Antiretroviral Drug Resistance Testing in Adult HIV-1 Infection: 2008 Recommendations of an International AIDS Society–USA Panel

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Resistance to antiretroviral drugs remains an important limitation to successful human immunodeficiency virus type 1 (HIV-1) therapy. Resistance testing can improve treatment outcomes for infected individuals. The availability of new drugs from various classes, standardization of resistance assays, and the development of viral tropism tests necessitate new guidelines for resistance testing. The International AIDS Society–USA convened a panel of physicians and scientists with expertise in drug-resistant HIV-1, drug management, and patient care to review recently published data and presentations at scientific conferences and to provide updated recommendations. Whenever possible, resistance testing is recommended at the time of HIV infection diagnosis as part of the initial comprehensive patient assessment, as well as in all cases of virologic failure. Tropism testing is recommended whenever the use of chemokine receptor 5 antagonists is contemplated. As the roll out of antiretroviral therapy continues in developing countries, drug resistance monitoring for both subtype B and non–subtype B strains of HIV will become increasingly important.

A panel of the International AIDS Society–USA published recommendations for HIV-1 drug resistance testing in HIV-1–infected adults in 1998, 2000, and 2003 [1–3]. Since the 2003 publication, drug resistance testing has become widespread in the developed world and has been accepted as an important adjunct to the management of patients with detectable plasma viremia who are receiving antiretroviral therapy. Moreover, person-to-person transmission of drug-resistant viruses occurs in a variety of settings, including between adults and from mother to child [4, 5], indicating that testing for drug resistance before initiating therapy may be useful even for treatment-naive patients. Novel resistance mutations that confer resistance to older drugs continue to be identified (figure 1), and newer-generation protease inhibitors (PIs) and reverse transcriptase inhibitors have been developed to counteract mutations that confer resistance to the older agents. Approval of agents from new classes, like integrase strand transfer inhibitors (INSTIs) and entry inhibitors, assure that drug resistance testing will become increasingly complex and important in case management in the years ahead. Testing methodologies have improved and are becoming more sensitive, and tests for viral coreceptor use (i.e., tropism) have been introduced.

With increasing patient access to antiretroviral drugs in the developing world, many of the same problems

MUTATIONS IN THE REVERSE TRANSCRIPTASE GENE ASSOCIATED WITH RESISTANCE TO REVERSE TRANSCRIPTASE INHIBITORS

Nucleoside and Nucleotide Reverse Transcriptase Inhibitors (nRTIs)

Multi-nRTI Resistance: 69 Insertion Complex (affects all nRTIs currently approved by the US FDA)

Multi-nRTI Resistance: 151 Complex (affects all nRTIs currently approved by the US FDA except tenofovir)

Multi-nRTI Resistance: Thymidine Analogue-associated Mutations (TAMs; affect all nRTIs currently approved by the US FDA)

Abacavir
Didanosine
Emtricitabine
Lamivudine
Stavudine
Tenofovir
Zidovudine

Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Efavirenz
Etravirine
Nevirapine

MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS

Atazanavir +/– ritonavir
Darunavir/ ritonavir
Fosamprenavir/ ritonavir
Indinavir/ ritonavir
Lopinavir/ ritonavir
Nelfinavir
Saquinavir/ ritonavir
Tipranavir/ ritonavir

MUTATIONS IN THE ENVELOPE GENE ASSOCIATED WITH RESISTANCE TO ENTRY INHIBITORS

Enfuvirtide
Maraviroc — See User Note (ref7)

MUTATIONS IN THE INTEGRASE GENE ASSOCIATED WITH RESISTANCE TO INTEGRASE INHIBITORS

Raltegravir

MUTATIONS

Insertion
Amino acid, wild-type
Amino acid position
Major (boldface type; protease only)
Amino acid substitution conferring resistance
Minor (lightface type; protease only)

Figure 1. Mutations in HIV-1 that affect susceptibility to antiretroviral drugs, by HIV gene target. The letter above each position is the wild-type amino acid and the letter(s) below each position indicate the substitution(s) that are associated with drug resistance. Reprinted with permission from [6]. Detailed user notes and regular updates are available at the International AIDS Society–USA Web site [7]. FDA, Food and Drug Administration.

Table 1. Strength of recommendation and quality of evidence rating scale.

Category, grade	Definition
Strength of recommendation	
A	Strong evidence to support the recommendation
B	Moderate evidence to support the recommendation
C	Insufficient evidence to support a recommendation
Quality of evidence	
I[a,b]	Evidence from ≥1 randomized, controlled clinical trial
II[a,b]	Evidence from nonrandomized clinical trials; cohort or case-control studies
III	Recommendation based on the panel's analysis of accumulated evidence (expert opinion)

[a] Peer-reviewed publications.
[b] Presented in abstract form at peer-reviewed scientific meetings.

involving drug-resistant virus that are common in the developed world have begun to occur in the developing world, as well. In addition, resistance patterns among the non–subtype B strains of HIV-1, which are circulating more widely in developing countries, may differ from those seen among subtype B strains in North America and Europe. Although resistance testing is not yet widely employed in developing countries because of the costs involved, drug resistance will predictably emerge as antiretroviral therapy is more widely introduced, and the need for appropriate testing will increase. Expert advice is important in the management of patients with drug-resistant infection [8], but because it is not always available, this report will attempt to provide updated recommendations for HIV-1 drug resistance testing and tropism testing in developed countries, with the anticipation that, over time, such recommendations will also prove useful in developing countries.

METHODS

The panel was first convened by the International AIDS Society–USA (which is not related to the worldwide International AIDS Society) in 1997 to develop evidence-based recommendations for the assessment of HIV-1 drug susceptibility and the management of drug-resistant HIV-1 infection in clinical practices in the developed world [1–3]. Panel members are not compensated, and there is a process for panel member rotation. Updated reports are initiated when enough new published or presented information in the field accumulates to warrant revising previous recommendations.

The panel was convened by conference call in mid-2007 and met regularly thereafter to discuss new data published or presented at scientific conferences since its previous report [3]. Topic areas included new information about the prevalence of drug resistance worldwide; new data on mechanisms of resistance by drug class, including INSTI and entry inhibitor classes that have become available since the previous report; developments in assays to determine viral tropism and replication capacity; issues related to non–subtype B HIV-1; and updated recommendations for the clinical use of HIV-1 drug resistance

and tropism testing. Individual panel members were appointed to review topics to be considered. In some cases, pharmaceutical or assay manufacturers were contacted to obtain relevant information in the public domain. Data on file, unpublished observations, personal communications, and other forms of data not previously published or presented in a scientific, public forum were not considered for this report. Discussions of drugs focused on those approved by the US Food and Drug Administration. Clinical recommendations were made by panel consensus.

The quality and strength of the evidence were rated according to a scale (table 1) that was modified in 2006 [9] and originally adapted from published rating scales used by other organizations (e.g., the American Heart Association [10], American Association for the Study of Liver Diseases [11], National Institutes of Health [12], and Infectious Diseases Society of America [13]).

TRANSMISSION AND EPIDEMIOLOGY OF DRUG-RESISTANT HIV-1

Transmission and prevalence of drug resistance in developed countries. Transmission of drug-resistant HIV-1 has been observed in most countries where antiretroviral treatment is available [14–22], and it jeopardizes the success of antiretroviral therapy. Indeed, transmitted drug resistance generally leads to a delay in virologic suppression [18, 23] and to an increased risk of earlier virologic failure [24, 25]. Long-term persistence of transmitted drug resistance in the absence of drug pressure has been documented for many types of mutations [26–30], as have specific revertant mutations for thymidine analogue reverse-transcriptase inhibitor–associated resistance mutations (TAMs) [29]. In contrast with patients with acquired drug-resistant virus that emerged during therapy, patients with transmitted drug-resistant virus do not have a reservoir of drug-susceptible virus. Consequently, transmitted drug-resistant virus can only change to drug-susceptible virus by back mutation [30], and it will do so rapidly only if a substantial fitness benefit occurs with reversion of the drug resistance mutation,

as in the case of the M184V mutation in reverse transcriptase. This mutation can revert relatively early after transmission [26], in contrast with the delayed reversion that occurs with most other mutations [26, 30]. This delayed reversion is different from the reversion associated with acquired drug resistance, in which archived, drug-susceptible wild-type virus reemerges within weeks of withdrawal of the selective pressure of drug treatment [31–34].

The prevalence of newly transmitted drug-resistant HIV-1 strains (primary HIV-1 drug resistance) varies widely with location, transmission risk group, and the sampling time after infection [14, 15, 18, 20–23, 35–37]. Variations in prevalence are multifactorial and reflect different treatment exposures at the population level, potential selection bias caused by non-representative sampling of certain transmission risk groups, different definitions of resistance [38, 39], different sampling times after infection, and different risk behavior and access to therapy among transmission risk groups. A large increase in overall primary resistance, from 13.2% for the period 1995–1998 to 24.1% for the period 2003–2004, was reported in New York, New York, and the rate of transmitted multidrug resistance increased from 2.6% to 9.8% over the same period [36]. A British group also reported high rates of primary resistance in 2003: 19.2% for any drug, 12.4% for nucleoside analogue reverse-transcriptase inhibitors (NRTIs), 8.1% for nonnucleoside analogue reverse-transcriptase inhibitors (NNRTIs), and 6.6% for PIs. High-level resistance was found in 9.3%. In contrast, a representative 10-year transmission surveillance study (1996–2005), conducted by the Swiss HIV Cohort Study, showed considerably lower rates: 7.7% for any drug, 5.5% for NRTIs, 1.9% for NNRTIs, and 2.7% for PIs. Dual– or triple–drug class resistance was observed in only 2% of patients [21]. The rate of transmission, including the transmission of multidrug-resistant virus, was stable over a 10-year period, with the exception of NNRTI-resistant virus transmission, which—as has been reported by other groups—increased in 2005 [16, 18, 20, 36, 40]. These examples demonstrate that specific countries and regions require separate surveillance systems to monitor transmitted HIV drug resistance, because extrapolation from foreign data may be misleading.

The CASCADE study [41] has reported the longest follow-up time for patients with transmitted drug resistance to date; this study found higher initial CD4+ T cell counts in patients infected with drug-resistant virus than in patients infected with wild-type virus. This initial higher CD4+ T cell count was followed by a faster decrease in CD4+ T cell count, such that initial differences in CD4+ T cell counts were lost over the 5-year observation period. Thus, the effects of transmitted drug-resistant HIV on the infection's natural history before treatment are not great.

Drug resistance acquired during antiretroviral therapy is much more common than transmitted drug resistance. Cross-sectional studies involving patients who have been treated but who are viremic yielded probabilities for the presence of at least 1 drug resistance mutation of 76%–90% [42–47]. However, considerable methodologic challenges exist in evaluating such prevalence data. Drug resistance testing can be reliably performed only if plasma HIV-1 RNA levels are >500 copies/mL. This is particularly important if a patient's plasma HIV-1 RNA level was suppressed to below the level of detection before the time that drug resistance testing became available, when patients initially received suboptimal treatment, such as single- or double-NRTI–only regimens. Estimates of prevalence are confounded, because the denominator of all treated patients is often not known, the practice of obtaining drug resistance test results has changed over time, and cross-sectional analyses may underestimate the prevalence of drug resistance [48]. In a recent 10-year longitudinal study from the Swiss HIV Cohort Study, which took into account shifts in population size and in which the denominator of treated subjects was known, the prevalence of drug-resistant virus among antiretroviral therapy–exposed patients was estimated to be 50%–60% in 1999 and decreased to 39%–53% in 2006 [49]. The prevalence of triple drug-resistant virus remained stable at 5%. These lower numbers are likely to be attributable to the improving efficacy of treatment.

Transmission and prevalence of drug resistance in developing countries. Access to antiretroviral drugs in the developing world is increasing rapidly, although only a fraction of individuals who need therapy are currently receiving it. As in the developed world, an increase in transmitted resistance will lag behind an increase in acquired drug resistance. Nevertheless, with (1) >3 million people receiving antiretroviral therapy; (2) treatment failure defined by clinical end points [50]; and (3) limited availability of assays for routine determination of plasma HIV-1 RNA levels and for detecting drug resistance, acquired and transmitted drug resistance in resource-limited settings will present formidable challenges. Even more extensive drug resistance may emerge in this setting than in the developed world [50]. Some studies have already demonstrated the presence of drug-resistant virus in patients with recent infections in developing countries [51]. The World Health Organization is developing a surveillance program to provide early warning of increasing rates of transmitted resistance and to facilitate additional treatment options [52].

Single-dose nevirapine is widely used in the developing world to prevent mother-to-child transmission of HIV-1, but it selects for nevirapine-resistant HIV-1 in 40%–60% of mothers, as detected by population sequencing within 6–8 weeks of administration [53], and this resistance may compromise subsequent response to nevirapine-containing regimens [51, 52, 54, 55]. Children who are born with infection despite nevirapine prophylaxis have a high risk of developing resistance to nevirapine,

which limits their future treatment options [54]. Coadministration of other antiretroviral drugs with nevirapine may reduce the risk of drug-resistant infection in adults and children [56].

A number of characteristics of antiretroviral use in resource-poor settings will affect the level of acquired drug resistance among treated patients. For example, in a Thai cohort receiving a fixed-dose combination of stavudine-lamivudine-nevirapine and infrequent monitoring, virologic failure was associated with more resistance (>90% of isolates had NNRTI- and lamivudine-resistance–associated mutations, and >30% had TAMs) [57] than would be expected in the developed world at the time of first viral rebound [50], although less resistance was observed in cohorts with more-intensive plasma HIV-1 RNA level monitoring [58, 59]. In the absence of real-time viral load monitoring, >55% of patients in Uganda who received zidovudine-lamivudine-abacavir within the Development of Antiretroviral Therapy in Africa study and who had detectable viremia at week 48 had 1–4 TAMs, as well as the M184V mutation [60]. Limited access to antiretroviral drug programs may encourage some infected individuals to share their antiretroviral drugs with others, which may lead to suboptimal dosing. Such undisclosed therapy is likely to be the cause of drug resistance in certain populations entering antiretroviral therapy rollout programs. Pretreatment drug resistant infection was detected in ~10% of a subset of the Development of Antiretroviral Therapy in Africa study recipients in Uganda and Zimbabwe [61].

MECHANISMS OF ANTIRETROVIRAL DRUG RESISTANCE

Since the previous guidelines were published [3], several new drugs have been approved, and novel mechanisms of resistance have been elucidated. This section will review some of these advances.

Reverse-Transcriptase Inhibitors

Etravirine. Etravirine (TMC125) is a second-generation NNRTI that exhibits activity against many viruses that are resistant to first-line NNRTIs. Etravirine has favorable safety, pharmacokinetic, and antiviral activity profiles in heavily treatment-experienced HIV-1–infected patients [62–67]. The impact of pretreatment phenotype and genotype on the virologic response to etravirine at week 24 was examined in the DUET-1 and DUET-2 clinical trials [68–71]. Thirteen baseline HIV-1 reverse-transcriptase mutations were associated with resistance to etravirine in the DUET analyses: V90I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V, and G190S/A [70, 71]; of note, V179T was also identified in a separate US Food and Drug Administration analysis [72]. In the pooled DUET study results, 70% of subjects had 0 or 1 baseline etravirine mutation, whereas 15% had ≥3 baseline etravirine mutations. Notably, the reverse-transcriptase K103N mutation, which is often seen

in virus obtained from patients who experience virologic failure during efavirenz and nevirapine treatment and which confers broad cross-resistance within the first-generation NNRTI class, was not associated with etravirine resistance [72].

Virologic responses were seen in the DUET trials despite the presence of single etravirine mutations [70, 71]. The impact of most of these etravirine mutations depended on the simultaneous presence of Y181C; however, Y181C had an impact only when present with ≥1 additional mutation [70, 71, 73]. Having a greater number of baseline etravirine-related mutations was associated with a decreasing virologic response to etravirine, particularly when ≥3 mutations were present [70, 74]. The impact of specific etravirine mutational patterns on clinical virologic responses has not been fully defined. No phenotypic "cutoff" levels for clinical responses to etravirine are currently available.

Antagonism among specific reverse-transcriptase mutations. A potentially relevant and mechanistically interesting antagonism among various thymidine analogue NRTIs, TAMs, and the emergence of the tenofovir-associated K65R mutation has been elucidated [75]. TAMs selected by zidovudine or stavudine counteract the selection of the K65R mutation, although TAMs and the K65R mutation do not appear on the same genome because of competing mutational pathways. Thymidine analogue NRTIs, such as zidovudine, may protect against the emergence of the K65R mutation when combined with tenofovir, leading some clinicians to combine these agents. In contrast to HIV-1 drug resistance patterns described for tenofovir and for the triple-NRTI regimen tenofovir-abacavir-lamivudine, which selects for the K65R mutation more frequently, a quadruple-drug regimen of tenofovir plus zidovudine-abacavir-lamivudine in the COL40263 trial selected predominantly for NRTI-associated TAMs in virus from patients in whom therapy had failed [76].

Mutations in the connection and RNase H domains of reverse transcriptase. Mutations in the connection (E312Q, G335C/D, N348I, A360I/V, V365I, T369I, A371V, A376S, and E399D) and RNase H (Q509L) domains of reverse transcriptase are selected by NRTI therapy (in addition to TAMs); these newly recognized mutations, which are located outside of reverse transcriptase regions covered by standard genotype assays, substantially increase resistance to zidovudine when TAMs are also present [77–83]. They can increase cross-resistance to lamivudine, abacavir, and tenofovir (although to a much lesser extent) but do not increase resistance to stavudine or didanosine [77–79]. NNRTIs (mainly nevirapine) are also affected [80, 82].

The Q509L and A371V/Q509L mutations with TAMs impair the formation of RNase H cleavage products, which increases zidovudine-monophosphate excision on RNA/DNA duplexes by reducing template degradation. Q509L and A371V/Q509L also increase the efficiency of excision of short RNA/DNA du-

plexes [81]. The N348I mutation in the reverse-transcriptase connection domain confers dual zidovudine-nevirapine resistance via 2 interrelated mechanisms [82]. First, N348I decreases the ability of nevirapine to inhibit HIV-1 reverse transcriptase; second, N348I substantially decreases the rate of RNase H cleavage, which increases zidovudine-monophosphate excision by reducing RNA/DNA template degradation. Furthermore, the ability of nevirapine to stimulate RNase H is substantially reduced, compared with the wild-type enzyme. The N348I and A360V mutations, in combination with TAMs, decrease the efficiency of RNase H cleavage and increase the amount of rescued reaction product after ATP-dependent excision. Mutations N348I and A360V promote reverse-transcriptase dissociation from an RNase H–competent complex, thereby reducing RNA/DNA template degradation [83]. The N348I mutation occurs relatively frequently and can emerge early during therapy with regimens containing zidovudine and nevirapine [82]. The clinical impact of connection and RNase H domain reverse-transcriptase mutations on virologic response has not been determined.

PIs

Improved understanding of the importance of drug exposure in PI activity and resistance has led to widespread use of low-dose ritonavir boosting to increase drug levels, resulting in more effective competition with viral substrates and reduced impact of single mutations on drug activity. Large cohort data confirm superior virologic suppression with ritonavir-boosted PI-containing regimens, compared with unboosted PI-containing regimens, in drug-naive patients [50]. The genetic barrier to resistance (i.e., the number of mutations required for resistance to develop combined with difficulty in their selection) is generally greater for ritonavir-boosted PI-containing regimens than it is for unboosted PI-containing regimens. Resistance to ritonavir-boosted PIs requires multiple mutations that vary among PIs, and the degree of resistance depends on the number, as well as the type, of mutations present [50, 73, 84, 85]. The large number of mutations required for resistance makes the selection of resistance to boosted PI regimens uncommon, compared with the selection of resistance to NNRTI-containing regimens, among drug-naive patients who are experiencing a first regimen failure [50, 86]. Recently approved drugs, such as darunavir and tipranavir, have improved virologic activity in patients harboring PI-resistant HIV-1 [87–89].

Studies relating baseline (i.e., pretreatment) PI susceptibility or mutations to virologic outcome have been performed for boosted PI-containing regimens. Predictions of virologic success can be made by measuring fold-change in susceptibility (phenotype) or number and type of mutations (genotype). Genotypic resistance scores and phenotypic clinical cutoff levels derived from virologic outcome data are now available for dif-

ferent boosted PI-containing regimens [87–93]. Among these regimens, the number and codon positions of PI mutations in the resistance scores vary, with partial overlap among different drugs. For example, the resistance scores of tipranavir-ritonavir and darunavir-ritonavir both include mutations I84V and L33F but differ with respect to many other mutations. Therefore, virus harboring multiple PI mutations will show some degree of reduced susceptibility to all boosted PI regimens, and the clinical usefulness of each regimen may vary greatly. Phenotypic resistance testing may be particularly beneficial in this setting. The extent of both resistance and drug exposure affects PI activity. Thus, considering both parameters might improve predictions of viral inhibition. The inhibitory quotient (i.e., the ratio of the measured plasma minimum concentration value divided by the IC_{50} value or IC_{90} value) characterizes this relationship. Nevertheless, studies evaluating whether assays for drug exposure add to the effectiveness of assays for resistance have yielded conflicting results [94–99]. Inhibitory quotient values better predict PI activity than do resistance or drug levels alone in some, but not all, clinical studies. No randomized studies have yet shown an overall benefit to using inhibitory quotient calculations for PI dose adjustment in place of standard resistance testing.

In addition to mutations in the protease gene associated with PI resistance, mutations in the *gag* cleavage site region, especially p7/p1 and p1/p6, can increase the viral fitness of viruses resistant to PIs [100–103]. These mutations typically occur in conjunction with drug resistance mutations in the protease gene [104–106]. Specific patterns of cleavage-site mutations have been described for certain PIs (e.g., A431V with L24I-M46I/L-I54V-V82A, I437V with I54V-V82F/T/S, L449V with I54M/L/S/T/A, and L449F/R452S/P453L with D30N-I84V). In contrast, mutation P453L and the emergence of V82A were negatively correlated [107]. Mutations in the C-terminal region of the viral *gag* gene (K436E and/or I437T/V), located outside the actual NC/p1 cleavage site, can be selected in vitro in the absence of conventional PI resistance mutations, and they can confer resistance by merely changing the substrate of protease and thereby increasing processivity of the enzyme [108]. These mutations were also present in clinical isolates that had reduced susceptibility to PIs but lacked major protease mutations. Whether these cleavage-site mutations are of clinical relevance and should be included in drug resistance assays remains to be determined.

Entry Inhibitors

HIV-1 entry involves the interaction of gp120 with its primary receptor, CD4, followed by binding to 1 of 2 chemokine receptors (chemokine receptor 5 [CCR5] or CXC chemokine receptor 4 [CXCR4]) that serve as coreceptors [109]. Engagement of the coreceptor triggers the assembly of the 2 heptad

repeats (HR-1 and HR-2) in the trimeric gp41 into a 6-helix bundle that leads to the approximation and fusion of the cell and virus membranes [110]. The third variable (V3) loop is the major structural element of gp120 that determines coreceptor recognition and specificity, but regions outside of V3 contribute, as well [111–113].

Enfuvirtide. Enfuvirtide is a synthetic 36–amino acid oligopeptide that inhibits HIV-1 entry by preventing the assembly of HR-1 and HR-2 in the trimeric gp41 into a 6-helix bundle [114]. The drug binds to the trimeric HR-1 complex, thereby inhibiting fusion and blocking virus entry [115]. Resistance to enfuvirtide is mediated by amino acid substitutions within HR-1 at amino acid positions 36–45 of gp41 [116, 117]. The substitutions most frequently associated with resistance to enfuvirtide include G36D/S/V/E, V38A/E/M, Q40H, N42T, and N43D [118–120]. These mutations confer substantially reduced binding of enfuvirtide to HR-1 and a substantial decrease in antiviral activity in vitro [119]. In addition, the N126K and S138A mutations in HR-2 may contribute to reduced susceptibility to enfuvirtide [118]. Viruses carrying enfuvirtide resistance mutations show reduced viral fitness in vitro in the absence of enfuvirtide [121]. Clonal analysis of plasma HIV-1 RNA obtained from patients receiving enfuvirtide in the absence of a fully suppressive antiretroviral regimen showed rapid emergence of enfuvirtide resistance mutations [122]. The earlier emergence of mutants with gp41 substitutions at amino acid positions 36 and 38 suggests that these mutants may have an initial fitness advantage over mutants with substitutions at codons 40 and 43, which tended to emerge later.

Chemokine receptor antagonists. Small-molecule antagonists of the gp120-CCR5 interaction, such as maraviroc and the investigational drug vicriviroc, are allosteric, noncompetitive antagonists that bind to a similar site on CCR5 [123–125]. These drugs are potent inhibitors of HIV-1 [126, 127]. Maraviroc is now approved in the United States for use in treatment-experienced patients with exclusively R5 virus strains. Phase III trials of vicriviroc are under way.

Resistance to CCR5 antagonists selected in vitro is mediated by changes in HIV-1 gp120 that allow the envelope glycoprotein to interact with the drug-bound form of CCR5. A variety of amino acid substitutions associated with maraviroc and vicriviroc resistance have been described throughout the *env* gene; most involve V3, but their effect on drug susceptibility depends on the *env* backbone into which they are introduced [128, 129]. Phenotypically, resistance to the CCR5 antagonists manifests not as a classic rightward shift of the IC_{50} curve but, rather, as a plateau in the maximum achievable suppression of viral replication (figure 2). This plateau, referred to as the percent maximal inhibition, correlates with viral adaptation to use the inhibitor-bound form of CCR5 for entry [129, 130].

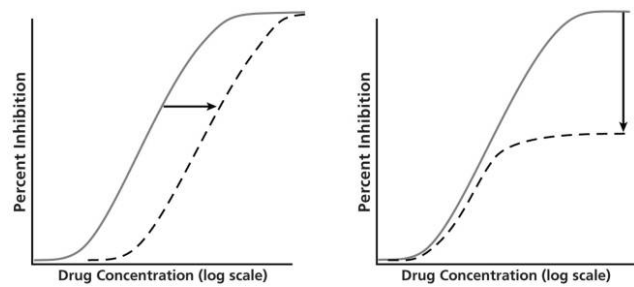Few clinical isolates that are resistant to maraviroc or vicri-

Figure 2. The left panel shows a typical inhibition curve of a susceptible virus *(solid line)* with a typical competitive inhibitor (e.g., a protease inhibitor). The IC_{50} value of the resistant virus *(dotted line)* is shifted to the right *(arrow)*. The right panel shows an example of a noncompetitive inhibitor (e.g., a chemokine receptor 5 antagonist). The susceptible virus *(solid line)* shows a typical inhibition curve, but in this case, the resistant virus *(dotted line)* reaches a plateau. The maximum achievable percent inhibition is shifted downward *(arrow)*, but the curve does not shift to the right; hence, the IC_{50} value remains unchanged.

viroc have been reported [131–133]. Overall, resistance of R5 virus appears to emerge slowly and is associated with mutations in the V3 loop stem (and possibly elsewhere in the *env* gene), similar to resistance that arises during in vitro passage experiments. The particular mutations observed vary from isolate to isolate. Thus, it is not yet possible to identify resistance to CCR5 antagonists on the basis of specific *env* mutations. Although no signature mutations associated with maraviroc resistance have yet been identified, changes at positions 13 and 26 in the middle of the V3 loop appear to be important [130, 132, 134]. Likewise, data are insufficient to determine the extent of cross-resistance within the class. In clinical trials, virologic failure of CCR5 antagonists has been more frequently attributed to the emergence and outgrowth of CXCR4-using viruses that pre-existed as minority populations before the initiation of CCR5 antagonist therapy.

INSTIs

The INSTIs are a new class of antiretroviral drugs that selectively target the HIV-1 integrase enzyme. Integrase catalyzes numerous steps within the cytoplasm and nucleus of host cells that allow proviral DNA to enter the nucleus and integrate into host cellular DNA. This entire process is required for productive HIV-1 replication. The approved drug raltegravir and other candidate drugs that are in development inhibit the strand transfer reaction.

Resistance to integrase inhibitors can emerge during treatment failure. Phenotypic and genotypic assays have been described that detect HIV-1 integrase resistance [135–137], but plasma-based phenotypic and genotypic assays to detect HIV-1 integrase inhibitor resistance are not yet clinically available. As with PI resistance mutations, INSTI resistance mutations are classified as either major or minor. Major mutations tend

to be the primary contact residues for drug binding, based on crystal structures, and have an effect on the drug susceptibility phenotype. Major mutations are those selected first in the presence of the drug or those shown at the biochemical or virologic level to lead to an alteration in drug binding or an inhibition of viral activity or viral replication. Minor mutations generally emerge later than major mutations and enhance the degree of resistance or improve replicative fitness of the virus that contains major mutations [73].

Raltegravir is a hydroxypyrimidinone carboxamide derivative of the diketobutanoic acid family. It is approved in the United States and in Europe for use in treatment-experienced HIV-1–infected patients in combination antiretroviral regimens, based on favorable safety, pharmacokinetic, and efficacy parameters [138–143]. In phase II and III studies, virologic failure occurred infrequently and was generally associated with the emergence of resistance mutations [136]. In Merck protocol 005, a phase II study of HIV-1–infected subjects with triple-class drug resistance, genotyping by population sequencing was performed at treatment failure. Phenotyping of patient-derived sequences and site-directed mutants employed single-cycle infection assays on long-terminal repeat-responsive reporter cell lines.

Raltegravir failure was associated with integrase mutations in 2 distinct genetic pathways, defined by ≥2 mutations, including (1) a signature (major) mutation at either Q148H/K/R or N155H and (2) ≥1 minor mutations unique to each pathway. The major mutations all reduced susceptibility to raltegravir and decreased viral replication capacity. Minor mutations described in the Q148H/K/R pathway included L74M, E138A, E138K, or G140S. These minor mutations consistently enhanced the level of resistance to raltegravir and, when combined with major mutations, improved viral replication capacity in a subset of combinations. The most common mutational pattern in this pathway is Q148H plus G140S. This Q148H-plus-G140S pattern exhibited the greatest decrease of drug susceptibility (>100-fold phenotypic resistance) and was the fittest variant seen (i.e., it had the highest replication capacity in both infectivity and multiple-cycle replication assays).

Mutations described in the N155H pathway include this major mutation plus 1 of either L74M, E92Q, T97A, E92Q plus T97A, Y143H, G163K/R, V151I, or D232N [73, 136]. Mutations observed in raltegravir protocols were similar to those selected with different integrase inhibitors in cell culture [144, 145]. The impact of specific raltegravir mutational patterns on clinical virologic responses has not been elucidated fully; thus, no phenotypic cutoff levels for clinical response have been determined for raltegravir. The most important prognostic factor that decreased the likelihood of virologic failure and drug resistance was having a genotypic susceptibility score or phenotypic susceptibility score >0 for optimized background reg-

imen; thus, integrase inhibitors should always be paired with other active agents in an HIV-1 treatment regimen [136].

HIV-1 DRUG RESISTANCE ASSAYS

There are 2 general types of resistance assays used in clinical practice: genotypic assays (i.e., HIV-1 gene sequencing to detect mutations that confer HIV-1 drug resistance) and phenotypic assays (i.e., cell culture–based viral replication assays in the absence or presence of drugs). Genotypic testing can be performed with commercial assay kits or in-house protocols. Blinded quality assurance programs indicate a very high concordance between kits and in-house methods used [146–148]. The ability to detect drug-resistance mutations, however, can vary substantially among laboratories [146, 149]. This wide variation results from difficulties in recognizing viral mixtures, particularly in heavily treatment-experienced patient populations [147, 150, 151], sequencing specimens with low viral loads, and testing non–subtype B HIV [149]. Performance also relates to the level of experience among laboratory personnel [149], which suggests that appropriate operator training, certification, and periodic proficiency testing are important for accurate genotyping. Resistance testing laboratories, therefore, need to participate in quality assurance programs [146, 147, 149–152].

Despite numerous studies, appropriate interpretation of genotypic and phenotypic drug resistance testing remains challenging. Results of genotypic tests use lists of predefined drug resistance mutations [73] or classifications by computerized, rules-based algorithms to characterize virus as "susceptible," "possibly resistant," or "resistant" to each antiretroviral drug [153–156]. The creation of rule-based algorithms is a difficult and lengthy process, and the algorithms require frequent updating. Algorithms vary considerably in the classification of expected drug activity [150, 151, 156–159]. Differences appear to be lowest for lamivudine and NNRTIs and highest for NRTIs and PIs [150, 151]. The most stringent approach to building algorithms is to evaluate the impact of mutational patterns at the initiation of treatment with a specific drug with regard to treatment response (e.g., decrease of plasma HIV-1 RNA levels according to specific genotypic patterns). Considerable progress has been made in identifying mutational baseline patterns that predict clinical failure for specific drugs. These patterns are currently available for the combinations lopinavir-ritonavir, atazanavir-ritonavir, tipranavir-ritonavir, darunavir-ritonavir, and amprenavir-ritonavir, as well as for zidovudine, stavudine, didanosine, lamivudine, tenofovir, efavirenz, nevirapine, and etravirine [70–72, 91–93, 157, 160–168]. The vast majority of genotypic algorithms are based on data that were obtained using subtype B viruses. Although no large differences exist with regard to interpretation of drug resistance in non–subtype B HIV, discrepancies between genotype and phenotype have been ob-

served for abacavir and subtype CRF02_AG, atazanavir and subtype C, and NNRTIs and subtype CRF01_AE [169]. HIV-1 proteases in drug-naive West African patients appear to be generally less sensitive to PIs [170]. More in vivo and in vitro resistance data are clearly needed for non–subtype B HIV.

An alternative approach for the interpretation of genotypic drug resistance information is to correlate genotypic data regarding the plasma HIV-1 RNA of a candidate gene with a large database of paired phenotypes and genotypes [171–174]. Such linkage then permits generation of a "virtual phenotype" by assigning calculated fold-changes in IC_{50}. Although actual and virtual phenotypes show excellent correlation for most drugs, superiority of virtual phenotype over genotype alone could not be demonstrated in predicting clinical response to salvage regimens [173, 175, 176]. Virtual phenotype is an approach to genotype interpretation, and its main limitation is that predictive power depends on the number of matched datasets available. Thus, variation is frequently higher in smaller datasets; consequently, variation is frequently higher for newer drugs. Furthermore, matches are based on preselected codons, not on the entire nucleotide sequence.

Standard phenotypic testing, using recombinant virus assays, is performed by few commercial laboratories. Current assays amplify HIV-1 protease, a part of the HIV-1 reverse transcriptase, as well as the 3'-terminus of *gag*, as a unit from plasma virus, and they generate a recombinant virus pseudovirus with other HIV-1 genes derived from a laboratory construct [177–181]. A comparison between 2 different assays showed an overall concordance of 86.9%, with the highest concordance for PIs (93.4%) and the lowest concordance for NRTIs (79.8%) [150]. However, even within drug classes, concordance can vary widely among specific drugs (e.g., lamivudine has a very high concordance of 93%, but abacavir has a concordance of only 74% [150]). This recombinant technology is being modified so that it can also test for susceptibility to INSTIs, fusion inhibitors, and chemokine receptor antagonists [136, 182].

The results of phenotypic testing are usually presented as the fold-change in susceptibility of the test sample compared with a laboratory control isolate. The initial "technical" cutoff values, representing the interassay variation of cloned virus controls, did not accurately reflect the inherent variation in susceptibility encountered in circulating viruses from drug-naive patients. The normal distribution of susceptibility to a given drug for wild-type isolates from treatment-naive individuals (i.e., the "biologic" cutoff) was then adopted. Although clinical cutoffs have been defined for many drugs, the relationship between viral susceptibility and drug response is a continuum in which progressively reduced viral phenotypic susceptibility to a particular drug results in progressively blunted reductions in plasma HIV-1 RNA levels. For practical application, 2 different clinical phenotypic cutoff values should be defined: one above

which clinical responses perceptibly diminish, compared with those of wild-type virus ("intermediate" resistance), and one above which no clinical response can be expected ("full" resistance). Even partial activity can be useful when treatment options are limited [183]. In evaluation of phenotypic cutoffs, drug-specific susceptibility needs to be compared at baseline, before switching to a new drug regimen, and with the drug-specific treatment response (e.g., decrease in plasma HIV-1 RNA levels) that occurs after initiation of new therapy. Since 2003, clinical trial and cohort data have led to a substantial increase in available clinical cutoff values, and such values are now available for most approved drugs.

In addition to standard genotypic and phenotypic testing used in clinical practice, other resistance testing assays may prove to be useful in the future. The allele-specific PCR assay [184–189] and the single-genome [190] and ultra-deep sequencing [191] assays are currently used to investigate the role of minority variants harboring drug resistance that are present below the level of detection by bulk plasma viral population sequencing approaches. Studies involving treatment-naive and treatment-experienced patients have shown strong associations between the detection of low-frequency drug-resistant variants, particularly those encoding resistance to NNRTIs, and subsequent treatment failure [192–194]. Further studies are required to define the clinically relevant frequency of variants in the virus population. Improvements in assay throughput and reductions in cost are necessary before such assays are available for patient management.

Replication capacity assays, which are designed to measure in vivo fitness of a virus, remain an interesting research tool, but they have not found a role in patient management [195]. A relatively simple and inexpensive alternative for estimating the reduction of in vivo fitness induced by a given nonsuppressive antiretroviral treatment regimen is to determine the differences in plasma HIV-1 RNA levels between pretreatment and on-treatment periods (e.g., for patients lacking fully suppressive treatment options); such information may be useful in optimizing a nonsuppressive treatment regimen [196]. Finally, the cost-effectiveness of using resistance testing assays for treatment-naive patients and for patients for whom antiretroviral treatment has failed has been demonstrated in various countries [197–202].

VIRAL CORECEPTOR USE TESTING

Phenotypic assays to determine coreceptor use (i.e., tropism testing) require the amplification of *env* sequences from plasma HIV-1 RNA and the construction of viral pseudotypes or infectious recombinant viruses that express the patient-derived *env* sequences along with a reporter gene [182]. These pseudotyped viruses or viral recombinants are then inoculated onto cells that express CD4 along with CCR5 or CXCR4. The pres-

ence of infection is detected by assays that determine reporter-gene activity. HIV-1 isolates that use CCR5 exclusively are termed R5 viruses, those that use only CXCR4 are termed X4 viruses, and those that use both are termed R5/X4, or dual-tropic viruses. Because these assays do not distinguish between the presence of truly dual-tropic viruses and a mixture of R5 and X4 viruses, samples that can infect both CCR5- and CXCR4-expressing cells are often termed dual-mixed viruses. As with commercially available resistance tests, tropism testing generally requires a plasma sample with an HIV-1 level of ≥1000 copies/mL. The assay used in most clinical trials of CCR5 antagonists can detect the presence of CXCR4-using virus when they constitute at least 5%–10% of the virus population as minor variants [182]. Assays with improved sensitivity for detection of 0.3% CXCR4-using or dual-mixed virus are now available [203].

Genotypic approaches to determining coreceptor use depend on sequencing the V3 loop and applying one of a variety of predictive algorithms. The 2 most commonly used measures for predicting CXCR4 use include (1) the presence of positively charged amino acids at positions 11 and 25, often referred to as the "11/25 rule," and (2) the total charge of V3 loop amino acid residues of +5 or greater [204, 205]. Bioinformatic approaches include the use of position-specific scoring matrices [206], neural networks [207], or machine-learning techniques [208]. The heteroduplex tracking assay has also been used to detect the presence of CXCR4-using virus [209]. In this assay, the electrophoretic mobility of PCR-amplified *env* genes is assayed after hybridization to V3-coding sequences from viruses with phenotypically defined coreceptor use. When verified against phenotypic assays, genotypic approaches showed excellent specificity but poor sensitivity for detecting the presence of dual-mixed or CXCR4 viruses in clinical samples [210]. The low sensitivity of these methods is explained, in part, by the extensive heterogeneity of HIV-1 *env* genes in plasma virus populations, which makes it difficult to obtain coherent sequence data with population-based sequencing approaches. Another contributing factor is that not all determinants of viral tropism reside in the V3 loop. For these reasons, genotypic approaches cannot be recommended at present for identifying patients who may be suitable candidates for CCR5 antagonist therapy. Other limitations of genotyping for coreceptor tropism include the inability of population sequencing to detect variants that comprise <25% of the virus population and the lack of interpretation algorithms for sequences that are not from subtype B.

Drug Resistance in Non–Subtype B HIV

Antiretroviral drug design, resistance research, and interpretation systems have been largely based on HIV-1 subtype B viruses, which have historically been the most prevalent subtype in North America, Western Europe, and Australia. However, subtype B viruses account for only ∼12% of the worldwide HIV-1 infections, with subtype C viruses being the most prevalent, accounting for ∼50% of cases [211]. An increasing number of individuals who are infected with many of the non–subtype B virus strains now receive antiretroviral therapy because of rollout programs in the resource-limited world and because of increasing migration to the developed world, particularly to countries in Europe. It is essential to appreciate how HIV-1 genetic variation alters the characteristics of drug susceptibility and drug resistance. The differences in the natural polymorphisms between HIV-1 subtype B and non–subtype B viruses have been well documented [212, 213]. The effects of viral subtype on resistance are expressed in 2 broad cases: in genetic routes to, and frequency of, specific mutations and in the algorithms developed for interpreting drug resistance.

Genetic routes to and frequency of specific resistance mutations. Viruses from patients infected with subtypes C, G, or CRF_01 AE for whom a first-line nelfinavir-containing regimen is failing preferentially select the L90M mutation, rather than D30N, which more frequently occurs with subtype B virus [214, 215]. This preferential selection of L90M in protease may stem from methionine polymorphism in these non–subtype B viruses at position 89, rather than from lysine in subtype B [216].

Synonymous differences are also responsible for different resistance mutations. For example, valine at position 106 of reverse transcriptase is coded by a GTG codon in subtype C, in contrast with coding by a GTA codon in subtype B. Thus, subtype C viruses more readily select methionine in the presence of efavirenz (a 1-nucleotide change), whereas subtype B viruses change to an alanine (a 1-nucleotide change) [217]. The V106M mutation is responsible for broad cross-resistance to older NNRTIs [218]. A further example is at reverse transcriptase position 210; subtype F viruses require 2 nucleotide substitutions for L210W to emerge, compared with the 1 mutation required by other subtypes. This difference explains the lower prevalence of L210W in cohorts of patients with subtype F infection [219]. An even more intriguing finding is the increased prevalence of K65R in reverse transcriptase from subtype C virus–infected patients receiving tenofovir or didanosine-containing regimens, compared with the prevalence in reverse transcriptase from patients infected with other virus subtypes. Although synonymous differences at reverse transcriptase position 65 exist, these do not change the number of substitutions required for the K65R mutation. One possible explanation for this is that the longer string of adenines in the reverse-transcriptase gene preceding the codon that codes for K65 in subtype C viruses favors increased slippage of the reverse-transcriptase enzyme during transcription, thus encouraging mutations at position 65 [220, 221].

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

Clinical setting	Comments
Before initiation of therapy	
Primary (acute and early) infection	Resistance testing is recommended. Initial therapy may be altered based on resistance test results.
First evaluation of chronic HIV-1 infection	Resistance testing is recommended, including for patients for whom therapy is delayed, because plasma wild-type isolates may replace drug-resistant virus with time in the absence of treatment.
Treatment initiation for chronic HIV-1 infection	Resistance testing is recommended because of a rising prevalence of baseline HIV-1 drug resistance in untreated patients with chronic infection [25], unless preexisting data or stored samples for testing are available.
In antiretroviral-treated patients	
Treatment failure	Resistance testing is recommended. The decision to change therapy should integrate treatment history, new and prior resistance results (if available), and evaluation of adherence and possible drug interactions.
In specific settings	
Pregnancy[a]	Resistance testing is recommended before initiation of therapy to effectively treat the mother and prevent mother-to-child transmission.
Other considerations and general recommendations	Postexposure prophylaxis should consider treatment history and resistance data from the source, when available; A sudden increase in HIV-1 plasma RNA may reflect superinfection, possibly with drug-resistant virus; Plasma samples to be tested for drug resistance should contain at least 500 HIV-1 RNA copies/mL to ensure successful PCR amplification required for all sequencing approaches; It is preferable that the blood sample for resistance testing be obtained while the patient is receiving the failing regimen, if possible; 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 knowledgeable in antiretroviral therapy and drug resistance patterns; Inhibitory quotient testing is not recommended for clinical decision making.

[a] If resistance test results are available from before the pregnancy, clinical judgment should guide whether retesting for resistance is necessary.

Drug resistance algorithms for non–subtype B HIV. The consensus sequence used for many algorithms, against which changes are identified in sequences from tested patients, is a subtype B sequence. This becomes problematic for subtypes in which some "resistance" mutations actually reflect the wild-type consensus for that subtype. This disparity is particularly apparent for minor protease mutations. For instance, 6 subtype-specific polymorphisms in protease (at positions 10, 20, 33, 36, 82, and 93) occur at sites known to be associated with drug resistance in subtype B viruses. These amino acid substitutions may represent the consensus sequence for more than 1 non–subtype B virus, such as M36I in subtypes A, C, D, F, G, CRF01, and CRF02. Most of the polymorphisms are located outside the active site of the protease [222], such as M36I, which is situated in the hinge region of the enzyme flap. Only in subtype G is a polymorphism present in the active site of the enzyme (V82I).

Several reports have suggested similar in vitro susceptibilities to antiretroviral drugs among different group M subtypes [223, 224]. No differences in response to therapy among these subtypes have been reported [195, 225]. The practical implication of these polymorphisms is that resistance algorithms may over-interpret resistance to specific drugs because of the inclusion of these polymorphisms in the total number of identified resistance mutations. This applies particularly to PIs, for which the total number of mutations is often used to ascribe susceptibility. Therefore, subtype determination should be included in genotypic resistance testing. Because the selection of mutations is incompletely characterized in non–subtype B viruses, it would be beneficial to compare their sequences before treatment and at treatment failure.

CLINICAL APPLICATIONS AND RECOMMENDATIONS

Clinical applications and recommendations are shown in table 2. The essential strategy behind the use of resistance testing for individual patient management is to provide information to assist in the selection of antiretroviral regimens that achieve and maintain virologic suppression—that is, plasma HIV-1 RNA levels below the lower limits of detection of the most sensitive assays available for routine clinical use (50 copies/mL).

Untreated, Established HIV-1 Infection

Because of the prevalence of primary HIV-1 resistance to antiretroviral drugs in developed countries, resistance testing is recommended for all patients at the time of diagnosis of HIV-1 infection as part of the initial, comprehensive assessment [**AII**]. This recommendation is not restricted to patients for whom initiation of antiretroviral treatment is being considered on the basis of clinical, immunologic, or virologic criteria. The persistence of detectable mutations acquired at the time of HIV-1 infection varies, with some (e.g., the reverse-transcriptase K103N mutation) having the potential to persist for years and others (e.g., the reverse-transcriptase M184V mutation) having a greater potential for reversion [30]. Thus, establishing a drug-susceptibility profile for a patient's virus at the time of diagnosis can be helpful in antiretroviral decision-making years later because of the ability of archived drug-resistant virus to reemerge in cases of suboptimal therapy. Genotypic testing is recommended [**AIII**] for the initial evaluation, because mutations that may not yet have accumulated enough to affect phenotypic susceptibility can be detected, HIV-1 subtype can be determined, and the cost of genotypic testing is lower than that for phenotypic testing.

Treatment Failure

First or second treatment failure. Because of the high prevalence of infection due to drug-resistant virus among antiretroviral-treated patients with confirmed, detectable plasma virus, drug resistance testing should be performed in all cases of treatment failure [**AI**] (defined as an insufficient decrease or an increase in plasma HIV-1 RNA level after 1–2 months of treatment or a confirmed viral breakthrough in a patient with previously undetectable virus). However, genotypic and phenotypic resistance assays both have low amplification success rates in specimens with plasma HIV-1 RNA levels <500 copies/mL. Resistance to specific drugs may not always be detected if treatment with the drug has been discontinued before the sample is obtained, because resistant strains are often less fit than wild-type virus and may persist only as undetectable minor subspecies in the absence of drug pressure. Therefore, a specimen for resistance testing should be obtained before treatment has been discontinued or changed, and if treatment has been discontinued, the specimen should be obtained as quickly as possible thereafter. In addition, antiretroviral treatment history is important, along with resistance data, in choosing the subsequent treatment regimen, especially if the treatment has been discontinued for several weeks.

Resistance is not the only cause of treatment failure. Insufficient drug exposure, often resulting from incomplete adherence to therapy, is the most common reason for failure of an initial treatment regimen. However, continuing therapy in the context of treatment failure will often lead to the emergence and accumulation of additional resistance mutations. This applies particularly to drugs with low genetic barriers to resistance (e.g., the NNRTIs, lamivudine, emtricitabine, enfuvirtide, and raltegravir). Clinical resistance to ritonavir-boosted PIs often emerges at a slower pace, requiring several mutations. Therefore, rapid evaluation and prompt action should occur at the time of treatment failure. For first and second treatment failures, genotypic resistance testing usually suffices, unless the patient had initially acquired multidrug-resistant virus. Current commercially available resistance assays report results for NRTIs, NNRTIs, and PIs. Viral tropism assays that determine whether a patient's virus population is predominantly R5, X4, or dual-mixed are used to indicate whether CCR5 antagonists (e.g., maraviroc) may be an appropriate choice for patients with treatment failure. Tropism determination may be useful in this circumstance [**BIII**]. It is expected that susceptibility testing for INSTIs (e.g., raltegravir) will be clinically available in the near future.

Multiple treatment failures (advanced treatment failure). In patients who experience multiple treatment failures, the virus is often resistant to NRTIs, NNRTIs, and PIs. Until recently, constructing potent alternative regimens that combined 2 or 3 fully active drugs was often impossible, despite the use of enfuvirtide, because of the high level of intraclass cross-resistance. The increased availability of new drugs—including drugs from existing classes but with low levels of intraclass cross-resistance, such as darunavir and etravirine, and drugs from new classes, such as maraviroc and raltegravir—have made the goal of attaining undetectable viral loads more realistic for patients with numerous treatment failures [9]. In this situation, tropism determination is recommended [**AI**], and phenotypic testing may be a useful addition to genotypic testing, because the number of mutations and the complexity of mutational interactions may make genotypic interpretation challenging. However, phenotypic testing may not be available because of cost. Genotypic testing is recommended for patients with multiple treatment failures [**AI**], along with phenotypic testing, if available [**AI**]. The decision to change therapy and the selection of the new regimen should be discussed with experts who are knowledgeable in antiretroviral therapy, antiretroviral pharmacology, and resistance patterns.

Special Circumstances

Acute and early phase HIV-1 infection. Genotypic resistance testing is recommended for any patient who presents within several months after HIV-1 infection because of the high reported rates of transmitted drug resistance [**AII**]. If immediate antiretroviral intervention is indicated, the initiation of treatment should not be delayed until this result is available, because the turnaround time may be ≥2 weeks; rather, treatment should be modified if the result demonstrates resistance to ≥1 com-

ponent of the regimen. The initial choice of treatment should also take into account the treatment history of the source patient and resistance data for that patient's virus, if available. If treatment is not initiated during the acute or early phases of infection, the resistance test results will still be helpful in the future, because early testing provides the best opportunity to detect transmitted drug resistant virus that has been archived and replaced by more-susceptible virus but which could emerge later during treatment.

Pregnancy. Genotypic resistance testing is recommended for all HIV-1–infected pregnant women with detectable plasma virus, both for their own health and for the health of their infants [**AII**]. This information will assist with treatment choices for the mother, as well as with choices to prevent mother-to-child HIV-1 transmission (including multidrug-resistant HIV-1 transmission) by selecting a regimen that will be effective and safe for the fetus.

Postexposure prophylaxis. The question of whether to initiate postexposure prophylaxis occurs primarily in 2 settings: accidental exposure of a health care worker and high-risk sexual exposure. In the former circumstance, drug resistance data from the source may be useful in constructing a prophylactic regimen. In the case of high-risk sexual exposure, data on the source are usually not available.

SUMMARY AND FUTURE DIRECTIONS

Antiretroviral drug resistance is present wherever antiretroviral drugs are widely used, and as treatment rollout continues in developing countries, the range of resistance will expand. The incidence, prevalence, and transmission of drug-resistant viruses vary from country to country, and programs for worldwide drug resistance surveillance should increase rapidly to meet the emerging need. In developing countries, methods to simplify specimen collection, storage, and testing should be explored to facilitate better monitoring of individual patients and community patterns.

The approval of several new antiretroviral drugs from different classes since our previous recommendations [3] has increased the complexity of resistance testing and interpretation in the developed world. Resistance testing for entry inhibitors and INSTIs should be incorporated into routine management when such testing becomes available and validated.

Techniques for resistance testing have become more standardized since our previous recommendations and have been widely incorporated into routine management in the developed world. Coreceptor tropism testing has also become available. Future efforts should be made to increase the sensitivity of these assays to better detect minor variants that may be of clinical significance. Moreover, as resistance testing becomes increasingly employed in developing countries, attention should be given to detecting resistance patterns for non–sub-

type B strains of HIV and to establishing algorithms for evaluating their importance.

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