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2004: Which HIV-1 Drug Resistance Mutations are Common in Clinical Practice?

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Abstract

The emergence of drug resistance remains a major problem for the treatment of HIV-infected patients. However, the variety of mutational patterns that evolve in clinical practice have made the application of resistance data to clinical decision-making challenging. Despite (or because of) an abundance of drug-resistance data from disparate sources, there is only limited information available describing the patterns of drug resistance which usually appear in the clinic. Here we attempt to address this issue by reviewing HIV drug resistance in the population of patients failing antiretroviral therapy in British Columbia, Canada from June 1996 to December 2003 as an example. Our findings suggest that, although hundreds of mutations have been associated with resistance, relatively few key mutations occur at a high frequency. For example, only the nucleoside reverse transcriptase inhibitor (NRTI) mutations M184V, M41L T215Y, D67N, K70R and L210W, non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations K103N and Y181C, and protease inhibitor (PI) mutation L90M, occur in more than 10% of samples tested for resistance in this population.
The introduction of new drugs allows for the selection of new mutations. Trends in the prevalence of resistance-associated mutations have generally followed trends in drug usage, but have not always mirrored them. The phenomenon of cross-resistance can play an important role in the efficacy of new antiretroviral agents, even before they become available. The extent of this cross-resistance depends in part on the prevalence of specific mutations in the population of individuals who have previously received antiretroviral therapy. Hence there is a need to determine which mutations are prevalent in the treated population. The tremendous capacity of HIV to adapt means that common resistance pathways are likely to change over time, and new pathways to resistance are likely to continue to be discovered in the future. **(AIDS Reviews 2004;6:107-16)**

Key words

Drug resistance. Antiretroviral therapy. Mutations. HIV.

Background

In recent years, it has become clear that drug-resistant HIV strains are commonly selected in patients taking antiretroviral agents. Approximately 20-40% of patients harbor at least some drug-resistant HIV within two years of initiating therapy[1-3]. The accumulation of drug-resistance mutations – or in some cases a single mutation – can significantly compromise the ability of individual antiretroviral agents to inhibit viral replication. As such, drug resistance is well recognized as a major obstacle to achieving the widely accepted clinical goal of sustained suppression of HIV at undetectable levels[4]. Although drug-resistant HIV strains are found in untreated HIV patients (primary resistance), most drug resistance is secondary to antiretroviral therapy, resulting from drug selection pressure when HIV replication is incompletely suppressed. While there have been a plethora of studies on the prevalence of

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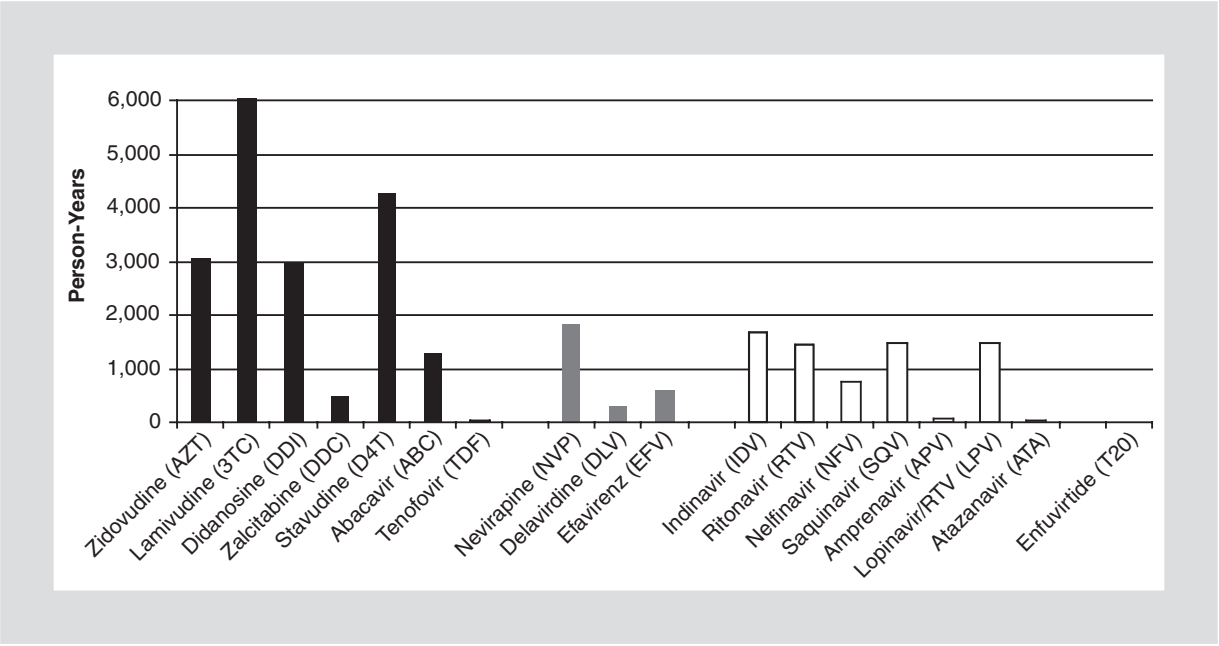

Figure 1. *Cumulative Drug Exposure. The total cumulative number of person-years of each antiretroviral therapy used by the 1910 individuals who have been genotyped for HIV drug resistance in British Columbia, Canada (Fig. 2) to the date of their latest on-therapy resistance test. Nucleos(t)ide analogues (nRTIs) are indicated by filled bars, non-nucleoside reverse transcriptase inhibitors (NNRTIs) by striped bars and protease inhibitors (PIs) by open bars. To date, there has been very little use of fusion inhibitors.*

resistance among recently infected individuals, surprisingly few studies have been published on the prevalence of HIV drug resistance to antiretrovirals in treated populations, in part because of the difficulty of achieving representative sampling[5].

Several organizations collect HIV sequence data, review mutations associated with resistance and provide access to their databases via publications and/or the internet[6-10]. For example, the Los Alamos Database compiles and annotates sequences and provides interactive programs that facilitate sequence alignment, rapid analysis of mutation patterns, clade identification, and more[7]. One of the most useful of these is the Stanford HIV Drug Resistance Database, a relational database which allows users to address HIV-resistance queries from HIV sequence data by using a number of methods, including comparing different resistance algorithms[8]. The mutation lists provided by these organizations are updated regularly; however, they are not universal. There is presently no standardized interpretation system for estimating drug resistance from HIV genotype. To complicate matters, new mutations associated with drug resistance are being identified at a rapid pace. For example, Shafer, et al. have recently described nine previously unreported mutations associated with nucleoside reverse transcriptase inhibitor (NRTI) therapy[11] and 22 previously unreported mutations associated with protease inhibitor (PI) therapy[12]. Furthermore, Chen, et al.[13] have identified more than 160 new amino acid mutations in the protease and reverse transcriptase (RT) genes that may potentially alter viral fitness and/or resistance, using an automated mutation-analysis system based upon the nucleic acid sequence variation of over 40,000 isolates. The clinical relevance of each of these mutations and the myriad combinations in which they can arise, as well as their prevalence at the population level, remain to be established. The complex mutational patterns that may evolve in clinical practice have made the application of resistance data to clinical decision-making challenging. The evaluation of drug resistance and treatment strategies used in the Western world has been the subject of a number of recent reviews[14-18].

Treatment of HIV infection is largely confined to HIV-1 subtype B, the strain of HIV that predominates in North America and other Western countries where highly active antiretroviral therapy (HAART) has been widely available for almost a decade. Recent studies have shown that non-B subtype protease and RT mutations are usually very similar to those observed from B subtype viruses[19,20]. However, it is worth noting that HIV patients with B and non-B subtype variants that are treated with similar PI or non-nucleoside reverse transcriptase inhibitor (NNRTI) regimens do not necessar-

ily present with exactly the same mutation patterns upon failure of therapy[21,22].

At least 5000 publications on HIV drug resistance appear in Medline, representing a massive effort by the scientific community to elucidate the complexity of this constantly evolving battle between the adaptability of HIV and antiretroviral regimens. Despite substantial amounts of drug-resistance data, there is only limited information available describing the patterns of drug resistance that emerge in the routine clinical setting. Here, we attempt to review the common mutations that confer HIV drug resistance, using the population of patients failing antiretroviral therapy in British Columbia (BC), Canada as an example. This population has a number of natural advantages: treatment history is known due to a centralized, population-based system of drug distribution and therapy monitoring and the therapeutic approach generally reflect those in international guidelines. As importantly, a large amount of matched genotypic-resistance data is also maintained in this centralized system, and this data is available to the authors.

We focus on resistance data from three populations: from all patients with any genotype data; from those whose first therapy was HAART; and from those with recent tests (to show the latest trends).

The BC HIV/AIDS Drug Treatment Program

The BC Centre for Excellence in HIV/AIDS (the Centre) provides any resident of British Columbia who is infected with HIV the opportunity to enter the provincial drug distribution program known as the BC HIV/AIDS Drug Treatment Program. As a participant, individuals receive anti-HIV care including antiretroviral agents free of charge. Antiretroviral medication is prescribed according to specific guidelines generated by the BC Therapeutic Guidelines committee. In June 1996, the Centre adopted plasma viral load driven antiretroviral therapy guidelines, consistent with those put forward by the International AIDS Society – USA (IAS-USA)[23]. In brief, antiretroviral therapy-naive individuals with a pVL > 100,000 copies/ml were offered triple therapy (i.e. two nucleosides plus a PI, or a NNRTI), whereas those with pVL from 5,000-100,000 copies/ml were offered dual nucleoside therapy. In July of 1997, the guidelines were revised to recommend triple combination therapy for all antiretroviral-naive individuals with pVL ≥ 5,000 copies/ml, or a CD4 cell count < 500 cells/μl. Participants are typically followed at three-month intervals, at which time routine viral load testing is performed. Drug-resistance testing of HIV patients virologically failing antiretroviral therapy was recommended in the Therapeutic Guidelines in 1999, and is widely available upon physician request, *including retrospective testing of stored samples*.

Mutation patterns among HIV patients who ever received antiretroviral therapy in British Columbia

Blood samples from all HIV-infected patients who have enrolled in the Drug Treatment Program have been routinely collected and stored since the adoption of plasma load driven antiretroviral therapy in June 1996. For the purposes of this analysis, we have included all HIV-infected patients who were prescribed any antiretroviral therapy (with one or more agents) with a documented treatment history, for whom a physician ordered at least one drug resistance test (n = 1,910). Patients were followed up to December 30th, 2003. Overall, these patients represent more than 27,700 person-years of antiretroviral therapy and ~40% of the approximately 4,600 patients who ever received therapy during this period (Fig. 1). During this period, the NRTIs represented the most commonly used class, both because they were available earliest, and because they are used in combination in HAART. Lamivudine (3TC) was by far the most commonly prescribed agent. In 1996, there were approximately 1,500 active participants in the Drug Treatment Program[24]. At that time, only the five NRTIs (AZT, ddI, ddC, d4T and 3TC) and three PIs (saquinavir, indinavir, and ritonavir) were widely available. Of the patients subsequently tested for HIV drug resistance, most (60%) were taking dual nucleoside therapy, with ~90% being resistant to one or more antiretroviral drug class and 14% resistant to two or more drug classes (Fig. 2). The genotypic resistance is defined as the presence of major mutations specified by the IAS-USA mutation tables[16]. Not surprisingly, mutations conferring NRTI resistance were most prevalent in individuals failing therapy. The M184V mutations associated with high-level 3TC resistance was present in a majority of cases (75%)[25-27]. In subsequent years a variety of new drugs have been introduced, including the NNRTIs delavirdine (DLV) and nevirapine (NVP), and the PI nelfinavir (NFV) in 1998, the NRTI abacavir (ABC) and the NNRTI efavirenz (EFV) in 1999, amprenavir (APV) and lopinavir (LPV) (both PIs) in 2001, and most recently tenofovir (TDF), the first nucleotide reverse transcriptase inhibitor (NtRTI)[24]. These changes have coincided with steadily

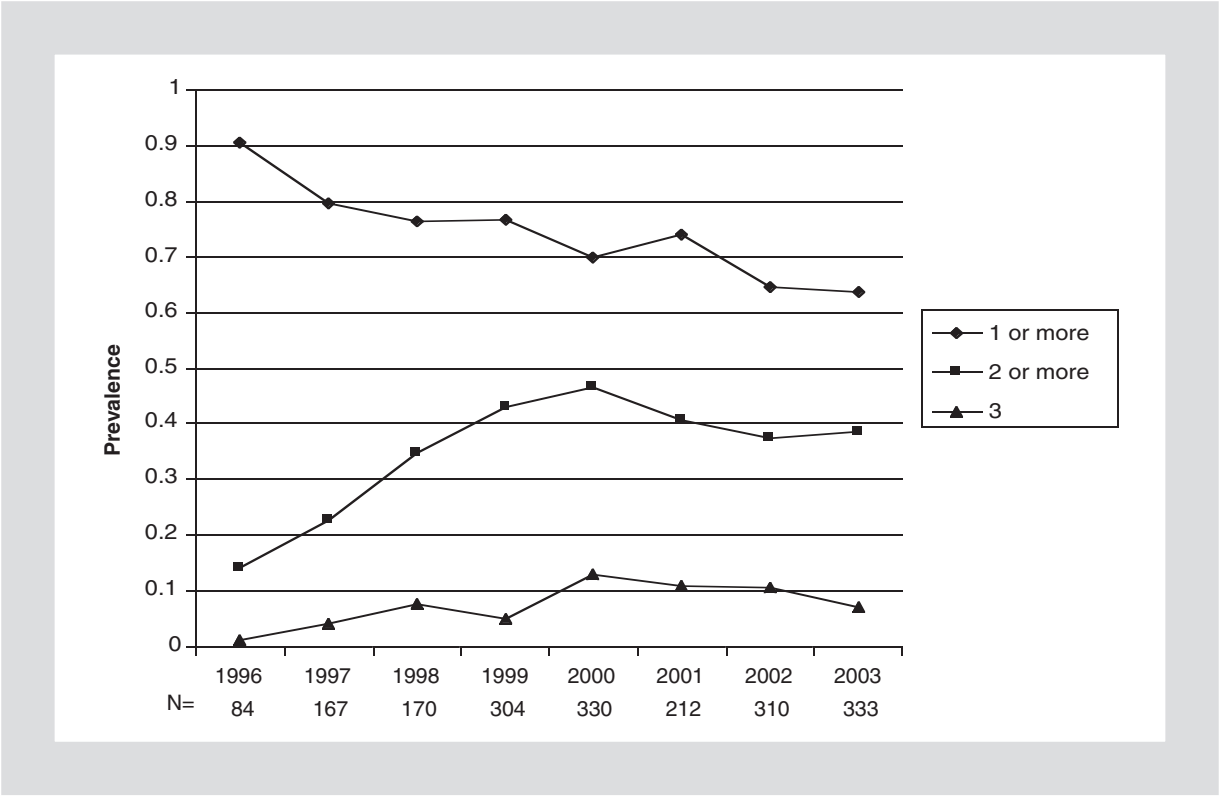

Figure 2. *Trends in drug resistance. The proportion of individuals with major mutations conferring resistance (IAS-USA definitions) to ≥1 (diamonds), ≥2 (squares) or 3 drug classes (triangles) is indicated for 1910 individuals from British Columbia, Canada. All patients were receiving therapy at the time of the genotypic test; where multiple samples were available per patient, the latest "on-therapy" genotype obtained from June 1996 to December 2003 was analyzed. The number of individuals is indicated below the graph. Note that many samples were tested retrospectively.*

increasing numbers of patients with resistance to multiple drug classes (Fig. 2). As in 1996, resistant variants continue to be detected in approximately 60% of patients failing antiretroviral therapy as defined by having a detectable plasma viral load despite therapy. By the end of 2003, however, 39% of patients harbor HIV variants that are resistant to two or more drug classes and a further 7% harbor variants that are resistant to all three drug classes (Fig. 2).

This change toward multi-class drug resistance has been driven primarily by the increase in prevalence of resistance to NNRTIs (Fig. 3b) as these agents became available and widely used. Since 1999, approximately 50% of treated patients in British Columbia have been prescribed NNRTIs. Subsequently, the prevalence of resistance to this drug class among failing patients increased rapidly, reaching approximately 50% by 2003. The low genetic barrier (requirement for a single mutation to confer resistance) and high degree of cross-resistance among NNRTIs most likely explain this rapid accumulation of NNRTI resistance in the treated population. It should be noted that most NNRTI use in British Columbia is NVP, rather than EFV.

Temporal trends in HIV drug resistance have not always been straightforward or predictable. For example, the prevalence of decreased susceptibility (defined using the Virco "Virtual Phenotype") among failing patients to the NRTI class as a whole dropped considerably between 1996 and 1998 (Fig. 3a). In particular, the prevalence of resistance to zidovudine (AZT) has continued to decrease over time. While a portion of this trend may largely reflect AZT usage, which has also steadily dropped from about 45% in 1996 to 20% in recent years, a larger contributor is likely the increasing use of triple therapy, resulting in the decreased selection of thymidine analogue mutations (TAMs). In addition, cross-resistance within each drug class may further complicate the prediction of drug resistance trends. For example, ABC has only been available since mid 1997. However, inferred ABC resistance was much more common in the British Columbia population failing therapy in 1996 (Fig. 3a) than it was in 2003. This observation appears to reflect the cross-resis-

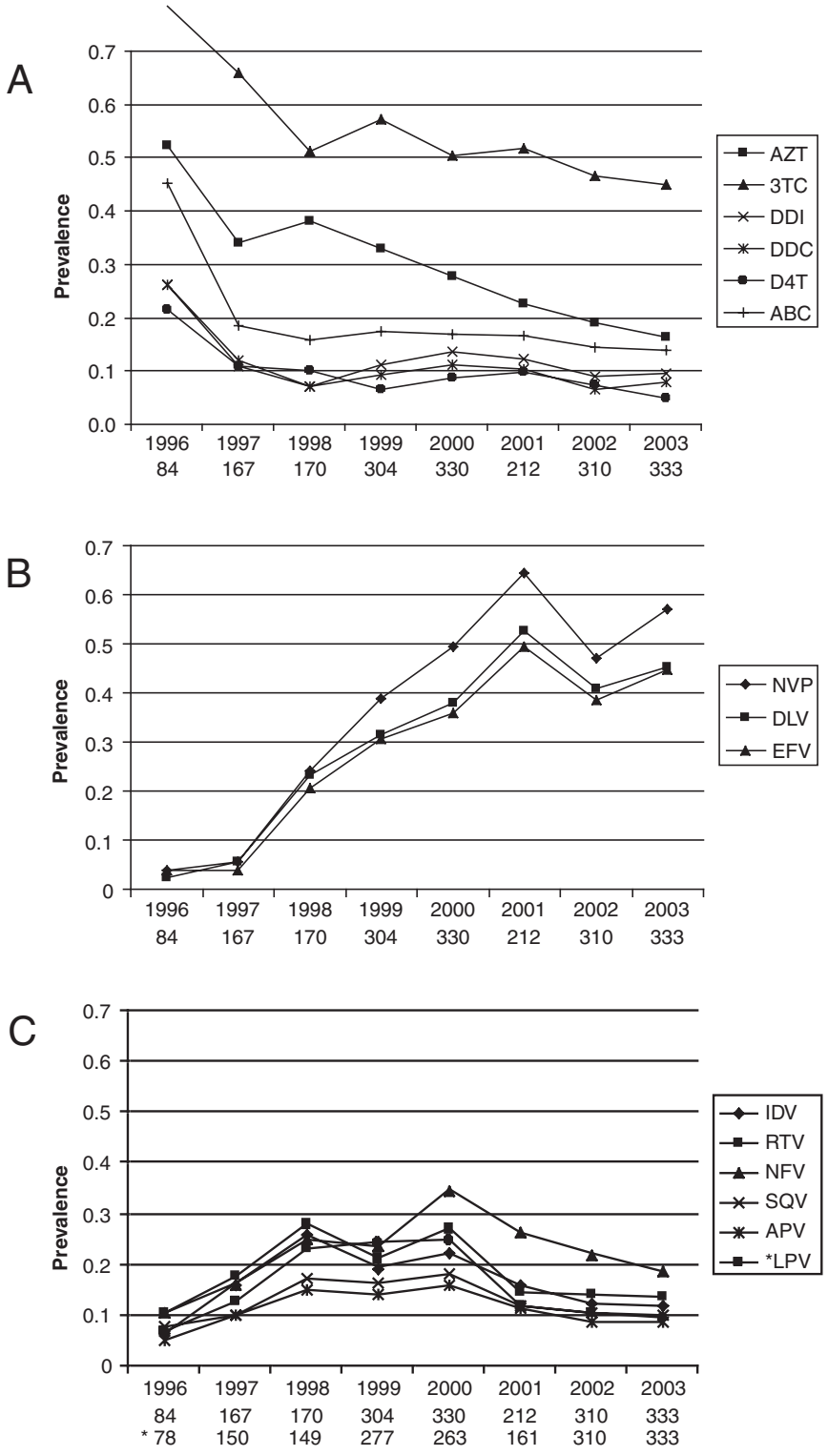

Figure 3. *Trends in drug resistance for each antiretroviral. As in figure 2, the proportion of individuals with decreased susceptibility as estimated by Virtual Phenotype for each antiretroviral is indicated for 1910 individuals from British Columbia, Canada whose last on-therapy sample genotyped was obtained from June 1996 to December 2003 for the nRTIs (panel A), NNRTIs (panel B) or PIs (panel C). The number of individuals is indicated below; note that Virtual Phenotypes for lopinavir were not available for all samples for earlier dates.*

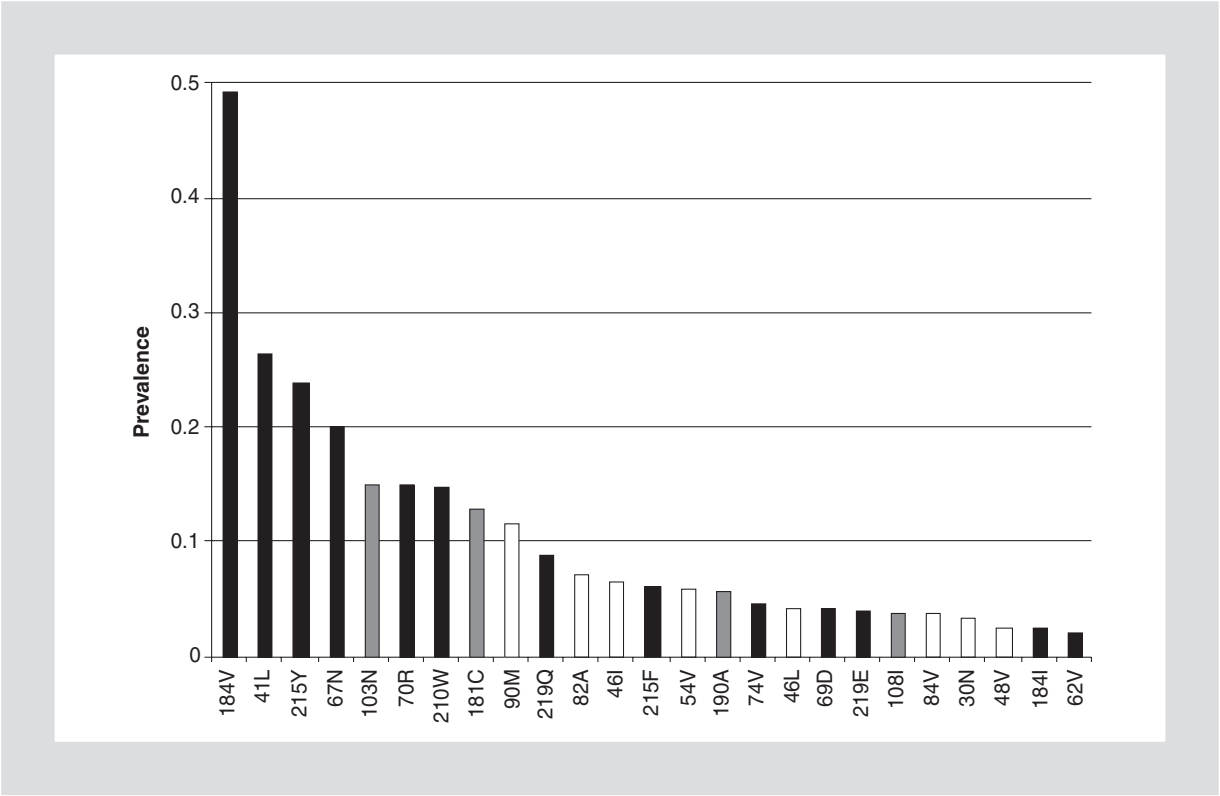

Figure 4. *Prevalence of major drug resistance mutations. Major mutations (IAS-USA definitions) conferring resistance to nucleos(t)ide analogues (nRTIs) are indicated by filled bars, non-nucleoside reverse transcriptase inhibitors (NNRTIs) by striped bars and protease inhibitors (PIs) by open bars. Data are generated from the last "on-therapy" sample tested for resistance mutations from 1910 individuals.*

tance between ABC and other commonly prescribed NRTIs.

The overall prevalence of resistance to PIs in failing patients has remained both relatively rare and relatively constant over time, at approximately 10-20% of tested samples (Fig. 3c). The prevalence of resistance was roughly similar for all PIs, consistent with the high degree of cross-resistance within this class. Slightly higher NFV resistance can be observed, which is consistent with the ability to select for resistance to only this agent via the D30N mutation. It should be noted that use of LPV has increased rapidly, and as of January 2003 it represented approximately 60% of PI usage by Drug Treatment Program participants tested for HIV drug resistance.

Although hundreds of HIV mutations have been described associated with resistance to antiretroviral agents and/or alterations in viral fitness, we have found relatively few that occur at high frequency in the population failing therapy (Fig. 4). The near absence of HIV RT mutations Q151M, M230L, P236L, and insertions at codon 69 is of particular note, as these mutations have received much attention in the literature[28-33]. Although they may occur more frequently in individuals infected with other HIV subtypes[34], we have found that they remain uncommon in treated patients infected with HIV-1 subtype B, even after years of follow-up (prevalence of 3.5, 0.3, 0.3 and 0.03% respectively), consistent with other smaller studies[28,35,36]. Thus, while such mutations are very relevant to individuals when they occur, they are not significant on a population basis.

Mutation patterns in the population which initiated therapy with HAART

The HOMER cohort comprises all antiretroviral-naive HIV-infected adults in British Columbia whose initial antiretroviral therapy was HAART, and started between August 1, 1996 and September 30, 1999 (n = 1,191). This patient population has been extensively characterized elsewhere[37]. In this analysis, patients were systematically genotyped at every viral load visit, up to 30 months following initiation of therapy where viral load was greater than 1,000 copies/ml[3]. Together, these patients represent a total of 6,600 person-years of antiretroviral therapy. The pattern of drug usage by

this subset was somewhat less varied than that of Drug Treatment Program participants as a whole, as the choice of drugs available was more limited during this period. Six antiretroviral drugs each accounted for more than 5% of the total antiretroviral usage: lamivudine (28%), stavudine (21%), indinavir (14.5%), zidovudine (10%), nevirapine (10%) and didanosine (5%). Together these represented 88% of the total antiretroviral drug usage by this population during the 30-month follow-up period. For most patients in this group, the initial HAART regimen comprised of an NRTI backbone of 3TC plus either stavudine (D4T) or AZT in combination with either the PI indinavir or the NNRTI nevirapine.

The IAS-USA mutation tables (see Johnson, et al.[15] for the October 2003 version, or the IAS-USA web-site (http://www.iasusa.org for regular updates) are a well recognized and valuable resource for assessing drug-resistance mutations. However, for understanding the populational influence of resistance mutations, this table can be somewhat misleading, as all mutations appear to be of equal relevance. In figure 5, we therefore have adapted the IAS-USA format, but relatively "scaled" the font size of mutations to represent the prevalence of drug usage and the prevalence of resistance mutations during the first 30 months of HAART. The widespread use of 3TC and its low genetic barrier – a single mutation (M184V) confers high level resistance – contributes to the exceptionally high frequency of M184V among this group. Using this presentation, few other mutations are visible other than K103N, Y181C and the minor protease mutations L10I and L71T/V, indicating graphically which mutations are most significant on a population basis. Similarly, the use of agents other than 3TC, D4T, AZT, NVP and indinavir (IDV) was insignificant during this time period.

The most recent trends in resistance

The choice of drugs which make up HAART regimens appear to be remarkably time-dependent. As newer drugs become available and different combinations of antiretroviral agents become possible, drug selection pressures change and the prevalence of individual mutations in the treated population evolves. The most recently approved antiretrovirals have been the PIs amprenavir, lopinavir/ritonavir, and atazanavir, the nucleotide analogue TDF and the fusion inhibitor enfuvirtide (T20). The introduction of these three new PIs has apparently had relatively little impact on the prevalence of PI mutations, though the number of protease codons which are known to be associated with resistance may be increasing[38,39]. In fact, only a single case of failure of lopinavir/ritonavir in a drug-naive individual leading to high-level resistance specific to LPV has been reported worldwide to date (as a result of protease mutations 32I and 47A)[40]. This rarity is likely due in part to a favorable pharmacokinetic profile, but probably also because such individuals are now rarely left on failing regimens without a change in treatment for long enough periods for PI resistance to develop. In addition, APV use in British Columbia has been sufficiently rare that it has had little impact on resistance. Similarly, atazanavir (ATA) and enfuvirtide (T20) have been introduced only very recently. As a result, the nominal "signature mutations" for these compounds have been detected exceedingly rarely to date.

In contrast, the previously rare K65R mutation associated with decreased susceptibility to abacavir, 3TC, ddI and TDF is now being reported more frequently in multiple datasets[41-44], particularly when these agents are used in the absence of AZT. Resistance to TDF appears to arise most commonly from either combinations of TAMs or from selection of K65R[45]. The role and impact of K65R in the context of TAMs and TDF resistance has been recently reviewed in detail[45]. Winston, et al. have reported a significant increase in K65R over a two-year period from 1.7% of tested samples in 2000 to 4% in 2002. This increase was associated with the use of the combination of TDF and ddI as a nucleoside backbone of triple therapy and especially with the triple nucleoside combination of TDF, ddI and ABC[46]. Similarly, MacArthur, et al. reported a doubling in the frequency of the K65R mutation between 2001 and 2002 in a sample of 900 genotypes from two large urban medical centers in the USA[47]. This trend was concurrent with an increase in the use of TDF and ABC[47]. An analysis of the VIRCO database (over 60,000 genotypes) also revealed a trend of increased frequency of K65R from a prevalence of 0.8% in 1998 to 3.8% in 2003[48]. Tenofovir was approved for the treatment of HIV-1 infection in Canada in March 2003, and has become a popular component of HAART. We have also observed a dramatic increase in the frequency of K65R in the British Columbia database, from a historical prevalence less than 1% before 1999 to more than 7.5% of recently genotyped samples (data not shown).

It has been proposed that K65R is preferentially selected with therapy which excludes thymidine nucleoside analogs compared with concurrent AZT use[44], and that the appearance of K65R is relatively rare in combination with TAMs[45,49]. These observations are

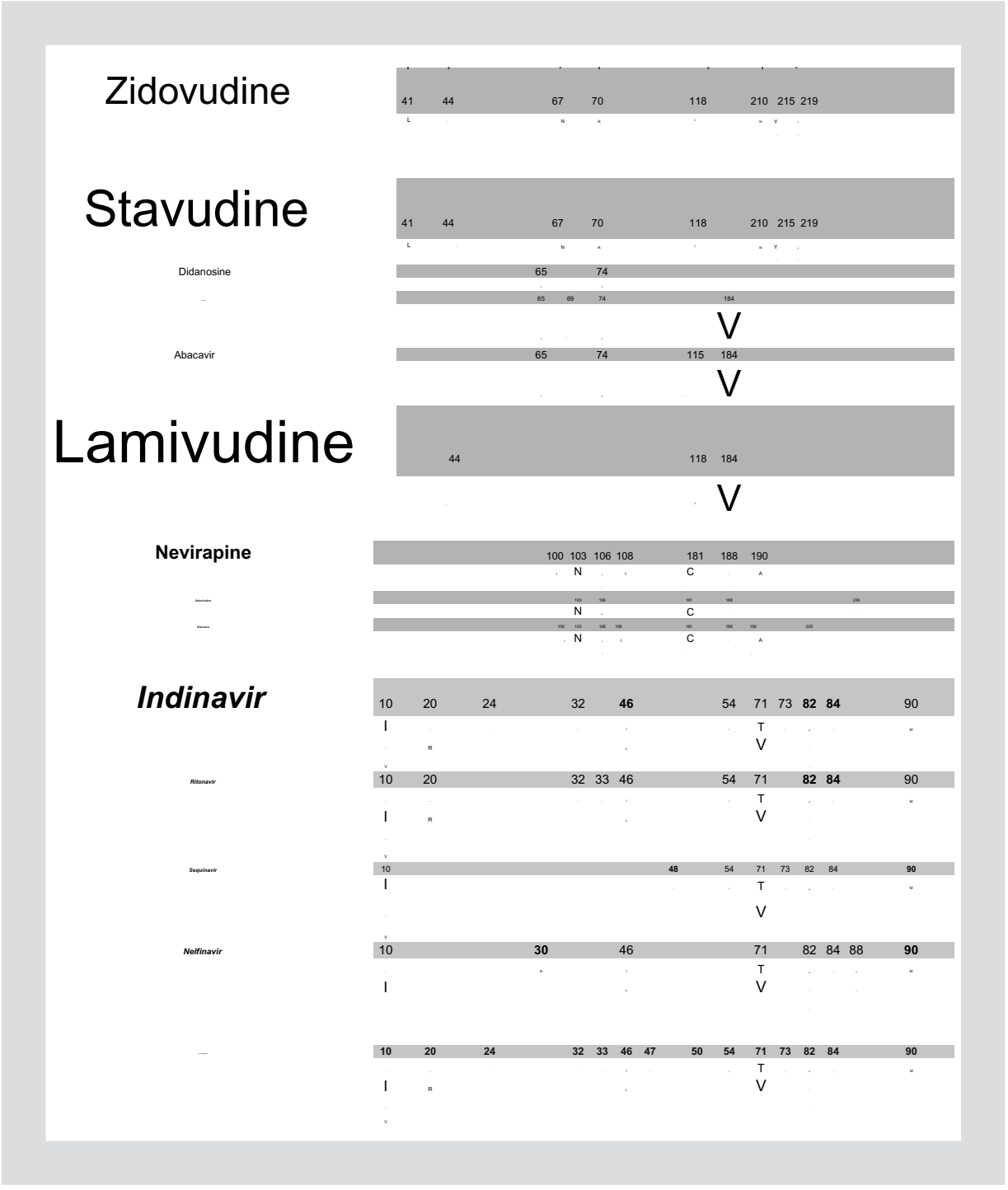

Figure 5. *"Weighted" version of IAS-USA resistance mutation table. The IAS-USA mutation table (available at http://www.iasusa.org) is adapted here to show both mutation prevalence and drug use to scale. The font size of the drug name is proportional to the amount of drug use, while the font size of each mutation is proportional to the prevalence of each mutation over the first thirty months after starting HAART therapy for individuals in the "HOMER" cohort.*

consistent with the results from *in vitro* mechanistic studies that have shown that a K65R variant was resistant to TDF, ddI and ABC, but appeared to be fully susceptible to AZT[33]. It is not clear whether D4T has the same negative impact on the selection of K65R as AZT, particularly since D4T itself can select for K65R[50]. It appears that K65R is compatible with other mutations which are less commonly observed with TAMs such as

L74V[51] or Y181C[unpublished observations], and that L74V may predispose patients to virological non-response and development of K65R taking TDF[52]. The K65R mutation does, however, appear in the context of the Q151M complex of mutations that confer broad nucleoside analogue resistance[11,49].

In addition, viruses containing the K65R and M184V mutations showed further decreases in susceptibility to ddI and ABC, but increased susceptibility to TDF compared to the susceptibilities of viruses with only K65R[53]. Roge, et al. have recently reported five of eight patients failing a triple NRTI combination comprised of ABC, ddI and D4T rapidly developed a K65R mutation in an otherwise wild-type background. This single mutation can easily emerge with triple therapy and compromise future treatment options[32]. Interestingly, phenotypic characterization of defined clonal mixtures of K65R and wild-type virus indicated that over 90% prevalence of the K65R mutation was required for this mutation to increase phenotypic susceptibility above the clinically defined cut-offs for TDF and 3TC[53]. This suggests that genotypic approaches are likely to be more sensitive than phenotypic approaches for detecting this resistance pathway, and emphasizes the complementary nature of these methods.

Summary

The thousands of publications in the scientific literature on HIV drug resistance over the last 15 to 20 years represent a major effort by the scientific community to keep up with the complexities of a highly dynamic epidemic. HIV/AIDS care has been revolutionized through the development of multiple new antiretroviral drugs, new drug classes, and new technologies for monitoring HIV disease. The data presented meaningful insight into how drug resistance is impacting on HIV-infected individuals who have accessed antiretroviral therapy at the population level. Although individualized HIV therapy is of the utmost importance to the continued improvement in the clinical care of HIV patients, a population-based perspective helps put resistance issues into perspective. Although hundreds of mutations have been associated with resistance, relatively few major mutations occur at a high frequency (i.e. more than 10%). Furthermore, while trends in the prevalence of mutations have followed trends in drug usage to some extent, and resistance is more common for the more commonly used agents, cross-resistance plays an important role in determining resistance of the population even to drugs which are not in common use. Finally, the introduction of new drugs allows for multiple new drug combinations. Interactions between mutations can alter the emerging genetic variation within the population. The tremendous capacity of HIV to mutate means that different resistance pathways are likely to evolve in the future. We must continue to monitor the outcomes of HIV drug resistance and the relative impact of new therapies within the continuum of treatment options, both at the individual and the population levels.

References

1. Zaccarelli M, Perno C, Forbici F, et al. Using a database of HIV patients undergoing genotypic resistance test after HAART failure to understand the dynamics of M184V mutation. Antivir Ther 2003;8:51-6.
2. Clotet B, Ruiz L, Martínez-Picado J, Negredo E, Hill A, Popescu M. Prevalence of HIV protease mutations on failure of nelfinavir-containing HAART: a retrospective analysis of four clinical studies and two observational cohorts. HIV Clin Trials 2002;3:316-23.
3. Harrigan P, Dong W, Alexander C, et al. The association between drug resistance and adherence determined by two independent methods in a large cohort of drug-naive individuals starting triple therapy. 2nd IAS Conference on HIV Pathogenesis and Treatment. Paris, France, July 13-16, 2003 [abstract LB12].
4. Hirsch M, Brun-Vezinet F, Clotet B, et al. Antiretroviral drug resistance testing in adults infected with HIV type 1: 2003 recommendations of an International AIDS Society-USA Panel. Clin Infect Dis 2003;37:113-28.
5. Richman D, Morton S, Wrin T, et al. The prevalence of antiretroviral drug resistance in the United States. AIDS 2004;18:1393-401.
6. International AIDS Society (IAS) USA. http://www.iasusa.org.
7. Los Alamos HIV Sequence Database. http://hiv-web.lanl.gov
8. Stanford HIV Drug Resistance Database. http://hivdb.stanford.edu
9. HIV Resistance web. http://www.resistanceweb.com
10. Kuiken C, Korber B, Shafer R. HIV sequence databases. AIDS Rev 2003;5:52-61.
11. Gonzales M, Wu T, Taylor J, et al. Extended spectrum of HIV-1 reverse transcriptase mutations in patients receiving multiple nucleoside analog inhibitors. AIDS 2003;17:791-9.
12. Wu T, Schiffer C, Gonzales M, et al. Mutation patterns and structural correlates in HIV type 1 protease following different protease inhibitor treatments. J Virol 2003;77:4836-47.
13. Chen L, Perlina A, Lee C. Positive selection detection in 40,000 HIV type 1 sequences automatically identifies drug resistance and positive fitness mutations in HIV protease and reverse transcriptase. J Virol 2004;78:3722-32.
14. Deeks S. Treatment of antiretroviral drug-resistant HIV-1 infection. Lancet 2003;362:2002-11.
15. Johnson V, Brun-Vezinet F, Clotet B, et al. IAS-USA Drug Resistance Mutations Group. Drug resistance mutations in HIV-1. Top HIV Med 2003;11:215-21.
16. Gallant J, Gerondelis P, Wainberg M, et al. Nucleoside and nucleotide analogue reverse transcriptase inhibitors: a clinical review of antiretroviral resistance. Antiviral Ther 2003;8:489-506.
17. Fumero E, Podzamczer D. New patterns of HIV-1 resistance during HAART. Clin Microbiol Infect 2003;9:1077-84.
18. Patarca R, Isava A, Campo R, et al. HIV-1 pharmacogenomics in clinical practice: relevance of HIV-1 drug resistance testing (part 2). J Environ Pathol Toxicol Oncol 2003;22:235-79.
19. Kantor R, Katzenstein D. Drug resistance in non-subtype B HIV-1. J Clin Virol 2004;29:152-9.
20. Kantor R, Katzenstein D, Camacho R, et al. Genotypic analyses of RT and protease sequences from persons infected with non-subtype B HIV-1. 10th Conference on Retroviruses and Opportunistic Infections. Boston, MA, February 10-14, 2003 [abstract 623].

21. Caride E, Hertogs K, Larder B, et al. Genotypic and phenotypic evidence of different drug-resistance mutation patterns between B and non-B subtype isolates of HIV type 1 found in Brazilian patients failing HAART. Virus Genes 2001;23:193-202.
22. Cane P, Osman H, Smit E. Effect of HIV-1 subtype on development of NNRTI resistance mutations in patients failing first line therapy. XIII International HIV Drug Resistance Workshop. Tenerife, June 8-12, 2004 [abstract 104]. Antiviral Ther 2004;9:115.
23. Carpenter C, Cooper D, Fischl M, et al. Antiretroviral therapy in adults: updated recommendations of the International AIDS Society-USA Panel. JAMA 2000;283:381-90.
24. British Columbia Centre for Excellence in HIV/AIDS. The HIV/AIDS Drug Treatment Program, Status Report. January 2003.
25. Larder B, Kemp S, Harrigan P. Potential mechanism for sustained antiretroviral efficacy of AZT-3TC combination therapy. Science 1995;269:696-9.
26. Boucher C, Cammack N, Schipper P, et al. High-level resistance to (–) enantiomeric 2'-deoxy-3'-thiacytidine *in vitro* is due to one amino acid substitution in the catalytic site of HIV type 1 reverse transcriptase. Antimicrob Agents Chemother 1993;37:2231-4.
27. Schuurman R, Nijhuis M, Van Leeuwen R, et al. Rapid changes in HIV type 1 RNA load and appearance of drug-resistant virus populations in persons treated with lamivudine (3TC). J Infect Dis 1995;171:1411-9.
28. Zaccarelli M, Perno C, Forbici F, et al. Q151M-mediated multinucleoside resistance: prevalence, risk factors, and response to salvage therapy. Clin Infect Dis 2004;38:433-7.
29. Matsumi S, Kosalaraksa P, Tsang H, Kavlick M, Harada S, Mitsuya H. Pathways for the emergence of multi-dideoxynucleoside-resistant HIV-1 variants. AIDS 2003;17:1127-37.
30. Meyer P, Lennerstrand J, Matsuura S, Larder B, Scott W. Effects of dipeptide insertions between codons 69 and 70 of HIV type 1 reverse transcriptase on primer unblocking, deoxynucleoside triphosphate inhibition, and DNA chain elongation. J Virol 2003;77:3871-7.
31. Miller M, Margot N, Lu B, et al. Genotypic and phenotypic predictors of the magnitude of response to tenofovir disoproxil fumarate treatment in antiretroviral-experienced patients. J Infect Dis 2004;189:837-46.
32. Roge B, Katzenstein T, Obel N, et al. K65R with and without S68: a new resistance profile *in vivo* detected in most patients failing abacavir, didanosine and stavudine. Antivir Ther 2003;8:173-82.
33. White K, Margot N, Wrin T, Petropoulos C, Miller M, Naeger L. Molecular mechanisms of resistance to HIV type 1 with reverse transcriptase mutations K65R and K65R + M184V and their effects on enzyme function and viral replication capacity. Antimicrob Agents Chemother 2002; 46:3437-46.
34. Sirivichayakul S, Ruxrungtham K, Ungsedhapand C, et al. Nucleoside analogue mutations and Q151M in HIV-1 subtype A/E infection treated with nucleoside reverse transcriptase inhibitors. AIDS 2003;17:1889-96.
35. Gallego O, De Mendoza C, Labarga P, et al. Long-term outcome of HIV-infected patients with multinucleoside-resistant genotypes. HIV Clin Trials 2003;4:372-81.
36. Quirós-Roldán E, Moretti F, Airoldi M, et al. Long-term benefit of genotypic-guided therapy and prevalence of multinucleoside resistance in an Italian group of antiretroviral multi-experienced patients. J Clin Lab Anal 2001;15:127-30.
37. Hogg R, Yip B, Chan K, et al. Rates of disease progression by baseline CD4 cell count and viral load after initiating triple-drug therapy. JAMA 2001;286:2568-77.
38. Wu T, Schiffer C, Gonzales M, et al. Mutation patterns and structural correlates in HIV type 1 protease following different protease inhibitor treatments. J Virol 2003;77:4836-47.
39. Kagan R, Winters M, Merigan T, Heseltine P. Large-scale cluster analysis of HIV-1 protease mutations in a clinical database. XIII International HIV Drug Resistance Workshop. Tenerife, June 8-12, 2004 [abstract 81]. Antiviral Ther 2004;9:81.
40. Parkin N, Petropoulos C, Chappey C, et al. Isolated lopinavir resistance after virological rebound of a lopinavir/ritonavir based regimen. XIII International HIV Drug Resistance Workshop. Tenerife, June 8-12, 2004 [abstract 70]. Antiviral Ther 2004;9:79.
41. Miller V, Ait-Khaled M, Stone C, et al. HIV-1 reverse transcriptase (RT) genotype and susceptibility to RT inhibitors during abacavir monotherapy and combination therapy. AIDS 2000;14:163-71.
42. Winters M, Shafer R, Jellinger R, et al. HIV-1 RT genotype and drug susceptibility changes in infected individuals receiving dideoxyinosine monotherapy for 1 to 2 years. Antimicrob Agents Chemother 1997;41:757-62.
43. Margot N, Isaacson E, McGowan I, Cheng A, Miller M. Extended treatment with tenofovir disoproxil fumarate in treatment-experienced patients: genotypic, phenotypic and rebound analyses. J Acquir Immun Def Syndr 2003;33:15-21.
44. Winston A, Mandalia S, Pillay D, Gazzard B, Pozniak A. The prevalence and determinants of the K65R mutation in HIV-1 RT in tenofovir-naive patients. AIDS 2002;16:2087-9.
45. Miller M. K65R, thymidine analogue mutations and tenofovir. AIDS Rev 2004;6:22-33.
46. Winston A, Pozniak A, Mandalia S, Gazzard B, Pillay D, Nelson M. Which nucleoside and nucleotide backbone combinations select for the K65R mutation in HIV-1 reverse transcriptase. AIDS 2004;18:949-51.
47. MacArthur R, Crane L, Álvarez D, Fairfax M, Richmond D, Curtis G. Factors associated with selection of the K65R mutation: a retrospective chart review. 2nd IAS Conference on HIV Pathogenesis and Treatment. Paris, France, July 13-16, 2003 [abstract 835].
48. Parikh N, Bacheler L. Prevalence, mechanism of resistance and mutational interactions in reviewing the VIRCO database. 11th CROI. San Francisco 2004 [abstract 54].
49. Trotta M, Bonfigli S, Ceccherinie F, et al. Clinical and genotypic correlates of K65R mutation in an unselected cohort of HIV-infected persons naive for tenofovir. XIII International Drug Resistance Workshop. Tenerife, June 8-12, 2004 [abstract 152]. Antiviral Ther 2004;9:168.
50. García-Lerma J, MacInnes H, Bennett D, et al. A novel genetic pathway of HIV type 1 resistance to stavudine mediated by the K65R mutation. J Virol 2003;77:5685-93.
51. Podzamczer D, Ferrer E, Gatell J, et al. Early virological failure and occurrence of resistance in naive patients receiving tenofovir, didanosine and efavirenz. XIII International HIV Drug Resistance Workshop. Tenerife, June 8-12, 2004 [abstract 156]. Antiviral Ther 2004;9:172.
52. Bae A, Waters J, Margot N, Borroto-Esoda K, Miller M. Pre-existing L74V is a risk factor for virological non-response and development of K65R in patients taking tenofovir DF (TDF). XIII International HIV Drug Resistance Workshop. Tenerife, June 8-12, 2004 [abstract 158]. Antiviral Ther 2004;9:174.
53. Underwood M, Ross L, Irlbeck D, et al. Sensitivity of phenotypic analyses for detection of K65R or M184V in mixtures with wild-type HIV-1. XIII International HIV Drug Resistance Workshop. Tenerife, June 8-12, 2004 [abstract 130]. Antiviral Ther 2004;9:144.