Topics in
HIV Medicine®

Perspectives

Reprint

The International AIDS Society–USA

About This Issue

This issue of *Topics in HIV* Medicine features 3 *Perspectives* articles based on and updated from spring 2003 lectures from International AIDS Society–USA (IAS–USA) courses in Los Angeles, San Francisco, and Washington, DC. Reporting on inquiries into enhancing immune reconstitution in HIV-infected patients, Laura A. Napolitano, MD, provides the latest update on interleukin-2, human growth hormone, and interleukin-7 studies. Michael S. Saag, MD, offers insights based on new data about how to select and when to initiate antiretroviral therapy. And Melanie M. Taylor, MD, MPH, provides a look at current recommendations for screening, diagnosing, and treating several sexually transmitted diseases that are currently experiencing a resurgence in the HIV-infected community.

As a special addition, a reprint of the IAS–USA panel's recommendations for antiretroviral drug resistance testing is included in this issue. Prepared by a volunteer panel of experts in the fields of HIV disease and antiretroviral drug resistance, the recommendations were published in the July 1 issue of *Clinical Infectious Diseases*.

The IAS–USA is delighted to announce the addition of 2 new members to the organization's board of directors. Judith S. Currier, MD, an associate professor of medicine at the University of California Los Angeles, has been involved with the IAS–USA for almost a decade. She has served as faculty on CME courses, as a member of the metabolic complications guidelines panel, and is on the *Topics in HIV Medicine* Editorial Board. William G. Powderly, MD, professor of medicine at the Washington University School of Medicine in St Louis, Missouri, has also been involved for almost 10 years, in the capacities of CME course faculty and on expert guidelines panels on cytomegalovirus infections and metabolic complications of HIV and its therapies. Please join us in welcoming our new Board members; we look forward to their contributions and insights for shaping the future of the IAS–USA programs.

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Perspective
Approaches to Immune Reconstitution in HIV Infection

Ongoing studies to identify antigen-nonspecific strategies for enhancing immune reconstitution in individuals with HIV infection include those focusing on the use of interleukin (IL)-2, human growth hormone, and IL-7. IL-2 has been shown to induce substantial CD4+ T-cell increases in HIV-infected patients receiving potent antiretroviral therapy. Although those with CD4+ cell counts greater than 300/μL have shown the greatest gain in CD4+ cells, IL-2 has also increased CD4+ cells in those with initial CD4+ cell counts of less than 200/μL. Ongoing phase 3 trials are evaluating whether these increases in CD4+ cell count are associated with improved clinical outcomes. A small study of human growth hormone suggests that the hormone can reverse thymic involution and improve thymic T-cell production in HIV-infected individuals. Larger studies are ongoing. IL-7 affects T-cell production and function at several stages of T-cell development. Animal studies indicate that administration of IL-7 is associated with marked increases in CD4+ cell counts, and human trials may be possible within the next 2 to 3 years. The mechanism of CD4+ cell increases—eg, expansion of peripheral cells versus enhanced de novo production in the thymus—may affect the potential immunologic and clinical benefit derived from these increases. Additional study is necessary to refine approaches to enhancing immune reconstitution in HIV infection. This article summarizes a presentation made by Laura A. Napolitano, MD, at the May 2003 International AIDS Society–USA course in San Francisco.

Overview

CD4+ T-cell decline in HIV infection can occur as the result of direct or indirect destruction of CD4+ T cells in the peripheral immune system. In addition, infection and destruction of developing CD4+ progenitor cells within the thymus gland may cause reduced synthesis of new CD4+ T cells. Given the normal biologic process of thymic involution, which results in greatly reduced de novo production of T cells in adult life, there is thus high potential for impaired new T-cell production in individuals with HIV infection. With the availability of potent antiretroviral therapy capable of limiting HIV replication, increased attention has been given to strategies for enhancing T-cell production to preserve or restore immune function in HIV-infected patients. Potential pharmacologic strategies include the use of interleukin (IL)-2, human growth hormone, or IL-7.

Dr Napolitano is Assistant Investigator at the Gladstone Institute of Virology and Immunology and an Assistant Professor of Medicine at the University of California San Francisco.

Interleukin-2

IL-2 is a central regulator of T-cell function that is produced by activated T cells. This cytokine induces proliferation of T lymphocytes (CD4+ cells more than CD8+ cells), promotes maturation and cytotoxicity of CD8+ T cells, stimulates the immune response to eliminate viruses and intracellular organisms (the T_H1 response), regulates the intensity of the immune response (eg, influences the number of activated cells and their removal through apoptosis), and potentiates the function of antigen-presenting cells (that stimulate function of T cells), natural killer cells, and B cells.

IL-2 treatment in HIV infection has been evaluated in a large number of studies over the past 2 decades. Experience with IL-2 treatment in HIV-infected patients can be summarized as follows. In the pre-potent antiretroviral era, IL-2 treatment was predominantly assessed in patients receiving single or dual nucleoside reverse transcriptase inhibitor antiretroviral therapy. Its use was associated with consistent improvement in CD4+ counts, with increases of approximately 200 to 500 cells/μL compared with controls being observed. No

CD4+ cell count increases in patients beginning IL-2 treatment with CD4+ cell counts less than 200/μL were observed. Increases in plasma HIV-1 RNA levels and worsening of clinical status were also observed in some patients beginning treatment at CD4+ cell counts less than 200/μL. In more recent studies, IL-2 administration along with initiation of potent antiretroviral therapy has been found to augment CD4+ cell count response by approximately 100 to 600/μL compared with antiretroviral therapy alone. When given together with potent antiretroviral therapy, CD4+ count increases are also observed in those patients with initial counts less than 200 cells/μL. In general, IL-2 does not appear to have a major effect on HIV-1 RNA levels in those taking potent antiretroviral therapy. However, transient increases in plasma HIV-1 RNA level have been observed during IL-2 treatment in some studies, and small declines in plasma HIV-1 RNA level (<1 log copies/mL) have also been reported.

Overall, IL-2 treatment results in large, sustained increases in CD4+ cell counts. The gains in the CD4+ cell count consist predominantly of increases in naive CD4+ cells (those not yet exposed to their antigen) versus memory cells (those so exposed). The expansion of the naive CD4+ cell pool appears to be attributable primarily to the proliferation and increased survival of existing naive cells in the peripheral circulation and lymph nodes. In most studies, IL-2 does not appear to stimulate the generation of new naive cells by the thymus, although the data are mixed. Currently, there are insufficient data to indicate whether the CD4+ cell count increases seen with IL-2 treatment actually improve immunity against recall antigens or HIV, include the generation of T cells that have normal function, or improve clinical outcomes. Use of IL-2 is approved in Europe for treatment for patients with persistently low CD4+ cell counts despite virologic suppression under

antiretroviral therapy, but IL-2 is not currently approved by the Food and Drug Administration for use in HIV disease in the United States. Important data on the potential clinical benefits of IL-2 treatment are expected from 2 ongoing phase 3 studies: the ESPRIT study in patients with initial CD4 + cell counts of 300/µL or higher and the SILCAAT study in patients with initial CD4 + cell counts of 50 to 299/µL

During initial studies in the 1980s, IL-2 was administered by continuous intravenous infusion at a dose of 18 million international units (MIU) per day for 5 days every 8 weeks. Administration in this form was associated with frequent moderately severe toxicities, including severe flu-like syndrome, fever, bone marrow toxicity, hypotension, phlebitis, gastrointestinal toxicity, renal insufficiency, and dermatologic reactions. Subsequently, subcutaneous administration of IL-2 was found to result in reduced (but still considerable) toxicity. Dosing of IL-2 in trials generally now consists of subcutaneous administration of 9 to 15 MIU per day (4.5 or 7.5 MIU twice daily) for 5 days every 4 to 8 weeks. In some cases, when IL-2 therapy leads to a prolonged increase in the CD4 + cell count, the dosing interval can be extended to 12 months or longer. IL-2 doses of less than 6 MIU per day appear to be less effective at increasing CD4 + cell counts. Because side effects of IL-2 are much decreased at these lower doses, some studies are still investigating the potential benefits of long-term, low-dose IL-2 therapy.

Human Growth Hormone

Human growth hormone (GH), which is produced in the anterior pituitary gland, acts directly or via insulin-like growth factor-1 (IGF-1) to regulate metabolism and tissue growth. Demonstrated effects of GH and IGF-1 include increases in lean body mass, lipolysis, and protein synthesis. GH also has important effects on the immune system. GH deficiency is associated with thymic hypoplasia in rodents (but not in humans), and animal studies have shown that GH and IGF-1 treatment in aging rodents reverses thymic atrophy and enhances T-cell production. Further,

both factors have been shown to augment immune reconstitution in immunodeficient rodents.

Based on these findings in animal studies, Napolitano and colleagues performed a small study in HIV-infected adults to determine if GH treatment might enhance T-cell production. Five HIV-infected male patients participating in a study of GH treatment for HIV-related fat accumulation were assessed for changes in the thymus and in thymopoiesis at 3-month intervals during

treatment with doses of 1.5 to 3.0 mg of GH per day for 6 to 12 months. A group of 7 HIV-infected persons participating in observational studies who had similar characteristics as the group receiving growth factor were also studied for comparative purposes. The 5 GH-treated patients had a mean age of 52 years, baseline (pre-GH treatment) plasma HIV-1 RNA level ranging from less than 50 to 5700 copies/mL, and baseline CD4 + counts ranging from 254 to 853 cells/µL; 4 of the 5 were on protease
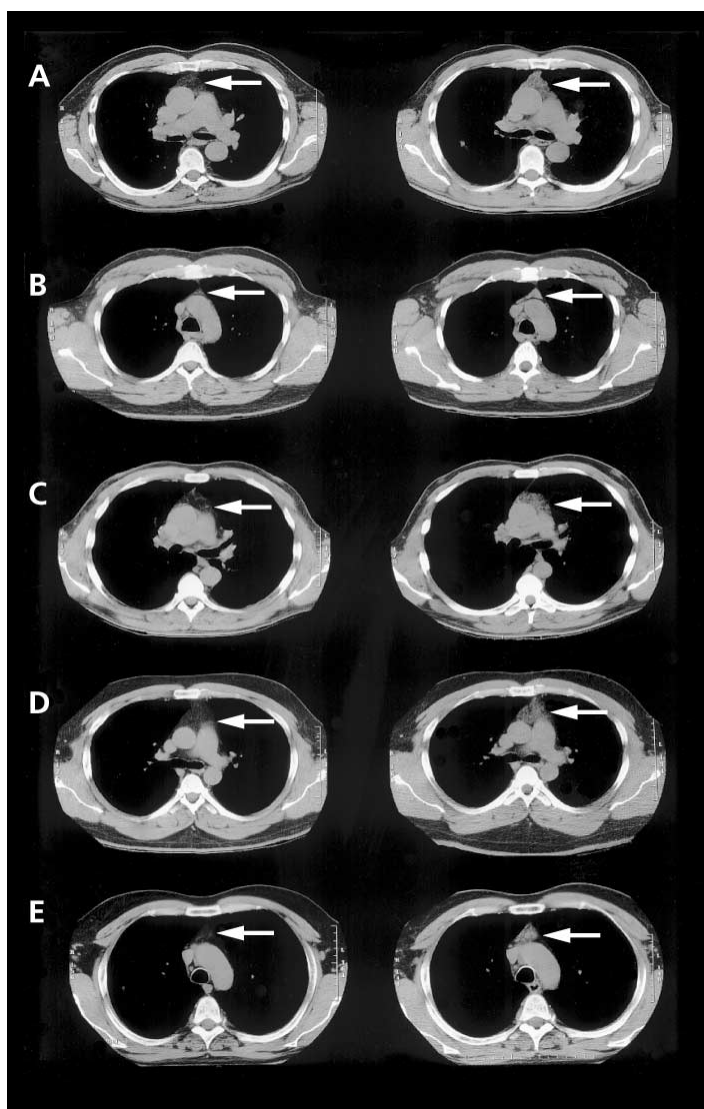
Figure **1.** Reversal of thymic atrophy by growth hormone. Thymus computed tomography scans were performed at baseline and 6 months after initiation of growth hormone (GH) therapy in 5 GH recipients (A-E). Prior to GH treatment, the thymus (left column, indicated by an arrow) was seen as relatively low attenuation tissue, nearly black in color, consistent with fat density. After GH treatment (right column) the density of the anterior mediastinum markedly increased. The formerly black, fat-density tissue was replaced by higher attenuation tissue consistent with cellular thymus. Adapted with permission from Napolitano et al, *AIDS,* 2002.

inhibitor-containing triple-drug therapy and 1 was receiving dual nucleoside reverse transcriptase inhibitor therapy. All 5 patients who received GH exhibited reversal of thymic atrophy during GH treatment, with computed tomography images that were consistent with regeneration of thymic tissue to produce a thymus resembling that typically found in adolescence (Figure 1). In the GH-treated group, thymic density and volume were both increased. Along with the reversal of thymic atrophy, a significant increase in circulating naive CD4+ T-cell percentage was observed in treated patients (Figure 2). Re-involution of the thymus was observed in 2 patients who underwent repeat computed tomography imaging after stopping GH therapy, but increases in naive CD4+ cell percentage persisted. During treatment, no significant changes in total CD4+ cells, total CD8+ cells, memory T-cell subsets, natural killer cells, or B cells were observed. Increases in CD4+ cell count were observed in some patients after discontinuation of GH, but no conclusions regarding such increases could be made in the absence of a true randomized control group. The specific targets of GH in the human immune system are not well understood; however, it appears likely that GH enhances T-cell production by stimulating bone marrow progenitors and facilitating their engraftment in the thymus. Overall, the findings in this small study suggest that GH treatment may enhance thymic function and suggest that de novo CD4+ cell production may be inducible in some HIV-infected individuals. GH treatment is associated with significant toxicities, including arthralgias, myalgias, edema, diabetes, and carpal tunnel syndrome. Additional studies of GH in the setting of immune reconstitution in HIV infection are under way, including a single-center study at the Gladstone Institute of Virology and Immunology at the University of California San Francisco and a multicenter study conducted by the AIDS Clinical Trials Group.

Interleukin-7

IL-7, which is produced by stromal cells of the thymus, bone marrow, and lymph nodes, is an essential regulator of lymphopoiesis that acts on both T cells and B-cell progenitors. The importance of IL-7 on T-cell production has been demonstrated in rodent models such as the IL-7-receptor knockout mouse, which exhibits massive reductions in thymus, spleen, and lymph node cellularity, with thymocyte levels approximately 1% of those seen in normal mice. IL-7 has effects on T-cell production at many stages of development in the bone marrow, thymus, and periphery: it stimulates expansion and export of progenitor cells from the bone marrow, inhibits apoptosis and enhances proliferation of developing thymocytes, and stimulates proliferation and enhances cytotoxicity of mature T cells. It is also believed to play an important role in T-cell homeostasis, with it being proposed that increased levels of IL-7 are part of a homeostatic response designed to increase T-cell production. Supporting lines of evidence include the findings that circulating IL-7 levels are increased in lymphopenia and that cellular production of IL-7 is increased in lymphocyte-depleted lymph nodes. The potent effects of IL-7 on T-cell development and homeostasis have made it an attractive therapeutic candidate to increase T-cell production during immunodeficiency.

IL-7 has been shown to promote T-cell production in several animal models of immunodeficiency. Murine studies demonstrate that IL-7 enhances immune reconstitution after myeloablation or T-cell depletion. In primate studies in simian immunodeficiency virus-infected or -uninfected cynomolgus monkeys and baboons undergoing bone marrow transplantation, IL-7 administration has resulted in marked increases in circulating CD4+ T cells, splenomegaly, and lymphadenopathy. The primate studies suggest that IL-7 induces expansion of existing T cells but that it does not appear to stimulate new T-cell production by the thymus. Additional animal studies are ongoing.

IL-7 therapy has not yet been administered to humans, although human trials of IL-7 may be possible within the next 2 to 3 years. Because laboratory studies have shown that IL-7 can enhance HIV replication, the use of IL-7 in HIV disease will need to be pursued with caution.
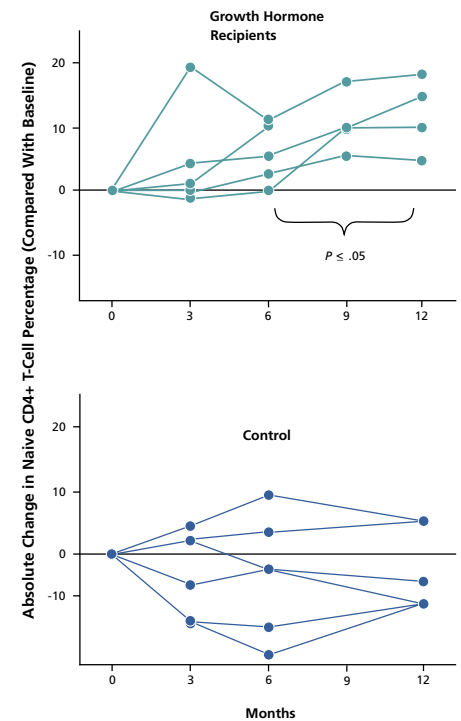
Figure **2.** Absolute change in naive CD4+ T-cell percentage in patients receiving human growth hormone and those not receiving growth hormone (control). Adapted with permission from Napolitano et al, *AIDS,* 2002.

Immune-based Therapies: Further Considerations

Exploration of immune-based therapies for the treatment of immunodeficiency is growing, but there is still much to be learned about the specific effects of these therapies on the recovery of the immune system. Laboratory and animal studies suggest that several potential therapies are capable of increasing de novo T-cell production by the thymus or expanding the numbers of existing T cells. Whereas IL-2 appears to predominantly act by increasing mature CD4+ T-cell populations, GH may act by stimulating new T-cell production by the thymus, and IL-7 may stimulate both de novo T-cell production and mature T-cell expansion. It is possible that the mechanism of T-cell increases may matter to efforts to promote immune system reconstitution in HIV-infected individuals. The normal T-cell repertoire consists of approximately 1×10^8 unique T-cell receptors. With HIV-related T-cell deple-

tion, much of this diversity can be lost (Figure 3). Expansion of existing T cells in the periphery increases the number of mature cells with existing antigen specificities, whereas de novo T-cell production by the thymus can replace elements in the T-cell receptor repertoire that are lost during HIV infection. It remains unclear, however, what the clinical consequences of differences in site and mechanism of T-cell increases might be. Ongoing studies will help to answer these and related questions and contribute to refining efforts to enhance immunologic reconstitution in HIV-infected persons.

Conclusion

Several regulators of T-cell production and function, including IL-2, GH, and IL-7, are being considered as potential immune-based therapies during HIV infection. IL-2 induces significant gains in CD4+ T cells in most HIV-infected patients in numerous studies. In preliminary studies, GH appears to reverse thymic involution and enhance T-cell production in some HIV-infected adults. IL-7, not yet studied in humans, appears to play an important role in T-cell production and homeostasis and may have future potential for the treatment of lymphopenic conditions.

These immune-based therapies offer several theoretical advantages in the management of HIV disease, such as improved immune restoration (increased CD4+ T-cell gains with effective antiretroviral therapy); improved immune preservation (delayed CD4+ T-cell decline); improved immune response against HIV or other antigens; improved breadth of the T-cell reper-
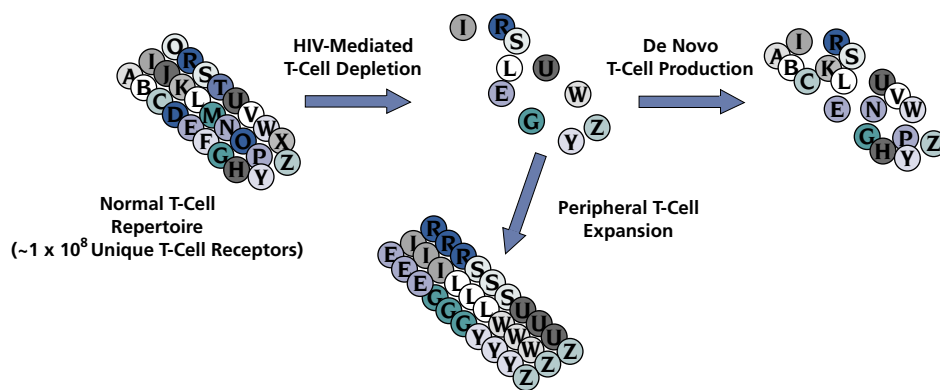
Figure 3. The source of T-cell increase affects T-cell diversity and antigen recognition. Losses in T-cell receptor repertoire due to HIV infection are not replaced by expansion of peripheral mature CD4+ T cells but can be replaced via de novo synthesis of T cells in the thymus. Figure courtesy of Dr Laura Napolitano.

toire; enhanced response to vaccination; and decreased opportunistic infection. However, these therapies also carry significant toxicities, and it remains to be determined whether these agents will offer any advantages beyond those provided by effective antiretroviral therapy alone. Additional clinical and immunologic investigations are ongoing to determine whether immune-based therapies provide clinical benefits to individuals infected with HIV.

Presented by Dr Napolitano in May 2003. First draft prepared from transcripts by Matthew Stenger. Reviewed and updated by Dr Napolitano in September 2003.

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Suggested Reading

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Perspective
Antiretroviral Therapy: Select Lessons From Recent Studies

Our understanding of HIV pathogenesis continues to evolve and form the foundation for antiretroviral treatment and drug development strategies. There is an increased focus on improving drug pharmacodynamics and tolerability and simplicity of drug regimens to optimize viral suppression and preserve immune function. In terms of antiretroviral therapy using current treatment options, the CD4+ cell count at initiation of treatment appears to be the most powerful discriminator of risk for disease progression. Newer drug regimens have better rates of virologic response to a plasma HIV-1 RNA level below 50 copies/mL on intent-to-treat analyses than prior standard regimens, which likely reflects improvements in simplicity, tolerability, and pharmacodynamics rather than improved potency per se. With regard to particular initial treatment choices, recent trials evaluating nevirapine (once or twice daily) with or without efavirenz, triple nucleoside reverse transcriptase inhibitor regimens, and didanosine EC/tenofovir, among others, have provided insights. This article summarizes a presentation given by Michael S. Saag, MD, at the May 2003 International AIDS Society–USA course in Washington, DC. The presentation focused on selected new data that provide insights in clinical management, particularly with regard to initiating antiretroviral therapy.

HIV Pathogenesis: The Background for Devising Treatment Strategies

Our understanding of HIV pathogenesis and its relationship to antiretroviral treatment has undergone continual evolution. The concepts of HIV pathogenesis affect strategies for antiretroviral therapy as well as strategies for drug development.

HIV-infected cells produce virions that predominantly target activated CD4+ T lymphocytes. After infection, an activated CD4+ T cell has a life span of approximately 1 day, during which it produces a multitude of virions, and after which it is essentially replaced by another newly infected CD4+ T cell from the activated cell pool. Antiretroviral drugs prevent new infection of activated CD4+ cells (whether by preventing formation of new virions as with nucleoside and nonnucleoside reverse transcriptase inhibitors, preventing infectivity of virus as with protease inhibitors, or blocking viral entry as with fusion inhibitors). Several years ago, it was believed that viral eradication would be possible if infection of

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new cells could be blocked completely (by 100%) and this blockade could be sustained for a sufficient period of time to allow complete loss of all previously infected cells. Based on the assumption that the half-life of latently infected CD4+ cells was 14 to 28 days, it was calculated that eradication could occur in 3 to 5 years. This provided much of the rationale for the very early and very aggressive use of antiretroviral therapy recommended at that time. It is now known that these latently infected cells live for anywhere from 6 to 40 or more months. Even with 100% prevention of new infection, complete eradication would require some 60 years of continuous treatment and thus, full eradication, or cure, currently is not a practical goal. Further, 100% blockade of new infection is an elusive goal, with a variety of data showing some degree of ongoing, yet low-level, viral replication even with profound viral suppression (Figure 1).

Several years ago, it was generally believed that the primary mechanism of
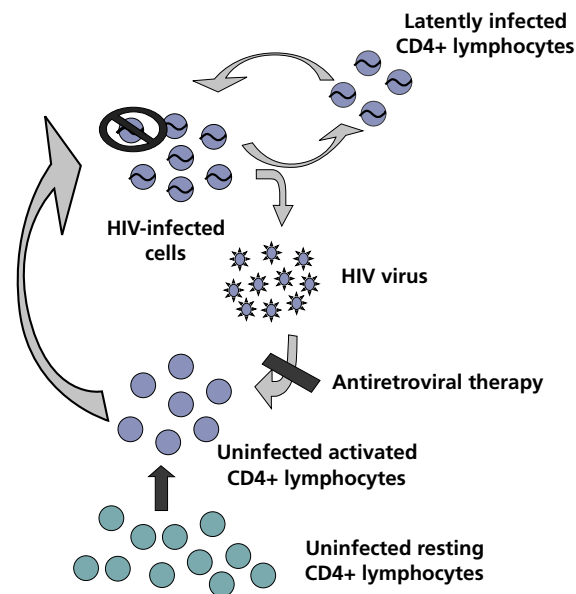
Figure **1.** HIV predominantly infects activated CD4+ T lymphocytes. An activated cell dies after approximately 1 day of productive infection and is replaced by another activated cell that is highly likely to be infected (since it is activated in a milieu teeming with virions). Effective antiretroviral therapy prevents infection of the activated cell, allowing replacement of the virus-producing cell with a cell that is not infected. Viral replication can be profoundly reduced but eradication is not a practical goal, given the extended survival of latently infected resting lymphocytes and the low-level viral replication in the presence of profound viral suppression. Figure courtesy of Dr Michael Saag.

cell death was a direct cytopathic effect of the virus. Although this mechanism likely contributes to cell death, recent evidence suggests that the predominant mechanism may be the action of CD8 + cytotoxic T cells and other effector cells in killing infected cells. With prevention of new infection under effective antiretroviral therapy, the processes resulting in cell lysis might also be downregulated. The rapid decay in viral kinetics under antiretroviral therapy, therefore, simply reflects the replacement of an infected cell that is actively producing virus with a newly infected cell. If therapy is stopped, production of virus is reestablished in activated cells no longer protected by antiretroviral therapy, with levels of viral replication usually returning to the set point observed previously.

This set point appears to be the product of the interaction of virus-producing cells and activity of cytotoxic CD8 + lymphocytes, which is governed by host genetics such as chemokine receptor genetics and HLA haplotypes. Infected activated cells therein produce multitudes of virions in an environment largely consisting of inactivated lymphocytes. At steady state, no matter how many virions an infected cell produces during its 1-day lifetime, it is effectively replaced with only a single newly infected activated cell at the time it dies. The viral set point thus represents an equilibrium between the rates of new cell infection and infected cell death. The plasma HIV RNA level directly reflects the total number of infected cells in an individual, with the level of replication remaining relatively constant as the number of newly infected cells remains constant. The HIV-1 RNA level as measured in plasma simply represents a spillover of virus from lymphatic tissue into the bloodstream and there is likely no replication occurring in the peripheral blood per se. Under conditions of antiretroviral therapy, the "replacement" lymphocyte is protected, resulting in the overall "loss" of a virus-producing cell and a proportionate decline in the number of virions produced. This decline in replication is reflected in the dramatic decrease in plasma HIV-1 RNA level, which accurately reflects the reduction in the level of replication in lymphatic tissues.

This conception of HIV pathogenesis is relevant to management strategies in a variety of ways. It sharpens focus on the importance of ensuring that antiretroviral drugs are penetrating into the cells that need protection or, as in the case of protease inhibitors, into cells that are already actively infected. Drug pharmacodynamics are crucial to the strength of protection provided to susceptible cells by antiretroviral therapy. Efforts are ongoing to better understand the pharmacokinetics and pharmacodynamics of drugs within cells to determine if there are ways to increase intracellular residence time by overcoming cellular mechanisms that retard drug entry or accelerate extrusion of drug from the intracellular compartment. It may be that virologic failure occurs in fully adherent patients because of induction of cellular pump systems over time by the antiretroviral drugs themselves.

This conception of pathogenesis also emphasizes the emergence of viral resistance as a chance phenomenon. Under conditions of incomplete viral suppression, a genetic variant of the virus that is resistant to a single drug can, by chance, be produced and infect an activated cell. The likelihood of such an occurrence increases with increasing levels of viral replication in the face of ongoing drug pressure on the virus, such as what might occur with intermittent adherence. In fact, the least amount of risk of resistance emergence is present when the plasma HIV-1 RNA is maintained at levels below 50 copies/mL. Triple-drug antiretroviral therapy provides additional protection for the activated cell via coverage for the chance development of a single mutation to 1 of the drugs in the regimen. Although it seems a heretical idea in the current treatment era with available drugs, effective monotherapy could be possible with optimized pharmacodynamics and thus is the subject of ongoing investigation. The pathogenesis of HIV suggests, however, that this is not likely to be a successful long-term strategy.

Initial Antiretroviral Strategies: Lessons From Recent Studies

Predicting Successful Response

Data are emerging that show that baseline CD4 + cell count may be the best predictor of response to initial antiretroviral therapy. The Multi-ART Cohort Collaboration Study observed more than 12,000 patients beginning antiretroviral therapy for long-term outcomes. Baseline CD4 + cell count was the best discriminator for predicting AIDS-free survival (Figure 2, left). By comparison, only plasma HIV-1 RNA level greater than 100,000 copies/mL provided any additional discrimination of risk (Figure 2, right). Hazard ratios for progression to AIDS or death using a multivariable Cox model for baseline predictive factors are shown in Table 1. Figure 3 shows the probability of progression to AIDS or death by year according to CD4 + cell count stratum and plasma HIV-1 RNA level stratum among patients with lower risk according to age, history of injection drug use, and Centers for Disease
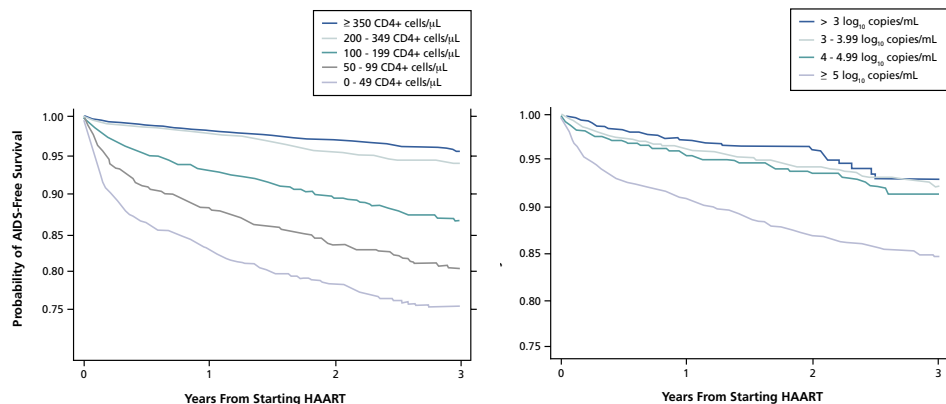

Figure 2. AIDS-free survival by baseline CD4+ cell count (left) and baseline plasma HIV-1 RNA level (right) in the Multi-ART Cohort Collaboration Study, covering a period of 3 years of patient treatment with antiretroviral therapy. Adapted from Hogg et al, *JAMA,* 2001.

Table **1.** Significant Discriminators of Risk for Progression to AIDS or Death in the Multi-ART Cohort Collaboration Study

Discriminator	Hazard Ratio (95% CI)
Age	
≥ 50 years	1
< 50 years	0.72 (0.62 to 0.84)
Risk Behavior	
Injection drug use	1
Other	0.68 (0.59 to 0.79)
CDC Disease Stage	
C	1
A/B	0.70 (0.61 to 0.81)
CD4+ cells/μL	
0-49	1
50-99	0.75 (0.63 to 0.90)
100-199	0.53 (0.44 to 0.63)
200-349	0.25 (0.20 to 0.30)
≥ 350	0.18 (0.14 to 0.22)
Log$_{10}$ HIV-1 RNA copies/mL	
≥ 5	1
< 5	0.75 (0.63 to 0.90)

CDC indicates Centers for Disease Control and Prevention; CI, confidence interval. Data are from Egger et al, *Lancet*, 2002.

Control and Prevention (CDC) disease stage. The highest probabilities of progression were observed in patients with the lowest CD4+ cell counts and higher HIV-1 RNA levels (12.4%, 17.0%, and 20.3% at 1, 2, and 3 years, respectively). The lowest risk of progression was observed in patients with a CD4+ cell count of 350/μL or greater and an HIV-1 RNA level below 5 log$_{10}$ copies/mL (1.5%, 2.5%, and 3.4% at 1, 2, and 3 years, respectively). Such findings have implications for when to start antiretroviral therapy. The CD4+ cell count has, over the last few years, become the primary trigger for when to begin antiretroviral therapy. Delaying therapy until the CD4+ cell count declines to 200/μL clearly is too late, but the optimal CD4+ count above 200/μL remains unknown. Current guidelines suggest a CD4+ cell count threshold somewhere between

200/μL and 350/μL, however, the above findings suggest that there is benefit to initiating therapy at higher CD4+ cell counts, especially among those with very high HIV-1 RNA levels. These findings are consistent with experimental data indicating that very high HIV-1 RNA levels are associated with reduced function of immune system effector cells, suggesting that the presence of large amounts of virus has an immunosuppressive effect. In recent years, there has been a trend toward delaying initiation of therapy until relatively low CD4+ cell counts owing to the low risk of opportunistic infections at moderately low cell counts. However, based on findings such as those in the current study, it appears that the pendulum is swinging back toward earlier initiation of therapy. Indeed, continued long-term outcome evaluations may indicate that it is best to initiate therapy when CD4+ cell counts are still above 400/μL.

Factors in Selecting Initial Therapy

Numerous factors are important in choosing the initial antiretroviral regimen, including potency, simplicity, tolerability, "forgivability" (ie, less deleterious effect of a single missed dose), and salvageability (ie, predicted resistance profile at time of virologic failure, which affects subsequent treatment options).

The single most important factor is potency. However, assuming equal potency of regimens, then tolerability and simplicity are the next primary considerations. Given the relatively equal potency of most current regimens, tolerability emerges as the most important factor in maintaining effective treatment on a day-to-day basis: drugs do not work if people do not take them. This brings the focus to "forgivability." It is human nature to miss doses of medications. Drugs with pharmacokinetic/pharmacodynamic properties that allow prolonged intracellular half-lives are less likely to lose antiretroviral pressure in the setting of a single missed dose and, therefore, would clearly be advantageous.

A meta-analysis performed several years ago indicated that only 46% of patients were able to achieve an HIV-1 RNA level below 50 copies/mL at 48 weeks using intent-to-treat analyses. More recent studies of newer regimens

Year	≥ 5	< 5	≥ 5	< 5	≥ 5	< 5	≥ 5	≥ 5	< 5
1	12.4	9.5	9.2	7.0	6.2	4.7	2.6	2.0 (1.6 to 2.4)	1.5 (1.2 to 1.9)
2	17.0	13.1	13.1	10.1	9.5	7.3	4.5	3.4 (2.8 to 4.1)	2.5 (2.1 to 3.0)
3	20.3	15.8	16.1	12.5	12.1	9.3	6.1	4.7 (3.9 to 5.6)	3.4 (2.8 to 4.1)
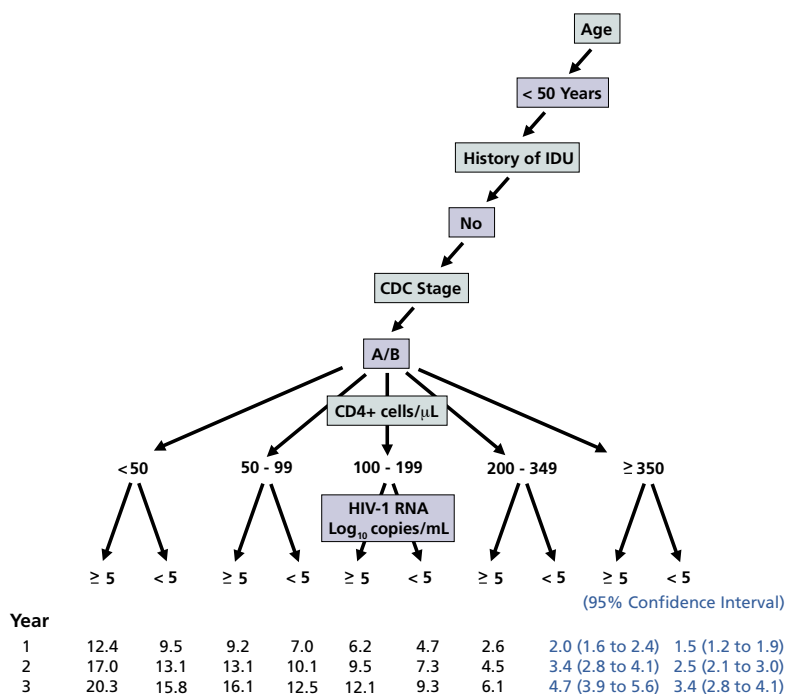
Figure **3.** The probability of progression to AIDS or death by year of study in patients aged less than 50 years, with no history of injection drug use (IDU), and with Centers for Disease Control and Prevention (CDC) disease stage A/B according to baseline CD4+ cell count and HIV-1 RNA level in the Multi-ART Cohort Collaboration Study. Data are, in part, published in Egger et al, *Lancet,* 2002; figure courtesy of Dr Matthias Egger.

show dramatically better 24-week response rates than those considered in the meta-analysis (Figure 4). These newer regimens are probably not more potent than previously studied protease inhibitor–based triple-drug regimens. Rather, it is more likely that improvements in tolerability and "forgivability" have accounted for the ability to achieve these greater levels of virologic response.

Relative Effectiveness of Nevirapine- and Efavirenz-Containing Regimens

The 2NN study compared nevirapine 400 mg once daily, nevirapine 200 mg twice daily, efavirenz 600 mg once daily, and the combination of nevirapine 400 mg/efavirenz 800 mg once daily along with stavudine/lamivudine in 1216 antiretroviral-naive patients with an HIV-1 RNA level above 5000 copies/mL and any CD4+ cell count. No significant differences in proportions of patients reaching an HIV-1 RNA level below 50 copies/mL were seen between any of the regimens on intent-to-treat analysis, with proportions of patients reaching this level ranging from 63% with the dual nonnucleoside reverse transcriptase inhibitor (NNRTI) combination to 70% with the nevirapine once-daily regimen and the efavirenz

regimen. However, there were differences in rates of discontinuation or interruption due to clinical adverse events: 15.5% in the efavirenz group, 21.2% in the nevirapine twice-daily group, 24.1% in the nevirapine once-daily group, and 29.7% in the combination group ($P < .001$). There was also an overall difference in rates of laboratory grade 3 or 4 hepato-biliary toxicities among groups: 4.5% in the efavirenz group, 7.8% in the nevirapine twice-daily group, 8.6% in the combination group, and 13.2% in the nevirapine once-daily group ($P = .002$). These findings suggest no added benefit and worse tolerance with the dual NNRTI nevirapine/efavirenz combination, at least at the doses studied, and suggest that nevirapine twice daily may be safer and better tolerated than the once-daily nevirapine regimen.

Relative Effectiveness of Abacavir-Containing Triple-Nucleoside Initial Therapy

ACTG 5095 compared efavirenz, abacavir, or the efavirenz/abacavir combination along with zidovudine/lamivudine as initial antiretroviral therapy. Virologic failure was defined as an HIV-1 RNA level above 200 copies/mL at week 16. The trial used rejection of a

non-inferiority null hypothesis with O'Brien-Fleming stopping rules. At the second Data and Safety Monitoring Board review of the study, there were no safety issues with any of the regimens. However, the trial was stopped because of demonstrated inferiority of the abacavir-containing triple-drug regimen compared with the other 2 regimens on the basis of rates of and time to virologic failure. Although this trial provides evidence that the lamivudine/zidovudine/abacavir (fixed dosage) combination is inferior as initial therapy, the response rate with the combination was still quite high (74% less than 50 copies/mL at 24 weeks), higher in fact than any response rate previously reported with the combination and much higher than the 46% response rate reported in the meta-analysis study reported above. Therefore, the combination might still be advocated for use in initial treatment in selected patient populations on the basis of simplicity of use. Further, the results of this study do not clearly support changing the regimen or enhancing it by adding efavirenz if patients are already responding to the lamivudine/zidovudine/abacavir combination with an HIV-1 RNA level below 50 copies/mL. Rather, the results from this study should be discussed with such patients and individual decisions made between the patient and the treating provider.

Combined Use of Didanosine EC and Tenofovir

There is confusion in the clinical setting about how didanosine EC and tenofovir should be used together. Tenofovir significantly boosts didanosine concentrations, and therefore the use of didanosine EC 400 mg with tenofovir 300 mg may markedly increase risk of pancreatitis. Pharmacokinetics studies evaluating didanosine alone at 400 mg and didanosine (at various doses)/tenofovir combinations with and without food indicate that the appropriate dose of the combination is didanosine EC 250 mg/tenofovir 300 mg once daily, for patients weighing more than 50 kg; those patients weighing less than 50 kg should have the didanosine dose reduced to 200 mg/per day. The combination can be given with food or fasting.

Regimen (Study or Author)

Regimen	%
Meta-Analysis (Bartlett et al)	54%
Zidovudine/Lamivudine/Efavirenz (DMP-006)	57%
Stavudine/Lamivudine/Lopinavir/Ritonavir (Abbott 863)	65%
Stavudine/Didanosine/Efavirenz (DMP-044)	65%
Stavudine/Didanosine/Efavirenz (FTC-301)	70%
Tenofovir/Lamivudine/Efavirenz (Gilead 903)	80%
Emtricitabine/Didanosine/Efavirenz (FTC-301)	81%
Stavudine/Lamivudine/Efavirenz (Gilead 903)	82%

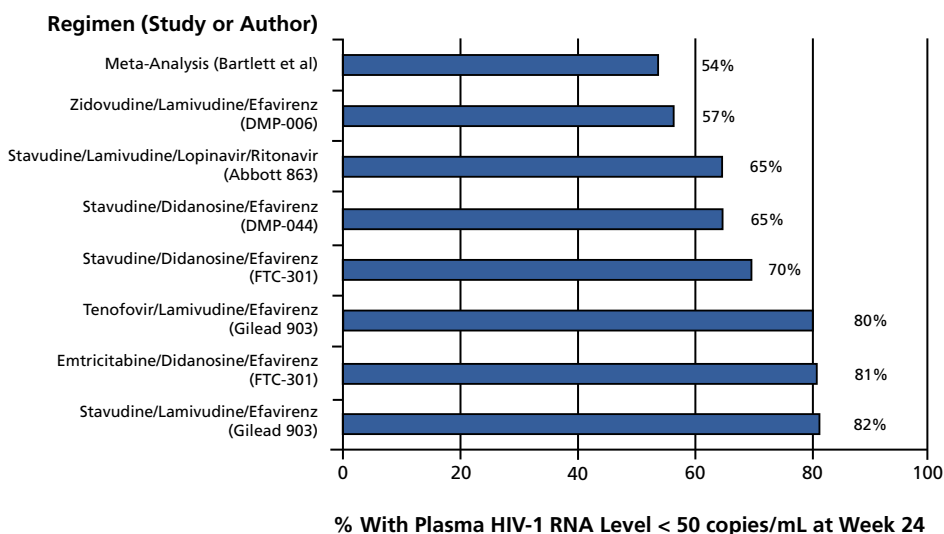**% With Plasma HIV-1 RNA Level < 50 copies/mL at Week 24**

Figure **4.** Proportions of patients achieving an HIV-1 RNA level below 50 copies/mL at 24 weeks on intent-to-treat analyses (non completer = failure) in recent studies compared with an earlier meta-analysis. Figure courtesy of Dr Michael Saag.

Combined Use of Tenofovir and Abacavir

At the 2nd International AIDS Society Conference on HIV Pathogenesis and Treatment in Paris, Charles Farthing presented results of a small pilot study evaluating the relative activity of a novel, once-daily regimen of abacavir/lamivudine/tenofovir in patients naive to antiretroviral therapy. Surprisingly, only 8 of 19 (42%) patients had a successful virologic response, defined as a greater than 2-log drop in HIV RNA by week 8, or loss of this effect (virologic rebound) after initial suppression.

Soon after this presentation, GlaxoSmithKline released a "Dear Healthcare Provider" letter reporting data from an ongoing study, EES 30009, that demonstrated similar findings. In EES 30009, patients naive to antiretroviral therapy were randomized (1:1) to receive abacavir/lamivudine with either efavirenz or tenofovir. An unplanned interim evaluation of the data revealed a surprisingly large difference in virologic outcomes: only 5 of 92 patients (5%) in the efavirenz arm experienced virologic failure compared with 50 of 102 patients (49%) in the tenofovir arm. Taking these data together with the Farthing study results, there appears to be a negative interaction among abacavir, lamivudine, and tenofovir. However, since numerous studies comparing abacavir and lamivudine together with other agents, as well as various studies comparing lamivudine and tenofovir combined with other agents, have not demonstrated such high rates of failure, it appears that the principal negative interaction might be between abacavir and tenofovir. This interaction could take the form of interference with drug absorption or metabolism, yet the most likely interaction seems to be at the level of intracellular concentrations of active drug(s) in the triphosphate form (similar to the type of negative interaction seen between zidovudine and stavudine).

Further studies are needed to fully elucidate the mechanism(s) responsible for these relatively poor virologic outcomes. Until then, patients on combination regimens containing abacavir and tenofovir, with or without lamivudine, should be monitored very carefully and this combination should be avoided in new regimens.

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Perspective

The Increasing Importance of Sexually Transmitted Diseases in HIV-Infected Persons

Increases in the incidence of sexually transmitted disease (STD) in some locales indicate that safe sex practices are declining and raise concerns about a potential increase in the incidence of HIV infection. Practitioners should renew their vigilance in sexual risk assessment and counseling and be aware of current recommendations for diagnosis and treatment of STDs in HIV-infected patients. This article reviews current recommendations for screening, diagnosis, and management of genital herpes simplex virus infection, chlamydial and gonococcal infections, syphilis, and human papillomavirus–associated neoplasia in HIV-infected patients. The article summarizes a presentation given by Melanie M. Taylor, MD, MPH, at the March 2003 International AIDS Society–USA course in Los Angeles.

There has been an increase in sexual behaviors placing individuals at risk of HIV infection and other sexually transmitted diseases (STDs) in many locales during the recent past, as indicated both by clinicians' findings in risk assessments and by increases in incidence of a number of STDs (Chen et al, *Am J Public Health,* 2002; Ciesielski, *Curr Infect Dis Rep*, 2003; Flaks et al, *Sex Transm Dis,* 2003; Rietmeijer et al, *Sex Transm Dis,* 2003). The increase in risk behaviors among HIV-seropositive men who have sex with men (MSM) may be related to gains in health status and sense of well-being achieved with effective antiretroviral therapy, as well as to general burnout over messages regarding the need for safe sex practices (Dilley et al, *N Engl J Med,* 1997; Flaks et al, *Sex Transm Dis,* 2003). It is important that physicians providing HIV-related care revisit recommendations for evaluating patients for unsafe sex and STDs, and for treating STDs. A summary of the Centers for Disease Control and Prevention's treatment guidelines appears in the Appendix.

STD Evaluation and Prevention Methods

It is crucial that physicians take responsibility for thoroughly discussing sexual

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risk behaviors with their patients and for encouraging their HIV-infected patients to take responsibility for their HIV disease, which includes informing their sex partners of their infection status. Patients must be informed that presence of STDs is associated with increased risk of HIV transmission. Additional components of STD risk-reduction counseling include performing a thorough STD/HIV sexual risk assessment (including history of anonymous partners, number of sexual partners, discussion of HIV serostatus, and use of drugs associated with heightened sexual activity); providing client-centered prevention counseling; and providing education about appropriate condom use and recognition of STD symptoms. In some locales, including Los Angeles County, MSM report a high frequency of sex with anonymous partners (partners without identifying or contact information) met in commercial sex venues, such as bathhouses and sex clubs, as well as through the Internet, presenting a significant challenge for disease control.

Counseling on prevention methods should include review of appropriate and inappropriate spermicide use, with emphasis on the fact that spermicides alone are not recommended for STD/HIV infection prevention. Nonoxynol-9 vaginal spermicides are not effective in preventing chlamydial, gonococcal, or HIV infections and should not be used as a microbicide or lubricant during vaginal or anal intercourse. In addition, frequent use of spermicides/nonoxynol-9 has been

associated with genital lesions. Latex condoms, when used consistently and correctly, are highly effective in preventing transmission of HIV. In addition, correct and consistent use of latex condoms can reduce the risk of other sexually transmitted diseases, including discharge diseases such as chlamydia, gonorrhea, and trichomoniasis as well as genital ulcer diseases, such as herpes simplex virus (HSV), syphilis, and chancroid when the infected area or site of potential exposure is protected. While the effect of condoms in preventing human papillomavirus (HPV) is unknown, condom use has been associated with a lower rate of cervical cancer, an HPV-associated disease.

Patients with newly diagnosed HIV infection should be thoroughly evaluated for STDs, including screening for gonococcal and chlamydial infections, and obtaining syphilis and viral hepatitis serologies. STD screening should be performed at least annually in sexually active persons and every 3 to 6 months in highest-risk MSM (eg, those who acknowledge having multiple anonymous partners or having sex in conjunction with illicit drug use and patients whose sex partners participate in these activities, and persons with previous history of STD or belonging to a patient population with a high prevalence of STDs), including syphilis serology, screening for gonococcal and chlamydial urethral infection by culture or nucleic acid amplification tests, and screening for pharyngeal or rectal gonococcal or chlamydial infections by culture if there is a history of oral-genital or receptive anal intercourse.

Genital HSV Infection

HIV-infected patients with genital HSV infection may have prolonged or severe episodes of reactivation with extensive genital or perianal disease. Episodic or suppressive antiviral therapy often is

beneficial for treating genital HSV infections. For severe cases, or those with complications requiring hospitalization, treatment with acyclovir 5 to 10 mg/kg intravenously (IV) every 8 hours may be necessary. Recommendations for episodic therapy consist of acyclovir 400 mg 3 times a day, famciclovir 500 mg twice a day, or valacyclovir 1 g twice a day for 5 to 10 days. Daily suppressive therapy can be performed with acyclovir 400 to 800 mg 2 or 3 times a day, famciclovir 500 mg twice a day, or valacyclovir 500 mg twice a day. Antiviral-resistant HSV is found in approximately 5 % of immunocompromised patients receiving suppressive therapy. Persistent or recurrent lesions during treatment should prompt susceptibility testing of viral isolates, although recurrence of outbreaks does not warrant cessation of suppressive therapy. Acyclovir-resistant HSV is also resistant to valacyclovir and usually is resistant to famciclovir. Alternatives include foscarnet (40 mg/kg IV every 8 hours) or cidofovir gel 1 % (daily for 5 days).

Screening for Gonococcal and Chlamydial Infections

Screening for *Chlamydia trachomatis* infection in women can be performed with nucleic acid amplification tests on endocervical swabs or urine specimens. Nucleic acid amplification tests on urine offer ease of sample collection as well as comparable sensitivity to that of the same test performed on endocervical samples. Alternative tests consist of DNA probes, enzyme immunoassay, or direct fluorescent antibody (DFA) testing on endocervical swab specimens or culture of endocervical specimens. These tests are less sensitive than nucleic acid amplification tests but may offer improved specificity. Screening for gonorrhea using endocervical swab specimens should be performed by culture due to the continuing need for antimicrobial-resistance monitoring. If culture is not available, nucleic acid amplification or DNA probe testing of endocervical specimens can be performed. Nucleic acid amplification tests can be used for urine specimens and offer similar benefits to that of chlamydia testing. Similarly, in screening for urethral chlamydial infections in men, it is rec-

ommended that nucleic acid amplification tests be used on urethral or urine specimens. Alternatively, non-nucleic acid amplification tests or culture can be used on urethral specimens. For gonococcal infection, culture of urethral swab specimens is recommended, with use of nucleic acid amplification tests or DNA probes on urethral specimens or nucleic acid amplification tests on urine specimens constituting alternative methods.

Rates of *Neisseria gonorrhoeae* resistance to fluoroquinolones, which constitute standard treatment for gonorrhea, are increasing in many locales. Data from the Gonococcal Isolate Surveillance Project indicate a steady increase in proportion of strains with resistance or decreased susceptibility to ciprofloxacin between 1996 and 2000 (Figure 1). There is, however, significant geographic variation in resistance. In most of the United States, strains of *N. gonorrhoeae* remain susceptible to fluoroquinolones. Increasing fluoroquinolone resistance rates are reported in Southeast Asia, the Pacific Islands, and in Hawaii and California in the United States; current STD treatment guidelines recommend fluoroquinolone treatment not be used for gonorrhea in California and Hawaii. Thus far, no gonococcal resistance to ceftriaxone has been reported.

HIV-infected women and men can be screened for pharyngeal and rectal chlamydial and gonococcal infections. For chlamydial infection, pharyngeal

or rectal culture (or DFA, using a *C. trachomatis* major outer membrane protein-specific stain may be acceptable as an alternative) can be used. For gonococcal infection, culture with additional testing of presumptively positive colonies (ie, typical morphology, oxidase-positive, Gram-negative diplococci) can be performed.

Syphilis

Of the more than 700 cases of syphilis reported in Los Angeles in 2002, approximately 70 % occurred in MSM; of those, approximately 60 % occurred in MSM with HIV infection (Figure 2). These data strongly indicate that some subgroups of MSM, including those with HIV disease, are engaging in high-risk sexual behaviors and suggest the potential for an increase in incidence of HIV infection. Indeed, preliminary data from the Los Angeles County Office of AIDS Programs and Policy indicate a rising incidence of HIV infection among MSM in Los Angeles. It is unclear the extent to which the syphilis epidemic in Los Angeles has contributed to the projected increase in incidence among MSM; nevertheless, heightened attention to screening for syphilis is warranted.

Numerous treatment regimens for syphilis are available. Data on treatment success in HIV-infected patients are conflicting, and there are some data indicating that HIV-infected patients progress more rapidly through stages of
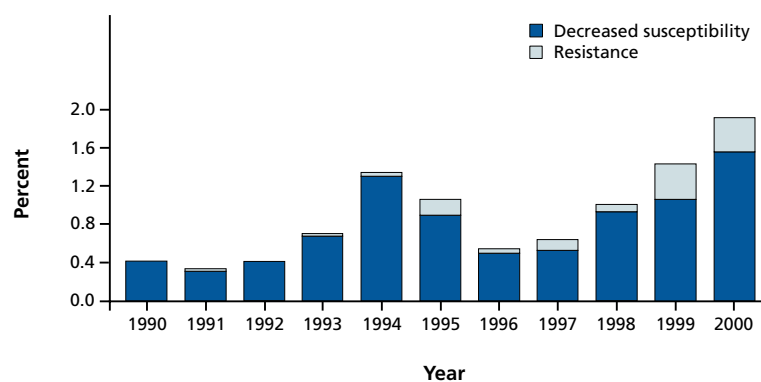

Figure **1**. Percent of *Neisseria gonorrhoeae* isolates with decreased susceptibility (minimum inhibitory concentrations 0.125 to 0.5 µg/mL) or resistance (minimum inhibitory concentrations ≥1 µg/mL) to ciprofloxacin, according to the Gonococcal Isolate Surveillance Project. Adapted from materials from the Centers for Disease Control and Prevention. Available at www.cdc.gov/std/gisp/.
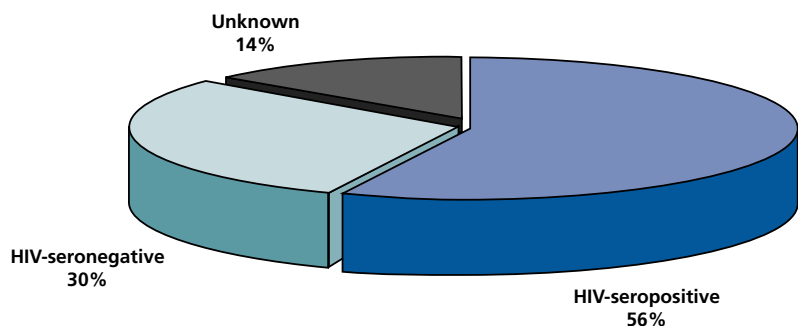
Figure **2.** HIV serostatus in 406 cases of syphilis among men who have sex with men in Los Angeles County in 2002 (provisional data). Adapted from Sexually Transmitted Disease Program, Los Angeles County Department of Health Services, Early Syphilis Surveillance Summary, 2003.

syphilis than do patients without HIV infection. Thus, rigorous follow-up of HIV-infected patients is required regardless of the chosen treatment regimen. Repeated serologies are necessary to ensure that treatment has been successful. If initial treatment is not successful, patients should undergo workup for neurosyphilis and treatment should be repeated.

In most HIV-infected patients, primary, secondary, and early latent (<1 year of exposure), syphilis responds appropriately to benzathine penicillin 2.4 mU intramuscularly (IM) times 1 dose. Benzathine penicillin should not be confused with Bicillin formulations (containing short-acting procaine penicillin) that are not appropriate for use in treating persons with syphilis. Some experts recommend performing cerebrospinal fluid (CSF) examination prior to therapy, and if CSF is normal, providing additional treatment—eg, weekly benzathine penicillin IM for 3 weeks—in HIV-infected patients. Clinical and serologic evaluation should be performed at 3, 6, 9, 12, and 24 months after treatment, with some experts performing CSF examination at 6 months. Those patients with clinical or serologic failure (eg, the absence of a 4-fold decline in serum RPR titer) at 6 to 12 months after initial treatment should undergo CSF examination and be retreated with benzathine penicillin 2.4 mU IM weekly for 3 weeks in the absence of neurosyphilis.

HIV-seropositive patients with late latent syphilis (>1 year of exposure) or syphilis of unknown duration should undergo CSF examination before treatment. Those with normal CSF results should receive benzathine penicillin 2.4 mU IM weekly for 3 weeks and undergo clinical and serologic evaluation at 6, 12, 18, and 24 months after treatment. CSF examination and treatment should be repeated if symptoms develop or if a rise of 4-fold or greater in antibody titer is observed. CSF examination and treatment should also be repeated if the nontreponemal antibody test titer does not decline within 12 to 24 months. Diagnosis of neurosyphilis is based on CSF white blood cell count and protein levels and a positive non-treponemal (usually Venereal Disease Research Laboratory, VDRL) test performed on CSF. Sensitivity in diagnosis can be improved with use of fluorescent treponemal antibody absorbption (FTA-ABS) testing on CSF, although false-positive findings occur with this method when CSF is contaminated with blood. Diagnosis presents some difficulty in the case of HIV-infected patients with negative CSF-VDRL results, since HIV infection alone is associated with CSF abnormalities, including pleocytosis and elevated protein, in approximately 30% of patients. A high index of suspicion for neurosyphilis should be maintained in patients with CSF abnormalities and negative VDRL test results, since many of the neurologic complications of disease can be permanent. The recommended treatment for neurosyphilis is

aqueous crystalline penicillin G 18 to 24 mU given at 3 to 4 mU IV every 4 hours for 10 to 14 days. An alternative regimen is procaine penicillin 2.4 mU IM daily plus probenecid 500 mg orally 4 times daily for 10 to 14 days. Some experts also recommend treatment with benzathine penicillin 2.4 mU IM weekly for 3 weeks following completion of these regimens to provide duration of treatment comparable to that for latent syphilis. Recent experience indicates that treatment with ceftriaxone 2 g IV for 10 to 14 days may be effective in treating neurosyphilis, as well. As is true for other HIV-infected patients with syphilis, patients receiving treatment for neurosyphilis should be followed closely after treatment to ensure treatment success.

HPV Infection

Women with HIV infection should be screened for cervical cancer associated with HPV infection. There is an increased prevalence of squamous intraepithelial lesions in HIV-infected women. Pap testing should be performed in all women twice in the first year of HIV infection diagnosis and annually thereafter if initial findings are normal. Management in the case of abnormal findings on Pap testing should follow the Interim Guidelines for Management of Abnormal Cervical Cytology provided by a National Cancer Institute Consensus Panel (Kurman et al, *JAMA*, 1994). Women with high-grade squamous intraepithelial lesions or squamous cell carcinoma with cytology should be referred for colposcopy and biopsy.

In men, HPV types 16 and 18 account for the majority of HPV infections in the anal canal and the majority of cases of anal intraepithelial neoplasia, which is a precursor to anal squamous cell cancer. The prevalence of anal intraepithelial neoplasia increases with decreasing CD4+ cell count in HIV-infected men. Although anal Pap testing is very sensitive in detecting such neoplasia, therapeutic strategies remain undefined due to an absence of sufficient data on treatment (Mathews, *Top HIV Med,* 2003). Thus, the Centers for Disease Control and Prevention currently does not have a recommendation

with regard to routine screening. More frequent biopsies may be considered in patients with recurrent lesions because of the increased risk of progression with recurrence. Currently, only local therapies applied by the patient or health care provider are recommended.

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Appendix. Summary of 2002 CDC Guidelines for Selected Sexually Transmitted Diseases

Disease	Recommended Rx	Dose/Route	Alternatives
Genital Herpes Simplex			
First clinical episode of genital herpes	acyclovir OR acyclovir OR famciclovir[1] OR valacyclovir[1]	400 mg orally 3x/day for 7-10 days[2] 200 mg orally 5x/day for 7-10 days[2] 250 mg orally 3x/day for 7-10 days[2] 1 g orally 2x/day for 7-10 days[2]	
Episodic therapy for recurrent genital herpes	acyclovir OR acyclovir OR acyclovir OR famciclovir[1] OR valacyclovir[1] OR valacyclovir[1]	400 mg orally 3x/day for 5 days 200 mg orally 5x/day for 5 days 800 mg orally 2x/day for 5 days 125 mg orally 2x/day for 5 days 500 mg orally 2x/day for 3-5 days 1 g orally 1x/day for 5 days	
Suppressive therapy[3] for recurrent genital herpes	acyclovir OR famciclovir[1] OR valacyclovir[1] OR valacyclovir[1]	400 mg orally 2x/day 250 mg orally 2x/day 500 mg orally 1x/day 1 g orally 1x/day	
Recommended regimens for episodic infection in persons infected with HIV	acyclovir OR acyclovir OR famciclovir[1] OR valacyclovir[1]	400 mg orally 3x/day for 5-10 days 200 mg orally 5x/day for 5-10 days 500 mg orally 2x/day for 5-10 days 1 g orally 2x/day for 5-10 days	
Recommended regimens for daily suppressive therapy in persons infected with HIV	acyclovir OR famciclovir[1] OR valacyclovir[1]	400-800 mg orally 2-3x/day 500 mg orally 2x/day 500 mg orally 2x/day	
Gonococcal Infections[4]			
Adults/adolescents/children ≥ 45 kg: urogenital, rectal	cefixime[5] OR ceftriaxone OR ciprofloxacin[6, 7] OR ofloxacin[5, 6, 7] OR levofloxacin[5, 7] PLUS	400 mg orally in a single dose 125 mg IM in a single dose 500 mg orally in a single dose 400 mg orally in a single dose 250 mg orally in a single dose	spectinomycin[10] 2 g IM in a single dose OR single-dose cephalosporin[11] OR single-dose quinolone[6, 11]
IF CHLAMYDIAL INFECTION IS NOT RULED OUT:	azithromycin[8] OR doxycycline[9]	1 g orally in a single dose 100 mg orally 2x/day for 7 days	
Pregnant women[11]	See complete CDC guidelines at www.cdc.gov/std/treatment		
Adults/adolescents: conjunctivitis	ceftriaxone	1 g IM in a single dose Irrigate infected eye with saline solution once	
Children (< 45 kg): urogenital, rectal, pharyngeal	ceftriaxone	125 mg IM in a single dose	spectinomycin[10] 40 mg/kg IM once (maximum 2 g)

Appendix (Continued)

Disease	Recommended Rx	Dose/Route	Alternatives
Gonococcal Infections[4] (continued)			
Neonates: Opthalmia neonatorum,[12] infants born to infected mothers	ceftriaxone[13]	25-50 mg/kg IV or IM in a single dose (maximum 125 mg)	
Chlamydial Infections			
Adults/adolescents	azithromycin[8] OR doxycycline[9]	1 g orally in a single dose 100 mg orally 2x/day for 7 days	erythromycin base[14, 15] 500 mg orally 4x/day for 7 days OR erythromycin ethylsuccinate[16] 800 mg orally 4x/day for 7 days OR ofloxacin[6] 300 mg orally 2x/day for 7 days OR levofloxacin 500 mg orally for 7 days
Pregnant women[11]	erythromycin base[14] OR amoxicillin	500 mg orally 4x/day for 7 days 500 mg orally 3x/day for 7 days	erythromycin base 250 mg orally 4x/day for 14 days OR erythromycin ethylsuccinate 800 mg orally 4x/day for 7 days OR erythromycin ethylsuccinate 400 mg orally 4x/day for 14 days OR azithromycin[8] 1 g orally single dose
Children (< 45 kg): urogenital, rectal	erythromycin base[17]	50 mg/kg/day orally (4 divided doses) daily for 14 days	
Neonates: opthalmia neonatorum, pneumonia	erythromycin base[17] OR erythromycin	50 mg/kg/day orally (4 divided doses) daily for 14 days 50 mg/kg/day orally (4 divided doses) daily for 14 days	
Syphilis			
Early-primary, secondary, or latent < 1 year	benzathine penicillin G	2.4 million units IM in a single dose	doxycycline[9, 18] 100 mg 2x/day for 14 days OR tetracycline[9, 18] 500 mg orally 4x/day for 14 days
Latent > 1 year, latent of unknown duration, late (cardiovascular, gumma)	benzathine penicillin G	2.4 million units IM each at 1 week intervals (7.2 million units total)	doxycycline[9, 18] 100 mg 2x/day for 28 days OR tetracycline[9, 18] 500 mg orally 4x/day for 28 days
Pregnant women[11]	See complete CDC guidelines at www.cdc.gov/std/treatment		
Neurosyphilis[12]	aqueous crystalline penicillin G	3 to 4 million units IV every 4 hours for 10-14 days (18-24 million units/day)	procaine penicillin G 2.4 MU IM 1x daily PLUS probenecid 500 mg orally 4x/day, both for 10-14 days
Congenital syphilis[11, 12]	aqueous crystalline penicillin G OR procaine penicillin G	100,000-150,000 units/kg/day (50,000 units/kg/dose IV every 12 hours) during the first 7 days of life and every 8 hours thereafter for a total of 10 days 50,000 units/kg/dose IM in a single dose for 10 days	

Appendix (Continued)

Disease	Recommended Rx	Dose/Route	Alternatives
Syphilis (continued)			
Children: early-primary, secondary, or latent < 1 year	benzathine penicillin G	50,000 units/kg IM in a single dose (maximum 2.4 million units)	
Children: latent > 1 year, latent of unknown duration, late	benzathine penicillin G	50,000 units/kg IM for 3 doses at 1-week intervals (maximum total 7.2 million units)	
Human Papillomavirus[19]			
External genital and perianal warts			
Patient applied:	podofilox 0.5%[1] solution or gel OR imiquimod 5%[1] cream OR	Apply to visible warts 2x/day for 3 days, rest 4 days, 4 cycles max. Apply once h.s., wash off after 6-10 hours 3x/week QOD, 16 weeks max.	Intralesional interferon OR Laser surgery
Provider administered:	liquid nitrogen or cryoprobe OR podophyllin resin 10%-25%[1] OR trichloroacetic acid or bichloroacetic acid 80%-90% OR surgical removal	Apply small amount, dry, wash off in 1-4 hours. Repeat weekly if necessary. Apply small amount, dry, apply weekly if necessary.	

Adapted from Centers for Disease Control and Prevention, *Sexually Transmitted Diseases: Summary of 2002 CDC Treatment Guidelines*. Complete guidelines can be downloaded from www.cdc.gov/std/treatment or by calling 1-888-232-3228.

[1] No definitive information available on prenatal exposure.

[2] Treatment may be extended if healing is incomplete after 10 days of therapy.

[3] Consider discontinuation of treatment after 1 year to assess frequency of recurrence.

[4] Patients with gonococcal infection should receive co-treatment for chlamydial infection.

[5] Not effective against pharyngeal gonococcal infection.

[6] Contraindicated for pregnant or lactating women.

[7] Quinolones should not be used for infections acquired in Asia, Pacific, Hawaii, or California.

[8] Safety of azithromycin has not been determined for pregnant or lactating women.

[9] Should not be administered during pregnancy, lactation, or to children < 8 years of age.

[10] For patients who cannot tolerate cephalosporins or quinolones; unreliable against pharyngeal gonococcal infection.

[11] Please refer to the complete 2002 CDC Guidelines for recommended regimens.

[12] Hospitalization recommended.

[13] Use with caution in hyperbilirubinemic infants, especially those born prematurely.

[14] Erythromycin estolate is contraindicated during pregnancy.

[15] If patient cannot tolerate high-dose erythromycin base schedules, change to 250 mg 4x/day for 14 days.

[16] If patient cannot tolerate high-dose erythromycin ethylsuccinate schedules, change to 400 mg orally 4x/day for 14 days.

[17] Effectiveness of erythromycin treatment is approximately 80%; a second course of therapy may be required.

[18] Pregnant patients allergic to penicillin should be treated with penicillin after desensitization.

[19] Vaginal, cervical, urethral meatal, oral, and anal warts may require referral to an appropriate specialist. Evaluate effectiveness after 3 treatments.

h.s. indicates at bedtime; IM, intramuscular; IV, intravenous; QOD, every other day

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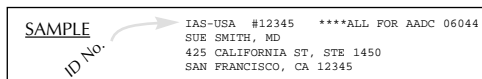
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Antiretroviral Drug Resistance Testing in Adults Infected with Human Immunodeficiency Virus Type 1: 2003 Recommendations of an International AIDS Society–USA Panel

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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-

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 ≥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 ≥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 ⩾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 — Degree of change, $\log_{10}$ copies/mL	P	HIV-1 RNA level less than the limit of detection — 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., fail-ure 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 resis-

tance 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 interrup-

tions 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 reevaluate 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.

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