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About This Issue

This issue provides our review of the 14th annual Conference on Retroviruses and Opportunistic Infections, in Los Angeles, California, from February 25 to 28, 2007. Mario Stevenson, PhD, reviews developments in and highlights of basic science research, including cellular restriction factors, virus replication regulation, and HIV pathogenesis. Susan Buchbinder, MD, presents recent findings in HIV epidemiology and prevention interventions, including updates on the European epidemic, the US epidemic, and the global epidemic in men who have sex with men. Scott Letendre, MD, Beau Ances, MD, PhD, Sarah Gibson, BS, and Ronald J. Ellis, MD, PhD, explore neurologic complications of HIV disease and their treatments. Judith S. Currier, MD, and Diane V. Havlir, MD, examine recent findings on complications of HIV disease and therapy, including antiretroviral-associated toxicities in resource-limited settings. Finally, Joyce Jones, MD, Barbara Taylor, MD, Timothy Wilkin, MD, MPH, and Scott M. Hammer, MD, review new findings in antiretroviral therapy, treatment strategies, and drug resistance.

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**Basic Science Highlights**

**Mario Stevenson, PhD**

The 14th Conference on Retroviruses and Opportunistic Infections generated a lot of excitement with the announcement of clinical studies employing the use of 2 new classes of antiretroviral drugs that target the viral integrase enzyme and the viral coreceptor CCR5. In addition, a number of presentations on cellular restriction factors provided surprises regarding the mechanism by which cellular restrictions antagonize viral infection. There was also much interest in studies presenting novel cellular cofactors of HIV-1 infection. The conference illustrated how basic science research is paying off. Essential steps in the viral life cycle, uncovered through basic research, are now being targeted by new classes of antiviral agents. In addition, basic science is unveiling potential new targets of antiretroviral therapy.

**Bench to Bedside**

A number of presentations, including the plenary given by Edward Berger of the National Institutes of Health (NIH) (Abstract 12a) who was this year’s recipient of the Bernard Field’s Memorial Lectureship, illustrated how basic science has uncovered aspects of the biology of HIV-1 that are now being exploited through antiretroviral therapy. Dr Berger discussed his laboratory’s role in identification of key cell surface molecules that are essential for HIV-1 infection. Shortly after the discovery of HIV-1 as the causative agent of AIDS, several research groups identified CD4 as an important cell surface protein that was needed for infection of a cell by HIV-1. However, it was apparent that although CD4 was necessary for HIV-1 infection of a cell, it was not sufficient and this suggested the existence of other cell surface proteins that may be required for virus infection.

In 1996, Dr Berger’s laboratory identified a chemokine receptor named CXCR4 as a cell surface protein that, in addition to CD4, was required for HIV-1 infection. Research examining the infectivity of different HIV-1 isolates from lymphocytes and macrophages suggested that there may be more than 1 type of coreceptor. Around that time, research by Russo, Gallo, and colleagues suggested that chemokines prevent HIV-1 infection of cells, including macrophages. Subsequently, Dr Berger’s research group and independently several other research groups identified a chemokine receptor known as CCR5 as a principle coreceptor that, in addition to CD4, was required for infection of certain cell types including primary macrophages.

These research discoveries have had a fundamental impact on the field. The identification of viral coreceptors has furthered the understanding of how HIV-1 targets specific cell types and has provided important clues to underlying mechanisms that underscore viral pathogenicity. Importantly, the identification of these coreceptors revealed novel targets for therapeutic intervention of HIV-1 infection. In particular, CCR5 has long been recognized as a particularly attractive therapeutic target. Shortly after the identification of CCR5 as a viral coreceptor, research from the Aaron Diamond Research Center in New York demonstrated that individuals harboring a homozygous 32-base pair deletion in the CCR5 gene were highly resistant to HIV-1 infection. In addition, the lack of a functional CCR5 gene did not appear to affect the health of the individual. Therefore, since CCR5 was apparently dispensable, this provided hope that antiviral strategies that block CCR5 function would be well-tolerated by the host.

Presentations in session 33 provided exciting evidence that the CCR5 inhibitor maraviroc is very effective in impacting viral replication in antiretroviral therapy-experienced patients (Abstracts 104aLB, 104bLB). There was also much excitement regarding salvage trials using another novel class of antiretroviral agent that targets the viral integrase enzyme. Integrase is encoded by the viral polymerase gene and integrase catalyzes the reaction by which viral cDNA is inserted into the host cell genome. Basic research demonstrated that this enzyme was essential for viral replication and also provided detailed insight into the mechanism by which integrase inserts viral cDNA into the cellular genome. This multistep process includes 2 catalytic reactions. The first involves 3′-endo nucleolytic processing of the free ends of the viral cDNA and, in a second reaction, the viral cDNA is joined to cellular DNA.

Initial screening assays that interfered with the formation of complexes between integrase and specific DNA sequences in the viral long terminal repeat identified compounds that, unfortunately, were inactive against HIV-1 in culture. Second generation screening assays recapitulated the strand transfer reaction and inhibitors identified in these assays exhibited antiviral activity in culture.

The fruits of these efforts were revealed in presentations in session 33 (Abstracts 105aLB, 105bLB) and in session 40 (Abstract 143LB) where results of the effect of integrase inhibitors on viral replication in antiretroviral therapy-experienced patients were presented. Clinical trials with the CCR5 and the the integrase inhibitors in antiretroviral therapy-experienced patients demonstrated that these agents are effective against viruses which are highly resistant to other classes of inhibitors. These results have generated optimism in the field since they represent new additions to the armamentarium being used in the treatment of HIV-1 infection.

**Cellular Restrictions**

Primate lentiviruses like HIV-1 operate on a limited genetic budget. Therefore, these viruses commandeer cellular func-

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tions that serve as cofactors at various stages in the viral replication cycle. However, it is clear that some cellular proteins with which the virus interacts do not cooperate in viral replication but rather, antagonize viral replication. APOBEC 3G was the first cellular protein shown to antagonize primate lentivirus replication. A number of APOBEC 3 family members and in particular, APOBEC 3G and 3F, have been shown to antagonize replication of a variety of lentiviruses and retroviruses as well as unrelated viruses such as hepatitis B virus. In the case of primate lentiviruses, APOBEC 3G exhibits potent antiretroviral activity but this is counteracted by the viral vif gene. Vif antagonizes the antiviral activity of APOBEC by promoting its ubiquitinylation and subsequent proteasomal degradation.

APOBEC 3G restriction has received a considerable amount of research attention. As a result, detailed insight has been gathered into how Vif promotes ubiquitylation of APOBEC proteins. However, the picture is less clear regarding the mechanism by which APOBEC proteins antagonize viral replication. At the conference, a number of presentations focused on the interplay between APOBEC and Vif (Abstracts 20, 49, 107, 184, 188, 325, and others). APOBEC 3G and 3F are cytidine deaminases that are packaged into viral particles during virus assembly. When those particles infect a new cell, and viral cDNA is being reverse transcribed, it is thought that these enzymes edit the viral genome during the reverse transcription process such that the synthesized cDNA contains extensive G-A mutations. These mutations are then thought to compromise the integrity of the viral cDNA both in terms of stability and its ability to encode functional viral transcripts. This mechanism of antiviral activity by the APOBEC proteins was questioned by the demonstration that APOBEC 3G mutants lacking a functional enzymatic site still retained antiviral activity.

Abstracts 204 and 211 extended on the theme that the antiviral activity of APOBEC 3 proteins is independent of cytidine deaminase activity. Abstract 211 examined mutant APOBEC 3G and 3F proteins with altered N-terminal or C-terminal cytidine deaminase motifs. APOBEC 3G and 3F mutants lacking cytidine deaminase activity were still fully able to function as antiviral factors. Importantly, the antiviral activity was strongly correlated with inhibition of reverse transcription. Similarly, Abstract 204 examined the cytidine deaminase activity and antiviral activity of a large panel of APOBEC 3G mutants in order to identify regions of APOBEC 3G which are important for antiviral activity. Collectively, these studies suggest that the antiviral activity of APOBEC 3 proteins might involve inhibition of reverse transcript accumulation and that this inhibition is independent of cytidine deamination.

A number of APOBEC 3 proteins have now been demonstrated to inhibit retrotransposition of LTR and non-LTR retrotransposons. Abstract 188 examined whether the ability of APOBEC 3 proteins to inhibit retrotransposition required a catalytically functional active site on the APOBEC 3 protein. Mutations in 1 or both of the active sites of human APOBEC 3B, 3F and 3G were examined for cytidine deaminase activity and ability to inhibit retrotransposition. This study concluded that the active sites of APOBEC 3 proteins are required to inhibit retrotransposition. Abstract 19 presented evidence that HIV-1 infection of primary CD4+ T-cells in vitro actually induced retrotransposition and the progressive accumulation of both LTR and non-LTR gene elements. Future studies should determine whether the induction of retrotransposition activity by HIV-1 infection plays a role in HIV-1 mediated immunopathogenesis.

Several studies focused on cellular factors that play a role in Vif-APOBEC interplay. Abstract 20 provided evidence that 7SLRNA interacts with an APOBEC 3G and is preferentially packaged into HIV-1 particles as a result of this interaction. Reduction of 7SLRNA packaging impaired the packaging of APOBEC 3G and its antiviral function. To exert their antiviral activity, APOBEC 3 proteins must be packaged within virus particles. However, precise information on the mechanism by which APOBEC 3 proteins are packaged within virions is not yet available. Furthermore, retroviruses such as murine leukemia virus (MLV) are sensitive to inhibition by mouse APOBEC 3. However, MLV does not harbor a known vif gene. Nevertheless, MLV appears to avoid restriction by mouse APOBEC 3 by excluding it from the virion.

Abstract 206 examined determinants that are important for APOBEC 3 packaging. The study demonstrated that both mouse and human APOBEC 3 proteins can be incorporated into HIV-1 Gag virus-like particles and that the nucleocapsid sequence within HIV-1 Gag is important for this packaging. Interestingly, chimeric proteins in which mouse APOBEC 3 fragments were fused to human APOBEC 3 fragments were packaged within MLV particles. Therefore, these chimeric proteins were able to overcome the mouse APOBEC 3 exclusion mechanism that is present in MLV. Abstract 49 characterized the subcellular localization of APOBEC 3G. This study presented evidence that APOBEC 3G localizes to specialized cytoplasmic compartments of mammalian cells, which are known as mRNA processing (P) bodies. P bodies are involved in degradation and storage of cellular mRNA. The study examined the colocalization of P body components with APOBEC 3G. The study concluded that the strong degree of colocalization between APOBEC 3G and P body components assigns a P body localization to APOBEC 3. Additional studies are underway to determine whether APOBEC 3G proteins that are contained within P bodies exhibit antiviral effects.

The cytoplasmic body component TRIM 5α was identified 3 years ago as a protein that restricts HIV-1 infection of Old World monkey cells. Since that finding, a number of investigators have turned their attention to deciphering the mechanism by which TRIM 5α proteins impact virus infection, and a number of presentations on this topic were featured at the conference.

Abstract 16 presented insight regarding the antiviral mechanism of TRIM 5α restriction. Current models suggest that TRIM 5α interacts with
capsid proteins of incoming virions. This interaction is thought to interrupt efficient uncoating of viral nucleic acid that subsequently leads to an inhibition of reverse transcription. Data presented in Abstract 16 presented evidence for a role of the proteasome in the antiviral mechanism of TRIM 5α restriction. The authors examined the fate of GFP-VPR-labeled virus particles in cells over-expressing monkey TRIM 5α and observed that proteasome inhibition led to the association of TRIM 5α cytoplasmic bodies with ubiquitin and proteasomal subunits. The authors proposed a model in which TRIM 5α proteins encapsulate the incoming viral core and alter trafficking away from the nucleus and to the proteasomal degradation pathway.

In Abstract 216, the effect of TRIM 5α on the fate of capsid during early steps of the infection was examined. Previous studies have suggested that TRIM 5α accelerates uncoating of the viral core. Data presented in Abstract 216 suggested that in restrictive cells (which express TRIM 5α) capsid undergoes a slow yet measurable degradation whereas it remains intact in nonrestrictive cells. The authors suggest that TRIM 5α directs viral cores to a vesicular compartment for degradation.

In Abstract 185, evidence for the ability of human TRIM 5α to restrict an extinct retrovirus was presented. Throughout the last 30 million years, TRIM 5α has been evolving under extreme positive selection in the primate lineage. It has been proposed that this rapid evolution has been driven by conflicts with retroviruses. Chimpanzees contain approximately 150 copies of a retrovirus called PtERV that is now endogenous. However, this retrovirus is absent from the human genome. The authors examined whether the absence of PtERV from the human genome may have been due to possible protection of the human genome by TRIM 5α. The authors constructed chimeric viruses containing the P12 and capsid regions of PtERV in the backbone of MLV. They found that human TRIM 5α potently restricts PtERV capsid suggesting that the evolution of TRIM 5α may have protected humans from infection when PtERV was exogenous some 3 to 4 million years ago.

**Cellular Cofactors**

Presentations that provided evidence for novel cofactors of HIV-1 replication received a lot of interest at the conference. Several years ago, a couple of research groups identified LEDGF/p75 as a binding partner of HIV-1 integrase in human cells. In the past year, several groups have demonstrated a convincing role for this protein in viral replication. Abstract 15 presented an overview of how LEDGF/p75 may impact viral replication. The authors presented a model in which LEDGF/p75 helps to tether integrase within the viral pre-integration complex to chromosomes. The study provides the rationale for the development for small molecule inhibitors which interrupt the interaction between integrase and LEDGF/p75. In order for viruses to assemble at the plasma membrane, newly synthesized retroviral Gag polyproteins are directed to the plasma membrane as a result of the membrane binding activity of the matrix domain of Gag. This domain contains an N-terminal myristyl group that can exist in both sequestered and exposed conformations.

Abstract 46 presented evidence that phosphatidylinositol-(4,5)-bisphosphate acts as a trigger of the myristyl switch. This factor is abundant in the inner leaflet of the plasma membrane and the findings present a mechanism for the specific targeting of HIV-1 Gag to membranes enriched in this phosphoinositide.

Abstract 47 presented evidence for a new cellular cofactor (TIP47) as being essential for the incorporation of HIV-1 Env glycoprotein into viral particles. During virus assembly, viral Env glycoprotein is specifically incorporated into virions but the mechanism by which Env associates with Gag particles is unknown. Research presented in Abstract 47 indicated that TIP47 promotes physical association between HIV-1 Gag and Env proteins during virus assembly. siRNA-mediated silencing of TIP47 prevented the association of Gag with Env. The results further suggest that TIP47 interacts with the matrix domain of Gag. Since TIP47 appears essential for the incorporation of Env into virus particles and for HIV-1 infectivity, the interaction of TIP47 with Gag or Env is an attractive antiviral target.

Lentiviruses like HIV-1 can efficiently infect nondividing cells. However, the viral and cellular determinants that promote the nuclear uptake of viral reverse transcription complexes remain obscure. In Abstract 48, evidence was presented that a truncated form of the cellular protein CPSF6 exhibits an antiviral effect by opposing the nuclear entry of HIV-1. When HIV-1 variants resistant to the antiviral activity of CPSF6 were derived, mutations in capsid were found to confer resistance. Interestingly, some of these mutants were impaired in their ability to infect nondividing HeLa cells but only slightly affected in their ability to infect nondividing macrophages.

In contrast, other mutations in CPSF6-resistant HIV-1 CA did not affect infection of growth-arrested HeLa cells but blocked infection of macrophages. The authors speculate that these mutations could influence the ability of capsid to dissociate from HIV-1 reverse transcription complexes and that truncated CPSF6 or its cofactors aid in this dissociation. This model suggests that nuclear entry of HIV-1 is in part regulated by the dissociation of capsid from the reverse transcription complex. The study also illustrates that caution should be exercised when using artificially growth-arrested cell lines as surrogates for nondividing macrophages.

The genomes of metazoans, including humans, encode short regulatory RNAs known as Micro RNAs (miRNAs). These miRNAs are expressed as long, capped, polyadenylated transcripts that are processed by the RNase III enzymes Drosha and Dicer to generate mature 22-nt miRNAs. The miRNAs are then incorporated into the RNA-induced silencing complex (RISC) to guide RISC to complimentary mRNA. The target mRNA is then translationally repressed.

In his plenary, Dr Bryan Cullen (Abstract 112) overviewed what is cur-
rently known regarding the cellular machinery involved in the synthesis of cellular miRNA. He presented evidence that several DNA viruses including Epstein-Barr virus (EBV) and Kaposi’s sarcoma-associated herpes virus (KSHV) encode viral miRNAs that may target host genes involved in antiviral defense. Dr Cullen presented evidence that RNA viruses such as HIV-1 and hepatitis C virus do not generate detectable miRNAs in infected cells. The identification and characterization of viral miRNAs encoded by EBV and KSHV were further expanded upon in Abstract 18. Future studies will identify how these viral miRNAs regulate viral replication in the infected cell. Recent studies have suggested that HIV-1 Tat protein can functionally suppress RNA silencing. Data presented in Abstract 217 presented evidence to the contrary and argued that Tat and related human transactivators do not act as suppressors of the RNA silencing machinery.

Regulation of Virus Replication

The establishment of a latent infection in memory CD4+ T-lymphocytes has been proposed to represent the biggest obstacle to HIV-1 eradication in infected individuals. However, understanding the actual mechanism of latency has been difficult because of a lack of physiologic models which faithfully recapitulate viral latency in vitro.

Abstract 228 presented evidence that the maintenance of a latent state is governed by transcriptional interference. Several groups have presented evidence that latent viral genomes are located primarily within introns of active host genes in vivo. This raises a possibility that active genes may be transcriptionally interfering with the transcription of the integrated provirus. To examine this, the authors constructed an actively transcribed HPRT gene containing an HIV-1 vector within a specific intron. The authors observed that HPRT transcription negatively regulated HIV-1 gene expression implying that integration of proviruses into transcriptionally active genes may promote the establishment of a latent provirus.

Abstracts 274 and 275 examined conditions under which nonproliferating lymphocytes can become latently infected. In Abstract 274, the authors demonstrated that enhanced transduction and addition of deoxynucleosides enhanced the efficiency of integration in resting lymphocytes. This argues that limiting substrate rather than postentry blocks may limit integration into resting CD4+ lymphocytes. Abstract 275 examined how the level of CD4+ T-cell activation at the point of infection dictates whether a latent or persistently infected cell will subsequently be established. The results suggested that cells with the lowest division rate were more likely to survive infection and return to a quiescent state that harbored latent, persistent infection.

Abstracts 227 and 258 examined Toll-like receptor (TLR) ligands for their ability to modulate infection of CD4+ T-lymphocytes. Data presented in Abstract 227 presented evidence that TLR stimulation of CD4+ T-lymphocytes led to activation of viral gene expression. Therefore, TLR regulation, which has previously been shown to be important in macrophage function, may also impact the dynamics of T-cell reservoirs of HIV-1. In Abstract 258, CCR7 ligands CCL19 and CCL21 were found to render resting CD4+ T-lymphocytes highly permissive to HIV-1 infection. However, although there was a high degree of HIV-1 integration, virus production was at a low level. Therefore, these CCR7 ligands may contribute to the induction of a latent HIV-1 infection in resting CD4+ T-lymphocytes.

Although CD4+ T-lymphocytes and macrophages are considered the principle targets for HIV-1 infection in tissues, a variety of studies have suggested that other cell types may act as reservoirs of HIV-1 replication. Abstract 167 presented evidence that primary human eosinophils are susceptible to infection by X4-tropic HIV-1.

Abstract 273 presented evidence that CD34+ hematopoietic stem cells are susceptible to HIV-1 infection. The study showed that CD34+ stem cells were susceptible to HIV-1 infection and, despite infection, were able to survive and proliferate. The infection of such cells could have important implications for HIV-1 persistence and for strategies to eradicate HIV-1.

Abstract 276 characterized the cell subset in the CD16 monocyte population that is susceptible to HIV-1 infection. Previous studies have shown that viral DNA can be detected in monocytes from HIV-1 infected patients on antiretroviral therapy. Levels of viral DNA in CD16+ and CD16− monocytes were compared and the authors observed that CD16+ monocytes were preferentially infected at levels compatible to resting memory T-cells. The authors suggest that CD16− monocytes may restrict HIV-1 infection because they contain active low-molecular mass APOBEC 3G complexes and may also exhibit limiting amounts of CD4 and CCR5 expression as compared with CD16+ monocytes.

Some cell types may not be directly susceptible to HIV-1 infection but may assist in the dissemination of the virus to other cell types. Previous studies have shown that dendritic cells have the capacity to capture HIV-1 particles and present them to CD4+ T-cells.

Abstract 202 presented evidence that Langerhans cells (LCs), which are a subtype of dendritic cells, can capture HIV-1 particles and transmit internalized particles to CD4+ T-cells in trans. Evidence was presented that viral particles are directed to an intracellular compartment that was positive for tetraspan markers including CD81. The major histocompatibility complex-like protein CD1A appeared to play an enhancing role in HIV infectivity. This suggests that HIV traffics through a CD1A+ compartment in LCs and that this trafficking is important for transinfection.

Pathogenesis

Several presentations highlighted the role of viral determinants in disease pathogenesis. The viral accessory protein Nef has been shown to exhibit a variety of functions. Among these activities, Nef has been shown to down modulate T-cell receptor-CD3 complexes from infected cells thereby restrict-
ing their ability to respond to stimulation and activation-induced cell death.

In Abstract 156, data were presented to suggest that the ability to down-regulate TCR-CD3 and to block apoptosis is only exhibited by nef alleles of viruses that exhibit nonpathogenic infections. In contrast, nef alleles from HIV-1 and subset of closely related simian immunodeficiency virus (SIV) lack the ability to down-regulate TCR-CD3. The authors suggest that differences in nef function may contribute to the high levels of immune activation and apoptosis that characterize pathogenic HIV-1 infection. The authors further suggest that the ability of naturally infected monkeys to maintain high viral loads in the absence of pathogenicity may be due to the ability of Nef to prevent high levels of immune activation and apoptosis.

Individuals infected with HIV-1 variants containing defective alleles nef have been shown to exhibit a long-term, non-progressive HIV-1 infection. Abstract 250 examined 6 individuals with Nef-deleted viruses who had long-term, non-progressive infection, 4 of whom eventually had HIV disease progression. Individuals harboring Nef-defective viruses who subsequently exhibited HIV disease progression harbored viruses with extended coreceptor use to CXCR4. Therefore, despite low levels of viral replication in these individuals harboring Nef-defective viruses, there was sufficient ongoing replication to allow envelope evolution and expanded coreceptor use that may ultimately be associated with disease progression.

In Abstract 269, vpu and envelope genes in 240 HIV-1-infected women from a high-risk commercial sex worker cohort were examined. Of the 240 samples examined, 16 contained an incomplete vpu gene and 13 of these also lacked a complete envelope gene. In addition, the incidence of vpu and envelope defective proviruses increased between 2000 and 2004 suggesting that these isolates are becoming more prevalent. Patients harboring proviruses with defective vpu and envelope genes had higher CD4+ cell counts and lower plasma viral load than patients harboring intact proviruses. In Abstract 304, evidence was presented that TLR ligands induced the expression of activation markers on CD4+ and CD8+ T-cells. The authors propose that microbial products can induce T-cell activation and turnover. Therefore, heightened translocation of microbial products through gut mucosa could contribute to exaggerated immune activation that underscores chronic HIV-1 infection (see also Abstract 227).

In approximately 50% of patients, disease progression is reflected by an emergence of CXCR4-utilizing viruses. Abstract 252 examined the timing of HIV-1 coreceptor usage in subjects with rapid and slow rates of CD4+ T-lymphocyte depletion. Results indicate that the emergence of X4 viruses is associated with the rapid loss of CD4+ T-cells but that this emergence occurs after rapid CD4+ T-cells loss begins. This would suggest that the emergence of X4 variants is a result of rather than a cause of rapid CD4+ cell depletion.

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A list of all cited abstracts appears on pages 83 to 91.
HIV Epidemiology and Prevention Interventions

Susan Buchbinder, MD

Several presentations at the 2007 Conference on Retroviruses and Opportunistic Infections focused on the underlying factors driving the HIV epidemic in selected regions of the world and on selected populations. The conference also provided updated data on 1 of 2 successful adult male circumcision efficacy trials to prevent HIV acquisition, and a review of 1 of 2 unsuccessful efficacy trials of the microbicide cellulose sulfate. Presentations also focused on strategies to prevent HIV acquisition through pre-exposure prophylaxis, treatment of sexually transmitted diseases, and prevention of mother-to-child transmission through breastfeeding.

The European Epidemic

An overview of factors driving the European AIDS epidemic was presented by Johnson (Abstract 54). The European epidemic is really 3 separate epidemics: 1) western Europe experienced a dramatic decline in newly diagnosed AIDS cases with the introduction of potent antiretroviral therapy in the mid-1990s; 2) central Europe has had a low-level, stable number of AIDS cases since the early 1990s; and 3) eastern Europe experienced dramatic increases in newly diagnosed infections and AIDS cases beginning in the early 2000s. Johnson provided insight into the many social, behavioral, and biologic factors driving these different epidemics.

The eastern European epidemic, now accounting for approximately twice the number of newly diagnosed HIV cases as in western Europe, is primarily driven by injection drug use (IDU) and secondarily through the heterosexual partners of these drug users. This is likely the result of the many political and social changes confronting eastern Europe, including changes in drug trafficking routes and drug prices, leading to an increase in the size of the population using drugs and HIV transmission within drug-sharing and sexual networks.

The western European epidemic was previously largely focused within populations of men who have sex with men (MSM), where rates continue to be high, driven by increases in unprotected sex. Johnson speculated that the epidemic in IDU in western Europe may have been diminished through use of harm-reduction strategies, such as access to clean injection equipment and drug treatment programs. However, the predominant mode of HIV acquisition in western Europe is now heterosexual sex, with a substantial contribution from immigrant populations displaced by political and social upheaval in sub-Saharan Africa.

Each of the European epidemics is affected by late HIV diagnosis, as 54% of patients are diagnosed when CD4+ counts are less than 200 cells/μL, attenuating the effectiveness of antiretroviral therapy for them, and leading to potential increase in transmission to partners when HIV-seropositive individuals with late-stage disease are unaware of their infection status. Johnson called for increased HIV testing and evidence-based prevention interventions to populations at highest risk.

The US Epidemic

Jaffe reviewed the current status of the HIV epidemic in the United States (Abstract 63). By the end of 2005, nearly 1 million people had been diagnosed with AIDS in the United States, and more than 500,000 had died. The death rate of 58 cases per 1 million population in 2005 in the United States was twice that of any country in the European Union. Although the number of new AIDS cases has remained relatively stable throughout the early 2000s, there was an approximate 10% increase in AIDS cases in 2005, which may be an early indicator of an upward trend. MSM account for the majority of US HIV and AIDS cases, and their relative contribution to the total HIV and AIDS caseload in the United States continues to rise. African Americans make up half of all HIV and AIDS cases in the United States, and the HIV and AIDS case rate is 8-times higher in African Americans than whites in the United States.

Jaffe also reviewed prevention interventions that have led to substantial reductions in new HIV infections in the United States (eg, screening of donated blood, prevention of mother-to-child transmission [PMTCT], condoms, provision of clean injection equipment), and contrasted them with the government-supported approach of abstinence-only programs, for which scientific support is quite limited. A Cochrane review of 8 randomized, controlled studies of abstinence-only programs in youth in the United States found no statistically significant reduction in reported risk or biologic outcomes. A more recent unpublished study found a statistically significant reduction of sexual activity in African American youth (aged 10-15 years) randomized to receive an abstinence-only intervention, although 30% of the virgins in this arm of the study initiated sexual activity during the 24 month follow-up period. Jaffe made a plea to focus efforts on prevention interventions known to be successful, abandon those found not to work, and encourage leaders within at-risk communities to prioritize changes in behavior.

Several presentations focused on the need for increased HIV testing to detect HIV infections and thus reduce the risk of ongoing transmission. Weis and colleagues presented data on 4221 persons newly diagnosed with HIV infection in South Carolina from January 2001 to December 2005 (Abstract 957). Of this group, 73% had visited
a South Carolina health care facility at least once prior to testing HIV seropositive, with a median of 4 visits per patient. 42% of this sample developed an AIDS diagnosis within 1 year of their first HIV-seropositive test. Torian and colleagues presented data on very late HIV diagnosis (Abstract 964). Overall, 28% of all newly diagnosed AIDS cases in New York City in 2004 had received their first HIV diagnosis within the previous 31 days. Independent predictors of late presentation were older age, heterosexual or unknown risk, or being foreign-born. Both studies highlight the need to expand HIV testing in health care settings and through outreach to vulnerable populations.

Other studies highlighted the difficulty of implementing broad testing programs in health care settings. Smerd and colleagues conducted a pilot project from January 2006 to August 2006 encouraging HIV testing in inpatient medical wards, ambulatory care, and emergency room settings (Abstract 958). Although there was an overall increase of 54% in the HIV testing rate over this time and 60% of those testing HIV seropositive were newly identified, testing rates remained quite low overall (<10% in each of the settings). Bernstein conducted a survey of HIV testing practices among 7300 US physicians from 1999 to 2000, using the American Medical Association Master File (Abstract 960). More than 70% of providers responded, but only 28% of physicians reported screening asymptomatic men or non-pregnant women for HIV. At that time, independent predictors of providers offering tests to these patients were female sex, being black or Hispanic, provider practice being located in a large city or public clinic, and provider being a primary care specialist.

Other presentations focused on additional testing strategies to be implemented specifically for high-risk groups. Denning presented data from the 2003 to 2005 Centers for Disease Control and Prevention (CDC) National HIV Behavioral Surveillance survey and analyzed data from MSM offered HIV testing in 5 US cities (Abstract 956). Of the 1593 MSM who reported that their last HIV test was negative, 33% stated they had not had a follow-up HIV test within the previous year. HIV testing was then conducted and 48% of those testing seropositive had not known their HIV serostatus. Denning concluded that a strategy of targeting only persons without prior HIV testing would have reduced the fraction of unrecognized HIV infection from 48% to 41%, but a strategy of annual testing would have reduced the fraction to 14%. Klausner and colleagues recommended coupling pooled HIV RNA testing with rapid HIV antibody testing in high-risk settings; data from their study of patients attending a sexually transmitted disease (STD) clinic suggested that HIV RNA screening increased HIV case detection by 12% (Abstract 953).

The Global Epidemic in MSM

Several presentations focused on the continued epidemic in MSM in different parts of the world. Van Griensven presented an overview of the topic, starting with a reminder that MSM make up half of the total number of HIV and AIDS cases in all persons (men and women combined) in the United States (Abstract 55). MSM are the only risk group in the United States in whom the proportion of new HIV and AIDS cases is increasing. Ellen also presented data on the US adolescent population, where the most heavily impacted population is young MSM (Abstract 145). In a venue-based sample, HIV prevalence in 15- to 19-year-old men was 7%, comparable with rates in young women in South Africa. In the National HIV Behavioral Surveillance survey conducted in 17 cities, 14% of MSM aged 18 to 24 years were found to be HIV infected.

However, the MSM epidemic is not confined to the United States, and van Griensven reviewed this global epidemic (Abstract 55). Throughout many countries in Latin America, MSM are the major risk group affected by HIV and AIDS, and prevalence rates in MSM exceed those in female sex workers by 10 to 15 fold. In Africa, which has been long-assumed to have very low rates of MSM sexual activity, 3 new surveys suggest very high HIV prevalence among populations of MSM, ranging from 9% to 38%. Because these first studies have been conducted in populations with relatively low HIV prevalence rates in the general adult population, MSM account for up to 36% of the total number of HIV-infected persons in those cities. Similarly, through many areas of Asia and Southeast Asia the prevalence of HIV in MSM is quite high and MSM make up a sizable proportion of the total estimated HIV infections: 28% of HIV cases in Hanoi, 30% in Bangkok, 37% in Yangoon, and 69% in Beijing. Van Griensven pointed to the many individual-level (eg, unprotected anal sex, greater number of sex partners, substance use, STDs, lack of circumcision) and societal-level (eg, laws against MSM sex, discrimination, stigma, lack of access to prevention tools) factors contributing to the epidemic. He stated that the Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates that only 10% of MSM worldwide have access to prevention services.

Prevention of Sexual Transmission

The last few months have brought momentous changes to the field of HIV prevention, with 5 large efficacy trials of biomedical prevention interventions stopped early because of benefit or harm to trial volunteers and their partners. Several of these trials were reported on at this year’s conference, including reports on adult male circumcision and vaginal microbicides. Other data presented at the meeting lent support to the potential for antiretrovirals to prevent HIV acquisition when given to HIV-seronegative persons as pre-exposure prophylaxis (PrEP) and the potential for herpes simplex virus-2 (HSV-2) suppression to reduce HIV transmission.

Male Circumcision

Gray and colleagues provided an in-depth review of 1 of the 2 successful adult male circumcision efficacy trials in HIV-seronegative men (Abstracts 155aLB and 155bLB) recently published in Lancet (Gray et al., 2007). That study from Rakai, Uganda showed a halving of HIV
acquisition among men aged 15 to 49 years randomized to receive immediate circumcision compared with the wait-list control group (odds ratio [OR], 0.49 on intention-to-treat analysis; 95% confidence interval [CI], 0.28-0.84). This effect appeared to be strengthened among men with 2 or more partners (incidence rate ratio [IRR], 0.30), extramarital partners (IRR, 0.34), and during later follow-up periods (IRR, 0.25 for the second year of follow up).

Male circumcision was also associated with a statistically significant reduction in the rate of self-reported genital ulcer disease (IRR, 0.53) with no effect on the rate of self-reported urethral symptoms. Moderate or severe adverse events were relatively rare (3.6%) in this controlled clinical trial with highly trained staff; care will need to be given for prevention and management of surgery-associated complications if this intervention moves into community practice. Gray’s study also found no evidence that circumcised men increased their risk compared with uncircumcised men (ie, behavioral disinhibition) although the other published paper describing the second successful male circumcision trial found a modest increase in several risk behaviors in the circumcised compared with uncircumcised groups (Bailey et al, Lancet, 2007).

Several days after the conclusion of the conference data were released from an early evaluation of the trial of male circumcision among HIV-infected men in Uganda. This trial was stopped early because of a statistically significant increase in the risk of HIV transmission to HIV-uninfected female partners. Exploratory analyses suggest that the increase in HIV transmission was associated with resumption of sexual activity before full healing of the surgical wound, and that the HIV incidence was comparable in the 2 arms when this subgroup was excluded. At best, these data suggest no immediate benefit to HIV-seronegative women from circumcision of their HIV-seropositive male partners and at worst substantial harm may arise. More concrete recommendations are expected after further analyses of this trial.

No recommendations have been made regarding the role of male circumcision in prevention of HIV transmission among MSM. Although 1 prospective cohort study of HIV-seronegative men found a doubling in the risk of HIV acquisition among uncircumcised men (Buchbinder et al, JAIDS, 2005), the population attributable fraction for this risk factor was only 10%. Begley and colleagues surveyed MSM attending gay pride events in 5 different states and found that 80% of men were already circumcised and 54% of the remainder were willing to undergo circumcision if circumcision were found to protect against HIV acquisition (Abstract 983).

Microbicides

Two large microbicide efficacy trials of cellulose sulfate were also stopped in early 2006 after an early analysis of data from 1 trial suggested an increased risk of HIV acquisition among HIV-seronegative women using this candidate microbicide. The second trial of cellulose sulfate found no evidence of increased transmission in the interim analysis several months earlier, but stopped the trial in the interest of safety for study volunteers.

Doncel presented preclinical data supporting the decision to evaluate cellulose sulfate in clinical trials (Abstract 106LB). Cellulose sulfate is a large sulfated polysaccharide that blocks gp120 and CD4+ coreceptor interactions. In extensive preclinical evaluation, it has been shown to have anti-HIV activity against both X4 and R5 viruses at concentrations that are not cytotoxic. It appears to be active against numerous HIV clades and drug-resistant isolates at concentrations that are orders of magnitude lower than the clinical dose. The product has also been shown to have activity against a number of STDs. Cellulose sulfate did not appear to be cytotoxic to cervico-vaginal cells nor to trigger release of inflammatory cytokines. These findings are markedly different than those seen in preclinical studies of nonoxynol-9. In addition, cellulose sulfate appeared to provide good surface coverage in the vagina and was nonabsorbable, nontoxic to lactobacilli (to maintain a healthy microenvironment), and long-lasting.

Van Damme presented clinical safety data on cellulose sulfate (Abstract 106LB). Eleven safety studies have been undertaken in which product was administered up to 4 times daily, generally for short courses (only 1 lasted to 6 months). In each of these studies, there was no evidence of local or systemic toxicity. A phase II trial of cellulose sulfate as a contraceptive was successfully completed in 200 couples, again with no apparent toxicity. Two large HIV prevention efficacy trials of this product were initiated. Van Damme reported on a multicenter, randomized, placebo-controlled trial that opened in July 2005 with the intention of enrolling 2574 HIV-seronegative women at risk for HIV acquisition. An interim efficacy analysis was planned when half of the 66 anticipated HIV infections in the trial had occurred, with a plan to stop the trial if the 2-sided P value was less than 0.10 in the direction of a greater number of infections in the cellulose sulfate arm, as this would indicate either potential harm or futility to find a difference. This interim efficacy analysis occurred on January 26, 2007, with data available on 35 new HIV infections in trial participants. At the time of data analysis, 1333 study participants had enrolled but only 326 had completed the full 12 months of follow up. Van Damme reported that the prespecified boundary for potential harm had been surpassed, but she declined to provide the breakdown of number of infections in each of the 2 arms. She stated that these numbers would change as final data were cleaned and made available.

The trial investigators and study staff moved rapidly to verify product labeling and coding of the randomization, and to confirm these results. They halted the trial on January 29, 2007, notifying study staff who in turn contacted study participants to stop using the product. All final study visits will be completed in April 2007. The investigators anticipate having a final report available by December 2007. A separate efficacy trial of cellulose sulfate had recently undergone an interim analysis that found no basis for early stopping. This trial was also stopped.
in late January 2007 as a result of the finding of potential harm to study volunteers in the other efficacy trial.

Although the closure of these trials was a major blow to the field, several other large efficacy trials of different microbicides are currently underway, and results from those trials will be available in the next several years. Other presentations at the conference on microbicides focused on preclinical, in vitro, ex vitro, and modeling studies (Abstracts 988-1000). There is great interest in using topical antiretrovirals for prevention of HIV acquisition; this subject is covered below in the discussion of PrEP.

Pre-exposure Prophylaxis

Several presentations at this year’s conference focused on the use of antiretroviral agents before HIV exposure, also known as chemoprophylaxis or PrEP. Cranage and colleagues presented data from a study of rectally applied 1% tenofovir gel to prevent simian immunodeficiency virus (SIVmac239) infection from single high-dose rectal challenge (Abstract 29). In this study, all 4 untreated macaques, 3 of 4 macaques given placebo gel, and 2 of 3 macaques given tenofovir gel 2 hours after exposure became infected, with detection of virus beginning 1 week after challenge and persisting through 20 weeks of follow up. In contrast, only 2 of 6 macaques pretreated with rectal tenofovir gel 15 minutes before exposure and 1 of 3 animals pretreated 2 hours before exposure became infected, and virus detection was intermittent or delayed in these animals. It should be noted that pretreated animals had systemic absorption of tenofovir, and those animals with breakthrough infection had lower blood levels of tenofovir than animals who were protected. Because the dose per kilogram was higher in macaques than normally used in humans and the blood levels apparently higher than with vaginal administration in human trials (Mayer et al, AIDS, 2006), this experiment may have more closely approximated oral than topical PrEP trials. Cranage also presented data that 4 of 7 uninfected macaques who had received tenofovir gel before or after rectal challenge had detectable Gag-specific T-cells measured in peripheral blood by gamma-interferon enzyme-linked immunospot (ELISPOT).

There have been no efficacy trials completed to date of oral PrEP, although several safety and efficacy trials are underway (see www.prepwatch.org). Data published last year suggested that a substantial proportion of MSM might know about PrEP as a prevention strategy and that some might already be using it (Kellerman et al, JAIDS, 2006). These data were countered by a study of MSM in San Francisco presented at the International AIDS Conference in Toronto in July 2006 (see also Liu et al, JAMA, 2006), as well as by a presentation by Voetsch and colleagues from the CDC at this year’s CROI (Abstract 982). Voetsch and colleagues surveyed 397 MSM at minority gay pride events in 5 US cities who were HIV seronegative or of unknown serostatus. Of this group, only 19% had heard of either pre- or postexposure prophylaxis, and only 1 person reported ever using PrEP. One of the 60 HIV-seropositive men surveyed reported giving antiretrovirals to his HIV-seronegative sex partner to prevent infection. This study confirms that, at the present time, PrEP use is relatively rare.

Role of Sexually Transmitted Diseases

Wasserheit provided an overview of the role of control of STDs in preventing new HIV infections (Abstract 56). Although numerous observational trials have identified a 2- to 5-fold increase in the risk of HIV transmission and laboratory-based studies have identified mechanisms by which these infections could facilitate transmission, only 1 of 5 randomized controlled trials of STDs completed to date have reported lowered HIV infection rates. Wasserheit reviewed these 5 trials and the likely differences between the populations that may have accounted for these differences. The only trial in which STD treatment lowered HIV incidence rates was in Mwanza, Tanzania (Grosskurth et al, Lancet, 2000), where the epidemic was in its early stages. Early stage epidemics are more frequently driven by core populations at very high risk, where coinfection with bacterial STDs (eg, gonorrhea, chlamydia) may fuel the epidemic. In these situations, treatment of bacterial STDs successfully lowered HIV incidence. The other 4 randomized controlled trials all took place in generalized epidemics in which transmission more frequently occurs in stable partnerships and where bacterial STDs play a lesser role, but viral infections such as genital herpes simplex virus (HSV) may play a larger role. Wasserheit concluded that programs to reduce HIV infection should focus on treatment of symptomatic STDs (often associated with acute HIV infection), and treatment of bacterial STDs in high-risk groups in nascent or concentrated epidemics. She also pointed to the importance of 2 ongoing efficacy trials to evaluate the impact of HSV suppression in reducing HIV acquisition (Abstract 987) and transmission in mature epidemics.

Two studies lent modest support to the hypothesis that HSV suppressive therapy might reduce genital shedding of HIV and therefore lower HIV transmission. Dunne and colleagues presented data demonstrating a modest reduction in HIV shedding (0.44 log10 copies/mL) in cervico-vaginal lavage among Thai HIV-infected women randomized to receive chronic suppressive acyclovir therapy or a placebo in a randomized, placebo-controlled, cross-over design (Abstract 30). In contrast, Delany and colleagues found no statistically significant decrease in the quantity of genital HIV among women randomized to receive acyclovir suppressive therapy in a trial in Johannesburg, South Africa (Abstract 154LB). In their study, acyclovir decreased the proportion of visits in which HIV shedding was detected in cervico-vaginal lavage as well as lowering plasma HIV RNA levels by 0.37 log10 copies/mL.

Transmission Through Breastfeeding

This year’s conference featured more than 15 abstracts on strategies to reduce the risk of HIV transmission through HIV-infected mothers breastfeeding their newborns. Coovadia provided an excellent overview of the topic in a plenary lecture (Abstract 13). In
wealthy countries, perinatal transmission rates fell from 24.5% in 1993 to 1.5% in 2002, with the widespread uptake of strategies for PMTCT. Although substantial reductions in perinatal transmission have been achieved in resource-limited countries, efficacy of peripartum antiretroviral therapy has been substantially attenuated by breastfeeding, where the risk of transmission appears to be relatively constant at 0.74 transmissions per month of feeding through 24 months. However, the increased risk in HIV acquisition is offset by higher rates of diarrheal disease in non-breastfed infants in resource-limited settings, and this was highlighted in several presentations at the conference.

Creek and colleagues presented the findings from an investigation of a large outbreak of diarrhea among infants in Botswana in 2006 (Abstracts 9 and 770). Botswana implemented a national PMTCT program in 1999 that included antiretroviral therapy, free infant formula for 12 months, and advice to all women to formula feed. Transmission rates in infants born to HIV-infected mothers fell to 7% in 2005. After heavy rains beginning in November 2005, an increase in diarrheic cases was observed in Botswana in the first quarter of 2006, with a 4-fold increase in diarrhea and a 25-fold increase in diarrhea death in children under 5 years of age. On multivariate analysis, the overwhelming risk factor for diarrheal illness was lack of breastfeeding of the infant (adjusted OR, 50). A cohort study of infants with diarrhea in Francistown confirmed the association of diarrhea with lack of breastfeeding, as 90% of infants under 2 years with diarrhea were not breastfed. Malnutrition in infants with diarrhea was high (42% developed marasmus and 20% developed kwashiorkor) as was overall mortality (22%) and investigation revealed that many malnourished children had not received sufficient supplies of formula. Other studies at the conference also demonstrated increased rates of diarrheal disease in infants during the rainy season (Abstract 772) and the association of diarrhea with weaning from breastfeeding (Abstracts 772-775).

The dilemma is this: at 18 months of age, HIV infection rates are higher in breastfed than formula-fed infants, but overall mortality is higher in the formula-fed group. Several studies provided data suggesting strategies to further reduce risk in the breastfeeding group. Coovadia presented data from many studies showing a clear benefit for exclusive breastfeeding compared with mixed breastfeeding, with evidence of a dose-response curve favoring breastfeeding (Abstract 13). Sinkala and colleagues presented data from a randomized, controlled trial of early weaning suggesting comparable rates of HIV-free survival at 24 months between the group randomized to wean at 4 months and those assigned to usual weaning practices (median duration of breastfeeding, 16 months). However, 24-month survival was substantially improved in the delayed weaning group for the subset of children born to asymptomatic mothers with CD4+ counts above 350 cells/μL. Survival was also significantly improved in infants found to be HIV-infected before 4 months if they received longer periods of breastfeeding. These data suggest promotion of exclusive breastfeeding, breastfeeding beyond 6 months, and prevention of late-stage disease in women of childbearing potential will improve infant morbidity and mortality in resource-limited countries. Attention and resources should be devoted to protecting clean water supplies and to training health care providers to monitor infant growth. Additional studies are underway evaluating the role of infant chemoprophylaxis and immuno-prophylaxis in preventing HIV acquisition during breastfeeding.

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A list of all cited abstracts appears on pages 83-91.

Additional Suggested Reading


Educational Programs of the International AIDS Society–USA

Established in 1992, the International AIDS Society–USA is a not-for-profit, HIV clinical specialist-education organization. The mission of the International AIDS Society–USA is to improve the treatment, care, and quality of life of persons with HIV and AIDS through balanced, relevant, innovative, and state-of-the-art education and information for practitioners who are actively involved in HIV and AIDS care. The organization’s educational activities are particularly intended to bridge clinical research and patient care.

Cases on the Web – www.iasusa.org/cow
Cases on the Web is an ongoing series of case-based, advanced online CME activities produced by the International AIDS Society–USA.

CURRENT PRESENTATIONS

Preconception Health Care for HIV-infected Women
Erika Aaron, MSN, CRNP
CME Credit Available: 1 hour

Current Issues in HIV Disease and Substance Abuse
Chinazo Cunningham, MD, and Hillary V. Kunins, MD
CME Credit Available: 1.5 hours

Diagnosis and Management of Immune Reconstitution Syndrome in HIV-infected Patients
Jaime C. Roberston, MD, and Carl J. Fichtenbaum, MD
CME Credit Available: 1 hour

The Importance of Viral Fitness and Drug Resistance in Chronic and Recent HIV Infection
Mark A. Wainberg, PhD, and Dan Turner, MD
CME Credit Available: 1 hour

2007 CME Course Schedule

Improving the Management of HIV Disease,® now entering its 15th year, continues to focus on cutting-edge, scientifically rigorous agendas presented by leading experts in the field.

Atlanta, GA
April 27, 2007
Westin Peachtree Plaza
Chairs: Michael S. Saag, MD, and Jeffrey L. Lennox, MD

Chicago, IL
May 7, 2007
Marriott Downtown Chicago
Chairs: John P. Phair, MD, and Harold A. Kessler, MD

Washington, DC
May 23, 2007
JW Marriott on Pennsylvania
Chairs: Henry Masur, MD, and Michael S. Saag, MD

San Francisco, CA
May 31, 2007
Grand Hyatt San Francisco
Chairs: Robert T. Schooley, MD, and Stephan E. Follansbee, MD

10th Annual Ryan White CARE Act Clinical Update
Phoenix, AZ
For clinicians in RWCA-funded clinics only
June 14-16, 2007
Hyatt Regency Phoenix
Chairs: Laura W. Cheever, MD, ScM, Michael S. Saag, MD, and J. Kevin Carmichael, MD

New York, NY
October 19, 2007
New York Marriott Marquis
Chairs: Douglas T. Dieterich, MD, and Roy M. Gulick, MD, MPH

NEW: An Intensive Workshop on Antiretroviral Strategies: New Drugs, Antiretroviral Failure, and Resistance Testing
These new small-group interactive CME workshops are designed for experienced HIV clinical decision makers and are led by HIV experts. Formatted to enhance professional development and improve clinical practice, they provide excellent intensive preparation for the topics covered in the annual full-day CME courses.

Los Angeles, CA
Tuesday, March 27, 2007
Workshop Leaders: Steven J. Deeks, MD, and Richard H. Haubrich, MD

Chicago, IL
Tuesday, May 8, 2007 (day after annual Chicago course)
Workshop Leaders: Eric S. Daar, MD, and Jeffrey L. Lennox, MD

San Francisco, CA
Wednesday, May 30, 2007
Workshop Leaders: Steven J. Deeks, MD, and Andrew R. Zolopa, MD

For information about any of these programs, please contact the International AIDS Society–USA.
Phone: (415) 544-9400 • Fax: (415) 544-9401 • E-mail: info2007“at”iasusa.org • Web Site: www.iasusa.org
Neurologic Complications of HIV Disease and Their Treatment

Scott Letendre, MD, Beau Ances, MD, PhD, Sarah Gibson, BS, and Ronald J. Ellis, MD, PhD

Important new information regarding neurologic complications of HIV disease was presented at the 2007 Conference on Retroviruses and Opportunistic Infections. In addition to presentations on pathogenesis and treatment of neurologic complications, the conference included findings that have implications for the management of HIV disease outside the nervous system. Key findings included that the distribution of antiretrovirals into the central nervous system may influence the effectiveness of treatment outside this protected compartment; that postponing initiation of therapy until blood CD4+ counts fall to 300 cells/µL may increase the risk for HIV-associated neurocognitive impairment but interruption of antiretroviral therapy in individuals with high CD4+ counts may have neuropsychologic benefits; that substantial changes, including macrophage activation and neuronal injury can occur shortly after HIV transmission; that HIV can influence neural progenitor cells to decrease neuronal differentiation; that newer neuroimaging technologies, such as diffusion tensor imaging and blood oxygen level-dependent functional magnetic resonance imaging can identify important effects of HIV on the brain such as alterations in cerebral oxygen consumption; that serotonin reuptake inhibitors and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors may reduce HIV RNA levels in cerebral spinal fluid; and that erythropoietin and the non-immunosuppressive immunophilin ligand, GPI-1046, may improve HIV-associated injury of peripheral nerves. The conference also included an important focus on JC virus encephalitis (also known as progressive multifocal leukoencephalopathy).}

Reports with Implications for Systemic Disease

Important new information regarding neurologic complications of HIV disease was presented at the 2007 Conference on Retroviruses and Opportunistic Infections. Reports on host and viral influences on pathogenesis, treatment of neurologic complications, newer neuroimaging methods, peripheral neuropathy, and JC virus encephalitis (JCV-E), also known as progressive multifocal leukoencephalopathy (PML), were included, as well as findings that have implications for the management of HIV disease outside the nervous system. Two studies examined the relationship between characteristics associated with antiretroviral distribution into the central nervous system (CNS) and HIV RNA levels in plasma. The relevant hypothesis for each of these analyses was that characteristics favoring better distribution of antiretroviral drugs throughout the body, including the nervous system, would lead to better control of HIV replication in plasma because (a) better distribution of antiretrovirals is associated with suppression of HIV RNA in cerebrospinal fluid (CSF) and (b) reductions of HIV RNA in CSF are associated with reductions of HIV RNA in plasma.

In the AIDS Clinical Trials Group (ACTG) 736 study, serial veni- and lumbar punctures (LP) were performed in 101 HIV-infected individuals before and after a change in antiretroviral therapy (Abstract 382b). The study confirmed that antiretroviral therapy regimens that contained a greater number of drugs with better distribution characteristics were more likely to suppress HIV RNA levels in CSF (odds ratio [OR], 5.0; P = .05). Notably, these same regimens were more likely to suppress HIV RNA levels in plasma as well (OR, 2.6; P = .03). The investigators also identified that previously untreated individuals were more likely to suppress HIV RNA levels in CSF and plasma (OR, 2.9; P = .02). Together, these findings extend the commonly held notion that the first regimen is the most likely to successfully reduce viral replication. Selection of an initial regimen that includes antiretroviral drugs with better distribution characteristics may further improve the chances for success.

An analysis from the Italian Cohort Naïve Antiretrovirals Group (ICONA) provided data complementary to these findings by analyzing the relationship between antiretroviral distribution characteristics and durability of the treatment response in plasma (Abstract 382a). This large cohort study assessed previously untreated individuals before and after initiating combination antiretroviral therapy. The investigators identified 2785 individuals who started antiretroviral therapy between 1997 and 2006 (the median number of individuals was enrolled in 2000) and who had suppressed HIV RNA levels below 80 copies/mL. This group identified those who experienced rebound replication (defined as 2 consecutive HIV RNA values above 400 copies/mL), calculated the rate of rebound per 100 person-years of follow up, and determined the relationships of this measure with CD4+ cell counts and the CNS Penetration-Effectiveness (CPE) score. They found that lower CPE scores (a reflection of worse distribution characteristics) seemed to be associated with higher rates of rebound among individuals.
with CD4 + counts below 200 cells/µL. In a multivariate Poisson regression analysis that adjusted for 11 cofactors, individuals with CD4 + counts below 200 cells/µL who took regimens with lower CPE scores trended toward a higher risk of rebound (adjusted relative risk [RR], 0.04; 95% confidence interval [CI], 0.01-1.61). Among individuals with CD4 + counts above 350 cells/µL, however, higher CPE scores were associated with a higher rate of rebound (adjusted RR, 4.54; 95% CI; 1.30-15.89). The observed relationships should be confirmed and their mechanisms explained but the data stand as provocative evidence that antiretroviral distribution may influence the durability of treatment response, perhaps for the better in some but for the worse in others.

Current guidelines recommend that the decision to initiate antiretroviral therapy in asymptomatic persons be individualized when the CD4 + count falls between 200 and 350 cells/µL.® The decision to start therapy typically involves discussion with the patient regarding the different treatment options available, dosing preferences, toxicities, adherence, and drug resistance. Reducing the risk of brain injury, however, is typically not included in these discussions. Although potent combination antiretroviral therapy has generally reduced the incidence of HIV-associated neurocognitive impairment (HNCI), few studies have quantified the impact of postponing therapy on it. If initiating therapy at CD4 + counts above 300 cells/µL had benefits for the nervous system, it would be another important consideration for patients. Muñoz-Moreno and colleagues attempted to answer this question by comparing the prevalence of HNCI with the CD4 + counts at which individuals first initiated antiretroviral therapy (Abstract 383). The analysis was small (n = 64) and descriptive but the investigators identified that fewer individuals had impaired neuropsychologic (NP) performance when antiretroviral therapy was initiated at higher CD4 + counts (>300 cells/µL, 56%; ≤300 cells/µL, 64%; >250 cells/µL, 53%; ≤250 cells/µL, 67%; >200 cells/µL, 53%; ≤200 cells/µL, 73%). This finding is consistent with larger studies that have identified associations between higher HNCI prevalence and lower CD4 + count nadirs and argues that patients should be counseled that delaying therapy may increase their risk for HIV-associated brain injury.

Treatment interruption is no longer routinely recommended since it increases the risk of HIV disease progression and death. Despite this, many individuals continue to interrupt therapy for a variety of reasons, including toxicities (eg, dyslipidemias, hepatitides) and psychosocial factors. Because stopping antiretroviral therapy results in rebounds in HIV replication and loss of CD4 + cells, it may also carry a potential risk for neurocognitive decline. Arguing against this, however, were data from ACTG 5170 study that indicate that stopping therapy may actually have NP benefits (Abstract 113). Robertson and colleagues prospectively studied individuals before and after treatment interruption as well as some individuals who reinitiated antiretroviral therapy. These individuals had generally not experienced advanced immunosuppression (median CD4 + nadir, 436 cells/µL) and had received antiretroviral therapy for a median of 4.5 years. Using a brief NP testing battery, changes in performance were evaluated at baseline and at 4 time points up to 96 weeks after antiretroviral therapy was stopped. The investigators saw small but statistically significant neurocognitive improvements at each follow-up evaluation. Because efavirenz is associated with CNS side effects, the investigators stratified the analyses by efavirenz use. Improvements in NP performance did not differ between those who did or did not use efavirenz. To determine if improvements over time were related to practice (improved performance resulting from familiarity with testing instruments) the investigators specifically evaluated improvement after the third testing session, after which the benefits of practice are minimal. Even after the third testing, NP performance continued to improve. In comparison, a subgroup of 46 individuals who restarted antiretroviral therapy before week 26 and had no statistically significant deterioration or improvement in NP performance. Taken together with the findings of Muñoz-Moreno, delaying antiretroviral therapy until CD4 + counts fall below 300 cells/µL may increase risk for HIV-associated CNS injury but initiating antiretroviral therapy at CD4 + counts closer to 500 cells/µL may increase risk for antiretroviral therapy-associated CNS injury. These conclusions, however, should be confirmed before they influence treatment decisions.

Two other abstracts had treatment-related implications. To reduce the cost and toxicity of antiretroviral therapy, clinical investigators studied the use of potent, single-agent therapy (SAT), typically a ritonavir-boosted protease inhibitor (PI), such as atazanavir or lopinavir. PIs are usually extensively bound to plasma proteins, and only the unbound fraction is available for distribution to protected compartments. This practice thus raises concern regarding effectiveness in the CNS.

Yeh and colleagues provided evidence that SAT with lopinavir/ritonavir controlled HIV replication in the CSF of most individuals (Abstract 381). Only 12 individuals were included in this analysis; they enrolled in the parent study of SAT, Integrated Minority AIDS Network Incorporated-2 (IMANI-2), had plasma HIV RNA levels below 75 copies/mL after 24 weeks of therapy, and consented to LP. Among the 11 persons in the analysis (1 was excluded because of a traumatic LP), 10 (91%) had HIV RNA levels in CSF below 50 copies/mL. One individual, however, had up to 747 copies/mL of HIV RNA in CSF despite having the highest CSF lopinavir concentrations in the group and no evidence of mutations associated with reduced susceptibility to lopinavir in CSF. This individual had very high levels of monocyte chemotactic protein-1 (MCP-1) in CSF, a potently chemotactic protein that is associated with the CNS complications of HIV, arguing that this individual was predisposed to having a greater migration of replication-competent lymphocytes and monocytes into the CNS. The clini-
Cal consequences of these elevated HIV RNA levels and MCP-1 levels are undetermined.

Clapham and others previously identified that HIV may adapt to the nervous system by increasing tropism for macrophages, in part by evolving an envelope that enables entry of cells that express only low levels of CD4 or CCR5. When adaptation occurs, it may have implications for the ability of 2 investigational classes of antiretrovirals, both monoclonal antibodies to CD4 and CCR5 antagonists, to control replication in the CNS. The investigators prepared pseudovirions from 10-brain- and 9-lymph node-derived envelopes by cotransfecting 293T cells with env + pSVIIIenv and env – pNL4.3 vectors, and then performed neutralization and inhibition experiments with a variety of proteins. They identified that better macrophage tropism was associated with reduced sensitivity to inhibition by CD4 monoclonal antibody. In addition, pseudovirions generated using the brain tissue of 2 individuals were more sensitive to TAK779 (a CCR5 antagonist) and b12 (an antibody for the CD4 binding site). No increased or decreased susceptibility to these or other inhibitors was seen for the other pseudovirions (Abstract 170).

Central Nervous System Complications

Host Pathogenesis

Many theories have been posited to explain vulnerability to CNS complications of HIV disease. These theories include elements attributable to the host (eg, production of inflammatory proteins, mediators of oxidative stress, and excitotoxic glutamate) and to the virus (eg, neuroadaptation and production of neurotoxic viral proteins). By better understanding pathogenesis, we hope to identify at-risk individuals and prevent neurologic complications from occurring. Once they occur, however, the goal is to understand mechanisms of recovery to enable selection of the best treatments. Investigations of neural progenitor cells (NPCs) may provide important insights into HIV neuropathogenesis.

The production of new neurons, or neurogenesis, is increased during neurodegenerative disorders. Stimulation of neurogenesis is being explored as a potential therapy for a variety of neurodegenerative disorders. The effects of HIV infection on NPCs are not known. Peng and colleagues studied human monocye-derived macrophages (MDMs) infected with HIV-1 macrophage-tropic HIV strains or immune-activated with lipopoly saccharide (LPS) (Abstract 353). HIV-1-infected MDMs activated by LPS released soluble factors that substantially increased NPC proliferation. Despite overall increases in proliferation of NPCs, neuronal differentiation was inhibited and astrocyte differentiation was increased. Thus, the combined effects of HIV infection and immune stimulation in this model would be reduced neurogenesis and increased gliosis, which is consistent with the neuropathologic findings of HNCI. If applicable to humans, these data suggest that inhibiting the effects of HIV on neurogenesis may help to ameliorate brain injury in HIV infection.

Measurements of host biomarkers can play numerous roles in understanding and treating neuroAIDS, including identifying new pathways for in vitro investigation, providing confirmatory evidence of laboratory observations, identifying at-risk individuals, and monitoring therapy. Studies performed primarily before the era of potent antiretroviral therapy validated several biomarkers that were associated with risk, including neopterin, beta-2-microglobulin, and MCP-1. None of these have consistently been associated with neuroAIDS in more recent studies, so there is a pressing need for new biomarker investigations of treated, impaired individuals.

Three studies used different approaches to identify relationships between host biomarkers and neurologic outcomes. Researchers from the University of Nebraska and Puerto Rico used an elegant but complex and labor-intensive proteomics approach to identify differential protein expression between neurologically symptomatic and asymptomatic individuals (Abstract 388). The investigators used 3 primary methods to accomplish this. First, they used 2-dimensional electrophoresis with differential gel electrophoresis (DIGE) technology to identify protein differences between the CSF of individuals with HNCI and those without it. Once these experiments identified proteins that differed between the groups, the second step was to use liquid chromatography and tandem mass spectrometry along with specialized software to identify the proteins. Once they identified the differential proteins, they used Western blot to confirm the presence of the protein in 1 group of specimens and its absence in the other group. This approach identified 90 protein spots with statistically significant differential expression. Fifty-two of these were selected for further analysis and this yielded high confidence identification of 19 proteins, including complement C3, gelsolin, vitamin D binding protein, procollagen C endopeptidase enhancer, clusterin, cystatin C, and neuronal cell adhesion molecule.

As demonstrated in these experiments, CSF from well-characterized research volunteers can be a very useful tool for understanding neuroAIDS. Since volumes of CSF are typically very limited and biomarker concentrations are often lower than in serum or plasma, sensitive assays that use only small volumes of CSF can be very valuable tools. A bead-based immunoassay system has these characteristics along with the substantial added ability to measure numerous analytes at once. Researchers from the United States, Sweden, and Italy used this system to simultaneously measure 29 proteins in CSF and blood specimens from 72 HIV-seropositive individuals without neurologic symptoms, 43 with AIDS dementia complex (ADC), 15 with early HIV infection, and 20 HIV-seronegative controls (Abstract 387). The team confirmed associations between ADC and MCP-1 and identified that another chemokine previously associated with ADC, interferon-inducible protein (IP)-10, was associated only with HIV serostatus but not ADC. They also identified associations between ADC
and 2 other proteins, interleukin-6 and interleukin-1 receptor antagonists in CSF. Interleukins have previously been linked to ADC<sup>+</sup>)<sup>+</sup> but this study provides a more recent validation of these findings and indicates that the bead-based immunoassay system may be a useful tool for future discovery and hypothesis-driven investigations.

The third study, from the CNS Antiretroviral Therapy Effects Research (CHARTER) study, measured 2 chemokines, MCP-1 and stromal cell-derived factor-1 (SDF-1), in CSF using routine plate-based immunoassays (Abstract 370). This analysis was distinctive in comparing chemokine concentrations in CSF with findings from morphometric analyses of magnetic resonance imaging (MRI) of the brain to build hypotheses about their relationship to NP performance. Higher MCP-1 levels were associated with greater volumes of abnormal white matter and higher SDF-1 levels were associated with greater volumes of cortical grey matter. These findings suggested that the 2 chemokines had opposing effects on cognition. When levels of these chemokines were compared with NP performance, this hypothesis was confirmed: Higher MCP-1 and lower SDF-1 levels were associated with worse NP performance. The association of MCP-1 with HNICI is well known, but the observation that SDF-1 may modify this effect is new.

Several other neuroimaging studies investigated links between HIV disease and brain injury. Similar to the CHARTER study, Tate and colleagues also evaluated white matter. Instead of morphometric methods, they used diffusion tensor imaging (DTI) to compare the fiber tract integrity of HIV-infected individuals with HIV-seronegative controls (Abstract 117). The control group had the highest fractional anisotropy (FA) and lowest mean diffusivity (MD) values (indicating maintenance of white matter integrity), although HIV-seropositive individuals with CD4+ counts above 350 cells/µL had intermediate FA and MD values, and those HIV-seropositive individuals with CD4+ counts below 350 cells/µL had the worst FA and MD values (indicating disruption of white matter tracts). DTI differences were most pronounced in the anterior regions of the corpus callosum with changes in FA and MD values being associated with neuropsychological measures of motor speed, semantic fluency, and free memory recall. DTI may thus have possible utility in studying the structural impact of HIV within the brain.

Ances and colleagues used single voxel magnetic resonance spectroscopy (MRS) to measure N-acetyl aspartate, choline, and creatine, as well as 2 novel markers—lactate, a marker of inflammation and anaerobic glycolysis, and lipid, an indicator of cell membrane turnover due to oxidative stress within the lenticular nuclei of the basal ganglia of individuals with HNICI and HIV-seronegative controls (Abstract 116). The groups did not differ in lenticular nuclei volume, N-acetyl aspartate and creatine, or choline and creatine. In contrast, the lactate/ creatine ratio was significantly higher in individuals with HNICI and the ratio of the sum of lipid and lactate to creatine was significantly higher among all HIV-seropositive groups than in seronegative controls. The study identifies that HIV-associated inflammation and oxidative stress can be detected by measurement of lactate and lipids using MRS.

A second study from Ances and colleagues assessed HIV-infected individuals using blood oxygenation level-dependent functional MRI (BOLD-fMRI) (Abstract 377). Participants viewed a fixed number pattern in the center of a screen that corresponded to finger taps on a 4-button box within a 3-Tesla scanner. Changes in cerebral blood flow and cerebral metabolic rate of oxygen consumption (CMRO2) were studied within the lenticular nuclei of the basal ganglia of HIV-seropositive patients and seronegative controls. Both early and chronically HIV-infected individuals who had normal NP performance each had greater functional changes in cerebral blood flow and CMRO2 than seronegative controls, suggesting that even neurocognitively normal individuals may have derangements in presynaptic recycling of glutamate. Spudich and colleagues also studied individuals with early HIV infection using MRS (Abstract 115). N-acetyl aspartate, a marker of neuronal integrity, was significantly decreased in individuals with early infection compared with seronegative controls. Consistent with this observation, the researchers also identified that some individuals with early HIV infection, particularly those with neurologic symptoms, had elevated levels in CSF of biomarkers reflecting neuronal injury (total tau and neurofilament-light). An imaging marker of inflammation, choline, was increased and correlated with elevations of inflammatory biomarkers in CSF, such as neopterin. Together, these neuroimaging studies support that inflammation and injury of the brain occurs early in the course of HIV disease and before the onset of advanced immunosuppression.

**HIV Pathogenesis**

A number of reports focused on differences in HIV between individuals who experience neurologic complications and those who do not. Three of these focused on evidence of quantitative differences in HIV replication. In the same series of analyses of early HIV infection mentioned in the previous section, Spudich and colleagues also reported on HIV RNA levels in CSF in 42 individuals, identifying that they were substantially elevated with a median of 1700 copies/mL (Abstract 115). A quarter of individuals had levels exceeding 63,096 copies/mL, a very high value for CSF. Correlational analyses supported that high viral load was linked to high plasma HIV RNA levels (increasing by 0.85 log<sub>10</sub> copies/mL for each 1-log<sub>10</sub> increase in plasma; P < .001) and to high leukocyte counts in CSF (P < .01). The high quality of their case definition data enabled the investigators to identify slow declines of HIV RNA levels in CSF of only 0.04 log<sub>10</sub> copies/mL for each 10 days postexposure, suggesting that injury attributable to this substantial early replication of HIV in the CNS may be slow to resolve.

Several studies have demonstrated that higher HIV RNA levels in CSF, but not in plasma, were associated with
increased risk for current HNCI and predicted risk for future HNCI. Most of these studies, however, were performed in Western populations that were likely infected with clade B HIV, and before the widespread use of combination antiretroviral therapy. More recent studies have not as strongly linked evidence of greater HIV replication within the nervous system to neurologic outcomes, possibly because effective antiretroviral therapy reduces HIV RNA levels in CSF below the quantity limit of current commercial assays. Since ongoing, low-level HIV replication may be responsible for the high prevalence of HNCI in treatment-experienced individuals, different approaches are needed to better understand the contribution of HIV to brain injury in this population.

Shiramizu and colleagues used one approach to address this challenge: measurement of HIV DNA in peripheral blood mononuclear cells (PBMCs) (Abstract 114). This group has previously reported on the association between higher HIV DNA in PBMCs and HNCI, but in this analysis, higher HIV DNA levels were present even in cognitively impaired, treatment-experienced individuals and only true in a subset of activated monocytes and macrophages (CD14+ and CD16+) that have been linked to HIV neuropathogenesis, integrating complementary elements of 2 models of HIV neuropathogenesis.

Another approach to detecting ongoing HIV replication in treatment-experienced individuals is to use a more sensitive assay for HIV quantification in CSF. Using this approach, 1 recent study showed that 8 of 13 (62%) antiretroviral therapy-experienced subjects had more than 2 copies/mL of plasma HIV RNA but none surpassed this level in CSF. In contrast, a second study showed that a minority of subjects (13 of 47, or 28%) taking successful antiretroviral therapy had more than 2.5 copies/mL of HIV RNA in CSF. At the conference, the CHARTR Group identified that 62 of 125 (49.6%) CSF specimens that had less than 50 copies/mL of HIV RNA when assayed with an ultrasensitive assay still had detectable HIV RNA levels when assayed using a modified HIV-1 assay that had sensitivity levels of 2.5 copies/mL of HIV RNA (Abstract 369). Among a clinically relevant subgroup of 40 individuals who had fewer than 50 copies/mL of HIV RNA in both plasma and CSF, 17 (42%) still had detectable HIV RNA in CSF and, importantly, this was associated with poorer estimated antiretroviral distribution (median CPE score 1.5 vs 2.0; P = .01). These findings support the conclusion that the high prevalence of HNCI in treatment-experienced populations may be attributable to ongoing, low-level replication and that this may be due to poor distribution of antiretroviral drugs into the CNS.

Much of the clinical research in neuroAIDS to date has been performed in North American, European, and Australian populations, most of which are infected with clade B virus. Other clades, such as clade C, however, account for most of the HIV infections worldwide and some laboratory data suggest that they may be less neuropathogenic. For example, clade C viruses may not replicate as well in microglia or brain macrophages. Infection with clade C may lead to differing cellular expression of proteins or toxins. Proteins encoded by clade C virus may have differing properties from those encoded by clade B.

Most of the laboratory findings to date have focused on events outside the nervous system. This is important because recent data indicate that East Asian, South Asian, and African populations have high, not low, prevalences of cognitive impairment. Jialin Zheng and colleagues performed experiments to investigate differences in neurotoxic glutamate production from MDMs infected with strains of either clade B and clade C (Abstract 354). Infection of MDMs with clinical clade B isolates was associated with greater production of glutamate than infection with clinical clade C isolates. Inter-clade differences in glutamate production were linked to inter-clade differences in reverse transcriptase activity. Conditioned media from MDM cultures were incubated with primary rat neuronal cultures, identifying that

the observed differences in glutamate production were associated with inter-clade differences in neurotoxicity, ie, media from clade B-infected MDMs were associated with greater neuronal injury than those from clade C-infected MDMs. These results stand in contrast to existing clinical observations but may indicate that even though prevalent cognitive syndromes are similar in clade B- and clade C-infected populations, the underlying mechanisms by which the brain is injured may differ, which has important implications for the treatment of neuroAIDS in international settings.

**Treatment**

The underlying premise of optimizing distribution of antiretroviral drugs into protected compartments is that improving control of HIV has benefits for the host. Recent efforts have focused on selecting the antiretroviral drugs to which the host’s virus is susceptible and that also have the best distribution characteristics. Another approach is to improve distribution by redesigning antiretroviral formulations to target particular tissues. This has been attempted in the past by use of liposomal forms of antiretrovirals. Bosket and colleagues developed and evaluated another delivery system in a mouse model. Experimental HIV encephalitis was first induced in severe combined immune-deficient mice by injecting their brains with HIV-infected macrophages. The investigators then used MRI to inject superparamagnetic iron oxide nanoparticles as a tag to track monocytes as they crossed the blood-brain barrier. Some of the mice were also administered macrophages laden with indinavir nanoparticles. The investigators were able to demonstrate in vivo migration of macrophages to brain tissue, and also measured indinavir levels of 10 to 15 nanomolar 5 days after a single intravenous injection, a level that exceeds the median inhibitory concentration (IC50) for wild-type virus. The brains of nanoparticle-treated mice also showed reduced p24 staining, suggesting improvements in HIV encephalitis. This approach would
be even more impressive if it could improve delivery of antiretroviral drugs that penetrate more poorly than indinavir, which has the best distribution to the CNS among the PI class.

Another approach to treating the CNS would be to reduce migration of HIV-producing cells across the blood-brain barrier. Effective antiretroviral therapy typically reduces replication but may be incompletely effective and does not target specific cell types such as the CD14+ and CD16+ monocytes that have been linked to HNCI. Williams and colleagues reasoned that depletion of these highly activated cells would reduce their migration across the blood-brain barrier, reduce delivery of HIV and toxic inflammatory products to the CNS, and ultimately limit brain injury (Abstract 364). To test this theory, they administered to simian immunodeficiency virus (SIV)-infected rhesus macaques a polyamine biosynthesis inhibitor, PA-001 that selectively kills CD14+ and CD16+ monocytes. Escalating doses resulted in near complete depletion of CD14+ and CD16+ monocytes by 16 days of treatment. No inflammation was histologically detected in the CNS and treated animals did not develop SIV encephalitis, in contrast to untreated animals. Gastrointestinal toxicity did occur at higher doses (400 mg/m²) but lower doses (250 mg/m²) were still effective and were not associated with gastrointestinal toxicity.

These experimental treatments may provide important options to affected individuals in the future but effective therapies are still needed. Clinical trials thus far have not provided consistently effective adjunctive therapies for HNCI but some drugs that have been approved by the US Food and Drug Administration for other indications and are already used in the HIV-infected population may have secondary benefits for the CNS. For example, lithium may protect neurons by modulating glycogen synthase kinase-3 beta, certain serotonin reuptake inhibitors (SRIs) can reduce HIV replication by uncertain mechanisms, and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), may reduce HIV replication via numerous mechanisms including reduction of chemokine receptor-containing membrane lipid rafts, adhesion molecule expression, and Rho guanosine triphosphatase activity. Since SRIs and statins are commonly used in clinic populations, the CHARM-TER Group examined the impact of their use on HIV RNA levels in CSF and performance on NP testing (Abstract 384). SRI users were less likely to have HIV RNA levels above 50 copies/mL in CSF (29% vs 37% in non-SRI users; OR, 0.69; P=.05). This association was most evident for 3 of the 7 SRIs (citalopram, sertraline, and trazodone; combined 25% vs 38% in non-SRI users; OR, 0.56; P=0.01) and was limited to those not taking concomitant antiretroviral therapy (61% vs 83%; OR, 0.31; P=0.01). Users of these 3 SRIs also performed better on NP tests (median global deficit score, 0.37 vs 0.47; P=.04). Statin users were also less likely to have HIV RNA levels in CSF above 50 copies/mL (16% vs 37%; P<.001), but in contrast to SRIs, statins showed the strongest association in those using antiretroviral therapy (2% vs 18%; P<.001) and statin use was not associated with better NP performance. These data support a role for SRIs and perhaps statins in the treatment of HNCI but these observations require confirmation and the mechanisms of their effects need to be more clearly identified before the adoption of their use in clinical practice.

Selected Other Topics

Distal Sensory Polyneuropathy

Contrary to popular belief, peripheral nerves are dynamic, plastic structures that may undergo both injury and regeneration. Most current treatment of polyneuropathy in HIV infection is focused on symptom relief, neglecting the underlying neuropathogenesis and permitting potential continued damage to peripheral nerves. Jack and colleagues studied an in vivo model relevant to HIV infection in humans (Abstract 363). Transgenic mice expressing HIV viral envelope protein, gp120, under the control of a glial fibrillary acidic protein promoter were administered oral didanosine. This reliably produced a reduction in intraepidermal unmethylated small sensory fibers in the foot pads of the mice. By administering either recombinant human erythropoietin (rhEPO) or the non-immunosuppressive immunophilin ligand, GPI-1046, the investigators were able to partially block the neuropathic effects seen in the animal model. These findings are of interest because assessments of intraepidermal nerve fiber layer density in humans are currently being studied for their potential clinical utility in diagnosis and monitoring polyneuropathies that have a small fiber component, which includes HIV distal sensory polyneuropathy (DSPN). A clinical trial of rhEPO administration in humans with DSPN is also underway.

JC Virus Encephalitis

David M. Clifford delivered a state-of-the-art talk on JCV-E, or PML. He reviewed the evidence indicating that JCV-E continues to cause substantial morbidity and mortality among people living with HIV, even though combination antiretroviral therapy has improved its prognosis. Unfortunately, 50% of patients still die within 6 months of onset.

Two studies provided evidence that survival of persons diagnosed with JCV-E could be improved. Gasnault and colleagues reported preliminary results of individuals with JCV-E who were treated under protocol Agence Nationale de Recherches Sur le Sida (ANRS) 125, which provided intensified antiretroviral therapy regimen within 90 days of diagnosis (Abstract 379). The 6-month cumulative probability of survival was 77% (95% CI, 63%-95%), supporting that early, intensive antiretroviral therapy may greatly benefit persons with JCV-E. Survival was associated with recovery of anti-JCV CD4+ memory T cell responses, detection of anti-JCV interferon-gamma producing CD8+ T cell effectors, and reduction of JCV DNA in CSF to levels below detection.

Published studies have reported that interferon alfa treatment can delay progression and prolong survival...
of individuals with JCV-E, although it may provide little added benefit when combined with potent antiretroviral therapy. Verma and colleagues used in vitro methods to identify 1 reason for lack of interferon benefit: Interferon beta may more potently inhibit JCV replication than does interferon alpha (Abstract 359). To demonstrate this, they infected primary human fetal glial cells with JCV, incubated the infected cells with interferon alpha and interferon beta, and measured T antigen DNA, mRNA transcripts, and interferon-stimulated gene mRNA transcripts. Interferon beta reduced JCV replication more effectively than interferon alpha and the inhibition was reversed with anti-interferon antibodies. These findings argue that people with JCV-E may benefit by combining more intensive antiretroviral therapy along with interferon alpha.

Neurosyphilis

Syphilis and neurosyphilis are frequent comorbidities in HIV-infected individuals. The success of antitreponemal therapy is conventionally evaluated by performing serial LPs to determine if CSF leukocyte counts are reduced after treatment. Having a method to assess treatment success in neurosyphilis that does not require repeated LPs would simplify the management of this condition and potentially increase patient adherence to therapy. Marra and colleagues evaluated one such approach (Abstract 372). In 68 HIV-infected subjects with CSF leukocyte counts above 20 cells/µL who received treatment for a first episode of neurosyphilis, CSF Venereal Disease Research Laboratory (VDRL) test was reactive in 29 (43%). Normalization of the serum Rapid Plasma Reagin test (RPR) was associated with reduction of the CSF leukocyte count to below 20 cells/µL. After 7 months of treatment, RPR normalization correctly predicted CSF normalization 88% of the time and, after 13 months of treatment, RPR normalization correctly predicted CSF normalization 96% of the time.

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A list of all cited abstracts appears on pages 83 to 91.

Additional References

22. Riedel D, Ghiante M, Nene M, et al. Screen-


Complications of HIV Disease and Therapy

Judith S. Currier, MD, and Diane V. Havlir, MD

Complications and consequences of untreated and treated HIV infection in domestic and international settings continue to be a major focus of HIV clinical research efforts. In this review major findings in these areas are highlighted, with a focus on studies that have application to clinical practice. As the field of clinical research in HIV continues to mature, we are repeatedly surprised by the findings from well-designed studies.

Opportunistic (and Non-opportunistic) Clinical Events

AIDS-defining conditions have been included as major endpoints of clinical trials evaluating the efficacy of antiretroviral therapies with the assumption that all AIDS-defining conditions carry a similar impact on outcome. Mocroft and colleagues analyzed data on antiretroviral-naive patients starting antiretroviral therapy from 15 HIV cohort studies in Europe and North America and compared mortality rates associated with different AIDS-defining conditions (Abstract 80). This large study examined nearly 2,500 AIDS-defining endpoints among more than 30,000 patients with a median of 3.5 years on antiretroviral therapy. The greatest hazard of death was following a diagnosis of non-Hodgkin’s lymphoma (NHL; hazard ratio [HR], 19.31), followed by progressive multifocal leukoencephalopathy (PML; HR, 9.56), cryptococcosis (HR, 9.00), toxoplasmosis (HR, 5.10), and Mycobacterium avium complex (MAC; HR, 5.07). Although this study did not determine whether the AIDS-defining condition was the cause of death, these results suggest that some consideration should be given to the impact of different clinical endpoints when comparing therapeutic interventions.

As rates of traditional opportunistic events continue to decline among populations with access to antiretroviral therapy, other sources of morbidity and mortality grow in importance. Investigators from the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) cohort examined the contribution of both AIDS-defining and non-AIDS-defining malignancies as causes of death for nearly 24,000 patients on treatment (Abstract 84). It is important to recognize that this report only considered malignancies as causes of death and may underestimate the contribution of malignancies to morbidity. The most common non-AIDS-defining malignancies were lung cancer, cancers of the gastrointestinal tract and anal canal, and hematologic malignancies. As expected, CD4+ counts less than 50 cells/μL were associated with higher incidence rates for AIDS-defining malignancies than non-AIDS-defining malignancies; however, overall the non-AIDS-defining malignancies were more common than AIDS-defining malignancies as causes of death in this treated population. These results stress the importance of cancer screening and prevention efforts in a population of aging patients successfully treated for HIV infection.

It has long been known that the risk of different opportunistic AIDS-defining conditions relates to the level of immunodeficiency as measured by CD4+ cell counts. However, there has recently been more focus on the spectrum of clinical events that occur in patients with varying levels of immunodeficiency. Investigators from the Terry Beirn Community Programs for Clinical Research on AIDS (CPCRA) examined the relationship between current CD4+ counts and risk of opportunistic diseases and non-opportunistic diseases among patients enrolled in a clinical trial of antiretroviral therapy (Abstract 37). Fatal and non-fatal opportunistic diseases and non-opportunistic diseases were examined over a 5-year median follow up. Non-opportunistic disease events included: liver events (cirrhosis and grade 4 transaminase elevations), cardiovascular events, non-opportunistic cancers, and renal events (end-stage renal disease [ESRD] or renal insufficiency). After adjusting for age, sex, race, baseline CD4+ count, and viral load, the authors reported that the relationship between latest CD4+ count and risk of opportunistic disease was strongest (for every 100 CD4+ cells/μL increase the risk of opportunistic disease was reduced by 42%). However, the risk of non-opportunistic disease also appeared to fall by 17% per 100 CD4+ cells/μL increase, suggesting that immunodeficiency may contribute to the risk of these non-opportunistic events as well. These results add to the growing interest in consideration of earlier initiation of antiretroviral therapy with the hopes of reducing morbidity from both opportunistic and non-opportunistic diseases.

Opportunistic Infections In Resource-limited Settings

Although antiretroviral therapy is associated with dramatic reductions in morbidity and mortality in resource-limited settings, morbidity and mortality from opportunistic infections remains high early in the course of antiretroviral therapy. Moore examined determinants of mortality in 1,120 subjects followed for a median of 2 years receiving antiretroviral therapy in the Tororo, Uganda cohort (Abstract 34). Median CD4+ count at entry was 127 cells/μL. Cumulative mortality was 3.3% at 3 months and 7.5% at 12 months. Tuberculosis (TB) accounted for 21% of the deaths, cryptococcosis for 11%, candidiasis for 11%, and Pneumocystis jiroveci pneumonia (PCP) for 9%. In 41% of deaths, no etiology could be identified. Low CD4+
count, low body mass index, and hemoglobin at baseline were the strongest predictors of mortality. Authors suggested that interventions such as food supplements, treatment of anemia, and preventive antibiotic approaches should be evaluated to reduce early mortality. A retrospective review of 76,989 patients from 4 continents receiving antiretroviral therapy and co-trimoxazole through Médecins Sans Frontières reported high rates of opportunistic infections among the first 6 months of antiretroviral therapy (Abstract 859). Bacterial pneumonias, TB, and candidiasis were the most frequently reported opportunistic infections. Rates of Kaposi’s sarcoma (KS) were highest in Africa, and rates of cryptococcal disease were highest in South America. Similar to other reports, rates of all opportunistic infections among patients receiving antiretroviral therapy in Africa, Asia, and Latin America decreased over time.

The benefits of co-trimoxazole prophylaxis among children and adults were highlighted in several presentations. The first study quantified the benefit of co-trimoxazole among adults receiving antiretroviral therapy. In this study conducted in Malawi, clinical outcomes among 1295 patients receiving antiretroviral therapy with or without co-trimoxazole were examined (Abstract 83). The striking finding of this study was that co-trimoxazole was associated with a 46% reduction in mortality among patients receiving antiretroviral therapy. There were also data about the added benefit of co-trimoxazole to prevent malaria in pediatric populations. Gasaseira and colleagues compared rates of malaria between HIV-seropositive children receiving co-trimoxazole plus insecticide-treated bednets with rates of malaria among HIV-seronegative children living in Uganda (Abstract 78). Co-trimoxazole plus bednets are associated with a 97% reduction in risk for malaria. Only 4% of fevers in the HIV-seropositive children compared with 33% of fevers in the HIV-seronegative children were due to malaria. Thus, combined co-trimoxazole and insecticide-treated bednets is highly effective for malaria prevention among HIV-infected children living in Uganda.

**Tuberculosis And HIV Coinfection**

Increased mortality among HIV-infected patients with TB coinfection, including those starting antiretroviral therapy in resource-limited settings, has been reported in several cohorts. What is the contribution of TB to the immediate cause of death in these patients? Martinson and colleagues attempted to address this question by taking on the difficult task of obtaining complete autopsies for 47 patients who died during hospitalization for HIV and TB coinfection in Soweto, South Africa (Abstract 82). Pulmonary TB was identified as the cause of death in 19 patients and bacterial pneumonia in 4. Among 28 patients, disseminated TB was considered a contributory cause of death. Salmonella species were identified among 11 patients. Other opportunistic infections such as PCP and cytomegalovirus (CMV) accounted for the remainder of the cases. All but 2 subjects had more than 1 major opportunistic infection. This report suggests that extensive mycobacterial disease, a high burden of bacterial infections, and opportunistic infection all contribute to mortality in seriously ill HIV and TB coinfected patients.

After noting that mortality rates were 2-fold higher among HIV-infected patients with TB who were starting antiretroviral therapy than those without TB in his Capetown, South Africa, program, Lawn evaluated predictors of outcome among 213 HIV-infected patients with TB and 675 without (Abstract 81). In a multivariate analysis, a CD4+ count below 100 cells/µL and World Health Organization (WHO) stage IV disease (adjusted HR, 2.9; 95% confidence interval [CI], 1.8-4.8) were the only independent predictors of death. Among patients with TB, 70% of the deaths occurred before antiretroviral therapy was initiated. The authors hypothesized that delays in accessing antiretroviral therapy among patients with TB contributed to the high mortality rates, and they recommended earlier initiation of antiretroviral therapy particularly in patients with advanced immune deficiency.

One of the concerns about early initiation of antiretroviral therapy among patients with TB is exaggerated inflammatory reactions, called paradoxical reactions or immune reconstitution disease. Lawn and colleagues reported the morbidity, mortality, and hospitalizations attributed to immune reconstitution disease in 160 TB patients starting antiretroviral therapy in the Capetown cohort (Abstract 863). Antiretroviral therapy was begun a median of 105 days after TB treatment. Twelve percent of patients had immune reconstitution disease. Starting antiretroviral therapy earlier after TB therapy was associated with a higher risk of immune reconstitution disease. Only 4% of patients required hospitalization, and there were only 2 deaths. Thus, in this nonrandomized study, early antiretroviral therapy initiation was associated with higher risk of immune reconstitution disease, but hospitalizations and mortality were rare. Randomized trials to address optimal timing of antiretroviral therapy initiation in this setting are ongoing.

There were several reports evaluating algorithms to diagnose TB in resource-limited settings. Conradie screened 650 HIV-infected adults with a 5-point symptom screen. Excluding patients with smear-positive TB, there were 38 patients in whom TB was suspected (Abstract 852). Among these, 2 of 3 were given presumptive TB treatment, and 14 were culture confirmed. One case of TB was diagnosed among the patients not started on TB therapy. Authors concluded that their clinical algorithm was 96% sensitive and 85% specific. A second report by Were and colleagues found that in a Ugandan cohort initiating antiretroviral therapy, a clinical index was highly sensitive but not very specific (66%) for TB (Abstract 848).

Isoniazid preventive therapy (INH) is recommended for HIV-infected persons in TB endemic settings. One of the main obstacles to intermittent preventive treatment (IPT) implementation is the exclusion of active TB. Samandari and colleagues reported the TB screening results from a large randomized study of INH preventive therapy in Botswana (Abstract 861). Among 4328 adults screened, 2608 of the asymptomatic patients had a chest radiograph. Twelve
percent of subjects had an abnormal chest radiograph, and 31 individuals had active TB. Thus, in a small but statistically significant proportion of asymptomatic individuals, chest radiographs can help identify persons with active TB during screening for preventive therapy. Phillips reported preliminary findings from a large IPT program in Kenya (Abstract 852). Among 561 patients with WHO stage I or II disease who started IPT, 86% completed or will still be receiving IPT. Twelve percent of patients were lost to follow up. Only 2 patients were diagnosed with TB during IPT. The study is ongoing.

Approaches and trends in the prevention of TB in low-incidence settings were addressed in several posters. One report evaluated whether persons who presented with TB in an HIV comprehensive care clinic in Nashville, Tennessee, had been previously evaluated for IPT (Abstract 849). Investigators found that the majority of the TB cases had not had tuberculin skin testing prior to TB diagnosis despite being in routine care. In view of sensitivity of current diagnostic tests, the authors’ conclusion that 80% of cases were preventable could be considered an overstatement. However, the authors convincingly demonstrated that there are missed opportunities to prevent TB in low-incidence settings. Furrer and colleagues analyzed tuberculin skin testing, IPT, and antiretroviral therapy among patients in the Swiss cohort (Abstract 850). A positive tuberculin skin test without IPT was associated with the highest risk of TB, and similar to prior studies, antiretroviral therapy reduces the risk of TB.

Moreno reported TB incidence rates from 4269 HIV-infected patients receiving HIV care from clinics in Spain (Abstract 847). The median follow up was 3.8 years. The strongest predictor of TB was injection drug use and CD4+ count below 200 cells/µL. Antiretroviral therapy was associated with a decreased risk of TB. Interestingly, TB rates decreased among nonantiretroviral therapy-treated patients over time. The authors speculated that decreased transmission rates accounted for this decline. Golug and colleagues reported the 8-year follow up from a longitudinal study of HIV-infected and -uninfected injection drug users residing in Baltimore. This cohort had been offered tuberculin skin testing and IPT from 1990 to 1998. Overall, TB rates declined in the cohort, but reductions were not observed in the HIV population. IPT reduced rates of TB in those who took it, but adherence to the 6-month regimen was poor.

Emerging data on performance of quantiferon assays for use of diagnosis of latent TB infection were presented in a poster discussion session. The Centers for Disease Control and Prevention (CDC) currently recommends interferon gamma release assays for screening of latent TB in the United States, although there are no large studies of the performance of these assays among HIV-infected persons. Luetkemeyer and colleagues compared the quantiferon gold in-tube assay (QFT) with standard tuberculin skin testing in a cross-sectional study of 196 HIV-infected patients living in San Francisco (Abstract 860). The overall concordance of the 2 assays was 89%. The majority of the patients (85%) had a negative test by both assays. However, there was a low concordance among those with positive test by either assay. Only 28% were positive on both assays. Fifty-six percent of tuberculin skin test positive results occurred in QFT-negative subjects. In addition, the QFT assay, which depends on a positive control, was “indeterminate” or uninterpretable in 16%. Luetkemeyer concluded that replacing tuberculin skin testing with QFT may miss a small but concerning proportion of HIV-infected patients and that QFT performance may be limited in advanced HIV disease by an elevated rate of indeterminate results.

In a study evaluating the same assay among 111 adults commencing antiretroviral therapy in Cambodia, indeterminate results were reported in 18% of the assays (Abstract 859). Repeat assays among patients with indeterminate results did not become interpretable after 3 to 6 months of antiretroviral therapy.

Perhaps one of the biggest pieces of news in the HIV and TB arena for resource-limited settings was the recognition that drug-resistant TB is being transmitted among the HIV-seropositive population. Last year, highly resistant TB strains now designated as extensively drug-resistant (XDR) TB were identified among HIV-infected patients in Kwa Zulu Natal. HIV-infected patients presenting with this strain of TB had a median survival of 24 days. These XDR strains are resistant to first-line TB agents, to an injectable agent such as streptomycin, and to quinolones. The extent of XDR and multiple drug-resistant TB in sub-Saharan Africa is under intense investigation, but data collected to date suggest that community and nosocomial transmission are occurring. Although there were no primary data presented at this meeting, Paul Nunn from the WHO summarized this information (Abstract 8).

HIV and Hepatitis B Virus Coinfection

Entecavir is a guanosine analogue recently approved by the US Food and Drug Administration (FDA) for the treatment of hepatitis B virus infection (HBV). In patients without an indication for antiretroviral therapy, it is considered a preferable option for HBV treatment because unlike lamivudine, tenofovir, and adefovir, it was previously reported to have no activity against HIV. After making the clinical observation that HIV RNA levels decreased in a patient taking entecavir only for HBV, McMahon and colleagues conducted a series of studies to evaluate entecavir for potential anti-HIV activity (Abstract 136LB). In vitro experiments using primary CD4+ lymphocytes showed a dose-response curve for entecavir against HIV. Clonal analysis of polymerase chain reaction (PCR)-amplified HIV RNA from plasma from a patient receiving entecavir monotherapy showed an increased proportion of clones with M184V over time. Entecavir’s inhibitory activity against HIV with an M184V mutation in reverse transcriptase was reduced. Based on these data, the authors cautioned against the use of entecavir as monotherapy for HBV in HIV-infected
patients. On February 24, 2007, the FDA and the manufacturer announced a change to the label of entecavir to include the new information from these 3 cases. (see http://www.fda.gov/medwatch/safety/2007/safety07.htm#Baraclude)

HBV drug resistance mutations accumulate in patients receiving HBV active agents that fail to suppress viral replication. Sheldon performed genotypic analysis of HBV isolates from HIV-infected and -uninfected patients who were treated with numerous nucleoside and nucleotide agents (Abstract 135). They found that HBV genotype A was more common in HIV-infected persons in their cohort and more likely to select for mutations in HBV surface antigen than genotype D. HBV env mutations were also selected in both groups in the presence of HBV therapy, and may have implications for vaccine efficacy.

Adefovir and tenofovir antiviral potencies and viral dynamics were compared in a nonrandomized French cohort study of HIV- and HBV-coinfected patients (Abstract 945). Twenty-nine patients received adefovir and 56 patients received tenofovir. HBV RNA levels declined rapidly in both groups, but declines were more rapid in patients receiving tenofovir than in those receiving adefovir. In addition, hepatic transaminase declines were more frequent among the patients treated with tenofovir. These data are consistent with prior randomized studies demonstrating superiority of tenofovir over adefovir for HBV treatment. Lewin and colleagues also measured viral dynamics in Thai patients receiving lamivudine, tenofovir, or both as part of a combination antiretroviral therapy study (Abstract 949). They reported no differences in viral decay rates among the 3 treatment arms, and noted that estimates of viral clearance were very similar to HBV-monoinfected patients exposed to the same HBV regimen. Long-term viral HBV suppression rates in 44 patients receiving tenofovir and lamivudine were reported by de Vries-Sluijs (Abstract 939). After a median follow-up of 56 months, the HBV viral suppression rate was 86%. Loss of HBeAg was reported in 57% of patients.

In HBV-infected patients receiving antiretroviral therapy with HBV active drugs, transient elevations in hepatic transaminases occur frequently. Chauvel described rates of transaminase elevations (5 per 100 person-years of observation) or cholestasis (6.7 per 100 person-years of observation) among French patients followed up in a 3-year prospective cohort (Abstract 938). Independent risk factors included hepatitis delta virus, HBV genotype G, older age, alcohol use, and longer duration of HBV infection. Protease inhibitors (PIs) were associated with higher rates of cholestasis.

The prevalence of HBV among HIV-infected populations was reported for 2 African countries. In South Africa, 5.6% of patients were HBV surface antigen positive, and two-thirds of these patients had elevations in liver transaminases (Abstract 919). In contrast, hepatitis C virus (HCV) was present in only 1%. In Nigeria, 11% of patients in a cohort of 1968 HIV-infected persons were HBV-infected (Abstract 920). In this cohort, 6-month HIV RNA suppression rates were the same between patients with and without HBV infection. Hepatotoxicity occurred in 4.3% of the HBV-infected patients and 0.4% of the non-HBV-infected group.

HIV and Hepatitis C Virus Coinfection

HCV seroconversion has been reported among cohorts of HIV-infected men having sex with men (MSM), and has been attributed to high-risk sexual practices. Fisher evaluated HCV seroconversion among a cohort of MSM that included both HIV-infected and -uninfected participants (Abstract 130). HCV seroconversion rates ranged from 40 to 60 per 100 person-years of observation in both HIV-infected and -uninfected men. Seroconversion rates in both groups increased between 2001 and 2005. It was difficult to compare HIV-infected and -uninfected rates, because there was a third group of unknown HCV serostatus. The authors suggested that routine HCV screening should be considered for MSM presenting to sexually transmitted disease centers.

A small proportion of HIV-infected patients spontaneously clear HCV after acute infection. Schnuriger and colleagues conducted immunologic studies to identify predictors of spontaneous clearance among this group (Abstract 887). Consistent with previous reports, less than 5% of patients spontaneously cleared acute HCV. In patients without spontaneous clearance, HCV-specific T cell responses asessed by enzyme-linked immunospot (ELISPOT) were low. Patients with the best responses to HCV treatment developed the most robust T-cell responses over time. Interestingly, the authors found the T-cell responses, which evolved among patients with good responses to HCV, were of similar magnitude to those among patients who exhibited spontaneous clearance.

Another interesting report on patients with acute HCV examined liver biopsies within 5 months of seroconversion (Abstract 889). Three of the 4 patients had stage 2 fibrosis with no other identifiable infectious or toxic cause. More data are needed to corroborate this finding, and to determine if patients with this profile of liver pathology exhibit more rapid clinical progression of liver disease.

To explore mechanisms to explain why HCV progresses more quickly in HIV-infected versus uninfected patients, Yeu and colleagues examined lymphocyte subsets in liver biopsies among HCV patients with (n = 14) and without HIV (n = 6) coinfection (Abstract 133). They hypothesized that HIV-specific T cells producing tumor necrosis factor alfa (TNFα) promote hepatic inflammation through bystander activation. Coinfected patients had lower levels of lymphocytes, but the frequencies of HCV-specific cells were similar. The combined frequency of TNFα-producing HIV and HCV cells was higher in the coinfected than the monoinfected patients. Antiretroviral therapy was associated in a reduction of TNFα-producing lymphocytes. These intriguing findings support but do not conclusively prove the authors’ hypothesis.

There was continued discussion at this year’s conference on how to pre-
dict which patients are likely to respond to HCV treatment, and the use of prolonged HCV therapy in nonresponders, but no data on new treatments. There were 2 reports showing that early virologic responses (4 weeks) could predict HCV treatment success (Abstracts 891, 894). In the report by Mira, the failure of HCV RNA to decrease by 0.6 log copies/mL by week 4 was highly predictive (96%) of treatment failure (Abstract 891). Hernandez evaluated transcription-mediated amplification (TMA) to quantify HCV at the end of a treatment course (interferon alfa plus ribavirin) to determine if it could detect low-level viremia and predict relapse (Abstract 892). The threshold for detecting HCV was 5 IU/mL for the TMA assay compared with 50 IU/mL for the PCR assay. All patients in this study had undetectable HCV by the PCR assays at the end of treatment. Eighty percent of the patients with HCV detectable by TMA relapsed compared with 11% with negative TMA.

TMA of HCV may help predict patients likely to relapse after completing treatment, but data from Nunez and colleagues suggest that extended treatment in these patients is unlikely to be successful (Abstract 899). In a multicenter study of interferon alfa plus ribavirin conducted in Spain, patients with genotypes 1 or 4 were offered extended 18-month treatment regimens. Many patients dropped out during the study, but overall sustained virologic response did not differ between the 12- and 18-month arms. Response rates for genotypes 1 and 4 remained disappointingly low, in the 20% to 30% range.

Disappointing HCV treatment responses were also observed among HIV-infected patients who had received a liver transplant for HCV and required HCV post-transplant treatment (Abstract 890). These 33 patients had a mean CD4 + count of 288 cells/µL, and all but 1 patient had undetectable HIV RNA levels. The early (2-log copies/mL drop at 12 weeks) and sustained virologic responses were seen in 56% and 25% of persons, respectively. Thirty-seven percent of the patients stopped HCV treatment due to toxicity. Among the patients who did not respond to HCV treatment, 50% died due to HCV-related graft loss. This sobering study underscores the need for new HCV drugs.

**Complications of Therapy**

**Body Fat Changes**

Lipoatrophy continues to be a major concern as a complication of long-term HIV treatment. Thymidine nucleoside analogues have been shown to contribute to the development of lipoatrophy (stavudine and zidovudine) and substitution of these agents with non-thymidine nucleoside analogues (abacavir or tenofovir) appears to improve lipoatrophy. Two new studies presented at this year’s conference confirm and extend the observations from earlier work in this area. In both studies lipoatrophy was defined as a 20% loss of limb fat as measured by dual-energy x-ray absorptiometry (DEXA) scan. Cameron presented the results of a study demonstrating that maintenance therapy with lopinavir/ritonavir monotherapy (after suppression with a 3-drug regimen including zidovudine for 24 weeks) was associated with a lower rate of lipoatrophy (5%) than maintenance on zidovudine/lamivudine/efavirenz (43%) for the same period of time, demonstrating the contribution of ongoing therapy with zidovudine to the development of lipoatrophy (Abstract 44L). Investigators from the AIDS Clinical Trials Group (ACTG) randomized treatment-naive patients to receive lopinavir/ritonavir plus nucleoside analogue reverse transcriptase inhibitors (nRTIs), efavirenz plus nRTIs, or the nRTI-sparing combination of lopinavir/ritonavir/ efavirenz (Abstract 58).

The assignment to specific nRTIs was not randomized within the trial, but it was well-balanced. As expected, the nRTI-sparing combination had lower rates (9%) of lipoatrophy at 96 weeks. In this group (lopinavir/ritonavir/efavirenz) limb fat increased by a median of 1 kg at week 96. Median change in limb fat also appeared to be greater than 0 in the other 2 groups; however, a surprising finding was that the proportion of patients with protocol-defined lipoatrophy was twice as high in the efavirenz group than in the lopinavir/nRTIs group. In both nRTI-treated study arms, rates of lipoatrophy were highest for those on stavudine (51% efavirenz, 35% lopinavir/ritonavir) and zidovudine (40% efavirenz, 16% lopinavir/ritonavir). However, even among the patients on tenofovir the rate of lipoatrophy was twice as high in the efavirenz treated group (12% efavirenz/tenofovir 6% lopinavir/ritonavir/tenofovir). No data on weight gain were reported; nonetheless, these results suggest that lopinavir/ritonavir was less likely than efavirenz to contribute to limb fat loss over 96 weeks. These surprising results remind us that we have more to learn about the optimal treatment of HIV infection, leaving clinicians and patients with the challenges of balancing virologic and metabolic outcomes over the long term.

Lipohypertrophy, specifically trunk fat gain, has also been documented in treatment-naive studies of a variety of regimens. Diet and exercise interventions remain the mainstay of treatment. Grinspoon reported the results of a phase III study comparing an injectable novel growth hormone-releasing factor analogue TH9507 with placebo in patients on stable antiretroviral therapy with evidence of abdominal fat accumulation (Abstract 45LB). After 26 weeks of treatment, visceral adipose tissue as measured by computed tomography (CT) decreased by 15% in the TH9507 arm compared with an increase of 5% in the placebo arm. No significant changes in limb fat were observed and notably lipids also improved in the TH9507 group. The treatment was well tolerated, with 2% of the TH9507-treated group developing hypersensitivity reactions. The magnitude of reduction in visceral adipose tissue seen with TH9507 is comparable with what was previously reported with human growth hormone. Whether the benefits of treatment will persist after the drug is stopped is currently being evaluated.

**Diabetes**

Diabetes, an important cause of morbidity and a known risk factor for car-
diabetes (CVD), continues to be a concern in the management of HIV infection. The Swiss cohort study reported the incidence of diabetes was 4.4 per 1000 person years of observation among participants and identified older age, male sex, non-white race or ethnicity, and obesity as risk factors for developing diabetes. Current treatment with PIs and nRTIs was also marginally associated with the risk of diabetes in this treated cohort. Of note, no association was seen between risk of diabetes and coinfection with HCV or HBV in this study. PIs appear to vary in their ability to induce changes in glucose metabolism in carefully conducted metabolic studies. Moyle reported that saquinavir/ritonavir (2000 mg/100 mg daily) was associated with a modest reduction in glucose disposal (~10%) compared with a 2% increase with atazanavir/ritonavir (300 mg/100 mg daily) in treatment-naive patients.

**Cardiovascular Disease**

Options to manage cardiovascular risk include smoking cessation, diet modification, the use of lipid-lowering therapy, and avoidance of antiretroviral regimens associated with the development of lipid abnormalities. Keogh reported on the dietary intake of a group of HIV-seropositive patients compared with an age-matched community sample and found that the HIV-seropositive group had a higher intake of total fat, saturated fat, and trans fat than controls, correlating with higher levels of total cholesterol, triglycerides, and lower high-density lipoprotein (HDL) in the HIV-seropositive group (Abstract 813). These results provide support for a greater role for dietary interventions among patients with HIV. Intermittent use of antiretroviral therapy, as studied in the SMART study, was shown to be associated with a marginally higher risk of CVD (Abstract 41). After further analysis of these data, it appeared that the group at greatest risk for a CVD event among those in the intermittent treatment group were the patients who were not on antiretroviral therapy at the start of the study, suggesting that untreated HIV infection may play some role in overall cardiovascular risk in HIV infection. However, there was no association between CD4+ cell count or HIV RNA level and CVD events. Reductions in HDL cholesterol off antiretroviral therapy appeared to be an important contributor to CVD in this study.

Use of lipid-lowering therapy was examined in several cohorts. Among patients with a CVD endpoint in the DAD study, less than half had started lipid-lowering treatment 6 months after the first event (Abstract 816). Among patients with diabetes in this study, only 20% had started a lipid-lowering drug. Overall use of lipid-lowering therapy was comparable among dyslipidemia HIV-seropositive and -seronegative Kaiser enrollees (Abstract 814). However, the magnitude of triglyceride- and total cholesterol-lowering effects of treatment appeared blunted in the HIV-seropositive group. The authors speculate that drug interactions between antiretroviral therapy and statins limit the options for lipid lowering in the HIV-infected population. Ezetimibe, a drug that inhibits intestinal absorption of dietary and biliary cholesterol was shown to be well tolerated and to have a modest impact on low-density lipoprotein (LDL) cholesterol when used as monotherapy compared with a placebo in HIV-infected patients (Abstract 39). Rosuvastatin is a newer statin that is not metabolized by CYP 3A4, however, it has not been well studied in HIV-infected patients. Hoody and colleagues conducted a formal pharmacokinetic study of rosuvastatin and lopinavir/ritonavir in HIV-uninfected volunteers and reported an unexpected 2- and 4-fold increase in rosvastatin area under the concentration curve and maximum concentration (Cmax), respectively, with 1 case of a grade 4 creatine phosphokinase level (Abstract 564). These authors concluded that rosvastatin should be used with caution in patients treated with lopinavir/ritonavir until more is known about this interaction.

Rates, risk factors, and clinical features of cardiovascular events and subclinical atherosclerosis continue to be examined by several groups (Abstracts 807, 808, 810, 811). Within the Kaiser population rates of hospitalization for myocardial infarction (MI) or coronary heart disease (CHD), although higher for HIV-seropositive patients than matched controls, appear to be stable over time. Women with HIV infection appeared to have an even greater risk than controls, something not previously reported from this database (Abstract 807). Angiographic features of acute coronary syndromes did not appear to differ between 100 HIV-seropositive and 84 HIV-seronegative patients undergoing cardiac catheterization, suggesting that the patterns of atherosclerosis in the HIV patient population are likely to be similar to that observed in the general population (Abstract 811). A cross-sectional study of carotid intima-media thickness (IMT) and coronary calcium scores (as measured by CT) among 657 HIV-infected patients identified traditional risk factors (age, hypertension, obesity), but not specific to antiretroviral therapy as predictors of increased carotid IMT. Notably the majority of HIV-infected patients (78%) had no measurable calcium by CT, suggesting that this non-invasive testing modality may have limited value in HIV-infected patients (Abstract 810).

**Renal Disease**

Renal disease is an important cause of morbidity among African Americans with HIV disease. ESRD among African Americans were reported to be 12-fold higher in those with HIV infection than in those without and do not appear to be declining with the availability of antiretroviral therapy (Abstract 839). Specific etiologies for ESRD in this study were not reported. It has previously been shown that treatment of HIV infection can improve renal function and this was demonstrated again among patients with low glomerular filtration rate (GFR) at the time of initiation of antiretroviral therapy in the ACTG Longitudinal Linked Randomized Trials (ALLRT) cohort study.

The contributions of certain antiretroviral therapy regimens, and specifically those containing tenofovir, to the development of renal dysfunction re-
mains an active area of investigation. Owing to the higher rates of renal disease among African Americans, some clinicians are cautious about the use of tenofovir in this population. However, Gallant and colleagues presented results of a subanalysis of African American patients enrolled in treatment-naive studies comparing tenofovir-containing regimens with thymidine nRTIs (Abstract 505). Results from this pooled analysis demonstrated that race did not alter the beneficial effects of tenofovir compared with thymidine nRTI therapy. Virologic response rates were superior among tenofovir-treated patients and renal function remained stable and similar between treatment groups.

Cohort studies continue to produce somewhat conflicting results regarding risk factors for renal insufficiency and use of tenofovir. Treatment-experienced patients and patients with a history of AIDS appear to be at greater risk for declines in renal function than treatment-naive patients (Abstracts 852, 854). In addition ritonavir-boosted PIs appear to contribute to renal impairment in some studies (Abstracts 833, 835). Whether the use of ritonavir-boosted PIs is a marker for more advanced HIV disease could not be determined from the collective group of studies. Studies using more sensitive markers of renal function such as the measurement of cystatin C, which is not dependent on weight, may yield more consistent results in future studies (Abstract 830).

**Bone Diseases**

Higher rates of osteopenia among HIV patients continue to be reported from cohort studies (Abstract 836). The impact of specific antiretroviral drugs on osteopenia remains unclear. Modarrisi (Abstract 838) reported an in vitro study suggesting that low-dose ritonavir alters the expression of genes important for osteoclast differentiation and activity. The clinical significance of this finding is unknown. In the Study to Understand the Natural History of HIV/AIDS in the Era of Effective Therapy (SUN) study, risk factors for osteopenia in this group include older age, lower body mass index, lower baseline CD4+ cell count, and prolonged duration of HIV infection. Alendronate, a bisphosphonate approved by the FDA for the treatment of osteoporosis along with vitamin D and calcium supplementation was compared with vitamin D and calcium alone in 82 HIV-infected patients with osteoporosis. Treatment with alendronate was well tolerated and associated with significant increases in bone mineral density in the lumbar spine, hip, and trochanter after 48 weeks of treatment.

**Antiretroviral-associated Toxicities in Resource-limited Settings**

Several presentations at this year’s conference focused extended information on antiretroviral therapy toxicities associated with first-line regimens being used in resource-limited settings. Although expanding access to antiretroviral therapy is the highest programmatic priority in resource-limited settings, several studies underscored the drug-associated morbidity and mortality associated with widely used stavudine regimens and the need for resource-limited countries to have access to affordable, coformulated regimens with less-toxic drugs.

Predictors and outcomes in patients with severe lactic acidosis were evaluated in a case-controlled study reported by Osler and colleagues (Abstract 792). The group performed a retrospective chart review of all case patients presenting to a referral center for 6 antiretroviral therapy clinics in Capetown, South Africa. Patients referred to this center were being treated with stavudine, lamivudine and an NNRTI. The authors identified 73 cases of lactic acidosis. Fifteen percent of patients died acutely, and 2 patients died subsequently. A low serum bicarbonate was the only risk factor associated with mortality. In a nested, case-controlled study, female sex, low CD4+ count nadir, and baseline weight greater than 60 kg were independent risk factors for lactic acidosis. In 29 patients who survived, only 1 patient developed recurrent lactic acidosis during a median follow up of 10 months. At the poster discussion session, the authors also pointed out that among women, weight gain after starting antiretroviral therapy was predictive of a higher risk for lactic acidosis. One potential explanation for this finding is that the stavudine dosage was higher during this period based on current dosing algorithms (40 mg for those weighing more than 60 kg). Lactic acidosis was also a major toxicity reported from a retrospective chart review of 305 patients receiving stavudine, lamivudine and efavirenz in a public-sector antiretroviral therapy program in Johannesburg, South Africa (Abstract 795). The most common adverse events in this cohort were peripheral neuropathy (52%), lipodystrophy (8.5%), gynecomastia (8.9%) and lactic acidosis (6.6%). In this cohort 19.7% of patients had a treatment-limiting side effect. Treatment changes were made after a median of 14 months of follow up.

Amoroso evaluated antiretroviral therapy switches for dose-limiting toxicity among patients receiving antiretroviral therapy in the US President’s Emergencypathy. The authors also examined the possibility of switching to stavudine in patients with dose-limiting toxicity. Switching to stavudine in patients who start therapy with stavudine before they reach a dose-limiting toxicity owing to stavudine to avoid cumulative stavudine toxicity has also been raised as a potential therapeutic strategy. Investigators from the CDC reviewed stavudine tolerance among 261 patients in the Tororo, Uganda, cohort who switched from stavudine to zidovudine for dose-limiting toxicity (Abstract 793). After switching to zidovudine, rates of anemia and leukopenia increased slightly. However, 95% of
patients successfully tolerated a switch from stavudine to zidovudine.

Peters and colleagues found that renal function actually improved among antiretroviral treatment-experienced patients with advanced HIV disease living in rural Uganda (Abstract 791). In 507 patients receiving antiretroviral therapy in the Tororo, Uganda, cohort creatinine clearance was measured over time using the Cockcroft-Gault equation. At baseline, 21% of patients had creatinine clearance of less than 50 mL/min/1.73 m². After 24 months of antiretroviral therapy, renal function of the cohort improved, and only 6% had clearance of less than 50 mL/min/1.73 m². Although low creatinine clearances may be due to numerous reasons in this population, these data support current thinking that antiretroviral therapy can improve kidney function in patients with HIV-associated renal disease.

There were 2 abstracts on lipid and metabolic changes associated with antiretroviral therapy in resource-limited settings. In the Tororo cohort, where patients received an NNRTI plus 2 nRTIs, fasting lipid measurements from baseline and 24 months after initiation of antiretroviral therapy were compared (Abstract 790). Total cholesterol (TC) increased by 24%, HDL by 62%, and LDL by 54%. Triglycerides decreased by 24%. TC was greater than 200 mg/dL for 11% of patients after 24 months compared with 3% at baseline. Studies with fasting lipid specimens linked to nutritional evaluations status will be needed to interpret these kinds of data sets in the future. A second study followed up 43 patients prospectively in Johannesburg (Abstract 796). Lipodystrophy was based on patient and physician assessment. This group reported that 39% of patients receiving stavudine/efavirenz/lamivudine developed lipodystrophy. Changes were first detected after 18 months of follow up. Lipodystrophy was associated with increases in waist-to-hip ratio and glucose and triglyceride levels. Elevations in TC, LDL, and HDL were reported in patients with and without lipodystrophy. Although this study was small and relied on subjective measures, the cumulative increase in lipodystrophy was striking in this cohort.

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Advances in Antiretroviral Therapy

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The 14th Conference on Retroviruses and Opportunistic Infections provided a forum for presentation of state-of-the-art research on antiretroviral therapy. This year’s conference marked the first public presentation of phase III trials of the lead compounds in 2 new drug classes: maraviroc (a CCR5 inhibitor) and raltegravir (an HIV-1 integrase inhibitor). These agents are likely to be approved by the US Food and Drug Administration this year and should provide major new options for treatment-experienced patients with multidrug resistant virus. Other dominant themes of the conference were the impressive number of presentations describing outcomes of antiretroviral therapy programs in resource-limited settings and new information on mechanisms of drug resistance. Among the latter, the importance of drug resistance mutations occurring in the RNase H and connection domains of the HIV-1 reverse transcriptase was of special note. In addition, substantial new information was presented on other new antiretroviral agents, studies in treatment-naïve patients, antiretroviral therapy strategies, prevention of mother-to-child transmission, predictors of clinical response to therapy, and antiretroviral pharmacokinetics. Research in antiretroviral therapy remains dynamic and advances in the field continue to improve our ability to maintain long-term control of HIV-1 replication in infected persons.

New Antiretrovirals

A major focus of this year’s conference was the presentation of phase III studies of entry and integrase inhibitors, which showed good clinical outcomes with acceptable side effect profiles. (see Table 1) These drugs are likely to be approved by the US Food and Drug Administration (FDA) in 2007 and will provide new options for treatment-experienced patients with multidrug resistant HIV-1. Studies on antiretrovirals in early-phase and preclinical development highlighted promising drugs that will hopefully further expand antiretroviral regimens available to HIV-seropositive patients.

Antiretrovirals in Late-phase Clinical Development

Entry Inhibitors: Maraviroc. Outcomes of 2 phase IIib/III studies of maraviroc, an investigational CCR5 inhibitor, were presented: MOTIVATE 1 (Abstract 104aLB) and MOTIVATE 2 (Abstract 104bLB). The studies were of identical design but conducted in different countries. Eligible subjects were 3-class experienced patients with plasma HIV-1 RNA levels above 5000 copies/mL and exclusive use of CCR5 coreceptor for entry as determined by tropism testing. Subjects were randomized 1:2:2 to placebo, maraviroc 150 mg once daily, and maraviroc 150 mg twice daily, all given with an optimized background regimen (OBR) of 3 to 6 antiretrovirals. If subjects were not receiving a ritonavir-boosted protease inhibitor (PI), they received maraviroc 300 mg once or twice daily. Both studies had similar participant baseline characteristics.

In MOTIVATE 1, 585 subjects were enrolled (approximately 82% were white and 90% were male). Across groups the median ranges were baseline CD4+ counts of 150 to 168 cells/µL and baseline HIV-1 RNA of 4.8 to 4.9 log10 copies/mL. The primary endpoint, a reduction in plasma HIV-1 RNA at 24 weeks, was statistically significantly greater in the once-daily (1.82 log10 copies/mL) and twice-daily arms (1.95 log10 copies/mL) than in the placebo arm (1.03 log10 copies/mL). Subjects in the once- and twice-daily arms were more likely to achieve plasma HIV-1 RNA levels below 50 copies/mL at week 24 than subjects in the placebo arm (42% and 49% vs 25%, respectively), and had a greater increase in CD4+ counts (107 and 111 vs 52 cells/µL, respectively). There were no marked safety issues and the rates of adverse events in the maraviroc arm were not statistically significantly different from rates in the placebo arm, including liver-related adverse events and malignancies.

In MOTIVATE 2, 464 subjects were enrolled (approximately 85% of subjects were white and 84% were male). Across groups the median ranges were baseline CD4+ counts of 174 to 182 cells/µL and baseline HIV-1 RNA of 4.8 to 4.9 log10 copies/mL. The primary endpoint, reduction in plasma HIV-1 RNA at 24 weeks, was significantly greater in once-daily (1.95 log10 copies/mL) and twice-daily arms (1.97 log10 copies/mL) than in the placebo arm (0.93 log10 copies/mL). Subjects in the once- and twice-daily arms were more likely to achieve a plasma HIV-1 RNA level below 50 copies/mL at week 24 than subjects in the placebo arm (41% and 46% vs 21%, respectively), and had a greater increase in CD4+ count (112 and 102 vs 64 cells/µL, respectively). There were no marked safety issues and rates of adverse events in the maraviroc arm were not statistically significantly different from rates in the placebo arm, including liver-related adverse events and malignancies.

The combined analysis from these 2 trials showed that 56% of subjects who screened for this study had HIV-1 that utilized only the CCR5 coreceptor for
entry (R5 HIV). Eight percent of subjects who had R5 HIV at screening had dual or mixed HIV-1 populations that utilized both CCR5 and CXCR4 coreceptors for entry at baseline, prior to receiving maraviroc. The dual or mixed HIV subjects who received maraviroc had a poorer virologic response than R5 HIV subjects who received maraviroc. These results are similar to what was observed in a previously presented trial of maraviroc in dual or mixed HIV subjects. The number of active drugs in the OBR was found to be a predictor of suppression to less than 50 HIV-1 RNA copies/mL and baseline HIV-1 RNA viral load was not related. The proportion of patients achieving HIV-1 RNA below 50 copies/mL in the once- and twice-daily arms were 18% and 29%, respectively, among patients with no active drugs in the OBR, 43% and 43% with 1 active drug in the OBR, 52% and 53% with 2 active drugs in the OBR, and 61% and 58% with 3 or more active drugs in the OBR, indicating that difference in viral load outcomes between once- and twice-daily maraviroc was apparent only among patients with no active drugs in the OBR.

Among patients with virologic failure, change in coreceptor usage from R5 HIV to dual or mixed HIV was noted in 4 of 84 (5%) subjects in the placebo groups, 31 of 49 (63%) subjects in the once-daily groups, and 32 of 49 (65%) of subjects in the twice-daily groups.
<table>
<thead>
<tr>
<th>Follow-up Time</th>
<th>HIV-1 RNA Response</th>
<th>Comments</th>
</tr>
</thead>
</table>
| 24 weeks       | -1.03 to -1.82 and -1.95 log_{10} copies/mL | Combined analyses:
The qd and bid dosing groups appeared no different when having 1 or more active drugs in OBR; in 31/49 subjects in whom maraviroc failed, there was a change in coreceptor usage. |
| 24 weeks       | -0.93 to -1.95 and -1.97 log_{10} copies/mL | 25% vs 42% and 49% < 50 copies/mL |
| 16 weeks       | 77% vs 41% < 400 copies/mL | Combined analyses:
32/41 subjects in whom raltegravir failed had mutations in integrase; 98% of subjects receiving raltegravir and enfuvirtide for the first time had < 400 HIV-1 RNA copies/mL at week 16. |
| 16 weeks       | 77% vs 43% < 400 copies/mL | 61% vs 33% < 50 copies/mL |
| 16 weeks       | 50-mg arm, -1.5 log_{10} copies/mL | Elvitegravir led to rapid declines in plasma HIV-1 RNA at week 2 that were sustained only if there were other active drugs in the OBR. |
|                | 125-mg arm, -1.7 log_{10} copies/mL | 20-mg arm stopped early for virologic failure vs control arm, -1.2 log_{10} copies/mL |

There were no obvious adverse effects that resulted from change in coreceptor usage. CD4+ cell counts were generally well preserved in subjects who experienced change in coreceptor usage.

**Integrate Inhibitors: Raltegravir and GS-9137.** Investigators presented data from 2 phase III studies of the investigational HIV-1 integrase inhibitor raltegravir (MK-0518), BENCHMRK-1 and BENCHMRK-2 (Abstracts 105aLB, 105bLB). The 2 studies were identical in design. Inclusion criteria included evidence of genotypic or phenotypic resistance to at least 1 drug from each of 3 classes and plasma HIV-1 RNA levels of above 1000 copies/mL. Subjects were randomized 2:1 to raltegravir 400 mg twice daily or placebo in each study.

In BENCHMRK-1 (Abstract 105aLB), 350 subjects were enrolled (approximately 78% of subjects were white and 85% were male). Across groups the median ranges were baseline CD4+ counts of 153 to 156 cells/µL and baseline HIV-1 RNA of 4.5 to 4.6 log_{10} copies/mL. The OBR contained 0 or 1 active drug in 48% to 51% of subjects; 20% to 21% received enfuvirtide for the first time and 25% to 27% received darunavir for the first time. More subjects in the raltegravir group achieved plasma HIV-1 RNA levels below 400 copies/mL (77%) and below 50 copies/mL (61%) at week 16 than subjects in the placebo group (41% and 33%, respectively). Subjects receiving raltegravir had a greater increase in CD4+ count than those in the placebo group (83 cells/µL vs 31 cells/µL).

Similar results were observed in BENCHMRK-2 (Abstract 105bLB). Of the 349 subjects enrolled, approximately 60% were white and 90% were male. Across groups the median ranges were baseline CD4+ counts of 146 to 163 cells/µL and baseline HIV-1 RNA of 4.5 to 4.6 log_{10} copies/mL. The OBR contained 0 or 1 active drug in 44% to 46% of subjects. Nineteen percent to 20% received enfuvirtide for the first time and 45% to 50% received darunavir for the first time. More subjects in the raltegravir group achieved plasma HIV-1 RNA levels below 400 copies/mL (77%) and below 50 copies/mL (62%) at week 16 than did subjects in the placebo group (43% and 32%, respectively). Subjects receiving raltegravir had a greater increase in CD4+ count than those in the placebo group (86 cells/µL vs 40 cells/µL).

The authors presented data on combined analysis of both studies. Genotypic resistance data were available for 41 subjects who had virologic failure while receiving raltegravir. Thirty-two of 41 had resistance-associated mutations in integrase, and mutations fell generally into one of 2 mutational pathways: N155H or Q148K/R/H. One of these mutations was usually present with additional integrase mutations. These pathways were expected based on in vitro data. Subgroup analysis showed that 98% of subjects receiving raltegravir with de novo use of both enfuvirtide and darunavir achieved HIV-1 RNA levels below 400 copies/mL at 16 weeks.
and 90% of subjects receiving raltegravir with de novo use of either enfuvirtide or darunavir achieved HIV-1 RNA levels below 400 copies/mL at week 16. The overall rate of serious drug-related adverse events was low in both studies and no specific adverse events were associated with raltegravir use.

Zolopa and colleagues (Abstract 143LB) presented data from a phase II dose-finding study of the investigational integrase inhibitor GS-9137. Eligible subjects had plasma HIV-1 RNA levels above 1000 copies/mL and 1 or more PI mutations. There were 278 subjects randomized to 1 of 3 doses of GS-9137 (20 mg, 50 mg, or 125 mg with 100 mg ritonavir once daily) or best available PI. The subjects were 90% male and 73% white, and 23% used enfuvirtide for the first time. The median baseline CD4+ count was 157 to 243 cells/µL and mean baseline HIV-1 RNA level was 4.5 to 4.7 log_{10} copies/mL. The 20-mg arm was stopped early due to inferior virologic performance. Through 16 weeks, the control arm had an average virologic reduction of 1.2 log_{10} HIV-1 RNA copies/mL compared with 1.5 log_{10} copies/mL in the 50-mg group and 1.7 log_{10} copies/mL in the 125-mg group. Although this was a noninferiority study design, the 125-mg group had a statistically significantly greater viral load reduction than the control group. Thirty-eight percent and 40% of subjects receiving the 2 higher doses achieved below 50 HIV-1 RNA copies/mL at week 16 compared with 30% of subjects in the control arm. Subjects with no active drugs in the OBR tended to have a rapid drop in plasma HIV-1 RNA at week 2 followed by virologic rebound, whereas subjects with 1 or more active drugs in the OBR had more sustained virologic suppression. Thus, GS-9137 was well tolerated and no marked safety concerns were identified. This study highlights the importance of supporting integrase inhibitor use with other active drugs in the regimen to avoid the rapid emergence of drug resistance to this new class.

**New Reverse Transcriptase Inhibitors: Rilpivirine (TMC278) and (±)-ß-2’, 3’-dideoxy-3’-thia-5-fluorocyto-

sine ([±]-FTC) Pozniak and colleagues (Abstract 144LB) presented data from the TMC278-C204 trial, a phase IIb study of an investigational non-nucleoside reverse transcriptase inhibitor (NNRTI) with retained antiviral activity against HIV and resistant to currently FDA-approved NNRTIs, in treatment-naive subjects. Subjects were randomized to 1 of 3 doses of rilpivirine or efavirenz once daily, given with fixed-dose tenofovir/emtricitabine or zidovudine/lamivudine. The median baseline plasma HIV-1 RNA was 4.9 log_{10} copies/mL and the median CD4+ count was 203 cells/µL. Three hundred sixty-eight subjects (33% women and 53%-56% nonwhite) were randomized. In an intention-to-treat analysis, 77% to 81% of subjects in the rilpivirine arms achieved the primary endpoint of below 50 HIV-1 RNA copies/mL compared with 81% in the efavirenz arm, a difference that was not statistically significant. Increases in CD4+ count were similar in both arms (123-145 cells/µL in the rilpivirine arms vs 125 cells/µL in the efavirenz arm; P = not significant). There were, however, better overall lipid profiles and significantly lower rates of rash and central nervous (CNS) system side effects in the rilpivirine arms than in the efavirenz arm. The rates of serious adverse events were low and there was not a statistically significant difference between groups. Complete resistance analysis is underway and was not available at this presentation.

(±)-FTC is an investigational mixture of emtricitabine plus its D-enantiomer. Cahn and colleagues (Abstract 488) presented data on a novel peptidomimetic PI, GS-8374. In vitro studies showed potent activity against a range of clinical isolates including those with resistance to darunavir and brecanavir. Development of resistance to this compound did not occur after 6 months of serial passage experiments despite development of resistance to lopinavir, atazanavir, and darunavir in parallel experiments. GS-8374 showed minimal effects on lipid accumulation and insulin-stimulated glucose uptake in adipocytes.

**Antiretrovirals in Phase I and Preclinical Development**

**Nucleoside Reverse Transcriptase Inhibitors: IDX12899 and IDX12989.** Richman and colleagues (Abstract 489) presented data on 2 investigational NNRTIs: IDX12899 and IDX12989. Both compounds exhibited potent activity against a broad range of clinical isolates including isolates with single- and double-NNRTI mutations. The pharmacokinetic profiles in various animal models supported once-daily dosing. No adverse events were noted in acute toxicity models. Serial passage studies exhibited a longer time for development of resistance to these compounds than to efavirenz.

**Protease Inhibitors: GS-8374.** Callebaut and colleagues (Abstract 491) presented data on a novel peptidomimetic PI, GS-8374. In vitro studies showed potent activity against a range of clinical isolates including those with resistance to darunavir and brecanavir. Development of resistance to this compound did not occur after 6 months of serial passage experiments despite development of resistance to lopinavir, atazanavir, and darunavir in parallel experiments. GS-8374 showed minimal effects on lipid accumulation and insulin-stimulated glucose uptake in adipocytes.

**Integrase Inhibitors: MK-2048 and GSK364735.** Wai and colleagues (Abstract 87) presented data on a new series of compounds that inhibit the strand transfer reaction of HIV integrase and are similar to 2 integrase inhibitors in late-stage clinical development (napthyridine [L-870810] and pyrimidinone [MK-0518, raltegravir]), but were designed to have a higher genetic barrier to resistance and limited cross-resistance to previously described compounds. One compound, MK-2048, was found to have a 95% inhibitory concentration (IC_{50}) of 41 nM
and a pharmacokinetic profile in dogs and rats that suggests once-daily dosing in humans is possible, and demonstrated retained activity against HIV-1 strains resistant to integrase inhibitors currently in clinical development.

Reddy and colleagues (Abstract 562) presented data on the safety and pharmacokinetics of an HIV-1 integrase inhibitor, GS-364735, in HIV-uninfected subjects. Among 79 subjects, they found only mild adverse events except for 1 moderate headache. No grade 2 or higher laboratory events were noted. Food increased the bioavailability of this compound by 30% to 100%, and aluminum and magnesium hydroxide decreased bioavailability by 50%. The compound did not markedly alter CYP450 enzymes except for weak inhibition of CYP1A2 and the pharmacokinetic profile was not affected by ritonavir. The pharmacokinetic data supported twice-daily dosing in future phase II studies.

**CXCR4 Inhibitors: AMD11070.** Two studies evaluated AMD11070, an investigational oral CXCR4 inhibitor: the X4 Antagonist Concept Trial (XACT), presented by Mylove and colleagues (Abstract 511), and AIDS Clinical Trials Group (ACTG) A5210 presented by Saag and colleagues (Abstract 512). Both were phase I studies that evaluated exposure to 10 days of AMD11070 given twice daily. Eligible subjects were off antiretroviral therapy, had plasma HIV-1 RNA levels above 5000 copies/mL, and above 2000 relative luminescence units (RLU) of CXCR4 usage in the trofile assay. Nearly all subjects received 200 mg of AMD11070 twice daily for 10 days. Four of 9 in XACT and 3 of 6 in ACTG 5210 subjects had at least a 1-log reduction in X4 RLU indicating a decrease of plasma HIV-1 using CXCR4 for entry in CD4+ cells) after 10 days of monotherapy. Both studies concluded that proof-of-concept has been established and that further studies are warranted. The clinical development of AMD11070 has been put on hold due to abnormal liver histology in long-term toxicology studies in animals.

**Morphilino Antisense Oligonucleotides.** Phosphorodiamidate morpholino oligomers (PMOs) are water-soluble antisense oligonucleotide analogues that block complementary RNA sequences. Bestwick and colleagues (Abstract 499) investigated the potential for PMO to act against the highly conserved start codon region of the HIV-1 vir gene and the Tar stem-loop. They found that the optimal vir PMO generated was able to inhibit viral replication with a median effective concentration (EC₅₀) of 260 nM whereas the Tar stem-loop PMO inhibited with an EC₅₀ of 3.6 μM. These data support pursuing this strategy for antiretroviral drug development.

**Histone Deacetylase Inhibitors.** Latently infected, resting memory CD4 + T cells are a major reservoir for HIV and represent a significant barrier to eradication of HIV from infected individuals. Histone deacetylases (HDACs), of which there are 3 classes (I, II, and III), are important in maintaining viral latency by causing the DNA to become tightly bound, thus preventing access to HIV DNA by nuclear transcription factors. HDAC inhibitors may lead to expression of HIV genes resulting in productive infection, which would then expose the previously latent HIV-infected cells to immune surveillance and the effects of antiretroviral drugs. Valproic acid is a known nonspecific HDAC inhibitor. Weinman and colleagues (Abstract 500) presented data on several small molecules that inhibit class I HDACs and showed that these molecules promoted histone acetylation. Archin and colleagues (Abstract 501) then tested these HDAC inhibitors on latently infected CD4+ cells and showed that they increased viral transcription and effectively withdrew cells from latency.

**Vpu Ion Channels.** Vpu has been shown to have 2 important functions in the lifecycle of HIV-1: virion assembly and release, and CD4+ degradation. It associates in pentamers to form a cation-specific ion channel. Luscombe and colleagues (Abstract 502) presented data on several inhibitors of the Vpu ion channel. Such inhibitors should be effective at interfering with viral replication in macrophages, a reservoir not targeted by currently available drugs. The lead compound, BIT225, showed potent antiretroviral activity at an EC₅₀ of 1.1 μM in HIV-1-infected macrophages. The 50% cytotoxicity concentration was 212 μM, suggesting a favorable antiviral index. It showed broad activity against a range of clinical isolates and appeared to be synergistic with several antiretrovirals.

**Clinical Trials in Treatment-naive Patients**

**Trials in Treatment-naive Patients with Established HIV-1 Infection**

Results of selected treatment trials in antiretroviral-naive patients are summarized in Table 2. Mildvan and colleagues (Abstract 138) presented results of the A5073 trial, a 48-week, multicenter, 3-arm, open-label trial that compared lopinavir 400 mg/ritonavir 100 mg soft gel capsule self-administered twice daily, lopinavir 800 mg/ritonavir 200 mg self-administered once daily, and lopinavir 800 mg/ritonavir 200 mg via directly observed therapy once daily. Participants were antiretroviral-naive with HIV-1 RNA levels above 3.3 log₁₀ copies/mL and were randomized in a 2:2:1 ratio stratified by screening HIV-1 RNA levels above or below 5.0 log₁₀ copies/mL. All patients received emtricitabine 200 mg with extended-release stavudine 100 mg or tenofovir 300 mg once daily. The 402 patients enrolled had a baseline median CD4+ count of 197 cells/µL and median HIV-1 RNA level of 4.8 log₁₀ copies/mL. The difference in plasma HIV-1 RNA at 48 weeks between the once- and twice-daily groups was 0.03 log₁₀ copies/mL (95% confidence interval [CI], −0.07-0.12). However, in the higher HIV-1 RNA level stratum, the probability of sustained virologic response at 48 weeks was statistically significantly higher in the twice-daily group than in the once-daily self-administered group (0.89; 95% CI, 0.79-0.94 vs 0.76; 95% CI, 0.64-0.84, respectively). Probabilities of sustained virologic response at 24 and 48 weeks between the directly observed therapy and once-daily self-administered arms were not statistically significantly different.

Rey and colleagues (Abstract 503)
presented the preliminary results of the DAUFIN study, a randomized, open-label, multicenter, noninferiority trial of once-daily lamivudine 300 mg, tenofovir 245 mg, and nevirapine 400 mg versus twice-daily zidovudine 300 mg/lamivudine 150 mg and nevirapine 200 mg. The trial was stopped by the steering committee after 12-week data showed 7 early nonresponses (defined as plasma HIV-1 RNA with a less than 2.0 log copies/mL decrease or rebound of more than 1.0 log copies/mL after initial decrease) in the once-daily arm and no early nonres-ponses in the twice-daily arm. The early nonresponders had higher baseline median plasma HIV-1 RNA levels and lower median CD4+ count, and all had 1 or more NNRTI mutations. Viral genotypes showed that 6 of the 9 individuals who did not respond to therapy in the once-daily arm also had K65R mutations.

Walker and colleagues (Abstract 506) presented 48-week results of the Evaluation of Nevirapine or Abacavir (NORA) trial, a randomized substudy of the Development of Anti-retroviral Therapy in Africa (DART) trial in Uganda of 600 antiretroviral-naive patients with CD4+ counts below 200 cells/µL. The trial compared zidovudine 500 mg/lamivudine 150 mg plus abacavir 300 mg twice daily with zidovudine 300 mg/lamivudine 150 mg plus nevirapine 200 mg twice daily and was placebo-controlled for the first 24 weeks. Baseline median CD4+ count was 99 cells/µL and mean plasma HIV-1 RNA was 5.4 log copies/mL. At 48 weeks, 77% of the nevirapine arm and 62% of the abacavir arm had plasma HIV-1 RNA levels below 1.7 log copies/mL (P < .001). The CD4+ cell count increase at 48 weeks was statistically significantly higher in the nevirapine arm (173 cells/µL) than in the abacavir arm (147 cells/µL; P = .006). Despite the greater HIV-1 RNA level decreases and CD4+ cell increases, the authors noted a trend suggesting clinical outcome superiority of the abacavir arm at 48 weeks, with 29 (10%) of individuals in the nevirapine arm developing new World Health Organiza-tion (WHO) stage IV events or death, compared with 17 (6%) in the abacavir arm (P = .06). A similar trend was observed for WHO stage III events, but was not statistically significant.

Busmann and colleagues (Abstract 507) presented preliminary results from the Tshepo Study, an open-label, randomized, ongoing study in Botswana examining 3 different nucleoside reverse transcriptase inhibitor (nRTI) regimens, 2 NNRTI regimens, and community-based directly observed therapy versus standard of care. The study enrolled 650 antiretroviral-naive patients between December 2002 and December 2004. Sixty-nine percent of the patients were female and baseline patient age, CD4+ count, and median follow up were 35.9 years, 199 cells/µL, and 89.7 weeks, respectively. The patients were randomized in a 3 × 2 × 2 factorial design to: A) zidovudine/lamivudine, zidovudine/didanosine, or stavudine/lamivudine; B) efavirenz or nevirapine; and C) community-based directly observed therapy or standard of care. There were also 2 balanced strata of CD4+ cell counts: below 201 cells/µL with any viral load, and between 201 and 350 cells/µL with plasma HIV-1 RNA above 4.74 log copies/mL. The analysis was undertaken after the Data and Safety Monitoring Board (DSMB) suspended the zidovudine/didanosine-containing arm owing to inferiority in the primary endpoint of virologic failure. The authors pooled data from the 2 NNRTI arms and compared patients receiving zidovudine/didanosine with those receiving zidovudine/lamivudine or stavudine/lamivudine. The rate of virologic failure with genotypic resistance was 11% in the zidovudine/didanosine arm and 2% in the zidovudine/lamivudine or stavudine/lamivudine arm (P = .0002). No difference in death rate or time to first treatment-limiting toxicity was observed in either nRTI arm. Unexpectedly, there were no differences between the community-based directly observed therapy and standard-of-care arms, which the authors suggested was due to overall low rates of virologic failure. No results were given comparing efavirenz and nevirapine groups.

Trials in Treatment-naive Patients with Acute HIV-1 Infection

Estes and colleagues (Abstract 67) examined the impact of antiretroviral treatment on CD4+ cell populations in lymph nodes, Peyer’s patches, and the lamina propria by obtaining inguinal lymph node and ileum biopsies in 32 HIV-1-infected and 11 HIV-seronegative individuals. They obtained follow-up biopsies in 15 of the HIV-1-infected individuals 6 months after initiation of potent antiretroviral therapy. All compartments were significantly depleted of CD4+ cells in HIV-1-infected patients and, after initiation of antiretroviral therapy, there was an increase in CD4+ cells in peripheral blood and lymph nodes, but not in the lamina propria. In patients who began antiretroviral therapy in the acute and early stages of infection, the mean increase in peripheral blood CD4+ counts was 388 cells/µL and the parafollicular T-cell zone was 12.5% occupied. In patients who initiated therapy with established infection in the presymptomatic phase the increase in peripheral blood CD4+ count was 176 cells/µL and the parafollicular T-cell zone was 13.65% occupied. Earlier initiation of antiretroviral therapy was associated with greater increases in the central memory cell pop-ulation of the Peyer’s patches. There was no change in measured T-cell subsets in patients who initiated antiretroviral therapy after being diagnosed with AIDS.

Thus far, studies of the immunologic and virologic outcomes of early treatment of primary HIV disease have been contradictory.13 Four abstracts at this year’s conference used data from large cohort studies to examine this, but did not end controversy. Similar definitions of primary infection were used in each of the following studies.

Steingrover and colleagues (Abstract 124LB) used data from 2 large cohorts of HIV-seropositive patients in Dutch treatment centers, the Amsterdam Cohort Study and the Athena cohort, to examine the immunologic and virologic outcomes of initiating potent antiretroviral therapy within 6 months of HIV seroconversion. Of the 332 patients identified with primary HIV-1 infection, 64 were treated with potent antiretrovi-
Table 2. Selected Trials of Antiretroviral Therapy in Treatment-naive Patients

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Abstract No.</th>
<th>Description</th>
<th>Population</th>
<th>Regimen(s)</th>
<th>Baseline CD4+ cells/μL, Log₁₀ copies of HIV RNA/mL</th>
<th>Follow-up Time</th>
<th>Response</th>
<th>Comments</th>
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<tbody>
<tr>
<td>TMC278-C204</td>
<td>Abstract 144LB</td>
<td>Efficacy and safety testing of 3 doses of rilpivirine (TMC278), an investigational NNRTI with activity against HIV-1 resistant to currently available NNRTIs</td>
<td>33% female 53%-56% nonwhite</td>
<td>Randomized to: rilpivirine 25 mg qd, rilpivirine 75 mg qd, rilpivirine 150 mg qd or efavirenz 600 mg qd plus: zidovudine/ lamivudine (76%) or tenofovir/ emtricitabine (24%) (n=368)</td>
<td>203 (median) 4.9 (median)</td>
<td>48 weeks</td>
<td>Primary endpoint of intent-to-treat analysis: Rilpivirine arms: 77%-81% with &lt;50 copies/mL Efavirenz arm: 81% with &lt;50 copies/mL P = ns CD4+ count rise: Rilpivirine arms: 123-145 cells/μL Efavirenz arm: 125 cells/μL P = ns</td>
<td>Some differences in side-effect profiles: Incidence of rash and central nervous system side effects were significantly lower in the rilpivirine arms. Rilpivirine associated with lower total cholesterol, lower LDL, and lower triglycerides, although the efavirenz group had a higher HDL. Complete resistance analysis was not available.</td>
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<tr>
<td>ACTG A5073</td>
<td>Abstract 138</td>
<td>Comparison of qd (with or without DOT) versus bid lopinavir/ritonavir</td>
<td>Plasma HIV-1 RNA &gt;3.3 log₁₀ copies/mL or &lt; or ≥5.0 log₁₀ copies/mL Non-blinded</td>
<td>Lopinavir 400 mg/ ritonavir 100 mg soft gel capsule 400/100 mg, self-administered bid Lopinavir 800 mg/ ritonavir 200 mg, self-administered qd Lopinavir 800 mg/ ritonavir 200 mg via DOT qd plus: emtricitabine/ stavudine or emtricitabine/ tenofovir (n=402)</td>
<td>197 (median) 4.8 (median)</td>
<td>48 weeks</td>
<td>Difference in plasma HIV-1 RNA at 48 weeks between qd and bid groups was 0.03 log₁₀ copies/mL (95% CI, -0.07-0.12)</td>
<td>No significant difference in probability of sustained virologic response between DOT and qd, self-administered arms at 24 and 48 weeks Probability of sustained virologic response at 48 weeks in higher stratum was higher in bid group (0.89; 95% CI, 0.79-0.94) than in the qd self-administered group (0.76; 95% CI, 0.64-0.84).</td>
</tr>
<tr>
<td>DAUFIN Study</td>
<td>Abstract 503</td>
<td>Non-inferiority trial of qd nevirapine vs bid nevirapine Preliminary results</td>
<td>CD4+ count &lt;350 cells/μL Non-blinded (n=71)</td>
<td>Lamivudine 300 mg/ tenofovir 245 mg/ nevirapine 400 mg qd (n=36)</td>
<td>207 (median) 4.85 (median)</td>
<td>12 weeks</td>
<td>7 early non-responses 19% early non-response rate</td>
<td>The trial steering committee stopped the study. The definition of early non-response was plasma HIV-1 RNA with either &lt;2.0 log₁₀ copies/mL decrease or rebound of &gt;1.0 log₁₀ copies/mL 1 or more NNRTI resistance mutations seen in all cases.</td>
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<tr>
<td></td>
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<td></td>
<td>Zidovudine 300 mg/ lamivudine 150 mg/ nevirapine 200 mg bid (n=35)</td>
<td></td>
<td>209 (median) 4.94 (median)</td>
<td>12 weeks</td>
<td>0 early non-responses</td>
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(Continued on next page)
Table 2. Selected Trials of Antiretroviral Therapy in Treatment-naive Patients (cont’d)

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Population</th>
<th>Regimen(s)</th>
<th>Baseline CD4+ cells/μL, Log₁₀ copies of HIV RNA/mL</th>
<th>Follow-up Time</th>
<th>Response</th>
<th>Comments</th>
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<tr>
<td>NORA Trial, nested substudy within DART Abstract 506</td>
<td>Ugandan adults 72% women median age, 36 19% WHO stage IV CD4+ count &lt;200 cells/μL (n=600)</td>
<td>Zidovudine 300 mg/ lamivudine 150 mg, abacavir 300 mg bid</td>
<td>99 (median) 5.4 (mean)</td>
<td>48 weeks</td>
<td>HIV-1 RNA &lt;50 copies/mL at week 48: 62%</td>
<td>More subjects in the nevirapine arm died or developed WHO grade IV events: 17 (6%) vs 29 (10%) P = .06</td>
</tr>
<tr>
<td>Tshepo Study Abstract 507</td>
<td>Botswanan adults 69.4% female median age, 35.9 Stratified to CD4+ count &lt;201 or 201-350 cells/μL with plasma HIV-1 RNA &gt; 4.74 log₁₀ copies/mL (n=650)</td>
<td>Zidovudine/ didanosine + NNRTI or stavudine/lamivudine + NNRTI</td>
<td>199 (median) 5.3 (mean)</td>
<td>89.7 weeks (median)</td>
<td>Rates of virologic failure with genotypic resistance: 11%</td>
<td>Increase in CD4+ cells, percentage of patients with undetectable plasma HIV-1 RNA, death rates, and time to first treatment-modifying toxicity were equivalent in the 2 arms. Zidovudine/ didanosine-containing arms were suspended by the DSMB.</td>
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</table>

NNRTI indicates nonnucleoside analogue reverse transcriptase inhibitor; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; ACTG, AIDS Clinical Trials Group; CI, confidence interval; DOT, directly observed therapy; WHO, World Health Organization; nRTI nucleoside analogue reverse transcriptase inhibitor; DSMB, Data Safety Monitoring Board; qd, once-daily; bid, twice-daily.

Antiretroviral therapy within 180 days of seroconversion, and 32 subsequently stopped the therapy. Higher plasma HIV-1 RNA levels at seroconversion independently predicted initiation of early antiretroviral therapy. Viral load at 7 weeks post-treatment interruption was lower in the treatment interruption group than at 7 weeks post-seroconversion among patients who did not initiate treatment (−0.6 log₁₀ copies/mL; P < .001). This difference decreased over time, and at approximately 100 weeks the plasma HIV-1 RNA levels were indistinguishable between the 2 groups. There was no difference in the CD4+ count decline between the untreated patients and patients after treatment interruption.

Koegl and colleagues (Abstract 125LB) performed a similar analysis using data from 2 German cohorts of patients with primary HIV-1 infection who were or were not treated with early antiretroviral therapy (the Prime-DAG cohort and the Ac-DAG cohort, respectively) and found differences in both viral load and CD4+ count in treated versus untreated groups. Of the 200 cases of primary HIV-1 infection, 95.5% were men and 144 started antiretroviral therapy either before or during seroconversion. The median CD4+ cell count was lower and the median plasma HIV-1 RNA level was higher in the treatment group than in the non-treatment group: 453 versus 629 cells/μL (P = .001), and above 5.7 versus 5.38 log₁₀ copies/mL (P < .001), respectively. One hundred of the 144 patients who initiated treatment stopped antiretroviral therapy after a median time of 9.5 months (range, 2.1-28.7 months). Although the plasma HIV-1 RNA levels...
differed significantly between treated and untreated patients at 6 months (4.4 log_{10} copies/mL vs 5.0 log_{10} copies/mL; P = .01), they did not differ significantly at 12 months (4.58 vs 4.72 log_{10} copies/mL; P = ns). The absolute CD4+ counts were not significantly different at 6 or 12 months. However, 12 months after treatment cessation, the CD4+ count had increased by 60 cells/µL in the treated group, and 12 months after seroconversion, the CD4+ count had decreased by 86 cells/µL in the untreated group, a statistically significant difference (P = .01).

Seng and colleagues (Abstract 347) found no difference in CD4+ count trends in patients in the Agence Nationale de Recherches Sur le Sida (ANRS) PRIMO cohort with primary HIV-1 infection after interruption of potent antiretroviral therapy compared with patients in the ANRS SEROCO cohort with primary HIV-1 infection who never received antiretroviral therapy. The 170 patients included from the ANRS PRIMO cohort began antiretroviral therapy within 3 months of their HIV-1 diagnosis. All responded with plasma HIV-1 RNA levels below 2.7 log_{10} copies/mL within 6 months, continued treatment for at least 6 months (average duration, 19 months), and discontinued treatment for at least 3 months (average duration, 21 months). Mean CD4+ count at 56 months after treatment interruption in the PRIMO cohort (n = 170) and after HIV-1 infection in the SEROCO cohort (n = 123) were equal at 416 cells/µL (95% CI for PRIMO, 369-464; for SEROCO, 360-476). Transient antiretroviral therapy initiated during primary HIV-1 infection did not extend time spent off treatment or time to a CD4+ count of below 350 cells/µL.

In a case-controlled study of patients with primary HIV-1 infection who received potent antiretroviral therapy (Abstract 348), early antiretroviral treatment correlated with immunologic but not virologic outcomes. Eighty-nine patients with primary HIV-1 infection who received 3 months of antiretroviral therapy in the St. Mary’s cohort were matched for age, sex, HIV-1 risk group, year of estimated seroconversion, and seroconversion window interval, with 179 patients from the Concerted Action on Seroconversion to AIDS and Death in Europe (CASCADE) cohort. After adjusting for confounders, rate of CD4+ cell decline over the 3 years following seroconversion was greater in the untreated controls than in the treated case patients, with a mean decrease of 51 cells/µL per year in the case patients (95% CI; 32-69) and 77 cells/µL per year in the controls (95% CI; 65-89). The estimated hazard ratio (HR) for the combined events of antiretroviral treatment initiation and a CD4+ count below 350 cells/µL was 1.445 (95% CI, 1.020-2.054, P = .039) for the untreated controls compared with the treated cases. Plasma HIV-1 RNA levels did not show a statistically significant difference between the 2 groups either at 18 months or 2 years after seroconversion. It is unclear what effect, if any, the significantly longer follow-up time for the case patient group (2.45 years) compared with the controls (1.20 years, P < .001) had on this analysis.

The above studies indicate that early antiretroviral treatment in primary HIV-1 infection may have beneficial virologic and immunologic effects, though the results are inconsistent and limited by the studies’ observational nature. The question of whether early antiretroviral treatment in primary HIV-1 infection should become standard-of-care awaits results from randomized, controlled trials that are currently underway.

Antiretroviral Treatment Strategies
Lopinavir/Ritonavir Monotherapy as a De-escalation Strategy
In the OK and OK04 trials (Abstracts 513 and 638) patients on a regimen of lopinavir/ritonavir plus 2 nRTIs with plasma HIV-1 RNA levels below 1.7 log_{10} copies/mL for at least 6 months and no history of virologic failure on PIs were randomized to either continue treatment with lopinavir/ritonavir plus 2 nRTIs or to lopinavir/ritonavir monotherapy. Data from 121 participants in both open-label trials assigned to lopinavir/ritonavir monotherapy were analyzed to identify risk factors for loss of virologic suppression, defined as a plasma HIV-1 RNA level above 1.7 log_{10} copies/mL at 48 weeks (Abstract 513). Baseline CD4+ counts for the patients from the OK and OK04 trials were 662 cells/µL and 474 cells/µL, respectively, and baseline plasma HIV-1 RNA levels before receiving antiretrovirals were 5.1 log_{10} copies/mL in both trials. Of 121 patients, 15 had loss of virologic suppression by week 48, with a Kaplan-Meier probability of loss of virologic suppression of 12.7%. Independent factors associated with loss of virologic suppression were low adherence (HR, 6.3; 95% CI, 2.0-19.6; P = .002), lower baseline hemoglobin (HR per g/dL, 0.68; 95% CI, 0.50-0.92; P = .013) and nadir CD4+ count below 100 cells/µL (HR, 4.1; 95% CI, 1.3-13.5; P = .02).

Arribas and colleagues (Abstract 638) reported drug resistance outcomes from the OK04 trial. During the trial, genotype testing was performed on all plasma samples in which HIV-1 RNA was greater than 2.7 log_{10} copies/mL. Eleven subjects in the monotherapy arm and 4 subjects in the control arm qualified for genotype by these criteria (P = .07), and 2 isolates in the monotherapy arm and 1 in the control arm had major PI mutations as defined by the International AIDS Society-USA 2006 guidelines (V82A or M46I). The authors concluded that the incidence of PI resistance over the first 48 weeks of the OK04 study was low, and similar between the 2 treatment arms. They also noted that 5 of 11 patients with plasma HIV-1 RNA levels above 2.7 log_{10} copies/mL did not have resistance mutations, remained on monotherapy, and had re-suppressed their plasma HIV-1 RNA to less than 1.7 log_{10} copies/mL.

Campos and colleagues (Abstract 514) reported results from the M03-613 trial, which randomized 155 antiretroviral-naive patients with HIV-1 infection in a 2:1 ratio to: lopinavir/ritonavir plus zidovudine/lamivudine induction therapy for at least 24 weeks, followed by maintenance with lopinavir/ritonavir monotherapy after 3 consecutive months of plasma HIV-1 RNA levels below 1.7 log_{10} copies/mL, or efavirenz plus zidovudine/lamivudine. Eligible subjects had plasma HIV-1 RNA levels above 1000 copies/mL, were antiretroviral naive, and had no evidence of resistance to study drugs. At 96 weeks, 60% of the lopinavir/ritonavir mono-
therapy arm and 63% of the efavirenz plus zidovudine/lamivudine arm had plasma HIV-1 RNA levels below 1.7 log_{10} copies/mL. In subjects who deintensified to monotherapy, 32 had confirmed plasma HIV-1 RNA levels above 1.7 log_{10} copies/mL. Time to loss of virologic response to a level above 1.7 log_{10} HIV-1 RNA copies/mL was statistically significantly different between the lopinavir/ritonavir (approximately 60% maintaining response at 64 weeks) and efavirenz arms (approximately 90% maintaining response at 64 weeks; P < .0001). When the virologic threshold for time to loss of virologic response was raised to greater than 2.7 log_{10} HIV-1 RNA copies/mL, there was no statistically significant difference between the groups. Low adherence, defined as a subject reporting at least 1 missed lopinavir/ritonavir dose (P = .07), and baseline CD4+ cell count (P = .06) predicted virologic rebound in the monotherapy group.

**Treatment Interruptions**

In contrast to last year’s conference, few new antiretroviral therapy outcome data were presented on treatment interruption strategies. Some further analysis of complications arising from interruption is reviewed elsewhere in this issue (see “Complications of HIV Disease and Antiretroviral Therapy”), and selected trials on outcomes are below.

The Experienced (E)-184V study (Abstract 516) is a prospective, open-label study. Patients in whom lamivudine-containing antiretroviral regimens were failing and who had documented M184V mutations, CD4+ counts above 500 cells/µL, and plasma HIV-1 RNA levels above 1000 copies/mL, and who requested treatment interruption, were randomized to either treatment interruption or lamivudine 300 mg daily as monotherapy. Data were presented for 144 weeks and the 29 patients in each group did not differ in baseline parameters, including CD4+ count (566 cells/µL in the treatment interruption group vs 580 cells/µL in the lamivudine monotherapy group) or HIV-1 RNA level (3.7 log_{10} copies/mL in the treatment interruption group vs 3.8 log_{10} copies/mL in the lamivudine group). Time to immunologic or clinical failure (defined as CD4+ count below 350 cells/µL or Centers for Disease Control and Prevention (CDC) class B or C diagnosis) took a median of 20 weeks (interquartile range [IQR], 16-84 weeks) for the treatment interruption group and 84 weeks (IQR, 36-144 weeks) for the lamivudine monotherapy group. In analysis of variance (ANOVA), the treatment interruption group had greater declines in CD4+ count over time than the lamivudine monotherapy group (P = .0095), as did patients with baseline CD4+ counts of 700 cells/µL or lower compared with patients with baseline CD4+ counts of greater than 700 cells/µL (P < .0001). The plasma HIV-1 RNA trend did not differ statistically significantly over time between the 2 groups. By 144 weeks, 93% of patients in the treatment interruption group and 90% of patients in the lamivudine monotherapy group had discontinued the study. There were more grade III and IV adverse events in the treatment interruption group than in the lamivudine monotherapy group (8 vs 1; P = .012). Lamivudine monotherapy in this study of treatment-experienced patients led to persistently better clinical and virologic outcomes than complete treatment interruption.

Watts and colleagues (Abstract 751) examined the effects of treatment interruption after pregnancy in the Women and Infant Transmission Study (WITS) cohort. The 206 women included in the analysis were antiretroviral-naïve, had CD4+ counts above 350 cells/µL, and were similar in baseline characteristics to the larger WITS cohort. One hundred and forty-seven women continued antiretrovirals post-partum, and 59 stopped therapy. Those continuing therapy were slightly older (mean ages 27.7 years vs 25.9 years; P = .04) and a smaller percentage of them had CD4+ counts above 500 cells/µL (54.4% vs 71.2%; P = .03). Neither the slopes of the CD4+ cell count changes, plasma HIV-1 RNA levels, nor the number of class B events differed between the 2 groups during the first postpartum year. Although the data are reassuring that stopping antiretroviral therapy after delivery does not lead to a more rapid decline in CD4+ count, studies with larger sample sizes and longer follow-up periods are needed.

**Interleukin-7-based Therapies**

Interleukin-7 (IL-7) is a cytokine that induces T-cell development, homeostasis, thymopoiesis, and T-cell maturation. It has been shown to cause dose-dependent increases in T cells in patients undergoing chemotherapy for cancer.

In an evaluation the safety and biologic effects of recombinant human IL-7 in patients chronically infected with HIV-1 (Abstract 127), 6 patients with CD4+ counts between 100 and 400 cells/µL and plasma HIV-1 RNA levels below 1.7 log_{10} copies/mL received 8 subcutaneous injections of 3µg/kg IL-7 over 18 days. Plasma HIV-1 RNA levels remained below 1.7 log_{10} copies/mL for all patients and no biologic adverse effects above grade II were observed. The median CD4+ count increased from 210 cells/µL at baseline to 405 cells/µL at day 21 and remained above baseline at 300 cells/µL at week 12 (P < .01). An expansion of CD8+ cells expressing CD28 was also noted, suggesting that IL-7 may promote CD8+ cell maturation in vivo. The trial continues to examine the efficacy and safety of higher doses of IL-7.

Sereti and colleagues (Abstract 128) report the results of ACTG A5214, a randomized, placebo-controlled, double-blind phase I dose escalation study of IL-7. Sixteen participants (12 active, 4 placebo) with a median CD4+ count of 601 cells/µL and plasma HIV-1 RNA level of below 1.7 log_{10} copies/mL were stratified by plasma HIV-1 RNA into 1 of 2 groups, below 1.7 log_{10} copies/mL or 1.7 to 4.7 log_{10} copies/mL. They were then randomized in a 3-to-1 fashion to receive one dose of 3, 10, 30, or 60 µg/kg of IL-7 or placebo subcutaneously. Two dose-limiting toxicities were seen at the 60 µg/kg dose, therefore the maximum tolerated dose was set at 30 µg/kg. For the placebo group, no statistically significant increase in
within-subject change in CD4+ count from baseline was seen at day 1, 4, 14, or 28. For the IL-7 group, a statistically significant within-subject CD4+ count change from baseline occurred at day 1 (decrease of 426 cells/µL; \( P = 423 \)) and day 14 (increase of 186 cells/µL; \( P = .04 \)). At day 28, the within-subject CD4+ count in the IL-7 group had increased by 213 cells/µL, but this was not statistically significant.

**Antiretroviral Therapy in Resource-limited Settings**

One of the most exciting aspects of this year’s conference was the increasingly global nature of the event. Several plenary sessions and many abstracts presented data from resource-limited settings, and the level of commitment to HIV/AIDS treatment in resource-limited settings was inspiring. Results from selected studies in resource-limited settings are summarized in Table 3.

**Adult Treatment Outcomes in Large Cohorts**

Matthias Egger delivered a plenary talk (Abstract 62) entitled “Outcomes of Antiretroviral Therapy in Resource-limited Settings.” He pooled data from several sources, including the Antiretroviral Therapy Cohort Collaboration (ART-CC), a network of European and North American cohorts, and the International Epidemiological Databases to Evaluate AIDS (IeDEA), a collaborative effort to establish regional networks of treatment sites throughout Africa, Latin America, and Asia. Using data from 33,008 treatment-naïve patients across 42 countries and 176 treatment sites, Egger and colleagues determined that the median CD4+ count in selected countries at initiation of therapy ranged from 164 to 187 cells/µL in North America, 87 to 125 cells/µL in sub-Saharan Africa, and 53 to 206 cells/µL in Asia. Although the trend in median CD4+ count increased from 2001 to 2005 in sub-Saharan Africa, it remains significantly lower than in European and North American cohorts. The investigators found that all areas except for Western Europe used a combination of 2 nRTIs and a NNRTI as the most frequent first-line regimen. However, the number of possible first-line regimens used to treat 90% of antiretroviral-naïve patients is 59 in North America, 47 in Western Europe, and 3 in all regions of Africa and Asia.

In an analysis of loss to follow up, Egger presented data from the Antiretroviral Therapy in Lower Income Countries (ART-LINC) collaboration on 16 treatment programs in resource-limited settings, 12 of which use active tracing of patients. He included 5575 adult patients (46% women) initiating antiretroviral therapy with a median age of 35 years, and found that 4% of patients did not return after their first visit and 17% were lost to follow up after the first 6 months. The HR for loss to follow up increased in each calendar year since 2000. In 2001 and 2002 the HR was 2.77 (95% CI, 1.69-4.55) and in 2003 and 2004 it was 7.86 (95% CI, 4.71-13.1). Patients with CD4+ counts below 50 cells/µL were also more likely to be lost to follow up, highlighting the need for active follow up in the determination of mortality estimates. Curves for virologic response, defined as plasma HIV-1 RNA below 2.7 log₁₀ copies/mL, were developed using data from the Swiss HIV Cohort and the Gugulethu and Khayelitsha township cohorts in South Africa; responses were similar between the cohorts, as were the rates of virologic rebound. In contrast, rates of treatment change varied dramatically between the cohorts: at 24 months approximately 60% of the Swiss cohort had changed regimens, compared with approximately 35% of the South African cohorts. The majority of this difference was attributed to changes for toxicity and by patient request, not for treatment failure.

An analysis of the 4 sub-Saharan Africa cohorts in the IeDEA collaboration and the ART-CC data revealed that tuberculosis (TB) is currently the most common opportunistic infection in both resource-limited and industrialized settings, although the incidence of TB is much higher in sub-Saharan Africa (approximately 250 cases per 1000 person-years) than in Europe and North America (approximately 25 cases per 1000 person-years). Crude mortality at 4 years was approximately 15% in sub-Saharan Africa, compared with approximately 5% in North America and Europe. A rapid increase in cumulative mortality in the first few months of treatment in sub-Saharan Africa was not observed in the ART-CC cohort. Breaking down mortality rates within the first year of treatment by cohort, and adjusting for baseline age, sex, CD4+ cell count, year, and disease stage, a wide range of mortality was observed in the North American and European cohorts, some with rates of 4% to 6%, similar to those observed in sub-Saharan Africa. Egger concluded that many patients in resource-limited settings are starting antiretroviral therapy later than recommended, but that virologic and immunologic responses are similar across regions. He highlighted the problem of loss to follow up in programs, which likely leads to underestimates of mortality, and the need for continued monitoring in the setting of rapid scale-up in resource-limited settings. Finally, he noted that although mortality rates are higher, particularly in the first few months of treatment, it is possible to achieve mortality rates in resource-limited settings that are comparable with some cohorts in North America and Europe.

El-Sadr and colleagues (Abstract 534) presented data on 171,259 patients receiving care from International Center for AIDS Care and Treatment Programs (ICAP)-sponsored sites in 7 different African countries, 71,482 of whom initiated potent antiretroviral therapy between July 2004 and December 2006. The investigators noted wide variations in populations, treatment outcomes, loss to follow up, and mortality among patients enrolled at each treatment site. Of the 116,609 patients who received HIV care from October 2006 to December 2006, 15% were eligible for antiretroviral treatment. Initiation of therapy varied from site to site, with 47% of eligible patients initiating antiretroviral therapy within 3 months in South Africa compared with 100% of eligible patients in Rwanda. Baseline CD4+ counts were low and ranged from 104 to 198 cells/µL. Most adult patients initi-
iated treatment with stavudine/lamivudine/nevirapine, except in South Africa, where stavudine/lamivudine/efavirenz was the most common regimen. After 12 months on therapy, the average median CD4+ count was 291 cells/µL, and in the overall cohort 98% of adults and 93% of children remained on first-line regimens. The proportion of patients known to have died without active case-finding ranged from 5% to 6% when averaged by country, but some sites in Ethiopia and Mozambique reported that more than 15% of patients on antiretrovirals were known to have died. The proportion of patients lost to follow up without active case-finding ranged from 1% in Rwanda to 17% in Kenya.

The importance of active case-finding of patients who are lost to follow up was highlighted in a retrospective cohort study among 410 HIV-1-infected adults consecutively presenting to an urban clinic in Botswana (Abstract 537). Standard of care in the clinic was active case-finding for all patients lost to follow up and involved telephone calls and home visits if needed. Patient outcomes were retrospectively classified by passive case-finding, in which patients were classified as dead if their death was recorded in the clinic chart, and lost to follow up if their last clinic contact was more than 30 days past their last visit. Median duration of follow up was 44 weeks among the 410 patients initiating antiretroviral therapy during the study period. Passive case-finding classified 29 patients (7%) as dead and 68 of 410 patients (17%) as lost to follow up. Active case-finding classified 69 patients as dead (17%) and 22 (5%) as lost to follow up. The 52-week Kaplan-Meier survival estimates differed significantly: 0.93 (95% CI, 0.88-0.94) for passive and 0.79 (95% CI, 0.74-0.81) for active case-finding. Baseline CD4+ count below 100 cells/µL and male sex were independently associated with death in the first 12 months of antiretroviral therapy.

Nacenga and colleagues (Abstract 33) examined the effectiveness of efavirenz- and nevirapine-based antiretroviral regimens in a cohort of 2821 patients on antiretroviral therapy in 9 Southern African countries between January 1999 and March 2005. Mean age was 37 years and median follow-up time was 2.2 years. All patients initiated first-line antiretroviral treatment; 64.6% received efavirenz-based regimens (60% female) and 35.4% received nevirapine-based regimens (68% female). The baseline median CD4+ count was 146 cells/µL for the efavirenz group and 167 cells/µL for the nevirapine group, and plasma HIV-1 RNA level was above 5.0 log$_{10}$ copies/mL for 61% and 55% of the efavirenz and nevirapine groups, respectively. CD4+ count outcomes data were not presented, but in a multivariate analysis controlling for adherence and other baseline variables, the HR for time to virologic failure after initial suppression was 0.72 (95% CI, 0.59-0.88) for efavirenz compared with nevirapine. Low adherence based on pharmacy claims data, low baseline CD4+ count, and high baseline plasma HIV-1 RNA correlated with decreased time to virologic failure.

Determinants of mortality were evaluated in a cohort of 1120 antiretroviral-naive patients enrolled in a home-based treatment program in Uganda (Abstract 34). Median age was 38 years, 73% were female, and 39% were WHO stage III or IV when they initiated therapy. Median baseline CD4+ count and HIV-1 RNA level were 127 cells/µL and 5.3 log$_{10}$ copies/mL, respectively. Early mortality, defined as death occurring within 3 months of treatment initiation, was 16.4 per 100 person-years of observation in the cohort. Mortality decreased in each time period to a low of 1.3 per 100 person-years of observation at 18 to 24 months on treatment. Baseline factors associated with mortality were low CD4+ count, hemoglobin less than 10 g/dL, body mass index (BMI) less than 18 kg/m$^2$, and a history of prior TB. Adherence to antiretroviral treatment strongly correlated with mortality in reported adherence of less than 90% in the first 6 months (HR, 3.3; $P < .001$) and after the first 6 months (HR, 7.4; $P < .001$). The most common conditions associated with death in first 3 months on treatment were no diagnosis, TB, cryptococcal disease, and oropharyngeal candidiasis.

Van Custen and colleagues (Abstract 535) presented 5 years of data from one of the longest-running treatment cohorts in a resource-limited setting, the Khayelitsha township program in Cape Town, South Africa. A total of 2565 antiretroviral-naive patients initiated therapy between 2001 and mid-2006, 70% of whom were female. Median CD4+ count at treatment initiation increased over time from 44 cells/µL in 2001 to 2002, to 99 cells/µL in 2005. After 3 years of antiretroviral therapy, median CD4+ count was 422 cells/µL and plasma HIV-1 RNA was below 2.6 log$_{10}$ copies/mL in 80% of the patients. Mortality at 6 months after antiretroviral initiation decreased each year, and was 12.4% in 2001 and 5.4% in 2005. Determinants of mortality included an initial CD4+ count of less than 50 cells/µL, WHO stage IV disease, and a history of or current Kaposi’s sarcoma (KS). At 48 months on treatment 14% had started second-line therapy, and the proportion of patients remaining in care at 54 months (comparing mortality and loss to follow up) was 78%.

Two abstracts presented data on responses to second-line therapy in resource-limited settings from Médecins Sans Frontières-supported programs. The first, presented by Calmy and colleagues (Abstract 35), detailed outcomes from 22 countries in Africa, Asia, and Central America. Of more than 80,000 antiretroviral-naive patients first initiating antiretroviral therapy since 2001 at a Médecins Sans Frontières site, 354 (0.4%) had changed regimens. The median age of those switching was 35 years, 57% were female, and 87% were WHO stage III or IV at therapy initiation. Stavudine/lamivudine/nevirapine was the initial regimen for 91% of the patients. Of the second-line regimens, 47% were nelfinavir-based and 46% were lopinavir/ritonavir-based. The median CD4+ count at switch was 99 cells/µL and the median plasma HIV-1 RNA level was 4.64 log$_{10}$ copies/mL for those programs with virologic testing. The median increase in CD4+ count was 91 cells/µL at 6 months and 113 cells/µL at 12 months. Approximately 6% were lost to follow up at 5 months, and 7% of the patients had died by 6 months. Probabilities of survival within the cohort, including death and loss to follow up, were 0.91 at 6 months.
(IQR, 0.88-0.95 months) and 0.86 at 12 months (IQR, 0.82-0.91 months).

Ferradini and colleagues (Abstract 36LB) examined the efficacy of lopinavir/ritonavir-based second-line therapy at several Médecins Sans Frontières sites in Cambodia. One hundred and thirteen patients who had been followed up for at least 6 months on second-line therapy were identified; 50% were female and median age was 38 years. The decision to stop first-line therapy was based on immunologic and virologic criteria in 35 and 78 patients, respectively. Median CD4+ count at regimen switch was 70 cells/µL and median plasma HIV-1 RNA level was 4.7 log_{10} copies/ml. The most frequent second-line regimens were didanosine/lamivudine/lopinavir/ritonavir (n = 47) and didanosine/zidovudine/lopinavir/ritonavir (n = 21), and the median duration of second-line therapy at the time of the evaluation was 10.2 months. Median CD4+ count increase was 105 cells/µL at 6 months and 180 cells/µL at 12 months. Plasma HIV-1 RNA at evaluation was below 2.6 log_{10} copies/mL for 101 patients (89.4%), between 2.7 and 3 log_{10} copies/mL for 6 patients (5.3%), between 3 and 4 log_{10} copies/mL for 2 patients (2.7%), and more than 4 log_{10} copies/mL for 4 patients (3.5%). Genotypic analysis in all patients with plasma HIV-1 RNA above 2.7 log_{10} copies/mL did not reveal any protease mutations, but 100% had NNRTI resistance and 91.5% had an M184V mutation. Other than low CD4+ cell count at switch, there were no predictors of second-line therapy failure. The authors concluded that short-term outcomes of empiric, second-line lopinavir/ritonavir-based regimens were successful and that adherence, although not measured in this study, was a main determinant of second-line regimen failure.

**Treatment Outcomes for Children in Large Cohorts**

Kline and colleagues (Abstract 79) pooled database and medical record information for 11,926 children, including 5151 children receiving antiretroviral treatment through January 31, 2007, at 5 Baylor College of Medicine-supported sites in Botswana, Uganda, Lesotho, Swaziland, and Malawi. Mean age at antiretroviral initiation ranged among sites from 5.1 to 7.8 years, 50% were female, and 50% to 92% had WHO stage III or IV disease. The vast majority of children were on first-line antiretroviral regimens, with 50% having received zidovudine/lamivudine/nevirapine. In the Botswana clinic, the median CD4+ cell percentage at baseline was 15%, compared with 8 in Uganda. In Botswana (n = 880), the CD4+ cell percentage was 27 at 6 months, 30 at 12 months, and 32 at 36 months. In Uganda, the CD4+ cell percentage was 18 at 6 months, 23 at 12 months, and 26 at 24 months. Plasma HIV-1 RNA data were available in the Botswana clinic, with levels below 400 copies/mL in 79%, 81%, and 71% of patients at 6, 12, and 24 months, respectively. The crude mortality of the entire Botswana clinic population, both on and off antiretroviral therapy, decreased dramatically from 4.7% in 2004 to 0.3% in 2006. At 2.5 years of follow up the Botswana clinic had 10% of its patient population on second-line regimens and 93% still alive and on antiretroviral therapy.

Arrivé and colleagues (Abstract 727) presented data from 8 clinical centers participating in the KIDS-ART-LINC collaboration. Of 2142 children initiating antiretroviral therapy between 1 and 15 years of age, 53.5% were 5 to 15 years old, 16.5% were 36 to 59 months old, 20.9% were 12 to 35 months old, and 9.1% were less than 1 year old. Based on CD4+ cell percentage, 65.7% of the children met WHO criteria for severe immunodeficiency. PI-based regimens were used initially in 57.8% and NNRTI-based regimens in 37.0%. After 1 year of antiretroviral treatment without active case-finding, 4.4% were dead and 12.2% were lost to follow up. Estimated cumulative mortality was 5.5% (95% CI, 4.3-7.1) at 6 months and 6.5% (95% CI, 5.1-8.2) at 1 year. The probability of death varied dramatically by age group: 16.8% mortality (95% CI, 11.7-23.7) among children initiating antiretrovirals before 12 months of age compared with 4.0%, 1.8%, and 4.3% mortality for those initiating at 12 to 35 months, 36 to 59 months, and more than 60 months, respectively. Severe immunodeficiency and severe anemia were associated with increased risk of death.

Outcomes of 370 children receiving potent antiretrovirals with NNRTIs (48.6%) and NRTIs (51.4%) were compared in a clinic in Cape Town, South Africa (Abstract 728). The NNRTI group was 41% female compared with 53% female in the PI group (P = .02), and the median age was 54.3 months and 21.8 months in the NNRTI- and PI-based groups, respectively (P < .01). Duration on therapy was longer for the NNRTI group (3 years) than for the PI group (1.2 years; P < .001). Baseline CD4+ cell percentage was 13 and plasma HIV-1 RNA was 5.5 log_{10} copies/mL for both groups. At 24 months there was no difference between groups in median CD4+ percentage (26.3) and survival curves were superimposable. However, plasma HIV-1 RNA levels differed, with 43% of the NNRTI group achieving virologic suppression at 24 months compared with 60% of the PI group (P = .05), and median plasma HIV-1 RNA was higher in the NNRTI group than in the PI group (3.8 log_{10} copies/mL vs 2.6 log_{10} copies/mL, respectively, P = .05). The authors speculated that the effectiveness of NNRTI-based regimens could be impaired by drug-drug interactions, suboptimal dosing, or pre-existing resistance as a result of prior exposure through prevention of mother-to-child transmission (PMTCT) programs.

Kamya and colleagues (Abstract 732) examined predictors of long-term virologic failure in a prospective cohort of 250 children and 526 adults initiating first-line antiretroviral treatment in Uganda between April 2004 and June 2005. The adult population was 69% female, had a mean age of 57 years, and 88% had WHO stage III and IV disease. The children were 48% female, had a mean age of 9.2 years, and 98% had WHO stage III and IV disease. All initiated NNRTI-based regimens, with stavudine/lamivudine/nevirapine being the most common in adults (75%) and zidovudine/lamivudine/efavirenz the most common in children (55%). Median CD4+ cell count was 99 cells/µL in
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<td><strong>Efavirenz vs Nevirapine-based ART Regimens: Adherence and Virologic Outcomes</strong>&lt;br&gt;Abstract 33</td>
<td>9 countries in southern Africa, Private-sector Aid for AIDS Disease Management Program&lt;br&gt;Jan 1999-Mar 2003; median, 2.2 y</td>
<td>nRTI + efavirenz (64.6%) vs nRTI + nevirapine (35.4%)&lt;br&gt;(n=2821)</td>
<td>37.0 y, efavirenz: 60% female, nevirapine: 68% female, antiretroviral treatment-naive</td>
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<td><strong>Determinants of Mortality among HIV-infected Individuals Receiving Home-based ART in Rural Uganda</strong>&lt;br&gt;Abstract 34</td>
<td>Uganda, Global AIDS Program&lt;br&gt;Median, 2 y</td>
<td>Nevirapine/famivudine/stavudine (96%)&lt;br&gt;Efavirenz/famivudine/stavudine (4%)&lt;br&gt;(n=1120)</td>
<td>Median 38.0 y, 73% female, 39% WHO Stage III or IV, antiretroviral treatment-naive</td>
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<td><strong>Variability in Populations Enrolled and Their Outcomes in HIV Care and Treatment Programs Across Countries in Sub-Saharan Africa</strong>&lt;br&gt;Abstract 534</td>
<td>Ethiopia, Kenya, Mozambique, Nigeria, Rwanda, South Africa, Tanzania, ICAP-supported, PEPFAR-funded&lt;br&gt;Sep 2004-Jun 2006</td>
<td>Majority on stavudine/lamivudine/nevirapine, except South Africa: stavudine/lamivudine/efavirenz (86%)&lt;br&gt;(n=116,284 total; 71,482 on antiretrovirals)</td>
<td>58% female (of those on antiretrovirals), antiretroviral treatment-naive</td>
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<td><strong>Clinical Outcomes and Emerging Challenges after 5 Years of ART in a South African Township</strong>&lt;br&gt;Abstract 535</td>
<td>South Africa, government-sponsored program&lt;br&gt;2001-mid-2006</td>
<td>Stavudine/lamivudine/efavirenz (37%)&lt;br&gt;Stavudine/lamivudine/nevirapine (43%)&lt;br&gt;(n=2565)</td>
<td>Median 32 y, 70% female, 90% WHO Stage III and IV, antiretroviral treatment-naive</td>
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<td><strong>Field Effectiveness of HAART in HIV-infected Adults in West Africa: The Aconda/ISPED/EGPAP Program, Abidjan, Côte d’Ivoire 2004-06</strong>&lt;br&gt;Abstract 541</td>
<td>Côte d’Ivoire, PEPFAR-sponsored&lt;br&gt;Mar 2004-Aug 2006</td>
<td>Stavudine/lamivudine/nevirapine (50%)&lt;br&gt;Stavudine/lamivudine/nevirapine (22%)&lt;br&gt;Zidovudine/lamivudine/nevirapine (21%)&lt;br&gt;(n=7862)</td>
<td>34 y, 71% female, 77% WHO Stage III or IV, antiretroviral treatment-naive</td>
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<tr>
<td><strong>Catalyzing the Care and Treatment of HIV-infected Children in Sub-Saharan Africa: Early Outcomes from 5 Baylor College of Medicine Centers</strong>&lt;br&gt;Abstract 79</td>
<td>Botswana, Uganda, Lesotho, Swaziland, and Malawi, joint Baylor, CDC, industry foundations, and government-sponsored&lt;br&gt;Dec 2001-Jan 2007</td>
<td>First-line therapy with zidovudine/lamivudine/nevirapine&lt;br&gt;(n=5151)</td>
<td>5.1-7.8 y (mean), 50% female, 50%-92% with WHO Stage III or IV, antiretroviral treatment-naive</td>
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<tr>
<td><strong>Outcomes of Adults Receiving Second-line ART in Médecins Sans Frontières-supported Projects in Resource-limited Countries</strong>&lt;br&gt;Abstract 35</td>
<td>22 countries in Africa, Asia, and Central America, MSF-supported programs&lt;br&gt;Median time from antiretroviral initiation to switch 20 mos, median follow-up post-switch was 7 mos</td>
<td>91% had first-line stavudine/lamivudine/nevirapine&lt;br&gt;47% had second-line nelfinavir-based, 46% had second-line lopinavir/ritonavir-based&lt;br&gt;59% had didanosine-containing&lt;br&gt;(n=352, 0.4% of adults initiating antiretroviral treatment with MSF since 2001)</td>
<td>Median, 35 y, 57% female, at first-line antiretroviral initiation: 87% WHO Stage III or IV, included only patients entering program who were antiretroviral treatment-naive</td>
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BMI indicates body mass index; CDC, Centers for Disease Control and Prevention; HAART, highly active antiretroviral therapy; Hgb, hemoglobin; ICAP, International Center for AIDS Care and Treatment Programs; MSF, Médecins Sans Frontières; n.a., not available; PEP-
<table>
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<tr>
<th>Baseline CD4+ cells/μL</th>
<th>CD4+ cells/μL Response</th>
<th>Response in Plasma HIV RNA copies/mL</th>
<th>Mortality</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Efavirenz 146 (mean)  
Nevirapine 167 (mean)  
>5.0 efavirenz: 61%  
>5.0 nevirapine: 55% | Data not presented | In multivariate analysis controlling for adherence, hazard ratio for time to virologic failure after initial suppression was 0.72 (0.59-0.88) for efavirenz vs nevirapine | Data not presented | Low adherence, low baseline CD4+ count, and high baseline plasma HIV RNA levels correlate with decreased time to virologic failure  
Results could be due to efavirenz superiority or unmeasured confounders |
| 127 (median)  
5.3 (median) | Data not presented | Data not presented | Defined early mortality as ≤ 3 mos, 16.4 per 100 person-years  
Mortality decreased in each time period to 1.3 per 100 person-years from 18-24 mos | Significantly more deaths from wasting in 0-3 mos  
Most common conditions contributing to death in first 3 mos were: no diagnosis, TB, cryptococcal disease, op/candida  
Baseline factors associated with mortality: CD4+ count 50-199 cells/μL, Hgb < 10g/dL, BMI < 18 kg/m², prior Tb, and depression index score |
| 104-198 | At 6 mos: 246 (range, 223-290)  
At 12 mos: 291 (range, 277-305) | No data available | Mortality varied from 5% in Rwanda, Mozambique, and Tanzania to 15% in individual sites in Mozambique and Ethiopia (without active case finding) | The proportion of patients lost to follow up, without active case-finding, ranged from 1% in Rwanda to 17% in Kenya  
98% of adult and 93% of children remained on first-line regimens |
| 44 (median, 2001-2002)  
99 (median, 2005) | At 3 years: 422 (median) | At 3 years: 80% with <400 | Mortality at 6 mos (year of antiretroviral start):  
12.4% (2001)  
5.4% (2005)  
Proportion remaining in care at 54 mos: 78% | Determinants of mortality: initial CD4+ count < 50 cells/μL, WHO stage IV disease, Kaposi's sarcoma at any point  
14% started second-line therapy by 48 mos |
| 116 (median) | At 6 mos: +136  
At 12 mos: +163  
At 18 mos: +213 (in those still on treatment) | No data available | 12-mo survival probability (baseline CD4+ count):  
77% (≤ 50 cells/μL)  
86% (51-100 cells/μL)  
89% (101-150 cells/μL)  
93% (>150 cells/μL) | Lost to follow up at 12 mos: 19%  
Factors associated with mortality: baseline CD4+ count <50 cells/μL, male sex, older age, low baseline Hgb, baseline BMI <18.5 kg/m², baseline WHO stage III or IV, care center |
| Botswana: CD4+ percentage: 15  
0%<400 copies/mL  
Uganda: CD4+ percentage: 8  
HIV RNA n.a. | Botswana: (n=880)  
at 6 mos: +27%  
at 12 mos: +30%  
at 36 mos: +32%  
Uganda: at 6 mos: +18%  
at 12 mos: +23%  
at 24 mos: +26% | Botswana: at 6 mos: 79% <400  
at 12 mos: 81% <400  
at 36 mos: 71% <400 | Botswana: crude mortality of entire clinic (on and off antiretrovirals):  
2003: 4.7%  
2004: 2.1%  
2005: 1.1%  
2006: 0.3% | Botswana at 2.5 y:  
10% switched to second or third-line treatment  
93% alive and on treatment |
| At first-line antiretroviral initiation:  
63 (median), and at antiretroviral switch: 99 (median) | At 6 mos: + 91 (median) | No data available | At 6 mos: ~7% died  
Probabilities of survival (death plus lost to follow-up):  
At 6 mos: 0.91  
At 12 mos: 0.86 | At 5.0 mos: 6% lost to follow up  
Factors associated with progression to death: CD4+ nadir <50 cells/μL  
Authors' speculation: low rate of second-line therapy use may be due to lack of availability, provider hesitation due to clinical status |

FAR, United States President’s Emergency Plan for AIDS Relief; TB, Tuberculosis; WHO, World Health Organization. *CD4+ counts and HIV RNA are in units stated unless indicated otherwise.
adults and 272 cells/µL (CD4+ cell percentage, 8.6%) in children; mean plasma HIV-1 RNA level at baseline was 5.3 log$_{10}$ copies/mL in both children and adults. Nevirapine or zidovudine had been used for PACTCT in 21 of the adults and 9 children. Children were almost twice as likely as adults to have plasma HIV-1 RNA above 2.6 log$_{10}$ copies/mL (26% vs 14%, respectively; \( P = .0001 \)). Predictors of virologic failure in children were male sex (odds ratio [OR], 2.54; 95% CI, 1.18-5.57), CD4+ percentage below 5 (OR, 3.99; 95% CI, 1.75-9.07), and initial antiretroviral regimen of stavudine/lamivudine/nevirapine versus zidovudine/lamivudine/efavirenz (OR, 3.33; 95% CI, 1.51-7.36). The same initial antiretroviral regimen predicted failure in adults.

Nkengasong and colleagues (Abstract 729) analyzed data from 134 children receiving their first antiretroviral treatment regimen between August 1998 and September 2005 in Côte d’Ivoire. At baseline, the median age was 7 years, 80% of the children had a CD4+ percentage of below 15, and the median plasma HIV-1 RNA level was 5.6 log$_{10}$ copies/mL. After 1 year of antiretroviral therapy, 54% of children had an undetectable plasma HIV-1 RNA and cumulative probability of developing any class of drug resistance was 0.44 (95% CI, 0.35-0.53). The magnitude of the virologic response was associated with emergence of drug resistance, as were smaller increases in CD4+ count from baseline, and dual-drug regimens.

**Adherence in Resource-limited Settings**

Although the need for strict adherence to antiretroviral medications is well documented in the literature, factors affecting antiretroviral treatment discontinuation and modification in resource-limited settings are not well described. Three abstracts took different approaches to adherence evaluation in Africa.

Kiguba and colleagues (Abstract 530) conducted a cross-sectional study of 686 individuals on antiretroviral therapy in 2 treatment centers in Kampala, Uganda. The median age of participants was 36 years, median CD4+ count was 175 cells/µL, and 70% were female. The majority of the patients (83.8%) were receiving NNRTI-based regimens. Adherence was assessed by self-report using semistructured quantitative and unstructured qualitative interviews. Ninety-four patients (13.7%) had at least 1 episode of discontinuation (defined as simultaneous discontinuation of all antiretroviral drugs for at least 1 month). There were 175 patients (25.5%) who reported modifying therapy by changing or switching at least 1 antiretroviral medication. The most common reason for discontinuation was drug cost (43%), and for modification was avoidance of adverse events (71.8%). In a multivariate logistic regression analysis adjusting for baseline parameters, factors associated with discontinuation were duration of antiretrovirals of less than 1 year (OR, 11.11; 95% CI, 5.00-25.00), year of initiation between 2004 or earlier (OR, 4.42; 95% CI, 1.90-10.47), prior antiretroviral experience (OR, 3.70; 95% CI, 2.13-6.25), history of hospitalization (OR, 2.36; 95% CI, 1.32-4.20), and use of alternative medicines (OR, 2.18; 95% CI, 1.06-4.47).

Mosoko and colleagues (Abstract 536) used a retrospective cohort analysis to examine adherence to antiretroviral therapy and loss to follow up during 2 time periods in Limbe, Cameroon. The investigators compared data for patients before and after October 2004, when the government reduced the cost of antiretrovirals from approximately US $30 per month to US $6 per month. The annual gross domestic product per capita in Cameroon is US $2400 (2006 estimate), but the population served by the clinic is highly economically disadvantaged and costs for antiretroviral therapy remain a large portion of household income even after the price reduction. A total of 2920 patients were included in the study; the median age was 35 years, 62% were female, and the median follow-up time was 6.2 months. Some 55.7% had “good” access to the clinic, defined as living less than 40 kilometers away. Probability of remaining alive and in care at 15 months without active case-finding was not statistically significantly different between the 2 time periods (HR, 1.1; 95% CI, 1.0-1.2). Multivariate analysis revealed several factors associated with remaining in care, including female sex (HR, 1.2; 95% CI, 1.1-1.3; \( P = .003 \)), good access to the clinic (HR, 1.5; 95% CI, 1.3-1.8; \( P < .001 \)), and treatment paid by a funding program or employer (HR, 3.6; 95% CI, 2.2-6.0; \( P < .001 \)). The investigators found that mean enrollment per month increased significantly with the decrease in antiretroviral cost: 46.5 persons enrolled per month in the first time period and 95.5 persons per month in the second (\( P < .001 \)). Nachega and colleagues (Abstract 548) performed an analysis of direct health care costs associated with patient demographic characteristics, baseline CD4+ cell count, and level of antiretroviral adherence as determined by pharmacy claims data in 5455 HIV-1-infected adults initiating NNRTI-based antiretroviral therapy in southern Africa between 1998 and 2003. The cohort had a mean age of 37.1 years, and was 59.6% female. Mean baseline CD4+ count was 152.6 cells/µL and the mean follow-up period was 27.3 months. The investigators noted a statistically significant dose-response relationship between nonantiretroviral therapy-related health expenditures (eg, consultations, hospitalizations, investigations, and medications other than antiretrovirals) and adherence. In those patients whose adherence rate was 95% or higher, nonantiretroviral therapy costs were US $152 per month (95% CI, 146-157), and in those with lower than 50% adherence, costs were US $200 per month (95% CI, 189-211). Total monthly health care costs decreased with time on antiretroviral therapy by approximately US $9 per month. Poor adherence, low baseline...
CD4+ cell count, older age, and black race were all associated with higher total expenditures.

**Laboratory Monitoring in Resource-limited Settings**

Plasma HIV-1 RNA measurements are frequently unavailable in resource-limited countries and low-cost surrogates for virologic response would be a great benefit to monitoring antiretroviral therapy in these settings. In an attempt to predict virologic failure with CD4+ count change, Wood and colleagues (Abstract 538) examined a cohort of 161 patients receiving antiretroviral therapy in Capetown, South Africa, who had experienced at least 1 episode of virologic failure, defined as a single episode of plasma HIV-1 RNA above 5.0 log_{10} copies/mL following a first plasma HIV-1 RNA of below 2.6 log_{10} copies/mL. Risk of virologic failure was independently associated with baseline CD4+ count (relative risk [RR] 2.48; 95% CI, 1.07-5.74, \( P = .04 \)) and with CD4+ count increases of less than 100 cells/μL during follow up (RR, 2.54; 95% CI, 1.01-6.43, \( P = .03 \)). However, a negative CD4+ count slope in values 3 to 2 months prior to failure had a sensitivity for detecting virologic failure of only 55.3% (95% CI, 47.2-63.1), a specificity of 61.5% (95% CI, 59.6-63.3), a positive predictive value of 7.8% (95% CI, 6.3-9.5), and a negative predictive value of 95.9% (95% CI, 94.8-96.8) indicating that change or negative slope in CD4+ cell count was a poor predictor of virologic failure.

Meya and colleagues (Abstract 673) expanded on the above strategy in a cross-sectional study of 496 patients in Uganda on NNRTI-based antiretroviral regimens. They searched for treatment, adherence, clinical, and laboratory parameters that could predict virologic failure, defined as a plasma HIV-1 RNA level above 2.6 log_{10} copies/mL. The cohort was 65% female and had a median age of 38 years, and 63% were on a nevirapine-based regimen. One hundred and seventeen patients had plasma HIV-1 RNA above 2.6 log_{10} copies/mL. Predictors of virologic failure in multivariate analysis were CD4+ count below baseline with a fall of greater than 30% from the peak value achieved on antiretroviral treatment (OR, 3.7; 95% CI, 1.4-9.4, \( P = .007 \)) and any treatment interruption of more than 2 days (OR, 5.6; 95% CI, 1.7-7.4, \( P = .001 \)). A failure score was then developed using these 2 parameters with 1 point for each parameter. If both parameters were absent (score = 0) the test had a sensitivity of 57% (95% CI, 42-71), specificity of 81% (95% CI, 77-84), a positive predictive value of 25% (95% CI, 17-34), and negative predictive value of 95% (95% CI, 91-96) for predicting plasma HIV-1 RNA below 2.6 log_{10} copies/mL. Applying this monitoring algorithm to the cohort, 112 patients would have had a failure score of 1 or 2 and been assigned to virologic testing, and 384 would have been assigned as score of 0 and not tested, resulting in 21 missed failures.

Iqbal and colleagues (Abstract 673) compared the performance of a non-nucleic acid-based plasma HIV-1 RNA assay to a gold standard nucleic acid-based assay on 121 plasma specimens from 107 HIV-1 subtype C-infected patients. The sensitivity of the non-nucleic acid-based assay to detect HIV-1 RNA below 2.6 log_{10} copies/mL (equivalent to <5500 copies/mL) was 100%. The difference seen in the non-nucleic acid-based assay compared with standard polymerase chain reaction (PCR) was a decrease of 0.23 log_{10} copies/mL (95% CI, -0.91-0.45). This study suggested that this less expensive and technically simpler method should be evaluated further as a substitute for current plasma HIV-1 RNA tests.

Waters and colleagues (Abstract 674) conducted an analysis of filter paper transfer of whole blood and plasma samples from resource-limited settings to a European laboratory for plasma HIV-1 RNA determinations in patients on antiretroviral therapy. Blood samples from 402 patients in Uganda underwent local testing using a standard reverse transcriptase PCR-based assay. Blood droplets were simultaneously transferred to filter paper that was sent to Holland every 3 weeks for RNA extraction and testing using a real-time reverse transcriptase PCR assay with a lower limit of detection of 2.7 log_{10} copies/mL. Using the local testing as the gold standard, the whole-blood filter-paper transfer assay had a sensitivity of 86% (99% CI, 67-100), a specificity of 77% (99% CI, 69-85), a negative predictive value of 27% (99% CI, 14-40), and a positive predictive value of 98% (99% CI, 95-100). The plasma filter paper transfer assay showed improved performance, with a sensitivity of 100% (99% CI, 84-100), specificity of 99% (99% CI, 97-100), negative predictive value of 95% (99% CI, 77-99) and positive predictive value of 100% (99% CI, 98-100) compared with the local gold standard. Filter paper transfer of plasma specimens could be a reliable means of virologic testing in resource-limited settings. Whole-blood filter-paper transfer testing was limited by a high number of false-positive results.

Dried blood spots are used in resource-limited settings as an alternative to plasma for drug resistance testing. Masciotra and colleagues (Abstract 629) evaluated the level of concordance between resistance detected in plasma versus dried blood spot. Specimens from 60 patients infected with HIV-1 subtype B virus were collected, and successfully RNA genotyped in 50 patients. There was good correlation between plasma and dried blood spot identification of resistance mutations with dried blood spot detecting 97% (306 of 316) of mutations identified in plasma, and plasma detecting 95% (306 of 322) of mutations identified in dried blood spot. A majority of discrepancies were secondary to mixtures containing minor protease position alterations and unusual amino acids substitutions in reverse transcriptase. Only 2 major mutations were absent in dried blood spots (M46L and K103N). In this small study, dried blood spots appeared to be a feasible and reliable alternative to plasma for resistance mutation detection in resource-limited settings (see also).
Prevention of Maternal-to-child Transmission

Butlers presented an overview regarding the status of PMTCT and the reasons it continues to be inadequate in resource-limited settings (Abstract 11). Rates of maternal-to-child transmission (MTCT) of HIV without intervention are 15% to 45% but can be reduced to 1% using antiretroviral prophylaxis, caesarian delivery, and avoidance of breastfeeding. In industrialized nations, these interventions have been successful at reducing rates of MTCT to around 2% or less but in resource-limited settings, particularly sub-Saharan Africa, rates of MTCT remain close to rates without intervention. According to United Nations International Children’s Emergency Fund (UNICEF) global estimates for 2005, there were 21 million HIV-seropositive pregnant women worldwide, of whom only 22% were identified as infected during pregnancy or delivery; 10% of mothers received antiretroviral prophylaxis and 8% of HIV-exposed infants received antiretroviral prophylaxis. Butlers suggested that PMTCT continues to fail in resource-limited settings. Lack of resources is a key limitation to successful PMTCT, and includes inadequate antenatal care infrastructures, limited availability of rapid HIV test kits and antiretroviral medications, a disconnection between PMTCT programs that identify HIV-seropositive pregnant women and their families and programs that provide long-term HIV care and antiretroviral therapy, and competing health priorities in the face of decreasing health care resources. Lack of access to clean water and good alternatives to breastfeeding counteract decreased rates of HIV transmission in resource-limited settings due to increased rates of mortality in formula-fed infants (see “HIV Epidemiology and Prevention Interventions” in this issue). Butlers concluded that the United Nations goal to decrease new HIV infections globally by 50% can be met but only by radically increasing access to and implementation of PMTCT. He suggested that full integration of maternal and child health care, incorporation of non-professional trained birth attendants into PMTCT programs, recognition of PMTCT as a crucial entry point to care for HIV-seropositive women and their families, and improvement of linkages between PMTCT and long-term antiretroviral services are key strategies to decrease rates of MTCT. The following abstracts reported on current efforts and barriers to decreasing MTCT.

Antiretroviral Prophylaxis for Prevention of Mother-to-Child Transmission Among Women Ineligible for Antiretroviral Treatment

In the Drug Resource Enhancement against AIDS and Malnutrition (DREAM) protocol, all HIV-seropositive pregnant women received potent antiretroviral therapy predelivery and postpartum regardless of virologic and immunologic status. In Abstract 747, outcomes of 341 HIV-seropositive women in Mozambique from the DREAM study who received triple-antiretroviral prophylaxis for PMTCT with either zidovudine/lamivudine/nevirapine or stavudine/lamivudine/nevirapine at 25 weeks gestation through 6 months postpartum were presented. At baseline, mean age, CD4+ count, viral load, and percent in clinical class WHO stage III to IV were 26 years, 422 cells/µL, 3.94 log₁₀ copies/mL, and 6, respectively. Median time of antiretroviral therapy predelivery was 87 days. At 1 month postpartum, 4 of 341 (1.2%) infants tested were HIV-seropositive and at 6 months an additional 2 of 251 (0.8%) infants tested were HIV seropositive (98 infants were not yet 6 months old and 8 infants were lost to follow up). Seven infants died, all of whom were HIV seronegative at 1 month (mortality rate 28.5% child-years). Risk of MTCT was not associated with baseline CD4+ count or viral load. There was a trend of decreased transmission among women with longer predelivery exposure to antiretrovirals (129 days among nontransmitters vs 79 days among transmitters) but the difference was not statistically significant (P = .58).

In a cost-effectiveness analysis of the DREAM study, costs for infections averted and Disability Adjusted Life Years (DALY) saved were calculated according to the Joint United Nations Programme on HIV/AIDS (UNAIDS) guidelines for intervention evaluation (Abstract 762). Of 6175 pregnant women who received antenatal HIV testing, 1862 tested HIV seropositive; 1594 of these HIV-seropositive pregnant women entered the program. The majority of program costs were spent on laboratory analyses (30%), medications (24%), and personnel (21%). Infection rates at 1 month and 6 months were 5.8% and 1.5%, respectively, resulting in an estimated 481 averted infections. The efficacy of the intervention was calculated to be 68.53% avoided infections through 6 months postpartum, with a calculated cost of US $518 per infection averted and US $22 per DALY saved. Subtracting the cost of care for HIV-seropositive children (US $369 per HIV-seropositive child), cost per infection was US $149 per infection averted and US $6 per DALY saved. This study showed that PMTCT with potent antiretroviral prophylaxis is cost-effective. Additional benefits not reflected in this analysis include decreasing the number of orphans by increasing the life expectancy of the mother, supporting the health sector by training of local personnel, and decreasing stigma by improving the quality of life of HIV-seropositive adults and their families thereby facilitating other HIV and AIDS-related public health interventions.

Antiretroviral Treatment of HIV-seropositive Pregnant Women

Providing long-term potent antiretroviral therapy for eligible HIV-seropositive women is an important global public health initiative both for the women who require antiretroviral drugs and for PMTCT. The following studies evaluated outcomes among women who initiated antiretroviral therapy during pregnancy.

The DART study is a randomized trial of antiretroviral-monitoring strategies among adults with symptomatic HIV infection and CD4+ counts below 200 cells/µL in Uganda and Zimbabwe.
Outcomes of 221 pregnancies in 198 women were assessed over a median follow-up period of 2.4 years. Median CD4+ count was 11.5 cells/µL and 18% were WHO Stage IV at baseline. Most of the women were on a regimen of zidovudine/lamivudine plus tenofovir (70%), nevirapine (15%), or abacavir (4%). Among the 164 women with a known outcome there were 91 live births (55%), 11 stillbirths (7%), and 62 terminations (38%). No infants were diagnosed with HIV infection. Four of the women died and 3 infants had congenital abnormalities. This is the largest data set on in utero exposure to triple antiretroviral therapy in resource-limited settings, the only data set evaluating in utero exposure to tenofovir to date, and is reassuring given the absence of perinatal transmission detected and rates of congenital abnormalities similar to other studies. Analysis of maternal and infant outcomes is ongoing.

A prospective study of HIV-seropositive, antiretroviral-naïve (from prior single-dose nevirapine for PMTCT) pregnant women with CD4+ counts below 350 cells/µL was conducted at a prenatal care clinic in Mozambique (Abstract 756). Antiretroviral therapy with nevirapine/lamivudine plus zidovudine or stavudine was initiated 3 weeks after enrollment and continued until at least 6 months postpartum. Infants were given single-dose nevirapine within 48 hours of delivery. Of 163 women who enrolled in the study, 148 received antenatal antiretroviral therapy and 146 were followed up through delivery. These 146 women delivered 149 infants, of whom 17 died prior to HIV testing and 26 were not tested. Seven of 106 (6.6%) tested infants were HIV-1 seropositive. Maternal and infant characteristics, including maternal baseline CD4+ count, HIV-1 RNA viral load, duration of antiretroviral therapy, and feeding strategy did not correlate with perinatal transmission. This relatively high rate of perinatal transmission in the setting of potent antiretroviral therapy could have resulted from subtherapeutic concentrations of antiretroviral drugs in the setting of pregnancy and nursing or nonadherence, neither of which were assessed in this study.

**Suppression of HIV-1 RNA During Prevention of Mother-to-Child Transmission**

The European Collaborative Study is a cohort of HIV-1-infected pregnant women and their infants from 10 European countries (Abstract 758). An analysis of time to virologic suppression was conducted in 240 women from this cohort who had their initial diagnosis of HIV during pregnancy or documented non-receipt of prior antiretroviral therapy. All women received potent antiretroviral therapy; 156 (65%) initiated a PI-based regimen (80% nevirapinavir and 84 (35%) initiated a nevirapine-based regimen. Fifty-nine percent were black, 90% were born in Africa, and 64% were diagnosed with HIV during pregnancy. At time of delivery, 73% achieved virologic suppression. Antiretroviral regimen did not correlate with virologic suppression but time to undetectable HIV-1 RNA was faster among patients on a nevirapine-based regimen (HR, 1.54), who had a country of origin in West Africa (HR, 1.90), and whose baseline HIV-1 RNA was below 3.81 log₁₀ copies/mL (HR, 2.76). Among women with baseline CD4+ count below 250 cells/µL, 82.4% on nevirapine-based regimens achieved virologic suppression at 8.5 weeks compared with 50.4% on PI-based regimens. Faster time to virologic suppression may have been due to the suboptimal efficacy of non-boosted PI nelfinavir pharmacokinetics during pregnancy leading to subtherapeutic levels, or nonadherence in the PI group, neither of which were assessed. Faster virologic suppression among women from West Africa may have been the result of differences in HIV-1 subtype or host biologic or genetic differences.

In a retrospective cohort of 114 women and infant pairs exposed to potent antiretroviral therapy in Vancouver, British Columbia (Abstract 759), 80% of women had achieved virologic suppression at time of delivery with no difference in probability of suppression between women on a PI-based regimen (n = 57) versus an NNRTI-based regimen (n = 34); 1 woman on a salvage regimen was not included. Women who had prior history of antiretroviral therapy had a longer time to achieving virologic suppression than did women with no prior history of antiretroviral therapy (58 vs 34 days, respectively). Adherence based on pharmacy records correlated with proportion of patients achieving virologic suppression: 90.2% of patients who had virologic suppression had “excellent adherence” compared with 54.5% of patients who did not achieve virologic suppression.

**Antiretroviral Prevention of Mother-to-Child Transmission: Effects on Resistance in Mothers and Infants**

Coffie and colleagues (Abstract 93LB) presented a prospective cohort study of 247 women in Côte d’Ivoire with at least 1 prior pregnancy who initiated antiretroviral treatment with stavudine or zidovudine plus lamivudine plus nevirapine or efavirenz. Virologic and immunologic responses were evaluated 12 months after initiation. Eighty-six women had previous exposure to single-dose nevirapine and short-course zidovudine with lamivudine for PMTCT, 52 had previous exposure to single-dose nevirapine and short-course zidovudine, and 109 women had no history of antiretroviral exposure for PMTCT. Of women who received nevirapine plus zidovudine/lamivudine, 11 of 73 had baseline resistance to lamivudine (15.1%) and 3 of 70 (4.3%) had baseline resistance to nevirapine. Sixteen of 42 (38.1%) in the nevirapine plus zidovudine group had baseline resistance to nevirapine. Neither group had evidence of zidovudine resistance. The overall rate of virologic failure (HIV-1 RNA > 500 copies/mL) at 12 months was 19% (42 of 219). Fifty percent of women with baseline lamivudine resistance had failure, compared with 18.9% of women exposed to lamivudine but with no evidence of baseline resistance. Among women with baseline nevirapine resistance 27.8% had failure, compared with 18.6% among women exposed to nevirapine but with no evidence of baseline nevirapine resistance. Women with no history of
antiretroviral exposure for PMTCT had a 16% rate of virologic failure. In multivariate analysis, baseline CD4+ count below 200 cells/µL, baseline lamivudine resistance and, especially, poor adherence were associated with virologic failure with ORs of 0.34, 6.86, and 12.68, respectively. PMTCT-acquired lamivudine resistance was associated with poorer 12-month virologic outcomes. However, time of initiation and duration of antiretroviral therapy may have confounded the outcomes in the lamivudine group, as time to initiation of antiretroviral therapy post-PMTCT was shorter among the lamivudine-exposed group than in women who received PMTCT prophylaxis without lamivudine (median 15 months vs 28 months), and time of exposure to antiretrovirals during PMTCT prophylaxis was longer in the lamivudine-exposed group (median 56 days vs 30 days).

A study of infants born to HIV-seropositive women in Mozambique (Abstract 99) compared rates of NNRTI resistance among infants infected in utero (HIV-1-seropositive by PCR at birth up to 2 weeks postpartum) with rates among infants infected intrapartum or early postpartum (HIV-1-PCR-positive 2-8 weeks postpartum). Standard of care for PMTCT in this setting is initiation of potent antiretroviral therapy in all HIV-seropositive pregnant women with CD4+ counts below 350 cells/µL and short-course zidovudine at 34 weeks gestation followed by single-dose nevirapine in the mother at labor and in the infant at birth for HIV-seropositive pregnant women with CD4+ counts above 350 cells/µL. Data for 330 infants who have enrolled thus far were presented. Twenty-two of 330 infants (6.7%) were infected in utero (7 have died, 8 are lost to follow up, and 7 continue follow up), 14 of 199 infants (7.0%) were infected peripartum (excluding infants who were HIV-1 seronegative at birth but status at 8-week follow up was unknown) and in 131 of 330, HIV status at birth was unknown. Six-month mortality of infants infected in utero and intra-peripartum was 6 of 16 (37.5%) and 0 of 6 (0%), respectively. Oligonucleotide ligation assay specific for detecting the NNRTI mutations K103N, Y181C, and G190A was conducted in samples from 29 infants. Four of 16 infants infected in utero and 4 of 13 infants infected intra-peripartum had evidence of nevirapine resistance. The 2 infants infected in utero who had wild-type virus received single-dose nevirapine at birth but their mothers did not receive nevirapine. Infants infected in utero who had resistance mutations had a mixture of mutant and wild-type virus, whereas infants infected intra-peripartum had either 100% resistant virus or 100% wild type. The dichotomy of virus (all resistant or all wild type) among infants infected intra-peripartum might result in persistent nevirapine resistance among infants infected with mutant strains. In contrast, the mixture of resistant and wild-type virus in infants infected in utero could result in a better chance of reversion to wild type. The high mortality associated with in utero infection indicates a need for earlier treatment. However, the high rates of nevirapine resistance in this group would require a non-nevirapine-based regimen. This study is ongoing and will assess rates of MTCT through breastfeeding and whether resistance mutations fade over time.

Antiretroviral drug resistance

**Transmitted Drug Resistance**

**Epidemiology of Transmitted Drug Resistance.** Rates of transmitted drug resistance (TDR) in Europe, Australia, and the United States range from 11% to 15% among patients with primary HIV-1 infection and 7% to 11% among patients with newly diagnosed HIV-1 in whom time of infection is unknown (Abstract 60). Data from 11 states in the US Variant, Atypical, and Resistant HIV Surveillance group (VARHS) were analyzed to estimate the prevalence of TDR and distribution of subtypes (Abstract 648). Specimens from 3130 antiretroviral-naive individuals, newly diagnosed with HIV-1 infection from March 2003 to October 2006 were analyzed, and 10.4% had virus with drug resistance mutations. Resistance to NNRTIs, nRTIs, and PIs were present in 6.9%, 3.6%, and 2.4%, respectively, and 1.9% had multi-class resistance. Predominant mutations were K103N for NNRTI (70.1%), M41L for nRTI (45.1%), and L90M for PIs (40.0%). Non subtype B or recombinant forms were found in 5.1% of patients.

Two large intervention studies that recruited HIV-seronegative MSM evaluated the prevalence of drug resistance among men who seroconverted during study participation. In the EXPLORE study (Abstract 650), men were randomized to a behavioral intervention to prevent HIV-1 acquisition. Two hundred and fifty-nine men seroconverted and 195 had genotyp-
ing results available for analysis. A total of 15.9% (31 of 195) had resistance mutations, 3.6% had multi-class resistance, and 5 men had CXCR4-tropic virus. In multivariate analysis, there was no association between resistance and demographic, clinical, or risk-factor data. In the gp120 vaccine efficacy trial (in which gp120 was found ineffective in prevention of HIV infection), 5095 HIV-seronegative MSM and 508 women at high risk for HIV were enrolled, and 362 men and 6 women seroconverted during the study (Abstract 653). Two hundred and eighty-six samples were available for sequencing, of which 16% had at least 1 resistance mutation and 7% had multi-class resistance. In this study, having an HIV-seropositive partner (OR, 4.6; 95% CI, 1.08-5.2, \( P = 0.03 \)), reporting unprotected anal sex (OR, 5.5; 95% CI, 1.3-14.9, \( P = 0.02 \)) and marijuana use (OR, 4.0; 95% CI, 1.02-4.2, \( P = 0.04 \)) were independently associated with TDR.

Nambiar and colleagues (Abstract 657) found that TDR among individuals with primary HIV-1 infection was associated with diagnosis of a sexually transmitted disease (STD) within 3 months of HIV diagnosis. The overall rate of TDR was 15% (28 of 185) and of the 124 individuals screened for an STD, 45% were diagnosed with an STD; 68% had TDR versus 31% with wild-type virus (\( P = 0.03 \)). The authors concluded that this correlation between TDR and presence of an STD could have resulted from facilitated transmission of less fit virus through mucosal breakdown in the setting of STDs or poor drug adherence among HIV-seropositive individuals in the sexual networks of people engaging in high-risk sexual behavior.

An analysis comparing cohorts of early acute, HIV-1-infected, antiretroviral-naive individuals in 2003 to 2004 and 2005 to 2006 in New York City showed a decrease in TDR from 24.1% (27 of 112) to 12% (13 of 108), \( P = 0.02 \) (Abstract 651). The reason for this decrease is unclear, although the authors hypothesize that better drug adherence with less viral breakthrough in the potential transmitter population may be a factor. Sentinel-site surveillance in STD clinics and HIV testing facilities in San Francisco (Abstract 652) showed that rates of TDR remained stable from 2004 to 2006 with an overall rate of 15.7% (55 of 402). Whether these trends continue and will be similar in other cohorts requires further evaluation.

**Natural History of Transmitted Drug Resistance.** In the setting of secondary drug resistance, it takes an average of 12 to 16 weeks for a population of predominantly mutant virus to convert to majority wild-type virus after removal of drug pressure. In the case of TDR, it takes an average of 3 years or more for reversion to wild type, as the mutant virus is not competing with existing wild-type strains. Evaluating a group of 14 acutely infected HIV-1-seropositive patients with TDR, Little and colleagues (Abstract 60) determined the mean time to first appearance of a wild-type and resistant mixture to be 103 weeks (about 2.0 years; 95% CI, 49-216 weeks). They subsequently evaluated mean time to last wild-type and resistant mixture (ie, no evidence of resistant virus) and found that 13 of 14 patients had pure resistant virus or persistence of mixture; time to complete reversion by population sequencing in the 1 patient that converted to wild-type virus was 2.7 years. These patients continue to be followed up, and at 4 years of follow up, many patients continue to have pure or mixed resistant virus. Mean replication capacity of the TDR virus was 87%, which was not statistically significantly different from reference wild-type virus. In this cohort, detection of wild-type virus after acquisition of TDR virus takes on average of 2 years and complete reversion to wild-type virus theoretically might never occur. The high fitness displayed by these TDR viruses may be due to selected transmission of more fit resistant variants.

Among patients enrolled in the Acute Infection and Early Disease Research Program (AIEDRP) DACS 003 study from June 1993 to January 2007, the rate of TDR among recently HIV-1-infected individuals was 10.2% (93 of 913) (Abstract 60). There was no statistically significant difference in baseline HIV-1 RNA level in patients with TDR compared with patients with susceptible virus, but when stratified by resistance class, individuals with NNRTI resistance had a baseline HIV-1 RNA level of 0.4 \( \log_{10} \) copies/mL higher than patients with susceptible virus (\( P = 0.005 \)). Patients with nRTI resistance had a mean baseline HIV-1 RNA level of 0.7 \( \log_{10} \) copies/mL lower than patients with susceptible virus (\( P = 0.001 \)). There was a trend toward lower baseline HIV-1 RNA level in patients with PI-resistance mutations than in patients with susceptible virus but the difference was not statistically significantly different. These differences in viral load continued for patients with NNRTI- and nRTI-resistance mutations at 1 and 3 years of follow up but the PI trend disappeared. The clinical significance of these differences in viral load is unclear and needs further confirmation in larger cohort studies.

Seven hundred and ninety-six patients with recent HIV infection were enrolled in AIEDRP DACS 002 from 1995 to 2006 and received antiretroviral treatment within 7 months of the estimated date of infection (Abstract 60). Of these patients 84 had TDR, and, compared with patients with susceptible virus, there was no statistically significant difference in time to reach HIV-1 RNA levels below 50 copies/mL. However, when evaluated by class, individuals with PI-resistance mutations had a longer time to reach HIV-1 RNA levels below 50 copies/mL than patients with susceptible virus (\( P = 0.002 \)). This was likely due to the fact that from 1996 to 2000, resistance testing was not routinely performed prior to antiretroviral therapy initiation and 50% of patients with baseline PI resistance were initiated on a PI-based regimen. Complete viral suppression failed to occur in 45% of patients (38 of 84), of whom 70% had fewer than 3 active antiretroviral medications in their regimen. The difference in time to suppression was likely related to whether antiretrovirals to which the virus is susceptible
were used. Given the relatively high rates of TDR in more developed settings, the authors emphasized the importance of performing baseline resistance testing on newly diagnosed, antiretroviral-naive patients in these areas, reaffirming current consensus recommendations.

**Low-frequency Resistance Variants.**

Each HIV-infected patient is infected with a strain of HIV that, over time, develops into a swarm of viruses containing different polymorphisms (ie, intra-patient viral diversity). Some of these polymorphisms are a result of the natural evolution of wild-type virus, and other polymorphisms represent resistance mutations that carry clinical significance. Conventional bulk resistance testing detects resistant variants that occur at a frequency greater than 20% above “background” wild-type polymorphisms. Low-frequency resistance variants are populations of resistant virus that occur at a frequency that is below the level detectable by bulk sequencing but above the background wild-type polymorphisms. Many studies have documented the existence of these low-frequency resistance variants among individuals with virologic failure but have no evidence of resistance based on bulk-resistance testing (Abstract 61). Johnson and colleagues (Abstracts 61, 639) used a real-time PCR detection assay to assess the prevalence of resistance among antiretroviral-naive patients who had no detectable resistance by conventional sequencing and to evaluate the level of baseline low-frequency resistance among antiretroviral-naive patients with known resistance by conventional sequencing. They found that 15% (30 of 205) of patients with wild-type virus by conventional sequencing had at least 1 major mutation detected by the more sensitive assay, 2% of whom had dual-class resistance. In a separate cohort of patients, 7% (21 of 302) of patients with known baseline resistance gained resistance to another drug class, based on real-time PCR testing. Rates of triple-class resistance doubled in this group to 5%.

Paredes and colleagues compared the detection of M184V and D30N resistance mutations using standard, population-based genotype-sequencing versus allele-specific PCR among 61 antiretroviral-naive pregnant women enrolled in the WITS. M184V was detected 1.5 times more frequently using allele-specific PCR than using standard genotype sequencing (13.3% vs 8.8%, respectively); D30N was detected 3 times more frequently in using allele-specific PCR than using standard genotype sequencing (6.7% vs 2.2%, respectively).

Each of these studies supports the conclusion that low-frequency resistance mutations are missed by conventional sequencing, but do these low-frequency resistant variants carry clinical significance, as has been suggested in previous studies? Peuchant and colleagues (Abstract 666) evaluated the effect of resistance detected by conventional and sensitive resistance testing on virologic and immunologic outcomes. Of 172 antiretroviral-naive, recent HIV-1 seroconverters, 9.3% had resistance to at least 1 class at baseline. Baseline resistance was related to a lower baseline HIV-1 RNA level (3.76 log₁₀ copies/mL vs 4.59 log₁₀ copies/mL for resistant and wild-type virus, respectively; \( P = .002 \)), higher baseline CD4+ counts (557 vs 425 cells/µL for resistant and wild-type virus, respectively; \( P = .03 \)) and a less steep decrease in viral load after 1 month of treatment. In a study of 78 patients, they found no effect of the presence of low-frequency resistant mutants on virologic or immunologic response to therapy. The authors were unable to conclude that detection of low-frequency resistant mutants correlated with virologic or immunologic outcomes but the study was limited by small sample size, potential selection bias, and the limited number of mutants that could be detected by the sensitive assay employed.

Johnson and colleagues (Abstracts 61, 639) conducted a retrospective analysis of antiretroviral-naive patients who participated in the treatment trials CNA 30021 and 30024 and received efavirenz and lamivudine with either abacavir or zidovudine. Of 316 patients, 95 had virologic failure (HIV-1 RNA level > 50 copies/mL) by 48 weeks, and 221 had suppressed viral loads (HIV-1 RNA level < 50 copies/mL) within 48 weeks. Using allele-specific PCR testing for K103N, Y181C, and M184V, 9 patients had low-frequency variants at baseline, 7 of whom (78%) had virologic failure. Five of 6 of the genotypes available at failure had the same mutations that were present at baseline. One patient who experienced failure within 2 months of treatment was found to have dual-class resistance (K103N, Y184V) at baseline. In logistic regression, presence of low-frequency variants at baseline was associated with virologic failure (OR, 11.0; 95% CI, 2.2-58.8, \( P = .004 \)), but baseline viral load and baseline CD4+ count were not associated with virologic failure (\( P = .43 \) and \( P = .30 \), respectively). The authors concluded that there are clinical consequences to harboring low-frequency resistant variants and advocated for baseline sensitive testing, especially among antiretroviral-naive individuals.

**Global Perspectives on Antiretroviral Resistance**

Schapiro (Abstract 59) presented an overview of the impact of HIV-1 subtype on drug resistance. A majority of drug development and resistance data have focused on HIV-1 subtype B, yet subtype B represents a minority of the infections worldwide (10%); other subtypes and circulating recombinant forms constitute the rest of infections worldwide. Furthermore, rates of non-subtype B infections are increasing in Europe and the United States (Abstracts 630, 648). Subtypes differ in genetic variability, which can lead to differences in response to antiretrovirals and development of drug resistance. Schapiro highlighted several examples of how this genetic variability before antiretroviral exposure can affect development of drug resistance mutations. Patients with subtype B who experience virologic failure in the context of nelfinavir treatment are more likely to develop the D30N PI resistance mutation than they are to develop the L90M mutation, whereas patients with subtype C are more likely to develop L90M.
Subtypes B and C differ in consensus sequence at position 89 (89L and 89M, respectively). When the D30N mutation is inserted into a virus with 89M, there is no viral replication, whereas insertion of L90M allows for a replication capacity of 78.9%. This implies that 89M strains, and therefore a majority of subtype-C virus strains, do not replicate successfully with D30N mutation and as a result the L90M mutation is found more commonly than D30N among subtype C patients in whom nelfinavir-containing treatment is failing.9 Subtype genetic variability can also affect resistance through different codons that code for the same amino acid at key resistance positions. At position 106, both subtype B and C consensus sequences are V106, however, at the nucleotide level, subtype B sequence is G1A whereas subtype C is GTG. The NNRTI resistance mutation V106M is encoded by ATG. In order for subtype B V106 (G1A) to convert to V106M (ATG) it would require a 2-step conversion through GTG. In contrast, subtype C requires only a 1-step conversion: GTG to ATG. The hypothesis that the preferred pathway of resistance in subtype C at V106 is V106M is strengthened by the observation that clinically, among patients in whom efavirenz is failing the V106M resistance mutation is found more frequently in those with subtype C virus (24%) than those patients with subtype B virus (0.3%).10

Subtype C and Drug Resistance. Wallis and colleagues (Abstract 661) evaluated resistance patterns in 115 patients from 2 clinics in Johannesburg, South Africa, in whom antiretroviral therapy was failing, 94% of whom had subtype C virus. They found that resistance patterns were similar to those found in patients with subtype B virus with the exception of higher rates of V106M, K65R, G19A, and P225H mutations. The impact of these resistance mutations on second-line treatment is unclear and the authors suggest that these findings should be confirmed in larger controlled studies.

The nRTI resistance mutation K65R is relatively rare in subtype B and causes decreased replication capacity that can be augmented or decreased in combination with other resistance mutations (Abstracts 591, 592). In areas where subtype C is prevalent, K65R is found at relatively higher rates11 and is selected more rapidly in culture by subtype C than other subtypes.12 Cousins and colleagues (Abstract 585) evaluated whether the effects of K65R mutation on reverse transcriptase in subtype C differ from its effects in subtype B. The authors found that subtype C recombinant virus with the K65R mutation had decreased replication capacity that is enhanced by M184V mutation and that M184V resensitizes K65R to tenofovir. These results are similar to what is found in subtype B.

Subtype G and Resistance. In Nigeria, subtype G and CRF 02-A/G are the predominant subtypes. Idigbe and colleagues (Abstract 641) evaluated the prevalence of resistance mutations among patients in Nigeria in whom a regimen of stavudine/lamivudine/nevirapine was failing. Of 125 patients, a majority were subtype G (43%) or CRF 02 (42%). Twenty-two (17.6%) were susceptible to all antiretrovirals, indicating likely non- or poor adherence as the cause of virologic failure, and 7 (5.6%) had resistance only to NNRTIs and 93 (74.4%) had resistance to NNRTI with TAMs or lamivudine resistance. The most common resistance mutations were M184V, Y181C, and K103N and there were no statistically significant differences in prevalence of resistance between treatment-experienced and treatment-naïve patients nor among subtypes. There did, however, appear to be a higher frequency of TAMs (K70R and D67N) in patients with subtype G than in those CRF 02. Patterns of resistance in subtype G virus are similar to those described in subtype B infection but there was a higher prevalence of TAMs in subtype G than in CRF 02.

Schapiro (Abstract 59) concluded that although it is clear that genetic variability between subtypes impacts resistance, the clinical significance of this impact depends on the drug and the subtype. Thus far, subtype does not appear to influence antiretroviral therapy success or failure and should not dictate a particular antiretroviral regimen. Resistance databases should continue to be expanded to include new information regarding resistance mutations in a variety of subtypes and algorithms should be updated accordingly. Furthermore, as new antiretroviral medications are developed, the potential effect of subtype variability on response to treatment should continue to be evaluated (Abstract 624).

Resistance in Resource-limited Settings. Marconi and colleagues (Abstract 94) presented rates of resistance after virologic failure among a cohort of patients in Durban, South Africa. One hundred and forty-one patients who had been on antiretroviral therapy for at least 24 weeks, were on their first regimen of potent antiretrovirals, or had history of prior mono- or dual-antiretroviral therapy, and who had virologic failure (defined as an HIV-1 RNA level >1000 copies/mL, failure to achieve at least a 1-log10 copies/mL decrease in viral load after 4 weeks, or rebound after virologic suppression) were included in this cross-sectional study. Of these patients, 47.6% were on a regimen of stavudine/lamivudine plus either efavirenz (43.3%) or nevirapine (4.3%), and 31.2% were on a regimen of zidovudine/lamivudine plus either efavirenz (21.3%) or nevirapine (9.9%). In 71.6%, at least 1 significant resistance mutation emerged (63% nRTI, 66% NNRTI, 4% PI) and in 53.9%, dual-class mutations were present. Of patients with no prior history of antiretroviral therapy, those on a regimen of zidovudine/lamivudine/NNRTI had statistically significantly higher rates of mono- and dual-class resistance (approximately 85% and 69%, respectively) than patients on a stavudine/lamivudine/NNRTI regimen (approximately 70% and 53%, respectively). Patients who had a history of antiretroviral therapy had no statistically significant difference in presence of at least 1 resistance mutation but patients on a regimen of ritonavir-boosted lopinavir and 2 nRTIs had statistically significantly lower rates of dual-class resistance than patients on an NNRTI-based regimen (approximately 20% vs
80%, respectively). The most prevalent resistance mutation was M184V (54.6%) followed by K103N (43.3%), with K103N and M184V mutations appearing together at a rate of 35%. TAMs were present in 29% of patients with resistance with the TAM 2 pathway representing a majority of these mutations (17.7%). In multivariate analysis, presence of at least 1 resistance mutation and virologic failure were significantly associated with recent or history of opportunistic infection (OR, 3.10; 95% CI, 1.27-7.58) and history of HIV-1 RNA level below 300,000 copies/mL at enrollment (OR, 5.96; 95% CI, 1.08-32.8). Resistance was not associated with adherence but this may have been due to under-reporting of nonadherence, which was measured by self-report.

Chaux and colleagues (Abstract 646) presented data on a large cohort of patients in Côte d’Ivoire who received continuous antiretroviral therapy and were followed up for 6 months as part of a prerandomization phase for structured treatment interruption. The majority of patients were women (76%) and initiated a regimen of zidovudine/lamivudine/efavirenz (90%). At 6-month follow up, 10 patients had died, 1 was lost to follow up, and 15 had no viral load data available. One hundred seventeen patients (15%) had detectable HIV-1 RNA levels (above 300 copies/mL) of which samples from 112 were successfully amplified. Rates of resistance were 4.2% overall, 3.9% in patients on an NNRTI-based regimen, and 6.9% in patients on a PI-based regimen. Women who received zidovudine/nevirapine for PMTCT had high rates of resistance (20.4%) compared with women who received zidovudine/lamivudine/nevirapine (0%) or zidovudine alone (0%) for PMTCT. This cohort demonstrated a high rate of virologic success (85%) at 6 months, with low rates of virologic resistance (4.2%). However, the prevalence of low-frequency variants among patients in whom treatment failed but who did not have detectable resistance (70%) was not assessed.

In a cross-sectional analysis of HIV-1-infected pregnant women in the Gauteng Provence of South Africa (Abstract 640), 65 of 128 plasma samples from the year 2002 and 48 of 117 samples from the year 2004 were successfully amplified and evaluated for resistance mutations. No resistance mutations were identified among samples from 2002 and only 2 patients had resistance mutations (T69D, K70R) in samples obtained from 2004. Using the sensitive allele-specific real-time PCR assay, 1 additional sample was found to contain K103N. Prevalence of TDR was less than 5%. Although prevalence of resistance was low, it decreased from 2002 to 2004. As availability of antiretroviral treatment increases, national surveys evaluating prevalence of drug resistance will be important in assessing trends in drug resistance.

In a cohort of 106 patients on antiretroviral therapy in Côte d’Ivoire (Abstract 645), patients were followed up for a median of 2 months to evaluate the correlation between baseline characteristics, including resistance, serious morbidity (WHO stage III-IV classification, hospitalization, or death), and immunologic failure (CD4+ count below 200 cells/µL). At baseline, 54% were on 2 nRTIs and a PI, 44% were on 2 nRTIs and an NNRTI, 58% had detectable viral loads (HIV-1 RNA level above 300 copies/mL), 20% had detectable viral loads without major resistance mutations, and 22% had detectable viral loads with at least 1 major resistance mutation. The most common mutations were M184V, D67N, M41L, K103N, and L90M. Detectable viral load with or without evidence of resistance was not predictive of serious morbidity, but presence of at least 1 resistance mutation was associated with immunologic failure (HR, 4.32; 95% CI, 1.38-13.57).

Sungkanuparph and colleagues (Abstract 663) evaluated rates and predictors of tenofovir resistance among tenofovir-naive patients in whom a first-line regimen of stavudine/lamivudine/nevirapine was failing. Ninety-eight patients met inclusion criteria, which included history of undetectable viral load at 4 to 6 months after antiretroviral initiation and at least 2 subsequent HIV-1 RNA levels of above 1000 copies/mL. Ten patients were noted to have tenofovir resistance (6 with K65R and 4 with at least 3 TAMs). All 10 patients with tenofovir resistance had concurrent NNRTI-resistance mutations. Factors associated with presence of tenofovir resistance included longer duration of antiretroviral therapy prior to detection of failure (OR, 1.12; 95% CI, 1.03-1.21) and higher level of HIV-1 RNA at time of failure (OR, 10.48; 95% CI, 1.77-62.13).

**Resistance to New Antiretrovirals**

**Resistance to Tipranavir and Darunavir and Cross-resistance to PIs.** Koh and colleagues (Abstract 606) conducted an in vitro analysis of emergence of darunavir resistance, comparing a wild-type strain of HIV-1 with polyclonal strains of HIV-1 obtained from 8 patients with known treatment failure of 9 to 11 antiretrovirals. They found that it was difficult to develop darunavir resistance in the wild-type strain but in the polyclonal multi-resistant strains, mutants with high levels of resistance to darunavir, saquinavir, amprenavir, indinavir, nelfinavir, ritonavir, and lopinavir emerged. The authors suggested that there is a significant barrier to development of darunavir resistance; however, in the setting of multi-resistant virus, high levels of resistance to darunavir can emerge.

In Abstract 607, investigators assessed darunavir cross-resistance to ritonavir-boosted amprenavir, tipranavir, and lopinavir by evaluating resistance profiles of 2682 patients with at least 1 major PI mutation. There was minimal evidence of cross-resistance between darunavir and atazanavir or tipranavir. There was more of a correlation of darunavir resistance with lopinavir resistance, and the strongest correlation was with amprenavir resistance. Patient-derived viral strains susceptible to darunavir were also susceptible to amprenavir, tipranavir, and lopinavir at rates of 100%, 89%, and 99.7%, respectively. Among patient strains with amprenavir resistance, 89% retained at least partial susceptibility to darunavir (39% remained completely susceptible to darunavir). In 84% and 88% of strains with tipranavir and lopinavir resistance, respectively, at least partial susceptibility to darunavir was
Abstract

These resistance CI, darunavir and coreceptor resistance permitted to develop effect from the optimizing utilizations of response mechanisms to multi-PI-resistant PI-susceptibility mutations (95% and 98% for V72I and E92Q). T66I decreased sensitivity to elvitegravir 15-fold but had no effect on susceptibility to raltegravir. E92Q decreased susceptibility to both elvitegravir (30-fold) and raltegravir (6-fold). Minor mutations including F121Y and S147G were identified to have low-level resistance to elvitegravir and augment resistance conferred by T66I and E92Q. All 4 of these resistance mutations have been observed to cause resistance to other integrase inhibitors and have no effect on susceptibility to other classes of antiretrovirals including nRTIs, NNRTIs, and PIs.

Several studies evaluated the natural occurrence of integrase-inhibitor resistance in antiretroviral-naive and treatment-experienced individuals. Integrase sequences from 2081 patients (1744 of whom were treatment-naive) from the GenBank database were evaluated (Abstract 623) and included strains that were representative of a variety of subtypes including subtype C (n = 504), subtype A (n = 288), and subtype B (n = 274). Of 288 amino acid positions, 162 were polymorphic, all of which were in the non-catalytic region of integrase.

There were low rates of conservative polymorphisms at extended active residues 141, 151, 155 and 156. Similarly, researchers from the Aaron Diamond AIDS Research Center (Abstract 625) found few polymorphisms associated with integrase-inhibitor resistance in vitro among 13 patients with multi-drug resistance virus and 103 recently infected, antiretroviral-naive patients. Yerly and colleagues (Abstract 626) evaluated prevalence of integrase polymorphisms in 35 patients with subtype B and 54 patients with non-subtype B virus and found that polymorphisms associated with integrase-inhibitor resistance were present at similar levels in subtype B and non-subtype B isolates. The most commonly occurring integrase-inhibitor-associated resistance mutations were V72I (17% of subtype B, 19% non-subtype B), V201I (11% subtype B, 17% non-subtype B), V165I (11% subtype B, 9% non-subtype B), and T206S (9% subtype B, 47% non-subtype B).

The clinical significance of naturally occurring resistance to integrase inhibitors is an area of ongoing research.

Resistance to Entry Inhibitors. Baseline susceptibility to CCR5 blockers as HIV-1 entry inhibitors depends on the presence of a predominant CCR5-tropic virus population in an individual, and change in tropism may be one avenue for HIV-1 to escape from CCR5 blockade. Tropism was initially thought to be the major source of resistance to entry inhibitors. However, the major mechanism of resistance to CCR5 blockers, such as maraviroc, appears to result from mutations that permit HIV-1 to utilize the CCR5 coreceptor despite the presence of bound inhibitor (Abstract 108). The following 2 abstracts conducted clinical and in vitro studies to evaluate factors associated with tropism switch in HIV-1 strains.

The rate and predictors of tropism switch among chronically infected patients with known antiretroviral drug resistance was evaluated among patients in the Study on the Consequences of the Protease Inhibitor Era (SCOPE) cohort (Abstract 619). Sixty-six patients met inclusion criteria, which included baseline plasma HIV-1 RNA level above 1000 copies/mL, presence of at least 1 major or 1 minor genotypic resistance mutation, and use of stable antiretroviral regimen for 120 days or more before baseline. Patients were followed up until regimen change. At baseline, 52, 22, and 2 patients had CCR5-, dual and mixed-, and CXCR4-tropic virus, respectively. Risk of progression from CCR5 to dual and mixed tropism at 1 year was 12% (95% CI, 6-26%) and in multivariate analysis, presence of CCR5 Δ32 heterozygosity was independently predictive of switch (P = 0.24). Risk of switch from mixed and dual to pure CXCR4 and to CCR5 was 8% (95% CI, 1-43%) and 11% (95% CI, 3-37%), respectively.

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2 CXCR4-tropic viruses identified at baseline had observed tropism switch. Of populations noted to have a tropism switch, 50% showed small changes in CXCR4 at entry as measured by reductions in relative light units, implying that the clinical significance of switch in these populations may be limited. This study implies that deferring a change in antiretroviral treatment among patients with known resistance may carry a small risk of losing CCR5 tropism (approximately 10%).

Moncunill and colleagues (Abstract 618) conducted in vitro studies of CCR5 coreceptor switches in the presence and absence of CCR5, CXCR4, and reverse transcriptase inhibitors (RTIs). They found that in the absence of drug pressure, 3 of 6 strains switched from CCR5 to CXCR4 and demonstrated increased rates of replication. In the presence of CCR5 antagonists, CXCR4-using virus emerged more quickly in the presence of TAK-799 but was prevented by plerixafor (AMD3100). These studies indicate that cell culture models can be useful in predicting the propensity of clinical isolates to develop entry inhibitor resistance in the setting of antiretroviral drug pressure.

Antiretroviral Resistance Mutations: Interactions and Effects on Fitness

Resistance to Nucleoside Reverse Transcriptase Inhibitors. Sluis-Cremer presented an overview of mechanisms of resistance to nRTIs and interactions among RTI resistance mutations (Abstract 58). HIV-1 reverse transcriptase is essential for transcribing an RNA template into DNA. The nRTIs are nucleoside or nucleotide analogues that compete with the naturally occurring deoxynucleotide triphosphate (dNTP) and cause chain termination that prevents formation of HIV DNA. Previous studies have shown that resistance to nRTI occurs due to preferential incorporation dNTP over the nRTI (a process known as discrimination) or increased excision of the nRTI. Kinetic data have shown that with the K65R, K70E, L74V, M184V, and Q151M mutations, resistance occurs through discrimination, whereas TAM-mediated resistance occurs through facilitated excision of the nRTI. These mechanisms of resistance appear to be antagonistic; there is evidence that when M184V, L74V, or K70E occur in conjunction with TAMs, the virus or enzyme is less susceptible to zidovudine. K65R and TAMs (of the TAM 67 pathway) appear to have bidirectional antagonism: clinical isolates with both mutations are infrequently observed. The presence of K65R with TAM 67 decreases the resistance to zidovudine in the presence TAM 67 alone from 54-fold to 1.5-fold, and TAM 67 with K65R decreases the resistance to tenofovir in the presence of K65R alone from 4.2-fold to 2.4-fold.

Sluis-Cremer also provided evidence that antagonism among resistance mutations affects virologic response. In the ESS30009 study13, at 12 weeks 49% (50 of 102) of patients randomized to tenofovir/abacavir/lamivudine were virologic nonresponders. Of these virologic nonresponders, 98% had M184V/I, either in combination with (44%) or without (53.5%) K65R. This relatively high rate of virologic nonresponse could be the result of a low genetic barrier to emergence of M184V and K65R and the fact that K65R confers resistance to all of the medications in the regimen.14 This is in contrast to the A5095 study15 in which patients randomized to receive zidovudine/abacavir/lamivudine had a rate of virologic nonresponse of only 21% (82 of 382). Of these nonresponders, 70% had evidence of resistance: 51% (29 of 57) with M184V alone, 14% (8 of 57) with TAMs and M184V in combination, and 3.5% (2 of 57) with TAMs alone. Sluis-Cremer hypothesized that the relatively low rate of virologic nonresponse could be attributed to the higher genetic barrier to TAMs and M184V, as well as the antagonism between TAMs and M184V.

K65R. The K65R mutation is associated with decreased susceptibility to nRTIs and decreased replication capacity. In a database analysis of patients in whom tenofovir therapy failed (Abstract 591), K65R was often accompanied by A62V and S68G mutations. Site-directed mutagenesis was conducted to create K65R, K65R/A62V, K65R/S68G, and K65R/A62V/S68G mutants, and their fitnesses were compared with wild-type virus in the presence and absence of tenofovir. The results suggested that A62V and S68G act as partial compensatory mutations for K65R by increasing replication capacity. In the absence of tenofovir, wild-type virus was more fit than the K65R/A62V/S68G mutant, but in the presence of greater than 7μM of tenofovir, the triple mutant was more fit. Incorporation kinetics showed that the K65R mutants with A62V/S68G exhibited more efficient incorporation of dATP and dGTP than mutants with K65R alone.

In contrast to A62V and S68G, the K70E mutation, which is associated with patients in whom tenofovir-containing regimens are failing, is rarely found in conjunction with K65R (Abstract 584). Site-directed mutagenesis has shown very poor replication capacity among K70E/K65R double mutants, suggesting antagonism between these mutations.16 Using molecular dynamic simulations, Kagan and colleagues (Abstract 592) evaluated whether there was a structural basis to this antagonism. In wild-type virus, the K65 was found to stabilize the triphosphate moiety of the dNTP ligand but with K65R mutation the stabilization was lost. The K70 in wild type appears to compensate for the loss of stabilization conferred by the K65R mutant. The double mutant K65R+K70E has no compensation for this loss of stabilization, which leads to a more severe defect, thus providing a structural basis for the antagonism between the K65R and K70E mutants. Molecular dynamic simulation may be a useful tool to evaluate other antagonistic mutation interactions.

Resistance to Enfuvirtide. Enfuvirtide blocks fusion of HIV with CD4 through competitive interactions with the HR1 and HR2 helices of the gp41 transmembrane protein. Genotypic changes in HR1 and HR2 have been associated with resistance to enfuvirtide. Resistance mutations in the HR1 (N43D) and HR2 (E137K) sequences among 5 enfuvirtide-experienced patients were
identified, cloned, and evaluated for their effect on fitness and response to enfuvirtide through in vitro assays of viral envelope fusogenicity and infectivity (Abstract 620). N43D single mutants and N43D/E137K double mutants had decreased susceptibility to enfuvirtide compared with wild type at magnitudes of 28-fold and 32-fold, respectively. However, the N43D single mutant had decreased fitness compared with wild type by 92 %, and the N43D/E137K double mutants had no statistically significant difference in fitness compared with wild type. The E137K single mutant conferred no statistically significant difference in sensitivity to enfuvirtide or in fitness compared with wild type. The N43D mutation appeared to decrease susceptibility to enfuvirtide, but at a substantial cost to fitness, for which the E137K mutation can compensate. An analysis of previously published data regarding prevalence of resistance mutations to enfuvirtide supported this hypothesis: the N43D mutation occurred at higher rates among patients with E137K/Q mutations than those without E137K/Q mutations.

Resistance to Protease Inhibitors. Previous studies have shown that amino acid inserts near the cleavage and noncleavage sites in Gag compensate for decreased viral fitness secondary to PI-resistance associated mutations. Aoki and colleagues (Abstract 601) evaluated the effect of a 7 amino acid insert (TTNTRNS) near the p17/p24 cleavage site in a heavily drug-experienced, HIV-infected patient. Samples were obtained throughout the course of treatment that included a regimen of zidovudine/lamivudine/nelfinavir followed by stavudine/ritonavir-booster saquinavir and then stavudine/ritonavir-booster saquinavir/abacavir. Seventy percent of clones from the patient contained the TTNTRNS insert and were found early in the course of antiretroviral treatment. The presence of the insert had no effect on the propensity to acquire nelfinavir mutations. Introduction of the insert to wild-type virus resulted in decreased fitness. However, clones with saquinavir-, indinavir-, nelfinavir-, or amprenavir-associated mutations containing the insert had increased fitness. In this study, presence of TTNTRNS insert near the p17/p24 cleavage sites improved the fitness of otherwise replication-compromised PI-resistant mutants.

Hypersusceptibility to Non-nucleoside Reverse Transcriptase Inhibitors. The nRTI induction of NNRTI hypersusceptibility and accompanying influences on virologic response have been previously described but the mechanism of this mutational interaction has remained unclear. Clark and colleagues (Abstract 598) evaluated 3 nRTI mutants (118I, 208Y, and 215Y) that are strongly associated with NNRTI hypersusceptibility and their effects in vitro on fitness and reverse transcriptase activity. Replication capacity varied depending on the combination of mutations present: 208Y/215Y, 118I/208Y/215Y, and 118I/215Y mutants had replication capacities of 40 %, 55 %, and equal to wild type, respectively. The effect of mutation combination on reverse transcriptase activity and polymerase activity of reverse transcriptase was similar to its effect on replication capacity: 208Y/215Y, 118I/208Y/215Y, and 118I/215Y exhibited 47 %, 30 %, and equal to reverse transcriptase activity compared with wild type, respectively. It was hypothesized that hypersusceptibility to NNRTIs conferred by 208Y/215Y and 118I/208Y/215Y mutants may be related to altered gag-pol processing and the mechanism of hypersusceptibility conferred by 118I/215Y double mutants is distinct and remains to be determined.

Fading of Resistance Mutations with Treatment Interruption. Ceccherini-Silberstein and colleagues (Abstract 587) evaluated the evolution of resistance mutations among 138 patients experiencing virologic failure who underwent treatment interruption for at least 1 month. Genotypes were available at the time of virologic failure and at least once during treatment interruption. Disappearance of resistance mutations correlated with known effects of resistance on fitness. M184V, Y115F, and K65R had completely disappeared within 4 months of treatment interruption and were associated with the fastest viral load increase, which is consistent with their detrimental effects on fitness. In contrast, TAMs, L74V, E44D, and H208Y progressively declined during treatment interruption but were still present in more than 10 % of individuals 9 months post-treatment interruption. E44D, V181I, E203K, and H208Y were all associated with maintenance of TAM 1 pathway mutations and K20R and D218E were associated with persistence of TAM 2 pathway mutations. The dynamics of resistance mutation evolution in the absence of drug pressure appears to be related to the relative fitness of the virus conferred by resistance mutations and resistance mutation combinations. Prevalence of low-frequency resistance variants, however, was not assessed.

Charpentier and colleagues (Abstract 622) evaluated the disappearance of mutations associated with resistance to enfuvirtide after treatment discontinuation. Bulk sequencing was used to detect resistance mutations in 7 patients who had immunovirologic failure while on enfuvirtide at baseline, during treatment, and after treatment. Molecular cloning was used to detect low-frequency resistance variants. Median medication duration was 6 to 4 months. Of 7 patients, 3 had complete reversion to wild type, 2 had persistence of enfuvirtide-resistant virus as a minority population, and 2 had persistence of resistance as a majority population. A high proportion of patients in whom resistance persisted were those who received enfuvirtide for long periods of time (> 6 months). There was no association between enfuvirtide resistance mutations and the kinetics of mutation disappearance. Further investigation is required to determine the clinical significance of persistent enfuvirtide resistance.

Mutations in the Connection and RNase H Domains of Reverse Transcriptase

The HIV-1 reverse transcriptase is composed of 3 domains of the p66 subunit
(polymerase, connection, and RNase H) and 2 domains of the p51 subunit (polymerase and connection). Most resistance assays sequence only the polymerase domain and do not evaluate for resistance mutations in the connection and RNase H domains. Several studies at this year’s conference presented evidence that mutations in these infrequently analyzed domains may be clinically significant.

**Mutations in the RNase H Domain.**

The function of RNase H is to cleave the RNA moiety of RNA and DNA hybrids during reverse transcription. Recent studies have shown that HIV-1 reverse transcriptase mutations in the RNase H domain can increase nRTI resistance, presumably by decreasing RNase H activity, allowing more time for excision of the incorporated nRTI monophosphate.\(^1\)

To evaluate mutations in reverse transcriptase outside of the catalytic domain, selections of zidovudine-resistant HIV-1 were made in vivo and the entire coding region of reverse transcriptase was sequenced (Abstract 90). Sequencing identified the A371V mutation (located in the connection domain) and Q509L mutation (located in the RNase H domain). Using site-directed mutagenesis, A371V and Q509L mutants were found to be 1.7-times more resistant to zidovudine than wild type, but in conjunction with TAMs (D67N and K70R), resistance increased to 39-fold that of wild type (compared with a 4.6-fold increase with TAMs alone). The mechanism of augmented resistance by A371V and Q509L appeared to be a decrease in RNase H cleavage activity, which may lead to facilitated zidovudine excision in the presence of TAMs. RNase H is also being evaluated as a novel target for antiretroviral therapy (Abstract 89), further emphasizing the need to evaluate the effects of RNase H activity on resistance and fitness.

**Mutations in the Connection Domain: N348I.** Abstracts 593 and 594 described the resistance mutation N348I, which is found in the connection domain and confers resistance to NNRTIs and nRTIs. Hachiya and colleagues (Abstract 593) identified 2 clinical isolates that were phenotypically more resistant to nevirapine and delavirdine despite a lack of NNRTI-associated resistance mutations. Sequencing of the entire reverse transcriptase domain of these isolates identified the N348I. Compared with wild type, N348I mutants conferred increased resistance to zidovudine (8.6-fold), didanosine (5-fold), nevirapine (22-fold), and delavirdine (4.3-fold).

Yap and colleagues (Abstract 594) performed genotyping on 1377 treatment-naive and treatment-experienced patients and identified N348I as the ninth most prevalent mutation, occurring 11.3-times more frequently in treatment-experienced patients than in treatment-naive patients. N348I appeared relatively early in virologic failure, before the appearance of TAMs and about the same time as NNRTI-resistant mutations, and was associated with zidovudine and combination zidovudine/nevirapine treatment. N348I decreased susceptibility to zidovudine between 2-fold alone and 4-fold in combination with TAMs, did not antagonize M184V resistance to zidovudine, conferred resistance to efavirenz and nevirapine as a single mutant, and augmented K103N-related resistance to efavirenz and nevirapine. Molecular dynamics simulation suggested that the N348I mutation inhibits movement of the thumb region of the polymerase domain, thereby allowing more time for zidovudine excision. These studies indicate that this novel N348I has clinical relevance and suggest that genotypic and phenotypic analysis of the entire reverse transcriptase should be conducted to identify the prevalence of other clinically-significant resistance mutations outside of the polymerase domain.

**Predictors of Immunologic, Virologic, and Clinical Outcomes**

From the first days of the Multicenter AIDS Cohort Study (MACS), investigators have gained insights by following up groups of HIV-infected individuals over time. The following section is a selected review of predictors of clinical outcomes from cohort studies at this year’s conference.

Sabin and colleagues (Abstract 528) presented an analysis from the Collaboration of Observational HIV Epidemiological Research in Europe (COHERE), a collaboration of 33 cohorts from 30 European countries contributing data on 827 children under 17 years of age and 49,094 adults. Patients eligible for inclusion in this study (n = 49,921) were antiretroviral therapy naive, aged 6 years or older, had initiated antiretroviral therapy between 1998 and 2006, and had more than 1 CD4 + cell count and plasma HIV-1 RNA measurement taken pre-antiretroviral initiation and during follow up. Baseline characteristics included median age of 37 years, 29% female, CD4 + count of 210 cells/µL, and plasma HIV-1 RNA level of 4.9 log\(_{10}\) copies/mL. Thirty-eight percent had initiated an NNRTI-based regimen and 28% had initiated non-ritonavir-boosted PI regimens.

Age was found to be a predictor of immunologic, virologic, and clinical outcome. Immunologic responses to therapy in the cohort were appropriate, with 59.2% achieving a confirmed CD4 + cell response by 1 year, but the probability of an immunologic response was higher in younger individuals (particularly those under 12 years of age) and reduced in those over 60 years of age. In the first year of therapy, 53.7% of patients achieved a virologic response to antiretroviral treatment, but the probability of response was lower in those aged 6 to 17 years, and higher in those aged 50 years and older than those aged 30 to 39 years. Older individuals were more likely to develop an AIDS-defining event or death in the first year on antiretrovirals, with adjusted HRs of 1.19 (95% CI, 1.05-1.34) and 1.34 (95% CI, 1.19-1.51) for those aged 55 to 59 years and over 60 years.

Braithwaite and colleagues (Abstract 520) examined the relationship between antiretroviral regimen, adherence, and virologic response in a cohort of 6394 patients initiating antiretroviral therapy within the Veterans
Affairs Healthcare System. Initiation of treatment with NNRTI-based regimens was compared with initiation of ritonavir-boosted PI-based regimens. Adherence, estimated from pharmacy refill data, was statistically significantly greater with efavirenz-based regimens (67 %) and nevirapine-based regimens (65 %) than with boosted (59 %) or unboosted (61 %) PI-based regimens (P < .001). In multivariate analyses, plasma HIV-1 RNA suppression was inferior in nevirapine-based regimens (OR, 0.60), boosted PI-OR (OR, 0.57), and unboosted PI-based regimens (OR 0.48) compared with efavirenz (all, P < .001).

Using data from the Swiss HIV Cohort Study, predictors for long-term CD4+ count increases in 2860 patients initiating first-line antiretroviral therapy were evaluated (Abstract 518). Sixty-three percent achieved virologic suppression, and median CD4+ count increases were 87, 52, and 19 cells/μL in the 3 time periods examined: 1, 2 to 3, and 4 to 5 years after plasma HIV-1 RNA suppression, respectively. In multivariate modeling, median CD4+ count increase was statistically significantly higher for patients with female sex (P < .001), lower age (P < .001), higher plasma HIV-1 RNA at start of antiretroviral therapy (P = .002), and CD4+ count of below 650 cells/μL at start of the period (P = .010).

Another evaluation of the Swiss HIV Cohort Study determined the predictive value of longitudinal self-reported adherence assessment for virologic rebound, defined as 2 consecutive plasma HIV-1 RNA measurements above 2.7 log10 copies/mL (Abstract 523). Patients were included in the analysis if they were on antiretroviral therapy, over 16 years of age, had plasma HIV-1 RNA below 1.7 log10 copies/mL over the previous 3 months, and had completed adherence questionnaires before June 1, 2006. Among the 2638 subjects who met inclusion criteria, the median follow up was 2.5 years. Patients reported missing 1 or more doses at 25 % of visits, and missing more than 2 doses at 9.9 % of visits. A total of 97 patients (3.7 %) experienced virologic rebound. In an unadjusted analysis, there was no difference in rates of virologic failure between patients who reported perfect adherence and those who reported missing 1 dose. HRs for 2 missed doses and more than 2 missed doses were 1.89 (95 % CI, 1.24-2.88) and 3.88 (95 % CI, 2.74-5.48), respectively. In a multivariate analysis, nonadherence, defined as a self-report of missing 2 or more doses of medication in the previous 28 days, was associated with an increased risk of virologic rebound (HR, 2.82; 95 % CI, 1.76-4.50). Other risk factors for virologic rebound were having more than 5 previous antiretroviral regimens (HR, 2.75; 95 % CI, 1.65-4.61) and comedication for cardiovascular problems, opportunistic infections, or hepatitis C virus infection (HR, 2.42; 95 % CI, 1.54-3.82).

Gibb and colleagues (Abstract 701) combined data from 11 studies of HIV-1-infected children in resource-limited settings to examine factors predicting mortality for these children. Ten African studies and 1 Brazilian study of untreated children older than 12 months were used to form a retrospective cohort of 2510 children, 3769 person-years of observation, and 557 deaths. The majority (81 %) of the follow up occurred after the initiation of co-trimoxazole therapy. The first available data points were used as baselines, and the investigators found a median age of 4.0 years, CD4+ cell percentage of 15, and weight-for-age z-score of −1.9. Predictors of mortality were CD4+ cell percentage, CD4+ count, weight-for-age, and hemoglobin level. Children with weight-for-age z-scores lower than −3 and hemoglobin level of below 8 mg/dL had a mortality rate of 55.2 per 100 person-years compared with 1.4 per 100 person-years when weight-for-age z-score was 1 or higher and hemoglobin level was 10 mg/dL or higher. This trend was seen even in children who had a baseline CD4+ cell percentage of more than 15. This report highlights the need for consideration of weight-for-age and hemoglobin level in decisions regarding antiretroviral therapy initiation in resource-limited settings, as is recommended in the most recent WHO antiretroviral treatment guidelines.

Clinical Outcomes Associated with Resistance

Phillips and colleagues (Abstract 532) presented data from the UK Collaborative HIV Cohort Study (UK CHIC) on the cumulative risk of extensive triple-class failure. They defined failure of a drug as plasma HIV-1 RNA above 2.6 log10 copies/mL after more than 4 months of continuous use of that drug. Extensive failure of the nRTI class was failure of at least 1 drug from each of the following sub-classes: zidovudine and stavudine; lamivudine and emtricitabine; and didanosine, tenofovir, and abacavir. Extensive failure of NNRTIs was determined by failure of either nevirapine or efavirenz, and extensive failure of PIs involved virologic failure of at least 1 ritonavir-boosted PI. Extensive triple-class failure was defined as failure of all 3 classes. Of 10,603 patients evaluated, 25 % were female, and median age, CD4+ count, and viral load were 36 years, 185 cells/μL, and 4.96 log10 copies/mL, respectively. During 58,190 person-years of observation, 169 patients developed extensive triple-class failure, 70 % of whom had at least 1 prior measurement of plasma HIV-1 RNA below 1.7 log10 copies/mL. Of 169 patients, 95 (56 %) subsequently had at least 1 plasma HIV-1 RNA measurement below 1.7 log10 copies/mL. A baseline CD4+ count of below 200 cells/μL carried a HR of 2.2 for extensive triple-class failure (P < .0001), and for those with a baseline CD4+ count above 200 cells/μL, the cumulative risk of extensive triple-class failure was 4 % (95 % CI, 2-6).

The correlation between resistance and clinical outcomes was analyzed among 1929 antiretroviral-naïve patients in the Swiss HIV Cohort Study who initiated treatment with at least 2 nRTIs plus an NNRTI or ritonavir-boosted PI (n = 518) from January 1999 to December 2005 (Abstract 667). Sixty-nine of 805 (8.6 %) patients in the NNRTI group and 24 of 518 (4.6 %) in the PI/ritonavir group experienced virologic failure, which was defined as viral load of 500 copies/mL or higher after at least 180 days on continuous treatment. Although discontinuation of
antiretroviral therapy due to virologic failure was not statistically significantly different between the groups, discontinuation due to an adverse event was more common among patients on an NNRTI than among patients on PI/rainavir (n = 189 vs n = 122, respectively, log rank P = .0241). Among patients for whom genotypic resistance testing was available (n = 1323), patients on a PI/rainavir regimen had higher rates of susceptibility to lamivudine/emtricitabine (75% vs 42.1%, P = .026) and to a third antiretroviral drug (90% vs 52.6%, P < .001) than patients on an NNRTI-based regimen. There was no statistically significant difference in susceptibility to nRTIs. Although rates of virologic failure were not different between PI/rainavir-based regimens and NNRTI-based regimens, rates of adverse events and resistance were higher among patients initiated on an NNRTI-based regimen.

Predictors of mortality were evaluated among patients enrolled the Danish HIV Cohort study who experienced triple-class virologic failure from 1995 to 2004. Triple-class failure was defined as viral load above 1000 copies/mL for a total of 120 days while on antiretroviral treatment, and median time of follow up after triple-class failure was 4.3 years. One hundred and seventy patients experienced triple-class failure, 133 of whom received resistance testing. The median number of resistance mutations was 8 and 61% (81 of 133) had resistance to 3 major classes. Mortality from time of triple-class failure was 70 (95% CI, 54-92) per 1000 person-years in patients who experienced triple-class failure compared with 29 (95% CI, 26-32) per 1000 person-years from time of antiretroviral initiation in all patients in the cohort. In multivariate analysis, mortality rate ratio (MRR) was associated with presence of 9 or more resistance mutations (MRR, 2.3; 95% CI, 1.1-4.8), presence of T215Y (MRR, 3.4; 95% CI, 1.6-6.66), G190AS (MRR, 3.2; 95% CI, 1.6-6.6), or V82F/A/T/S (MRR, 2.5; 95% CI, 1.2-5.3). After adjusting for latest CD4+ count, only presence of T215Y and latest CD4+ count remained associated with mortality. The authors concluded that a majority of resistance mutations among patients with triple-class failure accumulated during suboptimal treatment in the 1990s and that perhaps T215Y is a marker of earlier development of immunodeficiency. With the availability of newer drug classes, it remains to be seen if multi-class failure to RTIs and PIs will remain associated with mortality.

The relationship between mortality and use of resistance testing was analyzed using data from the HIV Outpatient Study (HOPS) (Abstract 660). Of patients enrolled in the cohort since January 1999, 3202 were evaluated and had median follow up of 3.3 years. Resistance testing was performed in 1110 of these patients. Patients who were white, had private insurance, had a lower CD4+ count, and whose risk behavior was MSM were more likely to have had resistance testing performed. Among patients who had received potent antiretroviral therapy (n = 2107), receiving a resistance test and private health insurance were associated with decreased mortality even after adjusting for stage of HIV disease, demographics, age, and year (HR, 0.60 and 0.63, respectively). Among patients who were antiretroviral naive with CD4+ counts below 200 cells/µL (n = 257), resistance testing before initiation of antiretroviral therapy was also protective (HR, 0.22, although 95% CI approaches 1.0). Although limited by the retrospective nature of the study design, this analysis provides evidence that antiretroviral therapy guided by resistance testing is associated with a substantial clinical benefit.

**Host-factor Influence on Response to Therapy**

The human leukocyte antigen (HLA) variants Bw4 and Bw6 help determine HLA interactions with natural killer cells, and Bw4 has been associated with enhanced control of HIV infection. Rauch and colleagues (Abstract 141) combined data from the Swiss HIV Cohort Study and the Western Australia HIV Cohort Study to examine the effects of Bw4 on immunologic and virologic responses to antiretroviral therapy. Data from 161 Bw4+ adult, white men initiating antiretroviral treatment between 1997 and 2002 were analyzed. In the Australian cohort, baseline mean age of Bw4+ individuals was 4 years older than Bw4– individuals, and in the Swiss cohort, mean CD4+ count of Bw4+ individuals was 90 cells/µL lower than Bw4– individuals. Baseline plasma HIV-1 RNA, follow-up time, initial antiretroviral regimen, and mode of infection did not differ significantly between Bw4+ and Bw4– individuals in either cohort. CD4+ percentage and absolute CD4+ counts were consistently lower in Bw4+ carriers than in the remainder of the cohort from 1 to 5 years on antiretrovirals. CD4 + counts were approximately 85 cells/µL lower in Bw4+ than Bw4– individuals in the Swiss cohort and 55 cells/µL lower in the Australian cohort (P = .005 and P = .01, respectively). These differences were more profound in patients carrying the variant Bw4-80T, and remained statistically significant after adjusting for virologic response rates.

Rosignoli and colleagues (Abstract 451) presented data on the effect of antiretroviral therapy on expression of CD279, also known as programmed death 1 (PD-1), and its ligand (PD-L1), which has been implicated in promoting and regulating anergy of HIV-1-specific CD8+ cells. They examined PD-1 and PD-L1 activity in 22 HIV-1-infected individuals on antiretroviral therapy who had plasma HIV-1 RNA below 1.7 log10 copies/mL and a median CD4+ count of 547 cells/µL, and compared it with levels in 10 uninfected controls. There were no statistically significant differences in levels of PD-1, even after stratification by antiretroviral treatment regimen. Previous studies have shown higher levels of PD-1 in untreated viremic patients, so this may represent a normalization of PD-1 levels with antiretroviral therapy. The mean fluorescence intensity of PD-L1 on T cells was higher in HIV-1-infected individuals regardless of their antiretroviral therapy status. Evidence of PD-1 activity could be a signature of the persistent anergic state.

Liptrott and colleagues (Abstract 452) also explored the relationship
between PD-1 and response to antiretroviral treatment, but they focused on PD-1.3, an allele of the PD-1 gene that has been shown to alter the regulation of PD-1 gene expression. PD-1.3 genotyping and an assay for CCR5 Δ32 were conducted on samples from 77 antiretroviral-naive patients initiating efavirenz-based regimens. Median CD4+ count at baseline was 202 cells/μL and median plasma HIV-1 RNA level was 4.9 log_{10} copies/mL. Fifteen heterozygotes and 1 homozygote for PD-1.3 and 7 heterozygotes for CCR5 Δ32 were identified. Baseline median CD4+ counts were statistically significantly lower in patients with PD-1.3 alleles (121 cells/μL, range 5-335) than patients with wild type (187 cells/μL; range 10-760; P = .02 for the difference between the 2 CD4+ counts). CD4+ counts were also significantly lower in individuals with the PD-1.3 allele at 2, 4, 6, and 8 months after initiation of antiretroviral therapy. There were no significant differences in virologic response between the 2 groups, and CCR5 Δ32 did not have an appreciable effect on response to therapy. The results were limited by small sample size, but suggest that further investigations are needed.

Selected Pharmacokinetic Presentations

Antiretroviral Drug Interactions

Waters and colleagues (Abstract 557) presented data on the interaction of abacavir with atazanavir/ritonavir and lopinavir/ritonavir in HIV-infected patients. The pharmacokinetics of abacavir before and after adding 2 weeks of either atazanavir/ritonavir or lopinavir/ritonavir and the pharmacokinetics of atazanavir/ritonavir or lopinavir/ritonavir before and after adding abacavir were evaluated. Atazanavir/ritonavir and lopinavir/ritonavir levels were not affected by the addition of abacavir, however, the AUC (area under the concentration curve) of abacavir was decreased by 17% after the addition of atazanavir/ritonavir and by 32% after the addition of lopinavir/ritonavir. The mechanism of this interaction and the clinical significance are not clear.

Kakuda and colleagues (Abstract 560) presented data from TMC125-C223, a phase II study of etravirine (an investigational NNRTI) in treatment-experienced subjects. The pharmacokinetic parameters of etravirine were decreased by coadministration of either a PI or tenofovir. Etravirine trough concentrations, AUC, and de novo enfuvirtide use were associated with improved virologic response. The association between pharmacokinetic parameters and virologic response appeared to be relevant in the lower but not the higher dose group.

Bertz and colleagues presented pharmacokinetic and pharmacodynamic data from BMS 424-089, a study comparing ritonavir-boosted atazanavir to unboosted atazanavir with 2 nRTIs in treatment-naive subjects (Abstract 565). As expected, trough concentration of atazanavir was lower in subjects receiving unboosted atazanavir than subjects receiving boosted atazavir (125 ng/mL and 663 ng/mL, respectively). The trough concentration correlated with probability of having a plasma HIV-1 RNA level below 50 copies/mL at week 48 and higher bilirubin levels, but did not correlate with changes in lipid parameters. The authors suggested that the relative adverse lipid effects seen with boosted atazanavir versus unboosted atazanavir were due to ritonavir administration, not due to higher atazanavir levels per se.

Tebas and colleagues (Abstract 572) presented data on the pharmacokinetics of enfuvirtide in patients with severe renal impairment (defined as calculated creatinine clearance of 11 to 35 mL/minute) and patients on hemodialysis. They found that the AUC of enfuvirtide was higher in patients with renal disease (80.5 μg·h/mL in patients with severe renal impairment and 71.1 μg·h/mL in patients with end-stage renal disease [ESRD]) than in controls with normal renal function (49.6 μg·h/mL). Despite the higher exposure in patients with renal disease, all patients tolerated enfuvirtide well and no safety concerns were identified, therefore no dose adjustment was recommended.

Interactions Between Antiretroviral and Non-antiretroviral Medications

Hoody and colleagues (Abstract 564) presented data on the interaction of lopinavir/ritonavir and rosuvastatin, an HMG Co-A reductase inhibitor. A drug-drug interaction was not expected as rosuvastatin is not a substrate for CYP3A4. However, investigators found that rosuvastatin AUC was increased 2.1 fold and maximum concentration (C_{max}) by 4.7 fold. The authors suggested that a dose separation strategy should be tested to overcome this interaction.

Agarwala and colleagues (Abstract 568) investigated several strategies to coadminister famotidine with atazanavir/ritonavir and tenofovir through pharmacokinetic studies with HIV-seronegative volunteers. Four different famotidine dosing strategies led to modest reductions in the minimum concentration (C_{min}) of atazanavir: 20 mg orally twice daily (morning dose of famotidine and atazanavir/ritonavir coadministered) led to a 19% reduction, 20 mg orally twice daily (atazanavir/ritonavir and famotidine separated by 2 hours) led to an 18% reduction, 40 mg orally once daily (separated by 12 hours from atazanavir/ritonavir) led to a 23% reduction, and 40 mg orally twice daily led to a 28% reduction.

Rifampin is known to decrease levels of PIs, precluding their coadministration. Acosta and colleagues (Abstract 575) presented data from ACTG A5213, which studied higher doses of atazanavir without ritonavir to overcome this interaction. Regimens studied included atazanavir 300 mg twice daily, atazanavir 400 mg twice daily, and atazanavir 600 mg twice daily. Even at the highest dose, the trough concentration of atazanavir (55 ng/mL) was statistically significantly lower than that of historic controls receiving 400 mg daily (159 ng/mL). Coadministration of these drugs is therefore not recommended.

German and colleagues (Abstract 577) presented data on the interaction of efavirenz and a leading antimalarial treatment, artesunate and amodiaquine. The study was stopped early af-
### Table 4: Key Findings and Potential Clinical Implications

<table>
<thead>
<tr>
<th>Clinical Trials of Antiretroviral Agents</th>
<th>Potential Clinical Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment of antiretroviral-experienced patients</strong> (Abstracts 104aLB, 104bLB, 105aLB, 105bLB)</td>
<td>Achieving complete virologic suppression (plasma HIV-1 RNA &lt;50 copies/mL) is a realistic goal for all patients initiating or changing antiretroviral therapy. New agents, especially those in new drug classes, are optimally given when there is at least 1 other agent to which the patient is sensitive.</td>
</tr>
<tr>
<td>2 studies of maraviroc and 2 studies of raltegravir achieved excellent virologic suppression in highly treatment-experienced subjects when using these new agents. The best responses occurred when there were 1 or more active drugs in the optimized background regimen. These drugs appeared safe and well tolerated.</td>
<td></td>
</tr>
</tbody>
</table>

| **Treatment of antiretroviral-naive patients** (Abstracts 138, 503, 506, 507) | No new once-daily options were evident from the data presented, although once-daily lopinavir/ritonavir regimens were comparable to twice-daily regimens in individuals with plasma HIV-1 RNA <5.0 \( \log_{10} \) copies/mL. |
| 2 trials examining once-daily regimens, 1 with a once-daily lopinavir/ritonavir-based regimen and 1 with once-daily nevirapine regimen had suboptimal virologic response profiles. |  |

| **Antiretroviral treatment strategies** (Abstracts 513, 514, 516, 638) | Treatment interruption is associated with serious adverse events and de-escalation to monotherapy with lopinavir/ritonavir or lamivudine may be alternative short-term options for patients on long-term antiretroviral therapy who request treatment interruption. |
| Lopinavir/ritonavir de-escalation was associated with continued suppression of plasma HIV-1 RNA for >1 year, but nonadherence consistently predicted loss of virologic suppression. Lamivudine monotherapy allowed patients with M184V mutations to remain off combination antiretroviral therapy for a longer period of time and was associated with fewer adverse events than treatment interruption. |  |

<table>
<thead>
<tr>
<th><strong>Antiretroviral Resistance</strong></th>
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<tbody>
<tr>
<td><strong>Transmitted drug resistance</strong> (TDR; Abstracts 60, 648, 650, 653, 657)</td>
<td>Baseline resistance testing in treatment-naive individuals should precede and guide initiation of antiretroviral therapy. The natural history of TDR and its impact on clinical outcomes deserve further evaluation.</td>
</tr>
<tr>
<td>TDR occurs at relatively high frequencies in industrialized countries and if undetected can lead to initiation of inactive antiretroviral medications.</td>
<td></td>
</tr>
</tbody>
</table>

| **Low-frequency resistance variants** (Abstracts 61, 639, 658, 666) | Additional studies should be conducted to determine the impact of low-frequency variants on outcome, and clinical cut-offs for sensitive resistance tests should be established. Future standard-of-care may include detection of low-frequency resistance variants to guide antiretroviral therapy but this is too labor intensive and too costly to be incorporated into practice in the near future. |
| Low-frequency resistance variants are not detected by standard resistance testing but appear to affect antiretroviral therapy response. |  |

| **Effect of subtype on resistance** (Abstracts 59, 585, 624, 661, 664) | Clinicians should know the HIV-1 subtype of their patients, be familiar with resistance mutations associated with this subtype, and take these factors into consideration when choosing antiretroviral regimens. Additional research should be conducted to evaluate prevalence of resistance mutations among non-B subtypes, and their clinical implications, and databases and resistance algorithms should be updated accordingly. |
| Genetic variability of subtypes affects development of resistance mutations. |  |
Novel mutations in HIV-1 reverse transcriptase (Abstracts 90, 593, 594)

Mutations in the connection and RNase H domains of HIV-1 reverse transcriptase have only recently been evaluated and may represent novel resistance mutations. Further studies should evaluate the impact of these mutations on resistance and fitness. If these mutations prove to have clinical significance in addition to mutations already identified in the polymerase domain, routine sequencing of the entire HIV-1 reverse transcriptase may become standard in resistance testing.

Antiretroviral Treatment in Resource-limited Settings

<table>
<thead>
<tr>
<th>Summary</th>
<th>Potential Clinical Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment outcomes in large adult cohorts (Abstracts 33, 34, 35, 36LB, 62, 531, 535, 537)</td>
<td>Addressing early mortality and loss to follow up remain important challenges in resource-limited settings. Further options for second-line therapy are urgently needed.</td>
</tr>
<tr>
<td>Treatment outcomes in large pediatric cohorts (Abstracts 79, 727, 728, 729, 732)</td>
<td>Increased access to and earlier initiation of treatment are needed for children in resource-limited settings. As recommended by the most recent World Health Organization guidelines, anemia and malnutrition should be considered in the decision to initiate antiretroviral treatment.</td>
</tr>
<tr>
<td>Laboratory monitoring in resource-limited settings (Abstracts 538, 531, 629, 673, 674)</td>
<td>No clear alternative to monitoring plasma HIV-1 RNA in resource-limited settings exists. Further development of less expensive, simpler assays is needed.</td>
</tr>
<tr>
<td>Adherence in resource-limited settings (Abstracts 530, 536, 548)</td>
<td>Nonadherence to antiretroviral treatment is high in some resource-limited settings. In addition to factors traditionally associated with decreased adherence in non-resource-limited settings, discontinuation of antiretroviral therapy in resource-limited settings may be related to transportation, other health care costs, and depression. Formal screening for adherence and addressing costs such as transportation and additional health care expenditures may improve adherence in resource-limited settings.</td>
</tr>
</tbody>
</table>

Table 4: Key Findings and Potential Clinical Implications (continued)

Nonadherence to antiretroviral treatment is high in some resource-limited settings. In addition to factors traditionally associated with decreased adherence in non-resource-limited settings, discontinuation of antiretroviral therapy in resource-limited settings may be related to transportation, other health care costs, and depression.

Antiretroviral Exposure in Pregnancy.

Peytavin and colleagues (Abstract 579) conducted a case-controlled study to evaluate the effect of pregnancy on lopinavir/ritonavir pharmacokinetics. Lopinavir trough levels were monitored in 100 HIV-infected women in the second and third trimester, respectively, 5122 ng/mL in the second and third trimester, respectively. Lower levels have
been associated with inadequate virologic suppression. In contrast, Khoung-Jones and colleagues (Abstract 743) found that lopinavir levels were adequate (C_{min}, 5300 ng/mL) in 36 pregnant women who received the tablet formulation of lopinavir/ritonavir.

Burger and colleagues (Abstract 741) conducted an uncontrolled study of 14 pregnant women receiving saquinavir 1000 mg/ritonavir 100 mg twice daily plus 2 nRTIs. They found that all 14 women achieved adequate levels of saquinavir that were comparable with published data.

Ripamonti and colleagues (Abstract 742) presented data on 9 pregnant women receiving atazanavir/ritonavir during the third trimester of pregnancy. The pharmacokinetic parameters obtained during the third trimester were similar to those seen at 8 to 16 weeks postpartum. Cord blood levels of atazanavir were 220 ng/mL, approximately 10% that of concurrent maternal plasma levels. Natha and colleagues (Abstract 750) collected trough concentrations of atazanavir from 15 women receiving atazanavir/ritonavir during pregnancy and found a mean trough level of 421 ng/mL. All but 1 woman were above the minimum target level of 100 ng/mL.

Read and colleagues (Abstract 740) investigated the pharmacokinetics of nelfinavir among women in the third trimester of pregnancy compared with postpartum levels. Trough concentrations were lower during pregnancy and were suboptimal for most women at both time points. In addition, the metabolism of nelfinavir was statistically significantly altered during pregnancy. The AUC of M8, the virologically active metabolite of nelfinavir, was 80% lower during pregnancy than postpartum, further compromising the efficacy of this drug.

Antiretroviral Concentrations in Breastfeeding Infants. Substantial evidence was presented at this year’s conference that formula feeding as a strategy for PMTCT of HIV is associated with a higher rate of mortality than is breastfeeding (see “HIV Epidemiology and Prevention Interventions” in this issue). The Kisumu breastfeeding study in Kenya (Abstract 72) is a phase II, open label study providing nevirapine, lamivudine, and zidovudine in HIV-1-seropositive women for PMTCT during breastfeeding. An analysis of concentrations of antiretrovirals in maternal plasma, breast milk, and infant plasma in the 67 mother-infant pairs enrolled showed statistically significant variability of concentrations in each compartment. Maternal plasma and breast-milk concentrations of zidovudine were low (medians of 25 and 9 ng/mL, respectively) and median plasma concentration in infants was below the assay level of detection. Lamivudine concentrations were higher in breast milk and maternal plasma and the median plasma concentration in infants was 25 ng/mL, equal to the median inhibitory concentration (IC_{50}) but less than the optimal dose for virologic suppression. Nevirapine concentrations in maternal breast milk and plasma were even higher than that of lamivudine, and median infant plasma concentrations (911 ng/mL) were well above the IC_{50} but below the target dose for virologic suppression. The authors concluded that providing antiretroviral prophylaxis for mothers of breastfeeding infants may be effective for PMTCT but there are risks of adverse effects and the possibility of resistance in infants who become infected. The variable pharmacokinetics of antiretroviral use in nursing mothers warrants further research.

Conclusion

This year’s conference maintained its reputation as the premier forum for presentation of new information in the field of antiretroviral therapeutics (see table 4). The likely additions of maraviroc and raltegravir, representing 2 new drug classes, to the list of FDA-approved antiretroviral drugs will improve our ability to maintain maximum virus suppression even in highly treatment-experienced patients. In addition, further clinical research involving these agents and others in their classes may well change current paradigms of therapy. Although the complexity of antiretroviral therapy and HIV-1 drug resistance is increasing, data presented at this year’s conference clearly demonstrate that public health approaches to delivery of antiretroviral therapy in resource-limited settings can be highly successful. Challenges in the field remain formidable but the basic and clinical research horizons in antiretroviral therapeutics are bright, and provide hope that these challenges can be met for the benefit of the nearly 40 million HIV-1-infected persons worldwide.

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Additional References


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20. 7SL RNA is a Cofactor of the Antiviral Cytidine Deaminase APOBEC3G. Tao Wang, C Tian, K Luo, T Sarkis, Y Yu, and XF Yu.


46. Structural Basis for Membrane Targeting by the HIV-1 Gag Protein. Michael Summers, JS Saad, J Miller, J Tai, A Kim, and RH Ghanam.

47. TIP47, a Cellular Cofactor Involved in HIV-1 Envelope Incorporation into Virions. Clarisse Berloiz-Torrent.


49. Functions of APOBEC3 Complexes and P Bodies. Tariq Rana.

54. What’s Driving the European HIV Epidemic? Anne Johnson.


56. The STD-HIV Connection from Research to Action: Are We Lost in Translation? Judith Wasserheit.


72. Plasma ARV Concentrations in Breastfeeding Infants Whose Mothers Are Receiving HAART. Mark Mirochnich, T Thomas, E Capparelli, C Zeh, D Holland, R Masaba, P Odhiambo, M Fowler, P Weidle, and M Thuggen.

78. The Effect of Cotrimoxazole Prophylaxis and Insecticide-treated Bednets on the Risk of Malaria among HIV-infected Ugandan Children. Anne Gasasira, M Kamya, J Achan, T


90. 3'-Azido-5'-Dideoxythymidine Selects Mutations in the Connection (A371V) and RNase H (Q509L) Domains of Reverse Transcriptase that Increase AZT Resistance in Combination with Thymidine Analog Mutations without Affecting the Rate of AZT Excision on a DNA/DNA Template/Primer. Jessica Brehm, D Koonzt, S Zelina, N Sluis-Cremer, and J Melors.


105aLB. Results of BENCHMRK-1, a Phase III Study Evaluating the Efficacy and Safety of MK-0518, a Novel HIV-1 Integrase Inhibitor, in Patients with Triple-class Resistant Virus. David Cooper, J Gatell, J Rockstroh, C Katlama, P Yeni, A Lazzarin, J Chen, R Isaacs, H Teppier, B Nguyen, for the BENCHMRK-1 Study Group.

105bLB. Results of BENCHMRK-2, a Phase III Study Evaluating the Efficacy of MK-0518, a Novel HIV-1 Integrase Inhibitor, in Patients with Triple-class Resistant Virus. R Steigbigel, P Kumar, J Ervin, M Schecter, M Markowitz, M Loufy, J Zhao, R Isaacs, B Nguyen, H Teppier, for the BENCHMRK-2 Study Group.

106LB. Update on the CONRAD Cellulosic Sulfate Trial. Gustavo Doncel and Lut van Damme.


113. This Is Your Brain On Drugs: Neurocognitive Function before and after ART Discontinuation in Patients with High CD4 Nadir (ACTG A5170). Kevin Robertson, Z Su, A Krambrink, S Evans, D Havlir, D Margolis, D Skiest, and ACTG 5170 team.

114. HIV-1-associated Dementia Associated with HIV DNA within Monocytes in Subjects Treated or Not Treated with Antiretroviral Medications. Bruce Shiramizu, C Shikuma, S Ratto-Kim, J Anaranwianich, and V Valcar.


128. rHIL-7 in HIV-1-infected Subjects with CD4 T-cell Count < 100cells/µL and Viral Load < 50,000 copies/mL: Results from a Randomized, Placebo-controlled, Double-blind Study (ACTG5214). Irini Sereti, E Aga, J Spritzer, A Landay, S Pawha, M Fischl, D Asmuth, A Tenorio, R Buffet, M Lederman, and ACTG 5214 team.

130. Acute Hepatitis C in Men Who Have Sex With Men Is Not Confined to Those Infected with HIV, and Their Number Continues to Increase. Martin Fisher, D Richardson, and C Sabin.

133. HIV-specific T Cells Accumulate in the Liver in HCV/HIV Co-infection. F Yue, B Valli, D Wong, J Heathcote, C Kovacs, M Loffaty, R Gray, R Halpenney, and Mario Ostrowski.


145. HIV among Adolescents and Young Adults in the US and Implications for Biomedical Prevention Trials. Jonathan Ellen.


155bLB. The Effects of Male Circumcision on Genital Ulcer Disease and Urthral Symptoms, and on HIV Acquisition: An RCT in Rakai, Uganda. Maria Wawer, R Gray, G Kigozi, F Nalugoda, T Quinn, F Makumbi, D Serwadda.

156. The Role of Nef in Primate Lentiviral Pathogenesis. Frank Kirchhoff.

167. Primary Human Eosinophils Are Highly Susceptible to Productive Infection by X4 HIV-1. Dawn Wooley and J Marathe.


188. Inhibition of Retrortransposition by APOBEC3 Proteins Requires a Catalytically Functional Active Site. Hui Chen, Y Hakata, N Sunseri, and N Landau.


204. Comprehensive Mutational Analysis of APOBEC3G. Melissa Farrow, E Geoghegan, D Espinola, S McKerman, and A Sheehy.

206. The Incorporation of APOBEC Proteins into HIV-1 and MLV Li Zhang and S Cen.

211. Further Insight into the Antiviral Activities of APOBEC3G and APOBEC3F. Rebecca Holmes, F Koning, K Bishop, and M Malim.

216. Examination of TRIM5α Effect on HIV-1 Capsid Core Uncoating and Degradation Using a Synchronized Viral Entry Assay. U Chatjeri, M Bobhardt, and Philippe Gallay.

217. HIV-1 Does Not Induce an Intrinsinc RNai Antiviral Response in Mammalian Cells. Jennifer Lin and B Cullen.

227. TLR Stimulation Increases HIV-1 Replication in Memory CD4+ T Cells. Sandra Thibault, M Tariff, and M Tremblay.

228. Transcriptional Interference: A Molecular Mechanism of HIV-1 Latency. Yefei Han and R Silicano.

250. Extension of gp120 Env Co-receptor Use to CXCR4 Is Associated with Disease Progression in UNPH Infection with rDefective HIV-1. A Crotti, S Ghezzi, E Santagostino, and Elisa Vicenzi.


258. The CCR7 Ligands CCL19 and CCL21 Increase Permissiveness of Resting CD4+ T Cells to HIV Infection. Saha Saleh, A Solomon, P Wightman, M Xhilaga, P Cameron, and S Lewin.


274. HIV-1 Integration into Resting CD4+ T Cells Can Be Enhanced and Provides a System to Study Viral Latency. Gabriela Plesa, J Riley, J Dai, C June, and O’Doherty.


304. Microbial Products that Bind Toll-like Receptors Promote T-cell Activation and Turn-over—a Model for HIV Immunopathogenesis. Nicholas Funderburg, A Luciano, W Jiang, S Sieg, and M Lederman.

325. G to A Changes Years after Primary HIV Infection Are Associated with Baseline Viral Activity. I Abowede, B Mangeat, I Kaiser, D Trono, L Perrin, and Sabine Yerly.


348. Slower CD4 Cell Decline following Cessation of a 3-Month Course of HAART in Primary HIV Infection. Sarah Fidler, G Touloumi, J Fox, N Fantazis, K Porter, A Babiker, J Weber, and CASCADE Collaboration.


C Jack, C Zhou, and Ahmet Hoke.


388. A Proteomic Fingerprint of Cerebrospinal Fluids from Individuals with HIV-1-associated Dementia. W Rozek, S Holloway, M Ricardo-Dukelow, L Melerendez, and Pawel Ciborowski.


477. The TILT Trial — A Pilot Trial of ART Intervention with and without the Use of Interleukin-2. Brian Angus, F Lampe, G Tambussi, C Katlama, M Youle, I Williams, B Clotet, M Fisher, F Post, A Babiker, and TILT Trial Steering Committee.


500. Small Molecule Inhibitors of Histone Deacetylases as a Means to Induce HIV Expression from Latently Infected CD4 T Cells. Shannon Weiman, V Terry, A Espejeth, D Haza, D Richman, and C Spina.

501. Expression of Latent HIV Induced by a Selective Class I HDAC Inhibitor Nancy Archin, A Espejeth, A Duff, M Chee, D Parker, D Hazuda, and D Margolis.


512. Proof of Concept of Antiretroviral Activity of AMD11070 (an Orally Administered CXCR4 Entry Inhibitor): Results of the First Dosing Cohort A Studied in ACTG Protocol A5210. Michael Saag, S Rosenkrantz, S Becker, K Kling-


144-Week Clinical and Immunological Outcome of HIV-1-infected Subjects Receiving Lamivudine Monotherapy or Treatment Interruption. Antonella Castagna, A Danise, L Galli, S Tiberi, N Gianotti, C Vinci, G Fusetti, E Semirani, A Sorra, and A Lazzarin.

Predictors for CD4 Cell Count Increase for Patients with Sustained Viral Load Suppression within 1 Year after Start of cART: The Swiss HIV Cohort Study. M Wolbers, M Battegay, B Hirschl, H Furrer, M Cavasini, R Weber, P Vernazza, E Bernasconi, G Kaufmann, Heiner Burcher, and The Swiss HIV cohort study (SHCS).


Response to Combination ART: Variation by Age. Caroline Sabin and the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) Study.


Cumulative Risk of Extensive Triple-class Virologic Failure in 10,603 Patients followed as Long as 10 Years from the Start of ART: Andrew Phillips, A Wilson, C Leen, and The UK CHIC Study Group.

Clinical Outcomes and Emerging Challenges after 5 Years of ART in a South African Township. Giles Van Cutsem, K Hilderbrand, D Coetzee, E Goemaere, and A Boulle.


Usefulness of CD4 Cell Count Changes in Predicting Virological Failure in Patients Receiving HAART in a Resource-poor Setting. M Badri, S Lawn, and Robin Wood.


Assessment of Pharmacokinetic/Pharmacodynamic Relationships through 48 Weeks from a Study in HIV+ ART-naive Subjects Receiving ARV Regimens Containing Atazanavir 400 mg or Atazanavir/Ritonavir 300/100 mg Once Daily. Richard Bertz, Y Wang, L Mahnke, A Persson, E Chung, M Mathew, S Agarwala, D Filoramo, J Hammond, and D Grasela.


Dynamics of NNRTI-resistance Mutations during Treatment Interruption after Failure of NNRTI-containing Regimens. Francesca Ceccherini-Silberstein, V Svicher, M Zaccarelli, M Santoro, M Triggiani, E Bourin, P De Longis, P Narciso, A Antonino, and CF Perno.


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601. TTNTRNS: An Amino Acid Insert near the p17/p24 Gag Cleavage Site Associated with Resistance to PI. Manabu Aoki, H Aoki, T Miyakawa, and H Mitsu ya.

602. An Investigation into the Influence of the T184I-associated V82L/G83A Mutations on the Susceptibility to Darunavir and Breccanavir. R Elston, Daniel Kuritzkes, and R Bellh.

606. Selection in vitro of HIV-1 Variants Highly Resistant to Darunavir Using a Mixture of HIV-1 Isolates Resistant to Multiple PI. Yasuhiro Koh, T Towara, A Ghash, and H Mitsu ya.


609. Prior Utilization or Resistance to Amprenavir at Screening Has Minimal Effect on the 48-Week Response to Darunavir/ritonavir in the POWER 1, 2, and 3 Studies. Gaston Picchio, T Yangeneuglid, B Van Baelen, E Lefebvre, D Miralles, and M de Bethune.


618. HIV-1 Co-receptor Switch Induced by Antagonism to CCR5. Gemma Moncunill, M Armand-Ugín, B Cilot, and J Esté.


625. Natural Variation of HIV-1 Group M Integrase: Implications for Integrase Inhibitor Therapy. R Zion, S Rhee, T Liu, and R Shafer.


627. Resistance Profile of HIV-1 Mutations in vitro Selected by the HIV-1 Integrase Inhibitor, GS-9137 (JTK-503). G Jones, R Ledford, F Yu, M Miller, M Tsang, and Damian McColl.


657. Acquisition of Transmitted Drug-resistant HIV Infection Is Associated with the Presence of Sexually Transmitted Infections. Kate Namhia, M Fisher, D Pao, D Sudarshi, J Reeves, G Dean, G Murphy, J Parry, and D Pillay.


661. Emerging ART Drug Resistance in Subtype C. Experience from the 2 Clinics in Jo-
hanneshburg, South Africa. Carole Wallis, C Bell, R Bouline, I Sanne, F Venter, M Papathanasopoulos, and W Stevens.


701. Markers for Predicting Mortality in HIV-infected Children in Resource-limited Settings. Diana Gibb, T Duong, and 5Cs4Kids Cohort Collaboration.


795. High Rates of Non-fatal Toxicities in a
24-Month Cohort Receiving Publicly Funded HAART in South Africa. Emily B Wong, D Murdoch, J Wing, C Feldman, and W Venter.


854. Tenofivir Treatment Is Associated with a Decrease in Calculated Glomerular Filtra- tion Rates in a Large Observational Cohort. C Fux, M Simcock, M Wolbers, H Bucher, M Cavassini, M Opravil, P Vernazza, B Hirschel, E Bernasconi, Hansjakob Furrer, and Swiss HIV Cohort Study (SHCS).

855. Increased Renal Impairment in Patients Receiving TDF + PT vs TDF + NRTI. Miguel Goicoechea, S Liu, B Best, S Sun, S Jain, C Kemper, M Witt, C Diamond, R Haubrich, S Louie, and the California Collaborative Treatment Group (CCTG) 578 Team.


858. Microarray Analysis of Primary Human Osteoclast Differentiation and Activity Identifies Signaling Pathways Altered by Low Levels of Ritonavir, and Restored by Pharmacologic Levels of IFN-gamma: Relationship to HIV Therapy-mediated Osteopenia. Rozbeh Modarresi, Z Xiang, and J Laurence.


956. Regular HIV Testing is Critical for Reducing the Number of MSM with Undiagnosed HIV Infection. Paul Denning.


992. Safety Concerns for the Potential Use of Cyanovirin as a Microbicial Anti-HIV Agent. Dana Hukins, K van den Ende, C Xilong, Y Liang, D Shands, and D Schols.


996. Co-receptor Perturbation as a Possible Mechanism Underlying the Immediate and Persistent Anti-HIV-1 Activity of the Microbical Compound PEHMB. Nina Thakkar, S Miller, L Schlipf, B Wigdahl, M Labib, R Rando, T Kish-Catalone, and F Krebs.

997. Epitope Mapping of the Candidate Microbicide PRO 2000 against HIV gp120. Darpan Dhawan, B Zerouni-Layachi, M Ortega, M Tuen, C Hsie, and M Klotman.


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