JAC antiviral

Journal of Antimicrobial Chemotherapy (2004) **53**, 555–557
DOI: 10.1093/jac/dkh157
Advance Access publication 10 March 2004

Benefits and limitations of testing for resistance to HIV drugs

Douglas D. Richman*

Veterans Affairs San Diego Healthcare System and University of California San Diego, Departments of Pathology and Medicine, 0679, 9500 Gilman Drive, La Jolla, CA 92093–0679, USA

Since drug resistance was first described in HIV in 1989, great progress has been made in our understanding of its genetic basis and molecular mechanisms. Despite these advances and the introduction of many new antiretroviral drugs, resistance remains prevalent. The types of drug resistance tests and their relative advantages and disadvantages are reviewed. How and when these tests should be used to inform HIV clinical practice and their place in anti-HIV drug development are considered.

Keywords: antiretroviral drugs, HIV drug resistance, resistance testing

When HIV drug resistance was first described,[1] there was only one approved antiretroviral drug, assays for drug resistance were slow and cumbersome, and the genetic basis and the molecular mechanisms of drug resistance were still being investigated. Now 20 drugs have been approved for use, targeting envelope, protease and reverse transcriptase. The genetic basis of drug resistance, with dozens of well-characterized mutations, is broadly appreciated.[2] The mechanisms of drug resistance are well characterized.[3] Drug resistance tests, based on high-throughput and well-characterized assays for both phenotype and genotype, are commercially available.[4] These assays are now part of standard practice in the management of antiretroviral chemotherapeutics.[3–5]

The pathophysiological basis for the emergence of HIV drug resistance has been well delineated.[5] The magnitude of HIV replication in patients is massive with 10^{10}–10^{11} virions generated daily.[6] With an error-prone reverse transcriptase and the absence of a proofreading mechanism, virtually all possible mutations must be generated daily. Thus the selective pressure imposed by antiretroviral therapy guarantees the emergence of drug-resistant virus unless the regimen is sufficiently potent to suppress virus replication.

The development of regimens over the past few years with sufficient potency, modest side-effect profiles and convenience of use (few pills administered once or twice daily) has resulted in 90% success rates at 1 year, with the failures often attributable to discontinuation of drugs or loss to follow-up, rather than to true drug failure. These results are encouraging since the most effective way to deal with drug resistance is to prevent it.

Resistance is prevalent, however, due to the historical use of sequential regimens, the prescription by some physicians of suboptimal combinations, the difficulty for some patients to adhere to their regimens and more recently the transmission of drug-resistant virus.[7,8] This prevalence of drug resistance is best managed with the use of drug resistance testing. Cost-benefit analyses have argued for the utility of drug resistance tests.[9] They avoid the costs and toxicity of drugs not likely to work and identify drugs most likely to have sustained benefit. Several prospective, randomized trials have demonstrated the benefit of drug resistance tests; however, these trials can usually be sustained only for short durations and have become very difficult to design and conduct because drug resistance testing has become the standard of practice.[3] One of the most compelling documentations of the utility of both genotypic and phenotypic drug resistance testing resulted from the registrational trials of enfuvirtide, in which the efficacy of the background antiretroviral regimen was assessed with these assays.[10,11]

Both phenotypic and genotypic assays are available to test drug resistance. Phenotypic assays measure the actual inhibition of virus replication by a drug. The initial assays were slow and cumbersome. Now high-throughput, automated, recombinant virus assays are available commercially to provide phenotypic drug susceptibility results.[12,13] The advantage of such assays is that they are as easy to interpret as standard antimicrobial drug resistance assays, they are quantitative and are 'open-minded' in the sense that they detect resistance to new drugs or new mutations conferring resistance to old drugs. Their disadvantage is their cost (~$900 US to test 18 drugs) and turnaround time (~2 weeks).

Genotypic assays report mutations known to be associated with drug resistance after performing nucleotide sequencing of the genes of interest. Their relative benefits are cost (~$400 US) and turnaround time. They can be performed in a few days, but if they are sent out the results usually are returned in about 1 week. In contrast to phenotypic tests, genotypic tests can only interrogate what is known. Although mutations are the basis of the phenotype, the identification of the mutations that are important for conferring drug resistance requires confirmation with phenotypic tests with viruses containing selected mutations prepared by site-directed mutagenesis. Thus genotypic tests are less interpretable with new drugs or newer mutations selected by older drugs.

*Tel: +1-858-552-7439; Fax: +1-858-552-7445; E-mail: drichman@ucsd.edu

A second disadvantage of genotypic tests is the difficulty of interpreting the results, which requires experience and effort in keeping up with the published literature and unpublished studies. Updates are maintained by expert panels[2] (www.iasusa.org). Interpretation schemes are also often provided with test results. There are many such schemes, as well as various computer-based strategies of interpretation, for example the Virtual Phenotype, which is an approach to genotype interpretation. None has proven superior to another or to interpretation by experts.[3,4]

Access to both phenotypic and genotypic assay results would be ideal. When cost is an issue, this author resorts to the following approach. A genotype assay is obtained first. The absence of drug resistance or the presence of easily interpretable resistance patterns (M184V or mutations to non-nucleoside reverse transcriptase inhibitors in reverse transcriptase) is often sufficient. However, with certain complex patterns of resistance to nucleosides and certainly to protease inhibitors when quantitative differences in resistance might be important in selecting a regimen, then a phenotypic test is obtained. For example, ritonavir-boosted protease inhibitors have been shown to exert activity against certain degrees of protease inhibitor resistance. These intermediate levels of resistance of five- to 50-fold are difficult to ascertain with genotypic tests. Improvements will be forthcoming in drug resistance technologies, but it is not clear what these will be. The utility of therapeutic drug monitoring as a component of management remains under active investigation.

When should drug resistance tests be obtained? Initially the indications were for patients who failed a regimen. The absence of drug resistance is often informative about poor patient adherence. Except in recently infected patients, drug resistance tests cannot be reliably interpreted if a patient has discontinued his or her regimen for more than a few weeks.[14] Standard drug resistance assays do not detect minority species in the circulation, or variants (either wild-type or resistant) that are archived in the lymphoid system. Moreover, variants may be replicating in the central nervous system and genital tract that are poorly represented in the blood. More recently, many practitioners are performing drug resistance tests on patients who have not yet initiated treatment. Recent studies indicate that in many populations primary HIV drug resistance is being transmitted in 5%–20% or more of newly infected patients.[7,8] Drug resistance testing is indicated with prevalence of drug resistance of at least 5% in either recently infected or even chronically infected drug-naive patients. Such rates have now been documented in many areas of North America and Europe. Furthermore, transmitted drug resistance, in contrast to acquired drug resistance, persists in the patient for years after infection, since wild-type virus is not archived in these patients to re-emerge in the absence of the selective pressure of drug treatment.

The first years of experience with antiretroviral therapy almost exclusively involved clade B infections in Europe and North America. With the increasing proportion of non-clade B infections in Europe and Israel and the significant extension of treatment to more resource-constrained countries, the issue of differences in resistance patterns and the utility of resistance assays becomes a concern. An excellent recent review of the early data suggests that non-clade B group M HIV-1 appears to exhibit similar patterns of susceptibility to antiretroviral drugs; however, differences in nucleotide sequence may result in distinctive genetic pathways to resistance in non-clade B viruses.[15] More data regarding patterns of both wild-type susceptibility and patterns of resistance in these highly prevalent non-B clades certainly will be generated over the coming years.

Drug resistance testing is an integral part not only of patient management but also of new drug development. Candidate compounds in all the classes of currently approved drugs are now being designed to inhibit drug-resistant variants of HIV, since these are highly prevalent and drugs in these classes for wild-type viruses are already approved. This also provides the impetus for identifying new classes of antiretroviral drugs, such as entry and integrase inhibitors. During the drug development process, characterizing the activity of a candidate drug against viruses from different clades and with different drug resistance patterns is now standard. Moreover, the approval process is expedited if a candidate drug can be shown to have efficacy against virus from patients with limited options due to drug resistance. In fact, the lopinavir/ritonavir co-formulation was the first drug approved with a specified indication for the treatment of patients infected with HIV having certain levels of protease inhibitor resistance.

Drug resistance testing thus has become a standard component of both drug development and patient management. The rapid evolution of the field of antiretroviral chemotherapy, not to mention of the virus itself, guarantees that the technology and the information regarding drug resistance testing will be rapidly changing as well. This provides challenges for drug development, assay development, regulatory oversight and healthcare providers. For the practitioner, the demands to keep up are appreciable and the stakes are high, but the opportunity to make a difference in patient outcome is great.

Acknowledgements

This work was supported by grants AI 27670, AI 38858, AI 43638, UCSD Centers for AIDS Research (AI 36214), AI 29164, from the National Institutes of Health and the Research Center for AIDS and HIV Infection of the San Diego Veterans Affairs Healthcare System.

References

1. Larder, B. A., Darby, G. & Richman, D. D. (1989). HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* **243**, 1731–4.

2. Johnson, V. A., Brun-Vezinet, F., Clotet, B. *et al.* (2003). Drug resistance mutations in HIV-1. *Topics in HIV Medicine* **11**, 215–21.

3. Hirsch, M. S., Brun-Vezinet, F., Clotet, B. *et al.* (2003). Antiretroviral drug resistance testing in adults infected with human immunodeficiency virus type 1: 2003 recommendations of an International AIDS Society-USA panel. *Clinical Infectious Diseases* **37**, 113–28.

4. Hirsch, M. S., Brun-Vezinet, F., D'Aquila, R. T. *et al.* (2000). Antiretroviral drug resistance testing in adult HIV-1 infection. *Journal of the American Medical Association* **283**, 2417–26.

5. Hirsch, M. S., Conway, B., D'Aquila, R. T. *et al.* (1998). Antiretroviral drug resistance testing in adults with HIV infection. *Journal of the American Medical Association* **279**, 1984–91.

6. Perelson, A. S., Neumann, A. U., Markowitz, M. *et al.* (1996). HIV-1 dynamics in vivo: virion clearance rate, infected cell lifetime, and viral generation time. *Science* **271**, 1582–6.

7. UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance. (2001). Analysis of prevalence of HIV-1 drug resistance in primary infections in the United Kingdom. *British Medical Journal* **322**, 1087–8.

8. Little, S. J., Holte, S., Routy, J. P. *et al.* (2002). Antiretroviral-drug resistance among patients recently infected with HIV. *New England Journal of Medicine* **347**, 385–94.

9. Weinstein, M. C., Goldie, S. J., Losina, E. *et al.* (2001). Use of genotypic resistance testing to guide HIV therapy: clinical impact and cost-effectiveness. *Annals of Internal Medicine* **134**, 440–50.

10. Lalezari, J. P., Henry, K., O'Hearn, M. *et al.* (2003). Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. *New England Journal of Medicine* **348**, 2175–85.

11. Lazzarin, A., Clotet, B., Cooper, D. *et al.* (2003). Efficacy of enfuvirtide in patients infected with drug-resistant HIV-1 in Europe and Australia. *New England Journal of Medicine* **348**, 2186–95.

12. Petropoulos, C. J., Parkin, N. T., Limoli, K. L. *et al.* (2000). A novel phenotypic drug susceptibility assay for human immunodeficiency virus type 1. *Antimicrobial Agents and Chemotherapy* **44**, 920–8.

13. Vingerhoets, J., Bloor, S., Michiels, L. *et al.* (1999). The accuracy and reproducibility of high throughput genotypic and phenotypic HIV-1 resistance testing under EN45001 and CLIA accreditation labels. *Antiviral Therapy* **4**, *Suppl. 1*, Abstract 53.

14. Deeks, S. G., Wrin, T., Liegler, T. *et al.* (2001). Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. *New England Journal of Medicine* **344**, 472–80.

15. Parkin, N. T. & Schapiro, J. M. (2004). Antiretroviral drug resistance in non-subtype B HIV-1, HIV-2 and SIV. *Antiviral Therapy* **9**, 3–12.