Using HIV resistance tests in clinical practice

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Genotypic resistance testing is now a standard of care in HIV management. Although there are clear, published guidelines to recommend the appropriate use of these tests, clinicians and scientists still struggle to determine the optimal use of resistance tests given the finite budgets and time constraints under which they work. In this article we discuss some ‘real-life’ clinical situations and aim to provide a useful insight into when and where genotypic resistance testing can be optimally applied in the management of HIV-positive adults.

Keywords: human immunodeficiency virus, genotype, genotypic resistance testing

Introduction

Through the remarkable development of combination antiretroviral therapy (cART), the last 25 years have witnessed the transformation of HIV infection in the developed world from a uniformly terminal disease to a manageable, chronic infection. However, in spite of this rapid progress, cART still fails to achieve its goals in a proportion of patients due to toxicity issues, problems with long-term adherence, pharmacological factors and the evolution and transmission of drug-resistant viruses. Whatever the precipitating cause of a loss of virological control, it is an inalienable truth that if viral replication is allowed to continue in the presence of an active drug, the selection of drug-resistant viruses will occur. The principle aim of treatment is hence to achieve maximum and durable suppression of viral replication using a combination of antiretroviral agents to which the virus is susceptible. Failure to achieve this results in the development of drug-resistant variants, the emergence of which is significantly associated with poorer survival. One study highlighted a 3-fold increased risk of death following the diagnosis of multidrug resistance, compared with the risk observed overall in HIV-infected individuals.1

In the UK, 52% of treatment-experienced patients in 2006 were shown to have some degree of drug resistance.2 Fortunately this figure has been in decline over recent years due in part to improvement in effective combination therapies, but also through a better understanding of HIV drug resistance and the subsequent refinement in the way clinicians choose, avoid and combine drugs.

The British, American and European HIV management guidelines all advocate the use of resistance testing in the management of HIV-positive patients.3–5 Notwithstanding this, the interpretation and optimal application of resistance testing sometimes causes uncertainty. What follows is a view from the ‘shop floor’ written by HIV physicians and virologists who use resistance testing technologies extensively and have found ‘clinically directed’ resistance testing to be an invaluable tool in optimizing patient care. The corollary, however, is also true; uninformed, indiscriminate use of these technologies is not cost-effective and the results can mislead rather than inform clinical decision making.

We have limited our discussion to genotypic resistance testing as this is the predominant modality used in the UK, phenotypic testing being reserved for a much smaller minority of highly treatment-experienced patients.

When does resistance testing have most clinical utility?

(i) Baseline resistance testing

Current treatment guidelines advocate ‘baseline’ resistance testing prior to the commencement of cART. The prevalence of transmitted drug resistance (TDR) in the UK is ~6–7% overall.2 Local prevalence, however, differs from clinic to clinic according to the make-up of its HIV population. Men who have sex with men in the UK have traditionally had higher rates of TDR compared with those acquiring their HIV in sub-Saharan Africa, although this may change as access to antiretrovirals becomes more widespread. Health economic studies have shown that baseline resistance testing is a cost-effective intervention where the prevalence of transmitted resistance exceeds 5%.6

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The probability of detecting TDR is at its highest close to the time of primary infection (Figure 1a). Following primary infection, the number of ‘detectable’ resistant variants will progressively fall with time, expressed as a proportion of the entire viral population. Clinicians who elect to delay baseline resistance testing until the time of treatment initiation may miss the opportunity to detect drug-resistant virus acquired at the time of transmission. Furthermore, there exists considerable variation in laboratory practice/policy with regard to the duration of storage of HIV-positive plasma samples, which may preclude the retrospective testing of stored samples for this purpose. It is hence advisable to perform baseline resistance testing on all newly diagnosed patients using the nearest sample available to the time of primary infection.

If samples from, or near to, the time of transmission (primary infection) are not available it is still appropriate to test the ‘earliest’ sample stored. This is due to the more recent understanding that some transmitted variants can persist for at least 2–3 years and occasionally longer, even in the absence of ART. These TDR variants often represent that individual’s
'wild-type' virus. This term is often used to describe a virus that does not carry resistance mutations, but in the context of TDR, the 'wild-type' virus is that which predominates within the host and is best adapted to that particular environment. This is irrespective of whether or not it carries resistance mutations. If a resistant strain is the only virus transmitted, it is by default the fittest virus in its new host. A daughter virus that has lost these resistance mutations will only 'outgrow' the resistant virus if it is (i) generated by the random mutational processes inherent within the replication cycle of HIV, and (ii) the resulting virus is fitter than the original resistant virus. This may explain why certain TDR viruses can sometimes persist for years instead of weeks.

It is also important to note that the absence of resistant variants does not equate to a lack of transmission of resistance. Commercially available population sequencing assays may fail to detect resistant variants if they are present at <20% of the total quasispecies in circulation (see Figure 1b).

Some TDR mutations are known as 'revertants' or reversion mutations. This concept is best explained by the example of viruses carrying mutations at position 215 of the reverse transcriptase (RT) gene. A zidovudine-resistant virus usually carries a Y (tyrosine) at this position and is two mutational steps removed from the non-resistant virus carrying a T (threonine) at position 215. Viruses carrying 215Y mutations have reduced susceptibility to zidovudine but are also less virologically 'fit' compared with non-resistant viruses. If a virus harbouring a 215Y mutation is transmitted sexually and is the only virus transmitted, it will by default become that patient's dominant viral population (despite its reduced fitness). In the new host, in the absence of drug pressure, random mutational events can give rise to variants with further changes at position 215 (i.e. T215D/S/N). These viruses are known as 'revertants'. While they do not confer resistance to zidovudine per se, they are fitter than the resistant 215Y viruses and hence outgrow and replace the resistant strains (Figure 1a). Although these 'reversion mutations' do not confer high-level resistance in their own right, their presence should warn the clinician that the patient may have previously been infected with resistant virus that may readily be selected out upon the introduction of ART. It is our view that any patient who presents with viruses carrying genotypic changes, or polymorphisms, at sites usually implicated with drug resistance, should be carefully monitored following the start of ART in case TDR viruses are present at levels below the limit of detection of conventional assays (further discussed below).

Some HIV drug resistance mutations, such as K103N, do not carry a significant replication disadvantage (in terms of 'replication capacity') over non-resistant viruses and as such may co-exist even if non-resistant viruses evolve within, or are co-transmitted to, a newly infected person. More sensitive resistance testing techniques have recently shown that low-level transmission of viruses harbouring the K103N or Y181C mutation may be far more common than previously recognized. Furthermore, they may contribute to early virological failure when baseline resistance is not detected by standard resistance testing methods.

Baseline resistance testing is currently recommended for nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs) and protease inhibitors (PIs). As integrase inhibitors become more widely used and more patients fail treatment with resistance to these agents, baseline testing for resistance to integrase inhibitors may also become pertinent.

(ii) Poor response to treatment following commencement or re-commencement of cART

Some TDR variants present as archived virus or quasispecies below the detection limit of genotypic resistance testing, may only be detected by more sensitive assays such as allele-specific PCR (ASPCR). Unfortunately ASPCR is not routinely available. We therefore adopt a practical approach by maintaining a high index of suspicion and a low threshold for the early use of standard genotypic resistance testing in those patients who do not demonstrate an adequate viral load response. As a general rule, one should expect a 2 log reduction in HIV viral load after 2–4 weeks of cART. We have found that in patients who fail to achieve this, resistance testing at week 4 may detect mutations that were probably present as minority drug-resistant quasispecies but only became detectable under selective drug pressure. This allows for an early treatment switch before resistance to the other drugs in the cART regimen has had time to evolve. Inadequate responses should, of course, prompt the clinician to consider other factors associated with a suboptimal virological response, of which drug resistance is only one.

(iii) Following viral load rebound

Patients on cART with a fully suppressed viral load should be assessed every 3–4 months accompanied by a viral load measurement. Amongst the various explanations for loss of virological control, the development of drug resistance has the greatest impact on future therapeutic success. Viruses replicating in the continued presence of drug will further accumulate resistance mutations, which in turn will compromise future treatment options. It is hence important to modify ART regimens as soon as resistance has been detected. Our policy is to perform resistance testing when the viral load on treatment rises to >400 copies/mL on consecutive samples (2 weeks apart), rather than waiting for it to exceed 1000 copies/mL as many older guidelines advocate.

(iv) Treatment decisions in patients with complex treatment histories: using a cumulative resistance interpretation

Many patients who started ART during the 1990s were prescribed mono or dual drug therapy and agents from newly emerging drug classes were commonly added to failing regimens. Many also had extensive exposure to unboosted PIs with the consequence that a significant proportion of patients developed extensive triple class (NRTI, NNRTI and PI) resistance. Genotypic resistance tests are invaluable in patients with triple class resistance in order to design an effective new regimen with as many active drugs as possible (often in combination with agents from newer drug classes).

Resistance testing in the context of virological failure is most useful when the patient is taking their ART. The selective pressure exerted by ongoing drug exposure will make it more likely that resistance mutations, if present, will be detected. As in the case of TDR, in the absence of drugs, resistant viruses or archived mutations may exist at a level just below the limit of detection of conventional genotypic resistance tests (Figure 1b). Studies have shown that on cessation of drug therapy in chronically infected individuals who have developed resistance mutations the mutant viruses generally disappear from the...
population in <2 weeks (Figure 1b).10 This is in contrast to those infected with TDR described above. It is hence vitally important that resistance testing be performed on samples taken whilst patients remain on drug therapy with evidence of virological failure. We often find it useful to retrospectively test stored plasma samples from timepoints when the patient had detectable viraemia whilst taking a particular drug or class of drug. As well as informing us whether virological rebound can be attributed to drug resistance, we may also be able to infer what the maximum level of resistance to a drug or class of drugs was likely to have been before that drug was discontinued. Once resistance mutations have evolved, they remain archived in T memory cells. Consequently these mutations may be reselected by a particular drug at a later stage. In view of this, we employ a ‘cumulative’ resistance interpretation when selecting a new regimen. This is produced by amalgamating all the mutations ‘ever detected’ on the patient’s previous resistance tests and reanalysing them using online resistance algorithms such as the rule-based interpretation algorithm hosted by Stanford University (www.hivdb.stanford.edu). The ensuing ‘cumulative’ resistance interpretation contains all the possible current and previously archived resistance mutations that have been detected in the patient and hence gives the clinician an indication of the ‘worst case resistance scenario’. This approach should prevent inappropriate use of drugs to which the virus is already resistant as this may not be apparent from the latest resistance assay or by drug treatment history alone especially if the patient is no longer taking the drug in question.

Resistance interpretation can be complex and we would recommend involvement of clinicians or virologists with expertise in this area as there are many subtleties and caveats to the interpretation of the different mutational patterns seen. Drugs described as having ‘intermediate’ resistance may have differing degrees of activity depending on factors such as the existence of antagonism between resistance mutations in vivo. There is also the possibility of exploiting the phenotypic phenomenon of hypersusceptibility or the viral fitness handicap that certain mutations confer. For example, the resistance assay report for the M184V mutation will read ‘high-level resistance’ to lamivudine and emtricitabine. Viruses carrying this mutation may, however, also have reduced levels of viral fitness, reduced fidelity of reverse transcription and may have increased susceptibility to zidovudine and tenofovir. The use of lamivudine or emtricitabine in the ART regimen despite the presence of genotypic resistance may hence be valuable. We advocate discussion of complex cases and resistance reports in a multidisciplinary ‘virtual clinic’ where patient factors such as adherence and drug interactions can also be addressed. The virtual clinic is a useful way of combining expertise and diagnostics in order to maximize the likelihood of an effective virological response for the patient.

When might resistance testing be clinically less useful?

(i) Resistance testing in patients who have stopped therapy

Unfortunately, patients sometimes present to clinic months after having stopped therapy on their own accord. The chance of detecting resistance mutations in patients whose viral load has returned to baseline is minimal11 (Figure 1b). Therefore a resistance test performed whilst a patient is ‘off’ therapy can be grossly misleading as it may miss mutations selected during previous therapy failure. In patients who experience virological failure due to problems with adherence, it is important to perform resistance testing on the sample in which the viruses are most likely to have been under selective drug pressure. The key indication that non-adherence is the likely cause of virological rebound is a rapid and significant rise in viral load (i.e. >10000 copies/mL) after having previously been undetectable. Resistance tests performed on these samples often fail to show resistance (Figure 1b). If restarting the same regimen, more frequent viral load monitoring is required and genotypic resistance testing should be undertaken should the subsequent viral load response be slow.

(ii) Super-infection

The British HIV Association HIV guidelines do not advocate the routine re-genotyping of patients prior to commencement of ART. In theory, patients who engage in high-risk behaviour may be at risk of super-infection and may consequently have a different genotype prior to starting treatment compared with that at baseline. Should the ‘super-infecting virus’ confer TDR then this could lead to treatment failure. In reality, clinically significant super-infection with resistant virus is difficult to detect if the new ‘super-infecting’ virus is not significantly more pathological or virulent than the original virus. Therefore, we do not routinely repeat resistance testing prior to restarting cART unless the patient has a sudden change in viral load/CD4 count with an appropriate risk history.

(iii) Virological failure in those on boosted PIs

Evidence and clinical practice suggest that previously PI-naive patients who fail on boosted PIs are unlikely to have PI mutations detected within the protease gene. Resistance testing is, however, useful to determine resistance to other agents in the regimen and to look for PI mutations in those previously exposed to unboosted PIs, or those with PI mutations prior to starting boosted PI therapy. It remains unclear whether or not mutations at a site distant from the protease gene (e.g. the GAG cleavage site) are an important mechanism of failure for boosted PIs.

Conclusions

Genotypic resistance testing is now a routine clinical tool in HIV management. We see it as crucial that HIV clinicians and virologists/microbiologists understand the role of, and implications for, resistance testing in everyday clinical practice in order to achieve maximal and sustained suppression of HIV within the existing constraints of finite budgets and time.

Transparency declarations

S. T. has received unrestricted educational travel grants and/or has acted as a medical advisor/speaker for Gilead, BMS, Abbott, Tibotec, MSD, GSK, BI and Roche pharmaceuticals. A. J. has received unrestricted educational travel grants from GSK, BMS,
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