIs] and protease inhibitors [PIs]), detected using only the last test (defined as current GRT) as compared with all three resistance tests executed (defined as historical GRT).

Definition of extended resistance
To assess the degree of resistance to antiretrovirals, we used the genotypic sensitivity score (GSS) as a standard definition and a non-standard definition, defined as CWR, which is based on mutations indicated as primary mutations by the International AIDS Society–USA consensus. Although this definition is non-standard and possibly updated, it can be useful as a comparison term.

Genotypic sensitivity score
At the third GRT, two further analyses using a standard interpretation system (Rega version 7.1; Rega Institute, Katholieke Universiteit Leuven, Leuven, Belgium) were also performed.

The overall number of drugs predicted to be active at the time of third GRT was assessed, taking into account the availability of the drug at that date through an early access programme or, in absence of an early access programme, at the date of license in Italy. The absence of any active drug (GSS<1) in any of the three major drug classes was tested as predictive of failure (GSS total). Furthermore, the number of drugs predicted to be active...
among those received as salvage regimen after the third GRT was also tested as predictive of failure (GSS).

**Class-wide resistance**

CWR for the major drug classes was set as ≥3 major mutations for NRTIs and PIs and ≥1 for NNRTIs. Specifically, for NRTIs these included ≥3 mutations from among M41L, E44D, D67N, K70R, V118I, L210W, T215Y/F and K219Q/E (nucleoside analogue mutations); L74V (resistance to didanosine); M184V/I (resistance to lamivudine and emtricitabine); and K65R, Q151M and T69I (resistance to >1 NRTI). For PIs, these included ≥3 mutations among D30N, V32I, L33F, M46I/I, I47V/A, G48V, I50L/V, I54L/M, V82A/F/T/S, L89G and L90M. For NNRTIs, these included ≥1 mutation among L100I, K103N, V106A/M, Y181C/I, Y188L and G190A.

**Statistical analyses**

The predictive role of GSS and CWR at current and historical GRT for treatment failure was calculated using a Kaplan–Meier survival analysis and log-rank test (unadjusted analysis) and with a Cox proportional hazard model (adjusted for CD4+ T-cell count, HIV RNA and AIDS diagnosis at third resistance test).

**Results**

Overall, 177 patients who were tested 3× for resistance after treatment failure were included. The characteristics of patients are described in Table 1. Patients were treatment-experienced: the median previous treatment time was close to 4 years and the median number of previous drugs used was eight.

The frequency of extended resistance measured with GSS was higher using the historical GRT in comparison with the current GRT. In particular, the proportion of patients without any active drug in all three classes (GSS total) detected with the historical GRT was 25% higher than with the current GRT (5.1% versus 4.0%, respectively); but it was lower than that observed using the CWR method.

The number of active drugs used in the salvage regimen after the third resistance test (GSS) was lower considering mutations detected at historical GRT (median 2 versus 2.5 at current GRT). Similarly, the proportion of patients carrying CWR to all three drug classes was found at a higher frequency (29.4% at historical GRT versus 13% at current GRT).

Considering single resistance mutations, M184V was the most represented for NRTIs, with the largest difference between historical and current GRT (75.7% versus 39.3%, respectively), followed by M41L (53.1% versus 35.1%), T215Y (52.0% versus 31.1%), D67N (49.2% versus 36.2%), L210W (33.3% versus 24.3%) and K70R (31.1% versus 20.9%). The K65R mutation, although infrequently found, showed the largest difference of proportion between historical and current GRT (4.6% versus 1.2%).

Among NNRTI resistance mutations, the highest frequency was associated with the K103N mutation, which also showed the largest difference between historical and current GRT (48.0% versus 23.3%, respectively), followed by Y181C (23.1% versus 13.5%) and G190A (20.3% versus 11.3%).

Among PI resistance mutations, L90M was found to be the most represented mutation (50.9% versus 35.1%)
for historical and current GRT, respectively), followed by V82A (32.8% versus 19.8%), P46I (31.6% versus 20.3%), I84V (19.8% versus 14.2%) and L33F (10.8% versus 7.1%).

A treatment interruption of >30 days for any reason was recorded for 43 patients (24.3%), whereas 30 patients (16.9%) had a wild-type GRT after an interruption of treatment >3 months. This resistance test was not considered in order to assess historical resistance. Both treatment interruption (odds ratio [OR] 0.8, 95% confidence interval [CI] 0.5–1.2; P<0.001) and wild-type reversion (OR 1.1, 95% CI 0.7–1.7; P<0.001) were not significant in the univariate analysis and were not included in the multivariate analysis.

After a mean observation of 13 months (interquartile range 6–72), viral rebound was observed in 143 patients (80.8%) and the overall probability of treatment failure was 69% after 1 year in the survival analysis.

Using the GSS total method, the absence of detection of any active drug in any drug class was associated with a higher probability of failure at survival analysis after 1 year (100% versus 70% for current GRT and 100% versus 68% for historical GRT). This association was significant in the multivariate analysis both for current (hazard ratio [HR] 4.67, 95% CI 1.92–11.35; P<0.001) and historical (HR 2.23, 95% CI 1.09–4.33; P<0.027) GRT as shown in model 1 of Tables 2 and 3.

The number of active drugs in the cART regimen after the third resistance test, used as a continuous variable, was also predictive of failure (model 2, Tables 2 and 3).

Table 2. Probability of failure for GSS total, GSS and CWR at current GRT using the Cox proportional hazard model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Model 1 (GSS total)</th>
<th>Model 2 (GSS)</th>
<th>Model 3 (CWR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Adjusted</td>
<td>Adjusted</td>
<td>Adjusted</td>
</tr>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
<td>HR (95% CI)</td>
<td>P-value</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSS total</td>
<td>4.33 (1.98–9.48)</td>
<td>&lt;0.001</td>
<td>4.67 (1.92–11.35)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>0.74 (0.64–0.85)</td>
<td>&lt;0.001</td>
<td></td>
<td>0.71 (0.59–0.84)</td>
</tr>
<tr>
<td>CWR, 3 versus &lt;3</td>
<td>1.55 (0.97–2.47)</td>
<td>0.064</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CDC C stage</td>
<td>1.45 (1.04–2.02)</td>
<td>0.028</td>
<td>1.15 (1.00–2.29)</td>
<td>0.048</td>
</tr>
<tr>
<td>Log_{10} HIV RNA at third GRT</td>
<td>1.17</td>
<td>0.056</td>
<td>1.07 (0.88–1.31)</td>
<td>0.93–1.43</td>
</tr>
<tr>
<td>CD4+ T-cell count at third GRT</td>
<td>0–200 cells/mm³</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>&gt;201–350 cells/mm³</td>
<td>1.18</td>
<td>0.187</td>
<td>1.00</td>
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<tr>
<td></td>
<td>&gt;350 cells/mm³</td>
<td>0.98</td>
<td>0.850</td>
<td>1.06</td>
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</table>

Failure was considered as time to first of two consecutive HIV RNA>50 copies/ml within 6 months after third genotype resistance test (GRT). Data in bold are statistically significant. No drug class with ≥1 active drug available at genotypic sensitivity score (GSS) versus ≥1 drug class with ≥1 drug available. HR, hazard ratio.

Discussion

Our results suggest that extended resistance is a crucial marker of virological failure in treatment-experienced patients, overcoming the importance of viral load, immunological and clinical status, thus indicating that the availability of active drugs is the key issue for treatment success.

Our analysis shows that there is a considerable difference between resistance-associated mutations.
found in current genotypes and those found in historical genotypes, confirming that background treatment information is vital for full characterization of the HIV antiretroviral drug resistance profile of a patient. Indeed, it is important to note that current guidelines correctly indicate that the results of prior genotype resistance tests should be fully considered before changing HAART because of virological failure [18].

Furthermore, although extended resistance detected in the latest GRT remained the main predictor of failure, our analysis also indicated that the identification of the archived mutations is useful to assess the effective regimen after failure. This can be done in clinical practice by considering the whole treatment history, including the resistance mutations detected from previous treatment failures.

It has long been recognized that latent forms of resistant viruses might persist in resting CD4+ T-cells. This reservoir of latently infected CD4+ T-cells corresponds to an archive of the viral genotypes that have been produced in an infected individual during the course of the disease. Patients with multiple virological failures maintain, in this latent reservoir, viruses with drug resistance mutations that have been generated during the treatment history of previous failing regimens [19].

Archived viruses that harbour resistance mutations are able to reinitiate active viral replication and to re-emerge in the setting of changed selective pressure. Apparent losses in resistance mutations and gains in phenotypic susceptibilities in patients with detectable viral load on HAART have already been described [20].

However, because resistance mutations are known to persist indefinitely at low levels or in the latent reservoirs, these mutations might quickly re-emerge if the new therapy is not fully suppressive. For instance, our findings confirm results of an earlier Canadian study [17] that analysed current and historical mutations in 1,734 treatment-experienced patients with ≥3 GRT between 1996 and 2004. They found that the most recent GRT consistently underestimated historical resistance mutation prevalence, especially for nucleoside analogue mutations. For example, current genotypes detected the M184V/I mutations in 25.5% of samples versus 58.8%.

Table 3. Probability of failure for GSS total, GSS and CWR at historical GRT using the Cox proportional hazard model

<table>
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<tr>
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<td></td>
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<td>(95% CI)</td>
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<tr>
<td>GSS totalb</td>
<td>2.63</td>
<td>0.006</td>
<td>2.23</td>
<td>0.027</td>
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<tr>
<td></td>
<td>(1.32–5.22)</td>
<td>(1.09–4.53)</td>
<td>2.65</td>
<td>0.021</td>
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<td>GSSa</td>
<td>0.80</td>
<td>0.014</td>
<td>0.80</td>
<td>0.039</td>
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<td></td>
<td>(0.67–0.96)</td>
<td>(0.65–0.99)</td>
<td>0.79</td>
<td>0.035</td>
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<td>CWR, 3 versus &lt;3</td>
<td>1.87</td>
<td>0.001</td>
<td>1.48</td>
<td>0.060</td>
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<td></td>
<td>(1.31–2.66)</td>
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<td>(1.01–2.35)</td>
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<td>(0.86–1.29)</td>
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<td>(0.84–1.26)</td>
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Failure was considered as time to first of two consecutive HIV RNA >50 copies/ml within 6 months after third genotype resistance test (GRT). Data in bold are statistically significant. No drug class with ≥1 active drug available at genotypic sensitivity score (GSS) versus ≥1 drug class with ≥1 drug available. For each active drug in the post-GRT regimen. CI, confidence interval; CWR, class-wide resistance; HR, hazard ratio.

Historical resistance helps to predict failure.
that this definition is flawed or outdated; however, these findings also suggest that further analyses are necessary to better define the role of actual and archived mutations for failure.

Moreover, because the re-emergence of the extended resistance cannot be overcome with potent and complex treatment regimens, an aggressive management must be used [22,23].

In our cohort of treatment-experienced patients, the proportion of patients failing treatment after 6 months of a third genotype-guided therapy were considerably high and increased with extended resistance to all three major antiretroviral classes. However, our rate of failure is comparable to that found in clinical trials performed in treatment-experienced patients previous to the availability of new drug classes that occurred in the past 2 years [22].

For this purpose, an extraordinary number of new antiretroviral drugs and new drug classes came into use during 2007 and 2008, including boosted PIs and NNRTIs with high genetic barriers [23–25] as well as drugs belonging to three new drug classes (entry inhibitors, CCR5 inhibitors and integrase inhibitors [26–29]) that were generally not available for patients included in this study at the time of failure.

Our study might have some limitations. First, data were obtained from an observational database of a single centre and not from a clinical trial. However, the quality of data collected retrospectively was accurate, as all the information regarding the clinical history for each patient was routinely collected and input in the database. Second, the genotype-guided regimen was chosen for each patient by the reference physician, which could have led to different treatment approaches, although the discussion of most complex genotype tests, which is routine in our centre, was useful to uniform the treatment decisions. Finally, the study lacks a validated measure of adherence. Poor adherence was associated with an enhanced probability of virological failure, increased probability of most complex genotype tests, which is routine in this study at the time of failure.

Our results provide an epidemiological demonstration that analysis of a combined current and historical GRT, which also takes into consideration archived mutations, might allow better identification of the more virologically impaired patients in order to assess the best salvage treatment.

Disclosure statement

The authors declare no competing interests.

Additional file

An additional file listing contributing members of the Collaborative Group for Clinical Use of HIV Genotype Resistance Test (GRT) at National Institute for Infectious Diseases Lazzaro Spallanzani in Rome can be accessed at www.intmedpress.com

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