Antiretroviral Drug Resistance Testing in Adults Infected with Human Immunodeficiency Virus Type 1: 2003 Recommendations of an International AIDS Society–USA Panel

Martin S. Hirsch,[1] Françoise Brun-Vézinet,[10] Bonaventura Clotet,[11] Brian Conway,[12] Daniel R. Kuritzkes,[2] Richard T. D'Aquila,[3] Lisa M. Demeter,[4] Scott M. Hammer,[5] Victoria A. Johnson,[6] Clive Loveday,[13] John W. Mellors,[7] Donna M. Jacobsen,[8] and Douglas D. Richman[9]

[1]Harvard Medical School and [2]Brigham and Women's Hospital, Boston, Massachusetts; [3]Vanderbilt University Medical Center, Nashville, Tennessee; [4]University of Rochester and [5]Columbia University College of Physicians and Surgeons, New York; [6]Birmingham Veterans Affairs Medical Center and the University of Alabama at Birmingham School of Medicine; [7]University of Pittsburgh and Veterans Affairs Medical Center, Pittsburgh, Pennsylvania; [8]International AIDS Society–USA, San Francisco, and [9]University of California San Diego and Veterans Affairs San Diego Healthcare System, California; [10]Hôpital Bichat-Claude Bernard, Paris, France; [11]Fundacio irsiCAIXA and HIV Unit, Hospital Universitari (UAB) Germans Trias i Pujol, Barcelona, Spain; [12]University of British Columbia, Vancouver; and [13]International Clinical Virology Centre, Buckinghamshire, England, United Kingdom

New information about the benefits and limitations of testing for resistance to human immunodeficiency virus (HIV) type 1 (HIV-1) drugs has emerged. The International AIDS Society–USA convened a panel of physicians and scientists with expertise in antiretroviral drug management, HIV-1 drug resistance, and patient care to provide updated recommendations for HIV-1 resistance testing. Published data and presentations at scientific conferences, as well as strength of the evidence, were considered. Properly used resistance testing can improve virological outcome among HIV-infected individuals. Resistance testing is recommended in cases of acute or recent HIV infection, for certain patients who have been infected as long as 2 years or more prior to initiating therapy, in cases of antiretroviral failure, and during pregnancy. Limitations of resistance testing remain, and more study is needed to refine optimal use and interpretation.

Recommendations of the International AIDS Society–USA panel regarding HIV-1 drug resistance testing were published in 1998 and 2000 [1, 2]. At the time of our most recent report, many issues remained unclear with respect to the use of these assays in various clinical situations. These included the relative merits of phenotypic and genotypic testing, criteria to define the likelihood of clinical response, long-term clinical benefits of testing, and the cost-effectiveness of resistance testing as a routine part of patient monitoring. Numerous studies have now addressed many of these issues. Moreover, data have emerged documenting the seriousness of the problem of HIV-1 drug resistance in previously treated and untreated patient populations. This new information emphasizes the need for better education on how to use resistance testing and for updated guidelines on how to use antiretroviral drug combinations most effectively to prevent or treat drug resistance.

In addition, subsequent studies have identified concepts not addressed in our previous reports. These include the importance of hypersusceptibility in predict-

Received 12 December 2002; accepted 5 March 2003; electronically published 23 June 2003.

Financial support: The International AIDS Society–USA is funded through a reserve fund independent of commercial company support.

Reprints or correspondence: Dr. Martin S. Hirsch, Massachusetts General Hospital, Infectious Disease Div., 65 Landsdowne St., Rm. 419, Cambridge, MA 02139 (mshirsch@partners.org).

Clinical Infectious Diseases 2003; 37:113–28

1058-4838/2003/3701-0016$15.00

ing response to nonnucleoside reverse-transcriptase inhibitors (NNRTIs) [3, 4], the impact of HIV-1 subtype and human leukocyte antigen type on patterns of HIV-1 drug resistance [5–7], the extent of cross-resistance among antiretroviral drugs [8], and the utility of ratios of trough level to IC_{50} in predicting response to antiretroviral regimens [9]. These concepts are more fully explored in this report.

MATERIALS AND METHODS

In 1997, the International AIDS Society–USA selected a panel of experts to develop consensus recommendations on the potential clinical role and limitations of drug resistance testing. The panel membership comprises physicians and scientists with expertise in basic science, clinical research, and patient care related to antiretroviral therapy and HIV drug resistance. Balance in perspective, US and international clinical and research experience with different assay methodologies, and a broad range of views on the roles and limitations of drug resistance testing were considerations in the selection of members.

For its initial reports [1, 2], the panel considered data from the published literature and abstracts from relevant scientific conferences since the recognition of HIV drug resistance in 1989 [10]. For this updated report, the panel members reviewed newly available published and presented information regarding HIV drug resistance since 2000. Evidence strengths were considered according to parameters such as type of study (e.g., randomized prospective trial, cohort study, and case reports), number of subjects, duration of follow-up, and publication source. For example, published prospective studies were given high priority. Evidence from abstracts of scientific meetings that had not been published within 2 years of presentation were generally excluded. Extrapolations from basic science data and expert opinion of the panel members were included. The recommendations focus on resistance regarding drugs that had been approved by the US Food and Drug Administration at the time of the report.

The panel was divided into writing committees for sections on mechanisms of drug resistance, drug resistance assays, prospective study results, clinical management issues, and updated recommendations. Each section committee met to identify relevant data and prepare draft recommendations for the sections, which were reviewed and discussed by the full panel. Draft sections with supporting data and preliminary recommendations were combined and circulated to the entire panel and discussed by full panel conference calls. The recommendations reflect unanimous agreement of the panel members that there is sufficient evidence for incorporating these recommendations into clinical practice.

MECHANISMS OF ANTIRETROVIRAL DRUG RESISTANCE

Antiretroviral resistance develops when viral replication continues in the presence of the selective pressure of drug exposure. For some drugs, such as the nucleoside reverse-transcriptase inhibitor (NRTI) lamivudine and all available NNRTIs, a single mutation induces high-grade resistance in a predictable manner. For others such as zidovudine, abacavir, tenofovir, and most of the protease inhibitors (PIs), high-grade resistance requires the serial accumulation of multiple mutations and is thus slower to emerge. Some other drugs, including didanosine and stavudine, are associated only with low levels of resistance as measured in phenotypic assays, despite the presence of $\geqslant 1$ key mutation. Clinical trial data now show that low-level resistance to didanosine and stavudine predict decreased efficacy [11, 12]. Resistance cutoffs for phenotypic assays for these drugs have been lowered to reflect this [13].

Nucleoside and nucleotide reverse-transcriptase inhibitors. Although most of the mutations associated with NRTI resistance are not at the active site of the enzyme, they do lead to conformational changes that affect the active site aspartate residues [14]. Different mutations lead to 2 different mechanisms for resistance: decreased substrate binding and increased phosphorolysis (removal of the chain-terminating substrate that has already been incorporated into the growing proviral DNA chain). Both mechanisms lead to an overall net decrease in termination of the elongating chain of HIV DNA by the NRTI [15, 16].

Three patterns of multi-NRTI resistance mutations have now been identified [17, 18]. One is the Q151M complex [19–22]. Another is the 69 insertion complex, consisting of a mutation at codon 69 (typically T69S) followed by an insertion of $\geqslant 2$ amino acids (S-S, S-A, S-G, or others) [23–25]. The 69 insertion is often accompanied by mutations at other sites. Some other amino acid changes from the wild-type threonine (T) in codon 69 without the insertion may also be associated with broad NRTI resistance [26]. The third pattern of multi-NRTI resistance involves NRTI-associated mutations (NAMs). These include the reverse-transcriptase mutations M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E, which were initially recognized after zidovudine therapy [27–29]. Although NAMs are selected for only by thymidine NRTIs (zidovudine and stavudine), they are associated to varying degrees with reduced susceptibility to all NRTIs. The NAMs cause resistance by improving excision of the chain terminator by phosphorolysis [30, 31] rather than the common mechanism for other reverse-transcriptase and protease mutations, which is by decreasing binding of the inhibitor to the target. Some other mutations, such as the 69 insertion and the RT K65R mutation, also appear to cause resistance by the excision mechanism.

In heavily pretreated patients, resistance patterns may be difficult to interpret, owing in part to multiple interactions among resistance mutations. Certain single reverse-transcriptase mutations may confer resistance to one drug and yet enhance phenotypic susceptibility to another. For example, M184V [32] and L74V [33] are associated with resistance to lamivudine and didanosine, respectively, and each leads to enhanced sensitivity to zidovudine. Recent studies have elucidated the underlying mechanisms for this sensitization. The lamivudine-associated M184V mutation causes decreased phosphorolysis, which counters the NAM effect of increasing phosphorolytic excision [34]. In early studies of the combination of zidovudine and lamivudine as double therapy, the emergence of zidovudine resistance due to NAMs was considerably delayed [32]. Although the M184V mutant may partially "reverse" phenotypic zidovudine resistance conferred by mutations in codons 41, 67, 70, 210, 215, and 219, [32] this effect is limited by the emergence of other mutations that restore zidovudine resistance and may thus lead to zidovudine and lamivudine coresistance [35–37]. The M184V also restores susceptibility to stavudine and tenofovir because NAMs cause resistance to all chain-terminating inhibitors by improving their excision [38].

The presence of NAMs related to zidovudine resistance is associated with increased resistance to stavudine, abacavir, lamivudine, didanosine, and tenofovir [17, 39–41]. An additional M184V or M184I mutation occurring with multiple NAMs may restore in vitro susceptibility to zidovudine, tenofovir, and stavudine; however, it increases resistance to lamivudine, abacavir, and perhaps didanosine. The availability of new drugs like tenofovir may partially overcome the cross-resistance caused by the accumulation of NAMs. Tenofovir often retains some activity against isolates that are resistant to zidovudine, didanosine, zalcitabine, and abacavir and against the multi-NRTI drug-resistant variants carrying the Q151M mutation. When resistance to tenofovir exceeds the upper limit of the range of susceptibility for wild-type virus, activity begins to diminish, and when resistance is >4-fold that of wild-type virus, any response to tenofovir therapy is probably lost [42]. Genotypic analysis in this setting has demonstrated that resistance to tenofovir in vivo is associated with the presence of K65R, T69S insertion, or ⩾3 NAMs, including M41L or L210W. A set of ⩾3 NAMs that do not include M41L or L210W has not been associated with tenofovir resistance.

NNRTIs. Two patterns of multi-NNRTI resistance have been described. One is the K103N reverse-transcriptase mutation. This single mutation confers resistance to all currently available NNRTIs, presumably by stabilizing the closed-pocket form of the enzyme, thus inhibiting the binding of the drug to its target [43]. The fact that all available agents in this class bind to the same domain explains the broad pattern of cross-resistance and has prompted the development of new agents that interact with this domain more favorably. Cross-resistance across this entire class may not be absolute. In practice, ⩾20% of patients in whom nevirapine resistance emerges may still have isolates that are sensitive to efavirenz [44]. However, subsequent exposure to efavirenz may lead to more rapid emergence of resistance than if the baseline isolate were wild-type, limiting the possibility of sequencing drugs within this class. Indeed, another pattern of multi-NNRTI resistance is the accumulation of multiple mutations, including L100I, V106A, Y181C, G190S/A, and M230L. Rarely, Y188L causes multi-NNRTI resistance.

Enhanced susceptibility to NNRTIs (i.e., hypersusceptibility) has been described in association with multiple mutations conferring broad cross-resistance to NRTIs and a lack of NNRTI resistance mutations [3, 45]. In patients with no prior NNRTI use, the prevalence of hypersusceptibility to NNRTIs (defined as an IC_{50} of >2.5-fold less than that of a wild-type reference strain) was 18%–24% [3]. Longer duration of NRTI use, prior use of zidovudine, and abacavir or zidovudine resistance have all been associated with hypersusceptibility. This phenomenon appears to have biological significance, with its presence enhancing the response to efavirenz-based regimens [3, 4, 46]. A significantly greater short-term reduction in the plasma HIV-1 RNA level was noted in patients showing hypersusceptibility to efavirenz who received that drug for salvage therapy. NNRTI hypersusceptibility in patients with extensive prior NRTI experience may help explain the value of these drugs in salvage regimens for patients naive to NNRTIs [3, 4]. However, the presence of hypersusceptibility did not appear to delay the emergence of delavirdine resistance or antiretroviral failure in one controlled study [47].

PIs. The sequential use of certain PIs may be possible in some situations, because several drugs in this class have distinctive major resistance mutations. This is particularly true for nelfinavir [48] and has been suggested for atazanavir. All other PIs retain activity in vitro and in vivo against D30N isolates selected by nelfinavir. Less commonly, nelfinavir failure is associated with L90M, which is more likely to add to cross-resistance to other PIs. The I50V amprenavir resistance mutation alters the hydrophobic interaction with the target and had been thought to only minimally alter the binding of other drugs in this class. However, amprenavir-selected genotypes do confer cross-resistance to lopinavir or ritonavir [49]. Clinical evidence to support particular PI sequencing, except that for nelfinavir, is lacking.

The presence of ⩾2 key mutations (e.g., D30N, G48V, I50V, V82A/F/T/S, I84V, and L90M) generally confers broad cross-resistance to most currently available PIs [50, 51]. One strategy to avoid the accumulation of multiple mutations is to use low-

dose ritonavir to increase the circulating levels (or "boost") other PIs (e.g., lopinavir, indinavir, amprenavir, and saquinavir), which may result in higher and more prolonged drug concentrations and greater suppression of viral variants that contain a limited number of mutations. Thus, resistance depends not only on intrinsic properties of the virus but also on the achievable plasma levels of the drug.

Hypersusceptibility has also been demonstrated in association with some protease mutations. Patients whose infections failed to respond to certain PI regimens may harbor HIV with the protease D30N and N88S mutations, which confer in vitro hypersusceptibility to other PIs [52–54]. In addition, viral constructs containing the indinavir-associated V82T mutation are less fit than wild-type virus and are hypersusceptible to saquinavir [55]. Interactions among other mutations, (e.g., at protease Gag cleavage sites) may also affect PI susceptibility. Of note, currently available genotypic resistance tests do not examine the *gag* region, and further research is needed to define the relationships between mutations in the Gag cleavage sites and in the protease gene as they affect virological and clinical outcome.

Entry inhibitors. Entry of HIV-1 into target cells is a multistep process involving attachment (mediated by gp120 binding to CD4), chemokine coreceptor binding, and association of 2 trimeric helical coils (HR-1 and HR-2) located in the ectodomain of gp41 into a 6-helix bundle that brings the virus and cell membranes into close approximation, allowing membrane fusion to occur. A number of drugs currently in development block HIV-1 infection by interfering with $\geqslant 1$ of these steps. The recently approved fusion inhibitor enfuvirtide (known as T-20) blocks the association of HR-1 with HR-2 by binding to the trimeric HR-1 complex, thereby inhibiting fusion and blocking virus entry [56]. Mutations in HR-1 that reduce enfuvirtide susceptibility are selected by in vitro passage of HIV-1 in the presence of the drug and have been identified in isolates obtained from patients receiving enfuvirtide in clinical trials [57, 58]. In particular, amino acid substitutions at gp41 codons 36–45 are found in virus samples recovered from patients experiencing protocol-defined treatment failure of enfuvirtide and are associated with an average 20-fold increase from the baseline IC_{50} of enfuvirtide [17]. The 500-fold range of enfuvirtide susceptibility among pretreatment isolates with wild-type sequences in HR-1 suggests that sequence variation in other regions of the HIV-1 envelope modulate susceptibility to this drug.

HIV REPLICATION CAPACITY

Several studies have demonstrated that drug-resistant mutants have reduced replication capacity (a component of relative viral fitness) compared with drug-susceptible HIV-1 variants in vitro [59–63]. These reductions in replication capacity can often be correlated with biochemical abnormalities in protease or reverse-transcriptase [59, 61, 62, 64]. Reductions in replication capacity can persist in clinical protease and reverse-transcriptase sequences [65], although mutations outside of protease and reverse-transcriptase may compensate for reductions in replication capacity conferred by resistance mutations [64].

Some studies suggest that the extent to which replication capacity is reduced influences the likelihood of the next mutant emerging during treatment failure [61, 62]. Reductions in replication capacity may also influence clinical outcome. In one study, the overgrowth of drug-resistant variants by drug-susceptible virus with improved replication capacity was associated with an increase in the plasma HIV-1 RNA level and a decrease in the CD4 cell count [66], suggesting that persistence of drug-resistant variants with reduced replication capacity may offer some clinical benefit. Another study found a correlation with replication capacity and clinical outcome, although the number of isolates studied was small [67]. A measure of HIV replication capacity is now being offered as part of one phenotypic resistance assay, although there is no consensus yet on how to measure replication capacity optimally or how to incorporate this information into clinical management.

RESISTANCE TESTING ASSAYS

There are 2 general types of resistance testing assays: genotypic assays (i.e., HIV gene sequencing to detect mutations that confer HIV drug resistance) and phenotypic assays (i.e., drug susceptibility testing of plasma virus). Genotypic testing to detect mutations associated with drug resistance may be performed using assay kits or in-house techniques. There is a high level of concordance (97.8%) between 2 commercial assay kits when tests are performed by the same laboratory for detection of resistance mutations [68, 69]. In 80% of cases, discordance was due to differences in detection of mixed wild-type and mutant populations by the 2 assays [68]. Earlier quality assurance evaluations have demonstrated underdiagnosis of resistance mutations and interlaboratory variation in the quality of genotyping, independent of the technology used, especially when mixtures of wild-type and mutant virus were present [70–72]. The frequencies of false-positive and false-negative test results were low (0.3% and 6.4%, respectively [70]), and 52% of laboratories were unable to detect all 10 mutations present in a sample in which mutant virus constituted 50% of the population [71]. Performance was related to the experience level of the laboratory, suggesting that appropriate operator training, certification, and periodic proficiency testing are essential for proper genotyping. Some regulatory authorities now require such training.

Appropriate interpretation of the results of HIV-1 drug re-

sistance testing remains a challenging problem for both phenotypic and genotypic assays. Results of genotypic tests are interpreted by individual judgment by consulting lists of drug resistance mutations [17, 18] or by computerized rules-based algorithms that classify the virus as "susceptible," "possibly resistant," or "resistant" to each antiretroviral agent. The construction of rules-based algorithms for interpretation of genotype is a lengthy and difficult process that requires frequent updating. Extensive variations exist among the different available algorithms in the classification of expected drug activity [73–75]. This variation appears to be drug related and more important for the NRTIs and PIs [73, 74]. Differences in how drug resistance is scored complicate comparisons among the algorithms. Ideally, algorithms for interpretation of genotype should be based on studies correlating the viral genotypic profile at baseline with the virological response to treatment (e.g., a decrease in the plasma HIV RNA level). The mutational profiles that predict a lack of virological response have been developed only for a few drugs [76–82].

An alternative approach to interpretation of genotype is the "virtual" phenotype, which uses genotypic data to determine the likely in vitro drug susceptibility of a particular virus on the basis of data from matching viruses in a large database of virus samples with paired genotypic and phenotypic data. Viruses in the database with genotypes that match the test virus are identified, and the average phenotype for all the available matches in the database is calculated. With a sufficiently large database, there is a high likelihood that a reasonable number of matches can be found for most genotypes encountered in practice. The actual and virtual phenotypes show excellent correlation ($R^2 > 0.8$) for most drugs [83]. A potential limitation of this approach is that the level of confidence placed in the result depends on the number of matching genotypes in the database and on selecting the appropriate codons to incorporate into the search. Matches are based on positions preselected as relevant for each drug, not the entire sequence. Correlation between actual and virtual phenotype most likely will be weaker for newer drugs or in cases in which there are fewer matches because of unusual genotypes.

Standard phenotypic testing by recombinant virus assays remains restricted to 3 commercial laboratories. Current assays amplify HIV protease and reverse-transcriptase as well as the 3′-terminus of *gag* as a unit from plasma virus and generate a recombinant virus with other HIV genes from a laboratory construct [84]. A comparison between 2 of these phenotypic assays showed 92.2% overall concordance [85]. However, only a small fraction of the samples tested had significant levels of drug-resistant mutations. Comparison between different methods by use of plasma samples from drug-experienced patients demonstrated a significant correlation overall ($R^2 = 0.61$; $P < .001$), but this did not reach significance for abacavir, stavudine, didanosine, or amprenavir [86]. A third study showed that test results were highly correlated for all 3 assays, although the strength of the correlation was weaker for stavudine and didanosine [87]. This technology has been modified to allow measurement of viral susceptibility to integrase inhibitors, fusion inhibitors (e.g., enfuvirtide), and chemokine receptor inhibitors [88].

Results of phenotypic testing usually are expressed as the fold-change in susceptibility of the test sample compared with a laboratory control isolate. Previously, cutoffs for defining "susceptible" and "resistant" viruses were based on the interassay variation of the controls (the "technical" cutoff). Testing laboratories have shifted to the use of "biologic" cutoffs, which are based on the normal distribution of susceptibility to a given drug for wild-type isolates from therapy-naive individuals. The key question, however, is whether a patient is likely to respond to a particular drug. Consequently, the most relevant approach for interpreting the phenotype results is to define "clinical" cutoffs by using data from clinical trials or cohort studies to determine the change in susceptibility that results in a reduction in virological response to the drug in question. To date, clinical cutoffs have been defined for relatively few drugs (e.g., abacavir, tenofovir, and lopinavir-ritonavir) [76, 77, 82]. The results of several studies underscore the difficulty in determining appropriate susceptibility cutoffs for many drugs. For example, data from the NARVAL trial show a continuous inverse relationship between fold-resistance and response rate for saquinavir and efavirenz (i.e., the higher the fold-resistance, the lower the rate of viral suppression); thresholds above which no response was observed were noted for stavudine, didanosine, abacavir, and amprenavir [89]. No correlation between fold-resistance and treatment response was observed, however, for zidovudine, lamivudine, nelfinavir, and nevirapine. Analyses of the activity of individual drugs are confounded by the presence of other drugs in the regimen to which the virus remains susceptible. Analysis in one study showed no predictive value of NRTI phenotype for virological success at 6 months by using a cutoff of 2.5-fold to define resistance [90]. The predictive value of NRTI phenotype improved when lower cutoffs (i.e., 1.7-fold) were used for didanosine and stavudine.

Two different clinical phenotypic cutoff values should be considered: one at which clinical responses diminish, compared with wild-type virus, and one at which no clinical response can be expected. Even partial activity may be clinically useful when treatment options are limited. For example, 60% of PI-experienced, NNRTI-naive patients with virus isolates resistant to lopinavir-ritonavir still achieved virological reduction at week 24 while receiving a lopinavir-ritonavir–containing regimen [77]. The definitions of clinically validated criteria for resistance phenotypes and genotypes require analyses that account for confounding factors, such as the type and duration of previous

antiretroviral therapy and the activity of the other drugs in the regimen.

The benefit of resistance testing results in guiding therapy also depends on drug exposure [91]. Thus, phenotypic cutoffs for defining drug resistance may need to consider drug concentrations in an individual. For example, boosting plasma levels of most PIs with low doses of ritonavir will change the definition or cutoff of resistance for the boosted drugs. One approach to relating drug exposure and drug susceptibility is the inhibitory quotient (IQ), which is the ratio of the measured plasma C_{min} divided by the IC_{50} or IC_{90}, corrected for 50% human serum. In one study, IQ predicted virological response over 48 weeks to ritonavir boosting of patients receiving an indinavir-containing regimen [92]. This concept needs to be evaluated for other PIs and NNRTIs, as does its application to genotypic testing (pertaining to number of mutations rather than IC_{50} or IC_{90}).

PROSPECTIVE STUDIES OF DRUG RESISTANCE TESTING

Randomized studies of the clinical utility of drug resistance testing have generally supported its use as a guide to selecting antiretroviral therapy in patients whose infections have failed to respond to previous regimens [75, 90, 93–97]. The studies differ in several important design features, including the extent of prior treatment experience of the study population, the particular resistance test used, whether expert advice was provided in addition to the test results, duration of follow-up, and the definition of virological success or failure used as the primary study end point. It is not surprising, therefore, that not all studies provide concordant results.

Three trials have shown an advantage for the use of genotypic testing over standard of care in the selection of regimens for patients whose infections fail to respond to antiretroviral therapy [93–95]. The genotyping arms of these studies had average decreases in plasma HIV-1 RNA levels that were significantly greater than those for the standard-of-care arms at 8–24 weeks. Subjects in the genotyping arms were also more likely to achieve plasma HIV-1 RNA levels that were less than the limits of assay detection. In a fourth trial, however, the advantages of genotyping proved to be short lived [75]. Although a significantly greater proportion of patients in the genotyping arm had plasma HIV-1 RNA levels that were less than the limit of detection at week 12, this difference was not statistically significant at week 24. Additional analysis of 2 of these trials demonstrated the importance of achieving adequate plasma drug levels for optimal treatment response, even after taking into account the benefits of genotypic testing [91, 98].

Expert advice also plays a significant role as an adjunct to resistance testing in influencing the outcome of salvage therapy. HIV practitioners' knowledge of HIV resistance patterns is incomplete [99]. One study that compared the utility of genotypic resistance testing, expert advice, or both with standard of care in selecting regimens for patients whose infections fail to respond to antiretroviral therapy showed that genotypic testing and expert advice each resulted in significantly better virological responses [95]. The best response rates were observed in patients who received both genotypic testing and expert advice. These results suggest that although expert advice is helpful, the availability of genotypic assays leads to further improvements in virological outcome in the setting of antiretroviral failure.

Trials of phenotypic testing versus standard of care have produced mixed results. A 16-week pilot study in NNRTI-naive patients with extensive NRTI and PI experience found no significant difference between phenotyping and standard-of-care arms [100]. In a subsequent study of patients whose illness failed to respond to the first PI-containing regimen, patients in the phenotypic testing arm had a significantly greater reduction in plasma HIV-1 RNA level by week 16 than did patients in the standard of care arm [96]. Of note, very few patients entering this trial had prior NNRTI experience. Overall, patients in the phenotypic testing arm received significantly more new drugs to which their virus was susceptible than did patients in the control arm. By contrast, one trial failed to show an advantage of phenotypic testing over the standard of care [90]. However, a significant difference favoring the phenotypic testing arm emerged in analysis of a subgroup of patients with virus resistant to >3 PIs. Despite the negative result in the study as a whole, the number of PIs and NNRTIs in the new regimen to which the virus was predicted to be susceptible was associated with the likelihood of maintaining plasma HIV-1 RNA levels of <400 copies/mL at 6 and 12 months after controlling for baseline $CD4^+$ cell count and HIV-1 RNA level.

The utility of phenotypic and genotypic testing was examined in the NARVAL study in which patients whose infections failed to respond to a 3-drug, PI-containing regimen were randomized to genotype testing, phenotype testing, or standard-of-care arms. Most patients were heavily pretreated, having received a median of 7 antiretroviral agents before study entry. No significant difference between arms was found at week 12 for either the percentage of patients with plasma HIV-1 RNA levels of <200 copies/mL or for the percentage of patients showing a $\geqslant 1$ $\log_{10}$ decrease in the HIV-1 RNA level from baseline [101]. In a secondary analysis of a subgroup of 179 patients whose disease failed to respond to a first PI, the virological response was significantly better in the genotypic testing arm than in the phenotypic testing and standard-of-care arms. Multivariate logistic regression analysis showed that randomization to the genotypic testing arm as well as use of efavirenz in patients naive to NNRTIs, or abacavir or lamivudine in the salvage regimen were significant independent predictors of virological

success, whereas a high number of resistance mutations, long duration of prior PI treatment, high baseline HIV-1 RNA level, and use of nelfinavir in the salvage regimen were significant independent predictors of virological failure [102]. This analysis highlights the many factors that contribute to determining outcome of salvage therapy and complicate the design and interpretation of randomized trials of resistance testing. Retrospective analyses of both the CCTG 575 and NARVAL trials suggested that inappropriately high cutoffs for stavudine and didanosine and inappropriately low cutoffs for abacavir resulted in suboptimal NRTI drug selection in the phenotypic arms.

The CERT study also compared genotype, phenotype, and standard of care [103]. As in the NARVAL study, there was no overall difference in the time to study end point (persistent virologic failure) between the study arms. An advantage of genotyping and phenotyping over standard of care was found among patients with a history of treatment with >4 antiretroviral agents before study enrollment. Interpretation of results from the genotype arm is clouded somewhat by the fact that part way through the study, patients in the genotype arm began receiving virtual phenotype reports in place of routine genotype reports. In the VIHRES study in heavily pretreated patients, there was no statistically significant difference in virologic outcome between patients whose next regimens were guided by genotypic testing versus those whose regimens were guided by phenotypic testing [104].

The clinical utility of the virtual phenotype was compared with standard drug susceptibility testing in the RealVirfen study [105]. Patients whose infections failed to respond to a triple-drug regimen were randomized to receive resistance test results based on the virtual phenotype or an actual measured phenotype. At week 24, the reduction in plasma HIV-1 RNA from baseline was significantly greater in patients in whom salvage therapy was selected with the aid of the virtual phenotype than those in which the standard phenotypic assay was used. A greater proportion of patients in the virtual phenotype arm achieved plasma HIV-1 RNA levels of <400 copies/mL than those in the standard phenotype arm, but this difference was not statistically significant.

Collectively, these prospective randomized trial data indicate at least short-term virologic benefits for both genotypic and phenotypic drug resistance testing, although evidence is strongest for genotypic testing. Table 1 summarizes published prospective trial results. Numerous factors contribute to determining outcome of salvage therapy and complicate the design and interpretation of randomized trials of resistance testing. In the absence of data from comparative trials, there is insufficient evidence to favor one resistance testing approach over the other. It is possible that, in some complicated situations, phenotypic and genotypic drug resistance testing provide complementary information helpful in patient management [106].

Further evidence supporting the clinical utility of drug resistance testing is provided by results of the phase III clinical trials of enfuvirtide [107]. Although not trials of resistance testing per se, phenotypic and genotypic resistance testing were performed at study entry in order to guide selection of optimized background (OB) regimens for this group of highly treatment-experienced patients whose infections failed to respond to current antiretroviral therapy. The number of drugs in the OB regimen to which the virus was susceptible, as judged by the results of resistance testing, was a significant independent factor in determining the magnitude of viral suppression [108].

The cost-effectiveness of resistance testing has been modeled by using data from the GART [94] and VIRADAPT [93] trials together with data from the HIV Cost and Services Utilization Survey and the 1998 *Red Book* to determine the cost of HIV-1 infection–related care [109]. The incremental increase in cost per quality-adjusted life-year (QALY) ranged from $16,300 to $17,900. These results compared favorably to prophylaxis for *Mycobacterium avium* complex (increase in cost per QALY, $35,000), fungal (increase in cost per QALY, $100,000), and cytomegalovirus infections (increase in cost per QALY, $314,000). By use of the same model, the cost-effectiveness of resistance testing for patients with acute or recent HIV-1 infection was shown to increase in parallel with increasing rates of the transmission of drug resistant HIV-1. Thus, the cost-effectiveness of genotypic resistance testing is similar to or better than that of many generally recommended interventions for HIV-1–infected patients.

CLINICAL MANAGEMENT AND RECOMMENDATIONS

Specimen collection. An important issue in resistance testing is the optimal time to obtain the plasma sample for analysis. False-negative results may occur if blood samples are obtained after therapy is changed or stopped because susceptible variants may outgrow the resistant mutants in the absence of drug pressure. For example, M184V predominance may be lost within a few weeks after withdrawal of lamivudine therapy [110–112]. Certain mutations may persist up to 2 years or longer in patients with transmitted (primary) resistance and for variable periods of time in those with acquired resistance [113–115]. Nevertheless, blood samples should optimally be obtained for resistance testing after virologic failure is confirmed and before the failing drug regimen is changed. Furthermore, the plasma sample should contain 500–1000 HIV RNA copies/mL, to allow successful PCR amplification for either genotyping or phenotyping.

Acute or recent HIV-1 infection. Several reports indicate that rates of transmission of drug-resistant HIV-1 variants (termed "primary resistance") may be increasing, although es-

Table 1. Summary of published prospective trials of drug resistance testing.

Study	No. of patients	Duration	Threshold of HIV-1 RNA detection, copies/mL	Study arm	Patient had change in HIV-1 RNA level		HIV-1 RNA level less than the limit of detection	
					Degree of change, $\log_{10}$ copies/mL	*P*	Percentage of patients[a]	*P*
VIRADAPT [93]	108	3 months	<200	Genotype	−1.04		29	
				Standard of care	−0.46	.01	14	.017
		6 months	<200	Genotype	−1.15		32	
				Standard of care	−0.67	.05	14	.067
GART [94]	153	8 weeks	<500	Genotype plus expert advice	−1.19[b]		55	
				Standard of care	−0.61	<.001	25	<.001
Havana [95]	326	24 weeks	<400	Genotype	−0.84		48.5	
				No genotype	−0.63	<.05	36.2	<.05
				Expert advice	−0.75		47.2	
				No expert advice	−0.73	NS	37.4	NS
VIRA 3001 [96]	272	16 weeks	<400	Phenotype	−1.23		46	
				Standard of care	−0.87	.005	34	.079
ARGENTA [75]	174	3 months	<500	Genotype	−0.62		27	
				Standard of care	−0.38	.12	12	.01
		6 months	<500	Genotype	−0.57		21	
				Standard of care	−0.39	.28	17	.47
NARVAL [97]	541	12 weeks	<400	Genotype	−0.95	.215[c]	44	.918[c]
				Phenotype	−0.93	.274[d]	35	.120[d]
				Standard of care	−0.76		36	
CCTG 575 [90]	238	6 months	<400	Phenotype	−0.71		48	
				Standard of care	−0.69	NS	48	NS

[a] All analyses are intention-to-treat, missing = failure. NS, not significant.
[b] Average change in plasma HIV-1 RNA level at weeks 4 and 8.
[c] For genotype versus standard of care.
[d] For phenotype versus standard of care.

timates of the prevalence among populations from different geographic regions vary [116–122]. Studies in North America, Germany, and the United Kingdom suggest that drug-resistant virus is being increasingly transmitted, whereas studies from some other European countries have indicated stable or decreased transmission [122].

Resistance testing is recommended for patients presenting with acute or recent (i.e., within 12 months) HIV infection, particularly if the source patient is known to be taking antiretroviral drugs. The goal of therapy in these patients is to suppress viral replication quickly to preserve HIV-specific $CD4^+$ cell helper responses and improve long-term outcomes. The ability of therapy to achieve this goal is under investigation, but preliminary data are promising [123, 124]. Initiation of therapy for patients with acute or recent infection should not await the results of resistance testing. Regimens can be adjusted within a few weeks if resistance to any drug is detected. Suboptimal HIV-1 RNA response to an initial regimen (e.g., failure to attain virus load less than detectable levels by 8–12 weeks of therapy) should also prompt consideration of resistance testing.

Established infection. The prevalence of drug-resistant virus in patients with established HIV infection before starting an initial regimen has been assessed [125–130]. It was expected that, even if resistant mutants were initially present, wild-type viruses would eventually predominate because of better replicative capacity [66]. However, newer studies suggest a difference in the persistence of resistant mutants after treatment failure, compared with after primary infection with resistant virus. Resistance mutations in the plasma HIV RNA of untreated patients have been reported to persist for >12 months [131, 132] and, more recently, for >2 years after infection [115]. These data support a recommendation for resistance testing for subjects initiating therapy who have been infected for up to 2 years and perhaps longer. It is often difficult to ascertain how long an individual has been infected, and consideration should be

given to testing when the duration is uncertain and the expected regional prevalence of resistance is ⩾5%. Analyses suggest that resistance testing is cost-effective if the prevalence of resistance is >5% [109]. In such situations, in contrast to that in acute or recent infection, therapy can generally be delayed until resistance test results are available. In addition, drug-resistant variants, persisting as minority species, might not be detected by current assays but could emerge rapidly when antiretroviral therapy is initiated. In cases where treatment is delayed because of high $CD4^+$ cell counts or low HIV-1 RNA levels, resistance tests should be considered to detect the possibility of transmitted resistance for future treatment planning.

Use of resistance testing for changing therapy. The estimated prevalence of any drug-resistant virus in US adults under care during the first 3 years of antiretroviral therapy (1999) in one study was 78% [116]. Resistance prevalence varied by drug class: 70% for NRTIs, 31% for NNRTIs, and 42% for PIs. The likelihood of resistance was higher with more advanced HIV disease and lower reported $CD4^+$ cell counts, but not with current $CD4^+$ cell count [133]. Similar results have been reported from Spain [125]. These results have implications for the potential efficacy of treatment interventions and for transmission of drug resistant HIV. Data from retrospective and prospective studies provide evidence that resistance testing in the setting of virological failure is useful for selecting an alternative antiretroviral regimen [50, 75, 93, 95, 96, 134–142].

Early virological failure of indinavir-zidovudine-lamivudine or amprenavir-zidovudine-lamivudine therapy is associated with the lamivudine-associated M184V mutation present in most patients [143–145]. Similarly, early failure to respond to regimens containing NNRTIs characteristically showed mutations associated with these drugs [114, 146]. This suggests that differential "genetic barriers" to resistance may in part determine the temporal pattern of HIV-1 drug resistance and that it may not always be necessary to change all the drugs in a failing regimen. Continuation of ⩾1 of the components, combined with other new drugs, may prove to be a successful strategy in certain settings; however, this approach has not yet been clinically validated. Single-drug substitutions should generally be avoided and current therapy guidelines followed [147]. Clinicians must be cautious about the potential existence of undetected minority resistant subspecies that could emerge quickly during receipt of a nonsuppressive regimen. Even if the entire regimen is changed, the knowledge gained from resistance testing may prove useful when a subsequent regimen fails, fewer options are available, and the issue of recycling of drugs arises. It should be noted that the absence of resistance in patients whose illness fails to respond to therapy most often indicates poor adherence to the regimen [143, 144].

First regimen failure. Initial regimen failure should prompt a review of adherence and recommendation for resistance testing. Pharmacokinetic reasons for failure should also be considered. Assuming a high degree of adherence and adequate drug absorption, the settings in which resistance testing is likely to prove helpful are: (1) soon after therapy initiation if only a minimal decrease in the plasma HIV-1 RNA level occurs during the first 4–12 weeks, suggesting a suboptimal treatment response; (2) during early virus breakthrough (i.e., a confirmed plasma HIV-1 RNA level of >500–1000 copies/mL that indicates therapy should be changed, after levels less than the detection limit have been attained); and (3) during more prolonged viral replication in which more extensive resistance might be suspected.

Multiple regimen failures. Drug resistance testing is recommended to help guide management after numerous regimens have failed [147]. Retrospective studies have shown that resistance is strongly predictive of lack of response to therapy, and prospective studies have demonstrated the clinical utility of resistance tests plus expert advice in individuals with advanced disease [75, 93–95]. Given the limited drug options available when multiple regimens have failed, incorporating resistance testing into patient management should provide physicians and patients with data that will permit the most effective use of approved or investigational drugs and may help to avoid the inconvenience, cost, and toxicity of drugs in a regimen with little likelihood of conferring benefit. Because resistant virus is archived, review of the cumulative results of prior resistance tests may be useful.

Pregnancy. Current guidelines recommend that zidovudine be included as a component of all regimens designed to prevent mother-to-child transmission [148–150]. However, transmission of zidovudine-resistant HIV-1 to newborns has been documented, and in cases in which it is suspected that a pregnant woman may harbor zidovudine-resistant virus, other drugs that are safe in pregnancy are preferable to zidovudine [151].

Nevirapine alone may also be useful in the maternal-newborn setting, although studies of nevirapine prophylaxis in Uganda showed that the K103M mutation could be selected after a single dose of this drug [152, 153]. According to current experience, it seems reasonable to avoid monotherapy or dual therapy in pregnant HIV-infected women if triple therapy is available. Current treatment guidelines discourage withholding combination antiretroviral therapy from pregnant women if otherwise indicated [149]. Efavirenz should be avoided in pregnancy because of potential teratogenicity [149, 154, 155].

Mother-to-child transmission of multidrug-resistant HIV-1 has been reported with incomplete suppression of maternal plasma viremia and extensive prior antiretroviral exposure [156]. If viremia is present in the mother, resistance testing should be performed on maternal virus, particularly when there has been prior antiretroviral exposure or when prevalence of

Table 2. Summary of clinical situations in which resistance testing is recommended.

Clinical setting	Rationale/comments
Acute or recent HIV infection	
Acute infection[a]	Detect transmission of drug-resistant virus; change therapy to provide optimal antiretroviral activity and preserve HIV-1–specific $CD4^+$ cell helper responses.
HIV infection within previous 12 months (if known)	Detect transmission of drug-resistant virus, although this may not always be possible with current tests.
Suboptimal HIV-1 RNA response to therapy	Failure to attain HIV-1 RNA level less than the detection limit by 8–12 weeks of therapy may suggest preexistence of drug resistance.
Before initiation of antiretroviral therapy in established HIV infection[b]	
Patients infected within previous 2 years and possibly longer	Detect prior transmission of drug-resistant HIV, although this may not always be possible with current tests.
First regimen failure	Document drug(s) to which resistance has emerged; select a new regimen of maximally active drugs. Possible poor regimen adherence and pharmacologic factors responsible for resistance should be assessed. See "Other" below.
Multiple regimen failure	Guide the selection of active drugs in the next regimen, excluding drugs to which response is unlikely. Review of the cumulative results of prior resistance results may be useful. See "Other" below.
Pregnancy, if the mother has detectable plasma HIV-1 RNA level	Optimize the treatment regimen for the mother and prophylaxis for neonate.
Other general recommendations	Plasma samples to be tested for drug resistance should contain at least 500–1000 HIV-1 RNA copies/mL to ensure successful PCR amplification.
	Given the absence of data from comparative trials, no one resistance testing method is recommended over another. Phenotypic testing may be particularly useful in complex cases with multiple resistance mutations.
	In patients in whom an antiretroviral regimen is failing (including suboptimal virologic response as long as HIV RNA level is greater than 500–1000 copies/mL, to allow resistance testing), it is preferable that the blood sample for resistance testing be obtained while the patient is taking the failing regimen, if possible.
	Measures of HIV replication capacity are under study but cannot be generally recommended at this time because of lack of consensus on how to optimally measure or how this information should be incorporated into patient management.
	Resistance testing should be performed by laboratories that have appropriate operator training, certification, and periodic proficiency assurance.
	Genotypic and phenotypic test results should be interpreted by individuals who are knowledgeable in antiretroviral therapy and drug resistance patterns.

[a] Therapy should not be delayed for resistance testing results.
[b] In untreated, established infection, wild-type isolates may replace drug-resistant quasi species over time.

resistant virus in the community is high. Optimally active drugs can then be identified for the pregnant woman, and regimen adjustments can be made to maximize prevention of mother-to-child transmission.

Treatment interruptions. An inducible archive of virus persists in resting memory $CD4^+$ cells harboring latent proviral genomes [157, 158]. One study supported the clinical utility of periods "off" therapy in patients with advanced HIV disease to select for reversion of the virus population from resistant mutants to wild-type and thus increase the response to subsequent antiretroviral therapy [159]. However, other studies have shown no apparent benefit associated with such treatment interruptions [154, 160, 161]. Moreover, treatment interruptions may be associated with deleterious consequences, including reemergence of acute retroviral syndromes [162, 163], selection for drug-resistant virus [162, 164, 165], and precipitous decreases in $CD4^+$ cell counts, resulting in an increased risk for the appearance of opportunistic infections and death. The risk is higher among patients whose latest $CD4^+$ cell count is <200 cells/μL [160, 166].

Non-B subtypes. Much of the knowledge about the development of drug resistance to HIV-1 is based on the study of clade B isolates. Different HIV-1 subtypes may develop drug resistance through different mutational pathways, which may affect cross-resistance [5, 6]. It may thus be necessary to re-evaluate some aspects of knowledge regarding drug resistance

as larger numbers of patients receiving antiretroviral treatment for non–clade B isolates are encountered. Results may be influenced by the primers used for amplification and sequencing. Although studies that use either of the 2 widely available commercial methods (Visible Genetics and Applied Biosystems) did not identify difficulties at the time of sequencing non-B HIV subtypes, one method [167] uses a novel set of RT-PCR primers in this setting and may improve the sequence efficiency [168–172]. Two commercially available phenotypic assays have demonstrated satisfactory performance with a limited number of specimens from patients infected with non–clade B virus.

SUMMARY AND FUTURE DIRECTIONS

Since the previous recommendations of this panel were published [2], considerable progress has been made in defining the indications for resistance testing and determining the cost-effectiveness of strategies that use testing in the management of HIV-infected individuals. Prospective randomized trials have shown at least short-term virological benefits for both genotypic and phenotypic resistance testing in a variety of situations. Moreover, emerging data indicate that viral drug resistance is a problem wherever treatment is used, and it may be increasing in importance. It has also become clear that knowledge concerning patterns of resistance and cross-resistance is critical to the development of successful sequencing of antiretroviral regimens.

Although much has been learned regarding mutational interactions and their effects on drug susceptibility, knowledge in this area is incomplete, and further studies are essential. Defining clinical cutoffs to determine viral resistance to individual drugs and drug combinations is imperative to guide the appropriate interpretation of test results. Evaluating susceptibility patterns among non–clade B HIV isolates should also be a high priority, because these viruses are the most prevalent around the world. In addition, it will be important to further define pharmacologic and virological interactions for individual drugs and combinations and to evaluate how these interactions can best be exploited to provide drug levels sufficient to inhibit partially resistant viruses.

Given the complexities of drug regimens, mutational interactions, and resistance testing, expert interpretation of both genotypic and phenotypic test results is highly recommended. Assessment of the many clinical and biological factors that affect interpretation of resistance test results (including the patient's previous treatment history) requires the input of individuals experienced with antiretroviral therapy and knowledgeable of drug resistance patterns. These guidelines (table 2) should help clinicians make appropriate decisions on how best to incorporate drug resistance testing into the management of HIV-infected individuals.

Acknowledgment

We thank Michelle Tayag for administrative support in preparing the manuscript.

References

1. Hirsch MS, Conway B, D'Aquila RT, et al. Antiretroviral drug resistance testing in adults with HIV infection: implications for clinical management. International AIDS Society–USA Panel. JAMA **1998**; 279:1984–91.
2. Hirsch MS, Brun-Vézinet F, D'Aquila RT, et al. Antiretroviral drug resistance testing in adult HIV-1 infection: recommendations of an International AIDS Society–USA Panel. JAMA **2000**; 283:2417–26.
3. Haubrich RH, Kemper CA, Hellmann NS, et al. The clinical relevance of non-nucleoside reverse transcriptase inhibitor hypersusceptibility: a prospective cohort analysis. AIDS **2002**; 16:F33–40.
4. Mellors J, Vaida F, Bennet K, Hellmann NS, DeGruttola V, Hammer S. Efavirenz hypersusceptibility improves virologic response to multidrug salvage regimens in ACTG 398 [abstract 45]. In: Program and abstracts of the 9th Conference on Retroviruses and Opportunistic Infections (Seattle). Alexandria, VA: Foundation for Retrovirology and Human Health, **2002**:69.
5. Gomes P, Diogo I, Goncalves MF, et al. Different pathways to nelfinavir genotypic resistance in HIV-1 subtypes B and G [abstract 46]. In: Program and abstracts of the 9th Conference on Retroviruses and Opportunistic Infections (Seattle). Alexandria, VA: Foundation for Retrovirology and Human Health,**2002**:69.
6. Brenner B, Turner D, Oliveira M, et al. A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to non-nucleoside reverse transcriptase inhibitors. AIDS **2003**; 17:F1–5.
7. Moore CB, John M, James IR, Christiansen FT, Witt CS, Mallal SA. Evidence of HIV-1 adaptation to HLA-restricted immune responses at a population level. Science **2002**; 296:1439–43.
8. Whitcomb JM, Paxinos E, Huang W, et al. The presence of nucleoside analogue mutations (NAMs) is highly correlated with reduced susceptibility to all nRTIs [abstract 569-T]. In: Program and abstracts of the 9th Conference on Retroviruses and Opportunistic Infections (Seattle). Alexandria, VA: Foundation for Retrovirology and Human Health, **2002**:264.
9. Back D, Gatti G, Fletcher C, et al. Therapeutic drug monitoring in HIV infection: current status and future directions. AIDS **2002**; 16: S5–37.
10. Larder BA, Darby G, Richman DD. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Science **1989**; 243:1731–4.
11. Shulman N, Hughes MD, Winters M, et al. Subtle decreases in stavudine phenotypic susceptibility predict poor virologic response to stavudine monotherapy in zidovudine-experienced patients. J Acquir Immune Defic Syndr **2002**; 31:121–7.
12. Calvez V, Costagliola D, Descamps D, et al. Impact of stavudine phenotype and thymidine analogues mutations on viral response to stavudine plus lamivudine in ALTIS 2 ANRS trail. Antivir Ther **2002**; 7:211–8.
13. Miller V, Larder BA. Mutational patterns in the HIV genome and cross-resistance following nucleoside and nucleotide analogue drug exposure. Antivir Ther **2001**; 6(Suppl 3):25–44.
14. Ren J, Esnouf RM, Hopkins AL, et al. 3′-Azido-3′-deoxythymidine drug resistance mutations in HIV-1 reverse transcriptase can induce

long range conformational changes. Proc Natl Acad Sci USA **1998**; 95:9518–23.
15. Arion D, Kaushik N, McCormick S, Borkow G, Parniak MA. Phenotypic mechanism of HIV-1 resistance to 3′-azido-3′-deoxythymidine (AZT): increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. Biochemistry **1998**; 37:15908–17.
16. Arion D, Sluis-Cremer N, Parniak MA. Mechanism by which phosphonoformic acid resistance mutations restore 3′-azido-3′-deoxythymidine (AZT) sensitivity to AZT-resistant HIV-1 reverse transcriptase. J Biol Chem **2000**; 275:9251–5.
17. International AIDS Society–USA Resistance Mutations Project Panel. Drug resistance mutations in HIV-1. Top HIV Med **2002**; 10:21–5.
18. International AIDS Society–USA Resistance Mutations Project Panel. Drug resistance mutations in HIV-1. Available at: http://www.iasusa.org/resistance_mutations/resistance.pdf. Accessed 3 March **2003**.
19. Shirasaka T, Kavlick MF, Ueno T. Emergence of human immunodeficiency virus type-1 variants with resistance to multiple dideoxynucleosides in patients receiving therapy with dideoxynucleosides. Proc Natl Acad Sci USA **1995**; 92:2398–402.
20. Iversen AK, Shafer RW, Wehrly K, et al. Multidrug-resistant human immunodeficiency virus type 1 strains resulting from combination antiretroviral therapy. J Virol **1996**; 70:1086–90.
21. Schmit JC, Van Laethem K, Ruiz L, et al. Multiple dideoxynucleoside analogue-resistant (MddNR) HIV-1 strains isolated from patients from different European countries. AIDS **1998**; 12:2007–15.
22. Kavlick MF, Wyvill K, Yarchoan R, Mitsuya H. Emergence of multidideoxynucleoside–resistant human immunodeficiency virus type 1 variants, viral sequence variation, and disease progression in patients receiving antiretroviral chemotherapy. J Infect Dis **1998**; 177:1506–13.
23. Larder BA, Bloor S, Kemp SD, et al. A family of insertion mutations between codons 67 and 70 of human immunodeficiency virus type 1 reverse transcriptase confer multinucleoside analog resistance. Antimicrob Agents Chemother **1999**; 43:1961–7.
24. de Jong JJ, Goudsmit J, Lukashov VV, et al. Insertion of two amino acids combined with changes in reverse transcriptase containing tyrosine-215 of HIV-1 resistant to multiple nucleoside analogs. AIDS **1999**; 13:75–80.
25. Winters MA, Coolley KL, Girard YA, et al. 6-Basepair insert in the reverse transcriptase gene of human immunodeficiency virus type 1 confers resistance to multiple nucleoside inhibitors. J Clin Invest **1998**; 102:1769–75.
26. Winters MA, Merigan TC. Variants other than aspartic acid at codon 69 of the human immunodeficiency virus type 1 reverse transcriptase gene affect susceptibility to nucleoside analogs. Antimicrob Agents Chemother **2001**; 45:2276–9.
27. Boucher CAB, O'Sullivan E, Mulder JW, et al. Ordered appearance of zidovudine resistance mutations during treatment of 18 human immunodeficiency virus–positive subjects. J Infect Dis **1992**; 165: 105–10.
28. Larder BA, Kemp SD. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). Science **1989**; 246:1155–8.
29. Kellam P, Boucher CA, Larder BA. Fifth mutation in human immunodeficiency virus type 1 reverse transcriptase contributes to the development of high-level resistance to zidovudine. Proc Natl Acad Sci USA **1992**; 89:1934–8.
30. Lennerstrand J, Hertogs K, Stammers DK, Larder BA. Correlation between viral resistance to zidovudine and resistance at the reverse transcriptase level for a panel of human immunodeficiency virus type 1 mutants. J Virol **2001**; 75:7202–5.
31. Mas A, Parera M, Briones C, et al. Role of a dipeptide insertion between codons 69 and 70 of HIV-1 reverse transcriptase in the mechanism of AZT resistance. EMBO J **2000**; 19:5752–61.
32. Larder BA, Kemp SD, Harrigan PR. Potential mechanism for sustained antiretroviral efficacy of AZT-3TC combination therapy. Science **1995**; 269:696–9.
33. St Clair MH, Martin JL, Tudor-Williams G, et al. Resistance to ddI and sensitivity to AZT induced by a mutation in HIV-1 reverse transcriptase. Science **1991**; 253:1557–9.
34. Gotte M, Arion D, Parniak MA, Wainberg MA. The M184V mutation in the reverse transcriptase of human immunodeficiency virus type 1 impairs rescue of chain-terminated DNA synthesis. J Virol **2000**; 74:3579–85.
35. Nijhuis M, Schuurman R, de Jong D, et al. Lamivudine-resistant human immunodeficiency virus type 1 variants (184V) require multiple amino acid changes to become co-resistant to zidovudine in vivo. J Infect Dis **1997**; 176:398–405.
36. Kemp SD, Shi C, Bloor S, Harrigan PR, Mellors JW, Larder BA. Novel polymorphism at codon 333 of human immunodeficiency virus type 1 reverse transcriptase can facilitate dual resistance to AZT and 3TC. J Virol **1998**; 72:5093–8.
37. Kuritzkes D, Shugarts D, Bakhtiari M, et al. Emergence of dual resistance to zidovudine and lamivudine in HIV-1–infected patients treated with zidovudine plus lamivudine as initial therapy. J Acquir Immune Defic Syndr **2000**; 23:26–34.
38. Naeger LK, Margot NA, Miller MD. Increased drug susceptibility of HIV-1 reverse transcriptase mutants containing M184V and zidovudine-associated mutations: analysis of enzyme processivity, chain-terminator removal and viral replication. Antivir Ther **2001**; 6:115–26.
39. Ross L, Scarsella A, Raffanti S, et al. Thymidine analog and multinucleoside resistance mutations are associated with decreased phenotypic susceptibility to stavudine in HIV type 1 isolated from zidovudine-naive patients experiencing viremia on stavudine-containing regimens. AIDS Res Hum Retroviruses **2001**; 17:1107–15.
40. Miller MD, McKenna P, Larder BA, Harrigan PR. Characteristics of tenofovir phenotypic susceptibility [abstract 8]. Antivir Ther **2001**; 6(Suppl 1):7.
41. Scott WA. The enzymatic basis for thymidine analogue resistance in HIV-1. AIDS Rev **2001**; 3:194–200. Available at: http://www.aidsreviews.com/fr_2001.html.
42. Miller MD, Zhong L, Chen S, Margot NA, Wulfsohn M. Multivariate analyses of antiviral response to tenofovir DF therapy in antiretroviral-experienced patients. Antivir Ther **2002**; 7(Suppl 1):S16.
43. Hsiou Y, Ding J, Das K, et al. The Lys103Asn mutation of HIV-1 RT: a novel mechanism of drug resistance. J Mol Biol **2001**; 309:437–45.
44. Delaugerre C, Rohban R, Simon A, et al. Resistance profile and cross-resistance to HIV-1 among patients failing a non-nucleoside reverse transcriptase inhibitor–containing regimen. J Med Virol **2001**; 65: 445–8.
45. Whitcomb JM, Huang W, Limoli K, et al. Hypersusceptibility to non-nucleoside reverse transcriptase inhibitors in HIV-1: clinical, phenotypic and genotypic correlates. AIDS **2002**; 16:F41–7.
46. Shulman N, Zolopa AR, Passaro D, et al. Phenotypic hypersusceptibility to non-nucleoside reverse transcriptase inhibitors in treatment-experienced HIV-infected patients: impact on virological response to efavirenz-based therapy. AIDS **2001**; 15:1125–32.
47. Swanstrom R, Katzenstein D, Hellmann NS, et al. Selection for delavirdine (DLV) resistance is not associated with loss of nucleoside analogue (NRTI) resistance mutations in subjects with non-nucleoside analogue (NNRTI) hypersusceptibility—results from ACTG 359 [abstract 567-T]. In: Program and abstracts of the 9th Conference on Retroviruses and Opportunistic Infections (Seattle). Alexandria, VA: Foundation for Retrovirology and Human Health, **2002**:263.
48. Kemper CA, Witt MD, Keiser PH, et al. Sequencing of protease inhibitor therapy: insights from an analysis of HIV phenotypic resistance in patients failing protease inhibitors. AIDS **2001**; 15:609–15.
49. Maguire M, Shortino D, Klein A, et al. Emergence of resistance to protease inhibitor amprenavir in human immunodeficiency virus type 1–infected patients: selection of four alternative viral protease genotypes and influence of viral susceptibility to coadministered reverse

transcriptase nucleoside inhibitors. Antimicrob Agents Chemother **2002**; 46:731–8.
50. Lorenzi P, Opravil M, Hirschel B, et al. Impact of drug resistance mutations on virologic response to salvage therapy. AIDS **1999**; 13: F17–21.
51. Condra JH, Schleif WA, Blahy OM. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. Nature **1995**; 374: 569–71.
52. Ziermann R, Limoli K, Das K, Arnold E, Petropoulos CJ, Parkin NT. A mutation in human immunodeficiency virus type 1 protease, N88S, that causes in vitro hypersensitivity to amprenavir. J Virol **2000**; 74: 4414–9.
53. Zachary KC, Hanna GJ, D'Aquila RT. Human immunodeficiency virus type 1 hypersusceptibility to amprenavir in vitro can be associated with virus load response to treatment in vivo. Clin Infect Dis **2001**; 33:2075–7.
54. Obry V, Race E, Vray M, et al. Hypersusceptibility to protease inhibitors associated with mutated proteases at codons 30 and 88 in treated patients [abstract 557-T]. In: Program and abstracts of the 9th Conference on Retroviruses and Opportunistic Infections (Seattle). Alexandria, VA: Foundation for Retrovirology and Human Health, **2002**: 260.
55. Martinez-Picado J, Savara AV, Shi L, Sutton L, D'Aquila RT. Fitness of human immunodeficiency virus type 1 protease inhibitor–selected single mutants. Virology **2000**; 275:318–22.
56. Kilby JM, Hopkins S, Venetta TM, et al. Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. Nat Med **1998**; 4:1302–7.
57. Rimsky LT, Shugars DC, Matthews TJ. Determinants of human immunodeficiency virus type 1 resistance to gp41-derived inhibitory peptides. J Virol **1998**; 72:986–93.
58. Greeberg ML, Melby T, Sista P, et al. Baseline and on-treatment susceptibility to enfuvirtide seen in TORO 1 and TORO 2 to 24 weeks [abstract 141]. In: Program and abstracts of the 10th Conference on Retroviruses and Opportunistic Infections (Boston). Alexandria, VA: Foundation for Retrovirology and Human Health, **2003**:108.
59. Back NK, Nijhuis M, Keulen W, et al. Reduced replication of 3TC-resistant HIV-1 variants in primary cells due to a processivity defect of the reverse transcriptase enzyme. EMBO J **1996**; 15:4040–9.
60. Croteau G, Doyon L, Thibeault D, McKercher G, Pilote L, Lamarre D. Impaired fitness of human immunodeficiency virus type 1 variants with high-level resistance to protease inhibitors. J Virol **1997**; 71: 1089–96.
61. Gerondelis P, Archer RH, Palaniappan C, et al. The P236L delavirdine-resistant human immunodeficiency virus type 1 mutant is replication defective and demonstrates alterations in both RNA 5′-end– and DNA 3′-end–directed RNase H activities. J Virol **1999**; 73:5803–13.
62. Archer RH, Dykes C, Gerondelis P, et al. Mutants of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase resistant to nonnucleoside reverse transcriptase inhibitors demonstrate altered rates of RNase H cleavage that correlate with HIV-1 replication fitness in cell culture. J Virol **2000**; 74:8390–401.
63. Martinez-Picado J, Savara AV, Sutton L, D'Aquila RT. Replicative fitness of protease inhibitor–resistant mutants of human immunodeficiency virus type 1. J Virol **1999**; 73:3744–52.
64. Bleiber G, Munoz M, Ciuffi A, Meylan P, Telenti A. Individual contributions of mutant protease and reverse transcriptase to viral infectivity, replication, and protein maturation of antiretroviral drug-resistant human immunodeficiency virus type 1. J Virol **2001**; 75: 3291–300.
65. Dykes C, Fox K, Lloyd A, Chiulli M, Morse E, Demeter LM. Impact of clinical reverse transcriptase sequences on the replication capacity of HIV-1 drug–resistant mutants. Virology **2001**; 285:193–203.
66. Deeks SG, Wrin T, Liegler T, et al. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. N Engl J Med **2001**; 344:472–80.
67. Quinones-Mateu ME, Ball SC, Marozsan AJ, et al. A dual infection/competition assay shows a correlation between ex vivo human immunodeficiency virus type 1 fitness and disease progression. J Virol **2000**; 74:9222–33.
68. Collin G, Descamps D, Telles F, et al. Differences in protease and reverse transcriptase sequences between the TruGene HIV-1 genotyping kit (Visible Genetics) and the ViroSeq genotyping system (PE Applied Biosystems) [abstract 68]. Antivir Ther **2000**; 5(Suppl 3):53.
69. Hoover ML, Wentworth DN, Neaton JD, et al. A blinded comparison of two sequencing assays for resistance mutations in HIV-1 protease and reverse transcriptase genes in the GART Study (CPCRA 046) [abstract 78]. Antivir Ther **2000**; 5(Suppl 4):60.
70. Brun-Vezinet F, Descamps D, Calvez V, et al. Quality control assessment for HIV-1 drug resistance sequencing [abstract 157]. Antivir Ther **2001**; 6(Suppl 1):121.
71. Keulen W, Brambilla D, Buimer M, et al. A study on HIV-1 genotyping proficiency in 125 laboratories, using the ENVA-3 panel [abstract 166]. Antivir Ther **2001**; 6(Suppl 1):127.
72. Schuurman R, Demeter L, Reichelderfer P, Tijnagel J, de Groot T, Boucher C. Worldwide evaluation of DNA sequencing approaches for identification of drug resistance mutations in the human immunodeficiency virus type 1 reverse transcriptase. J Clin Microbiol **1999**; 37:2291–6.
73. Wensing AM, Keulen W, Buimer M, Brambilla D, Schuurman R, Boucher C. Analysis of the world-wide evaluation study on HIV-1 genotype interpretation; ENVA-3 [abstract 133]. Antivir Ther **2001**; 6(Suppl 1):101.
74. Shafer RW, Gonzales MJ, Brun-Vezinet F. Online comparison of HIV-1 drug resistance algorithms identifies rates and causes of discordant interpretations [abstract 134]. Antivir Ther **2001**; 6(Suppl 1):101.
75. Cingolani A, Antinori A, Rizzo MG, et al. Usefulness of monitoring HIV drug resistance and adherence in individuals failing highly active antiretroviral therapy: a randomized study (ARGENTA). AIDS **2002**; 16:369–79.
76. Lanier ER, Hellman N, Scott J, et al. Determination of a clinically relevant phenotypic resistance "cutoff" for abacavir using the PhenoSense assay [abstract 254]. In: Program and abstracts of the 8th Conference on Retroviruses and Opportunistic Infections (Chicago). Alexandria, VA: Foundation for Retrovirology and Human Health, **2001**: 117.
77. Kempf D, Brun S, Rode R, et al. Identification of clinically relevant phenotypic and genotypic break-points for ABT-378/r in multiple PI-experienced, NNRTI-naive patients [abstract 89]. Antivir Ther **2000**; 5(Suppl 3):70.
78. Calvez V, Costagliola D, Descamps D, et al. Resistance and viral response to stavudine/lamivudine combination in zidovudine, didanosine and zalcitabine experienced patients in the ALTIS 2 ANRS trial [abstract 107]. Antivir Ther **2000**; 5(Suppl 3):83.
79. Shulman N, Shafer R, Winters M, et al. Genotypic predictors of virologic response to stavudine after zidovudine monotherapy (ACTG 302) [abstract 437]. In: Program and abstracts of the 8th Conference on Retroviruses and Opportunistic Infections (Chicago). Alexandria, VA: Foundation for Retrovirology and Human Health, **2001**:173.
80. Calvez V, Cohen-Codar I, Marcelin AG, et al. Identification of individual mutations in HIV protease associated with virological response to lopinavir/ritonavir therapy [abstract 82]. Antivir Ther **2001**; 6(Suppl 1):64.
81. Costagliola D, Descamps D, Calvez V, et al. Presence of thymidine-associated mutations and response to d4T, abacavir and ddI in the control arm of the Narval ANRS 088 trial [abstract 450]. In: Program and abstracts of the 8th Conference on Retroviruses and Opportunistic Infections (Chicago). Alexandria, VA: Foundation for Retrovirology and Human Health, **2001**:177.
82. Miller MD, Margot N, Coakley D, Cheng A. Anti-HIV responses and development of RT mutations in antiretroviral-experienced patients adding tenofovir DF (TDF) therapy: baseline and week 24 genotypic analyses of study 907 [abstract 1928]. In: Program and abstracts of

the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy (Chicago). Washington, DC: American Society for Microbiology, **2001**:345.
83. Larder BA, Kemp SD, Hertogs K. Quantitative prediction of HIV-1 phenotypic drug resistance from genotypes: the virtual phenotype (VirtualPhenotype) [abstract 63]. Antivir Ther **2000**; 5(Suppl 3):49.
84. Paxinos EE, Sartoris MM, Dawson K, et al. Natural variation in susceptibility to nonnucleoside reverse transcriptase inhibitors predates drug availability [abstract 156]. Antivir Ther **2000**; 5(Suppl 3):123.
85. Qari SH, Respess R, Weinstock H, et al. A comparative analysis of Virco Antivirogram and ViroLogic PhenoSense phenotypic assays for drug susceptibility of HIV-1 [abstract 62]. Antivir Ther **2000**; 5(Suppl 3):49.
86. Dam E, Clavel F, Calvez V, et al. Comparison of HIV-1 resistance phenotypes obtained by two different assay systems [abstract 158]. Antivir Ther **2001**; 6(Suppl 1):122.
87. Miller V, Schuurman R, Clavel F, et al. Comparison of HIV-1 drug susceptibility (phenotype) results reported by three major laboratories [abstract 169]. Antivir Ther **2001**; 6(Suppl 1):129.
88. Wrin T, Huang W, Yap J, et al. Evaluating HIV-1 co-receptor usage and inhibitors of virus entry using recombinant virus assays [abstract 1]. Antivir Ther **2001**; 6(Suppl 1):3.
89. Obry V, Costagliola D, Race E, et al. The extent of association between resistance phenotype and treatment response is highly dependent upon the drug [abstract 78]. Antivir Ther **2001**; 6(Suppl 1):61.
90. Haubrich R, Keiser P, Kemper C, et al. CCTG 575: a randomized, prospective study of phenotype testing versus standard of care for patients failing antiretroviral therapy [abstract 80]. Antivir Ther **2001**; 6(Suppl 1):63.
91. Durant J, Clevenbergh P, Garraffo R, et al. Importance of protease inhibitor plasma levels in HIV-infected patients treated with genotypic-guided therapy: pharmacological data from the VIRADAPT Study. AIDS **2000**; 14:1333–9.
92. Kempf D, Hsu A, Jiang P, et al. Response to ritonavir (RTV) intensification in indinavir (IDV) recipients is highly correlated with virtual inhibitory quotient [abstract 523]. In: Program and abstracts of the 8th Conference on Retroviruses and Opportunistic Infections (Chicago). Alexandria, VA: Foundation for Retrovirology and Human Health, **2001**:200.
93. Durant J, Clevenbergh P, Halfon P, et al. Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial. Lancet **1999**; 353:2195–9.
94. Baxter JD, Mayers DL, Wentworth DN, et al. A randomized study of antiretroviral management based on plasma genotypic antiretroviral resistance testing in patients failing therapy. CPCRA 046 Study Team for the Terry Beirn Community Programs for Clinical Research on AIDS. AIDS **2000**; 14:F83–93.
95. 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.
96. Cohen C, Hunt S, Sension M, et al. A randomized trial assessing the impact of phenotypic resistance testing on antiretroviral therapy. AIDS **2002**; 16:579–88.
97. Meynard JL, Vray M, Morand-Joubert L, et al. Phenotypic or genotypic resistance testing for choosing antiretroviral therapy after treatment failure: a randomized trial. AIDS **2002**; 16:727–36.
98. Mayers D. Both antiretroviral drug levels and drug resistance are associated with short-term virologic responses to subsequent drug regimens in CPCRA 046 (GART Study) [abstract 124]. In: Program and abstracts of the 1st International AIDS Society Conference on HIV Pathogenesis and Treatment (Buenos Aires). Stockholm: International AIDS Society, **2001**:122.
99. Salama C, Policar M, Cervera C. Knowledge of genotypic resistance mutations among providers of care to patients with human immunodeficiency virus. Clin Infect Dis **2003**; 36:101–4.
100. Melnick D, Rosenthal J, Cameron M, et al. Impact of phenotypic antiretroviral drug resistance testing on the response to salvage antiretroviral therapy (ART) in heavily experienced patients [abstract 786]. In: Program and abstracts of the 7th Conference on Retroviruses and Opportunistic Infections (San Francisco). Alexandria, VA: Foundation for Retrovirology and Human Health, **2000**:222.
101. Meynard JL, Vray M, Morand-Joubert L, et al. Phenotypic or genotypic resistance testing for choosing antiretroviral therapy after treatment failure. AIDS **2002**; 16:727–36
102. Vray M, Meynard JL, Dalban C, et al. Multivariate logistic regression analysis of factors predictive of the virological response in the Narval trial [abstract B1387]. In: Program and abstracts of the 14th International AIDS Conference (Barcelona). Barcelona: Prous Science, S.A., **2002**:377.
103. Wegner S, Wallace M, Tasker S, et al. Long-term clinical efficacy of resistance testing: results of the CERT trial [abstract 158]. Antivir Ther **2002**; 7:S129.
104. Blanco JL, Valdecillos G, Arroyo JR, et al. A prospective randomized study on the usefulness of genotypic resistance tests versus real phenotypic resistance tests in heavily pretreated patients with virological failure (VIHRES Study) [abstract TuPeB4624]. In: Program and abstracts of the 14th International AIDS Conference (Barcelona). Barcelona: Prous Science, S.A., **2002**:421.
105. Perez-Elias MJ, Garcia I, Munoz V, et al. A randomized, prospective study of phenotype (P) versus virtual phenotype (virtualP) testing for patients failing antiretroviral therapy (ARVT): final analysis [abstract H-1079]. In: Program and abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy (San Diego). Washington, DC: American Society for Microbiology, **2002**:268.
106. Parkin N, Chappey C, Maroldo L, et al. Phenotypic and genotypic HIV-1 drug resistance assays provide complementary information. J Acquir Immune Defic Syndr **2002**; 31:128–36.
107. Lalezari J, Henry K, O'Hearn M, et al. Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. N Engl J Med **2003**; 348:2175–85.
108. Lazzarin A, Clotet B, Cooper D, et al. Efficacy of enfuvirtide in patients infected with drug-resistant HIV-1 in Europe and Australia. N Engl J Med **2003**; 348:2186–95.
109. Weinstein MC, Goldie SJ, Losina E, et al. Use of genotypic resistance testing to guide HIV therapy: clinical impact and cost-effectiveness. Ann Intern Med **2001**; 134:440–50.
110. Verhofstede C, Van Wanzeele F, Van Der Gucht B, De Cabooter N, Plum J. Interruption of reverse transcriptase inhibitors or a switch from reverse transcriptase to protease inhibitors in a fast reappearance of virus strains with a reverse transcriptase inhibitor–sensitive genotype. AIDS **1999**; 13:2541–6.
111. Miller V, Sabin C, Hertogs K, et al. Virological and immunological effects of treatment interruptions in HIV-1 infected patients with treatment failure. AIDS **2000**; 14:2857–67.
112. Devereux HL, Youle M, Johnson MA, Loveday C. Rapid decline in detectability of HIV-1 drug resistance mutations after stopping therapy. AIDS **1999**; 13:F123–7.
113. Joly V, Descamps D, Zeng F, et al. Evolution of HIV-1 resistance mutations to nonnucleoside reverse transcriptase inhibitors (NNRTIs) following withdrawal [abstract 123]. In: Program and abstracts of the 1st International AIDS Society Conference on HIV Pathogenesis and Treatment (Buenos Aires). Stockholm: International AIDS Society, **2001**:122.
114. Richman DD, Havlir D, Corbeil J, et al. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J Virol **1994**; 68:1660–6.
115. Little SJ, Daar ES, Holte S, et al. Persistence of transmitted drug resistance among subjects with primary HIV infection not receiving antiretroviral therapy [abstract 95]. In: Program and abstracts of the 9th Conference on Retroviruses and Opportunistic Infections (Seattle). Alexandria, VA: Foundation for Retrovirology and Human Health, **2002**:83.
116. Little SJ, Holte S, Routy JP, et al. Antiretroviral-drug resistance among patients recently infected with HIV. N Engl J Med **2002**; 347:385–94.
117. Salomon H, Wainberg MA, Brenner B, et al. Prevalence of HIV-1

resistant to antiretroviral drugs in 81 individuals newly infected by sexual contact or injecting drug use. Investigators of the Quebec Primary Infection Study. AIDS **2000**; 14:F17–23.
118. Duwe S, Brunn M, Altmann D, et al. Frequency of genotypic and phenotypic drug-resistant HIV-1 among therapy-naive patients of the German Seroconverter Study. J Acquir Immune Defic Syndr **2001**; 26:266–73.
119. UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance. Analysis of prevalence of HIV-1 drug resistance in primary infections in the United Kingdom. BMJ **2001**; 322:1087–8.
120. Zaidi I, Weinstock H, Woods T, Thomas J, Heneine W, Kaplan J. Prevalence of mutations associated with antiretroviral drug resistance among HIV-1–infected persons in 10 US cities, 1997–2000 [abstract 155]. Antivir Ther **2001**; 6(Suppl 1):118.
121. Briones C, Perez-Olmeda M, Rodriguez C, del Romero J, Hertogs K, Soriano V. Primary genotypic and phenotypic HIV-1 drug resistance in recent seroconverters in Madrid. J Acquir Immune Defic Syndr **2001**; 26:145–50.
122. Yerly S, Vora S, Rizzardi P, et al. Acute HIV infection: impact on the spread of HIV and transmission of drug resistance. AIDS **2001**; 15: 2287–92.
123. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1–specific $CD4^+$ T cell responses associated with control of viremia. Science **1997**; 278:1447–50.
124. Musey LK, Krieger JN, Hughes JP, Schacker TW, Corey L, McElrath MJ. Early and persistent human immunodeficiency virus type 1 (HIV-1)–specific T helper dysfunction in blood and lymph nodes following acute HIV-1 infection. J Infect Dis **1999**; 180:278–84.
125. Gallego O, Ruiz L, Vallejo A, et al. Changes in the rate of genotypic resistance to antiretroviral drugs in Spain. AIDS **2001**; 15:1894–6.
126. Hanna GJ, Balaguera HU, Freedberg KA, et al. Drug-selected resistance mutations and non–B subtypes in antiretroviral-naive adults with established human immunodeficiency virus infection. J Infect Dis (in press).
127. Wegner S, Brodine S, Mascola J, et al. High frequency of antiretroviral drug resistance in HIV-1 from recently infected therapy-naive individuals [abstract 119]. Antivir Ther **1999**; 4(Suppl 1):85.
128. Verbiest W, Schel P, Conant M, et al. An epidemiological perspective survey assessing the prevalence of HIV-1 drug resistance in 230 HIV-1–positive antiretroviral-naive patients from the USA [abstract 122]. Antivir Ther **1999**; 4(Suppl 1):86–7.
129. Descamps D, Costagliola D, Glaude G, et al. Prevalence of resistance mutations in antiretroviral-naive patients: French National Study [abstract 123]. Antivir Ther **1999**; 4(Suppl 1):87.
130. Harrigan PR, Alexander C, Dong W, Jahnke N, O'Shaughnessy M, Montaner JS. Prevalence of resistance-associated mutations in patients starting antivirals: virological response after approximately 1 year of therapy [abstract 124]. Antivir Ther **1999**; 4(Suppl 1):88.
131. Brenner BG, Routy JP, Petrella M, et al. Persistence and fitness of multidrug-resistant human immunodeficiency virus type 1 acquired in primary infection. J Virol **2002**; 76:1753–61.
132. De Ronde A, van Dooren M, van der Hoek L, et al. Establishment of new transmissible and drug-sensitive human immunodeficiency virus type 1 wild types due to transmission of nucleoside analogue–resistant virus. J Virol **2001**; 75:595–602.
133. Richman DD, Bozzette S, Morton S, et al. The prevalence of antiretroviral drug resistance in the US [abstract LB-17]. In: Program and abstracts of the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy (Chicago). Washington, DC: American Society for Microbiology. **2001**:S129.
134. D'Aquila RT, Johnson VA, Welles SL, et al. Zidovudine resistance and HIV-1 disease progression during antiretroviral therapy. Ann Intern Med **1995**; 122:401–8.
135. Call SA, Saag MS, Westfall AO, et al. Phenotypic drug susceptibility testing predicts long-term virologic suppression better than treatment history in patients with human immunodeficiency virus infection. J Infect Dis **2001**; 183:401–8.
136. Japour AJ, Welles S, D'Aquila RT, et al. Prevalence and clinical significance of zidovudine (ZDV) resistance mutations in human immunodeficiency virus isolated from patients following long-term zidovudine treatment. J Infect Dis **1995**; 171:1172–9.
137. Miller V, Phillips A, Rottmann C, Staszewski S. Dual resistance to zidovudine (ZDV) and lamivudine (3TC) in patients treated with ZDV/3TC combination therapy: association with therapy failure. J Infect Dis **1998**; 177:1521–32.
138. Harrigan PR, Montaner JS, Hogg RS, et al. Baseline resistance profile predicts response to ritonavir/saquinavir therapy in a community setting [abstract 55]. Antivir Ther **1998**; 3(Suppl 1):38.
139. Zolopa AR, Shafer RW, Warford A, et al. HIV-1 genotype resistance patterns predict response to saquinavir-ritonavir therapy in patients in whom previous protease inhibitor therapy had failed. Ann Intern Med **1999**; 131:813–21.
140. Deeks SG, Hellman NS, Grant RM, et al. Novel four-drug salvage treatment regimens after failure of a human immunodeficiency virus type 1 protease inhibitor–containing regimen: antiviral activity and correlation of baseline phenotypic drug susceptibility with virologic outcome. J Infect Dis **1999**; 179:1375–81.
141. Clevenbergh P, Durant J, Halfon P, et al. Persisting long-term benefit of antiretroviral genotypic guided treatment for HIV-infected patients failing HAART: the VIRADAPT study, week 48 follow-up [abstract 60]. Antivir Ther **1999**; 4(Suppl 1):42.
142. Baxter JD, Mayers DL, Wentworth DN, Neaton JD, Merigan TC, CPRCA 046 Study Team. Final results of CPRCA 046: a pilot study of antiretroviral management based on plasma genotypic antiretroviral resistance testing (GART) in patients failing antiretroviral therapy [abstract 61]. Antivir Ther **1999**; 4(Suppl 1):43.
143. Havlir DV, Hellmann NS, Petropoulos CJ, et al. Drug susceptibility in HIV infection after viral rebound in patients receiving indinavir-containing regimens. JAMA **2000**; 283:229–34.
144. Descamps D, Flandre P, Calvez V, et al. Mechanisms of virologic failure in previously untreated HIV-1 infected patients from a trial of induction-maintenance therapy. Trilege (Agence Nationale de Recherches sur le SIDA 072) Study Team [see comments]. JAMA **2000**; 283:205–11.
145. De Pasquale MP, Murphy R, Kuritzkes D, et al. Resistance during early virological rebound on amprenavir plus zidovudine plus lamivudine triple therapy or amprenavir monotherapy in ACTG protocol 347 [abstract 71]. Antivir Ther **1998**; 3(Suppl 1):50–1.
146. Bacheler L, Ploughman L, Hertogs K, Larder B. Impact of baseline NNRTI resistance on the efficacy of efavirenz combination therapy in NNRTI therapy–naive patients (study DMP 266–006) [abstract 88]. Antivir Ther **2000**; 5(Suppl 3):70.
147. Yeni PG, Hammer SM, Carpenter CCJ, et al. Antiretroviral treatment for adult HIV-1 infection in 2002: updated recommendations of the International AIDS Society–USA panel. JAMA **2002**; 288:222–35.
148. Fiscus SA, Adimora AA, Schoenbach VJ, et al. Trends in human immunodeficiency virus (HIV) counseling, testing, and antiretroviral treatment of HIV-infected women and perinatal transmission in North Carolina. J Infect Dis **1999**; 180:99–105.
149. US Public Health Service. Public Health Service Task Force recommendations for the use of antiretroviral drugs in pregnant HIV-1 infected women for maternal health and interventions to reduce perinatal HIV-1 transmission in the United States. Updated 4 February **2002**. Available at: http://www.aidsinfo.nih.gov/guidelines/perinatal/Perinatal.pdf. Accessed 28 February 2003.
150. US Department of Health and Human Services Panel on Clinical Practices for Treatment of HIV Infection. Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. 4 February **2003**. Available at: http://www.aidsinfo.nih.gov/guidelines/adult/AAMay23.pdf. Accessed 28 February 2002.
151. Masquelier B, Chaix ML, Burgard M, et al. Zidovudine genotypic resistance in HIV-1–infected newborns in the French perinatal cohort. J Acquir Immune Defic Syndr **2001**; 27:99–104.
152. Jackson JB, Becker-Pergola G, Guay LA, et al. Identification of the

K103N resistance mutation in Ugandan women receiving nevirapine to prevent HIV-1 vertical transmission. AIDS **2000**; 14:F111–5.
153. Eshleman SH, Mracna M, Guay LA, et al. Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). AIDS **2001**; 15: 1951–7.
154. Lawrence J, Mayers D, Huppler-Hullsiek K, et al. CPCRA 064: a randomized trial examining structured treatment interruption for patients failing therapy with multi-drug resistant HIV [abstract 67]. In: Program and abstracts of the 10th Conference on Retroviruses and Opportunistic Infections (Boston). Alexandria, VA: Foundation for Retrovirology and Human Health, **2003**:81.
155. Sperling RS, Shapiro DE, Coombs RW, et al. Maternal viral load, zidovudine treatment, and the risk of transmission of human immunodeficiency virus type 1 from mother to infant. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N Engl J Med **1996**; 335:1621–9.
156. Johnson VA, Petropoulos CJ, Woods CR, et al. Vertical transmission of multidrug-resistant human immunodeficiency virus type 1 (HIV-1) and continued evolution of drug resistance in an HIV-1–infected infant. J Infect Dis **2001**; 183:1688–93.
157. Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science **1997**; 278:1295–300.
158. Wong JK, Hezareh M, Gunthard HF, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science **1997**; 278:1291–5.
159. Katlama C, Dominguez S, Duvivier C, et al. GIGHAART (ANRS 097): a prospective randomized trial comparing the efficacy of a salvage regimen administered with or without treatment interruption in patients with severe biological failure and extensive prior therapy [abstract 16]. In: Program and abstracts of the 8th European Conference on Clinical Aspects and Treatment of HIV-Infection (Athens). Athens: European AIDS Clinical Society, **2001**:81.
160. Deeks SG, Wrin T, Liegler T, et al. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. N Engl J Med **2001**; 344:472–80.
161. Ruiz L, Ribera E, Bonjoch A, et al. Virologic and immunologic benefit of a salvage therapy that includes Kaletra plus Fortovase preceded or not by antiretroviral therapy interruption (TI) in advanced HIV-infected patients (6-month-follow-up) [abstract 421]. In: Program and abstracts of the 9th Conference on Retroviruses and Opportunistic Infections (Seattle). Alexandria, VA: Foundation for Retrovirology and Human Health, **2002**:212.
162. Daar ES, Bai J, Hausner MA, Majchrowicz M, Tamaddon M, Giorgi JV. Acute HIV syndrome after discontinuation of antiretroviral therapy in a patient treated before seroconversion. Ann Intern Med **1998**; 128:827–9.
163. Kilby JM, Goepfert PA, Miller AP, et al. Recurrence of the acute HIV syndrome after interruption of antiretroviral therapy in a patient with chronic HIV infection: a case report. Ann Intern Med **2000**; 133: 435–8.
164. Tremblay CL, Hicks JL, Sutton L, et al. Antiretroviral resistance associated with supervised treatment interruptions in treated acute HIV-1 infection. AIDS **2003**; 17:1086–9.
165. Martinez-Picado J, Morales-Lopetegi K, Wrin T, et al. Selection of drug-resistant HIV-1 mutants in response to repeated structured treatment interruptions. AIDS **2002**; 16:895–9.
166. Lundgren JD, Vella S, Paddam L, et al. Interruption/stopping antiretroviral therapy and the risk of clinical disease: results from the EuroSIDA study [abstract 48]. In: Program and abstracts of the 9th Conference on Retroviruses and Opportunistic Infections (Seattle). Alexandria, VA: Foundation for Retrovirology and Human Health, **2002**:70.
167. Fiette P, Monnet A, Lloyd R, Naudin C. Use of novel set of RT-CPR primers in conjunction with Trugene HIV-1 genotyping kit provides reliable genotypes of difficult patient specimens [abstract 263]. In: Program and abstracts of the 8th European Conference on Clinical Aspects and Treatment of HIV Infection (Athens). Athens: European AIDS Clinical Society, **2001**:169.
168. Grossman Z, Vardinon N, Chemtob D, et al. Mutations in naive and treated clade C patients [abstract 162]. Antivir Ther **2000**; 5(Suppl 3): 127.
169. Boulmé R, Dugas O, Halfon P, Diaz R, Schmit JC. Analysis of mutational patterns associated with resistance to tenofovir DF and lopinavir/ritonavir [abstract P352]. In: Program and abstracts of the 8th European Conference on Clinical Aspects and Treatment of HIV Infection (Athens). Athens: European AIDS Clinical Society, **2001**: 213.
170. Barlotta C, Facchi G, Violin M. High prevalence of HIV-1 circulating recombinant forms among non–clade B strains in Italy [abstract P354]. In: Program and abstracts of the 8th European Conference on Clinical Aspects and Treatment of HIV Infection (Athens). Athens: European AIDS Clinical Society, **2001**:214.
171. Caride E, Hertogs K, Larder B, et al. Genotyping and phenotyping analysis of B and non-B HIV-1 subtypes from Brazilian patients under HAART [abstract 164]. Antivir Ther **2000**; 5(Suppl 3):129.
172. Lambert C, Fontaine E, Servais J, et al. Drug resistance–related mutations in the pol gene of non-subtype B HIV-1 patient strains [abstract 165]. Antivir Ther **2000**; 5(Suppl 3):129.