



## Monitoring Antiretroviral Drug Resistance

**Chris Birch**

Virus Laboratory

Victorian Infectious Diseases Reference Laboratory, Australia

**T**reatment of HIV infection with antiretroviral (ARV) drugs has had a significant impact on the morbidity and mortality associated with the virus. Although the availability of these drugs remains poor in underdeveloped countries, in developed countries up to 16 drugs exist within either the nucleoside reverse transcriptase inhibitor (NRTI), nonnucleoside RT inhibitor (NNRTI) or protease inhibitor classes. A fusion inhibitor, T-20 (enfuvirtide) is also at an advanced stage of clinical investigation.

Despite the positive impact of ARV drugs, failure of therapy occurs in a significant proportion of patients. The causes are multifactorial, but usually involve interactions between the patient, the drugs themselves and the virus. For example, poor adherence by the patient because of a high pill burden or concomitant drug-associated adverse side-effects, combined with the mutation frequency of HIV during replication, often leads to drug failure because of the development of resistance.

Tests to detect ARV drug resistance are becoming widely available. These tests involve either nucleotide sequencing (genotyping), direct evaluation of the replicative capacity of the virus in the presence of the ARV drug (phenotyping), or a combination of both (virtual phenotyping). When genotyping is performed, the amino acid sequence of the HIV RT, protease and gp41 (for T-20) is obtained and interpreted with respect to the likely susceptibility of the virus to drugs targeting these proteins. Results are reported for each drug as resistant (clear-cut evidence of mutations known to cause resistance), potentially resistant (mutations present which are likely to predispose to resistance) or sensitive. Quality assurance programs to monitor genotyping standards are in place, and are essential to maintain the quality of sequencing, the ability to detect mixtures of nucleotides that may impact on drug susceptibility, the appropriate interpretation of sequences, and timely reporting of the results to the treating doctor.

Phenotyping has largely become the domain of commercial organisations. Phenotyping methods are roboticised to some extent, and usually involve the removal of the RT and protease genes from the plasma derived HIV strain of interest, followed by the insertion of these genes into an RT/protease-deleted strain otherwise capable of replication in susceptible cells. This genetically engineered virus, which contains the sequences of interest, is then allowed to replicate in the presence of the ARV drugs within each class that are available clinically. A 50% inhibitory concentration ( $IC_{50}$ ) for each drug is calculated and a resistance 'cut-off' value established for each drug by reference to a panel of susceptible HIV strains. Cut-off values vary for each of the drugs within a class. However, in general an  $IC_{50}$  value greater than 3-fold that of wild-type viruses indicates resistance to a particular NRTI. The corresponding values for NNRTIs and protease inhibitors are approximately 8-fold and 3-fold, respectively.

Virtual phenotyping relates the RT and protease sequences of the virus of interest to identical or near-identical sequences of HIV strains whose phenotype (measured as an IC<sub>50</sub>) has been previously established. This information is held within a large proprietary database. While sequencing of the RT and protease genes is required, replication of the virus in the presence of ARV drugs is not.

A considerable amount of clinically-based evidence is accumulating that shows drug resistance testing provides virological, and by implication clinical, benefit. The pioneering investigations in this regard were the GART and Viradapt studies.<sup>1,2</sup> In particular, genotyping has been compared to standard of care (SOC) treatment in at least 4 studies. Only one, the NARVAL study,<sup>3</sup> did not find evidence of improved virological response in patients who received genotyping rather than SOC. When genotyping is undertaken using a commercial assay, the data generated is usually interpreted using a supplied software package. Although at least one study (Havana<sup>4</sup>) has shown that genotyping results interpreted by the treating physician provide virologic benefit, it is generally thought that expert interpretation of these results provides additional benefit. The cost benefit of resistance testing, even if only short term virological responses are gained as a result, is arguably strong.

Most resistance testing performed at present involves genotyping, and most of this is performed in-house (that is, without recourse to commercial assays). However, the list of commercial organisations providing genotyping and phenotyping is growing. The Visible Genetics 'TRUEGENE HIV-1 Genotyping Kit' was the first assay to gain US FDA approval for use in clinical practice. It is important that the manufacturers of such assays regularly update the algorithms used to provide information on ARV drug sensitivity, either through reference to peer-reviewed literature or presentations at meetings relevant to the issue of drug resistance.

Problems associated with drug resistance testing should not be underestimated. These are technically complex assays. For example, genotyping of plasma-associated HIV involves RNA extraction, reverse transcription, multiple polymerase chain reactions, DNA purification, sequencing, sequence analysis and interpretation. This leads to considerable expense in terms of labour, consumables, kit costs and equipment. Another problem is the overall lack of sensitivity of both phenotyping and genotyping. Not only does the virus load in the test plasma sample need to be approximately 1000 copies per ml, but the ability of both assays to detect mixtures of susceptible and resistant HIV strains within the one population is also limited.

Neither phenotyping nor genotyping has the ability to detect viruses or mutations associated with past ARV drug failure. The need for an accurate drug history to be maintained for all patients is therefore of great importance. Finally, appropriate interpretation of the results, particularly those for genotype, is essential but may not always be available to treating doctors. Incorrect interpretation of results may lead to wasted opportunities for some patients. Correct interpretation of genotyping results is therefore one of the most important aspects of drug resistance testing. Fortunately, the application of rules-based genotype interpretation can be generalised within the HIV M strains and, for most drugs, across the HIV-2 and group O strains also. The exception is the lack of efficacy of NNRTIs against HIV-2 and group O viruses because of a naturally occurring Y181-C mutation in the RT. Within the M strain subtypes, major resistance mutations do not occur as polymorphisms, although some minor mutations in the protease gene may predispose to resistance in some cases.

It should not be assumed that genotyping and phenotyping results on the same specimen will always be identical. This is partly because the interpreted sequence of the RT or protease may be more predictive of future outcomes than the phenotype. That is, the sequence may contain mutations that predispose to resistance, and this is incorporated into the genotyping interpretive algorithm. An example is the L90-M mutation generated by

saquinavir (and nelfinavir). Often, viruses containing this mutation will be phenotyped as susceptible to saquinavir, but most genotyping algorithms assume resistance. In a sense both results are correct. Other differences in interpretation arise as a result of the ability of the lamivudine induced M184-V mutation to resensitise viruses containing a T215-Y resistance mutation to zidovudine susceptibility. Phenotyping often reports M184-V/T215-Y mutants as lamivudine resistant/zidovudine sensitive, whereas genotyping calls them as resistant to both drugs. Overall, interpretation of sequences as part of the genotyping process is becoming increasingly difficult because of the above reasons and also because of the impact of other non-intuitive factors. These include the increased susceptibility to NNRTIs of patients with multiple NRTI exposure, the ability of mutations in the protease to cause cross-resistance, and the existence of ZDV resistance-associated mutations (also called thymidine associated mutations or TAMs) that cause significant cross-resistance within the NRTI class.

Cross-resistance within all drug classes is a significant treatment issue, and impacts unfavourably on the number of ARV drugs actually available clinically. Within the NRTI class, mutations that predispose to resistance to more than one drug include M184-V, the E44-D/V118-I combination and L74-V. These occur in more than 8% of the HIV strains of patients sampled in Melbourne, Australia. Certain combinations of TAMs are also common. Fortunately, K65-R and the classic multidrug resistant mutations (Q151-M and T69-S + insertions) are rare.

The basis of cross-resistance induced by TAMs has now been elucidated and involves the increased ability of viruses containing these RT mutations to remove chain terminating dideoxynucleotide monophosphates (ddNMP) from DNA as it is synthesised as a result of reverse transcription. This is known as excision or pyrophosphorolysis, and involves an interaction between the mutated enzyme, the chain-terminating ddNMP and cellular dATP to form a dinucleotide-tetraphosphate complex. As a result, depending on the TAMs present, certain NNRTIs are preferentially removed from the growing DNA chain, resulting in continued replication (and apparent resistance to that NNRTI).

Cross-resistance also occurs within the NNRTI class, where it is associated mainly with K103-N and Y181-C mutations, and the protease inhibitors, where a combination of more than one primary, inhibitor-specific mutation tends to generate resistance across the class.

Given that drug resistance testing provides clinical benefit, when should such testing be requested? There may be some indication for testing at the time of primary infection if the incidence of transmission of drug resistant virus continues to increase. In Melbourne in 2002, the predominant HIV strain in approximately 14% of new infections contained resistance mutations in the RT gene, and 3% also had mutations in the protease gene. Half of these patients were infected with virus containing the K103N mutation, effectively eliminating the NNRTI class as a treatment option. Otherwise, resistance testing is recommended towards the end of pregnancy as a means of optimising treatment for the mother and preventing infection of the neonate, and at the time of treatment failures. Testing at the time of the first failure documents ARV drug susceptibility at the time, and sets a baseline for future testing. At second and subsequent failures, resistance testing may indicate new mutations, some of which may cause cross-resistance.

## References

1. Baxter JD, Mayers DL, Wentworth DN, *et al.* A randomised study of antiretroviral management based on plasma genotypic antiretroviral resistance testing in patients failing therapy. *AIDS*. 2000;14:F83-93.
2. Clevenberg P, Durant J, Halfon P, *et al.* Persisting long-term benefit of genotype guided treatment for HIV-

- infected patients failing HAART. The Viradapt Study: week 48 follow-up. *Antivir Ther.* 2000;5:65-70.
3. Meynard J-L, Vray M, Morand-Joubert L, *et al.* Phenotypic or genotypic resistance testing for choosing anti-retroviral therapy after treatment failure: a randomised trial. *AIDS.* 2002;16:727-36.
  4. Tural C, Ruiz L, Holtzer C, *et al.* Clinical utility of HIV-1 genotyping and expert advice:the Havana trial. *AIDS.* 2002;16:209-18.