A publication of the International AIDS Society-USA

# IMPROVING THE MANAGEMENT OF HIV DISEASE

#### IN THIS ISSUE—

#### Recent Advances In...

- HIV Pathogenesis
- Assessing Occupational Risk of HIV Infection
- Continuing/Changing Antiretroviral Therapy
- Plasma HIV RNA Quantitation

#### Plus ...

- Drug/Drug Interactions with Protease Inhibitors
- Viral Load Guidelines: Special Insert

## International AIDS Society-USA BOARD OF DIRECTORS

Paul A. Volberding, MD

Professor of Medicine University of California San Francisco San Francisco, California

Margaret A. Fischl, MD

Professor of Medicine University of Miami School of Medicine Miami, Florida

Harold A. Kessler, MD

Professor of Medicine and Immunology/Microbiology Rush Medical College Chicago, Illinois

Douglas D. Richman, MD

Professor of Pathology and Medicine University of California San Diego and San Diego Veterans Affairs Medical Center San Diego, California

Michael S. Saag, MD

Associate Professor of Medicine University of Alabama at Birmingham Birmingham, Alabama

Robert T. Schooley, MD

Professor of Medicine University of Colorado School of Medicine Denver, Colorado

#### In This Issue

n this issue of *Improving the Management of HIV Disease* selected presentations at the International AIDS Society-USA advanced courses in New York and Atlanta are summarized. Dr David D. Ho summarizes the recent findings on the pathogenesis of HIV infection and the possible implications these findings have for the treatment of the disease. Dr David M. Bell discusses the risk of occupational exposure to HIV infection among health care workers and issues in post-exposure prophylaxis. Dr Daniel R. Kuritzkes presents an update on the applications of quantitative plasma HIV RNA assays in clinical research and patient management. And Dr Michael S. Saag and Dr Kuritzkes discuss approaches to changing therapeutic regimens in patients who have failed or who are intolerant to their current treatment, an area that has become much more challenging as a greater number of drugs and combinations have recently become available.

In addition to the summaries from the IAS-USA courses, this issue includes a review article provided by Dr John G. Gerber on the important topic of drug/drug interactions with the protease inhibitor class of antiretrovirals.

Finally, this issue includes a reprint of the interim recommendations for the use of plasma HIV RNA assays in clinical practice. A panel of experts in the field was convened by the International AIDS Society-USA, and the recommendations of the panel were published in the June 1996 issue of *Nature Medicine*.

This publication is part of the ongoing efforts by the International AIDS Society-USA to provide timely, relevant information for physicians involved in HIV/AIDS care, with the goal of improving the treatment, medical care, and quality of life for people with HIV/AIDS.

#### About The International AIDS Society-USA

The International AIDS Society-USA (IAS-USA) is a 501 (c)(3) not-for-profit organization committed to improving the treatment, care, and quality of life of persons with HIV disease by providing balanced and relevant information to physicians. The IAS-USA programs are particularly intended to bridge clinical research and patient care.

The IAS-USA has developed several strategies for reaching this goal. In addition to this publication, the IAS-USA sponsors the ongoing CME conference series, *Improving the Management of HIV Disease*, in cities around the country.

If you are not on our mailing list and would like to receive this publication and announcements concerning upcoming programs, please contact us.

International AIDS Society-USA

353 Kearny Street San Francisco, CA 94108

(415) 675-7430 (phone) (415) 675-7438 (fax)

IASUSA1@aol.com (e-mail)

#### **New York Program Chairpersons**

**Gerald H. Friedland, MD**Yale University School of Medicine

**Paul A. Volberding, MD**University of California San Francisco

#### **Atlanta Program Chairpersons**

**Michael S. Saag, MD**The University of Alabama at Birmingham

Melanie A. Thompson, MD
AIDS Research Consortium of Atlanta

## IMPROVING THE MANAGEMENT OF HIV DISEASE

A publication of the International AIDS Society-USA

VOLUME 4 ISSUE 2

**JUNE 1996** 

#### **Editorial Board**

This publication is produced by the International AIDS Society-USA (IAS-USA). The views and opinions expressed in this publication are those of the conference participants and authors and do not necessarily reflect the views or recommendations of the IAS-USA, the conference joint sponsors, or the commercial companies providing unrestricted grant support. Unrestricted educational grant support for this publication was received from Bristol-Myers Squibb, Glaxo Wellcome, and Roche Laboratories. Additional unrestricted funding was received from Merck US Human Health. All symposia faculty and publication contributors have provided disclosures of financial interests, and this information is available from IAS-USA by request. This publication may contain information about the investigational uses of drugs or products that are not approved by the US Food and Drug Administration. Please consult full prescribing information before using any medication or product mentioned in this publication.



© International AIDS Society-USA 353 Kearny Street San Francisco, CA 94108 Phone: 415 675-7430 Fax: 415 675-7438 e-mail: IASUSA1@aol.com

Printed in USA

lune 1996

## CONTENTS

Presentation Summaries
HIV Pathogenesis
HIV kinetics in vivo Clearance of plasma virion and productively infected cells Implications for genetic variability Implications for treatment
Occupational Risk of HIV Infection in Health Care Workers
Documented cases of occupational HIV infection in health care workers Seroprevalence studies in health care workers Risk factors for transmission and risk estimates Assessment of risk by type of exposure Exposure prevention Postexposure prophylaxis
Plasma HIV RNA Quantitation
Current quantitative plasma HIV RNA assays Correlation of viral load with disease stage Correlation of viral load with progression Correlation of changes in viral load with progression in treatment trials Plasma HIV RNA assays in clinical practice
Strategies for Continuing Antiretroviral Therapy
Treatment failure Viral load thresholds in treatment Factors in changing treatment Nucleoside reverse transcriptase inhibitors in continuing therapy Clinical trials including antiretroviral-experienced patients Other potential combinations Individualizing therapy Current options
Review Article
Drug Interactions with HIV Protease Inhibitors
Announcements
Viral Load Markers in Clinical Practice: Recommendations of an International AIDS Society-USA Panel
IAS-USA Symposium in Vancouver 24

## **HIV PATHOGENESIS**

HIV pathogenesis was discussed at the New York course by David D. Ho, MD, from the Aaron Diamond AIDS Research Center in New York.

he notion that viral replication is an ongoing and dynamic process during the period of clinical latency of HIV infection has been established over the past several years by findings from sensitive assays for viral RNA and histologic studies of lymphoid tissue. It has become increasingly clear that relatively steady levels of viral load are maintained for months to years during clinical latency but differ widely in individual patients. Maintenance of a relative steady state suggests that during these periods, viral replication approximates viral clearance. Dr Ho and colleagues recently performed a number of studies in which viral replication was abruptly curtailed by administration of a potent antiretroviral to determine viral clearance and, thus, replication rates.

#### **HIV Kinetics In Vivo**

These studies used the protease inhibitor ritonavir in 20 patients followed during acute treatment with a sensitive branched-DNA (bDNA) assay for plasma HIV RNA. In these patients plasma HIV RNA levels dropped rapidly for the first several weeks, with a first-order exponential decrease in plasma HIV RNA levels following the initiation of treatment (Figure 1). Measuring the slope of decay allowed determination of viral half-life, estimated at approximately 2 days. This estimation, how-

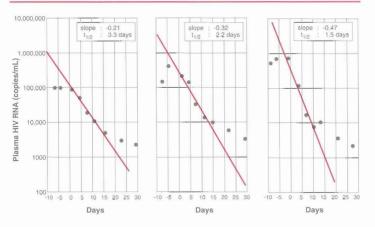


Figure 1. Plasma HIV RNA levels by duration of treatment in three patients. Adapted from Ho DD et al. Nature. 1995.

ever, is a composite of the half-life of the decay of free viral particles in the extracellular fluid space compartment and of the productively infected host cells (cellular compartment). A schematic summary of the kinetics of HIV infection in vivo in these compartments based on the findings in these initial studies is shown in Figure 2. Even with use of potent regimens, there is a residual viremia of approximately 0.1% to 1% of pretreatment levels, which indicates contribution to the viral pool by compartments that are as yet poorly understood.

#### Clearance of Plasma Virion and Productively Infected Cells

A subsequent study was performed in five patients to differentiate the free virion and infected cell clearance rates, with more frequent plasma HIV RNA measurements made to permit more detailed kinetic assessment. Mathematical modeling of these data showed that the half-life of free virus is 6 hours. This value was confirmed by an assay specific for infectious virus. Figure 3 compares findings from this assay with those measuring viral

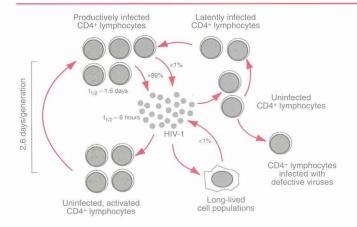


Figure 2. Schematic summary of HIV infection dynamics in vivo. The cell-free virion population sampled by plasma HIV RNA assays is shown in the center. Adapted from Perelson AS et al. Science. 1996.

particles in plasma. The decay of infectious particles is more rapid, although there is still a lag of several hours before decay is observed; Dr Ho believes that this delay is related to drug pharmacokinetics, with the effect on infectious virus being observed only after absorption and distribution of the drug into the intracellular compartment. Protease inhibitors act to prevent infectiousness of progeny virus. The noninfectious particles produced are counted in the plasma HIV RNA assay, but not in the infectious particle assay. Thus, clearance of only those infectious particles produced before drug administration is theoretically measured by the level of infectivity, reflecting what is believed to be the true viral clearance rate. Findings from this assay suggest that the half-life of virus particles in the extracellular fluid compartment is approximately 6 hours. On mathematical modeling, the decay half-life of infected cells actively producing virus is approximately 1.55 days, implying a prodigious turnover rate of susceptible host cells.

Rate of daily viral turnover. Using the body weight of the five subjects to derive plasma volume and extracellular fluid volume, the number of viral particles produced each day to maintain the quasi–steady state was calculated to be approximately 10 billion (10<sup>10</sup>). This figure is a minimum estimate since the calculated decay rate for the free virus reflects a minimum rate; moreover, this estimate reflects only those viral particles that are released into the extracellular fluid compartment (and are thus available for measurement), and not those virions produced within and never released from tissue. The number of particles

produced may be a few-fold or as many as 10- or even 100-fold higher. As noted, the viral levels during quasi-steady state differ in individual patients; recent data on the prognostic utility of plasma HIV RNA levels have shown a significant association between viral load and survival.

#### **Implications for Genetic Variabilty**

In the quasi-steady-state condition, there are successive generations of viral progeny, with each generation following the next by about 2.6 days. Approximately 140 generations of virions are produced over the course of a year and 1400 generations over the course of 10 years, allowing production of an extraordinary number of genetic variants. Some variants can provide preexisting drug-resistant forms or enable rapid development of resistance under drug pressure, and some enable the viral population to escape immune activity. The viral pool is estimated to be a minimum of 10<sup>10</sup> particles. It is known from experimental data that

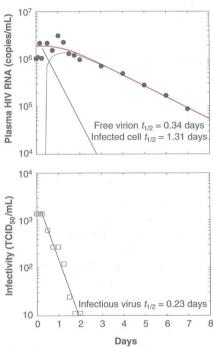


Figure 3. Assay of plasma viral particles (top) compared with assay of infectious virus (bottom). Adapted from Perelson AS et al. Science. 1996.

only 1 in 1000 particles is infectious, so the infectious viral pool may be on the order of  $10^7$  particles. At the reverse transcription stage, errors are made at the rate of 3 x  $10^{-5}$  per nucleotide per

cycle. With a genome of approximately 10<sup>4</sup> nucleotides, there is potential for generation of at least 10 million variants daily, ensuring that mutations will occur at every genome position on a daily basis.

#### **Implications for Treatment**

These findings have important implications for treatment. First, treatment should be initiated at a very early point in HIV disease to prevent the generation of genetic variants. Second, the data on the ability of the viral pool to develop mutations argue for the use of combination drug therapy rather than monotherapy. It is difficult for the virus to develop four or five mutations in a single genomic background simultaneously, and it thus makes sense to place the virus under pressure to do so by using a combination of drugs. Dr Ho and colleagues have recently begun to explore these implications clinically, by identifying acute seroconverters and either following them through the initial viremic phase or beginning aggressive treatment with combinations of protease inhibitors, zidovudine, and lamivudine. Although the data are very preliminary, they found that such treatment was capable of essentially eliminating the virus from the bloodstream in every patient who had been treated for at least 6 weeks. However, the 2- to 3log decreases in viral load that have been observed with combination treatment do not account for all of the virus present in an individual patient; there is a limit to the nadir achieved under treatment. This low-level residual viremia, as previously noted, may come from a number of different compartments. One potential source is latently infected CD4+ lymphocytes carrying infectious provirus; once activated, such cells become productively infected and the particles produced can be measured by quantitative assays. In addition, there may be cells that live for prolonged periods despite infection and that continuously produce measurable particles; such particles would be rendered noninfectious by protease inhibitor treatment (since these drugs act at the posttranscription stage) but would nevertheless be detected and measured by quantitative assays.

It is important for future studies to define the characteristics of decay in these viral compartments as well as in others that are less understood or have yet to be identified. Determination of the viral kinetics in these compartments is important because this virus appears to constitute the "embers" of infection, and can reignite robust replication if an antiretroviral regimen is withdrawn or becomes ineffective.

David D. Ho is Director of the Aaron Diamond AIDS Research Center and Professor, The Rockefeller University in New York, New York.

#### **Suggested Readings**

Clark SJ, Saag MS, Decker WD, et al. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *N Engl J Med.* 1991;324:954–960.

Embreton J, Zupancic M, Ribas JL, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature*. 1993;362:359–362.

Fauci AS. Multifactorial nature of human immunodeficiency virus disease: implications for therapy. *Science*. 1993;262:1011–1018.

Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N Engl J Med.* 1989;321:1621–1625.

Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature*. 1995;373:123–126.

Pantaleo G, Graziosi C, Demarest JF, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature*. 1993;362:355–358.

(continued)

Pantaleo G, Menzo S, Vaccarezza M, et al. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. *N Engl J Med.* 1995;332:209–216.

Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate Infected cell lifespan, and viral generation time. *Science*. 1996;271:1582–1586.

Piatak M Jr, Saag MS, Yang LC, et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science*. 1993;259:1749–1754.

Saag MS. What to do when zidovudine fails. Editorial. *N Engl J Med.* 1994;330:706–707.

Saag MS, Crain MJ, Decker WD, et al. High-level viremia in adults and children infected with human immunodeficiency virus: relation to disease stage and CD4+ lymphocyte levels. *J Infect Dis*. 1991;164:72–80.

Saag MS, Emini EA, Laskin OL, et al. A short-term clinical evaluation of L-697,661, a nonnucleoside inhibitor of HIV-1 reverse transcriptase. *N Engl J Med.* 1993;329:1065–1072.

Wain-Hobson S. AIDS: virological mayhem. *Nature*. 1995;373:102.

Wei X, Ghosh SK, Taylor ME, et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature*. 1995;373:117–122.

## OCCUPATIONAL RISK OF HIV INFECTION IN HEALTH CARE WORKERS

The following is a summary of the presentation on the occupational risk of HIV infection given at the Atlanta course by David M. Bell, MD, from the Centers for Disease Control and Prevention in Atlanta, Georgia.

## **Documented Cases of Occupational HIV Infection in Health Care Workers**

ccording to statistics from the Centers for Disease Control and Prevention (CDC) as of December 1994, 4.8% of adults with AIDS reported a history of employment in a health care setting. Since 7.7% of the labor force in the United States is employed in health services, health care workers do not appear to be overrepresented in cases of AIDS. As of December 1994, there were 42 documented cases of occupationally-acquired HIV infection in health care workers and 91 cases of possible occupational transmission, with the time or source of infection remaining undocumented in these persons (provisional numbers as of December 1995 are 49 documented cases and 102 possible cases). Of the 42 health care workers with documented cases as of 1994, 38 were exposed to infected blood, 2 were exposed to concentrated virus in a laboratory, 1 was exposed to visibly bloody body fluid, and 1 was exposed to an unspecified body fluid. In total, 36 cases involved percutaneous exposure, four involved mucocutaneous exposure, and one involved both types of exposure, with the exposure route in one laboratory transmission remaining uncertain. Of the 37 percutaneous exposures, 34

involved hollow-bore needles and one each involved a scalpel, a broken vial, and an unknown sharp object. The disease status of the 40 HIV infection-source patients consisted of AIDS in 29 (72%), asymptomatic infection in 4 (10%), symptomatic infection in 2 (5%), and unknown status in 5 (12%). Of the 42 health care workers with documented cases, 15 were clinical laboratory technicians (primarily phlebotomists), 13 were nurses, 6 were physicians, 2 were surgical technicians, 2 were nonclinical laboratory workers, 1 was a respiratory therapist, another was a health aide, 1 was a housekeeper/maintenance worker, and 1 was a dialysis technician.

#### Seroprevalence Studies in Health Care Workers

Seroprevalence studies in health care workers have consistently documented a very low positive rate when persons with known nonoccupational risk factors are excluded. Table 1 shows results of HIV seroprevalence studies in selected groups of health care workers. Extensive seroprevalence studies have also been conducted in dentists showing low rates of infection. One drawback of these seroprevalence studies is that the extent of exposure to HIV in most of the workers tested is unknown. These surveys are nonetheless helpful in that they do not suggest a high rate of previously undetected infection in those health care workers studied.

#### **Risk Factors for Transmission and Risk Estimates**

Another way to assess risk of occupationally-acquired infec-

TABLE 1. HIV SEROPREVALENCE IN SELECT GROUPS OF HEALTH CARE WORKERS.

Source	Worker group	No. tested	No. positive (%)	Prevalence* (%)
<b>Tokars.</b> <i>JAMA</i> . 1992.	Orthopedic surgeons	3420	2 (0.06)	0 (0)
Panlilio. J Am Coll Surgeons. 1995.	Surgeons in high-AIDS areas	770	1 (0.13)	1 (0.14)
Cowan. <i>JAMA</i> . 1991.	Physicians and dentists: US Army Reserve	3347	3 (0.09)	NA
5 Studies	Hemodialysis staff: NY, Paris, Chicago, Brussels, Florence	356	0 (0)	0 (0)
Chamberland. Ann Intern Med. 1994.	HCW blood donors: 6 urban US regions	8519	3 (0.04)	0-1 (0-0.01)
Turner. Am J Public Health. 1989. Gershon. Infect Control Hosp Epidemiol. 1995.	Morticians: Massachusetts, Maryland	259	2 (0.77)	0 (0)

<sup>\*</sup>Excluding health care workers with known risks.

tion is to construct estimates based on the prevalence of HIV infection in the patients with whom the health care worker is involved, the risk of infection transmission following a blood exposure, and the nature and frequency of such exposures. HIV seroprevalence varies widely in different patient populations. The CDC study by Janssen and colleagues involved testing of blood remaining from a sample of patients in 20 acute care hospitals in 15 cities in 1990, with seroprevalences ranging from 0.2% to 14.2%. The seroprevalence rate was 10.4

Table 2. HIV Seroconversion Rates in Health Care Workers **Enrolled in Prospective Studies After Percutaneous** Exposure to Infected Blood.

Source*	Location	No. enrolled	No. seroconverted	Seroconversion rate/100 HCWs
Cardo	United States	1391	4	0.29
Ippolito	Italy	1546	3	0.19
Lot	France	592	3	0.51
Arranz	Spain	883	2	0.23
23 Studies		6135	20	0.33 (95% CI: 0.20–0.50)

<sup>\*</sup>Presented as abstracts. HCWs, health care workers; CI, confidence interval.

times the rate of new diagnoses of AIDS per 1000 hospital discharges. This finding provides a way for acute care facilities to estimate seroprevalence in their respective populations; however, it must be remembered that the data from which this finding was derived are from 1990.

The risk of infection following a single percutaneous exposure to HIV-infected blood has been estimated in prospective studies to be about 1 in 300 (0.3%) (Table 2). In six studies involving 1143 mucous membrane exposures, 1 case of seroconversion (0.09%) was observed (95% CI, 0.002% to 0.50%). In a National Institutes of Health (NIH) study, none of 2712 workers who recalled skin contact with infected blood seroconverted, so that the rate was reported to be 0%, with a 95% CI of 0.0% to 0.11%.

The third determinant of occupational risk is frequency of exposure. Prospective studies in various occupations have provided summary estimates of percutaneous, mucocutaneous, or skin contacts with blood and the number of sharp object-related injuries per year. Using data such as these and a number of different methodologies, several authors have estimated cumulative occupational risks of HIV infection for various occupations. As shown in Table 3 these estimates include 1 worker every 3.5 years in the 130 Veterans Administration (VA) hospitals nationwide and rates of 0.008% to 0.026% per year in emergency department workers in high HIV prevalence locales. The estimate of 27 to 46 cases per 100,000 among residents and students at the Los Angeles County Medical Center was comparable to the estimated risk of death from motor vehicle accidents of 32 per 100,000 in the same age group.

It should be noted that these estimates are subject to many limitations, including simplified assumptions and incomplete data. Regarding the estimate by Henry and colleagues of 8 cases of occupationally-acquired HIV infection in hospital workers in 1990, Dr Bell noted that in that same year the CDC received reports of five hospital workers who had documented infection from hollow-bore needle injuries, and that the number of possible cases (the dates of which could not be precisely determined) may have brought the total number of cases for 1990 close to the estimated 8. It is the similarity in the order of magnitude that is important rather than the precise number of cases.

#### Assessment of Risk by Type of Exposure

The calculated average risk of percutaneous exposure underestimates the risk of some types of exposure and overestimates it for others. Prospective studies do not have sufficient statis-

tical power to analyze risk of infection based on such factors as volume of blood or disease stage of HIV-infected source patients. As an alternative, some investigators have performed laboratory studies to quantify the amount of blood that may be injected in different needle-stick accidents. One such study conducted by Gerberding and colleagues assessed the amount of blood transferred across a filter-paper barrier by a solid 2-0 suture needle (0.69 mm diameter) and by an 18-gauge hollow needle (1.27 mm diameter) penetrating no layers of gloves or 1 or 2 layers of gloves to a depth of 5 mm through the filter. With no gloves, hollow needles injected approximately twice the volume of blood as did solid needles. One glove reduced the injection volume by approximately 6-fold for the solid needle and by about one half for the hollow needle. A further marginal reduction was seen when a

Table 3. Estimates of Cumulative Occupational Risk of HIV Infection in US Health Care Workers.

Source	Population	Estimate
Henry. Minn Med. 1995.	Workers in US hospitals	8 cases in 1990
US General Accounting Office	Workers in 130 VA hospitals	1 worker every 3.5 y
<b>Gerberding.</b> N Engl J Med. 1990.	Surgical staff at San Francisco General Hospital	1 worker every 8 y
<b>Marcus.</b> Am J Med. 1993.	Emergency department workers: - high patient HIV prevalence - low patient HIV prevalence	0.008%-0.026%/y 0.005%-0.018%/y
O'Neill. Arch Intern Med. 1994.	Residents and students: Los Angeles County Medical Center	0.027%-0.046%/y

VA, Veterans Administration.

second layer of gloves was used with each type of needle. The investigators concluded that risk of infection was reduced when exposure involved solid needles and when gloves were worn, but noted that the amount of blood injected differed by less than a factor of 10. They further concluded that the amount of blood injected was likely to be less important as a determinant of risk than the concentration of HIV in the blood of the source patient.

Dr Bell and colleagues at the CDC recently published exposure risk estimates based on a case-control study that compared 31 cases of occupationally-acquired HIV infection in the United States, France, and the United Kingdom (January 1988 to August 1994) with 679 health care workers from a CDC needle-stick study who had percutaneous exposure to infected blood but did not seroconvert. The independent risk factors for HIV infection following exposure determined in a multivariate analysis are shown in Table 4. Factors associated with significantly increased risk of infection included deep injury (in most cases subjectively assessed), which was associated with the highest risk; visible blood on device prior to injury; and procedures involving a needle placed directly into a vein or artery of the source patient (eg, as opposed to a needle used in a heparin lock or IV tubing or in a subcutaneous injection). These three risk factors are likely surrogates for volume of blood injected. The other independent significant risk factor for infection was terminal illness in the source patient, with this factor likely being associated with high viral titer and perhaps characteristics of the virus in late-stage disease. A factor associated with a significantly decreased risk of infection was postexposure use of zidovudine, with an odds ratio of 0.21 indicating that such use was associated with a 79% reduction in risk following exposure.

#### **Exposure Prevention**

Prospective studies of injury during surgery have shown that at least one injury occurs in 1% to 7% of procedures and that approximately three fourths of injuries are associated with suture needles. A major intervention that has been studied by the CDC and other investigators is the use of blunt-tip suture needles. Blunt needles are useful for suturing a variety of tissues, but are less than optimal for suturing of skin and fragile tissue (eg, vascular tissue). In a CDC study of blunt needle use in gynecologic surgery (a specialty with one of the highest needle-stick rates), in three New York City hospitals use of a blunt needle increased markedly after an introductory period, with injuries per 100 procedures decreasing dramatically as use of a blunt needle increased. A number of other promising devices for surgical use have recently been developed, including electrocautery, stapling, and finger protective devices.

Other devices with safety features to prevent needle-stick injury have been introduced for procedures involving hollow needles, such as phlebotomy or starting an IV access. The risk of injury in phlebotomy, for example, is not limited to the person drawing the blood; most injuries in these settings occur after the needle has been used and is set aside or improperly disposed of, with housekeeping workers and others also being at risk of injury. Studies of these devices conducted by the CDC and other investigators have shown a potential for significant risk reduction. It should be noted, however, that these safety devices can be expen-

Table 4. Independent Risk Factors for HIV
Infection After Percutaneous Exposure
to Infected Blood.

Risk factor	Adjusted odds ratio (95% CI)	P-value
Deep injury	16.09 (6.11–44.57)	<.0001
Visible blood on device	5.22 (1.79–17.69)	.004
Insertion of needle directly into a vein or artery	5.11 (1.94–14.82)	.002
Terminal illness in source patient	6.39 (2.22–18.87)	.001
Postexposure use of zidovudine	0.21 (0.06–0.57)	.005

CI, confidence interval. Adapted from Centers for Disease Control and Prevention. *MMWR*. 1995.

sive, and workers have to be trained in their use. As an example of practical problems that can arise with introduction of novel devices, an increase in bloodstream infections has reportedly accompanied use of needleless IV systems. These infections have occurred in cases in which the device was left in place for prolonged periods; this problem appears not to be related to the device itself but to failing to institute or maintain infection control techniques appropriate for using the device.

#### Postexposure Prophylaxis

Results from animal studies of postexposure prophylaxis with zidovudine, the only antiretroviral agent to be studied in any detail to date in this regard, have not been conclusive. These studies have been hampered, however, by the absence of a good animal model of HIV infection and by the fact that the inocula given in existing models have been large (to ensure adequate challenge) and frequently delivered by routes not characteristic of occupational exposures in humans (eg, intrathymically). These animal studies, however, have revealed that if a postexposure agent is to work at all, it must be given promptly—eg, within several hours-after exposure. The data available indicate that treatment begun later than 24 to 48 hours after inoculation will prove ineffective. A second important finding is that treatment did not prevent infection in some animal studies, but suppressed it. The possibility of this phenomenon occurring in humans must also be considered.

Despite these findings from animal models, there is evidence (eg, in the case-control study mentioned above) that postexposure use of zidovudine in humans decreases risk of infection. Findings from ACTG protocol 076 regarding maternal-fetal transmis-

sion also documented a highly significant effect of zidovudine in preventing transmission. There is some evidence that this benefit was related to a protective effect from the drug as well as to a decrease in viral load achieved with treatment. Recommendations regarding chemoprophylaxis after occupational exposure to HIV are provided by an interagency working group composed of rep-

resentatives of the CDC, FDA, NIH, and HRSA (see *MMWR*, 1996 below).

David M. Bell is Chief of the HIV Infections Branch, Hospital Infections Program, of the Centers for Disease Control and Prevention in Atlanta, Georgia.

#### **Suggested Readings**

Centers for Disease Control and Prevention. Case-control study of HIV seroconversion in health-care workers after percutaneous exposure to HIV-infected blood—France, United Kingdom, and United States, January 1988–August 1994. MMWR. 1995:44:929-933.

Centers for Disease Control and Prevention. Public Health Service statement on management of occupational exposure to human immunodeficiency virus, including considerations regarding zidovudine postexposure use. *MMWR*. 1990;39.No. RR-1.

Centers for Disease Control and Prevention. Update: provisional Public Health Service recommendations for chemoprophylaxis after occupational exposure to HIV. MMWR. 1996;45:468–472.

Gerberding JL. Management of occupational exposures to blood-borne viruses. *N Engl J Med.* 1995;332:444–451.

Henderson DK, Fahey BJ, Willy M, et al. Risk for occupational transmission of human immunodeficiency virus type 1 (HIV-1) associated with clinical exposures: a prospective evaluation. *Ann Intern Med.* 1990;113:740–746.

Niu MT, Stein DS, Schnittmann SM. Primary human immunodeficiency virus type 1 infection: review of pathogenesis and early treatment interventions in humans and animal retrovirus infections. *J Infect Dis.* 1993;168:1490–1501.

Puro V, Ippolito G, Guzzanti E, et al. Zidovudine prophylaxis after accidental exposure to HIV: the Italian experience. *AIDS*. 1992;6:963–969.

Tokars JI, Marcus R, Culver DH, et al. Surveillance of HIV infection and zidovudine use among health care workers after occupational exposure to HIV-infected blood. *Ann Intern Med*. 1993;118:913-919.

## PLASMA HIV RNA QUANTITATION

Plasma HIV RNA quantitation was discussed at the New York course by Daniel R. Kuritzkes, MD, from the University of Colorado Health Sciences Center in Denver.

he results of recent studies on viral dynamics performed with plasma HIV RNA quantitative assays have had important implications for monitoring and treating HIV infection. Viral load is proportionate to viral production, which, in turn, is proportionate to CD4+ lymphocyte destruction, although the relationship may vary in individual patients. The importance of halting viral replication in treatment has been underscored by recent findings suggesting that there is a lifetime limit to the number of CD4+ cells that an individual can generate. Overall, data available on the correlation of viral load with disease progression suggest that suppressing viral replication as completely as possible and as early in infection as possible may be the most effective treatment strategy. At present, there are insufficient data, particularly from randomized clinical trials, to support specific treatment recommendations based on viral load levels; however, the data accumulated to date have provided a coherent picture of the implications of viral load measurements that permits general recommendations for treatment and monitoring. The potential uses of quantitative plasma HIV RNA assays in the clinical setting include assessing prognosis, monitoring drug activity following initiation of antiretroviral treatment, monitoring extent and duration of viral suppression, and assessing the effects of changes in

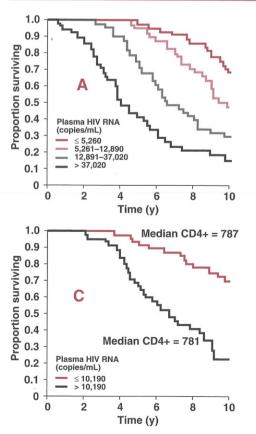
treatment. Interim guidelines for the use of viral load measurements in clinical practice have been developed by an International AIDS Society-USA appointed panel, and have been published in the June 1996 issue of *Nature Medicine*.

## **Current Quantitative Plasma HIV RNA Assays**

At present, there are three methods available for measuring plasma HIV RNA levels: reverse transcriptase polymerase chain reaction (RT-PCR), branched DNA (bDNA) assay, and nucleic acid sequence-based amplification (NASBA). In the RT-PCR assay (which was approved by the Food and Drug Administration [FDA] in June) HIV RNA is extracted from plasma, copied into DNA by RT, then amplified by PCR using HIVspecific primers. The amplified product is detected by hybridization and quantitated to yield the number of HIV RNA copies/mL in the original sample. In the bDNA assay, virus particles in plasma are pelleted by high-speed centrifugation; the RNA is extracted and captured by hybridization to oligonucleotides bound to a 96-well plate. The captured RNA is "decorated" with alkaline phosphatase molecules by several additional cycles of hybridization using branched enzyme-conjugated oligonucleotide probes. This complex is incubated with a chemiluminescent substrate and the liberated light signal is quantitated by a luminometer. The NASBA assay is similar in concept to the RT-PCR assay but uses somewhat different reaction components to amplify target sequence. In general, the results obtained with these three types of assays have been shown to be quite similar. Although each of these assays provides comparable results and reliability, direct comparisons of values from the different assays are difficult in the absence of a uniform standard.

#### Correlation of Viral Load With Disease Stage

Relatively stable plasma HIV RNA levels are maintained during the short term in the quasi-steady state; significant changes can be attributed to either therapeutic interventions or de facto increases in viral replication. The plasma HIV RNA assays have been shown to have prognostic value in several respects. Plasma HIV RNA levels correlate with HIV disease stage, inversely correlate (in general) with CD4+ cell count, to be predictive of disease progression after seroconversion, and to be independently predictive of disease progression in later disease. In the AIDS Clinical Trials Group (ACTG) protocol 116B/117,



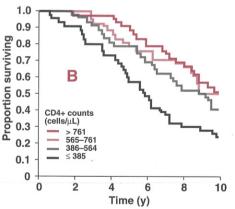


Figure 1. Kaplan-Meier survival curves of asymptomatic patients followed up from seroconversion. Patients were stratified by quartiles of the mean of the first two plasma HIV RNA measurements (A) and of the mean of the first two CD4+ cell counts (B). Kaplan-Meier survival curves shown in (C) are stratified by median plasma HIV RNA level in patients with baseline CD4+ cell counts ≥500/µL from a subset of the Multicenter AIDS Cohort Study (MACS). Adapted from Mellors JW et al. Science. 1996.

Table 1. Risk of Disease Progression Associated with Measured Changes in Plasma HIV RNA Levels as Reported in Major Clinical Trials.

Study/Marker effect (Reference)	No. of subjects	Median CD4+ count at entry (cells/µL)	Adjusted relative risk (95% CI)
ACTG 116B/117/ 2-fold (0.3 log <sub>10</sub> ) decrease* (Coombs et al. <i>J Infect Dis.</i> 1996.)	100	86	0.73 (0.52, 1.02)
ACTG 116A/ 2-fold (0.3 log <sub>10</sub> ) increase* (Welles et al. <i>J Infect Dis.</i> 1996.)	187	139	1.45 (1.02, 2.05)
VA CS298/ 4-fold (0.6 log <sub>10</sub> ) decrease* (O'Brien et al. <i>N Engl J Med.</i> 1996.)	270	350	0.44 (0.23–0.81)

<sup>\*</sup>Decrease/increase from pretreatment value. CI, confidence interval.

there were significant differences in HIV RNA copy numbers observed in asymptomatic patients (about 35,000 copies/mL), symptomatic patients (about 70,000 copies/mL), and in patients with clinically-defined AIDS (about 140,000 copies/mL). The intraindividual correlation (r) of repeated baseline plasma HIV RNA values in patients in the ACTG immunology protocol 209 was 0.89, indicating there was little variation in individual values over the short term. In addition, although there was a general correlation between initial plasma HIV RNA levels and CD4+ cell counts in patients enrolled in ACTG 209, there was significant splay of HIV RNA copy numbers at any given CD4+ cell count. This phenomenon has been repeatedly observed in studies correlating plasma HIV RNA level and CD4+ cell count, including analysis of patients in the virology substudy of ACTG protocol 175. Overall, it has been found that there is an approximately 3-log range in viral loads for each CD4+ count. This heterogeneity is one important reason why both plasma HIV RNA and

Table 2. Viral Load and HIV Disease Progression in Patients Enrolled in the NUCA 3001/3002 Studies.

Marker	Relative risk (95% CI)
Asymptomatic → CDC B/AIDS  Plasma HIV RNA (log <sub>10</sub> increase)  CD4+ cell count (50% decrease)	1.75 (1.23, 2.50) 1.39 (0.93, 2.04)
CDC B $\rightarrow$ AIDS Plasma HIV RNA ( $\log_{10}$ increase) CD4+ cell count (50% decrease)	3.19 (1.19, 8.57) 3.12 (1.45, 8.67)

CI, confidence interval.

Data presented by Phillips AN et al at Third Conference on Retroviruses and Opportunistic Infections; January 28–February 1, 1996; Washington, DC. Abstract 32.

CD4+ cell count, which have been found in other studies to have independent predictive values, are useful markers and should be used together to monitor HIV disease progression.

## **Correlation of Viral Load with Progression**

Plasma HIV RNA levels have been correlated with rates of HIV disease progression in a number of natural history and treatment studies. In a subset of the Multicenter AIDS Cohort Study (MACS) evaluated by Mellors and colleagues, asymptomatic subjects enrolled after sero-conversion were placed in quartiles on the basis of baseline plasma HIV RNA levels, and the rate of progression to AIDS and death over subsequent intervals was analyzed. In the quartiles ranging from the lowest (<4530 copies/mL) to the highest

baseline plasma HIV RNA values (>36,270 copies/mL), 8%, 26%, 49%, and 62% of patients progressed to AIDS by 5 years, respectively. The proportions of patients who died within 5 years were 5%, 10%, 25%, and 49%, respectively. Figure 1A shows a Kaplan-Meier analysis of survival by quartiles based on the mean plasma HIV RNA measurement from the first two clinic visits. These data indicate an extraordinary ability of initial plasma HIV RNA levels to discriminate risk of death very early in the course of disease. Also, the data show that even though patients with lower viral loads progress more slowly, progression still occurs. It does not appear that there is a specific threshold of plasma HIV RNA levels below which patients do not progress. A similar analysis by CD4+ cell count quartile (Figure 1B) showed that the quartile with the lowest counts could be discriminated on the basis of an increased risk, but that the remaining three quartiles could not be distinguished on this basis.

In another analysis, patients with baseline CD4+ counts greater than or equal to 500 cells/µL were grouped according to baseline viral loads above or below about 10,000 HIV RNA copies/mL, the median value for the subpopulation. The average CD4+ count was 780 cells/µL. As shown in Figure 1C, there was a significantly reduced rate of death in those patients with plasma HIV RNA levels less than about 10,000 copies/mL, with a median time to death not having been reached after 10 years, compared with a median survival time of 6.8 years in those HIV-infected patients with higher viral loads. According to Dr Kuritzkes, the exact progression rate beyond 10 years is difficult to determine reliably because of the small numbers of patients who have been followed for such a duration. Analysis of patients' plasma HIV RNA levels at given intervals before they developed AIDS showed an increase in median viral load values with a decrease in the interval of time to the development of AIDS; in contrast, patients whose disease did not progress exhibited relatively stable viral loads.

This correlation between pretreatment plasma HIV RNA levels and disease progression has also been demonstrated in large

Table 3. Use of the Plasma HIV RNA Assays in Clinical Practice: Interim Recommendations of an International AIDS Society-USA Panel.

Parameter	Recommendation
Plasma HIV RNA level that suggests initiation of treatment	<ul> <li>&gt;5,000–10,000 HIV RNA copies/mL and a CD4+ count/clinical status suggestive of progression</li> <li>&gt;30,000–50,000 HIV RNA copies/mL regardless of laboratory/ clinical status</li> </ul>
Target level of plasma HIV RNA after initiation of treatment	• Undetectable; <5,000 HIV RNA copies/mL is an acceptable target
Minimal decrease in plasma HIV RNA level indicative of antiretroviral activity	• >0.5-log decrease
Change in plasma HIV RNA level that suggests drug treatment failure	• Return toward (or within 0.3–0.5 log of) pretreatment value
Suggested frequency of plasma HIV RNA measurement	<ul> <li>At baseline, take two initial measurements 2 to 4 weeks apart</li> <li>Repeat assessments every 3 to 4 months, in conjunction with CD4+ cell counting</li> <li>Measure at shorter intervals as critical thresholds for decision points are neared</li> <li>Measure 3 or 4 weeks after initiating or changing treatment</li> </ul>
Optimal specimen handling	<ul> <li>All samples should be processed and frozen within 6 hours to ensure that no significant signal degradation occurs</li> <li>The anticoagulant recommended for the particular assay type should always be used and samples should always be of plasma (not serum)</li> <li>The same single assay type (eg, bDNA, RT-PCR, or NASBA) should always be used for the same individual patient</li> <li>Specimens for individual patients should always be handled in a consistent manner</li> </ul>

Adapted from Saag MS et al. Nature Med. 1996.

clinical trials in patients with advanced disease (ACTG 116B/117) and in those with more intermediate disease (ACTG 175).

## **Correlation of Changes in Viral Load with Progression in Treatment Trials**

Studies assessing the effect of changes in viral load on disease progression in HIV-infected patients given antiretroviral treatment have all yielded the same general conclusion: "Less virus is better for the patient and reducing viral load is good." The Veterans Affairs Cooperative Study 298 reported several years ago compared immediate with delayed zidovudine treatment in patients with minimal or no symptoms and CD4+ counts greater than 200 cells/µL. O'Brien and colleagues recently reported the virologic/immunologic analyses from this study, and detailed the effect of treatment-related changes in plasma HIV RNA levels and CD4+ cell counts on the risk of progression. Multivariate analysis of changes between pretreatment and week 8 of treatment showed that each 4-fold (75%, or 0.6-log) decrease in plasma HIV RNA levels was associated with a 50% to

55% decreased risk of disease progression (P = .0086); similarly, each 10% increase in CD4+ cell count was associated with a 50% reduction in the risk of progression (P = .0069). These two factors together accounted for virtually all of the beneficial effects of treatment noted in delaying disease progression; once the effects of these two factors had been accounted for, the timing of initiation of treatment proved to have no effect on risk of progression (relative risk for early vs delayed treatment, 1.06; P = .76). In ACTG protocols 116A and 116B/117, each 50% decrease in viral load in response to treatment was associated with a 33% reduction in the rate of disease progression; CD4+ cell count increases were also a strong predictor of favorable clinical response to treatment. Similarly, in ACTG protocol 175, a 1-log decrease in plasma HIV RNA was associated with a statistically significant 70% to 80% reduction in disease progression independent of the actual pretreatment plasma HIV RNA level, pretreatment CD4+ cell count, and change in CD4+ cell count. In contrast, changes in CD4+ cell counts noted up to week 8 of treatment

were not significantly associated with changes in the risk of HIV disease progression.

Table 1 summarizes the findings in three major trials that examined the effects of treatment-related changes in plasma HIV RNA on progression. Overall, the data from ACTG protocols 116A and 116B/117 (represented in the table as risk of progression in the absence of plasma HIV RNA reduction of a given magnitude) indicate a 30% reduction in the risk of clinical progression with each 2-fold (0.3-log) decrease in plasma HIV RNA levels. The data from The Veterans Affairs Cooperative Study 298 show a 55% decrease in risk of disease progression for each 0.6-log decrease in viral load. In addition to these studies, data from ACTG protocol 175 indicate an approximately 65% decrease for each 10-fold (1-log) decrease in viral load. The data from these studies demonstrate that the degree of clinical benefit can be predicted by the magnitude of reduction in plasma HIV RNA levels. The magnitudes of reduction used in analyzing these clinical trial populations generally were those that were convenient to examine statistically, particularly given the statistical power generated by having large numbers of data points from large numbers of patients. Focusing on the magnitude of decreases for which the statistical associations were reported in these study patients is not necessary in clinical practice—ie, at present, there is no reason to believe that a 50% decrease in viral load represents a specific 'threshold.' It is necessary, however, to remember that because of intraassay and biologic variation, an approximately 0.5-log decrease in plasma HIV RNA levels is taken as minimal evidence of a true antiretroviral effect.

Increases in viral load during treatment have been associated with an increased risk of disease progression. In an analysis of patients from NUCA 3001/3002, the North American trials that evaluated zidovudine and lamivudine monotherapy and combination therapy, the effects of increases in plasma HIV RNA levels from the lowest levels observed after treatment initiation on

clinical progression were examined (Table 2). Progression from asymptomatic disease to the Centers for Disease Control and Prevention (CDC) stage B HIV disease and from CDC stage B HIV disease to AIDS was assessed at 24 to 52 weeks following the treatment-associated plasma HIV RNA nadir value. It was found that there was a 3-fold increased risk of progression for each 1log increase in plasma HIV RNA from the nadir value.

#### Plasma HIV RNA Assays in Clinical Practice

Issues that require more detailed investigation include: (1) Is there a threshold plasma HIV RNA level for starting treatment? (2) Is there a threshold reduction for treatment benefit? (3) Is there a threshold loss of suppression for withdrawing treatment ie, in late-stage patients in whom no antiretroviral regimen appears to reduce viral load?

Despite the need for increasingly refined guidelines, sufficient data are now available to suggest that plasma HIV RNA assays should be performed routinely as part of the initial evaluation of HIV-infected patients to aid in assessing their stage of disease and risk of progression, and may prove useful in assessing response to treatment in individual patients (see Table 3). The data available support the contention that treatment should be considered in patients with viral loads greater than 5000 to 10,000 HIV RNA copies/mL of plasma and CD4+ counts/clinical status suggestive of progression. Therapy should be initiated in patients with greater than 30,000 to 50,000 HIV RNA copies/mL, regardless of clinical or laboratory status. According to Dr Kuritzkes, the important issue in deciding on treatment goals with assay use is not determining the minimal effect necessary for benefit, but to strive to achieve the maximal level of viral suppression (ie, viral loads below the limits of assay detection).

Daniel R. Kuritzkes is Associate Professor of Medicine and Microbiology at the University of Colorado Health Sciences Center in Denver.

#### **Suggested Readings**

Cao Y, Ho DD, Todd J, et al. Clinical evaluation of branched DNA signal amplification for quantifying HIV type 1 in human plasma. AIDS Res Hum Retroviruses. 1995;11:353-361.

Coombs RW, Welles SL, Hooper C, et al. Association of plasma human immunodeficiency virus type-1 RNA level with risk of clinical progression in patients with advanced infection. J Infect Dis. 1996. (In press.)

Embreton J, Zupancic M, Ribas JL, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature. 1993;362:359-362.

Fauci AS. Multifactorial nature of human immunodeficiency virus disease: implications for therapy. Science. 1993;262:1011-1018.

Hammer SM, Katzenstein DA, Hughes MD, et al. Virologic markers and outcome in ACTG 175. Presented at Third Conference on Retroviruses and Opportunistic Infections; January 28-February 1, 1996; Washington, DC.

Havlir DV, Richman, DD. Viral dynamics of HIV: implications for drug development and therapeutic strategies. Ann Intern Med. 1996;124:984-994.

Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N Engl J Med. 1989;321:1621-1625.

Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature. 1995;373:123-126.

Holodniy M, Katzenstein DA, Sengupta S, et al. Detection and quantification of human immunodeficiency virus RNA in patient serum by use of the polymerase chain reaction. I Infect Dis. 1991;163:862-866.

Holodniy M, Mole L, Winters M, Merigan TC. Diurnal and shortterm stability of HIV virus load as measured by gene amplification. J Acquir Immune Defic Syndr.1994;7:363-368.

Kappes JC, Saag MD, Shaw GM, et al. Assessment of antiretroviral therapy by plasma viral load testing: standard and ICD HIV-1 p24 antigen and viral RNA (QC-PCR) assays compared. Acquir Immune Defic Syndr Hum Retrovir. 1995;10:139-149.

Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science. 1996;272:1167-1170.

(continued)

## HIV viral load markers in clinical practice

Plasma HIV RNA determinations are an important prognostic marker of disease progression and, when used appropriately, provide a valuable tool for the management of individual patients. But what constitutes appropriate use?

The development of new molecular techniques designed to detect circulating virion-associated HIV RNA in plasma has created an opportunity to study viral dynamics and HIV pathogenesis in substantial detail. Prior beliefs, based on the concept of

a prolonged phase of relative virologic latency in the period before symptoms become evident have been replaced by a new paradigm of ongoing, high-level viral replication from the time of initial infection until death (Fig. 1). Indeed, as many as 10 billion new HIV virions are produced per day, with a half-life in plasma of 6 hours<sup>1,2,3</sup>. CD4<sup>+</sup> lymphocytes, one of the principal cell targets responsible for viral replication *in vivo*, are also produced in high numbers and, once productively infected, have a half-life of about 1.6 days. The life-cycle of the virus, from infection of one cell to the production of new progeny, which infects the next cell, is 2.6 days<sup>3</sup>. This extraordinarily high level of viral replication, cell destruction and cell replacement has led to a dramatic shift in clinical management of HIV-infected patients, and, in particular, the use of antiretroviral therapy.

Before the development of these new molecular techniques, quantitative culture of peripheral blood mononuclear cells (PBMCs) or plasma was used to estimate the infectious titer of HIV in the blood<sup>4,5</sup>. Increasing plasma virus titers were associated with clinical progression while decreases in plasma virus accompanied treatment with active drugs. However, fewer than 50% of patients with CD4<sup>+</sup> counts greater than 200 cells/µl had positive plasma cultures, and inherent biologic variability in virus quantitation required that a 25-fold (approximately 1.4 log) increase was seen before it was likely to be clinically meaningful. In contrast, HIV RNA detection techniques revealed measurable virus in the plasma of virtually all HIV-infected patients regardless of clinical stage<sup>6</sup>. Moreover, plasma RNA levels exhibited a wide dynamic range, correlated significantly with clinical stage, and fell rapidly following the initiation of effec-

tive antiretroviral therapy. Despite these promising attributes, it was not known whether plasma virus levels could be accurately and reproducibly measured in a clinical setting or whether they would be predictive of clinical outcome. Recent findings indicate that plasma HIV RNA determinations are an important prognostic marker of disease progression and provide a valuable tool for the management of individual patients.

#### The assays

Three commercially available plasma HIV RNA assays — branched DNA (bDNA),

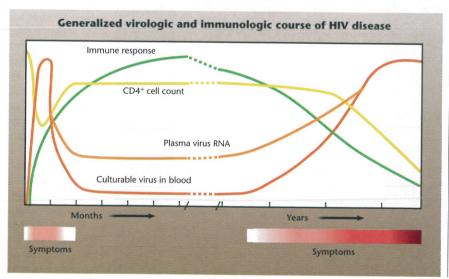
Fig. 1 Schematic representation of natural history of HIV disease.

M.S. Saag, M. Holodniy, D.R. Kuritzkes, W.A. O'Brien, R.W. Coombs, M.E. Poscher, D.M. Jacobsen, G.M. Shaw, D.D. Richman & P.A. Volberding RT-PCR, and Nucleic Acid Sequence-Based Amplification (NASBA) — have not yet been approved by the US Food and Drug Administration for use in routine patient management. The bDNA technique amplifies the signal from a captured viral

RNA target by sequential olignonucleotide hybridization steps while RT-PCR and NASBA use enzymatic methods to amplify target HIV RNA into measurable amounts of nucleic acid product7,8,9. Target RNA sequences in plasma are quantified by comparison with internal or external reference standards, depending on the assay used. Despite the differences in methodologic approach, plasma HIV RNA measurements obtained with the three assays are strongly correlated ( $R \sim 0.90$ )<sup>10,11</sup> and each has low intra-assay sample variability (approximately 0.12 to 0.2 log on repeated testing of single samples)10-12 (Table 1). Plasma HIV RNA levels are relatively stable on a week-toweek or month-to-month basis in clinically stable patients, as long as antiretroviral therapy is not instituted or changed (biologic variability ~0.3 log)13. Therefore, sustained changes in the plasma HIV RNA levels of >0.5 log (that is, greater than threefold) generally reflect biologically relevant changes in the level of viral replication.

#### Correlation of HIV RNA levels to stage of disease

Early studies demonstrated a clear association between the titer of culturable virus in the plasma and the clinical stage of disease<sup>4,5,14</sup>. In patients with the acute seroconversion syndrome and those with advanced HIV disease (<100 CD4<sup>+</sup> lymphocytes/µl), plasma viral titers were generally in the range of 100 to 10,000 tissue culture infectious doses (TCID)/ml while in asymptomatic patients with higher CD4<sup>+</sup> lymphocyte counts (>300 cells/µl) plasma titers were low or non-detectable. The use of quantitative HIV RNA techniques has further defined the association between plasma virus levels and clinical disease sta-



tus. Most importantly, in virtually all untreated patients, viral RNA is detectable in plasma regardless of clinical stage<sup>6,10,15</sup>. Plasma HIV RNA levels are highest in acute (primary) infection (former CDC stage I) and late-stage disease (CDC IV) and are intermediate in titer in earlier clinical stages (CDC II and III). The relative correlation between plasma virus titer (by culture methods) and quantitative PCR values is shown in Fig. 2.

## Ability of plasma viral RNA to predict clinical outcome

CD4<sup>+</sup> lymphocyte counts have been viewed as the best predictor of the risk of developing AIDS-related complications. The risk of developing HIV disease or dying over the next 24 months is <5% among individuals with CD4<sup>+</sup> counts above 500 cells/µl and >70% among those having fewer than 50 cells/µl (ref. 16). Despite its value as a gen-

eral marker of disease stage, the CD4\* count alone is inadequate as a means of measuring prognosis and response to antiretroviral therapy. CD4\* counts are subject to substantial biologic variability and exhibit a limited dynamic range (approximately 2 log)<sup>17,18</sup>. Most importantly, decreases in CD4\* cell counts occur as a result of viral replication and, in that sense, represent a clinical endpoint rather than a "surrogate marker" of disease activity. It is this very process — HIV-mediated lymphocyte destruction — that physicians attempt to prevent rather than observe.

Higher HIV RNA levels correlate with lower baseline CD4<sup>+</sup> counts, more-rapid declines in CD4<sup>+</sup> counts, and more rapid disease progression<sup>19-23</sup>. Patients with >100,000 HIV RNA copies/ml of plasma within six months of seroconversion were tenfold more likely to progress to AIDS over five years than

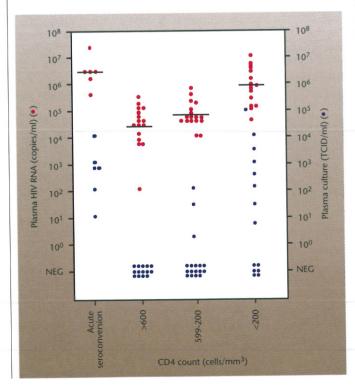


Table 1 Characteristics of Plasma HIV RNA Assays

Linear Dynamic range (copies/ml)	Observed intra-assay standard deviation range (log10) <sup>a</sup>	Preferred anticoagulant
$10^{2.6} - 10^{5.9}$	<0.15-0.33	ACD/EDTA
1 × 10 <sup>4</sup> –1.6 × 10 <sup>6</sup>	0.12–0.2	EDTA
$4\times10^24\times10^7$	0.13-0.23	ACD/EDTA/HEP
	range (copies/ml) $10^{2.6}-10^{5.9}$ $1 \times 10^{4}-1.6 \times 10^{6}$	range intra-assay (copies/ml) standard deviation range (log10) <sup>a</sup> $10^{2.6}-10^{5.9} < 0.15-0.33$ $1 \times 10^{4}-1.6 \times 10^{6} \qquad 0.12-0.2$

Higher values can be measured with dilution of the specimen into the linear dynamic range for each assay. \*Ranges are representative of an ongoing HIV RNA certification program sponsored by the National Institutes of Health, Division of AIDS, Virology Quality Assurance Program<sup>12</sup>. ACD = acid citrate dextran (citrate; yellow-top tube) EDTA = ethylenediaminetetraacetic acid (purple-top tube). HEP = heparin (green-top tube).

were those with <100,000 copies/ml (ref. 22). Patients who consistently maintained an HIV RNA copy number of less than 10,000/ml did not progress to AIDS during the next five years; HIV RNA levels tended to increase among progressors. Thus, maintenance of plasma HIV RNA levels below 10,000/ml in early HIV disease appears to be associated with decreased risk of progression to AIDS. However, in patients with more advanced disease (median CD4\* cell count, 89/ $\mu$ l), disease progression occurred in up to 30% of patients with fewer than 10,000 HIV RNA copies/ml (ref. 20, 21).

In a recent study, a single determination of plasma viral RNA in 181 seropositive individuals provided important prognostic information concerning time to AIDS and death<sup>23</sup>. Subjects were stratified by plasma HIV RNA level (quartiles of <4,530; 4,531–13,020; 13,021–36,270; and >36,271 HIV-1 RNA copies/ml plasma) and followed for as long as 11.2 years. Substantial increases in disease progression rates were associated with higher baseline viral levels. For patients with CD4 $^{+}$  cell counts above 500/µl (median, 780/µl), >70% progressed to AIDS and died within 10 years if their baseline RNA level was >10,200; in contrast, <30% of those with <10,200 copies/ml died within 10 years. A threefold higher baseline HIV RNA level was predictive of a 60% increased hazard of death. A single plasma virus RNA determination predicted clinical events oc-

Fig. 2 Comparison of plasma HIV levels as measured by infectious titer in tissue culture (tissue culture infective dose/ml (TCID/ml); blue circles) versus amplified genomic RNA via QC-PCR (copies/ml; red circles) in patients at different stages of HIV disease. Mean values of HIV RNA levels are indicated by horizontal bars. All values obtained for each assay were determined from paired specimens, as reported in Piatak *et al.*<sup>6</sup> The observed discrepancy between total virus levels determined by direct RNA measurements and those determined by culture (generally 100–10,000 to 1) is typical of retroviruses, which are known to exhibit high frequencies of genetic and phenotypic defectiveness. The fact that direct bDNA, RT-PCR and NASBA methods detect primarily virus that is non-culturable is not relevant to their clinical utility since plasma virus, infectious or not, is a direct measure of virus production and the processes sustaining HIV infection and pathogenesis.

Fig. 3 Composite of plasma HIV RNA and CD4<sup>+</sup> cell count responses among a group of patients receiving three different antiretroviral regimens: zidovudine monotherapy (ZDV, 600 mg/day; solid lines), zidovudine plus lamivudine (ZDV/3TC, 600 mg/day, 300 mg/day, respectively; long-dashed lines), and the protease inhibitor, indinavir (MK-639, 2400 mg/day; short-dashed lines). All patients were naive to their respective treatment regimens. For each treatment group, the relative HIV RNA and CD4<sup>+</sup> count treatment responses appear inversely proportional, although individual exceptions to this association exist.

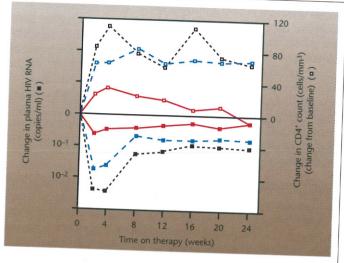
curring as long as 10 years later, similar to predictions determined through the staging of certain malignancies (for example, Hodgkin's disease). Placed in the context of clinical practice, plasma HIV RNA levels appear to be the best predictor of long-term clinical outcome while CD4<sup>+</sup> lymphocyte counts remain the best predictor of immediate or short-term risk of developing a new opportunistic disease process.

## Response of plasma HIV RNA to antiretroviral therapy

Plasma HIV RNA measurements are useful for rapidly evaluating the relative antiretroviral effect of new or available drugs or regimens in clinical trials 1,19,24,25. Effective antiretroviral therapy significantly decreases HIV RNA levels in plasma within one week of the start of treatment. No significant decrease in the plasma levels within this period suggests that the regimen has no antiretroviral activity. Zidovudine monotherapy results in a median 0.7 log decrease in the plasma HIV RNA level within two weeks, which returns toward baseline values by 24 weeks19,20. Lower HIV RNA troughs (~1.5 log decreases) and higher CD4<sup>+</sup> lymphocyte peaks are observed with nucleoside combinations and the responses are generally more durable, often persisting for more than one year<sup>26</sup> (Fig. 3). Protease inhibitors in combination with nucleoside therapy results in dramatic and sustained reductions in plasma viral RNA (~2.0 log) and, in one trial, treatment-associated reductions were associated with a survival benefit in patients with advanced disease<sup>1,2,27-29</sup>. Decreases in plasma HIV RNA

levels generally correlate with increases in the CD4<sup>+</sup> lymphocyte count in patients in whom effective antiretroviral therapy is initiated. In patients in whom plasma HIV RNA levels initially decline but return to pretreatment values, the loss of antiviral effect has been associated with the emergence of drug-resistant strains of HIV (ref. 1, 24, 30, 31).

Antiretroviral induced changes in plasma HIV RNA level and CD4+ lymphocyte count are both independent predictors of disease progression. In one study, each twofold (0.3 log) decrease in the HIV RNA level during treatment was correlated with a 27% reduction in the relative hazard of progression<sup>20</sup>. In another study, each three-fold (0.5 log) decrease in HIV RNA level was associated



with a 63% reduction in relative hazard of progression (P = 0.02)<sup>19</sup>. In a third study, a 1.0 log treatment-induced reduction in HIV RNA was associated with an 80% reduction in relative risk of disease progression and was a more powerful predictor of clinical outcome than CD4<sup>+</sup> cell counts or other virologic measures<sup>25</sup>. In general, a decrease in plasma HIV RNA level along with an increase in the CD4<sup>+</sup> lymphocyte count explain a significant part of the treatment effect observed in these studies.

### The effect of emerging data on clinical practice

As a result of the overlapping nature of clinical research and patient care and the rapid translation of clinical research findings (based on plasma HIV RNA endpoints) to patient management, many clinicians are using HIV RNA assays in their practices. As an example, among 915 HIV clinicians attending one of five International AIDS Society–USA-sponsored, advanced courses on the management of HIV disease, in spring 1995, 20% (172) used HIV RNA measurements in their practice. Yet despite increasing evidence demonstrating the value of viral load determinations, many practitioners are uncertain about the op-

### Table 2 Summary of interim recommendations

#### Parameter

- Plasma HIV RNA level that suggests initiation of treatment
- Target level of HIV RNA after initiation of treatment
- Minimal decrease in HIV RNA indicative of antiviral activity
- Change in HIV RNA that suggests drug treatment failure
- Suggested frequency of HIV RNA measurement

#### Recommendation

- More than 5,000–10,000 copies/ml and a CD4<sup>+</sup> count/clinical status suggestive of progression;
   >30,000–50,000 regardless of laboratory/clinical status
- Undetectable; <5,000 copies/ml is an acceptable target
- >0.5 log decrease
- Return to (or within 0.3 to 0.5 log of) pretreatment value
- At baseline: 2 measurements, 2–4 weeks apart
- Every 3 to 4 months or in conjunction with CD4<sup>+</sup> counts
- Shorter intervals as critical decision points are neared
- 3–4 weeks after initiating/changing therapy

timal use of these tests and are forced to make therapeutic decisions on the basis of anecdotal experience. Because of the need for guidelines to assist practitioners in the use of plasma HIV RNA measurements, the IAS-USA convened an *ad hoc* panel of investigators and clinicians to make recommendations for the use of these assays in clinical practice. The recommendations of the panel were developed in conjunction with a series of questions related to the natural history of HIV disease, current understanding of correlations between plasma HIV RNA levels and prognosis or response to antiretroviral therapy, and the performance characteristics of the assays.

#### Should plasma HIV RNA levels be used routinely in practice?

Yes. Monitoring plasma HIV RNA levels adds important information for patient management, including information on risk of disease progression, when to initiate therapy, the degree of initial antiretroviral effect achieved and when a drug regimen is failing. Although there are no published data from controlled clinical trials as yet, new data from controlled treatment trials and natural history studies strongly support their use in routine practice. The CD4\* lymphocyte count remains an essential index for making decisions regarding prophylaxis for opportunistic infections and for evaluating the immunologic effects of antiretroviral therapy. Since plasma HIV RNA levels and CD4\* lymphocyte count determinations are independent predictors of clinical outcome, their combined use provides a more complete picture of an individual patient's status and response to therapy.

#### When should antiretroviral therapy be initiated?

The goals of antiretroviral therapy are to limit or delay disease progression and increase survival. Given the dynamic interaction between viral replication and CD4\* lymphocyte destruction and the results of clinical studies showing increased survival in association with significant reduction in plasma RNA levels, the best way to achieve these goals is by minimizing viral replication. Steady-state plasma viral RNA levels are directly related to rates of virus production in lymphoreticular tissues and changes in plasma viral load can be used to assess antiretroviral drug effects in otherwise inaccessible tissue compartments. Ideally, the goals of therapy are to reduce the plasma HIV RNA level as much as possible and for as long as possible.

Recent disease progression cohort data and clinical trial results showed that there is a continuum of increased risk for AIDS and death as HIV RNA levels increase. Patients with very low HIV RNA levels (for example, <5,000-10,000 copies/ml) have a better clinical prognosis than those individuals with only modest elevations in RNA levels (for example, 10,000-25,000 copies/ml). Some investigators, including the authors, have concluded that plasma HIV RNA levels >30,000-50,000 copies/ml warrant the initiation therapy, regardless of CD4+ cell count or clinical status. In patients with plasma HIV RNA levels >5,000-10,000 copies/ml but less than 30,000, the decision to start therapy should be made in conjunction with CD4\* cell counts and clinical status. If antiretroviral treatment is initiated according to current clinical guidelines (based solely on CD4\* count values), plasma HIV RNA determinations may be helpful for patients who have CD4\* counts near the current threshold values (for example, 500 CD4\* cells/µl). A high viral load in such patients might provide additional impetus for initiation of treatment.

#### What level of plasma HIV RNA should be sought?

Ideally, undetectable levels of plasma HIV RNA should be sought. However, maximal clinical benefit might be achieved by maintaining plasma HIV RNA levels below 5,000 copies/ml. It has not been shown whether plasma HIV RNA reduced to a particular level by antiretroviral therapy carries the same risk of clinical progression as that same HIV RNA level without antiretroviral therapy; prospective clinical trials are urgently needed to address this question. Because sustained suppression below 5,000 copies/ml may not be achievable for many patients using the currently available therapies, this recommendation represents a reasonable "target" level for some patients, but certainly less than "ideal" in others.

#### For a patient, what suggests a drug is working?

A three-fold or greater sustained reduction (>0.5 log) of the plasma HIV RNA levels is the minimal response indicative of an antiviral effect, given within-assay variation (~0.15–0.2 log) and natural biologic variation of plasma HIV RNA in vivo (~0.3 log). Reductions of this magnitude have been associated with clinical benefit in treatment trials. It is not known whether a reduction in plasma viral RNA of any given magnitude has the same significance in terms of clinical benefit irrespective of the initial pretreatment RNA value. That is, it is not clear whether a 1.0 log (tenfold) reduction in virus load in a patient with a pretreatment level of 1,000,000 copies/ml has the same clinical significance as 1.0 log reduction in a patient with an initial pretreatment level of 10,000 or 100,000 copies/ml. It is likely that the clinical benefits of antiretroviral therapy are related to the duration as well as the magnitude of HIV suppression (that is, the area under the curve), although the precise duration of HIV suppression necessary to result in measurable clinical benefit still needs to be clearly defined.

With existing antiretroviral drug regimens, it is not realistic to expect that lowest plasma HIV RNA levels achieved can be maintained indefinitely. Thus, the return of HIV RNA levels to pretreatment values (or to within 0.3–0.5 log of the pretreatment value), confirmed by at least two measurements, is indicative of drug failure and should prompt consideration of alternative treatment regimens. Decisions to institute changes in therapy should be made using the plasma HIV RNA value in conjunction with CD4+ lymphocyte count and clinical status.

#### How often should plasma HIV RNA levels be measured?

For the initial determinations of the HIV RNA plasma level, two measurements should be obtained 2-4 weeks apart. Subsequently, we suggest that measurements might be obtained along with the CD4\* lymphocyte count (every 3-4 months, according to current convention), since serial determination of both markers simultaneously provide useful information. Viral load assessments may be made at shorter intervals (for example, every 4 weeks) as critical clinical decision points — such as the return of the viral load level to baseline values — are approached. Ideally, plasma HIV RNA levels should be measured 3-4 weeks after initiating or changing antiretroviral treatment to determine the magnitude of the response. Because of the effects of immune activation on viral load, HIV RNA levels should not be measured within a month of acute illnesses or within a month after influenza and pneumococcus immunizations. Increases in HIV RNA levels in blood of as much as 300-fold have been observed within two weeks of routine immunizations against influenza, tetanus, or pneumococcus<sup>32-33</sup>. These increases are transient, returning to preimmunization levels within four weeks of immunization. Increases are also associated with reactivated genital herpes and tuberculosis, and presumably occur with other acute illnesses.

### How should samples be stored, handled and processed?

Optimal procedures for storage, handling, and processing of patient samples have yet to be fully defined. Practitioners should be familiar with the particular assay they have chosen for HIV RNA quantification and the specific sample-handling factors necessary for that assay. Each provider should adopt consistent procedures for handling specimens, including using the same collection tube and anticoagulant, processing techniques, transport and storage procedures, and the same assay for every sample from the same patient. To minimize signal degradation, all plasma specimens should be separated and frozen within 6 hours of collection. If this approach is not possible, the plasma should be removed and refrigerated. Less desirably, the whole blood could be refrigerated, but not for more than 24 hours before separation and freezing are completed.

Plasma HIV RNA quantitation has provided valuable insights into HIV pathogenesis and the activity of antiretroviral regimens. The optimal use of plasma HIV RNA assays will become better understood as more data become available. Of obvious importance is the continued demonstration of clinical benefit in association with treatment strategies that focus on suppressing the viral load as the primary objective of therapy, as is better characterization of the types of events that cause perturbations in plasma HIV RNA levels. Until these issues are further elucidated, these recommendations provide a reference point for the use of plasma HIV RNA as a marker today.

#### Acknowledgments

The authors and the International AIDS Society—USA are grateful to Margaret Chesney and Donald Chambers for their assistance in development and data analysis of the International AIDS Society—USA survey, to Jane Garrison and Matthew Stenger for editorial assistance, to William Moore and Jennifer B. Wilson for assistance with the figures and to John Mellors for careful review of the manuscript.

- Wei, X. et al. Viral dynamics in human immunodeficiency virus type 1 infection. Nature 373, 117 (1995).
- Ho, D.D. et al. Rapid turnover of plasma virions and CD4+ lymphocytes in HIV-1 infection. Nature 373, 123–126 (1995).
- Perelson, A.S., Neumann, A.U., Markowitz, M., Leonard, J.M. & Ho, D.D. HIV-1 dynamics in vivo: Virlon clearnace rate, infected cell life-span, and viral generation time. Science 271, 1582–1586 (1996).
- Ho, D.D., Moudgil, T. & Alam, M. Qunatitation of human immunodeficiency virus type 1 in the blood of infected persons. N. Engl. J. Med. 321, 1621–1625 (1989).
- Coombs, R.W. et al. Plasma viremia in human immunodeficiency virus infection. N. Engl. J. Med. 321, 1626–1631 (1989).
- Piatak, M. Jr et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science 259, 1749–1754 (1993).
- Pachl, C. et al. Rapid and precise quantification of HIV-1 RNA in plasma using a branched DNA signal amplification assay. J. Acquir. Immune Defic. Syndr. Hum. Retrovir. 8, 446–454 (1995).
- Muider, J. et al. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: Application to acute retroviral infection. J. Clin. Microbiol. 32, 292–300 (1994).
- Klevits, T. et al. NASBA isothermal enzymatic in vitro nucleic acid amplification optimized for the diagnosis of HIV-1 infection. J. Virol. Methods 35, 273-286 (1991).
- Cao, Y. et al. Clinical evaluation of branched DNA signal amplification for quantifying HIV type 1 in human plasma. AIDS Res. Hum. Retrovir. 11, 353-361 (1995).
- Lin, H.J. et al. Multicenter evaluation of quantification methods for plasma human immunodeficiency virus type 1 RNA. J. Infect. Dis. 170, 553-562 (1994).
- Brambilla, D. et al. Variation in estimates of HIV-1 plasma RNA concentration among laboratories and assay kits. 3rd Conference on Retroviruses and

- Opportunistic Infections, Jan. 28-Feb. 1, 1996, 155, abst. 550 (Washington, DC).
  13. Holodniy, M., Mole, L., Winters, M. & Merigan, T.C. Diurnal and short-term stability of HIV virus load as measured by gene amplification. J. Acquir. Immune Defic. Syndr. 7, 363-368 (1994).
- Clark, S.J. et al. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. N. Engl. J. Med. 324, 954 (1991).
- Holodniy, M. et al. Detection and quantification of human immunodeficlency virus RNA in patient serum by use of the polymerase chain reaction. J. Infect. Dis. 163, 862–866 (1991).
- Stein, D.S., Korvick, J.A. & Vermund, S.H. CD4+ lymphocyte cell enumeration for prediction of clinical course of human immunodeficiency virus disease: a review. J. Infect. Dis. 165, 352-363 (1992).
- Raboud, R.M. et al. Quantification of the variation due to laboratory and physiologic sources in CD4 lymphocyte counts of clinically stable HIV-infected individuals. J. Acquir. Immune Defic. Syndr. Hum. Retrovir. 10 (suppl. 2), S67–S73 (1995).
- Hoover, D.R. et al. Effect of CD4+ cell count measurement variability on staging HIV-1 infection. J. AIDS 5, 794–802 (1992).
- O'Brien, W.A. et al. Changes in plasma HIV-1 RNA and CD4+ Lymphocyte counts and the risk of progression to AIDS. N. Engl. J. Med. 334, 425-431 (1996).
- Coombs, R.W. et al. Association of plasma human immunodeficiency virus type-1 RNA level with risk of clinical progression in patients with advanced infection. J. Infect. Dis. (in the press).
- Welles, S.L. et al. Prognostic value of plasma HIV-1 RNA levels in patients with advanced HIV-1 disease and with little or no zidovudine therapy. J. Infect. Dis. (in the press).
- Mellors, J.W. et al. Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. Ann. Intem. Med. 122, 573–579 (1995).
- Mellors, J.W. et al. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science (in the press).
- Kappes, J.C. et al. Assessment of antiretroviral therapy by plasma viral load testing: standard and ICD HIV-1 p24 antigen and viral RNA j(QC-PCR) assays compared. J. Acquir. Immune Defic. Syndr. Hum. Retrovir. 10, 139–149 (1995).
- Hammer, S.M., Katzenstein, D.A., Hughes, M.D., Hirsch, M.S., Merigan, T.C. Virologic markers and outcome in ACTG 175. 3rd Conference on Retroviruses and Opportunistic Infections, Jan. 28

   Feb. 1, 1996, 175, abst. S24 (Washington, DC).
- Eron, J. et al. Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4 cells per cubic millimeter. N. Engl. J. Med. 333, 1662–1669 (1995).
- Danner, S.A. et al. A short-term study of the safety, pharmacokinetics, and efficacy of ritonavir, an inhibitor of HIV-1 protease. N. Engl. J. Med. 333, 1528–1533 (1995).
- Markowitz, M. et al. A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection. N. Engl. J. Med. 333, 1534–1539 (1995).
- Heath-Chiozzi, M. et al. Anti-HIV activity and lymphocyte surrogate marker response dynamics to ritonavir therapy in advanced HIV immunodeficiency. 3rd Conference on Retroviruses and Opportunistic Infections, Jan. 28–Feb. 1, 1996, 162, abst. LB6b (Washington, DC).
- 30. Havlir, D., McLaughlin, M.M. & Richman, D.D. A pilot study to evaluate the development of resistance to nevirapine in asymptomatic human asymptomatic human immunodeficiency virus-infected patients with CD4+ cell counts of >500/mm³: AIDS Clinical Trials Group Protocol 208. J. Infect. Dis. 172, 1379–1383 (1995).
- Holodniy, M. et al. Determination of human immunodeficiency virus RNA in plasma and cellular viral DNA genotypic zidovudine resistance and viral load during zidovudine-didanosine combination therapy. J. Virol. 69, 3510–3516 (1995).
- Staprans, S.I. et al. Activation of virus replication after vaccination of HIV-1 infected individuals. J. Exp. Med. 182, 1727–1737 (1995).
- O'Brien, W.A. et al. Human immunodeficiency virus- type 1 replication can be increased in peripheral blood of seropositive patients after influenza vaccination. Blood 86, 1082–1089 (1995).

From the University of Alabama at Birmingham (M.S.S. & G.M.S.); Stanford University and the Palo Alto Veterans Affairs Health Care System (M.H.); the University of Colorado Health Sciences Center (D.R.K.); the University of California Los Angeles School of Medicine and West Los Angeles Veterans Affairs Medical Center (W.A.O.); the University of Washington (R.W.C.); the University of California San Francisco School of Medicine (M.E.P. & P.A.V.); the International AIDS Society—USA (D.M.J.); and the University of California San Diego and San Diego Veterans Affairs Medical Center (D.D.R.).

Correspondence should be addressed to M.S.S. at the University of Alabama at Birmingham, 908 20th Street South Birmingham, Alabama 35294-2050, USA Mellors JW, Kingsley LA, Rinaldo CR Jr, et al. Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. *Ann Intern Med.* 1995;122:573-579.

Merluzzi VJ, Hargrave KD, Labadia M, et al. Inhibition of HIV-1 replication by a nonnucleoside reverse transcriptase inhibitor. *Science*. 1991:251:363.

O'Brien WA, Grovit-Ferbas K, Namazi A, et al. Human immunodeficiency virus-type 1 replication can be increased in peripheral blood of seropositive patients after influenza vaccination. *Blood*. 1995;86:1082-1089.

O'Brien WA, Hartigan PM, Martin D, et al. Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. *N Engl J Med.* 1996;334:426-431.

Pachl C, Todd JA, Kern DG, et al. Rapid and precise quantification of HIV-1 RNA in plasma using a branched DNA signal amplification assay. *J Acquir Immune Defic Syndr Hum Retrovir*. 1995;8:446–454.

Pantaleo G, Graziosi C, Demarest JF, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature*. 1993;362:355-358.

Perelson AS, Neumann AU, Markovitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell lifespan, and viral generation time. *Science*. 1996;271:1582–1586.

Piatak M Jr, Saag MS, Yang LC, et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science*. 1993;259:1749-1754.

Saag MS. What to do when zidovudine fails. Editorial. *N Engl J Med.* 1994;330:706–707.

Saag MS, Crain MJ, Decker WD, et al. High-level viremia in adults and children infected with human immunodeficiency virus: relation to disease stage and CD4+ lymphocyte levels. *J Infect Dis*. 1991:164:72–80.

Saag MS, Emini EA, Laskin OL, et al. A short term clinical evaluation of L-697,661, a nonnucleoside inhibitor of HIV-1 reverse transcriptase. *N Engl J Med.* 1993;329:1065–1072.

Saag MS, Holodniy M, Kuritzkes DR, et al. HIV viral load markers in clinical practice. *Nature Med.* 1996;2:625–629.

Schnittman SM, Greenhouse JJ, Psallidopoulos MC, et al. Increasing viral burden in CD4+ T cells from patients with human immunodeficiency virus (HIV) infection reflects rapidly progressive immunosuppression and clinical disease. *Ann Intern Med.* 1990;113:438–443.

Wain-Hobson S. AIDS: virological mayhem. *Nature*. 1995;373:102.

Wathen LK, Nickens DJ, Chuang-Stein CJ, et al. HIV-1 RNA viral burden at baseline or it's reduction following antiretroviral therapy is highly correlated with reduced HIV-1 disease progression. Presented at Third Conference on Retroviruses and Opportunistic Infections; January 28–February 1, 1996; Washington, DC.

Wei X, Ghosh SK, Taylor ME, et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature*. 1995;373:117–122.

Welles SL, Jackson JB, Yen-Lieberman B, et al. Prognostic value of plasma HIV-1 RNA levels in patients with advanced HIV-1 disease and with little or no zidovudine therapy. *J Infect Dis*. 1996. (In press.)

## STRATEGIES FOR CONTINUING ANTIRETROVIRAL THERAPY

Strategies for continuing antiretroviral therapy were discussed at the New York courses by Michael S. Saag, MD, from the University of Alabama at Birmingham, and Daniel R. Kuritzkes, MD, from the University of Colorado Health Sciences Center in Denver.

Iffective strategies for continuing the benefits of treatment currently rely on the appropriate use of the growing number of available antiretroviral drugs and on the availability of plasma HIV RNA assays to follow patient response to treatment, as noted by both Dr Saag and Dr Kuritzkes. Overall, the goal of antiretroviral therapy is to keep viral load as low as possible for as long as possible (see Table 1). The majority of patients will not remain on their initial antiretroviral treatment for extended periods of time. Plasma HIV RNA assays provide a means for following the effect of treatment on viral load and are becoming routinely used in clinical practice along with measuring CD4+ cell counts for evaluating the response to therapy and identifying failure of the initial regimen. Drs Saag and Kuritzkes both focused their talks on nucleoside drugs and combinations, and noted that for many patients, the additional use of a protease inhibitor for these subsequent regimens would be appropriate. (For a review of protease inhibitors, see the April 1996 issue of Improving the Management of HIV Disease.)

#### **Treatment Failure**

Antiretroviral failure can be defined as drug intolerance or toxic effects that warrant discontinuation of therapy or as loss of antiretroviral effect. The return of plasma HIV RNA levels toward pretreatment values (whether pretreatment or values at the time of last treatment alteration) is considered to be an indication of the loss of antiretroviral effect. Factors involved in the loss of antiretroviral effect include incomplete suppression of HIV, emergence of drug-resistant variants, emergence of syncytium-inducing variants, and progressive immunologic decline. Incomplete viral suppression has been characteristic of prior widely used antiretroviral regimens. With the employment of new drugs and combinations, viral load can often be reduced, at least over

## Table 1. Guiding Principles of Antiretroviral Therapy.

- Maximum suppression of HIV replication should be the goal of therapy
- Suppression must be continuous to maximize the benefits of therapy
- Treatment regimens will need to change in response to emergence of drug resistance
- Antiretroviral therapy alone may not be sufficient for immune restoration, particularly in patients with advanced disease

the short term, to undetectable (ie, below the detection limits of the assay used [usually 200 to 500 copies/mL]) or to nearly undetectable levels. However, residual low-level viremia may persist even when maximal drug activity is achieved. Such incomplete viral suppression may be associated with persistent reservoirs of virus within chronically-infected, virus-producing cells (eg, macrophages) by incomplete penetration of drug into tissue compartments, or by changes in intracellular metabolism, resulting in reduced active drug levels.

Therapy should be changed at the first sign of treatment failure, such as clinical progression (new or recurrent opportunistic infections, persistent or worsening symptoms, or wasting syndrome), continuous CD4+ cell count decline, or an increase in plasma HIV RNA levels toward (or within 0.3 to 0.5 log of) baseline. While clinical progression should be taken as an indication of treatment failure, use of viral markers allows changing "ineffective" treatment before the lack of efficacy results in a clinical event. There are still many patients who are being given zidovudine monotherapy, and there is now a wealth of data that indicate that this is not an optimal regimen. All patients on zidovudine monotherapy should be reevaluated as to whether their treatment needs to be changed.

#### **Viral Load Thresholds in Treatment**

Given the intraassay and biologic variance of the plasma HIV RNA assays defined in clinical studies, the minimum decrease in viral burden indicating an antiretroviral effect is 0.5-log (or 3-fold) decrease. With regard to optimum decrease in viral burden, the goal of both initial treatment and subsequent alterations in treatment should be a reduction of viral load to at least 5,000 to 10,000 HIV RNA copies/mL, if achievable. The confirmed return to (ie, from 2 measurements) of plasma HIV RNA levels to within 0.3 log to 0.5 log of the pretreatment value is indicative of treatment failure. The return of CD4+ cell counts or CD4+ percentage to the pretreatment count also suggests an alteration in therapy is needed.

#### **Factors in Changing Treatment**

Factors to be considered when changing treatment because of antiretroviral failure include (1) the current regimen; (2) type of failure—ie, whether failure due to toxic effects or to loss of antiretroviral effect; (3) previous therapy—ie, whether resistance patterns resulting from prior use of a drug may still persist; (4) stage of underlying disease; (5) concomitant medications, to avoid or minimize potential adverse drug interactions; (6) availability of/access to drugs; (7) cost of drugs; and (8) philosophy of the treating physician and the patient (ie, how aggressive does the physician or patient want to be). Desirable characteristics of the new regimens include (1) greater potency; (2) different mechanism of action; (3) absence of cross-resistance or the reversal of resistance (eg, in the case of lamivudine and zidovudine); and (4) minimal drug interactions.

## Nucleoside Reverse Transcriptase Inhibitors in Continuing Therapy

The potent protease inhibitors may eventually be included in virtually all antiretroviral regimens. In this "new" era of treatment (ie, in which there are a range of potent drugs to choose from and in which therapy can be tailored using viral markers), nucleoside reverse transcriptase inhibitors will continue to have an important role alone or in combination with other drugs. Recent trials have demonstrated the efficacy of the nucleoside drugs in continuing therapy. In addition, when protease inhibitors are used alone the development of resistance ultimately limits their usefulness in this regard. Finally, it has been found that the proportion of patients rendered "aviremic" (ie, with viral loads that have fallen below assay detection limits) is maximized with combinations of protease inhibitors and nucleoside analogues.

#### **Clinical Trials Including Antiretroviral-experienced Patients**

In AIDS Clinical Trials Group (ACTG) Study 175, patients with CD4+ cell counts between 200/µL and 500/µL (median. 350/μL) and without AIDS were randomized to zidovudine alone, zidovudine plus didanosine, zidovudine plus zalcitabine, or didanosine alone. Study endpoints consisted of a 50% decrease in CD4+ cell count, AIDS-related opportunistic infection or cancer, death, and grades 3 or 4 toxic effects. The data on antiretroviralnaive patients were discussed in the previous issue of this publication (See Improving the Management of HIV Disease, 1996;4[1]:4-6). In the zidovudine-experienced population of the study, patients who continued on zidovudine monotherapy exhibited a loss of CD4+ cells from the start of study treatment, whereas those in the three remaining groups experienced an initial increase. Those who were given zidovudine/didanosine showed a particularly marked increase in CD4+ cell counts, with the mean level approaching the pretreatment level only by week 48. In the zidovudine-experienced population, patients who were given zidovudine/didanosine or didanosine monotherapy had significantly lower rates of progression to all three endpoints compared with those who were given zidovudine alone. The zidovudine/zalcitabine arm had a significantly lower rate only for progression to the CD4+ cell count/death aggregate endpoint.

The European/Australian Delta study was similar in design to ACTG 175 except that the patients studied had somewhat more advanced disease and no didanosine monotherapy arm was included in the trial. Patients with AIDS-related complex (ARC) or AIDS and CD4+ cell counts greater than 50/μL and patients with asymptomatic disease and CD4+ cell counts less than 350/µL were randomized to continued zidovudine monotherapy, zidovudine/didanosine or zidovudine/zalcitabine. In Delta 2, the portion of the study in zidovudine-experienced patients, 50% of patients were asymptomatic and 17% had AIDS; the median CD4+ cell count was 189/µL. In the final Delta 2 study analysis, clinical progression, defined as a new AIDS-defining event or death, occurred in 44% of zidovudine monotherapy recipients, 39% of zidovudine/didanosine recipients, and 43% of zidovudine/zalcitabine recipients. Death occurred in 27% of zidovudine alone recipients, 23% of zidovudine/didanosine recipients, and 26% of zidovudine/zalcitabine recipients. An interim analysis had indi-

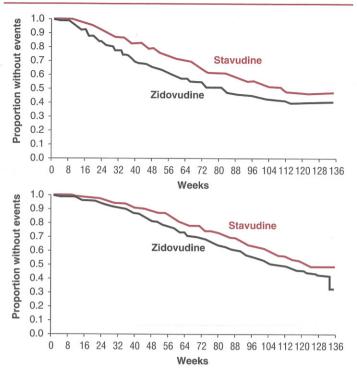


Figure 1. Time to treatment failure (top) and time to clinical progression (bottom) in patients enrolled in Bristol-Myers Squibb (BMS) Study 019. Adapted from Bristol-Myers Squibb Co. Stavudine (Zerit), Also Known as d4T: Final Results of the Study BMS-019 Phase III Comparative Trial. 1995.

cated that there were no differences among the treatment groups with regard to progression in the zidovudine-experienced patients; however, final analysis indicated a significant reduction in the rate of clinical progression in patients who were given zidovudine/didanosine, but not in those given zidovudine/zalcitabine.

In the Community Programs for Clinical Research on AIDS (CPCRA) 007 NuCombo trial, patients with CD4+ cell counts less than 200/µL (median 92/µL), 75% of whom had received prior zidovudine therapy, were randomized (partially doubleblind) to zidovudine, zidovudine/didanosine, or zidovudine/zalcitabine. Results showed superior CD4+ cell count changes in the zidovudine-experienced patients in the two combination therapy groups, but no significant differences in the rate of clinical progression or mortality. Given the advanced disease of patients in this trial and the substantial time spent on zidovudine prior to the study, zidovudine likely had little effect in the combination regimens, which were thus tantamount to didanosine or zalcitabine monotherapy. These three trials indicate a gradient of effect of nucleoside combinations in zidovudine-experienced patients: (1) a marked effect in patients with relatively early disease; (2) some effect, particularly on the part of zidovudine/didanosine in those patients with somewhat more advanced disease; and (3) little effect in those with even more advanced disease. These results argue for the earlier introduction of combination therapy. The effects of the protease inhibitors have been most notable in these latter patient groups.

Stavudine monotherapy is another regimen that has been studied in zidovudine-experienced patients. In Bristol-Myers

## Table 2. Possible Nucleoside Regimens for Continuing Antiretroviral Therapy.

#### Choices in zidovudine-intolerant patients

- Didanosine monotherapy
- · Stavudine monotherapy
- Didanosine/stayudine?
- · Didanosine/lamivudine?
- Stavudine/lamivudine?

#### **Combination therapies**

#### Useful combinations

- · Zidovudine/didanosine
- · Zidovudine/lamivudine
- Zidovudine/zalcitabine<sup>†</sup>
   (†in zidovudine-naive patients only)

#### Possible combinations

- Stavudine/didanosine
- Zidovudine/didanosine/nevirapine
- · Zidovudine/delavirdine

#### Unknown combinations

- Zidovudine/stavudine
- Stavudine/lamivudine
- Lamivudine/didanosine

#### Combinations to avoid

- Stavudine/zalcitabine
- Didanosine/zalcitabine

\*For many patients, nucleoside regimens will be used in combination with protease inhibitors.

Squibb (BMS) study 019, patients with CD4+ cell counts between 50/µL and 500/µL who had been given more than 6 months of prior zidovudine therapy were randomized to continued zidovudine or stavudine. The CD4+ cell counts in the stavudine group remained above the pretreatment level for more than 20 weeks, compared with less than 4 weeks in the zidovudine group; a persistent significant difference in the rate of treatment failure was observed in favor of stavudine (Figure 1[top]). A difference in the rate of clinical progression was noted also in favor of stavudine (Figure 1[bottom]). However, there was no difference between the two groups with regard to survival. Stavudine is associated with peripheral neuropathy; in this study, most of the cases were observed in the second year of treatment, with the incidence in stavudine patients becoming significantly greater than that in the zidovudine patients at this time. Although recent data on the combination of stavudine and didanosine (also associated with peripheral neuropathy) have yielded encouraging results with regard to limited neuropathy, these data have not included longer term follow-ups. Moreover, this combination regimen was studied in a relatively healthy population.

Another nucleoside combination that is generating much enthusiasm is zidovudine/lamivudine, in part because of the combination's potential benefit of reversing zidovudine resistance. In NUCA 3002, an evaluation of the lamivudine-zidovudine combination in patients with advanced disease, zidovudineexperienced patients with CD4+ cell counts between 100/µL and 300/µL were randomized to zidovudine plus either lamivudine 150 mg bid or lamivudine 300 mg bid or zidovudine plus zalcitabine. The peak median decreases in plasma HIV RNA levels were 0.7 log (approximately 5-fold) with the addition of zalcitabine and 1.5 log with either of the lamivudine dosages. Differences in plasma HIV RNA reduction disappeared as resistance to lamivudine emerged; with all three regimens, plasma HIV RNA levels remained below baseline for at least 48 weeks. It is of interest that whereas both lamivudine regimens resulted in marked increases in mean CD4+ cell counts, no increase was observed in the zidovudine/zalcitabine recipients. Results from ACTG 175, the Delta 2 study, and the NUCA trial all indicate that the addition of zalcitabine to zidovudine in zidovudine-experienced patients does not appear to produce the same benefit as addition of other drugs (eg, didanosine or lamivudine), and that this phenomenon may warrant closer examination.

The role of nonnucleoside reverse transcriptase inhibitors (NNRTIs) remains unclear. (SEE NOTE, END OF TEXT.) Nevirapine is associated with a prompt and dramatic reduction in viral load that is rapidly reversed in up to 80% of recipients, in association with the onset of high-level resistance. However, a significant proportion (20% to 30%) of patients exhibit a persistent response despite the development of nevirapine resistance. A recent ACTG study (protocol 260) showed that delavirdine resistance resulted in loss of activity by 4 to 8 weeks even at very high doses. Other studies have shown that delavirdine resistance is somewhat delayed when the drug is used in combination with zidovudine, but not when combined with didanosine.

The NNRTIs will likely be used in combination with nucleoside analogues. Until drug interaction studies are completed, the use of these drugs in combination with protease inhibitors should be avoided. In ACTG 241, patients who had been on zidovudine or didanosine for 25 months and who had a median CD4+ cell count of 130/µL were given either zidovudine/didanosine or the triple combination of zidovudine, didanosine, and nevirapine. At week 48, the three-drug combination was associated with a 25% greater increase in CD4+ cell count and a 50% greater decrease in viral infectivity (on quantitative microculture) and in plasma HIV RNA levels than was the two-drug combination.

#### **Other Potential Combinations**

There has been significant concern about potential antagonism of zidovudine and stavudine given that these drugs compete for the same enzyme for intracellular phosphorylation. A recent in vitro study, however, has indicated that the two drugs are synergistic against zidovudine-susceptible virus; however, the combination was antagonistic when tested against zidovudine-resistant virus. This combination is currently being evaluated in two clinical trials.

Lamivudine and stavudine is an attractive, promising combination because it might avoid the gastrointestinal intolerance associated with didanosine and zidovudine and the hematologic toxic effects associated with zidovudine. In vitro studies have indicated synergy of the combination, and there is no known ad-

verse or favorable interaction of resistance mutations. An important issue is whether this combination provides the same durability of effect as does lamivudine/zidovudine. Lamivudine/stavudine is currently being evaluated in an ACTG trial, and firmer data on how it compares with the lamivudine/zidovudine are expected before the end of the year.

#### **Individualizing Therapy**

The availability of the new assays for plasma HIV RNA permits truly individualized therapy. The choice of a replacement regimen will be guided by sequential viral load measurements, replacing reliance on group data from clinical trials. The clinical trial setting is artificial in that there are prescribed criteria for entry, treatment, and management that often do not coincide with how a patient would be managed in the clinical setting. Perhaps more important is that intent-to-treat analyses of efficacy, commonly employed in clinical trials, obscure individual treatment responses. A patient may demonstrate an antiviral effect (as measured by viral markers) for only 2 weeks of treatment with a regimen, and yet analysis at, for example, 2 years includes that patient as though he or she had been responding to the study regimen for the entire treatment duration. Group results thus include good re-

sponders and poor responders without differentiating between them. Use of the viral markers currently available provide an opportunity to tailor treatment to the individual patient.

#### **Current Options**

Table 2 shows proposed options for continuing antiretroviral therapy. The nucleoside analogue combinations that have the most clinical trial experience are zidovudine/didanosine, zidovudine/zalcitabine, and zidovudine/lamivudine. Ongoing studies will define the activities of lamivudine/didanosine, lamivudine/stavudine, and didanosine/stavudine.

Note: Preliminary data presented to the FDA in June suggest the suppression of plasma HIV RNA to levels below detection was achieved in the majority of previously untreated patients who received the combination of zidovudine/didanosine/nevirapine.

Michael S. Saag is Associate Professor of Medicine at the University of Alabama at Birmingham.

Daniel R. Kuritzkes is Associate Professor of Medicine and Microbiology at the University of Colorado Health Sciences Center in Denver.

#### **Suggested Readings**

Abrams DI, Goldman AI, Launer C, et al. A comparative trial of didanosine or zalcitabine after treatment with zidovudine in patients with human immunodeficiency virus infection. *N Engl J Med*. 1994;330:657–662.

Collier AC, Coombs RW, Schoenfeld DA, et al. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. *N Engl J Med*. 1996;334:1011–1017.

Danner SA, Carr A, Leonard JM, et al. A short-term study of the safety, pharmacokinetics, and efficacy of ritonavir, an inhibitor of HIV-1 protease. *N Engl J Med.* 1995;333:1528–1533.

Eron JJ, Benoit SL, Jemsek J, et al. Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4+cells per cubic millimeter. *N Engl J Med.* 1995;333:1662–1669.

Fischl MA, Stanley K, Collier AC, et al. Combination and monotherapy with zidovudine and zalcitabine in patients with advanced disease. *Ann Intern Med.* 1995;122:24–32.

Havlir D, Cheeseman SH, McLaughlin M, et al. High-dose nevirapine: safety, pharmacokinetics, and antiviral effect in patients with human immunodeficiency virus infection. *J Infect Dis.* 1995;171:537–545.

Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science*. 1996;272:1167-1170.

Montaner JSG, Schechter MT, Rachlis A, et al. Didanosine compared with continued zidovudine therapy for HIV-infected patients with 200 to 500 CD4 cells/mm<sup>3</sup>. A double-blind, randomized, controlled trial. *Ann Intern Med.* 1995;123:561-571.

O'Brien WA, Hartigan PM, Martin D, et al. Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. *N Engl J Med.* 1996;334:426–431.

Pavia AT, Gathe J, BMS-019 Study Group Investigators, et al. Clinical efficacy of stavudine (d4T, Zerit) compared to zidovudine (ZDV, Retrovir) in ZDV-pretreated HIV positive patients. Presented at Thirty-fifth Interscience Conference on Antimicrobial Agents and Chemotherapy; September 17–20, 1995; San Francisco, Calif.

Yeni P, on behalf of the International Coordinating Committee. Preliminary results of the European/Australian Delta trial: Based on data up to 31st May 1995. Presented at Fifth European Conference on Clinical Aspects and Treatment of HIV Infection; September 1995; Copenhagen, Denmark.

## DRUG INTERACTIONS WITH HIV PROTEASE INHIBITORS

#### JOHN G. GERBER, MD

The rapid approval by the Food and Drug Administration (FDA) of the HIV protease inhibitors was celebrated by both people with HIV and health care providers alike because of the potent antiviral activity of these drugs. However, because of the lipophilic nature of these molecules, and the large dose that needs to be administered to observe an antiviral effect, interaction with a host of xenobiotic metabolizing enzymes has been observed, which can result in adverse drug interactions with drugs that use those enzymes for metabolism.

Because of the short time period from development to approval, almost all the drug interaction studies with the protease inhibitors are unpublished or have yet to be performed; therefore, it is difficult to make rational decisions about how to use many of those drugs concomitantly with protease inhibitors. Since people with advanced HIV disease use perhaps as many as 8 to 10 drugs for the treatment or prophylaxis of opportunistic infections and for the treatment of HIV itself and the psychiatric problems that arise with a chronic fatal disease, significant drug interactions resulting in toxic effects are unavoidable.

For practicing clinicians it is more important than ever that before adding a protease inhibitor to the therapeutic regimen, the prescriber reviews the drug list of the patient. It is crucial to understand how those drugs are eliminated (renal vs hepatic, and, if hepatic, conjugation vs oxidation), so as to make rational choices about those drugs by altering doses as necessary to reduce the likelihood of toxic effects or loss of efficacy.

#### **Pharmacokinetic Principles**

To understand drug interactions it is important to review some pharmacokinetic principles of hepatic drug metabolism, and certain aspects about the cytochrome P450 isozymes involved with xenobiotic metabolism. Hepatic clearance of a drug is determined by two independent processes. One is hepatic blood flow and the other is the intrinsic capacity of the liver to metabolize a drug, known as intrinsic clearance. The capacity of the liver to metabolize a drug (intrinsic hepatic clearance) is determined by the avidity of the drug to the metabolizing enzyme and the concentration of the enzyme in the liver. If the liver has a large capacity to metabolize a drugand thus a very large intrinsic hepatic clearance-the rate-limiting step to its metabolism is the hepatic blood flow (the rate of delivery), and

small changes in the metabolizing enzymes will not have as profound an effect on hepatic clearance as will alterations in hepatic blood flow. These drugs are described as having perfusion-limited metabolism. If the liver has a limited capacity to metabolize a drug, hepatic clearance is determined exclusively by hepatic enzymatic activity. Alterations in hepatic enzymatic activity will thus result in profound changes in hepatic clearance. These drugs are described as having perfusion-independent metabolism. Many drugs fall within these two extremes, and both hepatic blood flow and enzymatic activity determine hepatic clearance.

Because most of the drugs used in clinical medicine are administered orally and are thus subject to first-pass metabolism in the bowel and the liver, drugs with high avidity to metabolizing enzymes (ie, drugs with high intrinsic hepatic clearance) have large first-pass metabolism and thus decreased bioavailability to systemic circulation. Therefore, if the activity of these enzymes is perturbed by administering another drug, the amount of the orally administered drug that reaches the systemic circulation may be greatly altered. Compounds that inhibit the metabolizing enzymes of drugs will have an effect on the kinetics of drugs with either high or low intrinsic clearance although by different mechanisms. Drugs with high intrinsic clearance will have greatly increased bioavailability secondary to inhibition of first-pass metabolism (thus a greater amount of the drug will reach the systemic circulation); while drugs with low intrinsic clearance will have greatly reduced hepatic clearance (elimination) of the drug. The overall effect will be an increase in plasma concentration at steady state and an increase in the area-under-the-time concentration curve (AUC) for those drugs, with consequent toxic effects of the drugs with low therapeutic ratios.

This concept is important to understand because both the potent protease inhibitors ritonavir and indinavir produce significant inhibitory effects on some of the cytochrome P450 isozymes involved in drug metabolism. Cytochrome P450 constitutes a superfamily of hemoproteins involved in monooxygenase reactions of a host of endogenous compounds as well as xenobiotics. The reason for adding an oxygen molecule to a xenobiotic is to make the compound more hydrophilic so that it can be excreted by the kidney or through the biliary system. Although hundreds of cytochrome P450

genes have been characterized, only three gene families—CYP1, CYP2, and CYP3—are thought to be responsible for the hepatic metabolism of xenobiotics. Within these families there are subfamilies as established by a capital letter, which is followed by an arabic numeral referring to the specific enzyme. Of these P450 enzymes, six represent the most important enzymes involved in drug metabolism. As shown in the table these six enzymes are 1A2, 2C9, 2C19, 2D6, 2E1, and 3A3. Of all these enzymes, the 3A is the most abundant and represents 30% of all the P450 proteins found in the liver. Because these are microsomal (membrane-bound) enzymes, they cannot be crystallized, and thus the three dimensional structures have not been definitively established. These enzymes have overlapping substrate specificity, but numerous drugs use primarily a single isoform for metabolism. Since drug inhibitors and inducers are frequently

Both of the protease inhibitors, ritonavir and indinavir, have significant inhibitory effects on some of the cytochrome P450 isozymes involved in drug metabolism.

isozyme specific, drugs that use a single isozyme for metabolism would be much more affected than would drugs that use numerous isozymes for metabolism.

Drug metabolism is frequently stereospecific; thus, the components of a racemic mixture of drugs may use different isozymes for metabolism. A good example is warfarin, which is a racemic mixture of both the S- and R-enantiomer; however, only S-warfarin is the substrate for the 2C9 isozyme, and many important drug interactions occur at that isozyme (eg, metronidazole, sulfamethoxazole) because S-warfarin is the more potent anticoagulant.

Both ritonavir and indinavir are extremely lipophilic and use primarily the P4503A isozyme for metabolism. Ritonavir is significantly more lipophilic than is indinavir as demonstrated by the more extensive protein binding of and almost undetectable renal excretion of the former. Both drugs in in vitro studies have demonstrated a ca-

Table 1. P450 Isozymes That Have Been Shown to Metabolize Clinically Important Drugs.

Gene	Enzyme	Substrates	Inducers	Antimicrobial Inhibitors
CYP1A2	P4501A2	caffeine, theophylline, acetaminophen, imipramine	cigarette smoke charcoal food omeprazole	ciprofloxacin
CYP2C9	P4502C9	phenytoin, S-warfarin, tolbutamide, ibuprofen, naproxen		fluconazole sulfamethoxazole metronidazole
CYP2C19	P4502C19	omeprazole, diazepam, demethyldiazepam, proguanil, imipramine, propranolol	genetic polymorphism	
CYP2D6	P4502D6*	codeine, hydrocodone, flecainide, encainide, fluoxetine, imipramine, amitriptyline, haloperidol, metoprolol, desipramine, nortryptiline	genetic polymorphism	quinidine quinine
CYP2E1	P4502E1	acetaminophen, ethanol	isoniazid, ethanol	
СҮРЗА	P4503A*†	cyclosporine, nifedipine, diltiazem, lidocaine, lovastatin, erythromycin, clarithromycin, quinidine, terfenadine, verapamil, midazolam, triazolam, astemizole, taxol, cisapride, ketoconazole, itraconazole, estrogens, corticosteroids, and others	rifampin phenobarb phenytoin dexamethasone	erythromycin clarithromycin ketoconazole itraconazole fluconazole

\*Ritonavir inhibits this isozyme. †Indinavir inhibits this isozyme.

pacity to inhibit P4503A isozyme in a competitive manner; ritonavir also inhibits P4502D6 but with higher affinity toward the P4503A isozyme. There are more published data on ritonavir's mechanism of inhibition than that of indinavir. Ritonavir competitively inhibits P4503A and P4502D6 by interacting with high affinity at the substrate binding site. In addition, its thiazole side chain interacts with the heme component of the P450 isozyme, thus preventing the attachment of oxygen. This is called a type II binding and it has been well described for the lipophilic imidazole compounds such as ketoconazole. The mechanism of inhibition of P4503A by indinavir has not been published, but present data suggest that there is no type II binding to P450 isozymes and that the drug only interacts competitively at the substrate binding site.

In terms of drug interaction, more data are available for ritonavir than for indinavir, and it is almost impossible to directly compare these two drugs until more complete data are published. However, ritonavir clearly appears to have different activity in the liver than indinavir. In addition to competitively inhibiting the P4503A and 2D6 isozymes, ritonavir induces the P4501A2 and 3A isozymes as demonstrated by the lowered theo-

phylline AUC with ritonavir and the lowered plasma concentration of ritonavir with continued use. In fact, the improved adverse reaction profile of ritonavir with continued use is thought to be due to autoinduction and lowered plasma concentration over time. In addition, ritonavir induces the family of enzymes involved with glucuronidation as demonstrated by the 25% decrease in the zidovudine AUC. Indinavir does not appear to have the capacity to induce hepatic enzymes, and blood concentrations of the drug remain stable over time. The suggestion that ritonavir is a more potent inhibitor of cytochrome P4503A than indinavir stems from data reported by Kempf et al at the Third Conference on Retroviruses and Opportunistic Infections held in Washington, DC, that showed that when indinavir was administered concomitantly with ritonavir in rats, the indinavir AUC increased 800%; in contrast, the ritonavir AUC remained unchanged. Similar data are not available in humans.

A number of drugs are metabolized by the cytochrome P4503A isozymes. A partial list is provided in the table. The P4502D6 isozyme also has a varied substrate specificity. The P4502D6 isozyme is one of the drug metaboliz-

ing enzymes that demonstrates genetic polymorphism. Genetic polymorphism refers to the observation that approximately 5% to 10% of Caucasians do not show phenotypic expression of this isozyme and are termed poor metabolizers. Thus, in this subgroup an inhibitor would not be expected to produce any additional effect on the metabolism of drugs that use P4502D6.

It is important to distinguish drug interactions with protease inhibitors that have been performed and reported from *potential* drug interactions based on the knowledge of the specific cytochrome P450 involved in the drug's metabolism. For the latter, drugs with very low therapeutic indices must not be used with protease inhibitors since the outcome may prove to be fatal. Some of these examples are described below.

#### Studies of Drug Interactions with Protease Inhibitors

Clarithromycin. Ritonavir increases the plasma concentration of clarithromycin by an average of 77%, with a range of 56% to 103%. Although this interaction does not seem to be very significant, the generation of the 14(R)OH clarithromycin was completely inhibited by ritonavir. This hydroxylated metabolite of clarithromycin is

generated by the P4503A isozyme, suggesting nearly complete shutdown of this pathway by ritonavir. Because 14(R)OH clarithromycin is also active, and since some of the gram-negative activity of clarithromycin is secondary to the synergistic interaction of the 14(R)OH metabolite with clarithromycin, the optimal benefits of using clarithromycin in treating *Haemophilus sp.* infection may be lost when ritonavir is administered concurrently. The unchanged form of clarithromycin is the active drug against *Mycobacterium avium* complex (MAC).

Indinavir also increases the plasma concentration of clarithromycin by 53%; however, the 14(R)OH clarithromycin AUC decreases only by 50%. These data again suggest a much more potent effect with ritonavir on the P4503A iso-

# Drugs with very low therapeutic indices must not be used with protease inhibitors because the outcome could be fatal.

zymes. Clarithromycin increases plasma concentration of both ritonavir and indinavir by only minor increments.

Rifabutin. Ritonavir significantly inhibits the metabolism of rifabutin. Rifabutin is metabolized in the liver by two main pathways. One is a deacetylation step that uses an esterase to form desacetyl rifabutin, and the other pathway involves the P4503A isozymes, resulting in a more soluble demethylated metabolite (see Figure 1). Desacetyl rifabutin is as active as rifabutin against MAC, but normally its plasma concentration is only about 10% of that of the parent drug. Ritonavir increases rifabutin AUC by approximately 4-fold, but increases the desacetyl rifabutin AUC by 35-fold. Since the metabolism of desacetyl rifabutin uses the P4503A isozyme almost exclusively, these data suggest a profound inhibition of P4503A isozyme by ritonavir.

Because rifabutin toxic effects may be secondary to both the parent drug and the metabolite, at present concomitant use of rifabutin and ritonavir is contraindicated. Two-way interaction may well be present, but there are no published data on the effect of rifabutin on ritonavir kinetics.

Indinavir also inhibits the metabolism of rifabutin but to a significantly lesser extent. Indinavir increases rifabutin AUC by a mean of 2.73-fold; however, the increase in the desacetyl rifabutin AUC is only 4.76-fold above baseline. This again suggests significantly less inhibition of the P4503A isozymes by indinavir compared with ritonavir. The package insert recommends a 50%



Figure 1. Rifabutin is metabolized in the liver by two main pathways—via an esterase, resulting in the formation of desacetyl rifabutin, and via the P4503A isozymes, resulting in a more soluble demethylated metabolite.

reduction in the dose of rifabutin when used concomitantly with indinavir, but this combination still should be used with caution until data on toxic effects are generated. Rifabutin has been shown to have a significant effect on indinavir kinetics by reducing indinavir AUC by 32%.

Fluconazole. Indinavir has been shown to have no significant effect on fluconazole kinetics, which is not surprising since to a large extent fluconazole is renally eliminated, and has been shown to produce nonsignificant changes in ritonavir and indinavir AUC. This finding demonstrates that fluconazole is a fairly weak inhibitor of the P4503A isozyme. In contrast, ketoconazole has been shown to increase indinavir AUC by 68%, consistent with the more potent effect of ketoconazole on the P4503A isozymes.

Zidovudine, didanosine. Since most of the nucleoside analogue reverse transcriptase inhibitors are prodrugs that require extensive intracellular metabolism to the active triphosphate metabolite, any change in the kinetics of the parent drug may not necessarily translate into changes in clinical efficacy. Indinavir has been shown to have no significant interaction with the nucleoside analogues, but ritonavir has been shown to reduce zidovudine AUC because of its ability to induce glucuronidation. In addition, the combination of ritonavir and didanosine should be used with considerable caution because both drugs increase serum uric acid levels and can increase serum triglyceride concentrations, possibly potentiating didanosine to cause pancreatitis.

Oral contraceptives. Ritonavir and indinavir have opposite effects on the kinetics of ethinyl estradiol. Administration of indinavir has been shown to produce a 24% increase in ethinyl estradiol AUC; in contrast, ritonavir has reportedly resulted in a 40% decrease in ethinyl estradiol AUC. The most likely explanation for these findings may reside in the ability of ritonavir to induce glucuronidation and other cytochrome P450 isozymes involved in ethinyl estradiol metabolism.

*Trimethoprim/sulfamethoxazole.* Neither ritonavir nor indinavir has had clinically significant effect on the kinetics of these drugs.

Theophylline. Ritonavir has been shown to decrease theophylline AUC by 43% because of its ability to induce the P4501A2 isozyme. Whether this interaction occurs to a lesser extent in smokers is an important issue to be addressed because smoking is also a potent inducer of the P4501A2 isozyme. Indinavir has not demonstrated a significant pharmacokinetic interaction with theophylline, but this finding needs to be specifically verified in non-P4501A2—induced subjects because P4503A is responsible for a fraction of theophylline's metabolism.

Desipramine. Ritonavir has increased desipramine AUC by 145%. Since desipramine metabolism uses primarily the P4502D6 isozyme, these data suggest that the effect of ritonavir on P4502D6 is not insignificant. Desipramine is also a high hepatic clearance drug, and the effect of ritonavir on desipramine is most likely secondary to inhibition of first-pass metabolism and, therefore, to an increase in bioavailability. There are no interactive pharmacokinetic data on indinavir and desipramine. However, based on in vitro data showing very little inhibition of the P4502D6 by indinavir, major interaction seems unlikely.

#### Potential Drug Interactions with Protease Inhibitors and Drugs with a Low Therapeutic Index

Although the package inserts specify drugs that are contraindicated, it is worth reviewing some of these drugs to clarify the mechanisms by which this interaction may occur.

Terfenadine (Seldane). This non-sedating antihistaminic drug has a very potent effect on rectifying potassium (K+) channels in cardiac tissue, thus resulting in the prolongation of Q-T intervals on electrocardiogram (ECG) with consequent torsade de pointes arrhythmia. Under normal circumstances, terfenadine is completely metabolized by P4503A isozymes during its first pass through the liver to the active carboxylate metabolite, which has no cardiotoxic effect. Thus, systemic blood concentration of terfenadine cannot be measured. With inhibitors of P4503A, terfenadine concentrations can be detected in plasma, with consequent effect on cardiac repolarization. Both ritonavir and indinavir

inhibit P4503A activity and thus would increase terfenadine bioavailability. However, because the outcome of this drug interaction is potentially fatal, it would be unethical to confirm this by performing such studies in humans. Thus, terfenadine should never be given to patients on either ritonavir or indinavir.

Other proarrhythmic drugs. The mechanism of toxicity of astemizole (Hismanal) is simi-

In general, drugs that are renally excreted or mainly conjugated by the liver are safe to use with protease inhibitors. Drugs metabolized by P4503A are at highest risk; those that use P4502D6 have less interaction.

lar to that of terfenadine and thus the same restrictions apply to this drug. Loratadine (Claritin) does not seem to have an effect on rectifying K+ channels in vitro or in vivo so this drug can probably be used safely. There are a host of other drugs that interact with rectifying K+ channels in the cardiac tissue and that are metabolized by the P4503A or P4502D6 isozymes and whose use is thus contraindicated or restricted. Cisapride (Propulsid) and many of the antiarrhythmic drugs are contraindicated with concomitant use of ritonavir or indinavir. Tricyclic antidepressants should be used with considerable caution, and concentrations of these drugs should be measured, if possible, when used along with ritonavir. Diphenhydramine hydrochloride (Benadryl) also affects K+ channels and its metabolism may be affected by protease inhibitors.

Midazolam (Versed). Many of the benzodiazepine sedative/hypnotic drugs use P4503A for metabolism and thus their use is contraindicated in patients taking ritonavir or indinavir. Midazolam metabolism has been studied best, and this

drug is metabolized exclusively by P4503A isozymes. Because the hepatic clearance of this drug is approximately 400 mL/min, both liver blood flow and hepatic enzymatic activity contribute to its overall clearance; thus, prolonged sedation is to be expected when this drug is administered intravenously in patients taking protease inhibitors. Triazolam (Halcion) is also a potent substrate for P4503A isozymes, and thus interaction similar to that observed with midazolam is to be expected. Lorazepam (Ativan), temazepam (Restoril), and oxazepam (Serax) are metabolized either solely or primarily by conjugation, and thus pharmacokinetic interaction is not to be expected with concomitant use of these drugs with protease inhibitors.

Calcium channel blockers. Essentially all of the calcium channel blockers are metabolized by the P4503A isozymes, and many of these drugs have high hepatic clearance and thus undergo extensive first-pass metabolism. Both ritonavir and indinavir can be expected to increase the systemic bioavailability of these drugs and decrease their hepatic clearance. Because the therapeutic effect of calcium channel blockers can be measured by monitoring blood pressure, pulse rate, and ECGs, these parameters must be followed closely if protease inhibitors are given to patients on calcium channel blockers. It is suspected that these drugs may be very difficult to use concomitantly with potent protease inhibitors.

Lovastatin. Because this HMG-CoA reductase inhibitor and its active metabolites use P4503A isozymes for further metabolism, use of lovastatin with protease inhibitors may result in a potentially fatal drug interaction. It is important to bear this in mind because severe toxicologic drug interactions have been observed with the concomitant use of imidazole antifungal drugs and lovastatin (ie, severe rhabdomyolysis and one death). Although much less is known about the metabolism of other HMG-CoA reductase inhibitors, all these drugs should be used with caution. The structure of simvastatin is very similar to that of lovastatin and thus its metabolic pathway is probably similar to that of lovastatin.

Codeine. There is evidence suggesting that the analgesic effect of codeine is secondary to the generation of the active O-demethylated metabolite, morphine. Patients who do not express the cytochrome P4502D6 isozyme, or who have inhibited P4502D6, do not obtain significant pain relief from codeine. Hydrocodone is also metabolized to the potent opioid hydromorphone by the cytochrome P4502D6 isozyme. Thus, a drug interaction resulting in the loss of these drugs' analgesic efficacy may follow concomitant use with ritonavir.

The addition of protease inhibitors to the list of available antiretrovirals is an important advance. However, because of the lipophilic nature of these molecules, they have major effects on the activity of the cytochrome P450 isozymes, resulting in toxic drug interactions. The elucidation of these interactions is at an early stage, and an increasing number of studies will be published in the near future, helping practitioners to understand how to use other drugs with protease inhibitors optimally and safely. In addition, the effect of protease inhibitors on protein binding of other drugs needs to be elucidated because altered protein binding may have major effects on the activity of some drugs. In contrast with indinavir, ritonavir is very highly protein bound. As a general principle, drugs that are renally excreted or that are mainly conjugated by the liver are safe to use concomitantly with protease inhibitors. Drugs that are metabolized by the P4503A isozymes are the most at risk for interaction, with less interaction with drugs that use P4502D6. In high concentrations, ritonavir may have an effect on P4502C9; however, there are no correlates in vivo. Although at first glance drug interaction issues are daunting, with a thorough understanding of how drugs that are used in the treatment of HIV disease are metabolized and what their therapeutic indices are, toxic drug interactions can be prospectively identified and averted.

John G. Gerber is Professor of Medicine and Pharmacology, Divisions of Clinical Pharmacology and Infectious Diseases, at the University of Colorado Health Sciences Center in Denver.

#### **Suggested Readings**

Chiba M, Hensleigh M, Nishime JA, Balani SK, Lin JH. Role of cytochrome P450 3A4 in human metabolism of MK-639, a potent human immunodeficiency virus protease inhibitor. *Drug Metab Dispos.* 1996;24:307–314.

Cholerton S, Daly AK, Idle JR. The role of individual human cytochrome p450 in drug metabolism and clini-

cal response. *Trends in Pharmacol Sci.* 1992;13:434–439.

Kempf D, Marsh K, Denissen J, et al. Coadministration with ritonavir enhances the plasma levels of HIV protease inhibitors by inhibition of cytochrome P450. Presented at Third Conference on Retroviruses and Opportunistic Infections; January 28–February 1, 1996; Washington, DC.

Kumar GN, Rodrigues AD, Buko AM, Denissen JE Cytochrome P450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes. *J Pharmacol Exp Ther.* 1996;277:423—431.

Murray M. P450 enzymes: inhibition mechanisms, genetic regulation and effects of liver disease. *Clin Pharmacokinet*. 1992;23:132–146.

Announcing an Important Symposium at the XI International Conference on AIDS in Vancouver...



# uidelines for Antiretroviral Therapy: Bringing the State-of-the-Art to Clinical Practice

Sponsored by

The International AIDS Society-USA and Pacific Region, Canadian HIV Trials Network



The symposium will comprise lectures and extensive audience discussion/question-and-answer sessions. The current standards for the use of antiretroviral therapies and regimens will be addressed based on the increasing understanding of the pathogenesis of HIV disease and clinical research in the field

In January of this year, the IAS-USA convened an international panel to develop current guidelines for the use of antiretroviral therapy in HIV disease. This panel will reconvene at the XI International Conference on AIDS in Vancouver to discuss how the new research and information presented will further revise those *Guidelines*. Attendees will be encouraged to participate in the discussions in order to assist the panel in updating the *Guidelines*.

Who should attend The symposium is targeted to physicians and other health care providers involved in HIV/AIDS medical care and attendance is open to all registrants of the XI International Conference on AIDS in Vancouver.

Issues to be Discussed The agenda will be designed around four basic questions in antiretroviral management: When to Start Therapy; What to Start With; When to Change Therapy, including clinical status and lab markers, issues of resistance, and definitions of treatment failure; and What to Change to, including prior treatment history, stage of disease, and time on therapy. Postexposure prophylaxis and vertical transmission prevention will also be discussed.



Wednesday, July 10

6:30-7:30 pm Reception
Atrium Lobby and Terrace,
Pan Pacific Hotel

7:30-10:30 pm Academic Program
Ballroom A/B
Vancouver Trade and Convention Centre

## Gidelines Panelists/Symposium Faculty

#### **Symposium Co-Chairs:**

Julio Montaner, MD St. Paul's Hospital Vancouver, BC **Stefano Vella, MD** Istituto Superiore di Sanità, Rome, Italy **Paul Volberding, MD** University of California San Francisco, CA, USA

#### **Speakers:**

Charles Carpenter, MD Panel Chairperson Brown University Providence, RI, USA

Martin Hirsch, MD Harvard Medical School Boston, MA, USA

**Douglas Richman, MD** University of California, San Diego, CA, USA Margaret Fischl, MD University of Miami Miami, FL, USA

Melanie Thompson, MD AIDS Research Consortium of Atlanta, GA, USA

Michael Saag, MD University of Alabama at Birmingham, AL, USA Scott Hammer, MD Harvard Medical School Boston, MA, USA

David Katzenstein, MD Stanford University Stanford, CA, USA

**Robert Schooley, MD** University of Colorado HSC Denver, CO, USA

Patrick Yeni, MD
X. Bichat Medical School
Paris, France

## egistration/Videotapes

For more information about the symposium or to order unedited tapes of the symposium AND FOR REGISTRATION INFORMATION, please contact the International AIDS Society-USA:

By Mail: International AIDS Society-USA
Attn: Vancouver Symposium

353 Kearny Street San Francisco, CA 94108

By Fax: 415-675-7438 By e-mail: IASUSA1@aol.com



## AUDIOTAPES OF IAS-USA PRESENTATIONS: Improving the Management of HIV Disease

Based on numerous requests from symposium participants, audiotapes of the symposium presentations are available for purchase. Each unedited individual presentation audiotape is approximately 30 minutes in length. To order, check the boxes corresponding to the tapes you wish to purchase and send the completed order form with payment to the address listed on the back of this page.

LOS ANGELES, CALIFORNIA	NEW ORLEANS, LOUISIANA
FEBRUARY 10, 1996	MARCH 9, 1996
Chair: RONALD T. MITSUYASU, MD	Chair: ROBERT T. SCHOOLEY, MD
Co-chair: PAUL A. VOLBERDING, MD	Co-chair: NEWTON HYSLOP, Jr, MD
HIV Pathogenesis	Viral Dynamics and Virus Load as Determinants of
H. Clifford Lane, MD	HIV Pathogenesis Daniel R. Kuritzkes, MD.
William A. O'Brien, MD.	Humoral Immunity to HIV Infection: Implications for Vaccine Development
Initiation of Antiretroviral Therapy Paul A. Volberding, MD	John P. Moore, PhD
Protease Inhibitors Steven A. Miles, MD	Initiation of Antiretroviral Therapy Paul A. Volberding, MD
Strategies for Continuing Benefit of Antiretroviral Therapy Michael A. Saag, MD	Strategies for Continuing Benefit from Antiretroviral Therapy
Advances in Prevention and Treatment of Pediatric	Michael S. Saag, MD
HIV Infection	Prevention and Treatment of <i>Pneumocystis carinii</i> and
Yvonne J. Bryson, MD	Toxoplasma Infections William G. Powderly, MD.
Immune-based Therapies for HIV Infection	Disseminated Mycobacterium avium Complex Disease in
Ronald T. Mitsuyasu, MD	Patients with AIDS
HIV Disease in the Era of Managed Care	Constance A. Benson, MD
Mark D. Smith, MD, MBA	Cutaneous Manifestations of HIV Infection Clay J. Cockerell, MD
ATLANTA, GEORGIA	Management Issues in Late-stage Disease: Case Studies
FEBRUARY 21, 1996	and Discussion
Chair: MICHAEL S. SAAG, MD	Perry Pate, MD
Co-chair: MELANIE A. THOMPSON, MD	NEW YORK, NEW YORK
HIV Pathogenesis	MARCH 22 & 23, 1996
Michael S. Saag, MD.	Chair: GERALD H. FRIEDLAND, MD
The Role of HIV Plasma RNA in Clinical Decision Making Melanie A. Thompson, MD	Co-chair: PAULA. VOLBERDING, MD
Initiating Antiretroviral Therapy	HIV Pathogenesis
Jeffrey L. Lennox, MD	David D. Ho, MD.
Protease Inhibitors: The Future Is Here	Plasma HIV RNA Quantitation: Is It Ready for the Big Time?
Kathleen E. Squires, MD.	Daniel R. Kuritzkes, MD
Continuing the Benefits of Antiretroviral Therapy: What to Do Next?	Initiation of Antiretroviral Therapy
Daniel R. Kuritzkes, MD	Paul A. Volberding, MD
Selected Issues in Primary Prophylaxis of	Donna Mildvan, MD
Opportunistic Infections	Progress and Pitfalls in OI Prophylaxis
C. Robert Horsburgh, Jr, MD	Judith Feinberg, MD
Novel Therapies for HIV-1 Infection  Robert T. Schooley, MD	Strategies for Continuing Antiretroviral Therapy Michael S. Saag, MD
Occupational Risk of HIV Infection	Pediatric HIV Disease:Prevention and Treatment
David M. Bell, MD	Stephen A. Spector, MD
Integrating Standard and Complementary Therapies for	HIV Disease in the Era of Managed Care Mark D. Smith, MD, MBA
People Living with HIV Charles Steinberg, MD	Prevention of HIV Infection Gerald H. Friedland, MD

SAN FRANCISCO, CALIFORNIA APRIL 20, 1996	Antiretroviral Therapy in the Untreated Patient John P. Phair, MD		
Chair: PAUL A. VOLBERDING, MD Co-chair: STEPHEN E. FOLLANSBEE, MD	Douglas D. Richman, MD		
HIV Variation, Natural Immune Response, and Long-term Survival	Antiretroviral Therapy Harold A. Kessler, MD		
Jay A. Levy, MD  Use of Virologic Markers in Clinical Practice Michael S. Saag, MD.  Novel Therapies for HIV Infection Robert T. Schooley, MD.	Advances in Prevention and Treatment of Pediatric HIV  Infection  Yvonne J. Bryson, MD  Molecular Genetic Interventions for AIDS Treatment Gary J. Nabel, MD, PhD		
Insights into the Molecular Regulation of HIV Growth and Pathogenesis	SAN FRANCISCO, CALIFORNIA		
Warner C. Greene, MD, PhD	MAY 4, 1996		
Kaposi's Sarcoma-associated Herpesvirus Donald E. Ganem, MD	Chair: MARK A. JACOBSON, MD Co-chair: RICHARD A. WOLITZ, MD		
New Agents and Combination Therapy Joseph J. Eron, MD	Disseminated Mycobacterium avium Complex Disease in		
Toxicities and Drug Interactions of the Protease Inhibitors Daniel S. Stein, MD	Patients with AIDS Constance A. Benson, MD		
Alternative Therapies  Donald I. Abrams, MD	with AIDS William G. Powderly, MD.		
Leveraging the Internet for HIV Management William A. Whitaker	Systemic Therapy for CMV End-Organ Disease in AIDS Mark A. Jacobson, MD		
CHICAGO, ILLINOIS	Intraocular Treatment for CMV Retinitis Richard A. Wolitz, MD		
APRIL 24, 1996	Prophylaxis for CMV Retinitis		
Chair: JOHN P. PHAIR, MD	Judith Feinberg, MD		
Co-chair: HAROLD A. KESSLER, MD Viral Pathogenesis	Michael J. Clement, MD		
Steven Wolinsky, MD.  Viral and Cellular Dynamics in HIV-1 Infection George M. Shaw, MD, PhD.	Pathogenesis and Treatment of HIV-associated Wasting Morris Schambelan, MD		
	otapes IDS Society-USA reet, Fourth Floor, San Francisco, CA 94108		
Name	To order audiotapes of IAS-USA program		
	presentations, please check correspond-		
Address			
City/State			
Zip Code	\$9.00 per 30-minute tape plus shipping and handling and applicable sales tax.  Do not send cash. Please allow three to		
Phone ()	four weeks for delivery.		
No. of Tapes S&H Charges No. of tapes X \$9.00 pe	er tape = Subtotal =		
2 tapes \$2.00	add 8.5% sales tax		
3 tapes \$3.00 4 tapes \$4.00 5–10 tapes \$5.00	handling (see left)		
0.000	OTAL ENCLOSED		

International AIDS Society-USA NON-PROFIT ORG. U.S. POSTAGE PAID San Francisco, CA 94108 PERMIT NO. 3842 SAN FRANCISCO, CA

353 Kearny Street

