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Highlights of the 12th Conference on Retroviruses and Opportunistic Infections

Special Contribution

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 22 to 25 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. Next, R. Paul Johnson, MD, discusses advances in HIV pathogenesis research and vaccine development. In the third article, Judith S. Currier, MD, and Diane V. Havlir, MD, examine new findings on metabolic complications of HIV disease and antiretroviral therapy. Finally, Magdalena E. Sobieszczyk, MD, Angela K. Talley, MD, Timothy Wilkin, MD, MPH, and Scott M. Hammer, MD, highlight new findings in antiretroviral therapy, treatment strategies, and drug resistance. This last topic is also 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.

The IAS—USA would also like to welcome 3 new members to its board of directors: Carlos del Rio, MD, Joel E. Gallant, MD, MPH, and Roy M. Gulick, MD, MPH.

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Highlights of the 12th Conference on Retroviruses and Opportunistic Infections

Special Contribution

Announcements

Developments in Basic Science Research

Mario Stevenson, PhD

Basic science research constitutes a steadily increasing part of the Conference on Retroviruses and Opportunistic Infections, and the 12th conference arguably had the strongest showing of basic science research. Presentations on cellular defense factors dominated the basic science category, and some of the presentations provided a few surprises with regard to the mechanism by which the cellular defense factor APOBEC counteracts HIV-1 infection. A second cellular defense factor has been identified in the past year and a number of presentations focused on its mode of action. Similarly, evidence for new cellular factors that positively promote viral replication were presented. Novel findings relating to viral pathogenesis were featured at the meeting and some clarity was brought to the issue of the underlying role of viral pathogenicity and immune activation in lymphocyte depletion. The crystal structure of an unliganded SIV envelope was presented and has broad significance in terms of understanding viral infection and generating better immunogens for vaccines.

Cellular Defense Factors

The initial discovery by the Malim laboratory[1] of the cellular defense protein APOBEC highlights the conflict that exists between viruses and their host at the cellular level. Before the identification of APOBEC, the cellular and humoral arms of the immune system were thought to be the major host defenses against infections by viruses such as HIV-1. Since the discovery of APOBEC and more recently, the discovery of another cellular restriction (TRIM 5α) by the Sodroski research group,[2] it is now becoming apparent that cellular defenses exhibit antiviral activities that oppose viral replication far more effectively than the immune system. As such, viruses have had to evolve mechanisms that allow them to circumvent these defenses so that they can replicate in monkey and human hosts. Although APOBEC was identified 3 years ago, how it blocks viral infection and how the virus defends itself from this restriction is still generating surprises. APOBEC is a cytidine deaminase that converts cytosines within the minus strand of the viral cDNA to uracils. When replicated, this results in G-to-A hypermutations in the plus strand of the cDNA. This conversion is so severe that the viral cDNA loses its integrity both in terms of stability and in terms of its ability to encode functional viral proteins.[3]

Given the presence of this potent cellular defense protein, why do people get infected? The answer is that the virus has evolved a countermeasure, namely the Vif protein, the function of which is to remove APOBEC from the cell by dragging it to the proteasome, where it is degraded. To exert antiviral activity, APOBEC must be packaged within virus particles in order to subsequently influence the reverse transcription step in newly infected cells, since the expression of Vif in target cells does not ameliorate the antiviral effects of APOBEC. Until recently, the antiviral activity of APOBEC was thought to be a consequence of its enzymatic activity and that Vif was efficient in clearing the way for viral replication by depleting APOBEC from the cell and preventing its incorporation into newly budded virions. Results presented at the conference challenged these views, and it now appears that APOBEC may have antiviral activity that is independent of cytidine deaminase. This cytidine deaminase–independent antiviral activity may be active against a variety of viruses unrelated to primary lentiviruses and, in addition, the Vif countermeasure may be imperfect given the frequent presence of G-to-A hypermutations in viruses sequenced directly from patient specimens.

In his Bernard Fields Memorial lecture, Malim (Abstract 5) presented evidence that APOBEC 3G mutants that lack cytidine deaminase activity are nonetheless capable of inhibiting HIV-1 replication. There are at least 11 APOBEC-related cytosine deaminases in the human genome. APOBEC 3G and APOBEC 3F have thus far been shown to inhibit HIV-1 replication. In addition, these APOBEC proteins are expressed in cells relevant to HIV-1 replication, namely lymphocytes and macrophages. APOBEC protein family members contain consensus cytidine deaminase motifs, and specific residues that are important for zinc coordination and proton transfer, which are crucial to enzymatic activity, have been identified. APOBEC 3G contains 2 such cytidine deaminase motifs. Malim presented evidence that only the C-terminal active site was necessary for the DNA-mutating activity of APOBEC 3C. Nevertheless, APOBEC 3G lacking this C-terminal active site potently suppressed HIV-1 infection without inducing G-to-A hypermutation. Although the mechanism through which APOBEC 3G suppresses HIV-1 infection independently of cytidine deaminase activity remains to be determined, this antiviral activity appears to require the packaging of APOBEC proteins into virions. As such, Vif is still important in order to inhibit the cytidine deaminase–independent antiviral activities of APOBEC.

There were more surprises regarding the antiviral activity of APOBEC in one study (Abstract 30). The study, conducted by the Green research group, originally set out to determine why cytoplasmic APOBEC is not active against incoming virions. APOBEC 3G was found to exist in a high molecular weight ribonucleoprotein complex that does not contain cytidine deaminase activity. When this complex was treated with RNAase, it was converted to a low molecular weight complex that exhibited cytidine deaminase activity. The investigators then looked at the form in which APOBEC 3G exists in resting CD4+ T lymphocytes and in primary undifferen-

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tiated monocytes. These cells have long been known to be refractory to HIV-1 infection, and an important question in the field regards the nature of the block that exists in these cells. Surprisingly, APOBEC 3G in resting lymphocytes and monocytes was present in a low molecular weight complex, which was subsequently converted to a high molecular weight complex when resting lymphocytes were activated or when monocytes were induced to differentiate into macrophages. The investigators then used the technique of RNA interference to modulate the expression of APOBEC 3G in resting lymphocytes. When small interfering RNAase specific for APOBEC 3G were introduced into resting cells, the block to HIV-1 infection was relieved. This may shed light on the mechanism by which HIV-1 infection is restricted in resting lymphocytes and in monocytes. It remains to be determined whether the mechanism by which the low molecular weight APOBEC complex in resting cells blocks HIV-1 infection is analogous to the mechanism by which cytidine deaminase–deficient mutants of APOBEC 3G block HIV-1 infection.

Continuing the theme of the mechanism by which APOBEC modulates HIV-1 infection, another study (Abstract 241) characterized which APOBEC 3 family members influence HIV-1 infection. There are 7 APOBEC 3 genes (A3A through A3G). A3B, in addition to A3F and A3G, exhibited antiviral activity and were specifically packaged into virions. Surprisingly, A3F and A3G were suppressed by HIV-1 Vif, but A3B was not. The caveat to this study is that A3B does not appear to be expressed in tissues relevant to HIV-1 infection and as such may not be able to influence viral replication in vivo.

Studies attempting to gain insight into the mechanism by which Vif blocks the antiviral activity of APOBEC were also featured at the conference. Two (Abstracts 31, 32) presented some of the cellular factors that are involved in Vif-mediated targeting of APOBEC to the proteasome. It is now becoming clear that HIV-1 harbors a suppressor of cytokine signaling (SOCS)-box–like motif that mediates interaction with an E3 ligase complex that is ultimately targeted to proteasome. This information could be valuable in terms of designing small-molecule inhibitors that prevent Vif from interacting with the E3 ubiquitin ligase complex, since this would prevent APOBEC degradation and allow it to exert antiviral activity even in the presence of Vif.

It is clear that the Vif protein of primate lentiviruses has evolved to potently suppress the antiviral activity of APOBEC, but there is accumulating evidence that the ability of Vif to counteract APOBEC is not absolute and as such, viral genomes are influenced by APOBEC proteins. The number of G-to-A hypermutations in viral plus-strand cDNA in the presence of Vif is higher than in cells that do not express APOBEC 3G (Abstract 5). Therefore, even in the presence of Vif, APOBEC 3G is still able to effect hypermutation of viral cDNA. Another study (Abstract 29) presented evidence that the virus may have an additional mechanism to further reduce the effects of APOBEC that escapes Vif-mediated degradation. Within cells, DNA repair enzymes such as uracil DNA glycosylase (UDG) prevent misincorporation of uracil into DNA by targeting hypermutated transcripts for degradation. UDG has previously been shown to bind to the accessory protein Vpr of HIV-1, although the functional significance of this interaction is not clear.

Schröfelbauer and colleagues (Abstract 29) demonstrated that HIV-1 Vpr, through its interaction with UDG, limits C-U deamination by APOBEC 3 so as to increase the fidelity of viral reverse transcription. These data illustrate that primate lentiviruses have gone to extraordinary measures to limit susceptibility to cytidine deaminase imposed by APOBEC. Continuing this theme, a study by Bourara and colleagues (Abstract 236) presented evidence that the hypermutational activity of APOBEC 3 on viral cDNA may facilitate viral evolution in vivo. Primate lentiviruses rapidly evolve and, as a result, can adapt to changes in the environment as imposed, for example, by antiviral pressure or immune surveillance. Traditionally, the ability of HIV-1 to evolve is principally considered to be a result of error-prone reverse transcription. However, by direct sequencing of viral sequences in clinical specimens, it is clear that there is an overrepresentation of G-to-A mutations in viral genomes. As such, these could contribute to accelerated viral evolution and may assist in allowing the virus to rapidly evolve drug resistance or escape from cytotoxic T cells or neutralizing antibodies.

Adding to the excitement in the field generated by the discovery of the defense factor APOBEC 3G was the discovery in the past year of a second cellular defense called TRIM 5α by Sodroski's laboratory. As expected, research on the mechanism by which TRIM 5α opposes viral replication was well represented at the conference (Abstracts 34, 152LB, 174, 175, 231, 234). A central feature of retroviruses and primate lentiviruses regards their host-species specificity, as illustrated by the fact that these viruses frequently encounter early blocks to infection after entering cells from different species. For example, HIV-1 does not efficiently infect monkey cells, and with the discovery of TRIM 5α, part of the mechanism by which monkey cells restrict HIV-1 infection is becoming clearer. Research from a number of groups had established that HIV infection of monkey cells is impaired early after infection to the extent that there is very little reverse transcription in these cells. Therefore, it was suspected that cellular proteins in these monkey cells were impairing an early step such as HIV-1 uncoating. To identify the factor responsible, the Sodroski group expressed a monkey cDNA library in human cells and then challenged those cells with an HIV-1 variant that carries a fluorescent marker. They then identified the genes that were expressed in cells that resisted HIV infection (and that presumably expressed the monkey restriction factor). This strategy led to the identification of TRIM 5α.

The details by which this protein blocks HIV-1 infection are still being worked out, but it is believed that TRIM 5α targets the viral capsid to interfere with uncoating of the viral core and release of genomic viral RNA following infection. TRIM 5α belongs to a family of proteins defined by a tripartite motif consisting of RING, B-box 2, and coiled-coil domains. The TRIM 5α contains a C-terminal B30.2 (SPRY) domain and within this domain are variable regions that differ depending upon the species of origin. Research presented at the meeting (Abstract 34) has shed light on why monkey but not human TRIM 5α blocks HIV-1 infection. When only 3 amino acids

within the first variable region of the human TRIM 5α B30.2 domain were altered to resemble monkey TRIM 5α, the human chimeric TRIM 5α, which still was more than 98% identical to the human protein, potently suppressed HIV-1 infection. Correspondingly, a single amino-acid change in this region of human TRIM 5α resulted in a protein that potently restricted SIV infection. These studies demonstrate the potent effect of host genetics on viral replication and, further, how cellular defense factors have shaped viral genomes. The hope is that this information will ultimately provide the rationale for novel therapeutics that may accentuate the ability of human TRIM 5α to interact with HIV-1 capsid and block viral replication.

A number of groups previously demonstrated that the species-specific restriction to HIV-1 infection could be modulated by a cellular protein called cyclophilin A. It is now clear that cyclophilin A is required for TRIM 5α-mediated restriction of HIV-1 in cells from Old World monkeys (Abstracts 175, 231, 232). Cyclophilin A is a peptidyl-proliyl isomerase first identified through its ability to interact with HIV-1 capsid. Studies in which cyclophilin A was inhibited by RNAase interference or was knocked out by gene deletion revealed that although cyclophilin A is packaged within virions through its interactions with the viral capsid, cyclophilin A in the target cells and not in the producer cell modulates viral infectivity, such that in the absence of cyclophilin A, viral replication is enhanced in cells that manifest TRIM 5α restriction.

Vpu is an accessory protein that is specific to HIV-1 and a few SIV variants. The role of Vpu in HIV-1 replication is not fully understood. Its previously reported activities include enhancement of viral particle release and cooperation with the viral envelope glycoprotein to downregulate CD4 from the cell surface. Studies presented at the meeting (Abstract 178) suggest that Vpu overcomes a dominant restriction to virus assembly that exists specifically in human cells. Thus, in heterokaryons formed between human and African Green monkey cells, HIV-1 assembly was inefficient and Vpu enhanced assembly. The human protein that inhibits viral assembly and that is targeted by Vpu awaits identification.

Positive Cellular Cofactors

On the flip side of cellular factors that oppose viral replication were presentations dealing with cellular factors that promote viral replication. Over the past 2 to 3 years, perhaps the greatest understanding regarding the cellular machinery that drives distinct steps in the viral replication cycle have dealt with the viral-budding step. During the process of virus budding, a membrane fission event allows the fully assembled virion to detach from the plasma membrane of the host cell. Cellular factors that are required for this fission event were discussed in 2 studies presented (Abstracts 176, 177). Some of the cellular factors that participate in virion budding also play an important role in membrane invagination events that are required for detachment of a phagocytic vacuole from the plasma membrane or for formation of multivesicular bodies that are involved in trafficking of processed antigens through the cell (Abstract 116). The identification of critical interactions between structural Gag proteins of the virus and cellular proteins involved in vacuolar biogenesis points to novel targets for therapeutic infection.

Cellular factors that may play a role in orienting the virus within the cell prior to integration were also presented at the meeting (Abstract 35). Arguably, the least-understood aspect of HIV-1 replication regards factors that aid in its translocation from the point of entry at the cell membrane to cellular DNA within the nucleus. To put this into perspective, if one considers a cell the size of a football field, the viral cDNA with its associated viral proteins (for example, reverse transcriptase and integrase) would be the size of a football. Therefore, there has been suspicion that the virus may use a roadmap in order to ensure that it follows the appropriate route to the center of the cell. Some data (Abstract 35) indicated that proteins of the inner nuclear envelope may be used by HIV-1 as part of this roadmap. Evidence was presented that the nuclear envelope itself is required for efficient HIV-1 infection but not for infection by other retroviruses such as murine leukemia virus (MLV). RNA interference was used to silence the expression of a number of inner nuclear envelope proteins. Silencing of 2 nuclear envelope proteins, namely barrier-to-autointegration factor (BAF) and emerin, did not interfere with the ability of the virus to localize the nucleus, but specifically interfered with the ability of the virus to integrate. Inefficient integration was accompanied by an increased formation of episomal cDNA, which are dead end products of viral infection to the point that almost all viral cDNAs in the nucleus were in the form of nonfunctional episomes. A similar effect is seen when infection is blocked by small-molecule inhibitors of the integration step. The authors propose that the inner nuclear envelope facilitates the interaction of incoming viral cDNA with cellular chromatin in order to prevent inactivation of the viral cDNA by recombinases and ligases within the nucleus that promote the circularization of viral cDNA. The study also suggests that HIV-1 replication may be restricted to nondividing cells.

RNA interference is widely exploited as a tool to regulate gene expression. As such, it offers a valuable approach to validating cellular cofactors of HIV-1 replication, since one can silence the expression of individual cellular genes and gauge whether, in the absence of that gene, viral replication is affected. Even so, it is becoming clear that HIV-1 cofactor validation is not trivial. A poster discussion session (Abstracts 220-227) was devoted to making sense of the role of LEDGF in HIV-1 replication. LEDGF is a transcriptional coactivator that was found to interact with lentiviral integrases in mammalian cells and was suggested to act as a cofactor for viral integration. However, the field has been unable to reach a consensus on whether LEDGF is a bona fide cofactor for viral integration. Silencing LEDGF by RNA interference has produced conflicting results ranging from a large impact on viral infection to negligible impact, even though biochemical data convincingly demonstrate that LEDGF tightly interacts with the viral integrase in mammalian cells. The take-home message is that the LEDGF-integrase association may be required in a viral context that is not reproduced with in vitro replication assays. For example, data from the Bushman laboratory indicate that HIV-1 preferentially integrates into active cellular genes (Abstract 251). Although the underlying mechanism by which HIV

favors particular integration sites over others is not understood, one possibility is that components of the viral preintegration complex, such as LEDGF, may play a role in integration site selection. If this is the case, the absence of LEDGF may not result in measurable differences in viral replication in simple in vitro culture systems.

Some presentations (Abstracts 257, 259, 260, 261) were aimed at understanding the factors that differentially influence endosomal versus plasma membrane budding of HIV-1. It has been previously demonstrated that in cells such as macrophages, viruses bud more selectively at cytoplasmic membranes than at the plasma membrane. These studies underscore the notion that endosomal assembly of HIV-1 may be a regulated process that HIV-1 has evolved to exploit. It remains to be determined whether intracellular budding of HIV-1 offers a survival advantage by, for example, providing a compartment in which virions evade immune surveillance.

Studies describing the structure of an unliganded SIV envelope (Abstracts 7, 28) generated a lot of interest. The structure of the core domain of HIV-1 gp120 in a CD4-bound confirmation has previously been described. Now, with the structural characterization of the core of a CD4-nonbound SIV gp120, a better understanding of the conformational changes imposed by receptor binding can be revealed. In the case of HIV-1, envelope binding to CD4 causes a major conformational change that exposes coreceptor binding domains in the protein. This may be a mechanism that HIV-1 has adopted in order to protect coreceptor binding domains from recognition by the host antibody response so that these domains are exposed immediately prior to coreceptor contact. Therefore, structural characterization of CD4-bound and -nonbound envelope glycoproteins has important implications not only for the understanding of the viral infection process but also for the design of vaccine immunogens that induce neutralizing antibodies to sensitive coreceptor binding sites.

Pathogenesis

A long-standing controversy in AIDS pathogenesis regards the relative roles of virus-mediated cell killing and indirect cell killing in lymphocyte depletion. Although HIV-1 is clearly recognized as a cytopathic virus that rapidly destroys CD4+ T lymphocytes in vitro, other processes such as immune activation have been proposed to play an important role in accelerated lymphocyte turnover in vivo. One presentation (Abstract 127) reinforced the case for direct virus-mediated cytopathicity in lymphocyte depletion by pathogenic primate lentiviruses. Studies in SIV-infected monkeys previously established that the gastrointestinal tract is a principal site for viral replication. More recently, by characterizing lymphocyte subsets in the gastrointestinal tract and lymph node in acutely infected individuals, it has been observed that lymphocytes are rapidly depleted by massive viral replication, which corresponds with acute high-level viremia.

In his plenary presentation, Douek presented evidence that lymphocyte depletion is directly due to viral cytopathicity. Using polymerase chain reaction (PCR) to quantitate the frequency of HIV-1 infected cells, it was demonstrated that during lymphocyte depletion in acute infection, the majority of lymphocytes harbor viral DNA. These findings, although not unexpected, have important implications for the treatment of HIV-1 infection, since the unrestricted viral replication that occurs during acute infection may be a defining event in the natural progression of disease that dictates viral set point (the steady-state level of viremia that is achieved following acute infection) and perhaps time to onset of disease. As such, it will be important to evaluate whether prophylactic or early therapeutic intervention prior to that interrupts this profound acute replication, preserves the integrity of gastrointestinal lymphocytes, and affects viral set point and time to onset of disease.

Several studies (Abstracts 152, 153, 154, 155) investigated characteristics of SIV infection in natural and nonnatural host species that might explain the differential manifestations of disease in these systems. In naturally infected monkeys such as sooty mangabeys, SIV replicates to extraordinarily high titers and promotes accelerated lymphocyte turnover. Despite this, there is no evidence for AIDS-like symptoms and the mechanism for lack of disease in these animals has been a central question in the field. Recent studies have suggested that high-level SIV replication occurs in the absence of generalized immune activation; in pathogenic infections including HIV-1–infected humans or SIV-infected rhesus macaques, high-level viral replication is accompanied by a generalized immune activation. Staprans and colleagues (Abstract 152) presented evidence that differences in dendritic cell (DC) function may partially contribute to the differential immune activation that is seen in pathogenic and nonpathogenic primate lentivirus infections. There were marked differences in DC maturation between pathogenic and nonpathogenic infection, which was reflected by increased expression of CCR7 (which is reported to play a role in immune cell homing to lymphoid tissue). The authors propose that this leads to accumulation of mature DCs in the lymph nodes, which leads to more extensive T-cell proliferation in acutely infected rhesus macaques. The authors also noted differences in the response of rhesus and sooty mangabey DCs to CpG and to activation by SIV. Collectively, these results suggest that DC responsiveness may play an important role in determining the magnitude of the inflammatory response that ultimately plays a role in bystander cell damage characteristic of pathogenic lentivirus infection.

The relationship between host-cell activation and susceptibility to HIV-1 infection is a well-recognized feature of primate lentivirus replication. Quiescent lymphocytes are refractory to infection and, upon entry into cell cycle (G1 phase and beyond), these cells acquire susceptibility to infection. As a result, factors that affect the cell-cycle state of CD4+ lymphocytes can directly affect the extent of viral replication. Both macrophages and lymphocytes have previously been reported to provide signals in trans that promote the ability of neighboring lymphocytes to replicate HIV-1. Dendritic cells capture virions and transmit capture virions to neighboring T cells while at the same time providing a signal that induces those cells to go into cell cycle. Infected macrophages produce virions and soluble factors that increase susceptibility of neighboring lymphocytes to infection by virions released from the infected macrophages. In an extension of this theme, one study (Abstract 148) demon-

strated that CD16+ monocytes, which are refractory to productive HIV-1 infection, can nevertheless transmit HIV-1 to T cells in trans and at the same time release soluble factors that increase their susceptibility to infection. The authors demonstrated that CD16+ monocytes more efficiently capture and transmit virions to activated CD4+ T cells during cell-cell contact and release eotaxin-2 and MCP-1, which activate resting CD4+ T cells, thereby rendering them permissive to viral infection.

One issue that has attracted the interest of a number of groups is whether the HIV-1 envelope, upon engaging receptor and core receptor molecules, can engender signals that influence susceptibility of the cell to infection. In support of this, Balabanian and colleagues (Abstract 200) presented evidence that the viral envelope glycoprotein exhibits similar signaling characteristics to the CXCR4 ligand SDF-1. The authors demonstrated that a CXCR4-tropic gp120, when added to primary CD4+ T cells, induced calcium mobilization and activation of PI3K and MAP kinases, as well as actin cytoskeleton rearrangements and membrane ruffling. Importantly, these effects were also mediated by inactivated HIV-1 virions containing conformationally intact envelope glycoproteins. The authors propose that envelope may mimic the action of chemokines such as SDF1 so as to promote the recruitment of target cells and, through the cytoskeleton effects, facilitate the uptake of viruses by target cells. In a similar vein, another presentation (Abstract 279) demonstrated that HIV-1 gp120 can elicit release of tumor necrosis factor (TNF)-α by human macrophages and that this stimulation is CCR5 dependent and mediated through a PI3 kinase– and MAPK kinase–dependent signaling pathway. The authors propose that induction of TNF-α in this way may play a role in processes such as AIDS-related dementia in which HIV-1–infected microglia have been reported to induce neuronal dysfunction through the release of soluble neurotoxins. One study (Abstract 278) suggested that CCR5 signaling by R5-tropic virions increases the efficiency of the viral replication upon infection. Another (Abstract 146) presented evidence that variation in gene copy number of CCL3L1, an agonist of the viral core receptor CCR5, influenced susceptibility to HIV-1 infection. Of note, CCL3L1 copy number was only important in the context of CCR5 genotype such that low CCL3L1 copy number combined with specific CCR5 (detrimental) genotypes were associated with 4-fold differences in the risk of acquiring HIV in the context of vertical transmission.

Swingler and colleagues (Abstract 149) demonstrated that soluble factors released by HIV-1–infected macrophages not only influence CD4+ lymphocyte function but can also directly impact B lymphocyte proliferation and differentiation. The authors presented evidence that the viral *nef* gene induces release of ferritin light chain, a protein with roles in iron metabolism, and that L-ferritin induces B-cell proliferation and differentiation to immunoglobulin-secreting plasma cells. The authors further demonstrated a direct correlation between plasma ferritin levels in HIV-1–infected individuals exhibiting hypergammaglobulinemia but not in SIV-infected monkeys that do not exhibit hypergammaglobulinemia. This correlated with the differential ability of HIV and SIV *nef* to induce ferritin from infected macrophages in vitro. Hypergammaglobulinemia is a well-recognized but poorly understood manifestation of HIV-1 infection. The authors propose that B-cell dysfunctions in AIDS may be attributable to the action of the viral Nef protein in infected macrophages.

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HIV Pathogenesis and Vaccine Development

R. Paul Johnson, MD

New information on the crystal structures of the HIV and the simian immunodeficiency virus (SIV) envelopes represented one of the scientific highlights of the 12th Annual Conference on Retroviruses and Opportunistic Infections. Numerous presentations also underscored the increasing recognition of the central role of gut-associated lymphoid tissue in AIDS pathogenesis and helped reveal a better understanding of the multiple mechanisms underlying CD4+ T lymphocyte depletion in AIDS. Progress on vaccine development was largely incremental but was strongly influenced by the impact of an expanding array of flow cytometric assays that have revealed significant functional and phenotypic differences in virus-specific CD8+ cells. The interplay between host cellular and humoral immune responses and virus evolution was another prominent theme, and it underscored the challenge facing host immune responses and vaccine developers in attempting to thwart an ever-mutating virus.

New Insights into Envelope Structure and HIV-specific Neutralizing Antibodies

Designing strategies to foil the ability of HIV to evade neutralizing antibodies represents one of the primary (and still elusive) goals of HIV vaccine research. The crystal structure of HIV-1 gp120 published in 1998 analyzed the conformation of a modified envelope stripped of most of its sugar residues and bound to CD4 and to an antibody that mimicked the chemokine coreceptor binding site (ie, a liganded envelope). Although this structure has been quite valuable for advancing our understanding of HIV envelope function, it does not provide information on several key points, including the structure of the envelope in its native state prior to binding to CD4, the point at which it is most susceptible to neutralizing antibodies.

Using crystals of a fully glycosolated SIV gp120 (which was more stable than the HIV version) stripped of the V1-V2 and V3 variable loops, the Harrison lab determined the crystal structure of the unliganded envelope (Abstract 7). The most notable finding was the dramatic conformational shift observed in the inner domain of the unliganded envelope compared with the previously determined liganded envelope structure. In the unliganded form, the tip of the CD4 binding site is exposed at the top of envelope. Following initial contact with CD4, the resulting conformational change brings together the other residues of the CD4 binding site as well as the chemokine receptor binding site, locking in the remodeled envelope around CD4, and exposing the newly formed chemokine receptor binding site. These changes shift a portion of the V1-V2 loop that interacts with gp41, thereby helping to release gp41 for subsequent fusion with the host cell.

The investigators also identified a deep hydrophobic pocket in the inner domain of the unliganded envelope where resistance mutations have been mapped for a newly identified antiviral compound (BMS-78806) that has been shown to inhibit HIV entry. This class of inhibitor is therefore likely to inhibit entry by binding to this pocket and stabilizing the unliganded form of gp120. Although significant conformational changes in the envelope were clearly anticipated prior to the determination of this structure, the availability of a 3-dimensional model to visualize these changes should help guide efforts to develop new means to interfere with viral entry by pharmacologic or immunologic means.

Another gap in our understanding of envelope structure has been the lack of solid information on the V3 loop, which was deleted in the molecule used for the previously reported crystal structure. Kwong (Abstract 110) described recent success in generating crystals of HIV-1 envelopes containing an intact V3 loop. After screening different combinations of gp120 core proteins with an intact V3 loop complexed with CD4 and different antibodies able to bind to the envelope only after CD4 binding (CD4-induced antibodies), the Kwong laboratory identified a crystal that could be used for structural analysis. Several features of the resulting predicted structure of the V3 loop in the context of an envelope trimer help explain its relative immunogenicity, including the fact that this loop is relatively exposed in the envelope trimer and the lack of intermolecular hydrogen bonds, a feature that confers more flexibility on the loop to interact with antibodies.

Only a handful of monoclonal antibodies are able to provide relatively broad neutralization of primary HIV isolates. Based on the premise that elucidation of the structure of these rare antibodies could provide clues to the design of modified envelope immunogens that would be more effective in inducing broadly neutralizing antibodies, the structures of several of these antibodies have been determined over the past several years. However, to date, the hope that this information would lead to better vaccines has yet to be realized. Most of these antibodies (notably b12 and 2G12) have relatively unusual structures that are likely to be difficult to elicit by immunization. However, Kwong highlighted the fact that the recently determined structure of the 2F5 monoclonal antibody might provide a logical path to a better immunogen. The antigen-binding region of 2F5 contains an extended hydrophobic region that allows the antibody to bind to a hydrophobic region of gp41 that is relatively close to the virion surface. In fact, binding of 2F5 to the HIV envelope is strongly enhanced by the presence of membrane, suggesting that presentation of the 2F5 epitope in the context of membrane may be a key factor in eliciting similar antibodies.

This observation suggests that use of a modified envelope immunogen, which is locked into place (by introduced disulfide bonds) and presented in the context of a membrane (eg, by a virus-like parti-

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cle or proteoliposome), might be a more effective means to induce antibodies to this epitope.

Alternative approaches to understand how to elicit broadly neutralizing antibodies have focused on analysis of antibodies in HIV-infected people that bind to conserved functional targets, such as the chemokine coreceptor binding site. Decker (Abstract 87) utilized a novel approach to screen for antibodies in patient sera able to bind to the chemokine receptor binding site by preincubating either HIV-1 or HIV-2 reporter viruses with soluble CD4 to expose the coreceptor binding site.

These CD4-induced neutralizing antibodies were able to mediate neutralization across numerous HIV-1 clades and even neutralization of HIV-2 at titers of up to 1:100,000 or more. Competition experiments with an antibody (19c) known to bind to the chemokine receptor binding site confirmed that this antibody activity largely or exclusively reflected antibodies to the chemokine receptor binding site. These results probably have greater significance for understanding of envelope function and evolution than vaccine design, because CD4-inducible neutralizing activity is only detected after the virus binds to CD4. Thus, the access of antibodies to this binding site is stearically inhibited following the interaction between HIV and CD4+ target cells. However, these data suggest that viruses are largely constrained to CD4 dependence in order to avoid neutralization by these relatively potent antibodies.

Virtually all studies that have attempted to understand protective immunity against HIV in humans have relied on correlative studies, and there have been relatively few opportunities to examine the ability of specific immune responses to block HIV replication in vivo. Trkola (Abstract 94LB) investigated whether 3 potent neutralizing antibodies (2G12, 2F5, and 4E10) could suppress viral rebound in HIV-infected subjects with either acute (n = 6) or chronic (n = 8) infection who underwent interruption of their antiretroviral therapy. These antibodies represent 3 of the 5 well-characterized antibodies with broadly neutralizing activity against HIV-1: 2G12 recognizes a carbohydrate epitope on gp120; 2F5 and 4E10 both recognize epitopes on highly conserved regions of gp41. As noted above, these antibodies are atypical, both with respect to their structure and ability to mediate relatively broad neutralization of multiple HIV strains, and are not representative of neutralizing antibodies found in most HIV-infected subjects. The subjects, who were preselected for HIV isolates sensitive to these antibodies, received all 3 neutralizing antibodies 1 day prior to discontinuation of antiretroviral therapy and received weekly infusions for a total of 12 weeks. The 8 chronically infected patients had undergone previous treatment interruptions, thereby allowing a comparison of the increase in viremia following monoclonal antibody treatment with that observed following previous interruptions.

A significant delay in the rebound of viremia was observed in 2 of the 8 chronically infected patients. The 6 acutely infected patients had such rebound significantly later than did a retrospective control group of patients with acute HIV infection who underwent treatment interruption and were not treated with neutralizing antibodies. Interestingly, the development of resistance in the resurgent virus to the 2G12 monoclonal antibody was observed in 11 of 13 patients. Overall, 7 of 14 patients had either a delayed or a decreased rebound in viremia after interruption of therapy. These data provide a proof-of-principle demonstration of the ability of neutralizing antibodies to help contain viral replication in a subset of HIV-infected patients. However, the fact that relatively high amounts of 3 potent neutralizing antibodies in a highly idealized setting had no effect on viral rebound in at least half of the study subjects serves as a compelling reminder of the challenge of neutralizing HIV in vivo. Also, the observation that evolution of resistance to 2G12 occurred in some patients without evidence of significant suppression of viremia suggests the possibility that neutralizing antibodies might exert selective pressure on envelope sequence diversity while mediating only modest suppression of viral replication.

The Guts of HIV Pathogenesis

Although CD4+ cell depletion has long been recognized as the defining feature of AIDS, the mechanisms that mediate this relentless depletion have remained controversial. In a plenary lecture, Douek (Abstract 127) provided an insightful and entertaining review of different mechanisms contributing to CD4+ cell depletion at different stages of HIV infection. Several studies in SIV-infected monkeys had previously documented depletion of CD4+ cells in the gut within 2 weeks after infection. However, there were some who interpreted these studies as representing peculiarities of the SIV/macaque model rather than insights into HIV pathogenesis. Recent studies in HIV-infected patients, including one from the Douek laboratory, have provided compelling evidence for rapid and profound depletion of CCR5+CD4+ cells in the gut of patients in the first several weeks of HIV infection. This depletion of activated CCR5+CD4+ cells occurs most noticeably in the gut, the major reservoir for activated CD4+ cells in the body, but based on studies in macaques, also occurs at other mucosal sites, including the pulmonary tract and female reproductive tract. Whether these cells are killed by direct infection or indirect mechanisms has been much debated. Douek presented data from acutely infected macaques documenting SIV infection of 30% to 60% of memory CD4+ cells. Although this high level of infection was most striking in the gut, it was also observed in peripheral blood lymphocytes as well. Interestingly, memory CD4+ cells that appeared to lack surface expression of CCR5 were also found to be infected with SIV at relatively high rates. These extraordinary rates of infection are over 100-fold higher than those observed in chronic infection. These data document the remarkable finding that approximately 50% of all the body's memory CD4+ cells are killed within 2 weeks after infection, a loss which appears to be largely, if not exclusively, due to the direct effects of viral infection.

However, distinct mechanisms appear to contribute to the depletion of CD4+ cells during chronic HIV infection, in which levels of infected memory CD4+ cells are much lower. Several mechanisms contribute to the progressive CD4+ cell depletion in chronic HIV infection, including the induction of an inflammatory state in lymph nodes associated with the deposition of collagen, suppression of thymic output, and chronic immune activation with its asso-

ciated increased rates of CD4+ and CD8+ cell apoptosis. Douek also proposed that breakdown of local immune responses at mucosal sites could contribute to chronic immune activation. Taken together, these observations paint a complex picture of CD4+ cell depletion in AIDS, in which direct infection of CD4+ cells leads to a substantial depletion of memory CD4+ cells within the first couple weeks of infection and in which multiple direct and indirect mechanisms contribute to the depletion of CD4+ cells during the chronic phase.

Protective Immunity Against HIV: Lessons from Macaques

Lack of solid information on protective immunity against HIV remains one of the major impediments to the development of an AIDS vaccine. Watkins (Abstract 95) provided an overview of insights into protective immunity from studies in macaques. He rejected the notion that it was realistic to expect that an AIDS vaccine could provide sterile protection and instead highlighted the importance of trying to suppress viral replication in the postacute phase of infection to less than 1000 copies/mL, a benchmark that would reduce transmission and significantly delay disease progression.

He also highlighted the fact that macaques infected with attenuated viruses such as SIVΔnef, which has provided some of the strongest protection in the SIV/macaque model to date, are distinguished not so much by high-frequency CD8+ cell responses but by their relatively high-frequency and broadly directed SIV-specific CD4+ cell responses. He further postulated that the induction of strong virus-specific CD4+ cell responses would likely prove to be a crucial characteristic of an effective AIDS vaccine, especially with regard to the ability of CD4+ cell responses to provide help for antibody and CD8+ cell responses. He also highlighted insights into the role of CD8+ cell responses in controlling SIV replication gleaned from the study of 161 macaques at the University of Wisconsin. Of these animals, 11 were termed elite controllers: animals able to achieve sustained control of plasma viremia to less than 1000 copies/mL. Seven of these 11 animals express the relatively infrequent major histocompatibility class I allele Mamu-B*17, which has been shown to present a number of relatively conserved SIV CD8+ cell epitopes. These animals do possess relatively broadly directed SIV-specific CD4+ and CD8+ cell responses, but the frequency of these responses does not necessarily distinguish these animals from other animals who have not achieved long-term control of SIV replication. Watkins postulated that there may be distinct differences in the ability of SIV-specific CD8+ lymphocytes specific for different epitopes to suppress SIV replication both in vitro and in vivo, and that these differences may be observed in settings where no cytotoxic T lymphocyte (CTL) escape has occurred.

Escape from HIV- and SIV-specific CTL Responses

The ability of HIV and SIV to rapidly mutate to escape CTL responses has been well documented in numerous settings. There is increasing evidence that immune selection pressure is shaping the sequence of HIV on a population level as well.

Altfeld (Abstract 91) analyzed CTL recognition of an HLA-A2–restricted epitope in Vpr. Most HLA-A2–positive individuals do not generate a response to the consensus sequence of this epitope. However, a subset of individuals infected with a less-frequent variant ($I_{60}L$) did mount a response against this epitope that was detectable relatively early in the course of infection. This $I_{60}L$ variant epitope had a higher affinity for HLA-A2 and was better recognized by CTL than the consensus sequence, suggesting that this immunodominant epitope may have already been lost due to virus escape driven by the relatively common HLA-A2 allele.

A similar scenario was presented by Leslie (Abstract 92), who demonstrated a negative association between the presence of HLA-B*57/*5801 and conservation of a consensus glycine at residue 83 of Nef. Most previous studies on immune escape from CTL have focused on the documentation of a positive association between the presence of a specific HLA allele and a change from the consensus HIV-1 sequence, whereas these authors reported the opposite effect—that is, the presence of a specific HLA allele was associated with a preservation of the consensus sequence. They went on to demonstrate that the Nef 83 residue lies in a previously undefined B*57/B*5801-restricted epitope and appears to represent an escape mutation that has increased in frequency to become the consensus sequence. A similar situation was demonstrated for an HLA-B*51-restricted epitope. In several instances, they documented transmission of these escape sequences to subjects who lacked the restricting allele and did not observe reversion of the sequence, an observation that strongly suggests that the presence of this mutation does not exact a cost to the virus in terms of decreased fitness.

The dynamic relationship between CTL escape and replication fitness was reinforced by macaque studies reported by Barouch (Abstract 131). Using a virus stock derived from monkeys that expressed the Mamu-A*01 major histocompatibility complex (MHC) class I allele and contained an escape mutation in the dominant A*01-restricted Gag epitope p11c, Barouch observed consistent reversion to wild-type p11c epitope sequences in monkeys that did not express A*01. These data confirm prior reports that escape mutations in this epitope exact a significant fitness cost to the virus. Interestingly, in A*01-positive animals, transient reversions to wild-type p11c sequences were observed, which then triggered an increase in the frequency of CTL specific for this epitope, followed by an increase in the frequency of CTL escape variants.

Taken together, these data reinforce the notion that HIV and SIV have evolved both in individuals and on a population level to develop mutations in immunodominant CTL epitopes. The implications for vaccine design would be that vaccines should attempt to elicit responses to epitopes for which escape mutations exact a significant fitness cost to the virus and are therefore less likely to become fixed in the general population.

Natural Hosts of Primate Lentiviruses

Natural hosts of primate lentiviruses, such as sooty mangabeys and African green monkeys, rarely develop immunodeficiency, despite viral loads that are often as high as those in HIV-infected people or SIV-infected macaques with

AIDS. Notwithstanding the intense interest surrounding this topic, the mechanisms that underlie the lack of disease progression in natural hosts of SIV remain obscure. Previous studies from several groups have shown that species such as sooty mangabeys lack the generalized immune activation and increased T-cell turnover found in HIV-infected humans or SIVmac-infected macaques.

One interpretation of the absence of generalized immune activation in natural hosts of SIV is that this reflects a marked attenuation of the host response to SIV and would be associated with weak or absent SIV-specific CD8+ cell responses. Two oral presentations presented differing views of this basic question.

Silvestri (Abstract 155) described the results of intracellular cytokine staining using lymphocytes from 74 infected sooty mangabeys that were stimulated with peptide pools corresponding to the SIVmac239 Gag, Pol, Env, and Nef proteins. Although SIV-specific CD8+ cell responses were detected in 65% of all animals tested, responses were low (<0.2%) or absent in 78% of all animals tested. No correlation between the magnitude of the CD8+ cell response and plasma viremia was found.

In contrast, Wang (Abstract 154) described a more robust SIV-specific CD8+ cell response in naturally infected sooty mangabeys using a combination of enzyme-linked immunospot (ELISPOT) and intracellular cytokine staining assays. Positive ELISPOT responses to SIV peptide pools were detected in 25 of 25 SIV-infected mangabeys studied, with the highest responses observed to SIV structural proteins. The overall magnitude of the total SIV-specific ELISPOT response ranged from 240 to 5200 spot-forming cells per 10^6 peripheral blood mononuclear cells, a value comparable to that obtained in SIV-infected rhesus macaques.

Reasons for the apparently discrepant results were not immediately clear but may be related to the greater sensitivity of ELISPOT assays in the detection of responses in sooty mangabeys. Although this group also noted no correlation between the overall magnitude of the response and the control of viremia, these results are in fact similar to those observed in SIV-infected macaques or HIV-infected humans. Although these 2 groups arrived at different conclusions, their data on the magnitude of SIV-specific CD8+ cell responses were in fact overlapping rather than diametrically opposed and clearly documented that most infected animals generate a significant virus-specific CD8+ cell response. This debate highlighted the limitations of trying to draw conclusions on the role of virus-specific immune response by correlative and phenotypic studies and emphasized the need for additional interventional studies such as CD8+ lymphocyte depletion in these animals to better assess the role of CD8+ cells.

An alternative hypothesis to explain the lack of immune activation and CD4+ cell depletion in naturally infected hosts suggests that the early interaction of SIV with the host innate immune responses and dendritic cells may differ fundamentally from those observed in susceptible hosts. Staprans (Abstract 152) analyzed differences in myeloid and plasmacytoid dendritic cell subsets during acute SIV infection of sooty mangabeys and rhesus macaques. Acutely infected macaques had significantly higher levels of expression of the chemokine receptor CCR7 on myeloid and plasmacytoid dendritic cells, but little CCR7 was observed on either dendritic subtype in acutely infected mangabeys. Consistent with the known role of CCR7 in mediating homing of lymphoid cells to secondary lymphoid tissue, there was a preferential accumulation of dendritic cells in lymph nodes of acutely infected rhesus macaques but not in mangabeys.

A significant difference among species was also observed in regard to the response of their dendritic cells to SIV stimulation. Both rhesus macaque and human dendritic cells produced relatively large amounts of interferon (IFN)-α following stimulation with SIV, but sooty mangabey dendritic cells had relatively little response. These data suggest that the lack of dendritic cell activation and type 1 IFN production in SIV-infected sooty mangabeys may lead to an attenuated inflammatory response and a significant reduction of the indirect bystander effects of SIV infection. The relative lack of induction of a type 1 IFN response to SIV infection was also verified by microarray analysis of peripheral blood lymphocytes from uninfected and infected mangabeys (Abstract 323). In contrast, activation of type 1 IFN responses was observed in lymphocytes from HIV-infected subjects.

Previous work from Veazey and colleagues has demonstrated that the intestinal tract is a major target for SIV infection and CD4+ cell depletion in SIV-infected macaques. Could the relative absence of these activated T cells in gut-associated lymphoid tissue of natural hosts in part underlie their resistance to immunodeficiency?

To address this issue, Veazey (Abstract 153), compared CD4 and CCR5 expression in lymphocytes from the intestinal tract, lymph nodes, and blood of uninfected sooty mangabeys, African green monkeys, and rhesus macaques. As previously reported, approximately 50% of T cells in the intestines of macaques express CD4 and 50% of these CD4+ cells express CCR5. However, less than 10% of T cells in the intestines of African green monkeys express CD4 and of these, only approximately 12% also express CCR5. The frequency of CD4+ cells in the gut of sooty mangabeys was higher than in African green monkeys, but essentially none of these cells express CCR5.

These observations suggest the hypothesis that natural hosts of SIV infection may have evolved to reduce the number of CD4+CCR5+ cells in gut-associated lymphoid tissue that serve as the primary fuel for SIV replication. Although this observation would initially appear to be at odds with the fact that many of the natural hosts have viral loads that are as high or higher than those observed in susceptible hosts, it is possible that viral replication in gut-associated lymphoid tissue may be responsible for initiating the indirect effects of CD4+ lymphocyte depletion. As suggested by Douek's plenary talk, this process may be initiated by immune activation induced by increased mucosal inflammation associated with the depletion of mucosal CD4+ lymphocytes.

Update on the Quest for a Safe and Effective AIDS Vaccine

Recent advances in HIV vaccine research were highlighted in a symposium encompassing basic science, nonhuman primate trials, and human clinical trials. The past decade has witnessed a significant expansion in the number of viral vectors able to induce cell-mediated immune

responses. Johnson (Abstract 111) summarized the strengths and shortcomings of viral vectors currently under study as candidate AIDS vaccines.

Notable advantages of these vectors include their track record in inducing relatively robust cellular immune responses in mice and nonhuman primates, as well as their ability to deliver multiple viral antigens. However, he highlighted the challenge of overcoming immune responses to these vectors induced by prior administration or natural infection. This limitation has been well documented in a trial of an adenovirus serotype 5 (Ad5) HIV-1 Gag vaccine, which demonstrated a significant decrease in the frequency of ELISPOT responses in subjects with high levels of titers of neutralizing antibodies to the Ad5 vector backbone, especially when administered at lower doses.

Several different options for overcoming preexisting or induced antivector immunity are being pursued. In some cases, the effect of preexisting immunity can be overcome by increasing the vector dose, as has been demonstrated for the adenovirus vectors. However, the necessity of increasing the vaccine dose by 100-fold to 1000-fold has a significant impact on vaccine cost and production, as well as reactogenicity.

Alternative strategies that are being pursued include the use of less-frequent serotypes of viral vectors (eg, adenovirus serotypes 11 and 35 or adenoviruses from other species, such as chimpanzees) and the use of viral vectors derived from viruses that infrequently infect humans (eg, vesicular stomatitis virus or Venezuelan equine encephalitis virus). A final strategy that is being pursued is that of boosting with heterologous viral vectors, an approach that is currently being pursued in a collaborative trial that is examining the immunogenicity of a combined canarypox (ALVAC) and adenovirus vaccine regimen.

McElrath (Abstract 112) summarized CD8+ cell responses elicited in several recent candidate AIDS vaccine trials. A trial jointly sponsored by Oxford University and the International AIDS Vaccine Initiative (IAVI) examined the safety and immunogenicity of an HIV-1 clade-A DNA vaccine followed by boosting with an HIV-1 clade-A modified vaccinia Ankara (MVA) vaccine in seronegative volunteers. Although preclinical trials with a comparable SIV construct yielded significant levels of SIV-specific CD8+ cell responses in nonhuman primates, the overall level of immunogenicity of the HIV-1 clade-A constructs was disappointing: regardless of the DNA dose or the MVA boosting schedule, only 14% to 20% of volunteers had a positive ELISPOT response, and the majority of these responses were not sustained. More encouraging results have been obtained with the human trials of adenovirus vectors. McElrath particularly highlighted recent results from the Ad5 Gag/Pol/Nef vaccine, which were also reported in detail in a subsequent oral presentation (Abstract 135). This vaccine was immunogenic in approximately 70% of subjects, and the majority of responders developed a response to more than 1 vaccine antigen.

McElrath also addressed the issue of heterogeneity of virus-specific CD8+ cell function. Although much of recent AIDS vaccine development has been focused on increasing the magnitude of HIV-specific CD8+ cells induced by vaccination, she highlighted a number of qualitative aspects of CD8+ cell function that might affect efficacy, including the breadth of responses, epitope avidity, and their ability to home to mucosal sites, as well as to proliferate following antigenic stimulation.

As one approach to determine what parameters of HIV-specific CD8+ cell function might correlate with control of viral set point after acute infection, 21 patients with primary HIV infection were studied in detail with respect to the magnitude, breadth, and avidity of their HIV-specific CD8+ cell response. In fact, none of these parameters correlated with viral set point, and the functional avidity of individual epitopes did not correlate with the magnitude of the response for a given epitope.

Although it remains unclear which parameters of CD8+ cell function best correlate with control of HIV replication, McElrath underscored the finding that HIV-specific CD8+ cells from long-term nonprogressors typically maintain a high perforin content and the ability to proliferate following antigen stimulation. Thus, although HIV-specific CD8+ cells generally persist with disease progression and retain the ability to secrete IFN-γ or tumor necrosis factor (TNF)-α, their functional characteristics with respect to proliferation and cytolytic ability are often impaired.

Although nonhuman primates have served as a primary platform for preclinical evaluation of AIDS vaccines, the ability of monkey studies to predict results in humans has been a source of recurring debate. Letvin (Abstract 113) reviewed evidence as to whether results from nonhuman primate studies are likely to be predictive of either the immunogenicity or efficacy of AIDS vaccines in humans. He highlighted 2 studies—the Oxford/IAVI DNA/MVA study described in McElrath's talk and a DNA/recombinant Ad5 study sponsored by the National Institutes of Health Vaccine Research Center—in which disappointing immunogenicity results had been obtained in humans. In each case, although initial testing of similar SIV vaccines had yielded promising results in macaques, subsequent testing of the HIV vaccine constructs that had yielded poor results in humans also demonstrated low levels of cell-mediated immune responses in macaques. These results highlight the fact that HIV immunogens should be evaluated for immunogenicity in macaques even without the ability to carry out an effective challenge.

Answering the question of whether vaccine efficacy trials in macaques will predict efficacy results in humans will await results from additional phase 2B or phase 3 efficacy studies of candidate AIDS vaccines. There has been considerable controversy as to whether the use of CXCR4-tropic simian/human immunodeficiency virus (SHIV) strains such as SHIV 89.6p, which induces rapidly progressive depletion of naive CD4+ cells in 2 to 4 weeks, are likely to serve as a better predictor of vaccine efficacy in humans than the use of CCR5-tropic viruses such as SIVmac239 or SIVmac251, which induce a slower progression to AIDS over a period of 1 to 2 years.

Letvin presented data from a DNA prime/adenovirus boost vaccine trial in SIV-infected macaques using a regimen that had previously generated significant protection from disease induced by SHIV 89.6p. When challenged with SIVmac251, vaccinated animals had an approximate 1-$\log_{10}$ decrease in viral load and improved survival. However, only a modest and marginally statistically significant effect against CD4+ cell

depletion was observed, in contrast to dramatic protection against CD4+ cell depletion induced by a similar vaccine following challenge with SHIV 89.6p.

These results provide some encouragement that T-cell–based vaccines may provide protection against disease in a setting of challenge viruses such as SIVmac251, which appear likely to provide a better model of HIV-induced disease in people. However, they still leave open the question of which of the different SIV and SHIV challenge models is most likely to predict results of vaccine efficacy in humans.

Most vaccine trials in nonhuman primates have focused on the use of a single high-dose challenge. However, there has been increasing interest in the use of repeated low-dose mucosal challenges that may better model the relative inefficient transmission of HIV through sexual contact observed in humans. Butera and colleagues (Abstract 134) analyzed the ability of a DNA prime/MVA boost regimen to protect against repeated low-dose rectal challenge. Animals were vaccinated with the Gag, Pol, and Env antigens of an HIV-1 CRF02_AG primary isolate using a DNA/MVA regimen previously shown to induce relatively strong cell-mediated immune responses. Vaccinated animals and naive controls then had a repeated low-dose rectal challenge with a recombinant SHIV isolate expressing an R5 clade-B envelope (SHIV SF162p3).

Although most vaccinated animals were ultimately infected after repeated intrarectal exposure to SHIV, there was a significant increase in the number of intrarectal exposures required to infect the vaccine recipients as opposed to naive controls. Naive controls were infected after an average of 3.5 rectal exposures, but 44% of vaccinated animals remained virus free after 10 intrarectal exposures and 5 of these remained uninfected after a total of 18 exposures.

Vaccine Alternatives: Microbicides and Preexposure Prophylaxis

In light of the clear challenges to developing an effective HIV vaccine in the next decade, there has been a resurgence of interest in microbicides. Veazey (Abstract 128) reported on the ability of a CCR5 coreceptor inhibitor (CMPD-167, which is no longer in the clinical pipeline) to block vaginal infection of macaques with the R5 SHIV strain 162p3. Previous studies of a lower dose of this compound had demonstrated that a low intravaginal dose (0.6 mg) of CMPD-167 was able to decrease peak viremia in macaques but was only rarely able to induce complete protection. Following a reformulation of the compound to allow a 5-fold increase in dose, the investigators observed complete protection from SHIV infection in 7 of 8 animals studied, whereas all 5 placebo-treated control animals were infected. The relatively high doses of this compound (and other vaginal microbicides such as PSC-RANTES) that are required suggest that diffusion of these compounds into the vaginal epithelium or submucosa may be required for inhibition of viral entry.

Promising preliminary findings for an alternative microbicide approach were reported by Cristofaro (Abstract 129), who demonstrated the ability of liposomal delivery of small interfering RNA (siRNA) to downregulate vaginal expression of the nuclear membrane protein lamin A/C (which was selected as a proof-of-principle target) or CCR5 in mice. Sustained downregulation was observed for up to 7 days after a single application. Although these results provide an encouraging demonstration of the feasibility of using siRNAs as a microbicide, practical questions related to toxicity, cost, and optimal dosing schedules will have to be addressed, including nonhuman primate studies, before this strategy can move forward to clinical trials.

Although the concept of providing preexposure prophylaxis to people at increased risk for acquisition of HIV infection has been controversial, given the grim prospects of developing an AIDS vaccine, this approach has received increasing attention and provoked ongoing controversy. Grant (Abstract 137) reviewed the rationale behind preexposure prophylaxis. The planned or recently initiated clinical trials of preexposure prophylaxis have employed tenofovir, which has a number of characteristics well suited for this purpose, including once-daily dosing and an excellent safety profile.

Several trials of tenofovir in macaques employing relatively high doses (20-30 mg/kg) had demonstrated essentially complete protection against mucosal or even intravenous infection with SIV. However, more recent studies using lower doses of tenofovir (4-10 mg/kg/day) have demonstrated more modest protection against oral or rectal challenge, although they have still demonstrated a statistically significant reduction in the risk of infection per exposure. Although several clinical trials of preexposure prophylaxis employing tenofovir have been planned or initiated, controversy regarding these studies has resulted in some of these studies being cancelled, notably in Cambodia, Cameroon, and Nigeria. Grant highlighted the fact that the assessment of the effects of preexposure prophylaxis should extend beyond simply measuring acquisition rates of HIV infection and include assessment of the development of viral resistance to the drug(s), potential effects of drug resistance mutations on viral replication and transmission fitness, potential effects on risk-taking behavior, and whether abortive infections might induce immune responses that could subsequently protect against infection (as has been observed in macaque studies).

Given the controversy and difficult ethical questions that have surrounded these trials, it is also clear that involvement of community members in the planning stages of future studies of preexposure prophylaxis will be a key factor in their successful execution.

Therapeutic Vaccination

The prospect that immunization of HIV-infected individuals could improve their ability to contain viral replication has generally offered more promise than hard results. Two studies provided support for the ability of therapeutic immunization regimens to improve control over viremia but also highlighted the significant challenges that this approach faces. In a late breaker (Abstract 133LB), Levy offered a report on the ANRS 093 trial through 100 weeks of follow-up.

In this trial, HIV-infected subjects with CD4+ counts greater than 350 cells/µl and HIV-1 RNA levels less than 50 copies/mL were randomized to antiretroviral therapy alone or to antiretroviral therapy plus immunization with the canarypox vector ALVAC vCP1433 (which expresses HIV-1 *gag*, *pol, env,* and *nef*) and HIV-1 lipopeptides in association

with low-dose subcutaneous interleukin (IL)-2. All subjects then underwent a treatment interruption at week 40 and were restarted on therapy if HIV-1 RNA levels rose to more than 50,000 copies/mL at 4 weeks or more than 10,000 copies/mL at subsequent time points. In the second phase of this trial, patients who had resumed antiretroviral therapy underwent a second treatment interruption with the restart criteria as defined above. Vaccinated patients did have a significant increase in their time off treatment as compared with controls (177 days vs 89 days) and also had slightly lower levels of viremia during the interruptions. Better control was associated with a positive lymphoproliferative response to one of the HIV peptides used for vaccination and with higher HIV-specific ELISPOT responses.

Although these results clearly document the potential of this therapeutic vaccination regimen to enhance control of viral replication in the setting of treatment interruptions, the magnitude and duration of benefit was relatively modest.

Additional evidence for the potential of therapeutic immunization was provided by a presentation from Pavlakis (Abstract 132) on SIV-infected macaques that received an SIV DNA vaccine while on antiviral therapy. SIVmac-infected macaques were treated with a combination of tenofovir, didanosine, and stavudine for 13 to 23 weeks. During treatment, the animals received intramuscular injections with optimized DNA vectors, and in a subset of animals, with an IL-15 DNA vector as well. Compared with unvaccinated controls that underwent a similar treatment interruption regimen, vaccinated animals had an approximate 1-$\log_{10}$ decrease in viral load off therapy, and a subset of 3 animals had prolonged control of viremia off therapy. Animals with better control had relatively high levels of Gag and envelope-specific ELISPOT responses. Although these results also suggested that the benefit of therapeutic immunization is generally short lived, they do offer some hope that more potent therapeutic immunization regimens may be able to offer an extended drug-free period to at least a subset of patients. Whether this increased period of time off therapy will justify the effort of therapeutic immunization is at present unclear.

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Complications of HIV Disease and Antiretroviral Therapy

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Metabolic, opportunistic, and other infectious complications of HIV infection and antiretroviral therapy continue to be major areas of active investigation. This year's Conference on Retroviruses and Opportunistic Infections included many important presentations on the clinical aspects of HIV complications. In each successive year, the studies reported in the area of complications have matured and now include more randomized trials evaluating interventions for the management of HIV complications and more well-designed observational studies with long-term follow-up. This article will review new data presented on metabolic complications, including cardiovascular risk, lipid disorders and lipoatrophy, renal complications, hepatic complications (hepatitis B and C virus infections), tuberculosis, and other bacterial infections.

Cardiovascular Risk

There continues to be great interest in examining the relationship between treatment with combination antiretroviral therapy and the risk of atherosclerosis. Several important analyses from the D:A:D study, the largest prospective study of cardiovascular risk in HIV-infected patients, were presented at this year's conference. The mean exposure time to combination antiretroviral therapy in this cohort is now 4.46 years. With more than 76,577 person-years of follow-up, 277 patients have experienced a myocardial infarction (MI). The risk of MI continues to increase with longer exposure to therapy. The MI incidence increased from 1.39/1000 person-years of observation in those not exposed to therapy, to 6.07/1000 person-years in those exposed for 6 years or more (relative risk [RR] compared with no exposure, 4.38 [95% confidence interval (CI), 2.39-8.04], $P = .0001$). The overall adjusted risk of MI per additional year of combination antiretroviral therapy exposure is estimated to be 17% (1.17-fold [95% CI, 1.11-1.24]). The MI risk associated with treatment was similar in men and women, and the relationship was similar in younger and older patients (men > 45 years and women > 55 years). Adjustment for lipid levels (total cholesterol, high-density lipoprotein [HDL], and triglycerides) reduced the association of an additional year of combined antiretroviral therapy with myocardial infarction to 1.10 (95% CI, 1.01-1.19). This finding suggests that some, but not all of the relationship between combination antiretroviral therapy and MI risk is explained by dyslipidemia. Of note, these researchers found no association between CD4+ cell count nadir or lipodystrophy and future risk of MI (Abstract 42). In another analysis, D:A:D investigators noted that the prevalence of cardiovascular risk factors does not appear to be declining in the cohort, but the incidence of MI appears to be on the decline when examined by calendar year; this suggests that higher-risk patients may be adopting interventions to reduce the rate of MI (Abstract 866).

A number of studies examined risk factors for subclinical atherosclerosis using noninvasive imaging such as carotid intima-media thickness (IMT) or coronary calcium as measured by computed tomography (CT) scan. Mangili and investigators from the Nutrition for Healthy Living (NFHL) study investigated the relationship between metabolic syndrome (MXS) and IMT and coronary calcium scores in 327 HIV-infected subjects in a cross-sectional analysis. MXS was defined as having at least 3 of the following: abdominal obesity (waist circumference > 102 cm for men, > 88 cm for women); hypertriglyceridemia (> 150 mg/dL); low HDL cholesterol (< 40 mg/dL for men, < 50 mg/dL for women); high blood pressure (≥130/85 mm Hg); or high fasting glucose (≥110 mg/dL). The prevalence of MXS in this cohort was 23%, similar to the prevalence in other cohorts of HIV-infected patients (26%; Abstract 867). A higher proportion of the group with metabolic syndrome (17%) had carotid IMT values greater than 0.8 mm, than did those without it (7%). In addition, the presence of any coronary calcium was also greater in the MXS group, leading these authors to suggest that interventions to reduce cardiovascular risk should be targeted with patients with evidence of MXS.

Longitudinal studies that include measures of subclinical atherosclerosis may help to determine which factors are associated with progression (or regression) of disease over time. Thiebaut and colleagues from the Agence Nationale de Recherches sur le SIDA (ANRS) in France reported on a 3-year study of carotid IMT in 233 HIV-infected subjects. At baseline, 59% of the cohort smoked and the majority were on a potent antiretroviral regimen. After 12 months of follow-up, the median carotid IMT increased from 0.55 mm to 0.57 mm ($P < .001$). After 2 more years of follow-up, the median carotid IMT had significantly decreased to 0.53 mm. During the 3 years of the study, a total of 94 subjects discontinued protease inhibitor (PI) therapy, 46 added lipid-lowering treatments, and 24 quit smoking. In the last 2 years of the study, smoking cessation was associated with improvement in carotid IMT in the univariate analysis only. These results suggest that interventions to reduce the prevalence of dyslipidemia may reduce the risk of cardiovascular disease in the HIV population and also highlight the importance of smoking cessation as effective intervention. This study also underscores the importance of controlling for smoking and other traditional risk factors when assessing the relationship between HIV treatments and atherosclerosis (Abstract 871).

Maggi and colleagues previously reported a higher prevalence of carotid plaque, as measured by ultrasound, in PI-treated patients than in nonnucleoside reverse transcriptase inhibitor (NNRTI)-treated patients. This group now reports follow-up in these same subjects 1 year later (Abstract 863).

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They found that a higher percentage of the subjects who had abnormal measures at baseline in the PI group (24%) showed new lesions, than did those with previously normal measures in the NNRTI group. No difference in the prevalence of new lesions was seen among subjects who had normal measures at baseline in the 2 treatment groups. The role of PI therapy was also examined by Knobel in a cross-sectional study that measured carotid IMT (Abstract 862). In this study, equal numbers of HIV-infected subjects who were deemed to be at low risk (<5%), moderate risk (5%-10%), and high risk (>10%) of coronary events within 10 years using the Framingham scoring system, were evaluated with ultrasound of the carotid artery. Subclinical atherosclerosis was defined as the presence of plaques in 1 or more sites. The prevalence of subclinical atherosclerosis in relation to the Framingham risk score group was 34.4% in the low-risk group, 70.6% in the moderate-risk group, and 94.1% in the high-risk group ($P = .0001$). In a multivariate analysis, PI use was identified as an independent risk factor for the presence of plaques after controlling for Framingham score.

Interventions for Hyperlipidemia

PI Switching

Prospective studies have suggested a lack of lipid elevations with the use of atazanavir; however, there are limited data on the safety and efficacy of switching therapy in patients whose virus is suppressed on a PI regimen (including those containing ritonavir) to atazanavir. Sension and colleagues reported the preliminary results of a trial in which subjects on a PI regimen with HIV RNA levels below 50 copies/mL and low-density lipoprotein (LDL) levels above 130 mg/dL were randomized to receive unboosted atazanavir (400 mg/d) or to continue their initial therapy (Abstract 858). The primary endpoint was evaluated at week 12. The decrease in lipid levels was greater for the atazanavir group than for the control group for LDL cholesterol, total cholesterol, and triglycerides, as well as apolipoprotein B and lipoprotein a. Virologic suppression was maintained, with exceptions in 2 atazanavir recipients and 1 control recipient. Further follow-up of this trial is continuing to 48 weeks.

In a complementary nonrandomized trial, Martinez and colleagues described their experience in substituting boosted atazanavir for other PIs among 162 subjects with 1 or more lipid abnormalities (fasting triglycerides >500 mg/dL, total cholesterol >200 mg/dL, or LDL >130 mg/dL; Abstract 850). The majority of subjects were receiving another boosted PI at entry. The proportion of patients with triglyceride levels above 500 mg/dL decreased from 33% to 10% ($P < .0001$), the proportion with total cholesterol levels above 200 mg/dL decreased from 90% to 51% ($P < .0001$), and the proportion with LDL levels above 130 mg/dL decreased from 65% to 36% ($P < .0001$). Patients were not required to have an undetectable viral load at entry, and after 6 months the proportion with HIV RNA levels below 500 copies/mL increased from 45% to 58%. Together these trials confirm that substitution with atazanavir (boosted or not) is a viable strategy for improving elevated lipid levels; however, a significant proportion of subjects remain above current National Cholesterol Education Program (NCEP) thresholds after this change, suggesting that other interventions may be needed.

Nucleoside Reverse Transcriptase Inhibitor Substitutions

Nucleoside reverse transcriptase inhibitor (nRTI) substitutions have been examined in an effort to reverse lipoatrophy. Moyle reported the results of a study of 105 subjects with lipoatrophy while on zidovudine- or stavudine-containing regimens (Abstract 44LB). The primary endpoint of the study was change in limb fat as assessed by dual-energy x-ray absorptiometry (DEXA) scan. Lipid changes were examined as a secondary endpoint. Virologic suppression was maintained in both treatment arms. After 48 weeks of follow-up, limb fat increased in both study arms and there was no difference between arms. Mean changes in total cholesterol, LDL, and triglyceride levels were significantly more favorable in the tenofovir arm than in the abacavir arm.

Spanish investigators reported the results of a small study of 56 patients that compared the impact of dose reduction of stavudine (from 40 mg BID to 30 mg BID) with tenofovir substitution or maintenance of full-dose stavudine on lipid parameters and subcutaneous fat (Abstract 857). Significant mean changes in lipids were only detected in triglycerides (+19 mg/dL for stavudine 40 mg, –40 mg/dL for stavudine 30 mg, –133 mg/dL for tenofovir; $P = .02$) and total cholesterol (+4 mg/dL for stavudine 40 mg, –4 mg/dL for stavudine 30 mg, –28 mg/dL for tenofovir; $P = .04$). Mean changes in total and limb fat were also seen in the low-dose stavudine and tenofovir arms, but the magnitude of the improvement in limb fat was greater for those randomized to tenofovir. Mean changes in total fat (–597 g for stavudine 40 mg, +332 g for stavudine 30 mg, +1005 g for tenofovir; $P = .04$) and limb fat (–247 g for stavudine 40 mg, +77 g for stavudine 30 mg, +440 g for tenofovir; $P = .008$) significantly differed among groups. Viral load remained suppressed in all subjects except for 1 on the full-dose stavudine arm. Previous studies have suggested a modest benefit of adding the statin pravastatin or a fibric acid drug to PI therapy for the treatment of hypercholesterolemia (Aberg, CROI 2004). Calza conducted the first randomized trial in which the addition of lipid-lowering therapy was directly compared with antiretroviral substitution (Abstract 859). Subjects with viral suppression (<50 HIV RNA copies/mL) on a PI-containing regimen (N = 142) with mixed hyperlipidemia were randomized to 1 of 4 arms: add pravastatin, add bezafibrate, switch to nevirapine, or switch to efavirenz. In an as-treated analysis, after 12 months of follow-up, a greater reduction in total cholesterol and triglyceride levels was observed in the pravastatin and bezafibrate arms than in the 2 NNRTI arms. Triglycerides decreased by 41% and 47% for the pravastatin and bezafibrate arms, respectively, compared with 25% for nevirapine and 9% for efavirenz. Total cholesterol decreased by 46% and 37% for pravastatin and bezafibrate, respectively, compared with 27% and 10% for nevirapine and efavirenz, respectively. When grouped together, the lipid-lowering agents had a significantly greater impact on both triglyceride and cholesterol levels than did the NNRTI-substitutions. Additionally, nevirapine substitution led to a greater decrease in both cholesterol and triglyceride levels

than efavirenz did. This is the first clinical trial that has directly compared the approach of adding lipid-lowering therapy with a change in antiretrovirals, and it appears that the lipid-lowering therapy was more effective. The magnitude of the decrease in cholesterol and triglyceride levels (~40%) observed with lipid-lowering therapy in this trial appears greater than previously described in other trials.

Fish oils containing omega-3 polyunsaturated fatty acids have been proposed as a possible treatment for isolated hypertrigylceridemia in patients on antiretroviral therapy. De Truchis and colleagues reported the results of a randomized double-blind trial in 122 antiretroviral therapy–treated patients who had triglyceride levels greater than 200 mg/dL (Abstract 39). Patients received 2 gm of a fish oil preparation 3 times per day or a placebo. Fish oil preparations vary in the content of omega-3 polyunsaturated fatty acids; each 1-gm capsule of this particular preparation contains 18% eicosapentaenoic acid (EPA) and 12% docosahexaenoic acid (DHA), which are standard amounts. The study design compared the fish oil preparation with placebo, and was followed by an 8-week open-label treatment period. In an intent-to-treat analysis, treatment with fish oil was associated with a 25% median decrease in triglyceride level, compared with a 1% increase with placebo ($P = .0033$). Additionally, triglyceride levels normalized (<200 mg) in 22.4% in the fish oil arm, compared with 6.5% in the placebo group. The initial decrease in triglyceride levels was maintained during the open-label period and no significant safety concerns were identified.

Lipodystrophy

Patterns of Fat Changes

Ideally, studies designed to examine the timing and patterns of peripheral and central fat changes related to antiretroviral therapy should be longitudinal evaluations conducted in the context of randomized antiretroviral treatment, and they should also include a population-based control group. Two important longitudinal studies were presented, each of which fulfills one of these criteria (Abstracts 38, 849). Mulligan described patterns of peripheral and central fat changes in the 64-week metabolic substudy of the large AIDS Clinical Trials Group (ACTG) treatment-naive study 384 (ACTG 384). Measurements included waist and hip circumference in all subjects and DEXA scans in a subset. The proportion of subjects with an elevated waist-hip ratio increased from 35% at baseline to 47% at week 64, with significantly more subjects experiencing a gain over time. The patterns of change were mixed: one quarter of subjects had an increase in waist measurement and a decrease in hip measurement; one half of subjects had an increase in waist circumference and no change in hip circumference; and one quarter had a decrease in hip circumference only. This mixed pattern of changes in regional fat was also seen with the DEXA results. Equal proportions (35%) of subjects *gained* in both regions or *lost* fat in both regions, but only 26% had the previously defined lipodystrophy phenotype of central-fat gain with limb-fat loss. These results suggest that there are several distinct patterns of fat change associated with antiretroviral therapy. The changes reported in this study were averaged over a 64-week period, and it is possible that certain types of changes occur at different points in time. Further work is needed to identify the factors that determine the pattern of change in fat over time in individual patients.

The second study reported on 4-year follow-up data from men who have sex with men (MSM) in the Multicenter AIDS Cohort Study (MACS) who received antiretroviral treatment compared with a control group of HIV-uninfected MSM also followed prospectively. Measurements in this study included body mass index (BMI) and circumference measurements of waist, hip, and limbs (arm and thigh). During the follow-up, BMI increased in the control group but did not change in the HIV group. Waist circumference increased similarly in both groups; however, hip circumference increased more slowly in the HIV group, yielding a greater increase in waist-to-hip ratio in the HIV group. Thigh circumference increased in the control group but decreased in the HIV group. In a multivariate analysis, cumulative exposure to nRTIs was associated with decreases in circumference in waist, hip, thigh, and arm, independent of PI use. These results confirm some earlier observations suggesting that some of the increase in waist circumference observed in patients treated with antiretroviral therapy is due to normal aging, and lipoatrophy is associated with nRTI treatment.

Interventions for Lipoatrophy

Previous studies have demonstrated improvement in lipoatrophy when stavudine (and to a lesser extent zidovudine) is replaced with abacavir (Carr et al, *JAMA*, 2002; McComsey et al, *AIDS,* 2005). As noted previously, Moyle (Abstract 44LB) reported the results of a randomized open-label 48-week study evaluating changes in limb fat following substitution of zidovudine (n = 34) or stavudine (n = 71) with abacavir or tenofovir in 105 virologically suppressed patients on antiretroviral therapy. Objective assessments of limb fat were obtained using DEXA scans. After 48 weeks of follow-up, there was a statistically significant increase in limb fat in both the abacavir- and tenofovir-treated groups but no difference between the study arms. Bone density was also evaluated and no differences were seen between the treatment groups. A smaller, uncontrolled trial also suggested an improvement in facial fat after stavudine was changed to tenofovir (Abstract 860). The results of this trial suggest that these nonthymidine nRTIs are effective in improving established lipoatrophy, albeit slowly.

Further evidence to support the notion that abacavir is less likely than stavudine to cause lipoatrophy was seen in a randomized trial in which subjects received either abacavir or stavudine in combination with lamivudine/efavirenz (Abstract 587). In a subset of subjects who had DEXA scans performed at baseline and at week 96, those randomized to abacavir had significantly more limb fat than did the stavudine recipients. In addition, triglyceride levels were lower in the abacavir-treated patients. Virologic outcomes favored abacavir in the intent-to-treat analysis, but no difference was seen between the study arms in the as-treated analysis.

Given the central role of nRTIs in the development of lipoatrophy, it follows that regimens that do not include drugs from this class might be expected to improve lipoatrophy. Tebas reported the

results of an ACTG study of antiretroviral patients who were randomized to the "nRTI-sparing" regimen of lopinavir/ritonavir and efavirenz or to efavirenz plus 2 nRTIs (Abstract 40). After a mean of 104 weeks, the median change in limb fat in the nRTI-sparing regimen was a 782 g gain, compared with a 900 g loss in the nRTI arm ($P = .0002$). Unfortunately, patients randomized to the nRTI-sparing combination of lopinavir/ritonavir/efavirenz experienced significantly greater increases in triglyceride and total cholesterol levels and had higher rates of virologic failure, as defined by a combined endpoint. No differences in bone density were noted between the arms of this study. This proof-of-concept study demonstrates that nRTI-sparing antiretroviral regimens may help to reverse lipoatrophy; however, more work is needed to identify regimens that are more lipid friendly.

Further evidence to support the efficacy of nRTI-sparing regimens in reversing lipoatrophy were presented by Murphy (Abstract 45LB). This ACTG study compared changing virologically suppressed patients with lipoatrophy on an antiretroviral regimen including either zidovudine or stavudine to an nRTI-sparing combination of lopinavir/ritonavir/nevirapine with the strategy of substituting zidovudine or stavudine with abacavir. In this trial, subcutaneous thigh fat and subcutaneous adipose tissue, as measured by CT scan, improved significantly by week 24 in both groups. Longer follow-up is ongoing to determine whether there is a difference in the impact of these 2 approaches on reversal of lipoatrophy. Collectively, these 2 studies provide the first evidence in randomized trials to support the concept of nRTI-sparing regimens for improving lipoatrophy.

Several previous randomized trials have evaluated rosiglitazone for the treatment of lipoatrophy. Although they have varied by inclusion criteria, dose, and duration of follow-up, the majority have not suggested a significant change in limb fat with this approach. A Canadian trial of rosiglitazone performed in subjects with lipoatrophy, which did not require documentation of insulin resistance, failed to demonstrate any evidence of a slower rate of limb fat loss with rosiglitazone (Abstract 854). Mallon presented data that may help to explain the lack of efficacy of rosiglitazone in the presence of continued thymidine nRTIs from his fat biopsy substudy of the Australian rosiglitazone trial for lipoatrophy (Abstract 41). In the original trial, subjects were allowed to modify nRTI therapy, an approach that is now known to lead to improvement in subcutaneous limb fat. He compared the impact of rosiglitazone on peroxisome proliferator-activated receptor γ (PPAR- γ) expression in subcutaneous adipose tissue in a group of subjects who continued thymidine nRTIs, compared with those who had stopped the drugs. At week 2, only those randomized to rosiglitazone in the no-thymidine nRTI group experienced a significant rise in PPAR-γ expression ($P = .046$). Similar significant increases in PPAR-γ coactivator 1 (PGC-1) expression were also observed in the rosiglitazone no-thymidine nRTI group. Of note at week 48, PPAR-γ expression was significantly higher only in the no-thymidine nRTI group, independent of rosiglitazone treatment. These results suggest that ongoing thymidine nRTI therapy may hinder the ability of rosiglitazone to increase PPAR-γ expression. Additionally this study suggests that nRTIs may have a direct effect on PPAR-γ as a mechanism underlying the development of lipoatrophy.

In August 2004, L-polylactic acid was approved by the US Food and Drug Administration (FDA) for treatment of facial lipoatrophy in patients with HIV infection. This absorbable material is injected into areas of facial fat loss, and short-term studies suggest that the procedure leads to improvement in the appearance of lipoatrophy. Mijch and colleagues reported on a 6-month open-label study of polylactic acid designed to quantify the impact of this treatment on facial fat using photography, quality of life measures, and spiral CT after injection of L-polylactic acid (Abstract 851). Improvements in psychological and emotional distress correlated with improvement by photography. Local pain was a common adverse event, but no subjects discontinued treatment and overall the procedures were well tolerated. Longer-term follow-up data are needed to ensure the long-term efficacy of this approach; however, the short-term results of this approach remain very promising and offer the best immediate improvement for patients suffering from the effects of facial lipoatrophy. Unfortunately, cost remains prohibitive for the majority of patients.

Hypertension and Renal Disease

Uncontrolled studies have previously suggested a relationship between antiretroviral therapy and the development of hypertension. MACS investigators examined the relationship between initiation of antiretroviral therapy and change in systolic and diastolic blood pressure in men with known normal pre-therapy blood pressure values and prospective follow-up measurements. They found that initiating antiretroviral therapy resulted in increased systolic blood pressure; each year of therapy was associated with a 0.6-mm increase in systolic blood pressure. The risk for an increase in blood pressure was greatest for men with CD4+ cell counts below 200/μL (Abstract 872). A retrospective analysis of factors related to the development of 10-mm Hg increases in systolic or diastolic blood pressure was reported by a group of University of Washington researchers. Within a cohort of 607 patients who had initiated therapy, 10% developed a 10-mm Hg increase in blood pressure or started antihypertensive therapy during follow-up. In contrast to the previous study, they found no relationship between CD4+ count nadir and risk for hypertension, but they did identify treatment with efavirenz or lopinavir/ritonavir as independent risk factors for hypertension (Abstract 873). These studies add to the growing body of evidence suggesting a relationship between certain types of antiretroviral therapy and the risk for hypertension; however, more work is needed to sort out the contributions of specific antiretroviral drugs to hypertension risk.

In the pre-potent antiretroviral therapy era, there were several reports that suggested a link between chronic HIV infection and the development of pulmonary arterial hypertension (PAH), with some early case reports suggesting that antiretroviral therapy might reduce the risk of PAH. Rosenkranz and colleagues studied a consecutive sample of patients treated with antiretroviral therapy to determine the prevalence of PAH using 2D and Doppler echocardiography. PAH was defined as mean pul-

monary arterial pressure above 25 mm Hg or systolic right ventricular pressure above 30 mm Hg at rest. Surprisingly, PAH was diagnosed in 15 of the 200 patients evaluated (7.5%), 8 of whom were completely asymptomatic. These findings suggest that clinicians should have a low threshold to screen patients with echocardiography who have symptoms of unexplained dyspnea. Larger studies are needed to determine whether more widespread screening of asymptomatic subjects should be recommended and to identify risk factors for the development of PAH in the setting of HIV infection (Abstract 874).

Microalbuminuria (MA) is a well-described marker of renal disease that has been noted to be common among HIV-infected individuals. Investigators from the Fat Redistribution and Metabolic Change in HIV Infection Study (FRAM) examined the prevalence of MA in a random sample of 1027 HIV-infected individuals, compared with a population-based control group. MA was present in 8% of HIV-infected patients, but only 2% of controls ($P < .001$). In a multivariate analysis, HIV infection was found to be an independent risk factor for MA (adjusted odds ratio [OR], 4.5). Within the group of HIV-infected patients, elevated systolic blood pressure and African American race were predictors of MA. The relationship between MA and future cardiovascular risk in HIV patients remains to be determined (Abstract 821).

A number of groups continue to investigate the risk of renal dysfunction in patients receiving antiretroviral therapy, with a special focus on tenofovir-containing regimens. Gallant examined the change in creatinine clearance among 344 tenofovir recipients compared with 314 patients who received other nRTIs. In this study, creatinine clearance was calculated using the Cockcroft-Gault equation. Median serum creatinine increased by 0.15 mg/dL and 0.10 mg/dL in the tenofovir and nRTI groups, respectively ($P = .01$). There was a statistically significantly greater decline in the median creatinine clearance in the tenofovir group (13.35 mL/min decline) compared with the other nRTI group (7.5 mL/min decline; $P = .005$). Longer duration of therapy and CD4+ cell count below 50/µL were risk factors for decline in renal function. The authors of this study noted that the change in creatinine clearance, although statistically significant, was small in size and of unclear clinical significance (Abstract 820). Becker and the CHORUS investigators examined rates of renal dysfunction among tenofovir recipients by calculating glomerular filtration rate using the following formula: GFR = (196)*(serum creatinine mg/dL-1.154)*(age-0.203)*(0.742 if female)*(1.212 if African American). They reported that this may be a more sensitive way to follow renal disease in HIV-infected patients (Abstract 819). Finally, MACS investigators examined rates of chronic kidney disease (creatinine clearance < 60 mL/min) in HIV-infected patients treated with antiretroviral therapy, compared with treatment-naive HIV-infected MSM. They identified a higher rate of chronic kidney disease in patients on therapy than in untreated MSM or HIV-uninfected controls. Among those on therapy, tenofovir use appeared to be associated with a higher risk of creatinine clearance below 60 mL/min (Abstract 818).

Hepatitis C Virus Infection

Sulkowski reported that the progression of liver fibrosis was much higher than expected among a cohort of hepatitis C virus (HCV)-infected patients with little or no fibrosis on initial liver biopsy (Abstract 121). In this study, liver fibrosis was evaluated on 2 biopsies a mean of 2.8 years apart in patients without cirrhosis. Of these subjects, 84% had Ishak fibrosis stage 0 or 1 upon first biopsy. Subjects had a median age of 44 years, 21% had CD4+ counts below 200 cells/µL, 57% had HIV RNA levels below 400 copies/mL, and the estimated duration of HCV infection was 23 years. Some of the patients had received prior HCV treatment for brief periods of time. A single pathologist read all biopsy slides in a blinded fashion. At the second biopsy, 13% of subjects had 2-stage progression, and 14% had 3 or more stage progression. Higher baseline HIV RNA and elevated alanine aminotransferase (ALT) levels were associated with increased risk of progression. In a separate study, hepatic steatosis did not progress in patients over a similar time interval (Abstract 831). It is surprising that such high rates of fibrosis progression were observed among this cohort with minimal disease at baseline, and it will be important to identify the factors that identify the most rapid progressors. These findings challenge current clinical practice of delaying HCV therapy and performing liver biopsy at intervals of 3 to 5 years in patients with stage 1 or no fibrosis on initial biopsy.

Although liver biopsies are considered the gold standard for evaluation of the severity of liver disease, noninvasive tests would be preferable, particularly if tests are required at frequent intervals. Sterling and colleagues evaluated the performance of noninvasive tests to predict liver histology from patients entering the AIDS PEGASYS Ribavirin International Co-infection Trial (APRICOT) data set, a randomized trial of 3 HCV treatment regimens presented at last year's conference (Abstract 120). Five hundred fifty-five of the subjects were assigned to a "training set" and 257 subjects were assigned to a "validation set." Fibrosis stage was collapsed into 3 categories: mild (0-1 Ishak), moderate (2-3 Ishak), and severe (4-6 Ishak). In the multivariate analysis, aspartate aminotransferase (AST) level, international normalized ratio (INR), and platelet count distinguished subjects in the 3 groups. The positive and negative predictive value of various cutoffs of an index called "FIB-4" was evaluated in the validation set. A cut-off higher than 3.25 had a positive predictive value of 65% and specificity of 97% for severe liver disease. The FIB-4 value did not perform as well in distinguishing patients with fibrosis scores ranging from 2 to 6. Clinicians and patients are eager for noninvasive tests to assess liver disease, but much more work is needed on these approaches before they are ready to replace liver biopsy in making important decisions for clinical management.

Accumulating data suggest that control of HIV disease with antiretroviral therapy may slow HCV progression (Abstract 947). In a prospective study of 231 injection drug users in an urban cohort, risk factors associated with liver-related morbidity and mortality were examined from 2002 to 2004. There were 22 events during the study period (5.1/100 person-years of observation). In a univariate analysis, Hispanic race and CD4+ count nadir below 100 cells/µL were associated with an increased rate of HCV disease progression; HIV RNA level below 75 copies/mL was associated

with a decreased rate of progression. In the multivariate analysis, CD4+ count nadir below 100 cells/μL was associated with a 20-fold increase in the rate of hepatic events. Starting effective antiretroviral therapy before the CD4+ nadir is below 200 cells/μL is associated not only with AIDS-related complications but with increased risk of liver-related morbidity and mortality in the HCV population. The important clinical question of the optimal timing of antiretroviral and HCV therapy in coinfected patients will require large, randomized clinical trials.

Studies of HCV-monoinfected and HIV-coinfected patients suggest that HCV causes neuropsychiatric changes. Tucker hypothesized that treatment of HIV could improve neurocognitive deficits attributed to HCV in coinfected patients (Abstract 949). Investigators performed repeated measures of neurocognitive functioning tests before and after 6 months of antiretroviral therapy in 32 subjects with HIV infection and 14 subjects with HIV/HCV coinfection. HCV/HIV-coinfected patients had higher rates of impaired visual memory and cognitive function than HIV-monoinfected subjects at baseline. Antiretroviral therapy did not cause a statistically significant improvement in neurocognitive function in this small study with relatively short follow-up. The individual and combined benefits of HIV and HCV therapy on neurocognitive function merit further study.

Standard treatment for HCV includes pegylated interferon alfa and ribavirin. Clinical trials have utilized various dosing regimens for ribavirin, and the optimal dose to maximize efficacy and minimize toxicity is not clear. Renden and colleagues measured ribavirin levels at week 4 and 12 in patients receiving weekly peginterferon alfa-2a plus 800 mg to 1200 mg of ribavirin daily (Abstract 929). Ribavirin levels showed significant interpatient variation but were stable within patients between week 4 and 12. Ribavirin dose was associated with serum levels only when adjusted for weight. Higher levels of ribavirin were associated with greater short-term virologic response, but also with greater drops in hemoglobin. Zidovudine was also an independent predictor of anemia. This study supports weight-based dosing of ribavirin and suggests that ribavirin exerts important early virologic activity.

In another presentation evaluating optimal ribavirin plasma levels in patients receiving HCV treatment, Breigh and colleagues measured plasma concentrations of ribavirin 6 and 12 hours post doses at 8 time points over a year of therapy (Abstract 928). They extrapolated the maximum concentrations (C_{max}), minimum concentrations (C_{min}), and areas under the concentration curve (AUC) from these measurements. In a multivariate analysis, higher C_{min} was associated with higher virologic response (viral suppression 24 weeks after discontinuation of HCV treatment). Based on this data set, the authors proposed that ribavirin should be dosed by weight and that plasma concentrations should be maintained above 1 ug/mL.

Higher ribavirin levels are associated with better virologic response rates during HCV treatment, but also with higher rates of toxicity, which may necessitate dose reduction. Alvarez and colleagues retrospectively evaluated the records of 217 patients receiving weekly peginterferon alfa-2a plus 800 mg to 1200 mg of ribavirin daily to determine if anemia and epoetin alfa use were higher in zidovudine recipients (Abstract 927). Hemoglobin-level declines of more than 5 g/dL were significantly more frequent in zidovudine recipients (13%) than in controls (3%; $P < .01$). Ribavirin dose reduction was also more common in zidovudine recipients (47%) than in controls (17%; $P < .0001$). By week 12, 47% of zidovudine recipients were receiving epoetin alfa, compared with 12% of controls. Hemoglobin levels were similar in both groups at week 12, as were week-12 virologic suppression rates. This study shows that hemoglobin can be maintained with a combined strategy of ribavirin dose reduction and use of epoetin alfa in zidovudine recipients. However, in view of the importance of ribavirin levels to both short- and long-term virologic response, additional studies are needed to determine the optimal clinical approach to patients who require both zidovudine and ribavirin, including those with low CD4+ cell counts.

Treatment of acute HCV in HIV-uninfected patients is associated with treatment response rates greater than those seen during chronic infection. There were 2 conflicting reports on acute HCV treatment outcomes among HIV-infected subjects at the conference this year. Chaix reported outcomes in 12 patients with acute HCV. One important epidemiologic point from this study was that all patients in this series reported MSM as their only risk factor for HCV (Abstract 122). Ten of these 12 had genotype 4d virus, which clustered to single variant on phylogenetic analysis. Ten of 12 patients were asymptomatic. The patients received a variety of treatment regimens, including interferon alfa or pegylated interferon alfa, sometimes in combination with ribavirin. Treatment was started a mean of 50 days from time of diagnosis. Four patients stopped therapy for toxicity before week 12, and none of the patients had a sustained virologic response.

In a second report by Vogel, 17 patients with acute HCV were treated with pegylated interferon alfa for 6 months. Ribavirin 800 mg daily was added in patients with genotype 1 or 4 virus (Abstract 922). The mean CD4+ cell count in the study population was 426/μL. HCV RNA level was undetectable at the end of treatment in 14 of 17 patients. Ten of 14 patients had sustained virologic responses. The authors emphasized that detectable HIV RNA at week 4 or 8 predicted nonresponders. The treatment responses in this German cohort are much more favorable than in the Chaix study. The most obvious difference between the 2 studies was that the treatment regimens in the latter study were more consistent and aggressive. Based on these 2 reports, the jury is still out on the optimal composition, dosing, timing, and utility of treatment during acute HCV infection. The results from the Vogel study are encouraging, but more data are needed in this patient population.

Hepatitis B Virus Infection

Entecavir is an inhibitor of hepatitis B virus (HBV) polymerase that received a unanimous vote of approval for initial and second-line therapy of HBV by the FDA Drug Advisory Committee on March 11, 2005. Most studies have been conducted on HBV-monoinfected patients, but there are limited encouraging data for HIV-coinfected persons. Pessoa presented the results of an ongoing double-blind trial evaluating treatment of HIV-infected subjects who had lamivudine exposure and detectable HBV viremia (Abstract 123). At baseline 88% of sub-

jects had at least 1 lamivudine resistance mutation and a mean HBV DNA level of 9.1 $\log_{10}$ copies/mL. Patients were randomized to entecavir 1 mg per day (51 subjects) or placebo (17 subjects). At 24 weeks, all patients received entecavir. Lamivudine was continued during the study. The mean reduction in HBV DNA level was 3.7 $\log_{10}$ copies/mL among entecavir recipients, compared with an increase of 0.1 $\log_{10}$ copies/mL in the placebo recipients ($P < .0001$). In the entecavir group, 80% of subjects had HBV DNA levels below 400 copies/mL or a 2-log reduction in HBV DNA, compared with 0% in the placebo group. ALT normalization occurred in 49% of the entecavir group and 17% of the placebo group. Entecavir appears to be well tolerated and does not have HIV activity that could lead to HIV drug resistance. Entecavir is efficacious for patients in whom lamivudine is failing and will likely become an important treatment option for chronic HBV infection.

Following the entecavir presentation, Peters reported the results of ACTG 5127, a randomized, double-blind, placebo-controlled study comparing tenofovir (300 mg) with adefovir (10 mg) for the treatment of chronic HBV (Abstract 124). Ninety-two percent of the patients were male, they had a median CD4+ cell count of 467/µL at entry, and the median serum HBV DNA level was 8.7 $\log_{10}$ copies/mL. More than 70% of subjects were lamivudine experienced. Three quarters of the patients had HIV RNA levels below 50 copies/mL on their current antiretroviral regimen. At 48 weeks, the mean reduction in HBV DNA was 5.7 $\log_{10}$ copies/mL in the tenofovir arm and 4.0 $\log_{10}$ copies/mL in the adefovir arm. The therapy was generally well tolerated, with only 4 treatment discontinuations, and none of these discontinuations were for nephrotoxicity. The authors concluded that tenofovir and adefovir are active against HBV in HIV-infected patients, and that tenofovir is not inferior to adefovir. It is notable that HBV reductions were lower in the tenofovir group than in the adefovir group, and that the HIV activity of tenofovir makes it a logical choice for co-infected patients requiring HIV treatment. Information on drug resistance to HBV agents generated during treatment is anticipated from trials such as ACTG 5127, and will be important in designing long-term treatment strategies for both infections.

Organ Transplantation

For patients in whom therapy for HBV or HCV is failing who have liver failure, liver transplantation is an option being carefully evaluated in several specialty centers around the world. Vogel described the clinical experience of 10 patients awaiting orthoptic liver transplantation in Europe (Abstract 931). Six patients had liver disease due to HCV, 3 due to HBV, and 1 due to both. Six of these patients had hemophilia. Two patients died while waiting for liver transplant, and a third patient had improvement of liver disease with the initiation of antiretroviral therapy. Among the 7 patients who received a transplant, 1 died at day 84 due to intrathoracic hemorrhage. The other patients are all alive, and only 1 acute organ rejection episode has occurred. The median follow-up for the cohort is 620 days. The authors highlighted several points of the courses of these patients. HCV reoccurred in all patients with underlying disease but responded to aggressive therapy. Kaposi's sarcoma in conjunction with Castleman's disease was a serious complication in 1 patient. Drug interactions with cyclosporine and antiretroviral agents required careful attention and dose adjustments.

Clinical outcomes in both liver and renal transplantation in the United States were presented by Roland (Abstract 953). There were 11 liver transplants performed. Indications for transplantation were HCV in 45%, HBV in 36%, and both in 9%. There were 2 deaths for recurrent HCV among the liver transplants; the 1- and 3-year estimated survival rates were 91% and 82%, respectively. The cumulative rejection rate among liver transplant patients was only 10%. The 3-year estimated survival for the 18 kidney recipients was 94%. Indications for renal transplantation were HIV-associated nephropathy (44%), hypertension (54%), and diabetes (11%). Rejections were more common among kidney transplant recipients, with cumulative incidence of 67% at 2 years. However, there were only 2 graft losses, both occurring shortly after transplant occurred. Patients were aggressively managed with antiretroviral therapy, and only 3 opportunistic infections associated with HIV disease occurred. Overall patient survival from this ongoing cohort is favorable, compared with HIV-uninfected populations.

Bacterial Infections

Mathews presented an interesting analysis of the incidence trends of methicillin-resistant *Staphylococcus aureus* (MRSA) in a cohort of 3445 HIV-infected adults from a university hospital–based HIV clinic (Abstract 142). Between 2000 and 2003, there were 94 episodes; 83% were skin and soft-tissue infections and 10% were blood infections. There was an estimated 6-fold increase in the rate of MRSA infection from 2000 to 2003. In a multivariate analysis, CD4+ count below 50 cells/µL and increasing levels of HIV RNA were associated with increased risk of MRSA infections. Consistent with these observations, antiretroviral use within the past 6 months was protective against MRSA. Soft-tissue infections with MRSA appeared to be a significant problem in this university hospital–based clinic, particularly among patients with low CD4+ cell counts and poor virologic control of HIV disease. Antiretroviral therapy appears to be an intervention to reduce this complication.

The source of MRSA causing soft-tissue infections among MSM were examined in a prospective survey in a Los Angeles-based HIV clinic. Rieg tested the hypothesis that nasal colonization of MRSA was the source of the increasing incidence of community-acquired MRSA in their population (Abstract 877). Investigators found that 43 of 158 subjects had nasal colonization of *Staphylococcus aureus*, but of these 43, only 7 (16%) were MRSA. In patients with recent MRSA skin infections, there were no trends toward colonization with MRSA. These and other epidemiologic data from HIV-uninfected patients suggest that skin-to-skin contact is a likely mode of transmission of MRSA in MSM with soft-tissue infections.

Trends in invasive pneumococcal disease (IPD) spanning the eras before and after potent antiretroviral therapy were the focus of an oral presentation by Lucas and colleagues (Abstract 139). Invasive pneumococcal disease was defined as bacteremia with *Staphylococcus pneumoniae*. Investigators estimated

that the incidence of IPD in the Johns Hopkins cohort from 1990 to 2003 was 379 cases per 100 person-years of observation. This high incidence rate did not decrease during the potent antiretroviral therapy era, and was estimated at 410 cases per 100 person-years of observation between 1998 and 2004. Using a nested case-control design, authors identified female sex, injection drug use, African-American race, and HCV infection as risk factors of IPD. Variables that surprisingly had no protective effect included trimethoprim/sulfamethoxazole prophylaxis, antiretroviral therapy, and pneumococcal vaccine. There was no protective effect of vaccination, even when only those who received vaccination at CD4+ cell counts above 300/µL were included in the analysis. CD4+ cell count and HIV RNA level were borderline significant in the analysis. Even in the potent antiretroviral therapy era, rates of IPD in this population remain high. Additional information, such as serovar of the pneumococcal strain, may be helpful in explaining these findings.

With IPD remaining a significant risk for HIV-infected patients, Lesprit and colleagues evaluated whether immune responses to the standard 23-valent pneumococcus polysaccharide vaccine (PPV) could be improved with a prime vaccination with the 7-valent pneumococcal conjugate vaccine (PCV; Abstract 140). Subjects with CD4+ counts of 200 to 500 cells/µL were randomized to receive either standard PPV at week 4, or PCV at time 0 and PPV at week 4. At 8 weeks, immunologic response to the prime-boost vaccine strategy was superior to the standard vaccination strategy. The clinical significance of these findings will require much larger studies.

Tuberculosis

Swaminathan presented interim results of an ongoing randomized trial evaluating a 6-month versus a 9-month treatment course for *Mycobacterium tuberculosis* in Chennai, India (Abstract 141). The study population included patients with culture-confirmed *M tuberculosis*. At baseline, the mean CD4+ cell count was 201/µL, and 77% of subjects were smear-positive for acid-fast bacilli. None of the patients were receiving antiretroviral therapy. Of the 122 patients with end-of-treatment data available, cultures were negative in 99% of the patients receiving a 6-month course of treatment and 95% of subjects receiving a 9-month course. Eleven patients died during treatment. There was relapse of tuberculosis in 7 cases in each arm. Final conclusions from this trial await its completion; interim results support current practice of a 6-month course of tuberculosis treatment in HIV-infected persons.

In a study of 20 patients receiving antiretroviral therapy in conjunction with rifampin-based tuberculosis therapy in South Africa, Friedland and colleagues reported on trough efavirenz levels measured during the course of treatment (Abstract 891). All subjects were receiving efavirenz at a dose of 600 mg per day as part of their antiretroviral regimen. There was significant interpatient variation among patients in this study, although higher efavirenz trough levels were associated with lower weight. In this small study, efavirenz trough levels were not statistically associated with treatment outcome or toxicity. Previous data from European studies have suggested that efavirenz dosing should be increased to 800 mg in the presence of rifampin, but defining the optimal dose in the African and Asian setting will require larger studies.

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Advances in Antiretroviral Therapy

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Antiretroviral therapy was a dominant theme of the 12th Conference on Retroviruses and Opportunistic Infections. Key focus areas were new drug advances, management strategies for treatment-naive and treatment-experienced patients, the growing experience with antiretrovirals in the developing world, prevention of mother-to-child transmission of HIV, and the implications of HIV resistance. This review will highlight the major findings relevant to clinicians and clinical investigators.

Investigational and New Antiretroviral Agents

A summary of select investigational drugs is presented in Table 1.

Entry Inhibitors

CCR5 Antagonists. Demarest and colleagues presented data on GSK 873140, a CCR5 antagonist in phase 2 studies (Abstract 77). The short-term virologic efficacy data were presented previously (Demarest et al, ICAAC, 2004). The drug resulted in a 1.7-$\log_{10}$ decline after being administered for 10 days, and the antiviral effect persisted 2 days after the compound was stopped. The authors examined samples from 31 participants in that study who received 1 of 4 doses tested (200 mg qd, 200 mg bid, 400 mg qd, and 600 mg bid) as well as 8 HIV-uninfected participants who received the compound (600 mg bid) for 7 days. The authors assessed the occupancy of the CCR5 receptors by GSK 873140 using a competitive monoclonal antibody to CCR5. They found that more than 98% of CCR5 receptors were occupied immediately after the last dose. The half-life for receptor occupancy was 122 hours across the different doses tested and was longest at the highest doses. It did not differ between HIV-infected and HIV-uninfected participants. The prolonged receptor occupancy provides a reasonable explanation for the antiviral effect seen after the drug had been stopped and plasma levels of GSK 873140 were undetectable.

TAK-779 was a potent CCR5 antagonist in vitro whose clinical development was abandoned due to poor bioavailability of the drug. TAK-652 is an orally bioavailable derivative of TAK-779. Baba and colleagues presented data on the single-dose pharmacokinetics in HIV-uninfected volunteers (Abstract 541). The compound was well tolerated and achieved good plasma levels. They also found that TAK-652 had in vitro activity (50% inhibitory concentration [IC_{50}] < 1 nM) against a panel of CCR5-utilizing HIV-1 viruses that were resistant to either protease inhibitors (PIs) or reverse transcriptase inhibitors (RTIs) and viruses that were subtypes A through G. TAK-652 did not inhibit CXCR4-utilizing viruses. Tremblay and colleagues assessed the interaction of TAK-652 and other antiretrovirals in vitro (Abstract 542). They found that TAK-652 was additive with nucleoside RTIs (nRTIs), nonnucleoside RTIs (NNRTIs), and PIs. Interestingly, it was synergistic with enfuvirtide, suggesting a potential therapeutic benefit by targeting multiple steps of HIV entry.

Attachment Inhibitors. Attachment inhibitors are hypothesized to work by preventing the binding of gp120 to the CD4 receptor. Lin and colleagues presented a series of experiments that supported this mechanism of action (Abstract 544). They showed that a series of compounds, including BMS-488043, bound gp120 and prevented both the binding of soluble CD4 (sCD4) and the exposure of gp41. They also demonstrated conformational changes in gp120 after binding BMS-488043. Finally, gp120 variants with mutations in the CD4 binding pocket were severely defective in compound binding.

Nonnucleoside Reverse Transcriptase Inhibitors

TMC278. Goebel and colleagues presented data on TMC278, a novel investigational NNRTI that is active against HIV-1 isolates resistant to currently available NNRTIs (Abstract 160). This was a double-blind, randomized, placebo-controlled trial of TMC278 given as monotherapy for 7 days. They enrolled 47 participants who were antiretroviral naive, had a median CD4+ count of 255 cells/μL and plasma HIV-1 RNA level of 4.5 $\log_{10}$ copies/mL. Participants started a standard combination antiretroviral therapy after completing 7 days of TMC278. The compound was well tolerated and no serious safety concerns were identified. The median change in plasma HIV-1 RNA at day 8 was 1.2 $\log_{10}$ copies/mL across all dose groups, and no dose relationship was observed. The CD4+ count increased by an average of 55 cells/μL in the TMC278 groups, and no evidence of genotypic resistance was seen at day 8. This study supports further development of TMC278 as an addition to the NNRTI class of antiretroviral medications.

BILR 355 BS. Bonneau and colleagues presented data on BILR 355 BS, an NNRTI with potent in vitro activity (Abstract 558). They tested this compound against wild-type viruses and recombinant viruses with 1 or more NNRTI-associated resistance mutations. BILR 355 BS had a 50% effective concentration (EC_{50}) of 0.25 ng/mL against wild-type virus. The EC_{50} ranged from 1.5 ng/mL to 13 ng/mL against the NNRTI-resistant recombinant viruses. It was also active against subtypes A through G, but was inactive against HIV-2 similar to other NNRTIs. The investigators also presented data on the pharmacokinetic profile in HIV-uninfected volunteers given either a single

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Table **1**. Summary of Selected Investigational Drug Studies

Drug	Abstract Nos.	Drug Class	Development Stage	Results
BMS-488043	Abstract 544	Attachment inhibitor	Preclinical	Binding of drug to gp120 alters conformation of the envelope and inhibits CD4 attachment.
873140	Abstract 77	CCR5 antagonist	Phase 2	Drug occupies receptors with a half-life of 122 hours, which correlates with antiviral activity as opposed to plasma levels.
TAK-652	Abstracts 541, 542	CCR5 antagonist	Preclinical/Phase 1	EC_{50}<1 nM against a panel of clinical isolates; active against recombinant viruses of subtypes A-G; orally bioavailable.
KMMP05	Abstract 157	RNAase H inhibitor	Preclinical	IC_{50} 500 nM; binds near NNRTI binding site, not at RNAse active site.
Compound-1	Abstract 156	Nucleotide-competing reverse transcriptase inhibitor	Preclinical	EC_{50} 30 nM; binds at active site of reverse transcriptase but is not incorporated into DNA.
Amdoxovir	Abstract 553	nRTI	No longer in development*	Safe and well tolerated.
Dioxolane thymine	Abstract 554	nRTI	Preclinical	Increased activity against nRTI-resistant HIV, compared with wild type.
TMC278	Abstracts 160, 556	NNRTI	Phase 2a	Median decline in plasma HIV-1 RNA level was 1.2 log_{10} copies/mL after 7 days of monotherapy.
Capravirine	Abstract 555	NNRTI	Phase 2	See Table 3.
BILR 355 BS	Abstract 558	NNRTI	Preclinical	EC_{50} 0.26 ng/mL against wild-type HIV EC_{50}.
L-000870810	Abstract 161	Integrase inhibitor	No longer in development	1.7-log_{10} copies/mL reduction in plasma HIV-1 RNA level after 7 days of monotherapy.
Styrylquinolines derivatives	Abstract 547	Integrase inhibitor	Preclinical	Synergistic with reverse transcriptase inhibitors and diketo acid integrase inhibitors.
TMC114	Abstract 164LB	Protease inhibitor	Phase 2b	See Table 3.
Tipranavir	Abstracts 104, 560, 654	Protease inhibitor	Phase 3	See Table 3.
UIC-02031	Abstract 562	Protease inhibitor	Preclinical	Active against multiple PI-resistant strains of HIV with IC_{50} of 15-38 nM.
640385	Abstract 563	Protease inhibitor	Phase 1	Safe and well tolerated in non-HIV-infected volunteers. When boosted with ritonavir, achieves target plasma levels.
PA-457	Abstracts 159, 551	Maturation inhibitor	Phase 1	8 of 12 participants at 2 highest doses had 0.5-log_{10} copies/mL drop in HIV-1 RNA level after a single dose.

EC_{50} indicates 50% effective concentration; gp120, glycoprotein 120; IC_{50}, 50% inhibitory concentration; NNRTI, nonnucleoside reverse transcriptase inhibitor; nRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RNAase H, ribonuclease H.

*Although amdoxovir is no longer in development, the license has been returned to the original developer who may pursue further studies.

dose or multiple doses. The plasma half-life and maximum concentration (Cmax) were increased by 3-fold to 5-fold with the coadministration of 100 mg of ritonavir. There was one transient grade 3 elevation of alanine aminotransferase (ALT), and no other safety concerns were noted. These data support further development of this compound.

Nucleoside Reverse Transcriptase Inhibitors

Amdoxovir. Gripshover and colleagues presented data on A5118: a randomized, placebo-controlled study of amdoxovir given with enfuvirtide plus an optimized background regimen (Abstract 553). The study was stopped early after a Data and Safety Monitoring Board review and the decision by the manufacturer not to develop the drug further. At that point, 9 patients were enrolled in each arm. The median baseline CD4+ count and plasma HIV-1 RNA level were 36 cells/μL and 4.8 $\log_{10}$ copies/mL. Several patients stopped the study early because of poor virologic response and enfuvirtide injection-site reactions. The time-averaged change in HIV-1 RNA level was −1.1 $\log_{10}$ copies/mL in the amdoxovir arm and −0.8 $\log_{10}$ copies/mL in the placebo arm (P = not significant). The changes in creatinine clearance were similar between arms and no patients developed lens opacities, both potential toxicities of amdoxovir.

Dioxalane Thymine. Chu and colleagues presented data on dioxalane thymine, an nRTI with a dioxalane sugar moiety (Abstract 554). They found that this compound had an EC_{50} of 0.43 μM against wild-type HIV. The EC_{50} was lower against nRTI-resistant strains, including those with K65R (0.21 μM), L74V (0.33 μM), and M184V (0.2 μM) mutations. The authors concluded that these data support further clinical development.

Other Reverse Transcriptase Inhibitors

Nucleotide Competing Reverse Transcriptase Inhibitors (Compound-1) Jochmans and colleagues (Abstract 156) presented data on a potential new class of reverse transcriptase inhibitors: nucleotide-competing reverse transcriptase inhibitors. Compound-1 from this class was shown to be a competitive inhibitor of reverse transcriptase with an EC_{50} of 30 nM against HIV-1. Its structure is not similar to that of nucleotide reverse transcriptase inhibitors or nRTIs and is not incorporated into the DNA strand. It seems to bind to the active site of reverse transcription, thereby preventing the binding and incorporation of nucleotides into the growing DNA strand.

RNAase H Inhibitors. HIV requires a double-stranded DNA intermediate in order to replicate. Reverse transcriptase forms a complementary (-) DNA strand from a (+) RNA template. It also must form a (+) DNA strand from a (-) DNA strand to create the double-stranded DNA intermediate. RNAase H is the portion of reverse transcriptase that facilitates this transition from reading the (+) RNA strand to reading the (-) DNA strand, and is essential for HIV replication. Its activities include degrading the viral RNA strand. Progress in finding drugs that target RNAase H has been limited by the lack of a high-throughput assay that can easily screen thousands of compounds to find potential candidates. Parniak reviewed RNAase H and the process of finding RNAase H inhibitors in a conference symposium, which can be viewed on the Web site (http://www.retroconference.org/2005/Pages/webcasts.htm).

Himmel and colleagues presented data on the crystal structure of a candidate RNAase H inhibitor bound to the enzyme (Abstract 157). The compound, KMMP05, exhibited in vitro activity against RNAase H, but not reverse transcriptase, at an IC_{50} of 500 nM. This compound bound at a site adjacent to the active site of RNAase H. Based on this, they speculated that this compound exerts it effect by diverting the RNA and preventing it from reaching the active site or by preventing other processes of the enzyme without directly binding in the active site. These data provide support for pursuing RNAase H inhibitors as a therapeutic class.

Integrase Inhibitors

L-000870810. HIV integrase is an important target for antiretroviral drug development. Little and colleagues presented data on a candidate integrase inhibitor, L-000870810, that inhibits HIV-1 integrase strand transfer in vitro (Abstract 161). This was a double-blind, randomized, placebo-controlled trial of this compound given for 7 days. The study was stopped early because of toxicity seen in ongoing animal studies. Thirty patients who were not on antiretroviral therapy were enrolled. The participants who received 200 mg bid (n = 7) and 400 mg bid (n = 17) had a mean baseline plasma HIV-1 RNA level of 4.7 $\log_{10}$ copies/mL and 4.6 $\log_{10}$ copies/mL, respectively. They observed reductions in plasma HIV-1 RNA levels in the 2 groups of 1.7 $\log_{10}$ copies/mL and 1.8 $\log_{10}$ copies/mL, respectively, at day 8. The doses were well tolerated and no serious adverse events were noted. Although this compound is no longer in development, this study provides the first proof of concept for the antiviral activity of HIV integrase inhibitors, and a backup compound is now under development.

Styrylquinoline Derivatives. Styrylquinoline derivatives are integrase inhibitors that act on the preintegration step of HIV, most likely by preventing the formation of the preintegration complex. Chéret and colleagues presented data on the interaction of styrylquinoline derivatives with RTIs and diketo acids that inhibit the strand-transfer step of HIV integration (Abstract 547). These combinations were synergistic in vitro in supporting the use of styrylquinoline derivatives in multidrug regimens and the potential to target numerous steps in the integration process.

Protease Inhibitors

UIC-02031. Koh and colleagues presented data on UIC-02031, a nonpeptidic PI (Abstract 562). UIC-02031 was active against primary clinical isolates of HIV subtypes A, B, C, and E; clinical isolates resistant to available PIs; and PI-resistant laboratory isolates selected by suboptimal exposure to other PIs. UIC-02031–resistant isolates generated in vitro had L33F, M46I, V82I, and I84V mutations among others in the protease gene, and several cleavage site mutations in Gag.

640385. 640385 is a PI with potent in vitro activity against several PI-resistant isolates. Ford and colleagues presented data on the safety and pharmacokinetics of 640385 given to HIV-uninfected volunteers in a double-blind, randomized,

placebo-controlled, dose-escalating study (Abstract 563). They found that the pharmacokinetic profile was greatly enhanced by coadministration with ritonavir and supported twice-daily dosing. This compound appeared safe and well tolerated. All adverse events were listed as mild or moderate, and further studies in PI-experienced HIV-infected subjects are anticipated.

Maturation Inhibitors

PA-457. PA-457 is the first maturation inhibitor for the treatment of HIV infection to reach the clinical development phase. It inhibits the conversion of the HIV capsid precursor (p25) into the final capsid protein (p24), and results in noninfectious virions. Martin and colleagues presented the results of the first trial in HIV-infected individuals: a double-blind, placebo-controlled study of a single oral dose of PA-457 (Abstract 159). Participants were on no antiretroviral medications and had CD4+ counts above 200 cells/μL and plasma HIV-1 RNA levels between 5000 copies/mL and 250,000 copies/mL. They compared 3 doses of PA-457 (75 mg, 150 mg, or 250 mg) with placebo, with 6 participants in each group. All doses were well tolerated and no major safety concerns were identified. Eight of 12 participants in the 2 highest doses had at least a 0.3- $\log_{10}$ copies/mL reduction in plasma HIV-1 RNA, and five had at least a 0.5-$\log_{10}$ copies/mL reduction. The largest reduction was 0.7 $\log_{10}$ copies/mL. Martin and colleagues also presented data from a separate study of 8 HIV-uninfected volunteers who received PA-457 (25, 50, or 100 mg qd) or placebo for 10 days (Abstract 551). They found that the doses were well tolerated and the pharmacokinetic profile supported once-daily dosing. Further development of this compound is planned. Freed reviewed the mechanism of action of PA-457 in an excellent talk about targeting the assembly and release of HIV (Abstract 116; http://www.retroconference.org/2005/Pages/webcasts.htm).

Treatment of Antiretroviral-Naive Patients

Bartlett and colleagues (Abstract 586) presented results of a metaanalysis of triple-combination therapy in antiretroviral-naive individuals. Forty-nine clinical trials, conducted between 1994 and 2004 and including 13,147 subjects in 85 independent treatment arms, were analyzed. Triple-combination therapy trials of at least 24 weeks in duration that had 30 or more chronically HIV-infected, antiretroviral-naive subjects, were included. The trials were selected from database searches and conference presentations. Triple-drug combinations included 2 nRTIs plus a PI, a boosted PI, an NNRTI, or a third nRTI. The primary endpoints were increase in CD4+ cell counts and proportion of subjects with plasma HIV-1 RNA levels below 50 copies/mL at week 48, as evaluated by intention-to-treat analysis. Overall, 57% of patients achieved an HIV-1 RNA level below 50 copies/mL at week 48 and an increase in CD4+ count of 177 cells/μL, both increased from the 2001 meta-analysis results of 45% and 158 cells/μL, respectively. Multivariate analysis of factors associated with response to therapy showed that lower pill count was no longer linked to improved response, and that boosted PI- and NNRTI-containing regimens were associated with virologic responses superior to nRTI-only or unboosted-PI regimens. Further, CD4+ response rates favored the boosted PI-containing regimens, with a significantly greater increase in CD4+ count (+209 cells/μL) than with the NNRTI- (+174 cells/μL), the triple-nRTI- (+150 cells/μL), or the unboosted-PI-containing regimens (+178 cells/μL). The investigators concluded that virologic response rates have been improving over time as better treatment regimens became available. Regimen potency, but not pill count, was associated with virologic response. Boosted PI and NNRTI regimens were associated with virologic suppression at 48 weeks superior to that of PI and nRTI regimens, and boosted PI regimens were associated with the greatest increases in CD4+ cell count.

INITIO Trial

The long-awaited results of the INITIO trial were presented by Cooper and Yeni for the INITIO Study Group (Abstract 165LB). The study compared the efficacy of a 3-drug regimen containing an NNRTI followed by a PI or a PI followed by an NNRTI with a 4-drug therapy containing an NNRTI and a PI in antiretroviral-naive patients. This open-label, multicenter study randomized 915 people to receive: stavudine/didanosine/efavirenz followed by zidovudine/lamivudine/abacavir/nelfinavir after virologic failure; stavudine/didanosine/nelfinavir followed by zidovudine/lamivudine/abacavir/efavirenz after virologic failure; or stavudine/didanosine/efavirenz/nelfinavir with no specified second regimen after virologic failure. Drug switches were allowed for viral load rebound and for adverse events.

The primary outcomes were the proportion of patients with HIV-1 RNA below 50 copies/mL and change in CD4+ cell count from baseline at 3 years. Secondary outcomes measured included change from baseline in HIV-1 RNA at 3 years, progression to AIDS events or death, and incidence of adverse events. Subjects had been followed for a mean of 3.7 years when the trial closed in June 2004. The overall median baseline CD4+ count and mean plasma HIV-1 RNA level were 220 cells/μL and 4.93 $\log_{10}$ copies/mL, respectively. Results analyzed on an intention-to-treat basis at 3 years favored the efavirenz arm, with virologic response rates of 74% (efavirenz), 62% (nelfinavir), and 62% (efavirenz/nelfinavir 4-drug arm) of patients with HIV-1 RNA levels below 50 copies/mL ($P = .004$) at 3 years. Proportions of time on the initial regimen were 74%, 63%, and 51%, respectively. No significant differences were found between groups in CD4+ cell response (mean increase 315 cells/μL, 289 cells/μL , and 274 cells/μL, respectively), in progression to a new AIDS event or death, in number of patients with serious adverse events, or number of patients with at least 1 adverse event leading to discontinuation of 1 or more drugs. Overall, 61% of patients stopped their initial regimen, usually in the setting of adverse events rather than virologic failure. However, patients in the efavirenz arm spent a longer proportion of time on the initial regimen and were least likely to be exposed to 3 drug classes over the 3-year period. Within-class switches occurred in 39% of all patients; the most common switch was from didanosine/stavudine to zidovudine/lamivudine. Thus, the findings of the INITIO trial support previous evidence from the AIDS Clinical Trials Group (ACTG) 384 study that initiating antiretroviral therapy with a 3-drug/2-class regimen containing efavirenz is

superior to starting treatment with similar regimens containing nelfinavir. Likewise, there remains no clear evidence to support the use of 4-drug/3-class therapy for the initial treatment of HIV infection. The nRTI backbone didanosine/stavudine was poorly tolerated, supporting the more favorable combination of zidovudine/lamivudine for use in initial treatment regimens.

Rizzardini and colleagues (Abstract 601b) compared 3-drug and 4-drug regimens in treatment-naive patients with respect to CD4+ count response, plasma HIV-1 RNA level, peripheral blood mononuclear cell (PBMC) proliferation, and cytokine production. Seventy-six treatment-naive individuals received zidovudine and were randomized to 1 of 6 arms: didanosine/abacavir, lamivudine/abacavir, didanosine/efavirenz, lamivudine/efavirenz, didanosine/indinavir/ritonavir, or lamivudine/indinavir/ritonavir. At 6 months, all regimens resulted in increased CD4+ cell counts and suppression of plasma HIV-1 RNA levels. The abacavir-containing regimens were associated with the best CD4+ cell response after 6 months of therapy, and the combination of zidovudine/lamivudine/abacavir resulted in better suppression of HIV-1 RNA levels. Triple-nRTI regimens overall resulted in higher increases in CD4+ cell counts than boosted PI regimens, yet the latter resulted in a more robust immune response as measured by interferon (IFN)-γ and PBMC proliferation.

ABCDE Study

Podzamczer and colleagues (Abstract 587) compared the efficacy and safety of 2 different nRTIs combined with lamivudine/efavirenz in treatment-naive subjects. This prospective, multicenter, open-label trial enrolled 237 patients with plasma HIV-1 RNA levels above 1500 copies/mL, who were randomized to receive either abacavir or stavudine in combination with lamivudine/efavirenz. The primary endpoint was lipodystrophy and mitochondrial toxicity, and the secondary endpoints were virologic, immunologic, and clinical efficacy and tolerability. Virologic success was determined by reduction of HIV-1 RNA level to below 50 copies/mL. Subgroup analysis was carried out at weeks 48 and 96 to assess further parameters of lipodystrophy and mitochondrial toxicity including venous lactate, dual-energy x-ray absorptiometry (DEXA) scan, blood lipoproteins, mitochondrial DNA/nuclear DNA (mtDNA/nDNA) ratio. Baseline characteristics were similar between both groups, with a median CD4+ count of 213 cells/μL and HIV-1 RNA level of 5.2 $\log_{10}$ copies/mL. At 96 weeks, abacavir was superior to stavudine in virologic response in the intent-to-treat analysis (60.9% vs. 47.5%; $P = .05$, but not in the on-treatment analysis (87.5% vs. 85.3%; $P = .81$) and demonstrated less-subjective and clinically-measured lipodystrophy (4.8% vs. 39.2%; $P < .0001$). These results were reinforced by the subgroup analyses that demonstrated superiority of abacavir in DEXA scan evaluation and lipid profiles, specifically lower triglyceride levels, greater high-density lipoprotein (HDL) and apolipoprotein A1 levels, and a greater reduction in total cholesterol/ HDL ratio. There were no differences in lactate levels, total cholesterol levels, low-density lipoprotein (LDL) levels, LDL/HDL ratio, or mtDNA/nDNA ratio between the 2 groups. The mean CD4+ cell count increases were likewise similar in both groups. The authors concluded, in this first head-to-head comparison of abacavir and stavudine, that abacavir is better tolerated, with less associated lipoatrophy, and they note that the lower treatment discontinuation rate in the abacavir group may account for the associated superiority in virologic response in this cohort. These results confirm findings of previous studies demonstrating the relationship between stavudine and mitochondrial toxicity.

nRTI Regimens in Treatment-Naive Patients

Current guidelines caution against initiating therapy with triple- nRTI regimens because of high rates of early virologic failure reported in treatment-naive patients treated with regimens containing tenofovir with either lamivudine/didanosine or lamivudine/abacavir. Recent data, however, suggest that this recommendation deserves ongoing evaluation (DeJesus et al, ICAAC, 2004, and Moyle et al, ICAAC, 2004).

Two studies evaluating nRTI-only regimens containing tenofovir plus fixed-dose combination zidovudine/lamivudine were presented at the conference. Rey and colleagues reported data from a pilot, prospective, single-arm cohort study conducted at the University of Strasbourg (Abstract 599). Forty-two treatment-naive patients with CD4+ cell counts below 350 cells/μL received a fixed-dose combination of zidovudine/lamivudine (300 mg/150 mg bid) plus tenofovir (300 mg qd). Plasma HIV-1 RNA levels and CD4+ cell counts were assessed at 1 and 2 months and then every 2 months for 48 weeks; evaluation for early virologic response was assessed at weeks 1 or 2 of treatment. The median baseline CD4+ count was 233 cells/μL, and the median plasma HIV-1 RNA level was 4.88 $\log_{10}$ copies/mL; 40% of patients had CD4+ counts below 200 cells/μL and 45% had plasma HIV-1 RNA levels above 5 $\log_{10}$ copies/mL. The median time of follow-up was 8 months. On-treatment analysis showed median plasma HIV-1 RNA decreases of 1.56 $\log_{10}$ copies/mL and 2.28 $\log_{10}$ copies/mL at weeks 2 and 4, respectively. At week 4, 86% of subjects achieved plasma HIV-1 RNA levels below 1000 copies/mL; the median time to HIV-1 RNA level below 50 copies/mL was 10 weeks. The median increase in CD4+ count at 48 weeks was 82 cells/μL. Ninety-three percent and 78% of subjects had HIV-1 RNA levels below 50 copies/mL at weeks 24, and 48, respectively. Five patients (12%) discontinued the study regimen due to side effects (abdominal pain and nausea in 3 and anemia in 2, probably due to zidovudine). Four virologic failures occurred due to poor adherence. Genotypic analysis demonstrated the K65R mutation in 1 patient, the M184V plus 2 or 3 thymidine analogue mutations (TAMs) in 2 patients, and 2 TAMs in 1 patient present at baseline. The authors concluded that the combination of zidovudine/lamivudine plus tenofovir in treatment-naive HIV-infected patients induces a rapid and sustained virologic response and is associated with good immunologic response and safety profiles. The tenofovir-associated K65R mutation was not detected alongside TAMs in patients with virologic failure, suggesting that salvage options are available with alternate classes in this triple-nRTI regimen and the potential of triple-nRTI regimens merits further evaluation.

DART Substudy. Mutuluuza and colleagues (Abstract 22) presented results from the DART (Development of Anti-Retroviral Therapy in Africa) study supporting the viability of nRTI-only regimens for initial therapy. The DART trial is a large, randomized, controlled clinical trial of 3300 patients at 3 sites in Uganda and Zimbabwe comparing intensive vs clinical monitoring and continuous vs intermittent therapy in treatment-naive individuals with a CD4+ below 200 cells/μL. Seventy-six percent of patients received fixed-dose zidovudine/lamivudine with tenofovir. Investigators evaluated virologic response to this regimen in a subset of 300 patients with advanced disease enrolled from early 2003 through October 2004. The median baseline CD4+ count was 100 cells/μL and the mean baseline HIV-1 RNA level was 300,000 copies/mL. At week 24, according to the intent-to-treat analysis, the mean decrease in plasma HIV-1 RNA level was 3.7 $\log_{10}$ copies/mL and the median increase in CD4+ count was 106 cells/μL. Eleven patients died before week 24, and 47 of 281 (17%) had HIV-1 RNA levels above 1000 copies/mL at 24 weeks. Overall, after 24 weeks of therapy, 71% and 53% of subjects achieved an HIV-1 RNA level below 400 copies/mL and below 50 copies/mL, respectively. On-treatment analysis demonstrated virologic suppression in 83% of patients in whom treatment was not interrupted. The investigators concluded that this regimen has similar efficacy to PI-based or NNRTI-based regimens used in resource-unlimited settings. The authors also suggested that this triple-nRTI regimen may be particularly efficacious and well tolerated in populations with high rates of tuberculosis coinfection (27% in the DART study).

Treatment for Antiretroviral-Experienced Patients

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

TMC114 vs Control PI

Haubrich and colleagues presented data on TMC114, an investigational PI that is active against a broad range of HIV isolates with resistance to currently available PIs (Abstract 164LB). These data were from a planned interim analysis of 2 ongoing dose-finding trials. Patients were 3-drug class experienced, had 1 or more major PI mutations, and had plasma HIV-1 RNA levels above 1000 copies/mL at screening. Subjects received TMC114 with ritonavir at 1 of 4 doses (400 mg/100 mg qd, 800 mg/100 mg qd, 400 mg/100 mg bid or 600 mg/100 mg bid) or a control PI chosen based on treatment history and resistance testing results. Participants also received an optimized background of nRTIs with or without enfuvirtide. The primary endpoint was change in plasma HIV-1 RNA level at week 24. Four hundred ninety-seven patients were included in the analysis. The median baseline plasma HIV-1 RNA level and CD4+ count were 4.6 $\log_{10}$ copies/mL and 141 cells/μL. Participants had a median of 8 PI mutations and 3 primary PI mutations. Phenotypic resistance to all currently available PIs was seen in 66% of patients. Forty-seven percent of patients used enfuvirtide in their optimized background regimen.

All doses showed significant declines in HIV-1 RNA levels compared with placebo. A dose-response relationship was seen, and the greatest HIV-1 RNA level decline, 1.85 $\log_{10}$, was seen in the 600 mg/100 mg twice-daily group. In addition, 47% of participants in this group had a plasma HIV-1 RNA level below 50 copies/mL at week 24, compared with 10% of participants in the control PI group. Among those participants receiving the highest dose of TMC114/ritonavir, 67% of those who also used enfuvirtide for the first time had a plasma HIV-1 RNA level below 50 copies/mL at week 24, compared with 37% who did not use enfuvirtide. These virologic responses were impressive given the level of treatment experience of the study population, but the durability of the response will need to be demonstrated. Further clinical development will use twice-daily doses of 600 mg of TMC114 given with 100 mg of ritonavir.

Tipranavir/ritonavir vs Lopinavir/ritonavir: A Subgroup Analysis of RESIST Trials

The RESIST-1 and RESIST-2 trials showed that the investigational PI tipranavir, boosted with low-dose ritonavir, given with an optimized background antiretroviral regimen was superior to an antiretroviral regimen using a currently available PI chosen based on history and resistance testing results. Patients were 3-drug class experienced, including 2 or more PI-based regimens. Enfuvirtide was allowed in the optimized background regimen. Cooper and colleagues presented a subgroup analysis of the trials that included only those participants choosing lopinavir/ritonavir for their comparator antiretroviral regimen (Abstract 560). Half of these participants were randomized subsequently to receive tipranavir/ritonavir. The baseline median CD4+ cell count and plasma HIV-1 RNA level were 162 cells/μL and 4.8 $\log_{10}$ copies/mL. There were 293 participants included in the tipranavir/ritonavir arm and 290 in the lopinavir/ritonavir arm.

The proportion of subjects with greater than 1 $\log_{10}$ copies/mL declines in plasma HIV-1 RNA level from baseline at week 24 was higher in the tipranavir group (40% vs 21%, respectively; $P < .05$). This difference was most apparent among those participants who had received lopinavir/ritonavir previously (35% vs 11%; $P < .05$) and those who had virus that was resistant to lopinavir/ritonavir (36% vs 13%, respectively; $P < .05$). The probability of a treatment response increased with the number of antiretroviral drugs to which the patients' isolates were susceptible, as has been noted in several other studies.

Placebo-Controlled Trial of Capravirine

Capravirine is an investigational NNRTI that is active in vitro against HIV-1 isolates that are resistant to currently available NNRTIs. Pharmacokinetic studies suggest that nelfinavir raises the plasma levels of capravirine. Pesano and colleagues presented the 48-week data from a phase 2, randomized trial of twice-daily capravirine 700 mg, capravirine 1400 mg, or placebo given with nelfinavir and 2 investigator-selected nRTIs (Abstract 555). Subjects were NNRTI-experienced and PI-naive, and 60 subjects were enrolled in each arm. Mean baseline plasma HIV-1 RNA levels were 4.5 $\log_{10}$ copies/mL, 4.4 $\log_{10}$ copies/mL, and 4.4 $\log_{10}$ copies/mL in the placebo, capravirine 700 mg, and capravirine 1400 mg groups, respectively, and median baseline CD4+ cell counts were

Table **2**. Selected Antiretroviral Studies in Treatment-Experienced Patients

Abstract Number	Comparison	Baseline CD4+ count (cells/μL)	Plasma HIV RNA (copies/mL)	Length of follow-up (weeks)	Results
Abstract 164	TMC114/r (n=397)* vs investigator-selected control PI given with an optimized background regimen (n=100)	141	4.6 $\log_{10}$	24	TMC114/r 600 mg/100 mg bid was the optimal dose: 47% had a plasma HIV-1 RNA <50 copies/mL vs 10% in the control PI group
Abstract 577	Fosamprenavir/r or lopinavir/r (n=28) vs fosamprenavir+lopinavir/r (n=28) given with tenofovir and 1-2 nRTIs	188	4.5 $\log_{10}$	24	75% vs 61% with >1-$\log_{10}$ decline (*P*=.17) 54% vs 46% with HIV RNA <50 copies/mL
Abstract 578	Dual boosted PIs without RTIs (Group 1: resistant to RTIs, n=41; group 2: intolerant of RTIs, n=41)	240 (Group1) 294 (Group 2)	4.1 $\log_{10}$ (Group1) <400 (Group 2)	24	Overall at week 24, 84% and 91% had HIV-1 RNA levels below 400 copies/mL by intent-to-treat and on treatment analyses, respectively
Abstract 555	Capravirine 700 mg, 1400 mg or placebo given with nelfinavir and 2 nRTIs in NNRTI-experienced and PI-naive subjects (n=60 in each arm)	248 249 208	4.4 $\log_{10}$ 4.4 $\log_{10}$ 4.5 $\log_{10}$	48	43%, 58%, and 46% had a plasma HIV RNA <400 copies/mL
Abstract 560	Subgroup analysis of RESIST-1 and -2: tipranavir/r vs lopinavir/r given with an optimized background regimen	163	4.8 $\log_{10}$	24	40% vs 21% with >1-$\log_{10}$ decline in plasma HIV RNA

*4 different doses of TMC114/ritonavir were studied (400 mg/100 mg qd, n=100; 800 mg/100 mg qd, n=100; 400 mg/100 mg bid, n=98; 600 mg/100 mg bid, n=99)

NNRTI indicates nonnucleoside reverse transcriptase inhibitor; nRTI, nucleoside (or nucleotide) reverse transcriptase inhibitor; PI, protease inhibitor; r, boosted ritonavir; RTI, reverse transcriptase inhibitor.

208/μL, 248/μL, and 249/μL, respectively. The time to virologic failure did not differ between study arms. The proportions of subjects with plasma HIV-1 RNA levels below 400 copies/mL at week 48 were 46%, 43%, and 58% (*P* = not significant). The most common side effects were diarrhea, nausea, and vomiting, and did not differ between treatment arms.

Lopinavir/ritonavir or Fosamprenavir/ritonavir vs. Fosamprenavir plus Lopinavir/ritonavir

A prior report from A5143 showed that combining fosamprenavir and lopinavir/ritonavir led to a significant reduction of both lopinavir and fosamprenavir levels, compared with giving these drugs separately. Collier and colleagues presented the virologic data from that trial (Abstract 577). This study tested whether lopinavir/ritonavir 400 mg/100 mg plus fosamprenavir 700 mg twice daily (double PI) leads to HIV-1 RNA response superior to lopinavir/ritonavir or fosamprenavir/ritonavir (single PI) in persons with virologic failure to PI-based therapy. This was an open-label study that selectively randomized patients based on prior PI experience so that all patients received at least 1 new PI. All patients received tenofovir and 1 or 2 additional nRTIs chosen based on resistance testing results and antiretroviral history.

The median entry CD4+ cell count and plasma HIV-1 RNA level were 188/μL and 4.5 $\log_{10}$ copies/mL, respectively. The study was stopped early based on the pharmacokinetic data, when 56 subjects (28 in the double-PI arm and 28 in the single-PI arm) were enrolled out of a planned sample size of 216 subjects. Seventy-five percent of participants in the double-PI arms had drops in HIV-1 RNA levels greater than 1 $\log_{10}$ copies/mL from baseline at week 24, compared with 61% of participants in the single-PI arms (*P* = .17) in the intent-to-treat analysis, and 100% and 64% in the on-treatment analysis (*P* = .02). HIV-1 RNA levels were below 50 copies/mL at week 24 in 54% and 46% of the double-PI and single-PI subjects in the intent-to-treat analysis (*P* = .37) and in 75% and 48% in the on-treatment analysis. Although the virologic responses were not significantly different in the intent-to-treat analysis, the trends in the on-treatment analysis suggest that the reduction in plasma levels was not associated with adverse virologic outcomes. The question of whether dual-boosted PIs are superior to single boosted PI regimens remains unanswered.

Saquinavir/Ritonavir vs Indinavir/Ritonavir

Harris and colleagues presented data from the Simplified Protease Inhibitor Trial (SPRINT; Abstract 574). They compared once-daily saquinavir/ritonavir with twice-daily indinavir/ritonavir, each with 2 RTIs in patients who were either PI-naive or had no evidence of PI resistance. They randomized 164 subjects to receive either saquinavir/ritonavir 1600 mg/100 mg once daily or indinavir/ritonavir 800 mg/100 mg twice daily, with either 2 nRTIs or 1 nRTI plus 1 NNRTI as selected by the treating physician. The efficacy analysis included 147 patients. Seventy-nine percent were male, 80% were PI-naive, 62% had a plasma HIV-1 RNA levels above 5 $\log_{10}$ copies/mL, and the median CD4+ count was 130 cells/μL. Background regimens included an NNRTI in 3 patients in the indinavir/ritonavir arm and none in the saquinavir/ritonavir arm. The proportion of patients with a plasma HIV-1 RNA level below 50 copies/mL in an intent-to-treat analysis were 56% and 49% in the saquinavir/ritonavir and indinavir/ritonavir arms ($P = .44$) at week 24, respectively, and 50% and 45% ($P = .7$) at week 48. CD4+ cell count increases to week 48 were also similar between arms (saquinavir/ritonavir, 147/μL increase; indinavir/ritonavir, 131/μL increase; $P = .60$). Patients taking indinavir/ritonavir were more likely to discontinue their PI because of toxicity (29 of 77 patients or 38%) than patients taking saquinavir/ritonavir (10 of 70 patients or 14%).

Dual-Boosted Protease Inhibitors Without nRTIs

Duvivier and colleagues presented data on a novel approach to antiretroviral therapy for treatment experienced patients: dual boosted protease inhibitors without use of reverse transcriptase inhibitors (Abstract 578). Subjects were either resistant to nRTIs and NNRTIs (group 1, n = 41) or were intolerant to those drugs (group 2, n = 41). The median baseline plasma HIV RNA was 4.1 $\log_{10}$ copies/mL and the median baseline CD4+ count was 240 cells/μL in group 1. Seventy-one percent of those in group 2 had an HIV-1 RNA level below 400 copies/mL and 29% were on a treatment interruption. The median baseline CD4+ count was 294 cells/μL in group 2. Subjects in both groups had prior exposure to a median of 2 PIs. Ten subjects in group 1 and 7 subjects in group 2 were PI-naive. The most frequent regimens used were saquinavir 800 mg/lopinavir 400 mg/ritonavir 100 mg bid (40%), indinavir 400 mg/lopinavir 400 mg/ritonavir 100 mg bid (30%), and indinavir 400 mg/amprenavir 600 mg/ritonavir 100 mg bid (17%). Overall at week 24, 84% (69 of 82 patients) and 91% (67 of 76 patients) had HIV-1 RNA levels below 400 copies/mL by intent-to-treat and on-treatment analyses, respectively. Seven patients discontinued the study regimen before week 24 (5 in group 1 and 2 in group 2) and 5 patients experienced virologic failure at week 24 (4 in group 1 and 1 in group 2). This study adds to a series of data suggesting that boosted PIs without nRTIs or NNRTIs may be an adequate alternative to standard antiretroviral regimens in some settings.

Acute HIV Infection

Fiscus and colleagues (Abstract 20) evaluated methods of detecting acute HIV infection (AHI) among 1440 individuals evaluated between February 2003 and January 2004 in a sexually transmitted disease (STD) clinic in Malawi. Following 2 rapid tests, negative or discordant results were tested for HIV p24 antigen and pooled 1:10:50 for HIV-1 RNA testing. Acute infection was defined as the presence of HIV-1 RNA positive results and HIV-1 antibody negative results. Thirty-eight and a half percent of patients had established HIV-1 infection and 1.4% (20 patients) had AHI. Of these, only 50% had clinical signs and symptoms of AHI, with a median baseline plasma HIV-1 RNA level of 599,994 copies/mL. Sensitivity of two rapid tests was only 35% compared to 100% for HIV-1 RNA. The authors concluded that parallel rapid antibody testing and p24 antigen assay can detect up to 75% of cases of AHI.

Hightow and colleagues (Abstract 565) assessed risk behavior and clinical symptoms of 29 individuals with AHI identified through North Carolina Screening and Training Active Transmission program (STAT) between January 2003 and December 2004. Twenty three (85%) of the subjects reported having an acute illness in the 3 months prior to testing; 19 of them sought medical treatment. The most common symptoms were fever (62%), anorexia (60%), sore throat (58%), and skin rash (38%). AHI was considered at only 3 (15%) of these initial evaluations. Sixty-two percent and 44% of subjects reported unprotected sex and symptoms of illness, respectively, as a reason for HIV-1 testing. The authors also reported (Abstract 566) that 38% of men were in college at the time of diagnosis, and 56% met their sex partners over the Internet.

Markowitz and colleagues (Abstract 973B) reported a case of a recent HIV infection by a multidrug-resistant and dual-tropic virus in a man who had sex with men and used methamphetamines. HIV-1 infection was confirmed in December 2004. The patient reported symptoms of pharyngitis, fever, and malaise in the 6 weeks preceding the diagnosis. Time of exposure was estimated to be between 4 and 20 months. The course of his illness over 4 months was characterized by presentation with symptomatic AIDS, a CD4+ count of 39 cells/μL and a plasma HIV-1 RNA of 232,000 copies/mL. The virus was noted to be resistant to nRTIs, some NNRTIs, and PIs; sensitive to enfuvirtide and possess a replication capacity of 136% and the ability to induce syncytia in PBMCs. Plasma virus utilizing both CCR5 and CXCR4 as the coreceptor were present.

The potential variations in the natural history of early HIV infection were highlighted by 2 presentations at the conference. Gange and Munoz (Session 31B) presented data on behalf of the Multicenter AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS). MACS and WIHS are prospective cohort studies of HIV-infected and high-risk individuals initiated in 1984 and 1994 that have enrolled 6,973 men and 3,768 women, respectively. In the MACS cohort, the median time to AIDS was estimated to be 8.3 years, and cumulative incidence of AIDS was 1.1% and 2.1% within 12 and 24 months of seroconversion, respectively. The authors used the MACS data to fit a model to estimate that the number of individuals progressing to AIDS at month 6, 12, and 24 of seroconversion would be 7, 45, and 262 per 10,000, respectively. Using both cohorts, the authors further estimated that the expected number of indi-

viduals with CD4+ counts below 200 cells/μL within 6 months of seroconversion would be 10 per 10,000.

In an analogous presentation, on behalf of the Tri-Service HIV/AIDS Consortium, Dolan described the variations in natural history of HIV-1 infection in a cohort of 4,500 United States military personnel. Since 1985 there have been 2,700 seroconverters. Of these, 12% achieved the endpoint of CD4+ count below 200 cells/μL or AIDS within 12 months of seroconversion.

Acute HIV Infection: Response to Treatment

It has been proposed that coadministration of cyclosporin A with antiretroviral agents during AHI leads to decreased immune activation (Rizzardi et al, *J Clin Invest*, 2002). Khonkarly and colleagues (Abstract 567) conducted an open label, prospective trial of 77 adults with AHI who were randomized to starting therapy (n=43) or starting therapy and an 8-week course of cyclosporin A (n=34). The mean baseline HIV-1 RNA level in the cyclosporin A and no- cyclosporin A arms were 5.8 $\log_{10}$ copies/mL and 5.64 $\log_{10}$ copies/mL, respectively and the mean baseline CD4+ counts were 480 and 461 cells/μL, respectively. At week 36 the proportion of patients with plasma HIV-1 RNA below 50 copies/mL was higher in cyclosporin A group (96% vs. 70%, respectively, $P=.011$). Comparing the 2 study arms, there were no differences in HIV-1 RNA changes during 120 weeks of follow up. The CD4+/CD8+ count ratio normalized more rapidly in the cyclosporin A arm ($P=.001$).

Benson and colleagues (Abstract 570) presented data on the safety and efficacy of antiretroviral therapy initiated during AHI and early (<3 months of seroconversion) HIV-1 infection. Fifty-five subjects were divided into 2 groups: starting atazanavir 600 mg (once daily)/didanosine/stavudine (n=37, 12 with AHI); and receiving no therapy (n=18, 3 with AHI). The overall median baseline HIV-1 RNA level was 50,950 copies/mL. At week 48, 66% of the treated group achieved plasma HIV-1 RNA levels below 50 copies/mL, the difference was not statistically significant between those initiating therapy during early infection versus AHI. At week 48, the median CD4+ counts were 725 cells/μL and 496 cells/μL for treated and untreated individuals, respectively ($P=.018$). Twenty-nine of 37 treated individuals (78.4%) developed grade 3 or 4 elevations of total bilirubin. Five had to discontinue atazanavir.

Primary Infection: Treatment Interruptions

Bloch and colleagues (Abstract 569) evaluated virologic response following structured treatment interruptions (STI) in patients receiving hydroxyurea. Sixty-nine patients with AHI (42%) and early infection were randomized into 2 groups starting indinavir/ritonavir/didanosine/lamivudine with or without hydroxyurea, followed by up to 3 STIs. The overall median baseline HIV-1 RNA level was 5.73 $\log_{10}$ copies/mL. Fifty-nine patients underwent up to 3 STIs; of these, 18 maintained plasma HIV-1 RNA below 5000 copies/mL for 6 months off therapy. There were no statistically significant differences between treatment arms in the proportions of patients maintaining plasma HIV-1 RNA level below 50 copies/mL during an STI. During antiretroviral therapy, the mean CD4+ count increase from baseline was greater in the no-hydroxyurea arm (195 cells/μL) than in the hydroxyurea arm (100 cells/μL, $P=.014$). The number of serious adverse events was greater in the hydroxyurea arm (26% vs. 9%, $P=.11$). The authors concluded that hydroxyurea did not improve virologic outcome.

Superinfection

Grant and colleagues (Abstract 287) attempted to determine the frequency of HIV-1 sequentially expressed dual infections among 104 recently infected individuals. Highly divergent viral sequences appeared in 4 cases over time (incidence of 2.1/100 person years of observation). The authors used heteroduplex mobility assays and inspected electropherograms to confirm that the subsequent virus was not present in the baseline specimen; these methods have sensitivity of 1.5 to 3.0% for detection of minor sequence variants. Source partner recruitment will be attempted to determine if dual infection arose from sequential exposure and superinfections.

Plantier and colleagues (Abstract 288) described 1 patient initially infected with an HIV-1 group O variant who was superinfected with an HIV-1 group M strain within 3 months of seroconversion. Analysis of the *env* regions of the virus demonstrated that the patient was infected with a group M CRF02_AG recombinant strain phylogenetically related to the partner's viral *env* region.

Treatment Strategies

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

Fischl and colleagues (Abstract 162) presented the results of ACTG A5116, a randomized, open-label study enrolling 236 individuals with plasma HIV-1 RNA levels of 200 copies/mL or lower, previous PI or NNRTI experience, and no resistance mutations. Subjects were randomized to 1 of 2 treatment arms: lopinavir/ritonavir/efavirenz or efavirenz with 2 nRTIs. The composite endpoint was virologic failure (confirmed HIV-1 RNA above 2000 copies/mL) or toxicity-related treatment discontinuation. In the nRTI arm, 78% of patients received zidovudine/lamivudine, and 19% received stavudine/lamivudine. The median baseline CD4+ count overall was 475 cells/μL. The median follow-up time was 110 weeks. Overall, 70% (165) of subjects achieved plasma HIV-1 RNA level below 50 copies/mL: 74% in the nRTI arm and 66% in the lopinavir/ritonavir arm. Compared with the nRTI arm, there was a trend toward a higher rate of virologic failure ($P=.088$) and toxicity-related endpoints (17% vs 5%; $P=.002$) in the lopinavir/ritonavir arm, by intent-to-treat analysis.

Drug Reduction Strategy

Molina and colleagues (Abstract 573) presented the 3-year follow up of the ALIZE-ANRS 099 trial. This was an open-label, multicenter, noninferiority study enrolling 355 subjects with plasma HIV-1 RNA levels below 400 copies/mL who were on a stable PI-based regimen. Subjects were randomized to continue the initial regimen (n=177) or to switch to emtricitabine/didanosine/efavirenz once daily (simplification therapy, n=178). In the simplification arm, 152 patients (85%) completed week 48 of the study; of these, 147 (85%) were followed up until year

Table **3**. Treatment Strategies in Antiretroviral-Experienced Patients

Study Name (Abstract No.) Description	Regimen/ Study Arm (No. of Patients)	Baseline HIV-1 RNA (copies/mL)	Baseline CD4+ (cells/μL)	Plasma HIV-1 RNA Response (copies/mL)	CD4+ Change (cells/μL)	Comments
ESS30008 (Abstract 572) 48-week multicenter, open-label, randomized, comparative trial. Virologic failure was defined as week 48 plasma HIV RNA 0.5 $\log_{10}$ copies/mL increase over 400 copies/mL. All patients were abacavir/lamivudine experienced.	Abacavir/lamivudine fixed dose combination qd (n=130)	<50 (median)	565 (median)	81% with <50 at week 48	Not available	Most common third agent was efavirenz (62%), fosamprenavir/ ritonavir (17%), and nelfinavir (14%). Grade 2-4 AEs were similar in both arms, and no abacavir hypersensitivity reactions were reported. 39% in the qd arm vs 31% in bid arm achieved >95% adherence.
	Abacavir, lamivudine bid (n=130)	<50 (median)	549 (median)	82% with <50 at week 48	Not available	
SPRINT (Abstract 574) 48-week prospective, randomized, open-label comparative trial. Primary endpoint was week 24 and week 48 plasma HIV-1 RNA <50 copies/mL. Background regimen included 2 reverse transcriptase inhibitors.	Saquinavir 1600 mg/ ritonavir 100 mg qd (n=70)	5 $\log_{10}$ (median)	152 (median)	56% with <50 at week 24, and 50% at week 48	+147 (median)	The indinavir arm had a higher discontinuation rate (51% vs 34%), most commonly due to renal and gastrointestinal AEs. Both arms sustained similar changes in lipid and glucose levels.
	Indinavir 800 mg/ ritonavir 100 mg bid	5 $\log_{10}$ (median)	122 (median)	49% with <50 at week 24, and 45% at week 48	+131 (median)	
CPCRA 064 (Abstract 579) Randomized, prospective, clinical endpoint trial evaluating the impact of STI on HIV disease progression in patients with virologic failure. Median length of follow-up was 36 months.	4-month STI (n=140)	5 $\log_{10}$ (mean)	183.3 (mean)	-0.8 $\log_{10}$ at month 24 (mean)	-3.2 (mean)	Total number of deaths were similar in both arms (30 in STI vs 33 in control); however, there were more progression of disease events in the STI arm (adjusted hazard ratio=1.66, *P*=.04).
	No STI (n=134)	5 $\log_{10}$ (mean)	177.5 (mean)	-0.8 $\log_{10}$ at month 24 (mean)	+39.6* (mean)	
CTN 164 (Abstract 580) 60-week, multicenter, open-label randomized trial to evaluate the impact of STI vs immediate salvage (IS) therapy on virologic outcome in patients with virologic failure. Study was terminated by SERC because of slow recruitment.	12-week STI (n=67)	3.9 $\log_{10}$ (median)	320 (median)	-1.7 $\log_{10}$ at week 60 (median)	+25 (median)	There was no difference in proportion of patients maintaining plasma HIV-1 RNA level <50 copies/mL for 3 months (69% in IS vs 55% in STI arm, *P*=.11).
	IS (n=67)	3.9 $\log_{10}$ (median)	360 (median)	-1.7 $\log_{10}$ at wk 60 (median)	+94.5† (median)	

continued, next page

Table **3**. Treatment Strategies in Antiretroviral-Experienced Patients (continued)

Study Name (Abstract No.) Description	Regimen/ Study Arm (No. of Patients)	Baseline HIV-1 RNA (copies/mL)	Baseline CD4+ (cells/μL)	Plasma HIV-1 RNA Response (copies/mL)	CD4+ Change (cells/μL)	Comments
Enfuvirtide Salvage (Abstract 581) 48-week pilot study to evaluate virologic outcome of STI prior to enfuvirtide-based salvage regimen Patients had plasma HIV-1 RNA level >500 copies/mL and resistance to >2 PIs, >2 nRTIs, >1 NNRTIs.	16-week STI followed by enfuvirtide/ optimized background (n=15)	4.79 $\log_{10}$ (median)	47 (median)	36% with <75 at week 24 and 48	Not available	Patients with virologic failure (return to <0.5 $\log_{10}$ copies/mL below baseline by week 16) had enfuvirtide resistance mutations in HR-1 sequence within 2 to 4 weeks of treatment.
	Immediate enfuvirtide/ optimized background (n=15)	4.62 $\log_{10}$ (median)	26 (median)	53% with <75 at week 24 and 48‡	Not available	

AEs indicates adverse events; bid, twice daily; HR1, helical region 1; NNRTI, nonnucleoside reverse transcriptase inhibitor; nRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; qd, once daily; SERC, Safety and Efficacy Review Committee; STI, structured treatment interruption.
*P=.07.
†P=.04.
‡Difference in virologic outcomes between study arms was not statistically significant.

3. The proportion of subjects with plasma HIV-1 RNA levels below 400 copies/mL in the simplification arm was 23% by intent-to-treat and 6% by on-treatment analysis. From month 12 to month 36, 27 patients in the simplification arm discontinued antiretroviral therapy due to patient choice (5 patients), treatment failure (2 patients), and adverse effect (8 patients). After 36 months of simplification therapy, there was no increase in total cholesterol level or LDL cholesterol level; 42% of subjects had plasma HDL cholesterol levels above 60 mg/dL. The authors concluded that the once daily regimen of emtricitabine/efavirenz/didanosine was not inferior to the PI-based regimen.

Sosa and colleagues (Abstract 572) presented results of ESS30008, a 48-week, randomized, open label, multicenter study comparing fixed-dose abacavir/lamivudine to twice-daily abacavir and lamivudine. The study enrolled 260 individuals with plasma HIV-1 RNA levels below 400 copies/mL and CD4+ counts of 50 cells/μL or above who were receiving abacavir/lamivudine twice daily with a PI or NNRTI. Subjects were randomized to continue abacavir and lamivudine twice daily or start fixed-dose abacavir/lamivudine. Virologic failure was defined a as plasma HIV-1 RNA 0.5 $\log_{10}$ copies/mL increase over 400 copies/mL (plasma HIV-1 RNA ≥ 1265 copies/mL). The overall median baseline plasma HIV-1 RNA was below 50 copies/mL; the median baseline CD4+ counts were 565 cells/μL and 549 cells/μL in the once-daily and twice-daily arms, respectively. Ninety-two percent of subjects in the once-daily arm and 90% in the twice-daily arm completed the study. The most common third drugs were efavirenz (62%), fosamprenavir/ritonavir (17%), and nelfinavir (14%). Comparing the 2 study arms, there were no statistically significant differences in median CD4+ counts during follow-up, nor were there such differences in the proportions of patients with virologic failure, with HIV-1 RNA level below 50 copies/mL at week 48 (81% in the once-daily arm vs 82% in the twice-daily arm; P=.76). There were also no such differences in grades 2 to 4 adverse events: 2 subjects in the twice-daily arm withdrew due to weight gain and lymphoma that were not treatment related. Two subjects in the once-daily and 4 in the twice-daily arm met criteria for virologic failure after documented antiretroviral nonadherence. A greater proportion of subjects in the once-daily arm (39%) than the twice-daily arms (31%) maintained at least 95% adherence to the antiretroviral regimen.

Structured Treatment Interruptions in Antiretroviral-Experienced Persons

Lawrence and colleagues (Abstract 579) presented the final results of the CPCRA 064 study, a randomized, prospective study enrolling 274 individuals with virologic failure that evaluated the impact of STI on HIV disease progression. Subjects on a stable antiretroviral regimen who had plasma HIV-1 RNA levels above 5000 copies/mL and multi-drug-resistant HIV documented by genotypic testing were randomized to a 4-month STI followed by a salvage regimen (n=140) or immediate initiation of a salvage regimen (n=134). The primary endpoint was progression of HIV disease or death. The overall mean baseline CD4+ count and plasma HIV-1 RNA level were 180 cells/μL and 5.0 $\log_{10}$ copies/mL, respectively; 58% of subjects had developed a prior opportunistic infection. At study entry, 96.7% of subjects had 3-class drug exposure, a median of 11 prior antiretroviral drugs and a mean of 9.7 drug resistance mutations. The median follow-up time was 36 months. At month 4, the mean changes in plasma HIV-1 RNA were a 0.4 $\log_{10}$ copies/mL increase in the STI arm and 0.8 $\log_{10}$ copies/mL decrease in the no-STI arm (P<.0001). Differences in plasma HIV-1 RNA levels at 24 months were not statistically significant. The mean change in

CD4+ count at months 4 and 24 were greater in the STI arm than in the no-STI arm; the difference persisted after 24 months (39.6 cells/μL increase vs 3.2 cells/μL decrease, respectively; $P = .007$). Subjects in the STI arm experienced a reduction in the number of mutations assessed (from 10 to 5.1) and 27% of subjects in the STI arm lost all mutations as assessed by standard sequencing of plasma present at study entry. There were no differences between the treatment arms in the total number of deaths: 30 in the STI and 33 in the no-STI arm ($P = .91$). The STI arm experienced more progression of disease events than did the no-STI arm, with 44 events in the STI arm and 29 in the no-STI arm (hazard ration [HR] = 1.66; $P = .04$). The most common progression of disease event was esophageal candidiasis (26.3% and 10.4%, respectively) and *pneumocystis* pneumonia (12.3% and 6.3%, respectively). The authors confirmed that there was no benefit conferred by STI as part of salvage therapy in patients with advanced disease and multidrug-resistant HIV.

Henry and colleagues (Abstract 582) presented the results of ACTG A5102. This was a randomized pilot study enrolling antiretroviral experienced individuals with CD4+ counts above 500 cells/μL and plasma HIV-1 RNA levels below 200 copies/mL. Subjects were randomized to 1 of 2 arms: continue antiretroviral therapy with 3 5-day cycles of interleukin-2 (IL-2) administered every 8 weeks (n = 23); or continue antiretroviral therapy for 18 weeks. At the end of treatment period, subjects with CD4+ counts above 500 cells/μL underwent a treatment interruption (TI) until the CD4+ count dropped below 350 cells/μL. The median follow-up on TI was 78 weeks; the median baseline CD4+ count overall was approximately 750 cells/μL. Subjects in the IL-2 arm maintained higher CD4+ counts for 72 weeks during the TI. A higher CD4+ count before antiretroviral therapy was associated with a longer time to CD4+ count below 350 cells/μL. Higher plasma HIV-1 RNA level in the beginning of TI was associated with shorter time to CD4+ count below 350 cells/μL (HR = 3.88; $P = .042$).

Deeks and colleagues (Abstract 680) evaluated the effect of stopping enfuvirtide therapy in patients with enfuvirtide resistance. There were 22 subjects in whom enfuvirtide was failing. The mean baseline plasma HIV-1 RNA level was 5.08 $\log_{10}$ copies/mL, and mean duration of enfuvirtide therapy was 28.6 weeks; these subjects stopped enfuvirtide while continuing background antiretroviral therapy. At weeks 2 and 4, the mean increases in plasma HIV-1 RNA level were 0.11 $\log_{10}$ copies/mL ($P = .3$) and 0.27 $\log_{10}$ copies/mL ($P = .03$), respectively. At baseline, clonal analysis of HR-1 sequences documented the presence of enfuvirtide-associated mutations in 21 of 22 patients. At week 16, enfuvirtide mutations were no longer detectable in clones from the majority of subjects ($P = .003$). The authors concluded that this is consistent with a fitness cost associated with enfuvirtide resistance.

The Emergence of Resistance During STIs

Ceccherini-Silberstein and colleagues (Abstract 681) investigated the disappearance of PI mutations during TI in 88 patients with at least 2 genotypes within 1 year: 1 during PI-containing regimen failure and 1 during TI (median length, 4 months). Mutations L33F, I47V, F53L, G73S, N88D, M46I/L and L90M disappeared in 33%, 66%, and 99% of patients at 6-, 9-, and 12-month intervals during TI, respectively. Mutations K20T, K43T, Q58E, T74S, I85V, Q92K, and C95F disappeared at a similar rate during TI. Mutations M36I, L63P, V77I were maintained in more than 75% of patients during TI. At month 3 of TI (n = 23), major PI mutations reverted back to wild type in 14 out of 23 patients (61%); among these, there was a trend toward lower plasma HIV-1 RNA level ($P = .08$). The disappearance of M46I and L90M was associated with greater plasma HIV-1 RNA increase and greater CD4+ cell decrease ($P < .001$). Maintenance of M36I, L63P, and V77I compensatory mutations was associated with higher plasma HIV-1 RNA increase ($P < .001$). The authors concluded that M46I and L90M confer a disadvantage for viral fitness.

Antiretroviral Drug Resistance and Replicative Capacity

Transmission of Drug-Resistant Virus

On behalf of the Duke-University of North Carolina-Emory Acute HIV consortium and the STAT program, Hicks (Abstract 673) presented data on genotypic resistance profiles of virus from 127 individuals with AHI and early infection diagnosed between January 1998 and December 2004. Overall, major resistance mutations were detected in 19.7% of cases and they did not differ significantly among the 3 major classes of antiretroviral agents: 7.1% for nRTI, 6.3% for NNRTI, 3.1% for PI. Prevalence of resistance mutations was similar among AHI and early infections.

In a complementary presentation, De Mendoza and colleagues (Abstract 672) documented a reduction in the rate of transmitted drug resistance among 198 consecutive newly HIV-infected individuals seen between January 1997 and December 2004 in 11 hospitals in Spain. The overall rate of genotypic resistance in this population was 12.1%, and 9.3%, 4.1%, and 2.1% had nRTI, NNRTI, or PI resistance mutations, respectively. The rates of drug resistance in 1997, 2003, and 2004 were 33.3%, 10.4% and 8.2%, respectively. The authors also noted a rise in the transmission of non-B subtypes of HIV-1.

Shet and colleagues (Abstract 289) described the transmission of drug-resistant HIV among 112 individuals with AHI presenting to the Aaron Diamond AIDS Research Center in New York between 2003 and 2004. The median duration of infection was 58 days, 98% were men who have sex with men (MSM). Of them, 27 individuals (24.1%) had drug-resistant HIV-1. Compared with the period between 1995 and 1998, NNRTI resistance increased from 2.6% to 13.4% in 2003 through 2004 ($P = .04$), PI resistance increased from 1.3% to 7.1% in 2003 through 2004 ($P = .09$). The prevalence of nRTI-associated mutations has remained stable since 2000.

The role of genital tract shedding in transmission of drug resistance was highlighted by several presentations at the conference. Katzenstein and colleagues (Abstract 670) presented the results from A5077, a 96-week, multicenter, longitudinal study evaluating genotype of plasma virus associated with genital tract shedding. The study enrolled 88 individuals with plasma HIV-1 RNA levels more than 2000 copies/mL, with matched genital tract samples for whom genotypic susceptibility score (GSS) was obtained within 10 days (n = 29) or 90

days (n = 53) of starting antiretroviral therapy. Overall, subjects had a median of 5.6 years of prior antiretroviral therapy and a median plasma HIV-1 RNA level of 4.7 $\log_{10}$ copies/mL. Among these highly drug-experienced patients, more than 50% of women had resistance to more than 2 NNRTIs drugs; men tended to have less NNRTI resistance and more PI resistance (GSS, 2.05 and 1.45, respectively; $P = .08$). Overall, the frequency of genital tract HIV shedding was 10-fold higher among men receiving antiretroviral therapy than women. The authors concluded that these observations may explain the increasing frequency of drug resistance among newly infected MSM.

Newstein and colleagues (Abstract 671) aimed to describe drug resistance in plasma and genital tracts of NNRTI-experienced women failing antiretroviral therapy. In 7 out of 8 women, NNRTI mutations were detected in both plasma and genital samples; K103N was detected in both sites in 4 out of 5 patients who had been off therapy for up to 28 months. The authors concluded that NNRTI mutations are stable in the female genital tract in the absence of drug selection.

Treatment-Experienced Patients

Didanosine. Bates and colleagues (Abstract 105) described the relationship between baseline phenotypic susceptibility and week-4 HIV-1 RNA level response to didanosine in the JAGUAR study. This was a multicenter, randomized, double-blind, placebo-controlled study of 168 individuals with plasma HIV-1 RNA levels between 3 and 5 $\log_{10}$ copies/mL and CD4+ counts above 100 cells/μL, who were randomized to add didanosine (n = 111) or placebo (n = 57) to a current, stable regimen. At week 4, the median changes in HIV-1 RNA in the didanosine and control arms were a 0.56 $\log_{10}$ copies/mL decrease and a 0.07 $\log_{10}$ copies/mL increase, respectively ($P < 0.0001$). The highest mean $\log_{10}$ change from baseline in plasma HIV-1 RNA levels occurred in individuals with a didanosine fold change (FC) less than 1.3 (-1.01 $\log_{10}$ copies/mL), compared with those with a didanosine fold change greater than 2.2 (-0.24 $\log_{10}$ copies/mL; $P < 0.001$). At a didanosine fold change less than 1.3, individuals experienced high response rates, while at a fold change greater than 2.2, the response rates were considerably lower. The authors proposed clinical cutoffs at 1.3 and 2.2 for didanosine to identify a zone identifying the probability of an intermediate virologic response.

nRTI-Associated K65R Mutation. The K65R mutation reduces TAM-mediated excision of zidovudine and is responsible for reversal of zidovudine resistance. Parikh and colleagues (Abstract 98) attempted to demonstrate that K65R and 215Y/F do not exist on the same genome. A total of 59,262 samples from a commercial database with K65R in combinations with 2 or more TAMs were analyzed. Of these, 3.2% had K65R and 14.2% had 215Y/F or 2 or more TAMs. The expected frequency of having both K65R and TAMs was 0.5% and the actual frequency of both was 0.04%. Single genome sequencing was performed on 173 genomes from 10 samples; K65R and any TAM occurred together in less than 10% of the samples and among these K65R was never found on the same genome with T215FY/F/I.

Atazanavir. Coakley and colleagues (Abstract 716) described a PI-naive patient who developed the N88S mutation without the sentinel atazanavir mutation I50L on atazanavir/ritonavir therapy. The patient had a baseline plasma HIV-1 RNA level of 6547 copies/mL, replicative capacity of 96%, and no baseline PI mutations when he started on atazanavir/tenofovir/abacavir/lamivudine. Ritonavir was added at month 3, plasma HIV-1 RNA level less than 50 copies/mL at month 4, and increased to 7535 copies/mL at month 11. The genotype at that time demonstrated K20T, M36I/V, L63P, A71T, and N88S mutations. The fold changes to atazanavir, amprenavir, indinavir, lopinavir, nelfinavir, and saquinavir were 56, 0.3, 13, 4.3, 68, and 4.2 respectively. The replicative capacity was 14%. In one commercial database, the N88S mutation was associated with resistance to atazanavir, nelfinavir, and indinavir, susceptibility to lopinavir, and ritonavir, and hypersusceptibility to amprenavir. Isolates with I50L mutations, on the other hand, demonstrated atazanavir-specific resistance, susceptibility, and hypersusceptibility to ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, and saquinavir. Surveillance of the database revealed that among samples with at least 1 major PI mutation, the prevalence of N88S and I50L increased from 1.04% and 0.15% in December 2003, to 2.86% and 3.65% in January 2005, respectively.

Tipranavir/Ritonavir. Schapiro and colleagues (Abstract 104) evaluated the resistance profile of tipranavir/ritonavir in the RESIST-1 and RESIST-2 trials. Patients with 3-class experience and 1 or more major PI mutations (among 30N, 46I/L, 48V, 50V, 82A/F/L/T, 84V, and 90M), were randomized to tipranavir/ritonavir (n = 582) or a comparator PI/ritonavir (CPI/r; n = 847). At baseline, 59% of patients in the tipranavir arm and 61% in the CPI/r arm had 3 or 4 primary PI mutations. The primary endpoint was defined as a confirmed 1 $\log_{10}$ copy/mL decrease in plasma HIV-1 RNA level from baseline. At week 24, patients in the tipranavir/ritonavir arm had better treatment responses than those in the CPI/r arm regardless of the number of baseline protease gene mutations. Among subjects with 12 or less and 19 or more protease gene mutations, 50.4% versus 29.8% and 31.7% versus 7.7% of subjects in the tipranavir/ritonavir and CPI/r arms, respectively, reached the primary endpoint of more than 1 $\log_{10}$ copy/mL decrease in plasma HIV-1 RNA from baseline. Twenty individuals had what was described as PI-resistance–associated mutations (PRAMs): L33W/F, V82A/F/L/T, I84V and L90M. Among these there was no difference in virologic response between arms in subjects with 0 PRAMs; however, with 1 and 2 PRAMS, 44% versus 25% and 41% versus 15% in the tipranavir/r and CPI/r arms, respectively, achieved treatment response.

Enfuvirtide. Enfuvirtide resistance is associated with changes at amino acids positions 36 to 45 of the N-terminal region of the first heptad repeat (HR1) in the envelope gene. Labrosse and colleagues (Abstract 97) investigated the evolution of enfuvirtide resistance in 6 patients with different degrees of susceptibility on enfuvirtide-containing salvage therapy. Baseline susceptibilities to enfuvirtide ranged from 56 ng/mL to 756 ng/mL. Mutations emerged at positions 36, 38, and 43 in the context of *env* quasispecies different from dominant

baseline populations, and were not accompanied by loss of *env*-dependent activity. In 4 patients, HIV-1 fitness increased from baseline. The authors concluded that mutations conferring enfuvirtide resistance are selected repeatedly in different *env* genetic backgrounds, resulting in replacement of dominant virus populations over time.

Maroldo and colleagues (Abstract 717) described the rapid selection of enfuvirtide resistance due to a single V38E mutation within the enfuvirtide binding site, appearing on day 9 of therapy. Mutations G36G/D and V38V/E/A were noted in the HR1 domain; clonal analysis of the gp160 *env* demonstrated that the G36D mutation was present on distinct strains from those carrying V38A or V38E. Samples from day 580 of therapy demonstrated persistence of only the V38E mutation. High level phenotypic resistance and reduced viral fitness were previously described with the V38E mutation in the context of N42S (Lu et al, *J Virol*, 2004). The authors concluded that the rapid outgrowth and persistence of V38E is consistent with a high selective advantage of this mutation.

Cabrera and colleagues (Abstract 718) described sequential accumulation of mutations in the gp41 *env* region after long-term enfuvirtide therapy. Samples were derived from 15 highly treatment-experienced individuals with virologic failure on enfuvirtide-containing salvage regimens. Mutations in HR1 and HR2 were analyzed by population-based sequencing. Mutations G36D/S, V38A, and N43D appeared 2 to 4 weeks after initiation of enfuvirtide. Double mutations V38A/G36V, V38A/N43D, G36V/N43D were established early in the treatment but never coexisted in the same viral genome.

UK-427,857. UK-427,857 is an investigational CCR5 blocker that binds to CCR5 through a triazole group. Virus resistant to UK-427,857 has mutations in the V3 loop. Westby and colleagues (Abstract 96) identified 8 CCR5 antagonists structurally related to UK-427,857 and investigated their binding sites and potential for developing cross-resistance. These compounds bind CCR5 in a pocket formed by the transmembrane helices and extracellular loop 2. UK-427,857-resistant variants are cross-resistant to compounds with a triazole functional group, but not with an imidazopiperidine group. The authors concluded that resistance to CCR5 antagonists may not lead to drug-class resistance.

Interactions Among RTI Resistance Mutations

Two presentations focused on interactions of L74V with other mutations. Frankel and colleagues (Abstract 698) used real-time polymerase chain reaction (PCR) to demonstrate that L74V was associated with a 50% reduction in the efficiency of synthesis of viral DNA and excision of zidovudine-terminated DNA synthesis. The presence of M184V further potentiates this effect. The authors concluded that M184V, K65R, and L74V share a common mechanism of resistance to nRTIs and their effects on reverse transcriptase.

Miranda and colleagues (Abstract 699) demonstrated that L74V interferes with the primer unblocking in the presence of TAMs, though to a lesser extent than M184V. The effect of M184V on primer unblocking was greatest in viruses with M41L, L210W, T215Y, and impaired in the presence of 6 TAMs. The authors concluded that this is the mechanism by which L74V partially reverses TAM-mediated zidovudine resistance.

Several posters presented data about the effects of interactions between nRTI and NNRTI mutations on HIV-1 replicative capacity and fitness. It was previously noted in ACTG 368 that K103N plus L100I were frequently associated with L74V in the nRTI-sparing arm of the study. L100I is selected for during efavirenz therapy and adds to the resistance of K103N. Koval and Demeter (Abstract 700) used growth competition experiments in H9 cells to elucidate whether L74V compensates for the replication fitness defect seen in the K103N/L100I double mutant. The K103N/L100I double mutation was associated with reduced fitness compared with K103N ($P < .0001$), and the relative fitness of the L74V triple mutant (L74V/K103N/L100I) was significantly higher than the K103N/L100I double mutant.

Colson and colleagues (Abstract 701) investigated the frequency of the K65R/L74V double mutant in the Marseille database. This was a retrospective analysis of 3201 patients and 7151 sequences: 12 of 3201 patients carried the K65R/L74V combination of mutations and 4 of these had virologic failure on a nRTI-based regimen and no protease resistance mutations. This is contrary to in vitro data reporting diminished replicative capacity and dysfunctional reverse transcriptase in the presence of K65R/L74V.

Wirden and colleagues (Abstract 702) analyzed linkage of K65R mutation with TAMs and L74V in 5 samples from patients obtained after tenofovir failed. Clonal analysis revealed association of K65R with M41L, D67N, L210W, and K219E, but no linkage between K65R with L74V.

Coakley and colleagues (Abstract 704) evaluated the effect of non-thymidine analogue nucleoside analogue mutations (non-TA NAMs) on efavirenz hypersusceptibility, defined as fold change in IC_{50} of less than 0.4. Samples from a commercial database with unmixed nRTI mutations (K65R, T69X, l74I/V, V75X, M184I/V; X = any amino acid change) but no TAMs (ie, none of M41L, D67N, K70R, L210W, T215FY, K219X) and no Q151M, T69 insertions, or NNRTI mutations were identified. Isolates without nRTI, NNRTI, and PI mutations served as the control, wild-type group. The mean efavirenz fold change was lower in isolates containing non-TA NAMs or TAMs than in control isolates. Over 40% of isolates with K65R, K65R/M184V double mutants, or 3 TAMS (including T215Y) had fold change in efavirenz IC_{50} of less than 0.4. The authors concluded that efavirenz hypersusceptibility is associated with non-TA NAMs as well as TAMs.

Resistance in Non-Subtype B Infections

Grossman and colleagues (Abstract 719) compared virologic response and resistance to lopinavir/ritonavir between clade C and clade B HIV-1 viruses. Samples were obtained from 37 subtype-B and from 49 subtype-C individuals with mean PI-treatment duration overall of 20 months. The mean plasma HIV-1 RNA levels were 5.14 $\log_{10}$ copies/mL and 5.24 $\log_{10}$ copies/mL in subtype C and B groups, respectively. Mutations were present with the following prevalence in clade C and B patients, respectively: 49% and 57% with L10I/V/F; 55% and 30% with K20R ($P = .03$); 4% and 14%

with L24I ($P = .07$); 33% and 32% with M46I; 39% and 46% with I54V; 33% and 92% with L63P ($P < .001$); 22% and 41%, with A71V ($P < .001$); 43% and 35% with V82A; 10% and 24% with I84V; 27% and 14% with L90M; and 98% and 46% with L93I. Mean lopinavir/ritonavir mutational score was 3.29 in subtype-C and 4.05 in subtype B, and the difference was not statistically significant. The authors concluded that the prevalence of lopinavir-associated mutations is similar in clade B and C patients in whom therapy is failing. Differences in frequencies of mutations in codons 20, 24, and 63, may be due to baseline polymorphic variations between subtypes.

Bessong and colleagues (Abstract 721) analyzed resistance mutations in the protease region of 40 antiretroviral-naive, subtype C-infected individuals from South Africa. No major PI mutations were detected; M36I and I93L were detected in 90% of the sequences; the combination of K20R and M36I mutations conferring indinavir and ritonavir resistance were detected in 25% of isolates.

Fitness and Replicative Capacity

Hicks and colleagues (Abstract 345) described an inverse relationship between baseline replicative capacity and CD4+ count among 132 antiretroviral-naive individuals who achieved plasma HIV-1 RNA level below 400 copies/mL after 12 months of antiretroviral therapy. The mean baseline CD4+ count was 223 cells/μL and mean plasma HIV-1 RNA level 4.9 $\log_{10}$ copies/mL. There were 52% and 37% of individuals who received a combination of NNRTI with a nRTI or nRTI with a PI, respectively. Baseline replicative capacity was inversely correlated with baseline CD4+ count ($P = .003$). After adjusting for baseline CD4+ and plasma HIV RNA level, for every increase of 1% in the replicative capacity, there was a corresponding decrease in baseline CD4+ count of 0.88 cells/μL ($P = .049$).

Martinez-Picado and colleagues (Abstract 691) examined the relationship between hypersusceptibility phenotype to multiple PIs and HIV-1 replicative capacity among 12 patients undergoing 5 consecutive STIs. Samples were collected at the peak of viremia during each STI. Ten patients had at least 1 sample demonstrating hypersusceptibility to 1 or more PIs. The median drug susceptibility overall for amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir was 0.2 fold-change lower than the median drug susceptibility for wild-type virus from the a commercial database. The mean replicative capacity of recombinant virus containing the 3'-end of *gag*, the protease and reverse transcriptase regions of all the isolates was 53% lower than expected for wild-type virus, and there was significant correlation between susceptibility to PIs and replicative capacity in all 12 patients. Replicative capacity of the virus did not change during the STIs, and there was no apparent association between replicative capacity as measured in vitro and CD4+ count, CD8+ count, and plasma HIV-1 RNA level at each treatment interruption.

De Luca and colleagues (Abstract 692) evaluated the relationship between treatment outcome and HIV-1 replicative capacity in 139 patients from the ARGENTA trial. Patients included those in whom antiretroviral therapy was failing; the mean plasma HIV-1 RNA level was 4.28 $\log_{10}$ copies/mL and the median CD4+ count was 264 cells/μL. At baseline, the median replicative capacity was 59% and the median phenotypic susceptibility score (PSS) of the first salvage regimen was 2. At month 36, higher PSS predicted better virologic response; the mean decrease from baseline plasma HIV-1 RNA of 1.6 $\log_{10}$ copies/mL and 0.8 $\log_{10}$ copies/mL for PSS of 2 or greater and PSS less than 2, respectively ($P = .040$). Replicative capacity was inversely correlated with decreased susceptibility to PIs. Among 85 patients with persistent plasma HIV-1 RNA below 500 copies/mL, after adjusting for PSS, replicative capacity above 65% was associated with lower CD4+ count gains at month 3 (mean decrease of 58 cells/μL; $P = .04$); month 9 (mean decrease of 147 cells/μL; $P = .003$), and month 24 (mean decrease of 125 cells/μL; $P = .047$. Among individuals with PSS of 3 and on their first salvage regimen (n = 25), a 1-log higher replicative capacity was associated with higher plasma HIV-1 RNA levels at 3 months (mean plasma HIV-1 RNA increase from baseline of 1.52 $\log_{10}$ copies/mL; $P = .019$). In a univariate analysis, a log change in replicative capacity was not associated with clinical progression (HR, 0.79; $P = 0.66$). The authors concluded that in persistently viremic patients, higher replicative capacity predicted worse 3-month virologic and 24-month CD4+ count outcomes.

Predictors of Response

On behalf of the British Columbia Center for Excellence in HIV/AIDS, Hogg (Abstract 712) described the effect of increasing antiretroviral resistance on survival among 1388 individuals initiating antiretroviral therapy between August 1996 and July 2000. During a median follow-up time of 52.7 months, 238 individuals died (17.2%, all-cause mortality). Resistance testing was available on 3120 samples; of these, drug resistance to at least 1 class and all 3 classes of antiretrovirals was noted in 28.5% and 7.9%, respectively. After controlling for age, CD4+ count, and plasma HIV-1 RNA level, NNRTI and nRTI resistance other than the presence of M184V, was associated with an increased risk of death (odd ratios [ORs], 2.07 and 2.93, respectively), and development of PI resistance was protective (OR, 0.32).

Previous studies have suggested that TAMs develop by 1 of 2 distinct pathways described as TAM1 (41L, 210W, and 215Y) and TAM2 (67N, 70R, 219E/Q). The effects of a specific profile on virologic outcome were highlighted by several presentations at the conference.

Antinori and colleagues (Abstract 709) presented results of a retrospective analysis documenting the effect of TAM1 and K65R mutations on virologic outcome in 172 patients from 10 Italian centers initiating salvage therapy with tenofovir/stavudine. Of these, 88.4% and 77.3% of patients were previously exposed to zidovudine and stavudine, respectively. Virologic failure was defined as an HIV-1 plasma RNA level above 500 copies/mL. The median baseline plasma HIV-1 RNA level and CD4+ count were 4.26 $\log_{10}$ copies/mL and 229 cells/μL, respectively. At baseline, 19.1% of patients had TAM1, 10.3% TAM2, 76% M184V, 42% T215Y/F, 33% M41L, 29% D67N, and 22% L210W mutations. At month 12, 50% and 66% of patients achieved plasma HIV-1 RNA levels of below 50 copies/mL and below 500 copies/mL, respectively. Multivariate analysis revealed that presence of either 1 TAM1 or all TAM1 mutations was associ-

ated with a greater risk of virologic failure (adjusted HRs [AHRs], 1.65, $P = .003$ and 2.53; $P = .048$, respectively). M184V was associated with a greater reduction in plasma HIV-1 RNA level at month 6 and 12, AHR for virologic failure of 0.36 ($P = .024$) in multivariate analysis. Among 17 evaluable patients with virologic failure, no K65R was detected at baseline or month 12; the prevalence of stavudine associated mutations increased from baseline: D67N from 23% to 46%; L210W from 31% to 67%; K219Q from 7.7% to 15%. The authors concluded that accumulation of TAMs, not K65R, is most predictive of virologic failure on tenofovir/stavudine.

Landman and colleagues (Abstract 710) presented data on the virologic outcome of salvage antiretroviral therapy in the presence of the K65R mutation. Fourteen patients from the TONUS trial (antiretroviral-naive patients on tenofovir/lamivudine/abacavir) and 8 patients from GS 903 study (randomized study of antiretroviral-naive patients on tenofovir/lamivudine/efavirenz or stavudine/lamivudine/efavirenz) were included. Baseline mutations included K65R/M184V (n = 14), K65R/M184V/NNRTI mutant (n = 5) and K65R/NNRTI mutant (n = 3). The median baseline plasma HIV-1 RNA level was 6336 copies/mL. Salvage therapy was initiated: tenofovir and lamivudine were continued in 5 and 9 patients, respectively; zidovudine and didanosine were started in 13 and 11 patients, respectively; a PI was the third agent in 16 patients and an NNRTI in 6 patients. At week 48, 86% achieved a plasma HIV-1 RNA below 50 copies/mL and the mean replicative capacity was 54%. The K65R/M184V double mutants were hypersusceptible to efavirenz, nevirapine, and zidovudine. The authors concluded that this may have contributed to the virologic response in patients receiving efavirenz or zidovudine as part of salvage therapy. Johnson and colleagues (Abstract 711) studied the effect of the number of baseline PI mutations on virologic response at week 96 in a post-hoc analysis of the BMS AI424-045 study. Subjects (n = 358) were randomized to 1 of 3 arms: atazanavir 300 mg once daily/ritonavir 100 once daily (n = 120); lopinavir 400 twice daily/ritonavir 100 twice daily (n = 123); and atazanavir 400 once daily/saquinavir 1200 mg once daily (n = 115). All patients received tenofovir and an nRTI. The median baseline plasma HIV-1 RNA level and CD4+ count were 4.44 $\log_{10}$ copies/mL and 295 cells/μL, respectively. Median number of baseline PI and nRTI mutations were 2 and 3, respectively. In 33% of patients in the atazanavir arms and in 38% in the lopinavir arm, 4 or more PI mutations were present at baseline; among these, the mean decreases in plasma HIV-1 RNA levels at week 96 were 1.71 $\log_{10}$ copies/mL, 0.93 $\log_{10}$ copies/mL, 1.81 $\log_{10}$ copies/mL in the atazanavir/ritonavir, atazanavir/saquinavir, and lopinavir/ritonavir arms, respectively. Among individuals with fewer than 4 PI mutations at baseline, the mean decreases were 2.47 $\log_{10}$ copies/mL for atazanavir/ritonavir; 2.17 $\log_{10}$ copies/mL for atazanavir/saquinavir, and 2.21 $\log_{10}$ copies/mL for lopinavir/ritonavir. The atazanavir/saquinavir arm was the least effective virologically, irrespective of the number of PI mutations.

Pharmacology

Selected Drug-Drug Interactions

Enteric-coated Didanosine/Atazanavir. Enteric coated didanosine (didanosine EC) and atazanavir are potential components of a once-daily antiretroviral regimen. However, didanosine is usually given without food and atazanavir with food. Kaul and colleagues examined the interaction of these 2 drugs when given together with food in 35 healthy volunteers (Abstract 648). They found that the C_{max} and area under the concentration curve (AUC) of didanosine were reduced approximately 35% when given with food and either atazanavir 400 mg or atazanavir 300 mg/ritonavir 100 mg compared with being given in the fasted state. The pharmacokinetics of atazanavir were not affected by coadministration of didanosine.

Buprenorphine and Efavirenz. Buprenorphine is a partial opioid agonist that was recently approved by the US Food and Drug Administration (FDA) for the treatment of opioid dependence. Efavirenz lowers the plasma levels of methadone, potentially leading to a withdrawal syndrome. The effects of efavirenz on buprenorphine pharmacokinetics are unknown. McCance-Katz and colleagues studied buprenorphine pharmacokinetics before and after 1 week of efavirenz administration in 10 buprenorphine-maintained, HIV-seronegative volunteers (Abstract 653). They found that efavirenz lowered the exposure (ie, AUC) by 50% and increased the rate of buprenorphine clearance. However, efavirenz did not cause opioid withdrawal in these volunteers; the efavirenz levels were in the therapeutic range. This suggests that buprenorphine may be preferable to methadone in HIV-infected patients who are prescribed efavirenz.

Rifampin and Atazanavir. Burger and colleagues examined the interaction of rifampin and atazanavir in HIV-seronegative volunteers (Abstract 657). Rifampin reduces the levels of other PIs and they should not be coadministered. The investigators determined the atazanavir pharmacokinetics using 3 different once-daily doses of atazanavir/ritonavir (300 mg/100 mg, 300 mg/200 mg, 400 mg/ 200 mg) given with rifampin 600 mg once daily. They compared these doses with atazanavir given without rifampin (400 mg without ritonavir and 300 mg with ritonavir 100 mg). They found that all 3 doses given with rifampin yielded atazanavir levels significantly lower than levels seen with 300 mg/100 mg once-daily. Compared with atazanavir 400 mg once daily, only atazanavir/ritonavir 400 mg/200 mg yielded comparable 24-hour AUC and C_{max} values. However, all 3 doses given with rifampin yielded significantly lower minimum concentration (C_{min}). The authors concluded that atazanavir should not be co-administered with rifampin at the doses studied.

Omeprazole and Atazanavir. Proton pump inhibitors are known to lower levels of atazanavir. Agarwala and colleagues tried 2 different strategies to overcome this interaction in HIV-infected volunteers (n = 48) (Abstract 658). They first assessed atazanavir pharmacokinetics after giving atazanavir/ritonavir 300 mg/100 mg once daily for 10 days. They divided the cohort into 3 groups who all received omperazole 40 mg once daily. Group A had no other change, Group B took the medications with 8 ounces of cola, and Group C increased their atazanavir dose to 400 mg/100 mg once daily. All 3 groups had atazanavir AUC, C_{max}, and C_{min} levels that were reduced

by 56% to 79% compared with those without omeprazole. The authors concluded that atazanavir should not be given with proton pump inhibitors. Future studies will determine if similar interactions exist with H2-blockers and antacids.

Antiretrovirals and DMPA. Little is known about the interaction of depo-medroxyprogesterone acetate (DMPA) and antiretrovirals. Cohn and colleagues presented data from A5093, an open-label nonrandomized study of the effect of DMPA on the pharmacokinetics of selected PI and NNRTI therapies in HIV-infected women (Abstract 82). The participants were taking 2 nRTIs plus nevirapine (n = 13), efavirenz (n = 14), or nelfinavir (n = 20). Participants were followed up for 12 weeks after receiving an injection of DMPA. According to progesterone levels, none of the women ovulated during the study supporting the efficacy of DMPA. The AUCs for efavirenz, nelfinavir and M8 (the active metabolite of nelfinavir) were not affected by DMPA. There was a statistically, but not clinically, significant increase in the AUC of nevirapine. The DMPA was well tolerated and seems to be safe and effective in women taking these antiretrovirals.

Pharmacogenetics

CYP2B6 and Efavirenz. Investigators from the ACTG 5095 study have previously reported a relationship between genetic polymorphisms in the CYP2B6 gene and efavirenz pharmacokinetics. They found that the G516T change, especially being homozygous for that allele (T/T), was associated with delayed clearance of efavirenz and increased central nervous system side effects. Haas and colleagues extended this observation to speculate how this polymorphism would lead to selective pressure for the development of NNRTI resistance should efavirenz be stopped (Abstract 651). Observed data from ACTG 5095 study subjects showed that the half-life of efavirenz was 23, 28 and 53 hours among G/G, G/T, and T/T subjects. Based on this, they calculated that efavirenz concentrations would be above the IC_{50} in these subjects for 7, 9, and 19 days. People with the T/T alleles at position 516 in the CYP2B6 are predicted to be at the highest risk for developing NNRTI resistance when stopping efavirenz.

R-Novoa and colleagues confirmed these observations concerning the CYP2B6 polymorphisms in a separate cohort (Abstract 652). Among 111 white patients starting efavirenz, 49% had G/G, 44% had G/T, and 7% had T/T alleles. The T/T and G/T allele-patients had higher plasma levels, and 40% and 19% had "toxic" levels (> 4 µg/mL) compared to 0% with the G/G alleles. Twenty percent of G/G patients had subtherapeutic levels (< 1 µg/mL). Central nervous system symptoms were more common among T/T and G/T patients. The risk of virologic failure was not related to the polymorphism but follow-up was limited to 12 weeks. The authors concluded that genetic testing for the G516T polymorphism may be useful for tailoring efavirenz therapy. Haas and colleagues also found that the CYP2B6 G516T polymorphism was associated with higher efavirenz levels among subjects in ACTG 384 (Abstract 81). However, it was not related to subsequent virologic outcomes with 3 years of follow-up.

P-glycoprotein. P-glycoprotein and multidrug resistance protein-1 are cellular efflux transporters that have the potential to lower intracellular levels of antiretroviral medications. Chandler and colleagues examined the correlation of these transporters and the expression of CXCR4 in PBMCs (Abstract 665). They found that both of these transporters were significantly correlated with expression of CXCR4 when examining PBMCs as a whole and with CD4+ cells in particular. These findings suggest that high expression of CXCR4 in PBMCs and CD4+ cells may make HIV infection more difficult to treat. Hartkoorn and colleagues found that rifampin upregulates P-glycoprotein expression, but not multi-drug resistance protein-1 (Abstract 666). They also found that rifampin is a substrate for both of these efflux transporters. This study suggests that rifampin could lower intracellular levels of antiretrovirals by promoting expression of efflux transporters.

Hulgan and colleagues assessed P-glycoprotein activity in samples derived from a large clinical cohort (Abstract 667). P-glycoprotein activity was higher in naive CD4+ lymphocytes than in total CD4+ lymphocytes. P-glycoprotein activity was also higher among African Americans than among whites, and in women than among men.

Therapeutic Drug Monitoring

Researchers from the California Collaborative Treatment Group (CCTG) enrolled 199 patients into a therapeutic drug monitoring trial (CCTG 578). Patients were starting a new PI or NNRTI-based antiretroviral regimen (Abstract 640). After 2 weeks, blood levels of PIs or NNRTIs were measured. A panel of experts reviewed the drug levels along with treatment history, CD4+ cell count, plasma HIV-1 RNA level and clinical toxicities, to arrive at a recommendation to either change the PI or NNRTI dose, or to leave it the same. Patients were randomized to receive the recommendation or to remain on the same dose. The mean baseline CD4+ count was 189 cells/µL and the mean plasma HIV-1 RNA was 5.2 $\log_{10}$ copies/mL. The median age was 40 years. Twenty-nine percent were starting their first antiretroviral regimen.

Overall, the expert panel recommended changing the PI or NNRTI dose in 67 patients (38%). All but 3 recommendations were to increase the dose. The factors associated with needing to increase the dose were having a higher weight or body mass index, use of either efavirenz or lopinavir/ritonavir, and being non-Hispanic. In the adjusted analysis, weight and use of either efavirenz or lopinavir/ritonavir remained associated with a dose-adjustment recommendation. Interestingly, adherence, age, and sex were not associated. The main result of this study, whether dose adjustment improves virologic outcomes, has not been analyzed yet.

Nettles and colleagues did intensive blood sampling on 10 highly adherent patients (Abstract 642). They drew blood 3 times a week for at least 3 months to assess the frequency of "blips" in the plasma HIV-1 RNA level. They also drew plasma levels of the NNRTI or PI that the patient was taking. Low plasma levels did not coincide with the blips. The drug levels in a given individual varied by as much as 43% for PIs and 26% for NNRTIs. This indicates that assessment of numerous drug levels may be necessary before making any treatment decisions based on this information.

Mother-to-Child Transmission of HIV

Single-dose nevirapine received significant attention at this year's conference

as new data became available from several ongoing clinical trials in sub-Saharan Africa focusing on issues of resistance and treatment alternatives. Fueled by recent public controversy surrounding the conduct of the HIVNET 012 trial, the use of single-dose nevirapine has been highly scrutinized despite significant successes in decreasing the rates of mother to child transmission of HIV (MTCT). Half a million doses of nevirapine have been distributed worldwide since the results of the HIVNET 012 study demonstrated a 40% reduction in MTCT (Abstract 8). Data presented at this year's conference shed new light on the ongoing controversy surrounding the efficacy and the implications of resistance following single-dose nevirapine.

Towne-Gold and colleagues (Abstract 785) presented results from a study evaluating efficacy of 1 NNRTI with 2 nRTIs administered during pregnancy. In the MTCT-Plus program in Cote d'Ivoire, 205 women were enrolled; 88 of them with a median CD4+ count of 185 cells/μL received a regimen of zidovudine/lamivudine/nevirapine starting at 26 weeks; 114 women with median CD4+ count of 472 cells/μL received MTCT prophylaxis with zidovudine/lamivudine starting at 32 weeks until 3 days postpartum followed by an intrapartum single-dose nevirapine. Infants received 1 week of zidovudine and single-dose nevirapine on day 3 after birth. Infant plasma HIV-1 RNA was assessed at 4 and 6 weeks. Among infants of women receiving zidovudine/lamivudine/nevirapine, 69 of 80 live births tested to date revealed 1 infection in the setting of maternal nonadherence, giving an overall transmission rate of 1.45% (95% CI, 0.00-7.8) at 4 weeks. Among infants born to women receiving the MTCT regimen, 77 of 94 live births were tested, 3 infants were HIV infected (rate, 3.89%; 95% CI, 0.03-9.67) whose mothers had only taken single-dose nevirapine at delivery. Six mothers in the zidovudine/lamivudine/nevirapine group experienced grade 3 adverse events (rash, n = 6; hepatotoxicity, n = 1) requiring drug switch. The investigators note that these low rates of transmission of HIV-1 in mothers on potent antiretroviral therapy are similar to those seen in studies from high resource settings. Based on these preliminary data, they conclude that potent antiretroviral therapy during pregnancy can dramatically reduce the risk of MTCT in women with advanced disease in African populations. Several smaller studies drew similar conclusions regarding the efficacy of combination prophylactic therapy antenatally to prevent MTCT.

Jourdain and colleagues (Abstract 782) evaluated 137 HIV-1 infected pregnant women in Thailand receiving minimal or no antenatal care who were given emergency antiretroviral prophylaxis with zidovudine antenatally where possible followed by intrapartum single-dose nevirapine. Infants were given single-dose nevirapine and 4 weeks zidovudine after birth. Ninety women (66%) did not receive any zidovudine before labor and the remainder received the drug for less than 15 days. 95% of infants received zidovudine for 6 weeks. Overall transmission rates were 15.7% among 103 newborns in which single-dose nevirapine was administered to both the mother and infant and 23.2% in 29 cases in which only the newborn received nevirapine. The investigators concluded that compared with their recently published results of the Program for HIV Prevention and Treatment (PHPT)-2 study where women received zidovudine from 28 weeks gestation, the efficacy of peripartum single-dose nevirapine in this cohort was poor, emphasizing the need for extended combination therapy in the entire third trimester of pregnancy.

Tubiana and colleagues (Abstract 810) presented results from an observational, single-center study evaluating the safety and efficacy of indinavir/ritonavir in 32 French women. Twenty-one antiretroviral-experienced and 11 -naive pregnant women received indinavir/ritonavir (400 mg/100 mg bid) in combination with 2 nRTIs. The most common nRTI regimens were zidovudine/lamivudine (91%), stavudine/lamivudine (6%), and zidovudine/didanosine (3%). All women received intrapartum zidovudine infusion and infants received 6 weeks of zidovudine. The median exposure to indinavir/ritonavir was 24 weeks, with 87% completing their pregnancy on the regimen. Overall, by ITT analysis, 91% of women at delivery had plasma HIV-1 RNA levels below 400 copies/mL with median CD4 count of 352 cells/μL. Thirty-three live births were reported with 1 spontaneous miscarriage and 2 twin births; none were infected at 0, 3, and 6 months as assessed by HIV RNA and DNA PCR. No renal toxicity or hyperbilirubinemia was reported in any of the 33 infants. Four women discontinued indinavir/ritonavir prior to delivery, 1 for virologic failure and the remainder for intolerance (2 with xerosis and 1 with mild liver enzyme elevation). The investigators concluded that a boosted PI regimen may improve adherence, decrease MTCT, and preserve future therapeutic options for the mother, indicating that a larger prospective study is warranted.

Ngo-Giang-Huong and colleagues (Abstract 802) found decreased rates of nevirapine resistance among perinatally infected, nevirapine-exposed infants treated with zidovudine. Mothers in this subset of the PHPT-2 cohort from Thailand received single-dose nevirapine plus varying durations of prenatal zidovudine prophylaxis. Overall resistance rate in infants given single-dose nevirapine or placebo plus zidovudine for 1 to 6 weeks was 8%, lower than that described for most studies evaluating exposure to single-dose nevirapine alone.

The DREAM Study

Palombi and colleagues (Abstract 67) presented data evaluating the efficacy and safety of antiretroviral therapy among HIV-1-infected Mozambique women enrolled in the Drug Resource Enhancement against AIDS and Malnutrition (DREAM) study. A total of 778 pregnant women received nevirapine with either zidovudine/lamivudine or stavudine/lamivudine, starting at 25 weeks and continued to month 6 postpartum. The infants were not treated after birth. Women with CD4+ cell counts above 200 cells/μL stopped therapy 1 month post delivery. The baseline CD4+ count and plasma HIV-1 RNA level were 498 cells/μL and 4.15 log $_{10}$ copies/mL, respectively. The median duration of antiretroviral therapy prior to delivery was 74 days; 65 women were lost to follow-up. The authors found that, by ITT analysis, the cumulative transmission rate of HIV was 6.1% and 1.4% respectively, among infants breastfed in the first month. Factors associated with transmission were pre-antiretroviral therapy plasma HIV-1 RNA level, antenatal duration of therapy, presence of a sexually transmitted disease, and nonadherence to or discontinuation of treat-

ment. There were 42 samples available for genotypic testing 6 months after suspension of treatment; all were clade C virus and 88% had no evidence of resistance. K103N and G190S mutations were seen in 1 and 5 samples, respectively. Grade 3 or 4 hepatotoxicity occurred in 6% of women but there were no nevirapine related deaths. The investigators concluded that 3-drug antiretroviral therapy is effective, well tolerated and, compared to single-dose nevirapine, associated with lower rates of resistance and HIV transmission.

ANRS DITRAME Plus

Chaix and colleagues (Abstract 72 LB) described the resistance rates in the ANRS DITRAME Plus study. This was an open-label study enrolling 329 women receiving zidovudine/lamivudine starting at week 32, an additional intrapartum dose of zidovudine/lamivudine with single-dose nevirapine, and then 3 days of zidovudine/lamivudine. Infants received 1 week of zidovudine, and single-dose nevirapine on day 2. Six weeks after birth, the rate of MTCT was 4.7%, and transmission was associated with higher viral load and lower CD4+ cell count in the mothers. Genotypic analysis performed at baseline and 4 weeks postpartum on 16 transmitting mother/infant pairs and 80 non-transmitting mothers/infant pairs showed 1.14% and 8.3% rates of nevirapine and lamivudine resistance, respectively. Among the 16 HIV-infected infants, 1 developed K103N/Y181C and M184V mutations, and 3 had M184V despite never receiving lamivudine. Among the nontransmitting women, 1 had both K103N and M184V and 7 had M184V alone. Multivariate analysis demonstrated that the duration of zidovudine/lamivudine therapy was associated with development of M184V. The authors concluded that this regimen may reduce the rates of drug resistance seen after single-dose nevirapine.

MASHI

The resistance data from the MASHI trial in Botswana presented by Shapiro and colleagues (abstract 74LB) suggested that combination antiretroviral therapy may not always be effective at preventing the emergence of nevirapine resistance. This randomized, partially blinded, placebo-controlled study enrolled 1200 HIV-infected pregnant women to receive nevirapine at delivery with nevirapine for the infant, or placebo at delivery and placebo for the infant. All women and infants received 1 month of zidovudine. The study design was revised after 17 months to administer nevirapine to every infant at birth. Mother/infant pairs were further randomized to formula feed or breastfeed with 6 months of infant zidovudine prophylaxis. Antiretrovirals became available to women with AIDS in the second study period and were initiated by 71 of 694 women. This was initially designed as a superiority trial of maternal/infant nevirapine (N/N) versus maternal/infant placebo (P/P), but the revised study evaluated equivalence of P/N to N/N. Results of the 2 study periods were evaluated separately. Transmission rates in the first study period were higher in the P/P group (4.5% vs. 3.8% in the N/N group) and rose after 1 month (6.2% vs. 5.3% respectively, $P = .7$). Transmission rates in the second period were 3.8% versus 2.3% in those receiving placebo and nevirapine respectively, rising to 4.3% and 3.7% ($P = .70$), respectively, at 1 month after birth, meeting predetermined criteria for equivalence in the 2 arms. Transmission rates overall for both study periods were 4.0% at birth and 4.7% at 1 month. Analysis of feeding strategy favored the nevirapine arm in the first period, but not in the revised study. Resistance was found in 44% (69/157) of nevirapine exposed women tested at 1 month postpartum and 33% harbored the nevirapine associated K103N mutation. The investigators concluded that results from the initial period demonstrate nonsuperiority of nevirapine as background to zidovudine therapy, with a possible benefit seen in formula-fed infants. Likewise, results from the revised study period showed no advantage of adding maternal nevirapine in the context of infant nevirapine. Coupled with high rates of nevirapine resistance, they further conclude that nevirapine sparing strategies should be considered.

A further analysis of this cohort evaluating effect of feeding strategy on infant mortality and transmission rates was presented by Thior and colleagues (abstract 75LB). The 1179 infants in the MASHI trial were randomized as described above to either formula feeding with 1 month zidovudine or breast feeding during 6 months of infant zidovudine prophylaxis. Primary outcomes were cumulative rate of HIV infection at 7 months and 18-month HIV-free survival. As noted above, in the initial study design, HIV infection rates strongly favored the formula-fed infants in the nevirapine arm, but this advantage decreased in the revised study. Overall, cumulative HIV infection rates were slightly better the formula-fed arm (5.6% vs. 9.1%, $P = .04$) at 7 months although mortality at 7 months was higher in this group (9.3% vs. 4.9%; $P = .0003$). HIV-free survival was similar in both groups (86% vs. 84% for the formula-fed arm and breast-fed and zidovudine arm, respectively). Overall, the investigators concluded that both formula feeding and breast feeing/zidovudine are reasonable strategies in this population.

Standard genotype assays may miss mutations comprising less than 20% of the viral population. The utility of highly sensitive resistance testing following single-dose nevirapine was highlighted by 3 presentations at the conference.

Johnson and colleagues (Abstract 100) used real-time reverse transcriptase polymerase chain reaction (RT-PCR) to identify low-frequency mutations among pre- and postpartum samples from women enrolled in a South African MTCT study receiving single-dose nevirapine at labor. The nevirapine-associated K103N mutation was not detected by conventional sequencing methods in any of the pre-nevirapine specimens was detected in and 10 of 50 of specimens (20%) collected at 6 weeks to 36 weeks postpartum. Of the 40 specimen with no resistance mutations by conventional assays, the K103N was detected by the RT-PCR method in an additional 16 specimens (40%). Five samples had the Y181C mutation by RT-PCR analysis that was not detected by conventional sequencing. Clonal sequencing analysis of 5 positive samples confirmed the presence of detected mutations in all samples, with frequencies of 1.1% to 11% of total virus population. Thus by RT-PCR, overall resistance estimates in this cohort were revised upward to 65%, representing a 62% increase in incidence of nevirapine resistance compared with population-based sequencing detection methods.

Similar findings were presented by Palmer and colleagues (Abstract 101),

who used an allele specific RT-PCR with primers developed to detect NNRTI resistance associated mutations, K103N and Y181C. Investigators evaluated samples from 29 women from the South African MTCT trial who received single-dose nevirapine at the onset of labor. By standard genotypic analysis, 8 (27%) had NNRTI resistance at 6 weeks and 6 months post delivery but not at 12 months; 11 had resistance mutations at 6 weeks only, and 10 demonstrated no resistance at any time point. Using allele specific RT-PCR they were able to quantify resistance mutations in these samples at below 0.1% frequency. At 12 months after nevirapine exposure, resistance mutations were detected in 7 of 8 (88%) in group 1, with a median frequency of 3.2%. Similarly, resistance was detected in 100% of group 1, 80% of group 2, and 50% of group 3 samples at 6 months, with frequencies ranging from 0.9% to 10%. Extrapolation of these results to the total cohort in this trial indicates that 69% of women receiving single-dose nevirapine could have NNRTI resistance mutations at 4 months, 32% at 6 months, and 22% at 12 months. Thus the incidence of NNRTI resistance was double that reported by standard genotyping.

Loubser and colleagues (Abstract 102) reinforced these findings in a similar study assessing the frequency of the K103N mutation in 18 postpartum samples from women receiving single-dose nevirapine. Resistance mutations demonstrated in 50% of samples at 6 weeks postpartum by standard detection methods increased in prevalence to 89% by the RT-PCR method. Longitudinal follow-up of 16 women demonstrated that these mutations faded over time, detected in only 25% at 1 year.

Taken together, these 3 studies of RT-PCR detection of nevirapine resistance mutations in HIV-1 seropositive African women with subtype C virus indicate that nevirapine resistance is likely present in the majority of women following single-dose nevirapine exposure and resistance rates are highly underestimated by standard population-based sequencing. The frequency of resistance mutations declines over time but can remain above pretreatment levels for at least 1 year. Despite this evidence, RT-PCR remains cost prohibitive for widespread use in resource poor settings, yet these data emphasize the importance of assessing the clinical implications of resistant variants and should be employed in future clinical research studies.

Despite the mounting concerns about single-dose nevirapine for prevention of MTCT of HIV, data presented by Martinson and colleagues (Abstract 103) suggest that the use of single-dose nevirapine in primigravid women does not necessarily limit its utility in subsequent pregnancies. Investigators in Soweto enrolled 318 infant/mother pairs from 13 prenatal clinics in this pilot case-controlled study with a primary aim to compare rates of MTCT of HIV in women exposed to single-dose nevirapine in 2 successive pregnancies by evaluating maternal viral load, CD4+ count, and HIV resistance prior to single-dose nevirapine and 6 weeks postpartum. Infection in infants was determined by PCR at 6 weeks. Preliminary results presented on 77 of 106 mother-infant pairs with prior nevirapine exposure and 140 nevirapine-naive matched controls demonstrated that although transmission rates were higher in the cohort receiving single-dose nevirapine for the second time (10.7% vs. 3.0%), the difference was not statistically significant. These rates are comparable to that observed in the general population exposed to single-dose nevirapine in a single pregnancy, leading investigators to conclude that the efficacy of single-dose nevirapine in second pregnancies is reasonably maintained, and in the setting of transmission rates of 25% in the absence of prophylaxis, single-dose nevirapine remains a significantly beneficial modality for prevention of MTCT of HIV. Resistance rates were comparable in the 2 groups. Implications of these results will be more apparent as results of the entire study population become available.

Eshelman and colleagues (Abstract 799) reported that resistance rates among African women given single-dose nevirapine may be clade specific. They found resistance rates as high as 67% (45/67) in Malawian women following single-dose nevirapine for MTCT in women with subtype C virus subjects from the NVAZ trial compared with 36% (28/147) of patients with subtype D and 19% (35/98) of patients with subtype A virus from Ugandan subjects from the HIVNET 012 trial. The proportion of women with 2 or more mutations associated with nevirapine resistance was also higher in women with subtype C virus (43%) than women with subtype D (16%) or A (8%). The most common mutations were K103N and Y181C. Multivariate analysis of risk factors associated with development of nevirapine resistance in this study demonstrated maternal viral load at delivery and viral subtype were independent predictors of nevirapine resistance (C vs A: OR, 8.38; 95% CI, 4.19-16.76. C vs D: OR, 3.27; 95% CI, 1.53-3.25), but age, parity, and nevirapine dosing time relative to delivery were not. The authors cautioned that as subjects in this study were taken from different cohorts (subtype C patients from NVAZ trial and subtype A and D from HIVNET 012 trial) other factors may contribute to the observed the differences in nevirapine resistance. However, these intriguing data indicate that further investigation of subtype dependent differences in nevirapine resistance rates is warranted.

SIMBA

Data on rates of drug resistance among breast-fed infants in the Ugandan SIMBA trial were presented by Giuliano and colleagues (Abstract 99). The SIMBA trial evaluated safety and efficacy of lamivudine and nevirapine in preventing MTCT of HIV in 404 infants of infected mothers receiving didanosine/zidovudine therapy from 36 weeks gestation to 1 week postpartum. Infants were randomized to receive either daily nevirapine or lamivudine throughout breastfeeding or until HIV infection was confirmed. Of the 30 infants who were infected during the study period, 26 were evaluated for resistance mutations in this substudy, half received nevirapine, and the other half lamivudine. There were no differences between the 2 arms in preventing transmission. Genotypic analysis demonstrated the lamivudine associated resistance mutation M184V in 9 of 13 (69%) of those in the lamivudine arm initially, yet this faded over time and was not present in samples collected 3 to 6 months later. Twelve of the 13 (92%) infants in the nevirapine arm had nevirapine resistance mutations, 9 with Y181C and 3 with multiple mutations; all persisted on follow up after discontinuation of prophylaxis. Two mothers in the lamivudine arm had K103N and

M41L mutations on enrollment and at delivery and these were demonstrated in their infants' samples. Two mothers in the nevirapine group had mutations G190A and V108I at baseline, but only the latter persisted in their infants' samples. No zidovudine/didanosine associated mutations were observed in the 22 mothers samples tested at delivery. The investigators pointed out that while the study numbers are limited, postpartum prophylaxis with either nevirapine or lamivudine invariably led to selection of resistance mutations in infected infants in this study, especially in the nevirapine arm. These results should be considered in development of prophylaxis regimens to be studied in future clinical trials.

Conclusions

The 2005 CROI solidified its status as the premier conference of the year providing state-of-the-art information on antiretroviral therapy from completed and ongoing studies and introducing new avenues of investigation. Presentations covered the range of important advances in HIV care and research, in particular the recent advances in new antiretroviral agents, experience with therapy in the international setting, and the debate about the use of single-dose nevirapine in developing countries.

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Additional Suggested Reading

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A list of all cited abstracts appears on pages 45 to 50.

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

5. Natural Resistance to HIV Infection: The Vif-APOBEC Interaction. Michael H Malim.

7. The HIV Envelope Glycoprotein: Interactions and Conformational Changes. Stephen C Harrison.

8. Controversies in the Use of Nevirapine for the Prevention of Mother-to-Child Transmission. James McIntyre.

20. Real-time Detection of Patients with Acute HIV Infection in Africa. Susan Fiscus, C Pilcher, W Miller, I Hoffman, M Price, D Chilongozi, C Mapanje, R Krysiak, M Hosseinipour, S Galvin, S Gama, F Martinson, and M Cohen.

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30. A New Mechanism of Antiviral Defense by APOBEC3G. Ya-Lin Chiu, V Soros, K Stopak, J Kreisberg, W Yonemoto, J Neidleman, and W Greene.

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35. Host Factors Affecting the Integration of HIV-1 in Non-dividing cells. Jean-Marc Jacqué and M Stevenson.

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39. Treatment of Hypertriglyceridemia in HIV-infected Patients under HAART, by (n-3) Polyunsaturated Fatty Acids: A Double-blind Randomized Prospective Trial in 122 Patients. Pierre De Truchis, M Kirstetter, A Perier, C Meunier, J Gardette, JC Melchior, and Maxepa-VIH Study Group.

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41. The Effect of Rosiglitazone on PPARγ Expression in Human Adipose Tissue Is Limited by Continued Exposure to Thymidine NRTI. Patrick Mallon, R Sedwell, G Rogers, D Nolan, P Unemori, H Wand, K Samaras, A Kelleher, S Emery, D Cooper, A Carr, and The Rosey Investigators.

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45LB. Switching to a Thymidine Analog-sparing or a Nucleoside-sparing Regimen Improves Lipoatrophy: 24-Week Results of a Prospective Randomized Clinical Trial, AACTG 5110. Robert Murphy, J Zhang, R Hafner, A Shevitz, K Tashima, K Yarasheski, J Forand, B Berzins, S Owens, S Evans, P Tebas, and AACTG 5110 Study Team.

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72LB. Addition of 3 Days of ZDV + 3TC Postpartum to a Short Course of ZDV + 3TC and Single-dose NVP Provides Low Rate of NVP Resistance Mutations and High Efficacy in Preventing Peripartum HIV-1 Transmission: ANRS DITRAME Plus, Abidjan, Côte d'Ivoire. M L Chaix, Francois Dabis, D Ekouevi, F Rouet, B Tonwe-Gold, I Viho, L Bequet, G Peytavin, H Toure, H Menan, V Leroy, and C Rouzioux.

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111. Viral Vectors as HIV Vaccines: Lessons Learned and Future Prospects. Phillip Johnson.

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113. Nonhuman Primate HIV Vaccine Studies: Will They Be Predictive? Norman L Letvin.

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139. The Incidence of Invasive Pneumococcal Disease in the Era of HAART Is the Same as that in the Pre-HAART Era. Gregory Lucas, J Keruly, K Gebo, and R Moore.

140. Immunologic Efficacy of a Prime-boost Strategy Combining a 7-Valent Pneumococcal Conjugate Vaccine followed by a 23-Valent Pneumococcal Polysaccharide Vaccine vs PPV Alone in HIV-infected Adults with 200 to 500 CD4 Cells/µL. Results of the ANRS 114 Study. Philippe Lesprit, G Pedrono, J M Molina, C Goujard, P M Girard, N Sarrazin, C Katlama, P Yéni, P Morineau, B Fritzell, J F Delfraissy, G Chène, Y Lévy, and A Study Group.

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154. Relationship between Cellular Immune Responses and Viral Load in Naturally SIV-infected Sooty Mangabeys. Zichun Wang, B Metcalf, D Lee, S Staprans, H McClure, and A Kaur.

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164LB. Efficacy of TMC114/r in 3-Class Experienced Patients with Limited Treatment Options: 24-Week Planned Interim Analysis of 2 96-Week Multinational Dose-finding Trials. C Katlama, D Berger, N Bellos, B Grinsztejn, Richard Haubrich, T Wilkin, J M Molina, C Steinhart, R Pedro, M P de Béthune, S De Meyer, R Hoetelmans, W Parys, T Vangeneuden, and E Lefebvre.

165LB. Virological and Immunological Outcomes at 3 Years following Initiation of ART with Regimens Containing a NNRTI or PI or both: The INITIO Trial. D Cooper and Patrick Yeni.

174. TRIM5α: Mediator of Innate Intracellular Immunity to Retroviruses. M Stremlau, B Song, M Perron, C Owens, H Javanbakht, W Ulm, R Mulligan, B Gold, C O'hUigin, C Winkler, M Dean, and Joseph Sodroski.

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177. Tsg101 and other Cellular Co-factors in HIV Assembly and Release. Carol Carter, L Ehrlich, A Goff, G Medina, Q Lu, S Cohen, and M Powell.

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862. High Prevalence of Subclinical Atherosclerosis in Low Cardiovascular Risk HIV Patients on HAART. Hernando Knobel, A Guelar, C Jericó, N Calvo, P Saballs, A González, J Gimeno, J López-Colomés, and J Pedro-Botet.

863. Rapid Progression of Carotid Lesions in HAART-treated HIV-1 Patients. Paolo Maggi, F Perilli, A Lillo, G Epifani, M Gargiulo, S Ferraro, B Grisorio, S Ferrara, C Pellegrino, V Carito, C Bellacosa, N Ladisa, G Pastore, A Chirianni, and G Regina.

871. The Changing Face of Cardiovascular Risk in HIV-infected Patients: ANRS Aquitaine Cohort, 2003. Rodolphe Thiébaut, V Aurillac-Lavignolle, F Bonnet, N Ibrahim, C Cipriano, D Neau, M Dupon, F Dabis, P Mercié, and Groupe d'Epidemiologie du SIDA en Aquitaine.

872. The Effect of HAART Initiation on Blood Pressure. Eric Seaberg, S Riddler, J Margolick, C Sutcliffe, R Sharrett, R Detels, C Williams, J Phair, and Multicenter AIDS Cohort Study.

873. The Association between Increasing Blood Pressure and Use of NNRTI and Lopinavir/Ritonavir. Heidi Crane, S Van Rompaey, and M Kitahata.

874. HIV-associated Pulmonary Hypertension in Patients on HAART. S Rosenkranz, H Steffen, D Vogel, M Werner, C Wyen, C Lehmann, N Schmeißer, and Gerd Fätkenheuer.

891. Efavirenz Levels and Clinical Outcomes in Patients with TB and HIV Treated Concomitantly with ART and Rifampin-containing TB Regimen. Gerald Friedland, C Jack, S Khoo, U Lalloo, and V Naidoo.

922. HCV/RNA 4 Weeks after Interferon/Ribavirin Therapy Predictive for End-of-treatment Response in Acute Hepatitis C. Martin Vogel, S Dupke, A Baumgarten, H Jessen, T Lutz, L Locher, S Fenske, B Bieniek, W Schmidt, A Theisen, D Schranz, S Mauss, T Seidel, E Voigt, J Rockstroh, and the German Hepatitis Group.

927. Effect of Zidovudine on Anemia and Epoetin-alfa Use during Pegylated Interferon/Ribavirin Therapy for HCV in HIV-infected Persons. Daniel Alvarez, L Moorehead, L Ball, and M Sulkowski.

928. Plasma Target Concentration of Ribavirin in HCV/HIV Co-infected Patients. Dominique Breilh, D Neau, S Djabarouti, P Trimoulet, J L Pellegrin, C Duprat, J M Ragnaud, M Dupon, and M C Saux.

929. Ribavirin Plasma Concentrations Predict Early Virological Response as Well as Anemia in Anti-hepatitis C Therapy. Ana Rendón, M Nuñez, M Romero, P Barreiro, L Martin-Carbonero, J García-Samaniego, I Jiménez-Nácher, J González-Lahoz, and V Soriano.

931. Orthotopic Liver Transplantation in HIV-positive Patients: Outcome of 10 Patients from the Bonn Cohort. Martin Vogel, E Voigt, J C Wasmuth, H Brackmann, G Goldmann, M Wolff, A Hirner, T Sauerbruch, U Spengler, and J Rockstroh.

947. Predictors of Liver Disease Progression in a Cohort of HIV/HCV-co-infected Drug Users. Sherri O Stuver, C Fleming, D Nunes, C Reed, S Tumilty, J Murray, C Graham, M Koziel, D Craven, P Skolnik, and C Horsburgh.

949. Neuropsychological Functioning in HCV/HIV-co-infected Subjects. K Tucker, T Parsons, A Parente, T Dickens, M Fried, J Eron, K Straits-Tröster, A Tröster, C Hall, and Kevin Robertson.

953. 1- to 3-Year Outcomes in HIV-infected Liver and Kidney Transplant Recipients. Michelle Roland, D Stablein, L Carlson, L Frasseto, B Murphy, M Keller, K Olthoff, E Blumberg, K Brayman, R Redfield, D Oldach, B Barin, and P Stock.

Update of the Drug Resistance Mutations in HIV-1: 2005

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Since 2000, the International AIDS Society–USA (IAS–USA) Drug Resistance Mutations Group has worked as an independent entity and forged a collaborative process to identify key HIV-1 drug resistance mutations. The goal of the group is to quickly deliver accurate and unbiased information to clinical practitioners on HIV-1 resistance. This April 2005 version of the IAS-USA Drug Resistance Mutations Figures replaces the version published in this journal in October 2004.

The IAS-USA Drug Resistance Mutations Figures are designed for use in identifying mutations associated with viral resistance to antiretroviral drugs and in making therapeutic decisions. Care should be taken when using this list of mutations for surveillance or epidemiologic studies of transmission of drug-resistant virus. A number of amino acid substitutions, particularly minor mutations, represent polymorphisms that, in isolation, may not reflect prior drug selective pressure or reduced drug susceptibility.

In the context of making clinical decisions regarding antiretroviral therapy, evaluating the results of HIV genotypic testing includes: (1) assessing whether the pattern or absence of a pattern in the mutations is consistent with the patient's antiretroviral history; (2) recognizing that in the absence of drug (selection pressure), resistant strains may be present at levels below the limit of detection of the test (analyzing stored samples, collected under selection pressure, could be useful in this setting); and (3) recognizing that virologic failure of the first regimen typically involves HIV-1 isolates with resistance to only 1 or 2 of the drugs in the regimen (in this setting, resistance most commonly develops to lamivudine or the nonnucleoside reverse transcriptase inhibitors).[1-5] This paradox may involve patient nonadherence, laboratory error, drug-drug interactions leading to subtherapeutic drug levels, and possibly compartmental issues, indicating that drugs may not reach optimal levels in specific cellular or tissue reservoirs.

A copy of the current recommendations for antiretroviral resistance testing from the IAS–USA HIV Resistance Testing Guidelines Panel[6] can be found on the IAS–USA Web site at www.iasusa.org.

Revisions to the Figures for the 2005 Update

Two major changes have been included in this April 2005 version of the figures and user notes. First, on the figures, the bars representing multidrug resistance mutations have been moved to the bottom of the drug class. The only exception is that the multi-nucleoside (or nucleotide) reverse transcriptase inhibitor (nRTI) bar remains at the top of the nRTI class, because of the pervasive effect of the thymidine analogue-associated mutations (TAMs), the E44D and the V118I in conferring resistance to all nRTIs.

In addition, user notes have been revised to focus on current, more clinically focused information. These new user notes provide additional detail, where necessary, to the information presented in the figures. Other changes to the figures and user notes are described below.

Nucleoside (or Nucleotide) Reverse Transcriptase Inhibitors

In this version, the definitions of nucleoside (or nucleotide)-associated mutations (NAMs) and TAMs have been clarified (see user note 2). In brief, the TAMs (M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E) are a subset of the NAMs. The TAMs are distinguished as bold mutations on the multi-nRTI bar; E44D and V118 are shown in normal weight type.

For lamivudine, the E44D and V118I have been removed from the list as specific lamivudine-associated mutations. Originally, the E44D and V118I were understood to confer a low level of resistance to lamivudine.[7] From subsequent studies, it has become evident that E44D and V118I are mutations that contribute to zidovudine resistance and thus contribute to cross resistance to the other nRTIs, including lamivudine.[8]

For zalcitabine, T69D now is marked in a lighter-weight type than the other mutations to indicate that it is rare and less important than other mutations, such as the TAMs, in conferring resistance to zalcitabine.[9] In the future, the group plans to weight the mutations in the nRTI and NNRTI classes, as is currently done with the major and minor protease inhibitor (PI) mutations.

The D67N has been deleted from the Multi-nRTI Resistance: 69 Insertion Complex bar, now located at the bottom of the nRTI list. A recent analysis found no evidence of the D67N in 200 sequences from clinical isolates containing the insertion.[10]

Nonnucleoside (or Nucleotide) Reverse Transcriptase Inhibitors

A new user note has been included referencing both the nRTIs and the nonnucleoside (or nucleotide) reverse transcriptase inhibitors (NNRTIs) on the issue of

(continued, page 56)

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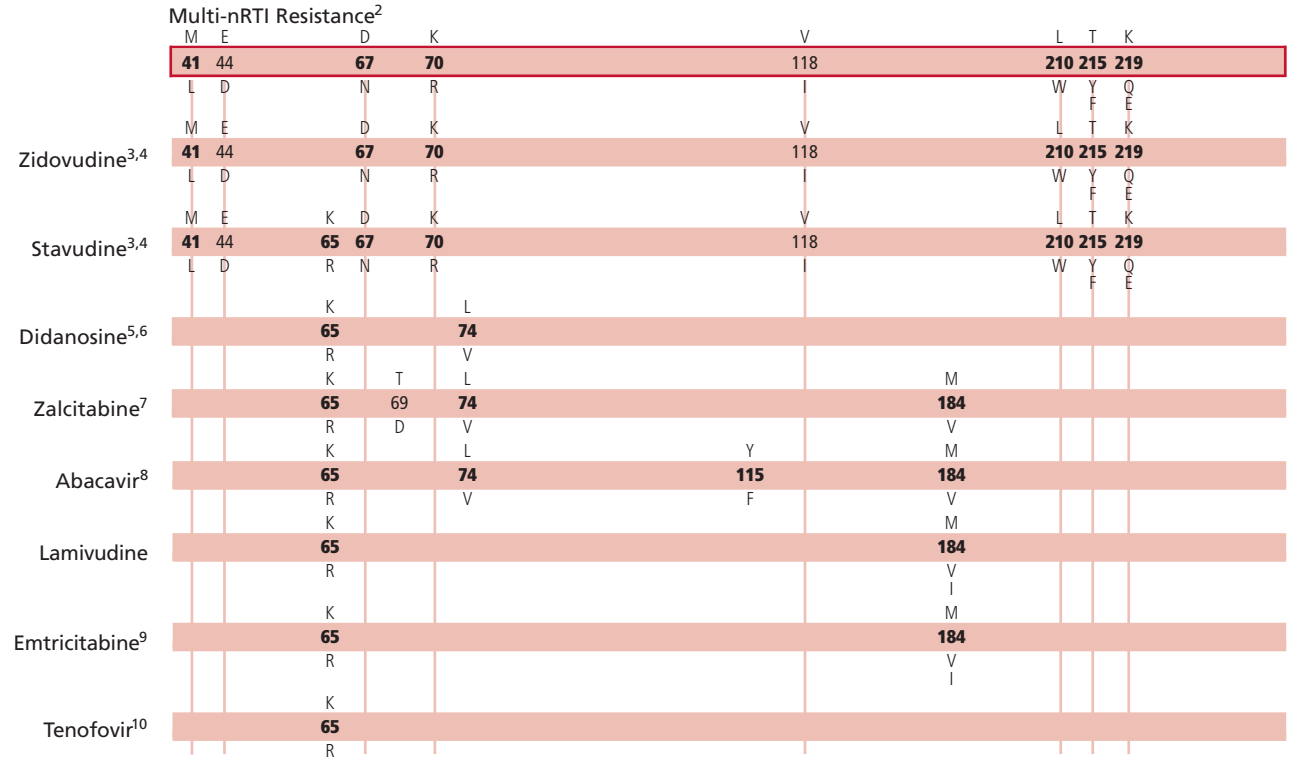
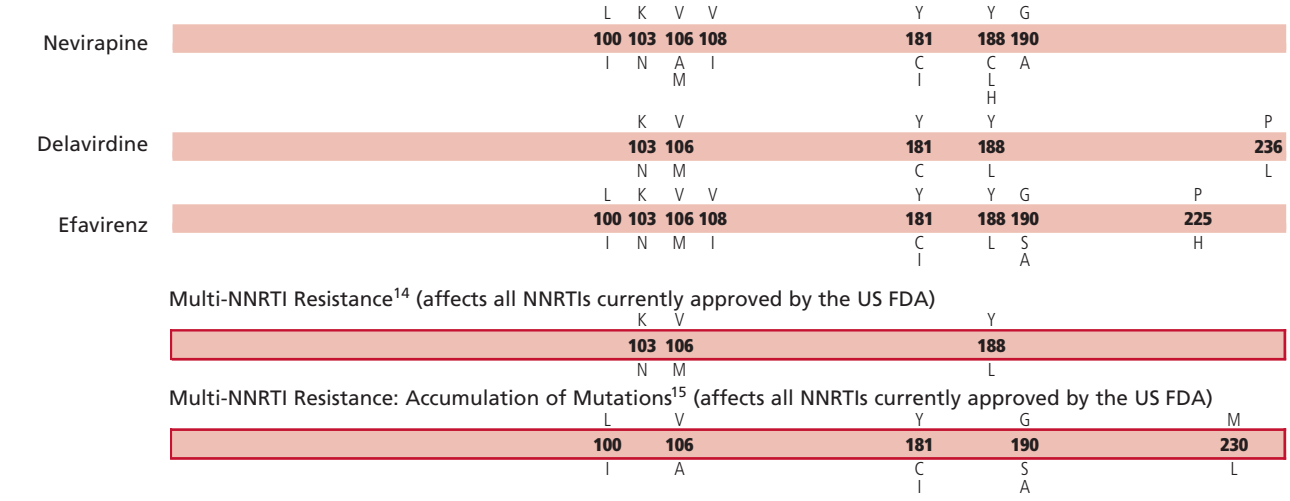
MUTATIONS IN THE REVERSE TRANSCRIPTASE GENE ASSOCIATED WITH RESISTANCE TO REVERSE TRANSCRIPTASE INHIBITORS

Nucleoside and Nucleotide Reverse Transcriptase Inhibitors (nRTIs)[1]



Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs)[1,13]



Date of Revision: March 2005

MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS[16,17]

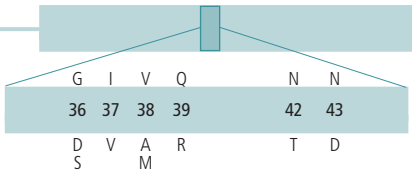
	10	20	24	32	33	36	46	47	48	50	53	54	63	71	73	77	82	84	88	90
Indinavir	L I R V	K M R	L I	V I		M I	M I L					I V		A V T	G S A	V I	V A F T	I V		L M
Ritonavir	L F I R V	K M R		V I	L F	M I	M I L					I V L		A V T		V I	V A F T S	I V		L M
Saquinavir	L I R V								G **48** V			I V L		A V T	G 73 S	V 77 I	V 82 A	I 84 V		L **90** M
Nelfinavir[18]	L 10 F I			D **30** N		M 36 I	M 46 I L							A 71 V T		V 77 I	V 82 A F T S	I 84 V	N 88 D S	L **90** M
(Fos) amprenavir	L 10 F I R V			V 32 I			M 46 I L	I 47 V		I **50** V		I 54 L V M			G 73 S			I **84** V		L 90 M
Lopinavir/ ritonavir[19]	L 10 F I R V	K 20 M R	L 24 I	V 32 I	L 33 F		M 46 I L	I 47 V A		I 50 V	F 53 L	I 54 V L A M T S	L 63 P	A 71 V T	G 73 S		V 82 A F T S	I 84 V		L 90 M
Atazanavir	L 10 I F V	K 20 R M I	L 24 I	V 32 I	L 33 I F	M 36 I L V	M 46 I		G 48 V	I **50** L		I 54 L		A 71 V	G 73 C S T A		V 82 A	I **84** V	N 88 S	L 90 M
Tipranavir/ ritonavir (expanded access)[20]	L 10 I V	K 20 M L T			L **33** I F		M 46 I					I 54 V					V **82** A F L T	I **84** V		L 90 M

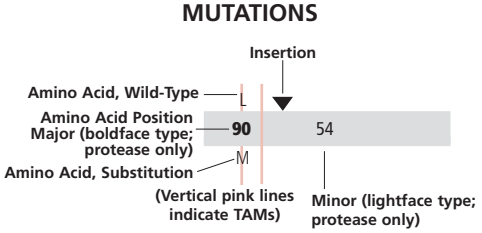
Multi-protease Inhibitor (PI) Resistance: Accumulation of Mutations[21] (affects all PIs currently approved by the US FDA)

	10	32	46	54	82	84	90
	L F I R V	V I	M I L	I V M L	V A F T S	I V A C	L M

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

Enfuvirtide[22]





The International AIDS Society–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 includes mutations that may contribute to a reduced virologic response to a drug.

The mutations listed have been identified by 1 or more of the following criteria: (1) in vitro passage experiments or validation of contribution to resistance by using site-directed mutagenesis; (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. In addition, the group only reviews data that have been published or have been presented at a scientific conference. Drugs that have been approved by the US Food and Drug Administration (FDA) or are available through expanded access protocols are included. User notes provide additional information as necessary. Although the Drug Resistance Mutations Group works to maintain a complete and current list of these mutations, it cannot be assumed that the list presented here is exhaustive. Readers are encouraged to consult the literature and experts in the field for clarification or more information about specific mutations and their clinical impact.

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; nRTI indicates nucleoside reverse transcriptase inhibitor; NAMs indicates nRTI-associated mutations; TAMs indicates thymidine-associated mutations; NNRTI indicates nonnucleoside reverse transcriptase inhibitor; PI indicates protease inhibitor.

User Notes

1. Numerous nucleoside (or nucleotide) reverse transcriptase inhibitor (nRTI) mutations, such as the M41L, L210W, and T215Y mutations, may lead to viral hypersusceptibility to the nonnucleoside reverse transcriptase inhibitors (NNRTIs) in nRTI-treated individuals. The presence of these mutations may improve subsequent virologic response to NNRTI-containing regimens in NNRTI treatment-naive individuals (Shulman et al, *AIDS*, 2004; Demeter et al, 11th CROI, 2004, Haubrich et al, 11th CROI, 2004; Tozzi, *J Infect Dis*, 2004; Katzenstein et al, *AIDS*, 2003).

2. Multi-nRTI resistance mutations, also known as nucleoside-associated mutations (NAMs), are associated with resistance to numerous nRTIs. The M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E are known as thymidine-associated mutations (TAMs; in bold type on the figure). TAMs are a subset of NAMs that are selected by the thymidine analogues zidovudine and stavudine and are associated with cross-resistance to all nRTIs currently approved by the US FDA (Larder et al, *Science*, 1989; Kellam et al, *Proc Natl Acad Sci USA*, 1992; Calvez et al, *Antivir Ther,* 2002; Kuritzkes et al, *JAIDS*, 2004). The E44D and the V118I increase the level of resistance to zidovudine and stavudine, and correspondingly increase cross-resistance to the other nRTIs. 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 presence of the M184V mutation appears to delay or prevent emergence of TAMs (Kuritzkes et al, *AIDS,* 1996). This effect may be overcome by an accumulation of TAMs or other mutations. The clinical significance of this effect of M184V is not known.

4. The T215A/C/D/E/G/H/I/L/N/S/V substitutions are revertant mutations at codon 215, conferring increased risk of virologic failure of zidovudine or stavudine in antiretroviral-naive patients (Riva et al, *Antivir Ther*, 2002; Chappey et al, *Antivir Ther*, 2003; Violin et al, *AIDS*, 2004). In vitro studies and preliminary clinical studies suggest that the T215Y mutant may emerge quickly from one of these mutations in the presence of zidovudine or stavudine (Garcia-Lerma et al, *J Virol*, 2004; Lanier et al, *Antivir Ther*, 2002; Riva et al, *Antivir Ther*, 2002).

5. The K65R mutation may be selected by didanosine and is associated in vitro with decreased susceptibility to the drug (Winters et al, *Antimicrob Agents Chemother*, 1997). The impact of the K65R in vivo is unclear.

6. The presence of 3 of the following—M41L, D67N, L210W, T215Y/F, and K219Q/E—has been associated with resistance to didanosine (Marcelin et al, *Antimicrob Agents Chemother*, in press). The K70R and M184V mutations are not associated with a decreased virologic response to didanosine in vivo (Molina et al, *J Infect Dis*, 2005).

7. Limited clinical use of zalcitabine has resulted in very limited data on resistance and cross-resistance. The K65R, L74V, and 184V mutations, alone or in combination with any of the TAMs, can lead to resistance to zalcitabine in vitro (Parikh et al, *Antivir Ther*, 2003; Gu et al, *Antimicrob Agents Chemother,* 1994; Zhang et al, *Antimicrob Agents Chemother* 1994). The T69D alone leads to a modest increase (2-fold change) in zalcitabine resistance (not bolded on the figure) (Fitzgibbon, *Antimicrob Agents Chemother*, 1992).

8. The M184V mutation alone does not appear to be associated with a reduced virologic response to abacavir in vivo (Harrigan et al, *J Infect Dis*, 2000; Lanier et al, *Antivir Ther*, 2004). When present with 2 or 3 TAMs, M184V contributes to reduced susceptibility to abacavir and is associated with impaired virologic response in vivo (Lanier et al, *Antivir Ther*, 2004). The M184V plus 4 or more TAMs resulted in no virologic response to abacavir in vivo (Lanier et al, *Antivir Ther*, 2004).

9. There are limited data on the effects of emtricitabine mutations in vivo. It is assumed that if resistance to emtricitabine emerges, the virus will also be resistant to lamivudine, and vice versa. New mutations that confer resistance or cross-resistance to emtricitabine may exist, but have not yet been described.

10. The K65R is associated with a reduced virologic response to tenofovir in vivo (Miller et al, *J Infect Dis*, 2004). A reduced response occurs in the presence of 3 or more TAMs inclusive of either M41L or L210W (Miller et al, *J Infect Dis*, 2004). Slightly increased treatment responses to tenofovir in vivo were observed if M184V was present (Miller et al, *J Infect Dis*, 2004).

11. The 69 insertion complex consists of a substitution at codon 69 (typically T69S) and an insertion of 2 or more amino acids (S-S, S-A, S-G, or others). The 69 insertion complex is associated with resistance to all nRTIs currently approved by the US FDA when present with 1 or more TAMs at codons 41, 210, or 215 (Miller et al, *J Infect Dis*, 2004). Some other amino acid changes from the wild-type T at codon 69 without the insertion may also be associated with broad nRTI resistance.

12. Tenofovir retains activity against the Q151M complex of mutations (Miller et al, *J Infect Dis*, 2004.

13. The long-term virologic response to sequential NNRTI use is poor, particularly when 2 or more mutations are present (Antinori et al, *AIDS Res Hum Retroviruses*, 2002; Lecossier et al, *J Acquir Immune Defic Syndr*, 2005).

14. The K103N or Y188L mutation alone can substantially reduce the clinical utility of all NNRTIs currently approved by the US FDA (Antinori et al, *AIDS Res Human Retroviruses*, 2002). The V106M mutation is more common in HIV-1 subtype C than in subtype B, and confers cross-resistance to all currently approved NNRTIs (Brenner et al, *AIDS*, 2003; Cane et al, *J Clin Micro*, 2001).

15. Accumulation of 2 or more of these mutations substantially reduces the clinical utility of all NNRTIs currently approved by the US FDA.

16. In general, the same mutations emerge whether or not the protease inhibitors (PIs) are boosted with low-dose ritonavir, although there is some difference in the relative frequency of various mutations. However, with regimens that include boosted PIs, multiple mutations may be required to result in less virologic activity. More data are needed to make specific comparisons between a particular boosted PI and a nonboosted PI.

17. Resistance mutations in the protease gene are classified as either "major" or "minor," if data are available.

Major mutations in the protease gene are defined in general either as those selected first in the presence of the drug; or those shown at the biochemical or virologic level to lead to an alteration in drug binding or an inhibition of viral activity or viral replication. Major mutations have an effect on drug susceptibility phenotype. In general, these mutations tend to be the primary contact residues for drug binding.

Minor mutations generally emerge 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 containing major mutations. However, some minor mutations are present as common polymorphic changes in HIV-1 nonsubtype B clades, such as K20I/R and M36I in protease.

18. In some HIV-1 non-B subtypes, D30N is selected less frequently than other PI mutations (Gonzalez et al, *Antivir Ther*, 2004).

19. Major and minor designations have not been assigned for mutations associated with lopinavir boosted with low-dose ritonavir (lopinavir/ritonavir) because no clear data yet define degrees of influence with this drug combination. However, the accumulation of 6 or more of these mutations is associated with a diminished response to lopinavir/ritonavir (Masquelier et al, *Antimicrob Agents Chemother*, 2002). The product information states that accumulation of 7 or 8 mutations confers resistance to the drug.

20. Tipranavir boosted with low-dose ritonavir (tipranavir/ritonavir) is not yet approved by the US FDA, but it is available through an expanded-access protocol. No substantial data are available regarding mutations associated with clinical failure of tipranavir/ritonavir when it is the first PI used. In PI-experienced patients, accumulation of mutations at positions 33, 82, 84, and 90 correlated with virologic response. Responses were greater when fewer than 3 of these mutations were present, but larger data sets did not confirm the role of the L90M in resistance to tipranavir. Subsequently, analyses of data from phase II studies in PI-experienced patients identified mutations associated with reduced susceptibility or virologic response. These include: I10V, I13V, K20 M/R/V, L33F, E35G, M36I, N43T, M46L, I47V, I54A/M/V, Q58E, H69K, T74P, V82L/T, N83D, and I84V. These data must be considered preliminary, and they require further confirmation and validation before their clinical utility can be considered (Schapiro et al, 12th CROI, 2005; Kohlbrenner et al, DART, 2004; Mayers et al, *Antivir Ther*, 2004; Kohlbrenner et al, *Antivir Ther*, 2004; Hall et al, *Antivir Ther*, 2003; McCallister et al, *Antivir Ther*, 2003).

21. Accumulation of these mutations contributes to broad multi-PI resistance (Palmer et al, *AIDS*, 1999; Shafer et al, *Ann Intern Med*, 1998). The genotypic threshold for resistance (ie, the number of mutations needed to have an impact) is higher with PIs boosted with low-dose ritonavir than with PIs that are not boosted.

22. Although resistance to enfuvirtide is associated primarily with mutations in the first heptad repeat (HR1) region of the gp41 envelope gene, wild-type viruses in the depicted HR1 region vary 500-fold in susceptibility. Such pretreatment susceptibility differences were not associated with differences in clinical responses (Labrosse et al, *J Virol*, 2003). Furthermore, mutations or polymorphisms in other regions in the envelope (eg, the HR-2 region or those yet to be identified) as well as coreceptor usage and density may affect susceptibility to enfuvirtide (Reeves et al, *Proc Natl Acad Sci USA*, 2002; Reeves et al, *J Virol*, 2004, Xu et al, *Antimicrob Agents Chemother*, 2005). Thus, testing to detect only the depicted HR1 mutations may not be adequate for clinical management of suspected failure (Reeves et al, *J Virol*, 2004; Menzo et al, *Antimicrob Agents Chemother*, 2004; Poveda et al, *J Med Virol*, 2004; Sista et al, *AIDS*, 2004; Su C et al, *Antivir Ther*, 2004).

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.

hypersusceptibility. As described in user note 1, numerous nRTI mutations may lead to viral hypersusceptibility to the NNRTIs and thus may improve subsequent virologic response to NNRTI-containing regimens.[11-15]

Protease Inhibitors

A comment has been added on the mutations that emerge with PIs that are boosted with low-dose ritonavir and those that emerge with PIs that are not boosted (see user note 16). In general: (1) the same mutations emerge whether or not the PIs are boosted, although there is some difference in the relative frequency of various mutations with boosted versus unboosted PIs; and (2) more mutations are required to impact susceptibility with regimens including a boosted PI.

The lopinavir/ritonavir mutations have been designated as minor mutations (see user note 19). Previously, the lopinavir/ritonavir mutations had not been assigned as either major or minor. The initial data analysis for this drug focused on the number of mutations associated with resistance rather than degree of impact for individual mutations. This approach, which complicates the major/minor designation, is likely to be used for future analyses of other PIs that are boosted with low-dose ritonavir.

Future Revisions of the Figures

As part of the recent revisions to the user notes, the IAS-USA Drug Resistance Mutations Group is developing a table for the IAS-USA Web site (www.iasusa.org) on emerging issues in HIV-1 resistance and available resistance data for drugs in development that have completed phase 2 trials. Other issues under discussion for future versions of the figures and notes include comments on transmitted drug resistance, and the indication of nonsubtype-B mutations.

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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 resistance2005“at”iasusa or by fax at 415-544-9401. Please include your name and institution.

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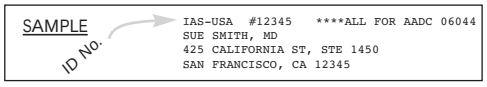

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