Highlights of the 13th Conference on Retroviruses and Opportunistic Infections

Developments in Basic Science Research

Mario Stevenson, PhD

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About This Issue

This issue provides our review of the 13th annual Conference on Retroviruses and Opportunistic Infections, held this year from February 5 to 8 in Denver, Colorado. Mario Stevenson, PhD, reviews developments in HIV basic science research. R. Paul Johnson, MD, discusses advances in HIV pathogenesis research and vaccine development. Susan Buchbinder, MD, presents recent findings in epidemiology and prevention. Scott Letendre, MD, and Ronald J. Ellis, MD, PhD, explore the neurologic complications of HIV disease and their treatments. Judith S. Currier, MD, and Diane V. Havlir, MD, examine new findings on metabolic complications of HIV disease and antiretroviral therapy. Finally, Magdalena E. Sobieszczyk, MD, Joyce Jones, MD, Timothy Wilkin, MD, MPH, and Scott M. Hammer, MD, highlight new findings in antiretroviral therapy, treatment strategies, and drug resistance.
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Developments in Basic Science Research

Mario Stevenson, PhD

The 13th Conference on Retroviruses and Opportunistic Infections featured a strong basic science program that provided new findings on the nature of HIV disease as well as surprises regarding the antiviral action of innate restriction factors.

Intrinsic Cellular Restrictions and Viral Replication

In the past couple of years, the identification of cellular proteins that potentially restrict infection of cells by primate lentiviruses has revealed the extent of the conflict between the virus and its host. The cellular and humoral arms of the immune system are generally considered to comprise the major defense strategy of the host against viruses such as HIV-1. However, it is now apparent that there are additional lines of defense against infection by primate lentiviruses and that cellular proteins including APOBEC 3 family members and TRIM 5α are major players in an intrinsic antiviral defense strategy commonly referred to as innate cellular restriction. APOBEC 3 family members, and in particular APOBEC 3G and 3F, are cytidine deaminases that are packaged into virions and upon infection of a cell introduce catastrophic G-to-A hypermutations that compromise the integrity of the viral cDNA. The primate lentiviral Vif proteins counteract the cytidine deaminases by targeting them for proteosomal degradation. As a result, there is very little APOBEC packaging in virions and viral reverse transcription is not compromised.

Research from a number of laboratories has established a working model in which the enzymatic activity of APOBEC is necessary for its antiviral effect. To exert an antiviral effect, APOBEC must be packaged within virions. Studies summarized by Greene and colleagues (Abstract 61) reveal a novel antiviral mechanism for APOBEC and one that may explain a poorly understood aspect of primate lentivirus biology. Dr Greene’s group has identified a low molecular mass (LMM) form of APOBEC 3G that is enzymatically active and is present in the cytoplasm of resting CD4+ T cells and peripheral blood monocytes. Truly quiescent (G0 stages of cell cycle) CD4+ T lymphocytes and peripheral blood monocytes have long been recognized as being refractory to productive HIV-1 infection. Studies presented by Dr Greene suggest that it is this enzymatically active LMM form of APOBEC 3G that accounts for the resistance of quiescent lymphocytes and monocytes to HIV-1 infection. When APOBEC 3G was silenced in quiescent lymphocytes using RNA interference, quiescent lymphocytes became susceptible to productive HIV-1 infection (Chiu et al, Nature, 2005). When quiescent lymphocytes are stimulated to enter cell cycle or when monocytes differentiate to macrophages, they become permissive to productive HIV-1 infection.

Dr Greene likewise demonstrated that lymphocyte stimulation and monocyte differentiation also result in incorporation of APOBEC 3G into a high molecular mass (HMM) RNA-protein complex that is enzymatically inactive. Resting tonsilar lymphocytes were also found to contain an HMM APOBEC 3G complex which would explain the ability of resting lymphocytes to support HIV-1 infection in vivo.

These experiments present a paradox. The current model is that APOBEC 3G must be packaged in virions to inhibit viral infectivity (by compromising reverse transcription). If APOBEC 3G is present as an HMM enzymatically inactive complex in cycling and permissive cells, why is it able to affect viral replication? The answer appears to be that APOBEC 3G is packaged as an HMM complex but the complex is subsequently degraded into an enzymatically active LMM form.

Dr Greene presented evidence that the viral RNAase H activity of reverse transcriptase is responsible for degrading the RNA that maintains the integrity of the HMM form of APOBEC 3G thereby reducing it to an LMM form that is enzymatically active. Dr Greene also presented the results of a proteomic approach that his laboratory has taken to characterize the components of the HMM complex of APOBEC 3G. More than 60 cellular proteins were associated with the HMM APOBEC 3G complex and were found to be similar in composition to a previously described high molecular weight complex known as a Staufen granule. Staufen is a protein that is involved in localizing small mRNAs during oogenesis and early central nervous system development in Drosophila. The mammalian Staufen protein harbors several conserved double-stranded mRNA-binding domains and forms granules that are transported to distal dendrites during neuronal maturation. These granules also colocalize with ribonuclear particles that transport small RNAs to the dendrites. Collectively, these observations provide new and intriguing insights into the defenses that are levied against primate lentivirus infection.

Several aspects of the mechanism by which APOBEC 3 proteins are packaged in virions remain unanswered. Several studies (Abstracts 212, 213, 214, and 218) focused on a potential mechanism for APOBEC 3 packaging. Two main models were described that involve the interaction of APOBEC 3 with genomic viral RNA or the interaction of APOBEC with...
structural virion proteins including nucleocapsid and Gag. Although HIV-1 Vif, which is the viral counter defense against the antiviral activity of APOBEC 3 proteins, is a highly attractive target for therapeutic intervention, this target is yet to be exploited.

Abstract 200 identified Vif and APOBEC 3G peptides that inhibit the interaction of APOBEC 3G with HIV-1 Vif in vitro. Although these peptides are being used to derive structural and functional information about the mechanism by which these proteins interact, these peptides will be important reagents in the design of small molecule inhibitor screens for the identification of novel HIV-1 therapies.

TRIM 5α is another recently identified cellular protein that restricts infection of a variety of viruses including HIV-1. The mechanism by which TRIM 5α restricts infection is not fully understood. However, TRIM 5α acts at an early stage in viral replication and most likely compromises the uncoating step of the virus in which genomic viral RNA is released into the cytoplasm after disassociation of the viral capsid core. Like APOBEC 3 proteins, TRIM 5α exhibits species-specific antiviral activity. For example, HIV-1 infection is inhibited by TRIM 5α from some monkey species but not by human TRIM 5α. Intriguingly, owl monkey TRIM 5α restricts HIV-1 because a portion of their TRIM 5α, the B30.2 domain, was replaced with cyclophilin A through a pseudogene insertion. Cyclophilin A has previously been demonstrated to interact with the capsid protein of HIV-1. Therefore, fusion of cyclophilin with owl monkey TRIM 5α allows cyclophilin A to tether TRIM 5α to HIV-1 Gag. Studies presented in Abstract 59 examined, therefore, whether the B30.2 domain may be involved in mediating the interaction of TRIM 5α proteins with capsid. By fusing different TRIM alleles to cyclophilin A, the investigators were able to examine the ability of other TRIM proteins to restrict HIV-1 infection. These studies also revealed that TRIM 5α may exhibit an antiviral effect at 2 different stages in HIV-1 infection and that restriction factor binding has different mechanistic outcomes.

Abstract 207 presented information on the significance of TRIM 5α cytoplasmic bodies. It has recently been suggested that TRIM 5α localizes to cytoplasmic pods perhaps as high molecular weight complexes. However, the significance of this to restriction of HIV-1 infection is unknown.

In a study from Dr Hope’s group, TRIM 5α was fused to a fluorescent protein in order to study its subcellular localization. In the presence of a proteosome inhibitor, there was an increase in the size and decrease in the number of cytoplasmic bodies formed by fluorescent TRIM 5α. However, expression and turnover of TRIM 5α was not affected. Therefore, the proteosome appears to regulate the biology of TRIM 5α.

Several studies (Abstracts 60, 201, 205, 206) presented evidence on the evolutionary consequences of TRIM 5α variation. Genes encoding proteins that influence susceptibility to pathogens are predicted to be subject to rapid evolution. Malik and colleagues noted that evolution of TRIM 5α as well as APOBEC 3G predates the evolutionary origin of primates (approximately 35 million years) and as a result predates the origin of primate lentiviruses. The authors suggest that endogenous retroviruses may have been responsible for the evolutionary pressure that drove TRIM 5α and APOBEC 3G evolution. Current attempts to screen human populations for genetic variation in TRIM 5α and other genes will lead to identification of novel restriction factors that provide defense against retroviruses and lentiviruses.

Primate lentiviruses and lentivirus vectors infect nondividing cells. As a consequence, primate lentiviruses can infect terminally differentiated macrophages in the tissues. In contrast, only dividing cells are permissive to murine leukemia virus (MLV) infection. The prevailing hypothesis is that the lentiviruses infect nondividing cells because they harbor nucleophilic proteins that allow the viral cDNA to translocate through the nuclear envelope in a nondividing cell. Although a number of nucleophilic viral proteins have been proposed (including Gag MA, Integrase, Vpr, and a triple-stranded cDNA intermediate known as the DNA flap), it is unclear which, if any, of these candidates dictates the ability of HIV-1 to infect nondividing cells. Studies presented in Abstract 58 provide evidence for an alternative model for the ability of HIV-1 to infect nondividing cells. Studies conducted by Emermen and colleagues demonstrated that transfer of the p12 and CA portions of Gag from MLV into HIV impaired the ability of HIV-1 to infect nondividing cells suggesting that p12 and CA distinguish this fundamental viral characteristic. The authors suggest a model in which capsid masks determinants within the viral genome that influence the ability to infect nondividing cells. They further suggest that the ability of HIV to infect nondividing cells may be dependent upon the uncoating step.

The identification of APOBEC 3G and TRIM 5α as potent primate lentivirus restrictions has provided the impetus for research aimed at identifying novel additional restriction factors. Abstract 135 presented evidence for a novel restriction that potently blocks HIV-1 infection. Kewalramani and colleagues expressed a cDNA library in cells permissive to HIV-1 infection in order to identify cells that acquired resistance to infection. One such HIV-1-resistant subclone contained a C-terminally truncated form of CPSF6 that restricted infection by X4- and R5-tropic HIV-1, HIV-2, and SIV but not by MLV. Propagation of HIV-1 in cells expressing truncated CPSF6 led to the emergence of CPSF6-resistant HIV-1 variants containing a mutation in gag. Truncated CPSF6 appeared to interfere with late steps in viral reverse transcription or with stability of nascent viral cDNA. The wild-type form of CPSF6 did not interfere with HIV-1 infection. Thus, the C-terminal truncation that was artificially created in CPSF6 during construction...
of the cDNA library conferred antiviral activity upon CPSF6. Nevertheless, further understanding of the mechanism of antiviral restriction by truncated CPSF6 will provide important insight into the regulation of viral reverse transcription or cDNA stability and may ultimately reveal novel therapeutic targets in the viral replication cycle.

### Viral Reservoirs and Mechanisms of Persistence and Latency

The ability of primate lentiviruses to persist within infected individuals reflects the life span of the cellular reservoirs that support HIV-1 replication. Although highly active antiretroviral therapy (HAART) has been effective in sustaining suppression of viral replication to below detectable levels for extended intervals, there is a rapid rebound of viremia if therapy is discontinued. The prevailing view is that rebound of viremia if therapy is discontinued for extended intervals, there is a rapid rebound of viremia if therapy is discontinued. The prevailing view is that rebound of viremia if therapy is discontinued. The prevailing view is that rebound of viremia if therapy is discontinued. The prevailing view is that rebound of viremia if therapy is discontinued. The prevailing view is that rebounds of viremia if therapy is discontinued. The prevailing view is that rebounds of viremia if therapy is discontinued.

Abstract 242 presented evidence that HIV-1 latency is determined post-transcriptionally. Primary resting CD4⁺ T lymphocytes were obtained from HIV-1-infected individuals on HAART who had undetectable viral loads. These cells were found to harbor low steady-state levels of full-length multiply spliced and unspliced HIV-1 RNA. Nevertheless, these cells did not produce virus. The authors found that the viral RNA was sequestered in the nucleus. The authors further demonstrated that expression of a polypyrimidine tract binding protein (PTB) in latently infected cells restored cytoplasmic accumulation of viral RNA and subsequent virus production. This study defines a novel mechanism for HIV-1 latency. This study may also aid in therapeutic strategies that attempt to purge latently infected cells by stimulating the exit of the virus from latency.

A somewhat different take on the mechanism by which HIV-1 persists in the presence of therapy was presented in Abstract 168. Chun and colleagues have been examining virus activity in aviremic individuals on long-term suppressive HAART. In contrast to current models suggesting that the virus is harbored in a latent state, Chun presented evidence that highly virologically suppressed individuals harbor activated lymphocytes that chronically produce infectious HIV-1. Furthermore, phylogenetic analysis of viral sequences in the activated lymphocyte population and in resting cells indicated that viruses released from activated cells were infecting resting cells even in the presence of HAART. In contrast to the current latency models, this suggests that viral replication may persist in the face of HAART and that ongoing infection may continually replenish viral reservoirs. This study has several important implications. The ability of the virus to replicate in the presence of HAART may indicate that current therapies are not completely suppressive. If this is the case, therapeutic intensification by, for example, incorporating the next generation of drugs (coreceptor inhibitors and integrase inhibitors) into regimens may begin to address the question of whether therapy intensification interrupts viral persistence.

Activated memory CD4⁺ T lymphocytes that coexpress CCR5 are considered to be the principle targets for HIV-1 and SIV replication in the host. In humans, the majority of these cells are contained within the intestinal tract and, as recently demonstrated by several groups (for a review, see Veazey et al., *Nat Med*, 2005), massive acute viral replication in the intestine profoundly depletes these cells within a few weeks of infection. As a consequence, acute infection and viremia is considered to be a pivotal event in the natural history of HIV-1-mediated immunodeficiency. Paradoxically, SIV infection of natural monkey hosts such as sooty mangabeys is nonpathogenic despite significant viremia and lymphocyte turnover. In contrast to humans, CCR5-expressing memory CD4⁺ cells are rare in the gut of sooty mangabeys. Therefore, to examine whether there was a rate-limiting number of permissive cells for SIV infection that might explain why they exhibit a nonpathogenic infection, several studies (Abstracts 36, 37, 40, and 167) examined whether nonpathogenic infection exhibited any of the profound and acute intestinal lymphocyte depletion characteristics of pathogenic HIV-1 infection. Remarkably, analysis of naturally SIV-infected sooty mangabeys after acute infection revealed a similar massive depletion of CD4⁺ T lymphocytes in the mucosal lymph node tissue. A similar pattern of massive intestinal lymphocyte depletion was observed in SIV-infected African green monkeys in which SIV is nonpathogenic. Therefore, lack of disease in natural SIV infections cannot be explained by limited availability of permissive substrates for viral replication in the gut, and profound and acute lymphocyte depletion is a characteristic of both nonpathogenic SIV infection as well as pathogenic SIV and HIV-1 infection. This research is sure to drive an intense investigation into why lymphocyte destruction can be tolerated in naturally infected monkeys since this will further our understanding of why HIV-1 is pathogenic.

In addition to CD4, HIV-1 infection of a cell requires coreceptor molecules of which CCR5 and CXCR4 are the most widely used. Viruses are classified according to X4 (CXCR4-using) or R5 (CCR5-using) variants with R5 variants being described as macrophage tropic. Abstract 134 presented evidence that challenges this.

Instead of virus isolation, Clapham and colleagues directly amplified envelope genes from various tissue sources of infected individuals and examined the in vitro tropism of viruses that contain these chimeric virus envelopes. The investigators demonstrated that viruses harboring R5 envelopes differed from one another by more than 1,000-fold in their ability to infect primary macrophages. Instead, the ability to exploit low levels of CD4 was a more accurate determinant of macrophage tropism. Furthermore, there was a
striking absence of macrophage-tropic viruses in lymph nodes, blood, and semen. Therefore, macrophage-tropic viruses may define a subset of viral variants that are specifically restricted to certain tissues such as the brain. In contrast, R5 tropism likely reflects tropism for lymphocytes that express varying amounts of CCR5. The classification of viruses on the basis of R5 and X4 tropism warrants re-evaluation.

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A list of all cited abstracts appears on pages 63 to 70.

Additional References


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HIV Pathogenesis and Vaccine Development

R. Paul Johnson, MD

Cautionous optimism was a recurring theme of many of the AIDS vaccine-related presentations at the 13th annual Conference on Retroviruses and Opportunistic Infections. Several investigators suggested that the ability of HIV to escape cytotoxic T-lymphocyte responses may be more limited than previously thought, and encouraging results were presented regarding the ability of consensus ancestral sequences or polyvalent vaccines to increase the breadth of induced immune responses. A number of studies highlighted the potential efficacy of neutralizing antibodies: data from 2 groups suggested that neutralizing antibodies may play a role in preventing superinfection and previously unrecognized neutralizing epitopes were identified in the membrane proximal external region of envelope. Two studies documented that immunization with polyvalent simian immunodeficiency virus vaccines can induce sustained control of viremia following repeated low-dose mucosal challenge with pathogenic SIVmac strains and provided hope for the potential of T-cell–based vaccines to slow disease progression.

HIV Sequence Variation and its Implication for Vaccine Design

The remarkable genetic diversity displayed by HIV represents one of the major barriers to the development of an AIDS vaccine. In the Bernard Fields Memorial Lecture, Korber highlighted the intricate interplay between HIV sequence variation and host immune responses (Abstract 13). Since its introduction into the human population approximately 70 years ago, HIV has developed tremendous genetic diversity, resulting in the evolution of multiple distinct clades of viruses that can vary by more than 38% in the envelope gene. The precise nature of the selective pressures that have fostered the evolution of these clades remains a matter of debate. One leading theory is that selective pressure exerted by T-cell responses may have been a major factor guiding the evolution of these distinct clades. By generating phylogenetic trees using only the silent bases in HIV that do not direct the synthesis of amino acids, Korber demonstrated that a similar clade structure is evident, thus strongly suggesting that selection by human leukocyte antigen (HLA) molecules (which would be expected to select for nonsynonymous substitutions encoding escape mutations) is not the major driving force in the evolution of these clades. However, Korber noted that this finding does not exclude a role for immune selective pressure on selected portions of the genome.

The fact that the current HIV diversity has arisen as a result of progressive divergence from common viral ancestors has important implications for the design of AIDS vaccines: this observation implies that a common ancestral sequence may be closer to divergent contemporaneous sequences than any of these contemporaneous sequences are to each other. This hypothesis has prompted intensive efforts over the past several years to develop consensus or central sequence vaccines that have been artificially constructed to mimic the predicted ancestral sequence. An envelope consensus sequence (designated Con6) has been created by Korber, Hahn, and colleagues for the M group clade. Initial results demonstrate that the Con6 envelope can bind to CD4 and CCR5, can mediate viral entry, and can be recognized by neutralizing antibodies. Recent unpublished results reveal that mice vaccinated with this M group consensus envelope develop significant levels of cross-clade T-cell responses and that the elicited neutralizing antibodies may have a greater breadth of neutralization than those induced by wild-type envelope.

Although the increased breadth of T-cell and antibody responses induced by the consensus vaccines to date are encouraging, they are modest and may only be partly effective in combating HIV sequence diversity. An alternative approach would be to generate a mosaic or polyvalent T-cell vaccine that contains a mixture of variant peptides at key positions that vary in natural infection, which Korber termed toggle peptides. The design of these toggle peptides is facilitated by the fact that in relatively conserved HIV proteins such as p24 or Pol, the variable amino acids are limited in repertoire and tend to occur in only a subset of positions. Use of these synthetic toggle peptides to detect T-cell responses in HIV-infected subjects resulted in a 2.5-fold increase compared with consensus peptides. Korber postulated that the creation of a mosaic cocktail of overlapping viral peptides might serve as a very effective means to generate a diverse array of immune responses. Although this approach clearly has theoretical advantages, the key test will be whether immunization with a mosaic vaccine is able to induce a T-cell response with greater breadth than monovalent vaccines.

Korber also provided a reanalysis of a previously reported association between HIV mutations in Pol and specific HLA alleles that suggested that T-cell–mediated selection pressure was a major force in the evolution of HIV diversity. Korber presented compelling data that many of these apparent associations between sequence mutations and HLA alleles were in fact the result of association with distinct clades rather than a result of HLA-driv-
en mutations. Of more than 500 potential sites of selective evolution, only 6 mutations were clearly identified as being likely to have been mediated by cytotoxic T-lymphocyte (CTL) escape. This reanalysis suggests that the complex array of selective pressures on HIV evolution, including fitness constraints and selection pressure exerted by diverse HLA alleles, is likely to minimize the probability of fixation of CTL escape mutations at the population level, an encouraging finding with respect to vaccine design.

Guts, Germs, and AIDS

Over the past several years, AIDS immunologists have progressively become more “gut-centric,” prompted by a number of high-profile reports that have vividly documented the fact that HIV and simian immunodeficiency virus (SIV) infections rapidly deplete CD4+ T cells from the gut and other mucosal sites early in the courses of infection. These reports have raised the question of whether the acute infection. These reports have raised the question of whether the acute infection. These reports have raised the question of whether the acute infection. These reports have raised the question of whether the acute infection. These reports have raised the question of whether the acute infection. These reports have raised the question of whether the acute infection. These reports have raised the question of whether the acute infection. These reports have raised the question of whether the acute infection. These reports have raised the question of whether the acute infection. 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roles of humoral and cellular immune responses.

Although there is now convincing evidence that many HIV-infected individuals generate neutralizing antibodies against autologous viral sequences, the role that neutralizing antibodies play in controlling viral replication in chronic HIV infection and their potential to protect against infection (or superinfection) remains unclear. Two presentations suggested that the presence of neutralizing antibody responses may help prevent against superinfection. Smith and colleagues (Abstract 91) performed a case-controlled study of 3 well-characterized subjects who were superinfected within the proceeding 13 months. The 3 subjects all had significantly lower titers of neutralizing antibodies to common laboratory strains of HIV (JRCSF and NL4-3) than 11 matched individuals who also had primary infection and ongoing sexual exposures. In addition, there was a trend for weaker neutralizing responses to autologous HIV sequences in the superinfected patients. These differences in the frequency of neutralizing antibody responses were not explained by unusual genetic dissimilarity of either the pre-existing or superinfecting sequences. Increases in both homologous and heterologous neutralizing antibody responses were observed following superinfection, but the levels of neutralizing antibodies still remained lower than in those subjects without superinfection. Comparable data were reported by Grant and colleagues (Abstract 92) who identified 4 cases of superinfection in a cohort of 104 patients with recent HIV infection. These 4 subjects had either no neutralization or only weak neutralization of autologous virus, whereas analysis of 12 individuals without superinfection revealed relatively strong autologous and heterologous neutralization. All 4 of these individuals were superinfected 1 to 3 years after their initial primary infection, leading the authors to propose that subjects who lack broad neutralizing activity may be particularly susceptible to superinfection early after primary infection. Superinfected individuals also had lower neutralization titers to laboratory strain JRCSF.

The frequent occurrence of HIV escape mutations that evade host CD8+ T-cell responses has cast doubts on whether T-cell–based vaccines would be able to mediate sustained control of HIV infection. Walker (Abstract 98) suggested that the predictable occurrence of immune escape might be exploited to facilitate the design of improved HIV vaccines. Underscoring the challenges inherent in studying HIV-infected individuals with a diverse array of infecting genotypes and host HLA alleles, Walker focused on several selected experimental settings. He first described analysis of HIV-specific CD8+ T-cell responses and immune escape in a pair of monozygotic twins who were infected simultaneously from the same donor. Genome-wide analysis of HIV-specific responses revealed a remarkable concordance of T-cell responses, which differed in only 2 of the 10 to 14 epitopes recognized by each subject. CD8+ T-cell responses were correlated not only in specificity but in magnitude as well. Both twins demonstrated characteristic evolution of escape mutations in the same immunodominant HLA-B*40-restricted response to a Pol epitope. Analysis of autologous neutralizing titers also revealed significant concordance between the infected twins. Overall, these data suggest that the evolution of HIV-specific humoral and cellular responses can be relatively predictable in the setting of a similar genetic background and viral inoculum. Transitioning from a microanalytic to a macroanalytic approach, Walker next described a detailed analysis of epitope recognition in a cohort of 515 HIV-infected subjects from South Africa. Through the combination of high-resolution HLA typing and precise epitope mapping, Walker and his colleagues were able to demonstrate that recognition of a subset of HIV peptides was highly associated with expression of specific HLA alleles such as HLA-B15. Analysis of the relationship between HLA alleles and plasma viremia revealed a strong association of B alleles with lower levels of viremia, whereas no significant association was noted with A alleles. To better understand the relationship of HLA alleles and control of viremia, Walker went on to analyze a cohort of 104 subjects with acute HIV infection who were tested 2 months after infection for recognition of a panel of HIV-optimal epitopes selected based on the donor’s HLA type. Of an average of 30 peptides tested per subject, only 10% to 15% of all defined optimal epitopes were recognized, suggesting that only a minority of potential epitopes are involved in control of acute infection. Interestingly, the immunodominance of specific epitopes was affected by the presence of other HLA alleles. Certain HLA alleles such as A24, which are normally responsible for presenting immunodominant peptides, could be “trumped” by the presence of even more immunodominant alleles such as B27 and B57. HLA alleles associated with prolonged survival (eg, B27, B57) contributed disproportionately to the early T-cell response, raising the question of which qualities were distinctive about these early responses associated with more effective control. Analysis of the affinity of T-cell responses to these epitopes revealed relatively high affinity responses in acute infection, whereas lower affinity responses were observed in chronic infection, a pattern of evolution that stands in contrast with what has been typically observed in murine infections. This suggests the possibility that the high-affinity clonotypes may be selectively lost over time in HIV-infected subjects.

Further pursuing the analysis of potential qualitative difference among CTL epitopes, Walker and colleagues performed a comprehensive analysis of more than 1000 South African individuals infected with HIV clade C viruses. They identified 8 immunodominant epitopes presented by high-frequency HLA alleles that were used to create HLA tetramers, and then correlated the frequency of tetramer-binding cells with plasma viremia in a subset of 115 subjects.
Remarkably, a negative association between the frequency of tetramer-binding cells and viral load was only observed for 1 of 8 of the epitopes. Two of the epitopes showed a positive correlation with viral load, and for 5 of the 8, no significant relationship was observed. Escape was only observed in 1 of the epitopes. These results suggest that a significant fraction of HIV-specific T-cell responses may be relatively ineffectual, as demonstrated by their inability to either mediate viral escape or suppress viral replication.

Walker highlighted another example of qualitative differences between epitopes: the distinct characteristics of CD8+ T-cell responses to an immunodominant Gag epitope that is recognized by B*5701- and B*5703-positive individuals. Evolution of escape in this Gag epitope is commonly observed in subjects who express B*5703 but not B*5701. Comprehensive analysis of the T-cell receptor (TCR) repertoire to this epitope revealed that the response was relatively narrow in subjects that express B*5701, and more diverse in subjects with B*5703. This apparently paradoxical result stands in contrast to previously reported results in SIV-infected macaques. Walker and colleagues postulate that the highly conserved TCRs in HLA-B*5701+ patients were better able to recognize the escape variants, whereas the more diverse repertoire in B*5703+ subjects is less able to recognize variants.

The Quest for Neutralizing Antibodies Against HIV—Is the Membrane Proximal External Region the Achilles Heel of HIV?

Only a handful of monoclonal antibodies are able to mediate significant neutralization activity against primary isolates of HIV-1, and many of these antibodies bind to a conserved region of gp41 that is close to the transmembrane region, termed the membrane proximal external region (MPER). An entire symposium at this year’s conference was dedicated to recent advances in the analysis of neutralizing antibodies to the HIV-1 MPER. Three neutralizing antibodies that bind to epitopes in the MPER have been characterized to date (2F5, 4E10, and Z13). Although the MPER represents a potentially attractive target for neutralizing antibodies, a number of issues have arisen in attempts to induce antibody responses to this region, including difficulties with accessibility, relatively low immunogenicity, the requirement that MPER epitopes be complexed with lipid for effective binding, and the question of whether generation of antibodies to MPER epitopes might be limited by self mimicry.

Zwick and colleagues (Abstract 111) presented new structural data on the nature of binding of the MPER-binding antibody Z13, which has been less studied than the 2F5 and the 4E10 antibodies. Detailed peptide-binding studies revealed that the Z13 epitope is similar to that recognized by 4E10 but shifted to the N-terminal region of the MPER. Using an in vitro affinity maturation technique, Zwick and colleagues were able to generate a variant antibody fragment (Fab), termed Z13 E1, with a 100-fold greater affinity than that of the wild-type Z13, which was associated with a corresponding increase in neutralization efficiency. Like the other MPER antibodies 4E10 and 2F5, Z13 E1 has a long hydrophobic CDR H3 loop, which may play a crucial role in allowing the antibody access to the hydrophobic environment close to the virion membrane. As with the other MPER-specific monoclonal antibodies, Z13 E1 does exhibit some non-specific binding to self-antigens such as cardiolipin, reinforcing the issues addressed by Haynes and colleagues (see below) regarding whether the potential mimic-ry of the MPER by host molecules may serve as a barrier to the induction of antibodies to this target. Detailed mapping of this specificity of Z13 E1 compared with 2F5 and 4E10 revealed significant differences, which reinforces the existence of multiple potential targets for neutralization within the MPER. However, the issue of how to reliably induce antibody responses to this epitope by immunization remains.

Although many groups have noted the difficulty in inducing neutralizing antibodies to the MPER by immunization, the mechanisms responsible for this lack of success have remained controversial. A provocative hypothesis advanced by Haynes and colleagues proposes that structural similarities between the MPER and other host antigens may in part underlie this difficulty (Abstract 112). Haynes considered several potential explanations for the difficulty in inducing anti-MPER antibodies, including holes in the antibody repertoire, the inability of immunogens to mimic the native confirmation of the MPER, the possibility that antibodies that recognize MPER may be structurally unusual and difficult to generate, and the possibility that they may be derived from a polyspecific pool of B cells that is usually either deleted or tolerized. Focusing on the latter possibility, Haynes referred to recently published work that documented that both 2F5 and 4E10 antibodies were able to bind several self-antigens, particularly lipid molecules such as cardiolipin. If polyspecificity and the ability to recognize self-antigens are common characteristics of MPER-specific neutralizing antibodies, then these antibodies might be deleted or tolerized during normal B-cell differentiation. Comparison of the 2F5 and 4E10 antibodies with 2 well-characterized antcardiolipin monoclonal antibodies revealed a number of significant binding and structural similarities, suggesting that the ability of MPER-specific antibodies to bind a subset of host antigens may be related to their neutralizing activity. Examination of the kinetics of association of the 2F5 and 4E10 antibodies with 2 well-characterized antcardiolipin monoclonal antibodies led Haynes to propose that these antibodies require a 2-step conformational change in order to efficiently neutralize HIV. In the first step, weak interactions with lipid bring the antibody in close proximity to the MPER; in the second step, a conformational change leads to a more stable, high-affinity interaction of the antibody with the envelope trimer.
Although elicitation of antibodies able to bind the MPER during HIV infection occurs commonly, there have not been good data on how frequently neutralizing antibodies directed to the MPER are generated during natural infection. Shaw and colleagues (Abstract 113) grafted epitopes, or various portions of the MPER, of HIV-1 into an HIV-2 backbone to address this question. Because of the relatively high sequence divergence between HIV-1 and HIV-2 and the lack of cross-neutralizing antibodies, the HIV-2 envelope provides a convenient scaffold to examine the effects of MPER sequences without the confounding effects of neutralizing antibodies to other envelope determinants. They examined neutralization of a panel of HIV-2 envelopes that were grafted with either the entire 23-amino-acid HIV-1 MPER or several truncations of the MPER, including truncations limited to the defined 2F5 or 4E10 epitopes. Plasma was obtained from 217 subjects infected with 10 different HIV-1 subtypes or circulating recombinant forms. Of these 217 individuals, only 3 had any significant neutralizing antibody titer against either the 2F5 or 4E10 epitopes, and these were only at low levels. The remarkable rarity of neutralizing antibodies to the 2F5 and the 4E10 epitopes was compatible with work presented by Johnson and colleagues (Abstract 94) who were unable to detect any specific neutralization of either the 2F5 or the 4E10 epitopes when grafted onto an SIV backbone. Interestingly, however, Shaw and colleagues did identify anti-HIV-1 MPER activity to regions other than those represented by the 4E10 and 2F5 epitopes in one third of study subjects. The specificity of these antibodies mapped to several distinct regions of the MPER and in some instances required the complete 23-amino-acid MPER sequence for recognition. The demonstration of these novel neutralizing epitopes in the MPER in a significant minority of HIV-infected patients suggests that this region may represent a valuable target for vaccines. However, a number of issues need to be addressed in more detail, including the relative potency of these antibodies, their breadth of neutralization, and the ability to induce immune responses to these novel MPER epitopes by vaccination.

The ultimate test of the efficacy of MPER antibodies should be reflected in their ability to suppress viral replication in HIV-infected patients. Such a trial was reported at last year’s CROI and subsequently published by Trkola (Trkola, Nat Med, 2005). Fourteen HIV-infected patients received infusions of the broadly neutralizing antibody 2G12—which recognizes a carbohydrate epitope in V3—and 2 MPER antibodies, 4E10 and 2F5, while receiving antiretroviral therapy. After interruption of antiretroviral therapy, only 3 patients had sustained suppression of viral replication until the time of antibody washout; the remainder of the patients had rebound viremia during the period when infused neutralizing antibodies were still present. Although the antibody infusions provided some delay in the rebound of viral replication, one of the major questions surrounding this trial was how the virus was able to replicate even in the presence of what appeared to be significant levels of neutralizing antibodies in vivo. At this year’s conference, Trkola and colleagues (Abstract 114) provided a detailed analysis of potential mechanisms that might be responsible for the limited efficacy of the MPER antibodies in vivo. Although evolution of viral strains resistant to the 2G12 antibody was observed in the majority of patients, no significant evolution of escape mutations in the 2F5 and the 4E10 epitopes was identified, a finding that was confirmed by sequencing of viral isolates as well as by sequencing of plasma viral RNA. This observation suggests that the MPER antibodies might be less effective in vivo than predicted based on their in vitro efficacy. Analysis of the pharmacokinetics of each of the 3 antibodies revealed that although the distribution half-lives of all of these antibodies were equal, the elimination half-lives of the 2 MPER antibodies were approximately 4-fold shorter than that of the 2G12 antibody. This was not due to the development of endogenous antibody response against the infused antibodies and may reflect the ability of the MPER antibodies to bind phospholipid or other self-antigens in vivo. Similar results were reported by Mehandru and colleagues (Abstract 178), who also observed consistent induction of viral variants resistant to 2G12 but not 4E10 and 2F5 in patients who received infusions of all 3 monoclonal antibodies and then underwent treatment interruption. To address the question of whether the observed concentrations of antibodies were effective in vivo, Trkola and colleagues calculated the relative contributions of the infused neutralizing antibodies against the patient’s endogenous neutralizing antibodies based on the measured antibody concentration in plasma and analysis of the ability of the monoclonal antibodies to neutralize each patient’s autologous viral sequence. In responding patients, a significant contribution of the infused antibodies to the total neutralizing antibody titer in plasma was observed, whereas in non-responding patients, there was little contribution of the infused antibody to total neutralization activity. An alternative explanation that the investigators pursued was that the fitness cost of escape mutations to the MPER antibodies was so great as to render the escape viruses nonviable. In vitro experiments demonstrated that escape to 2G12 rapidly occurs in vitro, as opposed to escape to the MPER antibodies, which was relatively infrequent and appeared to be associated with less replication-competent viruses. This conclusion was confirmed by examining the growth kinetics of in vitro viruses that had the 4E10 and 2F5 escape mutations inserted into their envelope sequences. Notably, the 4E10 escape mutation significantly increased the sensitivity of the envelope to autologous neutralizing antibodies. Thus, several factors appear to contribute to the infrequent observance of escape to the MPER antibod-
ies, including the fitness cost of these escape mutations, the low frequency of nonsynonymous mutations occurring in the 4E10 epitopes, and the fact that these escape mutations may increase the susceptibility of these viral variants to other neutralizing antibodies. Overall, passive immunization with these antibodies was only infrequently able to increase neutralization titers above the patients’ own autologous neutralizing antibody responses.

Preclinical AIDS Vaccine Studies—Is Less More?

In the majority of studies conducted to date, T-cell–based vaccines have had only limited success in protecting against infection or disease progression in macaques challenged with pathogenic SIVmac strains. (The primary exceptions to this general observation have been in macaques challenged with rapidly pathogenic, CXCR4-tropic simian-human immunodeficiency viruses, but there is controversy as to how well these strains are likely to model HIV pathogenesis.) Although these discouraging results using the SIVmac-challenge model have induced some pessimism about the potential use of T-cell–based AIDS vaccines, it has also been suggested that the use of high-dose challenges in nonhuman primates may overwhelm a potentially protective effect. There has therefore been increasing interest in the utility of repeated low-dose SIV challenges in nonhuman primate vaccine studies. Gauduin and colleagues (Abstract 174) reported the ability of a multigenic DNA prime, modified vaccinia Ankara (MVA)-boost regimen to protect against a repeated low-dose vaginal SIV challenge. Macaques were immunized with an optimized DNA vaccine encoding for 6 SIV proteins, followed by a peripheral boost with MVA vectors expressing a similar complement of SIV proteins. Levels of enzyme-linked immunospot (ELISPOT) responses induced by this regimen were robust and generally comparable to those induced by other regimens.

When examined 2 weeks after the MVA boost, this systemic immunization regimen was able to induce levels of SIV-specific CD8 + T cells in vaginal and rectal tissues similar to those in peripheral blood. Following a repeated low-dose challenge, the authors observed an approximately 30-fold reduction in peak viremia and a 100- to 300-fold reduction in viremia in vaccinees compared with controls, a difference that was sustained to more than 25 weeks after infection. Vaccinees also had significantly better preservation of CD4 + T-cell counts and lower mortality. Taken together with the results reported by Watkins (see below), these data suggest that the ability of T-cell–based vaccines to provide sustained protection against disease progression in nonhuman primates challenged with SIVmac may have been underestimated by traditional high-dose challenge models. However, whether these results will necessarily prove predictive of those obtained in human clinical trials awaits the results from ongoing phase Iib and III trials.

Watkins (Abstract 180) described similar results in macaques vaccinated with DNA encoding SIV Nef, Tat, and Rev followed by an adenovirus boost, which resulted in quite robust SIV-specific CD8 + T-cell responses comprising 1% to 24% of all CD8 + T cells at peak after boost. Following a repeated low-dose rectal challenge, the vaccinees had an approximately 1-log_{10} decrease in peak plasma SIV RNA level and had a sustained 30-fold decrease in set-point viremia, which was maintained almost 1 year after infection. This significant and sustained decrease in viral load was accompanied by preservation of total CD4 + T-cell counts and CD8 + memory cells.

Watkins went on to readdress the theme of qualitative difference in T-cell responses previously introduced in Walker’s presentation. Watkins’s model consisted of analysis of a subset of rhesus macaques that were able to control replication of the highly pathogenic SIVmac239 strain to less than 1000 plasma RNA copies/mL for several years. Control of viremia in these animals was highly statistically associated with specific major histocompatibility complex (MHC) class I alleles, primarily B*17 and, to a lesser extent, A*01. Compelling evidence for the role of CD8 + T-cell responses in controlling viral replication in these animals arose from the observation that following CD8 + T-lymphocyte depletion with a monoclonal antibody, viremia promptly rebounded by 2- to 4-log_{10} plasma RNA copies/mL, and subsequently came under control when the CD8 + T-cell responses returned. After analyzing the immunodominance of specific epitopes during initial viremia and during subsequent rebound following CD8 + T-cell depletion, Watkins concluded that recognition of only a subset of all CTL epitopes originally targeted were involved with control of viremia after CD8 + T-cell depletion. Moreover, most of these epitopes represented formerly subdominant responses and were commonly found in Nef and Vif. These data, coupled with the data from Watkins’ Nef/Tat/Rev vaccine trial, suggested that induction of T-cell responses against a subset of normally subdominant epitopes, particularly in proteins such as Nef, Vif, and Gag, may be an especially attractive strategy for vaccine development.

Immunity to adenovirus serotype 5 (Ad5) vectors represents a significant limitation to the otherwise notable successes of the adenovirus-based vectors. Barouch and colleagues (Abstract 179LB) described the construction of novel chimeric Ad5 vectors designed to circumvent anti-Ad5 humoral responses. Previous work has shown that much of the antibody response against the Ad5 vectors is directed against the Ad5 hexon protein. The authors replaced the short hypervariable regions of the Ad5 hexon protein with the corresponding hypervariable regions from the relatively rare adenovirus serotype 48. In mice and in monkeys, these recombinant chimeric Ad5 vectors displayed immunogenicity comparable to that induced by the Ad5 parent. Similar levels of immune responses were generated in Ad5-
seropositive animals, demonstrating that the chimeric vectors were able to bypass the effect of pre-existing antibodies. Previous attempts to utilize adenovirus vectors based on serotypes other than Ad5 have been limited in part by the suboptimal immunogenicity, whereas this strategy, if successful in humans, would retain the proven safety and immunogenicity of the Ad5 vectors while also offering the opportunity to bypass pre-existing immunity.

In their quest to develop a vaccine able to induce effective antibody responses against HIV, investigators have engineered a number of mutant envelopes, which have largely been unsuccessful in inducing broadly neutralizing antibodies. As an alternative approach, Frost and colleagues (Abstract 176) turned to natural HIV envelope sequences, hypothesizing that infection with neutralization-sensitive HIV strains may result in stronger neutralizing antibody responses. Drawing on a population of 38 recently HIV-infected individuals who were not treated with antiretroviral therapy, Frost and colleagues correlated the results of autologous neutralizing antibody titers to heterologous neutralization using benchmark sera which displayed broad cross-neutralization. Subjects who were infected with a virus that was more susceptible to neutralization by the broadly cross-neutralizing sera had an approximately 4-fold increase in the rate of neutralizing antibody responses, which was associated with an approximately $\frac{\text{half-log}_{10}}{\text{increase}}$ in set-point plasma viral RNA levels. The authors concluded that infection with inherently more neutralization-sensitive viruses is more effective in eliciting better neutralizing antibody responses and suggested that these envelope strains may prove useful in inducing neutralizing antibody responses.

**AIDS Vaccine Trials—Where Do We Stand?**

Corey, Director of the HIV Vaccine Trials Network (HVTN), provided a frank and comprehensive assessment of past progress and future challenges in the AIDS vaccine field (Abstract 56). Twenty-five years after the identification of HIV, much progress has been made: 23 distinct AIDS candidate vaccines are now in various phases of development and large-scale clinical trials are underway. However, progress toward an effective vaccine remains frustratingly slow. Corey started by highlighting disappointing results from a number of different vaccine candidates in phase I and II trials during 2005, including lackluster immunogenicity results observed with a number of peptide or lipopeptide vaccines, as well as several DNA vaccines. He also highlighted the need to define true maximal tolerated doses of candidate vaccines in phase I trials as opposed to the tendency to employ doses dictated by manufacturing limitations.

Despite these setbacks, the past year was also notable for the success of several vaccine strategies employing adenovirus vectors, either in the form of a recombinant Ad5 vector alone or when used as a boost following initial priming with DNA immunization. Ad5-based vaccines have proved effective in both nonhuman primate studies and phase II clinical trials in inducing immune T-cell responses in more than 60% of vaccinees. The factors associated with the proven immunogenicity of the adenovirus-based vectors are not well-defined but may be related in part to the ability to produce these vectors at sufficiently high titers to allow dosages of $10^{10}$ to $10^{12}$ infectious units per dose, which exceed the currently employed doses used for other viral vectors by 3 to 4 $\log_{10}$ units per dose. However, the presence of pre-existing immune responses to adenovirus vectors represents an important limitation. The presence of antibodies to Ad5, currently the most commonly used backbone employed in clinical trials, results in a decreased frequency of response and decreased breadth of the response in seropositive vaccinees. However, this diminution can be overcome in part by increasing the vector dose, albeit at the expense of increased vector reactivity. The use of a DNA prime and the use of adenovirus vectors other than Ad5 are also being pursued as alternative strategies to deal with pre-existing immunity. The potential effect of pre-existing immunity is particularly important in areas of high HIV prevalence where rates of Ad5 seropositivity are typically even higher than those observed in the United States.

Corey also elucidated the rationale for the current emphasis on proof-of-concept or phase Ib vaccine trials. The phase Ib trials are designed to demonstrate proof-of-principle (ie, can a T-cell-based vaccine reduce the risk of HIV infection or decrease set-point viremia in infected individuals) but is not of sufficient size to result in licensure. Distinct advantages of the Ib approach are the significant decrease in cost (10% to 30% of that for a standard phase III trial), the use of a significantly smaller study population, and the accelerated timeline, which can shave 1.5 to 2 years from the time required to complete a phase III trial. These features have been employed in the design of the currently ongoing HVTN 502 trial, which will examine the efficacy of an Ad5 trivalent vaccine expressing Gag, Pol, and Nef, and is currently enrolling a targeted number of 3000 patients at sites predominantly in North and South America and the Caribbean. One significant limitation of the HVTN 502 study design is the relatively small number of women who will be enrolled, a situation that is likely to be remedied by the plans to move forward with HVTN 503, which will examine the safety and efficacy of the Ad5 trivalent vaccine in South Africa. Encouraging results in phase II trials have also been obtained with a vaccine approach directed by the National Institutes of Health (NIH) Vaccine Research Center, which involves a polyvalent DNA prime with the gag, pol, and nef genes from clade B strains and the envelope from clade A, B, and C, followed by an Ad5 boost expressing the 3 different HIV-1 envelopes, Gag, and Pol. Initial analysis of T-cell responses in
subjects who have received a combination DNA prime and adenovirus boost are encouraging, and stand in contrast to the results observed using several other DNA vaccines. This approach is currently being investigated in phase II studies and is targeted to enter a phase IIb proof-of-concept trial in early 2007.

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Additional Reference


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Epidemiology and Prevention

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The 2006 Conference on Retroviruses and Opportunistic Infections marked the 13th year of this conference and the 25th anniversary of the first published cases of AIDS in gay men in Los Angeles. As noted in the opening plenary session, HIV has likely been present in the human population since the 1930s, and therefore represents a relatively recent disease for humans. However, by the end of 2005, more than 40 million persons were estimated to be living with HIV infection, and 5 million new infections are occurring annually. Investigators at this year’s conference presented a number of abstracts focused on the leading edge of the epidemic, HIV testing strategies, the role of acute versus chronic HIV infection in driving the epidemic, and the substantial progress being made in developing and testing a variety of biomedical prevention strategies.

The Global Epidemic

Sub-Saharan Africa continues to account for the majority of new and prevalent HIV infections worldwide. Rehle and colleagues presented data from the 2005 South African national household survey on HIV, behavior, and communication (Abstract 31LB). Among the 15,851 individuals for whom linked anonymous testing was performed, HIV prevalence in persons 2 years or older was 10.8%. As has been demonstrated in a number of other countries in sub-Saharan Africa, the prevalence is higher and peaks earlier in women than in men. For example, HIV prevalence was 4.4% in men 15 to 24 years of age, but was nearly 4 times higher in girls of that age group (16.9%). Because of recent concerns that BED-enzyme immunoassay (an IgG enzyme immunoassay assay to distinguish recent from chronic infection) may substantially overestimate HIV incidence rates, these investigators used a number of methods to estimate HIV seroincidence in the population. Estimates using a lower optical density ratio for BED (0.4 instead of 0.8 cutoff) resulted in similar estimates to those using a birth cohort method, with an overall rate of 1.9 per 100 person-years in adults aged 15 to 40 years. This methodologic issue is of crucial importance in projecting the growth of epidemics and for properly sizing prevention trials in these communities.

High HIV seroprevalence points to the need for HIV counseling and testing services throughout the developing world, particularly as antiretroviral therapy becomes available to larger populations. Weiser and colleagues (Abstract 25) conducted an assessment of attitudes toward HIV testing among a population-based survey of 1268 adults in 5 districts in Botswana. The majority of participants felt that routine testing would decrease barriers to testing (89%), HIV-related stigma (60%), and violence toward women (55%). A substantial minority, however, were concerned that routine testing would lead people to avoid medical care (45%) or were concerned that they could not refuse the test (32%), suggesting that more work needs to be done to reduce stigma and emphasize the voluntary nature of testing.

Although the HIV epidemic continues to be fueled largely by sexual transmission worldwide, there is a burgeoning epidemic of HIV in injection drug users (IDUs) in Eastern Europe and Asia. Two posters focused on this epidemic at different stages in different regions. Beyrer and colleagues presented data from Dushanbe, Tajikistan (Abstract 923). In 2001, HIV prevalence in a cross-sectional sample of IDUs there was 3.85%. By 2004, prevalence had increased to 12.1%. Solomon and colleagues presented data from IDUs in Chennai, India, where HIV prevalence had reached 35.6%, and incidence using the BED assay was 4.5% per year (95% confidence interval [CI], 0.6–8.5; Abstract 922). Prevention interventions must be designed and tested in IDUs as well as those at risk through sexual transmission.

This year’s international symposium focused on the intersection of HIV with other infectious diseases, including pneumococcal infection, tuberculosis (TB), and malaria. Klugman pointed out that the leading infectious cause of death worldwide is respiratory infection, with the leading 2 causes in HIV-infected persons being TB and pneumococcal disease (Abstract 10). Pneumococcal disease in HIV-infected adults and children is more likely to be caused by pediatric serotypes and more likely to be resistant to antibiotics. Although conjugate vaccine protects HIV-infected children from invasive disease and has lowered the burden of disease among adults through herd immunity, the total number of pneumococcal infections does not appear diminished in HIV-infected women, probably because women are getting infected through their exposure to children with other pneumococcal serotypes not covered by the conjugate vaccine. The 23-valent vaccine given to adults may protect HIV-infected persons on highly active antiretroviral therapy (HAART), but may not be immunogenic in untreated persons with advanced HIV disease. This highlights the need to detect and immunize HIV-infected patients with pneumococcal vaccine early in disease.

Harries provided an overview of the devastating interaction of HIV and

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reviewed the therapeutic implications of coinfection with both agents, and the opportunities for prevention and research through interaction between malaria control and HIV prevention and treatment programs.

The Epidemic in the United States

In the United States, the HIV epidemic continues to be concentrated most heavily in the African American population and in men who have sex with men (MSM). Durant presented some hopeful data from the Centers for Disease Control and Prevention (CDC), suggesting that new HIV diagnoses have declined from 2001 to 2004 among African Americans in the 33 states with named HIV reporting (Abstract 27). However, African Americans comprised 51% of the new HIV diagnoses during those years, despite making up only 13% of the population in those states. The one African American population in whom new diagnoses did not decline was MSM. Previously published CDC data on all racial and ethnic groups have found that MSM continue to account for the largest number of new HIV diagnoses, and are the only risk group with increasing rates of new diagnoses (CDC surveillance report, 2005).

Sifakis and colleagues provided a snapshot of the US MSM epidemic in Baltimore (Abstract 28). In an analysis of 891 men recruited from gay venues in Baltimore as part of the Behavioral Surveillance Research (BESURE) study, overall HIV seroprevalence was 52.2% and HIV seroincidence—calculated using the serologic testing algorithm for recent HIV seroconversion (STARHS or “detuned” assay)—was 9.2% per year (95% CI, 4.3–17.4). Among the 500 African American MSM in this study, prevalence was 45.6%, estimated seroincidence was 15.4% per year (95% CI, 8.7–25.2), and only 36% of the HIV-infected men reported knowing their HIV status. Although it is possible that detuned studies overestimate true population seroincidence rates, these data highlight the very substantial epidemic occurring in MSM, and particularly African American MSM, in the United States and point to the importance of increased access to HIV testing and prevention and care services.

One strategy that is being used by some MSM to reduce the risk of HIV transmission is serosorting, or intentionally selecting sex partners or deciding on condom use based on partner serostatus. Golden addressed the question of the frequency and potential utility of serosorting among MSM as a strategy for decreasing HIV transmission rates (Abstract 163). There is indirect evidence that serosorting is occurring in MSM populations, and that the practice may be increasing. Although serosorting may reduce the risk of HIV transmission, it is an imperfect strategy: several studies estimate that 15% to 30% of new HIV infections in MSM are occurring in men who report having unprotected anal sex exclusively with men believed to be HIV seronegative. Golden concluded that serosorting is likely a useful strategy only for men who are already engaging in high-risk activities, but could paradoxically increase risk in men who are already relatively safe. He recommended that clinicians urge their patients to both know and disclose their serostatus to their partners, and urged further evaluation of the public health utility of serosorting before recommendations are put into place.

HIV Testing Issues

Mastro presented the CDC’s new recommendations for routine “opt-out” HIV testing in clinical settings, currently under development (Abstract 164). He pointed out that of approximately 1.1 million persons living with HIV in the United States, one fourth are unaware of their infection. He reviewed the substantial benefits that accrue when individuals learn their HIV serostatus: opportunities for treatment with HAART leading to improved survival and quality of life, opportunities for treating pregnant women with antiretrovirals leading to dramatic reductions in the number of newly
infected infants, and substantial self-reported changes in risk behaviors after individuals learn their HIV-positive serostatus. Other recent data suggest that some of the most heavily affected populations, including African Americans and young people, may be significantly less likely to know their HIV serostatus. Nearly half of newly diagnosed AIDS cases had only learned of their HIV-positive serostatus within the 12 months prior to development of AIDS, limiting the benefits they may have received had antiretroviral therapy been started earlier in their course of disease.

The CDC is in the process of developing new testing guidelines that would recommend routine, voluntary HIV screening in healthcare settings for all patients aged 13 to 64 years, eliminating recommendations that only high-prevalence populations or individuals with certain risk behaviors be tested. At least annual screening of high-risk persons continues to be recommended. Patients would be given an opportunity to ask questions but consent would be covered under the general consent for receipt of medical care, and patients would need to “opt out” of HIV testing rather than “opt in” as is currently practiced in most settings.

Mastro presented data from Texas sexually transmitted diseases clinics in which a substantially larger proportion of patients were tested with an opt-in than an opt-out testing program. This change resulted in a 60% increase in the number of HIV-infected patients identified. Several analyses suggest that routine screening would be cost-effective, even with very low prevalence in a community (0.65%). However, the CDC is recommending that after routine screening is implemented, if prevalence in a particular setting is documented to be below 0.1%, routine screening could be stopped. As part of these guidelines, the CDC also suggests that clinical settings need not provide risk-reduction counseling as part of HIV counseling and testing, but that patients may instead be referred to prevention services in the community. There was a substantial amount of discussion after his presentation from both clinicians and community members, expressing concern about the lack of adequate healthcare infrastructure to handle increased demand once more HIV-infected persons are identified. Concerns were also raised about whether patients are likely to be adequately informed about the testing, and the extent to which true linkages will be made with local HIV prevention services.

Branson and colleagues presented data from a CDC investigation of reports of excessive false-positive test results from oral fluid rapid tests (Abstract 34LBb). A rapid HIV antibody test was approved for use with oral fluid in March 2004. In December 2005, there were several reports in the press suggesting that some clinical sites were experiencing an unusually high rate of false-positive tests, and the CDC investigated these concerns through a number of methods. Combined data from more than 12,000 participants enrolled in 4 prospective studies comparing whole blood and oral fluid testing from 2000 to 2005 showed excellent specificity for both (99.6% for oral fluid versus 99.9% for whole blood). Because a small number of sites recently experienced a slight drop in the specificity of test results, several sites participated in additional analyses to determine the etiology of the relative increase in the rate of false-positive test results. These analyses suggested that neither test lot nor device problems accounted for the difficulty. It seems more likely that the problems encountered were site-specific, but this hypothesis needs to be explored in more detail.

The CDC has developed an interim strategy to help sites deal with this issue that involves a second rapid test on a fingerstick specimen for patients with a positive result on an oral fluid specimen. All positive results require confirmation, but a negative result on a fingerstick specimen could indicate a false-positive result, and would help sites communicate the uncertainty of the screening result and the need for a confirmatory test.

**The Role of Acute HIV Infection**

Fraser and colleagues presented a very clear and reasoned overview of the role of primary (acute) HIV infection in driving the epidemic globally (Abstract 162). Early in the course of a local epidemic, when HIV infection rates are low overall, a substantial proportion of new infections occur through transmission from persons with early HIV infection. However, once an epidemic has matured, acute infection is likely to play only a minor role in new infections (eg, projected as 12% of new infections among heterosexuals in Rakai, Uganda), primarily because acute infection lasts a matter of several weeks to months, but chronic asymptomatic infection can last more than a decade. Fraser’s colleagues from the Imperial College in London also suggested that transmissions during acute infection alone cannot cause an epidemic (Abstract 913), and that there are not likely to be virologic “super-spreaders” of HIV (Abstract 910). Investigators at the University of North Carolina, on the other hand, have previously published statements to the effect that acute infection does account for a substantial number of new infections (Pilcher et al. J Infect Dis., 2004). At this conference, they presented several studies on the utility of pooled RNA testing to identify acutely infected persons (Abstracts 370, 371, 374.) In these studies, they were able to identify small numbers of acutely infected persons and interrupt small numbers of transmissions between sexual partners or from mother to child. The likely public health utility of this strategy of testing is not yet known, but may depend on the likelihood that acute infection is fueling the HIV epidemic and on the effectiveness of interventions during acute infection at preventing transmission.

**Biomedical Prevention Strategies**

This year’s conference featured a number of presentations on the state-of-the-art of biomedical prevention interventions. HIV vaccine research is
summarized in the review by Dr. Johnson in this issue.

Quinn presented the case for male circumcision in a plenary session, reviewing the long-standing history of what is likely the oldest and most common surgical procedure (Abstract 120). A number of ecologic studies have demonstrated an inverse relationship between circumcision rates and HIV prevalence in both Africa and Asia, and data from cross-sectional and longitudinal studies have also demonstrated an association of circumcision and lower HIV prevalence and incidence.

More recent data suggest a protective effect of circumcision on HIV transmission to uninfected female partners (Abstract 128), particularly for HIV-infected men with low viral loads. There is also biologic plausibility that circumcision could reduce HIV acquisition and transmission, as the inner surface of foreskin is a large nonkeratinized surface enriched with HIV target cells, and is prone to microtears that render it susceptible to other sexually transmitted infections. As reported at the International AIDS Society meeting in Brazil in July 2005, one randomized controlled trial of circumcision demonstrated a 60% reduction in HIV infection rates in circumcised men (Auvert et al, IAS, 2005). Two other trials are fully enrolled and due to provide data in the second half of 2007. One trial is evaluating the effect of circumcision on male HIV acquisition, and the other the effect of circumcision on transmission to uninfected female partners. If circumcision is proven to lower HIV infection rates in these trials, it is likely to be a cost-effective strategy that could have a dramatic impact on HIV infection rates in uncircumcised populations.

Much planning will be required to roll out this potentially cost-effective strategy for large populations that could minimize surgical risks, maximize acceptability, and counter the potential increases in risk behavior, which could lead to paradoxical increases in HIV infection rates.

A number of presentations focused on the use of antiretrovirals for prevention of HIV transmission, either initiated after a high-risk exposure (postexposure prophylaxis, or PEP) or through continual therapy (pre-exposure prophylaxis, or PrEP). As reviewed by Cohen (Abstract 54), there are no direct clinical data proving that PEP prevents HIV acquisition, and there have been reported cases of infection despite PEP. Nonetheless, there are abundant animal data suggesting that the approach may be efficacious, and the CDC has recommended PEP for occupational exposures since 1996 and for nonoccupational exposures since 2005. A World Health Organization study indicated that 98% of 41 developing countries surveyed had national PEP guidelines, and 20% of these recommend PEP for nonoccupational high-risk exposures (Abstract 904).

One of the major challenges of implementing PEP is getting treatment administered quickly after the high-risk exposure. Kindrick and colleagues reported that 55% of calls to the National Clinicians’ PEP Hotline occurred more than 24 hours after an exposure, and 25% occurred after 72 hours (Abstract 906). Because animal data suggest that efficacy is likely to be highest if PEP is initiated shortly after exposure, it is imperative that clinicians and patients understand that PEP should be initiated as soon as possible after exposure, rather than any time up to 72 hours.

Roland and colleagues presented data on 457 people presenting for PEP for sexual exposures, randomized to 2 versus 5 risk-reduction counseling sessions (Abstract 902). Although both groups had similar reductions in the reported number of sex acts at the 12-month visit compared with baseline, those in a higher risk group (more than 4 unprotected sex acts) had a greater reduction in risk with 5 counseling sessions than with 2 sessions. This highlights the need to provide more intensive counseling services for those at highest risk and emphasizes that the greatest effect of PEP may be in providing clinicians the opportunity to link high-risk patients with more intensive preventive services. Cohen also suggested that drugs for PEP should be selected, in part, based on their concentration in genital secretions, and presented a graphic indicating that nucleoside reverse transcriptase inhibitors (nRTIs) are generally superior to protease inhibitors (PIs) in concentration in genital secretions (Abstracts 54, 129).

Published data suggest that for some high-risk populations, the majority of seroconversions occur in persons with multiple episodes of risk, rather than isolated exposures (Celum et al., J Infect Dis, 2001). For these situations, use of PrEP has several theoretic advantages over PEP providing ongoing protection in which timing of the intervention does not need to be matched to self-identification of exposure. At this conference, Heneine and colleagues presented data from an animal model of PrEP, in which 6 macaques were given daily subcutaneous tenofovir and emtricitabine injections beginning 9 days prior to the first rectal challenge, through 28 days after the last challenge (Abstract 32LB). All 6 macaques were completely protected from weekly rectal simian HIV (SHIV) challenge, comparable in titer to viral levels in semen in acute infection. In contrast, 5 of 6 control animals were infected.

These are promising data from a challenge model that may more closely mimic human sexual exposure. Some have compared these results with data presented last year from the same group in which macaques given lower doses of oral tenofovir were all infected, albeit several weeks later than the control animals. What is not clear is whether the difference between last year’s and this year’s experiments was the addition of a second drug, or whether the problem in the earlier experiment was in giving the animals too low a dose of tenofovir, and administering it orally, when ingestion cannot be assured. Heneine also presented preliminary data from an ongoing study of emtricitabine alone in the macaque model, and in this trial, 1 macaque was infected after the fifth exposure and a second after
the tenth. The relevance of all of these data requires validation from human trials, which are currently underway.

Cohen summarized the state of clinical PrEP trials (Abstract 54) in which several trials have been closed or not allowed to open (Cambodia, Cameroon, Nigeria, Malawi). 1 trial site has been completed (Ghana), and several other studies are underway (Thailand, Botswana, San Francisco, Atlanta) or planned (Peru). Some of the factors leading to study-site closure were concerns expressed by community groups or governments about protection of study participants (access to medical care including antiretrovirals for participants becoming infected in trials and coverage for trial-related injuries) and concerns about emergence of drug resistance in the community. These studies have reinforced the importance of community involvement in the planning and implementation of trials, and of creating sufficient clinical and regulatory infrastructure in locations in which clinical trials are performed. Current trials are working closely with community groups and should provide important information on the safety of daily oral tenofovir in HIV-uninfected subjects, the impact of PrEP on risk behaviors, and preliminary estimates of PrEP efficacy.

Hillier presented an overview of physical and chemical barriers to protect women from HIV acquisition (Abstract 55). She noted that, in many countries, HIV seroprevalence is much higher for young women than for men of the same age, pointing to the need for methods of prevention controlled by women. The most promising physical barrier approach, apart from male and female condoms, is the use of diaphragms. A large efficacy trial of diaphragms (the Methods for Improving Reproductive health in Africa, or MIRA, study) is currently underway in South Africa and Zimbabwe, with results expected in 2007. A number of approaches are being taken to developing topical microbicides, and efficacy trials are currently evaluating 5 different products (cellulose sulfate, Pro 2000, C31G, carbopol 974P, and a lambda carrageenan microbicide).

Moore reviewed the spectrum of new products in early stages of development, including antiretroviral agents, monoclonal antibodies, small interfering RNA, chemokines, and live commensal bacteria (Abstract 121). There are limited nonhuman primate data supporting the efficacy of some of the investigational products and approaches, and of combination approaches, but few are yet in clinical trials. Hillier and Moore both pointed to major challenges that remain including the development of better animal models and their relevance for human experience; measuring the relative safety, effectiveness, and effect on resistance of antiretrovirals administered topically; the need to develop products that can be applied daily or less frequently (eg, weekly, monthly); and the cost and acceptability of products. A number of investigators are also developing better ex vivo explant models (Abstract 893) and validating biomarkers of vaginal mucosal integrity (Abstract 896) to allow for more rapid evaluation of products prior to entry into clinical trials.

Nagot presented data on the impact of herpes simplex virus (HSV)-suppressive therapy on HIV-1 genital tract and serum levels in HIV-infected women with high CD4 + cell counts, not requiring HAART (Abstract 33LB). Suppressive therapy with 1000 mg of valacyclovir daily led to a 0.39-log_{10} reduction of plasma HIV-1 RNA (compared with a 0.12-log_{10} increase in placebo recipients) and a 0.26-log_{10} reduction of plasma HIV-1 RNA in genital secretions (compared with a 0.09-log_{10} increase in placebo recipients). Valacyclovir also had a beneficial effect on HSV-2 shedding in these women. This is the first randomized controlled trial demonstrating a reduction in HIV levels in patients with HSV treated with HSV-suppressive therapy. Two large efficacy studies are currently underway to further test this approach in reducing the number of new HIV infections. One study is nearing enrollment completion of more than 3000 participants in the United States, Peru, and sub-Saharan Africa to evaluate the efficacy of twice-daily acyclovir in preventing HIV acquisition in HSV-2-infected men and women at risk for HIV infection. The second study is evaluating the impact of daily oral acyclovir in the prevention of HIV transmission from HIV-infected persons with HSV-2 to their HIV-uninfected partners. Data are expected from these trials by 2007 or 2008.

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A list of all cited abstracts appears on pages 63 to 70.

Additional References


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Neurologic Complications of HIV Disease and Their Treatments

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The 2006 Conference on Retroviruses and Opportunistic Infections was marked by a record number of presentations focused on the neurologic complications of HIV infection. Key findings included the identification of the high prevalence of HIV-associated neurocognitive impairment (HNCI) in Western and East Asian populations; biomarkers that may be clinically useful in determining risk and treatment of HNCI, such as neurofilament protein, insulin resistance, leptin, soluble Fas, and total protein levels in cerebrospinal fluid; host genotypes that were associated with HNCI as well as antiretroviral toxic neuropathy; HIV envelope signature positions that were associated with HNCI and reduced CD4 dependence; the importance of combined antiretroviral drug penetration into the central nervous system for control of HIV replication; and an effective treatment for painful sensory neuropathy (capsaicin) and provocative preclinical data on treatments for HNCI (rosiglitazone, glatiramer immunization). Together, these findings heighten concern for persistent neurologic diseases in antiretroviral therapy-treated individuals but provide guidance for their improved identification and treatment.

Central Nervous System Complications

Epidemiology

The use of potent antiretroviral therapy has led to declines in the incidence of the neurologic complications of HIV infection, including HIV-associated neurocognitive impairment (HNCI). Although its incidence has declined, HNCI continues to occur not only in untreated but in treated individuals as well. As HIV-infected individuals live longer, the prevalence of HNCI has risen; different clinical phenotypes have been recognized; and prior associations with biomarkers, such as HIV RNA levels in cerebrospinal fluid (CSF), have weakened. Several presentations at the 13th Conference on Retroviruses and Opportunistic Infections built on these prior observations.

Using a pooled dataset of 7923 seroconvertors enrolled in 22 cohorts from Europe, Australia, and Canada (in the Concerted Action on SeroConversion to AIDS and Death in Europe study, or CASCADE), Mussini and colleagues identified 152 subjects who were diagnosed with HNCI (Abstract 351). When stratified by assessment period, the relative risk (RR) of HNCI fell substantially from 1997 to 1999 (RR, .28) and 2000 to 2004 (RR, .19) compared with pre-1997, a finding that is consistent with prior reports. In contrast to this relatively low prevalence of HNCI, a more recent cohort (AIDS Clinical Trials Group [ACTG] 5001) of 1498 antiretroviral therapy-treated individuals demonstrated a high prevalence (43%) of impaired performance on a brief neuropsychologic battery at first testing (Abstract 362). Among the 853 initially unimpaired subjects, 19% subsequently became impaired during the observation period (median 93 weeks). As in these 2 reports, much of the data identifying epidemiologic shifts in HNCI have been derived from Western cohorts. Wright and colleagues sought to identify the prevalence of HNCI in the Asia Pacific NeuroAIDS Consortium (APNAC) cohort (Abstract 366). Of 129 consecutive HIV-infected outpatients from Indonesia, China, and Malaysia screened with a brief neuropsychologic battery, a surprising 71% had impairment, two thirds of which was mild to moderate and one third severe.

Clinical Biomarkers

Before the use of combination antiretroviral therapy, the risk of HNCI increased with declining CD4+ cell count. Among antiretroviral-treated individuals, some reports indicate that risk correlates more closely with nadir than with current CD4+ cell count. This shift may reflect incomplete normalization of neuroinflammation following immune reconstitution. The ACTG 5001 analysis confirmed that individuals who had a lower nadir CD4+ cell count (below 200/µL), but not lower current CD4+ cell count, had 23% increased odds of having HNCI. In contrast, Mussini showed that the risk of HNCI increased with declining current CD4+ cell count strata (RR, 4.5 for 200/µL to 349/µL, 11.9 for 100/µL to 199/µL, and 69.0 for 0/µL to 99/µL), compared with those with current CD4+ cell counts of at least 350/µL but not the nadir CD4+ cell count. This disagreement may be attributable in part to the inclusion in the CASCADE analysis of pre-antiretroviral therapy-treated subjects, in whom immune reconstitution was probably uncommon. Importantly, neither of these analyses addressed the prevalence of hepatitis C virus (HCV) coinfection, which may be important because numerous studies have now confirmed its association with HNCI and because, in a cohort of 87 HIV-infected individuals reported by Paul and colleagues, HCV coinfection was associated with both worse neuropsychologic performance and lower current CD4+ cell count than HIV infection alone (Abstract 880).

Arendt and colleagues identified
that the relationship between CSF RNA and neuropsychologic performance varied with both antiretroviral use and stage of disease (Abstract 360). For example, in 23 untreated individuals, higher CSF RNA levels correlated with worse global and motor performance regardless of disease stage. In contrast, in 61 treated individuals, higher CSF RNA correlated with worse motor performance, but only in individuals with earlier, not later, disease stage. This reduced predictive ability of CSF RNA is consistent with findings from large published studies and indicates that CSF RNA may still be a useful biomarker in untreated individuals. Shiramizu and colleagues avoided the impact of antiretroviral therapy seen in Western cohorts by measuring HIV RNA and DNA levels in antiretroviral-naive individuals in Thailand (Abstract 352). Consistent with their prior findings from the Hawaii Aging with HIV Cohort, they found that higher HIV DNA levels in circulating leukocytes were associated with the diagnosis of HNCI (HNCI: n = 12, mean 2.20 log10 HIV DNA copies/mL; non-HNCI: n = 12, mean 0.73 log10 HIV DNA copies/mL, P = .02). In contrast, HIV RNA levels in plasma did not differ between individuals with and without HNCI.

Investigators at the University of California San Diego (UCSD) sought to better understand the effects of antiretroviral therapy on the relationships between 12 CSF biomarkers and neuropsychologic performance by evaluating 29 individuals before and following a change in antiretroviral therapy (Abstract 346). Antiretroviral therapy variably modified these relationships and this variability depended in part on which biomarker was considered and the duration and effectiveness of antiretroviral therapy. Overall, of the 12 biomarkers measured, 4 (total protein, soluble Fas, urokinase-type plasminogen activator receptor, and interferon inducible protein-10) most consistently correlated with neuropsychologic performance. The relationships between neuropsychologic performance and 2 biomarkers, total protein and soluble Fas, was particularly strong. In a multivariate analysis, for example, higher soluble Fas levels in CSF were associated with worse neuropsychologic performance even after adjusting for duration of antiretroviral therapy and changes in CD4+ cell count and HIV plasma RNA level. Perhaps more importantly, the sensitivity and specificity of a total protein level in CSF above 36 mg/dL for the diagnosis of HNCI in successfully treated individuals were 100% and 78%, respectively, although the small and selected nature of the study may limit the generalizability of this finding. The median duration of antiretroviral therapy in this analysis was 15 weeks but Eden and colleagues determined the effects of a much longer duration of antiretroviral therapy on 2 measures of neuroinflammation (Abstract 355). Despite at least 4 years of effective antiretroviral therapy (plasma HIV RNA below 50 copies/mL, 13 of 16 (81%) HIV-infected treated individuals still had abnormal neopterin levels in CSF. A smaller, but still substantial, proportion of treated individuals (7 of 16 (44%)) had abnormal IgG indices. These abnormalities were not compared with HNCI diagnosis but do support the hypothesis that neuroinflammation can persist despite otherwise effective antiretroviral therapy.

Measurement of these neuroinflammatory and neurotoxic markers requires lumbar puncture and timely sampling, both of which can create challenges in the clinic. An alternative approach to identifying an individual's neuroinflammatory risk is to characterize genes that encode crucial proteins in the immune response. This approach has the additional advantage of avoiding fluctuations in protein expression associated, for example, with antiretroviral therapy use, HIV disease stage, or comorbid conditions. Thus far, published studies have identified that HNCI is associated with polymorphisms in genes encoding MCP-1, its receptor (CCR2), and other inflammation-associated proteins. Pemberton and colleagues characterized several potential susceptibility genes in 56 individuals diagnosed with HNCI and up to 203 HIV-infected and 204 -uninfected controls (Abstract 343). This analysis identified associations between HNCI and tumor necrosis factor alpha (TNFα)-308 and BAT1 (intron 10). Combining their data with prior reports, the investigators concluded that the currently supported host risk genotype for HNCI is TNFα-308*(2,2), BAT1 (intron 10)*(2,x), MCP1*2578G, and ApoE (4,4).

As suggested by the inclusion of ApoE in this genotype, noninflammatory processes can also increase risk for HNCI. Four studies evaluated relationships between noninflammatory biomarkers and HNCI. Li and colleagues investigated protein nitration, an indicator of nitrosative stress, by measuring 3 nitrotyrosine-modified proteins (3-NT) in 43 CSF samples from individuals enrolled in the North Eastern AIDS Dementia (NEAD) cohort (Abstract 347). Higher 3-NT levels distinguished individuals with active HNCI from those with inactive HNCI, an important clinical distinction. The investigators then used immunoprecipitation and mass spectrometry to identify 9 potential proteins with 3-NT modification, the most important of which seemed to be prostaglandin D2 synthase. In a large and well-designed study, Gisslen and colleagues measured CSF concentrations of the light subunit of the neurofilament protein (NFL), a major structural component of myelinated axons (Abstract 72). CSF NFL levels were higher in individuals with HNCI than in neuroasymptomatic individuals, but did not distinguish them from those who had central nervous system opportunistic infections. CSF NFL levels did distinguish milder and more severe forms of HNCI and intensive sampling of CSF identified that NFL levels declined in parallel with neuropsychologic improvements.

Metabolic complications of HIV disease and its treatments are well recognized. The final 2 biomarker studies investigated links between these metabolic derangements and HNCI. Among 145 individuals enrolled in the Hawaii Aging with HIV Cohort, Valcour and colleagues identified that greater
insulin resistance, as measured by the homeostasis model of assessment, was associated with both milder and more severe forms of HNCI, but only among older individuals (at least 50 years of age; Abstract 349). The relationship between the homeostasis model of assessment and HNCI remained statistically significant even after adjusting for CD4+ cell count, sex, ethnicity, and antiretroviral therapy use, although the effect size was modest. Huang measured leptin in serum and CSF specimens from 84 (42 HIV-infected, 42 HIV-uninfected) men who had undergone comprehensive, standardized neuropsychologic testing (Abstract 73). Leptin may link HIV- and antiretroviral drug-associated lipid disorders since it is produced by peripheral fat cells and penetrates into the brain, where it can regulate appetite and have multiple neuroprotective effects. Among all subjects, lower CSF-to-serum leptin ratios were associated with worse memory and learning performance. This relationship held among the HIV-infected individuals even after adjusting for CD4+ cell count, CSF RNA level, and disease stage.

**Treatment**

Antiretroviral therapy can effectively prevent HNCI in many individuals, as evidenced by its reduced incidence, but may not completely treat HNCI once it occurs, as supported by its high prevalence in treated populations. Tozzi and colleagues evaluated the reversibility of HNCI by assessing antiretroviral-associated improvements in 116 affected individuals (Abstract 354). Participants had a mean duration of antiretroviral therapy of 32 months and were assessed up to 6 times with standardized neuropsychologic testing. The investigators observed improvements in neuropsychologic performance at all evaluations (6, 12, 18, 24, 36, and 48 months) but, overall, only 30% of participants were considered to have fully reversible disease. Reversible disease was associated with better education, male sex, less severe impairment at baseline, and greater improvement at the first follow-up, but not age, CDC stage, or either baseline or change in CD4+ cell count or plasma HIV RNA level.

These findings support initiation of antiretroviral treatment as early in the HNCI disease course as possible. Another hypothesis is that those who are less impaired may be better able to adhere to complex antiretroviral regimens. An analysis from the University of North Carolina of 37 impaired individuals who changed antiretroviral treatment supports this hypothesis, finding that worse neuropsychologic performance before changing therapy (global, verbal memory, attention) predicted worse adherence at 6 months (Abstract 363).

Another explanation for incident or persistent HNCI in successfully treated individuals is poor antiretroviral penetration into the central nervous system, which could allow ongoing HIV replication. Three studies addressed this question by comparing measures of antiretroviral penetration to CSF RNA levels. In a longitudinal study of 101 individuals who initiated new antiretroviral regimens following a baseline assessment (ACTG 736), Marra and colleagues identified that greater antiretroviral penetration, as calculated by summing an ordinal measure of penetration for each drug in the regimen, was associated with greater reductions in CSF RNA ($r = -0.08; P = 0.4$), after adjusting for baseline CSF RNA level, change in plasma RNA levels and CD4+ cell count, and antiretroviral experience at baseline (Abstract 361). Letendre and colleagues reported a cross-sectional analysis from the CNS HIV Anti-Retroviral Therapy Effects Research cohort (CHARTER), a North American, 6-center cohort focused on antiretroviral therapy. The third investigative NeuroAIDS (IRINA; Abstract 359) found that a larger number of penetrating antiretrovirals correlated with lower CSF RNA level ($r = 0.17; P < 0.01$; Abstract 359). Similar to the other studies, this association withstood adjustment for other variables and the effect sizes were even larger. Compared with regimens containing no penetrating antiretrovirals, use of 2 (OR, 6.1) or at least 3 (OR, 7.1) penetrating antiretrovirals markedly improved the odds of having a CSF RNA level below 50 copies/mL.

The UCSD approach to categorization of antiretroviral penetration is hierarchical, weighting pharmacokinetic and pharmacodynamic data more heavily than drug characteristics. Atazanavir is an example of the importance of this approach. Atazanavir is 14% unbound to plasma proteins, which should enable penetration into the central nervous system in therapeutic concentrations. Best and colleagues presented findings from the CHARTER group identifying that atazanavir concentrations were actually lower in 76 CSF specimens than expected based on drug characteristics and atazanavir concentrations in matched plasma specimens (Abstract 576). Twenty (26%) CSF specimens were below the assay’s limit of detection (5 ng/mL) and 42 (55%) were below 11 ng/mL, a median inhibitory concentration of atazanavir for wild-type HIV. One explanation for these lower-than-expected atazanavir concentrations in the central nervous system may be that many antiretrovirals are substrates for multidrug resistance pumps, such as P-glycoprotein, that are expressed on brain endothelial cells. For example, Marque-Beck and colleagues presented findings compar-
ing the C3435T polymorphism in mdr1, the gene encoding P-glycoprotein, and indinavir concentrations in CSF and plasma from 37 individuals (Abstract 365). Wild-type homozygotes (C/C) had lower indinavir concentrations in CSF than wild-type mutants (T/T) and lower indinavir concentrations in CSF, in turn, correlated with higher CSF RNA levels (P = .03).

Therapeutic antiretroviral concentrations in plasma in the presence of subtherapeutic antiretroviral concentrations in CSF may lead to discordant resistance between compartments. Two studies addressed the prevalence of discordant resistance in HIV derived from plasma and CSF. Wong and colleagues from the CHARTER group presented data on genotypic resistance derived from 145 CSF-plasma pairs, identifying discordant resistance in 35 (37%; Abstract 75). Among these 35 CSF-plasma pairs, 20 (57%) had more resistance mutations in CSF than plasma and 5 (14%) had resistance mutations present in CSF when only wild-type HIV was identified in plasma. In a smaller analysis, Bush and colleagues identified the discordant resistance mutations in 3 of 7 (43%) CSF-plasma pairs, a prevalence very similar to the larger CHARTER study.

Currently, individuals who have HNCI but do not normalize with antiretroviral therapy have fewer therapeutic options. Findings from 1 clinical and 3 preclinical studies of approaches designed to address this were presented. The first was an analysis from Schifitto and colleagues of data from ACTG 5090, a randomized, placebo-controlled, multicenter clinical trial of selegiline, a monoamine oxidase-B inhibitor that has antioxidant and pronerveotrophic effects (Abstract 364). A total of 128 individuals with HNCI were randomized to 1 of 3 treatment arms and 96 completed 24 weeks of treatment. Selegiline was well tolerated but ineffective, demonstrating no between-arm differences in neuropsychologic performance (P = .91). Potula and colleagues from the University of Nebraska presented more hopeful animal data on rosiglitazone, a clinically available peroxisome proliferator-activated receptor (PPAR)-γ agonist that can regulate anti-inflammatory pathways (Abstract 336). Using an established murine model of HIV-1 encephalitis, mice treated with 2 weeks of rosiglitazone had reduced p24+ macrophages in brain tissue (23%) and a substantial reduction in HIV RNA level (15,597 copies/mL), compared with placebo-treated controls (40% and 6921 copies/mL, respectively; both P < .05). Even more provocatively, Gorantla and colleagues presented animal data on immunization with glatiramer, a modulator of microglial and T-cell activation (Abstract 335). In severe combined immunodeficiency disease /HIV encephalitis (SCID/HIVE) mice, glatiramer immunization was associated with reductions in microglial and astrocyte-induced inflammation and improved neuronal integrity. In HIV-1 vesicular stomatitis virus-HIV encephalitis (HIV/SV-HIVE) mice, which have intact adaptive immune responses, glatiramer immunization greatly enhanced control of macrophage-induced inflammation, a mechanism strongly implicated in HIV neuropathogenesis, and restored hippocampal neuroregeneration. From a safety standpoint, the immunizations seemed to be well tolerated by the animals. Finally, Agrawal and Strayer explored the effects of RNA interference on expression of CCR3, a chemokine receptor expressed by neural cells and implicated in neural adaptation of HIV (Abstract 332). Using a panel of small interfering RNAs, they identified R3-526 as the most effective in downregulating CCR3, leading to an almost 80% decrease in CCR3 expression and a 70% decrease in productive HIV infection in microglia.

Pathogenesis

Several basic studies advanced current knowledge on HIV neuroadaptation and the roles of specific cytokine pathways in neural injury. Building on data identifying position 308 in the V3 loop of gp120 as a possible indicator of neural-adapted HIV, Thomas and colleagues from the Gabuzda lab amplified full-length env genes from autopsy brain and lymphoid tissue from 4 patients with HNCI (Abstract 330). Sequence alignments and viral epidemiology signature pattern analysis confirmed residue 308 as a brain signature position. However, the mechanism by which 308 might be selected was not identified since mutagenesis experiments did not identify that a proline at 308 was associated (1) with improved ability to infect cells in low CD4 or CCR5 conditions or (2) with sensitivity to inhibition by a panel of entry inhibitors. Dunfee and colleagues, also from the Gabuzda lab, presented data on another position, 283, in env (Abstract 76), which did seem to be associated with reduced CD4 dependence. To characterize this position, they cloned Env from 2 primary brain isolates that had reduced CD4 dependence and used mutagenesis to identify amino acids that contributed to reduced CD4 dependence and virus replication in macrophages or microglia. These analyses demonstrated that asparagine, rather than consensus threonine, at position 283 in the C2 region of gp120 was associated with reduced CD4 dependence. The investigators also summarized that 5 published studies with matched brain- and lymphoid-derived Env from 31 patients identified that N283 was detected at a 5.5-fold higher frequency in brain-derived Env than lymphoid-derived Env, and that it occurred at a 5-fold higher frequency in brain-derived Env from individuals with HNCI than those without.

These studies provide strong evidence that HIV adaptation to neural cells is an important event in the pathogenesis of HNCI. Two others focused on host factors that may also contribute to it. Carroll-Anziger and colleagues focused on astrocytes, which are critically important in HIV neuropathogenesis but may only support restricted HIV replication (Abstract 339). After stimulating cells with interferon (IFN)-γ, HIV replication increased in human fetal astrocytes by 10-fold and in U87MG cells by 3-fold. IFN-γ also upregulated expression of CCR3, which is interesting in light of the findings of Agrawal and Strayer (Abstract 332). Astrocytes may also influence HIV disease by producing...
SDF-1, a neurotoxic protein that is also the ligand for the HIV coreceptor, CXCR4. Peng and colleagues from the Zheng lab stimulated astrocytes with media from activated macrophages and identified substantial increases in SDF-1 (Abstract 337). In particular, the investigators were able to link production of SDF-1 from astrocytes to interleukin (IL)-1β from activated macrophages by identifying parallel changes in their concentrations and by interfering with astrocyte SDF-1 production by treating macrophages with IL-1Ra and IL-1β small interfering RNAs.

**Peripheral Nervous System Complications**

Antiretroviral toxic neuropathy, typically related to exposure to the antiretroviral drugs stavudine or didanosine, represents an important neurologic complication among individuals with HIV infection in the United States and Europe. Additionally, due to the inclusion of these drugs in antiretroviral therapy regimens in the developing world, antiretroviral toxic neuropathy may become a global neurologic problem. Unfortunately, in many cases, neuropathic signs, symptoms, and disability do not disappear after these drugs are stopped. This year’s conference featured several presentations bearing on host vulnerability to antiretroviral toxic neuropathy, its clinical course, and new biomarkers and treatments.

In an observational study performed at a single clinical center (Ellis and colleagues, Abstract 368), neuropathic symptoms and signs were prospectively characterized in 2 groups of individuals: one consistently exposed to stavudine or didanosine (“d-drug” exposed, or DDE) over a period of 1 to 5 years (n = 252), and another never exposed to d-drugs (NDDE), either prior to or during the clinical follow-up period (n = 250). At the initial evaluation, DDE subjects were significantly less likely to have symptomatic antiretroviral toxic neuropathy than NDDE subjects. This bias was anticipated, since care providers are likely to switch out a d-drug for an alternative antiretroviral agent when neuropathic symptoms develop in an individual who is taking didanosine or stavudine. Among subjects who did not already have antiretroviral toxic neuropathy at baseline, the accumulation of neuropathic symptoms and signs was similar in DDE subjects (359 person-years of follow-up) to that in NDDE subjects (311 person-years of follow-up). Additionally, continued d-drug use did not result in an excess of worsening neuropathic signs or symptoms, even among those already suffering from symptomatic neuropathy at the initial visit. These findings re-emphasize that only a subset of individuals appear susceptible to the development of a dose-limiting neuropathic syndrome with exposure to d-drugs. In the remainder, continued d-drug use appears to be well tolerated.

Kallianpur and colleagues from Vanderbilt took advantage of a unique opportunity to evaluate potential genetic host susceptibility markers associated with the development of antiretroviral toxic neuropathy (Abstract 78). Specifically, in ACTG 384, HIV-infected subjects were randomized to receive an antiretroviral drug regimen that either did or did not include d-drugs (specifically, didanosine plus stavudine versus zidovudine plus lamivudine). The investigators targeted polymorphisms in the hemo-chromatosis (*hfe*) gene that alter intracellular iron disposition, and may thereby affect mitochondrial function. The parent study showed a clear excess of incident neuropathy symptoms among individuals randomized to d-drug—compared with non-d-drug—containing regimens. The incidence of antiretroviral toxic neuropathy was lower in individuals who had 1 of 2 *hfe* polymorphisms (C282Y and H63D), suggesting a protective effect. These polymorphisms were found in only a minority of study participants. In particular, C282Y was rare among African Americans. This study concluded that specific polymorphisms in the *hfe* gene might confer protection from the development of neuropathy with exposure to d-drugs. The putative mechanism by which this genetic marker acts to reduce susceptibility—through alterations in intracellular iron disposition and mitochondrial function—remains to be demonstrated. A significant limitation of this study is its case definition, which was based on an adverse events scale rather than on a systematic neurologic examination. As previously shown in studies such as NARC007, case ascertainment for neuropathy may be problematic, particularly in multicenter studies.

In another analysis from ACTG 384, Hulgan and colleagues compared the diagnosis of neuropathy to polymorphisms in mitochondrial genes (Abstract 350). In this case, the polymorphisms T4216C and A4917G conferred an increased risk for the development of neuropathy in individuals who were taking d-drugs. Since the same polymorphisms are known to alter amino acid sequence in mitochondrial complex I subunits, the authors inferred that the mechanism by which the polymorphisms confer increased risk involves alterations in mitochondrial oxidative phosphorylation.

Cherry and colleagues reported on associations between another panel of host genetic vulnerability factors and neuropathy (Abstract 344). The cohort examined in this study was somewhat different from those in previous studies, in that it was restricted to individuals who had previously been exposed to stavudine, didanosine, or zalcitabine. Subgroups were defined as individuals who developed neuropathic symptoms or signs on the d-drugs (antiretroviral toxic neuropathy; n = 40), and those who did not develop neuropathy despite at least 6 months of exposure to a d-drug (antiretroviral toxic neuropathy resistant; n = 28). Polymorphisms in genes encoding 2 inflammatory mediators (TNF-α-308 and IL-12B′UTR) were more frequent among individuals who developed neuropathy during d-drug exposure than among those who did not. The investigators concluded that inflammatory pathways might play a role in the development of neuropathy due to d-drugs. This study was limited by the absence of a comparison group without d-drug exposure, such that relative
risks cannot be assessed either for the development of neuropathy or for the effect of host chemokine polymorphisms on the development of neuropathy.

In an overview session, McArthur presented a review of emerging data on an important new surrogate endpoint for neuropathy in HIV infection: epidermal nerve fiber layer density (ENFL; Abstract 80). The ENFL represents the distal-most end of nerve fibers in the skin that transduce pain and temperature sensation. Recent studies have demonstrated that this layer is deficient in HIV-infected individuals with neuropathy—both primary HIV-associated neuropathy and antiretroviral toxic neuropathy—compared with HIV-infected individuals without neuropathy and with HIV-seronegative individuals. It has been shown that topical capsaicin, the active ingredient in hot chili peppers, dramatically reduces the ENFL at the site of injury. In HIV-seronegative individuals, the ENFL reconstitutes itself over several months, providing clear evidence of peripheral nerve regenerative capacity. These findings are of considerable interest with respect to the possibility of using ENFL density as a surrogate endpoint in clinical trials of neuroprotective and neuroregenerative agents.

Finally, Simpson and colleagues presented data from a randomized clinical trial of high-dose topical capsaicin dermal patch treatment for painful sensory neuropathy (Abstract 79). Although capsaicin depletes the ENFL, evidence to date suggests that any resulting sensory impairment produces no disability. In the capsaicin dermal patch trial, 307 individuals with painful neuropathy were randomized to receive either a low-concentration capsaicin patch or a high-dose capsaicin patch applied for 30, 60, or 90 minutes. The endpoint was reported pain on a standardized scale. The pooled high-dose capsaicin dermal patch groups experienced pain reduction of 23% lasting up to 12 weeks after a single capsaicin dermal patch application. This compared with only 11% pain reduction in the pooled control group. Despite transient pain increases during and shortly after capsaicin dermal patch application, tolerability was otherwise quite good. Studies of epidermal nerve fiber reconstitution following capsaicin dermal patch application are ongoing. This represents a novel technique to be added to the existing armamentarium for HIV-associated neuropathic pain, a condition often refractory to treatment.

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Complications of HIV Disease and Antiretroviral Therapy

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

As antiretroviral treatment regimens become more potent and easier to administer, differences in the rates of adverse events and complications associated with treatment will increasingly drive decisions regarding the selection of therapy. This year’s Conference on Retroviruses and Opportunistic Infections featured a wide range of research directed toward understanding the pathogenesis, treatment, and long-term consequences of complications associated with HIV infection and the use of antiretroviral therapy in both resource-limited settings and in the developed world. This review will summarize information on complications of antiretroviral therapy in resource-limited settings, hepatitis C virus, tuberculosis, and discussion of metabolic, cardiovascular, and renal complications.

Antiretroviral Drug Toxicity in the International Setting

Zidovudine, Stavudine, Nevirapine, and Abacavir

At this year’s conference, several international groups provided analyses of drug toxicities being observed in the global rollout efforts. These programs are utilizing World Health Organization first-line regimens including nevirapine or efavirenz and zidovudine or stavudine plus lamivudine. Although many of these analyses were limited by ascertainment bias, all data suggested that these populations were susceptible to the same significant toxicities—such as peripheral neuropathy and lactic acidosis—that have been observed in studies in the developed world.

Boule and colleagues presented follow-up on 1700 HIV treatment-naive adults in the Khayelitsha cohort from South Africa (Abstract 66). Initially patients in this cohort utilized zidovudine, lamivudine, and efavirenz, but then switched to a nevirapine- and stavudine-based regimen. By 2 years, similar proportions of patients had switched off of stavudine (8.5%), zidovudine (8.7%), and nevirapine (8.9%). For patients on stavudine, lactic acidosis and peripheral neuropathy were the main reasons for switching. The risk for lactic acidosis was particularly high in obese women. The risk of peripheral neuropathy was greatest among patients older than 50 years with CD4+ cell counts below 50/µL. At 36 months, 59% of patients in this cohort were still receiving their original antiretroviral regimen. Investigators concluded that switches early on were concentrated around zidovudine, for which anemia was the treatment-limiting toxicity. With a stavudine regimen, reasons for dose-limiting toxicity were peripheral neuropathy and lactic acidosis.

Forna and colleagues presented results from the Tororo, Uganda, cohort in which 1073 persons started antiretroviral therapy (Abstract 142). This was the same cohort in which tuberculosis (TB) outcomes were described (see below). The initial regimen for almost all patients (96%) was nevirapine/stavudine/lamivudine. Drug-related toxicities developed in 417 (39%) patients. Peripheral neuropathies developed in 31% of patients; 8% were classified as “severe.” Rash occurred in 6% of patients; 2% of patients had severe cases and 2% had a hypersensitivity reaction. Five percent of patients had acute hepatitis. The probability of remaining free of any toxicity at 6, 12, and 18 months was 76%, 59%, and 47%, respectively; the probability of remaining free of severe toxicities was 92%, 86%, and 84%, respectively.

Twenty-one percent of patients in the cohort had drug changes for toxicity: 181 patients switched from stavudine to zidovudine and 30 from nevirapine to efavirenz. There was no mention of lactic acidosis.

A cohort study from Nairobi, Kenya, included 284 patients with a median CD4+ cell count of 159/µL (Abstract 143). The most commonly used regimen was nevirapine/stavudine/lamivudine. Neuropathy was reported in 23%, rash in 20%, hepatitis toxicity in 1.4%, and lipodystrophy in 0.4%. Toxicity-free survival was reported at 6, 12, and 18 months and was 45%, 27%, and 21%, respectively. The probability of staying on the original regimen was 98%, 97%, and 96%, respectively. Patients in this cohort continued treatment in the face of moderate and mild toxicity. The investigators concluded that tolerance of antiretroviral therapy was not a barrier to care in this resource-poor setting. However, in spite of this optimistic conclusion, cumulative toxicity from stavudine remains a major concern with the regimen, and better outcomes may be achievable with other regimens, resources permitting.

Lipoatrophy

Very little data have been presented on lipoatrophy in resource-limited settings. Two presentations addressed this topic. Wanchu and colleagues reported results from a study in India of 500 patients (Abstract 562). Two hundred thirty-five patients had a nevirapine-based regimen, and 65 had an efavirenz-based regimen. The median duration of follow-up was 8 months. During follow-up, 47 patients (15.5%) changed at least 1 drug. The most common reason to change drug was lipoatrophy; other reasons for

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changing included peripheral neuropathy, TB, and anemia. In this cohort, more than 15% of patients had to change drug for toxicity. The authors reported that an additional 12.6% would have liked to change for various reasons, such as peripheral neuropathy, but were limited by the availability of alternatives. The authors concluded that more options are needed for patients with drug toxicity, including lipoatrophy.

The second study was specifically designed to look at lipoatrophy in the Doctors without Borders program in Kigali, Rwanda (Abstract 560a). The study used a standardized case definition and questionnaire to evaluate 141 patients presenting to clinic who had been on antiretroviral therapy for at least 1 year. The study population was 78% female, with 112 subjects on stavudine and 15 on zidovudine. Body fat changes were reported in 38 subjects, all of whom were on stavudine. Lipoatrophy was confirmed in 33 cases with a prevalence of 23%. Risk factors for lipoatrophy were female sex and receipt of stavudine. Based on these results, tenofovir is preferred over stavudine. The authors suggested that the body habitus changes associated with stavudine are likely to affect adherence and increase stigmatization.

**Tenofovir Experience**

Some early data on tolerance to tenofovir in West Africa were reported by Landman and colleagues (Abstract 543). Forty treatment-naïve subjects received tenofovir/emtricitabine/efavirenz. Median plasma HIV RNA level at baseline was more than 5 log_{10} copies/mL and the median CD4+ cell count was 122/µL. The viral suppression rate was 87.5% at 24 weeks. Two patients died, 2 developed TB, and 1 changed drug for pregnancy. One patient switched for dizziness, but otherwise no serious toxicities were reported. There was no report of renal toxicity. These results are encouraging, but tenofovir is not yet available in most resource-limited settings.

**Abacavir Compared with Nevirapine: Toxicity**

The first study providing systematic data on the safety of abacavir in an African population was presented by Munderi (Abstract 109LB). The 24-week results of the Nevirapine or Abacavir (NORA) study, a randomized substudy of the Development of Antiretroviral Therapy in Africa (DART) trial, were presented. There were 599 patients with a median CD4+ cell count of 99/µL. This was a double-blind comparison; all patients received zidovudine/lamivudine as the nucleoside analogue reverse transcriptase inhibitor (nRTI) backbone. Serious adverse events were reported in 20 patients, and 19 of 20 were consistent with hypersensitivity reactions. Fourteen patients on abacavir (4.7%) and 30 on nevirapine (10%) discontinued the drug. The clinical symptoms that resulted in the syndromes included fever, rash, and respiratory and constitutional symptoms. Hepatotoxicity rates were significantly higher in those who received nevirapine. Abacavir had a discontinuation rate of approximately 2% in this African population with advanced HIV disease. This rate was lower than the rate for nevirapine. Results of viral load testing are not yet available for this study.

**Hepatitis C Virus Infection**

The elegant descriptions of protease and polymerase inhibitors for hepatitis C virus (HCV), highlighted in the symposium by Kwong (Abstract 172) and Hazuda (173 Abstract), generated a great deal of excitement regarding HCV at the conference. Several HCV protease inhibitors (HCV PIs) are already in clinical development. With both HCV PIs and polymerase inhibitors, resistance emerges with selective pressure, and antiviral activity to polymerase inhibitors was heterogeneous. It is likely that combination therapies will be required for the best outcomes with these new drug classes.

**Refining Current Strategies for Hepatitis C Virus Treatment**

Attempts to refine current strategies with available drugs have met with limited success. Ruys and colleagues conducted a randomized study to evaluate if high-dose peginterferon alfa plus ribavirin would be more effective than the standard dose (Abstract 852). In this open-label study, patients were randomized with either (1) induction therapy (peginterferon alfa 3 µg/kg/week for the first 4 weeks, followed by 2 µg/kg/week for 4 weeks, and then followed by the standard 1.5 µg/kg/week for the remaining 40 weeks), or (2) with the standard-of-care (1.5 µg/kg/week dose throughout). Patients were stratified by genotype, CD4+ cell count, and plasma HCV RNA level. The primary endpoint was virologic response 24 weeks after the completion of therapy. There was no difference in response between the 2 arms. Neuropsychiatric events were more common in the patients receiving induction therapy.

Extension of therapy was likewise not an effective strategy. In the trial presented by the Hepatitis Resource Network Clinical Trials Group (Abstract 854), all patients were treated with standard dose peginterferon alfa and weight-based ribavirin. Patients with undetectable plasma HCV RNA at 24 weeks were randomized to a standard 48 weeks or 72 weeks of therapy. The study enrolled 177 patients, of whom 80% were infected with HCV genotype 1; 61 patients qualified for the randomization. The sustained virologic response rates were approximately 50% in both groups. More than half of the patients randomized to the extended therapy were unable to complete the therapy due to patient preference and toxicity.

More success was achieved in identifying predictors of treatment response. Dieterich and colleagues analyzed the APRICOT study looking at treatment success rates in subjects with a rapid virologic response defined as undetectable HCV RNA at week 4 (Abstract 856). In the APRICOT study,
22 of 289 patients (13%) had a rapid virologic response and 18 (82%) had a sustained virologic response. Peginterferon alfa plus ribavirin produced a sustained virologic response of 81% in genotype 1 regardless of baseline viral load. This was even higher than response rates in subjects with low viral loads. A small study looking at ribavirin levels as predictors of early virologic response found that higher ribavirin levels were associated with improved responses only for patients with genotypes 1 and 4 (Abstract 857).

Predictors of treatment response in acute HCV were also evaluated among a cohort of 21 patients who received treatment with interferon alfa (Abstract 83). Investigators looked at cytokine genotypes to interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), transforming growth factor-beta (TGF-β), interferon gamma (IFN-γ), and interleukin-10 (IL-10) as predictors of sustained virologic response to treatment. IL-6–high producers exhibited the best responses to treatment. Investigators speculated that IL-6–mediated activation of signal transducers and activators of transcription enhance activity of the anti-HCV effects of interferon alfa in hepatocytes.

When HCV therapy fails, or when patients present at an advanced stage of liver disease, liver transplantation may be the only option. Miró and colleagues reported on their 4-year experience (median follow-up time of 1 year) in Spain with liver transplantation in HIV-infected patients (Abstract 875). Of the 50 transplant patients, 49 had HCV. All patients were receiving highly active antiretroviral therapy (HAART), and 48 had undetectable plasma HIV RNA. Forty-eight percent of patients had an acute rejection episode. Ten patients died, and half of these deaths were attributed to HCV. HCV therapy was re instituted in 16 cases, but only 18% of patients achieved a sustained virologic response. The authors identified rejection episodes and HCV relapse as the major challenges of transplantation in this population.

**Hepatitis C Virus: Updates on Sexual and Mother-to-Child Transmission**

Accumulating evidence suggests that HCV is spreading within the men who have sex with men (MSM) community via a sexual route in some large urban cities. Coutinho and de Laar presented a molecular epidemiologic analysis of acute HCV among the MSM population in Amsterdam (Abstract 87). The study population included 1836 MSM participating in the Amsterdam cohort; acute cases of HCV were identified from hospital records. The main findings from this study were that in the period from 1985 to 2003, there was a dramatic increase in HCV among HIV-seropositive, but not -seronegative, men and that the cases of acute HCV clustered phylogenetically within a few specific subtypes (1a, 1b, 3a, and 4d). The authors speculated that HCV was introduced into the population during this period and that mucosal trauma during sexual events contributed to the high rates of transmission. A study from the United Kingdom also supported evidence of transmission of HCV within the MSM community (Abstract 86). The authors identified 111 HCV seroconverters from 2002 to 2005. Molecular analysis revealed multiple clusters of independent HCV lineages. Risk factors for new cases included multiple sexual partners and mucosal trauma. A poster of an analysis from the French PRIMO cohort was presented by Ghosn and colleagues (Abstract 843). In addition to finding increased incidence of HCV among MSM, these investigators reported cases of acute HCV among women for whom sexual exposure was the only identified risk factor. Although these and other studies support evidence of sexual transmission of HCV, the inability to completely exclude drug use as a risk factor has prevented complete consensus among experts in the field on the frequency of HCV sexual transmission.

The risk for mother-to-child transmission of HCV is increased in the setting of HIV. In Catalonia, Spain, where the HCV prevalence is over 60% among HIV-seropositive pregnant women, Fortuny and colleagues hypothesized that HAART or cesarean delivery would reduce HCV transmission (Abstract 841). Interestingly, cesarean delivery, but not antiretroviral therapy, was associated with reduced rates of HCV transmission. Children with HIV also had a higher rate of HCV acquisition.

**High-Risk Populations and Barriers to Hepatitis C Virus Treatment**

Injection drug users (IDUs) remain most at risk for HCV, and the high incidence rates among even the youngest IDUs were highlighted in a report from San Francisco. Page-Shafer and colleagues performed 2 different antibody tests (enzyme immunoassay [EIA] 2.0 and EIA 3.0) and measured plasma HCV RNA levels among a cohort of young IDUs (Abstract 844). Among the cohort of 321 youth, 93 new infections were identified, giving an estimated incidence rate of 26%. The median age of the infected persons was 22 years. More than a third of the new infections were detected before antibody conversion by measurement of plasma HCV RNA. The EIA 3.0 detected infection approximately 3 weeks earlier than the EIA 2.0. Thirty-nine percent of patients had elevations in liver function tests. Despite aggressive public health campaigns, new HCV infections among young IDUs occur at an alarming rate.

HCV treatment remains challenging, and out of the reach of many populations for various reasons. Two presentations underscored this point. Scott and colleagues looked at the outcomes in a cohort of 369 HIV and HCV coinfected patients in an urban Seattle hospital (Abstract 882). Only 28% of the Seattle cohort was evaluated for HCV; 5% received treatment and 1.6% had a sustained virologic response. Mehta and colleagues reported the experience from a similar study of 845 HIV and HCV coinfected patients in Baltimore (Abstract 884). Thirty-three percent of patients in this cohort were referred for evaluation. Approximately one third of patients completing an
Hepatitis B Virus: Combination Therapy and Entecavir Drug Resistance

As with HCV, treatment studies of hepatitis B virus (HBV) in HBV and HIV coinfected patients have lagged behind studies of HBV in HBV-monoinfected patients. Nevertheless there were 2 studies of interest presented at this year’s conference. Nelson and colleagues presented the results of an open-label 59-patient randomized study of tenofovir versus lamivudine versus tenofovir/entecavir (Abstract 831). Twenty-seven of the patients were lamivudine-naive at entry. In lamivudine-experienced patients, the median reductions in plasma HBV RNA level at 24 weeks were 3.41, 0.82, and 3.93 log_{10} copies/mL, respectively. Among lamivudine-naive patients, reductions were 4.66, 3.31, and 5.03 log_{10} copies/mL, respectively. Thus in lamivudine-naive patients, combination therapy with tenofovir plus lamivudine was superior to either monotherapy. For lamivudine experienced patients, adding or switching to tenofovir was superior to lamivudine only.

Entecavir is a recently approved drug for HBV treatment that has no activity against HIV. Lamivudine resistance predisposes to the development of entecavir resistance. Colonno and colleagues presented the results of resistance testing done on 50 subjects with lamivudine resistance at baseline randomized to entecavir or placebo (Abstract 832). The plasma HBV DNA level dropped by 4.2 log_{10} copies/mL at 48 weeks in patients treated with entecavir. There was no viral rebound, but entecavir resistance mutations were detectable in 2 patients. More sensitive assays performed on pre-treatment isolates detected minority entecavir-resistant variants. Entecavir treatment was effective, but also enriched for minor drug-resistant variants. With the high HBV viral loads that exist in most patients undergoing treatment, prevention of drug resistance is likely to be an important goal of treatment.

Insulin Resistance and Diabetes

The relationship between HIV infection, antiretroviral drugs, and the development of insulin resistance and diabetes remains an active area of investigation. Three groups examined the rates of diabetes or impaired glucose tolerance in HIV-infected adults compared with population-based uninfected controls (Abstracts 759, 760, 761). Howard and colleagues prospectively evaluated oral glucose tolerance tests in a diverse sample of 198 HIV-seropositive and 125 at-risk adults at 2 points 18 months apart and found no difference in the incidence of impaired glucose tolerance (IGT) or diabetes by HIV status. Within the group of HIV-seropositive subjects, the use of protease inhibitor (PI)-based HAART did not appear to increase rates of abnormal glucose tolerance. The independent risk factors for developing abnormal glucose tolerance or diabetes included only older age and obesity. Diabetes prevalence among antiretroviral therapy-naive HIV-infected subjects enrolled in Community Programs for Clinical Research on AIDS (CPCRA) studies was compared with population-based data from the National Health and Nutrition Examination Survey (NHANES) III. The prevalence of diabetes was lower in the HIV-infected group than in NHANES III and factors associated with the presence of diabetes included HCV coinfection, older age, and body mass index (BMI), emphasizing the importance of traditional risk factors for diabetes. Finally, the prevalence of diabetes was further assessed among 2000 HIV-seropositive subjects (mostly men) and a similar number of control men in the Veterans Aging Cohort Study (Abstract 761). The prevalence of diabetes was again lower in the HIV-infected group. Among the HIV-infected subjects, higher BMI and the use of a PI at any point were associated with an increased risk of diabetes, and a CD4+ cell count below 150/µL was associated with a lower risk. Collectively these results suggest that un-treated HIV infection may not increase the risk of diabetes; however, traditional risk factors, use of PI therapy, and possibly HCV coinfection may contribute to diabetes risk among patients with preserved CD4+ cell counts. These studies highlight the importance of control groups when assessing the contributions of HIV infection and antiretrovirals to metabolic complications.

Investigators from the Multicenter AIDS Cohort Study (MACS) previously reported a slightly higher prevalence of metabolic syndrome among men with HIV than controls (Abstract 747). At this year’s conference they extended these observations to include an analysis of the association between specific antiretrovirals and metabolic syndrome. Each additional year of HAART contributed to an increased risk of metabolic syndrome, in part driven by the increased risk of elevated triglycerides with PI use and efavirenz. In contrast to the diabetes data noted above, lower CD4+ cell counts appeared to correlate with increased risk of this syndrome, suggesting that perhaps chronic uncontrolled HIV infection may contribute to the development of metabolic syndrome.

Insulin resistance was examined in several studies as a marker for other vascular outcomes. In the Hawaii Aging with HIV Cohort, insulin resistance, independent of diabetes, was a marker for cognitive impairment in older but not younger HIV-infected patients (Abstract 349). Insulin level was also identified as a predictor of higher rates of progression of carotid intima media thickness in adults (Abstract 145), thicker carotid intima media thickness in children (Abstract 691), and progression of coronary calcification (Abstract 739).
Dyslipidemia and Cardiovascular Disease

Over the past several years there has been a growing focus on minimizing cardiovascular risk by addressing dyslipidemia and other modifiable risk factors among patients with HIV infection and we are beginning to see evidence that these efforts may be yielding a benefit. Several large cohort studies demonstrated that rates of myocardial infarction (MI) and coronary heart disease among HIV-infected adults seem to be stabilizing or declining (Abstracts 735,737). This change has been attributed to changing patterns of antiretroviral use and increased attention to lipid-lowering and antihypertensive therapy (Abstract 740). However there is room for improvement as noted in the Swiss HIV Cohort Study in which only one third of patients with dyslipidemia or hypertension were receiving lipid lowering or antihypertensive therapy.

Options for managing dyslipidemia range from antiretroviral drug substitution to the use of lipid lowering agents. Fish oil was shown to be effective in a small trial at last year’s meeting and at this year’s conference 2 additional randomized trials presented data on the use of fish oil for hypertriglyceridemia. Twelve weeks of treatment with salmon oil 3 gm/day reduced mean values for fasting triglycerides by 95 mg/dL (20%) compared with an increase of 26 mg/dL among placebo recipients in HAART-treated patients with elevated fasting triglycerides at baseline (Abstract 756). At the end of 24 weeks of treatment, the overall benefit of the salmon oil was more modest (59-mg/dL decrease) compared with baseline values. Fish oil was compared with fenofibrate in a randomized trial of 100 patients who had elevated triglycerides (but normal low-density lipoprotein) on HAART. After 8 weeks of therapy the median reduction in triglycerides was slightly greater in the fenofibrate group (58% versus 46%) and a higher proportion of patients achieved triglyceride levels below 200 mg/dL in the fenofibrate group (17% versus 9%). Combination therapy with fish oil and fenofibrate was administered to those with triglyceride levels above 200 mg/dL at week 8 and further reductions were seen with the combination. At the end of 18 weeks, median triglycerides fell by 65%, and 22% of subjects achieved a triglyceride level below 200 mg/dL. These results show the added benefit of combining fish oil and fenofibrate for those patients who do not respond to single-agent therapy with either drug.

Several studies at this year’s conference seemed to strengthen concerns about the relationship between dyslipidemia on antiretroviral therapy and cardiovascular risk in the setting of HIV infection while continuing to emphasize the importance of traditional risk factors in predicting cardiovascular events. The Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study had previously reported an association between longer exposure to combination antiretroviral therapy and risk of MI (Fris-Moller, N Engl J Med, 2003). At this year’s conference, observations from the DAD investigators were extended to include an analysis of exposure to specific classes of drugs and MI risk. With 94,469 person-years of follow-up, the risk of MI appeared to be stabilizing and not increasing over time. For each additional year of PI exposure, the risk of MI increased by 1.6%. After adjustment for lipid levels, this risk was diminished, suggesting that the lipid levels could mediate the MI risk. Of note, the risk of MI was also slightly increased with longer duration of nonnucleoside analogue reverse transcriptase inhibitor (NNRTI) exposure; however, this was not statistically significant after adjustment for nRTI use.

In a matched cohort of HIV-infected and -uninfected adults, 3-year rates of progression of carotid intima media thickness were not statistically greater in PI-treated patients than in non-PI–treated HIV-seropositive or -seronegative controls; however, among the HIV-infected patients there was a suggestion of a PI effect on greater rates of progression of carotid intima media thickness (Abstract 145). In another study examining predictors of increased intima media thickness, cytomegalovirus-specific T-cell responses and higher levels of high-sensitivity C-reactive protein, but not levels of T-cell activation, correlated with cross-sectional values for intima media thickness (Abstract 741).

Lipoatrophy and Fat Accumulation

Management of lipoatrophy and fat accumulation continue to be challenging clinical issues. Although objective measures of body fat are crucial to intervention studies these measurements are not practical in many clinical settings. McComsey and colleagues reported that patient self-report and physician exam correlated well with objective measures of limb fat by dual-energy x-ray absorptiometry using data from a study of lipoatrophy (Abstract 751). News from this year’s conference extends previous observations that a change from zidovudine or stavudine to either abacavir or to an nRTI-sparing regimen (Abstract 755) seems to be the best currently available option for managing lipoatrophy.

Pioglitazone, which is a thiazolidinedione and PPARγ agonist, was evaluated at a dose of 30 mg/day as a potential treatment for lipoatrophy in a randomized, placebo-controlled trial conducted in France (Abstract 151LB). The magnitude of the increase in limb fat (0.53 kg) was not noticeable by the subjects; however, it is within the range of increase noted by other interventions over a similar time period of follow-up. Of note, no increase in limb fat was observed among subjects who remained on stavudine, and the significant increase was driven by subjects who were not receiving stavudine during study follow-up time. “Normal values” for limb fat are approximately 6 kg and it is possible that further increases will accrue over longer follow-up in the absence of stavudine treatment.

Disappointing results were seen in studies evaluating treatments for fat accumulation. Metformin and rosiglitazone in combination or alone failed to
significantly reduce visceral fat in randomized controlled trials (Abstracts 147, 148) and a modest increase in limb fat was noted among the rosiglitazone-treated patients in one study (Abstract 147).

In obese HIV-uninfected men, testosterone replacement has been shown to reduce visceral fat. A randomized placebo-controlled trial was conducted to evaluate the use of supplemental testosterone (10 gm of topical gel applied daily) in men with low serum testosterone and increased waist circumference or an increased waist to hip ratio (above 0.95; Abstract 149). After 24 weeks of treatment a surprising finding of the study was a statistically significant decline in total body fat (and notably in extremity fat) in the testosterone-treated group with no change in visceral fat in the testosterone-treated group compared with placebo. Lean body mass did increase modestly in the testosterone-treated patients. The results of this study suggest that an unexpected outcome of testosterone replacement in this group of patients could be loss of limb fat. The magnitude of the decline (0.48 kg) is greater than the magnitude of increases seen in studies examining therapies for the treatment of lipoatrophy.

**Pulmonary Hypertension**

Pulmonary hypertension was a recognized complication of HIV infection prior to the HAART era and it appears that this problem has not diminished. Prospective studies by 2 groups confirmed that HIV-infected patients appear to be at increased risk for pulmonary arterial hypertension, and that the incidence (0.21%) does not appear to have decreased in recent years (Abstracts 743, 744). Treatment with the oral endothelin antagonist bosentan appears to be well tolerated in HIV-infected patients based on a report of more than 100 treated HIV-infected patients; no efficacy data were included (Abstract 745). Clinicians are reminded to consider pulmonary arterial hypertension in the differential diagnosis of dyspnea in the setting of HIV.

**Genetic Predisposition to Metabolic Complications**

The growing interest in the relationship between host genetics and HIV complications was evident at this year's conference. A poster discussion session moderated by Telenti and Mallal reviewed potential genetic markers for metabolic complications. The moderators emphasized the need to validate the findings of associations between genetic markers and adverse outcomes identified in one cohort to another; the importance of having a biologic mechanism to explain the association and follow-up of the association studies with in vivo work to explain the mechanism of the effect. Many of the studies presented at the conference could be categorized as first-time associations between the presence of a specific polymorphism and an adverse event.

For example, adverse metabolic changes were examined in AIDS Clinical Trials Group (ACTG) 384 study participants (Abstract 736). Subjects with similar baseline values for metabolic parameters were grouped based on metabolic results after 32 weeks of follow-up. Genetic markers were then compared between the high-risk group and a lower risk group based on values of BMI, total cholesterol, low-density lipoproteins, triglycerides, and the homeostasis model assessment of insulin resistance. A single nucleotide polymorphism (C-to-T) in the second intron of the resistance gene was associated with a significantly increased risk for developing a constellation of adverse metabolic changes on HAART (*P* = .001). Heterozygotes (*n* = 65; odds ratio [OR], 2.8; 95% confidence interval [CI], 1.4–5.7) and homozygotes (*n* = 5; OR, 19.4; 95% CI, 2–182) were at increased risk relative to wild type (*n* = 117) of being in this high-risk group. This polymorphism consistently increased the risk of adverse metabolic changes on HAART in all races. Confirmation of these findings in other cohorts is required.

In another study polymorphisms in genes involved in lipid metabolism were examined in a testing cohort and positive findings were examined in a second smaller validation dataset. Potentially clinically significant differences in high-density lipoprotein and triglyceride levels were found when these genotypes were examined (Abstract 764). For example, ritonavir recipients with the most unfavorable APOE/APOCS/APOA5/CETP/ABCA1 genotype had median triglyceride levels that were markedly higher than the triglyceride levels of those without ritonavir exposure and a favorable genotype. Likewise, patients receiving NNRTI-based treatment with a favorable CETP/APOA5 genotype had higher high-density lipoprotein levels than those on no antiretroviral therapy with an unfavorable genotype. Although not yet ready for clinical use, these types of genetic profiling studies suggest that at some point in the future we might be able to minimize treatment-related dyslipidemia.

**Bone Complications**

A cross-sectional study of menstrual irregularities, HIV infection, and bone mineral density found that both menstrual irregularities and HIV infection were independent predictors of low bone mineral density; however, HAART use was associated with higher bone mineral density. These results suggest that the detrimental effect of HIV on bone mineral density may be mediated by menstrual irregularities (Abstract 754).

**Renal Disease**

The prevalence of renal disorders was assessed among a large cohort of children with HIV disease with 7 years of follow-up (Abstract 699). Renal diagnoses were noted among 6% of the children followed up, and 22% had persistently abnormal lab results. Of the children who died during follow-up, 28% had persistent abnormal lab results. The authors concluded that renal pathology in HIV-infected children is a common complication associated with HIV-related morbidity and mortality.

Several presentations examined risk
factors for renal disease among HIV-infected adults with a focus on the relationship between tenofovir exposure and renal insufficiency. Previously reported data from clinical trials demonstrated a low rate of renal insufficiency among patients treated with tenofovir, and several presentations at this year’s conference attempted to extend the understanding of risk factors for renal insufficiency in cohort studies and postmarketing safety databases (Abstracts 778, 779, 780, 781). The 2 largest studies, one from the Centers for Disease Control and Prevention (CDC) and the other from the Gilead Early Access Program (EAP) and postmarketing surveillance, suggest that the rates of serious renal adverse events among patients receiving tenofovir remain low. The CDC analysis includes follow-up data from 9555 patients with 34,814 6-month person-observations (17,357 person-years) of follow-up. The percentage of persons with renal impairment as defined by categories of glomerular filtration rate were 40% with any impairment, 32% with mild impairment, 6.1% with moderate impairment, and 2.4% with severe impairment. Factors associated with renal impairment included lower CD4+ cell counts, lower hemoglobin, the presence of diabetes, and hypertension. The authors acknowledged that the relationship between the use of tenofovir and renal impairment in the analyses may have been confounded by the fact that patients with more advanced disease were using tenofovir during the first years the drug was available. In addition, data on co-administration of nephrotoxic drugs and information about appropriate dose reduction were not available. The Gilead analysis of the EAP program included 3700 person-years of follow-up and reported an incidence of 0.5% for serious renal adverse events. In addition, the postmarketing surveillance database included 455,392 person-years of follow-up with 43.3 serious renal adverse events per 100,000 person-years. Although the rates of serious renal events remain low, clinicians are reminded to estimate and follow creatinine clearance during HIV treatment, to avoid co-administration of nephrotoxic agents with tenofovir, and to dose-reduce tenofovir among patients with creatinine clearance below 50 ml/minute to avoid this complication.

### Opportunistic Infections

Opportunistic infections remain rare among patients responding to antiretroviral therapy. In a large cohort of treatment-naive patients who received antiretroviral therapy in controlled clinical trials, the median CD4+ cell count at the time of an opportunistic infection was 55/µL. A lower CD4+ cell count, higher viral load, history of a prior opportunistic infection, and female sex were independent predictors of an opportunistic infection during 3 years of follow-up (Abstract 782). There continues to be interest in the use of oral valganciclovir to prevent cytomegalovirus infection among patients with CD4+ cell counts below 100/µL and uncontrolled HIV infection. Wohl and ACTG colleagues conducted a randomized controlled trial designed to evaluate the utility of pre-emptive oral valganciclovir therapy to prevent end-organ disease (Abstract 150). Patients with low CD4+ cell counts who were not responding to antiretrovirals were monitored for cytomegalovirus viremia and then randomized to valganciclovir or placebo and followed-up closely for end-organ disease. An unexpected outcome of the trial was a very low rate of cytomegalovirus end-organ disease in the viremic patients rendering the study underpowered to detect an impact of the intervention. The results of this study suggest that there is a lingering benefit of antiretroviral therapy even among patients who appear to be failing both virologically and immunologically using standard markers.

### Tuberculosis

#### Tuberculosis Associated with High Rates of Mortality

TB in the setting of HIV was the central theme of an overview talk by Harries from Malawi in the opening symposium of the meeting (Abstract 9) and the topic of many of the international abstracts at this year’s conference. Several studies highlighted that TB is one of the most common causes of mortality in HIV-infected persons before and after antiretroviral therapy initiation in TB-endemic areas. The contribution of TB to mortality prior to antiretroviral therapy was highlighted in a study presented by Peters (Abstract 30). This report included a cohort of 1582 discordant HIV-infected couples living in Rwanda. This group developed a modified HIV disease staging classification, coined as the Kigali Combined Stage (KCS), which included BMI, erythrocyte sedimentation rate, and hematocrit. The 3-year mortality rates for KCS stages 1, 2, 3, and 4 were 12.7%, 11.4%, 24.7%, and 51.3%, respectively. TB and chronic gastrointestinal infections were primary causes of death accounting for 24% and 20%, respectively. Other AIDS-defining illness such as Kaposi’s sarcoma, cryptococcal meningitis, and candidal esophagitis were causes of mortality in 6.3% of deaths combined.

Several programs reported the mortality and morbidity rates after initiation of antiretroviral therapy, and TB rose to the top of the list. All presenters acknowledged the difficulty in precise classification of causes of mortality. In a prospective cohort of 404 adults treated with a PI- or NNRTI-based triple drug regimen in Senegal, with a follow-up of 46 months, mortality was 11.7%. Among the 76 cases where cause of death was ascertained, mycobacterial disease was the most common (Abstract 63). In the Antiretroviral Therapy in Lower Income Countries (ART-LINC) cohort study, which includes patients receiving antiretroviral therapy through care programs in Africa, Asia, and Brazil, there were 696 new HIV illness in 4655 patients over 12 months. TB was the most common cause of death with a rate of 5.8 per 11 person-years. The majority of these events occurred within the first 2 months of antiretroviral therapy initiation with rates of 13 per 100 person-years in the
first month. Predictors of TB were younger age, lower baseline CD4+ cell count, and a history of TB (Abstract 67).

**Multidrug-Resistant Tuberculosis Outbreak in South Africa—Contributor to Mortality?**

Drug-susceptibility testing for TB is not routinely performed in Africa. When an extraordinarily high rate of mortality was observed among patients with TB in a South African district who were receiving antiretroviral therapy, investigators asked the question as to whether TB was multidrug resistant (Abstract 795). Among 95 sputum cultures, 40 patients (43%) were infected with multidrug-resistant TB. Twenty-six of these patients had resistance to all first- and second-line TB therapies. The median survival for a patient was 25 days, echoing observations from the United States in the early 1990s when multidrug-resistant TB was nosocomially transmitted in urban hospitals. Preliminary molecular analysis from the South African study suggests that a single strain is associated with multidrug-resistant TB. It remains unclear whether this is an isolated event, or more widespread than previously appreciated.

**Antiretroviral Therapy as Prevention for Tuberculosis**

Lawn and colleagues presented findings that antiretroviral therapy is associated with an overall reduction in rates of TB, even taking into account that some of the early cases probably represent undiagnosed disease present prior to antiretroviral therapy initiation (Abstract 68). In a prospective, hospital-based cohort of 546 persons receiving antiretroviral therapy in Cape Town, South Africa, the incidence rate of TB for the first year was 3.5 cases per 100 person-years. This rate declined to 1.0 case per 100 person-years after 5 years of antiretroviral therapy. Risk of TB was 2-fold higher in patients with CD4+ cell counts below 100/µL and age under 33 years. In this study TB was not associated with plasma HIV RNA level, previous history of TB, low socioeconomic status, or gender. Another interesting observation from this study was that the CD4+ cell response to antiretroviral therapy was lower in patients with TB than in those who did not have TB.

The prevalence, incidence, and outcomes of TB in a cohort of 1044 patient treated with antiretroviral therapy in Tororo, Uganda, were presented by Moore and colleagues (Abstract 794). At baseline, all subjects were screened for TB with a questionnaire, exam, chest x-ray, and sputum smear. TB cultures were not performed as part of screening. Among the cohort of 1044 antiretroviral therapy-eligible subjects screened to start antiretroviral therapy, 75 subjects (7.2%) were identified as having TB during this screening process. Risk factors for TB included low BMI and prior history of TB. An additional 52 subjects were diagnosed with TB during a median of 1.4 years of follow-up. Mortality rates of those diagnosed with TB at baseline or follow-up was 17.9 per 100 person-years and 3.8 in those without TB. Investigators estimated that TB was associated with 36% of the deaths in this cohort. They also observed that rates of TB declined over time. Rates were 7.27 per 100 person-years, 2.29 per 100 person-years, and 1.77 per 100 person-years at 1 to 6, 7 to 12, and 13 to 18 months after antiretroviral therapy initiation, respectively.

**Diagnoses: Undiagnosed and Latent Tuberculosis**

There were 2 abstracts looking at identification and treatment of tuberculin skin test reactors in Thailand. In a Thai Mother-to-Child Transmission (MCTC) plus treatment cohort program with 621 participants, 456 of whom were women (Abstract 792), the tuberculin skin-test positive rate was 20%. Discordance between couples was 37%. After 2 years of follow-up, new tuberculin skin-test reactions in the absence of TB were found in 13.8%: a 7-fold increase in skin test conversion after 2 years. A positive skin test in a partner was the highest predictor of skin test conversion. It was not clear whether the increased risk of TB within households was due to risk of asymptomatic active disease transmission, or reflects similar exposure risk in the household members.

In a second Thai study of a TB screening program in a clinic among 1078 HIV-infected subjects (Abstract 799), 22% were currently receiving antiretroviral therapy. In a multivariate analysis, CD4+ cell count above 200/µL, male sex, and antiretroviral therapy use were associated with a positive tuberculin skin test. Of 250 persons with positive tuberculin skin tests, 92% received isoniazid. Active TB was diagnosed in 14 persons with a positive skin test. Ten of these were diagnosed during the initial process of TB evaluation following skin-test results. Three developed active TB during isoniazid prophylaxis, and 1 at a later time. The authors concluded that tuberculin skin testing combined with isoniazid prophylaxis was a useful strategy in this population.

In a complementary study from Cape Town, South Africa, Bekker and colleagues estimated age- and sex-specific TB trends among a community of 13,800 between 1996 and 2004. During this time, reported TB cases increased 2.4-fold. Highlighting the challenges of TB case finding, there were more individuals in the community with undiagnosed TB than individuals already in treatment. Undiagnosed cases were not limited to the HIV-infected population, and smear-negative cases among both groups represented a large pool of undiagnosed cases (Abstract 69).

**Immune Reconstitution Inflammatory Syndrome in Subjects with Tuberculosis**

Preliminary data were presented from a randomized trial of early (2 weeks) versus delayed (8 weeks) initiation of antiretroviral therapy in patients diagnosed with TB in Tanzania (Abstract 796). One of the main objectives of this study was to see if immune reconstitution inflammatory syndrome (IRIS) dif-
ferred between patients starting antiretroviral therapy early or late after TB treatment initiation. Data were reported on 70 patients with smear-positive pulmonary TB. Subjects in this study received lamivudine/zidovudine/abacavir (fixed-dose combination) for antiretroviral therapy. They were observed as inpatients for the first 8 weeks of the study. At entry, the median CD4+ cell count was 103/µL. Outcomes of this preliminary report included the death of 3 subjects not attributed to TB (disseminated Kaposi’s sarcoma, pneumonia, cerebral malaria). There were 6 changes in antiretroviral therapy: 2 for anemia due to zidovudine and 4 with suspected abacavir hypersensitivity. The surprising finding of this study was that there were no cases of IRIS despite 516 patient-months of follow-up and inpatient observation. Other African investigators commented that these findings differed from their clinical experience where IRIS occurred in HIV-infected, and to a lesser degree, HIV-uninfected patients.

An interesting study from the Autran lab in Paris looking at reconstitution of immune responses in patients with TB treated with antiretroviral therapy was presented by Bourgarit and colleagues (Abstract 797). In a prospective study of 22 patients starting TB therapy and antiretroviral therapy, 9 experienced IRIS. Purified protein derivative (PPD)-specific Th1 IFN-γ cells increased sharply during IRIS; a similar increase was not observed to cytomegalovirus antigens. These PPD-specific cells represented up to 22% of all cells, and all expressed human leukocyte antigen (HLA) DR. Only 3 IRIS patients had ESAT-6 responses. Those without IRIS did not develop acute PPD-specific responses. It is interesting that immune restoration to mycobacterial antigens containing tuberculin but not ESAT-6 was associated with expansion of IFN-γ producing cells.

The Tuberculosis Clinic as Entry Point for HIV Testing

HIV testing is recommended for all persons diagnosed with TB. Srikanitah and colleagues provided data to extend these recommendations to all patients presenting to a TB clinic (Abstract 798). Among 395 subjects referred to a TB clinic, 82% consented to voluntary HIV counseling and testing. TB diagnosis was based on clinical symptoms and acid-fast bacillus (AFB) smears; cultures were not available. The HIV seroprevalence rate was high both among those with a TB diagnosis (45%) and those without (54%). The presentation also highlighted an opportunity for HIV prevention as the majority of clients reported ongoing sexual activity with at least 1 partner and condom usage was reported at less than 10%.

Tuberculosis Diagnosis

TB diagnosis is one of the Achilles heels of TB control and treatment programs. There are increasing reports of HIV-infected patients who have TB confirmed by culture but who are chest-x-ray negative, smear negative, and who may even be asymptomatic. More sensitive rapid diagnostic tests are available that increase diagnostic yield (Abstract 793). In this study of 73 subjects with proven TB, polymerase chain reaction direct amplification was performed in 20 patients who each had 3 negative AFB smears. Direct amplification diagnosed 75% of smear-negative cases. These types of tests are needed but are not yet feasible in resource-limited settings where perhaps they could have greatest benefit.

Drug Interactions

The controversy regarding safety of using nevirapine plus rifampin in patients treated for TB continued at this year’s conference. Pharmacokinetic studies from the United States and Europe showed dramatically reduced levels of nevirapine in the presence of rifampin. However, recent data from Thailand suggest that the combined use of nevirapine and rifampin is effective and safe in HIV-infected patients with TB. Many programs in Africa are utilizing this combination because of its access, cost, and practicality. In a study of healthy volunteers from India, single-dose pharmacokinetics of nevirapine was assessed by Pujari and colleagues (Abstract 574). Pharmacokinetic studies were repeated after these volunteers took rifampin for 7 days. The pharmacokinetics of nevirapine after addition of rifampin showed no significant change in Cmax. However, C24 was reduced by 60%; area under the curve by 80%; and half-life by 66%. The authors concluded that this combination should not be used in HIV-infected patients with TB.

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Additional Reference


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Advances in Antiretroviral Therapy

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2006 is the 25th anniversary of the AIDS epidemic and the 10th anniversary of the potent antiretroviral therapy era. During this period, the annual Conference on Retroviruses and Opportunistic Infections (CROI) has grown to represent one of the most important meetings of the year with respect to providing a forum for investigators to present the latest information on developments in the antiretroviral therapeutic arena. This year’s conference maintained this tradition and was most notable for the presentations on new antiretroviral agents, therapeutic strategies including major results of treatment interruption studies, viral resistance, and encouraging results of antiretroviral rollout programs in the developing world which have brought us to the threshold of a new era in treatment and clinical investigation.

Investigational and New Antiretroviral Agents

Entry Inhibitors

TNX-355. Duensing and colleagues presented data on TNX-355, a humanized monoclonal antibody to CD4 (Abstract 158LB). They analyzed baseline samples from an ongoing phase II trial of TNX-355 in treatment-experienced subjects. All isolates were relatively sensitive to TNX-355 and sensitivity did not vary according to coreceptor tropism. They also examined paired samples from a phase I trial collected prior to treatment with TNX-355 and after 9 weeks of monotherapy. Resistance to TNX-355 was associated with a reduction in the maximal suppression achieved with TNX-355 rather than a shift of the inhibitory curve. TNX-355-resistant viruses became more sensitive to soluble CD4.

Peptide Fusion Inhibitors. Delmedico and colleagues presented data on candidate peptide fusion inhibitors (Abstract 48). The goal of their development was to match or improve on the efficacy of enfuvirtide and to improve patient convenience. They presented data on 2 peptides. Enfuvirtide and the candidate fusion inhibitors block the binding of the heptad repeat (HR) 2 domain to the HR1 domain of gp41 in the “coil-coil interaction” prior to HIV fusion. The authors started with what they considered the optimized “slice” of the HR2 domain of gp41 (ie, the region of HR2 most important for this interaction). By itself, this peptide was very potent in vitro but had poor pharmacokinetic properties. They determined which amino acid residues of this peptide sequence were crucial for binding, and then set out to modify the peptide sequence while maintaining the crucial residues. The first strategy was to add helix stabilization motifs to the noncrucial residues, which yielded the peptide TRI-1144. The second strategy was to covalently add a fatty acid residue to the peptide yielding TRI-999. This peptide is stable in the presence of serum proteases. The pharmacokinetic profiles of these peptides were much improved compared with both the unmodified optimized “slice” of gp41 and enfuvirtide. Subcutaneous administration of these peptides to monkeys supported once-weekly administration. These peptides were potent in vitro and retained activity against enfuvirtide-resistant isolates.

CXC4R Antagonists: KRH-3955 and KRH-3140. HIV entry requires binding of gp120 to a chemokine coreceptor (either CCR5 or CXC4R) after binding CD4. Utilization of the CXCR4 coreceptor becomes more common in treatment-experienced subjects and subjects with lower CD4+ cell counts. Tanaka and colleagues presented data on 2 candidate CXCR4 inhibitors (Abstract 49LB). They showed that these compounds bound CXCR4 but did not bind other chemokine receptors. They did not appear to cause any significant toxicity in rats and the compounds were orally bioavailable. These compounds seemed to be effective against CXCR4-tropic HIV in a severe combined immunodeficiency disease (SCID) mouse model.

PRO 140. Olson and colleagues presented data on PRO 140, a humanized IgG4 CCR5 monoclonal antibody (Abstract 515). They tested a single intravenous infusion of 0.1 to 5.0 mg/kg in healthy male subjects. The infusions developed in these patients and plasma levels of RANTES, one of the native chemokines that binds to CCR5, were unchanged.

Zinc Finger Protein Nucleases. Zinc finger proteins can be constructed to bind to specific DNA sequences of 9, 12, or 18 basepairs. Two zinc fingers coupled with FokI nucleases can pinpoint specific DNA sequences and introduce a targeted double-stranded break in the genome. Subsequent
repair can render the gene nonfunctional. Jouvenot and colleagues presented data using zinc finger nucleases to target the CCR5 gene (Abstract 51). The CCR5 coreceptor is required for entry of CCR5-tropic HIV into CD4+ cells. They showed that CCR5 could be disrupted in 2 different cell lines, and these cells were protected against infection with CCR5-tropic HIV. The practical introduction of the zinc finger nucleases into CD4+ cells for possible clinical use still needs to be optimized.

**Aprepitant.** Wang and colleagues presented in vitro data on aprepitant (Abstract 511). This drug has been approved by the US Food and Drug Administration (FDA) for the treatment of chemotherapy-related nausea. It is a neurokinin-1 receptor antagonist. In vitro studies suggest that it downregulates CCR5 expression. The authors tested the antiretroviral activity of aprepitant in an in vitro cell culture system. Consistent with the mechanism of action, they found that aprepitant inhibited HIV infection with CCR5-tropic (but zidovudine-resistant) HIV, and was additive or synergistic with other currently approved antiretrovirals. It had no activity against a CXCR4-tropic strain.

**Vicriviroc.** Greaves and colleagues presented data from a treatment-naive study comparing the CCR5 antagonist vicriviroc, with efavirenz—each given with fixed-dose zidovudine/lamivudine (Abstract 161LB). All subjects received 2 weeks of monotherapy with 1 of 3 doses of vicriviroc or placebo. Then all subjects received open-label zidovudine/lamivudine and subjects receiving placebo were changed to open-label efavirenz. The median CD4+ cell count at baseline was 290µL and the mean plasma HIV-1 RNA level was 4.8 log₁₀ copies/mL. After 14 days of monotherapy, all 3 doses of vicriviroc (25, 50, and 75 mg) were associated with a significantly greater decline in plasma HIV-1 RNA (0.8, 1.2, and 1.5) than with placebo (0.1; P < .001). The study was stopped early by the Data and Safety Monitoring Board because of increased virologic breakthrough in the vicriviroc arms. At the time the study was stopped, 56%, 41%, and 17% of subjects in the 3 vicriviroc arms experienced virologic breakthrough compared with 4% in the efavirenz arm. Subjects taking regimens that failed generally developed the M184V mutation (associated with lamivudine resistance). The authors did not present data on resistance to vicriviroc. Coreceptor tropism shifts were generally infrequent and occurred in both the vicriviroc and placebo arms. There were no significant safety concerns reported.

**Reverse Transcriptase Inhibitors**

**GS9148.** Cihlar and colleagues presented data on an adenosine-analogue nucleotide reverse transcriptase inhibitor (RTI), GS9148, and its prodrug (Abstract 45). These compounds have low cytotoxicity, low potential for mitochondrial toxicity, and achieve good intracellular levels in peripheral blood mononuclear cells (PBMCs). The median effective concentrations (EC₅₀) for these compounds are 3.5 and 0.09 µM, respectively. They are active in vitro against many different subtypes of HIV. GS9148 retained antiretroviral activity against clinical isolates with 4 to 5 thymidine analogue-associated mutations (TAMs; with and without M184V), K65R, and the T69 insert. A clinical isolate with Q151M was associated with a 3-fold change in susceptibility to GS9148. A serial passage experiment generated resistant isolates in vitro with the K70E mutation. The prodrug had good bioavailability in a dog model which favored once-daily dosing (Abstract 498).

**1-(β-D-Dioxolane) Thymine.** This compound is a substrate for thymidine kinase. Prior studies have shown that 1-(β-D-Dioxolane) Thymine (DOT) has low cytotoxicity, low potential for mitochondrial toxicity, and is orally bioavailable in monkeys (>95%). Lennerstrand and colleagues presented data on the in vitro activity of DOT against a panel of site-directed HIV drug-resistant mutants (Abstract 46). The EC₅₀ was not significantly changed from that of wild-type by the presence of M184V or 4 to 5 TAMs. Low-level resistance was seen with K65R. However, significant resistance was observed in the presence of Q151M.

**β-D-2,6-Diaminopurine Dioxolane.** Margolis and colleagues presented the results of AIDS Clinical Trials Group (ACTG) 5165, a phase I/II study of β-D-2,6-diaminopurine dioxolane (DAPD) with mycophenolate mofetil (MMF) or placebo in addition to optimized antiretroviral background (Abstract 517) in treatment-experienced persons. The primary endpoint was viral load change from baseline after a 2-week period of functional monotherapy. MMF did not enhance the activity of DAPD. After combining both groups, DAPD was associated with a 0.29-log₁₀ reduction in plasma HIV-1 RNA after 2 weeks. Three of 40 (8%) subjects maintained at least a 0.5-log₁₀ reduction in plasma HIV-1 RNA after 24 weeks of treatment with DAPD. DAPD appeared safe and well tolerated.

**Dexelvucitabine.** Erickson-Viitanen and colleagues presented the correlation of genotypic and phenotypic resistance with virologic efficacy in a study of dexelvucitabine (formerly DPC-817 or DFC; Abstract 632). The main results of study RVT-203 have been presented previously (Cohen, IAS, 2005). The study participants were treatment experienced and 58% had either 3 or 4 TAMs at baseline. The only mutation associated with baseline phenotypic resistance to dexelvucitabine and poorer virologic efficacy was the Q151M mutation. No specific mutations emerged in the subjects in whom dexelvucitabine was failing.

**Nucleoside Competing Reverse Transcriptase Inhibitors.** Nucleotide competing reverse transcriptase inhibitors (NcRTIs) bind to the active site of reverse transcriptase, but unlike nucleoside (or nucleotide) analogue reverse transcriptase inhibitors (nRTIs) they are not incorporated into the DNA chain and their structure is not similar to nucleotides. Prior data have shown...
in vitro activity of the prototype compound NcRTI-1. Ehteshami and colleagues provided more detailed information about the mechanism of action of this agent (Abstract 47). NcRTI-1 traps reverse transcriptase in the posttranslocation state (ie, after reverse transcriptase translocates by 1 base pair prior to binding a new nucleotide). This binding forms a stable dead-end complex. NcRTI-1 binds preferentially after the incorporation of a pyrimidine, but the nature of the template base pair does not appear to matter. Jochmans and colleagues reported on the in vitro activity of NcRTI-1 against a wide panel of HIV-1 isolates (n = 6000; Abstract 500). The antiretroviral activity of NcRTI-1 was diminished in the presence of the combination of M184V and Y115F. Hyper susceptibility was observed in the presence of K65R.

**Maturation Inhibitors**

**UK-201844.** Blair and colleagues presented data on a new maturation inhibitor. It prevents cleavage of gp160 and renders the virion noninfectious (Abstract 500LB). However, the in vitro antiviral activity is limited and it is not active against most clinical isolates. This compound, however, provides evidence of a potential new mechanism to inhibit maturation of HIV-1.

**PA-457.** PA-457 is a maturation inhibitor that blocks cleavage of the CA-SP1. Smith and colleagues presented pharmacokinetic and pharmacodynamic data from a 10-day monotherapy study in humans (Abstract 52). The primary results have been presented previously. The highest dose resulted in a median drop in plasma HIV RNA of 1.1 log_{10} copies/mL at day 10; however, several study subjects did not respond despite the presence of comparable drug levels in their plasma. The basis for variable responses among the study subjects remains to be explained. The pharmacokinetic data showed that the half-life was about 70 hours. Drug exposure (24-hour area under the curve, or AUC_{24}) accounted for 88% of the variability of antiviral response. The authors noted that the maximal antiviral response was not achieved with the highest dose studied suggesting that higher doses should be considered. Adamson and colleagues presented data on the generation of resistance to PA-457 in vitro (Abstract 156). Serial passage experiments selected mutations at the CA-SP1 cleavage site consistent with the purported mechanism of action. Further in vitro studies suggest that PA-457 is either additive or synergistic with other antiretrovirals (Abstract 509).

**Integrase Inhibitors**

**MK-0518.** Grinsztejn and colleagues presented data from a phase II study of MK-0518, an HIV-1 integrase inhibitor, in treatment-experienced subjects (Abstract 159LB). This study compared 3 doses of MK-0518 (200, 400, or 600 mg bid) or placebo given with an optimized background regimen. Subjects were required to have genotypic or phenotypic resistance to at least 1 drug from each of 3 classes. The mean baseline CD4+ counts were between 220 and 283 cells/µL in the 4 groups, and the mean baseline plasma HIV-1 RNA level was 4.6 to 4.8 log_{10} copies/mL. Forty to fifty percent of each group was phenotypically resistant to all antiretroviral drugs in the optimized background regimen. All 3 MK-0518 doses were significantly more likely than placebo to achieve an HIV-1 RNA level below 50 copies/mL at week 16 (56% to 72% for MK-0518 compared with 20% for placebo). Most side effects were mild to moderate and none appeared to be associated with MK-0518 compared with placebo.

**SPI-256.** Gulnik and colleagues presented data on a new protease inhibitor (PI), SPI-256 (Abstract 501). The in vitro activity of this PI was evaluated using phenotypic analysis. The median inhibitory concentration (IC_{50}) for SPI-256 was 0.3 nM against wild-type and 3.9 nM against PI-resistant viruses. SPI-256 was 4 to 50 times more potent than currently available PIs (tipranavir was not tested) against highly PI-resistant HIV.

**Trials with Current Antiretroviral Agents**

**Treatment of Antiretroviral-Naive Patients**

Malan and colleagues (Abstract 107LB) presented the 48-week results of a 96-week, open-label trial comparing the efficacy of atazanavir 300 mg/ritonavir 100 mg (n = 95) with atazanavir 400 mg (n = 105) in combination with lamivudine/stavudine in antiretroviral-naive HIV-1–infected subjects. At baseline, mean CD4+ count and plasma HIV-1 RNA level were 235 cells/µL and 4.95 log_{10} copies/mL, respectively, and were not statistically significantly dif-
ferent between the groups. In the intent-to-treat (ITT) analysis, 75% of individuals in the ritonavir-boosted (rT) atazanavir arm versus 70% in the atazanavir arm reached plasma HIV-1 RNA below 50 copies/mL (P, not significant). Adverse event-related discontinuations occurred more commonly in the atazanavir/r group (8%) than in the atazanavir group (1%), most commonly due to hyperbilirubinemia. Three patients in the atazanavir/r group and 10 in the atazanavir group experienced virologic failure, defined as a plasma HIV-1 RNA level above 400 copies/mL. Resistance testing showed no major PI mutations and 1 nRTI mutation (to lamivudine) in the atazanavir/r group. In the atazanavir group there was 1 major PI mutation and 7 lamivudine-associated mutations.

In ACTG 5095, 1147 treatment-naive patients were randomized to receive zidovudine/ lamivudine/abacavir; zidovudine/lamivudine with efavirenz; or zidovudine/lamivudine/abacavir with efavirenz. The zidovudine/lamivudine/abacavir arm was stopped early due to virologic inferiority compared with efavirenz-based regimens (Gulick et al, NEJM, 2004). Gulick and colleagues (Abstract 519) presented the results of an open-label rollover study of tenofovir or efavirenz intensification in 170 patients who received zidovudine/lamivudine/abacavir and had plasma HIV-1 RNA below 200 copies/mL. Baseline characteristics were similar between both groups: mean CD4+ cell count was 484/µL and 73% had plasma HIV-1 RNA below 50 copies/mL. Overall, treatment failure (2 successive plasma HIV-1 RNA levels > 200 copies/µL) occurred in 19% of subjects; there was no statistically significant difference between the 2 arms. Rate and time to virologic failure, rate and time to treatment discontinuation, CD4+ cell count response, new grade 3 or 4 adverse events, self-report of adherence, and emergence of viral resistance at the time of virologic failure were not statistically significantly different between the 2 groups over a median of 1.5 years of follow-up.

Optimal CD4+ Cell Count at Which to Initiate Antiretroviral Therapy

The optimal CD4+ cell count at which to initiate antiretroviral therapy remains unclear and currently there are no randomized controlled clinical trials underway to address this issue. Using data from a number of large cohort datasets, Sterne and colleagues (Abstract 525) attempted to address this question as others have done (eg, Egger et al, Lancet, 2002). The authors reviewed data from antiretroviral-naive individuals in the Antiretroviral Therapy Cohort Collaboration (ART-CC) and compared them with the pre-antiretroviral therapy data from the Multicenter AIDS Cohort Study (MACS). At initiation of antiretroviral therapy, CD4+ count was at or below 200 cells/µL, 201 to 350 cells/µL, and 351 to 500 cells/µL in 40%, 37%, and 23% of individuals, respectively. The median length of follow-up was 2.7 years. Individuals who initiated treatment with CD4+ count at or below 200 cells/µL had higher rates of progression to AIDS and death than those who initiated therapy between 201 and 350 cells/µL (hazard ratio [HR] for AIDS, 3.68; 95% confidence interval [CI], 3.01–4.51; HR for AIDS and death, 2.93; 95% CI, 2.41–3.57). Starting antiretroviral therapy with a CD4+ count between 351 and 500 cells/µL, compared with a CD4+ count between 201 and 350 cells/µL, correlated with an increased risk of AIDS in the latter group (HR, 1.52; 95% CI, 1.10–2.10). The authors concluded that rates of progression to AIDS were lower in individuals who initiated antiretroviral therapy at higher CD4+ cell counts. Additional data are needed before current guidelines for the initiation of therapy are changed but the field may be witnessing a pendulum shift to earlier initiation of therapy in the years ahead.

Differences between treatment regimens and the degree to which individuals must adhere to medications to maintain virologic suppression (“forgiveness”) is poorly understood. Gross and colleagues (Abstract 533) evaluated the relative forgiveness of PI-, non-nucleoside analogue reverse transcriptase inhibitor (NNRTI)-, and PI/r-based regimens by analyzing the association between adherence to different regimens and viral suppression. In the highly active antiretroviral therapy (HAART) Observational Medical Evaluation and Research (HOMER) Cohort, 1634 treatment-naive patients with at least 2 plasma HIV-1 RNA levels below 500 copies/mL were studied. At baseline, 46% of individuals were on PI-, 38.6% on NNRTI-, and 15% on PI/r-based regimens. The median baseline CD4+ cell count was 200/µL and the median time of follow-up was 29 months. Six hundred and six patients (37.1%) had virologic breakthrough, which was defined as a plasma HIV-1 RNA level above 1000 copies/mL. Risk of virologic breakthrough was most strongly associated with less than 95% adherence in the PI group (HR, 1.78; 95% CI, 1.41–2.29) followed by the NNRTI group (HR, 1.47; 95% CI, 1.01–2.14). Adherence above 95% was not associated with virologic breakthrough in the PI/r group. The authors concluded that boosted PIs are more forgiving than unboosted PI- and NNRTI-based regimens with respect to adherence—an expected result.

Treatment of Antiretroviral-Experienced Patients

Results of select treatment-strategy studies in antiretroviral-experienced individuals are summarized in Table 1.

Etravirine. Vingerhoets and colleagues presented the genotypic and phenotypic predictors of response to etravirine (formerly TMC125) in the TMC125-C223 trial (see Table 1; Abstract 154). Etravirine is a novel investigational NNRTI with antiretroviral activity against HIV resistant to currently available NNRTIs. The primary results of TMC125-C223 have been presented previously (Grossman, ICAAC, 2005). The etravirine arms (400 mg or 800 mg bid) had a significantly greater reduction in plasma HIV-1 RNA levels at week
### Table 1. Selected Antiretroviral Studies in Treatment-Experienced Patients

<table>
<thead>
<tr>
<th>Study Name (Abstract No.)</th>
<th>Description</th>
<th>Regimen(s) (No. of Patients)</th>
<th>Population</th>
<th>Baseline CD4+ Count (cells/µl)</th>
<th>Plasma HIV RNA (log10 copies/mL)</th>
<th>Follow-up</th>
<th>Response</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMC125-C223 (Abstract 154)</td>
<td>Analysis of baseline resistance in TMC125-C223</td>
<td>Best available PI plus nRTIs with or without enfuvirtide (n=40) versus etravirine 800 mg bid plus lopinavir/ritonavir plus nRTIs with or without enfuvirtide (n=79)</td>
<td>3 or more major PI mutations, NNRTI resistance at screening or by history</td>
<td>99 (median)</td>
<td>4.7 (median)</td>
<td>24 weeks</td>
<td>Plasma HIV RNA change –0.19 versus –1.18 log10 copies/mL (P &lt; .05)</td>
<td>V32I, L33F, I47V, and I54M were identified as key mutations for darunavir when present with many other PI mutations. They were associated with resistance when present at baseline or they emerged with loss of virologic response.</td>
</tr>
<tr>
<td>POWER 1 and POWER 2 (Abstract 157)</td>
<td>Analysis of baseline resistance in the combined POWER 1 and 2 studies</td>
<td>Best available PI plus darunavir 600 mg/r 100 mg twice daily plus nRTIs with or without enfuvirtide (n=112)</td>
<td>1 or more major PI mutation, 3-class experience</td>
<td>141 (median; Katlama, CROI, 2005)</td>
<td>4.6 (median)</td>
<td>24 weeks</td>
<td>47% had HIV RNA &lt;50 copies/mL versus 25% of control subjects receiving a sensitive PI (n=31) versus 9% receiving a resistant PI (n=81; P &lt; .001)</td>
<td></td>
</tr>
</tbody>
</table>

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bid indicates twice daily; nRTI, nucleoside (or nucleotide) analogue reverse transcriptase inhibitor; NNRTI, nonnucleoside analogue reverse transcriptase inhibitor; NS, not significant; PI, protease inhibitor; /r, ritonavir-boosted.
24 than the control group (1.04 and 1.18 log_{10} copies/mL versus 0.19; P < .05). The authors discussed the relationship of baseline genotypic resistance to virologic outcomes. The number of NNRTI mutations predicted the phenotypic resistance to etravirine ranging from 0.6-fold change with no NNRTI mutations to 3.1-fold change with 3 or more mutations. Twelve percent of baseline isolates had a greater than 10-fold change to etravirine. The number of NNRTI mutations present at baseline predicted virologic response: 1.8- with zero, 1.7- with 1, 1.0- with 2, and 0.7-log_{10} copies/mL decrease in plasma HIV-1 RNA with 3 or more NNRTI mutations.

**Daranavir.** De Béthune and colleagues presented the relationship of baseline and treatment-emergent genotypic mutations in HIV protease to the antiretroviral efficacy of darunavir-(formerly TMC114) containing regimens (Abstract 157). Darunavir is available through an expanded access protocol. This analysis used data from the previously presented POWER 1 and POWER 2 studies as well the POWER 3 study, which has not been presented. Only subjects receiving 600 mg of darunavir twice daily were included. All 3 of these studies enrolled highly treatment-experienced subjects. The virologic response to a darunavir-containing regimen appeared diminished in those subjects with 10 or more PI-associated resistance mutations at baseline. The V32I, L33F, 147V, I54L, and L89V mutations appear to be associated with decreased virologic efficacy of darunavir when present at baseline and these mutations emerged during virologic failure. These mutations had to be present with many other mutations to be associated with resistance. Isolates that were susceptible to tipranavir at baseline remained sensitive at the time of virologic failure on darunavir.

**Daranavir/Etravirine.** Boffito and colleagues reported on the combination of darunavir and etravirine given with an optimized background regimen in 10 highly treatment-experienced subjects (Abstract 575c). The pharmacokinetic profile of each drug was assessed after 28 days and compared with historic controls. They found that the profile for darunavir was not significantly different from the historic control, and the levels of etravirine were reduced by about 30%. The authors did not feel that this was clinically relevant. At week 12, all subjects had plasma HIV-1 RNA levels below 400 copies/mL and 8 of 10 had less than 50 copies/mL.

**Acute HIV Infection**

Patterson and colleagues (Abstract 370) presented the results of an enhanced HIV testing strategy to detect acute and established HIV infection in pregnant women in North Carolina as part of the Screening and Tracing Active Transmission (STAT) Program. The authors reported that among 187,135 women, 16 were found to have acute infection. Of these women, 5 were pregnant at the time of the diagnosis; no infants were infected. During the study period (from 2002 through 2005), 3 of 6 HIV-1-infected infants born in North Carolina were born to women who had been screened and were seronegative early in the pregnancy. To reduce residual mother-to-child transmission (MTCT), the authors suggested early testing in pregnancy, repeat testing in the third trimester, and pooled HIV-1 RNA screening of all antibody-negative women.

Brenner and colleagues (Abstract 373) reported the results of a population-based surveillance of primary HIV-1 infections in Quebec between 1997 and 2005. Fifty percent (298 of 598) of subtype B HIV-1 infections were present in 71 transmission clusters (2 to 14 persons per cluster). Thirty percent of transmission clusters included individuals with more than 2 partners 3 months prior to diagnosis. Nonsubtype B HIV-1 infections represented 16% of recent and 9% of primary infection in Quebec. The prevalence of resistance mutations to a single drug class and 2 or more drug classes was 10.4% and 3.8%, respectively, in transmission clusters.

Mendoza and colleagues (Abstract 383) examined viral tropism among 240 individuals with recent HIV infection in Spain. Mean time of infection was 7 months; 16.3% of individuals were infected with X4-tropic virus, and the rate of primary drug resistance was 10.7%; 8.2% of individuals were infected with nonsubtype B HIV-1. There was no association between HIV coreceptor tropism and plasma HIV-1 RNA level, HIV subtype, or drug resistance mutations. Persons infected with HIV-1 through intravenous drug use had a higher proportion of X4-tropic viruses than those infected through sexual contact (54% versus 14.3%, respectively; P < .01). Individuals infected with X4-tropic virus tended to have higher HIV-1 RNA levels 1 year after exposure than individuals infected with R5-tropic virus.

Cachay and colleagues (Abstract 392) examined the effect of HSV-2 infection on the plasma HIV-1 RNA level during early and acute HIV-1 infection. Among 295 individuals with early infection, 41.7% were seropositive for HSV-2 virus. Baseline and 6-month plasma HIV-1 RNA levels did not differ significantly between HSV-2 seropositive and seronegative individuals.

Kassutto and colleagues (Abstract 391) presented data on the efficacy of antiretroviral therapy initiated during primary and early HIV-1 infection (≤12 months after seroconversion) among 102 subjects. Median time of follow-up was 40 months; 99 (97%) individuals reached plasma HIV-1 RNA below 50 copies/mL. In 91% and 86% of subjects, plasma HIV-1 RNA level was maintained below 50 copies/mL at months 12 and 36, respectively. There were no differences in response between subjects who initiated antiretroviral treatment before or after seroconversion or by treatment regimen (PI- or NNRTI-based). Median time to plasma HIV-1 RNA below 50 copies/mL was 11.1 weeks. Median CD4+ cell count at 12 months was 702/µL.

Chaix and colleagues (Abstract 397) compared the response to antiretroviral therapy between patients with B or
non-B subtype HIV-1 infection who initiated treatment during acute infection (≤1 month after diagnosis). Between 1996 and 2005, 584 subjects were enrolled in the French PRIMO Cohort Study. Among 312 individuals who initiated treatment, 71 (23%) were infected with nonsubtype B strains. Since 2001, the proportion of patients infected with nonsubtype B HIV-1 increased from 16% to 27%. Baseline CD4+ cell counts were lower in the nonsubtype B group (442/µL versus 490/µL; \( P = .020 \)), and plasma HIV-1 RNA levels were similar between the 2 groups. Overall, 88% of individuals received PI-based and 12% received NNRTI-based therapy. After 12 months of therapy, the CD4+ cell counts did not differ significantly between the 2 groups and 73% of individuals with subtype B, compared with 82% of individuals with nonsubtype B virus, achieved plasma HIV-1 RNA levels below 400 copies/mL (\( \gamma \)).

**Treatment Strategies**

Results of select treatment-strategy studies in antiretroviral-experienced individuals are summarized in Table 2.

**NEFA Study**

Martínez and colleagues (Abstract 521) presented results from a multicenter, randomized, open-label study of 460 participants on PI-based regimens with HIV-1 RNA below 200 copies/mL randomized to switch PI to: nevirapine (\( n = 155 \)); efavirenz (\( n = 156 \)); or abacavir (\( n = 149 \)). All patients continued therapy with 2 nRTIs. At baseline, the most common regimens were stavudine/lamivudine/indinavir; zidovudine/lamivudine/indinavir; and stavudine/lamivudine/nelfinavir. Baseline median CD4+ cell counts were 508/µL, 558/µL, and 544/µL in the nevirapine, efavirenz, and abacavir arms, respectively. At 3 years, according to the ITT analysis, more patients switching from PI to abacavir reached the primary endpoint of death, virologic failure, or progression to AIDS: 34 (23%) compared with 23 (15%) and 17 (11%) in the efavirenz and nevirapine arms, respectively (\( P = .031 \)). Among individuals receiving prior mono- or dual therapy with nRTIs, those in the abacavir arm had a significantly greater risk of virologic failure than those in the nevirapine or efavirenz arms: 36% versus 11% and 8%, respectively (\( P < .001 \)). Individuals without prior suboptimal nRTI therapy had similar rates of virologic failure in all 3 arms (2% to 5%). There were fewer discontinuations in the abacavir arm due to clinical and laboratory adverse events. The most common adverse event in the efavirenz arm was neuropsychiatric symptomatology. Individuals in the abacavir arm had significantly lower median cholesterol levels than those in the nevirapine or efavirenz arms at 12, 24, and 36 months. Stopping the PI was associated with a decrease in body fat in all 3 arms: prevalence of moderate to severe lipohypertrophy decreased among all patients from 20% at baseline to 12% at 36 months (\( P = .017 \)); moderate to severe lipoatrophy significantly increased among all individuals from 27% at baseline to 45% at 36 months; there were no significant differences between treatment arms.

Nasta and colleagues (Abstract 523) presented results of a 48-week, open-label, randomized prospective study of individuals who were randomized to maintain current treatment (arm A, \( n = 102 \)) or add lopinavir/r (arm B, \( n = 99 \)). Subjects were lopinavir/r-naive, had a history of 2 or more failed regimens, and HIV-1 RNA level between 1000 and 20,000 copies/mL. Mean baseline CD4+ cell counts and plasma HIV-1 RNA levels were 422/µL and 3.6 log_{10} copies/mL, respectively. At week 48, mean plasma HIV-1 RNA decrease was 0.16 log_{10} copies/mL and 1.4 log_{10} copies/mL in arm A and B, respectively. Forty-nine percent of subjects in arm B reached a plasma HIV-1 RNA level below 50 copies/mL versus none in arm A. Eleven individuals (10%) in arm A versus 3 (3%) in arm B achieved plasma HIV-1 RNA levels above 30,000 copies/mL or CD4+ cell count above 200/µL (\( P = .03 \)). Sixty-nine percent of subjects in arm A were on a PI-sparing regimen. There was no change in the total number of mutations at week 48, and a small decrease in mean number of protease mutations (from 3.2 to 2.8; \( P = .006 \)).

Katlama and colleagues (Abstract 520) presented the combined week-48 results of the RESIST-1 and RESIST-2 trials. This study compared tipranavir/r to comparator PIs (CPIs) with optimized background in subjects with 3-drug class experience. Patients had 1 or more major protease mutations (D30N, M46I/L, G48V, 150V, V82P/F/L/T, 184V, L90M) and fewer than 3 mutations at codons 33, 53, 82, 84, and 90. At study entry, 746 individuals were randomized to the tipranavir/r arm and 737 to CPI/r arm. Baseline mean CD4+ cell counts were 196/µL and 195/µL, in the tipranavir/r and CPI/r arms, respectively; mean plasma HIV-1 RNA level was 4.73 log_{10} copies/mL in both arms. The CPIs included lopinavir/r, indinavir/r, saquinavir/r, and amprenavir/r.

The week-48 risk of not achieving a 1-log_{10} copies/mL or greater reduction in plasma HIV-1 RNA level was 39% lower in the tipranavir/r than in the CPI/r arm (HR, 0.63; \( P < .0001 \)). The proportion of subjects who reached plasma HIV-1 RNA levels below 50 copies/mL was higher in the tipranavir/r
### Table 2. Treatment Strategies in Antiretroviral-Experienced Patients

<table>
<thead>
<tr>
<th>Study Name (Abstract No.) Description</th>
<th>Study Arm (No. of Patients)</th>
<th>Baseline HIV-1 RNA (copies/mL)</th>
<th>Baseline CD4+ Count (cells/µL)</th>
<th>Plasma HIV-1 RNA Response (copies/mL)</th>
<th>CD4+ Count Change (cells/µL)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTG 5170 (Abstract 101) 96-week multicenter prospective study</td>
<td>STI (n=167)</td>
<td>71% with &lt;50</td>
<td>833 (median); 436 (median nadir)</td>
<td>+3 log₁₀ (mean) in first 8 weeks; no change thereafter</td>
<td>~20/week (mean) in first 8 weeks; ~1.7/week (mean) thereafter</td>
<td>Nadir CD4+ count &lt;400 cells/µL and baseline plasma HIV RNA &gt;400 copies/mL were associated with higher likelihood of reaching endpoint (HR=1.95 and 2.75, respectively). 46 patients restarted therapy. Of 5 deaths, 3 were coronary artery disease-related</td>
</tr>
<tr>
<td>STACCATO (Abstract 102) CD4+ count-guided STI compared with continuous therapy for a median of 21.9 months</td>
<td>STI (n=299)</td>
<td>4.72 log₁₀ (median)</td>
<td>470 (median)</td>
<td>NA</td>
<td>374 (median); 60.5% with &gt;350</td>
<td>After resuming therapy, 91% in STI and 92% in continuous therapy (P = .9) arms achieved plasma HIV-1 RNA &lt;50 copies/mL. 85.9% in the STI and 96.9% in the continuous therapy arms reached CD4+ count &gt;350 cells/µL. 17 patients (5.8%) in STI arm had acute retroviral syndrome. Of 2 deaths (1 in STI arm), none were AIDS-related.</td>
</tr>
<tr>
<td>ISS PART (Abstract 103) Randomized comparison of STI versus continuous antiretroviral therapy in patients with viral suppression</td>
<td>24-month continuous therapy (n=137)</td>
<td>&lt;400</td>
<td>768 (mean)</td>
<td>92.3% with &lt;400 at 24 months</td>
<td>+6 (median) 86.5% with &gt;500 at 24 months</td>
<td>Only 56 patients in STI arm at end of study. In ITT analysis, cumulative risk of plasma HIV RNA &gt;400 was 24% in continuous therapy and 26% in STI arms. Cumulative risk of resistance (≥1 mutation) at 24 months in STI arm was 30%. Unboosted PI and archived mutations in proviral DNA independently associated with higher risk of resistance during STI. Resistance associated with increased risk of failure (HR, 2.64).</td>
</tr>
<tr>
<td>ISS PART (Abstract 103) Randomized comparison of STI versus continuous antiretroviral therapy in patients with viral suppression</td>
<td>1- to 3-month STI (n=136)</td>
<td>&lt;400</td>
<td>714 (mean)</td>
<td>91.1% with &lt;400 at 24 months</td>
<td>~26 (median) 69.1% with &gt;500 at 24 months</td>
<td>Only 56 patients in STI arm at end of study. In ITT analysis, cumulative risk of plasma HIV RNA &gt;400 was 24% in continuous therapy and 26% in STI arms. Cumulative risk of resistance (≥1 mutation) at 24 months in STI arm was 30%. Unboosted PI and archived mutations in proviral DNA independently associated with higher risk of resistance during STI. Resistance associated with increased risk of failure (HR, 2.64).</td>
</tr>
</tbody>
</table>
Table 2. Treatment Strategies in Antiretroviral-Experienced Patients (continued)

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Description</th>
<th>Study Arm (No. of Patients)</th>
<th>Baseline HIV-1 RNA (copies/mL)</th>
<th>Baseline CD4+ Count (cells/µL)</th>
<th>Plasma HIV-1 RNA Response (copies/mL)</th>
<th>CD4+ Count Change (cells/µL)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANRS 106 WINDOW</td>
<td>A 96-week prospective randomized trial of IT in patients with CD4+ count &gt;450 cells/µL and plasma HIV RNA &lt;200 copies/mL.</td>
<td>Continuous therapy (n=194)</td>
<td>&lt;200 (median)</td>
<td>748 (median)</td>
<td>90% with &lt;400 at 96 weeks</td>
<td>-8 (median)</td>
<td>Incidence of serious morbidity was 5.7% in continuous therapy versus 17.6% in CD4+ count-guided therapy arm (mainly due to invasive bacterial infections). The fixed treatment interruption arm (2 months off/4 months on treatment) is ongoing.</td>
</tr>
<tr>
<td>ANRS 106 WINDOW</td>
<td>24-month randomized comparison of CD4+ count-guided therapy with continuous therapy. Patients had CD4+ count &gt;350 cells/µL and plasma HIV RNA &lt;300 copies/mL. Initiation and interruption threshold of 250 to 350 cells/µL, respectively. The CD4+ count-guided therapy arm was stopped early due to increased morbidity.</td>
<td>Continuous therapy (n=110)</td>
<td>&lt;300 (median)</td>
<td>461 (median)</td>
<td>NA</td>
<td>600 (mean)</td>
<td></td>
</tr>
<tr>
<td>SMART</td>
<td>Randomized comparison of episodic CD4+ count-guided therapy with continuous therapy. Initiation and interruption threshold of 250 to 350 cells/µL, respectively, in the drug conservation arm.</td>
<td>Continuous therapy (n=2736)</td>
<td>70.8% with &lt;400</td>
<td>599 (median)</td>
<td>NA</td>
<td>NA</td>
<td>33% versus 93% of follow-up time on therapy in the continuous versus drug conservation therapy arms. 3.1% versus 0.8% of follow-up time at CD4+ count &lt;200 cells/µL. Relative risks of clinical disease progression or death, nonfatal cardiovascular disease, and nonfatal renal events in the drug conservation versus continuous therapy arms were 2.5, 1.5, and 2.5, respectively (P &lt; .0001).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drug conservation (n=2736)</td>
<td>71.0% with &lt;400</td>
<td>596 (median)</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

HR indicates hazard ratio; IT, intermittent therapy; ITT, intent-to-treat analysis; NA, not available; STI, scheduled treatment interruption.
arm than in the CPI/r arm, regardless of the PI (23.9% in the tipranavir/r arm versus 16.8% in the lopinavir/r arm; 22.4% in the tipranavir/r arm versus 6.2% in the saquinavir/r arm), baseline CD4+ cell counts, or baseline plasma HIV-1 RNA levels. Individuals who initiated tipranavir/r with higher baseline CD4+ cell counts or lower baseline plasma HIV-1 RNA levels were more likely to achieve plasma HIV-1 RNA reduction than those with lower baseline CD4+ cell counts or higher plasma HIV-1 RNAs. Forty-two percent of subjects with baseline CD4+ cell count above 200/µL compared with 18% of individuals with baseline CD4+ cell counts below 50/µL achieved a 1-log10 decrease in plasma HIV-1 RNA levels. Individuals who initiated a baseline plasma HIV-1 RNA level above 400 copies/mL and had plasma HIV-1 RNA levels above 100,000 copies/mL discontinued antiretroviral therapy for up to 96 weeks. At study entry, the median time of antiretroviral therapy was 4.5 years; 37% of subjects were on PI-based and 36% on NNRTI-based therapy. Median CD4+ cell count was above 20/µL/week during the first 8 weeks of treatment interruption, and 1.7/µL/week thereafter. By week 96, 26 individuals reached the primary endpoint; there were 5 deaths and 3 were related to cardiovascular events. Forty-six participants restarted antiretroviral therapy; among them, 14 resumed the same regimen (64% reached plasma HIV-1 RNA levels <50 copies/mL), and 32 initiated a new regimen. Nadir CD4+ cell count below 400/µL and baseline plasma HIV-1 RNA level above 400 copies/mL were associated with increased risk of reaching the endpoint (HR, 1.95 and 2.75, respectively). Plasma HIV-1 RNA level prior to antiretroviral therapy was not predictive of reaching an endpoint.

**Treatment Interruptions**

**ACTG 5170.** Skiest and colleagues (Abstract 101) presented the results of ACTG 5170, a multicenter, prospective study that evaluated the safety of treatment interruption among subjects on stable antiretroviral therapy for 6 months or more. Primary endpoint was defined as time to CD4+ count at or above 250 cells/µL, AIDS-related event, or death. One hundred sixty-seven individuals with median CD4+ cell count of 833/µL, 71% of whom had plasma HIV-1 RNA below 50 copies/mL, discontinuing antiretroviral therapy for up to 96 weeks. At study entry, the median time of antiretroviral therapy was 4.5 years; 37% of subjects were on PI-based and 36% on NNRTI-based therapy. Median CD4+ cell-count decrease was 20/µL/week during the first 8 weeks of treatment interruption, and 1.7/µL/week thereafter. By week 96, 26 individuals reached the primary endpoint; there were 5 deaths and 3 were related to cardiovascular events. Forty-six participants restarted antiretroviral therapy; among them, 14 resumed the same regimen (64% reached plasma HIV-1 RNA levels <50 copies/mL), and 32 initiated a new regimen. Nadir CD4+ cell count below 400/µL and baseline plasma HIV-1 RNA level above 400 copies/mL were associated with increased risk of reaching the endpoint (HR, 1.95 and 2.75, respectively). Plasma HIV-1 RNA level prior to antiretroviral therapy was not predictive of reaching an endpoint.

**STACCATO.** Ananworanich and colleagues (Abstract 102) presented the results of a randomized, multicenter trial of CD4+ count-guided scheduled treatment interruptions compared with antiretroviral therapy. Five hundred forty-eight individuals with CD4+ cell counts above 250 cells/µL and plasma HIV-1 RNA levels below 50 copies/mL were randomized to the CD4+ count-guided treatment interruption arm (n = 299) or continuous therapy arm (n = 154). Individuals in the treatment interruption arm stopped antiretroviral therapy if the CD4+ count was above 350 cells/µL and resumed treatment if the CD4+ count was below 350 cells/µL. At study end all patients resumed continuous antiretroviral therapy for 12 to 24 weeks.

Baseline median CD4+ cell counts and plasma HIV-1 RNA levels were 470/µL and 4.72 log_{10} copies/mL versus 506/µL and 4.76 log_{10} copies/mL in the treatment interruption and continuous therapy arms, respectively. Median duration of prior antiretroviral therapy was 13.7 months in the treatment interruption and 15.6 months in the continuous therapy arm; 80% of individuals were on a saquinavir/r-based regimen. Participants were randomized for a median of 21.9 months. At the end of the study period, median CD4+ cell counts were 601/µL and 374/µL (P < 0.002) in the continuous therapy and treatment interruption arms, respectively. Adverse events related to HIV disease were more common in the treatment interruption than the continuous therapy arm and included oral candidiasis (3.5% versus 0%; P = 0.04), vaginal candidiasis (1.7% versus 0.6%; P > 0.05), self-reported lipodystrophy (7.9% versus 0.2%; P < 0.05), diarrhea and neuropathy were reported more often in the continuous therapy arm (23.8% versus 15.7% and 46% versus 1.9%, respectively). Seventeen patients (5.8%) in the treatment interruption arm had acute retroviral syndromes. Total cholesterol was lower in the treatment interruption arm at the end of the study period: 179.4 mg/dL compared with 195 mg/dL (P = 0.05), as was self-reported lipodystrophy (7.9% versus 13.5%; P = 0.05); triglycerides did not differ between the arms. After resuming continuous antiretroviral therapy, 91% in the treatment interruption arm and 92% in the continuous therapy arms (P = 0.9) achieved plasma HIV-1 RNA levels below 50 copies/mL; 85.9% in the treatment interruption and 96.9% in the continuous therapy arms maintained CD4+ counts above 350 cells/µL after 24 weeks of therapy.

In a separate presentation, Anan-
woranich and colleagues (Abstract 622b) reported data on the development of resistance mutations among 430 Staccato participants in the continuous therapy (n=146) or treatment interruption arms (n=284). A total of 79.1% of individuals received saquinavir/r in combination with 2 nRTIs (didanosine/stavudine or tenofovir/lamivudine). Genotyping was performed in individuals with plasma HIV-1 RNA levels above 500 copies/mL after 12 or more weeks of treatment (22 in continuous therapy arm and 10 in treatment interruption arm) or individuals with 2 or more treatment interruption cycles (n=111). There was no difference in the development of resistance between the arms (2%). The most prevalent mutation was M184V. The relative risk of resistance was 0.95 (95% CI, 0.11–7.91) for individuals on continuous therapy arm and 10 in treatment interruption arm) or individuals with 2 or more treatment interruption cycles (n=111). The incidence of serious morbidity in the CD4+ count-guided therapy arm was 2.6-times higher than in the continuous therapy arm (17.6 per 100 person-years of observation in the CD4+ count-guided therapy arm compared with 6.7 per 100 person-years in the continuous therapy arm; incidence rate ratio [IRR], 2.6; 95% CI, 1.3–5.6; \( P = .001 \)). These events included invasive bacterial infections (IRR, 15.9; 95% CI, 2.6–64.8); oropharyngeal candidiasis (IRR, 2.7; 95% CI, 0.9–11.0); and tuberculosis (IRR, 1.5; 95% CI, 0.5–6.5). Salmonella typhi and Streptococcus pneumoniae were the most commonly isolated bloodstream pathogens; 85% of bacterial infections were resistant to trimethoprim/sulfamethoxazole. The overall mortality rate was 0.6 per 100 person-years in the continuous therapy arm versus 1.2 per 100 person-years in the CD4+ count-guided therapy arm (\( P = .57 \)). The number of outpatient visits and days spent in the hospital were significantly higher in the CD4+ count-guided therapy arm than in the continuous therapy arm. Five percent of individuals in the continuous therapy arm compared with 11% in the CD4+ count-guided therapy arm, had virus strains emerge which were resistant to at least 1 drug.

**ANRS 106 (WINDOW).** Marchou and colleagues (Abstract 104) presented the results of a 96-week prospective randomized controlled trial evaluating the safety of fixed-time scheduled treatment interruption. Participants were randomized to an 8-weeks on/8-weeks off arm (intermittent therapy, n=197) or continuous therapy arm (n=194). Baseline median CD4+ cell counts were 739/µL and 748/µL. Individuals had a median of 5.2 and 5.1 years of antiretroviral therapy in the intermittent therapy and continuous therapy arms, respectively. Twenty-seven (17%) and 16 (8%) individuals in the intermittent therapy and continuous therapy arms, respectively, discontinued study participation (\( P = .09 \)). At week 96, according to the ITT analysis, 3.6% in the intermittent therapy and 1.5% in the continuous therapy arm reached CD4+ cell counts below 350 cells/µL; 81% in the intermittent therapy versus 90% in the continuous therapy arm maintained plasma HIV-1 RNA levels below 400 copies/mL (\( P = .02 \)). There were 2 deaths in the intermittent therapy arm (liver failure and violence). Median changes in CD4+ cell counts were –155/µL and –8/µL in the intermittent therapy and continuous therapy arms, respectively (\( P < .0001 \)). Adverse events included acute retroviral syndrome (5 in intermittent therapy arm), mucosal candidiasis (10 in intermittent therapy versus 6 in continuous therapy arm), and lymphadenopathy (10 in intermittent therapy versus 3 in continuous therapy arm). Nine subjects in the intermittent therapy arm and 2 in the continuous therapy arm developed grade 3 or 4 thrombocytopenia. Thirty-one participants (17 in intermittent therapy and 14 in continuous therapy arm) had plasma HIV-1 RNA levels above 1,000 copies/mL for longer despite more than 6 weeks of therapy. Of these, 14 in the intermittent therapy arm and 10 in the continuous therapy arm underwent genotypic testing; there were no differences in the number of PI, NNRTI, or nRTI resistance mutations between the 2 arms.

**ANRS 1269 (TRIVACAN).** Danel and colleagues (Abstract 105LB) presented preliminary results of a randomized controlled trial comparing 2 treatment interruption strategies with continuous therapy in sub-Saharan Africa. Individuals with plasma HIV-1 RNA levels below 300 copies/mL were randomized to 3 arms: continuous therapy arm (n=110); 2 months off/4 months on intermittent therapy arm (n=525); or CD4+ cell count-guided intermittent therapy arm (n=216). Participants in the CD4+ count-guided therapy arm stopped antiretroviral therapy when CD4+ cell counts were above 350µL and resumed when CD4+ cell counts declined below 250µL. The CD4+ count-guided therapy arm was stopped early due to increased morbidity; the other treatment interruption arm is currently ongoing. Baseline median CD4+ cell counts were 475/µL and 461/µL in the CD4+ count-guided therapy and continuous therapy arms, respectively. At study entry, 89% of individuals in the CD4+ count-guided therapy and 85% in the continuous therapy arms received 6 months or more of zidovudine/lamivudine/efavirenz; 7% in the CD4+ count-guided therapy and 10% in the continuous therapy arms received 6 months or more of zidovudine/lamivudine/indinavir. Mean follow-up time was 19.4 months in the continuous therapy arm and 19.2 months in the CD4+ count-guided therapy arm.

The incidence of serious morbidity in the CD4+ count-guided therapy arm was 2.6-times higher than in the continuous therapy arm (17.6 per 100 person-years of observation in the CD4+ count-guided therapy arm compared with 6.7 per 100 person-years in the continuous therapy arm; incidence rate ratio [IRR], 2.6; 95% CI, 1.3–5.6; \( P = .001 \)). These events included invasive bacterial infections (IRR, 15.9; 95% CI, 2.6–64.8); oropharyngeal candidiasis (IRR, 2.7; 95% CI, 0.9–11.0); and tuberculosis (IRR, 1.5; 95% CI, 0.5–6.5). Salmonella typhi and Streptococcus pneumoniae were the most commonly isolated bloodstream pathogens; 85% of bacterial infections were resistant to trimethoprim/sulfamethoxazole. The overall mortality rate was 0.6 per 100 person-years in the continuous therapy arm versus 1.2 per 100 person-years in the CD4+ count-guided therapy arm (\( P = .57 \)). The number of outpatient visits and days spent in the hospital were significantly higher in the CD4+ count-guided therapy arm than in the continuous therapy arm. Five percent of individuals in the continuous therapy arm compared with 11% in the CD4+ count-guided therapy arm, had virus strains emerge which were resistant to at least 1 drug.

**SMART.** El-Sadr and colleagues (Abstract 106LB) presented the results of the Community Program for Clinical Research on AIDS (CPCRA)-sponsored Strategies for Management of Antiretroviral Therapy (SMART) study. This was a prospective, randomized, multicenter study of 5472 individuals with CD4+ cell counts above 350/µL randomized to a continuous treatment virus suppression arm (n=2752) or drug conservation arm (n=2720). Individuals in the drug conservation arm stopped antiretroviral treatment when CD4+ cell counts reached above 350/µL and resumed treatment when...
CD4+ cell counts declined below 250/µL. The Data Safety Monitoring Board recommended stopping enrollment in January 2006 because of an increased risk of death or HIV-1 disease progression in the drug conservation arm. At baseline, median CD4+ cell counts in the virus suppression arm and the drug conservation arm were 599/µL and 596/µL, respectively; only 4.7% of individuals were antiretroviral-treatment naive. Median duration of antiretroviral therapy was 6 years in both groups. Individuals in the drug conservation arm had a 2.5-fold greater risk of HIV disease progression or death than in the virus suppression arm (95% CI, 1.8–3.6; P < .0001). The rate of HIV disease progression or death was 3.7 per 100 person-years in the drug conservation arm versus 1.5 per 100 person-years in the virus suppression arm. The relative risk (RR) of death due to cardiovascular, liver, or renal disease was 1.4 (95% CI, 0.7–2.8) in the drug conservation arm compared with participants in the virus suppression arm; the RR of nonfatal cardiovascular events was 1.5 (95% CI, 1.0–2.5), of nonfatal renal events was 2.5 (95% CI, 0.5–13) in the drug conservation arm compared with the virus suppression arm. The RR of disease progression or death was higher in the drug conservation arm than in the virus suppression arm, but there was no difference in the RR by nadir CD4+ cell count stratum: RR of disease progression or death for the below 50/µL stratum was 2.9 (95% CI, 1.0–8.0) versus 2.5 (95% CI, 1.2–5.0) in the 300 to 399/µL stratum. Risk of disease progression or death in the drug conservation versus virus suppression arms was 3.8-times higher for subjects with baseline plasma HIV-1 RNA levels above 400 copies/mL than for those with baseline plasma HIV-1 RNA levels below 400 copies/mL. Individuals in the drug conservation and virus suppression arms spent 3.1% versus 0.8% of follow-up time at CD4+ cell counts below 200/µL, respectively. The authors concluded that episodic, CD4+ count-guided treatment interruption was inferior to the continuous therapy with respect to both HIV disease progression and major cardiac, renal, and hepatic adverse events. The increased rate of the latter events in the drug conservation arm was unexpected and will require more investigation to explain from a pathogenetic perspective.

PART. Palmisano and colleagues (Abstract 103) presented results of a randomized trial of structured treatment interruptions of increasing length compared with continuous antiretroviral treatment. Participants with plasma HIV-1 RNA below 400 copies/mL were randomized to continuous treatment (n = 137) or treatment interruption arm (n = 136). Individuals in the treatment interruption arm underwent interruptions of increasing duration (1 to 3 months) separated by 3 months of treatment. Baseline mean CD4+ counts were 768 cells/µL and 714 cells/µL in the continuous treatment and treatment interruption arms, respectively. Over-all duration of prior antiretroviral therapy was 26 months. At 24 months, according to the on-treatment analysis, 86.5% in the continuous treatment arm compared with 69.1% in the treatment interruption arm maintained a CD4+ cell count above 500/µL. The study did not demonstrate noninferiority of the treatment interruption strategy. Male sex and nadir CD4+ cell count were independently associated with primary endpoint (defined as the proportion of patients with CD4+ count above 500 cells/µL at 24 months). In the ITT analysis, 92.3% in the continuous treatment arm and 91.1% in the treatment interruption arm achieved HIV-1 RNA levels below 400 copies/mL at 24 months. One individual in the treatment interruption arm had acute retroviral syndrome; 27 individuals in the continuous treatment and 12 in the treatment interruption arm developed grade 3 to 4 laboratory adverse events. Resistance mutations emerged in 38 of 136 individuals in the treatment interruption, corresponding to a cumulative 24-month risk of resistance of 30%.

The M184V/I mutations were detected in 17% of lamivudine-treated subjects; K103N was detected in 8% of NNRTI-treated individuals. Resistance mutations emerged in 50% of individuals on PI-based and 20% on NNRTI-based regimens. Baseline proviral DNA genotyping was performed in 82 subjects in the treatment interruption arm. Archived mutations were found in 9 of 27 individuals (33%) who subsequently developed resistance and in 1 of 55 subjects with wild-type virus. In a logistic regression model, mutations in proviral DNA and an (unboosted) PI-based regimen were associated with an increased risk of resistance during treatment interruption (P = .002 and P = .048, respectively). Resistance was associated with an increased risk of not reaching the primary endpoint (HR, 2.64; 95% CI, 1.2–5.9).

Antiretroviral Drug Resistance and Replicative Capacity

The K70E mutation in the HIV-1 reverse transcriptase has become more prevalent since the introduction of tenofovir. In a recent study, K70E was selected in 10% of antiretroviral-naive subjects on tenofovir, abacavir, and lamivudine (Ross et al, Antivir Ther, 2005). Sluis-Cremer and colleagues (Abstract 152) reported that the K70E mutation significantly impairs the reverse transcriptase’s ability to excise zidovudine monophosphate from the proviral DNA chain suggesting that this mutation may be antagonistic toward TAMs. The authors suggested that inclusion of zidovudine in the nRTI component of an antiretroviral regimen may reduce the selection of K70E.

Variations in the env variable regions and N-glycosylation sites are known to influence susceptibility to attachment inhibitors. Toma and colleagues (Abstract 153) demonstrated that minor genetic changes in constant regions of env and the variable regions of gp120 and gp41 can result in large changes to phenotype. This may affect susceptibility to anti-CD4 antibodies, CD4 binding-site inhibitors, and monoclonal antibodies that target either gp120 or gp41. These observations are consistent with a structurally integrat-ed model of the gp120-gp41 glycoprotein complex.
Drug Resistance Testing and Transmission of Drug-Resistant Virus

Palenlla and colleagues (Abstract 654) described the impact of HIV-1 genotypic and phenotypic susceptibility testing on the rate of survival among subjects in the HIV Outpatient Study (HOPS) between 1999 and 2005. Among 4186 individuals with plasma HIV-1 RNA levels above 1000 copies/mL, 25% underwent susceptibility testing. The median number of regimens before genotypic and phenotypic testing was 3. Subjects with 1 or more genotypic or phenotypic tests had a 59% improvement in survival (HR, 0.41; \( P < .01 \)). Individuals most likely to undergo HIV susceptibility testing had lower CD4 + cell counts, higher plasma HIV-1 RNA levels, were white, and were younger than 40 years of age.

To evaluate transmission of drug-resistant virus, a real-time polymerase chain reaction (PCR) point-mutation assay was developed and used to detect low-frequency mutations among 277 antiretroviral-naive individuals infected with drug-resistant HIV-1 (Abstract 642). The authors identified previously undetected resistant HIV-1 viruses at frequencies between 0.2% and 14%. Use of the real-time PCR assay increased the detected prevalence of L90M from 8% to 10%, M41L from 16% to 26%, and M184V from 9% to 11%. Identification of additional mutations resulted in an additional 5% of the samples being classified as having resistance to another drug class; the frequency of HIV-1 resistance to 2 or more drug classes increased from 17% to 22%.

Pilon and colleagues (Abstract 646) described transmission of drug-resistant virus among 537 newly diagnosed HIV-1–infected individuals in Canada between January and December 2004. The prevalence of drug resistance ranged from 5.6% to 18.4% and varied by region. Resistance to any drug class was 9.7%, and has been constant over the last several years. The prevalence of nonsubtype B HIV-1 strains among subjects varied by region and ranged from 8.7% to 36%. The overall prevalence of nonsubtype B HIV-1 infections in Canada had increased since 2001. Gatanaga and colleagues (Abstract 647) reported a lower rate of transmission of drug-resistant virus in Japan. Among 575 newly diagnosed individuals between 2004 and 2005, 5% were resistant to at least 1 drug. Of these individuals, 3.5%, 1.2%, and 0.9% had evidence of nRTI, NNRTI, and PI resistance mutations, respectively.

Hyersusceptibility, Fitness, and Replication Capacity

Prior studies have demonstrated the importance of T215Y and H208Y in inducing efavirenz hypersusceptibility. Shulman and colleagues (Abstract 624) postulated that hypersusceptible viruses have a reduced replication capacity compared with wild-type virus. They evaluated the impact of T215Y and H208Y mutations on the replication capacity of HIV-1. Mutants analyzed included T215Y, H208Y, T215Y+H208Y, T215Y+H208Y+V118I, and H208Y+V118I. All mutants, with the exception of those containing T215Y alone, were hypersusceptible to efavirenz. The presence of the H208Y mutation alone reduced the replication capacity to 9.3%; this was restored to 70% when T215Y was present.

Kitchen and colleagues (Abstract 626) identified the following positions in the protease gene at which mutations resulted in decreased replication capacity: 30, 36, 63, 77, 82, and 90. The D30N mutation conferred the greatest defect in replication capacity. Paredes et al (Abstract 628) evaluated the effect of the M184V mutation on viral fitness using the sensitive allele-specific PCR assay in 6 subjects who stopped all RTIs and remained on PIs. Lamivudine was stopped in 5 of 6 subjects. M184V was detected in all subjects discontinuing lamivudine. The proportion of M184V mutants remained stable for 16 weeks. After week 16, M184V decayed rapidly. The authors concluded that in the absence of drug pressure, M184V decreases the fitness of HIV in vivo, consistent with previously reported in vitro data.

Bezemere and colleagues (Abstract 630) described the evolution of mutations in the reverse transcriptase and protease gene among 20 recently infected antiretroviral-naive individuals from the Concentrated Action on SeroConversion to AIDS and Death in Europe (CASCADE) cohort. Individuals had genotypic testing at the time of diagnosis and after a median follow-up time of 15 months. The following mutations in reverse transcriptase did not evolve: M41L, T215D, and T215S; whereas K70R, M184V, T215Y, and T215F evolved or reverted to alternative codons. Mutations in the protease gene remained stable over 15 months. Twelve individuals demonstrated evolving resistance mutations and this was associated with slower CD4 + cell decline following seroconversion compared with individuals infected with HIV-1 that did not revert. The authors concluded that T215Y/F, K70R, or M184V may confer reduced fitness over the short term.

Cong and colleagues (Abstract 627) evaluated the impact of mutational interactions on viral fitness in HXB2-derived mutants and transmitted drug-resistant isolates. The lowest fitness cost was seen in mutants with K70R, L210W, Y181C, or M41L (0.4-, 0.9-, 1.3-, and 4-fold, respectively); the highest cost was seen in viruses with K65R, M184V, or T215Y (26-, 14-, and 11.5-fold, respectively). The fitness cost of mutations varied with the presence of additional reverse transcriptase mutations. The low fitness cost of the K70R mutation was found to increase in HXB2 viruses with D67N/K219Q (4.6-fold) and in viruses with only D67N (6-fold). Similarly, the high fitness cost of M184V was reduced in HXB2 viruses carrying the D67N/K70R/K219Q or M41L/L210W/T215Y genotypes (2.3- and 8.9-fold, respectively) but remained high in viruses with M41L/L210W/T215Y/K103N or D67N/K70R/K219Q/T215F (2.3- and 16.1-fold, respectively). A similarly wide range of fitness costs of M184V (from 2- to 20-fold) was seen in transmitted isolates carrying M184V alone or in association with K70R, K103N, or M41L/T215Y. The authors concluded that modulation of fitness cost may
play a role in the rate of reversion and persistence of transmitted resistance mutations. The authors concluded that a more fit mutant has a greater persistence potential in vivo and a less fit mutant may predict rapid reversion of the mutation(s).

**Resistance in Treatment-Experienced Patients**

**Enfuvirtide.** Mutations associated with enfuvirtide develop at residues 36 to 45 of HIV-1 gp41 HR1 domain. Aquaro and colleagues (Abstract 596) compared gp41 mutations selected during long-term enfuvirtide treatment with gp41 sequences in enfuvirtide-naive individuals. Enfuvirtide was added to failing therapy in 54 individuals with a median plasma HIV-1 RNA level of 5.1 log$_{10}$ copies/mL and a median CD4 + cell count of 48/µL. Individuals had a median number of 2 NNRTI mutations, 8 PI mutations, and 5 nRTI mutations. At week 8, median HIV-1 RNA level decreased from 5.1 log$_{10}$ copies/mL to 4.2 log$_{10}$ copies/mL. By week 24, however, HIV-1 RNA level increased to 4.8 log$_{10}$ copies/mL. By week 36, median CD4 + cell count increased to 136/µL. Enfuvirtide resistance mutations developed in 45 of 54 subjects, 28% of whom developed V38A/E. At week 24, 9% developed a Q40H plus L45M. V38A/E mutations were associated with a 4.5-fold increase in CD4 + cell count at week 24 compared with wild-type gp41 (94 versus −25/µL; $P = .004$). At week 24, the median CD4 + cell count decreased by 45/µL in subjects with the Q40H + L45M combination versus 25/µL in those with wild-type gp41 ($P = .02$). The N126K mutation was associated with a 2.1-fold increase in CD4 + cell count at 24 weeks. There was no association between enfuvirtide mutations and plasma HIV-1 RNA levels at week 24.

**Atazanavir.** Coakley and colleagues (Abstract 634) validated the phenotypic clinical cutoff for atazanavir/r based on data from the BMS 045 study. BMS 045 was a randomized, open-label study of patients randomized to tenofovir and either atazanavir/r 300/100 mg; lopinavir/r 400/100 mg; or atazanavir/saquinavir 400/1200 mg. Baseline fold change below 5.2 correlated with best response to atazanavir/r: at week 2, 89% of subjects with fold change below 5.2 reached at least 1.0-log$_{10}$ copies/mL decrease in plasma HIV-1 RNA level versus 26% of patients with a fold change of at least 5.2. This phenotypic cutoff was associated with virologic response regardless of the presence of baseline protease mutations.

**nRTI-associated K65R mutation.** Previous studies have shown reduced HIV-1 RNA responses to tenofovir in individuals with multiple TAMs, including either the M41L or L210W mutation (Miller et al, *J Infect Dis*, 2004). The L74V mutation has been associated with a reduced tenofovir response and development of the K65R mutation (Bae et al, *Antivir Ther*, 2004). Waters and colleagues (Abstract 653) evaluated the impact of TAMs and K65R on the response to tenofovir among individuals enrolled in the Gilead 907 and 902 trials. These were studies of individuals on stable antiretroviral regimens with plasma HIV RNA levels above 400 copies/mL randomized to the addition of either tenofovir or placebo. The previous baseline resistance analysis included 233 additional patients from 6 Italian centers. Subjects switched antiretroviral regimens after genotypic resistance testing. The median baseline plasma HIV-1 RNA level and CD4 + cell count were 4.17 log$_{10}$ copies/mL and 312/µL, respectively; 56.5% of individuals were on tenofovir, 56.5% on lamivudine, and 55.7% on didanosine. Among these individuals, 44.8% had M184V; 15.2% and 22.8% had mutations from TAM-1 (M41L, L210W, and T215Y) and TAM-2 (D67N, K70R, and K219Q/E) pathways, respectively. After genotypic resistance testing, individuals changed therapy to lamivudine (59.7%), zidovudine (29%), stavudine (27.4%). The best predictor of achieving a treatment response (defined as a plasma HIV-1 RNA level below 50 copies/mL at 12 months), was associated with the presence of the M184V mutation (HR, 1.97; 95% CI, 1.0–3.86; $P = .05$); and the addition of a thymidine analogue to the salvage regimen (HR, 2.55; 95% CI, 1.25–5.19; $P = .01$). Inclusion of zidovudine rather than stavudine was associated with a better outcome (HR, 2.66; $P = .04$). The authors concluded that development of K65R may not limit the effectiveness of salvage therapy.

**Evolution and Persistence of Resistance**

Wind-Rotolo and colleagues (Abstract 616) evaluated the persistence of tenofovir (0.13-log$_{10}$ copies/mL reduction in HIV-1 RNA level; $P = .0026$). The emergence of viruses containing both K65R and L74V appears to be relatively infrequent. To better characterize tenofovir resistance, Frankel and colleagues (Abstract 608) studied purified reverse transcriptase sequences containing K65R, L74V, or both. They reported that the co-occurrence of K65R and L74V may potentiate resistance to tenofovir. Addition of M184V resulted in resensitization to tenofovir but not to dideoxynucleoside adenosine triphosphate (ddATP). Antinori and colleagues (Abstract 636) evaluated the impact of the K65R mutation on response to salvage therapy among 145 individuals from 6 Italian centers. Subjects switched antiretroviral regimens after genotypic resistance testing. The median baseline plasma HIV-1 RNA level and CD4 + cell count were 4.17 log$_{10}$ copies/mL and 312/µL, respectively; 56.5% of individuals were on tenofovir, 56.5% on lamivudine, and 55.7% on didanosine. Among these individuals, 44.8% had M184V; 15.2% and 22.8% had mutations from TAM-1 (M41L, L210W, and T215Y) and TAM-2 (D67N, K70R, and K219Q/E) pathways, respectively. After genotypic resistance testing, individuals changed therapy to lamivudine (59.7%), zidovudine (29%), stavudine (27.4%). The best predictor of achieving a treatment response (defined as a plasma HIV-1 RNA level below 50 copies/mL at 12 months), was associated with the presence of the M184V mutation (HR, 1.97; 95% CI, 1.0–3.86; $P = .05$); and the addition of a thymidine analogue to the salvage regimen (HR, 2.55; 95% CI, 1.25–5.19; $P = .01$). Inclusion of zidovudine rather than stavudine was associated with a better outcome (HR, 2.66; $P = .04$). The authors concluded that development of K65R may not limit the effectiveness of salvage therapy.
NNRTI resistance mutations in resting CD4+ T cells in 6 individuals with previously documented resistance. Samples were obtained 36 to 64 months after stopping NNRTIs. HIV-1 clones with K103N or Y181C were found in the resting CD4+ T cells but not in the plasma in 3 of 5 individuals. NNRTI resistance mutations were present in proviral DNA sequences in 3 of 3 subjects with plasma HIV-1 RNA levels below 50 copies/ml.

Easterbrook and colleagues (Abstract 620) evaluated the prevalence of protease mutations in PI-naive and PI-experienced individuals infected with nonsubtype B virus. The United Kingdom HIV Drug Resistance Database collected 15,624 samples between 1996 and 2002, which were then reviewed. Of these, 11,692 samples were subtype B HIV-1. The most common non-B subtypes were C (n=2043), A (n=815), and D (n=428). The authors found differences in the amino acid sequences between PI-naive and PI-exposed individuals in non-B subtypes at the positions known to be associated with drug resistance in subtype B viruses: specifically, at codons 10, 20, 30, 46, 63, 71, 82, and 90. New protease mutations in non-B subtypes were rare (at codons 13, 6, 33, 37, 41, 57, 65, 72, 74, and 89). Significant association with PI exposure was found for more than 1 non-B subtype only at position M89I/V.

**Resistance to Entry Inhibitors**

**Maraviroc.** (UK-427,857) is a CCR5 antagonist currently in phase IIb/III clinical trials. The compound binds within the transmembrane region of the receptor while gp120 interacts with the N-terminus and extracellular loop of CCR5. Mosley and colleagues (Abstract 598) characterized phenotypic and genotypic determinants of maraviroc resistance. The emergence of maraviroc resistance was associated with A316T and I323V mutations in the V3 region of gp120. Both mutations were necessary and sufficient for the fully resistant phenotype. Resistance to maraviroc was not associated with cross-resistance to aplaviroc or enfuvirtide.

**Resistance Associations by Genotypic Database Analyses**

Ross and colleagues (Abstract 602) evaluated differences in susceptibility between lamivudine and emtricitabine. Samples from a commercial database that had susceptibility to both drugs did not have the M184I/V mutation, but did contain: K65R, L74I/V, Q151M, T69 insertions, 2 or 3 TAMs from those at positions 41, 210, or 215, 2 or 3 TAMs from positions 67, 70, or 219, any 3 or 4 TAMs, any 5 or 6 TAMs (with and without E44D/V118I), or K65R + Q151M were identified. In the presence of K65R, L74I/V, or Q151M without TAMs, there were no differences in the mean fold change in resistance between lamivudine and emtricitabine. In samples containing 2 or more TAMs, the mean fold change in resistance was higher for emtricitabine than for lamivudine (P < .001).

A tipranavir mutation score was derived from analysis of a limited number of samples from phase II and III clinical trials and took into account the following mutations in the protease: L10V, I13V, K20M/R/V, L35F, E35G, M36I, K43T, M46I, L47V, I54A/M/V, Q58E, H69K, T74P, V82L/T, N83D, and I84V. Each mutation is scored equally.

Parkin and colleagues (Abstract 637) used samples from a commercial database to evaluate the accuracy of the tipranavir mutation score in predicting phenotypic susceptibility. Tipranavir fold change significantly correlated with the tipranavir mutation score (P < .0001). Several new mutations were significantly associated with higher- than-expected tipranavir fold change: A71L, V11L, G73T, L89V, I84V, V52I, M36I, I66, D60E, K55R, L90M, M46I, and L10I. Additional mutations were significantly associated with lower-than-expected tipranavir fold change: I50V, D30N, L76V, L24I, V82I, I50L, I54L, N88D, and L10F. The authors proposed a revised tipranavir mutation score that incorporated new mutations and had greater correlation with measured phenotype, and lower phenotypic and genotypic discordance than the current mutation score.

**Pharmacology and Therapeutic Drug Monitoring**

**Selected Drug-Drug Interactions**

**P-glycoprotein.** P-glycoprotein is a cellular efflux transporter that can affect absorption, tissue penetration, and intracellular concentrations of certain antiretroviral agents. Although it is expressed in T lymphocytes, its relevance to antiretroviral effectiveness is unclear. Cyclosporine A is an inhibitor of P-glycoprotein in vitro. Hulgan and colleagues presented data on the inhibition of P-glycoprotein in vivo from a substudy of ACTG 5138, a randomized open-label trial of antiretroviral therapy with or without cyclosporine A (Abstract 564). Subjects initiated antiretroviral therapy with fixed-dose abacavir/lamivudine/zidovudine for 14 days after which efavirenz was added. Nine subjects received cyclosporine A in addition to antiretroviral therapy and 7 subjects received antiretroviral therapy alone. P-glycoprotein activity was measured at baseline, day 14, and week 4. Cyclosporine A plus 3 nRTIs was associated with an 8% decrease in P-glycoprotein activity compared with baseline (P = .03) and was positively associated with cyclosporine A trough levels. No difference was found in those not receiving cyclosporine A. At week 4 (2 weeks after efavirenz was added), P-glycoprotein activity was not different from baseline in either group. This study shows that targeted inhibition of P-glycoprotein activity is feasible. The relevance to antiretroviral activity remains to be shown.

Marzolini and colleagues investigated the effect of P-glycoprotein and other drug transporters on the distribution of efavirenz (Abstract 565). They administered radiolabeled efavirenz to mice that did or did not express P-glycoprotein. They found that tissue levels in the brain, liver, kidney, testes, and plasma were not
statistically significantly different. They also looked at cell cultures with varying expression of other transporters and did not find an effect on efavirenz transport. The authors concluded that pharmacologic alteration of drug transporters was unlikely to affect efavirenz disposition in vivo.

Dupuis and colleagues tested the interaction of diketo acid integrase inhibitors and P-glycoprotein (Abstract 566). They found that integrase inhibitors competed with other P-glycoprotein substrates for cellular efflux. Also, prolonged treatment of cell cultures that had a low expression of P-glycoprotein with integrase inhibitors resulted in increased P-glycoprotein expression and activity. This may have implications for modulation of the absorption of integrase inhibitors and their ability to penetrate the central nervous system and genital compartments over time.

**Tenofovir.** Kiser and colleagues hypothesized that ritonavir may decrease the renal clearance of tenofovir by affecting proximal renal tubular cells (Abstract 570). They compared 15 subjects receiving tenofovir and lopinavir/r with age-, race-, and sex-matched controls that were receiving tenofovir and nRTIs or an NNRTI. They found that the clearance of tenofovir was greater in those subjects not receiving ritonavir and that the clearance of tenofovir and glomerular filtration rate were associated with intracellular PBMC tenofovir diphosphate levels. However, no difference in intracellular levels was seen between groups. The authors concluded that the mechanism of this interaction merits further study.

**Efavirenz.** Prior data from ACTG 5095 implicated genetic polymorphisms in the CYP2B6 gene (the enzyme mainly responsible for metabolizing efavirenz) with efavirenz plasma levels and toxicities, which was confirmed with data from a second efavirenz-containing study, ACTG 384 (Haas et al., *AIDS*, 2004; Haas et al., *J Infect Dis.*, 2005). Polymorphisms in the *mdr1* gene, which encodes P-glycoprotein, were shown to be related to decreased virologic failure on an efavirenz-based regimen but not to plasma levels. Motsinger and colleagues presented further data on interactions between these polymorphisms and their relationship to plasma levels, toxicity, and virologic failure among subjects receiving efavirenz in ACTG 384 (Abstract 571). They were better able to predict higher plasma levels and AUC of efavirenz, efavirenz toxicity, and virologic failures by considering 2-gene interactions. However, the accuracy of these predictions and the specific polymorphisms involved varied according to race.

Gupta and colleagues presented data on the pharmacokinetics of lopinavir/r or efavirenz in subjects on hemodialysis compared with historic controls (Abstract 573). They found that the average AUC (90% CI) of efavirenz for subjects on hemodialysis was 135% (89%–170%) that of historic controls. The AUC of lopinavir and ritonavir were 77% (65%–91%) and 91% (73%–115%), respectively. Although the lopinavir AUC was reduced compared with controls, the lopinavir inhibitory quotient for wild-type HIV remained high. These data suggest that dose adjustment of these drugs may not be necessary for subjects on hemodialysis.

**Rifampin.** Pujari and colleagues investigated the effect of rifampin on the pharmacokinetics of nevirapine in non-HIV–infected subjects from India (Abstract 574). Consistent with studies in other populations, rifampin markedly reduced the AUC of nevirapine by 80% (P < .0001), suggesting that concomitant administration of these drugs is problematic. Both carbamazepine and efavirenz are substrates and inducers of CYP3A4 and CYP2B6. Kauf and colleagues examined the pharmacokinetic interactions of these drugs given in combination in 36 non-HIV–infected subjects in a crossover design (Abstract 575a). They found that the AUC of efavirenz was reduced by 34% when coadministered with carbamazepine (90% CI; 32%–40%). Similarly, the AUC of carbamazepine was reduced by 27% (20%–33%). This 2-way drug interaction suggests that coadministration of these drugs may compromise antiretroviral or anticonvulsant efficacy.

**Etravirine.** As noted earlier, etravirine is a novel investigational NNRTI that is active against HIV resistant to currently available NNRTIs, making it a potential option for treatment-experienced patients. Harris and colleagues examined the interactions between etravirine and 3 PIs: lopinavir, ritonavir, and saquinavir (Abstract 575b). They studied 15 HIV-seropositive subjects who were receiving lopinavir/r, saquinavir, and 2 nRTIs with plasma HIV RNA levels below 50 copies/mL. They assessed the pharmacokinetics of the PIs at baseline and after 2 weeks of coadministration with etravirine. The 12-hour AUCs for lopinavir, ritonavir, and saquinavir were reduced by 18%, 13%, and 13%, respectively. Only the change in lopinavir 12-hour AUC was statistically significant (P = .04). The HIV-1 RNA level remained below 50 copies/mL for all subjects.

Schöller and colleagues evaluated the interaction of tipranavir/r and etravirine in 24 subjects utilizing a crossover design (Abstract 583). They found that the AUC of tipranavir was increased by 18% after coadministration with etravirine compared with tipranavir alone and the AUC of ritonavir was increased by 23%. However, the AUC of etravirine was reduced by 76% (90% CI; 67%–82%) precluding coadministration of these drugs.

**Atazanavir.** Best and colleagues presented data on behalf of the Central Nervous System HIV Antiretroviral Therapy Effects Research (CHARTER) study (Abstract 576). They collected concomitant cerebrospinal fluid and plasma samples in 26 subjects who were receiving atazanavir/r. They found that the ratio of cerebrospinal fluid to plasma levels was often below the IC50 for wild-type virus. The mean plasma HIV RNA level was 2.7 log10 copies/mL and the mean cerebrospinal fluid HIV-1 RNA level was...
1.9 log_{10} copies/mL. The authors suggested that the observed cerebrospinal fluid levels may not be sufficient to control HIV replication in the central nervous system compartment.

Gastric acid suppression has been associated with reduced levels of atazanavir, but data on other PIs have been incomplete. Klein and colleagues investigated the pharmacokinetics of lopinavir/r with and without omeprazole and ranitidine (Abstract 578). They did not find a significant alteration with coadministration of either acid-reducing agent with lopinavir/r given once daily or twice daily.

**Vicriviroc.** The levels of vicriviroc, an investigational CCR5 antagonist, are markedly enhanced by coadministration with ritonavir. Sansone and colleagues studied HIV-seronegative subjects who received 14 days of vicriviroc and 100 mg of ritonavir once or twice daily depending on assigned PI cohort (Abstract 582). After this, they received indinavir, fosamprenavir, nelfinavir, or saquinavir soft gel capsules for 2 weeks or atazanavir for 1 week. The pharmacokinetic profile of vicriviroc was not altered after the addition of the second PI for any of the 5 cohorts.

Two studies examined the pharmacokinetic effect of atazanavir on other PIs without using ritonavir. King and colleagues compared saquinavir hard gel capsules and atazanavir (1000 mg/200 mg twice daily and 1500 mg/200 mg twice daily) with saquinavir/r (1000 mg/100 mg twice daily) in a crossover design in which each participant received all 3 regimens (Abstract 586). They found that the saquinavir pharmacokinetic profile (maximum concentration \([C_{\text{max}}]\), minimum concentration \([C_{\text{min}}]\), and 12-hr AUC) was markedly reduced compared with saquinavir/r. The atazanavir levels were comparable to levels reported for atazanavir 400 mg daily. They also noted that female subjects had higher drug levels than men even after controlling for differences in weight. Clay and colleagues performed a similar study of 21 subjects who first received fosamprenavir alone followed by atazanavir alone, followed by a combination of the 2 drugs (Abstract 587). The AUC and \(C_{\text{min}}\) of fosamprenavir were increased by 78% and 283% respectively when coadministered with atazanavir, but the AUC and \(C_{\text{min}}\) of atazanavir were reduced by 33% and 57%, respectively.

**Therapeutic Drug Monitoring**

Best and colleagues reported the results of California Collaborative Treatment Group 578 Study (Abstract 589). This involved both adherence interventions and therapeutic drug monitoring (TDM). Only the results of the TDM intervention were reported. Eligible subjects were receiving either an NNRTI- or PI-based regimen. One hundred ninety-nine subjects were randomized to standard dosing or to dose modifications by an expert panel based on drug levels and patient history; 137 subjects completed the study. At baseline, 81% were men and 29% were treatment naive. The median CD4+ count and plasma HIV-1 RNA level were 190 cells/µL and 5.2 log_{10} copies/mL, respectively. Of 647 TDM evaluations, 225 suggested a change in PI or NNRTI dose. In the TDM arm, 77% of recommendations were followed by the providers, and 60% of these subjects achieved the targeted drug levels compared with only 35% of subjects in the standard-of-care arm. This study showed that one third of patients receiving a PI- or NNRTI-based regimen have suboptimal drug levels, and that TDM followed by dose modification was able to achieve targeted drug levels twice as often as no dose modification.

Podsadecki and colleagues examined patterns of adherence among subjects in a study comparing once with twice-daily dosing of lopinavir/r soft gel capsules (Abstract 590). They measured adherence with self-report, lopinavir levels, and electronically captured dosing events (MEMS caps). They noted a phenomenon termed “white coat compliance.” It is described as excellent adherence for several days before a study visit coupled with suboptimal adherence at other times. The lopinavir levels in these individuals were generally within the therapeutic range. This occurred at 31% of visits and occurred at least once in 66% of patients. The opposite pattern, suboptimal adherence just before a visit coupled with excellent adherence at other times, was rare (1% of study visits).

Gandhi and colleagues examined a wide variety of factors affecting drug levels of nevirapine, efavirenz, or lopinavir/r among women in the Women’s Interagency HIV Study (WIHS) cohort (Abstract 592). They found that women coinfected with hepatitis C virus (HCV) had a nevirapine AUC 1.23-times higher than women without HCV. The AUCs of both nevirapine and efavirenz were greater with higher levels of hepatic transaminases. For all 3 drugs, an increase in lean body mass was associated with a reduced AUC.

**Mother-To-Child Transmission of HIV**

MTCT of HIV remains a significant problem in resource-limited settings. At this year’s conference, several areas of research regarding prevention of MTCT (pMTCT) were highlighted including implications of single-dose nevirapine for future pMTCT and development of resistance; results of combination PI- and NNRTI-based maternal prophylaxis; and prevention of breast milk-associated MTCT of HIV.

The HIV Network for Prevention Trials (HIVNET) 012 study demonstrated that single-dose nevirapine was efficacious in pMTCT but increased the risk of NNRTI resistance (Guay et al, *Lancet*, 1999; Eshleman et al, *J Acquir Immune Defic Syndr*, 2004). In resource-limited settings where the availability of combination antiretroviral therapy is limited, the implications of these findings are unclear as rates of resistance appear to decline and long-term resistance patterns and response to treatment in women and infants exposed to single-dose nevirapine are unknown (Coovadia, *N Engl J Med*, 2004). Two abstracts at this year’s conference (Abstracts 125 and 722) evaluated the effectiveness of single-dose
nevirapine in preventing HIV transmission in consecutive pregnancies.

Eure and colleagues (Abstract 125) retrospectively identified Ugandan women who had received single-dose nevirapine \( (n = 59) \) or short-course zidovudine \( (n = 41) \) through HIVNET 012 and received single-dose nevirapine for pMTCT during a subsequent pregnancy. A parallel, prospective study identified women who had received single-dose nevirapine in pregnancy. A parallel, prospective study identified women who had received single-dose nevirapine through HIVNET 012 and received single-dose nevirapine in pregnancy. A parallel, prospective study identified women who had received single-dose nevirapine in pregnancy. A parallel, prospective study identified women who had received single-dose nevirapine in pregnancy.

In the prospective group, there were no statistically significant differences in baseline median CD4+ counts \((463–486 \text{ cells/µL})\) or plasma HIV-1 RNA levels \((19,900–22,950 \text{ copies/mL})\) between nevirapine-experienced and nevirapine-naive women. Time between pregnancies for the majority of women \((97\%)\) was 12 months or more. In the retrospective and prospective groups, 6- to 9-month transmission rates among single-dose nevirapine-experienced versus single-dose nevirapine-naive women were not statistically different: 11.8% versus 17%, respectively, in the retrospective group and 18.4% versus 17.5%, respectively, in the prospective group. The authors concluded that single-dose nevirapine is an effective option for sequential pregnancies for pMTCT in resource-limited settings where more complex options are not yet feasible.

Martinson and colleagues (Abstract 721) presented an open-label, prospective study evaluating the efficacy and safety of a ritonavir-boosted, saquinavir-based versus a nelfinavir-based regimen with a zidovudine/lamivudine backbone for pMTCT. Sixty-six antiretroviral-naive pregnant women were enrolled from sites around the world \((\text{Ireland, sub-Saharan Africa, Eastern Europe})\) and initiated therapy during the third trimester for a minimum of 6 weeks prior to delivery. Women were randomized to receive an 8-tablet-per-day regimen of zidovudine/lamivudine/saquinavir/r \((n = 19)\), a 6-tablet-per-day regimen of zidovudine/lamivudine/nelfinavir \((n = 6)\), or a 12-tablet-per-day regimen of zidovudine/lamivudine/nelfinavir \((n = 42)\). Ninety-four percent of women in the saquinavir group \((14 \text{ of } 16)\) and 84% in the nelfinavir group \((42 \text{ of } 48)\) received intrapartum zidovudine. Mode of delivery in the saquinavir group was 56.3% spontaneous vaginal delivery, 6.2% elective caesarian delivery, and 37.5% emergency caesarian delivery. In the nelfinavir group, the above modes of delivery were 56.3%, 31.2%, and 12.5%, respectively. At 36 weeks, 87.5% of women in the saquinavir arm versus 56.3% in the nelfinavir arm reached plasma HIV-1 RNA levels below 50 copies/mL. One infant in the nelfinavir group seroconverted in the setting of maternal delivery at 36 weeks with ruptured membranes for more than 24 hours and maternal HIV RNA below 50 copies/mL. No infants in the saquinavir group seroconverted. At 6 weeks postpartum, genotypic testing was performed in both groups and no major PI resistance mutations were reported. Saquinavir peak and trough levels were evaluated in 4 patients, all of whom had adequate levels. The authors concluded that treatment with zidovudine/lamivudine/saquinavir/r achieved better virologic suppression than zidovudine/lamivudine/nelfinavir \((P < .01)\). The absence of PI mutations in either group post-treatment suggests that short-term PI administration has no detrimental effect on future antiretroviral therapy options.

Previous studies have noted that in the setting of single-dose nevirapine, a greater proportion of nevirapine resistance is found in subtype C virus than A or B and that the K103N mutation is the most common nevirapine resistance mutation \((\text{Eshleman, CROI, 2005}).\) Flies and colleagues (Abstract 726) reported the prevalence of K103N mutations among subtypes A, C, and D in women who had previously received single-dose nevirapine for pMTCT. A sensitive and quantitative point-mutation assay was conducted in samples from women collected 6 to 8 weeks after receiving single-dose nevirapine through the HIVNET 012 and the Nevirapine and Zidovudine plus programs were enrolled during their first pregnancies; all women received single-dose nevirapine with either zidovudine or zidovudine/lamivudine in both pregnancies. The median interdelivery time between pregnancy and CD4+ count during second pregnancy in the Soweto group were 21 months and 400 cells/µL versus 26 months and 462 cells/µL in the Abidjan group. The rate of cesarean deliveries during both births was greater in Soweto than Abidjan at 21% and 2% to 3%, respectively. The reported rates of HIV transmission for the first and second pregnancies were 10.9% \((10 \text{ of } 92)\) and 13.6% \((14 \text{ of } 103)\), respectively, in Soweto and 13.2% \((5 \text{ of } 38)\) and 5.49% \((2 \text{ of } 37)\), respectively, in Abidjan. Of note, infant HIV status was not known for all of the women for both pregnancies at the time of the presentation. Furthermore, infants were tested for HIV a median of 87 weeks \((\text{Soweto})\) and 74 weeks \((\text{Abidjan})\) earlier in the second pregnancies than in the first pregnancies. Rates of transmission in women with an interdelivery time of less than 12 months was 27% versus 7.7% in women with an interdelivery time of 12 months or more \((P = .032)\). The authors concluded that rates of transmission were similar to those in the first pregnancy at both sites. Additionally, they noted that increased interdelivery time might be protective against transmission in the setting of single-dose nevirapine, possibly because of reversion to wild-type virus in the absence of sustained nevirapine pressure.

Hanlon and colleagues (Abstract 721) conducted a similar study to evaluate the effectiveness of single-dose nevirapine in consecutive pregnancies in Soweto, South Africa, and Abidjan, Côte d’Ivoire. In Soweto \((n = 122)\), women who participated in HIVNET 012, had not breast fed their first infant, and had received single-dose nevirapine during both pregnancies were enrolled. In Abidjan \((n = 41)\), women from the MTCT-plus or DITRAMA-
Emergence of NNRTI resistance has been shown to be less frequent if nevirapine is administered with a short course of zidovudine and lamivudine (Chaix et al., CROI, 2005). Perez and colleagues (Abstract 725) evaluated 25 plasma samples from 20 pregnant women in whom zidovudine/lamivudine/nevirapine was initiated during their second or third trimester and continued until delivery. Prepregnancy, all women were either antiretroviral therapy naive or had received only zidovudine during a previous pregnancy. Eighty percent were infected with subtype B/F and 20% with subtype B HIV-1. Median plasma HIV-1 RNA level at labor was below 50 copies/mL (range, <50–108 copies/mL). Plasma samples were collected up to 15 months after discontinuation of therapy. Using standard bulk sequencing, there were no mutations associated with resistance to PIs or RTIs except for 1 M41L mutant. One sample was not amplified and there were no episodes of MTCT. The authors concluded that in this sample of women, zidovudine/lamivudine/nevirapine was highly effective in preventing MTCT and the risk of selection for NNRTI-resistant virus was low.

Karchava and colleagues from the New York State Department of Health (Abstract 724) presented rates of resistance in 51 perinatally infected infants diagnosed between 2001 and 2002. Of them, 42 had samples available for resistance testing. Eight (19%) had at least 1 drug resistance mutation of which 7.1% conferred nRTI resistance, 11.9% NNRTI resistance, and 2.4% PI resistance. Compared with a prevalence study from 1998 to 1999, rates of overall resistance increased from 12.1%, NNRTI resistance increased from 3.3%, and nRTI and PI resistance rates remained relatively stable. Four of 8 infants with resistance mutations had perinatal antiretroviral therapy exposure; of these, 1 was resistant to the drug administered. Phylogenetic analysis revealed 7 (17%) infants were infected with nonsubtype B virus (2 [4.8%] had subtype C and 5 [11.9%] had circulating recombinant form [CRFO2]).

Eshleman and colleagues (Abstract 719) evaluated the association between HIV replication capacity and MTCT. In a random subset of women from the NVAZ late-presenter trial in Malawi, replication capacity in 52 transmitters (women whose infants were HIV-seropositive at birth or 6 to 8 weeks postpartum) was compared with replication capacity in 48 nontransmitters (women whose infants were uninfected at 6 to 8 weeks postpartum). Fifty-four percent of infants had received single-dose nevirapine only and 46% received single-dose nevirapine followed by 1 week of zidovudine; mothers presented too late to receive antiretroviral therapy prophylaxis. All women had subtype C HIV and there were no cesarean deliveries or twins. All but one infant was breast fed at 6 to 8 weeks postpartum. Replication capacity was evaluated in maternal plasma samples obtained at delivery via a phenotypic assay. Mean replication capacity and maternal plasma HIV-1 RNA level at delivery were higher in transmitters than in nontransmitters: 35% versus 27.4% (P = .02) and 5.1 versus 4.6 log_{10} RNA copies/mL (P = .001), respectively. Maternal age, infant antiretroviral therapy regimen, and parity were not statistically significantly different. Adjusting for maternal viral load at delivery, maternal age, and infant regimen, replication capacity was significantly associated with transmission (OR, 6.60; 95% CI, 1.25–35.31). The authors concluded that replication capacity appears to be related to MTCT and should be further evaluated in different HIV-1 subtypes, clinical settings, and modes of transmission.

Handelsman and colleagues (Abstract 718) presented results from a matched, case-control study comparing rates of GB virus C (GBV-C) viremia among HIV-seropositive women in the Women and Infants Transmission Study (WITS) who had (n = 133) and had not (n = 266) transmitted HIV to their infants. GBV-C viremia has been associated with improved survival, nonprogression, and response to antiretroviral therapy in HIV-infected persons (Williams et al., N Engl J Med, 2004; Souza et al., HIV Med, 2006). Of 397 women, 11% had evidence of active GBV-C and 36% had past infection. There was a trend toward protection against MTCT in women with active GBV-C (OR, 0.79; 95% CI, 0.52–1.2). Women with GBV-C viremia had lower plasma HIV-1 RNA levels and higher CD4+ cell counts than women without GBV-C infection. As noted in other studies, low birth weight, lack of antiretroviral therapy, and maternal HIV-1 RNA level during pregnancy were associated with MTCT. The authors conclude that the association between GBV-C viremia and lower HIV viral load and higher CD4+ cells counts, rather than the presence of GBV-C viremia itself, may explain the association with decreased MTCT that has been reported in other studies. Although there was a trend toward an independent correlation between GBV-C viremia and decreased MTCT, this trend was not statistically significant. More and larger cohorts are necessary to further evaluate the relationship between GBV-C viremia and MTCT.

Kisin and colleagues (Abstract 127) analyzed a point-of-care rapid HIV testing program in St. Petersburg, Russia. The program was initiated in April, 2004 in 2 maternity hospitals where women in St. Petersburg with unknown HIV status (had either never been tested or had their last test before 34 weeks gestation) are referred for labor and delivery. All women with unknown HIV status are offered opt-out testing with a
rapid, HIV-1/HIV-2 test that has 100% sensitivity and 99.9% specificity. Standard enzyme immunoassay (EIA) and Western blot (WB) HIV testing is done in parallel with the rapid test. HIV-seropositive mothers and their infants receive single-dose nevirapine and infants receive replacement feeding. From April 2004 to April 2005, 4353 pregnant women presented to these hospitals and were eligible for rapid testing: 1408 women had never had an HIV test and 2945 had their last HIV test at before 34 weeks gestation; 2.3% of the women never tested and HIV test at before 34 weeks gestation; testing: 1408 women had never had.

From April 2004 to April 2005, 4353 pregnant women from 4 sub-Saharan African sites (Blantyre and Lilongwe, Malawi; Dar es Salaam, Tanzania; and Lusaka, Zambia) enrolled. All received single-dose nevirapine according to the HIVNET 012 regimen and no difference in rates of transmission was found either at birth or at 4 to 6 weeks in the antibiotic versus placebo groups. Inclusion criteria for the substudy were infants from HPTN 024 who were HIV-seronegative as of 4 to 6 weeks but were subsequently HIV-seropositive (presumably via breast milk transmission if breast fed) in whom follow-up information was available (n=1558). The cumulative incidence of HIV infection in the substudy group was 6.81 per 100 person-years of observation. Specific maternal socioeconomic factors (ie, literacy, parity, electricity in the house, body mass index [BMI]) and presence of breast infection were not associated with transmission. Higher levels of maternal hemoglobin and CD4+ cell count correlated with protection against transmission and higher plasma HIV-1 RNA levels and cervicovaginal HIV-1 RNA levels were associated with risk for transmission in univariate analysis. In multivariate analysis, high plasma viral load and low CD4+ cell count remained statistically significantly associated with transmission. Infant factors including Apgar score, birth weight, presence of oral thrush, and sex did not correlate with transmission. Incidence of transmission after 4 to 6 weeks varied significantly among study sites with Dar es Salaam having the lowest incidence (3.5%) and Lusaka having the highest (10.5%). Biologic factors did not account for this difference in rates and the authors hypothesized that length and intensity of breast feeding may have accounted for regional differences. At Dar es Salaam, 98% of infants are weaned at 6 months and at the other sites, a majority of infants continued to be breast fed at 12 months.

Four abstracts at this year’s conference (Abstracts 727, 728, 729, 730) evaluated the association between maternal factors and HIV-1 RNA level in breast milk.

Giuliano and colleagues from the Drug Resource Enhancement against AIDS and Malnutrition (DREAM) program (Abstract 727) in Mozambique conducted an observational study comparing levels of HIV-1 RNA in the breast milk of women who had or had not received prophylactic antiretroviral therapy for pMTCT. Through the DREAM program, pregnant, HIV-seropositive women received antiretroviral prophylaxis (zidovudine/lamivudine/nevirapine or stavudine/lamivudine/nevirapine) at 28 weeks gestation through 6 months postpartum and infants receive single-dose nevirapine within 72 hours of delivery. Levels of HIV-1 RNA in breast milk of 40 women enrolled in DREAM prepartum were compared with breast milk from 40 women diagnosed with HIV infection at delivery (who had therefore not received prophylaxis with potent antiretroviral therapy). Pretherapy median CD4+ count, HIV-1 RNA level, and time of therapy use in the treatment group was 538 cells/µL, 4.2 log_{10} copies/mL, and 83 days, respectively. At delivery and 7 days postpartum, HIV-1 RNA in breast milk of women who received antiretrovirals was lower than in women who did not: the median level was 2.3 log_{10} copies/ mL in the antiretroviral therapy group versus 5.4 log_{10} copies/mL in the nontherapy group at delivery and 1.9 log_{10} copies/mL versus 3.6 log_{10} copies/mL 7 days postpartum (P ≤ .001 for both). The proportion of women with HIV-1 RNA level below 400 copies/mL in breast milk was also higher among therapy-treated women at delivery and 7 days postpartum: 46% treated versus 15% not treated at both time points (P = .01). Use of antiretrovirals was the strongest predictor of an HIV-1

HIV-1 Transmission in Breast Milk

It is estimated that breast milk transmission of HIV-1 accounts for nearly one half of all pediatric HIV-1 infections thereby significantly reducing the effects of prenatal and perinatal pMTCT (John et al, E Afr Med J, 2001). The following studies evaluated factors associated with breast milk transmission of HIV in resource-limited settings where formula feeding is often associated with social stigma and other health risks (such as enteric infections due to contaminated water), and is frequently inaccessible due to cost.

Hoffman and colleagues (Abstract 730) presented results from a substudy of infants from HIV Prevention Trials Network (HPTN) 024. HPTN 024 was a multicenter, randomized, double-blind, placebo-controlled trial of antibiotics to prevent chorioamnionitis-associated perinatal transmission. There were 2128 pregnant women from 4 sub-Saharan African sites (Blantyre and Lilongwe, Malawi; Dar es Salaam, Tanzania; and Lusaka, Zambia) enrolled. All received single-dose nevirapine according to the HIVNET 012 regimen and no difference in rates of transmission was found either at birth or at 4 to 6 weeks in the antibiotic versus placebo groups. Inclusion criteria for the substudy were infants from HPTN 024 who were HIV-seronegative as of 4 to 6 weeks but were subsequently HIV-seropositive (presumably via breast milk transmission if breast fed) in whom follow-up information was available (n=1558). The cumulative incidence of HIV infection in the substudy group was 6.81 per 100 person-years of observation. Specific maternal socioeconomic factors (ie, literacy, parity, electricity in the house, body mass index [BMI]) and presence of breast infection were not associated with transmission. Higher levels of maternal hemoglobin and CD4+ cell count correlated with protection against transmission and higher plasma HIV-1 RNA levels and cervicovaginal HIV-1 RNA levels were associated with risk for transmission in univariate analysis. In multivariate analysis, high plasma viral load and low CD4+ cell count remained statistically significantly associated with transmission. Infant factors including Apgar score, birth weight, presence of oral thrush, and sex did not correlate with transmission. Incidence of transmission after 4 to 6 weeks varied significantly among study sites with Dar es Salaam having the lowest incidence (3.5%) and Lusaka having the highest (10.5%). Biologic factors did not account for this difference in rates and the authors hypothesized that length and intensity of breast feeding may have accounted for regional differences. At Dar es Salaam, 98% of infants are weaned at 6 months and at the other sites, a majority of infants continued to be breast fed at 12 months.

Four abstracts at this year’s conference (Abstracts 727, 728, 729, 730) evaluated the association between maternal factors and HIV-1 RNA level in breast milk.

Giuliano and colleagues from the Drug Resource Enhancement against AIDS and Malnutrition (DREAM) program (Abstract 727) in Mozambique conducted an observational study comparing levels of HIV-1 RNA in the breast milk of women who had or had not received prophylactic antiretroviral therapy for pMTCT. Through the DREAM program, pregnant, HIV-seropositive women received antiretroviral prophylaxis (zidovudine/lamivudine/nevirapine or stavudine/lamivudine/nevirapine) at 28 weeks gestation through 6 months postpartum and infants receive single-dose nevirapine within 72 hours of delivery. Levels of HIV-1 RNA in breast milk of 40 women enrolled in DREAM prepartum were compared with breast milk from 40 women diagnosed with HIV infection at delivery (who had therefore not received prophylaxis with potent antiretroviral therapy). Pretherapy median CD4+ count, HIV-1 RNA level, and time of therapy use in the treatment group was 538 cells/µL, 4.2 log_{10} copies/mL, and 83 days, respectively. At delivery and 7 days postpartum, HIV-1 RNA in breast milk of women who received antiretrovirals was lower than in women who did not: the median level was 2.3 log_{10} copies/ mL in the antiretroviral therapy group versus 5.4 log_{10} copies/mL in the nontherapy group at delivery and 1.9 log_{10} copies/mL versus 3.6 log_{10} copies/mL 7 days postpartum (P ≤ .001 for both). The proportion of women with HIV-1 RNA level below 400 copies/mL in breast milk was also higher among therapy-treated women at delivery and 7 days postpartum: 46% treated versus 15% not treated at both time points (P = .01). Use of antiretrovirals was the strongest predictor of an HIV-1

HIV-1 Transmission in Breast Milk

It is estimated that breast milk transmission of HIV-1 accounts for nearly one half of all pediatric HIV-1 infections thereby significantly reducing the effects of prenatal and perinatal pMTCT (John et al, E Afr Med J, 2001). The following studies evaluated factors associated with breast milk transmission of HIV in resource-limited settings where formula feeding is often associated with social stigma and other health risks (such as enteric infections due to contaminated water), and is frequently inaccessible due to cost.
RNA level below 400 copies/mL in breast milk. The authors concluded that these data support the role of maternal antiretroviral prophylaxis in prevention of breast-feeding associated HIV-1 transmission. Further studies are needed to determine if the observed decrease in breast milk viral load translates to a significant reduction in postnatal HIV-1 transmission.

Gantt and colleagues (Abstract 728) presented results from a prospective cross-sectional study of postpartum HIV-1–infected women in Zimbabwe to determine if infectious clinical and subclinical mastitis was associated with the level of detectable HIV-1 RNA in breast milk. Two hundred seventeen women were enrolled 1.5 to 7 months postpartum. Breast milk was aseptically collected, cultured for bacteria and fungi, and analyzed for HIV-1 RNA levels and absolute and differential white blood cell (WBC) counts. Seventeen of 217 (8%) women had symptoms consistent with clinical mastitis. Fifty of 428 samples of breast milk (12%) were culture positive for a bacterial or fungal pathogen, the most common pathogen being *Staphylococcus aureus*. Subclinical mastitis (defined as total WBC count >10⁶/mL breast milk) occurred in 60 of 217 women (28%). Positive culture was not associated with rate of detectable HIV-1 RNA, level of HIV-1 RNA, or clinical or subclinical mastitis. Presence of subclinical mastitis was not associated with symptoms. Absolute neutrophil count (ANC) was associated with detectable HIV-1 RNA in breast milk—the highest quartile of ANC (102 of 409 samples) had an OR of 3.64 for detectable HIV-1 RNA. The authors concluded that this elevated ANC, and therefore detectable level of HIV-1 RNA, could be associated with other infectious agents such as viruses, mycobacteria, or mycoplasma that were not looked for in this study.

### Antiretroviral Therapy in Resource-Limited Settings

Results of selected studies are presented in Table 3. Etard and colleagues representing the Agence Nationale de Recherches sur la Sida (ANRS; Abstract 63) presented results of a prospective cohort study of patients treated through the Senegalese antiretroviral drug-access initiative program, the first government-sponsored treatment program in Africa. Four hundred four patients were observed for a median of 46 months. At study entry, 5% were antiretroviral therapy-experienced, and 55% had Center for Disease Control and Prevention (CDC) Stage C disease. Baseline median CD4+ cell count and plasma HIV-1 RNA were 128/µL and 5.2 log₁₀ copies/mL, respectively. At treatment initiation, 41% received therapy free of charge, 42% started a PI-based regimen, and 79% were started on cotrimoxazole prophylaxis. At 60 months, the median CD4+ cell count increased by 300/µL, the median plasma HIV-1 RNA level decreased by 3 log₁₀ copies/mL, and 60% had HIV-1 RNA below the limits of detection. During the follow-up period, 93 patients died, a majority (n = 47) within the first year of antiretroviral initiation. The overall rate of death was 6.2 per 100 person-years (95% CI, 5.0–7.6). The death rate decreased over time with the cumulative probability of dying being 11.7% (95% CI; 8.5–15.3%) during the first year and 25.7% (95% CI; 21.1–31.0%) in year 6. Adjusting for baseline characteristics, independent predictors of survival were BMI at or above 19 kg/m² (HR for death, 0.54; 95% CI, 0.35–0.82); hemoglobin level at or above 10 g/dL (HR, 0.56; 95% CI, 0.37–0.85); and CD4+ count of 200 cells/µL or higher (HR, 0.43; 95% CI, 0.24–0.77). Age, sex, PI-containing regimen, financial participation, cotrimoxazole prophylaxis, and HCV or hepatitis B virus (HBV) carrier status were not statistically significant predictors of survival. Cause of death was ascertained in 76 instances through hospital records or postmortem interviews. Mycobacterial infections (n = 17), neurologic disorders (n = 16), and septicemia (n = 10) were the most frequent causes of death.

Sinkala and colleagues (Oral Abstract 64) presented 1-year clinical and immunologic outcomes from a rapid scale-up of antiretroviral treatment programs in 18 public and private clinical sites across 3 provinces in Zambia. This government-sponsored treatment program, which provides antiretrovirals at no cost, started in May of 2004 and as of December 2005, 36,566 HIV-seropositive adults and children had been enrolled in the program and 22,121 patients has been started on antiretroviral therapy. A cohort of 18,075 adults enrolled from April 2004 to August 2005 of whom 11,074 (61%) started antiretroviral therapy. Among individuals who initiated antiretroviral therapy, 61% were women, and the median age and mean CD4+ count were 35 years and 131 cells/µL, respectively. Ten percent had hemoglobin below 8.0 g/dL and 73% were at World Health Organization (WHO) stage III or IV. Forty-seven percent started zidovudine/lamivudine/nevirapine, 45% stavudine/lamivudine/nevirapine, 4% zidovudine/lamivudine/efavirenz, and 4% stavudine/lamivudine/efavirenz. Over 81,148 patient-months, 1269 patients died (crude death rate 0.016 deaths per patient-month). Forty-three percent of deaths occurred in patients with entry CD4+ counts at or below 50 cells/µL and 53% of deaths occurred within 60 days of enrollment. Adjusted for baseline characteristics, risk of death was associated with CD4+ count between 50 and 200 cells/µL (HR, 1.5; 95% CI, 1.1–2.0); CD4+ count at or below 50 cells/µL (HR, 2.1; 95% CI, 1.5–3.0); WHO stage III (HR, 2.0; 95% CI, 1.4–2.7); WHO stage IV (HR, 3.5; 95% CI, 2.3–4.9); BMI below 16 kg/m² (HR, 2.3; 95% CI, 1.8–3.0); and hemoglobin below 8.0 g/dL (HR, 3.1; 95% CI, 2.4–4.0); and nonadherence (90th percentile; HR, 3.1; 95% CI, 2.1–4.5). CD4+ cell count at 6 months was available for 11,854 individuals, 8284 of whom had started antiretroviral therapy. Individuals on antiretroviral therapy had a greater mean increase in CD4+ count at 6 months (61 versus 5 cells/µL; $P < .0001$) and at 12 months (85 versus ~23 cells/µL; $P < .0001$) than those not on therapy.

Semitala and colleagues with the Academic Alliance for AIDS Care and Prevention in Africa (Abstract 555) presented the 6-month results of a treat-
### Table 3. Selected Studies from Resource-Limited Settings

<table>
<thead>
<tr>
<th>Abstract Name (Abstract No.)</th>
<th>Country, Treatment Program Type, Years of Enrollment</th>
<th>Baseline Treatment Regimen (No. of Patients)</th>
<th>Baseline Age (years), Sex, Clinical Stage, Treatment Experience</th>
<th>Baseline CD4+ Count (cells/µL), Plasma HIV RNA (log_{10} copies/mL)</th>
<th>CD4+ Count (cells/µL) Response</th>
<th>Plasma HIV RNA (log_{10} copies/mL) Response</th>
<th>Mortality</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mortality and Causes of Death in Adults Receiving HAART in Senegal: A 7-Year Cohort Study (Abstract 63)</strong></td>
<td>Senegal Government-sponsored with ANRS</td>
<td>First-line nRTI plus an NNRTI or PI 42% PI-based at initiation (n=404)</td>
<td>37 (median)</td>
<td>128 (median)</td>
<td>At month 60:</td>
<td>At month 60:</td>
<td>93 documented deaths, 51% in first year of antiretroviral therapy</td>
<td>Leading causes of death: mycobacterial infections, neurologic conditions, or septicemia or other infectious disease.</td>
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<tr>
<td>Median follow-up of 46 months</td>
<td>41% of subjects received antiretrovirals at no cost at enrollment (100% of participants received antiretrovirals at no cost by 2003)</td>
<td></td>
<td></td>
<td>55% female</td>
<td>5.2 (median)</td>
<td>+300 (median)</td>
<td>60% with &lt;400</td>
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<td>August 1998 to April 2002</td>
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<td>55% CDC class C</td>
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<tr>
<td><strong>Rapid Scale-up of Antiretroviral Services in Zambia: 1-year Clinical and Immunologic Outcomes (Abstract 64)</strong></td>
<td>Zambia Government-sponsored</td>
<td>Zidovudine/lamivudine/nevirapine (47%)</td>
<td>35 (median)</td>
<td>131 (mean)</td>
<td>At 6 months:</td>
<td>At 6 months:</td>
<td>1269 documented deaths, 53% within 60 days, 43% CD4+ count &lt;50 cells/µL</td>
<td>Risk of death associated with CD4+ count &lt;200 cells/µL, WHO stage III or IV, hemoglobin &lt;8.0 g/dL, and nonadherence (90th percentile) at baseline.</td>
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<tr>
<td>Median follow-up was 165 days (range: 1 to 562 days)</td>
<td>100% of cohort received antiretrovirals at no cost</td>
<td>Stavudine/lamivudine/nevirapine (45%)</td>
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<td>61% female</td>
<td>HIV RNA not available</td>
<td>+61 (mean); at 12 months: +85 (mean)</td>
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<td></td>
<td>April 2004 to August 2005</td>
<td>Antiretroviral therapy experience not reported</td>
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<td>73% WHO stage III or IV</td>
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<td>Zidovudine/lamivudine/efavirenz (4%)</td>
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<td>At 12 months: +85 (mean) (n=8284)</td>
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<td>Stavudine/lamivudine/efavirenz (4%; n=11,074)</td>
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### Table 3. Selected Studies from Resource-Limited Settings (continued)

<table>
<thead>
<tr>
<th>Abstract Name (Abstract No.)</th>
<th>Country, Treatment Program Type, Years of Enrollment</th>
<th>Baseline Treatment Regimen (No. of Patients)</th>
<th>Baseline Age (years), Sex, Clinical Stage, Treatment Experience</th>
<th>Baseline CD4+ Count (cells/µL), Plasma HIV RNA (log_{10} copies/mL)</th>
<th>CD4+ Count Response</th>
<th>Plasma HIV RNA (log_{10} copies/mL) Response</th>
<th>Mortality</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Early Success of Antiretroviral Therapy in a Sub-Saharan African Cohort (Abstract 555) Follow-up of 6 months</td>
<td>Uganda</td>
<td>Infectious Diseases Institute of Makerere University</td>
<td>100% of cohort received antiretrovirals at no cost</td>
<td>Stavudine/ lamivudine/ nevirapine (fixed-dose combination)</td>
<td>37 (mean) 100 (median) 5.8 (median; n=448)</td>
<td>85% &lt;400 (n=448, not ITT)</td>
<td>58 documented deaths</td>
<td>No losses to follow-up or transfers.</td>
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<td>Uganda</td>
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<td>69% female 90% WHO stage III or IV</td>
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<td>No statistically significant differences in baseline characteristics of HIV RNA level &lt;400 copies/mL and ≥400 copies/mL.</td>
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<td>100% antiretroviral therapy-naive</td>
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<td>Baseline Karnofsky score, CD4+ cell count, and hemoglobin were predictors of survival.</td>
</tr>
<tr>
<td>Implementation of an Antiretroviral Therapy Access Program for HIV-Infected Individuals in Resource-limited Settings: Clinical Trial Results from 4 African Countries (Abstract 558) Follow-up of 24 months</td>
<td>Senegal, Côte d’Ivoire, Uganda, Kenya</td>
<td>Supported by PharmAccess International and industry partner</td>
<td>100% of subjects received antiretrovirals at no cost</td>
<td>Saquinavir/ ritonavir (fixed-dose combination) plus lamivudine plus zidovudine</td>
<td>206 subjects enrolled; 192 received treatment</td>
<td>36 (median) 62% female 78% CDC class B or C (n=206)</td>
<td>+198 (median)</td>
<td>16 deaths (7.8%) 6.3% lost to follow-up</td>
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<td>192 received treatment</td>
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<td>5.5 (median)</td>
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<td>5.3% withdrew (patient decision or inability to pick up medication).</td>
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<td>Exclusion criteria: investigator’s opinion that subject unlikely to complete cohort or adhere to medications, severe HIV-related illness or opportunistic infection at time of enrollment, and hemoglobin &lt;8 g/dL.</td>
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</table>

ANRS indicates Agence nationale de recherches sur le Sida; BMI, body mass index; CDC, Centers for Disease Control and Prevention; HAART, highly active antiretroviral therapy; ITT, intent-to-treat; nRTI, nucleoside (or nucleotide) analogue reverse transcriptase inhibitor; NNRTI, non-nucleoside analogue reverse transcriptase inhibitor; OT, on-treatment; PI, protease inhibitor; WHO, World Health Organization.
ment program in Uganda where antiretroviral therapy is available at no cost to patients. Between January 2004 and June 2005, 647 HIV-infected, antiretroviral therapy-naive adults were consecutively enrolled into an observational cohort at the Infectious Diseases Institute at Makerere University, Kampala, Uganda. Antiretroviral therapy was initiated shortly after enrollment in 582 patients and deferred in the rest. At baseline, 90% of individuals met the criteria for WHO clinical stage III or IV of disease. The initial regimen was stavudine/lamivudine/nevirapine (fixed-dose combination), zidovudine/lamivudine (fixed-dose combination)/efavirenz, or stavudine/lamivudine/efavirenz. By 6 months, 58 had died, 378 had plasma HIV-1 RNA levels at or below 400 copies/mL, 68 had plasma HIV-1 RNA levels above 400 copies/mL, and 78 were confirmed to be alive but had not completed the 6-month visit. There were no losses to follow-up and no transfers. Adjusting for baseline characteristics, predictors of plasma HIV-1 RNA level at or below 400 copies/mL were female sex (OR, 1.9; 95% CI, 1.10–3.30), Karnofsky score (OR, 0.97; 95% CI, 0.94–0.99), and stavudine dose (40 mg versus 30 mg: OR, 0.44; 95% CI, 0.22–0.88). Karnofsky score (OR for death, 0.97; 95% CI, 0.91–0.97), baseline CD4+ cell count (OR, 0.99; 95% CI, 0.99–0.999), and baseline hemoglobin (OR, 0.81; 95% CI, 0.68–0.95) were predictors of survival.

Results from an open-label treatment program conducted in 4 urban clinics in Senegal (Dakar), Côte d’Ivoire (Abidjan), Uganda (Kampala), and Kenya (Nairobi) were presented by Sow and colleagues (Abstract 558). Antiretrovirals were provided at no cost to patients and after participation in the study, patients were enrolled in government-sponsored antiretroviral provision programs; 206 individuals initiated therapy in 2002. Baseline characteristics were as follows: 78% CDC clinical category B or C, median CD4+ count 119 cells/µL, and median plasma HIV-1 RNA level 5.5 log_{10} copies/mL. At week 96, according to the ITT analysis, 52% of patients had plasma HIV-1 RNA levels below 400 copies/mL. Rates of viral load suppression varied by site: 40% in Nairobi, 46% in Dakar, 54% in Abidjan, and 69% in Kampala. The median increase in CD4+ count from baseline was 198 cells/µL (range 191 to 292 cells/µL) and did not differ significantly among sites. Of the 206 individuals who initiated therapy, 16 died (8%), 13 were lost to follow-up (6%), 11 discontinued due to patient decision, inability to pick up medications, or for unknown reasons (5%), and 166 (81%) are continuing treatment. Non-HIV-related serious adverse events were reported in 55 patients (27%); anemia and neutropenia were the most frequently reported (13 and 7 patients respectively). Thirty-five patients (17%) changed treatment due to toxicities.

Dillingham and colleagues (Abstract 556) conducted a retrospective analysis of 622 patients from Haiti who initiated antiretroviral therapy at the Groupe Haitien d’Etude du Sarcome de Kaposi et des Infections Opportunistes (GHESKIO) Centers between March 2003 and June 2005. Baseline characteristics were mean weight for women was 112.5 lb, mean weight for men was 126.1 lb, mean CD4+ cell count 129/µL, and mean hemoglobin 10.5 g/dL. During follow-up, 69 (11%) patients died, 51 (74%) within the first 6 months. One-year survival was 86%. In a univariate analysis, the presence of diarrhea at initiation of antiretroviral therapy, the presence of wasting (weight <25th percentile for sex), hemoglobin below 9.5 g/dL, and CD4+ cell count correlated with 1-year mortality. Sex, age, and tuberculosis at presentation were not significant predictors of survival. Adjusting for baseline characteristics, wasting (OR, 2.4; 95% CI, 1.4–3.9; P = .001) and hemoglobin below 9.5 g/dL (OR, 2.1; 95% CI, 1.3–3.5; P = .003) were the only independent risk factors for death in the first year. The authors concluded that intensive nutritional rehabilitation, micronutrient supplementation, and treatment of anemia could improve survival in resource-limited settings. It is also possible that low hemoglobin and presence of wasting are indicators of an undiagnosed underlying condition such as an opportunistic infection. The cause of death in this cohort was not assessed.

**Adherence**

Ensuring adherence to medications and clinic visits is essential to any successful antiretroviral treatment program. Several studies evaluated rates and predictors of adherence and tools to measure adherence in various resource-limited settings.

Marazzi and colleagues (Abstract 551) presented adherence results from the DREAM program in Matola, Mozambique. The DREAM program began in January 2002 and addresses specific psychosocial factors that interfere with adherence through the use of interventions such as health education and counseling for patients and staff, free antiretroviral treatment and laboratory tests (including CD4+ cell counts and viral load), nutritional support, and involvement of family and peer health educators in patient care. Therapy was initiated in 569 adult patients. At baseline, 3.7%, 19.5%, 35.1%, and 41.7% had been on antiretroviral therapy for more than 3 years, 2 to 3 years, 1 to 2 years, and 0 to 1 year, respectively. During the follow-up period, 28 patients (4.9%) died, 34 (6.0%) transferred to other clinics, and 20 (5.5%) abandoned the program, leaving 487 patients (85.6%) on treatment. Of those remaining on treatment, the last viral load was below 400 copies/mL in 359 (73.7%). Of the 128 with plasma HIV-1 RNA levels above 400 copies/mL, 100 (78.1%) kept 96.2% of their appointments (including visits for medical checkups, medication pickup, and laboratory tests) and were considered to have unsuppressed viral load due to problems unrelated to adherence. The other 28 with a detectable viral load (21.9%) kept fewer than 90% of their appointments, and were considered to be unsuppressed due to nonadherence. Using the definition of adherence as keeping more than 90% of all appointments (visits for medical checkups, medication pickup, and laboratory...
tests) overall, 90.5% of patients were adherent and 9.5% nonadherent. This study illustrates that with a multidisciplinary approach, high levels of adherence, and minimal loss to follow-up can be maintained in resource-limited settings.

Ramadhani and colleagues (Abstract 553) evaluated predictors of nonadherence among patients at the Kilimanjaro Christian Medical Center Adult HIV Clinic in northern Tanzania. One hundred fifty consecutive adult patients on stavudine/lamivudine/nevirapine for 6 months or longer enrolled in the study. A structured questionnaire regarding adherence to antiretroviral therapy, sociodemographics, economic conditions, knowledge, beliefs, disclosure relating to HIV and antiretroviral therapy, access to care, and mental health was administered. Of the 150 patients, 16% were nonadherent (defined as self-reported adherence of less than 100%), 57% had no formal education or had primary education only, and 73% had started antiretroviral therapy before it was available for free. Poor adherence was associated with sacrificing healthcare to pay for food, education, or housing; self-pay for antiretroviral therapy; having to walk more than 19 minutes to the clinic; and paying more than US$5.30 per month for medicines. Free antiretroviral therapy and discussion of antiretroviral therapy side effects with healthcare worker at initiation of treatment was protective against poor adherence. Adjusting for baseline characteristics, sacrificing healthcare to pay for food, education, or housing remained a risk factor for nonadherence (OR, 59.9; 95% CI, 7.6–46.2) as did needing to walk more than 19 minutes to the clinic (OR, 7.3; 95% CI, 1.9–27.7). Discussion of antiretroviral therapy side effects with a healthcare worker at initiation of treatment (OR, 0.15; 95% CI, 0.04–0.60) and disclosure of HIV status to persons other than healthcare workers (OR, 0.10; 95% CI, 0.02–0.06) protected against nonadherence.

Muhindo and colleagues (Abstract 557) conducted a retrospective chart review of the first 500 antiretroviral therapy-naive patients initiating a self-pay, fixed-dose combination stavudine/lamivudine/nevirapine. At baseline, 16% had a prior history of tuberculosis; mean weight was 57 kg and mean CD4+ cell count (available for 81% of patients) was 98×10^3/µL. The median follow-up time was 21.5 months and the median treatment time was 18 months. At 12 months, 63% were on the initial regimen, 3.8% changed regimens, 50% had documented discontinuation of regimen, 20.8% were lost to follow-up, and 6.4% had documented death. During the entire follow-up period, 6.2% (n=31) had discontinued their regimen, 45% due to treatment of tuberculosis and 42% due to cost. Predictors of treatment discontinuation (adjusted for baseline characteristics) were baseline weight (HR, 0.98 per kg; 95% CI, 0.95–1.0), chronic diarrhea (HR, 2.80; 95% CI, 1.32–5.91) and having children (HR, 0.48; 95% CI, 0.24–0.99). This study highlights the fact that self-pay for antiretroviral therapy plays an important role in the ability to maintain antiretroviral therapy and follow-up for care.

Prior studies have shown that pharmacy data are a simple and useful way to evaluate adherence (Grossberg et al, J Clin Epidemiol, 2004), which has been associated with survival (Nachega et al, CROI, 2005; Garcia de Olalla et al, J Acquir Immune Defic Syndr, 2002). Nachega and colleagues (Abstract 62) presented a study evaluating the correlation between pharmacy refill data and plasma HIV-1 RNA level below 400 copies/mL. Among 3325 South African antiretroviral therapy-naive individuals who had initiated NNRTI-based regimens, the median CD4+ count was 151 cells/µL and the median plasma HIV-1 RNA level was 5.1 log_{10} copies/µL. The median follow-up time was 2.4 years. Adherence to antiretroviral therapy was calculated as a percentage: number of months patients submitted claims divided by number of months since antiretroviral therapy initiation. A significant dose-response relationship was seen between viral load suppression and adherence across strata with viral suppression achieved in 22% of patients with less than 70% adherence, 56% with 70% to 79% adherence, 69% with 80% to 89% adherence, 76% with 90% to 94% adherence, and 80% with 95% or better adherence (P for trend < .001). Adjusting for baseline characteristics, high antiretroviral therapy adherence (≥95% versus <70%; OR, 15.6; 95% CI, 12.4–19.5), high baseline CD4+ cell count (>200×10^3/µL versus ≤50×10^3/µL: OR, 1.4; 95% CI, 1.1–1.7) and low baseline viral load (<10^5 log_{10} copies/mL versus >10^5 log_{10} copies/mL: OR, 1.3; 95% CI, 1.1–1.5) were significantly associated with viral suppression. The authors concluded that pharmacy records are a simple and valid tool to monitor adherence at the program level.

**Evaluating Response to Therapy in Resource-Limited Settings**

Plasma HIV-1 RNA levels are crucial to accurately assess response to antiretroviral therapy but are much more expensive and therefore even more difficult to obtain than CD4+ cell counts in resource-limited settings. More cost-effective and accessible viral load methodologies are under development. Several presentations evaluated the utility of changes in CD4+ cell counts to approximate changes in viral load and responses to antiretroviral treatment.

Schechter and colleagues from the Antiretroviral Treatment in Lower-Income Countries (ART-LINC) Collaboration (Abstract 559) analyzed discordant immunologic and virologic responses to antiretroviral therapy among treatment-naive adults initiating antiretroviral therapy in resource-constrained settings. The ART-LINC Collaboration is a multinational network of HIV treatment programs in Africa, Brazil, and Asia. Eligibility criteria were previously being treatment naive, being older than 16 years, initiating treatment with 3 or more antiretroviral drugs, and availability of CD4+ cell counts and HIV-1 RNA results at 6 months (±1.5 months). In total, 1916 patients from 15 centers were eligible. Complete responders
were defined as individuals with an increase in CD4+ count of 50 or more cells/µL and a plasma HIV-1 RNA level below 500 copies/mL at 6 months. Discordant responders had increases in CD4+ counts of 50 or more cells/µL but plasma HIV-1 RNA levels above 500 copies/mL (CD4+ /RNA-) or plasma HIV-1 RNA levels below 500 copies/mL but an increase in CD4+ count of 50 or less cells/µL (RNA +/CD4-). One thousand ninety-four individuals (57%) were complete responders, 365 (19%) were CD4+/RNA- discordant, 269 (14%) were RNA+/CD4- discordant, and 188 (10%) had no CD4+ cell count or viral load response and were excluded from the analysis. Older patients and those with higher baseline CD4+ cell counts were more likely to be CD4+/RNA- discordant. Younger patients, those with lower baseline CD4+ cell counts or higher baseline plasma HIV-1 RNA levels, and those who initiated a regimen that was not NNRTI-based were more likely to be RNA+/CD4- discordant.

Bisson and colleagues (Abstract 548) evaluated 384 treatment-naive patients at 2 clinics in Gaborone, Botswana to determine the accuracy of CD4+ cell count response in predicting plasma HIV-1 RNA levels at or below 400 copies/mL 6 months after initiation of antiretroviral therapy. Sixty-two percent were women and baseline median CD4+ count and plasma HIV-1 RNA level were 101 cells/µL and 5.5 log_{10} copies/mL, respectively. Eighty-three percent had HIV-1 RNA below the limits of detection at the 400 - copies/mL level at follow-up. Median increase in CD4+ cell count in those who became undetectable was 128/µL compared with 49/µL in those who were not undetectable (P < .0001). The discriminative ability of increases in CD4+ cell count from baseline to predict undetectable viral load at 6 months was determined by calculating the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each cutoff point and further evaluated using receiver-operator characteristic (ROC) curves. A CD4+ count increase of 50 or more cells/µL from baseline in individuals with a baseline CD4+ count above 100 cells/µL (n = 194) resulted in sensitivity, specificity, PPV, and NPV that predicted undetectable viral load of 75.1%, 41.2%, 85.4%, and 24.6%, respectively. These performance characteristics improved in individuals with a baseline CD4+ count of 100 or fewer cells/µL (n = 190) with sensitivity, specificity, PPV, and NPV of 93.1%, 91.3%, 92.5%, and 63.3%, respectively. In the cohort of individuals with a baseline CD4+ count of 100 or fewer cells/µL, restricting viral load tests to individuals with an increase in CD4+ count of fewer than 50 cells/µL would lead to a decrease in confirmatory testing of 93% of individuals with undetectable viral loads and would detect 61% of failures. If the cutoff in this same cohort were changed to an increase in CD4+ count of 150 cells/µL, confirmatory HIV-1 RNA would be avoided in 48% of individuals with a detection of 90% of failures. The authors concluded that using changes in CD4+ cell counts to prioritize the use of viral load tests in resource-limited settings should be considered. Ultimately, having universal access to cost-effective viral load testing should be the goal for resource-limited settings.

Moore and colleagues (Abstract 547) performed similar analyses in 1125 antiretroviral therapy-naive individuals in British Columbia. The median baseline CD4+ count was 90 cells/µL. Suppressed viral load was defined as plasma HIV-1 RNA at or below 500 copies/mL at 2 time points within the first year of primary initiation of antiretroviral therapy. At 6 months, median CD4+ count was 180 cells/µL reflecting a median increase of 100 cells/µL. Sixty percent (n = 674) had suppressed plasma HIV-1 RNA levels. Median time to viral load suppression was 2.4 months. Using criteria of no increase in CD4+ cell count by 6 months, sensitivity, specificity, PPV, and NPV to predict suppressed viral load were 54%, 94%, 75%, and 71%, respectively. Changing the threshold to no increase in CD4+ cell count by 12 months slightly improved these performance characteristics to 35%, 95%, 79%, and 73%, respectively. Using no increase in CD4+ cell count from baseline at 6 and 12 months, 21% and 25%, respectively would be incorrectly classified as failing treatment and only 34% to 35% of true treatment failures would be identified through these criteria.

Each of these studies highlights the significant discordance between CD4+ cell count and viral load responses to treatment and the effect that various patient characteristics have on the degree and direction of the discordance. In resource-constrained settings, there may be a role for CD4+ cell count in predicting virologic failure; however, more studies with larger cohorts are needed to further evaluate this relationship.

**Conclusions**

This year’s conference proved itself to be the premier meeting of the year with respect to updating the field on the state-of-the-art of antiretroviral therapy. The new antiretroviral agent pipeline remains robust and promising; the results of treatment interruption trials have immediate implications for clinical practice, and the data presented from resource-limited settings are encouraging as reflections of the reality of antiretroviral rollout programs and the importance of reporting clinical and translational research results from the areas of the world that carry more than 90% of the global HIV-1 disease burden. As we mark the 25th anniversary of the AIDS epidemic and the 10th anniversary of the potent antiretroviral therapy era, the opening of a new era of life-saving therapy in the developing world is welcome and overdue.

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A list of all cited abstracts appears on pages 63 to 70.

ADDITIONAL REFERENCES


Williams CF, Klinzman D, Yamashita T, et al.
Conference Abstracts Cited in This Issue

The full text of all abstracts is available online at www.retroconference.org.

8. HIV and Malaria—When Elephants Collide. Laurence Slutsker and B. Marston.


32LB. Prevention of Rectal SHIV Transmission in Macaques by Tenofovir/FTC Combination. J Garcia-Prevention of Rectal SHIV Transmission in Macaques by Tenofovir/FTC Combination. J Garcia-Patrick.


46. 1-(β-D-Dioxolane) Thymine is Effective Against HIV-1-containing TAM and M184V. Johan Bergen, G. Bloomling, M. Ruskull, M. Bennett, C. Chu, and R. Shinazi.


54. Antiretroviral Therapy to Prevent Sexual Transmission of HIV. Myron Cohen.

55. Beyond Condoms: Chemical and Physical Barriers to Protect Women from HIV. Sharon Hillier.


73. Leptin and Neuropsychological Performance: A New Biomarker for Cognitive Function? Jeannie Huang, S. Letendre, J. Beck, M. Chern, S. Kolakowski, R. Ellis, and HIV Neurobehavioral Res...
B Alston-Smith, M Basar, S Grinspoon, and the ACTG Protocol 5082 Team.


150. Randomized, Placebo-Controlled Trial of Valganciclovir to Prevent CMV End-organ Disease in Patients with HIV-1 Infection: Results from the ACTG Protocol 5082 Team. Thomas Duensing, M Fung, S Lewis, M Jacobson.


152. Molecular Mechanism of Tenofovir, Abacavir, and Lamivudine Resistance by the K70E Mutation in HIV-1 Reverse Transcriptase. Nicolas Sluis-Cremer, P Argoti Torres, J Grzybowski, U Panik, and J Mellors.


158LB. In vitro Characterization of HIV Isolated from Patients Treated with the Entry Inhibitor TNX-355. Thomas Duensing, M Fung, S Lewis, and S Weinheimer.

159LB. Potent Antiretroviral Effect of MK-0518, a Novel HIV-1 Integrase Inhibitor, in Patients with Triple-class Resistant Virus. Beatriz Grinsztejn, BY Nguyen, C Katlama, J Gatell, A Lazzarin, D Vittecoq, C Gonzalez, J Chen, R Isaacs, and the Protocol 005 Study Team.


162. Quantifying the Impact of Primary Infection on HIV Transmission and Control. Christophe Fraser, TD Hollingsworth, R Chapman, and RM Anderson.


175. HCV Polymerase Inhibitors: Multiple Shots on Goal? Daria Hazuda.


332. Role of CCR5 in CNS HIV-1 Infection. Lokesh Agrawal and D Strayer.


346. The Effects of Antiretroviral Therapy Use on Cognitive Function in Older HIV+ Adults, the Hawaii Aging with HIV Cohort. Victor Valcour, A Williams, M Watters, N Sacktor, O Selnes, B Shiramizu, R Paul, and C Shikuma.


355. Central Nervous System Immune Activation is Still Present after More than 4 Years of Effective HAART. Arold Eden, R Price, S Spudich, D Puchs, L Hagberg, and M Gisslen.

359. NNRTI more than Boosted PI Exposure Enhances Virological Control in Cerebrospinal Fluid of HIV-1 Infected Patients with Neurological Disorders. Maria Letizia Giancola, P Lorenzini, A Cingolani, D Larussa, S Bossolasco, M Bongiovanni, L Monno, A’Arminio Monforte, P Pinque, A Antinori, and Italian Registry Investigative Neuro AIDS Study Group.


551. Levels of Adherence to HAART in the DREAM Program in Mozambique. M Marazini, M Magnano San Lio, P Germano, S Mancinelli, G Liotta, G Guidotti, P Scarrcella, G Tintisona, P Narciso, and Leonardo Palombi.


556. Predictors of Early Mortality in Haitian Patients Treated with Antiretroviral Therapy in a Community Setting Rebecca Dillingham, R Pinkerton, P Leger, P Severe, J Pape, and D Fitzgerald.


566. HIV-1 Integrase Inhibitors Are Potent Substrates for the MDR1 Multidrug Transporter. Maria Luisa Dupuis, M Cianfroglia, R Costi, C Galuzzi, M Andreotti, A Roux, R Di Santo, and L Palmisano.


575b. Pharmacokinetics and Safety of Adding TMC125 to Stable Regimens of Saquinavir, Ritonavir, and NRTI in HIV+ Adults. Marianne Harris, C Zala, S Ramirez, B Woodfall, M Peeters, M Scholler, B Hoetelmans, and J Montaner.

575c. Pharmacokinetics and Antiretroviral Response to TMC141/4 and TMC125 Combination in Patients with High Level Viral Resistance. Marta Boffito, A Winston, C Fletcher, A Pozniak, M Nelson, G Moyle, I Tolowinska, R Hoetelmans, D Marrales, and B Gazzard.


582. Pharmacokinetics of Vicriviroc Are Not Affected in Combination with Five Different Protease Inhibitors Boosted by Ritonavir. Angela Sansone, A Keung, E Tetedeh, H Weisbrot, M Martinho, S Lang, R Keller, and M Kraan.


586. Pharmacokinetics of Saquinavir with Low-dose Ritonavir or Azatavir Twice Daily in Seronegative Volunteers: ASPIRE II. Jennifer King, S Paul Lundy, T Kakuda, S Becker, and A Acosta.


602. In a Paired-sample Comparison, the Presence of Thymidine Analogue Mutations, but not Other Common Nucleoside Reverse Transcriptase Inhibitor Mutations Has a Differential Effect on Entrimetabon and Lamivudine Susceptibility. Lisa Ross, N Parlin, M Underwood, P Gerondelis, M S Cier, and R Lanier.


620. Effect of HIV-1 Subtype on Genotypic
Resistance to Protease Inhibitors in the United Kingdom. Philippa Easterbrook and T Welz on behalf of the UK Collaborative Group on HIV Drug Resistance.


624. Replicative Capacity Effect of Mutations that Are Associated with Elavirience Hypersusceptibility. Nancy Shulman, J Mellors, S Clark, R Bosch, and N Parkin.


627. Mutation Interchange Modulate the Fitness Cost of Drug Resistance Mutations in Transmitted Isolates: Implications for Persistence of Transmitted Resistance. ME Cong, D Bennett, W Heneine, and J Gerardo Garcia-Lerma.


637. Protease Mutations Associated with Higher or Lower than Expected Tipranavir Susceptibility Based on the TPV Mutation Score. Neil Parkin and C Chappey.


742. Increased Carotid Intima-media Thickness in HIV-infected Patients Is Associated with Increased C-reactive Protein Levels and Increased Cytomegalovirus Specific T Cell Activation. Priscilla Hseu, P Hunt, D Waters, E Sinclair, J Martin, A Franklin, R Hoh, and S Deeks.


745. Long-term Safety Profile of Bosantan in Patients with Pulmonary Arterial Hypertension Associated with HIV. Results from the Tracleer PMS Database. M Humberd, M Hoepa, D Kiey, Eleanor Segal, and J Carlsen.


755. Peripheral and Visceral Fat Changes following a Treatment Switch to a Nonthymidine Analogue or a Nucleoside-sparing Regimen in Patients with Peripheral Lipopothy. 48-week Final Results of ACTG A5110. A prospective, Randomized, Multicenter Clinical Trial. Robert Murphy, J Zhang, R Haffer, K Yasashesi, K Tashima, B Berzins, S Owens, A Shevitz, S Evans, P Tebas, and ACTG A5110 Team.

759. A Prospective Study of Abnormal Glucose Tolerance among Older Adults with or at-risk for HIV. Andrea Howard, R Klein, M Flores-Moore, J Amstren, and E Schoenbaum.


777. Renal Impairment Associated with the Use of Tenofovir. James Heffelfinger, D Hanson, A Voetsch, A McNaghten, and P Sullivan.


788. Identification of a Multidrug Resistant Tuberculosis Cluster as a Cause of Death among HIV-infected Patients in Rural South Africa. A Moll, N Giviri, H Matsi, F Van der Elst, C Visser, and J Wieringa.


904. Guidelines for Post-exposure Prophylaxis for HIV in Developing Countries. Marco Vitoria, E Beck, S Mandalia, M Diepart, TT Sint, S Crowley, C Gilks, and Y Souteyrand.

906. HIV Post-exposure Prophylaxis following Sexual Exposure is Started Too Late for Optimal Benefit. Amy Kindrick, H Tang, C Sterkenberg, C Gruta, P Saberi, M Mahoney, J Cocohoba, D Alexander, J So, and R Goldschmidt.


913. Has the Role of Primary Infection in the Epidemiology of HIV been Overstated? Deirdre Hollingsworth, R Anderson, and C Fraser.


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