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Topics in HIV Medicine®

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The International AIDS Society–USA

About This Issue

This issue provides our annual review of the Conference on Retroviruses and Opportunistic Infections, held this year from February 10 to 14 in Boston, Massachusetts. In the first of 4 articles summarizing research presented at the conference, Mario Stevenson, PhD, reviews developments in HIV basic science research. Amitinder Kaur, MD, and R. Paul Johnson, MD, discuss advances in HIV pathogenesis research and vaccine development, while Diane V. Havlir, MD, and Judith S. Currier, MD, examine new findings on metabolic complications of antiretroviral therapy, HIV transmission, and coinfections. Finally, Mary A. Albrecht, MD, Timothy J. Wilkin, MD, Eoin P. G. Coakley, MD, and Scott M. Hammer, MD, highlight findings on antiretroviral therapy, treatment strategies, and drug resistance. This last topic is separately addressed by the International AIDS Society–USA (IAS–USA) Drug Resistance Mutations Group, which in a special contribution presents an updated list of mutations in HIV-1 associated with resistance to antiretroviral drugs.

Beginning this issue is the transcript of a powerful speech delivered at the conference's opening session by Zinhale Tabethe, a member of the Durban, South Africa-based Sinikithemba HIV + Choir. With other organizations, the IAS–USA has established a fund to care for HIV-seropositive members of the choir and an affiliated support group at Durban's McCord Hospital. Further information on these efforts can be found on page 68.

The Retrovirus Conference review articles are also available on the IAS–USA Web site at http://www.iasusa.org/pub/index.html#retrovirus.

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Opening Session: Welcome from the Sinikithemba Choir

At the opening session of the 10th Conference on Retroviruses and Opportunistic Infections, Zinhale Tabethe, a member of the Durban, South Africa–based Sinikithemba HIV+ Choir, spoke about living with HIV and her experiences with antiretroviral therapy. Ms Tabethe was asked to speak at the last minute, after travel difficulties prevented choir director and scheduled speaker Nonhlanhla Mhlongo from attending. A transcript of Ms Tabethe's powerful speech is reprinted below. A Webcast of the speech, which begins with a performance by the choir, is also available at http://www.retroconference.org/2003/webcast.htm.

The Sinikithemba Choir evolved from a support group for HIV-infected patients at Durban's McCord Hospital. To help meet the costs of their care, group members fashioned bead jewelry for sale, singing while they worked. Despite early reservations about disclosing their HIV status, members began to sing in public, and the Sinikithemba Choir now performs to raise awareness of the HIV/AIDS pandemic. The choir also sells beadwork made by support group members, with proceeds going toward medical care and counseling for members of the choir and the McCord Hospital support group.

According to clinicians at Boston's Partners AIDS Research Center (PARC), which works with McCord Hospital, many choir and support group members need immediate antiretroviral therapy. At an estimated annual cost of US $1500 per patient, however, the drugs are currently inaccessible for many. The South African government has recently indicated it may expand the availability of antiretroviral drugs, but this move, while welcome, also carries costs: McCord Hospital officials expect they will need funds to meet an increase in patients seeking support services. Upgrading the hospital's treatment and counseling services will be fundamental to helping patients stay committed to antiretroviral drug therapy.

The choir had hoped to use its trip to the United States as an opportunity to raise money for these needs through sales of its beadwork. With support from PARC and the Doris Duke Charitable Foundation, the choir had also produced a compact disc (CD) of its work, intending to sell the collection of traditional Zulu, gospel, and original a cappella pieces at the Retrovirus conference. But the group chose instead to give a free CD to each attendee—3800 in all—as thanks to those whose efforts have made care and treatment possible. The choir missed another chance to sell its work when a scheduled post-conference performance at New York's St. John the Divine Cathedral was cancelled due to a major snowstorm. As a result, group members generated little income from the trip.

In recent months, a growing number of Topics in HIV Medicine *readers and attendees at International AIDS Society–USA (IAS–USA) symposia have asked how they can help fight HIV/AIDS in the developing world. Assisting the Sinikithemba Choir and the McCord Hospital support group provides one opportunity to do so. PARC and the IAS–USA, among others moved by the choir's appearance at the conference, have established trusts to help care for choir and support group members. Donations to the PARC and IAS–USA funds are tax-deductible and go directly and completely to patients. Contributions may be sent to the address below. Please also include contact information, as support group members hope to individually thank those who help. In addition, the IAS–USA is offering the choir's CDs for sale for US $20 each; again, 100% of proceeds go directly to patient care. Donations can also be made and CDs purchased by contacting the IAS–USA at (415) 544-9400.*

Transcript of Welcome Remarks

I want to thank the conference organizers for giving Sinikithemba Choir the opportunity to be here with you tonight and share our voices and music with you. And to the president, Bill Clinton, who has committed himself to fighting against the AIDS pandemic, I'm so honored to share the stage with him.

For those of you who are looking at the programs this evening, you have been expecting to hear from Miss [Nonhlanhla] Mhlongo, who is the director of Sinikithemba HIV+ Choir and its support group for people who are living in Durban, South Africa. I pass my apologies for Miss Mhlongo. Unfortunately, she was unable to be with us this evening. As a member of Sinikithemba Choir, I was asked to stand in her place tonight.

My name is Zinhale. This week as I was preparing for this trip from home in Durban, I became very excited, thinking that I'm going to see your faces—faces of the world's best scientists and experts in HIV research. To look out and see the people who have been on the front lines in the laboratories and clinics and hospitals, figuring out that things like AZT would fight the virus and discovering triple therapy called HAART, that it would give people life much longer. I know you're way past that, and you're busy thinking up new drugs, and you're busy thinking about resistance. You're also busy thinking about the new strategies of fighting with HIV, but I am so very excited about this triple therapy, which is so amazing.

Like the members of the Sinikithemba Choir, I am HIV-positive. I'm just like them. Like them, I am living in a country with one of the highest HIV infection rates in the world. You know the [World Health Organization] statistics, but we ARE those statistics. On Saturdays, we go to funerals for our friends, our neighbors, and our families. And in our support group we have lost 10 members this past year to AIDS. At support groups on Tuesday, when you look around the room and see there is

no face, you become very worried if that person is sick or what's going on. We have watched each other lose weight, have the dark spots of KS appear on our skin, listened to the deep cough of TB, wiped each other's tears after one of us lost our 6-year-old child to AIDS.

Like most of the others in the choir, I have battled with opportunistic infections. I have had TB twice in the last 2 years. Eighteen months ago, I had cryptococcal meningitis. I had PCP, and I have had bacterial pneumonia, and I have had thrush. I lost weight until I no longer looked like the same person. I finally came face to face with the end of my life. I was in bed for 3 months. I was too weak to walk and could only crawl back and forth to the bathroom. It was the end of me, then.

But 10 months ago, I became different than most of the people at Sinikithemba. I got in a pilot study that provides antiretroviral drugs. Now I am not like them. I gained back all the weight that I lost. I have not been seriously sick for the past 6 months. My CD4+ count came up from 160 to 480. I wake up every morning at 6 AM and take my 3TC and d4T, and every night at 6 PM I take the same drugs. Before I go to bed, I take 3 efavirenz tablets. I'm never mad that I have to take those drugs. I know they are allowing me to live and to be able to think about tomorrow. But I am one of the few lucky people in South Africa. I am done asking myself, why me? Why did I have to be infected with HIV? Now I ask myself, why me? And why do I get to live when others next to me are dying without treatment?

Some people say that really poor countries should not get antiretroviral drugs. They say poor people, uneducated people, will not be able to be adherent to antiretroviral drugs. They say they would miss doses and end up with resistant virus that we would then spread. Some people say there are things more urgent that we need to take care of before HIV and AIDS. Things like poverty. Things like malnutrition. And things like high crime, unemployment, violence; diseases like TB, malaria, diarrheal diseases.

I am from a poor family. When I was at my most sick, I was living with my mother, with my 2 sisters, and their 4 children. I had been fired from work, from the job that I was doing, because they found out that I was HIV-infected. So my family was living without any income. Our house had no electricity and had no running water. When there was no money, there was no food. There are no people anywhere who live more basic than what I've just shared with you. But I am adherent to my ARVs. I can tell you that ever since I started medication, I have never missed a dose. Ever.

So what can I, as one of the HIV-positive persons and we, as the Sinikithemba Choir, say to you? Well, we want to say thank you. Thank you very much for the job that you have done so far. And I want to thank you—thank you in advance for the job that you're still going to do. The word *sinikithemba* means "give us hope." You do that with your job. You give us hope. You give us hope that we will, at one day, or some day, have treatment that will save our lives. Thank you.

Donation Information

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Developments in Basic Science Research

Mario Stevenson, PhD

A large percentage of the topics covered at the 10th Conference on Retroviruses and Opportunistic Infections in Boston were concerned with basic research aspects of HIV-1 and AIDS. Topics that generated particular interest included the identification of host factors that restrict retrovirus and lentivirus infection. In addition, a number of presentations concerned the use of RNA interference (RNAi) as a tool for understanding HIV biology, and several presentations discussed approaches to therapeutic exploitation of RNAi in HIV/AIDS.

Host Restriction Factors for Lentivirus Infection

In the past year, there has been significant progress in the identification of cellular mechanisms that impact the susceptibility of the host cell to retrovirus and lentivirus infection. This progress has included the identification of the cellular target for the HIV-1 Vif protein (Sheehy et al, *Nature,* 2002). In a plenary lecture (Abstract 5), Malim presented research on a protein called CEM-15, which is the cellular target for Vif. Research from the laboratories of Kabat and Malim had proposed that primate cells contain an inhibitor that blocks HIV-1 infection and that the Vif protein combats this cellular factor in order to allow primate cells to support viral infection. These initial studies provided the impetus for research aimed at identifying the inhibitory factor. Using a subtractive hybridization approach, Malim and colleagues identified a number of complementary DNA segments (cDNAs) that were unique to cells in which Vif was required for viral infection. After genetic and virologic characterization, one of these cDNAs, CEM-15, exhibited the predicted properties of the cellular inhibitor. For example, CEM-15 was expressed only in cells in which Vif was required for viral infection. In the presence of CEM-15, HIV-1 virions exhibited a normal morphology and composition yet were noninfectious. CEM-15 exhibits homology with a cellular protein called apobec 3G, which belongs to a small family of RNA editing proteins. Since some of these apobec proteins have cytidine deaminase activity, this homology suggests a model in which CEM-15 may edit viral RNA by deaminating cytidines to uridine to the extent that reverse transcription is stalled when genomic viral RNA modified by CEM-15 is used as a substrate.

In an extension of these studies, Landau and colleagues (Abstract 72) have been studying the influence of the murine CEM-15 homologue on viral replication. It was demonstrated that murine CEM-15 can potently inhibit HIV-1 infectivity to a greater extent than can human CEM-15. Although studies with HIV-1 have suggested the Vif defect acts at late steps in viral reverse transcription, in the presence of murine CEM-15, initiation of reverse transcription was blocked. There also appeared to be some differences in the sites of action for human CEM-15 and murine CEM-15. In human cells, HIV-1 Vif must be present in virus-producing cells in order to combat the action of CEM-15. However, murine CEM-15 could inhibit HIV-1 replication when expressed in target cells. Importantly, the inhibitory action of murine CEM-15 could not be impaired by HIV-1 or simian immunodeficiency virus (SIV) Vif. This finding suggests that CEM-15 possesses 2 functional domains, 1 that regulates its inhibitory activity on viral replication and 1 that is targeted by Vif. Takaori-Kondo and colleagues (Abstract 202) also suggested that the RNA editing activity of CEM-15 is required for its ability to suppress viral infectivity. Thus, a point mutant of CEM-15 that lacked RNA editing activity was unable to suppress the infectivity of a Vif-defective virus. It was also demonstrated that the subcellular localization of CEM-15 was not affected by Vif. Therefore, the mechanism through which Vif blocks the CEM-15 effect on viral infectivity remains undetermined. Taken together, these studies highlight the potential importance of Vif/CEM-15 interaction as a therapeutic target. In order to adapt to its new host, HIV-1 has evolved a Vif protein that, by an as-yet-uncharacterized mechanism, inactivates CEM-15. This relationship provides the rationale for therapeutic strategies that prevent CEM-15 inactivation by Vif either using small molecules that prevent their interaction or compounds that mimic the action of CEM-15.

Bieniasz (Abstract 111) discussed resistance factors other than CEM-15 that influence susceptibility of the cell to virus replication. For example, an inhibitory activity referred to as Ref 1, which acts at the level of gag to block an early step in viral infection, has been identified (Towers et al, *Proc Natl Acad Sci U S A,* 2000; Besnier et al, *Proc Natl Acad Sci U S A,* 2002). This inhibitory activity is manifest by certain monkey cell lines that are resistant to HIV-1 infection. This resistance factor is saturable in that infection can proceed at very high multiplicities of infection. Although the identity of the cellular resistance factor is unknown, it appears to target the gag protein, since virus-like particles minimally containing gag and protease are able to saturate the restriction factor. The step in the viral replication cycle that is inhibited by Ref 1 awaits identification but may be manifest at the uncoating step. Identification of the restriction factor should aid the development of therapeutic strategies that exploit these innate host restriction factors of lentivirus infection.

Accessory Proteins

Nef

A number of studies related to the biological activities of Nef were featured at the conference (Abstracts 203-211). One of the models proposed for the

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mechanism of action of Nef in enhancement of viral replication relates to the impact of the protein on host-cell activation status. Since virus replication is optimal in activated CD4+, a number of studies have suggested that HIV-1 Nef may enhance the activation state of the host cell. However, this model requires that Nef be expressed in cells that are refractory to productive infection. Studies from Wu and Marsh (*Science,* 2001) suggested that Nef may be expressed from unintegrated DNA, and presumably, this early expression of Nef augments host-cell activation status so as to promote completion of subsequent events in the viral life cycle. In an extension of this theme, Gillim and Klotman (Abstract 208) suggested that Nef expressed from unintegrated DNA is also able to effect down-regulation of CD4+. As alluded to above, numerous studies have focused on the possibility that Nef augments host-cell activation state to improve conditions for viral replication. Choi and colleagues (Abstract 207) presented evidence that the impact of Nef on viral replication depends on the stimulus with which CD4+ cells are induced to proliferate. When CD4+ T cells were incubated with human umbilical-vein–derived monolayers and treated with interferon γ, the T cells proliferated. The replication of wild-type virus in CD4+ T cells stimulated in this way was approximately 50-fold higher than replication for Nef-deleted virus. This study provides intriguing evidence that lymphocytes induced to proliferate by physiologic signals may be the preferred environment in which Nef enhances viral replication.

Previous studies have shown Nef to be incorporated into virions (Pandori et al, *J Virol,* 1996). Forshey and Aiken (Abstract 205) presented a study indicating that Nef remains associated with the viral reverse transcription complex following the fusion and uncoating steps. The authors proposed that this may allow Nef to influence viral infectivity at the reverse transcription step; it is also possible, however, that Nef that is introduced by incoming virions may influence the activation state of the host cell to promote conditions for viral infection. In addition, Nef has a well-documented ability to down-regulate CD4+ receptor expression on the cell surface. Lama and Argañaraz (Abstract 203) presented evidence that the relative abilities of different Nef alleles to down-regulate CD4+ strongly impact viral replication in cells with high levels of CD4+ expression and in primary lymphocytes. Furthermore, Nef alleles isolated from HIV-1-infected individuals at varying stages of disease exhibited differential impact on CD4+ receptor expression, and HIV-1 variants containing Nef alleles that more efficiently down-regulated CD4+ consequently replicated more efficiently in primary lymphocytes. Collectively, these findings suggest that CD4+ down-regulation may be an important determinant for HIV-1 pathogenesis. In a related study (Abstract 204), Lundquist and colleagues presented evidence that the impact of Nef on CD4+ down-regulation is context-dependent, in that deletion of Nef more profoundly impacted the infectivity of X4-tropic HIV-1 than of R5-tropic viruses. The authors proposed that the specific requirement for Nef in X4-tropic virus replication is a consequence of the more efficient envelope-mediated down-regulation of CD4+ by those viruses.

HIV-1 has been shown to bud selectively from lipid rafts. In addition, the composition of lipid rafts may impact the composition of virions and ultimately virion infectivity. Heaton and colleagues (Abstract 206) presented evidence that Nef is targeted to lipid rafts and that this targeting is important for the ability of Nef to down-regulate major histocompatibility complex (MHC) class 1 but less so for its ability to down-regulate CD4+. Further, increased replication capacity and pathogenicity of wild-type virus relative to virus with the *nef* gene deleted (ΔNef) has been demonstrated in a number of model systems, including SIV-infected macaques, the SCID-Hu PBL model of HIV-1 infection, and human thymic organ cultures. Agostin and Su (Abstract 209) demonstrated that the ability of Nef to effect thymocyte depletion in thymic organ cultures required neither the CD4+ or MHC class 1 down-regulating activities of Nef nor interaction of Nef with cellular kinases PAK1/2, RAF-1, or ASK-1, or the vacuolar adenosine triphosphatase V1H. Thus, a novel motif in Nef may involve its ability to direct thymocyte depletion.

Vpr

A number of studies (Abstracts 213-219) were concerned with various aspects of Vpr activity, particularly the contribution of Vpr to HIV-1 cytopathogenicity. Cytopathic effects of HIV-1 and CD4+ T lymphocytes are manifest both by syncytial structures and by single-cell killing, and the envelope glycoprotein is a critical determinant in both processes. However, it is becoming increasingly apparent that Vpr may also play a role in virus-mediated cell killing through the induction of apoptotic cell death. Bolton and Lenardo (Abstract 217) also presented evidence that virion-associated Vpr and expressed Vpr induce cell death by a nonapoptotic mechanism. Vpr-mediated cell death required the C-terminus of Vpr, in that viruses containing C-terminal Vpr deletions did not exhibit the characteristic cytopathic properties of wild-type virus. In a variation on this theme, induction of cell death by Vpr was found to occur by a DNA damage-signaling pathway (Abstract 213). The serine-threonine kinase ATR becomes activated in response to DNA damage. Expression of a dominant-negative ATR or disruption of ATR by RNA interference blocked the ability of Vpr to induce cell-cycle arrest. Thus, in addition to its possible role in Vpr-mediated cell death, the DNA damage-signaling pathway may also play a role in Vpr-mediated cell-cycle arrest.

Another study (Abstract 217) presented evidence that expression of viral transcripts from unintegrated viral DNA requires Vpr. When the levels of HIV-1 transcripts were compared for wild-type and integrase-defective viruses, expression was higher for virions containing an intact Vpr. The ability of Vpr to impact expression from unintegrated DNA was unrelated to the cell-cycle arrest activity of Vpr and was independent of Tat. Although it would be important to determine whether Vpr can influence the expression of unintegrated DNA that contains a functional integrase, this study may shed light on the mechanism through which unintegrated DNA serves as a template for the expression of viral proteins.

Vpu

Vpu genes are contained within the genomes of HIV-1 and SIV_{CPZ}; however, HIV-2 and other SIV lineages may contain a Vpu-like activity within the envelope glycoprotein, at least with regard to CD4+ down-regulation. Courgnaud and colleagues (Abstract 73) identified a novel SIV lineage with a Vpu gene (SIV_{MON}). This lineage was identified in a seroprevalence survey of wild-born monkeys in Cameroon and supports the hypothesis that the SIV_{CPZ} lineage is a recombinant of related SIV lineages. Vpu is a small transmembrane protein, although the impact of membrane localization on Vpu activity is not well-understood. Singh and colleagues (Abstract 212) compared the subcellular localization of Vpu proteins from HIV-1 subtype B and C isolates. Subtype B Vpu was localized within the endoplasmic reticulum/Golgi complex, but subtype C Vpu was localized at the cell surface, and the cytoplasmic domain was responsible for this membrane localization. Thus, although the Vpu proteins from subtype C and subtype B viruses may localize to different cellular compartments, it remains to be determined whether the biologic activities of Vpu from these subtypes exhibit different biologic properties.

Aspects of Virus Replication

Preintegration Events

Arguably, one of the least well-understood events in virus replication is the uncoating of the viral capsid and release of viral nucleic acids into the cytoplasm. Aiken and colleagues (Abstract 17) presented evidence that the fusogenic activity of HIV-1 envelope glycoprotein is coordinated with HIV-1 core maturation. Immature HIV-1 particles were unable to fuse efficiently with T cells, but truncation of the gp41 cytoplasmic tail or cleavage of the gag precursor Pr55 gag between the MA and NC domains rescued fusion. This finding suggests that HIV-1 fusion is coupled to core maturation through binding of the gp41 cytoplasmic domain to Pr55 gag. This study provides new targets for the development of strategies that interrupt HIV-1 entry and uncoating.

Virus entry requires colocalization of receptors and coreceptors at the point of virus contact. Steffens and Hope (Abstract 20) provided evidence that receptor and coreceptor molecules colocalize in the region of lipid rafts to facilitate the interactions that lead up to HIV-1 fusion. The study also found that receptor and coreceptor molecules were linked with actin-containing structures. This finding may reconcile independent observations on the role of the cytoskeleton and lipid rafts in increasing the efficiency of virus entry.

Several studies were concerned with analyzing the impact of receptor and coreceptor density and location on virion infectivity, susceptibility to entry inhibitors, and resistance to antibody-mediated neutralization. Although transmembrane envelope glycoproteins of lentiviruses contain long cytoplasmic tails, passage of SIV_{mac} in human cell lines selects for variants with truncated cytoplasmic tails. One study (Abstract 19) demonstrated that truncation of the cytoplasmic domain of gp41 dramatically increased envelope content on virions by 25- to 50-fold. Although the increased envelope content led to a modest increase in viral infectivity, it was accompanied by a dramatic reduction in sensitivity to neutralizing antibody. Thus, truncation of the gp41 cytoplasmic tail by in vitro passage favors emergence of variants with increased infectivity in vitro, but evolutionary pressure for long cytoplasmic domains in vivo favors increased neutralization resistance. In a related study (Abstract 23), Reeves and colleagues found that the density of coreceptor molecules on target cells influenced virus susceptibility to entry inhibitors such as enfuvirtide (T-20) and TAK-779. There was an inverse correlation between coreceptor expression levels and sensitivity to enfuvirtide. In addition, there was an inverse correlation between gp120/coreceptor affinity and sensitivity to entry inhibitors, presumably because the more rapid fusion kinetics conferred by greater envelope/coreceptor affinity reduces the time during which enfuvirtide is able to interact with the viral envelope.

A large number of coreceptor molecules for HIV-1 and SIV infection have been identified, but there is little definitive information that coreceptors other than CCR5 and CXCR4 are directly involved in HIV-1 replication and pathogenesis in vivo. Willey and colleagues (Abstract 273) presented evidence for an unidentified receptor for the chemokine vMIP-1 that may serve as a functional coreceptor for a number of HIV-1 and SIV variants. A subset of HIV-1, HIV-2, and SIVs were found to infect primary endothelial cells and astrocytes that lack CCR5 and CXCR4 expression by a CD4+-dependent mechanism that was resistant to inhibitors of CXCR4- and CCR5-dependent infection. This finding suggests the presence of an alternative functional coreceptor on these cells, which about 25% of the dual tropic HIV-1 strains tested were able to utilize. In addition, this coreceptor was active on primary peripheral blood mononuclear cells (PBMCs). The ability to block use of this coreceptor using vMIP-1 suggests that a vMIP-1 receptor was involved. Although CCR8, CXCR6, and GPR1 all bind vMIP-1, the expression of these receptors did not correlate with usage of the alternative coreceptor. Collectively, this suggests the presence of an alternative coreceptor, which is expressed on primary cells and which may be functionally involved in viral tropism in vivo.

Several studies have suggested that R5 viruses are selectively transmitted. This result has been interpreted as suggesting that cells such as macrophages, where infection is CCR5-dependent, may be early reservoirs for the establishment of infection following viral transmission. In order to gain further insight into the mechanism of R5 dominance, Harouse and colleagues (Abstract 125lb) compared the transmissibility of pathogenic SIV-HIV hybrid viruses (SHIVs) that exhibit X4 or R5 tropism. Both X4- and R5-specific SHIVs were detectable in the plasma of coinfected animals within the first 3 weeks of infection, but between 3 and 6 weeks, coincident with the onset of antiviral immunity, R5 viruses predominated. When transmission was compared in coinfected animals in which CD8+ cells had been depleted, X4 viruses predominated. This finding suggests that R5 viruses predominate after transmission because they are

less susceptible to inhibition by CD8+ suppressor cells. One interpretation of this result is that the infected macrophage reservoir may be less susceptible to cytotoxic T lymphocyte surveillance. An important question is whether a similar mechanism may explain the switch from R5 to X4 viruses that occurs in approximately 50% of patients during progression to late-stage disease.

It is now becoming apparent that DC-SIGN may be one of several attachment factors expressed on dendritic cells that mediate virus transmission to CD4+ T cells. KewalRamani (Abstract 110) presented evidence that the role of DC-SIGN in virus transmission is context-dependent and restricted in some cell types. A variety of cell lines expressing low levels of DC-SIGN were examined for ability to transmit HIV-1. Although all cell lines were able to bind HIV-1 at similar efficiencies, some cells did not efficiently transmit the virus. Therefore, there may be additional cellular factors that function collaboratively with DC-SIGN in virus transmission. Bobardt and colleagues (Abstract 270) presented evidence to suggest that cell-surface proteoglycans such as syndecans may capture HIV-1 on the surface of endothelium and serve as transreceptors for HIV-1 infection of T cells. By analogy, DC-SIGN expressed on dendritic cells has been suggested to act as a transreceptor involved in virion capture and transmission to neighboring T cells. Although the syndecans were able to serve as an attachment factor for a broad range of lentiviruses, the syndecans could not substitute for primary or coreceptors in virus entry. The authors proposed that the endothelium, via expression of syndecans, may trap virions and subsequently transmit virions to T cells when the T cells come into close contact with the capillary endothelium.

McDonald and colleagues (Abstract 113) presented evidence for a mechanism that promotes polarization of viral particles on dendritic cells at the point of contact with T cells. Using fluorescent microscopy visualization of HIV-1 particles incorporating green fluorescent protein, the investigators demonstrated that HIV-1 was recruited to sites of cell contact between monocyte-derived dendritic cells and T cells. The mechanism through which virions were localized to the dendritic-cell/T-cell contact point is unclear but likely represents an efficient mechanism through which captured virions are positioned to increase the likelihood of contacting a substrate T cell. This study raises some important questions for future research, such as whether it is possible that HIV-1 may have evolved the ability to exploit this mechanism by specifically activating the localization by, for example, polarization of the cytoskeletal network. In addition, as T cells are activated following contact with infected dendritic cells, it would be interesting to determine whether, in addition to virions, costimulatory molecules involved in T-cell stimulation are also polarized at the point of contact between the dendritic cell surface and the T cell.

It has been suggested that signals generated through receptor/coreceptor molecules enhance post-entry events in the viral replication cycle. Matthews and colleagues (Abstract 292) presented evidence that R5 HIV-1 binding to CCR5 elicits a signal that allows infection of resting cells. The authors also attempted to identify signaling intermediaries that are involved in this process. Treatment of resting memory T cells with either MIP-1β (CCR5 ligand) or R5 HIV-1 led to activation of the protein tyrosine kinase Pyk2. Replication of R5 HIV-1 in resting memory T cells was hindered by inhibitors of the signaling molecules PI3 kinase or c-Src kinase but not by G_{α} inhibitors. Thus, signals generated by R5 HIV-1 binding to CCR5 may involve PI3 kinase and c-Src kinase, which enhance conditions for subsequent steps in virus replication. It will be interesting to determine the fate of infected resting memory T cells and whether these cells are able to enter a latent state of infection.

Integration

A long-standing question in retrovirus biology regards whether there are selective sites for viral integration. Two studies presented opposing viewpoints regarding the selective integration of retroviruses into genes. In one study using a nonlentivirus system, Maxfield and colleagues (Abstract 69) examined retroviral integration into a highly inducible endogenous gene under induced and uninduced transcriptional states. When the frequencies of integration events downstream of the inducible gene were compared under induced and uninduced conditions, there were fewer integration events into the gene when actively transcribing. The authors proposed that active genes may be less desirable integration substrates because of the steric hindrance imposed by RNA polymerase II. Alternatively, double-stranded DNA may be a preferred substrate for the preintegration complex, or remodeling of chromatin by transcription may generate a less desirable substrate for integration. In the second study (Abstract 70), Mitchell and colleagues took a different approach to study lentivirus integration sites in primary T cells. The group had previously established that following infection of a T-cell line, HIV-1 integrates selectively within genes. In an extension of that study, the investigators looked at integration sites in primary lymphocytes and again concluded that genes were favored substrates for integration. It will be important to determine whether, in primary lymphocytes, the genes selected for integration are also transcriptionally active.

Emiliani and colleagues (Abstract 74lb) described the identification of a novel cellular factor that binds HIV-1 integrase. The protein, LEDGF/p75, is a chromatin-associated transcriptional coregulator that is involved in cell survival and the stress response. Inhibition of LEDGF/p75 expression by RNA interference resulted in impairment of HIV-1 infection. Although the studies are preliminary, they suggest that LEDGF/p75 may be a functional cofactor for HIV-1 integration and a potential target for therapeutic intervention.

Late Events

The last few years have seen significant advances in understanding of virus assembly and, in particular, viral and cellular determinants of the budding process. Retroviral and lentiviral gag proteins contain late domains that bind distinct cellular cofactors that promote virus budding. Several groups have identified TSG101, a component of the

vacuolar protein-sorting machinery, as required for the budding of viruses such as HIV-1 and Ebola (Garrus et al, *Cell,* 2001; VerPlank et al, *Proc Natl Acad Sci U S A,* 2001; Martin-Serrano et al, *Nat Med,* 2001). In an extension of these studies, Martin-Serrano and colleagues (Abstract 24) presented evidence that VPS28, a component of the TSG101-containing ESCRT-I complex, interacts with TSG101 and that the interaction is required for particle budding, as was TSG101 multimerization. In addition, a minimal TSG101 domain that contained multimerizing activity exhibited dominant-negative inhibitory activity on HIV-1 budding.

While significant strides are being made in the identification of cellular cofactors for virus budding, Wild and colleagues (Abstract 14) described a novel small-molecule inhibitor of HIV-1 budding. PA-457, a derivative of betulinic acid, exhibited 50% inhibitory concentration (IC_{50}) in the low nanomolar range. PA-457 appeared to interfere with gag processing and specifically with cleavage of p25 to p24. PA-457 did not affect HIV-1 protease activity and was active against nonnucleoside reverse transcriptase inhibitor-resistant and protease inhibitor-resistant viruses. Although the molecular basis of HIV-1 inhibition by PA-457 awaits identification, it may represent a potential new class of antiretrovirals.

As alluded to earlier, several studies have suggested that HIV-1 buds selectively from lipid rafts. When lipid rafts are disrupted in virus-producing cells, the resulting viral particles have a lower infectivity, although the point at which infectivity is impaired is unclear. Cavrois and colleagues (Abstract 18) presented evidence that HIV-1 fusion to target cells is markedly impaired when lipid rafts are disrupted in virus-producing cells. In addition, although Nef has been suggested to localize to lipid rafts, the enhancement of viral infectivity by Nef was independent of budding from lipid rafts. This finding suggests that the composition of proteins within the virion that is dictated by the site of virus budding does not impact enhancement of viral infectivity by Nef.

RNA Interference

RNA interference (RNAi) is a process by which 21- to 23-nucleotide RNAs act as guides to target homologous messenger RNAs (mRNAs) for degradation. First identified in plants, RNAi has been documented in a variety of species, including worms, flies, yeast, and mammals. In the worm, RNAi is initiated when double-stranded RNA is processed by the enzyme Dicer into discreet 21- to 23-nucleotide fragments. These processed interfering RNAs are incorporated into a multiprotein complex and then guide the complex to mRNAs to which the interfering RNAs are homologous. Proteins within this complex subsequently degrade target mRNAs. Because long, double-stranded RNA triggers a type 1 interferon response, initiating RNAi from long, double-stranded RNA is problematic in mammalian cells. However, synthetic 21- to 23-nucleotide double-stranded RNAs, also referred to as small interfering RNAs (siRNAs), bypass the requirement for Dicer and initiate the degradation of mRNAs bearing homologous sequences without activating interferon responses (Zamore, *Nat Struct Biol,* 2001). With this approach, it is possible to target almost any RNA for degradation. Since HIV-1 employs an RNA intermediate in its replication cycle, the virus represents a rather obvious target for manipulation by RNAi.

In the past year, several studies demonstrated the utility of siRNAs for inhibiting HIV-1 infection in vitro (Pomeranz, *Nature Med,* 2002). A number of studies (see presentations in sessions 10 and 37) described progress in the use of RNAi to modulate viral replication, to modulate expression of cellular cofactors for viral replication, and to adapt vector strategies for delivery of interfering RNAs to cells both in vitro and in vivo. The majority of studies described the in vitro activity of siRNAs against a variety of viral genes such as *tat/rev* (Abstract 52), Vif (Abstract 220), and cellular cofactors such as the coreceptors CXCR4 and CCR5 (Abstracts 221, 222, 223, 226, and 231A), as well as the primary receptor CD4+ (Abstract 227). Several studies demonstrated the inhibition of HIV-1 replication by siRNAs in primary cells including lymphocytes and macrophages (Abstracts 51, 220, and 225). In an exciting study, Lieberman (Abstract 51) demonstrated in vivo utility of siRNAs using a murine model of autoimmune hepatitis. The fas siRNAs injected intravenously were able to protect mice from fas-induced acute liver damage and death, suggesting that siRNAs are capable of direct uptake into hepatocytes. This finding has clear implications for potential treatment of pathogenic states involving the liver. Several studies (Abstracts 50, 226, 227, 228, and 229) also detailed progress in the development of viral vectors that express siRNAs under constitutive and inducible promoters.

Although the therapeutic applicability of RNAi to HIV/AIDS remains an open question, it nevertheless is an important tool with which to study HIV-1 biology. In particular, the ability of RNAi to inhibit the expression of cellular genes provides an opportunity to validate cellular factors that may play a role in virus replication and to prioritize those factors that are functionally involved in this process.

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Additional Suggested Reading

1. Besnier C, Takeuchi Y, Towers G. Restriction of lentivirus in monkeys. *Proc Natl Acad Sci U S A.* 2002;99:11549-11551.

2. Garrus JE, von Schwedler UK, Pornillos OW, et al. Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell.* 2001;107:55-65.

3. Martin-Serrano J, Zang T, Bieniasz PD. HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. *Nat Med.* 2001;7:1313-1319.

4. Pandori MW, Fitch NJ, Craig HM, Richman DD, Spina CA, Guatelli JC. Producer-cell modification of human immunodeficiency virus type 1: Nef is a virion protein. *J Virol.* 1996;70:4283-4290.

5. Pomerantz RJ. RNA interference meets HIV-1: will silence be golden? *Nat Med.* 2002;8:659-660.

6. Sheehy AM, Gaddis NC, Choi JD, Malim MH. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature.* 2002;418:441-442.

7. Towers G, Bock M, Martin S, Takeuchi Y, Stoye JP, Danos O. A conserved mechanism of retrovirus restriction in mammals. *Proc Natl Acad Sci U S A.* 2000;97:12295-12299.

8. VerPlank L, Bouamr F, LaGrassa TJ, et al. Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55(Gag). *Proc Natl Acad Sci U S A.* 2001;98:7724-7729.

9. Wu Y, Marsh JW. Selective transcription and modulation of resting T cell activity by preintegrated HIV DNA. *Science.* 2001;293:1503-1506.

10. Zamore PD. RNA interference: listening to the sound of silence. *Nat Struct Biol.* 2001;8:750.

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HIV Pathogenesis and Vaccine Development

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Continuing the trend of recent years, the 10th Conference on Retroviruses and Opportunistic Infections offered a strong and diverse array of presentations on AIDS pathogenesis and vaccine development. Recent advances in pathogenesis research highlighted efforts to understand how HIV persists in the face of a vigorous immune response and underscored the importance of assessing functional properties of cellular immune responses. This year's conference also witnessed a resurgence of interest in neutralizing antibody responses, and numerous efforts to translate new insights into the structure of the HIV-1 envelope and neutralizing antibodies to the development of new vaccine approaches able to induce broadly neutralizing antibodies. Nonhuman primate vaccine studies offered encouraging results of novel approaches able to induce protection against pathogenic simian immunodeficiency virus (SIV) isolates but also offered concerning caveats regarding the potential for viral escape and the impact of host genotype on vaccine protection.

Immune Control of HIV: Size is Not Enough

In the ongoing quest for an effective AIDS vaccine, advancing our understanding of the barriers that impede immune control of viral replication in HIV-infected individuals is of paramount importance. Although the association of long-term nonprogression (LTNP) with strong HIV-specific CD4+ and CD8+ T-cell responses is well-established, the factors associated with progressive HIV infection are not well-understood.

Using a panel of 410 overlapping peptides spanning all HIV proteins of a clade B consensus sequence, Kaufmann and Draenert presented results of a comprehensive investigation of the total HIV-specific CD4+ and CD8+ T-lymphocyte response in different cohorts of HIV-infected subjects. Kaufmann (Abstract 31) presented an analysis of HIV-specific CD4+ T-cell responses in 32 HIV-infected subjects who either were on highly active antiretroviral therapy (HAART) or were off therapy and had relatively low plasma HIV-1 RNA levels (median, 2850 copies/mL). HIV-specific CD4+ responses were focused on relatively few epitopes, predominantly found in Gag and Nef. CD4+ T-cell responses to at least 1 HIV peptide were detected in 78% of patients, and a median of 4.5 peptides were recognized by each subject. Despite the presence of disparate human leukocyte antigen (HLA) class II alleles, 7 peptides (5 in Gag and 2 in Nef) were recognized by 75% of the subjects, including 1 that was recognized in 56% of subjects. This finding suggests a relatively promiscuous presentation of CD4+ T-cell epitopes by different HLA class II alleles. In contrast to the CD4+ T-cell response, the CD8+ T-cell response in this cohort of HIV-infected subjects was 10-fold greater in magnitude and broadly directed, targeting reverse transcriptase (RT), integrase, and Env in addition to Gag and Nef. A similar pattern of vigorous and broad HIV-specific CD8+ T-cell responses was also observed by Draenert (Abstract 35) in 26 chronically HIV-infected subjects with late-stage HIV infection (mean plasma HIV-1 RNA level, 186,271 copies/mL, and mean CD4+ count, 141 cells/µl). A median of 13.5 peptides (range, 2-39) were recognized by individual progressors in this group, and the median magnitude of HIV-specific CD8+ T cells was 5705 spot-forming cells/10^6 peripheral blood mononuclear cells (PBMCs) (range, 185-25,000). Although the plasma HIV-1 RNA levels and CD4+ T-cell counts in this group showed a weak inverse correlation with the breadth and magnitude of the CD8+ T-cell response, a striking overlap in the magnitude and breadth of CD8+ T-cell responses was observed between progressors and LTNPs. Thus, in 25 LTNPs, recognition of a median of 16 peptides (range, 5-58) with magnitudes ranging between 1046 and 43,000 spot-forming cells/10^6 PBMCs (median, 7980) did not differ significantly from progressors.

Since T-lymphocyte responses to HIV have been routinely measured using nonautologous viral sequences, the real breadth and magnitude of the HIV-specific T-cell response as gauged by autologous virus sequences is likely to be even greater. Using peptide sets from a clade B consensus sequence, Altfeld and colleagues (Abstract 314) showed that the virus-specific T-cell response clustered in conserved regions of the genome. However, when responses to peptides spanning the consensus and autologous virus sequences of Tat, Vpr, and p24-Gag were compared, the autologous sequences demonstrated broader and significantly stronger responses, particularly those directed against the variable regulatory proteins. Thus, differences between controllers and noncontrollers of HIV infection may be better appreciated using autologous virus sequences. However, Migueles (Abstract 318) showed that the frequency of CD8+ T lymphocytes secreting interferon-γ in response to stimulation with autologous virus-infected CD4+ T cells was equivalent in LTNPs and progressors. In addition, variant peptides with autologous virus sequences were well-recognized, demonstrating that loss of immune control can occur in the presence of persistent recognition of autologous virus sequences.

These data highlight the paradox

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that despite the fact that HIV infection is highly immunogenic, induction of a strong and broad anti-HIV response is not in itself sufficient for effective immune control. This finding raises the possibility that factors other than the overall magnitude of cellular immune responses may be critical in the ability of the host to control HIV infection. Are there distinct functional properties of the HIV-specific T-cell response associated with an effective immune response? Presentations by Walker (Abstract 164) and Connors (Abstract 165) addressed this key issue.

The ability of cytotoxic T lymphocytes (CTLs) to control HIV replication may be dependent on a variety of factors, including genetic background, presence of CTL escape, and defects in CTL maturation or function. In his presentation, Walker (Abstract 164) proposed that one of the reasons underlying lack of effective viral control with strong CTL responses may be related to epitope-specific or allele-specific qualitative differences among CTLs. These variations may lead to differences in the propensity for and the consequences of CTL escape. Thus, loss of certain CTL responses may be more important than loss of others. One such example is the consequence of CTL escape in a single B27-restricted epitope (KK10). Although the presence of HLA-B27 is highly associated with nonprogression in HIV-infected individuals, escape in this particular CTL epitope is strongly correlated with loss of immune control of viral replication. Often, factors other than sequence variation in known CTL epitopes appear to be responsible for noncontrol. Thus, in a noncontroller, only 4 of 13 peptide responses identified by enzyme-linked immunospot (ELISPOT) assays differed from the viral sequence of the autologous virus. In longitudinal studies of sequence variation in the whole HIV genome in 2 HIV-infected subjects, Walker showed that although increasing sequence variation with time was associated with increasing plasma HIV-1 RNA levels, amino acid variation in as few as 15 amino acids of all HIV proteins was temporally associated with the loss of immune control. In both individuals, more than half of the amino acid changes were not at sites of previously defined CTL epitopes. These may represent novel CD8+ T-cell epitopes in the autologous virus or sites of escape from CD4+ T lymphocytes or antibodies.

Loss of immune control in the setting of a strong and broad HIV-specific T-lymphocyte response was also observed in subjects undergoing structured treatment interruptions (STIs) following institution of HAART in acute HIV infection. Walker presented an update on 14 such subjects. Substantial augmentation of cellular immunity was observed following each episode of STI. In 1 individual, the number of peptides recognized increased from 2 pre-STI to 25 at the end of the second STI. Post-STI responses were also broader, encompassing both structural and regulatory proteins. Although the pre-STI responses were exclusively in Gag, Env, or Nef, following STI, 100% of the responses targeted Nef, Env, and Pol; 88% targeted Gag; and 38% to 60% were directed toward regulatory proteins. However, even this augmentation in breadth was not sufficient to sustain inhibition of viral replication, and eventually immune control was lost. No benefit in survival was observed in an intent-to-treat analysis. However, there was some benefit in terms of plasma HIV-1 RNA levels, in that the time to reach greater than 30,000 HIV-1 RNA copies/mL in plasma was slowed in individuals on STIs.

What qualitative defects are correlates of poor immune control? In studies presented by Connors (Abstract 165), reduced proliferative capacity of HIV-specific CD8+ T cells was the single most dominant defect that differentiated progressors from nonprogressors, as well as distinguishing viremic and aviremic episodes in HIV-infected subjects on intermittent HAART. The HLA-B*5701 allele was present in 15 of 17 patients in a cohort of LTNPs. The magnitude of the HIV-specific CD8+ T-cell response, as assessed by interferon-γ secretion, did not differ between HLA-B*5701-positive and -negative progressors. Neither did it differ between HLA-B*5701-positive progressors and nonprogressors. The difference in immune control between the 2 groups was not due to escape of HLA-B57-restricted CTL epitopes from immune control. Instead, HLA-B57-restricted CTLs showed a marked inability to proliferate in response to antigen-specific stimulation in progressors but not in LTNPs. This defect in proliferative capacity was linked to a lack of perforin expression in the nonproliferating antigen-specific CD8+ T cells. A similar defect in proliferative capacity has been seen in HIV-specific CD4+ T cells in individuals on STI during viremic episodes.

Price and colleagues (Abstract 32) compared the physical enumeration of HIV-specific CD8+ T lymphocytes by tetramers or by clonotypic quantitative polymerase chain reaction for the T-cell receptor B (TCRB) locus of defined HIV-specific CTL clones with functional enumeration of interferon-γ producing cells during viremic and aviremic episodes in 133 HIV-infected subjects enrolled in the Swiss HIV Cohort Study. In this study, individuals were subjected to cycles of 8 weeks on and 2 weeks off therapy, and treatment was discontinued after 4 such cycles. During "off-treatment" phases, increases in plasma HIV-1 RNA levels were associated with decline in interferon-γ ELISPOT responses in the presence of stable or increased frequencies of tetramer-positive cells. In contrast, the frequency of tetramer-positive and interferon-γ-positive cells approximated each other in aviremic individuals. Functional and nonfunctional CD8+ T cells had the same clonality and were not preferentially infected with HIV.

A novel method of assessing the function of CD8+ T cells was presented by Betts and colleagues (Abstract 306). They assessed degranulation of cytotoxic granules in CTLs by flow-based measurement of lysosomal-associated membrane glycoproteins CD107a, CD107b, and CD63. These proteins are expressed only on the membranes of cells that have recently undergone degranulation following antigen-specific stimulation, and may serve as a surrogate marker for cytolytic ability of antigen-specific CD8+ T lymphocytes. Functional heterogeneity with regard to cytokine-secreting and degranulating ability existed within CD8+ T-cell clonotypes specific for a single antigen. How lack of function demonstrated by 1 technique correlates with lack of function by other techniques (eg, decreased cytokine secretion vs defec-

tive granulation vs decreased perforin vs decreased proliferative ability) remains to be determined.

Phenotypic differences among antigen-specific T lymphocytes that reflect distinct stages of maturation have been correlated with their functional ability. Harari and colleagues (Abstract 33) detected CD45RA+ CCR7– HIV-specific CD4+ T lymphocytes in LTNPs but not in progressors and observed an inverse correlation between viremia and frequency of CD45RA+ CCR7– HIV-specific CD4+ T lymphocytes. These differences appeared to be restricted to HIV-specific T lymphocytes, since cytomegalovirus (CMV)-specific CD4+ T lymphocytes with a CD45RA+ CCR7– phenotype were present in both LTNPs and progressors. Complementary findings for CD8+ T cells were observed by Draenert (Abstract 35), who showed an association between the presence of increased frequencies of CD45RA+ CCR7– (mature effector phenotype) HIV-specific CD8+ T cells and viral control.

Boutboul and colleagues (Abstract 34) used complementary DNA (cDNA) microarray analysis to compare gene expression in PBMCs from 5 healthy donors and 27 HIV-infected subjects at different stages of disease. Interferon-α and perforin genes were upregulated, while the interleukin-7 receptor (IL-7R) gene was downregulated in resting PBMCs from HIV-infected individuals with late-stage disease compared with PBMCs from HIV-seronegative individuals. The degree of upregulation or downregulation of these genes was comparable to that observed in normal PBMCs activated in vitro by CD3/CD28 stimulation. Flow cytometric analysis revealed that CD8+ T lymphocytes with high CD127 (IL-7R) expression were negative for perforin, and reciprocally, that cells expressing high levels of perforin were CD127-negative or -low. These findings suggest the possibility that low expression of IL-7R on CD8+ T lymphocytes in HIV infection may represent cells that are lytic but that have lost their ability to proliferate.

An additional determinant of immunologic control is the presence of adequate numbers of antigen-specific T lymphocytes at tissue sites of viral replication. Cromwell and colleagues (Abstract 30) analyzed the frequency and phenotype of SIV-specific CD8+ T lymphocytes targeting the Mamu-A*01-restricted SIV $Gag_{181-189}$ epitope in the peripheral blood and reproductive tissues of female SIV-infected rhesus macaques. The frequency of tetramer-positive cells was increased an average of 14-fold in genital mucosa compared with that in peripheral blood. Mucosal CD8+ T lymphocytes had a high frequency of CXCR3+ cells compared with peripheral blood. The increased expression of CXCR3 on vaginal CD8+ T lymphocytes correlated with expression of its ligand CXCL9 in lymphoid aggregates and lamina propria of the vaginal mucosa, and may be a mechanism for homing of antigen-specific T lymphocytes to mucosal sites of viral replication.

The inability of most HIV-infected subjects to generate potent neutralizing antibodies effective against primary HIV isolates has led many to question whether antibodies play a significant role in controlling viral replication. Shaw (Abstract 166) described analysis of neutralizing antibody responses using an assay in which autologous envelope sequences are used to generate pseudotyped HIV particles expressing a reporter gene. This process in turn allows analysis of neutralizing antibody titers to autologous viral sequences using a single-round infectivity assay. Using this assay, he was able to demonstrate emergence of autologous neutralizing antibodies as early as 72 days after seroconversion and titers that reached as high as 2500. However, variant viral sequences resistant to concurrent neutralizing antibodies rapidly emerged, such that neutralizing antibody titers to autologous envelope sequences at the time virus was isolated were relatively low. Analysis of sequence changes associated with the evolution of escape viruses supported the concept of a "glycan shield," in which an ever-shifting umbrella of sugar residues blocks the ability of antibodies to bind to envelope oligomers.

Effect of Viremia on Immune Function

It is now abundantly clear that in addition to depletion of CD4+ T cells, HIV induces widespread immune activation and dysfunction that affects numerous arms of the immune system. In a plenary lecture, Fauci (Abstract 119) outlined results from studies on the effect of HIV viremia on CD4+ T lymphocytes, B lymphocytes, and natural killer (NK) cells performed on HIV-infected individuals during viremic and aviremic stages of infection. In viremic subjects, a number of abnormalities in CD4+ cells, B cells, and NK cells were detected. Resting CD4+ T lymphocytes in viremic subjects had spontaneous virus production, whereas those in aviremic subjects did not. Microarray analysis revealed upregulation of 493 genes involved in transcriptional regulation and RNA processing in resting CD4+ T cells from viremic subjects. Fauci proposed that the effect of viremia on CD4+ T cells appears to be mediated by both cytokines and the HIV envelope.

Effects of viremia were also demonstrated on B cells and NK cells. B lymphocytes in HIV-viremic subjects were characterized by loss of CD21 expression and decreased proliferation in response to CD4+ T-cell help due to downregulation of CD25 expression. As reported in more detail in a subsequent oral presentation by Malaspina (Abstract 128), B cells from HIV-viremic subjects have a reduced capacity to stimulate CD4+ T cells through CD80/86-CD28 interactions. B cells of HIV-infected viremic subjects had decreased induction of CD80/86 on activated B cells and a reduced capacity to induce proliferation of allogenic CD4+ T cells from healthy HIV-seronegative subjects. This functional abnormality was corrected following reduction of plasma HIV-1 RNA levels by effective antiretroviral therapy and was associated with upregulation of CD80/86 on the B lymphocytes. NK cells from viremic HIV-seropositive subjects had a reduced ability to secrete beta-chemokines and suppress HIV replication in vitro. The activating NK receptors NKp46, NKp30, NKp44, and NKG2D were markedly reduced, and some killer inhibitory receptors were upregulated on NK cells of viremic as opposed to aviremic HIV-infected subjects.

A novel mechanism of viremia-induced immune evasion was proposed

by Brainard and colleagues (Abstract 29). Previous work from this laboratory had demonstrated that the chemokine SDF-1, while serving as a chemoattractant at low concentrations, was able to mediate repulsion of T lymphocytes (termed fugetaxis) in a CXCR4-receptor-mediated manner at higher concentrations. Their current data suggest that increased concentrations of gp120 may induce fugetaxis of HIV-specific CTLs. Fugetaxis induced by gp120 was CD4-independent and inhibited by anti-CXCR4 and pertussis toxin. By altering cell density and performing CTL assays in flat-bottomed wells, these investigators demonstrated decreased efficacy of cell killing at lower cell densities, suggesting the possibility that CTL migration may affect killing efficacy. According to this scenario, gp120-mediated inhibition of CTL migration toward sites of increased viral replication may reduce CTL efficacy in vivo.

Host Genetics

Host genetic polymorphisms can affect the outcome of HIV infection at the level of virus entry, virus dissemination, and immune control of viral replication. In a symposium, 4 presentations discussed the impact of host genetics on HIV-1 variability and outcome of HIV infection. In large population studies, Ahuja (Abstract 53) presented data on the impact of genetic variation in the CCR5 gene and MCP-1 gene on HIV-1 transmission and disease progression. In a cohort of 649 children exposed perinatally to HIV and followed for 4 years, multiple polymorphisms in the CCR5 gene other than the well-described Δ32 genotype were identified that were associated with either increased or decreased risk of mother-to-child transmission. CCR5 polymorphisms that were either deleterious or beneficial had a similar impact on disease progression in both adults and children. The prevalence of disease-modifying CCR5 haplotypes was different in populations of different ethnicities, emphasizing race as a confounding factor in therapeutic efficacy studies. Two polymorphisms and 3 haplotypes were also identified in the MCP-1 gene. A 2578G single-nucleotide polymorphism, although associated with a decreased risk of acquiring HIV infection, was linked to decreased survival and increased risk of AIDS-associated dementia once HIV infection was established. The deleterious effect of this polymorphism was possibly related to increased transcription of MCP-1 and increased monocyte recruitment to the brain. Kaslow (Abstract 56) summarized the current knowledge of host genetic polymorphisms that have consistently been shown to have an effect on HIV transmission or disease progression. At the level of HLA alleles, the most consistent deleterious effects on disease progression have been seen with HLA homozygosity at any locus and with the presence of certain allelic variants of HLA-B35 and HLA-B*5301. In contrast, alleles B*5701 and B*5703 are consistently associated with control and delayed progression of HIV-1 infection across HIV subtypes A, B, and C. With regard to impact on HIV acquisition, sharing of HLA alleles by transmitter and recipient at 1 or more loci can be associated with an increased risk of horizontal transmission. In a study of 221 serodiscordant couples in Zambia followed up for 5 years, there was a strong deleterious effect on transmission of sharing alleles at the HLA-B locus. The effect of the HLA-A locus on clinical outcome or HIV transmission has not been consistent across studies. The HLA-A2/A*6802 supertype has been associated with a decreased risk of horizontal and mother-to-child transmission in white and African American populations infected with clade A or B virus. Surprisingly, the same alleles were shown to be significantly associated with an increased risk of transmission in a Zambian cohort infected with clade C virus.

The effect of HLA polymorphisms in driving HIV-1 sequence variability was elegantly presented by John (Abstract 54). HIV adapts to the host at 2 levels. At 1 level the accumulation of mutations is driven by immune pressure, which for CTL epitopes is evident as HLA class I allele-specific polymorphisms at the population level. However, the emergence of mutations at each residue is modulated by variable genetic barriers, including functional or structural constraints or cost to replicative fitness. In a full-length HIV genome analysis in 175 patients, adaptation of HIV to HLA polymorphisms was strongly predictive of plasma HIV-1 RNA levels, with greater viral adaptation being associated with higher HIV-1 RNA level. Although univariate analysis showed a significant association between the presence of HLA B*5701 or HLA-B*2705 and low plasma HIV-1 RNA levels, this effect was no longer apparent on multivariate analysis. Instead, low plasma HIV-1 RNA level was strongly associated with less HIV adaptation. It is possible that the protective effect of HLA B*5701 and B*2705 is causally related to a decreased propensity for their CTL epitopes to accrue mutations because of the presence of genetic barriers.

Beneficial and deleterious effects of HLA class I haplotypes on HIV may also be mediated via its effect on innate immunity. Carrington (Abstract 55) presented data on associations between killer immunoglobulin-like receptors (KIRs) and HLA that can modulate HIV-1 disease. The KIR genes, located on chromosome 19q13.4 in the leukocyte receptor complex, encode a group of receptors that regulate inhibition and activation of NK cells. Binding of inhibitory KIR receptors on NK cells to major histocompatibility complex (MHC) class I molecules on target cells inhibits NK-mediated cytolysis, whereas the absence of MHC class I molecules on target cells is associated with NK cell activation and killing. The presence of 1 activating KIR allele, KIR3DS1, in combination with a specific subset of HLA-B alleles (those containing the Bw4 serological epitope that also have an isoleucine at position 80, designated as HLA-B Bw4-80I), was shown to have a highly protective effect against progression to AIDS. This protective effect was not observed with HLA-B Bw4-80I in the absence of KIR3DS1. Further, KIR3DS1 alone shows a deleterious effect on survival. HLA molecules expressing the Bw4 serologic epitope are a ligand for the inhibitory KIR receptor encoded by KIR3DL1. Since the extracellular domains of 3DL1 and 3DS1 are very similar, they may interact with similar ligands. The model to explain the protective synergistic effect of HLA-B Bw4-80I and KIR3DS1 proposes that KIR3DS1 may preferentially bind to Bw4-80I, leading to NK cell activation and killing of HIV-infected target cells.

Swift NK cell activation mediated through KIR molecules early in infection may positively impact viral control, not only for HIV, but also for other pathogens.

Dendritic Cells

Although DC-SIGN, a C-type lectin that is highly expressed in monocyte-derived dendritic cells (DCs), is believed to be a key molecule facilitating dendritic cell-mediated HIV infection of CD4+ T cells, the precise mechanisms underlying this function are not known. KewalRamani (Abstract 110) summarized research on DC-SIGN from his laboratory. Stable expression of DC-SIGN in the monocytic cell line THP-1 resulted in transmission of HIV-1 at efficiencies similar to those of primary immature DCs. In transwell experiments, cell contact between THP-1/DC-SIGN cells and CD4+ T lymphocytes was required for HIV-1 transmission. When other transformed cell lines (K562 and 3T3) stably transfected and expressing DC-SIGN at levels comparable to those of the THP-1/DC-SIGN cells were examined, they were not able to transmit HIV-1, even though their ability to bind HIV-1 or intracellular adhesion molecule 3 (ICAM3) was not impaired. This finding led to a search for cell molecules required for DC-SIGN-mediated HIV transmission. As observed for immature DCs, HLA-DR and leukocyte function antigen 1 (LFA-1) were expressed on THP-1 cells permissible for HIV transmission, but these molecules were not expressed on K562 and 3T3 cells. However, expression of HLA class II molecules and LFA-1, singly or in combination, in the nonpermissive K562 and 3T3 cell lines did not reverse the defect in HIV transmission, suggesting that DC-SIGN expression is not sufficient for efficient HIV-1 transmission and that other cofactors are likely to play a role. Identification of these specific cofactors should provide valuable insights into DC-SIGN-mediated transmission of HIV.

Studies using live-cell video microscopy to examine transfer of HIV from DCs to CD4+ T lymphocytes were presented by Hope (Abstract 113). Using fluorescent labeling of HIV by incorporation of a fusion protein of GFP with Vpr, investigators showed that in monocyte-derived DCs, HIV was recruited to sites of cell contact with T cells. At the same time, in the target cell, CD4 and CXCR4 were concentrated at the CD4+ T cell/DC interface. The localized concentration of HIV, CD4, and coreceptors (termed an "infectious synapse") may play a critical role in facilitating infection of T cells by dendritic cells.

Innate Immunity

In addition to acting as a conduit for HIV transmission, DCs are the principal antigen-presenting cells in the body and essential for mounting an effective adaptive immune response. Loré and colleagues (Abstract 81) demonstrated that innate immune recognition mediated by binding of the microbial pattern recognition Toll-like receptors (TLRs) on DCs to their natural ligands resulted in maturation of DCs and enhancement of adaptive virus-specific immune responses in vitro. CD11c+ myeloid DCs expressing TLRs 3, 4, and 7 and CD123+ plasmacytoid DCs expressing TLRs 7 and 9 were activated on exposure to their respective ligands: CpG (TLR9 receptor), imidazoquinolones (TLR7), LPS (TLR4), and poly I:C (TLR3). TLR-ligand-mediated activation of plasmacytoid or myeloid DCs resulted in an enhanced capacity to generate antigen-specific T lymphocytes. The ability to modulate adaptive immunity makes TLR ligands a potentially useful immunologic adjuvant for HIV vaccines.

Unutmaz (Abstract 112) outlined the role of natural killer T cells (NKT cells) in HIV infection. NKT cells are a distinct subset of human effector T lymphocytes that express an invariant T-cell receptor and recognize glycolipid antigens presented by the nonpolymorphic MHC class I molecule CD1d. Using flow cytometry to identify NKT cells in humans ($V\alpha24+V\beta11+$ or CD1d tetramer+$V\beta11+$), 20% to 90% of NKT cells were found to be CD4+ and to express CCR5. Autologous DCs pulsed with the glycolipid alpha-galactosylceramide were able to expand NKT cells, which secreted IL-4 and IL-5 and were highly susceptible to infection with R5-tropic HIV. HIV-infected subjects had marked declines in NKT cells, which may be mediated by direct infection. Decline in CD4+ NKT cells but not total NKT cells correlated inversely with plasma HIV-1 RNA level.

Natural Hosts of SIV Infection

An expanding number of nonhuman primates such as sooty mangabeys and African green monkeys serve as natural hosts for SIV infection. Despite lifelong infection and levels of viremia that can in some species equal or exceed those in HIV-infected subjects with AIDS, these hosts remain asymptomatic and do not develop AIDS. Increasing attention over the past several years has been devoted to trying to elucidate immunologic or virologic mechanisms associated with the lack of pathogenicity of primate lentiviruses in their natural hosts. Barry and colleagues (Abstract 120) proposed that a limited SIV-specific CD8+ T-cell response may play a causal role in the lack of immunodeficiency in sooty mangabeys naturally infected with SIVsm. Analysis of several markers of T-cell activation (eg, CD69, CD25, and HLA-DR) or cell proliferation (Ki67) revealed only minor increases in SIVsm-infected sooty mangabeys compared with those observed in uninfected mangabeys. Similar findings were observed even in the setting of acute SIVsm infection of sooty mangabeys, whereas acute infection of rhesus macaques with the same stock of SIVsm resulted in significant increases in T-cell activation, increased proliferation of CD8+ and CD4+ T cells, and progression to AIDS. Depletion of CD8+ T cells using a murine CD8-specific monoclonal antibody resulted in only 3-fold increases in plasma viremia, leading Barry and colleagues to conclude that a weak or absent SIV-specific CD8+ T-cell response may be an important factor in the lack of immunodeficiency in SIV-infected sooty mangabeys.

A contrasting view was reported by Kaur (Abstract 121), who described the results of a study in which CD8+ T cells were depleted in SIV-infected sooty mangabeys using a chimeric mouse-human CD8-specific monoclonal antibody (cM-T807). This antibody has been widely used in nonhuman primate

experiments and generally induces more prolonged and complete depletion of CD8+ T cells than has been obtained with murine monoclonal antibodies. Following a depletion of CD8+ T cells that often extended to 3 weeks, Kaur and colleagues observed up to 100-fold increases in plasma viremia in 5 of 6 animals studied, whereas no significant increases in viremia were observed in 4 animals that received a control chimeric monoclonal antibody. Further evidence for SIV-specific CD8+ T-cell responses was provided by the finding of significant ELISPOT responses to SIV proteins in most SIV-infected mangabeys. Reasons for the apparent discrepancy in findings between these groups was not immediately clear, but may relate to differences in the efficacy of CD8+ T-cell depletion induced by the 2 different monoclonal antibodies.

Prophylactic AIDS Vaccines

Neutralizing Antibody Responses

After several years of being overshadowed by research on cell-mediated immune responses to HIV, research on neutralizing antibodies has recently enjoyed a renaissance prompted by improved information on the structural basis for neutralization, new assays for measuring neutralizing antibodies, and continued hope that structurally modified envelope immunogens will enhance the induction of broadly reactive neutralizing antibodies to HIV. Leading off a symposium devoted solely to neutralizing antibodies, Burton (Abstract 184) reviewed the evidence that supports the view that neutralizing antibodies represent the small fraction of envelope-specific antibodies that are able to bind the oligomeric envelope spike. According to this viewpoint, broadly reactive neutralizing antibodies are therefore the subset of antibodies that are able to bind to conserved sites on the oligomer. Burton also noted that although passive transfer of neutralizing antibodies in animal models has provided compelling proof-of-principle demonstration of the ability of neutralizing antibodies to protect against primate lentiviruses, efforts to elicit broadly neutralizing antibodies by vaccination have consistently met with failure. Describing one approach to better understand why it has been so difficult to induce broadly neutralizing antibodies by vaccination, Burton discussed detailed structural studies of 2 neutralizing monoclonal antibodies, b12 and 2G12. The first is a well-characterized neutralizing antibody that was initially isolated by a phage display library derived from an asymptomatic HIV-infected individual. Previous work had demonstrated that b12 recognized a conserved site of gp120 that overlaps with the CD4 binding site. A subsequent analysis of the crystal structure of b12 revealed a long protrusion at the tip of the antibody that inserts into the recessed CD4 binding site of gp120. In an effort to enhance production of antibodies with similar structure to b12, Burton and colleagues have tried to block production of antibodies to other common epitopes on the gp120 oligomer by hyperglycosylation of regions such as the CD4 binding site and the V3 loop. Immunization studies with the hyperglycosylated gp120 molecule are in process. A similar structural analysis has been carried out for the 2G12 antibody, which recognizes an epitope in the C4/V4 region of gp120 and has been previously shown to recognize a cluster of oligomannose glycans in this region of gp120. Crystal structure analysis of 2G12 revealed that instead of forming the typical Y shape of most immunoglobulin G (IgG) molecules, the hypervariable heavy chains (V_H) formed a tight cluster in which the 2 V_H domains are directly juxtaposed, a phenomenon called domain swapping. The unusual structures of both b12 and 2G12 provide at least 1 reason for the difficulties in generating broadly neutralizing antibodies against HIV. Whether this understanding will ultimately facilitate elicitation of such antibodies by the use of modified immunogens will be a major challenge for research in this area for the next several years.

After binding to cellular receptors, gp120 undergoes a series of conformational changes ultimately resulting in insertion of an oligomeric form of gp41 into the host cell membrane and subsequent fusion of the viral particle with the host cell membrane. Whether antibodies can be successfully generated to these intermediate forms of envelope, thereby blocking virus entry, has been controversial. Weiss (Abstract 185) reviewed efforts to generate antibodies able to block viral fusion. She identified 3 distinct stages at which fusion inhibitors may inhibit virus entry: prevention of conformational changes, blocking close opposition of the virus and host cell membrane, and prevention of fusion pore assembly. One of the intermediate steps of viral entry involves conformational changes in the ectodomain of gp41, in which 2 heptad repeats of gp41 are brought together to make up the prehairpin intermediate. This hairpin intermediate subsequently generates a 6-helix hairpin structure that catalyzes formation of the fusion pore. Peptides mimicking the gp41 heptad repeats are able to potently inhibit HIV replication, which suggests that antibodies might also be able to block this critical step in virus entry. Inherent challenges to this approach include steric hindrance (ie, whether antibodies can fit in the small gap between the virus particle and the host cell membrane) and whether sufficient time exists during viral fusion to allow the antibody to bind. Immunization with recombinant immunogens that mimic either the prehairpin or 6-helix bundle conformation of gp41 resulted in antibodies able to bind either early intermediates or late intermediates, respectively, and that were able to inhibit viral entry. These data provide an important proof-of-principle demonstration of the feasibility of inducing antibodies able to bind to these fusion intermediates.

Generation of antibodies able to bind the chemokine coreceptor binding site of gp120 has been another leading approach for the induction of broadly neutralizing antibodies against HIV-1. Sodroski (Abstract 186) described recent efforts to define the structural characteristics of antibodies able to bind the chemokine receptor binding site. Two specific regions of gp120 make up the chemokine receptor binding site: the variable V3 loop and the beta19 strand, a more conserved region of the bridging sheet that is protected by the V3 loop. Because the length of IgG molecules (approximately 115 angstroms) is significantly longer than that of the space between gp120 and

the host cell membrane after initial virus attachment (approximately 85 angstroms), antibodies able to interact with the chemokine receptor binding site need to have a flexible and extended region to reach beyond the adjacent V3 and V1/V2 loops. Indeed, these are the characteristics of one of the best-characterized chemokine receptor binding antibodies, 17b. Another distinctive characteristic of many chemokine receptor binding antibodies (although not 17b) is the sulfation of tyrosine residues in the antigen binding site. As described in more detail in the presentation by Farzan (Abstract 28), this sulfation mimics the post-translational modification of tyrosine residues of CCR5 that interact with positively charged residues of gp120 and thus play a critical role in gp120-CCR5 interactions.

Despite the disappointing failure of efforts in the 1980s to generate neutralizing antibodies effective against primary isolates by V3 loop immunization, there has been continued interest in the potential utility of the V3 loop as a vaccine immunogen. Zolla-Pazner (Abstract 107) described efforts to elicit V3-specific antibodies with a broader range of neutralization. Previous studies using antibodies specific for linear determinants of V3 had demonstrated that neutralization mediated by these antibodies was quite type-specific. However, recent work from the Zolla-Pazner laboratory has demonstrated that monoclonal antibodies specific for conformational determinants of V3 were able to mediate broader neutralization of primary isolates. These data presented a paradox: given the variability of the V3 loop, how were V3-specific antibodies able to neutralize diverse HIV-1 isolates? The answer appears to lie in part in the structural constraints of the V3 loop. Nuclear magnetic resonance analysis of the structure of the V3 loop bound to the V3-specific antibody 447 suggested at least 2 alternative conformations of V3: 1 able to bind to CCR5, the other able to bind to CXCR4. Selection pressure on the V3 loop to maintain these 2 alternative conformations may also provide sufficient conservation to allow neutralization by conformation-sensitive V3-specific antibodies.

Nonhuman Primate Studies: The Good News

Much of the newfound optimism in the AIDS vaccine field over the past 2 years has arisen as a result of nonhuman primate studies employing the simian-human immunodeficiency virus (SHIV) 89.6p as a challenge strain. Although this strain is pathogenic and results in rapid CD4+ T-cell depletion over a matter of weeks, concerns have been raised that because of its atypical disease course and ease of neutralization, 89.6p may not be predictive of protection against clinical HIV isolates. Two presentations highlighted vaccine approaches able to induce partial protection against challenge with isolates from pathogenic SIV of macaques (SIVmac), against which it has been historically quite difficult to induce protection.

Robert-Guroff (Abstract 77) described the results of a trial in which macaques were vaccinated mucosally with a replication-competent adenovirus. Animals were vaccinated with a recombinant adenovirus expressing Env and Rev administered either by itself or in combination with recombinant adenoviruses expressing other SIV proteins (Gag or Nef) or a recombinant subunit boost. Relatively strong cell-mediated immune responses as determined by ELISPOT assays were observed against Gag and Env, with weaker responses observed against other SIV proteins. Neutralizing antibody responses were also observed in groups that received a recombinant subunit boost. Following intrarectal challenge with SIVmac251, a significant (20-fold) reduction of set-point viremia was observed in animals that had been immunized with the SIV Env/Rev construct in combination with one of the other SIV recombinants. A subset of animals was able to control viremia to undetectable levels. Protection correlated in part with ELISPOT responses on day of challenge, but other responses, such as proliferative responses, neutralizing antibody responses, and CD8+ antiviral responses, may have played a role in mediating protection as well.

Previous work from Shiver and colleagues had demonstrated impressive protection against SHIV 89.6p challenge in macaques immunized with a recombinant replication-defective adenovirus vector expressing SIV Gag either alone or when given following DNA priming. In this initial study, to facilitate evaluation of cellular immune responses using MHC tetramers, all macaques were selected to express a specific MHC class I allele (Mamu-A*01). Although these results were clearly encouraging, questions were raised as to whether they could be replicated using other challenge stocks and in animals with a more diverse MHC background. In a late-breaker abstract (Abstract 85lb), Shiver presented follow-up studies in 2 groups of macaques to address these questions. The first study involved Mamu-A*01-negative macaques that were immunized with adenovirus vectors encoding either SIV Gag, a heterologous envelope (JRFL), a homologous envelope (89.6p), or the combination of Gag and the heterologous envelope. Following intravenous challenge with SHIV 89.6p, immunized animals had at least a 100-fold decrease in set point viremia, and combined immunization with adenovirus vectors expressing Gag and Env appeared to give better protection than that obtained with either antigen alone, reinforcing observations previously made by the Robinson laboratory at Emory. Interestingly, the level of protection observed in this experiment appeared to be less complete than that observed in previous Mamu-A*01-positive vaccines. The second study involved immunization of Mamu-A*01-positive and -negative macaques with an adenovirus SIV-Gag vector with or without DNA priming. Following an intrarectal challenge with the pathogenic SIVmac239 virus, Mamu-A*01-positive animals that received DNA priming and the adenovirus Gag boost had a significant 10- to 30-fold reduction in plasma viremia at 150 days compared with controls. However, this effect was not observed in Mamu-A*01-positive animals that received adenovirus alone or in Mamu-A*01-negative animals that received the DNA prime/adenovirus-boost regimen. Although the demonstration of a significant protective effect on challenge with a pathogenic SIVmac strain in Mamu-A*01-positive animals is encouraging, the absence of a clear significant effect

in Mamu-A*01-negative animals is concerning. These results further illustrate the difficulties in interpreting challenge data derived from nonhuman primate models and raise numerous questions. Is SIVmac239 too rigorous a challenge? Which macaque model will ultimately prove to be the best predictor of efficacy in human clinical trials? Answers to these questions will be ultimately dependent on results from phase 3 clinical trials involving approaches able to induce potent cellular immune responses and are thus at least 4 to 7 years away.

Nonhuman Primate Studies: The Bad News

Even more grounds for caution was provided by oral presentations from Barouch and Staprans. Barouch (Abstract 76) analyzed the frequency of escape from CTL surveillance in vaccinated macaques. These studies were prompted in part by a report that appeared last year that described escape in a dominant CTL epitope in a single DNA-vaccinated animal that had been challenged with SHIV 89.6p. In the present study, Barouch described breakthrough viremia and disease progression in 3 of 4 Mamu-A*01-positive macaques that had been vaccinated with a DNA *gag* vaccine and challenged with the pathogenic SIV stock SIVsmE660. Emergence of CTL escape mutants in multiple epitopes correlated with decreases in epitope-specific responses as determined by MHC tetramers, increased viremia, and CD4+ T-cell depletion. These results reinforce the frequent occurrence of CTL escape in SIV-infected macaques previously described by other groups and suggest that initial gains in decreasing plasma viremia by vaccination may be lost over time due to viral escape.

Over the past several years, several investigators have raised the question of whether an ineffective AIDS vaccine might in fact worsen disease progression, either by production of antibodies that enhance instead of neutralize viral replication or by induction of virus-specific CD4+ T-cell responses that would serve to facilitate HIV replication. Although there has fortunately been little evidence to date for such an in vivo effect in macaque studies, Staprans (Abstract 80) described results of a study that raised a concern in this regard. In this study, macaques were immunized either with a live attenuated varicella-zoster virus (VZV-OKA) expressing the SIVsmH4 envelope or with the parental VZV-OKA strain. In immunized animals, the authors observed relatively disappointing SIV-specific immune responses, which consisted primarily of virus-binding antibodies and limited CTL responses but no neutralizing antibodies. After a prolonged rest, animals were reboosted and challenged rectally with SIVsmE660. A dramatic difference was observed between the groups. Animals that had been vaccinated with the VZV-OKA envelope vaccine developed significantly higher levels of plasma viremia and more rapid CD4+ T-cell depletion compared with controls immunized with the unmodified VZV-OKA. Increased rates of viral replication were correlated with increased percentages of proliferating CD4+ T cells (as assessed by expression of the proliferation marker Ki67) 3 days after challenge, although SIV-specific CD4+ T-cell responses were not directly measured. These results raise the concerning possibility that induction of virus-specific CD4+ T-cell responses in the absence of other effective immune responses might accelerate instead of impede disease progression. However, several significant caveats should be noted. There was no direct measurement of virus-specific CD4+ responses after challenge. Also, the spontaneous suppression of viremia in the control animals challenged with the pathogenic SIVsmE660 strain is unusual; most groups have observed significantly higher levels of viremia with this stock. Although this study raises important concerns, these results will have to be replicated with more intensive analysis of virus-specific immune responses before any hard conclusions regarding potential adverse effects of vaccination can be drawn.

Human Clinical Trials

In contrast to the broad array of novel findings presented from nonhuman primate AIDS vaccine studies, progress reported in human clinical trials was more modest. (The much-anticipated results from the phase 3 VaxGen study were not reported until 10 days after the close of the conference). Hammer (Abstract 108) provided a broad overview of the current status of HIV vaccine clinical trials. After detailing the numerous challenges facing the development and testing of HIV/AIDS vaccines, he highlighted results from several recently completed or ongoing vaccine trials. HVTN 203 examined the immunogenicity of a third-generation canarypox vector (vCP1452) that expressed HIV-1 Gag and Env. Induction of CTL responses to Gag or Env was disappointingly infrequent, occurring in only 13% and 7% of vaccines, respectively. This relative lack of immunogenicity was significantly below the milestone that had been set for progression of this approach to phase 3 clinical trials in the United States. Results from the ongoing Merck vaccine trials have been more encouraging. At the highest doses studied, 42% of subjects receiving a DNA *gag* vaccine had developed ELISPOT responses to Gag after 4 immunizations. Initial trials of the Merck replication-defective adenovirus vector expressing Gag are still being analyzed, but preliminary results indicate that 78% of subjects receiving the highest dose of adenovirus had positive ELISPOT responses after 2 doses. The National Institutes of Health Vaccine Research Center is pursuing a similar approach utilizing DNA priming with a recombinant adenovirus vector boost and will utilize as immunogens a clade B Gag/Pol/Nef given in combination with 3 different envelopes (clades A, B, and C). Although results from phase 3 efficacy trials of these approaches remain several years away, these early results demonstrating relatively robust induction of cellular immune responses offer some grounds for optimism.

As one approach to try to increase the immunogenicity of canarypox vectors, Goepfert and colleagues (Abstract 82) examined whether increasing the dose of the recombinant canarypox vector vCP1452 up to 10^8 50% tissue culture infectious dose ($TCID_{50}$) could improve induction of cellular immune responses. While previous lower doses

of this vector have been relatively well-tolerated, at the increased dose, the vast majority of subjects (> 85%) developed both local and systemic symptoms. No improvement in interferon-γ ELISPOT responses was observed, and the overall frequency of responding subjects remained disappointingly low (18-24%).

Novel Approaches

Although vaccinology in general (and the study of adjuvants in particular) has been criticized frequently for being a largely empirical science, numerous poster presentations highlighted novel approaches utilizing the rational use of molecular adjuvants. Huang et al (Abstract 396) analyzed the ability of the NKT cell ligand α-galactosylceramide (α-GalCer) to serve as an adjuvant for DNA vaccination in mice. When α-GalCer was administered in combination with suboptimal doses of DNA vaccines, a 5-fold increase in CD8+ T-cell responses and a 2-fold increase in CD4+ T-cell responses was observed. An alternative approach to increasing immunogenicity was presented by Zhang and colleagues (Abstract 397), who created a fusion protein of CTLA4 and SIV Gag in order to target B7-expressing, antigen-presenting cells such as dendritic cells. Immunization with CTLA4/SIV Gag resulted in a 100-fold increase in Gag-specific antibody responses. A complementary approach was described by Ross and colleagues (Abstract 400), in which DNA constructs were created that encoded fusions of Env with C3d. C3d is a component of the complement cascade that specifically binds to complement receptor 2 on B cells and therefore targets C3d fusion proteins to B cells. DNA immunizations with the Env-C3d complex resulted in 10^3- to 10^5-fold increases in antibody titers compared with results of immunizations with DNA expressing Env alone. Although it will be important to see whether these encouraging results in mice can be replicated in primates, the proliferation of these studies suggests that rational approaches to optimizing immunogenicity may ultimately bear fruit over the next several years.

A third approach to antigen targeting was reported by Rosati (Abstract 448). Codon-optimized SIV *gag* and *env* genes were fused to either the secreted chemokine MCP-3 (which directs proteins to the secretory pathway) or to the β-catenin peptide (which directs proteins to the proteasomal degradation pathway). Immunization of animals with the MCP/*gag*/*env* construct resulted in increased humoral responses. Macaques immunized with a combination of the unmodified MCP-3 and β-catenin fusion proteins showed a broad range of immune responses, including CD4+ T-cell, CTL, and antibody responses. After mucosal challenge with SIVmac251, vaccinated animals had significantly lower viral loads compared with those of naive animals, a difference that was maintained out to 30 weeks postchallenge.

Although DNA vaccination has proved to be a very effective means to induce cellular immune responses in rodents, results have been less impressive in macaques and even more disappointing in humans. Whether these differences reflect true species-specific differences or largely differences in the modes of administration is not clear. For instance, when expressed on a milligram per kilogram basis, doses of DNA administered to mice are significantly higher than those used in comparable studies in nonhuman primates or humans. To address the issue of whether modifications in the method of administration could affect immunogenicity, Gardiner and colleagues (Abstract 452) analyzed whether division of a constant amount of DNA among 1, 2, 3, or 4 limbs in mice altered cellular immune responses as determined by interferon-γ ELISPOT assays. Dividing a 20 μg DNA dose among 4 extremities significantly increased cellular immune responses to a level similar to that provided by a single 100 mg dose of DNA in one leg. These data suggest that very basic issues regarding amount of DNA and number of immunization sites need to be more carefully addressed in nonhuman primate and human studies.

Although live attenuated SIV vaccines have proved to be one of the most effective means to induce protection against infection with pathogenic SIV isolates in nonhuman primates, safety concerns have precluded progression of this approach to human clinical trials. Two presentations analyzed the use of single-cycle SIV or HIV constructs (essentially nonreplicating lentiviral vectors) as a potential vaccination approach. Evans (Abstract 78) described results of immunization with a single-cycle SIV generated by introduction of a nucleotide substitution in the *gag/pol* frameshift site and providing Gag-Pol expression in trans. A single intravenous injection of single-cycle SIV in macaques resulted in peak levels of 10^4 to 10^5 copies/mL of plasma viremia that rapidly decayed to undetectable levels and low but significant SIV Gag-specific responses detected by ELISPOT and MHC tetramers. A second inoculation resulted in significantly lower levels of viremia (presumably reflecting the effect of preexisting SIV-specific immune responses) and transient boosting of SIV-specific cellular immune responses. Tung (Abstract 79) described a complementary approach involving production of replication-defective, vesicular stomatitis virus G (VSV-G)-pseudotyped HIV vectors with a truncation of the *pol* gene. Systemic and mucosal inoculation resulted in virus-binding antibodies and virus-specific CTL responses. Challenge of animals with the SHIVku-2 strain resulted in lower levels of viremia in vaccines as compared with levels in a concurrent control animal. These data support further study of single-cycle lentiviruses as a vaccine modality in nonhuman primates, but it remains to be seen whether this approach will have sufficient advantages to outweigh the safety concerns regarding the use of an integrating virus for a preventive vaccine.

Evaluation of cell-mediated immune responses in HIV/AIDS vaccine trials has increasingly relied upon assessment of secretion of cytokines such as interferon-γ using ELISPOT and intracellular cytokine staining assays. In many instances, whether secretion of interferon-γ accurately reflects the majority of antigen-specific cells has not been well-addressed. Using 12-color flow cytometry, De Rosa (Abstract 405) analyzed the range of cytokines secreted by antigen-specific cells following either tetanus or hepatitis B immunization. Remarkably, cells secreting interferon-γ represented only a small minority of the antigen-

specific response. With fresh cells, IL-2 was the dominant response, whereas with frozen cells, cells producing MIP-1β were the dominant response, exceeding the frequency of cells secreting interferon-γ by 10-fold or more. These provocative results will need to be confirmed with other antigens, but if validated, would have significant implications for evaluation of antigen-specific responses in both vaccine trials and HIV/AIDS pathogenesis studies.

Therapeutic Vaccination

Since immune control of HIV replication is not achieved in most subjects, despite the presence of a readily detectable HIV-specific immune response, could therapeutic augmentation of HIV-specific immunity by vaccination potentiate existing immune responses and generate novel responses sufficient to induce immune control?

Walker (Abstract 164) presented results of a pilot vaccine trial using a whole inactivated Env-depleted vaccine of clade A/G Zairian isolate in an adjuvant to immunize chronic HIV-infected subjects. Using stringent criteria for augmentation of immunity (5-fold increase in stimulation index and at least an increase of 500 counts per minute in at least 2 antigens on numerous occasions), a significant increase in HIV-specific proliferative responses, but not CD8+ T-cell responses, was observed in vaccinated subjects. Unfortunately, this augmentation was not associated with any clinical benefit. Several presentations (Abstracts 60, 61, and 62) discussed results of therapeutic vaccination using modified vaccinia Ankara (MVA) or recombinant canarypox-HIV (ALVAC) vectors in HIV-infected subjects on HAART. In a phase 1 trial of a MVA vector expressing the HIV-1 *nef* gene, administration of 3 doses of the vaccine to 14 subjects on HAART with plasma HIV-1 RNA levels below 50 copies/mL and CD4+ counts above 400 cells/μl induced CD8+ and CD4+ T cells to recognize new epitopes in 10 of 16 subjects (Harrer, Abstract 60). Interruption of HAART resulted in rebound viremia in all subjects, although the plasma HIV-1 RNA levels remained lower than pre-HAART levels in 7 of 14 vaccinated subjects. In a randomized control study (Levy, Abstract 62), 70 patients on HAART for at least 1 year were randomized to continue HAART alone (n = 37) or to receive HAART in combination with vaccination with ALVAC HIV vCP1433 (a recombinant canarypox expressing HIV-1 *env, gag,* and *nef* genes) and Lipo-6T (consisting of 6 peptides in Nef, Gag, Env, and Pol and a helper TT epitope). Five doses were administered at monthly intervals. Following the last dose, the vaccinated group had a significantly higher frequency of subjects with detectable proliferative responses to p24 antigen. Four weeks after stopping HAART, 2 of 37 patients in the control group and 8 of 33 patients in the vaccinated group had controlled viremia. However, this effect was not sustained. At present, although some boosting of immune responses has been observed following therapeutic vaccination, clear clinical benefits have yet to be realized.

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Complications of HIV Infection and Antiretroviral Therapy

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Metabolic Complications

Atherosclerosis and HIV Infection

More attention has increasingly been focused recently on determining whether patients with HIV infection who are treated with highly active antiretroviral therapy (HAART) regimens are at an increased risk of cardiovascular or cerebrovascular (CVD) events. Several presentations at this year's conference addressed this issue. The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study group presented the preliminary results of the first prospective study to examine the incidence of myocardial infarction (MI) among more than 23,000 patients being followed up in 11 cohorts in Europe, Australia, and North America (Abstract 130). Monitoring for rates of MI began in 1999. As of September 2002, the D:A:D investigators noted a 26% increase in the MI rate per year of exposure to combination antiretroviral therapy (nonnucleoside reverse transcriptase inhibitor [NNRTI] and protease inhibitor [PI] regimens were considered together). The overall incidence of MI remains low at 3.5 events per 1000 person-years. Importantly, risk factors for MI in addition to time on combination therapy included age, male sex, smoking (60% were never smokers), and previous cardiovascular disease. In addition, elevated cholesterol level, diabetes, and hypertension were also predictors of increased risk. Surprisingly, a clinical diagnosis of lipodystrophy conferred a decreased risk of MI. Data from the Johns Hopkins cohort were also examined for trends in the rates of both coronary heart disease (CHD) and cerebrovascular events (Abstract 132). In that retrospective review, the rates of CHD and CVD were 5.9 and 5.0 per 1000, respectively. A case-control analysis identified PI use and stavudine use as independent predictors of CHD, and investigators noted that the overall rates of both CHD and CVD were higher than those reported for an age-, sex-, and race-matched group in the general population. Continued follow-up from the Northern California Kaiser Permanente cohort continues to show an increased risk of hospitalization for MI among HIV-seropositive enrollees compared with HIV-seronegative controls; however, no association with PI use has been noted (Abstract 747). A retrospective assessment of 8 US cohorts suggested an increased risk of MI among PI-exposed patients; however, PI use was no longer a statistically significant predictor of MI after the analysis was adjusted for other cardiovascular risk factors (Abstract 746).

Data from 2 studies of carotid intima medial thickness (IMT) (measured by ultrasound) as a marker of subclinical atherosclerosis were reported (Abstracts 131 and 139Ib). The AIDS Clinical Trials Group (ACTG) 5078 study investigators reported baseline results on the first prospectively matched cohort of HIV-seropositive PI-treated patients with both HIV-seropositive (but non-PI-treated) and HIV-seronegative controls (Abstract 131). The groups in this study were matched for race, age, sex, smoking status, and blood pressure measurement. With a mean of almost 4 years of PI use at baseline, and despite increased values for cholesterol and triglycerides in the PI group, no difference in baseline carotid IMT was seen between the PI and non-PI groups or between the HIV-seropositive and HIV-seronegative groups.

An uncontrolled study of 104 HIV-seropositive patients suggested that age, low-density lipoprotein cholesterol (LDL-C), hypertension, and nadir CD4+ cell count of 200/µL or below were associated with greater carotid IMT (Abstract 139lb). In a preliminary analysis of the first 21 patients with 1 year of follow-up, age and duration of PI therapy were predictive of the rate of progression of carotid IMT. Compared with age-matched historical controls, the HIV-seropositive patients had increased carotid IMT and greater rates of carotid IMT progression within this small group. More data on the relationship among duration of HIV infection, antiretroviral treatment, and rates of carotid IMT progression from both of those studies are eagerly awaited.

Collectively, these cohort studies and clinical trials indicate an association between long-term use of combination antiretroviral therapy and an increase in the relative risk for CHD. The absolute risk of CHD, however, remains low and needs to be balanced against the known benefits of treatment for HIV infection. In addition, other modifiable risk factors, such as smoking, that may be more common in some HIV cohorts than in the general population must be accounted for when determining the strength of the association among HIV infection, HIV therapy, and CHD. Appropriate control for known risk factors for CHD, such as smoking, family history, and diabetes (pre-HIV infection), needs to be considered when attributing an increased risk of cardiovascular disease to HIV infection or its treatment.

Dyslipidemia and Hypertension

Further light was shed on the relationship among HIV infection, HAART therapy, and the development of dyslipidemia and hypertension using the Multicenter AIDS Cohort Study (MACS) data (Abstracts 744 and 750). Seaberg and colleagues reported that compared with HIV-seronegative subjects not taking antiretroviral therapy (or monotherapy or combination therapy only), MACS participants infected with HIV were less likely to have systolic hyper-

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tension than those without HIV infection. After 2 years of HAART, the risk of systolic (but not diastolic) hypertension in the HIV-infected group exceeded that in the HIV-uninfected group (Abstract 744). These data suggest an increased risk of systolic hypertension following HAART exposure.

Riddler and colleagues compared lipid profiles from men in the MACS who had nonfasting blood samples available preseroconversion, postseroconversion, and pre- and post-HAART and made several important observations (Abstract 750). After HIV seroconversion, the levels of most lipid parameters fell, with decreases in total cholesterol (31 mg/dL decrease), high-density lipoprotein cholesterol (HDL-C) (12 mg/dL decrease), and LDL-C (21mg/dL decrease) compared with preseroconversion values. Investigators observed significant increases in lipid levels after 3 years of HAART (most were PI-treated); however, HDL-C levels did not rise above preinfection values, and the magnitude of the increase in total cholesterol beyond preseroconversion values (20 mg/dL increase) was consistent with what would be expected because of aging. These results suggest that a portion of the lipid increases observed after the initiation of HIV therapy may represent a "return to health" associated with treating chronic HIV infection.

Lipid changes associated with the use of NNRTIs have generally been more favorable than those seen with first-generation PIs, although there have been no studies that directly compared the lipid changes in patients treated with efavirenz with the changes in patients treated with nevirapine. The 2NN study randomized patients to receive nevirapine, efavirenz, or both, with a backbone of stavudine/lamivudine in all arms. After 48 weeks, the mean increase in HDL-C was slightly greater in the nevirapine-treated group (14 mg/dL) than in the efavirenz-treated group (9 mg/dL), and the mean increase in triglycerides was higher in the efavirenz-treated group (33 mg/dL) than in the nevirapine-treated group (11 mg/dL). Although both of these differences reached statistical significance, the clinical significance is not likely to be great for most patients.

The lipid and glucose effects of lopinavir/ritonavir were evaluated by Lee and colleagues in a study of 10 HIV-seronegative healthy men (Abstract 748). After 4 weeks of treatment with lopinavir/ritonavir monotherapy, triglyceride and free fatty acid levels increased. The increase in triglycerides was 65 mg/dL, which is slightly less than what has been reported in treatment-naive HIV-infected subjects. The 2-hour glucose tolerance testing showed a statistically significant increase in 2-hour glucose and insulin levels after 4 weeks of lopinavir/ritonavir, but there were no significant changes in the insulin-mediated glucose disposal rate (as has been seen with indinavir) by euglycemic, hyperinsulinemic clamp studies. These results highlight the concept that individual drugs within a class may have divergent impacts on glucose and lipid metabolism. It is hoped that studies such as this will become incorporated into drug development in the future. These data also demonstrate the direct effect of lopinavir/ritonavir on triglyceride, but not cholesterol, levels in HIV-uninfected subjects.

The minimal impact of atazanavir on lipids appears to be sustained with longer-term follow-up data out to 108 weeks (Abstract 555). In a randomized trial, patients treated with the amprenavir prodrug GW433908 experienced lipid changes that were comparable to those who received nelfinavir, with a mean increase in total cholesterol of 45 mg/dL and LDL-C of 33 mg/dL after 48 weeks of treatment. In both groups, the values generally remained below the National Cholesterol Education Program (NCEP) thresholds for intervention. A greater rise in triglyceride level was noted in the nelfinavir group.

Lipodystrophy

The relationship among mitochondrial DNA levels in fat, lipoatrophy, and specific drug exposure continues to be explored. This year's conference was notable for several reports of studies attempting to quantify mitochondrial DNA (mtDNA) from fat biopsy samples as well as from blood mononuclear cells. It appears that fat biopsies, although more difficult to obtain than peripheral blood mononuclear cells (PBMCs), may be a better source of tissue for examining the pathogenesis of lipoatrophy.

In 1 study, Thompson and colleagues assessed adipose tissue apoptosis, mtDNA quantification, and improvement in lipoatrophy among 14 patients who substituted stavudine with either abacavir (n = 13) or zidovudine (n = 1) (Abstract 728). Despite the small numbers of patients in this study, there appeared to be clear trends in the improvement in adipose tissue apoptosis, adipose mtDNA levels, and fat (as measured by dual-energy x-ray absorptiometry scanning [DEXA]) in the legs, arms, and trunk 48 weeks after the nucleoside reverse transcriptase inhibitor (nRTI) substitution. Cherry and colleagues presented data from 232 fat biopsies performed in independent studies with paired mtDNA PBMC samples and/or clinical data that suggested that mtDNA levels in fat were lower among patients who were current users of "d-drugs" (ie, stavudine, didanosine, or zalcitabine) compared with those taking zidovudine or abacavir (Abstract 133). Notably, levels of mtDNA also were lower in patients receiving zidovudine compared with those not receiving nRTIs. In a small subset of patients who had discontinued nRTI therapy, mtDNA levels appeared to increase rapidly (much sooner than changes in limb fat were observed in this study). Taken together with prior data from randomized trials and cohort studies, it appears that the process of subcutaneous fat loss is progressive over time and that it is associated with exposure to nRTI therapy.

Several studies at this conference (Abstracts 728, 733, and 739) and in the past suggest that the rate of fat loss may be faster with exposure to stavudine than with zidovudine, but that fat loss occurs to some degree with both drugs. The relationship between mtDNA levels in fat cells and adipose tissue apoptosis (and other markers of mitochondrial function) to changes in subcutaneous fat wasting merits further prospective investigation.

If nRTIs are a key etiologic agent in fat wasting, it would be expected that the process might reverse when that therapy is withdrawn. This notion has led to the investigation of "nucleoside-sparing" regimens. Boyd and colleagues

reported the 48-week results of a single-arm open-label study in which patients in whom nRTI therapy was failing changed to a regimen of indinavir/ritonavir and efavirenz (Abstract 738). The percentage of body fat (body fat as percent of mass) measured by DEXA increased significantly in the legs (1.7%), arms (2.8%), and trunk (1.8%). In addition, computed tomography showed an increase in square centimeters of both visceral and subcutaneous fat in the abdomen as well as mid-thigh fat. By patient assessment only, the increases in abdominal fat were apparent. Further controlled trials are needed to more fully assess the impact of "nucleoside-sparing" regimens on the process of fat wasting, both in terms of the initial development of fat wasting and as a means for reversing the problem once it has developed.

In a comprehensive and elegant conference presentation, Capeau discussed recent developments in our understanding of the pathogenesis of lipodystrophy by focusing on the role of adipose tissue in that process (Abstract 160). Capeau reviewed data demonstrating the impact of PIs and nRTIs on adipose tissue both in vitro and from patient samples. Her presentation included discussion of data suggesting that PIs may impair the nuclear localization of sterol regulatory element-binding protein-1 (SREBP-1) through an interaction with lamin A/B, and she described how disruption of this process could lead to abnormal adipose cell differentiation (with altered adipocytokine levels) and insulin resistance. She went on to show that PIs may induce the secretion of tumor necrosis factor (TNF) alpha, which could further impact fat-cell differentiation and promote insulin resistance as well as alter mitochondrial function. Coupled with the effects of nRTIs on mtDNA and consequently mitochondrial function, she built a case for the differential but synergistic effects of both classes of drugs that could conspire to produce peripheral lipoatrophy, insulin resistance, and potentially, visceral adipose hypertrophy (due to increased cortisol synthesis intracellularly).

Following Capeau's presentation was a thoughtful review by Grinspoon of clinical data that confirm a central role for insulin resistance in the pathogenesis of lipodystrophy (Abstract 161). Other clinical studies this year also focused on the relationship between adipocyte hormones and lipodystrophy (Abstracts 754 and 760). Adipocytokines may regulate fat metabolism, lipid and glucose homeostasis, and insulin sensitivity. As initially reported by Kosmiski and colleagues at last year's conference (Abstract 40), levels of adiponectin correlated inversely with insulin resistance in patients with lipodystrophy (Abstracts 754 and 760).

Clinical studies of phenotype of lipodystrophy involving women from the Women's Interagency HIV Study (WIHS) and involving men from the Fat Redistribution and Metabolic Change (FRAM) Study were reported. In a longitudinal study of 1057 HIV-seropositive and HIV-seronegative women with semiannual self-reports of body-shape changes (confirmed by anthropometric measurements) the incidence rates of peripheral and central lipoatrophy among the HIV-seropositive women were twice the rates among the HIV-seronegative women (Abstract 736). The incidences of peripheral lipohypertrophy were lower among the HIV-seropositive group, and, in contrast to what has previously been reported, the rates of central lipohypertrophy were no different between the 2 groups. The predominant syndrome that distinguished the HIV-seropositive women from controls was the presence of both central and peripheral lipoatrophy (pLA).

A smaller study of body-shape changes (using DEXA scans) among women in a cohort that included 45% African American and 36% Hispanic women was reported by Howard and colleagues (Abstract 735). As a group, the HIV-seropositive women had a lower percentage of body fat and limb fat by DEXA compared with controls. However, the percentage of trunk fat was higher among the HIV-seropositive women in the study than in controls. Of note, African American women had a decreased trunk-fat percentage compared with all other women. In a multivariate analysis that included only the HIV-seropositive women, stavudine use and non-African American race were independently associated with increased truncal fat and decreased limb fat, and PI use was not. These results suggest that racial differences in changes in body fat need to continue to be examined in future prospective studies.

Cross-sectional data from the FRAM study (that had previously been reported in preliminary form in an oral presentation at the XIV International AIDS Conference in 2002 in Barcelona) demonstrated that lipoatrophy was the predominant abnormality in HIV-seropositive men compared with the control group (Abstracts 733 and 732). In this study report, both DEXA scans and total-body magnetic resonance imaging (MRI) were used to assess body composition among men who reported pLA compared with those who did not. Body mass index, median kilograms of limb fat as measured by DEXA, and median liters of both upper- and lower-trunk subcutaneous fat as measured by MRI were all *lower* among HIV-seropositive men with pLA than among those those without pLA and lower than among controls. Importantly, median liters of visceral fat assessed by MRI was also *lower* (but not statistically significantly different) among the men with pLA than among both the HIV-seropositive men without pLA and controls. In addition, men with HIV who did not report pLA (and for whom it was not noted on their exam) also had decreased subcutaneous fat compared with the control group. These findings demonstrate a predominant syndrome of subcutaneous fat loss associated with HIV infection and suggest that the degree of fat loss may not be appreciated by exam alone.

A second report from FRAM provided further details on the prevalence and character of buffalo humps among men with HIV infection compared with a control group that provided data on subcutaneous fat by MRI (Abstract 734). Although the prevalence of buffalo hump was not statistically different in the HIV-infected patients (8%) compared with controls (11%), the buffalo humps in HIV-infected men were 2.5 times larger than those in controls and tended to occur in men with a higher body mass index. In addition, patients with a buffalo hump also tended to have a greater amount of visceral fat in both the HIV-seropositive and the control

groups than those without a buffalo hump, suggesting that the presence of a buffalo hump may be a surrogate marker for visceral fat.

The prevalence and optimal management of asymptomatic hyperlactatemia remains uncertain. In general, screening of asymptomatic patients has not been recommended. Wohl and ACTG colleagues examined the prevalence of hyperlactatemia among nRTI-treated patients with 1 or more risk factors for the disorder (Abstract 761). A standardized procedure developed by the ACTG for lactate collection was followed that does not allow the use of a tourniquet or fist clenching. Hyperlactatemia was defined as a value 1.5 times the upper limit of normal, and all abnormal values prompted repeat evaluation. After the assessment of 83 patients with risk factors for hyperlactatemia, none was found to have confirmed hyperlactatemia when the careful collection procedures were followed. These results suggest that the syndrome of asymptomatic hyperlactatemia is rare and confirm that routine measurement of lactate levels is unwarranted. In addition, this study highlights the importance of following careful collection procedures when obtaining lactate levels among patients with symptoms.

Bone Effects

There continues to be interest in the relationship between HIV infection, antiretroviral therapy, and the risk of osteopenia and osteoporosis. Two studies reported at this year's conference examined bone mineral density (BMD) data among HIV-infected women. In both studies, traditional risk factors—not HIV therapy or HIV infection—were associated with decreased BMD (Abstracts 102 and 103). Jacobson and colleagues reported longitudinal data on change in BMD in 141 women enrolled in the Nutrition for Healthy Living Study, in which they found that median BMD did not change over 2 years of follow-up (Abstract 102). Factors associated with loss of BMD included recent weight loss, smoking, and being Caucasian. In a cross-sectional study of women older than age 35 years, BMD was compared in HIV-seropositive (n = 140) and HIV-seronegative (n = 144) women (Abstract 103). After the data were controlled for traditional risk factors (weight, age, smoking, and physical activity), HIV infection was not associated with reduced BMD. In contrast to previous studies, these investigators found that PI use for more than 1 year appeared to protect against the development of reduced BMD among women over age 35. Finally, Mondy and colleagues reported the results of a small randomized trial of calcium and vitamin D alone or combined with alendronate as treatment for osteopenia or osteoporosis. They found a statistically significant increase in BMD for those who received alendronate compared with those who received only vitamin D and calcium (Abstract 134). The treatment appeared to be well-tolerated in this 48-week study of 31 subjects.

Tuberculosis

Chaisson reviewed the global epidemiology of the HIV/tuberculosis (TB) epidemic in a symposium addressing international models and perspectives on the topic (Abstract 46). He made a compelling argument that in the developing world, the traditional definition of TB "control"—70% diagnosis and 85% cure—needs to be reevaluated. TB control will only occur when the basic reproductive rate R_o is less than 1. Because of the copathogenesis of HIV and TB, the approach to the HIV/TB epidemic needs to include more aggressive TB case identification, more directly observed therapy (DOT) for TB, evaluation of TB prophylaxis strategies, and use of HAART. HAART is already in use for coinfected patients in regions in which TB is endemic.

Patel and colleagues reported about experience with HAART in 197 patients in Ahmedabad, India (Abstract 138). Patients were treated with standard 4-drug TB therapy, generic efavirenz, and 2 nRTIs. CD4+ counts increased from a median of 104 cells/μL to 306 cells/μL at 9 months. Ten percent of patients reported paradoxical reactions, or transient clinical worsening of TB, but HAART was safely continued.

Preliminary results using this same regimen in South Africa (Abstract 783) and in Brazil (Abstract 784) were encouraging. In the South Africa program, HAART was successfully administered through a modified DOT program 5 days a week. Longer follow-up of these cohorts examining virologic suppression rates and drug resistance will be of interest.

A US study involving 431 TB patients yielded several findings of note (Abstract 137). First, despite current recommendations, 33% of patients in this otherwise model TB-control program were not tested for HIV. Second, the rate of relapse of TB was 2.3-fold higher in HIV infected-patients than in HIV-uninfected patients and was associated with lower CD4+ cell count. In a second multicenter Centers for Disease Control and Prevention (CDC)-sponsored study presented by Burman (Abstract 136), 169 patients infected with HIV and TB were examined. As in previous studies, patients responded well to TB therapy (95% success rate). Eighty patients received HAART during the period of observation. Fifteen percent of patients had paradoxical reactions. Compared with a historical control of HIV-infected TB patients in the United States in the pre-HAART era, there was a 6-fold decrease in HIV progression among the patients treated with HAART.

In 2 US studies, drug interactions between HAART and TB treatment were examined. In the first, concomitant administration of 300 mg rifabutin and 600 mg efavirenz slightly lowered rifabutin, but not efavirenz levels, supporting recommendation of a dosage of 450 mg twice weekly of rifabutin when administered with efavirenz without the need to increase the dose of efavirenz (Abstract 785). In the second study, the hepatic enzyme effects of rifampin on indinavir could not be overcome with the addition of ritonavir, eliminating indinavir as an option for patients requiring TB treatment including rifampin (Abstract 542).

GBV-C Infection

There were several conference presentations on the relationship between the GB virus-C (GBV-C) and HIV disease progression. Initial cohort studies demonstrated a survival advantage in HIV-infected patients with evidence of GBV-C infection. Among the studies present-

ed this year, there was consensus that GBV-C viremia was cleared in many HIV-infected patients (Abstracts 157, 159lb, and 848) and that early GBV-C viremia did not predict HIV disease progression (Abstract 157). The precise relationship between GBV-C and HIV remained controversial, however, and more work is needed in this area.

Williams and colleagues reported that many patients in the MACS cohort cleared GBV-C viremia and became GBV-C E2 antibody-positive (Abstract 159lb). Early GBV-C infection was not associated with HIV disease progression, but patients with sustained GBV-C viremia had a survival advantage. Patients who cleared GBV-C viremia appeared to be at the highest risk for HIV disease progression. Aboulker and colleagues reported that GBV-C viremia was associated with a greater CD4+ cell response in early trials of single- and dual-nRTI regimens (Abstract 849). Xiang et al presented data from a series of experiments designed to explore the mechanisms by which GBV-C could decrease HIV replication (Abstract 156). In an in vitro system in which GBV-C-infected cells were challenged with HIV, expression of the CCR5 chemokines RANTES, MIP-1α, MIP-1β, and SDF-1 were upregulated and were associated with decreased HIV replication compared with cells not infected with GBV-C.

In another epidemiologic study from Sweden, GBV-C viremia at baseline was not associated with HIV disease progression (Abstract 157). Clearance of GBV-C viremia occurred in 11 of 44 patients without antibody seroconversion and was associated with accelerated HIV disease progression. Authors of this report concluded that GBV-C was secondary to HIV disease progression. In a report from the Viral Activation Transfusion Study (VATS), Busch and colleagues found that GBV-C was transmitted to 22% of HIV-infected patients during transfusion (Abstract 846). No cases of GBV-C viremia occurred among patients who were antibody-positive prior to transfusion.

Hepatitis and Liver Transplantation

In contrast to the 2002 CROI, no new major hepatitis therapeutic trials were presented this year. The importance of including detection for hepatitis B virus (HBV) core antibody in screening strategies was emphasized by Gandhi in a US study (Abstract 821). In patients infected with HIV and hepatitis C virus (HCV), history of HBV infection did not produce a detectable effect on liver histology (Abstract 822). Tenofovir use was associated with sustained responses to HBV in small cohort studies including patients with lamivudine-resistant HBV (Abstracts 824 and 825). In a large study of US veterans, Fultz and colleagues showed that HCV was associated with reduced survival in both the HIV-infected and the HIV-uninfected populations (Abstract 828). A second study of the Veterans Administration population suggested that HCV infection is associated with an increased risk for diabetes (Abstract 830). In a Spanish cohort, more advanced liver fibrosis (extensive portal fibrosis or cirrhosis) was more frequent in HIV- and HCV-coinfected patients compared with HCV-infected patients (Abstract 830). In a small prospective study, patients with HCV starting HAART showed no evidence of liver damage due to immune reconstitution (Abstract 831). Based on an in vitro model, Li suggested that morphine may enhance HCV replication (Abstract 158). Kim reported a higher frequency of CD8+ cell responses to HCV than previously appreciated (Abstract 837), and Graham noted Th1 responses were associated with milder inflammation and cirrhosis attributed to HCV (Abstract 839). A small, descriptive study of outcome in 23 HIV-infected liver transplant recipients suggested that outcome in patients tolerant of HAART and with CD4+ cell count greater than 200/µL posttransplant was similar to that in non-HIV-infected patients (Abstract 155).

HIV Prevention and Transmission

In the first plenary session of the conference, Valdiserri from the CDC gave an overview of the epidemiology of HIV infection in the United States (Abstract 4). Estimates of HIV cases in the United States have continued to rely on data from 25 states where HIV is a reportable disease. Despite the fact that 2 of the largest states—New York and California—are not included in these estimates, some important trends were evident. Among the 25 states, the number of cases reported increased by 8% from 1999 to 2001. Valdiserri cautioned that this increase might reflect a fluctuation of a steady rate and should not be overinterpreted. More notable, however, was the consistent and dramatic rise in rates of infection among African American men (15%) over the same time period.

Behavior and Virus Transmission

An interesting debate among modelers of the HIV epidemic has been around the effect of HAART on HIV transmission. Although it would seem intuitive that reducing HIV RNA plasma levels with HAART would reduce transmission rates, Valdiserri made the case that these benefits are lost if wider use of HAART results in an increase in high-risk behavior. He advocated a more intensive approach to risk reduction among HIV-infected patients receiving care through multidisciplinary clinic-based programs. Guidelines sponsored by the CDC and Infectious Diseases Society of America for risk-reduction counseling for HIV-infected patients in care will be released shortly.

It is estimated that at least one quarter of HIV-infected persons in the United States are unaware of their HIV serostatus. Freedberg and others have previously advocated offering HIV testing to all hospitalized patients in high-prevalence areas. "Think HIV" was a program designed by Freedberg's group to identify undiagnosed HIV-infected patients in Massachusetts presenting to urgent care centers (Abstract 39). Of patients presenting to care in a 7-month period, 1853 (31%) accepted HIV testing and 37 new HIV cases were identified. This program had a higher yield than self-referral testing in Massachusetts during the same time period and identified many patients who did not consider themselves at risk for HIV infection.

Henson and colleagues reported an update of HIV prevalence among patients presenting to the emergency department at Johns Hopkins University (Abstract 38). During a 2-month period

in 2001, HIV testing was performed on 1613 individuals or patients. The seroprevalence in 2001 was 11.3% (183 patients). Twenty-four percent of seropositive patients did not know they were infected. Eight of the 183 patients were recently infected, and 7 of those 8 were unaware of their serostatus. Among patients aware of their HIV diagnosis, 24% admitted to having engaged in unsafe sex and 6% to needle sharing.

High-risk behavior among persons known to be infected also was documented in patients being released from prison. Among a group of 80 HIV-infected persons released from prison, 24% had unprotected sex within 6 months of release from jail, and 31% thought it was likely that they would infect their partners (Abstract 36). In a different study, McConnell and Grant proposed that the effects of high-risk behavior on the HIV epidemic in the United States might be less than predicted because of serosorting, a disproportionate amount of high-risk behavior among HIV-seroconcordant contacts (Abstract 41).

Important insights into HIV transmission were made from a study of the Rakai cohort in Uganda presented by Wawer (Abstract 40). In this retrospective study of 240 serodiscordant couples followed up between 1995 and 1999, the risk of HIV transmission was highest during the first 5 months after HIV-infected index case seroconversion and during the period of 5 to 15 months before death. The strongest predictor of HIV transmission was the HIV RNA level in the index case. Although it has been postulated that recent HIV seroconverters are more likely to transmit HIV to uninfected sexual partners, this is the first study to provide systematically collected data on a cohort of this size. From a public health standpoint, identification of recent seroconverters needs to be a priority in HIV-prevention programs.

The findings of HIV case identification in Malawi reported by Pilcher and colleagues take on additional importance in view of the Rakai data (Abstract 154). In this cross-sectional study of 1361 men seen at a sexually transmitted disease (STD) or dermatology clinic, 24 participants had early, antibody-negative HIV infection. Presenting to the STD (vs dermatology) clinic was associated with a higher risk of primary HIV infection. Thus, antibody tests alone are insufficient to identify HIV-infected persons presenting to care, and additional testing in targeted populations appears to be warranted.

A third study regarding HIV transmission in South Africa was reported by Sanne (Abstract 42). Because of the high prevalence of HIV infection among adults and the frequency of sexual assault, a private health care facility in Johannesburg began providing zidovudine and lamivudine for postexposure prophylaxis in 1999. Of 858 patients, HIV infection was already present in greater than 14%. Among the 644 for whom postexposure prophylaxis was prescribed within 72 hours, over three fourths returned for testing, and only 1 HIV seroconversion was documented.

Mother-to-Child Transmission

Other important studies on mother-to-child HIV prevention programs shed light on the timing of HIV transmission and drug resistance among mothers treated with short-course therapy. In an analysis of 9 randomized, placebo-controlled trials with more than 5000 mother-infant pairs, it was estimated that over 40% of all HIV transmission occurred during breast-feeding at least 4 weeks after delivery (late postnatal transmission) (Abstract 97). There was a steady accumulation of HIV infection cases during the period of breast-feeding for more than a year. Having a CD4+ cell count less than 200/μL at the time of delivery was associated with an 8-fold increase in risk for late postnatal transmission. Female infants also had a diminished risk for acquiring HIV infection during breast-feeding compared with male infants.

Lee reported results of a study examining drug resistance among 33 pregnant women in Zimbabwe receiving single-dose nevirapine for prevention of perinatal transmission (Abstract 96). Although HIV-1 RNA levels in the plasma exceeded those in breast milk, there were more women with viral mutations associated with resistance to NNRTIs in the breast milk (65%) compared with in the plasma (40%) 8 weeks after nevirapine administration. The predominant NNRTI mutations differed between breast milk and plasma within individuals.

Eshleman presented additional studies on samples from mothers receiving single-dose nevirapine for perinatal transmission in the HIVNET 012 trial in Uganda. In the first study, Eshleman showed that the NNRTI Y181C mutation emerged within 1 week of a single dose of nevirapine and then was followed by the emergence of the K103N mutation as the predominant circulating drug-resistant variant within 8 weeks (Abstract 856). In the second study, women with HIV subtype D appeared to be at higher risk for selection for nevirapine resistance mutations than were women with subtype A (Abstract 857).

Written by Drs Havlir and Currier in March 2003.

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Drug Resistance Mutations in HIV-1

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The International AIDS Society–USA (IAS–USA) Drug Resistance Mutations Group reviews new data on HIV drug resistance with the goal of maintaining a current list of mutations associated with clinical resistance to HIV. This list, presented as the IAS–USA Mutations Figures, has been revised to include recently published data on the effects of the V106M mutation on nonnucleoside reverse transcriptase inhibitors (NNRTIs).[1]

The figures presented here (March 15, 2003) replace the November/December 2002 versions published in this journal and at www.iasusa.org.

New Addition to the Mutations Figures

This updated version of the figures includes the addition of the V106M mutation to all NNRTI bars—nevirapine, delavirdine, and efavirenz. The V106M mutation has also been added to the first multi-NNRTI resistance bar on the figures.

In the recently published study[1], investigators performed genotypic analysis to ascertain prevalence of V106 (GTG) and 106M (ATG) codons in clinical isolates. Most subtype B isolates harbored GTA (valine) at codon 106 (97%), and the GTG (valine) polymorphism was generally present in clade C viruses (94%).

In addition, cell-based phenotypic assays demonstrated that under conditions of efavirenz (but not nevirapine or delavirdine) pressure in tissue culture, clade C isolates developed the V106M mutation (GTG←ATG), conferring high-level (100- to 1000-fold IC_{50} change) cross-resistance to all NNRTIs.[1]

As stated in the new User Note 12, this mutation has been observed only in HIV clade C clinical isolates. However, site-directed mutagenesis indicates that the V106M mutation confers cross-resistance to all NNRTIs in HIV clade B virus.

The IAS–USA Mutations Figures are available on a pocket-sized folding card.

Copies of the card can be ordered by:

- **Calling the IAS–USA at (415) 544-9400**
- **Visiting www.iasusa.org/resistance_mutations/index.html, and printing out the request form and faxing to (415) 544-9401 or mailing to IAS–USA, 425 California Street, Suite 1450, San Francisco, CA, 94104-2120**
- **E-mailing resistance@iasusa.org for ordering information**

Also in this new version of the figures, enfuvirtide (T-20) is no longer listed as available only through expanded access. The drug was approved by the US Food and Drug Administration in March 2003, marking the first approval in the fusion inhibitor class of anti-HIV agents.

Comments?

The IAS–USA Drug Resistance Mutations Group welcomes comments on the mutations figures and user notes. Please send your evidence-based comments, including relevant reference citations, to the IAS–USA at resistance@iasusa.org or by fax at (415) 544-9401. Please include your name and institution.

Reference

1. Brenner B, Turner D, Oliveira M, et al. A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to nonnucleoside reverse transcriptase inhibitors. *AIDS.* 2003;17:F1-F5.

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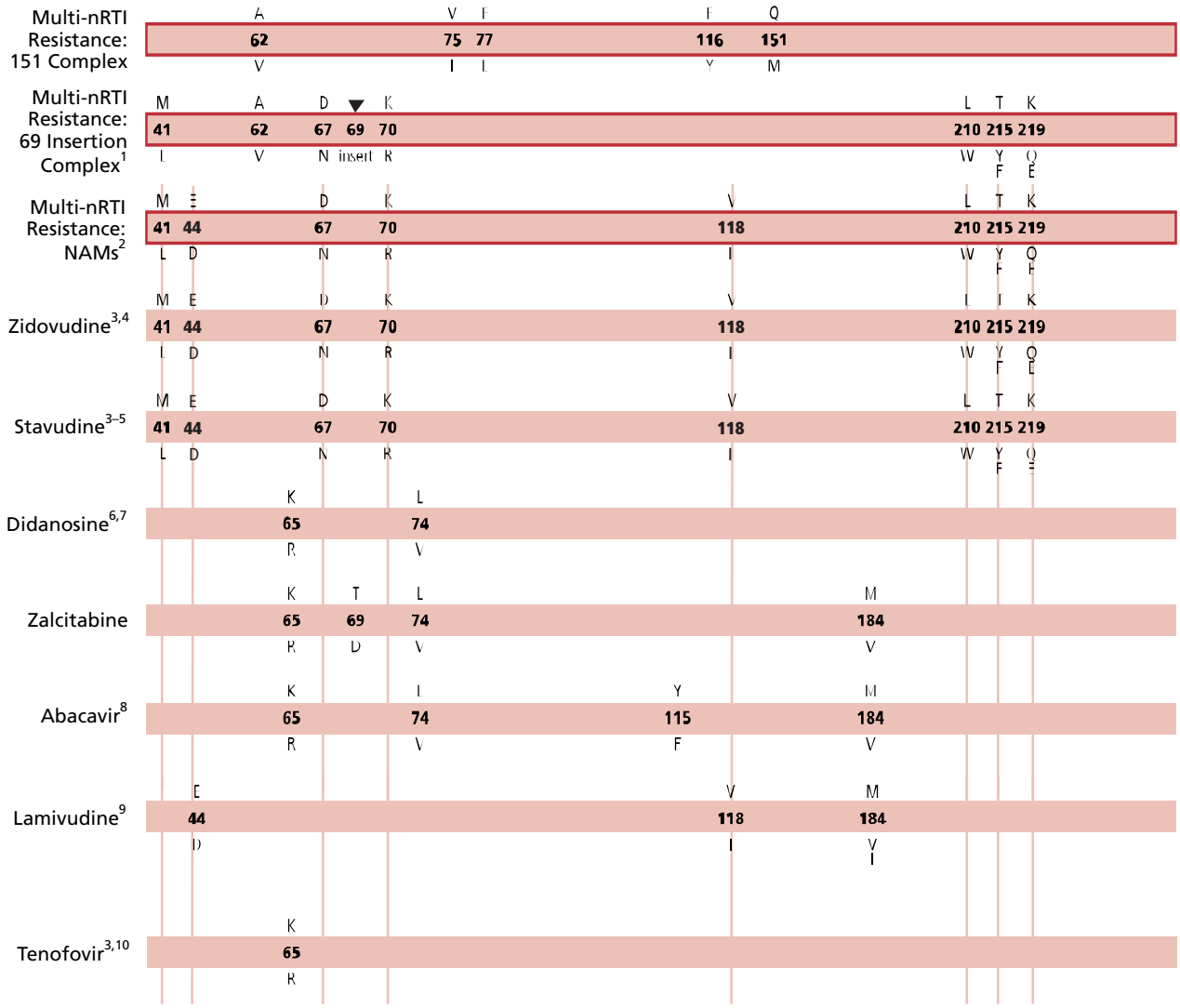
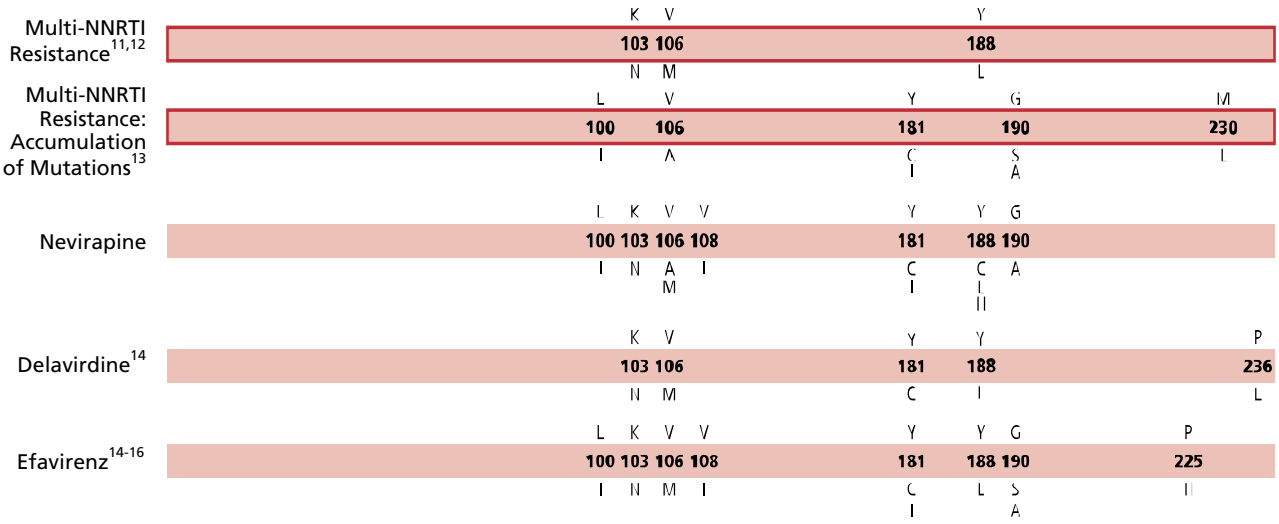
Date of Revision: March 15, 2003

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

Nucleoside and Nucleotide Reverse Transcriptase Inhibitors



Nonnucleoside Reverse Transcriptase Inhibitors



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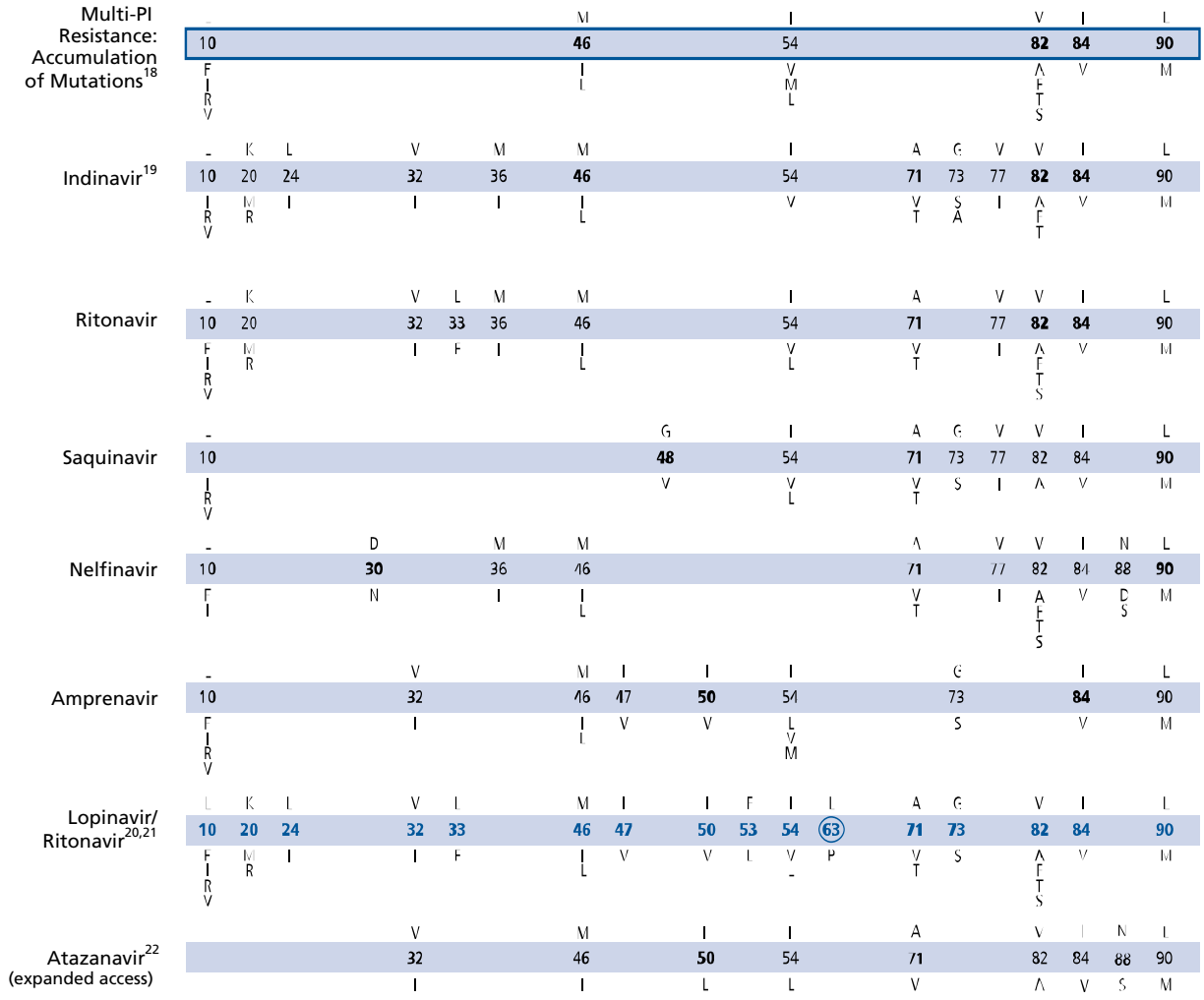
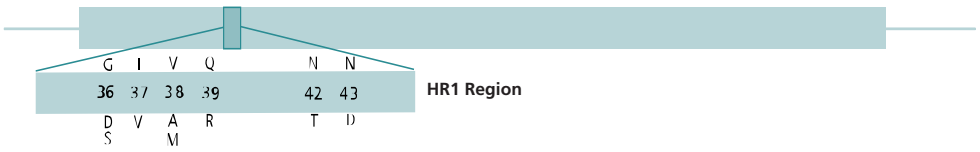
MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS

Protease Inhibitors[17]

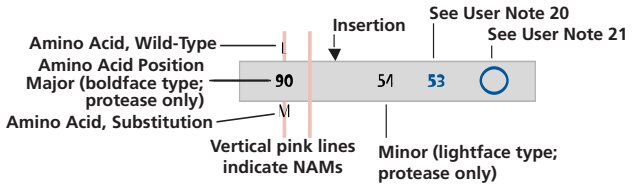


MUTATIONS IN THE GP41 ENVELOPE GENE ASSOCIATED WITH RESISTANCE TO ENTRY INHIBITORS

Enfuvirtide[23]



MUTATIONS



For each amino acid residue, the letter above the bar indicates the amino acid associated with wild-type virus and the letter(s) below indicate the substitution(s) that confer viral resistance. The number shows the position of the mutation in the protein. Mutations selected by protease inhibitors in Gag cleavage sites are not listed because their contribution to resistance is not yet fully defined. HR1 indicates first heptad repeat; NAMs indicates nRTI-associated mutations; nRTI indicates nucleoside reverse transcriptase inhibitor; NNRTI indicates nonnucleoside reverse transcriptase inhibitor; PI indicates protease inhibitor. The figures were last published in *Topics in HIV Medicine* in November/December 2002.

Amino acid abbreviations: A, alanine; C, cysteine; D, aspartate; E, glutamate; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

Date of Revision: March 15, 2003

User Notes

The IAS–USA Drug Resistance Mutations Group reviews new data on HIV drug resistance in order to maintain a current list of mutations associated with clinical resistance to HIV. This list, presented as the IAS–USA Mutations Figures, includes mutations that may contribute to a reduced virologic response to a drug. These mutations have been identified by one or more of the following criteria: (1) in vitro passage experiments; (2) susceptibility testing of laboratory or clinical isolates; (3) genetic sequencing of viruses from patients in whom the drug is failing; (4) correlation studies between genotype at baseline and virologic response in patients exposed to the drug. Drugs that have been approved by the US Food and Drug Administration (FDA) or are available through expanded access protocols are included. Additional information on the mutations is provided, where necessary, in these user notes.

1. The 69 insertion complex, consisting of a mutation at codon 69 (typically T69S) and followed by an insertion of 2 or more amino acids (S-S, S-A, S-G, or others), is associated with resistance to all FDA-approved nRTIs. The 69 insertion complex is often accompanied by mutations at other sites. Some other amino acid changes from the wild-type T in codon 69 without the insertion may also be associated with broad nRTI resistance.

2. The nRTI-associated mutations (NAMs), including M41L, E44D, D67N, K70R, V118I, L210W, T215Y/F, and K219Q/E, are associated with cross-resistance to nRTIs and are represented by vertical pink lines. Zidovudine and stavudine select for these mutations, and as such, the positions and mutations are indicated on the bars along with the pink lines. For other nRTIs, the NAMs are not commonly selected by those drugs, but the presence of the NAMs confers cross-resistance to the drugs. This is represented by pink lines only at the positions.

The E44D and V118I mutations are listed as NAMs. In a recent study, the E44D and V118I mutations were more common in virus from patients treated with zidovudine and lamivudine, and were associated with higher-level resistance to zidovudine (Kuritzkes et al, *Antimicrob Agents Chemother,* in press). When present together with other NAMs, the E44D and V118I mutations confer resistance to lamivudine. Analysis from the AIDS Clinical Trials Group (ACTG) study 136 has shown that the V118I mutation is commonly selected by a zidovudine/didanosine regimen (Shafer et al, *J Infect Dis,* 1995). Findings from ACTG study 241 have shown that the E44D mutation is commonly selected by zidovudine/didanosine (Hanna et al, *J Infect Dis,* 2002) and that the E44D mutation is associated with a significantly worse response to treatment with zidovudine and didanosine, with or without nevirapine (Precious et al, *AIDS,* 2000). The significance of E44D or V118I when each occurs in isolation is unknown (Romano et al, *J Infect Dis,* 2002; Walter et al, *Antimicrob Agents Chemother,* 2002; Girouard et al, *Antivir Ther,* 2002).

3. The M184V mutation may enhance susceptibility to zidovudine, stavudine, or tenofovir. This effect may be overcome by an accumulation of NAMs or other mutations. The clinical significance of this effect is not known.

4. Recent data on revertant mutations in codon 215 indicate that the T215D/C/S/E/N/A/V substitutions confer increased risk of virologic failure of zidovudine and stavudine in antiretroviral-naive adults starting therapy with these drugs (Riva et al, *Antivir Ther,* 2002). In vitro studies and preliminary clinical studies suggest that the T215Y mutant may emerge quickly from these mutations in the presence of zidovudine or stavudine (Garcia-Lerma et al, *Proc Natl Acad Sci U S A,* 2001; Lanier et al, *Antivir Ther,* 2002; Riva et al, *Antivir Ther,* 2002).

5. Mutations at codon 75 (V75T/M/S/A) have been observed in vitro and may confer a low-level change in susceptibility to stavudine (Lacey et al, *Antimicrob Agents Chemother,* 1994).

6. The K65R mutation or the L74V mutation, alone or in combination with the NAMs and/or T69D/N can lead to didanosine resistance.

7. Based on preliminary, yet-unpublished data, the M184V mutation does not appear to have a negative impact on in vivo responses to didanosine, even though the mutation reduces susceptibility in vitro (Winters et al, *Antivir Ther,* 2002; Eron et al, *Antivir Ther,* 2002; Pozniak et al, *Antivir Ther*, 2002).

8. When present with NAMs, the M184V mutation contributes to reduced susceptibility to abacavir and is associated with impaired response in vivo. However, when present alone, the M184V mutation does not appear to be associated with a reduced virologic response to abacavir in vivo (Harrigan et al, *J Infect Dis,* 2000).

9. The E44D and V118I mutations were reported to confer low-level resistance to lamivudine when accompanied by several other nRTI-associated mutations (M41L, D67N, L210W, T215Y/F, K219Q/E) in the absence of a concurrent M184V mutation (Hertogs et al, *Antimicrob Agents Chemother,* 2000). Data presented but not yet published (D'Arminio-Monforte et al, 8th CROI, 2001), reported no association over the short term between E44D or V118I and virologic response to a lamivudine-containing combination regimen. (See also User Note 2.)

10. The accumulation of NAMs (M41L, D67N, K70R, L210W, T215Y/F, K219Q/E [note: data here do not include E44D and V118I]) increases resistance to tenofovir. Mutations M41L and L210W contribute more than others. Therefore, the number and type of NAMs will determine the degree of reduced response. T69D/N/S may also contribute to a reduced response to tenofovir (Miller et al, *Antivir Ther,* 2002; Lu et al, *Antivir Ther,* 2002; Masquelier et al, *Antivir Ther,* 2002).

11. The K103N or Y188L mutation alone can substantially reduce the clinical utility of all currently approved NNRTIs.

12. The V106M mutation confers high-level resistance in vitro to nevirapine, delavirdine, and efavirenz (Brenner et al, *AIDS,* 2003). This mutation has been observed only in HIV clade C clinical isolates, although site-directed mutagenesis indicates that V106M confers cross-resistance to all NNRTIs in HIV clade B virus.

13. Accumulation of 2 or more of these mutations substantially reduces the clinical utility of all of the currently approved NNRTIs.

14. The prevalence of the Y318F mutation in clinical isolates along with mutations K103N, Y181C, or P236L was approximately 5%, 2%, and 15%, respectively (Kemp et al, *Antivir Ther,* 2001). In vitro this mutation confers resistance to nevirapine, delavirdine, and efavirenz.

15. The Y181C/I mutation is not selected by efavirenz, but its presence contributes to low-level cross-resistance to the drug. Clinical impact of this mutation may be overcome with a fully active antiretroviral combination regimen, although no clinical trial data yet address this question.

16. V108I and P225H each contribute to efavirenz resistance when present in combination with other NNRTI-associated mutations. Although V108I or P225H alone does not confer measurable resistance in labora-

tory strains of HIV-1, their presence in a clinical isolate may indicate prior selection for efavirenz-resistant variants.

17. Resistance mutations in the protease gene are classified as either "major" or "minor" (if known).

> Major: In general, major mutations are either (1) selected first in the presence of the drug; or (2) shown at the biochemical or virologic level to lead to an alteration in drug binding or an inhibition of viral activity or viral replication. By themselves, major mutations have an effect on phenotype. In general, these mutations tend to be the major contact residues for drug binding.
>
> Minor: In general, minor mutations appear later than major mutations, and by themselves do not have a significant effect on phenotype. In some cases, their effect may be to improve replicative fitness of virus carrying major mutations.

18. Accumulation of 4 or more of these mutations is likely to cause multi-PI resistance (Palmer et al, *AIDS,* 1999; Shafer et al, *Ann Intern Med,* 1998).

19. For indinavir, the mutations listed as major may not be the first mutations selected, but they are present in most clinical isolates in combination with other mutations.

20. Major and minor mutations have not been designated for lopinavir/ritonavir-associated resistance since currently there are no clear data defining degrees of influence with this drug combination. The accumulation of 6 or more of these mutations is associated with a diminished response to lopinavir/ritonavir. The product information states that accumulation of 7 or 8 mutations confers resistance to the drug. However, recent data suggest as few as 4 mutations can be associated with such high-level resistance (Prado et al, *AIDS,* 2002). Further clinical experience and research are needed to better define the mutations that affect the clinical effectiveness of lopinavir/ritonavir. It is reasonable to consider phenotyping to assess this in individual cases.

21. Protease mutation L63P is common in viruses that have never been exposed to PIs (Kozal et al, *Nat Med,* 1996) and may be more prevalent in viruses from patients in whom a PI-containing regimen has failed. However, by itself, L63P does not cause any appreciable increase in the IC_{50} for any PI. L63P is listed for lopinavir/ritonavir (and not any other PI) because studies have shown that this mutation, when present with multiple other mutations, is associated with clinical failure.

22. Atazanavir is currently available through an expanded access protocol and is not approved by the US FDA. When administered to patients as the initial PI, atazanavir selects for the mutations I50L and A71V (Colonno et al, *Antivir Ther,* 2002). When used as a subsequent PI in combination with saquinavir, atazanavir selects for I54L and I84V (Colonno et al, *Antivir Ther,* 2002). In vitro, atazanavir selects for V32I, M46I, I84V, and N88S (Gong et al, *Antimicrob Agents Chemother,* 2000). Although other major mutations, such as V82A and L90M, have not been selected for by atazanavir either in vitro or in vivo, these mutations have been shown to confer cross-resistance to atazanavir, particularly when present in combination with each other or with other known PI resistance mutations (Colonno et al, *Antivir Ther*, 2000).

23. To date, resistance mutations in the gp41 envelope gene have been identified primarily at positions 36 to 45 of the first heptad repeat (HR1) region. These mutations have been identified in viruses from patients treated with the drug and have been shown to confer resistance or reduced susceptibility (Wei et al, *Antimicrob Agents Chemother,* 2002; Sista et al, *Antivir Ther,* 2002; Mink et al, *Antivir Ther,* 2002). It is important to note that wild-type viruses in this region show a 500-fold range in susceptibility, and mutations in other regions in the envelope may affect susceptibility to enfuvirtide. Further research is needed to evaluate the clinical relevance of these mutations.

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Advances in Antiretroviral Therapy

Mary A. Albrecht, MD, Timothy J. Wilkin, MD, Eoin P. G. Coakley, MD, and Scott M. Hammer, MD

As witnessed in previous years, antiretroviral therapy was a dominant theme of the 10th Conference on Retroviruses and Opportunistic Infections, with important information for clinicians presented in the areas of new antiretroviral agents, management of treatment-naive and -experienced patients, treatment strategies (particularly treatment interruptions), and drug resistance. This review will highlight the major findings presented at the conference from studies performed in the developed world. One important new aspect of this year's meeting, however, was the reporting of experiences with antiretroviral agents in the developing world. These reports will not be summarized here, but readers may visit the conference Web site for more information concerning these sessions (www.retroconference.org).

Investigational Antiretroviral Agents

Results of select studies on investigational antiretroviral agents are summarized in Table 1.

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Entry Inhibitors

CCR5 Antagonists. AK-602 is a CCR5 inhibitor in preclinical development. In vitro studies suggest that it preferentially blocks the HIV-CCR5 interaction and has less effect on the interaction of CCR5 and chemokines such as RANTES and MIP-1β (Abstracts 10 and 564a). It suppresses HIV replication and is orally bioavailable in the SCID-Hu mouse model. Although CCR5 Δ32 homozygosity appears to have no untoward effects in humans, CCR5 inhibitors, such as SCH-C and TAK-779, that affect chemokine-CCR5 interactions are, in fact, being studied in organ transplant recipients as possible immune modulators. This immune modulation is probably not a desirable quality when treating HIV infection, suggesting that CCR5 inhibitors that do not affect chemokine-CCR5 binding may be preferable to those that do. AK-602 has potent activity against a wide panel of primary R5 and multidrug-resistant isolates (50% inhibitory concentration [IC_{50}] 0.2-0.6 nM).

TAK-220 is a CCR5 inhibitor that is orally bioavailable, unlike manufacturer Takeda Chemical Industries' previous CCR5 inhibitor, TAK-779 (Abstracts 11 and 562). The authors did not present the structure of the new compound but did say that it was not similar to TAK-779. TAK-220 appears to bind specifically to CCR5 (not CCR1, CCR3, or other chemokine receptors). It is active in vitro against primary R5 viruses, including those resistant to other available drugs (50% effective concentration [EC_{50}] 1.1 nM and 90% effective concentration [EC_{90}] 13 nM), and appears synergistic with other antiretrovirals against wild-type R5 virus.

UK-427,857 is a CCR5 inhibitor that has entered phase 1 studies. Studies suggest that it is specific for CCR5-virus interactions and, consistent with the mechanism, is not active against X4 viruses (Abstracts 12, 546a, and 547). It is active against a broad range of viral isolates in vitro, including non-B subtypes. Phase 1 safety studies in HIV-seronegative individuals showed no obvious toxicities (including QTc interval prolongation) and that it was well-tolerated at a range of doses given for up to 12 days. The IC_{50} for HIV replication is 0.2 nM, and the IC_{50} for binding of MIP-1β is 3 to 7 nM. Pharmacokinetic studies in humans showed good absorption, with a terminal half-life of 17 hours with repeated doses.

CXCR4 Antagonist. AMD070 is an orally bioavailable CXCR4 inhibitor active in vitro against a wide variety of X4 viruses and R5/X4 dual tropic viruses (EC_{50} 1-10 nM), but not R5 viruses (Abstract 563). It is being developed by Anormed, whose development of AMD-3100 was stopped because of suboptimal efficacy but did establish proof of concept for targeting CXCR4. AMD070 appears to be either additive to or synergistic with other antiretrovirals and does not interact with any other chemokine receptor tested. Phase 1 studies are planned.

Monoclonal Antibody to CD4. TNX-355 is a human monoclonal antibody (IgG4) to CD4 formerly known as Hu5A8 (Abstract 13). It does not prevent attachment of HIV to CD4 but does prevent subsequent interactions. No immunosuppressive effects were noted in previous studies with peripheral blood lymphocytes or rhesus macaques. In this study, HIV-infected individuals on stable antiretroviral therapy or no antiretroviral therapy, and with plasma HIV-1 RNA levels of greater than 5000 copies/mL and CD4+ counts of greater than 100 cells/μL, were given a single dose of the agent at increasing amounts with successive cohorts. The mean baseline CD4+ count and plasma HIV-1 RNA levels were 354 cells/μL and 4.78 $\log_{10}$ copies/mL, respectively. Mean drops in plasma HIV-1 RNA of 1.48 $\log_{10}$ and 1.09 $\log_{10}$ were achieved at the 2 highest doses (10 mg/kg and 25 mg/kg), respec-

Table **1.** New Antiretroviral Agents

Drug Name	Abstract Nos.	Mechanism	Development Stage	Results
AK-602	10, 564a	Entry inhibitor (CCR5)	Preclinical	IC_{50} 0.2-0.6 nM
TAK-220	11, 562	Entry inhibitor (CCR5)	Preclinical	EC_{50} 1.1 nM
UK-427,857	12, 546a, 547	Entry inhibitor (CCR5)	Preclinical; phase 1 studies in HIV-seronegative subjects	IC_{50} 0.2 nM
AMD070	563	Entry inhibitor (CXCR4)	Preclinical	EC_{50} 1-10 nM
TNX-355	13	Fusion inhibitor (human monoclonal antibody)	Phase 1 studies in HIV-infected subjects	1.09-1.5 $\log_{10}$ copies/mL drop in plasma HIV-1 RNA levels after single dose
T-1249	14lb	Fusion inhibitor	Phase 2 studies in HIV-infected subjects	1.1 $\log_{10}$ copies/mL drop in plasma HIV-1 RNA levels after 11 days as functional monotherapy[1]
Racivir	552	Nucleoside reverse transcriptase inhibitor	Phase 2 studies in HIV-infected subjects	2.1-2.6 $\log_{10}$ copies/mL drop in plasma HIV-1 RNA levels after 28 days (given with stavudine/efavirenz)
V-165	9, 556	Integrase inhibitor (inhibits initial interaction of integrase and DNA)	Preclinical	EC_{50} 8.9 μM
PA-457	14	Gag processing inhibitor (inhibits processing of p24 capsid protein)	Preclinical	Not available
RO-033-4649	7	Protease inhibitor	Preclinical	IC_{50} 17 nM for wild-type viruses; IC_{50} 100 nM for highly PI-resistant viruses
TMC114	8, 549, 553	Protease inhibitor boosted with low-dose ritonavir	Phase 2 studies in HIV-infected subjects	1.1-1.5 $\log_{10}$ copies/mL reduction in plasma HIV-1 RNA levels after 14 days as functional monotherapy[2]

[1]This study substituted T-1249 for enfuvirtide (T-20) as the sole change in a failing antiretroviral regimen. Better efficacy was seen with a shorter time on the failing regimen containing enfuvirtide. [2]Participants were multiple-PI-experienced, and their PI-containing regimen was failing; the median baseline plasma HIV-1 RNA was 4.3 $\log_{10}$ copies/mL. TMC114/ritonavir was substituted for the failing PI or PIs as the sole change in antiretroviral therapy for 14 days.

IC_{50} indicates 50% inhibitory concentration; EC_{50}, 50% effective concentration.

tively. The nadir plasma HIV-1 RNA level was achieved at days 14 and 21, respectively. These days coincided with the duration of antibody coating of CD4, giving further support for the proposed mechanism of action. No CD4+ cell depletion was noted; in fact, CD4+ cell count increases were seen for the 3 highest dose groups (3, 10, and 25 mg/kg). No resistance has been created to date.

Fusion Inhibitor. Miralles and colleagues presented interim results of a study evaluating T-1249 in patients in whom a regimen containing enfuvirtide (T-20) was failing (Abstract 14lb). T-1249 is a fusion inhibitor similar to enfuvirtide given by subcutaneous injection once daily. It was substituted for enfuvirtide for 10 days as the sole change in the antiretroviral regimen. The endpoint was change in plasma HIV-1 RNA level at day 11. Participants were eligible if they were in a phase 2 or 3 enfuvirtide trial and their enfuvirtide regimen was failing, with a plasma HIV-1 RNA level of between 5000 and 500,000 copies/mL. The baseline plasma HIV-1 RNA level was 5 $\log_{10}$ copies/mL and the median duration of enfuvirtide use was 70 weeks. Overall, the median drop in plasma HIV-1 RNA was 1.12 $\log_{10}$ copies/mL. The drop in plasma HIV-1 RNA levels after substitution with T-1249 was related to the time on a failing regimen containing enfuvirtide: 7 of 7 patients in whom enfuvirtide failed for 24 to 48 weeks achieved greater than 1 $\log_{10}$ drop in plasma HIV-1 RNA, compared with 8 of 17 in whom the drug failed for more than 48 weeks. Presumably, this difference was due to the accumulation of more enfuvirtide-associated resistance mutations with longer exposure to the drug.

Integrase Inhibitors

V-165. V-165 is a new type of integrase inhibitor from the pyranodipyrimidine class that inhibits the binding of DNA to integrase (Abstracts 9 and 556). It is structurally different from the 2 integrase inhibitors currently in phase 1 trials—S-1360 from Shinogi and L-870,810 from Merck. S-1360 and L-870,810 are diketoacid and naphthyridine compounds, respectively, but they overlap in their resistance profiles (Abstract 140). V-165 has a different resistance pattern than these agents and is active against isolates resistant to the diketoacids. It is also active against nonnucleoside reverse transcriptase inhibitor (NNRTI)-, nucleoside reverse transcriptase inhibitor (nRTI)-, and fusion inhibitor-resistant viruses and is synergistic with zidovudine and nelfinavir versus wild-type virus. The in vitro potency (EC_{50} 8.9 µM) is comparable to that for the Merck integrase inhibitor, L-870,810.

Gag Processing Inhibitor

PA-457. PA-457 appears to target a new point in the HIV-1 life cycle: Gag processing, or specifically, the conversion of capsid protein p25 to p24 (Abstract 14). Martin and colleagues presented data supporting this mechanism of action, including electron micrographs showing morphologically defective HIV virions similar to those known to have a defect in the processing of p25 to p24. PA-457 does not act at other points in the HIV life cycle, such as fusion, reverse transcriptase, integrase, or protease. The inhibitor's specific molecular target, however, is unknown. Previous work has shown that it is orally bioavailable in rats. PA-457 is effective in vitro at low nM concentrations against a wide panel of isolates, including wild-type and resistant viruses, and is synergistic with other classes of antiretrovirals.

Reverse Transcriptase Inhibitors

Emtricitabine. Emtricitabine (FTC) is a cytosine analogue that has demonstrated potent activity against HIV-1. Wakeford and colleagues presented long-term results from the combined FTC 303 and FTC 350 trials, which evaluated the efficacy and safety of emtricitabine in HIV-infected subjects who switched from a lamivudine-containing regimen (Abstract 550). The parent study, emtricitabine 303, was a randomized, open-label, 48-week trial comparing emtricitabine 200 mg once daily with lamivudine 150 mg twice daily in 440 HIV-infected subjects who had achieved plasma HIV-1 RNA suppression of less than 400 copies/mL on a lamivudine-containing triple-drug regimen for at least 12 weeks prior to study entry. Subjects were randomized either to continue lamivudine therapy (n = 146) or to switch to emtricitabine (n = 294) within their current antiretroviral drug regimen. Subjects who maintained plasma HIV-1 RNA suppression of less than 400 copies/mL at week 48 of the FTC 303 study were then offered the option of participating in a rollover extension study, FTC 350, which evaluated the use of emtricitabine 200 mg once daily. The baseline median plasma HIV-1 RNA level and CD4+ cell count were 1.7 $\log_{10}$ copies/mL and 484 cells/µL, respectively.

At week 48, 77% of the subjects (n = 227) randomized to emtricitabine therapy achieved suppression of plasma HIV-1 RNA to less than 400 copies/mL. Of these 227 patients, 215 chose to continue emtricitabine therapy in the emtricitabine 350 study. After a median time of 140 weeks of emtricitabine treatment, 164 (56%) of 294 subjects in FTC 303 had discontinued emtricitabine therapy or had chosen not to enroll in emtricitabine 350. The majority of the subjects in FTC 350 (79%) received a protease inhibitor (PI)-containing highly active antiretroviral therapy (HAART) regimen; the remaining 21% of subjects received an NNRTI-based antiretroviral therapy regimen. With the Kaplan-Meier method, the probability of experiencing virologic failure (defined as plasma HIV-1 RNA level of at least 400 copies/mL on 2 consecutive visits) at 4 years was estimated at 11%.

Amdoxovir. Amdoxovir (DAPD; -b-D-2,6-diaminopurine dioxolane), a dioxalane guanosine analogue, is a novel nRTI inhibitor of HIV-1 replication in vitro. Amdoxovir is deaminated by adenosine deaminase to produce (-)–b-D-dioxolane guanine (DXG), which is the active moiety that is the substrate for HIV reverse transcriptase. In vitro, DXG has antiviral activity against zidovudine/lamivudine- and stavudine/lamivudine-resistant strains of HIV and those with a mutation at the codon 69 insert multidrug-resistance locus. After multiple passages of the virus in the presence of amdoxovir, 2 mutations in the reverse transcriptase gene have emerged: K65R and L74V.

Thompson and colleagues presented

the preliminary results from the DAPD-150 trial, a 96-week, open-label, 2-arm, phase 1/2 clinical study. The study evaluated the efficacy and safety of amdoxovir at 2 different doses in combination with background antiretroviral therapy in heavily treatment-experienced subjects (Abstract 554). Patients with a screening plasma HIV-1 RNA level between 5000 and 250,000 copies/mL and a CD4+ count of at least 50 cells/μL, and who had virologic failure of a prior zidovudine/lamivudine- or stavudine/lamivudine-containing antiretroviral therapy regimen, were eligible. A total of 18 HIV-infected subjects (94% male) with a mean age of 40 years, a median duration of 8 years of prior exposure to a median of 10 antiretroviral therapy drugs, and a median number of 3 nRTI mutations were enrolled. Median baseline plasma HIV-1 RNA level and CD4+ count were 4.41 $\log_{10}$ copies/mL and 326 cells/μL, respectively. Subjects were randomized to receive amdoxovir 300 mg twice daily (n = 8) or amdoxovir 500 mg twice daily (n = 10) in combination with optimized background antiretroviral therapy.

Of the 18 patients enrolled in this study, 11 discontinued the study for the following reasons: lens opacity (5), virologic failure (4), and voluntary withdrawal/noncompliance (2). The 300 mg arm achieved a median decrease from baseline in plasma HIV-1 RNA of 1.53 $\log_{10}$ copies/mL, compared with a 0.75 $\log_{10}$ copies/mL decline in plasma HIV-1 RNA levels in the 500 mg arm. These decreases were maintained through week 24. A median rise in CD4+ count from baseline to week 12 of 55 cells/μL was observed in both study arms. There were no serious adverse events reported in either study arm, and amdoxovir was well-tolerated at both doses. In animal toxicology studies, high doses of amdoxovir were associated with obstructive uropathy in monkeys and rats and with the development of elevated serum glucose levels and cataract formation in some monkeys. As a result, the DAPD-150 protocol was amended to require complete ophthalmologic assessments, with slit-lamp examinations at baseline in newly enrolled subjects and on a bimonthly basis to identify lens opacities in patients receiving amdoxovir. Five patients were documented to have lens opacities on formal ophthalmologic exam and were discontinued from study treatment. Since none of these 5 patients had ophthalmologic exams performed at baseline, it was unclear whether the lens opacities in these subjects were related to amdoxovir exposure.

Racivir. Racivir ([±]-2-hydroxymethyl–5 [5-fluorocytosine-1-yl]-1,3-oxathiolane) is an investigational nRTI that exhibits potent, highly selective activity against HIV-1 and hepatitis B virus in cell cultures and in animal models. This drug is composed of a mixture of emtricitabine with its positive enantiomer. This drug has been well-tolerated in preclinical safety studies conducted in dogs and rats and possesses an excellent oral bioavailability profile in animals and humans, which makes once-daily dosing feasible. Otto and colleagues presented the results of a dose-ranging phase 1b/2a study (Abstract 552) evaluating the antiviral activity and safety of racivir used at 3 different doses in combination with stavudine and efavirenz in HIV-infected, treatment-naive men. Subjects with plasma HIV-1 RNA levels greater than 5000 copies/mL and CD4+ counts greater than 50 cells/μL received racivir at a dose of 200 mg, 400 mg, or 600 mg once daily plus stavudine 40 mg twice daily and efavirenz 600 mg once daily for 14 days. Patients in all 3 racivir dosing arms achieved an initial rapid decline in plasma HIV-1 RNA levels and sustained a mean reduction in those levels ranging from 1.13 to 1.42 $\log_{10}$ copies/mL by day 4. Mean reductions in plasma HIV-1 RNA level ranging from 2.02 to 2.43 $\log_{10}$ copies/mL were seen by day 14. After stopping antiretroviral drugs on day 15, all 3 racivir dose groups maintained suppression of plasma HIV-1 RNA for more than 2 weeks; mean plasma HIV-1 RNA declines ranged from 2.1 to 2.6 $\log_{10}$ below baseline though day 28. At day 35, plasma HIV-1 RNA levels remained more than 1.0 $\log_{10}$ copies/mL below baseline values. All 3 doses of racivir were well-tolerated.

PIs

GW433908. GW433908, the prodrug formulation of amprenavir, is an investigational PI with a distinct resistance profile and no food restrictions for dosing. Nadler and colleagues presented the results of the NEAT study, an open-label, randomized trial (Abstract 177) that compared the efficacy and safety of GW433908 with that of nelfinavir over 48 weeks in antiretroviral therapy-naive HIV-infected subjects. A total of 251 subjects with plasma HIV-1 RNA levels greater than 5000 copies/mL and no CD4+ entry criteria were randomized in a 1:2 fashion to GW433908 1400 mg twice daily or nelfinavir 1250 mg twice daily. All patients also received abacavir and lamivudine twice daily. The primary endpoint was suppression of HIV-1 RNA to less than 400 copies/mL. At baseline, median plasma HIV-1 RNA levels were 4.82 and 4.85 $\log_{10}$ copies/mL in the GW433908 and nelfinavir arms, respectively; 44% and 48% of subjects had plasma HIV-1 RNA levels greater than 100,000 copies/mL. The median baseline CD4+ counts were 214 and 212 cells/μL in the GW433908 and nelfinavir arms, respectively.

At week 48, 66% of subjects in the GW433908 arm had achieved suppression of HIV-1 RNA to less than 400 copies/mL compared with 51% of subjects in the nelfinavir arm, and 55% and 41%, respectively, had achieved suppression to less than 50 copies/mL (intent-to-treat [ITT] analysis, rebound = failure). Sixty-seven percent of subjects in the GW433908 arm with HIV-1 RNA greater than 100,000 copies/mL at study entry achieved plasma HIV-1 RNA suppression to less than 400 copies/mL, compared with 35% of subjects in the nelfinavir arm. A median CD4+ count increase of 201 cells/μL from baseline at week 48 was seen in the GW433908 arm, compared with an increase of 216 cells/μL in the nelfinavir arm.

The GW433908 arm sustained increases in mean total cholesterol (from 152 to 197 mg/dL) and low-density lipoprotein (LDL) cholesterol levels (86 to 119 mg/dL) from baseline to 48 weeks that were similar to those observed in the nelfinavir arm (total cholesterol, 153 to 202 mg/dL; LDL cholesterol, 89 to 122 mg/dL). The nelfinavir arm, however, sustained an increase in mean triglyceride levels (154 to 200 mg/dL) by week 48. The nelfi-

navir arm had a higher incidence of diarrhea than did the GW433908 arm (18% vs 5%; $P < .002$), whereas rash was observed more frequently in the GW433908 arm (7%) than in the nelfinavir arm (2%). In this study population of treatment-naive subjects with moderately advanced HIV disease, therefore, GW433908 therapy was well-tolerated and conferred superior plasma HIV-1 RNA suppression at week 48 than did nelfinavir.

DeJesus and colleagues presented the week 24 results of the multicenter, randomized, open-label CONTEXT study (Abstract 178), which compared the efficacy and safety of GW433908/ritonavir, dosed once daily or twice daily, with lopinavir 400 mg/ritonavir 100 mg twice daily in PI-experienced subjects over 48 weeks. Antiretroviral therapy-experienced subjects with prior exposure to 1 or 2 PIs who were NNRTI-naive or -experienced, had a screening HIV-1 RNA level of at least 1000 copies/mL, and had any CD4+ cell count were eligible for study participation.

A total of 320 patients with median baseline plasma HIV-1 RNA level and CD4+ cell count of 4.14 $\log_{10}$ copies/mL and 263 cells/µL, respectively, were randomized in 1:1:1 manner to GW433908 1400 mg/ritonavir 200 mg once daily (n = 105), GW433908 700 mg/ritonavir 100 mg twice daily (n = 107), or lopinavir 400 mg/ritonavir 100 mg twice daily (n = 103). Each regimen included 2 active nRTI agents selected on the basis of genotypic testing. The study population was extensively NNRTI-experienced, with 52%, 60%, and 60% of subjects in the GW433908/ritonavir once daily, GW433908/ritonavir twice daily, and lopinavir/ritonavir twice daily arms, respectively, having received an NNRTI prior to study entry. The study population also had extensive prior use of nRTIs.

Using the primary endpoint of plasma HIV-1 RNA reduction as measured by the mean time-averaged change from baseline, the week 24 plasma HIV-1 RNA decreases were as follows: 1.48 $\log_{10}$ in the GW433908 once daily arm, 1.50 $\log_{10}$ in the GW433908 twice daily arm, and 1.66 $\log_{10}$ in the lopinavir/ritonavir twice daily arm. At week 24, 58% [40%], 60% [42%], and 69% [48%], respectively, of subjects achieved plasma HIV-1 RNA of less than 400 [less than 50] copies/mL. The lopinavir/ritonavir arm experienced fewer virologic failures than the GW433908 study arms (34%, 27%, and 21% in the GW433908/ritonavir once daily, GW433908/ritonavir twice daily, and lopinavir/ritonavir twice daily arms, respectively.) Nonvirologic treatment failures (8%, 10%, and 9%, respectively) were similar in all 3 arms, however. The median increase from baseline in CD4+ count ranged from 62 to 72 cells/µL at week 24 in the 3 arms. The study drug regimens were generally well-tolerated.

Atazanavir. Atazanavir is an azapeptide investigational PI administered on a once-daily dosing schedule that does not result in elevations in serum lipids. Murphy and colleagues presented the long-term results of the rollover/switch BMS 044 study (Abstract 555). The study evaluated the efficacy and safety of extended-use atazanavir in combination with stavudine and lamivudine in HIV-infected patients who were originally treated with atazanavir in the BMS 008 study or who switched from a nelfinavir-containing regimen to atazanavir. A total of 346 patients with plasma HIV-1 RNA levels of less than 10,000 copies/mL who had completed the BMS 008 study were randomized to continue atazanavir treatment at 400 mg once daily (n = 139) or at 600 mg once daily (n = 144), or to switch from nelfinavir to atazanavir at 400 mg once daily (n = 63). At baseline, median plasma HIV-1 RNA level and CD4+ count were 1.73 $\log_{10}$ copies/mL and 495 cells/µL, respectively, and 75% of patients had plasma HIV-1 RNA levels of less than 400 copies/mL.

At week 24, the proportion of subjects who achieved HIV-1 RNA levels less than 400 copies/mL [50 copies/mL] were as follows in the 3 study arms: 80% [58%] in the continued atazanavir 400 mg arm; 82% [54%] in the continued atazanavir 600 mg arm; and 86% [59%] in the switch arm. The median increases in CD4+ count at week 24 were 39, 34, and 33 cells/µL, respectively. For the nelfinavir-to-atazanavir switch arm, there were significant changes in total cholesterol (-16%), high-density lipoprotein (HDL) cholesterol (+5%), fasting LDL cholesterol (-20%), and fasting triglyceride levels (-25%). A switch from nelfinavir to atazanavir was associated with a low incidence (2%) of diarrhea. Elevations in total bilirubin (predominantly unconjugated) were the most frequent laboratory abnormality, with 26%, 44%, and 13% of patients in the continued atazanavir 400 mg, continued atazanavir 600 mg, and switch arms, respectively, experiencing grade 3 or 4 elevations in serum bilirubin. The investigators concluded that extended use of atazanavir/stavudine/lamivudine in treatment-naive HIV-infected patients results in sustained virologic suppression and continued increases in CD4+ cell counts with minimal changes in cholesterol, fasting LDL, and fasting triglyceride levels.

Tipranavir. Tipranavir, a novel nonpeptidic PI, exhibits a unique resistance profile and has demonstrated potent antiviral activity against multiple-PI-resistant isolates in vitro. Gathe and colleagues presented the results of the BI 1182.52 trial (Abstracts 179 and 528), a multicenter, randomized, blinded, phase 2 dose-finding study that evaluated 3 different tipranavir/ritonavir doses in highly treatment-experienced HIV-infected subjects. Entry criteria required subjects to have HIV-1 RNA levels greater than 1000 copies/mL, any CD4+ cell count, prior exposure to all 3 initial antiretroviral therapy classes, virologic failure of at least 2 PI-based regimens, and the presence of at least one or more major mutations in the protease gene (D30N, M46I/L, G48V, I50V, V82A/F/L/T, I84V, or L90M) but not more than one of V82L/T, I84V, or L90M. Three doses of tipranavir/ritonavir were evaluated: 500 mg/100 mg; 500 mg/200 mg; and 750 mg/200 mg. At study entry, subjects' current PI therapy was replaced with tipranavir/ritonavir, which was continued for 2 weeks prior to optimizing background therapy. A total of 216 patients with baseline median plasma HIV-1 RNA level and CD4+ count of 4.53 $\log_{10}$ copies/mL and 153 cells/µL, respectively, were randomized. Baseline genotypic and phenotypic resistance testing were conduct-

ed, which confirmed that the viral isolates within this study population had extensive resistance to currently available PIs.

At day 14, plasma HIV-1 RNA responses were as follows: the 500 mg/100 mg arm achieved a reduction in plasma HIV-1 RNA of 0.87 $\log_{10}$ copies/mL; the 500 mg/200 mg arm, a reduction of 0.97 $\log_{10}$ copies/mL; and the 750 mg/200 mg arm, a reduction of 1.18 $\log_{10}$ copies/mL. At day 14, 20% of participants had no changes made to their antiretroviral therapy background regimen due to lack of available active agents. All study arms maintained at least a 1.0 $\log_{10}$ decrease in plasma HIV-1 RNA from baseline through day 56. All patients had at least 5 PI mutations at study entry. Patients were subsequently grouped according to the number of mutations: 6 to 10 mutations; 11 to 15 mutations; 15 to 20 mutations; or more than 20 mutations. The reductions in plasma HIV-1 RNA from baseline were at least 0.8 $\log_{10}$ copies/mL regardless of the number of baseline PI mutations and, in the 500 mg/200 mg and 750 mg/200 mg dosing arms, ranged to 1.2 $\log_{10}$ copies/mL. Patients in the 500 mg/100 mg dosing arm with more than 20 mutations, however, did not achieve substantial reductions in plasma HIV-1 RNA level, with an average decline of 0.2 $\log_{10}$ copies/mL.

The 3 study drug regimens were generally well-tolerated. However, the 750 mg/200 mg study dosing arm had the highest proportion of participants who discontinued the study due to adverse events. Based on the similar antiviral activity demonstrated by the 500 mg/200 mg and 750 mg/200 mg dosing arms in this study and the lower frequency of grade 3 or 4 adverse events reported in the lower-dose arm compared to the 750 mg/200 mg arm, the dose selected for use in phase 3 development was tipranavir 500 mg/ritonavir 200 mg.

RO-033-4649. RO-033-4649 is a PI developed through structure-activity analysis of HIV-1 protease containing 1-5 site-directed mutations (Abstract 7). The in vitro results show potent activity against a panel of 50 viral strains, each with 10-fold or greater resistance in a phenotypic drug resistance assay to 4 of 5 marketed PIs (median IC_{50} 100 nM) as well as wild-type viruses (median IC_{50} 17 nM). Previous work showed favorable pharmacokinetic profiles in 3 animal species. Phase 1 studies are beginning.

TMC114. Arasteh and colleagues presented the phase 2a data on TMC114 coadministered with ritonavir in multiple PI-experienced patients (Abstracts 8, 549, and 553). Patients with CD4+ counts greater than 50 cells/µL, plasma HIV-1 RNA greater than 2000 copies/mL, previous treatment with 2 to 4 PIs for more than 2 months each, virologic failure on the current regimen, and no NNRTI use in the baseline failing regimen were eligible for study participation. Fifty subjects were randomized to continue their background antiretroviral therapy regimen plus TMC114 300 mg/ritonavir 100 mg twice daily (n = 13); to receive TMC114 600 mg/ritonavir 100 mg twice daily (n = 12); to receive TMC114 900 mg/ritonavir 100 mg once daily (n = 13); or to continue their current PI-based regimen (control arm; n = 12). Median baseline plasma HIV-1 RNA and CD4+ cell count were 4.3 $\log_{10}$ copies/mL and 305 cells/µL, respectively. After 2 weeks, TMC114 was stopped and the antiretroviral regimen changed according to the practitioner's wishes. At day 14, according to an ITT analysis, the median change in plasma HIV RNA from baseline was -1.24 $\log_{10}$ copies/mL in the 300 mg/100 mg arm; -1.13 $\log_{10}$ copies/mL in the 600 mg/100 mg arm; -1.50 $\log_{10}$ copies/mL in the 900 mg/100 mg arm; and +0.02 $\log_{10}$ copies/mL in the control arm. The median plasma HIV-1 RNA change from baseline to day 14 for the 3 TMC114 study arms was significantly greater than for the control arm ($P < .001$). The proportion of subjects who achieved plasma HIV-1 RNA suppression to less than 400 copies/mL at day 14 was 46% in the 300 mg/100 mg arm; 31% in the 600 mg/100 mg arm; 43% in the 900 mg/100 mg arm; and 8% in the control arm. Treatment with TMC114 at all 3 doses was generally well-tolerated; most side effects were gastrointestinal, with 32% of subjects experiencing diarrhea. Headache and dizziness occurred in 16% and 11% of TMC114-treated patients, respectively. There was 1 grade 4 rash (eczema) that occurred in the 300 mg/100 mg arm, which was deemed to be possibly related to study drug. All other rashes in the remaining TMC114 study arms were judged to be grade 2 or less in severity. One serious adverse event of hepatotoxicity in the 600 mg/100 mg dose arm was reported.

Treatment of Antiretroviral-Naive Patients

Results of select studies in antiretroviral-naive patients are summarized in Table 2.

Gilead 903 Study

The Gilead 903 Study, presented by Staszewski and colleagues (Abstract 564b), compared the efficacy and safety of the nucleotide reverse transcriptase inhibitor (ntRTI) tenofovir disoproxil fumarate 300 mg once daily with the nRTI stavudine 40 mg twice daily, each used in combination with a background of efavirenz 600 mg twice daily and lamivudine 50 mg twice daily in antiretroviral therapy-naive patients over 144 weeks. This phase 3, multicenter, randomized, double-blind, active-controlled trial enrolled 600 subjects with plasma HIV-1 RNA levels greater than 5000 copies/mL and no CD4+ cell count criteria. At baseline, the mean plasma HIV-1 RNA levels were 81,300 copies/mL each in the tenofovir (n = 299) and stavudine (n = 301) study arms; 46% and 43% of subjects, respectively, had plasma HIV-1 RNA levels greater than 100,000 copies/mL at study entry. Mean CD4+ counts at study entry in the tenofovir and stavudine arms were 276 and 283 cells/µL, respectively.

Through week 96, 82% of subjects in the tenofovir arm achieved plasma HIV-1 RNA levels of less than 400 copies/mL compared with 78% in the stavudine arm; 78% and 74% of subjects, respectively, had suppressed plasma HIV-1 RNA levels less than 50 copies/mL. The mean increase in CD4+ count at week 96 was 261 cells/µL in the tenofovir arm and 266 cells/µL in the stavudine arm. The incidence of grade 3 or 4 clinical adverse events was similar in both study arms. The tenofovir arm sustained significantly lower lipid elevations (triglyceride, total cholesterol, and LDL cholesterol levels)

Table 2. Trials in Antiretroviral-Naive Subjects

Study (Abstract No.), Description	Regimen/Study Arm (No. Patients)	Baseline Values HIV-1 RNA (copies/mL)	Baseline Values CD4+ (cells/µL)	Changes in Values HIV-1 RNA (copies/mL)	Changes in Values CD4+ (cells/µL)
903 Study (564b) 96-wk, phase 2, randomized, double-blind, active-controlled comparative trial of tenofovir and stavudine.	Tenofovir/efavirenz/ lamivudine (299)	81,300 (mean) 46% >100,000	276 (mean)	82% <400 78% <50 (ITT analysis; M=F)	+261 (mean)
	Stavudine/efavirenz/ lamivudine (301)	81,300 (mean) 43% >100,000	283 (mean)	78% <400 74% <50	+266 (mean)
Comment: Grade 3 and 4 adverse events were similar in both arms. The tenofovir arm sustained lower mean increases in triglyceride and fasting LDL cholesterol levels than did the stavudine arm (*P* <.001; *P* <.001). Time to use of a first lipid-lowering agent was longer in the tenofovir arm than in the stavudine arm (*P* <.001).					
2NN Study (176) 48-wk, multicenter, open-label, randomized comparative trial. *All patients also received stavudine/lamivudine	Nevirapine 400 mg/ efavirenz 800 mg qd (209)*			62% virologic success (combined endpoint) 63% <50 (ITT analysis; M=F)	+150 (median)
	Efavirenz 600 mg qd (400)*	4.7 $\log_{10}$ (overall median)	190 (overall median)	68% virologic success (combined endpoint) 70% <50	+160
	Nevirapine 400 mg qd (220)*			65% virologic success (combined endpoint) 70% <50	+170
	Nevirapine 200 mg bid (387)*			64% virologic success (combined endpoint) 65% <50	+160
Comment: The nevirapine/efavirenz arm had a higher discontinuation rate (29%) than did the other arms (16%, 24%, and 21% in the efavirenz, nevirapine qd, and nevirapine bid arms, respectively) due to toxicity. Grade 3 and 4 hepatobiliary adverse events were as follows: nevirapine qd vs efavirenz, *P* <.001; efavirenz vs nevirapine/efavirenz, *P* <.04.					
NEAT Study (177) 48-wk, multicenter, open-label, randomized comparative trial.	GW433908[1] 1400 mg bid/ abacavir/lamivudine (166)	4.82 $\log_{10}$ (median)	214 (median)	66% <400 55% <50 (ITT analysis; rebound=failure)	201 (median)
	Nelfinavir 1250 mg bid/ abacavir/lamivudine (83)	4.85 $\log_{10}$	212	51% <400 41% <50	216
Comment: For patients with baseline plasma HIV-1 RNA >100,000 copies/mL, 67% in the GW433908 arm achieved plasma HIV-1 RNA levels <400 copies/mL vs 35% in the nelfinavir arm. In the GW433908 arm, 14% experienced virologic failure compared with 28% in the nelfinavir arm.					
BMS 008/044 Study (555) Rollover/switch study to assess the long-term safety/ efficacy of atazanavir[1]. Rollover phase, 24 wks; total study duration, 72 wks. *All patients also received stavudine/lamivudine	Continue atazanavir 400 mg qd (139)*	1.73 $\log_{10}$ (overall median) 75% <400	495 (overall median)	80% <400 58% <50 (ITT analysis; observed data)	+39 (median)
	Continue atazanavir 600 mg qd (144)*			82% <400 54% <50	+34
	Switch from nelfinavir to atazanavir 400 mg qd (63)*			86% <400 59% <50	+33
Comment: Grade 3 and 4 bilirubin elevations (indirect) were more frequent in the atazanavir 600 mg arm (44%) than in the atazanavir 400 mg (26%) and switch (13%) arms. The switch arm sustained mean reductions in total cholesterol (16%; *P* <.001) and LDL cholesterol (21%; *P* <.001) levels.					

Table **2.** Trials in Antiretroviral-Naive Subjects, Continued

Study (Abstract No.), Description	Regimen/Study Arm (No. Patients)	Baseline Values		Changes in Values	
		HIV-1 RNA (copies/mL)	CD4+ (cells/μL)	HIV-1 RNA (copies/mL)	CD4+ (cells/μL)
Racivir (552) 2-wk, dose-ranging, phase 1b/2a study of racivir[1].	6 men at each dose received racivir 200 mg qd, racivir 400 mg qd, or racivir 600 mg qd. All patients also received efavirenz/ stavudine (18).	>5000	>50	2.02–2.43 $\log_{10}$ (overall mean reduction)	Not available
Comment: After stopping antiretroviral drugs on day 15, all 3 arms maintained suppression of plasma HIV-1 RNA levels for >2 wks. Mean plasma HIV-1 RNA reductions at day 28 ranged from 2.1 to 2.6 $\log_{10}$ copies/mL in patients who stopped antiretroviral therapy at day 14. All 3 doses of racivir were well-tolerated. Based on potency and oral bioavailability, racivir is targeted for once-daily combination therapy.					
QUEST (520) 48-wk study to evaluate virologic and immunologic outcomes of HAART initiation in primary HIV-1 infection.	Fixed-dose lamivudine/zidovudine plus abacavir/amprenavir for 18 months, then randomization to continue HAART ± vaccine prior to stopping HAART (148; 90% male)	5.4 $\log_{10}$ (median)	517 (median)	64% <50 47% <10 34% <3 (ITT analysis; M=F)	+157 (median)
Comment: In patients remaining on HAART, messenger RNA and DNA were <3 copies/10^6 peripheral blood mononuclear cells in 37% and 12%, respectively. In predicting plasma HIV-1 RNA levels <3 copies/mL, CD8/38+ count (HR, 2.2; 95% CI [1.3, 3.9]) and messenger RNA levels (HR, 1.4; 95% CI [1.1, 1.8]) were strongly associated with plasma HIV-1 RNA decline.					

[1]Investigational drug; not approved by the US Food and Drug Administration.

ART indicates antiretroviral therapy; bid, twice daily; CI, confidence interval; HAART, highly active antiretroviral therapy; HR, hazard ratio; ITT, intent-to-treat analysis; LDL, low-density lipoprotein; M=F, missing data equals failure; qd, once daily.

at week 96 than did the stavudine arm. The tenofovir arm also experienced fewer toxicities associated with mitochondrial dysfunction (peripheral neuropathy and lactic acidosis) than did the stavudine arm at week 96: 4% (n = 11) versus 20% (n = 61; $P < .001$). In a subset of subjects evaluated for changes in body fat distribution by dual-energy x-ray absortiometry (DEXA) scan, those in the tenofovir arm had more preserved total limb fat ($P < .001$) and weight gain ($P = .002$) than did those in the stavudine arm at week 96.

2NN Study

Lange and colleagues presented the week-48 results from the 2NN clinical trial (Abstract 176). This was a multicenter, open-label, randomized trial that compared the antiviral activity of nevirapine, efavirenz, and the combination of nevirapine/efavirenz in treatment-naive HIV-infected patients. A total of 1216 subjects with screening plasma HIV-1 RNA levels greater than 5000 copies/mL and any CD4+ cell count, and at any stage of Centers for Disease Control and Prevention (CDC) classification of HIV/AIDS, were randomized to receive nevirapine 400 mg once daily (n = 220); nevirapine 200 mg twice daily (n = 387); efavirenz 600 mg once daily (n = 400); or nevirapine 400 mg/efavirenz 800 mg once daily (n = 209). All subjects received a nRTI backbone of stavudine/lamivudine. The median baseline plasma HIV-1 RNA level was 4.7 $\log_{10}$ copies/mL and median CD4+ count was 190 cells/μL.

At week 48, treatment success (a combined endpoint) achieved in each arm was as follows: nevirapine once daily, 56.4%; nevirapine twice daily, 56.3%; efavirenz once daily, 62.3%; and nevirapine/efavirenz, 46.9%. The only statistically significant difference was observed between the efavirenz and nevirapine/efavirenz arms ($P < .001$). The proportions of subjects in each arm who achieved plasma HIV-1 RNA suppression to less than 50 copies/mL at week 48 were as follows: nevirapine once daily, 70%; nevirapine twice daily, 65.4%; efavirenz once daily, 70%; and nevirapine/efavirenz once daily, 62.7%. No statistically significant differences were observed among the study arms with respect to the CD4+ cell count changes seen at week 48.

At week 48, the proportion of subjects in each study arm who experienced a grade 3 or 4 adverse clinical event was as follows: nevirapine once daily, 15%; nevirapine twice daily, 20.4%; efavirenz once daily, 18%;

and nevirapine/efavirenz once daily, 24.4%. The difference between the efavirenz and nevirapine/efavirenz arms was statistically significant ($P < .001$). Grade 3 or 4 clinical hepatotoxicity and laboratory hepatobiliary toxicity (elevation of transaminase levels) were noted, respectively, in each arm at week 48 as follows: nevirapine once daily, 1.4% and 13.2%; nevirapine twice daily, 2.1% and 7.8%; efavirenz once daily, 0.3% and 4.5%; and nevirapine/efavirenz once daily, 1.0% and 8.6%. Central nervous system toxicity and rash occurred, respectively, in each study arm as follows: nevirapine once daily, 1.4% and 4.1%; nevirapine twice daily, 3.5% and 3.1%; efavirenz once daily, 5.5% and 1.8%; and nevirapine/efavirenz once daily, 7.7% and 3.8%.

The proportion of subjects who discontinued the study in each arm over 48 weeks was nevirapine once daily, 24.1%; nevirapine twice daily, 21.2%; efavirenz once daily, 15.5%; and nevirapine/efavirenz once daily, 29%. There were 25 deaths during the study, and 2 were attributed to nevirapine: 1 female subject with no documented coinfection with hepatitis B or C virus developed toxic hepatitis, and 1 patient developed Stevens-Johnson syndrome complicated by sepsis. Both nevirapine regimens demonstrated similar potency to that of the efavirenz-based therapy at 48 weeks, and the nevirapine once-daily regimen had similar efficacy to that of the nevirapine twice-daily regimen. The use of dual-NNRTI therapy (nevirapine/efavirenz), however, resulted in substantial toxicity requiring treatment discontinuation and higher treatment failure rates.

Predictors of Response to Initial Antiretroviral Therapy

Defining immunologic, virologic, and host-cell factors that influence long-term outcomes of antiretroviral therapy is critical to providing effective individualized therapy for HIV-infected patients. Benson and colleagues analyzed baseline factors associated with treatment response at week 96 in treatment-naive HIV-infected patients enrolled in the A5001 (Adult AIDS Clinical Trials Group [AACTG] Longitudinal Linked Randomized Trials [ALLRT]) trial (Abstract 572). ALLRT is a prospective, planned series of meta- and cross-protocol analyses of patients enrolled in AACTG trials. This analysis was conducted in the 785 treatment-naive patients who were randomized to receive potent antiretroviral therapy (nRTIs plus a PI, an NNRTI, or both) in 3 clinical trials between 1998 and 2002.

At baseline, the median age of patients was 36 years; 83% were male; and 47% were white, 27% black, and 23% Hispanic. The median baseline CD4+ count and plasma HIV-1 RNA level were 222 cells/μL and 143,000 copies/mL, respectively. Forty-eight percent of patients had baseline CD4+ counts less than 200 cells/μL; 21% and 31% of patients, respectively, had baseline CD4+ counts between 200 and 350 and greater than 350 cells/μL. Fifty-seven percent of patients had baseline plasma HIV-1 levels greater than 100,000 copies/mL.

By week 96, 96% of patients achieved plasma HIV-1 RNA suppression to less than 50 copies/mL at least once. Higher baseline plasma HIV-1 RNA levels and younger age were associated with failure to achieve plasma HIV-1 RNA suppression. In regression models including baseline plasma HIV-1 RNA level and age, higher baseline hemoglobin level was associated with a greater probability of viral suppression, but sex was not.

The median rise in CD4+ count from baseline to week 96 was 237 cells/μL. Lower baseline plasma HIV-1 RNA level, older age, and male sex were each associated with smaller rises in CD4+ cell count at week 96. In regression models with baseline plasma HIV-1 RNA level, lower CD4+ cell count and higher percent of naive CD4+ cells were each significantly associated with greater increases in CD4+ cell count at week 96. Age and sex were not additionally predictive of CD4+ cell increases.

Treatment of Antiretroviral-Experienced Patients

Results of select studies in antiretroviral-experienced patients are summarized in Table 3.

HIV-NAT 009

The optimal antiretroviral therapy combination regimen for patients who experience virologic failure on nRTI therapy has not been defined. Boyd and colleagues presented the 48-week results from the HIV-NAT 009 trial (Abstract 566), a single-arm, open-label study that evaluated the use of an RTI-sparing regimen using ritonavir-boosted indinavir plus efavirenz for patients in whom nRTI-based therapy was failing. A total of 61 patients (38 men) with a mean duration of prior nRTI combination therapy of 4.1 years received indinavir 800 mg/ritonavir 100 mg twice daily plus efavirenz 600 mg once daily. At baseline, the median plasma HIV-1 RNA level and CD4+ count were 4.09 $\log_{10}$ copies/mL and 169 cells/μL, respectively. At week 48, the mean reduction in plasma HIV-1 RNA from baseline was 2.29 $\log_{10}$ copies/mL, and 53 (87%) of subjects achieved plasma HIV-1 RNA suppression to less than 50 copies/mL. The median increase in CD4+ count from baseline at week 48 was 116 (range, 47.5-179) cells/μL. The dual indinavir/ritonavir regimen in combination with efavirenz thus provided potent viral suppression and conferred robust immune responses in subjects with prior virologic failure of an nRTI-based therapy.

Primary HIV Infection

Response to Treatment

Vanhems and colleagues (Abstract 514) presented results from a prospective observational cohort of 99 patients starting antiretroviral therapy while either symptomatic from primary HIV infection, less than 6 months after primary HIV infection, or between 6 and 12 months after primary HIV infection. Those individuals starting antiretroviral therapy during true primary HIV infection reached a plasma HIV-1 RNA level below the limits of detection more often and had a higher CD4+ cell count 12 months after primary HIV infection than did the other groups. A similar study was performed by investigators from Boston, Massachusetts, and Sydney, Australia (Abstract 516). They evaluated

222 patients starting antiretroviral therapy during primary HIV infection (60%) or within 6 months of HIV seroconversion (40%). Seventy percent of participants reached a plasma HIV-1 RNA level below the limits of detection within the first year of infection. There was a greater likelihood of reaching a plasma HIV-1 RNA level below the limits of detection for those treated during primary HIV infection (hazard ratio, 0.73; $P = .057$) than for those in the other group.

QUEST Study

Kinloch and colleagues presented the week-48 preliminary results of the QUEST trial (Abstract 520), which evaluated the virologic and immunologic outcomes of patients with primary HIV-1 infection who initiated HAART for more than 18 months, followed by randomization to 6 months of continued HAART with or without vaccines before stopping antiretroviral therapy. Subjects with 3 or fewer bands on Western blot and with HIV viremia initiated fixed-combination zidovudine/lamivudine, abacavir, and amprenavir. The study enrolled 148 subjects (90% male) with a mean age of 33.9 years; median baseline plasma HIV-1 RNA level and CD4+ count were 5.4 $\log_{10}$ copies/mL and 517 cells/μL, respectively. At week 48, 28% of patients had stopped treatment and 59% had revised their initial HAART regimen. A median decrease in plasma HIV-1 RNA level from baseline to week 48 of 5.3 $\log_{10}$ copies/mL (range, 3.8 to 6.4 $\log_{10}$ copies/mL; $P < .001$) was sustained in this cohort and a median increase in CD4+ count from baseline to week 48 of 157 cells/μL (range, 0 to 290 cells/μL; $P < .001$) was observed. At 48 weeks, 83%, 61%, and 44% of subjects in this cohort with continued follow-up (n = 114) achieved suppression of plasma HIV-1 RNA levels to less than 50 copies/mL, less than 10 copies/mL, and less than 3 copies/mL, respectively. Using an ITT approach (missing data equals failure [M = F]), 64%, 47%, and 34% of subjects (n = 148) achieved suppression of plasma HIV-1 RNA levels to less than 50 copies/mL, less than 10 copies/mL, and less than 3 copies/mL, respectively. During follow-up, lower levels of CD8/38+ cells (activated CD8+ T lymphocytes), cellular messenger RNA (mRNA), and proviral DNA were associated with plasma HIV-1 RNA suppression. The patients with primary HIV-1 infection who were treated with HAART thus achieved a high rate of viral control as demonstrated by plasma HIV-1 RNA levels, mRNA, DNA, and CD8/38+ at week 48.

Interleukin-2 Plus Antiretroviral Therapy in Early HIV Infection

Hecht and colleagues examined virologic and immunologic outcomes in subjects with early HIV-1 infection who initiated HAART, had achieved suppression of plasma HIV-1 RNA to less than 500 copies/mL, and subsequently added interleukin-2 (IL-2) in an immediate versus delayed fashion (Abstract 649). The study enrolled 62 subjects who initiated fixed-combination zidovudine/lamivudine plus nelfinavir or other HAART regimens within 12 months of HIV infection. After achieving suppression of plasma HIV-1 RNA levels to less than 500 copies/mL, subjects were randomized to add IL-2 either immediately or after a delayed interval of 48 weeks. IL-2 was administered as 7.5 million units subcutaneous twice daily for 5 days every 8 weeks for 6 cycles. Of the 62 subjects, 29 were randomized to early IL-2 and 33 to the delayed group; 31 subjects had completed all 6 cycles of IL-2 (19 in the early IL-2 group and 12 in the delayed IL-2 group). From randomization to week 48, median CD4+ activation (CD38+) declined from 38.9 mean fluorescent intensity units to 5.5 in the early IL-2 group and from 115 to 3.7 in the delayed IL-2 group (difference between groups, $P = .12$). At week 12 of IL-2 therapy, the mean increases in percentage of naive and memory CD4+ cells were 2.1% and 0.8%, respectively. At 48 weeks after initiating IL-2 therapy, the median CD4+ count increased from 645 cells/μL to 1326 cells/μL in the early IL-2 group and from 629 to 1431 cells/μL in the delayed IL-2 group (difference between groups, $P = .81$).

Plasma HIV-1 RNA levels were suppressed to less than 50 copies/mL in 79% and 92% of the subjects randomized to the early and delayed IL-2 groups, respectively ($P = .62$). There were similar rises in CD4+ cell count when IL-2 was added to HAART in early HIV infection following suppression of plasma HIV-1 RNA levels to less than 500 copies/mL whether IL-2 was administered immediately or delayed by 48 weeks. Naive and memory CD4+ cells increased in equal proportion after IL-2 administration. CD4+/CD8+ activation declined on HAART with or without concurrent IL-2 administration. Unfortunately, no toxicity data were presented, which is important for this drug.

Treatment Interruptions

Hoen and colleagues (Abstract 512) presented interim results of the PRIMSTOP Pilot Trial from France. The 29 enrollees received a regimen of stavudine/didanosine/nelfinavir/hydroxyurea for 34 weeks, followed by a 50-week period of structured treatment interruptions (STIs), discontinuation of antiretroviral therapy at week 84, and follow-up to week 104. Of the 8 patients who completed the study, 2 maintained a plasma HIV-1 RNA level of less than 400 copies/mL and none reinitiated therapy. Of note, the use of stavudine and didanosine in initial therapy should be avoided due to higher rates of neuropathy and lactic acidosis, and combining these agents with hydroxyurea is associated with an unacceptably high rate of pancreatitis.

Lafeuillade and colleagues (Abstract 513) presented results of 30 patients treated with antiretroviral therapy during primary HIV infection: 15 with 3 nRTIs and 15 with stavudine, didanosine, nelfinavir, saquinavir, hydroxyurea, and 3 courses of IL-2. After 24 months, patients underwent 1 to 3 cycles of STI. Therapy was reinitiated when the plasma HIV-1 RNA level was consistently greater than 5000 copies/mL. Only 2 of 15 participants taking 3 nRTIs were able to maintain plasma HIV-1 RNA levels of less than 5000 copies/mL, compared with 12 of 15 on the dual-PI regimen. On multivariate analysis, the strength of proliferative responses to p24 antigen (9-fold for responders and 2-fold for nonresponders) and the level of proviral DNA (1.3 log copies/10^6 cells for responders vs 1.9 log copies/10^6 cells for nonresponders, $P = .01$) were related to

Table **3.** Trials in Antiretroviral-Experienced Subjects

Study (Abstract No.), Description	Regimen/Study Arm (No. Patients)	Baseline Values HIV-1 RNA (copies/mL)	Baseline Values CD4+ (cells/μL)	Changes in Values HIV-1 RNA (copies/mL)	Changes in Values CD4+ (cells/μL)
FTC 303/FTC 350 (550) Rollover study to determine the long-term efficacy and safety of emtricitabine[1] replacing lamivudine. Patients in FTC 303 (48 wks) with HIV-1 RNA <400 copies/mL were offered emtricitabine in FTC 350 (140 wks median).	**FTC 303** Emtricitabine + ART (294) Lamivudine + ART (146)	1.7 $\log_{10}$ (median)	484 (median)	77% <400 Using Kaplan-Meier method, probability of virologic failure (plasma HIV-1 RNA >400) at 4 yrs was 11%	Not available
	FTC 350 Continue or switch to emtricitabine + ART (215)				Not available
Comment: Of FTC 303 participants, 164 (56%) discontinued emtricitabine or did not roll over into FTC 350. Reasons for premature discontinuation of emtricitabine were patient request (20%); virologic failure (10%; n=28); or adverse event (8%; n=24). Tolerability failure (death or adverse event leading to permanent emtricitabine discontinuation) at 4 yrs was estimated at 13%.					
Amdoxovir (554) 24-wk, open-label, phase 1/2, 2-arm study evaluating efficacy and safety of amdoxovir[1].	Amdoxovir 300 mg bid + ART (8)	4.55 $\log_{10}$ (median)	310 (median)	-1.53 $\log_{10}$ (median change at wk 12)	+30 (wk 12) +70 (wk 24)
	Amdoxovir 500 mg bid + ART (10)	4.41 $\log_{10}$	329	-0.75 $\log_{10}$	+50 (wk 12) +150 (wk 24)
Comment: No serious adverse events were seen in either arm. 11 patients discontinued the study for lens opacity (5), virologic failure (4), or withdrawal/noncompliance (2). No grade 3 or 4 lab toxicities (except triglyceride-level elevations) were seen in either arm.					
ALIZE-ANRS 99 (551) 48-wk, prospective, randomized, open-label trial (n=355) to evaluate virologic outcomes of switch from PI to NNRTI qd regimen.	Emtricitabine/didanosine/efavirenz qd	1.7 $\log_{10}$ (median)	540 (median)	89% had no virologic failure (plasma HIV-1 RNA=400) to wk 48 (ITT [M=F]) 95% <50	+21 (median)
	Continue PI-based regimen			88% had no virologic failure 87% <50	+13
Comment: More patients in the qd arm achieved plasma HIV-1 RNA levels <50 copies/mL at week 48 than in the continue arm (P <.01). The once-daily arm sustained a greater increase in fasting HDL cholesterol levels than did the continue arm: +0.2 vs 0.0 nmol/L (P <.0001).					
CONTEXT (178) 24-wk comparative study of GW433908[1] with lopinavir/ritonavir in PI-experienced patients. Primary endpoint was the time-averaged change in plasma HIV-1 RNA level.	GW433908/ritonavir qd/ 2nRTIs (105)	4.53 $\log_{10}$ (overall median)	263 (overall median)	-1.48 $\log_{10}$ 40% <50	+62-72% (overall median)
	GW433908/ritonavir bid/ 2 nRTIs (107)			-1.50 $\log_{10}$ 42% <50	
	Lopinavir/ritonavir/ 2 nRTIs (103)			-1.66 $\log_{10}$ 48% <50	
Comment: Fewer virologic failures were sustained in the lopinavir/ritonavir arm (21%) than in the GW433908 arms (qd 34%; bid 27%). Regimens were well-tolerated. Cholesterol increases were minimal; grade 3 or 4 triglyceride elevations were seen in 4%-8% of patients in the GW433908 arms and in 4% in the lopinavir/ritonavir arm.					

Table **3.** Trials in Antiretroviral-Experienced Subjects, Continued

Study (Abstract No.), Description	Regimen/Study Arm (No. Patients)	Baseline Values		Changes in Values	
		HIV-1 RNA (copies/mL)	CD4+ (cells/µL)	HIV-1 RNA (copies/mL)	CD4+ (cells/µL)
BI 1182.52 (179) 2-wk, randomized, blinded, phase 2, dose-finding study (n=216) to evaluate 3 doses of tipranavir[1]/ritonavir. At entry, PIs were stopped and replaced with tipranavir/ritonavir for 2 weeks; ART was optimized at week 2.	Tipranavir 500 mg/ ritonavir 100 mg	4.53 $\log_{10}$ (overall median)	153 (overall median)	-0.87 $\log_{10}$ (ITT analysis; LOCF)	Not available
	Tipranavir 500 mg/ ritonavir 200 mg			-0.97 $\log_{10}$	
	Tipranavir 750 mg/ ritonavir 200 mg			-1.18 $\log_{10}$	
Comment: All study arms maintained a 1 $\log_{10}$ copies/mL decrease in HIV-1 RNA through day 56. 4 protease mutations (L33I/V/F, V82A, M184V, and L90M) were observed in the setting of PI cross-resistance. If 3 such mutations were present, the median HIV-1 RNA reductions were 0.19, 0.33, and 0.54 $\log_{10}$ copies/mL in the 500 mg/100 mg, 500 mg/200 mg, and 750 mg/200 mg dosing arms, respectively. The 750 mg/200 mg arm had the highest study discontinuation rate due to adverse events: 15% vs 5.6% in the 500 mg/200 mg arm and 2.7% in the 500 mg/100 mg arm.					
TMC114 (8) 2-wk, open-label, randomized, phase 2a study to evaluate the efficacy, safety, and pharmokinetic profile of TMC114[1] when given to PI-experienced patients at 3 different doses with ritonavir.	TMC114 300 mg/ ritonavir 100 mg bid (13)	4.3 $\log_{10}$ (overall median)	305 (overall median)	-1.24 $\log_{10}$ (median change) 46% <400 (ITT analysis)	Not available
	TMC114 600 mg/ ritonavir 100 mg bid (12)			-1.13 $\log_{10}$ 31% <400	
	TMC114 900 mg/ ritonavir 100 mg bid (13)			-1.50 $\log_{10}$ 43% <400	
	Continue current PI as control (12)			+0.02 $\log_{10}$ 8% <400	
Comment: No correlation was seen between baseline resistance to PIs and HIV-1 RNA response. Treatment with TMC114 at all 3 doses was well-tolerated. 32% of patients developed diarrhea. Central nervous system side effects included headache (16%) and dizziness (11%). 1 patient developed a grade 4 rash, and 1 patient had hepatotoxicity; liver function tests normalized with drug treatment interruption.					
HIV-NAT 009 (566) 48-wk, single-arm, open-label study that evaluated indinavir/ritonavir/efavirenz in patients in whom an nRTI-based regimen had failed. Mean duration of prior nRTI therapy was 4.1 yrs.	Indinavir 800 mg bid/ ritonavir 100 mg bid/ efavirenz 600 mg qd (61)	4.09 $\log_{10}$ (median)	169 (median)	-2.29 $\log_{10}$ (mean reduction) 53 (87%) <50	+116
Comment: Drug interruptions occurred in 16% of patients due to study drug-related events. The most frequent laboratory-related toxicity was elevated triglyceride levels in 9 (15%) patients. Rash occurred in 23 (38%) patients, but no patient interrupted drug therapy due to rash. 3 (5%) patients developed renal stones; 2 required study drug interruption and all 3 had recurrent stones/sludge.					

[1]Investigational drug; not approved by the US Food and Drug Administration.

ART indicates antiretroviral therapy; bid, twice daily; HDL, high-density lipoprotein; ITT, intent-to-treat analysis; LOCF, last observation carried forward; M=F, missing data equals failure; NNRTI, nonnucleoside reverse transcriptase inhibitor; nRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; qd, once daily.

maintaining a plasma HIV-1 RNA level of less than 5000 copies/mL.

Hecht and colleagues presented data on behalf of the Acute Infection Early Disease Research Program (Abstract 519). They compared those who started antiretroviral therapy within 6 months of seroconversion and later underwent STI with those choosing to defer antiretroviral therapy. After adjusting for baseline plasma HIV-1 RNA level and estimated number of weeks since infection, subjects starting antiretroviral therapy had a lower plasma HIV-1 RNA level and a higher CD4+ cell count off antiretroviral therapy than did those deferring antiretroviral therapy. However, the unadjusted analysis did not show a clear benefit, and the authors suggested that a randomized trial is necessary to definitively assess the role of treatment in primary HIV infection.

Superinfection

Several studies concerned so-called superinfection with viral variants phylogenetically distinct from preceding HIV isolates. Wong and colleagues (Abstract 485) described 2 cases in which new clade B strains were retrospectively identified by phylogenetic analysis of *env* clones in female sexworkers who were also injection drug users. Allen and colleagues (Abstract 307) described a single individual who received HAART at primary infection and later went on to 4 cycles of STI. Phylogenetic analysis of *gag* sequences from 4 time points demonstrated emergence of a strain with 12% heterogeneity from the strain at seroconversion. This second strain persisted during the second and third STIs despite the presence of an immunodominant CD8+ T-cell epitope in p24 common to both viruses. Subbarao and colleagues (Abstract 486) described cell-free HIV RNA and proviral DNA levels using a real-time reverse transcriptase polymerase chain reaction method adapted to distinctly quantify subtypes B and CRF01_AE in a coinfected individual. Initially, the plasma virus was a subtype B strain. Subtype CRF01_AE was detected at 8 and 20 months in plasma and proviral DNA, respectively. The plasma HIV-1 RNA level for subtype B ranged from 7484 to 237,649 copies/mL over 44 months but for CRF01_AE was consistently lower, ranging from 3320 to 18,348 copies/mL over 33 months, respectively.

Treatment Strategies

Results of select studies of STIs in antiretroviral-experienced patients are summarized in Table 4.

STIs in Persons with Virologic Suppression

Ananworanich and colleagues presented the 48-week results of HIV-NAT 001.4 (Abstract 64), a prospective, open-label, randomized trial conducted in Thailand that evaluated HIV disease progression and safety of a STI undertaken in patients with chronic HIV infection. A total of 74 Thai patients who had received 1 year of dual nRTIs followed by PI-based therapy (saquinavir soft gel capsule 1600 mg/ritonavir 100 mg once daily) for 3 years were enrolled. The trial examined STIs based upon a CD4+ count decline versus 1 week on/1 week off antiretroviral therapy. When CD4+ count was greater than 350 cells/μL and plasma HIV-1 RNA was less than 50 copies/mL for at least 6 months, subjects were randomized to 1 of 3 study arms: continuous antiretroviral therapy (n = 25), CD4+ count-guided arm (n = 23), or 1 week on/1 week off arm (n = 26). The STI in the CD4+ count-guided arm was based on a CD4+ count of 350 cells/μL or less or a 30% drop or rise in CD4+ cell count. Treatment failure was defined as plasma HIV-1 RNA levels greater than 1000 copies/mL or CD4+ count of 350 cells/μL or less in the continuous and week on/week off study arms. Baseline characteristics were similar between arms with regard to sex (49% male), mean age (34 years), and mean CD4+ count (644 cells/μL). Preantiretroviral therapy and pre-HAART plasma HIV-1 RNA levels were higher in the CD4+ count-guided arm (4.8 and 3.2 $\log_{10}$ copies/mL, respectively) and in the week on/week off arm (4.9 and 3.4 $\log_{10}$ copies/mL, respectively) than in the continuous arm (4.3 and 2.6 $\log_{10}$ copies/mL, respectively; $P < .05$).

At week 48, no differences between study arms in HIV disease progression (AIDS or death), adverse events, serum lipid levels, or quality of life were demonstrated. One subject in the CD4+ count-guided arm developed an acute retroviral syndrome while off therapy. Median changes in CD4+ count from baseline to week 48 were an increase of 5 cells/μL in the continuous arm, a decline of 178 cells/μL in the CD4+ count-guided arm, and a decline of 6 cells/μL in the week on/week off arm ($P < .05$). All patients (25/25) in the continuous arm maintained CD4+ counts greater than 350 cells/μL through week 48. The proportion of patients who maintained CD4+ counts greater than 350 cells/μL through week 48 in the other 2 arms was 87% (20/23) in the CD4+ count-guided arm and 96% (25/26) in the week on/week off arm. Twelve of the 23 patients in the CD4+ count-guided arm were off antiretroviral therapy at study analysis. No treatment failures occurred in the continuous or CD4+ count-guided study arms through 48 weeks of follow-up. There were 8 treatment failures in the week on/week off study arm: 7 subjects had virologic failure with HIV-1 RNA levels greater than 1000 copies/mL and 1 patient sustained a decrease in CD4+ count to less than 350 cells/μL. Two additional patients were lost to follow-up. The median time to virologic failure in the week on/week off study arm was 16 weeks. Of 9 subjects in the week on/week off arm for whom genotypic testing results were available, 4 had resistance mutations (3 in the reverse transcriptase gene and 1 in the protease gene). At week 48, the proportion of subjects in each study arm who achieved suppression of plasma HIV-1 RNA to less than 500 [50] copies/mL was 100% [96%] in the continuous arm; 100% [83%] in the CD4+ count-guided arm; and 54% [35%] in the week on/week off study arm (HIV-1 RNA < 500 copies/mL, $P < .05$; HIV-1 RNA < 50 copies/mL, $P < .05$). All subjects who had virologic failure in the week on/week off study arm achieved plasma HIV-1 RNA suppression of less than 50 copies/mL within a median of 12 weeks after resuming the same PI-based antiretroviral therapy regimen. The CD4+ count-guided study arm provided the best cost-saving strategy and had simi-

lar virologic outcomes to the continuous therapy arm, whereas the week on/week off study arm had unacceptably high rates of virologic failure.

Ruiz and colleagues presented the 48-week results of a multicenter, controlled, open-label, randomized clinical trial (Abstract 65) that evaluated the strategy of continuous versus intermittent antiretroviral therapy guided by CD4+ cell counts and plasma HIV-1 RNA levels. The primary objective of this study was to compare the safety of continuous versus intermittent antiretroviral therapy in chronically HIV-infected patients who had maintained viral suppression on the current regimen. Virologic and immune responses were assessed. Patients with plasma HIV-1 RNA of less than 50 copies/mL for 1 year or greater, CD4+ count greater than 500 cells/μL for at least 6 months, and nadir CD4+ count greater than 100 cells/μL were eligible. A total of 120 patients were randomized to either interrupt therapy (n = 59) or continue the same prior antiretroviral therapy (n = 61). The criteria for resuming antiretroviral therapy in the interrupt study arm included CD4+ count decline below 350 cells/μL, plasma HIV-1 RNA increase to greater than 100,000 copies/mL, or an AIDS-defining event. Those patients who had resumed antiretroviral therapy would stop treatment again when CD4+ counts had risen to greater than 500 cells/μL and plasma HIV-1 RNA levels had suppressed to less than 50 copies/mL. The median baseline CD4+ count was 851 cells/μL in the interrupt arm and 800 cells/μL in the continue arm; nadir CD4+ counts were 416 and 379 cells/μL in the interrupt and continue study arms, respectively. The median pretreatment plasma HIV-1 RNA level was 4.5 $\log_{10}$ copies/mL.

No AIDS-defining events occurred in either study arm; no subject sustained a decline in CD4+ count to less than 200 cells/μL. One subject in the interrupt arm sustained a CD4+ count decrease to between 200 and 250 cells/μL. Four patients in the interrupt arm experienced a decrease in CD4+ count to between 250 and 350 cells/μL, compared with no patients in the continue arm ($P = .02$). Six (10%) patients in the interrupt arm developed an acute seroconversion illness; the plasma HIV-1 RNA level threshold off antiretroviral therapy was not correlated with the risk of developing an acute seroconversion syndrome. In those patients who remained off treatment for 48 weeks, a CD4+ count decrease of 335 cells/μL was observed at week 48 and a loss of 33 CD4+ cells/μL/month was incurred. Of these 33 patients, 23 (70%) experienced plasma HIV-1 RNA rebound to greater than 100,000 copies/mL at a median of 8 weeks after stopping treatment; 2 (6%) sustained a decline in CD4+ count to 350 cells/μL or below; and 8 (24%) met both plasma HIV-1 RNA rebound and CD4+ criteria to resume treatment. The median time off antiretroviral therapy was 32 weeks. Those patients in the interrupt arm who remained off treatment (n = 26) had a higher CD4+ count nadir of 454 cells/μL than did those subjects who resumed treatment (n = 33), who had a CD4+ count nadir of 363 cells/μL ($P = .06$). A higher CD4+ cell count was associated with remaining off antiretroviral therapy for a longer period of time. In a multivariate analysis, CD4+ cell count nadir and preantiretroviral therapy plasma HIV-1 RNA level were both strongly associated with risk for resuming treatment ($P = .004$ and $P = .009$, respectively).

Vella and colleagues presented the first results of the ISS-PART Trial, sponsored by the Italian HIV Clinical Research Program (Abstract 66). This study randomized 273 subjects with plasma HIV-1 RNA levels of less than 400 copies/mL and CD4+ counts greater than 350 cells/μL to continue their current PI- or NNRTI-based therapy or to interrupt their therapy on an increasing schedule of 3 months on treatment alternating with 1, 1, 2, 2, and 3 months off. The median baseline CD4+ counts were 699 and 673 cells/μL in the 2 arms, respectively. After a median follow-up of 56 weeks, there were more dropouts in the intermittent therapy arm than in the continuous therapy arm (25 vs 5, respectively), mainly due to physician or patient request. Of those completing 12 months of follow-up, 42 (89%) of 47 subjects in the continuous therapy arm remained virologically suppressed; in the intermittent therapy arm 35 (97%) of 37 followed to this time point resuppressed plasma HIV-1 RNA levels to less than 400 copies/mL after the third cycle. CD4+ cell counts were well-maintained in the latter group. Over the period spanning the first 3 interruptions, 33 (24.2%) of 136 subjects demonstrated a drug-resistance mutation, with M184V most frequently detected. Twenty-four of these 33 were studied further, and 14 (58%) of these 24 were found to have mutations in their baseline peripheral blood mononuclear cell or plasma samples. Plasma HIV-1 RNA and CD4+ cell changes during each interruption did not correlate with whether mutations were detected. Importantly, subjects with mutations suppressed plasma HIV-1 RNA levels with reinstitution of the same therapy at rates of 91%, 88%, and 92% after the first, second, and third interruption, respectively. The suppression rates for those with no detectable mutations were 96%, 92%, and 100%, respectively. It will be important to see how the mutational frequency and virologic failure rates compare in the 2 arms of this study with longer follow-up.

Dybul and colleagues reported the National Institute of Allergies and Infectious Diseases' trial results with cyclic antiretroviral therapy involving 8 weeks on and 4 weeks off (Abstract 68lb). Fifty-two patients with plasma HIV-1 RNA levels of less than 50 copies/mL and CD4+ counts greater than 300 cells/μL were randomized to continue therapy or to interrupt therapy as noted. Enrollment was halted early because in the intermittent therapy arm, 3 patients on efavirenz-based therapy developed lamivudine- or NNRTI-associated mutations, and 1 patient receiving a PI-based regimen developed lamivudine resistance. Toxicity markers were not significantly improved in the intermittent therapy arm compared with in the continued therapy arm. Thus, no clear benefit and a higher risk of viral resistance was seen in the intermittent therapy arm. These data are in contrast to the previously published work of Dybul and colleagues reporting the efficacy of short-cycle therapy (7 days on, 7 off).

STIs in Treatment-Experienced Patients

CPCRA 064. Lawrence and colleagues presented the results of the CPCRA 064 study, a randomized, prospective, clinical endpoint trial conducted in HIV-infected patients with virologic failure that evaluated the impact on HIV disease progression of initiating an STI strategy prior to a salvage antiretroviral therapy regimen (Abstract 67). This multicenter study enrolled HIV-infected patients with advanced disease who had experienced virologic failure and continued on stable antiretroviral therapy regimens with screening plasma HIV-1 RNA levels greater than 5000 copies/mL. Multidrug-resistant HIV was documented by genotypic testing. Baseline genotypic and phenotypic testing was performed to help guide the selection of active drugs in the new salvage regimen. The primary endpoint was progression of HIV disease or death. A total of 270 patients with mean baseline CD4+ count and plasma HIV-1 RNA of 180 cells/μL and 5.0 $\log_{10}$ copies/mL, respectively, were randomized 1:1 to a 4-month STI followed by a new salvage regimen or to an immediate change in antiretroviral therapy regimen. At baseline, 9% of subjects were female; 26% had CD4+ count of 50 cells/μL or below, with median CD4+ count nadir of 69 cells/μL; and 56% had developed a prior opportunistic infection. This study population was extensively treatment-experienced, with prior exposure to a mean of 5 nRTIs, 1.5 NNRTIs, and 4.2 PIs. At study entry, 48% of patients had resistance to all 3 drug classes on testing. The total number of antiretroviral [active] drugs provided to each of the study arms was 3.6 [2.7] in the STI arm and 3.8 [2.8] in the no-STI arm.

A total of 34 clinical endpoints (HIV disease progression or death) were reached: 22 endpoints in the STI arm and 12 in the no-STI arm (hazard ratio, 2.6; 95% confidence interval, 1.2-5.5; $P < .01$). The STI arm experienced 17 progression-of-disease events: 7 (41%) patients developed esophageal candidiasis; 4 (24%), *Pneumocystis carinii* pneumonia (PCP); 3 (18%), cryptosporidiosis; 2 (12%), lymphoma; and 1 (6%) cytomegalovirus disease. Eight deaths occurred in each study arm. The mean difference in CD4+ count favored the no-STI arm over the STI arm, with 85 cells/μL ($P < .001$) for months 1 through 4 (STI phase), 47 cells/μL ($P < .001$) for months 5 through 8, and 31 cells/μL ($P = .11$) for months 12 through 20. In the STI arm, 52% sustained a greater than 50% decrease in CD4+ cell count. The mean changes in plasma HIV-1 RNA in the STI arm were +0.31 $\log_{10}$ copies/mL and -0.76 $\log_{10}$ copies/mL at 4 months and 12 months, respectively, compared with -0.75 $\log_{10}$ copies/mL and -0.66 $\log_{10}$ copies/mL, respectively, in the no-STI arm. At month 4, 64% of patients in the STI group had wild-type virus on genotypic testing and sustained plasma HIV-1 RNA suppression of 0.7 $\log_{10}$ copies/mL at 20 months. The study was closed prior to full accrual based upon recommendations from a data and safety monitoring board. Given the persistently inferior CD4+ cell count responses and higher number of clinical events in the STI arm, there was no clinical or immunologic benefit conferred by STI as a salvage strategy in this group of patients with advanced HIV disease and multidrug-resistant HIV.

ANRS 097. Katlama and colleagues presented the results of the GIGHAART ANRS 097 study, which evaluated the impact on virologic outcome of STI as a salvage strategy in patients with advanced HIV disease and multiple treatment failures (Abstract 68). This prospective, open-label, randomized trial enrolled 70 HIV-infected patients with screening plasma HIV-1 RNA greater than 50,000 copies/mL and CD4+ count of 200 cells/μL or below. Patients were randomized to an immediate therapy arm or to a deferred therapy arm initiated after an 8-week treatment interruption. The GIGHAART regimen consisted of 7 to 9 drugs: 3 or 4 nRTIs and 1 NNRTI with or without hydroxyurea 500 mg twice daily, in combination with ritonavir 400 mg twice daily and amprenavir 600 mg twice daily or lopinavir 400 mg/ritonavir 100 mg twice daily, and a third PI (indinavir 400 mg twice daily, saquinavir 600 mg twice daily, or nelfinavir 1250 mg twice daily). The primary endpoint was a decrease in plasma HIV-1 RNA levels of greater than 1 $\log_{10}$ copies/mL after 12 weeks of therapy. Sixty-eight of the 70 randomized patients initiated treatment; 63 were evaluated at weeks 12 and 24, and 64 at week 48. At baseline, median plasma HIV-1 RNA level and CD4+ count were 5.3 $\log_{10}$ copies/mL and 27 cells/μL, respectively. The median duration of prior antiretroviral therapy was 6.6 years with a median of 11 antiretroviral drugs. There was extensive 3-class antiretroviral therapy drug resistance present at baseline in this study population: 88% and 72% of subjects in the immediate and deferred arms, respectively, had more than 3 nRTI-associated mutations (NAMs), and 79% and 91% of subjects, respectively, had at least 1 major PI mutation. Genotypic reversion occurred in 48% of subjects following an 8-week treatment interruption.

The proportion of subjects who achieved a greater than 10-fold decrease in plasma HIV-1 RNA was 26% at week 12 and 24% at week 24 in the immediate arm, compared with 62% at week 12 and 50% at week 24 in the STI arm (week 12, $P = .007$; week 24, $P = .043$). The immediate arm sustained median decreases in plasma HIV-1 RNA from baseline of 0.37 $\log_{10}$ copies/mL at week 12; 0.29 $\log_{10}$ copies/mL at week 24; and 0.37 $\log_{10}$ copies/mL at week 48. By comparison, the STI arm sustained median plasma HIV-1 RNA decreases of 1.91 $\log_{10}$ copies/mL, 1.08 $\log_{10}$ copies/mL, and 0.79 $\log_{10}$ copies/mL, respectively. The proportion of subjects who achieved suppression of plasma HIV-1 RNA level to less than 400 copies/mL was 15% at week 12 and 12% at week 24 in the immediate arm versus 38% at week 12 and 32% at week 24 in the STI arm (week 12, $P = .053$; week 24, $P = .077$). The median increase in CD4+ count from baseline was 7 cells/μL at week 24 and 7 cells/μL at week 48 in the immediate arm versus 51 cells/μL and 69 cells/μL, respectively, in the STI arm. There were 2 deaths in each study arm. At week 48, 22% and 47% of subjects in the immediate and STI arms, respectively, remained on giga-HAART salvage therapy (more than 6 drugs). In a multivariate regression model evaluating baseline predictors of virologic success, 3 factors were associated with favorable

Table **4.** Structured Treatment Interruptions (STIs) in Antiretroviral-Experienced Patients

Study (Abstract No.), Description	Regimen/Study Arm (No. Patients)	Baseline Values: HIV-1 RNA (copies/mL)	Baseline Values: CD4+ (cells/μL)	Changes in Values: HIV-1 RNA (copies/mL)	Changes in Values: CD4+ (cells/μL)
HIV-NAT 001.4 (64) 48-wk, prospective, open-label, randomized trial conducted in Thailand that evaluated HIV progression and safety of STI in patients with HIV-1 RNA <50 copies/mL. Prior to entry, all patients had received at least 3 years PI-based therapy of saquinavir sgc 1600 mg/ritonavir 100 mg/2 nRTIs qd.	Continuous ART (25)	2.6 $\log_{10}$ (pre-HAART)	644 (overall mean)	96% <50	100% >350
	CD4+-guided STI (23)	3.2 $\log_{10}$		83% <50	87% >350
	1 wk on/1 wk off (26)	3.4 $\log_{10}$		35% <50	96% >350
Comment: No treatment failures occurred in the continuous arm vs 8 treatment failures in the wk on/wk off arm: 7 with HIV RNA >1000 copies/mL and 1 with CD4+ decrease <350. 2 patients were lost to follow-up. Median time to virologic failure in the wk on/wk off arm was 16 wks. One patient in the CD4+-guided arm had acute retroviral syndrome. The CD4+-guided arm had similar virologic outcomes as did the continuous arm. Time spent on ART was 33% in the CD4+-guided arm vs 59% in the wk on/wk off arm.					
Continuous vs Intermittent ART (65) 48-wk, multicenter, controlled, open-label, randomized trial that evaluated continuous vs intermittent HAART guided by CD4+ count and HIV-1 RNA levels in patients with HIV-1 RNA suppression <50 copies/mL. Criteria for resuming ART in the interrupt arm were CD4+ decrease to <350 cells/mL; a confirmed HIV-1 RNA increase to 100,000 copies/mL or greater or an AIDS-defining event.	Interrupt (59)	4.5 $\log_{10}$ (median)	851 (median)	4.1 $\log_{10}$ in patients remaining off ART; 33 (56%) resumed ART and of these, 23 (70%) had >100,000	96 (median decrease)
	Continue (61)	4.5 $\log_{10}$	800	97% <50	804 (median count)
Comment: 6 (10%) of patients in the interrupt arm developed acute seroconversion illness; HIV-1 RNA threshold was not correlated with risk of such illness. Median time off ART in the interrupt arm was 32 wks. Patients in the interrupt arm who remained off ART (n=26) had a higher nadir CD4+ count (454 cells/μL) than did those (n=26) who resumed ART (363 cells/μL). Of 33 patients in the interrupt arm with viral rebound, 2 (6%) had CD4+ cell counts <350; 8 (24%) met viral rebound and CD4+ criteria to resume ART. No AIDS-defining events occurred in either arm. CD4+ nadir (*P*=.004) and pre-ART HIV-1 RNA levels (*P*=.009) were associated with resuming ART.					
CPCRA 064 (67) Randomized, prospective, clinical endpoint trial in patients with virologic failure (n=270) that evaluated the impact on HIV disease progression of STI prior to devising salvage regimen. STI interval was 4 mos. Median wks of follow-up was 11.3 months; study closed prior to full accrual based on DSMB recommendation.	4-mo STI	5.0 $\log_{10}$ (mean)	180 (mean)	+0.31 $\log_{10}$ (4 mos) -0.76 $\log_{10}$ (12 mos)	Difference between no-STI and STI arms: 85 (1-4 mos) 47 (5-8 mos) 31 (12-20 mos)
	No STI			-0.75 $\log_{10}$ -0.66 $\log_{10}$	
Comment: At 4 mos, 64% of patients in the STI arm had wild-type virus and sustained HIV-1 RNA decrease of 0.7 $\log_{10}$ copies/mL at 20 months. 34 clinical endpoints (disease progression/death) were reached: 22 in the STI arm vs 12 in the no-STI arm (HR 2.6; 95% CI [1.2, 5.5]; *P* <.01). 8 deaths occurred in each arm. The STI arm had 17 disease events: 7 (41%) candidal esophagitis; 4 (24%) PCP; 32 (18%) cryptosporidiosis; 2 (12%) lymphoma; and 1 (6%) cytomegalovirus. In the STI arm, 52% of patients had a >50% decrease in CD4+ cell count.					

Table **4.** Structured Treatment Interruptions (STIs) in Antiretroviral-Experienced Patients, Continued

Study (Abstract No.), Description	Regimen/Study Arm (No. Patients)	Baseline Values		Changes in Values	
		HIV-1 RNA (copies/mL)	CD4+ (cells/μL)	HIV-1 RNA (copies/mL)	CD4+ (cells/μL)
GIGHAART ANRS 097 (68) 48-wk, prospective, open-label, randomized trial to evaluate the impact of STI vs immediate salvage therapy on virologic outcome in patients with multiple virologic failures (n=70). Primary endpoint was decrease in plasma HIV-1 RNA >1 $\log_{10}$ copies/mL after 12 weeks of salvage therapy.	Deferred therapy (8-week STI)	5.3 $\log_{10}$ (overall median)	27 (overall median)	Wk 12: -1.91 $\log_{10}$ 38% <400 (ITT analysis, M=F) Wk 24: -1.08 $\log_{10}$ 32% <400 Wk 48: -0.79 $\log_{10}$	Week 24: +51 Week 48: +69
	Immediate salvage: 3 or 4 nRTIs/1 NNRTI ± hydroxyurea 500 mg bid/ritonavir 400 mg bid/amprenavir 600 mg bid or lopinavir 400 mg/ritonavir 100 mg/third PI (saquinavir 600 mg bid, indinavir 400 mg bid, or nelfinavir 1250 mg bid)			Wk 12: -0.37 $\log_{10}$ 15% <400 Wk 24: -0.29 $\log_{10}$ 12% <400 Wk 48:-0.37 $\log_{10}$	Week 24: +7 Week 48: +7
Comment: On ITT analysis (M=F), 62% of patients in the deferred arm sustained a >1 $\log_{10}$ copies/mL decline in HIV-1 RNA levels at wk 12, compared with 26% in the immediate arm (*P*=.007). There were 2 deaths in each study arm. At wk 48, 22% and 47% of patients in the immediate and deferred arms, respectively, remained on mega-salvage therapy. In a multivariate regression model, 3 factors were predictive of favorable HIV-1 RNA response: reversion of resistance (RH, 12.4); adequate drug exposure (RH 5.6); and lopinavir use (RH 6.0).					
ISS-PART (66) 56-wk, prospective, multicenter, randomized trial of STI in subjects with chronic HIV infection and stable HIV replication (<400 copies/mL). Study endpoint was the proportion of patients with CD4+ count >500 cells/μL at 24 mos.	Continued ART (137)	<400	699	Not available	Not available
	5 STIs: 1, 1, 2, 2, and 3 months duration, each followed by 3 months on ART (136)	<400	673	After first STI: 88.9% <400 Second STI: 96.8% <400 Third STI: 100% <400	After first STI: -72 Second STI: -60 Third STI: -147
Comment: After ART was reinitiated, median CD4+ counts in the STI arm returned to baseline values. After the first STI, 33 (25%) of patients had no plasma HIV-1 RNA rebound, compared with 25 (27%) after the second STI and 3 (8.8%) after the third STI. Most patients achieved plasma HIV-1 RNA suppression <400 copies/mL with ART resumption after STI, but a trend of increased resistance mutations was noted with STI. Mutations were identified in 24% of STI patients; approximately 50% of these mutations were present at baseline (largely nRTI-associated mutations). M184V was the most frequently detected mutation. NNRTI- and PI-associated mutations were identified in 6% and 8% of patients, respectively, in the STI group.					

ART indicates antiretroviral therapy; bid, twice daily; CI, confidence interval; DSMB, Data and Safety Monitoring Board; HAART, highly active antiretroviral therapy; HR, hazard ratio; ITT, intent-to-treat analysis; M=F, missing data equals failure; NNRTI, nonnucleoside reverse transcriptase inhibitor; nRTI, nucleoside reverse transcriptase inhibitor; PCP, *Pneumocystis carinii* pneumonia; PI, protease inhibitor; RH, relative hazard; sgc, soft-gel capsule.

plasma HIV-1 RNA response: reversion of resistance, adequate drug exposure, and use of lopinavir/ritonavir.

The reasons for the apparently conflicting results of CPCRA 064 and ANRS 097 are not clear. The patients in the latter study, however, had more advanced HIV disease with a lower baseline CD4+ cell count than those in the former study, underwent a shorter length of treatment interruption, and were treated with a multidrug rescue therapy (mega- or giga-HAART).

Haubrich and colleagues presented a preliminary analysis of a California Collaborative Treatment Group (CCTG) 578 substudy (Abstract 565). This study evaluated the benefit of an STI in PI-experienced patients in whom HAART was failing. Subjects were treated with a lopinavir/ritonavir-inclusive regimen immediately (n = 16) or after an STI of more than 4 months (n = 20). The exploratory analyses did not demon-

strate differences in 6-week CD4+ counts, plasma HIV-1 RNA levels, or changes in CD4+ counts from baseline between the 2 arms.

Selective (Partial) Treatment Interruption

Deeks and colleagues evaluated the relative impacts of interrupting either the PI (n=15) or the nRTI (n=5) components of a stable HAART regimen while continuing the other antiretrovirals, a so-called partial treatment interruption (Abstract 640). The study population was a highly selected cohort of 20 antiretroviral-adherent individuals with stable on-treatment viremia and CD4+ counts but high levels of antiretroviral resistance. PI or nRTI therapy was interrupted based on patient-specific toxicity. In brief, there were immediate increases in plasma HIV-1 RNA levels with later reductions in CD4+ cell counts in the stop-nRTI/continue-PI group. Conversely, these values remained stable in the stop-PI/continue-nRTI group. In the latter group, the PI mutations persisted through week 24, and thus replication capacity remained reduced during this period. The implications of these findings for clinical management are not yet clear.

Switching/Simplification

Molina and colleagues presented the 48-week results from the ALIZE-ANRS 99 study, a prospective, randomized, open-label, multicenter, non-inferiority trial (Abstract 551). Investigators evaluated virologic outcomes in HIV-infected subjects with plasma HIV-1 RNA of less than 400 copies/mL on current PI-based therapy who either continued a PI-containing regimen or switched to a once-daily combination antiretroviral therapy regimen of the investigational drug emtricitabine/didanosine/efavirenz. Virologic failure was defined as a confirmed plasma HIV-1 RNA level of 400 copies/mL or greater.

A total of 355 patients with plasma HIV-1 RNA levels of less than 400 copies/mL were randomized; 86% were male, the median age was 41 years, and the median duration of PI therapy was 35 months. Baseline median CD4+ count was 540 cells/μL. The proportions of patients who achieved virologic success at week 48 using as-treated and ITT (M=F) analyses were 96% and 89%, respectively, in the once-daily study arm, compared with 93% and 88%, respectively, in the continued-PI therapy arm. At 48 weeks, 95% of patients in the once-daily arm had achieved suppression of plasma HIV-1 RNA to less than 50 copies/mL, compared with 87% of patients in the continued-PI therapy arm ($P < .01$). The median CD4+ count increase was similar at week 48 between the study arms (21 and 13 cells/μL in the once-daily and continued-PI therapy arms, respectively). A statistically significant increase in median fasting HDL cholesterol levels was observed in the once-daily arm compared with the continued-PI therapy arm: 0.2 vs 0.0 nmol/L, re-spectively ($P < .0001$). The substitution of a PI-based regimen with a once-daily NNRTI-based combination regimen of emtricitabine/didanosine/efavirenz thus demonstrated continued suppression of plasma HIV-1 RNA levels and conferred continued increases in CD4+ cell counts for 48 weeks.

Dalmau and colleagues described the 24-month outcomes in the NEFA study (Abstract 608). Subjects on at least 1 PI plus 2 nRTIs who had plasma HIV-1 RNA levels of less than 200 copies/mL for at least 6 months and who were randomly assigned to switch off the PI to either nevirapine (n=155), efavirenz (n=156), or abacavir (n=149) were evaluated. A history of mono- or dual-nRTI therapy was permitted in this study. Briefly, 11% of subjects had plasma HIV-1 RNA levels greater than 200 copies/mL at 24 months. In the nevirapine, efavirenz, and abacavir arms the failure rates were 15/155, 12/156, and 24/149, respectively. Genotypic testing demonstrated greater nRTI resistance in the abacavir arm than in the NNRTI arms. No differences in failure rates were observed between those with no prior nRTI exposures. These data support prior studies on the reduced potency of nRTI-only regimens in highly nRTI-experienced individuals.

Antiretroviral Drug Resistance and Replication Capacity

Results of select studies of antiretroviral drug resistance and replication capacity are summarized in the Appendix.

Treatment-Naive Patients

Little and colleagues presented data on behalf of the Acute HIV Infection and Early Disease Research Program (Abstract 152). They evaluated the relationship between virologic set point, drug susceptibility, and viral replication capacity in 194 subjects remaining treatment-naive after primary HIV infection (range, 5-24 months). No relationship was noted between the mean baseline replication capacity and the plasma HIV-1 RNA level or CD4+ cell count. The mean baseline CD4+ cell percent was higher in those with resistance to any drug compared with those with fully susceptible isolates, 34% and 28% ($P=.05$), respectively. The mean replication capacity for isolates with PI hypersusceptibility (<0.4-fold change to ≥1 PI) and for those with PI susceptibilities in the standard susceptibility range were 30% and 50%, respectively ($P < .0001$). PI hypersusceptibility was observed more commonly at primary infection than in established infection, 33% and 18%, respectively. The authors suggest that viruses with low replication capacities at primary infection may evolve to higher replication capacity in established infection. Among subjects with NNRTI resistance the mean plasma HIV-1 RNA set point was 0.6 $\log_{10}$ copies/mL higher than the mean set point in those without NNRTI resistance ($P=.005$).

Employing 188 isolates from this same data set, Leigh Brown (Abstract 594) analyzed the genotypic factors associated with variations in baseline PI susceptibility. Combinations of changes at codons in both protease codons 10, 13, 37, 57, 62, 63, and 73 and *gag* cleavage sites were identified that were associated with hypersusceptibility to ritonavir (n=22). A strong association was noted between increasing hypersusceptibility to ritonavir and replication capacities of 10%

or lower ($P < .0001$).

Grant and colleagues presented data from the Options Project, which follows HIV-serodiscordant couples (Abstract 505). Viral phylogenetic linkages were established in 35 partnerships. Investigators compared those individuals who transmitted virus to their partners (n = 33) with those who did not (n = 26). Transmitters were less likely to have PI mutations than were nontransmitters (9% vs 23%, $P = .09$), whereas the prevalence of nRTI and NNRTI mutations was similar in both, suggesting that PI mutations may be associated with decreased infectiousness. However, it is not clear whether the presence of PI mutations in the known HIV-seropositive partner was associated with a lower plasma HIV-1 RNA level leading to a decreased risk of transmission. Importantly, all transmitters with drug-resistant viremia (n = 9) transmitted drug-resistant virus to their partners, and all circulating mutations were transmitted in 7 of 9 cases. These data stand in contrast to the findings by other investigators of a low prevalence of drug-resistance mutations in newly infected individuals (Abstracts 502 and 504).

Barbour and colleagues evaluated the evolution of replication capacity and phenotypic drug susceptibility in 22 untreated HIV-infected adults within 6 months of seroconversion, who were followed up for a median of 1 year (Abstract 617). At baseline, the median replication capacity (percent control, not normalized), plasma HIV-1 RNA level, and CD4+ count were 47%, 3.79 $\log_{10}$ copies/mL, and 608 cells/µL, respectively. Eight of 22 (38%) isolates demonstrated PI hypersusceptibility (< 0.4-fold change to ≥1 PI) and 6 of 22 (27%) demonstrated resistance by phenotypic testing to more than 1 drug. The median replication capacity for the 6 drug-resistant isolates was lower than for the 16 wild-type isolates (21% and 61%, respectively; $P = .07$). In follow-up, modest but statistically significant decreases in replication capacity of 0.54% per month ($P = .02$) were observed.

Simon and colleagues (Abstract 504) compared viral resistance and phylogenetic profiles among HIV isolates derived from newly infected individuals over 3 time periods: 1995 to 1998 (n = 76), 1999 to 2000 (n = 71), and 2001 to 2002 (n = 102). Resistance to nRTIs was less frequent over time, and PI and NNRTI resistance was more common. Transmission of drug-resistant virus was observed in 3 clades representing 14.6% of all drug-resistant variants observed over the period from 1995 to 2002. The individual mutations observed were K70R and K103N in reverse transcriptase and L90M in protease.

Treatment-Experienced Patients

'Immune-Discordant' Patients. Linden and colleagues presented single-time-point observational data from a cohort of 50 subjects adherent to stable HAART regimens (Abstract 146). Twenty "immune discordant" subjects (10 on PIs, 10 on NNRTIs) had CD4+ counts of 200 cells/µL or greater, which were stable or increasing, and plasma HIV-1 RNA levels ranging from 500 to 10,000 copies/mL. Another group of 20 subjects (10 on PIs, 10 on NNRTIs) had plasma HIV-1 RNA levels of less than 50 copies/mL and CD4+ counts of above 200 cells/µL that were stable or increasing. A further 10 subjects in whom PI therapy was failing had increasing plasma HIV-1 RNA levels and falling CD4+ counts. Comparing the discordant and treatment failure groups, the following were observed more frequently in the discordant group: nonsyncytium-inducing virus (0/15 discordant and 7/9 treatment failure, respectively; $P < .007$), higher levels of HIV Gag-specific immune responses, and lower levels of CD38+ cells. Lower median replication capacities were observed among the discordant-on-PI group than among the discordant-on-NNRTI and PI-failing groups, at 12%, 27%, and 22%, respectively ($P = < .05$ for differences between discordant-on-PI and discordant-on-NNRTI). This study includes subjects with immunologic failure on HAART and enhances our understanding of immunologic failure in populations with stable virologic failure.

Lopinavir/ritonavir. Kempf and colleagues presented a resistance analysis of the 96-week data from M98-863 (Abstract 600). This study evaluated antiretroviral-naive subjects with virologic failure on either lopinavir/ritonavir or nelfinavir, each combined with stavudine/lamivudine. The findings demonstrated the absence of lopinavir resistance in those with extended periods of viremia on lopinavir/ritonavir. The study also described the lower incidence of lamivudine and stavudine resistance in those taking lopinavir/ ritonavir than in those taking nelfinavir. These results extend observations made by others of the lower frequency of viral resistance in antiretroviral-naive subjects on ritonavir-boosted PI regimens (eg, the SOLO study, discussed in Abstract 598).

Atazanavir. Colonno and colleagues described the prevalence of atazanavir resistance in 7 trials, including 3 studies of PI-naive subjects (Abstract 597). Atazanavir resistance was observed in 4% of all subjects and 14% of those with virologic failure. Data on 58 atazanavir-resistant isolates were presented. A unique protease mutation, I50L (typically occurring with A71V), was observed in 26 isolates (23 from PI-naive subjects). In 18 of 19 isolates with matched phenotypic data, I50L conferred a 4-fold or greater increase in atazanavir resistance from baseline but also conferred hypersusceptibility (fold change to 0.4-fold or lower) to at least one other PI. Thus, the I50L mutation appears to broadly enhance susceptibility to available PIs while conferring atazanavir resistance (as determined by phenotypic assay). In recombinant isolates bearing the I50L mutation, the A71V mutation increased the level of atazanavir resistance without an apparent effect on the broad enhancement of susceptibilities to other PIs. Conversely, in PI-experienced individuals, atazanavir resistance was associated with the presence of at least 5 of the following 14 specific mutations: L10I/V/F, K20/R/M/I, L24I, L33I/F/V, M36I/L/V, M46I/L, G48V, I54V/L, L63P, A71V/T/I, G73C/S/T/S, V82A/F/S/T, I84V, and L90M).

Tipranavir. Cooper and colleagues described the virologic response and baseline phenotypic susceptibilities to the investigational drug tipranavir and other PIs in isolates derived from 216 multiple PI-experienced individuals

entering BI 1182.52, a tipranavir/ritonavir dose-ranging study (Abstract 596). Genotypic screening was employed as part of the study entry criteria. Isolates at entry had at least 1 of the following protease mutations: D30N, M46I/L, G48V, I50V, V82A/F/L/T, I84V, and L90M. However, isolates did not have more than 1 of V82L/T, I84V, or L90M. At baseline, 41 of 216 (19%) subjects had isolates with genotypic changes associated with resistance to all available PIs, and 22% of individuals had isolates with 3 or more of the following mutations observed in the setting of cross-resistance among PIs: L33I/V/F, V82A/F/L/T, I84V, and/or L90M. At baseline, 42%, 27.4%, and 30.6% of isolates had a fold change of less than 1.0, between 1.0 and 2.0, and greater than 2.0, respectively. Presence of 3 PI cross-resistance mutations was associated with greater than 2-fold tipranavir resistance, which was in turn associated with diminished virologic response.

GW433908. MacManus and colleagues evaluated the relative frequency of drug resistance evolution in antiretroviral-naive subjects taking the investigational drug GW433908 (a prodrug of amprenavir) with or without low-dose ritonavir or taking nelfinavir twice daily. All subjects also received abacavir/lamivudine at standard doses (Abstract 598). Patients studied were enrolled in the SOLO or NEAT trials; details of the latter are reviewed earlier in this article (Abstract 177). Genotypic data were obtained from subjects with plasma HIV-1 RNA levels greater than 1000 copies/mL at 2 consecutive visits from 12 weeks. The NEAT study compared GW433908 1400 mg twice daily with nelfinavir 1250 mg twice daily, each with twice-daily abacavir/lamivudine. The SOLO study evaluated GW433908 1400 mg once daily/ritonavir 200 mg once daily versus nelfinavir 1250 mg twice daily, each with twice-daily abacavir/lamivudine. At virologic failure in the NEAT study, comparing the GW433908 and nelfinavir arms showed no statistically significant differences in the incidence of PI mutations (8/29 vs 8/26, respectively) or lamivudine mutations (16/29 vs 20/26, respectively). Conversely, in the SOLO study at virologic failure there were no PI mutations in the GW433908/ritonavir arm (0/32) compared with 27 of 54 developing PI mutations in the nelfinavir arm ($P < .001$). In the SOLO study the incidence of M184V was also significantly lower in the GW433908/ritonavir arm than in the nelfinavir arm (4/32 vs 30/54, $P < .001$). For subjects receiving unboosted GW433908, the following mutations associated with resistance to GW433908 were observed to emerge during treatment failure: I54L/M, V32I, I47V, and M46I. These observations extend prior studies describing the absence of PI evolution on ritonavir-boosted regimens after virologic failure.

Amprenavir. In ESS400006, Schooley and colleagues evaluated baseline susceptibility to amprenavir as a predictor of 24-week virologic outcomes in subjects who had 3 or more months of prior PI failure and who were treated with amprenavir/ritonavir salvage therapy (Abstract 143). At entry, subjects had plasma HIV-1 RNA levels greater than 1000 copies/mL, CD4+ counts greater than 50 cells/μL, 5-fold or lower change in IC_{50} for abacavir resistance, and 4-fold or lower change in IC_{50} for other nRTIs. Subjects received abacavir with 1 other active nRTI. NNRTI-naive subjects (n = 38) received efavirenz, and NNRTI-experienced subjects received tenofovir (n = 76; 90% with tenofovir fold change less than 2.5). Subjects were randomized to receive amprenavir/ritonavir at 900 mg/100 mg or 600 mg/100 mg twice daily. Among those receiving efavirenz, between 85% and 95% had plasma HIV-1 RNA levels of less than 200 copies/mL at 24 weeks. Among those on tenofovir, at baseline all isolates had less than 4-fold change in amprenavir IC_{50} and 84% had at least 1 mutation associated with drug resistance. Also, among those receiving tenofovir, multivariate analyses demonstrated that a baseline amprenavir fold change in IC_{50} of less than 0.66 ($P = .015$), the absolute amprenavir fold change ($P = .018$), and the baseline plasma HIV-1 RNA level ($P = .022$) were associated with increased odds of achieving a plasma HIV-1 RNA level of less than 200 copies/mL at 24 weeks.

***Gag-Pol* Mutations.** Lastere et al retrospectively evaluated the impact of alterations in the *gag-pol* cleavage sites CA-p2, p2-NC, p7-p1 (A431V), and p1-p6 (L499 and P453) on week-12 virologic response to amprenavir among 82 amprenavir-naive subjects in the NARVAL trial (ANRS 088; Abstract 599). Subjects were highly antiretroviral-experienced with prior exposure to a median of 5 nRTIs and 3 PIs but were amprenavir-naive. No association was observed between any mutation and week-12 plasma HIV-1 RNA level. The frequency of 1 or more cleavage site mutations was CA-p2, 12 of 82 (14.6%); p2-NC, 75 of 82 (91.5%); p7-p1 (A431V), 28 of 82 (34%); and p1-p6 (L499P/F), 16 of 82 (19.5%), and P453, 19 of 82 (23%). The following significant associations were noted: A431V (34%) with changes at protease codons 10, 30, 54, and 82 ($P < .05$) and P453L with changes at codons 20, 82, and 88 ($P < .05$). A variety of polymorphisms were seen in the $p6^{gag}$ region. Thirty-seven isolates had insertions at the PTAPP motif. Fourteen of these insertions were at position P459 and were associated with mutations at protease codon 82 ($P = .02$). The mean 12-week plasma HIV-1 RNA decreases in isolates with and without P459 insertions were 0.3 and 1.0 $\log_{10}$ copies/mL, respectively ($P = .006$).

Enfuvirtide. Delfraissy and colleagues presented pooled efficacy data from the TORO I and II trials, in which enfuvirtide was added to an optimized background regimen in an open-label, randomized (2:1) fashion (Abstract 568). In the TORO I (n = 661) and II (n = 334) arms, multiple regression analyses demonstrated the following as significant predictors of change in plasma HIV-1 RNA level at 24 weeks (ITT, last observation carried forward): entry CD4+ count ($P < .0001$); use of enfuvirtide ($P < .0001$); use of lopinavir/ritonavir in the optimized background ($P = .0037$); prior lopinavir/ritonavir use ($P < .0001$); and phenotypic sensitivity score per unit ($P < .0001$). Notably, there was a significantly greater incidence of bacterial pneumonia in the enfuvirtide plus optimized background arm than in the optimized background alone arm (4.5% and 0.3%, respectively; $P = .0094$).

Whitcomb and colleagues evaluated enfuvirtide susceptibilities in 612 baseline isolates derived from the subjects in the TORO I and II trials (Abstract 557). Isolates were found to use CCR5 or CXCR4 or to be dual tropic in 62%, 4%, and 34% of cases, respectively. X4 isolates had slightly higher IC_{50} values than did R5 isolates (2.8 and 1.2, respectively). Baseline gp41 substitutions in the 36 to 45 codon region were uncommon (2%). Among R5 viruses an N42S substitution (15%) was associated with modest but significantly greater enfuvirtide susceptibility than that seen in isolates that were wild-type at this codon ($P < .001$). In addition, Heil and colleagues described 5 patient-derived isolates for which resistance to enfuvirtide and the investigational agent T-1249 appeared to localize to the HR2 domain of gp41, distinct from changes at the HR1 domain typically associated with enfuvirtide resistance (Abstract 615).

The impact of baseline and on-treatment enfuvirtide susceptibilities on 24-week virologic outcomes in TORO I and II was described by Greenberg and colleagues (Abstract 141). They evaluated 612 baseline phenotypes and results for the 205 of 218 enfuvirtide failures for which complete resistance data were available. For all isolates the mean baseline enfuvirtide EC_{50} was 0.258 µg/mL (range 0.007-7.526 µg/mL [+2 standard deviations (SD) 1.956 µg/ml; n = 16, 2.6%]). The week-24 change in plasma HIV-1 RNA level was not predicted by the following baseline characteristics: enfuvirtide susceptibility (612 isolates); enfuvirtide susceptibility of isolates at +/-1 or +/-2 SD from mean; HIV subtype; or coreceptor tropism. Among the enfuvirtide failures, the mean EC_{50} was 5.67 µg/mL (21-fold increase from baseline [range, <1- to 422-fold]). Mutations at codons 36 to 45 were seen in 185 of 187 (99%) patients with 4-fold or greater enfurvirtide resistance, including changes at codons V38 (n = 27, 42-fold change) and N43 (n = 19, 29-fold change).

Reverse Transcriptase Inhibitors. Lanier and colleagues described the median phenotypic reverse transcriptase inhibitor susceptibilities of distinct reverse transcriptase mutation clusters within a commercial database (Abstract 586). The mean fold changes for zidovudine of M184V plus M41L-L210W-T215Y/F (n = 108) and M184V plus D67N-K70R-K219Q/E/N/R (n = 130) were 15.6 and 3.7; for stavudine, 2.0 and 1.3; for abacavir, 6.5 and 4.0; for didanosine, 1.8 and 1.5; and for tenofovir, 1.4 and 1.0. This study also described the median nRTI fold changes in IC_{50} of the mutations K65R, K65R-M184V, and L74V/I-M184V as follows: zidovudine (0.5, 0.4, and 0.3), stavudine (1.4, 0.9, and 0.8), didanosine (2.1, 2.9, and 2.2), abacavir (2.7, 6.7, and 6.8), and tenofovir (1.9, 1.4, and 0.4). Therefore, zidovudine/stavudine resistance was not conferred by these non-NAM mutations.

Other Factors

Subtypes and Resistance. There were a number of presentations describing HIV infection with nonsubtype B strains (Abstracts 623, 624, 625, 628, and 629). Grossman and colleagues described differences in NNRTI genotypic resistance profiles in 279 patient-derived subtype C isolates (73 drug-naive, 224 experienced), 141 subtype B isolates (28 drug-naive, 113 experienced), and 476 subtype B isolate sequences in the Stanford database (Abstract 624). In the clinical cohort no statistically significant differences in mean nRTI and NNRTI exposure times were observed. Compared with the Stanford database, 3 mutations were significantly more frequent in subtype C than B isolates after NNRTI exposure: A98G/S postefavirenz (C = 35%, B = 8%; $P < .0001$), 98G/S postnevirapine (C = 46%, B = 13%; $P < .0001$), V106M postefavirenz (C = 19%, B = 1%; $P < .0001$), and Y188H/L postnevirapine (C = 11%, B = 2%; $P = 0.039$).

Kantor and colleagues presented preliminary comparative genotypic analyses of non-B HIV subtypes derived from numerous patients worldwide (Abstract 623). The 2267 isolates were from 836 treated and 1431 drug-naive subjects and included subtypes A, C, D, F, G, H, J, and K. Among drug-naive individuals, subtype-specific polymorphisms included, in reverse transcriptase, G190A/R in 2% of A isolates, and in protease, M46I/L in 3% of G isolates and V82I in 5% of C isolates and 85% of G isolates. Among treated individuals, L90M (33/73) was more frequent than D30N (2/73) after nelfinavir failure in subtypes A, C, or G ($P = .0001$). Protease mutation I54V was more common at first protease failure in subtypes F (11/19) and G (20/66) than in B (60/456, $P = .001$). The authors note that, in general, functionally important drug-resistant mutations were preserved across subtypes.

Colson and colleagues evaluated the protease sequences in 32 HIV-2 infected subjects from Marseilles, France (Abstract 628). Natural polymorphisms were observed at codons 14, 40, 43, 46, 65, and 70. Among PI-naive subjects, mutations L10V, V32I, M36I, M46I, I47V, A71V, and G73A were observed. Among PI-treated subjects, changes at codons K7R, V62T/A, and L99F were observed. These data highlight the importance of understanding the resistance profiles in HIV-2 and non-B subtype HIV-1 isolates.

NAMs and Replication Capacity. Miller and colleagues compared the median normalized replication capacities of selected isolates without associated PI mutations from a commercial database (Abstract 616). The mutations studied (number, percent replication capacity) were as follows: M184V (n = 57, 75%), K65R (n = 8, 56%) K65R-M184V (n = 3, 29%), Q151M-M184V (n = 2, 30%), and T69 insertion-M184V (n = 3, 29%). The replication capacity analyses for NAM-bearing isolates (number, percent replication capacity without M184V; number, percent replication capacity with M184V) are as follows: 1 or 2 NAMs (n = 60, 80%; n = 61, 66%), 3 or 4 NAMs (n = 36, 73%; n = 68, 55%), and more than 4 NAMS (n = 9, 82%; n = 21, 47%).

Long-Term Nonprogressors and Replication Capacity. Rodes and colleagues described the replication capacities and other characteristics in a cohort of 19 untreated long-term nonprogressors with a median estimated duration of infection of 14 years (Abstract 469). The median CD4+ count was 891 cells/µL. Subjects were described as slow progressors if they had net CD4+ count declines (n = 7) and nonprogressors if they had stable or increasing CD4+

counts (n = 12). Comparing slow progressors and nonprogressors, the median plasma HIV-1 RNA levels were 1118 copies/mL and 85 copies/mL, respectively ($P \leq .007$). In 10 of 12 nonprogressors the plasma HIV-1 RNA level was consistently less than 50 copies/mL. Comparing slow progressors and nonprogressors, 3 of 7 and 0 of 12, respectively, were heterozygous for the Δ32 CCR5 genotype ($P = .036$); coreceptor use was by CCR5 in 5 of 5 and 2 of 2, respectively. Replication capacity values in 6 isolates (2 nonprogressors and 4 slow progressors) were low, ranging from 3% to 45%.

Low-Level Viremia and Viral Persistence

A number of studies highlighted ongoing viral replication in the setting of HAART, the natural history of viremia at the lowest levels, and associated viral resistance. These observations will be of growing importance as plasma HIV-1 RNA levels and resistance tests with increasing sensitivity become incorporated into clinical care (Abstracts 183, 465, 466, 494, 576, and 609). Maldarelli and colleagues described a novel plasma HIV-1 RNA quantitation assay employing 7.0 mL of plasma, which has a lower limit of quantification than has previously existed—as low as 1 copy/mL (Abstract 466). The plasma HIV-1 RNA level was quantified by bDNA assay (version 3.0) in 22 subjects with on-treatment plasma HIV-1 RNA levels sustained at 75 copies/mL or lower for at least 4 months. Of these, 7 of 22 had plasma HIV-1 RNA levels of 1 copy/mL or lower, 6 of 22 had between 1 and 5 copies/mL, and 9 had consistently quantifiable plasma HIV-1 RNA below 75 copies/mL for follow-up times ranging from 7 to 12 months.

The persistence of HIV replication in lymphoid tissue of patients on antiretroviral therapy was evaluated by Alós and colleagues (Abstract 465) and van Lunzen and colleagues (Abstract 183). Alós and colleagues compared tonsillar lymphoid tissue at baseline and after 1 year on HAART in subjects with plasma HIV-1 RNA levels of less than 20 copies/mL. Tissue samples were graded by architecture, p24 antigen staining, tissue CD4+ cells/CD8+ cells/cytotoxic T lymphocytes (CTLs), and lymphoid tissue HIV-1 RNA level. At baseline, 8 of 14 samples had an absence of lymphoid follicles and 12 of 14 had extensive and intense p24 staining cells. The mean lymphoid tissue HIV-1 RNA level at baseline and at 1 year were significantly different: 5.85 and 3.15 $\log_{10}$ copies/mg tissue, respectively ($P < .001$). At 1 year, 14 of 14 samples had lymphoid follicles and 0 of 8 samples positive for p24 had extensive and intense staining. Also at 1 year, inverse relationships were noted between the mean lymphoid tissue HIV-1 RNA level and the severity of histologic grading ($P = .03$) and the mean number and mean size of follicular centers ($P = .028$ and $P = .019$, respectively). Similar correlations were noted for p24 staining. Significant reductions in lymphoid CD8+ cells and CTLs and increases in CD4+ cells were also observed compared with baseline ($P < .001$), but these were significantly lower than cell counts in HIV-seronegative samples ($P < .001$). The authors suggest that although subjects had plasma HIV-1 RNA levels sustained below 20 copies/mL, ongoing local HIV replication was present and correlated with the degree of abnormal tissue histology.

Van Lunzen and colleagues evaluated axillary lymphoid tissue samples from 32 subjects with plasma HIV-1 levels suppressed to less than 25 copies/mL for a mean of 18 months on antiretroviral therapy. Treatment regimens comprised 2 or 3 nRTIs only (n = 7), 2 nRTIs plus a PI (n = 14), and 2 nRTIs plus an NNRTI (n = 11). In the nRTI, PI, and NNRTI groups, the proportions of samples with follicular virions trapped in dendritic cells were 3 of 6, 1 of 14, and 0 of 8, respectively; with detectable plasma HIV-1 RNA at germinal centers, 5 of 6, 6 of 12, and 5 of 8; and with staining for HIV at extrafollicular tissue, 3 of 6, 10 of 11, and 8 of 9. Coculture of HIV from lymph node was positive in 4 subjects, 3 of whom were on nRTIs only; viruses from these subjects variously demonstrated the M41L, M184V, and/or T215Y reverse transcriptase mutations. Lymphoid tissue from 1 subject on abacavir/zidovudine/lamivudine with transient viremic episodes or "blips" in plasma HIV-1 RNA levels yielded virus with the M184V mutation. These data extend prior observations on the potential for ongoing lymphoid viral replication while on antiretroviral therapy, especially among those on nRTI-only regimens.

Persaud and colleagues presented data derived from 12 children with plasma HIV-1 RNA levels below the limit of detection (ie, less than 50 or less than 20 copies/mL) for 1 to 6 years on a PI-inclusive regimen (Abstract 619). Three of 21 samples were taken during blips in plasma HIV-1 RNA level to less than 200 copies/mL. A highly sensitive plasma HIV-1 RNA assay (lower limit of detection, 2.5 copies/mL) demonstrated quantifiable plasma HIV-1 RNA in 11 of 12 subjects. In only 2 subjects on nelfinavir therapy, 199 clones from 54 positive amplifications of HIV protease demonstrated major protease mutations: 1 V32I in 2 of 6 clones during a blip to 241 copies/mL and 1 N88S in 1 of 6 clones in a subject with a plasma HIV-1 RNA level below 20 copies/mL. The authors note that viral replication appears to continue on effective antiretroviral therapy but is maintained largely by archival virus rather than by virus with ongoing evolution of resistance.

Di Mascio (Abstract 521) evaluated blips in 76 subjects treated within 6 months of primary infection with PI-inclusive HAART. Subjects had overall sustained suppression of HIV-1 RNA level to less than 50 copies/mL or below over the period of observation (mean 719 days), with plasma HIV-1 RNA testing every 23 days (mean). The mean and median frequencies of viral blips were 0.07 and 0.04 per sample, respectively. Only 32 patients (45%) did not show a viral blip during the entire period of plasma HIV-1 RNA suppression. The mean and median amplitudes of blips were 176 and 119 copies/mL, respectively. Pretherapy drug-resistance genotypic testing demonstrated mutations in 7 of 76 patients (9.2%). However, in only 3 cases did mutations confer resistance to drugs in the current regimen. No association was found between blip frequency and baseline drug resistance. However, blip frequency correlated with plasma HIV-1 RNA level at pretherapy setpoint ($P = .0009$).

Compartments

Several studies evaluated the plasma HIV-1 RNA levels and resistance profiles in different body compartments or fluids, including semen and prostate (Abstracts 454 and 459), female genital secretions collected by cervico-vaginal lavage (Abstracts 620 and 621), and breast milk (Abstract 96). Taylor and colleagues evaluated blood and semen samples from 72 HIV-seropositive men with detectable plasma HIV-1 RNA levels (Abstract 454). A significant correlation was noted between HIV-1 RNA levels in blood plasma and semen plasma (Spearman R = 0.53, $P < .0001$). Thirty percent of semen plasma HIV-1 RNA levels were less than 400 copies/mL. Median blood plasma HIV-1 RNA levels were significantly higher than corresponding semen plasma values: 4.70 $\log_{10}$ copies/mL and 3.56 $\log_{10}$ copies/mL, respectively ($P < .0001$). Nine subjects were identified with plasma HIV-1 RNA levels greater in semen than in blood. Urethritis was diagnosed in 3 of these 9 subjects (33%), compared with 3 of the 63 remaining subjects (4.8%; $P = .02$). Smith and colleagues evaluated HIV-1 RNA levels in weekly semen samples from 9 subjects (Abstract 459a). Subjects underwent prostate massage at weeks 5 to 10 prior to sample submission. HIV-1 RNA levels were more frequently detectable in semen samples obtained after prostate massage than without massage ($P = .035$).

Conley and colleagues compared the HIV genotypic profiles of concurrent plasma and cervico-vaginal lavage isolates from 22 women with stable or rebounding plasma HIV-1 RNA levels after an initial response to therapy (Abstract 620). Subjects were followed up for a median of 20 months and had a median of 3.5 samples taken. Resistance was present at baseline in 4 of the 22 women and emerged later in 10 of the 18 remaining women. Of these 10, only 5 had matching samples in both plasma and cervico-vaginal lavage, and in 4 of those 5 the resistance patterns were the same. Complete data were available on 13 women in whom resistance was observed to emerge over 29 visits. Resistance evolved over 9 of the 29 (31%) visits, but the resistance profiles were different in the 2 compartments at only 1 of the 9 (11%) visits. The authors suggest that in most women the resistance profiles in plasma and vaginal secretions are closely matched.

Pharmacology

Once-Daily Dosing

Didanosine with enteric coating (EC) and tenofovir are used together in once-daily antiretroviral therapy regimens. However, didanosine levels are elevated when administered with tenofovir, which prompted consideration of reducing the didanosine EC dose to 250 mg once daily. Kearney and colleagues presented pharmacokinetic data from healthy volunteers who were first given a single dose of didanosine EC 400 mg alone as a reference treatment. Subjects then received tenofovir 300 mg and a reduced dose of didanosine EC 250 mg in 3 different manners: staggered, with didanosine EC given in a fasted state 2 hours after tenofovir; simultaneously after a light meal (373 kcal, 20% fat); or simultaneously in a fasted state (Abstract 533). The tenofovir 300 mg/didanosine EC 250 mg 24-hour area under the curve (AUC_{24}) for all 3 groups was similar to the AUC_{24} of the single dose of didanosine EC 400 mg, with the former AUC_{24} nearly identical to the latter AUC_{24} for the staggered group, 11% lower for the light-meal group, and 14% higher for the fasted group. This finding suggests that staggering the doses results in acceptable drug levels but that other options exist for the 2 drugs to be taken simultaneously in a true once-daily regimen.

Kaul and colleagues also presented data on possible interactions between tenofovir and stavudine in extended-release format (XR) (Abstract 534). There were no differences seen in the value of maximum concentration (C_{max}), AUC_{24}, or median time of maximum concentration (T_{max}) of stavudine XR when given at 100 mg alone or with tenofovir 300 mg after a light meal (373 kcal). This result provides additional pharmacokinetic support for coadministering stavudine XR and tenofovir in once-daily regimens.

Drug-Drug Interactions

Efavirenz/Nelfinavir. Smith and colleagues evaluated the effect of efavirenz on the pharmacokinetics of nelfinavir (Abstract 148). Previous studies in healthy volunteers suggested that nelfinavir levels are increased by coadministration of efavirenz. This substudy of ACTG 384, however, found a trend in HIV-seropositive patients toward lower nelfinavir levels when the drug was coadministered with efavirenz at week 32 (ΔC_{max} $P = .08$, change in value of minimim concentration [ΔC_{min}] $P = .04$, ΔAUC_{12} $P = .07$). Although the combination of efavirenz and nelfinavir is rarely used in clinical practice, this study suggests that short-term pharmacokinetic data from healthy volunteers do not necessarily generalize to HIV-infected individuals.

Tenofovir/Ritonavir/Atazanavir. Taburet and colleagues presented data on a possible interaction between tenofovir and ritonavir/atazanavir (Abstract 537). They compared several pharmacokinetic parameters of ritonavir and atazanavir before and after addition of tenofovir. Although not all changes reached statistical significance, atazanavir and ritonavir levels were both reduced when the drugs were administered with tenofovir. The investigators felt that the reduced levels of atazanavir were likely due to reduced levels of ritonavir, the mechanism of which is unknown. Similar studies of tenofovir and atazanavir given without ritonavir are highly anticipated, as these 2 drugs will be an attractive combination for once-daily dosing.

Atazanavir/Efavirenz. Tackett and colleagues presented data on the interaction of atazanavir and efavirenz (Abstract 543). The investigators tested 2 strategies to overcome the reduction of atazanavir levels when the drug is coadministered with efavirenz. They compared pharmacokinetic parameters between atazanavir 400 mg once daily without efavirenz (standard dosing) and either atazanavir 300 mg/ritonavir 100 mg or atazanavir 600 mg, both with efavirenz 600 mg once daily. They found that the atazanavir AUC was increased by 39% when giving

atazanavir 300 mg/ritonavir 100 mg/efavirenz 600 mg and decreased by 21% when giving atazanavir 600 mg/efavirenz 600 mg. Further data are needed before firm recommendations can be made regarding coadministration of atazanavir and efavirenz.

Lopinavir/Ritonavir/Indinavir. Dual- and triple-PI combination regimens are used with increased frequency when devising salvage therapy in PI-experienced individuals with virologic failure. Pharmacokinetic drug interactions, however, have been challenging to overcome with regard to potential toxicities and subtherapeutic PI drug concentrations resulting from such drug combinations. Isaac and colleagues presented the results of the Protect Study of pharmacokinetic interactions, which evaluated the feasibility of combining lopinavir/ritonavir with indinavir (Abstract 531). Plasma drug concentrations of both drugs and determinations of cerebrospinal fluid (CSF) and seminal plasma concentrations were examined when these drugs were coadministered. Ten HIV-infected male subjects taking lopinavir 400 mg/ritonavir 100 mg twice daily in combination with at least one nRTI (with 3 subjects also taking nevirapine) were enrolled. Indinavir 400 mg twice daily was added to stable background therapy, and pharmacokinetic sampling was performed prior to and 2 weeks after adding indinavir therapy.

No significant differences in lopinavir pharmacokinetic parameters were demonstrated when lopinavir/ritonavir was coadministered with indinavir 400 mg twice daily, although marked interpatient variability was noted. Median lopinavir C_{max}, C_{min}, and AUC_{12} increased by 9%, 46%, and 20%, respectively, after the addition of indinavir ($P < .30$; $P < .33$; and $P < .06$; Wilcoxon test). Lopinavir trough concentrations in seminal plasma and CSF were low and comparable with published data. All lopinavir was below detection limits in all CSF samples before the addition of indinavir; following indinavir, 2 of 4 samples were above the nonprotein-bound IC_{50} for lopinavir of 11 ng/mL. Seminal plasma concentrations were significantly lower than blood concentrations, and only 2 of 5 semen samples had lopinavir concentrations in excess of the protein-corrected minimum effective concentration (MEC) for lopinavir of 700 ng/mL. Trough blood plasma indinavir concentrations were above the protein-corrected MEC for wild-type virus of 80 to 100 ng/mL in 7 (88%) of 8 patients. Indinavir seminal concentrations were above the plasma MEC of all samples and indinavir CSF concentrations were in excess of the nonprotein-corrected IC_{95} for indinavir (21 ng/mL) in all samples.

The lopinavir/ritonavir/indinavir PI combination regimen was well-tolerated. The addition of indinavir 400 mg twice daily to the lopinavir/ritonavir regimen did not significantly alter the median lopinavir pharmacokinetic parameters. Blood lopinavir plasma concentrations were therapeutic in all patients both before and after the addition of indinavir; CSF and seminal concentrations, however, were subtherapeutic in the majority of samples tested before and after coadministration of indinavir. In contrast, the indinavir concentrations in blood plasma (C_{min}), CSF, and seminal plasma were above the target concentrations in 7 of 8 plasma samples tested and in all CSF and seminal plasma samples collected.

Food Interactions

Petersen and colleagues examined how to optimize nelfinavir pharmacokinetics with food (Abstract 544). They found a dramatic variation in the C_{max} and AUC of nelfinavir according to different meals. The AUC_{12} and C_{max} were 4.3 and 3.3 times higher, respectively, when subjects took nelfinavir with a 100 kcal/50% fat meal than when they were fasting. In addition, the AUC_{12} and C_{max} levels of nelfinavir were 1.5 and 1.4 times higher, respectively, for a 1000 kcal/50% fat meal than for a 500 kcal/20% fat meal. More data are needed to understand the relative importance of total calories and fat content on nelfinavir pharmacokinetics.

Hepatitis B and C Virus Interactions

Bossi and colleagues presented interesting data from a GENOPHAR substudy evaluating indinavir pharmacokinetics in participants with either chronic hepatitis B virus or chronic hepatitis C virus infection (Abstract 546). Five of 6 patients with chronic hepatitis B or C virus had a C_{min} of indinavir (given at 400 mg with ritonavir 100 mg twice daily) above the therapeutic range, compared with 3 of 16 patients without either of these conditions. Normal drug levels were achieved in all patients with supratherapeutic levels by reducing the dose to indinavir 200 mg/ritonavir 100 mg twice daily. Given the high prevalence of chronic hepatitis in HIV-infected individuals, further pharmacokinetic studies in coinfected patients are warranted.

Conclusions

The conference once again demonstrated that it is the premier meeting of the year during which new developments in antiretroviral therapy are presented. Covering such topics as the promise of new drugs, the reality of simplified regimens, important data from randomized trials of treatment interruptions, and the increasing complexity of drug resistance, the meeting was again notable for moving the field forward in incremental but substantial ways. The conference embodies the nexus between clinical research and clinical practice in this rapidly moving field.

Written by Drs Albrecht, Wilkin, Coakley, and Hammer in March 2003.

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Appendix. Antiretroviral Drug Resistance and Replication Capacity

Authors (Abstract), Description	Results and Comments
Greenberg et al (141) Impact of baseline enfuvirtide susceptibilities on virologic outcomes to 24 wks in TORO I and II, using 612 baseline phenotypes and results from 205/218 enfuvirtide failures with complete resistance data.	**Enfuvirtide baseline EC_{50}** • Range: 0.007-7.526 µg/mL • Mean: 0.258 µg/mL • 2 SD: 1.956 µg/mL (n=16, 2.6%) **Wk-24 change in HIV-1 RNA levels not predicted by:** • Baseline enfuvirtide susceptibility (612 isolates) • Baseline enfuvirtide susceptibility of isolates at 1 or 2 SD from mean • Clade • CCR5/CXCR5 tropism **Enfuvirtide resistance** • Mean enfuvirtide EC_{50} = 5.67 µg/mL • 21-fold increase from baseline (<1-422-fold) • 185/187 (99%) with ≥4-fold enfuvirtide resistance had mutations at gp41 codons 36-45, including V38 (n=27, mean 42-fold change) and N43 (n=19, mean 29-fold change) **Comment:** There is a broad natural range of enfuvirtide susceptibilities, which in this analysis was not predictive of virologic response. Enfuvirtide resistance is closely linked to mutations at gp41 codons 36-45.
Schooley et al (143) ESS40006 study of baseline amprenavir susceptibility as a predictor of 24-wk virologic outcome in subjects receiving salvage therapy. Subjects received abacavir + 1 other nRTI with amprenavir/ritonavir 100 mg/900 mg bid or 100 mg/600 mg bid. NNRTI-naive subjects (n=38) received efavirenz; NNRTI-experienced subjects (n=76) received tenofovir (90% <2.5-fold change to tenofovir).	**Baseline Data** • HIV-1 RNA level ≥1000 copies/mL • CD4+ count >50 cells/µL • Resistance ≤5-fold resistance to abacavir ≤4-fold resistance to other NNRTIs **Wk 24** • HIV-1 RNA levels <200 copies/mL (%) (ITT observed) On efavirenz: 27/31 (87%) On tenofovir: 38/59 (64%) • For those on tenofovir, mean prior PI experience was 3.3 years; all isolates had <4-fold change to amprenavir • In multivariate analyses, the following were associated with increased odds of an HIV-1 RNA level <200 copies/mL: Baseline amprenavir fold change <0.66 (OR, 7.24; *P*=.015) Baseline HIV-1 RNA level (OR, 0.35, *P*=.022) **Comment:** The potency of NNRTIs in salvage is highlighted. Amprenavir hypersusceptibility was predictive of outcomes on pharmacokinetically boosted amprenavir regimens.
Miller et al (616) Evaluated median normalized RCs of isolates in a commercial database with specific RT mutations and without PI resistance.	**RT mutations: RC (no. patients)** • M184V: 57% (130) • K65R: 56% (8) • K65R + M184V: 29% (3) • Q151M + 184V: 30% (2) • T69insertion + M184V: 29% (3) • 1-2 NAMS [+ M184V]: 80% (61) [66% (60)] • 3-4 NAMS [+ M184V]: 73% (36) [55% (68)] • >4 NAMS [+ M184V]: 82% (9) [47% (21)] **Comment:** K65R and K65R + M184V were associated with large reductions in RC. The mean RC of representative wild-types viruses was 94%.

Appendix. Antiretroviral Drug Resistance and Replication Capacity, Continued

Authors (Abstract), Description	Results and Comments
Little et al (152) Evaluated the relationship between set point viremia, drug susceptibility, and RC in 194 treatment-naive subjects 5-24 months after primary infection. The mean baseline HIV-1 RNA level, CD4+ count, RC, and time from infection to evaluation were 4.5 $\log_{10}$ copies/mL, 531 cells/µL, 43%, and 123 days, respectively.	**Resistance data** • Prevalence of resistance nRTI (>4-fold): 4% NNRTI (>10-fold): 7% PI (>10-fold): 4% • Mean % CD4+ for baseline isolates Drug-resistant: 34% Drug-susceptible: 28% (*P*=.05) No difference in mean HIV-1 RNA levels between resistant and susceptible baseline isolates **RC data** • No differences in baseline HIV-1 RNA levels or CD4+ counts by baseline RC • Mean RCs PI-hypersusceptible[1] isolates: 50% PI-susceptible isolates: 30% (*P* <.0001) • PI hypersusceptibility among subjects infected with drug sensitive-virus Primary infection: 33% Established infection: 18% • Viremia set point 0.6 $\log_{10}$ copies/mL higher with NNRTI resistance than without (*P* =.005); no difference noted with other drugs **Comment:** No relationship was observed between RC and HIV-1 RNA levels. The authors suggest that low-RC virus at primary infection may evolve to higher-RC virus over time.
Simon et al (504) Surveillance study of 102 newly infected individuals. Resistance and phylogenetic analyses of isolates from 1995-1998 (n=76), 1999-2000 (n=71), and 2001-2002 (n=102) were conducted.	• Resistance was observed more frequently to nRTIs and less frequently to PIs and NNRTIs over time. • Phylogenetic clustering was seen in 54/241 (21%) patients. • Transmission of drug-resistant virus was seen in 3 clades (14.6%) of all resistant isolates. **Comment:** The reduced incidence of PI and nRTI resistance is notable. The transmission of drug-resistant virus is also highlighted.
Haubrich et al (565) CCTG 578 substudy of PI-experienced patients who either switched immediately to lopinavir/ritonavir or underwent a >4-month STI prior to starting lopinavir/ritonavir.	**Immediate change group (n=16)/treatment interruption group (n=14)** • Prior use of ≥2 PIs: 25%/38% • HIV-1 RNA levels (copies/mL): 4.5 $\log_{10}$/5.2 $\log_{10}$ (*P* <.05) • CD4+ cells/µL: 190/172 • Sensitive to ≥2 drugs: 73%/100% • Lopinavir C_{12}/IC_{50}: 17/26 **Wk 6** • HIV-1 RNA levels, CD4+ counts, and changes in CD4+ count from baseline not significantly different between arms. **Comment:** Exploratory analyses did not demonstrate a definitive benefit for STI.
Lanier et al (635) Queried a commercial HIV data warehouse for changes in resistance patterns on genotypic tests for the period 1/99-7/02.	• Wild-type: 29%⇒37% • K65R: 0.64%⇒1.69%* • Y115F: 0.59%⇒1.42%* • L74V: 9.77%⇒7.85% • T215Y: 32%⇒23% • D67N/K70R/K219Q/E: 13%⇒8%* • M41L/L210W/T215Y: 17%⇒11%* • K103N: 29%⇒30% • Y181C: 19%⇒12%* **Comment:** A relative reduction in nRTI resistance was noted. **P* <.0001

Appendix. Antiretroviral Drug Resistance and Replication Capacity, Continued

Authors (Abstract), Description	Results and Comments
Delfraissy et al (568) Pooled efficacy data from TORO I and II: open-label, randomized trials of OB alone vs OB + enfuvirtide.	**Baseline values: Enfuvirtide + OB (n=661) vs OB (n=334)** • Median HIV-1 RNA (log_{10} copies/mL): 5.2/5.1 • Median CD4+ cells/μL: 88/97 • Mean genotypic susceptibility score: 1.7/1.8 • Mean phenotypic susceptibility score: 1.6/1.6 **Outcomes: Enfuvirtide + OB vs OB** • % HIV-1 RNA <50 copies/mL: 15.9/6.3 (*P* <.0001) • % HIV-1 RNA <400 copies/mL: 32.7/15.0 (*P* <.0001) • Difference in HIV-1 RNA reduction (Enfuvirtide + OB) - OB: -0.846 log_{10} copies/mL • Difference in CD4+ count increase (Enfuvirtide + OB) - OB: 36.6 cells/μL **Regression analyses for change in HIV-1 RNA levels at 24 wks** • Use of enfuvirtide: -0.89* • Lopinavir/ritonavir in OB: -0.22* • History of lopinavir/ritonavir use: +0.83* • Phenotypic susceptibility score: -0.26* **P* values: .0037-<.0001 **Comment:** Bacterial pneumonia was more frequent in the enfuvirtide + OB arm (4.5%) than in the OB-only arm (0.3%), (*P*=.0094).
Parkin et al (585) HIV phenotypic assay sensitivity and reproducibility in 2000 wild-type viruses and 10 resistant clones derived from a commercial database.	**Median IC_{50}s of wild-type viruses** • Tenofovir: 0.86 • Zidovudine: 0.89 • Abacavir: 0.90 • Stavudine: 0.95 • Didanosine: 0.95 • Lamivudine: 1.01 • Nevirapine: 0.89 • Delavirdine: 1.25 • Efavirenz: 0.86 • Lopinavir: 0.69 • Amprenavir: 0.70 • Atazanavir[2]: 0.70 • Saquinavir: 0.70 • Indinavir: 0.78 • Ritonavir: 0.82 • Nelfinavir: 1.04 **Mean coefficients of variation of IC_{50}s for resistant isolates** • 10 resistant clonal isolates were phenotyped multiple times and coefficients of variation calculated: Zidovudine: 32% Other nRTIs: 12%-18% NNRTIs: 12%-15% PIs: 14%-17% **Comment:** In wild-type viruses, nelfinavir fold change >2.0 was associated with the following mutations: L10V/I, G16A, D60E, Q61N or A71V/T (OR=2.2-4.5, P <.0001- .0054). The median susceptibility for most wild-type isolates was <1.0.
Scott el al (631) Noted changes in the prevalence of antiretroviral resistance from 1998-2000 in clinics throughout Britain. Samples were taken from 1 calendar month for each year.	• Comparing 1998 with 2000, reductions in resistance to nRTIs (OR=0.17, *P* =.001) and PIs (OR=0.23, *P* =.02) were observed • Prevalence of the K103N mutation increased (OR=3.53, *P*=.005) concurrent with widespread use of NNRTIs **Comment:** A relative reduction in nRTI resistance was noted.

Appendix. Antiretroviral Drug Resistance and Replication Capacity, Continued

Authors (Abstract), Description	Results and Comments

Lanier et al (586)

Measured median nRTI susceptibilities of specific mutation clusters in a commercial database (susceptible isolates in italics).

Mutations	Zidovudine/stavudine fold changes	Didanosine/abacavir/ tenofovir fold changes
M184V + M41L-L210W-215Y/F (n=108)	15.6 / 2.0	1.8 / 6.5 / 1.4
M184V + D67N-K70R-K219Q/E/N/R (130)	3.7 / *1.3*	*1.5* / *4.0* / *1.0*
K65R (22)	*0.5* / *1.4*	2.1 / *2.7* / 1.9
K65R-M184V (7)	*0.4* / *0.9*	2.9 / 6.7 / 1.4
L74V/I-M184V (68)	*0.3* / *0.8*	2.2 / 6.8 / *0.4*

Comment: The M184V + M41L/L210W/215Y/F cluster conferred greater cross-resistance than did other clusters. Non-NAMs did not confer zidovudine/stavudine resistance.

Leigh Brown et al (594)

Analyzed genotypic profiles associated with ritonavir hypersusceptibility in 188 antiretroviral-naive individuals.

- Combinations of changes at protease codons 10/13/37/57/62/63/73 and *gag* cleavage sites were identified that were associated with ritonavir hypersusceptibility (n=22).
- A strong association was noted between increasing ritonavir hypersusceptibility and RCs ≤10% (Pearson correlation, 0.51; $P < 10^{-10}$).

Comment: Lower RC was closely associated with PI hypersusceptibility in antiretroviral-naive subjects.

Cooper et al (596)

BI 11823.52: A double-blind, randomized study of tipranavir[2]/ritonavir in highly PI-experienced subjects at the following doses: OB + tipranavir/ritonavir 500 mg/100 mg bid, 500 mg/200 mg bid, or 750 mg/200 mg bid.

Resistance at baseline

- Genotypic screening at baseline allowed isolates with ≥1 of the following PI mutations: D30N, M46I/L, G48V, I50V, V82A/F/L/T, I84V, and L90M; however, it did not allow ≥1 of V82L/T, I84V, or L90M
- 41/216 (19%) of baseline isolates had genotypic resistance to all available PIs
- 22% had ≥3 of the following PI cross-resistance mutations: L33I/V/F, V82A/F/L/T, I84V, and/or L90M

Median IC_{50}s of baseline isolates and those with 3 PI cross-resistance mutations (measured by commercial assay)

- All isolates, isolates with 3 PI cross-resistance mutations

Tipranavir:	1.1, 2.2
Lopinavir:	76.5, 102.8
Amprenavir:	8.7, 22.1
Saquinavir:	7.0, 32.9
Indinavir:	12.2, 17.1
Nelfinavir:	36.8, 43.4
Ritonavir:	94.2, 422.0

Overall median day 14 change in HIV-1 RNA by baseline tipranavir susceptibility

- Fold change: Change in HIV-1 RNA (log_{10} copies/mL)

<1.0:	-1.23
1.0-2.0:	-1.24
2.0-4.0:	-0.20
>4.0:	-0.19
>4.0:	0.0 in tipranavir/ritonavir dose arm 500 mg/100 mg
>4.0:	-0.58 in tipranavir/ritonavir dose arm 750 mg/200 mg

Comment: Three PI cross-resistance mutations were associated with >2-fold change to tipranavir, which in turn was associated with diminished virologic response.

Appendix. Antiretroviral Drug Resistance and Replication Capacity, Continued

Authors (Abstract), Description	Results and Comments
Colonno et al (597) Measured atazanavir resistance in 3 trials of PI-naive subjects and 4 of PI-experienced subjects.	• Unique protease mutation I50L (±A71V) seen in 26 isolates (23 from PI-naive subjects) • 18/19 I50L isolates with matched phenotypic data from baseline had a ≥4-fold change in atazanavir resistance and also had hypersusceptibility (≤0.4-fold change) to at least 1 PI • In I50L recombinants, the A71V mutation increased resistance to atazanavir without apparent effect on observed hypersusceptibility conferred to other PIs, including nelfinavir and amprenavir • In PI-experienced individuals, atazanavir resistance was associated with 5/14 mutations: L10I/V/F, K20/R/M/I, L24I, L33I/F/V, M36I/L/V, M46I/L, G48V, I54V/L, L63P, A71V/T/I, G73C/S/T/S, V82A/F/S/T, I84V, and L90M **Comment:** Protease mutation I50L (±A71V) appears to confer modest atazanavir resistance while broadly enhancing susceptibilities of available PIs.
Macmanus et al (598) NEAT and SOLO studies of resistance data for GW433908[2] vs nelfinavir. Subjects had HIV-1 RNA levels >1000 copies/mL at 2 consecutive visits after study wk 12.	**Regimens** • NEAT: GW433908 1400 mg bid + abacavir/lamivudine bid vs nelfinavir 1250 mg bid + abacavir/lamivudine bid • SOLO: GW433908 1400 mg/ritonavir 200 mg qd + abacavir/lamivudine bid vs nelfinavir 1250 mg bid + abacavir/lamivudine bid **Primary or secondary PI mutations at virologic failure** • NEAT GW433908: 8/29 Nelfinavir: 8/26 (*P*=NS) • SOLO GW433908: 0/32 Nelfinavir: 27/54 (*P* <.001) **M184V mutation at virologic failure** • NEAT GW433908: 16/29 Nelfinavir: 20/26 (*P*=NS) • SOLO GW433908: 4/32 Nelfinavir: 30/54 (*P* <.001) **Comment:** The absence of PI resistance in pharmacokinetically boosted GW433908 compliments the M98-863 study findings (Abstract 600; see below). D30N or L90M emerged in 28/80 (35%) in whom nelfinavir was failing. On "unboosted" GW433908, at codons I54L/M, V32I, I47V, and M46I emerged.
Lastere et al (599) Studied the impact of *gag/pol* cleavage site changes on week 12 HIV-1 RNA levels among 82 amprenavir-naive subjects treated with amprenavir in the NARVAL trial (ANRS 088).	• Cleavage site mutation frequencies CA-p2: 12/82 (14.6%) p2-NC: 75/82 (91.5%) p7-p1 A431V: 28/82 (34%) p1-p6 L499P/F/V 16/82 (19.5%); P453L 19/82 (23%) • No association observed between any mutation and wk 12 HIV-1 RNA changes A431V associated with changes at codons 10, 30, 54, and 82 (*P* <.05) P453L associated with PI mutations at codons 20, 30, 82, and 88 (*P* <.05) • 37 isolates had cleavage site insertions at the PTAPP motif; 14/37 were at position P459 • Mean wk-12 HIV-1 RNA change With P459 insertions: -0.3 $\log_{10}$ copies/mL Without insertions: -1.0 $\log_{10}$ copies/mL (*P*=.006) **Comment:** Insertions at position P459 may negatively impact PI activity in salvage regimens.

Appendix. Antiretroviral Drug Resistance and Replication Capacity, Continued

Authors (Abstract), Description	Results and Comments
Kempf et al (600) M98-863 study: 96-wk comparison of virologic failure (>400 copies/mL HIV-1 RNA) with lopinavir/ritonavir vs nelfinavir. The proportions of patients with virologic failure (with available genotypic test results) were 51/326 (15.6%) in the lopinavir/ritonavir arm and 96/327 (29%) in the nelfinavir arm.	**PI resistance** • Lopinavir/ritonavir + stavudine/lamivudine: 0/51 (0%) • Nelfinavir + stavudine/lamivudine: 46/96 (48%) (*P* <.001) **Lamivudine resistance** • Lopinavir/ritonavir arm: 19/51 (37%) • Nelfinavir arm: 79/96 (82%) (*P* <.001) **Risk of resistance in nelfinavir arm for those with 1 yr virologic failure after initial suppression** • Lamivudine: 100% • Nelfinavir: 74% • Stavudine: 15% **Comment:** In the lopinavir/ritonavir arm, 7/51 had mutations at codons 36, 10, or 71 but without change in lopinavir susceptibility. These findings compliment SOLO study resistance data (Abstract 598; see above).
Dalmau et al (608) NEFA trial: Examined the comparative 24-mo outcomes of 3 "switch-off" strategies in 460 subjects on 1 PI/2 nRTIs with HIV-1 RNA levels <200 copies/mL for ≥6 mos.	**Baseline** • Subjects switched off PI and randomized to start efavirenz (n=156), nevirapine (155), or abacavir (149). • History of mono- or bi-nRTI use permitted • Median mos on HAART (percent with prior nRTI-only therapy) Nevirapine arm: 29 (50%) Efavirenz arm: 31 (58%) Abacavir arm: 30 (46%) **At 24 mos** • 11% had HIV-1 RNA levels >200 copies/mL • Rates of failure Nevirapine: 15/155 Efavirenz: 12/156 Abacavir: 24/149 • No differences observed in failure rates among those with no prior nRTI exposure • Genotypic NRTI resistance was more frequent in those failing in the abacavir arm than in the other 2 arms **Comment:** Greater numbers in the abacavir arm had virologic failure. These data extend prior observations on the reduced potency of nRTI-only regimens in nRTI-experienced subjects.
Deeks et al (640) 16-wk outcomes of partial treatment interruption of either PI (n=15) or nRTI (n=5) therapy and continuation of other antiretroviral drugs in a highly selected cohort of subjects with ongoing viremia on a stable HAART regimen.	**Median baseline characteristics (n=20)** • CD4+ count: 336 cells/μL; change from pretherapy baseline: +245 cells/μL • HIV-1 RNA level: 3.9 $\log_{10}$ copies/mL; change from pretherapy: -1.2 $\log_{10}$ copies/mL • Abacavir fold change: 5.2; ritonavir fold change: 31.0 **Outcomes to wk 16: Stop PI (n=15)/stop nRTI (n=5)** • Mean HIV-1 RNA change ($\log_{10}$ copies/mL): 0.005/0.03 (*P* <.001*) • Mean change in CD4+ count (cells/μL/wk): 0.0/-3.5 (*P*=.006*) • No. with sustained HIV-1 RNA increase >0.5 $\log_{10}$ copies/mL: 2 of 15/5 of 5 **P* values reflect change in outcome vs change=0 **Comment:** The stop-PI group had greater HIV-1 RNA changes at wk 2 (*P* =.001) and significant reductions in wk 4 fasting lipids. Little short-term alteration in drug susceptibilities was noted in most subjects.

Appendix. Antiretroviral Drug Resistance and Replication Capacity, Continued

Authors (Abstract), Description	Results and Comments
Barbour et al (617) Measured evolution of RC (nonnormalized) and drug susceptibilities in 22 untreated subjects with early HIV infection followed up for median of 1 yr.	**Median baseline values** • HIV-1 RNA: 3.79 $\log_{10}$ copies/mL • CD4+ count: 608 cells/μL • PI hypersusceptibility (fold change ≤0.4 to ≥1 PI): 7/21 (33%) • Resistance to ≥1 drug: 6/22 (27%) • RC, all isolates (n=22): 47% • RC, wild-type isolates (n=16): 61% • RC, drug-resistant isolates (n=6): 21% (*P* =.07 vs wild-type RC) **Comment:** Modest but significant decreases in RC of 0.54% per month (*P* =.02) were observed in follow-up.

[1]PI hypersusceptibility is a fold change ≤ 0.4 to at least 1 PI.

[2]Investigational drug; not approved by the US Food and Drug Administration.

bid indicates twice daily; C_{12}, plasma concentration at 12 hours; EC_{50}, 50% effective concentration; HAART, highly active antiretroviral therapy; IC_{50}, 50% inhibitory concentration; NAM, nRTI-associated mutation; NNRTI, nonnucleoside reverse transcriptase inhibitor; nRTI, nucleoside reverse transcriptase inhibitor; OB, optimized background; OR, odds ratio; PI, protease inhibitor; RC, replication capacity; SD, standard deviation; STI, structured treatment interruption.

Conference Abstracts Cited in This Appendix

585. Distribution of Phenotypic Drug Susceptibility Among More than 2,000 Wild-type Viruses. N. T. Parkin, N. Hellmann, J. Whitcomb, L. Kiss, C. Chappey, C. J. Petropoulos.
608. NEFA Simplification Trial: Genotypic and Phenotypic Resistance Patterns Among Patients with Virological Failure. D. Dalmau, A. Ochoa de Echagüen, E. Martinez, M. Xercavins, M. Arnedo, J. A. Arnaiz, H. Knobel, E. Ribera, P. Domingo, B. Roson, M. Riera, F. Segura, J. M. Llibre, E. Pedrol, J. M. Gatell.
631. Surveillance of HIV-1 Drug Resistance Within the UK. P. Scott, E. Arnold, B. Evans, J. Shirley, P. Cane, D. Pillay.
635. Prevalence of Mutations Associated with Resistance to Antiretroviral Therapy from 1999-2002. E. R. Lanier, J. Scott, M. Ait-Khaled, C. Craig, T. Alcorn, D. Irlbeck, P. Gerondelis, R. Burgess, M. Underwood.

Conference Abstracts Cited in This Issue

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

4. Preventing New HIV Infections in the U.S.: What Can We Hope to Achieve? R. O. Valdiserri.
5. HIV Vif and the Evasion of Host Anti-viral Resistance. M. H. Malim.
7. RO033-4649: A New HIV-1 Protease Inhibitor Designed for Both Activity Against Resistant Virus Isolates and Favorable Pharmacokinetic Properties. N. Cammack, S. Swallow, G. Heilek-Snyder, Y. Lie, N. Parkin, A. Kohli.
8. First Clinical Results on Antiretroviral Activity, Pharmacokinetics, and Safety of TMC114, an HIV-1 Protease Inhibitor, in Multiple PI-experienced Patients. K. Arasteh, N. Clumeck, A. Pozniak, H. Jaeger, M. De Pauw, H. Muller, M. Peeters, R. Hoetelmans, S. De Meyer, I. van der Sandt, S. Comhaire, R. van der Geest.
9. Pyranodipyrimidines: A New Class of HIV Integrase Inhibitors That Block Viral Replication in Cell Culture. Z. Debyser, C. Pannecouque, W. Pluymers, B. Van Maele, J. Vercammen, V. Tetz, Y. Engelborghs, E. De Clercq, M. Witvrouw.
10. AK602: A Novel HIV-specific Spirodiketopiperazine CCR5 Inhibitor Potent Against a Wide Spectrum of R5-HIV. K. Maeda, H. Nakata, T. Miyakawa, H. Ogata, Y. Koh, S. Shibayama, K. Sagawa, Y. Takaoka, J. Moravek, Y. Koyanagi, H. Mitsuya.
11. Anti-HIV-1 Activity of TAK-220, a Small Molecule CCR5 Antagonist. Y. Iizawa, N. Kanzaki, K. Takashima, H. Miyake, Y. Tagawa, Y. Sugihara, M. Baba.
12. UK-427,857, a Novel Small Molecule HIV Entry Inhibitor Is a Specific Antagonist of the Chemokine Receptor CCR5. P. Dorr, M. Macartney, G. Rickett, C. Smith-Burchnell, S. Dobbs, J. Mori, P. Griffin, J. Lok, R. Irvine, M. Westby, C. Hitchcock, B. Stammen, D. Price, D. Armour, A. Wood, M. Perros.
13. Safety and Preliminary Anti-HIV Activity of an Anti-CD4 mAb (TNX-355; Formerly Hu5A8) in HIV-infected Patients. D. R. Kuritzkes, J. M. Jacobson, W. Powderly, E. Godofsky, E. DeJesus, F. Haas, K. A. Reimann, P. Yarbough, V. R. Curt, W. R. Shanahan.
14. PA-457 Is a Small Molecule Inhibitor of HIV-1 Budding/Maturation That Potently Inhibits Replication of Virus Isolates Resistant to All Classes of Approved Drugs. C. Wild, N. Kilgore, M. Reddick, F. Li, K Salzwedel, C. Matallana, G. Allaway, D. Martin.
14lb. T-1249 Demonstrates Potent Antiviral Activity over 10 Day Dosing in Most Patients Who Have Failed a Regimen Containing Enfuvirtide (ENF): Planned Interim Analysis of T1249-102, a Phase I/II Study. G. D. Miralles, J. P. Lalezari, N. Bellos, G. Richmond, Y. Zhang, H. Murchison, R. Spence, C. Raskino, R. A. DeMasi.
17. Inside-out Regulation of HIV-1 Particle Fusion. Christopher Aiken, Donald J. Wyma, Janet E. Lineberger, Michael D. Miller.
18. Disruption of Lipid Rafts in HIV-producing Cells Impairs Fusion of HIV-1 Virions to Target Cells. M. Cavrois, W. Yonemoto, D. Fenard, J. Neidleman, W. Greene.
19. Mutations in the Cytoplasmic Domain of the SIV Transmembrane Molecule Can Dramatically Increase Envelope Content in Virions, Infectivity, and Resistance to Antibody-mediated Neutralization. E. Yuste, R. Desrosiers.
20. Recruitment of CD4, CCR5, Rafts, and Ezrin in Actin-dependent Cell Surface Structures: Implications for HIV Fusion and Entry Events. C. M. Steffens, T. J. Hope.
23. Sensitivity of HIV-1 to Entry Inhibitors Correlates with Envelope: Co-receptor Affinity, Co-receptor Density, and Fusion Kinetics. J. Reeves, S. Gallo, N. Ahmad, J. Miamidian, P. Harvey, M. Sharron, S. Pöhlmann, T. Pierson, M. Biscone, J. Sfakianos, C. Derdeyn, G. Tomaras, R. Blumenthal, E. Hunter, R. Doms.
24. The Role of ESCRT-I in Retroviral Budding. J. Martin-Serrano, T. Zang, P. D. Bieniasz.
28. Tyrosine Sulfation of Human Antibodies that Bind the CCR5-binding Domain of HIV-1 gp120. H. Choe, W. Li, P. Wright, M. Moore, N. Vasilieva, J. Robinson, J. Sodroski, M. Farzan.
29. Migration of T-cells Away from HIV-1 gp120: A New Mechanism for Viral Immune Evasion. D. M. Brainard, W. G. Tharp, I. T. Olszak, A. Trocha, S. A. Kalams, B. D. Walker, R. Wyatt, J. Sodroski, M. C. Poznansky.
30. Expression of Lymphocyte Homing Receptors by SIV-specific CD8+ T-cells in the Female Reproductive Tract of Rhesus Macaques. M. Cromwell, X. Alvarez, J. Altman, S. Westmoreland, S. Klumpp, A.Luster, A. Lackner, P. Johnson.
31. Comprehensive Analysis of HIV-specific CD4 Responses by IFN-gamma ELISpot in Early Chronic HIV-1 Infection Shows Marked Immunodominance of gag and nef and the Presence of Broadly Recognized Peptides. D. Kaufmann, P. Bailey, L. Cosimi, P. Norris, M. Addo, M. Altfeld, H. Truong, M. Johnston, C. Brander, B. Walker, E. Rosenberg.
32. Functional Discrepancies in HIV-specific CD8+ T-lymphocyte Populations Reflect the Kinetics of Virus Exposure. D. A. Price, A. Oxenius, A. K. Sewell, S. J. Dawson, H. F. Guenthard, M. Fischer, G. M. Gillespie, S. L. Rowland-Jones, R. A. Koup, C. Fagard, B. Hirschel, D. C. Douek.
33. Relationship Between Functional and Qualitative Abnormalities Within the Pool of HIV-1 Specific Memory CD4 T-cells and Control of HIV-1 Disease. A. Harari, S. Petitpierre, M. Khonkarly, P. A. Bart, G. Pantaleo.
34. Systematic Microarray Analysis Reveals Counter Balance Between Perforin and Interleukin 7 Receptor with Progression of HIV Infection. F. Boutboul, D. Puthier, C. Nguyen, H. Ait Mohand, C. Katlama, G. Carcelain, P. Debré, I. Hirsch, B. Autran.
35. Vigorous HIV-specific CD8 T-cell Responses in Late Stage HIV Infection. R. Draenert, Y. Tang, C. Verrill, A. Wurcel, M. Boczanowski, A. Rathod, M. Addo, B. Walker.
36. HIV Transmission Risk Behaviors Among HIV-infected Individuals Released from Prison. D. A. Wohl, L. Shain, M. Adamian, B. L. Stephenson, R. Strauss, C. Golin, A. Kaplan.
38. HIV Incidence, Prevalence, and Behavior of Inner City Individuals Attending the Johns Hopkins Emergency Department. C. Henson, O. Laeyendecker, B. J. Horne, R. E. Rothman, G. D. Kelen, J. B. Shahan, K. Ketlogetswe, T. C. Quinn.
39. The High Yield of Routine HIV Screening in Urgent Care Sites in Massachusetts. R. P. Walensky, E. Losina, L. Malatesta, G. Barton, J. F. McGuire, K. A. Freedberg.
40. HIV-1 Transmission per Coital Act, by Stage of HIV Infection in the HIV+ Index Partner, in Discordant Couples, Rakai, Uganda. M. J. Wawer, D. Serwadda, X. Li, T. C. Quinn, N. K. Sewankambo, N. Kiwanuka, G. Kigozi, R. H. Gray.
41. Sorting Out Serosorting with Sexual Network Methods. J. McConnell, R. Grant.
42. Post-exposure Prophylaxis After Sexual Assault in South Africa. A. Wulfsohn, W. D. F. Venter, D. Schultze, M. Levey, I. M. Sanne.
46. Beyond DOTS: Approaches to Tuberculosis Control in Areas Where HIV Prevalence Is High. R. E. Chaisson.
50. Designing RNA I-based Therapeutic Strategies for HIV. J. J. Rossi, N-S. Lee, M. Li, H. Li, S. Gu, J. Kim, G. Bauer, D. Castanotto, G. Pfeifer, S. Tommasi, A. Bannerjie, R. Akkina.
51. Some Steps Towards Moving RNA Interference to the Clinic. J. Lieberman.
52. Using RNA Interference to Inhibit HIV-1 Replication and Infection. B. R. Cullen.
53. Genetic Condoms of HIV-1 Infection: Using Host Genetics to Dissect HIV-1 Pathogenesis. S. Ahuja.
54. HIV Adaptation to the Genetic Polymorphism of the Host. S. Mallal.
55. Influence of the KIR Genes on HIV-1 Disease. M. Carrington.
56. Comparison of Effects on Infection and Disease Progression. R. A. Kaslow, J. J. Tang, M. T. Dorak.
60. Phase-I Study with a Therapeutic MVA-BN-Nef Vaccine in HIV-1 Infected Patients on HAART. E. Harrer, M. Bäuerle, B. Ferstl, P. Chaplin, B. Petzold, S. Bergmann, M. Hamacher, J. R. Kalden, D. Willbold, T. Harrer.
61. Therapeutic Vaccination with ALVAC-HIV vCP1433: A Recombinant Canarypox Vaccine in Chronically HIV-1 Infected Patients Treated with HAART: VACCITER (ANRS 094). R. Tubiana, G. Carcelain, M. Vray, K. Gourlain, S.Hilpert, C. Dalban, A. Chermak, R. El Habib, M. Klein, D. Costagliola, V. Calvez, B. Autran, C. Katlama, Vacciter Study Group.
62. Immunological and Virological Efficacy of ALVAC-VIH 1433 and HIV Lipopeptides (Lipo-6T) Combined with SC IL-2 in Chronically HIV-infected Patients-Results of the ANRS 093 Randomized Study. Y. Levy, H. Gahery-Segard, C. Durier, A-S. Lascaux, V. Meiffrédy, C. Goujard, J-P. Cassuto, C. Rouzioux, R. Elhabib, J-G. Guillet, J-P. Aboulker, J-F. Delfraissy, ANRS 093 Study Group.
64. HIV-NAT 001.4: A Prospective Randomized Trial of Structured Treatment Interruption in Patients with Chronic HIV Infection. J. Ananworanich, P. Cardiello, P. Srasuebkul, T. Samor, E. Hassink, A. Mahanontharit, T. Boonmangum, W. Apateerapong, A. Hill, K. Ruxrungtham, D. Cooper, J. Lange, P. Phanuphak.

65. A Multi-center, Randomized Controlled Clinical Trial of Continuous vs Intermittent HAART Guided by CD4+ T-cell Counts and Plasma HIV-1 RNA Levels. L. Ruiz, L. Gómez, P. Domingo, J. Romeu, G. Tambussi, J. Martínez-Picado, J. Miranda, J. C. Martínez, J. M. Llibre, C. Tural, G. Sirera, F. Vidal, CR. Fumaz, E. Negredo, B. Clotet.

66. ISS-PART: A Prospective, Randomized, Multi-center Clinical Trial of Intermittent Therapy in HIV+ Subjects with Persistent Suppression of Viral Replication. S. Vella, M. Giuliano, L. Palmisano, R. Bucciardini, M. Andreotti, R. Arcieri, V. Fragola, C. Galluzzo, L. Weimer, M. F. Pirillo, R. Amici, E. Germinario, Italian ISS-PART Clinical Centers.

67. CPCRA 064: A Randomized Trial Examining Structured Treatment Interruption for Patients Failing Therapy with Multi-drug Resistant HIV. J. Lawrence, D. Mayers, K. Huppler Hullsiek, G. Collins, D. Abrams, R. Reisler, L. Crane, B. Schmetter, T. Dionne, J. Saldanha, M. Jones, J. Baxter, the CPCRA 064 Study Team of the Terry Beirn Community Programs for Clinical Research on AIDS.

68. Long-term Benefit of Treatment Interruption in Salvage Therapy (GIGHAART ANRS 097). C. Katlama, S. Dominguez, C. Duvivier, C. Delaugerre, G. Peytavin, M. Legrand, V. Calvez, K. Gourlain, D. Costagliola.

68lb. A Randomized, Controlled Trial of Long Cycle Structured Intermittent Versus Continuous ARV Therapy for Chronic HIV Infection. M. Dybul, E. Nies-Kraske, M. Daucher, C. Hallahan, G. Csako, C. Yoder, L. Ehler, P. Sklar, M. Belson, B. Hidalgo, J. Metcalf, R. Davey, D. Rock Kress, A. Fauci.

69. The Relationship Between Retroviral Integration and Host Cell Transcription 2. L. Maxfield, C. Dow, J. Coffin.

70. Selection of DNA Integration Sites by HIV in Human Peripheral Blood Mononuclear Cells. R. Mitchell, N. Portier, A. Schroder, P. Shinn, H. Chen, C. Berry, J. Ecker, F. Bushman.

72. The Murine Homologue of the VIF Cellular Co-factor, APOBEC3G/CEM15, is a Potent Inhibitor of HIV-1 Replication Whose Activity Is Not Blocked by HIV-1 or SIV VIF. R. Mariani, D. S. Chen, H. Nymark-McMahon , N. R. Landau.

73. Identification of a Novel SIV Lineage with a Vpu Gene in Cercopithecus Monkeys from Cameroon. Valerie Courgnaud, Severin Loul, Eitel Mpoudi, Florian Liegeois, Bernadette Abela, Xavier Pourrut, Eric Delaporte, Beatrice Hahn, Martine Peeters.

74lb. Identification of LEDGF/p75 as an Essential Factor for HIV-1 Replication that Binds Integrase: a Novel Target for Anti-HIV-1 Drug Discovery. S. Emiliani, J.C. Rain, M. Maroun, F. Moisant, E. Segeral, L. Selig, P. Legrain, R. Benarous.

76. High Frequency of Stereotypic Viral Escape from Dominant SIV Epitope-specific CTL in DNA-vaccinated Rhesus Monkeys. D. H. Barouch, J. Kunstman, J. Glowczwskie, K. Kunstman, M. Egan, S. Santra, F. Peyerl, M. Kuroda, J. Schmitz, S. M. Wolinsky, N. L. Letvin.

77. Protection Against Pathogenic SIVmac251 Infection Elicited by Vaccination of Rhesus Macaques with a Replication Competent Ad-recombinant Priming/Subunit Boosting Regimen. L. J. Patterson, N. Malkevitch, J. Pinczewski, Y. Lou, B. Peng, V. S. Kalyanaraman, P. D. Markham, R. Pal, G. N. Pavlakis, F. A. Robey, M. Robert-Guroff.

78. Virus-specific Immune Responses in Macaques Inoculated with Single-cycle SIV. D. Evans, J. Bricker, J. Lifson, S. Lang, R. Desrosiers.

79. Protective Immunity in Rhesus Monkeys Immunized with Replication-defective HIV-1 Vaccine. F. Tung, K. Cole, L. Tung, H. McClure, R. Montelaro.

80. Enhanced Simian Immunodeficiency Virus Replication and Accelerated Progression to AIDS in Macaques Primed to Mount a CD4 But Not a CD8 T-cell Response to the Viral Envelope Protein. S. I. Staprans, A. P. Barry, G. Silvestri, J. T. Safrit, N. Kozyr, H. McClure, D. Montefiori, J. L. Cohen, M. B. Feinberg.

81. Toll-like Receptor Ligands Can Modulate Dendritic Cell Functions to Augment Virus-specific T-cell Responses. K. Loré, R. A. Seder, S. Khojasteh, M. R. Betts, J. M. Brenchley, J. C. Kuruppu, S. P. Perfetto, M. Roederer, R. A. Koup.

82. Safety, Tolerability, and CD8+ T-cell Immunogenicity of High Dose Live Recombinant Canarypox ALVAC-HIV Vaccine (vCP1452) in Healthy, HIV-1 Uninfected Adults. P. Goepfert, H. Horton, J. McElrath, S. Surunathan, L. Mallet, F. Verdier, K. Weinhold, M. Allen, T. Shea, Y.-L. Chiu, T. Rossini, S. Self, L. Corey.

85lb. Replication-Defective Adenovirus Vector Vaccines Attenuate SIVmac239 and SHIV89.6P Challenge Infections: Effects of Challenge Virus, MHC Class I Expression and Multiple Vaccine Antigen Expression. J. W. Shiver, D. R. Casimiro, X. Liang, W. A. Schleif, F. Wang, Z. Zhang, A. Bett, M. Davies, L. G. Tussey, J. H. Condra, T. Fu, L. Handt, A. B. McDermott, D. I. Watkins, E. A. Emini.

96. Breast Milk Shedding of Drug-resistant Subtype C HIV-1 and Among Women Receiving Single-dose Nevirapine. E. Lee, R. Kantor, E. Johnston, P. Mateta, L. Zijenah, D. Katzenstein.

97. Late Postnatal Transmission of HIV in Breastfed Children: An Individual Patient Data Meta-analysis (The Breastfeeding and HIV International Transmission Study). J. S. Read, M-L. Newell, V. Leroy, F. Dabis.

102. Low Bone Mineral Density in HIV-infected Women. D. Jacobson, T. Knox, A. Shevitz, S. Gorbach.

103. HIV Infection and Protease Inhibitor Use Are Not Associated with Reduced Bone Mineral Density in Older HIV-infected Women. J. H. Arnsten, R. Freeman, N. Santoro, E. E. Schoenbaum.

107. The Role of Neutralizing Antibodies in the Prevention of HIV Infection. S. Zolla-Pazner.

108. Changes in AIDS, Death Rates, and Survival after AIDS in the EuroSIDA Study: 1994-2002. A. Mocroft, B. Ledergerber, C. Katlama, O. Kirk, P. Reiss, A. d'Arminio Monforte, B. Knysz, M. Dietrich, A. N. Phillips, J. D. Lundgren, the EuroSIDA study group.

110. DC-SIGN Function in HIV-1 Transmission. V. N. KewalRamani.

111. Cellular Defenses Against Retroviruses. P. Bieniasz.

112. Effector T-cells and HIV Infection. D. Unutmaz.

113. Enhancement of HIV Infection by Dendritic Cells: Transfer of HIV to Target Cells Through an Infectious Synapse. D. McDonald, L. Wu, S. M. Bohks, V. N. KewalRamani, D. Unutmaz, T. J. Hope.

119. Pathogenic Mechanisms of HIV Disease: The Multi-faceted Effects of Virus Replication and Viremia on the Host. A. S. Fauci.

120. Limited Role of Cellular Immune Responses in SIV-infected Sooty Mangabeys. A. P. Barry, B. Sumpter, G Silvestri, S. I. Staprans, M. B. Feinberg.

121. Increased SIV Viremia Following In Vivo CD8+ T-lymphocyte Depletion in Sooty Mangabeys with Natural SIV Infection. Z. Wang, N. Kassis, S. Staprans, M. Elliott, S. O'Neil, J E Schmitz, K. A. Reimann, H. M. McClure, R. P. Johnson, A. Kaur.

125lb. Mechansim of CCR5 Viral Dominance in Macaques Coinfected with CXCR4- and CCR5-SHIV. J.M. Harouse, C. Buckner, A. Gettie, R. Fuller, R. Bohm, J. Blanchard, C. Cheng-Mayer.

128. HIV-infected Patients Have a Defect in B-cell-CD4+ T-cell Interactions That Is Dependent on the Presence of Viremia. A. Malaspina, S. Moir, L. Ehler, S. Liu, M. Mclaughlin, A. Marie Planta, M. Dybul, T-W. Chun, A. Fauci.

130. Exposure to HAART Is Associated with an Increased Risk of Myocardial Infarction: The D:A:D Study. N. Friis-Møller, R. Weber, A. D'Arminio Monforte, W. El-Sadr, P. Reiss, F. Dabis, L. Morfeldt, S. De Wit, C. Pradier, G. Calvo1, M. Law, O. Kirk, C. Sabin, J. D. Lundgren.

131. Carotid Imtima-media Thickness in HIV-infected and Uninfected Adults: ACTG 5078. J. Currier, M. Kendall, K. Henry, F. Torriani, S. Storey, C. Shikuma, K. Mickelberg, B. Alston, M. Basar, R. Zackin, H. Hodis.

132. Increasing Incidence of Cardiovascular Disease in HIV-infected Persons in Care. R. D. Moore, J. C. Keruly, G. Lucas.

133. Longitudinal Associations Between Antiretroviral Treatments and Quantification of Tissue Mitochondrial DNA from Ambulatory Subjects with HIV Infection. C. Cherry, D. Nolan, I. James, S. Mallal, E. McKinnon, M. French, E. Hammond, M. Gahan, J. McArthur, S. Wesselingh.

134. Alendronate, Vitamin D, and Calcium for theTreatment of Osteopenia/Osteoporosis Associated with HIV Infection. K. Mondy, W. G. Powderly, S. A. Claxton, K. E. Yarasheski, J. S. Stoneman, M. E. Hoffmann, P. Tebas.

136. Use of Antiretroviral Therapy During Treatment of Active Tuberculosis with a Rifabutin-based Regimen. W. Burman, D. Benator, A. Vernon, A. Khan, W. El-Sadr, C. Silva, C. Lahart, S. Weis, B. Mangura, B. King, M. Weiner, B. Jones.

137. Tuberculosis Relapse and Acquired Rifamycin Resistance in HIV-1 Infected Persons Is Associated with Low CD4 Count, But Is Not More Common with Rifabutin Than Rifampin. R. Nettles, D. Mazo, K. Alwood, R. Gachuhi, G. Maltas, K. Wendel, W. Cronin, W. Bishai, T. Sterling.

138. To Study the Safety and Antiretroviral Efficacy of Concomitant Use of Rifampicin and Efavirenz in Antiretroviral-naïve Tuberculosis Co-infected HIV-1 Patients in India. A. Patel, K. Patel, J. Patel, N. Shah, B. Patel.

139lb. Increased Atherosclerotic Progression in Patients with HIV: The Role of Traditional and Immunologic Risk Factors. P. Hsue, J. Lo, A. Franklin, A. F. Bolger, S. G. Deeks, D. D. Waters.

140. The Potential for Cross Resistance Between

S-1360, L-870810 and Other Structurally Diverse Inhibitors of HIV-1 Integrase Strand Transfer. Daria Hazuda, The Integrase Inhibitor Discovery Team.

141. Baseline and On-treatment Susceptibility to Enfuvirtide Seen in TORO 1 and TORO 2 to 24 Weeks. M. L. Greenberg, T. Melby, P. Sista, R. DeMasi, N. Cammack, M. Salgo, J. Whitcomb, C. Petropoulos, T. J. Matthews.

143. Effect of Amprenavir Hyper-susceptibility on the Response to APV/Ritonavir-based Therapy in ART-experienced Adults Selected by Baseline Susceptibility (ESS40006): 24-week Data. R. Schooley, R. Haubrich, M. Thompson, D. Margolis, S. Schneider, D. Richman, K. Pappa, L. Yau, S. Hessenthaler, J. Hernandez.

146. Viral and Immune Correlates of Discordant CD4/VL Responses to NNRTI-based HAART and Comparison to a Discordant Cohort Receiving Protease Inhibitor-Based HAART. D. Linden, S. Sufka, G. Ferrari, S. Fiscus, T. Wrin, V. Gryszowka, B. Exley, K. Weinhold, N. Hellman, C. Petropoulous, C. Hicks.

148. Effect of Efavirenz on the Pharmacokinetics of Nelfinavir and M8 in Naïve, HIV-infected Patients Receiving Long-term HAART Therapy. P. F. Smith, G. Robbins, R. Shafer, H. Wu, S. Yu, M. Hirsch, T. Merigan, G. D. Morse, ACTG 384 Study Team.

152. High Replication Capacity Is Associated with High Baseline Viral Load in Untreated Subjects with Primary HIV Infection. S. J. Little, S. D. W. Frost, J. P. Routy, A. C. Collier, J. B. Margolick, E. S. Daar, R. A. Koup, B. Conway, L. Wang, T. Wrin, C. J. Petropoulos, N. S. Hellmann, D. D. Richman, S. Holte.

154. Frequent Detection of Acute Primary HIV Infection in Men in Malawi: Reconsideration of Counseling and Testing Approaches. C. D. Pilcher, M. A. Price, I. F. Hoffman, F. E. A. Martinson, P. N. Kazembe, J. J. Eron, W. C. Miller, S. A. Fiscus, M. S. Cohen.

155. Survival in HIV-infected Liver Transplant Recipients. M. Ragni, S. Belle, K. Im, G. Neff, M. Roland, P. Stock, N. Heaton, A. Humar, J. Fung.

156. GBV-C Infection Inhibits CCR5 and CXCR4 HIV Strains and Alters Chemokine and Cytokine Gene Expression in PBMC Cultures. J. Xiang, S. L. George, S. Wuenschmann, D. Klinzman, J. T. Stapleton.

157. GB Virus C Viremia During the Natural Course of HIV-1 Infection: Viremia at Diagnosis Does Not Predict Mortality. P. Björkman, L. Flamholc, A. Nauclér, E. Wallmark, A. Widell.

158. Morphine Enhances Hepatitis C Virus Replicon Expression. Y. Li, T. Zhang, S. D. Douglas, W. Z. Ho.

159lb. Persistent GBV-C virus Type C (GBV-C) Infection is Associated with Decreased Risk of Death in HIV-seroconvertors in the Multicenter AIDS Cohort Study (MACS). C. Williams, D. Klinzman, T. Yamashita, J. Xiang, P. Polgreen, C. Rinaldo, C. Liu, J. Phair, J. Margolick, D. Zdunek, G. Hess, J. Stapleton.

160. Progress in Understanding the Pathogenesis of HIV-1 Related Lipodystrophy. J. Capeau.

161. The Pathophysiology of HIV-1 Related Insulin Resistance and Dyslipemia. S. K. Grinspoon.

164. Immune Control and Immune Failure in HIV Infection. B. D. Walker, T. Allen, M. Altfeld, X. Yu, P. Goulder, M. Addo, R. Dranert, C. Brander, N. Frahm, M. Johnston, C. Corcoran, H. Trong, P. Lee, E. Maier, K. O'Sullivan, R. Eldridge, M. Johnston, E. Rosenberg, C. Hess, A. Luster.

165. Shifting the Paradigm of Immunologic Control of HIV. M. Connors.

166. Antibody Neutralization and Escape by HIV-1. G. M. Shaw.

176. Results of the 2NN Study: A Randomized Comparative Trial of First-line Antiretroviral Therapy with Regimens Containing Either Nevirapine Alone, Efavirenz Alone or Both Drugs Combined, Together with Stavudine and Lamivudine. F. van Leth, E. Hassink, P. Phanuphak, S. Miller, B. Gazzard, P. Cahn, R. Wood, K. Squires, C. Katlama, B. Santos, P. Robinson, R. van Leeuwen, F. Wit, J. Lange, for the 2NN study group.

177. The NEAT Study: GW433908 Efficacy and Safety in ART-naïve Subjects, Final 48-week Analysis. J. Nadler, A. Rodriguez-French, J. Millard, P. Wannamaker.

178. The Context Study: Efficacy and Safety of GW433908/RTV in PI-experienced Subjects with Virological Failure (24 Week Results). E. DeJesus, A. LaMarca, M. Sension, C. Beltran, P. Yeni.

179. Tipranavir/Ritonavir Demonstrates Potent Efficacy in Multiple Protease Inhibitor Experienced Patients: BI 1182.52. J. Gathe, V. M. Kohlbrenner, G. Pierone, K. Arasteh, R. Rubio, R. LaLonde, P. Piliero, S. McCallister, S. Garfinkel, R. Chaves, G. M. Mukwaya, C. Dohnanyi, S. Shaw, U. Drees, D. Mayers.

183. How Hard Is HAART? Residual Viral Replication and Evolution in Lymphoid Tissue During Sustained Control of Viremia. J. van Lunzen, B. Zöllner, H. J. Stellbrink, C. Schneider, O. Degen, S. Staszewski, S. Christensen, L. Ruiz, B. Clotet, K. Tenner-Racz, P. Racz.

184. Molecular Approaches to Immunogens Able to Elicit Broadly Neutralizing Anti-HIV-1 Antibodies. D. R. Burton, D. A. Calarese, R. A. Dwek, H. Katinger, R. Pantophlet, P. W. H. I. Parren, P Poignard, P. M. Rudd, E. O. Saphire, C. N. Scanlan, R. L. Stanfield, M. B. Zwick, I. A. Wilson.

185. Blocking of gp41-Mediated Fusion by Antibodies. C. D. Weiss, Y. He, R. Vassell, E. de Rosny, L. King, M. Zaitseva, P. Wingfield, H. Golding.

186. Attacking the Co-Receptor Binding Site on gp120. J. Sodroski, H. Choe, R. Wyatt, C. Grundner, S-H. Xiang, X. Yang, M. Venturi, A. Labrijn, P. Poignard, A. Raja, D. Burton, P. Kwong, M. Farzan.

202. Enzymatic Activity of CEM15 Is Essential for Its Function to Suppress the Infectivity of deltaVif Virion. A. Takaori-Kondo, K. Shindo, M. Kobayashi, A. Arukin, T. Uchiyama.

203. Enhanced CD4 Down-modulation Allows Efficient Replication of Late-stage HIV Strains. J. Lama, E Argañaraz.

204. Co-receptor Dependent Stimulation of HIV-1 Replication in Primary T-cells by Nef. C. A. Lundquist, J. Zhou, D. Unutmaz, C. Aiken.

205. Disassembly of HIV-1 Cores In Vitro Reveals an Association of Nef with the Subviral Ribonucleoprotein Complex. Brett M. Forshey, Christopher Aiken.

206. The Association of HIV-1 Nef with Lipid Rafts Is Functionally Important for CD4 and MHC Class I Down-modulation. M. A. Heaton, K. S. Ravichandran, M. L. Hammarskjold, D. Rekosh.

207. Vascular Endothelial Cells Induce Dramatically Higher Levels of HIV-1 Replication in a Nef-dependent Manner. J. Choi, J. Walker, J. S. Pober, L. Alexander.

208. Nef Expressed from Integrase Defective HIV-1 Down-regulates CD4 Expression. L. Gillim, M. Klotman.

209. Evidence of a Novel Nef Activity Involved in nef-Mediated Thymocyte Depletion in the Thymus. R. D'Agostin, L. Su.

210. HIV-1 Nef Impairs Generation of Thymic CD4+ T-cells. P. Chrobak, M.C. Simard, T.M. Ndolo, Z. Hanna, P. Jolicoeur.

211. Characterization of CD4+ and CD8+ T-lymphocytes in CD4C/HIV nef Transgenic Mice: Their Presence is Dispensable for the Development of a Severe AIDS-like Disease. P. Chrobak, X. Weng, E. Priceputu, J. Poudrier, D.G. Kay, Z. Hanna, T.W. Mak, P. Jolicoeur.

212. Identification of the Domain Within the Subtype C Vpu that Is Responsible for Efficient Transport to the Cell Surface. D. K. Singh, E. Pacyniak, D. Hout, E. B. Stephens.

213. Activation of the ATR-initiated DNA Damage Signaling Pathway by HIV-1 Vpr. V. Planelles, S. Murala, J. Walker, M. Roshal.

214. Glucocorticoid Receptor Modulates HIV-1 Replication Through Interaction with the Leucine Motifs in Helix I and III of Vpr. D Kumar, S Mahalingam, M Wagner, B Gray, SP Singh, A Srinivasan, P Gupta, V Ayyavoo.

215. HIV-1 Vpr Alters the Phophorylation of hVIP/mov34. Michelle L Janket, Sundrasamy Mahalingam, Velpandi Ayyavoo.

216. Structure of Purified Intracellular HIV-1 Reverse Transcription Complexes. Ariberto Fassati, Milan V. Nermut.

217. Molecular Basis of Human Immunodeficiency Virus Type 1 Vpr-associated Cytopathicity in Human CD4+ T-cells. D. L. Bolton, M. J. Lenardo.

218. Molecular Analysis of HIV-1 Induced CD4+ T-lymphocyte Cytopathicity. K. Sakai, J. Dimas, M. J. Lenardo.

219. Small Heat Shock Proteins Antagonize HIV-1 Vpr Activities Through a MAPK-independent Mechanism. Zigmond Benko, Jason Hou, Dong Liang, Karen Chiu, Paul G. Young, Lorena Taricani, Min Yu, Scott Innis, Patrick Reed, Paola DiMarzio, Michael Bukrinsky, Yuqi Zhao.

220. Modulating the HIV-1 Replication Cycle Through RNAi. J. M. Jacque, M. Stevenson.

221. RNA Interference of Chemokine Receptor Expression Allows for Inhibition of HIV-1 Replication. M. A. Martinez, A. Gutierrez, M. Armand-Ugon, J. Blanco, M. Parera, J. Gomez, B. Clotet, J. A. Este.

222. CXCR4 Gene Silencing with Multiple Small Interfering RNAs Inhibits HIV-1 Infection. J. Ji, P. Erb, T. Klimkait, M. Wernli.

223. Inhibition of CCR5 Expression Using RNA Interference Selectively Blocks Infection by R5-Tropic HIV-1. M. T. M. Lee, B. R. Cullen.

225. Sustained siRNA-mediated HIV Inhibition in Primary Macrophages. P. Shankar, E. Song, S. K. Lee, D. M. Dykxhoom, C. Novina, K. Crawford, J. Cerny, P. A. Sharp, J. Lieberman, M. N. Swamy.

226. Combination Therapies Targeting CCR5 Using RNAi, Antibody, and Ribozymes Delivered by rSV40 Vectors. P. Cordelier, B. A. Morse Jr, J. Kukowski, M. BouHamdan, R. J. Pomerantz, J. Rossi, D. S. Strayer.

227. Lentiviral Vectors Interfering with CD4 Down-modulation as an Anti-HIV Approach. B. Gröschel, M. J. Cortés, E. Argañaraz, J. Lama.
228. Lentivirus Packaging and Gene Transfer Vectors. S. K. Arya, J. E. Cho, A. Zaman.
229. The HIV-1 cPPT/CTS Fragment Mediates Efficient Transgene Expression from Lentiviral Vectors. J. van Griensven, B. Van Maele, E. De Clercq, Z. Debyser.
231a. Inhibition of HIV Infection in CD4 T-cells by Lentiviral-mediated Delivery of anti-CCR5 siRNA Is Influenced by CCR5 Expression. C. Butticaz, A. Ciuffi, M. Munoz, J. Thomas, R. Iggo, P. Meylan, A. Telenti.
270. Syndecans on the Vascular Endothelium Serve as a Major in Trans Viral Reservoir for HIV. M. Bobardt, A. Saphire, P. Gallay.
273. A Subset of HIV and SIVs That Use an Alternative Coreceptor to Infect Primary Human Cell Cultures. S. Willey, J. Reeves, A. McKnight, J. Bell, R. Hudson, K. Luzuriaga, P. Clapham.
292. HIV-1 Mediated Signal Transduction Through CCR5 Allows Infection of Resting Memory T-cells. A. Matthews, J. Vasudevan, D. Camerini.
306. Simultaneous Assessment of Degranulation and Cytokine Production in HIV- and CMV-specific CD8 + T-cells Ex Vivo. M. Betts, J. Brenchley, D. Ambrozak, D. Price, D. Douek, M. Roederer, R. Koup.
307. HIV-1 Superinfection Despite Broad CD8 + T-cell Responses Containing Replication of the Primary Virus. T. M. Allen, M. Altfeld, X. G. Yu, M. N. Johnston, D. Agrawal, B. T. Korber, D. C. Montefiori, D. H. O'Connor, B. T. Davis, P. K. Lee, E. L. Maier, P. J. R. Goulder, C. Brander, E. S. Rosenberg, B. D. Walker.
314. Underestimation of HIV-1 Specific T-cell Responses Directed Against the Autologous Virus using HIV-1 Clade B Consensus Sequences. M. Altfeld, M. M. Addo, P. K. Lee, R. Shankarappa, C. Brander, T. Allen, A. Rathod, X. G. Yu, J. Harlow, K. O'Sullivan, M. N. Johnston, D. Strick, J. I. Mullins, E. S. Rosenberg, B. D. Walker.
318. The Differential Ability of Long-term Nonprogressors and Progressors to Restrict HIV Replication is Not Caused by Loss of Recognition of Autologous Viral Sequences. S. A. Migueles, A. C. Laborico, H. Imamichi, W. L. Shupert, C. Royce, M. McLaughlin, L. Ehler, S. Liu, J. Metcalf, M. Connors.
396. Adjuvant Effects of an NKT Ligand, alpha-Galactosylceramide, on DNA Vaccination. Y. Huang, Z. Chen, W. Zhang, Y. Song, D. Gurner, D. Gardiner, A. Chen, M. Tsuji, D. D. Ho.
397. CTLA4-mediated APC Targeting Enhanced the Humoral and Cellular Immune Responses of an SIV DNA Vaccine in Mice. W. Zhang, Z. Chen, Y. Huang, Y. Song, D. D. Ho.
400. Enhancement of Immune Responses to HIV-1 by DNA Vaccination Using the Complement Protein, C3d. T. Ross, T. Green, J. Sodroski, J. Bower.
405. Vaccination in Humans Generates Broad T-cell Cytokine Responses. S. C. De Rosa, F. X. Lu, S. P. Perfetto, S. Moser, C. J. Miller, T. G. Evans, M. Roederer.
448. Novel Forms of DNA Vaccines Decrease Viremia Upon SIVmac251 Challenge. M. R. Rosati, P. Carney, A. von Gegerfelt, A. Valentin, E. Tryniszewska, G. Franchini, P. Markham, D. Venzon, N. Miller, B. K. Felber, G. N. Pavlakis.
452. The Effect of Single-site Versus Multiple-site DNA Vaccination on Immune Responses in Mice. D. Gardiner, Y. Huang, Z. Chen, D. Gurner, L. Leung, Y. Song, M. Tsuji, D. Ho.
454. Seminal Super Shedding of HIV: Implications for Sexual Transmission. S. Taylor, T. Sadiq, C. Sabin, D. White, P. Cane, S. Drake, D. Pillay.
459. Mucosal CCR5 Expression is Down-regulated in HIV Infection. I. McGowan, J. Elliott, P. Taing, M. Fuerst, J. Boscardin, P. Anton.
459a. The Prostate as a Reservoir for HIV-1. D. M. Smith, J. D. Kingery, C. C. Ignacio, J. K. Wong, D. D. Richman, S. J. Little.
465. The Persistence of Morphological and T-lymphocyte Subsets Abnormalities in Lymphoid Tissue in HIV-1 Infected Patients Successfully Treated with HAART Correlate with Lymphoid Tissue Viral Replication. L. Alós, V. Morente, F. García, M. Caballero, G. Mestre, A. Cruceta, M. Arnedo, T. Pumarola, M. Plana, T. Gallart, E. Martínez, J. Mallolas, J. L. Blanco, J. M. Miró, J. M. Gatell.
466. Persistence of Stable, Quantifiable Viremia in Patients on Antiretroviral Therapy Despite Suppression of Plasma HIV-1 RNA to less than 50 Copies/ml. F. Maldarelli, A. Wiegand, S. Palmer, M. Kearney, V. Boltz, M. Polis, J. Falloon, J. Mican, R. Davey, D. Rock, S. Liu, A. Planta, J. Metcalf, J. Mellors, J. Coffin.
469. Immunologic and Virologic Outcome in a Cohort of Long-term Non-progressors After More than 15 Years of HIV-1 Infection. B. Rodes, C. Toro, E. Paxinos, S.Fransen, T. Wrin, S. Bassani, E. Poveda, V. Soriano.
485. HIV Superinfection in HIV + Persons. T. Wong, S. Kravcik, J. Angel, T. Gleeson, M. Montpetit.
486. HIV-1 Superinfection with a CRF01_AE Strain: Cell-free RNA Levels Are Significantly Lower Than the Primary Infecting Subtype B Strain. S. Subbarao, A. Ramos, P. Srinivasan, D. Hu, S. Vanichseni, K. Choopanya, T. Chaowanachan, J. Tappero, T. Mastro, T. Folks.
494. HIV-1 Genetics of Intermittent Low-level Viremia. N. H. Tobin, Y. Wang, G. H. Learn, J. L. McKernan, G. M. Ellis, K. M. Mohan, S. E. Holte, S. M. De Vange, D. M. Pawluk, A. J. Melvin, P. L. Lewis, I. A. Beck, M. Mahalanabis, J. I. Mullins, L. M. Frenkel.
502. Antiretroviral Resistance Among Patients with Primary HIV Infection in the Southeastern U.S.-Impact on Treatment Outcome. C. Hicks, J. Eron, J. Lennox, C. Pilcher, S. Fiscus, A. Weintrob, J. Giner, P. Menezes, E. Patrick, T. Alcorn.
504. Frequency of Transmitted Drug Resistance and Identification of Phylogenetic Clusters in a Homogenous Cohort of Newly Infected Individuals. V. Simon, C. Hogan, M. Louie, S. Vasan, L. Rowe, A. Hurley, N. Padte, J. Vanderhoeven, K. Dawson, P. Balfe, M. Markowitz.
505. The Relative Transmissibility of Drug Resistant HIV-1 among Couples. R. M. Grant, R. Atchison, K .Franses, A .Rollins, M .Warmerdam, J. O. Kahn, F. M. Hecht.
512. Structured Treatment Interruptions in Acute HIV Seroconverters: Preliminary Results of the Multicenter Prospective PRIMSTOP Pilot Trial (ANRS 100). B. Hoen, C. Lacabaratz, I. Fournier, I. Charreau, M. Burgard, F. Raffi, P. Morlat, T. May, J. P. Aboulker, A. Venet, C. Rouzioux, Primstop Study Group.
513. Predictors of Plasma HIV RNA Control after Discontinuation of HAART Initiated at Acute Infection. A. Lafeuillade, E. Counillon, C. Poggi, G. Hittinger, D. Emilie.
514. The Early Effect of Highly Active Antiretroviral Therapy During Acute Retroviral Syndrome Is Associated with the Time of Starting and the Duration of Symptoms. P. Vanhems, N. Voirin, J. P. Routy, D. Baratin, M. Legault, C. Trépo, P. Miaihles, M. R. Boulassel, J. M. Livrozet, J. L. Touraine, J. Fabry, study collaborators.
516. Analysis of Treatment-associated Viral Load Change During Primary HIV Infection. P. Grey, M. Johnston, K. Petoumenos, T. Ramacciotti, P. Cunningham, E. Rosenberg, D. Smith, G. Robbins, R. Finlayson, D. Cooper, B. Walker, J. Kaldor, C. Corcoran, A. Carr.
519. Is HAART for Primary/Early HIV Infection Associated with Improved Outcomes After Treatment Discontinuation? F. M. Hecht, L. Wang, A. Collier, J. Margolick, S. Little, M. Kilby, C. Benson, B. Conway, E. Daar, M. Markowitz, S. Holte.
520. The QUEST Cohort: Treatment of Primary HIV Infection with Quadruple HAART: Week 48 Preliminary Results. S. Kinloch, D. Cooper, F. Lampe, D. Smith, G. Janossy, B. Hoen, A. Sonnerborg, C. Tsoukas, A. Phillips, L. Goh, L. Perrin.
521. Viral Blips During HAART in Primary HIV Infection. M. Di Mascio, M. Markowitz, M. Louie, C. Hogan, V. Simon, A. Hurley, D. D. Ho, A. S. Perelson.
528. Correlation of Viral Load Reduction and Plasma Levels in Multiple Protease Inhibitor Experienced Patients Taking Tipranavir/Ritonavir in a Phase IIB Trial: BI 1182.52. P. Yeni, T. MacGregor, J. Gathe, K. Arasteh, D. Jayaweera, J. Jemsek, T. Hawkins, W. Cameron, N. Bodsworth, S. McCallister, V. Kohlbrenner, A. Quinson, J. Leith, J. Sabo, D. Mayers.
531. Lopinavir/Ritonavir Combined with Twice-daily Indinavir: Pharmacokinetics in Blood, CSF, and Semen (The Protect Study). A. Isaac, S. Taylor, G. Rubin, S. Gibbon, D. White, S. Drake, D. Back.
533. Didanosine and Tenofovir DF Drug-drug Interaction: Assessment of Didanosine Dose Reduction. B. P. Kearney, E. Isaacson, J. Sayre, H. Namini, A. Cheng.
534. Lack of Interaction Between Stavudine Extended-release Formulation and Tenofovir Disoproxil Fumarate. S. Kaul, K. Bassi, B. Damle, R. Smith, J. Gale, E. O'Mara, U. Chaudhari, K. Ryan.
537. Pharmacokinetic Parameters of Atazanavir/Ritonavir When Combined to Tenofovir in HIV Infected Patients with Multiple Treatment Failures: A Sub-study of Puzzle2-ANRS 107 Trial. A. M. Taburet, C. Piketty, L. Gérard, I. Vincent, C. Chazallon, F. Clavel, V. Calvez, J. P. Aboulker, P.M. Girard.
542. Pharmacokinetic Interaction Between Rifampin and the Twice-daily Combination of Indinavir and Low-dose Ritonavir in HIV-infected Patients. U. Justesen, A. Andersen, N. Klitgaard, K. Brosen, J. Gerstoft, C. Pedersen.
543. Atazanavir: A Summary of Two Pharmacokinetic Drug Interaction Studies in Healthy Subjects. D. Tackett, M. Child, S. Agarwala, M. Geiger, M. Geraldes, B. Laura,

E. O'Mara.

544. Pharmacokinetics of Nelfinavir (Viracept 250 mg tablet): Effect of Food Intake on Single-dose PK Parameters. C. Petersen, E. Pun, R. Strada, E. Daniels, C. Bramson, E. Randinitis.

546. High Indinavir Plasma Concentrations in HIV-1 Patients Co-infected with Hepatitis B or C Virus Receiving Indinavir and Ritonavir Low Dosages: A GENOPHAR Substudy. P. Bossi, G. Peytavin, C. Lamotte, H. Ait-Mohand, M. Bonmarchand, N. Ktorza, A. Simon, C. Delagerre, V. Calvez, F. Bricaire, D. Costagliola, C. Katlama.

546a. The Preclinical Pharmacokinetics and Safety Pharmacology of the Anti-HIV CCR5 Antagonist, UK-427, 857. C. Napier, P. Dorr, R. Gladue, R. Halliday, D. Leishman, I. Machin, R. Mitchell, A. Nedderman, M. Perros, S. Roffey, D. Walker, R. Webster.

547. Pharmacokinetics of Single and Multiple Oral Doses of UK-427,857—A Novel CCR5 Antagonist in Healthy Volunteers. S. Abel, E. Van der Ryst, G. J. Muirhead, M. Rosario, A. Edgington, G. Weissgerber.

549. TMC114, a Next Generation HIV Protease Inhibitor: Pharmacokinetics and Safety Following Oral Administration of Multiple Doses with and Without Low Doses of Ritonavir in Healthy Volunteers. R. Hoetelmans, I. Van der Sandt, M. De Pauw, K. Struble, M. Peeters, R. Van der Geest.

550. Long-term Efficacy and Safety of Emtricitabine in HIV+ Adults Switching from a Lamivudine Containing HAART Regimen. C. Wakeford, G. Shen, L. Hulett, J. B. Quinn, F. Rousseau.

551. Once-daily Combination of Emtricitabine, Didanosine, and Efavirenz vs Continued PI-based HAART in HIV-infected Adults with Undetectable Plasma HIV-RNA: 48-week Results of a Prospective Randomized Multicenter Trial (ALIZE-ANRS 99). J. M. Molina, F. Ferchal, C. Rancinan, P. Yeni, W. Rozenbaum, V. Journot, L. Morand-Joubert, S. Fournier, P. Morlat, B. Dupont, J. F. Delfraissy, P. Dellamonica, I. Poizot-Martin, E. Rosenthal, G. Chene.

552. Sustained Anti-HIV-1 Effect of Racivir Combined with D4T and Sustiva Following a 14-day Treatment of Infected Volunteers. M. J. Otto, K. Arastèh, P. Kreckel, D. Drauz, A. Beard, L. Cartee, S.J. Hurwitz, D.C. Liotta, R.F. Schinazi, R. L. Murphy.

553. TMC114 (UIC96017): a Novel Nonpeptidic Protease Inhibitor Potent Against Multi-PI Resistant HIV in vitro. Y. Koh, H. Nakata, K. Maeda, J. F. Kincaid, G. Bilcer, D. Thippeswamy, A. K. Ghosh, H. Mitsuya.

554. Preliminary Results of Dosing of Amdoxovir in Treatment-experienced Patients. M. Thompson, G. Richmond, H. Kessler, A. Bae, J. Sorbel, N. Sista, N. Adda, F. Rousseau.

555. Long-term Efficacy and Safety of Atazanavir with Stavudine and Lamivudine in Patients Previously Treated with Nelfinavir or ATV: 108-week Results of BMS Study 008/044. R. Murphy, V. Pokrovsky, W. Rozenbaum, R. Wood, L. Percival, L. Odeshoo, M. Giordano.

556. Distinct Antiviral Resistance Profiles for the Authentic HIV Integrase Inhibitors; the Diketo Compounds L-708,906 and S-1360 and the Pyranodipyrimidine V-165. V. Fikkert, B. Van Maele, B. Van Remoortel, M. Michiels, J. Vercammen, C. Pannecouque, Y. Engelborghs, E. De Clercq, Z. Debyser, M. Witvrouw.

557. Analysis of Baseline Enfuvirtide (T20) Susceptibility and Co-receptor Tropism in Two-phase III Study Populations. J. M. Whitcomb, W. Huang, S. Fransen, T. Wrin, E. Paxinos, J. Toma, M. Greenberg, P. Sista, T. Melby, T. Matthews, R. DeMasi, G. Heilek-Snyder, N. Cammack, N. Hellmann, C. Petropoulos.

562. TAK-220, a Novel Small Molecule Inhibitor of CCR5 Has Favorable Anti-HIV Interactions with Other Antiretrovirals in vitro. C. L. Tremblay, F. Giguel, J. L. Hicks, T. C. Chou, Y. Lizawa, Y. Sugihara, M. S. Hirsch.

563. Anti-HIV Activity Profile of AMD070, an Orally Bioavailable CXCR4 Antagonist H. D. Schols, S. Claes, S. Hatse, K. Princen, K. Vermeire, E. De Clercq, R. Skerlj, G. Bridger, G. Calandra.

564a. Potent in vivo Anti-R5-HIV Effects of AK602, a Novel Spirodiketopiperazine (SDP)-containing HIV-specific CCR5 Inhibitor, in hu-PBMC-NOD-SCID Mice. H. Nakata, K. Maeda, Y. Kawano, T. Miyakawa, S. Shibayama, M. Matsuo, Y. Takaoka, Y. Koyanagi, H. Mitsuya.

564b. Efficacy and Safety of Tenofovir DF (TDF) Versus Stavudine (d4T) When Used in Combination with Lamivudine and Efavirenz in Antiretroviral-naïve Patients: 96-week Preliminary Interim Results. S. Staszewski, J. E. Gallant, A. L. Pozniak, J. M. A. H. Suleiman, E. DeJesus, B. Lu, J. Sayre, A. Cheng.

565. Response to LPVr in Experienced Patients: Effect of a Treatment Interruption. R. Haubrich, B. Best, C. Miller, M. Witt, C. Kemper, K. Squires, C. Diamond, P. Heseltine, N. Hellmann, A. Rigby, E. Capparelli, J. A. McCutchan, California Collaborative Treatment Group (CCTG).

566. Dual Therapy with Indinavir/Ritonavir 800/100 mg BID and Efavirenz 600 mg QD Effectively Treats Patients with Combination Nucleoside Analogue Failure: HIV-NAT 009 48-week Analysis. M. Boyd, C. Duncombe, P. Srasuebkul, E. Hassink, N. Chomchey, T. Methanukroh, S. Ubolyam, K. Ruxrungtham, M. Stek, J. Lange, D. Cooper, P. Phanuphak.

568. Summary of Pooled Efficacy and Safety Analyses of Enfuvirtide Treatment for 24 Weeks in TORO 1 and TORO 2 Phase III Trials in Highly Antiretroviral Treatment-experienced Patients. J-F. Delfraissy, J. Montaner, J. Eron, R. DeMasi, J. Chung, C. Drobnes, J. Delehanty, M. Salgo.

572. Pre-treatment Factors That Predict Responses to Potent Antiretroviral Therapy: Findings from AACTG A5001. C. A. Benson, A. C. Collier, R. Bosch, K. Bennett, R. Zackin, A5001 Protocol Team.

576. Evidence of Low-level Viral Replication (< 50 copies/ml) Predicts Eventual Virologic Failure. A. L. Pozniak, B. G. Gazzard, M. Yehya, D. Pillay, A. Wildfire, A. Cox.

586. Prediction of NRTI Options by Linking Reverse Transcriptase Genotype to Phenotypic Breakpoints. E. R. Lanier, D. Irlbeck, L. Ross, P. Gerondelis, M. Underwood, N. Parkin, C. Chappey, M. St Clair.

594. Genotypic Basis of Variation in Protease Susceptibility in Primary HIV Infection Analyzed with Machine Learning. A. J. Leigh Brown, S. D. W. Frost, B. Good, E. S. Daar, V. Simon, M. Markowitz, A. C. Collier, E. Connick, B. Conway, J. B. Margolick, J. P. Routy, J. Corbeil, N. Hellmann, D. D. Richman, S. J. Little.

596. Baseline Phenotypic Suceptibility to Tipranavir/Ritonavir Is Retained in Isolates from Patients with Multiple Protease Inhibitor Experience (BI 1182.52). D. Cooper, D. Hall, D. Jayaweera, S. Moreno, C. Katlama, S. Schneider, L. Minoli, P. Yeni, R. Steigbigel, S. McCallister, V. Kohlbrenner, E. Cuaresma, J. Sabo, D. Mayers.

597. Emergence of Atazanavir Resistance and Maintenance of Susceptibility to Other PIs Is Associated with an I50L Substitution in HIV Protease. R. Colonno, R. Rose, C. Cianci, G. Aldrovandi, N. Parkin, J. Friborg.

598. GW433908 in ART-naive Subjects: Absence of Resistance at 48 Weeks with Boosted Regimen and APV-like Resistance Profile with Unboosted Regimen. S. Macmanus, P. Yates, S. White, N. Richards, W. Snowden.

599. Impact of Amino-acid Insertions in HIV-1 p6 PTAP Region on the Virological Response to Amprenavir in the NARVAL Trial. S. Lastere, C. Dalban, G. Collin, D. Descamps, P. M. Girard, F. Clavel, D. Costagliola, F. Brun-Vezinet.

600. Comparative Incidence and Temporal Accumulation of PI and NRTI Resistance in HIV-infected Subjects Receiving Lopinavir/Ritonavir or Nelfinavir as Initial Therapy. D. Kempf, M. King, E. Bauer, J. Moseley, B. Bernstein, S. Brun, E. Sun.

609. Evolution of Protease and Reverse Transcriptase Inhibitor-associated Mutations in HIV-1 Protease Inhibitor-treated Patients with Persistent Low Viremia. X. Duval, S. Darmon, P. Longuet, D. Descamps, J. L. Ecobichon, S. Delarue, G. Peytavin, C. Leport, J. L. Vildé, F. Brun-Vezinet.

615. Analysis of Patient-derived HIV-1 Isolates Suggests a Novel Mechanism for Decreased Sensitivity to Inhibition by Enfuvirtide and T-649. M. Heil, J. Decker, D. T-. Chen, J. Sfakianos, G. Shaw, E. Hunter, C. Derdeyn.

616. Decreased Replication Capacity of HIV-1 Clinical Isolates Containing K65R or M184V RT Mutations. M. D. Miller, K. L. White, C. J. Petropoulos, N. T. Parkin.

617. Viral Pro/Pol Replication Capacity Declines Slowly Among Untreated, HIV-1 Infected Adults in Early Infection with and without Primary Drug Resistance. J. D. Barbour, T. Wrin, G. E. Spotts, C. J. Petropoulos, F. M. Hecht, R. M. Grant.

619. Plasma Viremia at < 20 copies/ml Is Comprised of Drug-Sensitive HIV-1 with Wild-type Protease Despite Up to 6 Years of Continuous HAART with PI-containing Regimens. D. Persaud, G. Siberry, A. Ahonkhai, D. Monie, N. Hutton, D. Watson, S. Ray, R.F.Siliciano.

620. Does Plasma Resistance to Antiretroviral Drugs Predict HIV Resistance Transmitted During Heterosexual Contact? L. J. Conley, T. J. Bush, J. L. Lennox, T. C. Wright, A. J. Uzgiris, T. Evans-Strickfaden, M. J. Vick, C. E. Hart, T. V. Ellerbrock.

621. Discordance Between Vaginal and Plasma HIV-1 Mutation Patterns in Puerto Rican Females Under HAART. G. Tirado, G. R. Jove, Y. Yamamura.

623. Genotypic Analyses of RT and Protease Sequences from Persons Infected with Non-subtype B HIV-1. R. Kantor, D. Katzenstein, R. Camacho, P. R. Harrigan, A. Tanuri, D. Pillay, A. M. Vandamme, P. Phanuphak, W. Sugiura, V. Soriano, L. Morris, Z. Grossman, L. F. Brigido, J. M. Schapiro, R. W. Shafer.

624. Greater Genetic Variation at NNRTI-associat-

ed Resistance Positions in Patients Infected by Subtype C. Z. Grossman, V. Istomin, D. Averbuck, I. Levi, K. Risenberg, M. Chowers, E. Shahar, M. Lorber, E. Mendelson, D. Ram, Z. Kra-Oz, M. Burke, Z. Bentwich, S. Maayan, J. M. Schapiro.

625. HIV-1 Protease Inhibitor Mutations in Drug-naive Patients and Subtype Distribution in Rural Villages of Five Provinces in Cameroon. F. Konings, P. Zhong, S. Burda, M. Urbanski, L. Zekeng, P. Nyambi.

628. Polymorphism Analysis and Drug Resistance Mutations in the Protease Gene of HIV-2 Infected Patients in Marseilles, France. P. Colson, M. Henry, C. Tourres, D. Lozachmeur, H. Gallais, J. Gastaut, J. Moreau, C. Tamalet.

629. Protease Sequences from HIV-2 Subtypes A and B Harbor Multiple Mutations Associated with Protease Inhibitor Resistance in HIV-1. D. Pieniazek, M. Rayfield, D. Hu, J. Nkengasong, V. Soriano, W. Heneine, C. Zeh, S. Agwale, Ch. Wambebe, L. Odama, M. Kalish.

640. Continued Reverse Transcriptase Inhibitor Therapy Is Sufficient to Maintain Short-Term Partial Suppression of Multi-drug Resistant Viremia. S. G. Deeks, J. N. Martin, R. Hoh, T. Wrin, C. Petropoulos, R. M. Grant.

649. Interleukin-2 in Conjunction with HAART in Early HIV Infection Increases Naïve and Memory CD4 Cells and Lowers Activation Markers. F. M. Hecht, C. B. Hare, M. S. McGrath, L. Liu, R. L. Gascon, J. O. Kahn, J. A. Levy.

728. Improvements in Body Fat and Mitochondrial DNA Levels Are Accompanied by Decreased Adipose Tissue Cell Apoptosis After Replacement of Stavudine Therapy with Either Abacavir or Zidovudine. K. Thompson, G. McComsey, D. Paulsen, C. Cherry, T. Lonergan, S. Hessenthaler, V. Williams, R. Fisher, S. Wesselingh, J. Hernandez, L. Ross.

732. Lipoatrophy Is the Dominant Feature of the Lipodystrophy Syndrome in HIV-infected Men. B. Gripshover, P. C. Tien, M. Saag, D. Osmond, P. Bacchetti, C. Grunfeld for the Investigators of the Fat Redistribution and Metabolic Change in HIV Infection (FRAM) Study.

733. Body Composition in HIV+ men With and Without Peripheral Lipoatrophy Is Different Than Controls. M. Saag, P. C. Tien, B. Gripshover, D. Osmond, P. Bacchetti, C. Grunfeld for the Investigators of the Fat Redistribution and Metabolic Change in HIV Infection (FRAM) Study.

734. Buffalo Hump in Men with HIV Infection: Larger, but Not More Common. A. Zolopa, C. Benson, P. Bacchetti, P. C. Tien, C. Grunfeld for the Investigators of the Fat Redistribution and Metabolic Change in HIV Infection (FRAM) Study.

735. Body Composition and Antiretroviral Use in Older HIV-infected Women. A. A. Howard, R. Freeman, N. Santoro, E. E. Schoenbaum.

736. Incidence of Lipoatrophy and Lipohypertrophy in the Women's Interagency HIV Study. P. C. Tien, S. R. Cole, C. M. Williams, R. Li, J. Justman, M. H. Cohen, M. Young, N. Rubin, M. Augenbraun, C. Grunfeld.

738. Lipodystrophy in Patients Switched to Indinavir/Ritonavir 800/100 mg BID and Efavirenz 600 mg QD After Failing Nucleoside Combination Therapy: A Prospective, 48-week Observational Sub-study of HIV-NAT 009. M. Boyd, D. Bien, P. van Warmerdam, E. Hassink, P. Srasuebkul, N. Chomchey, T. Methanukroh, B. Sopa, S. Wangsuphachart, A. Krisanachinda, K. Ruxrungtham, J. Lange, D. Cooper, P. Phanuphak, P. Reiss.

739. Relation Between Use of Nucleoside Reverse Transcriptase Inhibitors, Mitochondrial DNA Depletion, and Severity of Lipoatrophy: Results from a Randomized Trial Comparing Stavudine and Zidovudine-based Antiretroviral Therapy. M. van der Valk, M. Casula, J. P. Ruiter, C. van Kuijk, B. L. F. van Eck-Smit, G. J. Weverling, H. J. Hulsebosch, A. van Eeden, K. Brinkman, J. M. A. Lange, R. J. Wanders, A. de Ronde, P. Reiss.

744. HIV Infection, HAART, and Blood Pressure: Results from the Multi-center AIDS Cohort Study. E. C. Seaberg, A. Muñoz, M. Lu, R. Detels, J. Margolick, S. Riddler, C. Williams, J. Phair.

746. Protease Inhibitors May Increase Risk of Cardiovascular Disease in HIV-infected Patients. U. Iloeje, Y. Yuan, A. Tuomari, G. L'Italien, J. Mauskopf, R. Moore.

747. Hospitalizations for Coronary Heart Disease and Myocardial Infarction Among Men with HIV-1 Infection: Additional Follow-up. D. Klein, L. Hurley.

748. Metabolic Effects of Lopinavir/Ritonavir (Kaletra) in Healthy HIV-seronegative Men. G. A. Lee, T. Seneviratne, M. A. Noor, J. C. Lo, J-M. Schwarz, K. Mulligan, M. Schambelan, C. Grunfeld.

750. Serum Lipids and Antitretroviral Therapy Among HIV-infected Men. S. Riddler, E. Smit, R. Li, S. Cole, J. Chmiel, A. Dobs, F. Palella, B. Visscher, R. Evans, L. Kingsley.

754. Serum Adipocytokines Are Related to Lipodystrophy and Metabolic Alterations in HIV-infected Men Under PI-Based HAART. C. Vigouroux, M. Maachi, T-H. Nguyen, C. Coussieu, S. Gharakhanian, T. Funahashi, Y. Matsuzawa, I. Shimomura, W. Rozenbaum, J. Capeau, J-P. Bastard.

760. Evaluation of the Oxidative Metabolism in Skeletal Muscle Using Carbon-11 Acetat PET in HIV-infected Patients with Lipodystrophy Syndrome. G. M. N. Behrens, A-R. Boerner, K. Weber, J. van den Hoff, J. Ockenga, G. Brabant, R. E. Schmidt.

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