

HIV/AIDS

Antiretroviral

Newsletter



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The aim of this biannual newsletter is to provide health workers in the Region with a brief, up-to-date summary of the latest developments in antiretroviral therapies.

Drug Resistance: Part 1

What is it; how to measure and interpret the results?

Next issue: Drug Resistance: Part 2 Application to clinical practice

THE BASICS

Drug resistance is a major contributing factor to the failure of antiretroviral therapy. The development of drug resistance further complicates clinical management because of the high level of cross-resistance within all three drug classes, nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI). The nucleoside sequence that determines a gene is known as the genotype. The characteristics or properties of a virus are known as the phenotype, which is in turn determined by the genotype. Mutations in the genes that encode for the replicative enzymes, reverse transcriptase and protease, confer resistance to antiretroviral drugs.

Primary mutations cause decreased binding of the drug to its enzyme target and are the first mutations selected during therapy. This results in an increased amount of drug required to inhibit the enzyme. Secondary mutations contribute to drug resistance by improving the fitness of viruses carrying primary mutations. They have little direct effect on inhibitor binding or on the level of resistance in the absence of primary mutations. Genotypic resistance assays detect mutations in these key genes. Resistance mutations (such as the lamivudine

M184V mutation) are described using a number to denote the mutant codon with letters before and after the codon number denoting the amino acid associated with "wild-type" and mutant virus respectively. The tables presented below indicate the mutant codon position.

Phenotypic resistance assays measure susceptibility of the virus to antiretroviral drugs in terms of the concentration of drug required to inhibit viral replication *in vitro* by a defined amount such as 50% (IC₅₀) or 90% (IC₉₀).

The rapid turnover of HIV (estimated at 10⁹ new viral particles each day), and the high error rate of the reverse transcriptase enzyme during viral replication, results in an extensive genetic diversity of the virus population. HIV is a RNA virus and, as such, lacks the proof-reading mechanisms that DNA-based organisms possess to identify and correct genetic mutations that occur during replication. The result is that drug resistant mutants (quasi-species) pre-exist in any viral population. There is also definitive evidence that resistant strains of HIV, including multidrug resistant strains, can be transmitted. If viral replication is not completely suppressed, there is rapid selection of drug resistant mutants from the viral pool.

Table 1: Mutations in the protease gene selected by protease inhibitors

Drug	Associated Mutations													
	20	30	36	46	48	50	54	63	71	82	84	88	90	101
Amprenavir			Secondary			Primary	Secondary	Secondary	Secondary	Primary	Primary			Primary
Indinavir	Natural		Secondary	Primary			Secondary	Secondary	Secondary	Primary	Primary		Primary	
Nelfinavir		Primary	Secondary				Secondary	Secondary	Secondary			Natural	Primary	
Ritonavir	Natural		Secondary				Secondary	Secondary	Secondary	Primary				
Saquinavir			Secondary		Primary		Secondary	Secondary	Secondary	Primary			Primary	
Lopinavir	Secondary			Secondary			Secondary	Secondary	Secondary	Secondary			Secondary	Secondary







-  Primary mutations: clearly associated with drug resistance.
-  Secondary mutations: add to the resistance caused by primary mutations.
-  Natural variants: natural variants of the virus that can add to drug resistance.

Table 2: Mutations in the reverse transcriptase gene selected by NRTIs and NNRTIs

Drug	Associated Mutations																	
	41	65	67	70	74	75	103	115	116	151	181	184	188	210	215	219	333	
3TC																		
Abacavir																		
AZT																		
AZT + ddI/ddC																		
AZT + 3TC																		
d4T																		
ddI / ddC																		
MNR																		
Delavirdine																		
Efavirenz																		
Nevirapine																		

-  Primary mutations: clearly associated with drug resistance.
 -  Secondary mutations: add to the resistance caused by primary mutations.
 -  Multinucleoside resistance (MNR): also includes mutations at 62, 77 and 69
- These mutations confer resistance to AZT, ddI, ddC, d4T, 3TC and ABC

THE MECHANISM OF DRUG RESISTANCE

Antiretroviral resistance secondary to replicative mutations in HIV develops differently depending on the drug class and can evolve via different pathways for specific drugs. Single point mutations associated with the NNRTI drug class and with lamivudine (3TC) result in rapid, high-level resistance. Step-wise accumulation of mutations is required for high-level resistance to other drugs such as zidovudine (AZT) and PIs.

NRTIs

The resistance pathway for AZT starts with the selection of the K70R mutation, which is associated with an eight-fold decrease in sensitivity. Progressive mutations at codons 215 and 41 lead to rapid replacement of the K70R mutant with these two variants and a further 60-fold decrease in the IC₅₀ for AZT. Further viral evolution results in mutations at codons 67 and 219. The presence of all five mutations results in a 500-1000 fold reduction in sensitivity compared to wild type virus. At a molecular level, recent research suggests that a major mechanism of AZT resistance is enhancement of pyrophosphorolysis. NRTIs act as DNA chain terminators by inserting into the growing viral DNA chain as it is being copied by reverse transcriptase. Once inserted, further DNA synthesis is blocked. This process is called phosphorolysis. Pyrophosphorolysis (primer unblocking) is the reverse of this process

whereby the nucleoside (AZT-triphosphate) is removed from the chain, allowing reverse transcription to resume. Pyrophosphorolysis enhancing mutations are shared between AZT and stavudine (d4T) and this may, in part, explain the cross-resistance between these two NRTIs seen in clinical practice. That AZT and d4T, both thymidine nucleoside analogues, share common resistance mutations led to the coining of the term “TAMS” (thymidine analogue mutations).

However, this set of mutations, when combined with the M184V mutation, blunts the response to abacavir, a guanosine analogue. Some researchers now believe that resistance to NRTIs cannot be grouped into analogue-specific clusters. Sets of mutations have now been described that confer resistance to all of the available NRTIs. (See Table 2)

NNRTIs

All drugs in the NNRTI, although structurally different, bind to the same site on the reverse transcriptase enzyme. They do so by creating a binding pocket and acting as non-competitive inhibitors of the enzyme. Mutations are found in clusters between codons 100-108 and 179-190 and confer broad-based cross-resistance to all three available drugs in this class. The most commonly selected mutations are K103N (efavirenz, nevirapine and delavirdine) and Y181C (nevirapine and delavirdine). In a partially suppressive regimen, resistance emerges rapidly due to these point mutations.

PROTEASE INHIBITORS

Protease inhibitors compete with substrate for binding at the active site on the HIV-1 protease enzyme. Forty-two mutations occurring at 27 codons have been associated with PI resistance. Many of these map to the binding site of proteases and interfere with PI binding. Secondary mutations map to other regions and improve the activity of proteases without directly effecting inhibitor binding. Resistance to PIs results from step-wise accumulation of mutations, which occur rapidly if these drugs are administered at inadequate doses or as part of non-fully suppressive drug regimens. The presence of two or more of the key PI mutations (D30N, G48V, I50V, V82A, F, T or S, I184V, L90M) is likely to confer broad cross resistance to most currently available PIs.

However, studies have demonstrated that PI-resistant virus has reduced replicative capacity. This may explain why viral load levels can remain partially suppressed in the presence of high-level PI resistance. An understanding of PI resistance is complicated by extensive polymorphism (naturally occurring variations in sequences) found in viral isolates from PI-naïve patients. Additionally, PI resistance is not only dependent on drug susceptibility but also is strongly linked to achievable plasma drug concentrations. For example, recent data suggests that early resistance to indinavir can be overcome by pharmacoenhancement of indinavir plasma drug levels with low doses of ritonavir.

MEASURING DRUG RESISTANCE IN PRACTICE

Resistance testing in the management of HIV-infected patients is now considered standard in developed countries. In developing countries, resistance testing remains limited largely to the research setting.

Retrospective studies have shown that pre-treatment genotyping or phenotyping is significantly predictive of virological response. Prospective studies have shown that treatment decisions based on resistance assay data are associated with improved virological outcome. In the VIRADAPT study, patients on a PI-containing regimen with viral load >10,000 copies/ml were randomly assigned to have their therapy changed based on clinical judgement or with access to genotypic testing. In six months of follow-up, significantly more patients in the genotype guided group had viral load <200 copies/ml (32% versus 14%). The VIRA3001 study demonstrated a significantly greater reduction in viral load at week 16 in patients failing first line PI-containing therapy whose salvage regimen was based on phenotypic assay results. In the NARVAL study, patients were randomized to genotyping *versus* phenotyping *versus* a control arm. There was no significant benefit seen in terms of short-term virologic outcome between the randomized groups, but a trend favouring genotyping was noted.

Data from these and other studies have been used to perform cost-effectiveness analyses of resistance testing. Current costs in US\$ range from \$400-\$550 for sequencing of protease and reverse transcriptase to \$700-\$900 for phenotypic testing. Given the high cost of ARV and the savings that result from not using ineffective drugs, these assays are highly cost-effective in a developed country setting. The economics are not dissimilar in developing countries, especially when second-line drugs are employed. Despite recent ARV price reductions, the drugs used in salvage therapy remain generally expensive and should be used in the most cost-effective manner. More widespread use of resistance assays in the future seems likely in certain situations in developing countries.

Table 3: International AIDS Society Consensus Panel recommendations for antiretroviral resistance testing

Clinical situation	Rationale
Primary HIV infection	Detect transmission of drug resistant virus. Modify therapy to optimize response ¹ .
Established HIV infection ²	Detect prior transmission of drug resistant virus, although may not always be possible with current tests.
First regimen failure ³	Document drugs to which there is resistance to guide choice of second-line regimen.
Multiple regimen failures ³	Optimize the number of active drugs in the next regimen and exclude drugs to which response is unlikely.
Pregnancy	Optimize maternal treatment and prophylaxis for the neonate.

1. E.g., ritonavir boosting if viral suppression to below the level of detection is not rapidly achieved.
2. In untreated established infection, wild-type virus may replace drug resistant quasi-species over time. These quasi-species will rapidly be selected by drug pressure once the patient commences antiretroviral therapy. Drug resistance results should be interpreted with caution in this situation.
3. The results are most reliable for drugs that are being taken by the patient at the time of testing.

LIMITATIONS OF RESISTANCE ASSAYS

The use of genotypic and phenotypic resistance assays is complicated by a number of factors. In general, plasma samples with more than 500 to 1,000 HIVRNA copies/ml are needed to generate a result. Resistance data from the predominant species may not reflect important minority populations, since viral species constituting less than 20% to 30% of the amplified product may not be detected with currently available assays.

Drug selection pressure at the time of testing may also effect the result. False negative results may occur if blood is drawn after therapy is changed or stopped because susceptible wild-type variants outgrow the resistant virus. For example, the M184V mutation is rapidly lost after lamivudine withdrawal. Blood should be drawn for resistance testing before the failing drugs are stopped.

Interactions between resistance mutations may complicate interpretation of results. To use M184V again as an example, this mutation partially reverses resistance to AZT conferred by mutations in codons 41, 67, 70, 210, 215 and 219. A thorough knowledge of drug cross resistance and mutation interactions in all the classes of ARV is necessary to interpret results and to plan an appropriated change of therapy.

In addition to resistance testing, other important factors need to be considered when making a decision to switch regimens. These include adherence, drug treatment history, viral load, medication tolerance, concomitant medical conditions and medications.

NEW RESEARCH

VIRTUAL PHENOTYPE

Virtual phenotype is a new concept which employs a database with thousands of clinical isolates in which both the genotype and phenotype are known. The virtual (or derived) phenotype is determined by taking a patient's genotype and matching it with genotypes in the database. Given the complexity of interpreting genotypic assay results, this new technology may allow for an objective and quantifiable method to interpret the genotype in a consistent manner. In addition, since it is based on a genotypic test, virtual phenotype is likely (although not certain) to be cheaper and faster than phenotypic tests.

INHIBITORY QUOTIENTS AND BIOLOGICAL CUT-OFFS

There has been much interest recently in the use of inhibitory quotients (IQs) as a better measure of resistance. The IQ is based on the actual or expected minimum drug concentration (plasma C_{min}) divided by a measure of viral susceptibility to that drug (IC_{50}). The equation can be written $IQ = C_{min}/IC_{50}$.

Theoretically, the IQ is a better measure of resistance because, for most drugs, viral resistance is relative to drug concentrations. A measure that includes an assessment of drug concentration relative to the level of viral resistance

may more accurately predict response to that particular is a necessary component for the calculation of the inhibitory quotient. TDM is employed in many areas of medicine and will probably be used increasingly in the context of refining antiretroviral therapy.

Another difficulty in the interpretation of phenotypic test results is the lack of 'biological cut-offs' that truly define virologic response to a particular drug. These are an attempt to establish clinically relevant levels for individual drugs, which tell the clinician whether or not the virus is resistant to that drug. Commercial phenotypic assays have used arbitrary cut-offs (e.g. 2.5 or 4 fold IC_{50} increase) to indicate resistance to all antiretrovirals. However, studies have recently demonstrated that a 1.8 fold increase in IC_{50} to d4T is associated with a significantly blunted viral load response compared to wild-type virus. Two important cut-offs can be defined for any antiretroviral agent: the first point at which the response to the drug becomes attenuated but some antiviral activity still remains; a second point at which the drug has no meaningful activity. Researchers are working to define these cut-offs, individualized for each antiretroviral drug.

SUMMARY

At present, genotypic and phenotypic resistance assays are commercially available. The virtual phenotype shows promise as a clinically useful tool. Measures of IQ may eventually prove more useful than either the genotype or phenotype alone, but require that therapeutic drug monitoring be made part of clinical practice. This would require timed blood specimens, which add to the cost and complexity of patient management. However, knowing the IQ is only useful if one can manipulate the pharmacokinetic parameters of the drug in question. This can be done for many of the currently available PIs by means of ritonavir boosting, but may be less applicable for the other available antiretrovirals. Finally, the full potential of phenotyping will only be realized when clinically defined biological cut-offs have been determined for all the drugs used in practice.

FURTHER READING AND RESISTANCE WEBSITES

www.viral-resistance.com/
www.hivresistance.com/
<http://hivinsite.ucsf.edu/medical/resistance/2098.474c.html>
<http://hivdb.stanford.edu/>

Little SJ, Daar ES, D'Aquila RT, et al. Reduced antiretroviral drug susceptibility among patients with primary HIV infection. JAMA. 1999;282:1142-1149. Available at: <http://jama.ama-assn.org/issues/v282n12/abs/joc91355.html>

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