The use of human immunodeficiency virus resistance tests in clinical practice

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Abstract

Important progress has been made in recent years in the development and clinical use of drugs for the treatment of human immunodeficiency virus type 1 (HIV-1) infection. Nevertheless, when antiretroviral therapy fails to be fully suppressive, new viral variants emerge, thus allowing HIV-1 to escape from drug pressure by accumulating mutations. Between 50% and 70% of treated patients with virological rebound harbour some form of drug-resistant virus; transmitted drug resistance in drug-naïve populations has reached 5–20% in areas of the world with access to treatment. The emergence of drug-resistant viruses remains the limiting factor in HIV-1 management, being a major cause of treatment failure, and being associated with clinical progression and death. All international guidelines focus on the importance of tailoring antiretroviral therapy to the individual patient, on the basis on HIV-1 genetic data, integrated with clinical, laboratory and therapeutic information. The aim of this review is to provide useful information to clinicians and virologists about how and when to use genotypic resistance testing in clinical practice, especially in the management of the first stages of HIV-1 patient care and treatment decisions.

Keywords: Antiretroviral resistance, antiretroviral treatment, clinical practice, genotype, human immunodeficiency virus, review

Article published online: 20 August 2010

Clin Microbiol Infect 2010; 16: 1511–1517

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Introduction

From the 1980s, stunning advances in antiretroviral therapy have been made: human immunodeficiency virus type 1 (HIV-1)-infected patients are today experiencing increased longevity, reduced HIV-1-related complications, and improved quality of life. However, severe adverse effects, therapy adherence and the evolution of drug-resistant viruses are frequently compromising virological and clinical outcomes.

Today, the main aim of treatment is to achieve maximum and durable suppression of viral replication (HIV-1 viral load, <50 copies/mL), with a combination of antiretroviral agents to which the virus is susceptible [1–3]. Failure to achieve this result leads to the emergence of drug-resistant variants, which are significantly associated with poorer survival and increased risk of death [4,5]. Monitoring of drug resistance is thus of the utmost importance and, in the developed world, resistance testing is now a standard of care in HIV-1 infection management. All guidelines recommend appropriate use of these tests, with the aim of helping clinicians in setting up a correct and individualized therapeutic strategy. However, the widespread distribution of HIV-1 infection in different areas, with different economic and social settings, generates difficulties in defining optimized screening and treatment protocols, in terms of finite budgets, expertise and time constraints. The aim of this review is to provide useful information to clinicians and virologists regarding how and when to use genotypic resistance testing in clinical practice, especially in patients starting their first therapeutic regimen.

Use of Resistance Assays in Clinical Practice

The goal of resistance testing is to identify, in clinical samples, viral variants harbouring mutations causing or contributing to drug resistance, and thus to provide information to
assist in the selection of active antiretroviral regimen(s) that are more likely to achieve and to maintain viral suppression. Genotypic resistance testing is generally preferred because of the faster turn-around time, lower cost, and enhanced sensitivity in detecting mixtures of wild-type and resistant viruses. The test is based on traditional population (Sanger) sequencing, and is able to detect quasi-species representing, on average, at least 20% of a viral population. However, for patients with a complex treatment history, results derived from both genotypic resistance tests (GRTs) and phenotypic resistance tests might provide critical and complementary information to guide regimen changes [1]. Once the viral gene target sequence has been obtained, several interpretation algorithms are freely available with which to analyse the resistance for all approved nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and integrase inhibitors (INIs): the ANRS (National AIDS Research Agency) drug resistance interpretation algorithm (http://www.hivfrenchresistance.org/), the HIVdb drug resistance interpretation algorithm (http://hivdb.stanford.edu/), or the Rega Institute Drug-Resistance Interpretation Algorithm (http://www.rega.kuleuven.be).

Fig. 1 shows an example of a ‘real-life’ working routine in a virology centre. The histograms represent the number of GRTs performed in the last 10 years in our laboratory, the largest reference centre in Rome, and its surrounding area for HIV-1. Not surprisingly, and in line with the increased efficacy of antiviral regimens, the number of GRTs of patients treated with highly active antiretroviral therapy (HAART) has decreased in recent years (>600 performed in 2009 vs. ≥1000 in 2004–2006), although the number is now stable, confirming that failures continue to occur in clinical practice. Following guideline recommendations, a steady increase in GRTs requested for drug-naïve patients was also observed, starting from 2004 (with >600 being performed in 2009).

**Use of GRTs in Drug-naïve Patients**

HIV-1 is an extremely variable and highly evolving pathogen. One of the main consequences of its unique nature is that the selection of the first therapeutic regimen is crucial for the success of subsequent therapies, thus limiting the use of drugs against which the virus has already selected primary mutations. Transmission of drug-resistant HIV-1 strains is a well-documented phenomenon, and most studies agree that transmitted drug resistance (TDR) is associated with a higher risk of virological failure of first-line antiretroviral therapy [6,7], a higher risk of developing resistance even to those drugs in their regimen that were originally fully active [8], and faster progression to advanced disease [9].

In AIDS Clinical Trials Group A5095, it has been shown that baseline NNRTI resistance can more than double the risk of virological failure in response to an initial NNRTI-containing regimen (hazard ratio 2.27 (95% CI 1.15–4.49); p 0.018) [7]. Interestingly, one study from the CASCADE collaboration reported a steeper CD4+ cell count decline in subjects with primary resistance than in those without [9].

All over the world, TDR has been analysed in recent years. The risk of a transmitted virus being resistant to at least one antiretroviral drug has a remarkable difference in prevalence (2.2–24%), even in different areas of the same country. The major part of the variation is related to differences in access to therapy (universal vs. limited), with a range of 5–18% in the USA and Europe (Tables 1 and 2), 13.8% in Asia, and 2.2–24% in Africa (Sungkanuparp et al., 12th European AIDS Conference, 2009, Abstract 3.1/3; Kasang et al., 12th European AIDS Conference, 2009, Abstract 3.1/9; Kim et al., 17th Conference
Conflicting data regarding trends in the frequency of transmitted variants over time have also been reported, with some studies showing a stable TDR over time [12], some showing a decline (Poon et al., 17th Conference on Retroviruses and Opportunistic Infection, 2010, Abstract 583 [11]), and some showing an increase [13]. All together, these results indicate that differences in patient populations (in terms of risk behaviour, adherence and ethnicity) and in the types of antiretroviral regimen used can compromise data interpretation. Therefore, only local studies can provide a correct determination of the prevalence of resistance in drug-naive patients in that particular setting, avoiding erroneous conclusions with relevant clinical consequences.

Although previous models assumed that TDR reflects direct infection from treated individuals, the dynamics of transmission of drug-resistant HIV-1 strains are still unclear. Recently, through phylogenetic analysis of >10 000 HIV-1 pol gene sequences generated in the UK from both treatment-naive and treatment-experienced individuals, Huê et al. identified five treatment-independent viral clusters, generated in the late 1990s, but containing mutations conferring cross-resistance to antiretroviral drugs [14]. Phylogenetic analysis indicated that these reservoirs have persisted in the HIV-1-infected population for up to 8 years. The existence of sustained reservoirs of resistance in the absence of treatment has the capacity to threaten the long-term efficacy of antiretroviral therapy, and suggests that there is a limit to the decline of TDR. Given the current decrease in resistance transmitted from treated individuals, a greater proportion of resistance is likely to come from drug-naive lineages. These findings provide new clues to help in the development and management of treatment programmes in resource-rich and developing countries.

To date, all current guidelines recommend HIV-1 drug resistance testing for all HIV-1-infected individuals entering into clinical care, regardless of whether therapy will be initiated immediately or deferred. In addition, genotypic resistance testing is recommended for all pregnant women prior to therapy initiation and for those entering pregnancy with detectable HIV-1 RNA levels while on therapy [1,2,15,16]. Health economic studies have shown that in antiretroviral-naive persons, baseline genotypic resistance testing is a cost-effective intervention when the prevalence of TDR exceeds 5% [17], and currently involves genotypic resistance testing for mutations in the reverse transcriptase and protease genes. However, as pre-existing genotypic and phenotypic raltegravir resistance is completely absent or extremely rare in INI-naive patients, integrase genotyping in all patients before raltegravir treatment may not be cost-effective and should not be recommended at this point. Future evidence of TDR to INIs, and/or more detailed knowledge of the clinical relevance of integrase minor variants and/or polymorphisms, may lead experts to reconsider this option [18].

**TABLE 1. Prevalence of primary human immunodeficiency virus type 1 (HIV-1) drug resistance by drug class, from 2003 to 2008, in different parts of the world**

<table>
<thead>
<tr>
<th>Year</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (N)</td>
<td>58</td>
<td>54</td>
<td>43</td>
<td>29</td>
<td>40</td>
<td>42</td>
</tr>
<tr>
<td>Any resistance (%)</td>
<td>10</td>
<td>11</td>
<td>19</td>
<td>17</td>
<td>28</td>
<td>10</td>
</tr>
<tr>
<td>NRTI (%)</td>
<td>7</td>
<td>6</td>
<td>12</td>
<td>7</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>NNRTI (%)</td>
<td>2</td>
<td>6</td>
<td>9</td>
<td>10</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>PI (%)</td>
<td>9</td>
<td>4</td>
<td>0</td>
<td>7</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

*NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.*

*Patients enrolled in the Options Project, a longitudinal cohort study in San Francisco of acute/early HIV infection (<12 months) (Jain et al., 16th Conference on Retroviruses and Opportunistic Infection, 2009, Abstract P473).*

**TABLE 2. Prevalence of primary human immunodeficiency virus type 1 (HIV-1) drug resistance by drug class, from 2002 to 2008, in different parts of the world**

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Patients (N)</td>
<td>464</td>
<td>326</td>
<td>550</td>
<td>–</td>
</tr>
<tr>
<td>Any resistance (%)</td>
<td>16</td>
<td>14</td>
<td>14</td>
<td>–</td>
</tr>
<tr>
<td>NRTI (%)</td>
<td>14</td>
<td>10</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>NNRTI (%)</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>PI (%)</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>–</td>
</tr>
</tbody>
</table>

*NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.*

*The genotypic resistance tests were performed at each site on plasma samples collected before the initiation of antiretroviral therapy, and the FASTA sequences were uploaded to the Italian ARCA database [11].
In the absence of therapy, TDR mutations may revert to the wild type, although in a highly variable proportion of patients, and at discrete time-points. The M184V/I mutations revert more often and more rapidly than thymidine analogue mutations, T215 revertants, NNRTI mutations, or PI mutations (Jain et al., 16th Conference on Retroviruses and Opportunistic Infection, 2009, Abstract P672). This is probably attributable to reduced viral fitness of M184V/I variants, and a lower likelihood of being ‘fixed’ by compensatory mutations. When TDR is being analysed, reversion events need to be considered. This underlines the importance of performing resistance testing on patients as soon as they enter into clinical care, minimizing the chance that a resistance mutation will become undetectable.

Repeating a GRT just before treatment initiation should be considered for all patients. This is because of the chance of a superinfection with a drug-resistant virus during the time between entry into clinical care and initiation of antiretroviral therapy, especially for patients with high-risk behaviour. Recently, it has been shown that superinfection events can be detected in approximately 10% of HAART-naïve men who have sex with men, with a sudden increase in viral load during routine follow-up (Doyle et al., 8th European HIV Drug Resistance Workshop, 2010, Abstract 89). In addition, for patients with TDR, it is important to re-analyse the degree of resistance at the time of treatment initiation, together with historical GRTs, in order to assess the best first-line treatment, considering also archived mutations.

The presence of at least one surveillance drug resistance mutation [19] in a drug-naïve patient may suggest the presence of hidden minor species with other drug resistance mutations. Indeed, it is important to remember that resistant viruses may decline over time to below the detection limit of standard genotypic resistance testing, but still persist as minority species, and can be stored in pro-viral DNA of infected cells.

These minority species may become dominant during treatment, increasing the probability of virological failure of the first-line regimen [20,21]. Underestimating the burden of resistance in drug-naïve individuals can affect optimal antiretroviral therapy selection, especially for patients diagnosed long after becoming infected, when primary resistance mutations may have decayed [22,23].

In practice, some studies have shown that detection of pre-existing minority NNRTI-resistant variants in drug-naïve subjects increases by more than three-fold the risk of virological failure of first-line NNRTI-based regimens [24], especially with adequate treatment adherence [25,26]. On the other hand, some studies have not confirmed these findings, suggesting that this area requires further investigation [27–29].

**Use of Genotypic Resistance Testing in Patients with First Virological Failure**

Drug resistance acquired during suboptimal antiretroviral therapy is much more common than TDR. The prevalence of drug resistance in therapy-exposed subjects was estimated to be 39–53% in 2006 [8,30], with the prevalence of triple-drug-resistant virus being 5%. Fortunately, the degree of resistance has declined in recent years, thanks to the introduction of newer potent agents, improvements in effective drug combination, and, last but not least, a better understanding of HIV-1 drug resistance. In a systematic overview of 20 clinical trials that comprised 7970 adult patients receiving first-line HAART, which consisted of dual NRTIs combined with a third agent (either an NNRTI or a ritonavir-boosted PI), initial therapy with ritonavir-boosted PI regimens resulted in less resistance within and across drug classes. In this meta-analysis, virological failure rates at week 48 were comparable, but the incidence of M184V and K65R mutations in reverse transcriptase, as well as resistance to the third agent, were higher for subjects starting NNRTI-based HAART (Table 3) [31].

Moreover, from the ‘real world’ of medical practice, in a recent analysis of 7891 patients who started recommended treatments in the UK, using NRTIs plus either a ritonavir-boosted PI or an NNRTI, virological failure by 8 years was relatively common (28%), and was paralleled by an appreciable risk of resistance detection, although the detection rate

### TABLE 3. Pooled resistance data at week 48 of virological failure of first-line highly active antiretroviral therapy

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. of patients</th>
<th>No. (%) of patients with VF</th>
<th>No. of patients with available genotype data (% of those with VF)</th>
<th>No. of patients (% of genotype analysis group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No major resistance</td>
</tr>
<tr>
<td>Ritonavir-boosted PI</td>
<td>3063</td>
<td>216 (7.1)</td>
<td>180 (83)</td>
<td>123 (56.9)</td>
</tr>
<tr>
<td>NNRTI</td>
<td>4212</td>
<td>240 (7.6)</td>
<td>291 (80)</td>
<td>114 (38.0)</td>
</tr>
</tbody>
</table>

Modified from Gupta et al. [31].

NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TAM, thymidine analogue mutation; VF, viral failure.
for class-specific resistance was lower for those who started a ritonavir-boosted PI-based regimen. The cumulative probabilities of detecting any mutation, one or more major NRTI International AIDS Society-USA (IAS-USA) mutations, one or more major NNRTI IAS-USA mutations (in those starting an NNRTI) and one or more major PI IAS-USA mutations (in those starting a PI) were 17%, 14%, 15% and 7%, respectively, by 8 years [32,33]).

Current guidelines state that the goal of therapy is to achieve and maintain HIV-1 RNA below detectable levels, with recommendations to switch regimens upon virological failure, because of the adverse consequences of higher levels of viraemia. Virus continues to evolve if kept under pressure by failing antiviral therapy. Maintenance of unchanged antiviral therapy in subjects with virological failure leads to further resistance accumulation, an increase in cross-resistance, and decreased chances of efficacy of subsequent drugs and regimens. In an analysis of 106 chronically HIV-1-infected patients on a stable antiretroviral regimen for at least 120 days, with plasma HIV-1 RNA levels >1000 copies/mL and at least one genotypic resistance mutation, the risk of losing one fully suppressive drug or two partly suppressive drugs was estimated to be 32% at 1 year [34]. The risk of developing a new major protease mutation was 17%, and the risk of developing a new nucleoside-associated mutation at 1 year was 23%. Similarly, a EuroSIDA study based on 110 HIV-1-infected patients with plasma HIV-1 RNA levels >400 copies/mL found that, in patients kept on the same virologically failing HAART regimen for a median time of 6 months, there was a considerable accumulation of drug resistance mutations, particularly in patients with an initial low level of resistance to the failing regimen [35]. Recently, it was also observed that maintaining a failing first NNRTI regimen for 6–12 months was the only factor associated with the development of full resistance to etravirine (>4 etravirine Tibotec score [36]) (Zaccarelli et al, 9th International Congress on Drug Therapy in HIV Infection 2008, Abstract PO179). This suggests that quick withdrawal of a failing NNRTI regimen, possibly during the first 3 months, can maintain etravirine sensitivity, thus preserving a treatment option.

Therefore, in the framework of correct therapeutic sequencing, clinicians must prevent resistance accumulation by detecting virological failure early, and by quickly switching HAART to fully suppressive combinations. Guidelines recommend the performance of HIV-1 genotypic resistance testing when managing suboptimal viral load reduction, and to assist in the selection of active drugs when changing antiretroviral regimens in patients with virological failure and HIV-1 RNA levels >1000 copies/mL [1,2]. In persons with >500 but <1000 copies/mL, testing may be unsuccessful, but should still be considered. Genotypic resistance testing in the setting of virological failure should be performed while the patient is taking prescribed antiretroviral drugs, or, if this is not possible, within 4 weeks after discontinuing therapy [1].

The recent suggestion to perform genotypic resistance testing also at levels <1000 copies/mL reflects the wide variability of skill in performing resistance testing among different laboratories. In real life, many reports have shown the highly successful use of genotypic resistance testing in HIV-1-infected patients with detectable viraemia between 50 and 1000 copies/mL [37-39]. In addition, multiple drug resistance mutations can also be selected and detected at HIV-1 rebound with low viral loads [37,40]. Indeed, resistance mutations have been found to accumulate in 68–93% of patients with persistent viraemia between 50 and 1000 copies/mL [41]. Therefore, in patients with a suboptimal response, resistance testing at week 4 is recommended.

Genotype validity with plasma HIV-1 RNA level <75 copies/mL was also confirmed in 49 of 50 patients with a previous or follow-up genotype [42]. Overall, the belief that genotypic resistance testing is unreliable in samples with low-level viraemia should be reassessed.

Fig. 2 shows another example of a virology working routine. The histograms represent the number of GRTs performed at the first failure in relation to viral load and year of treatment start.

**FIG. 2.** Number of genotypic resistance tests (GRTs) performed at first treatment failure in relation to viral load and year of treatment start.

Conclusions

The importance of resistance testing in clinical practice has been widely recognized and acknowledged in guidelines,
although its implementation in clinical practice needs reassessment. The long-term clinical implications of resistance in today’s situation of multiple drug availability require further cohort studies. At the same time, the use of resistance testing before therapy initiation gives the chance of the best selection of antivirals to be used in the first regimen. The technical feasibility of resistance testing at first failures with low viraemia (a quite common situation) seems to be far greater than what is suggested by guidelines. Its implementation is relevant in preventing the further evolution of the virus and the selection of mutations causing multiple cross-resistance, which limits therapeutic options.

Advances in ultrasensitive technologies now allow the detection of resistant variants present at very low levels (<0.1%) in the quasi-species populations of infected patients. However, appropriate validation and standardization of such new technologies, together with significant cost reductions and assessment of the clinical relevance of minor variants, is mandatory before approval of their future use in diagnostic routine.

**Funding**

This work was financially supported by grants from the Italian National Institute of Health, the Ministry of University and Scientific Research, ANRS (National AIDS Research Agency) and the European Commission Framework 7 Program (CHAIN, Collaborative HIV and Anti-HIV Drug Resistance Network, Integrated Project no. 223131).

**Transparency Declaration**

F. Ceccherini-Silberstein has received funds for attending symposia, speaking and organizing educational activities from Abbott, Merck Sharp & Dohme, Janssen Cilag, and Virco. V. Calvez and C.-F. Perno have received funds for attending symposia, speaking, organizing educational activities, grant research support, consultancy and advisory board membership, from Abbott, Boehringer Ingelheim, Bristol Myers Squibb, Gilead, Merck Sharp & Dohme, Janssen Cilag, Pfizer, Tibotec, Roche, and ViIV. V. Cento has nothing to declare.

**References**