Reverse Transcriptase Inhibitors in HIV/AIDS Therapy

Edited by

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Infectious Disease

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Foreword

Although nucleoside analog reverse transcriptase inhibitors (NRTIs) were the first active antiretroviral agents that made it to the market–zidovudine, as early as 1987, less than four years after the discovery of HIV as the causative agent of AIDS–they remain a mainstay of anti-HIV therapy: the "backbone" of most highly active antiretroviral therapy (HAART) regimens still consists of a combination of two NRTIs or the nucleotide.

The dramatic impact that the introduction of HAART in the mid-1990s has had on HIV-related morbidity and mortality in the developed world is one of the success stories of modern medicine, a success story that was initially almost exclusively ascribed to the introduction of HIV protease inhibitors (PIs), but that to a greater extent was a consequence of the introduction of molecular techniques, such as PCR, to measure plasma HIV-1 load. The ability to quantify the HIV load led to the elucidation of HIV dynamics and an understanding of the necessity to suppress HIV replication to near minimal levels with a combination of drugs with nonoverlapping drug resistance patterns. When these principles were applied for the first time in the INCAS study, which used a non-nucleoside RT-inhibitor (NNRTI) instead of a PI as the third drug or "anchor drug" in a HAART regimen, NNRTIs—which were initially discarded because of rapid loss of activity due to the rapid development of viral drug resistance—were resurrected as a valuable antiretroviral drug class. In fact, most first line HAART regimens are now NNRTI- and not PI-based.

With all the excitement about drugs in development with new antiviral targets, such as HIV entry and proviral integration in the host genome, it is easy to forget the simple fact that most current first-line HAART regimens rely exclusively on inhibition of RT, albeit by two different mechanisms. Combinations of two N(t)RTIs and an NNRTI have proven to be exceptionally successful, from the perspectives of efficacy, tolerance, and ease of use.

Who would have predicted this in the early 1990s? Then, NNRTIs were cast aside because of the aforementioned low genetic barrier against resistance development, and NRTIs were considered by many to be useless drugs because of the outcomes of the ill-conceived and misinterpreted Concorde and ACTG155 studies.

Because they can be manufactured at low cost and can be coformulated, NRTI/NNRTI combinations have also become the dominant regimens used in the scale up of antiretroviral therapy in resource-poor settings.

The characterizations "timely" and "relevant" thus very much apply to this book, which covers all aspects of NRTIs, N(t)RTIs and NNRTIs, including

drug discovery, pharmacology, development of viral drug resistance, toxicity, and prevention of mother-to-child transmission of HIV. In recognition of the global distribution of HIV and the current momentum to increase access to antiretrovirals in resource-poor settings, and much to my satisfaction, *Reverse Transcriptase Inhibitors in HIV/AIDS Therapy* also dedicates a chapter to HIV therapies in the developing world, coauthored by my oldest African friend and collaborator Elly Katabira.

May the book enlighten, inspire, and guide those involved in antiviral drug discovery, and those involved in the care and treatment of persons living with a virus that is not only killing individuals on a massive scale, but also fueling a global tuberculosis epidemic and threatening the survival of whole societies.

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Preface

Inhibitors of nucleic acid biosynthesis have had a long and varied history as therapeutic agents. They have frequently provided the backbone of therapy in a wide variety of proliferative disorders ranging from infectious diseases to cancer. Because of the specialized and highly evolved synthetic chemistry in this area, the many analogs of nucleosides, nucleotides, and their biosynthetic precursors have found use as tools for basic research. It is not surprising that, upon discovery of the etiology of AIDS about twenty years ago—it is a syndrome associated with infection with a retrovirus—nucleoside analogs with potential antiviral activity against the virally encoded RNA-dependent DNA polymerase (reverse transcriptase) were among the first compounds to be screened.

Reverse Transcriptase Inhibitors in HIV/AIDS Therapy covers the discovery and development of this class of drugs and others inhibiting the same viral target from a therapeutic perspective. As the vanguard agents with efficacy in this disease, these nucleoside analogs were also the first to manifest the toxicities and resistance associated with chronic administration and inadequate single-agent potency. Nevertheless, they have retained their position as the backbone of therapy in the vast majority of newly treated and treatment-experienced patients. The discovery of several unrelated chemical classes of inhibitors, all binding to the same target, has meant for many patients that viral reverse transcriptase is the sole target for highly active drug combination therapy.

Human cells express many polymerases involved in essential functions. Therefore, there is every expectation that nonselective viral polymerase inhibitors would possess inescapable mechanism-based toxicities. The HIV reverse transcriptase, however, has no human counterpart, giving reason to believe that a wider safety margin might be achievable. This is still a challenging area of research.

The early chapters describe the role of reverse transcriptase in the viral life cycle and structural work that has led to a greater understanding of mechanism and resistance. The discovery and development of six nucleoside analogs are described in the next chapters. Among these are drugs representing milestones in treatment history, such as the benefit of combination therapy, as well as milestones in pharmaceutical manufacturing, such as coformulation. The inescapable topics of toxicities and resistance to this class are described in subsequent chapters.

The non-nucleoside reverse transcriptase inhibitors are described in a similar fashion in general terms, and two chapters discuss these agents with respect to pharmacokinetics and comparative clinical efficacy. New reverse transcriptase inhibitors in all classes in various stages of development are described in one chapter and the impact of the approved agents on treatment in general and on vertical transmission in the developing world are dealt with in the final chapters.

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Structural Studies on HIV Reverse Transcriptase Related to Drug Discovery

David K. Stammers and Jingshan Ren

REVERSE TRANSCRIPTASE: A DRUG TARGET FOR THE CHEMOTHERAPY OF HIV AND AIDS

The problems to human health posed by the AIDS epidemic have prompted wide-ranging research into the causative agent, HIV *(1)*. A greater knowledge of the virus, including a detailed understanding of the structure and function of HIV-encoded gene products is generally expected to be valuable in designing new therapies. HIV, a retroviridae family member, has a relatively small, single-stranded positive sense ribonucleic acid (RNA) genome that contains three main genes (*gag*, *pol*, and *env*) as well as regulatory (*tat* and *rev*) and accessory (*vif*, *nef*, *vpr*, and *vpu*) genes. Although certain of the gene products (such as gag-pol) are further processed to smaller proteins, there is a relatively limited number of potential virus-specific targets against which to develop drugs. The virus-encoded deoxyribonucleic acid (DNA) polymerase has been a cornerstone target for anti-HIV drug discovery because it produces copies of the viral genome, a key step in the replication of HIV. Retrovirus polymerases are referred to as reverse transcriptases (RTs) because the flow of genetic information is from RNA to DNA, the opposite direction to that normally specified. Because of its important role as the target for many anti-AIDS drugs, HIV RT (almost exclusively from the HIV-1 serotype) has been the subject of extensive structural biology studies, particularly studies using X-ray crystallography *(2–5)*. Such studies have been performed with a number of objectives in mind, but, in the context of drug discovery, the key areas of interest include understanding the binding properties of inhibitors, investigating the mechanisms of drug resistance at the molecular level, and structure-based drug design.

CATALYTIC PROPERTIES OF RT

RT is a multifunctional enzyme that catalyses at least three reactions: RNA-dependent DNA polymerase, DNA-dependent DNA polymerase, and

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ribonuclease H (RNase H) activity *(6)*. HIV RNase H is an endonuclease responsible for the degradation of the RNA/DNA heteroduplex after the initial copying of the viral RNA genome. Additionally, RTs are able to bind a specific transfer RNA (tRNA) that is used as the primer for the RNA-dependent DNA polymerase reaction. In the case of HIV-1 and HIV-2, the primer is $tRNA^{lys3}$, which has 18 bases from the 3′ end complementary to part of the HIV genome referred to as the primer-binding site. The initiation step from tRNA is the only stage at which RNA acts as both template and primer; the complex itself can include additional factors, such as the nucleocapsid protein, p7 *(7)*. After initiation of DNA synthesis, the conversion of virus genomic RNA into DNA is a multistep process involving enzymatic steps interspersed by a series of strand transfers. The end product of this process is proviral DNA, which is extended relative to the genomic RNA through a duplication of the long-terminal repeat region. Proviral DNA is incorporated into the host genome by HIV integrase.

THE HIV RT HETERODIMER

The coding sequence for RT is located within the *pol* gene, which also contains protease and integrase enzymes. The *pol* gene is translated as a gag-pol fusion protein, which is the result of a frame-shift event occurring at a frequency that gives a ratio of gag to gag-pol of 40:1. The protease then cleaves out itself as well as other gag and pol proteins, including RT, from the polyprotein. The translated HIV-1 RT contains 560 residues, resulting in a band that migrates on sodium dodecylsulfate polyacrylamide gel electrophoresis with an apparent molecular weight of 66 kDa (p66). Recombinant HIV RT can form a homodimer (p66/p66 for HIV-1) that undergoes further HIV protease-catalyzed cleavage of one subunit between residues 440 and 441 (Tyr-Phe), resulting in the removal of the C-terminal RNase H domain (p15) and yielding the stable heterodimer (p66/p51) *(8)*, which is the form found in the virion *(9)*. The released p15 fragment seems largely devoid of RNase H activity and apparently has no other function. As described in a later section (The Architecture of the HIV-1 RT Heterodimer), the p51 subunit has a radically different arrangement of its four domains compared with the corresponding region of p66, and is not an active DNA polymerase. There is evidence that the p51 subunit may have a role in binding the $tRNA^{lys3}$ required for priming the RT reaction *(10)*.

CLASSES OF DRUGS THAT TARGET HIV RT

Nucleoside Analog Inhibitors of RT

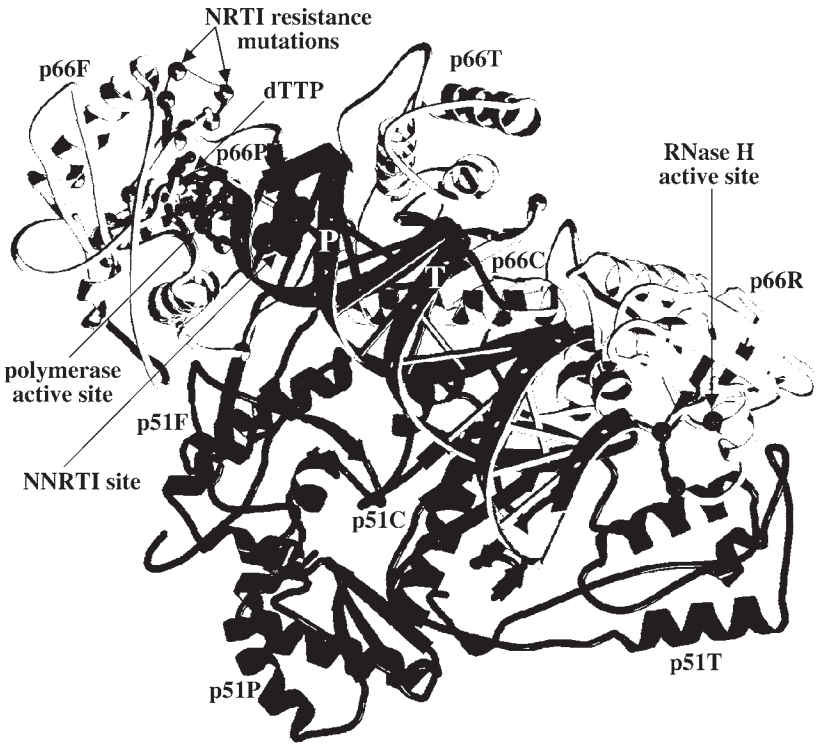
The development of antiviral agents before the HIV era was mainly focused on compounds active against the human herpesviruses. In vitro screening of compounds against herpesvirus in tissue culture successfully identified the

nucleoside analog, acyclovir, which became a widely used drug. Acyclovir is a guanosine analog containing an acyclic sugar chain, and its initial activation is via a selective phosphorylation by the thymidine kinases of herpesviruses such as herpes simplex 1 or varicella zoster *(11)*. Acyclovir triphosphate acts as a substrate for the herpesvirus-encoded DNA polymerase, leading to incorporation into the primer strand and chain termination. Thus, nucleoside analogs were obvious starting points in the search for anti-HIV drugs, although, in contrast to most herpesviruses, HIV does not encode a thymidine kinase, hence, the activation of the nucleoside is entirely via cellular kinases. Inhibitor screens against HIV in tissue culture identified a number of potent nucleosides, and zidovudine (azidothymidine) was rapidly approved for treating AIDS patients. Biochemical assays identified zidovudine triphosphate as a selective inhibitor of HIV RT compared with the cellular DNA polymerase-α *(12)*. Zidovudine-triphosphate acts as a competitive inhibitor of the substrate thymidine triphosphate (dTTP), but can itself also be incorporated into the primer strand and, thereby, act as a chain terminator because an azido group occupies the 3′-ribose position *(13)*. Further nucleoside analogs, such as didanosine, zalcitabine, lamivudine, stavudine, and abacavir, have been approved for treatment of HIV infection; in each case, they act as RT inhibitors by similar competition/chain terminating mechanisms. The nucleoside class of inhibitors of RT is referred to as the nucleoside analog RT inhibitors (NRTIs). Some NRTIs are not fully selective for HIV RT and can also inhibit certain cellular DNA polymerases, which is thought to contribute to clinical toxicities, such as neuropathy *(14)*.

Non-Nucleoside RT Inhibitors

First-Generation Non-Nucleoside RT Inhibitors

After the initial identification of NRTIs as therapeutic agents for treating HIV infection, a second distinct class of RT inhibitors, referred to as non-nucleoside RT inhibitors (initially abbreviated as NNIs, but more recently referred to as NNRTIs) was discovered *(14–16)*. NNRTIs were found by screening compound libraries against HIV-1 virus in tissue culture or against recombinant HIV-1 RT in enzyme asssays. As the name implies, NNRTIs generally are structurally distinct from nucleosides, they are hydrophobic molecules of diverse chemical structure that are generally highly specific for HIV-1 (Scheme 1). Kinetically, NNRTIs are noncompetitive with respect to deoxyribonucleoside triphosphates (dNTPs) and nucleic acid substrates. Crystal structures of RT in complexes with NNRTIs have revealed the inhibitor site on the p66 subunit to be distal to the polymerase active site. In the vast majority of cases, NNRTIs do not inhibit the HIV-2 virus or the RT from this HIV serotype. There are, however, some reported examples of weak inhibition of HIV-2 RT by NNRTIs, for example phenylethylthiazolylthiourea (PETT)-2 has an inhibitory concentration



Scheme 1. Structures of some NNRTI compounds.

of 50% (IC_{50}) of 2 μ*M* against HIV-2 RT, yet is almost three orders of magnitude more potent against HIV-1 RT *(17)*. First-generation compounds, such as hydroxyethoxymethylphenylthiothymine (HEPT), nevirapine, 9-chlorotetrahydroimidazo-benzodiazepin-2-one (Cl-TIBO), and delavirdine, are characterized by large reductions in potency for a wide range of single point mutations within RT selected by NNRTIs in either tissue culture or clinical use *(18)*. Thus, first-generation NNRTIs were of very limited use as monotherapies for the treatment of HIV infection, although, more recently, they have found a role in multidrug regimens used for highly active antiretroviral treatment. Two such NNRTIs approved for use in combination therapy with NRTIs or HIV protease inhibitors are first-generation compounds: nevirapine and delavirdine.

Second-Generation NNRTIs

Follow-up work after the discovery of nevirapine identified further chemical series of NNRTIs that demonstrated much greater resilience to the presence of many of the drug-resistance mutations in RT identified from earlier studies. For example, efavirenz shows only a 2-fold loss of activity against the Tyr181Cys mutation in vitro, whereas, by comparison, nevirapine has a 40-fold reduction in potency *(19)*. Compounds such as efavirenz are termed second-generation NNRTIs, and some of these inhibitors show dramatic improvements in their resistance profile, including the retention of significant activity against RTs containing two resistance mutations *(20)*. Efavirenz is, to date, the only second-generation NNRTI approved for clinical use. It has been shown in the clinic that resistance to these approved NNRTIs also gives rise to extensive cross-resistance to currently available NNRTIs. Additional second-generation NNRTIs, including capravirine (also known as S-1153), are in clinical trials *(20)*. An objective of a number of structural studies of HIV RT has been to understand the molecular basis of the differing resistance profiles of first- and second-generation NNRTIs.

DRUG RESISTANCE OF HIV RT TO INHIBITORS

Resistant forms of HIV are selected in vitro and during the clinical use of anti-HIV drugs. The rapid turnover of the virus is considered the most significant factor giving rise to the selection of such drug-resistant forms, although the errors generated by the RT (which contains no editing function) also contribute to drug resistance *(21)*. Resistance to zidovudine, the first NRTI used as monotherapy, emerged in the clinic during a period of weeks to months *(22,23)*, whereas for the NNRTI nevirapine, resistance was selected in a matter of days to weeks, making nevirapine useless as monotherapy *(24)*. Despite the introduction of highly active antiretroviral treatment, there is still emergence of drug resistance in HIV, which, in part, is caused by compliance problems associated with the side effects of the long-term treatment needed to treat this chronic infection. Thus, the selection and spread of drug-resistant virus remains one of the key issues in continued efforts to combat HIV and AIDS in Western countries. These preceding issues are discussed in much greater depth in subsequent chapters.

HIV RT CRYSTAL STRUCTURES

The Architecture of the HIV-1 RT Heterodimer

A number of crystal structures of HIV-1 RT have been published during the last 10 years; mainly of the full RT heterodimer. In addition, structures of some RT domains have been determined, including the C-terminal HIV-1 RNase H *(25)*

and N-terminal fragments from HIV-1 *(26)* and murine Moloney RTs *(27)*. The HIV-1 RT heterodimer structures determined, to date, can be divided into three general categories:

1. complexes containing nucleic acid, which include binary complexes with DNA or RNA/DNA with a bound Fab fragment *(3,28)*, a covalently linked double-stranded DNA (dsDNA) catalytic complex with bound dTTP *(29)*, and a complex with an RNA pseudoknot *(30)*;
2. unliganded forms *(5,31,32)*; and
3. complexes with NNRTIs, which have been mainly determined in two different crystal forms *(2,4)*, with a third form described more recently *(33)*.

In comparison with HIV protease, for which hundreds of structures have been reported, there are a more limited number of HIV RT structures available (currently <100 are deposited in the Protein Data Bank, www.pdb.org). The higher molecular weight of RT compared with protease (117 kDa vs 18 kDa) and the presence of many domains in RT that may give rise to flexibility probably account for the poor quality of many of the RT crystals studied. The result is that RT crystals show weak diffraction, often to only medium resolution, therefore, the collection of accurate X-ray data to sufficient resolution for full structural refinement is not always straightforward. Such technical difficulties, in turn, provide a limiting factor in attempting structure-based drug design approaches. Initial studies using a monoclinic crystal form of HIV-1 RT were carried out at 3.5-Å resolution *(2)* but a more favorable orthorhombic crystal form, capable of diffracting to 2.2 Å after a partial dehydration procedure *(34,35)*, has proved the most useful in yielding well-refined, high-resolution structures.

The first crystal structure of HIV-1 RT revealed the basic architecture of the p66/p51 heterodimer *(2)*. The N-terminal portion of the p66 subunit is arranged in a structure that is analogous to an open right hand containing three domains, referred to as fingers, palm, and thumb. The connection domain follows the thumb domain and leads finally to the C-terminal RNase H domain. One of the most surprising features of this structure was the radically different arrangement of the four domains within the p51 subunit when compared with the p66 subunit, such that the cleft is occluded in p51, therefore, this subunit cannot be an active polymerase (Fig. 1).

The assignment of secondary structure elements in RT models is varied in different reports *(2–4)*. This variation could be caused by some genuine changes between structures but is also the inevitable result of the limited resolution of the first RT structures determined, which meant that it was not possible to carry out full structural refinements, resulting in some incorrect assignments of secondary structure and alignments of amino acid sequences to the models. Some changes to the initial secondary structure nomenclature of Kohlstaedt et al. *(2)* were made for the RT/DNA/Fab complex *(3)*. However, the results from the first fully

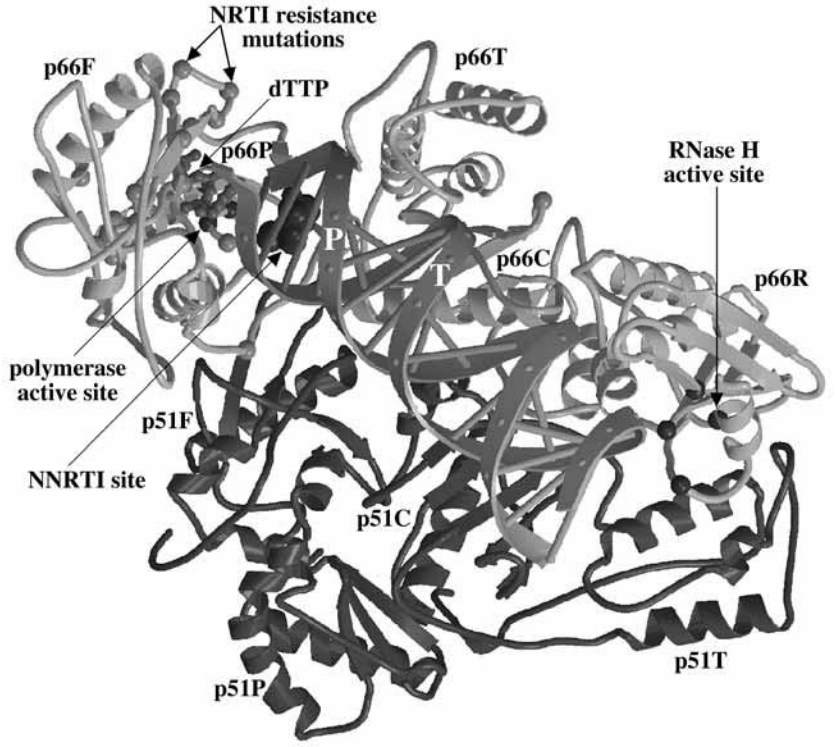


Fig. 1. Schematic diagram showing the overall structure of HIV-1 reverse transcriptase (RT). The protein chains are shown as ribbons and coils with the p66 subunit in light gray and the p51 subunit in dark gray. The double-stranded deoxyribonucleic acid in the structure of a catalytic complex *(29)* is drawn as spiral ladder with T and P marking the template and the primer, respectively, whereas the bound thymidine triphosphate (dTTP) is drawn in ball-and-stick representation. The key residues of the polymerase and ribonuclease H (RNase H) active sites are indicated as black spheres. The gray spheres represent the sites of nucleoside analog RT inhibitor (NRTI)-resistance mutations. The nevirapine molecule shown as a black space-filling model marks the non-nucleoside RT inhibitor (NNRTI) site.

refined, high-resolution HIV-1 RT structure indicated enough differences in secondary structure assignments to indicate that a significant revision of the nomenclature was necessary *(36)*; the revised nomenclature is used in this chapter.

Interactions Between RT and NRTIs

Nucleotide Binding in the RT Catalytic Complex

A significant step forward in understanding the binding of dNTP substrates to HIV-1 RT was the structure determination of a catalytic complex of the enzyme with bound dTTP and covalently linked, dsDNA *(29)*. A modified

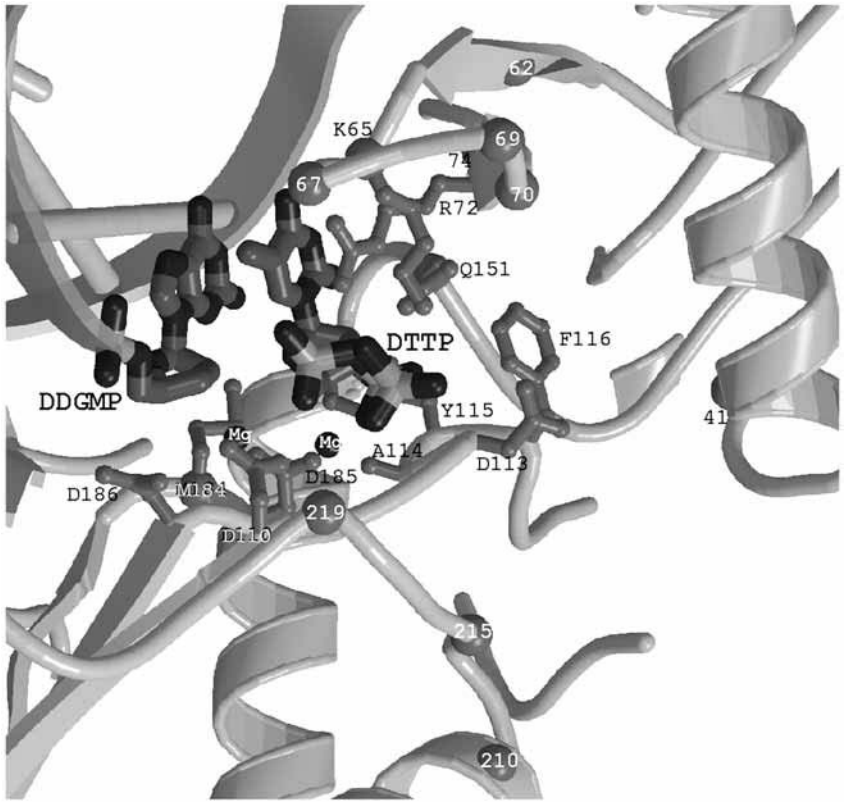


Fig. 2. The polymerase active site of the HIV-1 reverse transcriptase (RT) catalytic complex crystal structure *(29)*. The protein main chains are shown as ribbons and coils. The template and the primer strands are shown as a spiral ladder. The chain terminator (ddGMP) and the substrate thymidine triphosphate (dTTP) are highlighted in dark thicker bonds. The three active-site aspartic acids and residues that interact with the dTTP are drawn in ball-and-stick representation. The larger gray and small black spheres indicate the Cα positions of nucleoside analog RT inhibitor-resistant mutations and two Mg^{2+} ions, respectively.

guanosine base containing a sulfhydryl group was incorporated into the template strand, allowing linkage to a cysteine introduced at position 258 in the thumb domain of RT after rounds of dNTP incorporation and chain termination. Such covalent trapping is necessary to observe the catalytic complex because there is a lack of specific register between RT and the DNA substrate. The RT complex with covalently linked dsDNA has a dideoxyguanylate terminating the primer strand *(29)*. The next available base in the template is an adenine, allowing the adjacent dNTP site to be occupied by dTTP, but without further reaction (Fig. 2). This catalytic complex shows significant differences in conformation of the

protein compared with the binary RT-DNA structure *(37)*. There is a closure of the fingers and thumb domains, resulting in a more-constricted central cleft and closer protein contacts with the dNTP site. The template overhang is positioned outside the central cavity, rather than between the fingers and thumb domain, as had been previously inferred from modeling studies with the noncatalytic binary DNA complex *(38)*. dTTP is located at the primer terminus, with the thymine base stacked as if in a continuous DNA strand, whereas the side chains of Lys65 and Arg72 interact with its outer surface. The closure of the fingers domain allows side-chain interactions with the dTTP triphosphate group (Lys65 with the α-phosphate and Arg72 with the γ-phosphate). Additional interactions are via the main chain NH– groups of residues, 113 and 114, and via two magnesium ions, one of which links to two of the key catalytic aspartates, 110 and 185. The 3′-hydroxyl of dTTP projects into a pocket that contains the side chains of Asp113, Tyr115, Phe116, and Gln151, as well as the backbone of residues 113 to 115. This pocket has room to accommodate the 3′-azido group of zidovudine and, thus, is important for an understanding of the structure–activity relationships and mechanisms of resistance for this NRTI (Fig. 2).

Other nucleotide-binding positions have been inferred from model-building studies. These include lamivudine-triphosphate docked into an RT (Met184Ile)/DNA complex *(39)*.

Experimental and Modeling Studies Designed to Explain the Structural Basis for Drug-Resistance Mechanisms for NRTIs

When the first structure of HIV-1 RT was determined, it was immediately apparent that many of the NRTI-resistance mutations, particularly those for zidovudine, mapped to positions distal to the putative dNTP site defined by the three essential catalytic aspartates *(2)*. A number of hypotheses were put forward to explain the distal positioning of NRTI-resistance mutations, including template rearrangement *(38)* and long-range conformational changes *(40)*. A notable exception to the distal positioning of NRTI mutations from the active site is observed in the case of Met184Val, a mutation that confers a high-level resistance to lamivudine *(41,42)*. Residue 184 is part of the conserved YMDD active-site motif found in all immunodeficiency virus RTs. From model building and biochemical studies, it seems likely that the unusual stereochemistry of the sugar ring of lamivudine gives rise to a steric clash with a β-branched side chain, such as isoleucine or valine *(39)*. The crystal structure of the RT (Met184Ile) binary DNA complex at a 3.5-Å resolution also indicates that a repositioning of the template overhang occurs, which has been suggested as a contributing factor to the resistance mechanism *(39)*. However, others consider it not necessary to invoke such a template rearrangement, proposing that the

steric clash of the mutated side chain with lamivudine triphosphate is sufficient to explain resistance *(43)*.

Significant progress in resolving certain aspects of NRTI-resistance mechanisms resulted from the determination of the structure of a catalytic complex of RT in which dsDNA was covalently linked to the enzyme *(29)*. The presence of a chain terminator allowed a dNTP (in this case, dTTP) to be bound in the acceptor site without reacting further. This structure revealed a closing down of the fingers domain, such that certain residues moved nearer to the active site. Thus, the site of the characteristic resistance mutation for didanosine at residue 74 (Leu74Val), which is situated on the β-2 β-3 loop, is within 3.5 Å of the thymidine ring of dTTP. The catalytic complex also showed that Lys65, a resistance mutation for multinucleoside resistance, and Arg72 form direct contacts with the phosphates of dTTP. Thus, it could be inferred that the effect of the insertion mutant at position 69 is to cause perturbation of neighboring residues, which, in turn, alter interactions with the phosphate groups of the nucleotide. Gln151 shows a direct contact with the nucleotide, again via a phosphate group in the catalytic complex; thus, the Gln151Met change, a key multinucleotide-resistant mutation, can be envisaged as causing a selective disruption of the binding of NRTIs compared with the standard nucleotide triphosphates. Although elucidating the structure of the catalytic complex has been a major step forward in understanding NRTI-resistance mechanisms, it does not provide all of the answers to zidovudine resistance, because, in several cases (e.g., 215 and 219), mutations are still positioned at some distance from the dNTP site in the catalytic complex (Fig. 2).

An important breakthrough in the understanding of the possible biochemical mechanism of zidovudine resistance was the identification of a pyrophosphorolysis reaction that is capable of removing zidovudine monophosphate from the terminated primer strand. Such pyrophosphorolysis can be catalyzed by pyrophosphate or adenosine triphosphate (ATP). Both the affinity for the zidovudine monophosphate-blocked primer *(44)* and the rate of the pyrophosphorolysis reaction are increased for the zidovudine-resistant RT containing four mutations (Asp67Asn, Lys70Arg, Thr215Phe, Lys219Gln) *(45,46)*. ATP is considered the likely physiological ligand for this reaction because its cellular concentration (2 m*M*) is much higher than the pyrophosphate concentration *(47)*. It has also been shown that the pyrophosphorolysis reaction is increased for a number of RT mutants *(48,49)* and can release other NRTI chain terminators, including stavudine *(47,50)*.

To date, there have been no crystal structures of HIV-1 RT reported with bound ATP, although modeling studies have been undertaken *(51,52)*. Docking ATP into RT by overlapping the phosphate groups in the opposite sense to dTTP in the crystal structure of the catalytic complex of RT gives a plausible binding mode. There are indications that the side chain of the key zidovudine-

resistance mutation, Thr215Tyr/Phe, can form aromatic ring-stacking interactions with the adenine ring of ATP, presumed to give tighter binding of the nucleotide *(51,52)*. However, it is more difficult to envisage a direct interaction of ATP with residue 41 (Met41Leu in combination with Thr215Tyr gives high-level zidovudine resistance), and a significant conformational change would be required to allow contacts. Alternatively, an indirect mechanism might apply, for example, it has been noted that the side chain of residue 41 interacts with Phe116, and with the adjacent residue Tyr115, which in turn, forms hydrogen bonds with the phosphates of dTTP *(53)*.

It has long been recognized that there are interactions between different sets of resistance mutations in HIV-1 RT. Therefore, the Met184Val mutation that provides resistance to lamivudine *(41,42)* reverses the effect of the zidovudine-resistance mutations, such as those at codons 41 and 215 *(54)*. Similarly, the nevirapine-resistance mutation, Tyr181Cys, can also restore sensitivity to zidovudine in previously resistant HIV *(55)*, indicating communication between the NRTI- and NNRTI-binding sites, over relatively long distances. Kinetic studies of the effects of the Met184Val mutation suggest that this mutation attenuates the rate of the pyrophosphorolysis reaction, thus providing a biochemical rationalization of the reversal effect on zidovudine resistance *(56,57)*. The structural basis for the reversal effect, however, remains unclear.

Clearly, a number of crystal structures of mutant RTs with covalently linked DNA in the presence of different NRTIs or ATP are required to provide further understanding of the mechanism of drug resistance at the molecular level for this class of anti-HIV drugs.

The NNRTI-Binding Site

Architecture of the NNRTI-Binding Site and the Two-Ring Binding Mode

The NNRTI-binding site is sandwiched between two three-stranded β-sheets (β4, β7, and β8; and β9–β11) and is largely contained within the p66 subunit, the one main exception being the side chain of Glu138 from the p51 subunit (Fig. 3) *(2,4)*. The NNRTI site represents the largest cavity in the RT molecule (volume ~720 $Å^3$ for the complex with nevirapine). The internal surface of the pocket is largely hydrophobic in nature, and the loss of accessible surface area on binding inhibitors is variable but mainly involves shielding of Leu100, Tyr181, and Tyr188. Tyr181, Tyr188, and the invariant Trp229 form a subpocket at the "top" of the NNRTI-binding site. Further important contacts that various NNRTIs make with hydrophobic residues include Val106, Val179, Phe227, and Tyr318. Some polar residues also form part of the site, including Lys101, Lys103, and Glu138 (p51). These residues are all positioned at the periphery of the pocket, in a region that has access to bulk solvent. The alkyl regions of the side chains of Lys101, Lys103, and the carboxyl group of Glu138

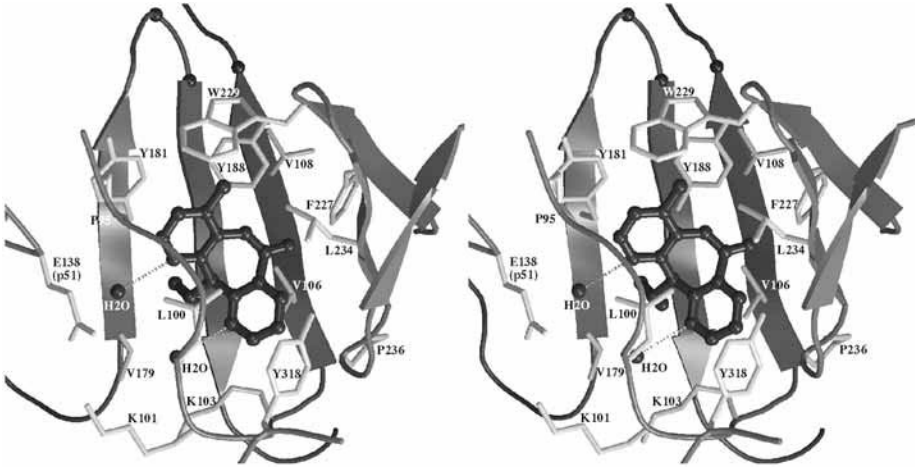


Fig. 3. Stereo diagram showing the non-nucleoside reverse transcriptase inhibitor (NNRTI)-binding pocket formed between two β-sheets, each containing three strands (β4, β7, and β8; and β9–β11). The protein backbone is shown as dark gray ribbons and coils. The side chains of residues lining the NNRTI pocket are drawn as light gray sticks. The nevirapine molecule is shown in black ball-and-stick representation. The broken lines represent hydrogen bonds formed from two water molecules (black spheres) to the nevirapine. The positions of the Cα atoms of the three polymerase active-site aspartic acid residues are marked by black spheres *(4)*.

are able to make contact with certain NNRTIs. The charged end groups of Lys101 and Lys103 have not been observed to interact with NNRTIs in any of the published structures. The bulky NNRTI, delavirdine (also known as *bis*(heteroaryl)piperazine [BHAP] or U-90152), is able to make a contact via its indole ring with the side chain of Pro236 positioned at the edge of the inhibitor pocket (Fig. 4), an interaction not accessible to smaller compounds *(58)*. The methylsulfonamide group of delavirdine projects from the NNRTI pocket into the bulk solvent, creating a channel between polypeptide segments 225/226 and 105/106. This has been suggested as a possible site of entry for inhibitors into the pocket *(58)*.

In addition to side-chain contacts, there are a number of hydrogen-bonding interactions between NNRTIs and protein main-chain atoms. The most commonly observed of such hydrogen bonds is with the carbonyl of Lys101, but hydrogen bonding with Lys103 and Pro236 main-chain atoms have also been reported *(58,59)*. Overlap of a core region of the RT palm domain allows detailed comparison of the binding modes for differing NNRTIs *(4)*, revealing a remarkable degree of inhibitor overlap between widely divergent chemical series (Fig. 5) *(4,58,60)*. The common theme is the presence of a "two-ring"

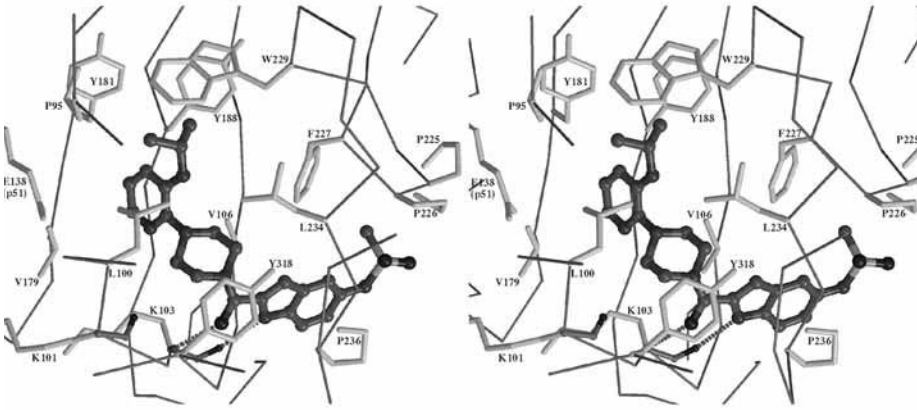


Fig. 4. Stereo diagram showing the non-nucleoside reverse transcriptase (RT) inhibitor-binding pocket for the RT/delavirdine (also known as *bis*(heteroaryl)piperazine [BHAP] or U-90152) complex *(58)*. The Cα backbone of the protein is draw as thinner, dark-gray bonds, the protein side chains surrounding the inhibitor are shown as thicker, light-gray bonds. The BHAP molecule is shown as a dark ball-and-stick representation. The inhibitor makes two hydrogen bonds (broken bonds) to the main-chain amide nitrogen and carbonyl oxygen atoms of residue Lys103.

system, with an angle of approx 120° (105–148°) between them. The tricyclic compound, Cl-TIBO, does not obviously conform to this mode; however, modeling studies predicted that the Cl-TIBO dimethylallyl group overlapped with one of the two ring moieties *(61)* and this was confirmed experimentally *(36,60)*. A further example of an acyclic group mimicking an aromatic ring has been observed for UC-781. Although UC-781 contains two rings and the naive assumption is that each should overlap with the NNRTI two-ring pharmacophore; in fact, crystallographic studies reveal that the 3-methylallyl group mimics the second ring in a manner similar to Cl-TIBO *(60,62)*. Thus, modeling NNRTIs into HIV-1 RT is not straightforward, and, indeed, such studies have had somewhat varying success. For example, one attempt at predicting the binding mode of certain carboxanilides was unsuccessful *(63)* because they were modeled assuming a *trans* conformation of the carboxanilide ring, whereas crystal structures revealed that they bound with a *cis* conformation *(62)*. The docking attempts for related carboxanilides *(64)* were much closer to the experimentally determined binding mode, however, the subtleties of the slightly different binding modes of the first-and second-generation versions of this compound series determined from the crystal structures were not predicted. A study aimed at predicting the bound conformation of certain thiazolo-isoindolinone NNRTIs was performed by overlapping them with Cl-TIBO and nevirapine *(61)*. A largely correct overlap with Cl-TIBO was obtained, but the

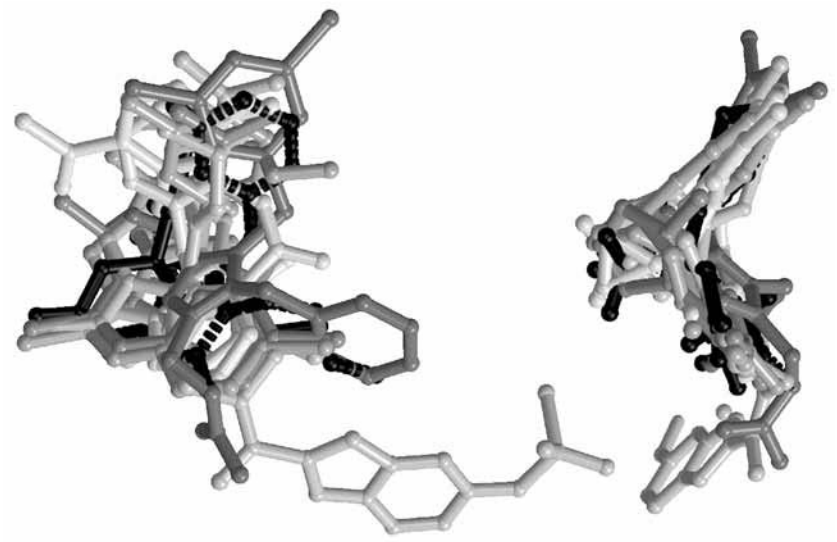


Fig. 5. The positions, orientations, and conformations adopted by the representative non-nucleoside reverse transcriptase (RT) inhibitors (NNRTIs) of 10 different chemical series in the crystal structures of RT/NNRTI complexes. The inhibitors shown are: nevirapine *(4)*; α-APA *(4)*; 9-Cl-TIBO *(36)*; delavirdine (also known as *bis*(heteroaryl)piperazine [BHAP] or U-90152) *(58)*; phenylethylthiazolylthiourea (PETT)-2 *(17)*; MKC-442 *(70)*; BM+21.1326 *(65)*; UC-781 *(62)*; efavirenz (also known as DMP-266) *(73)*; and capravirine (also known as S-1153 or AG1549) *(59)*. Two orthogonal views of the NNRTIs alone are shown in ball-and-stick representations with MKC-442, efavirenz, and capravirine highlighted in broken black bonds, dark gray, and black, respectively. The superimposition was performed using Cα atoms of the protein residues around the NNRTI site (residues 94–118, 156–215, and 225–243 from the palm domain; residues 317–319 from the connection domain; and residues 137–139 from the fingers domain of p51). α-APA, α-anilinophenylacetamide; 9-Cl-TIBO, 9-chloro-tetrahydroimidazo-benzodiazepin-2-one; MKC-442, UC-781, BM+21.1326, DMP-266 are pharmaceutical company designations.

pseudo-chiral center of nevirapine led to an incorrect overlap, as was later demonstrated by X-ray crystallography of the complex with HIV-1 RT *(65)*.

Mechanism of Inhibition of RT by NNRTIs

After the first structure determination of RT as a complex with nevirapine, it was suggested that the drug could be inhibiting by distorting the active-site geometry or by attenuating the conformational flexibility necessary for the correct functioning of the polymerase, thereby inducing "molecular arthritis" *(2)*. Comparison of structures of RT in the presence or absence of an NNRTI reveals significant local differences in protein conformation; in particular, the inhibitor

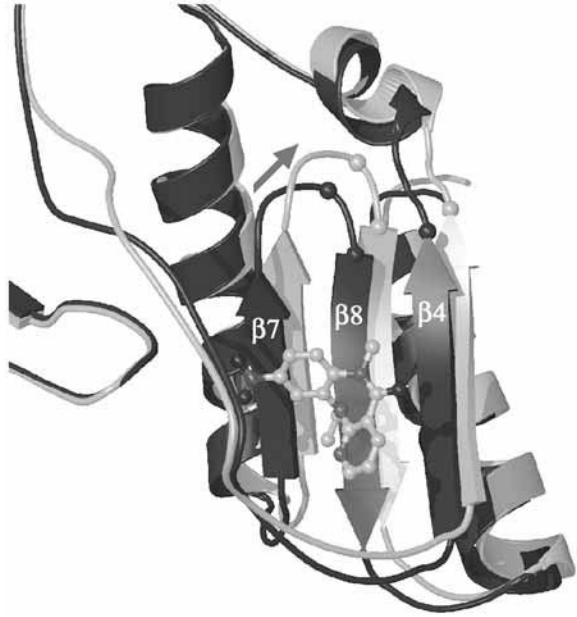


Fig. 6. Comparison of the polymerase active site and the non-nucleoside reverse transcriptase (RT) inhibitor (NNRTI)-binding site in the apo enzyme and in the inhibitor-bound form (RT/1051U91); both structures are in the same crystal form. A ball-and-stick representation is used for the 1051U91 molecule. Three segments of protein chain are shown (residues 83–125 and 146–215 from the p66 subunit, and residues 132–152 from the p51 subunit) with the apo structure drawn in darker gray. Strands β9–β11, which lead into the thumb domain, have been omitted from the diagram for clarity. The three aspartic acid residues contributing to the polymerase active site (residues 110, 185, and 186) are indicated by small spheres marking their Cα atoms. The three-stranded β-sheet comprising β4, β7, and β8 moves as a rigid body to create the NNRTI pocket, the direction of movement is indicated by an arrow. The displacement of Cα for Asp186 is 1.9 Å *(31)*.

site is collapsed in the unliganded RT form *(5,31,32)*. On binding an NNRTI, the side chains of Tyr181 and Tyr188 undergo a switch from a "down" position, which essentially fills the space occupied by the NNRTI, to an "up" position. The Tyr181 and Tyr188 then form two faces capable of interaction with inhibitors via aromatic ring stacking, a particularly feature of first-generation NNRTIs. In addition, there is a significant distortion of the β-sheet consisting of strands β4, β7, and β8, which contains the key catalytic aspartate residues (Asp110, Asp185, and Asp186) (Fig. 6). Because of the requirement for precise alignment of enzyme catalytic groups with the substrate to achieve enhancement of the rate of reaction, distortion of the active site by NNRTIs is likely to

provide the mechanism of inhibition of RT by these compounds. Pre-steady state kinetics of NNRTI binding to HIV-1 RT indicated that the inhibitor does not significantly alter the rate or equilibrium constant for conformational changes during the catalytic cycle, and these data indicate that there is direct communication between the NNRTI and polymerase sites *(66,67)*. The kinetic results are, therefore, entirely consistent with the structural mechanism of resistance proposed by Esnouf et al. *(31)*. Other structural proposals put forward to explain NNRTI inhibition include the movement of the β9–β11 sheet *(68)*, however, this region seems to be inherently flexible, because it is capable of adopting different conformations in a range of RT/NNRTI complexes and, thus, is a less likely candidate for such a role.

Structural Requirements for Tight-Binding NNRTIs

The structure determination of a series of HEPT analogs provided clues regarding structurally important features that are required for tight-binding NNRTIs. HEPT is a weak inhibitor of HIV-1 RT (IC_{50} of 17 μ*M*), whereas MKC-442, which has a bulkier isopropyl group at the 5-position in place of the methyl group of HEPT, binds orders of magnitude more potently, with a IC_{50} of 8 n*M* *(69)*. TNK-651 contains a larger substituent in the 1-position compared with MKC-442, and binds with an IC_{50} of 4 n*M*. Comparing the crystal structures of RT complexes with these compounds showed variations in protein conformation *(70)*. In the case of HEPT, Tyr181 is in a "down" position and the phenyl ring of the inhibitor is rotated compared with MKC-442 (Fig. 7). The complexes of both RT/MKC-442 and RT/TNK-651 have Tyr181 in the conventional "up" position, and the residues in the 236 loop were in a more-extended conformation. The observations can be rationalized by consideration of the effect of bulk at the 5-position of the pyrimidine ring. If the substituent is ethyl or isopropyl, then it is large enough to act as a "trigger" that correctly orients the side chain of Tyr181 in the "up" position, such that an energetically favorable aromatic ring stacking interaction can be made with the phenyl ring of MKC-442. The rearrangement of the 236 loop does not seem to contribute significantly to the binding energy of this compound series, because this movement does not correlate with the values of the dissociation constants. Structural studies of other NNRTI series show that the bulky group orienting Tyr181 does not have to be in a directly analogous position relative to the aromatic rings of the inhibitor. For example, with nevirapine, the cyclopropyl group provides this bulk; whereas, in the case of a series of 2-amino-6-arylsulfonylbenzonitriles, the sulfonyl group that forms the link between the two aromatic rings acts as the trigger that orients Tyr181 *(71)*.

Structural Features of Second-Generation NNRTIs

Structural studies of a range of second-generation inhibitors have been reported, including UC-781, HBY093, S-1153, and efavirenz *(59,62,72,73)*. Such

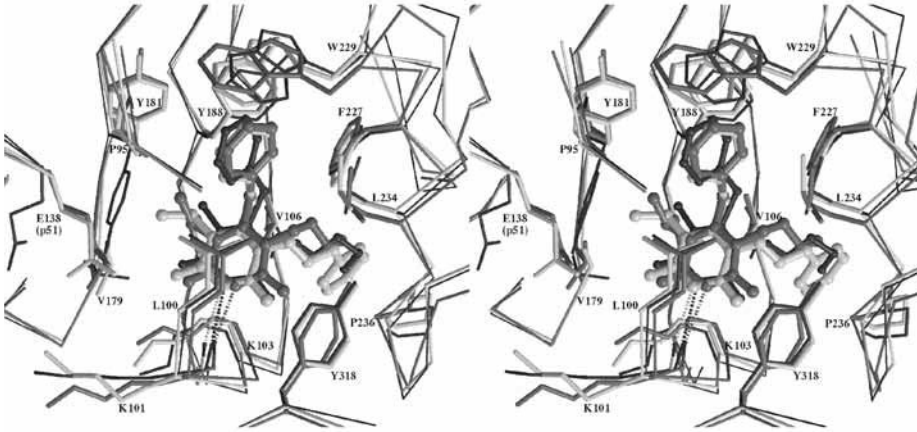


Fig. 7. Stereo diagram showing the superposed non-nucleoside reverse transcriptase (RT) inhibitor (NNRTI)-binding site for the RT complexes with hydroxyethoxymethylphenylthiothymine (black), MKC-442 (dark gray), and TNK-651 (light gray). The NNRTIs are shown as ball-and-stick representations, and the Cα backbones and side chains of the surrounding protein residues as sticks of the same color. Hydrogen bonds from inhibitors to the carbonyl oxygen of residue 101 are indicated as broken bonds *(70)*.

studies have suggested a number of factors that might contribute to the resilience of some of these second-generation compounds when challenged by mutations in HIV-1 RT that give high-level resistance to first-generation compounds. Crystal structures of a series of four carboxanilide NNRTIs with examples of both first- and second-generation properties allowed a direct comparison between structurally related compounds and provided some clues regarding factors contributing to the differing resistance profiles (Fig. 8) *(62)*. The four compounds all bind in an overall similar position and with similar bound conformations; however, there are some subtle differences that correlate with whether the inhibitor has first- or second-generation characteristics. It seems that the longer substituents of the two compounds with second-generation properties (UC-10 and UC-781) mean that these inhibitors have fewer contacts with Tyr181 and Tyr188 side chains than do the first-generation compounds (UC-38 and UC-84).

The crystal structure of the clinically approved drug, efavirenz, in complex with HIV-1 RT reveals that the compound does not form ring-stacking interactions with Tyr181 and Tyr188 *(73)*. In fact, contacts for efavirenz are more limited than in the case of nevirapine, and are formed via the propynyl-cyclopropyl group (particularly with Tyr188) (Fig. 9).

S-1153 is an experimental second-generation NNRTI currently undergoing clinical trials that has activity against a wide range of mutant RTs, including

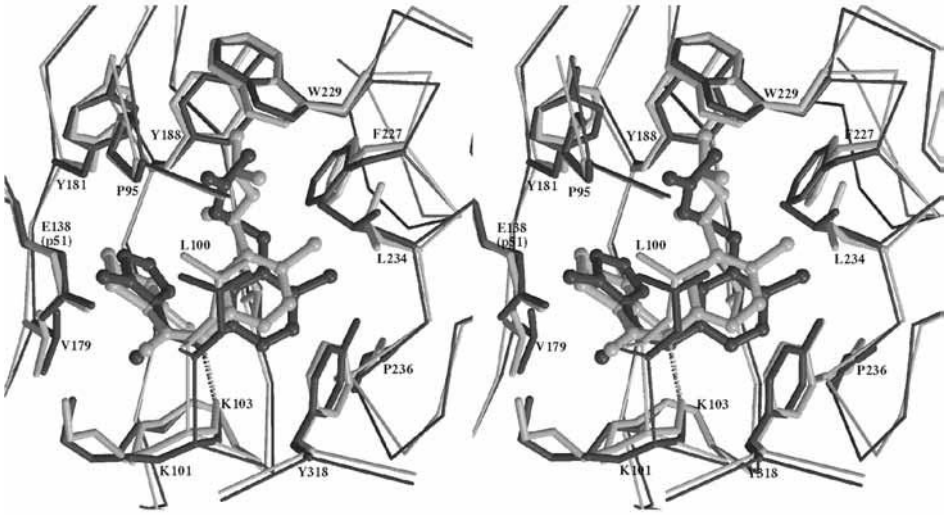


Fig. 8. Stereo diagram comparing the non-nucleoside reverse transcriptase (RT) inhibitor sites of RT/UC-781 and RT/UC-84 complexes *(62)*. The Cα backbones (thinner bonds), side chains (thicker bonds), and the inhibitors (ball-and-stick representations) are shown in darker gray and light gray for RT/UC-781 and RT/UC-84, respectively.

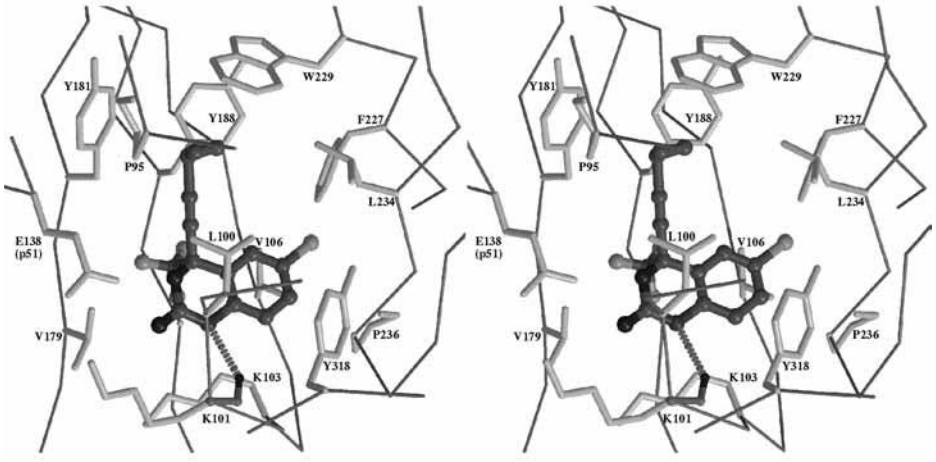


Fig. 9. Stereo diagram showing the non-nucleoside reverse transcriptase (RT) inhibitor-binding pocket for the RT/efavirenz (also known as DMP-266) complex *(73)*. The Cα backbone of the protein is draw as thinner, dark-gray bonds, the protein side chains surrounding the inhibitor are shown as thicker, light-gray bonds. The efavirenz molecule is shown as a dark ball-and-stick representation. The inhibitor makes a single hydrogen bond (broken bonds) to the main-chain amide nitrogen atom of residue Lys103.

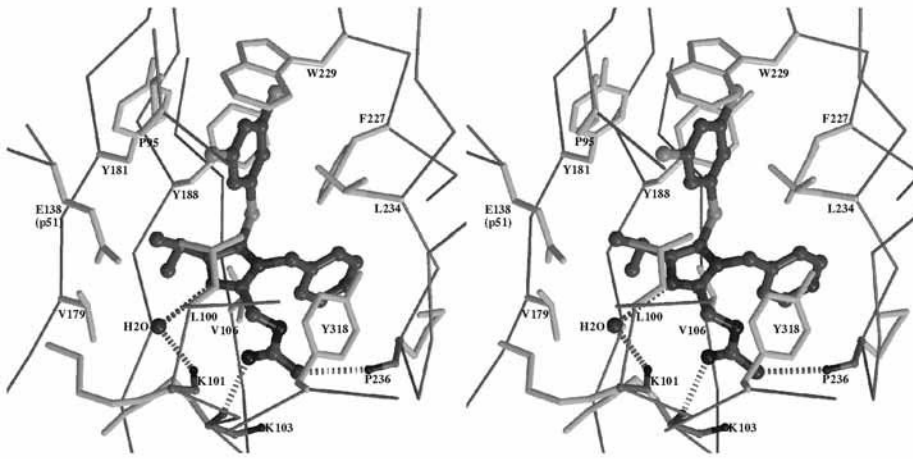


Fig. 10. Stereo diagram showing the non-nucleoside reverse transcriptase (RT) inhibitor-binding pocket for the RT/capravirine (also known as S-1153) complex *(59)*. The Cα backbone of the protein is draw as thinner, dark-gray bonds, the protein side chains surrounding the inhibitor are shown as thicker, light-gray bonds. The capravirine molecule is shown as a dark ball-and-stick representation. There are three hydrogen bonds from the inhibitor to protein main-chain atoms: amide nitrogen of Lys103, carbonyl oxygen of Pro236, and carbonyl oxygen of Lys101 via a water molecule.

some double mutants *(20)*. S-1153 shows some novel structural features in its interactions with HIV-1 RT that may help explain the favorable resistance profile *(59)*. The crystal structure reveals a series of three hydrogen bonds from the protein main chain to the compound (Fig. 10). For many other NNRTIs, there are either one or no such hydrogen-bonding interactions. Inhibitor interactions with the main chain are likely to be inherently less susceptible to the effects of side-chain mutation than compounds that interact with side chains. Interestingly, the structure of the RT/S-1153 complex shows that the phenyl ring of the inhibitor is positioned in the Tyr181, Tyr188, and Trp229 subpocket, which might be considered to be more characteristic of a first-generation NNRTI. However, for S-1153, the presence of the 3,5-dichloro substituents on the phenyl ring allows a closer interaction with the highly conserved Trp229 at the top of the pocket (Fig. 10) and less dependence on contacts with Tyr181 and Tyr188. A similar effect has also been demonstrated for GCA-186, which has 3,5-dimethyl substituents on the phenyl ring compared with the parent compound, MKC-442. GCA-186 has a 30-fold improved potency against RT (Tyr181Cys) compared with MKC-442.

From crystallographic studies of HBY093 binding to wild-type and mutant HIV-1 RT, it has been shown that there is some adjustment of the compound

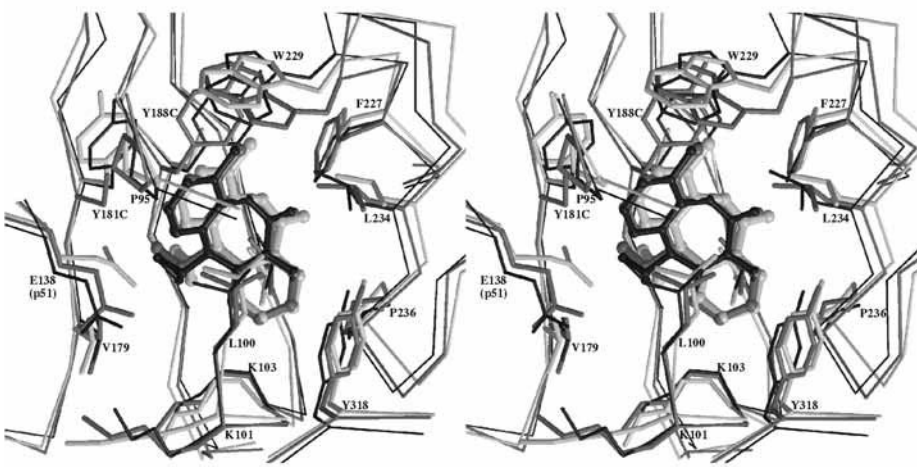


Fig. 11. Stereo diagram comparing non-nucleoside reverse transcriptase (RT) inhibitor sites of wild-type, Tyr181Cys mutant, and Tyr188Cys mutant RT/nevirapine complexes. The Cα backbone, side chains, and inhibitor in each structure are shown as thinner sticks, thicker sticks, and a ball-and-stick representation, respectively, with the wild type in black, the Tyr181Cys mutant in dark gray, and the Tyr188Cys mutant in light gray *(74)*.

conformation in response to the modified binding site and it has been suggested that flexibility might be a useful design criterion for second-generation inhibitors *(72)*. Comparison of crystal structures of efavirenz and nevirapine with wild-type RT and with a mutant RT form (Lys103Asn) led to the identification of three factors, which together may contribute to the second-generation properties of efavirenz *(73)*. The factors included main-chain hydrogen bonding, the ability to rearrange within the NNRTI-binding site, and the previously identified attenuated interactions with the key Tyr181 and Tyr188 residues. Further structural studies of NNRTIs in complex with drug-resistant mutant RTs are described in more detail next.

Structural Basis of Drug-Resistance Mechanisms for NNRTIs

Because of the proximal location of resistance mutations for NNRTIs, the mechanisms involved are generally more straightforward to rationalize than is the case for NRTIs. The aromatic residues Tyr181 and Tyr188 were among the first sites for NNRTI-selected resistance mutations to be reported and were the first studied crystallographically *(68,72,74)*. Structural studies revealed that there was indeed a loss of ring-stacking interactions with the first-generation compounds such as nevirapine (Fig. 11), resulting in a shift in the position of the drug molecule for the Tyr188Cys mutant. In contrast, the positioning of the second-generation inhibitors, such as UC-781 and efavirenz, were largely unperturbed

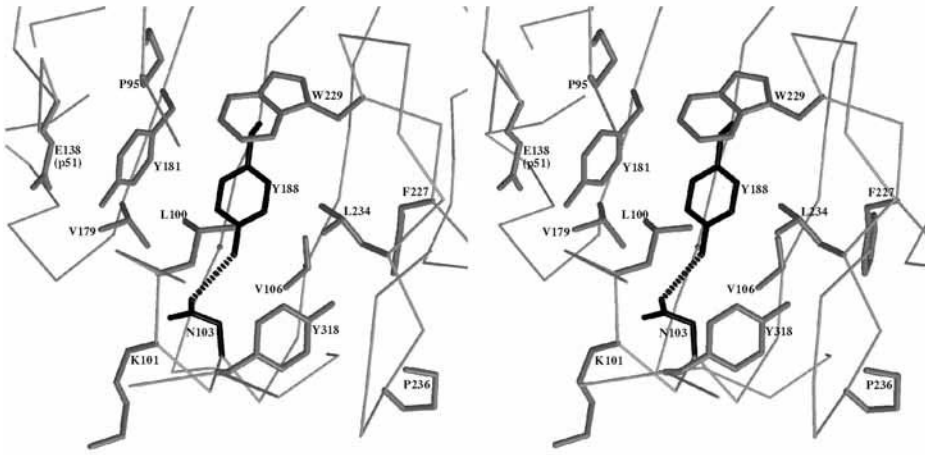


Fig. 12. Stereo diagram showing the non-nucleoside reverse transcriptase (RT) inhibitor site of Lys103Asn mutant RT. The Cα backbone and side chains are shown as thinner and thicker bonds, respectively. The broken bond indicates the hydrogen bond formed between Tyr188 and the mutated Asn103 *(75)*.

because of their much-attenuated contacts with the side chains of Tyr181 and Tyr188 *(74)*. In the case of complexes of RT (Tyr181Cys) with Cl-TIBO and RT (Tyr188Leu) with HBY093, the mutated side chains seemed disordered in each case *(68,72)*. In contrast, for seven structures of RT Tyr181Cys or Tyr188Cys as NNRTI complexes or an unliganded form, the mutated side chains were visible in every case *(74)*. Thus, the disordering of these side chains does not seem to provide a general mechanism for inducing resistance by these mutations.

The most commonly encountered resistance mutation identified from the clinical use of NNRTIs is Lys103Asn, which, therefore, has been the subject of a number of crystallographic reports. An early suggestion based on modeling the Lys103Asn mutation into the unliganded RT structure was that Asn103 could form a hydrogen bond to the hydroxyl group of the Tyr188 side chain, thereby acting to stabilize the apo-enzyme structure *(31)*. Experimental evidence for this proposal was obtained from the crystal structure of unliganded Lys103Asn RT, which revealed that the hydrogen bond was present as predicted, although the length at 3.1 Å indicated that it was a relatively weak interaction (Fig. 12) *(75)*.

From studies comparing the structures of complexes of efavirenz with wild-type and Lys103Asn RT, it was shown that the compound shifted position in the mutant RT and the pocket itself was rearranged such that the Tyr181 flipped to a “down” position resembling the conformation of the protein in the complex with HEPT (Fig. 13) *(73)*. A second report of a crystal structure of

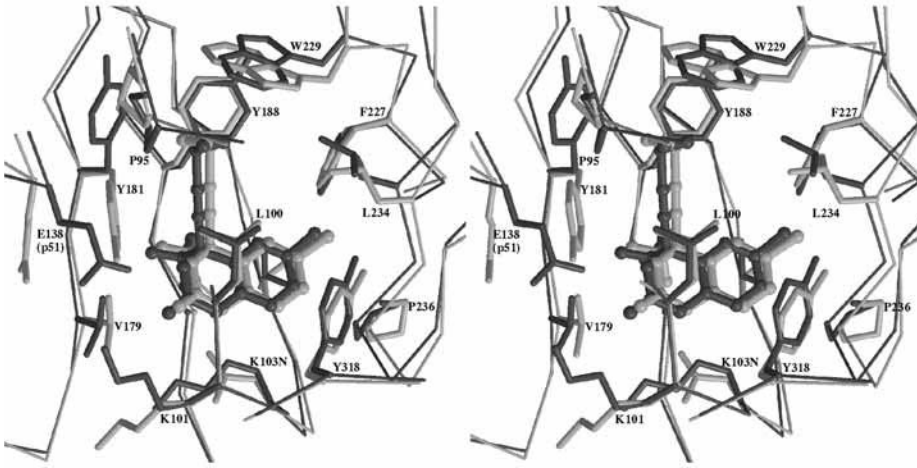


Fig. 13. Stereo diagram comparing non-nucleoside reverse transcriptase (RT) inhibitor sites of wild-type and Lys103Asn mutant RT/efavirenz complexes. The Cα backbone, side chains, and inhibitor in each structure are shown as thinner sticks, thicker sticks, and a ball-and-stick representation, respectively, with the wild type in black and the Lys103Asn mutant in gray *(73)*.

efavirenz with RT Lys103Asn showed that the mutant RT had the Tyr181 as well as the drug molecule itself in a wild-type conformation *(76)*. This ability of efavirenz to bind to different conformational states of mutant RT could be a further contributing factor to its resilience to certain mutations.

STRUCTURE-BASED DRUG DESIGN WITH HIV RT

Structure-based approaches have made a very significant contribution to the development of many of the currently approved drugs against HIV protease, including ritonavir, nelfinavir, amprenavir, and indinavir *(77)*. In contrast, structure-based design, to date, has not been nearly as successful for drugs against HIV RT. The reasons for this are many fold. NRTIs require activation by cellular kinases, thus, structure–activity relationships of the nucleoside/nucleotides are complex, involving a requirement for the active drug and various intermediates to bind at potentially four different enzyme active sites. Thus, an approach based on the HIV-1 RT active site alone, without taking into account cellular kinases, would be unlikely to be successful. In the case of NNRTIs, the structural requirements for binding are relatively loose, therefore, the identification of hits from screening is facile; hence, many different chemical series are readily available for optimization. Two further factors also suggest that structure-based approaches are not straightforward with RT. First, there is significant flexibility within the enzyme, as shown by domain movements, as well

as significant conformational variations within the NNRTI pocket. Both side-chain movements and main-chain rearrangements between different RT/NNRTI complexes are observed, making the prediction of binding modes using drug-design software problematic. The second factor relates to the difficulties associated with obtaining good crystals of HIV RT for structure determination. Validation of the predicted mode of binding for a newly synthesized inhibitor is required as one part of the drug-design cycle. Turnaround times in obtaining crystal structures are longer for RT than for HIV protease; potentially making less impact on the drug-design process. A number of studies have been published using known crystal structures of HIV-1 RT inhibitor complexes for docking in new NNRTIs *(63,64,78)*. Such hypothetical binding modes are, however, likely to have errors because of the difficulty in predicting movement of the protein and, thus, it is advisable to verify the predictions by experimentation using crystallography. In the case of a member of the PETT series, the intramolecular hydrogen bond and the overall binding mode of the compound were predicted *(79)* and later confirmed by crystal-structure determination *(17)*.

The use of the structure of HIV RT for compound design, followed by crystallographic studies to test the predicted binding modes has rarely been reported. An example of this has been in the case of two analogs of MKC-442, GCA-186 and TNK-6123, which were designed from the HIV RT structure to be more active against RT (Tyr181Cys). The binding modes of both compounds in complexes with RT were determined by X-ray crystallography and shown to be as predicted *(80)*.

CRYSTAL STRUCTURE OF HIV-2 RT

Until recently, there were no reports of a crystal structure of HIV-2 RT. HIV-2 is a less-widespread serotype than HIV-1 and is found most commonly in West Africa, although there is some evidence of the spread of the virus to other areas, including Europe and Asia. HIV-2 is considered less pathogenic than HIV-1 *(81)*. HIV-2 RT forms a heterodimer that has different apparent molecular weights than HIV-1, reported as either p68/p55 or p68/p58 for HIV-2, compared with p66/p51 for HIV-1 *(82)*. Although HIV-2 RT shows approx 60% sequence identity to HIV-1 RT, it has inherent resistance to most NNRTIs. There are some exceptions, however, such as PETT-2, which inhibits HIV-2 RT with an IC_{50} of 2 μ*M* *(17)*. The key aromatic residues of Tyr181 and Tyr188 involved in aromatic ring stacking with many inhibitors are changed to aliphatic side chains in HIV-2 RT (Ile181 and Leu188). HIV-2 RT has been purified and crystallized *(83)* and the structure determined to 2.35-Å resolution *(84)*. The overall fold of the unliganded HIV-2 RT is similar to that of the HIV-1 RT, with the thumb domain in a folded-down conformation. Comparison of the apo structures of HIV-1 and HIV-2 RT in the region of the NNRTI site shows differences

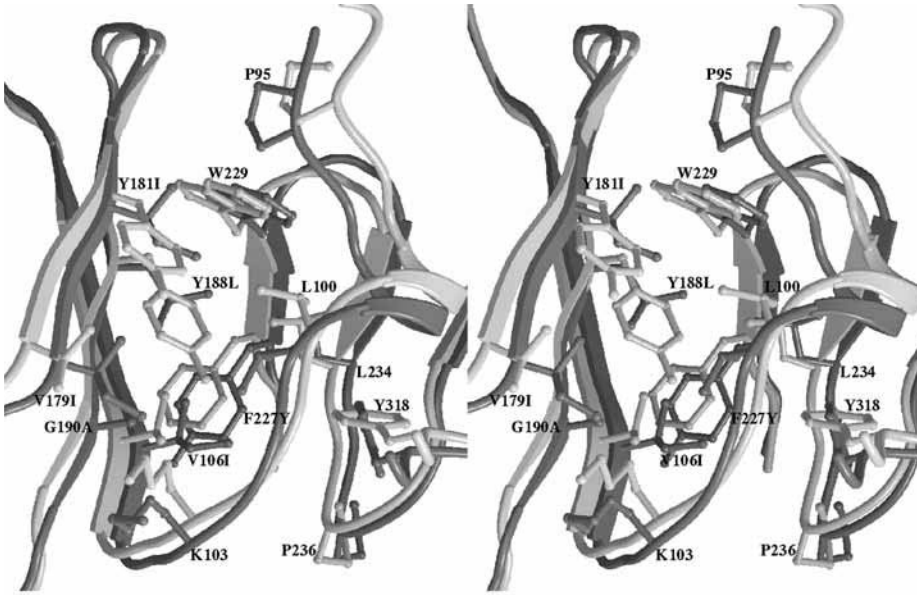


Fig. 14. Stereo diagram comparing the non-nucleoside reverse transcriptase (RT) inhibitor-binding region of apo HIV-1 RT with the corresponding region of HIV-2 RT. The protein backbones are shown as ribbons and coils and the side chains are shown as ball-and-stick representations, with HIV-1 RT in light gray and HIV-2 RT in darker gray. The first letters in the labels are the single letter code and always indicate the residue type of HIV-1 RT; a letter is added to the end of a label showing the residue type of HIV-2 RT if this is different between the two proteins *(84)*.

in position for both conserved and unconserved residues (Fig. 14) that could give rise to unfavorable inhibitor contacts or destabilization of the pocket. The conformation of Ile181 compared with Tyr181 in HIV-1 RT seems to be an important contribution to the inherent drug resistance of HIV-2 RT to NNRTIs. The less bulky side chains at residues 101 and 138 in HIV-2 RT compared with HIV-1 RT create a pocket that is occupied by glycerol in the crystal structure. It is possible that drug-like molecules can be designed for this pocket, which, although not causing distortion of the catalytic aspartates, could inhibit HIV-2 RT by interfering with relative domain movements of the protein.

STRUCTURE OF HIV RT AND DRUG DISCOVERY—THE FUTURE?

Because RT was the first anti-HIV target to be exploited for the development of drugs, the question arises regarding whether, in view of the widespread selection of drug resistance, it is worthwhile or possible to develop further anti-RT drugs. The fact that drug discovery programs aimed at developing follow-up NRTIs and NNRTIs with improved potency and resistance/cross-resistance

profiles are still underway indicates that many researchers believe this is still viable. However, because of the multifunctional nature of RT, there are potentially further target sites that might be exploited.

One avenue that has not been widely explored is the design of ligands that occupy the dNTP site that are not nucleotides. Such active site-directed NNRTIs would have the advantage over NRTIs of not requiring phosphorylation and, because they are more structurally divergent from nucleosides, they might have less affinity for host polymerases and, therefore, have lower toxicity. An attempt to discover such inhibitors has been made by fitting compounds into the active site using the earlier open-form structure of the RT/DNA complex *(85)*. Compounds identified from the study were biaryl acids, which were found to inhibit both HIV-1 and HIV-2 RTs. Crystal-soaking experiments indicated that the biaryl acids did not bind at the NNRTI site, consistent with the inhibitors interacting at a conserved site on HIV-1 and HIV-2 RT, such as the polymerase active site. Given that the more relevant structure of a catalytic form of RT is now available *(29)*, such an approach is worthy of re-examination.

One potential target that has been explored but, as yet, has not yielded drugs, is RNase H, the C-terminal domain of the p66 subunit of HIV-1 RT. RNase H has been shown to be essential for viral replication and, thus, a valid chemotherapeutic target *(86)*. Assays are available to screen enzyme inhibitors, and a number of RNase H inhibitors have been identified; however, only weak antiviral activity has been observed and, thus, these RNase H inhibitors have for the most part not progressed into the drug-development pipeline *(87)*. Other potential target sites include $tRNA^{lys3}$ binding, which is essential for the natural priming reaction of the reverse transcription of the HIV genome. Cordycepin analogs have been identified as RT inhibitors and have been proposed to act as $tRNA^{lys3}$ antagonists *(88)*. Biochemical studies of the site of binding for $tRNA^{lys3}$ have shown that it involves both the p66 and p51 subunits *(10)*; however, there are no crystal structures available of this complex to stimulate a structure-based ligand design approach. The many strand-transfer steps during reverse transcription are also potential targets for drug action *(89)*, however, assay methods are less straightforward than the measurement of DNA polymerase activity.

A further category of RT inhibitors acting at novel sites is based on molecules that disrupt the RT heterodimer. Peptides of up to 10 amino acids in length corresponding to the sequence of RT residues 395–404 have been shown to block dimerization of RT in vitro and to have antiviral activity in HIV-infected cells *(90)*. However, problems of uptake and peptide stability in the gastrointestinal tract remain to be overcome before such inhibitors could be used as orally active drugs against HIV. The NNRTI compound, TSAO, or [2′,5′-*bis*-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5′′-(4′′-amino-1′′,2′′-oxathiole-2′′,2′′-dioxide), has been shown to be capable of

disrupting the RT dimer *(91)*. TSAO has been modeled into the interface region of the RT heterodimer, and this may stimulate ideas for new compounds that could have potential for the development as anti-RT drugs *(92)*. To date, a crystal structure of TSAO bound to HIV-1 RT is not available.

It is frequently pointed out that it would have been impossible to design NNRTIs *de novo* from any of the unliganded or DNA-bound HIV-1 RT structures that have been determined, because a large conformational change is involved in the inhibitor site formation, which only occurs once the ligand has bound. The current state of the art with ligand-docking software is such that, although some limited movement of the protein can be allowed, it is not possible to accurately predict extensive conformational changes resulting from ligand binding *(93)*. It is clear that structural studies of HIV RT do not provide an easy answer to the design of novel anti-AIDS drugs to target virus resistant to current therapies. Nevertheless, the combination of compound screening, virology, and medicinal chemistry together with structure-based design and protein crystallography to test predicted ligand-binding modes brings together a powerful set of tools for developing new HIV RT inhibitors. Such multifaceted approaches are likely to provide the greatest hope of discovering novel anti-RT drugs that will be useful in combating the ever-present threat of drug-resistant HIV. Such drugs are of vital importance in the continuing fight against HIV and AIDS.

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2

Zidovudine, Lamivudine, and Abacavir

Monica Carten and Harold Kessler

ZIDOVUDINE

Introduction

The history of the discovery and development of zidovudine (ZDV; or 3′-azido-3′-deoxythymidine, AZT, or Retrovir™, formerly BW A509U) is fascinating not only because it was the first Food and Drug Administration (FDA)-approved agent for the treatment of HIV, but for the unprecedented speed with which this drug moved through the new-drug approval process (Table 1). In March 1987, ZDV was approved by the FDA for use in HIV-infected individuals with a previous episode of *Pneumocystis carinii* pneumonia (PCP) and/or a CD4 cell count of less than 200 cells/mm^3. The use of ZDV in asymptomatic or symptomatic patients with CD4 cell counts greater than 500 cells/mm^3 was approved in March 1990. ZDV was initially approved as a monotherapy. Subsequently, ZDV was approved for use in combination regimens with zalcitabine and lamivudine (3TC, Epivir®). Although early studies demonstrated clinical and survival benefits of ZDV alone or in combination with other nucleoside analogs, these benefits were of limited durability because of incomplete virological suppression and the emergence of resistant HIV strains. ZDV is currently approved for the treatment of HIV infection in combination regimens with potent antiretroviral agents, including HIV protease inhibitors (PIs); nonnucleoside reverse transcriptase inhibitors (NNRTIs); and potent nucleoside reverse transcriptase inhibitors (NRTIs), such as abacavir (ABC).

Preclinical Development

ZDV was initially synthesized as a potential antineoplastic agent by Horwitz and colleagues in 1964 *(1)*. However, it was never approved for use in humans as a cancer chemotherapy. In 1974, Ostertag et al. were the first to describe the antiretroviral activity of ZDV by demonstrating inhibition of the spleen focus-forming virus, a murine type C retrovirus *(2)*. In 1985, Mitsuya and collaborators showed that ZDV was a potent inhibitor of the in vitro

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Table 1
AZT Timeline

1964	Compound synthesized for its antineoplastic activity
1974	AZT found to have activity against retroviruses
1985	AZT demonstrated to have activity against HIV
June 14, 1985	Burroughs-Wellcome files for IND1
June 21, 1985	IND granted by FDA2
July 3, 1985	First patient treated with AZT in phase I trial
February 1986	Phase II studies begun
September 1986	Decreased mortality seen with AZT, placebo arm discontinued
October–March 1987	Compassionate Use program for AZT
March 1987	FDA approves AZT for patients with previous PCP3 or CD4 cell count <200 cells/mm^3
March 1990	FDA approves AZT for asymptomatic and symptomatic patentis with CD4 cell count <500 cells/mm^3

IND, investigational new drug; FDA, Food and Drug Administration; PCP, *Pneumocystis carinii* pneumonia

replication and cytopathic effect of HIV (then known as HTLV-III/LAV [human T-lymphotrophic virus type III/lymphadenopathy-associated virus) and suggested the development of ZDV as a potential treatment for HIV infection in humans *(3)*.

Mechanism of Action

ZDV is a thymidine-analog NRTI in which the 3′-hydroxy (-OH) group is replaced by an azido (-N) group (Fig. 1). It is converted intracellularly to its active triphosphate form by anabolic phosphorylation. ZDV triphosphate (ZDV-TP) acts by competitively inhibiting the use of deoxythymidine triphosphate, an essential substrate for the formation of proviral DNA by the HIV DNA polymerase (reverse transcriptase). In addition, ZDV-TP serves as a DNA chain terminator when incorporated into the proviral DNA chain because the 3′ N substitution impedes the 5′-3′ phosphodiester linkages necessary for chain growth. Human cellular DNA polymerase-α and -β are 100 times less susceptible to inhibition by ZDV-TP than is retroviral reverse transcriptase. In contrast, human polymerase-γ, responsible for mitochondrial DNA synthesis, is inhibited by concentrations of ZDV as low as 1 μ*M*, concentrations that are achieved in vivo. In addition, ZDV inhibits adenine nucleoside translocator-1 and this may also contribute to the mitochondrial toxicity seen with ZDV *(4)*. In cell culture drug-combination studies, ZDV demonstrated synergistic to additive inhibitor activity with other NRTIs (zalcitabine, didanosine, 3TC, and ABC), NNRTIs (efavirenz [EFV], delavirdine, and nevirapine) and PIs (saquinavir [SQV], ritonavir [RTV], indinavir [IDV], nelfinavir [NFV],

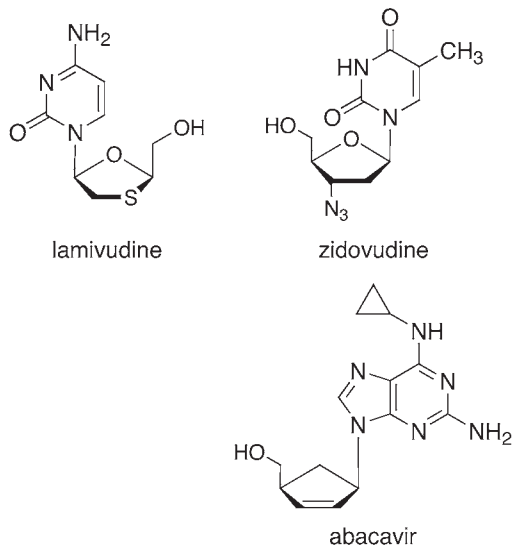

Fig. 1. Chemical structures of the drugs discussed in this chapter.

amprenavir, and lopinavir), and interferon-α *(5–11)*. The combination of ZDV and stavudine (d4T) shows antagonistic activity in vitro against several isolates of HIV *(12)*. Ribavirin antagonizes the antiviral activity of ZDV in vitro by inhibiting ZDV phosphorylation *(13)*.

ZDV Pharmacokinetics

ZDV crosses the blood–brain barrier and has a cerebrospinal fluid (CSF)-to-plasma ratio of 0.25 *(14)*. The compound is metabolized by the liver, primarily by glucuronidation, and then excreted by the kidneys *(15)*. It is well-absorbed in the gut, with an average bioavailability of approx 60%, and is approx 35% protein bound. After oral dosing, the peak serum concentration is achieved in 0.5 to 1.5 h. Food decreases peak plasma concentrations by more than 59%. However, total exposure, as reflected by the area under the concentration curve (AUC), is unchanged. The mean serum half-life is 1.1 h and the intracellular half-life is 3 h. ZDV 5′-triphosphate, however, has an intracellular half-life of approx 3 to 4 h, and, in treated patients, it is stable for 6 h after dosing. ZDV is present in breast milk and crosses the placenta *(16)*. ZDV is also detected in the semen, with a semen-to-serum ratio ranging from 1.3 to 20.0 *(17)*. No dose adjustment is required for patients with severe renal dysfunction. Hemodialysis and peritoneal dialysis seem to have a negligible effect on the removal of ZDV *(18)*. The pharmacokinetics of ZDV seem unchanged during pregnancy *(19)*. ZDV concentrations in newborns are equivalent to maternal levels.

Phase I/II Studies

On June 14, 1985, the Burroughs-Wellcome Company submitted an application for an investigational new drug (IND) exemption for the use of ZDV in humans. Seven days later, the FDA approved the exemption, and, on July 3, 1985, the first patient was treated with ZDV. The phase I study was a 6-wk trial of four ZDV dose regimens involving 19 patients with AIDS or AIDS-related complex (ARC) and was conducted at the National Cancer Institute and Duke University Medical Center *(20)*. ZDV was administered intravenously for 2 wk, then orally for 4 wk at twice the intravenous dose. ZDV was well-absorbed from the gut and crossed the blood-brain barrier. Therapeutic levels were maintained with either 5 mg/kg of ZDV administered intravenously or 10 mg/kg of ZDV administered orally, every 4 h. There were no treatment-limiting side effects. The most common side effects were headaches and depressed white blood cell counts, which were not dose related. Fifteen of the 19 patients had increases in their CD4 cell counts during therapy, 6 previously anergic patients showed restoration of delayed-type hypersensitivity skin test reactions, 2 patients had resolution of chronic fungal nailbed infections without specific anti-fungal therapy, and the entire cohort had an average weight gain of 2.2 kg.

ZDV Monotherapy

Advanced HIV Disease

In February 1986, a multicenter, double-blind, placebo-controlled trial of ZDV involving 282 patients was begun (Table 2) *(21)*. Patients were enrolled who were within 4 mo of diagnosis of PCP or had ARC. Patients were randomized to receive either 250 mg ZDV or placebo, every 4 h for a total of 24 wk. By September 1986, 19 patients taking placebo but only 1 patient taking ZDV had died. The placebo arm of the trial was discontinued and all patients previously administered placebo were offered open-label ZDV. Other findings of the study included a decreased incidence of opportunistic infections, increased CD4 cell counts, and weight gain in the ZDV group.

At that time, it was recognized that a mechanism was needed to provide ZDV to seriously ill HIV-infected patients while awaiting clinical trial data analysis and regulatory review. A compassionate plea program (Treatment IND) was established to provide ZDV to patients with a previous episode of PCP. The Treatment IND was in place from October 11, 1986 through March 24, 1987, during which time, 4805 patients received ZDV therapy. In late March 1987, ZDV was approved for patients with a previous episode of PCP or whose CD4 cell count was below 200 cells/mm^3.

Mildly Symptomatic HIV Disease

In a double-blind, placebo-controlled trial conducted by the newly formed AIDS Clinical Trials Group (ACTG) 016, 711 people with mildly symptomatic HIV disease were stratified by pretreatment CD4 cell counts of between 200 and 500 cells/mm^3, or at least 500 but fewer than 799 cells/mm^3*(22)*. Three hundred fifty-one subjects were assigned to placebo and 360 subjects were assigned to 200 mg ZDV every 4 h for a median duration of follow-up of 11 mo. Clinical endpoints were the development of AIDS, development of ARC, or death. In the subgroup of patients with CD4 cell counts between 200 and 500 cells/mm^3, 34 endpoints occurred in the placebo group and 12 in the ZDV-treated group. The ZDV-treated group had significant increases in CD4 cell counts and weight gain. No benefit in time to progression to a clinical endpoint was found in the subgroup with CD4 cell counts greater than 500 cells/mm^3; however, this group did have significant increases in the number of CD4 cells that persisted for 8 wk.

The Veteran Affairs Cooperative Study compared early vs late initiation of ZDV therapy in 338 patients with CD4 cell counts between 200 and 500 cells/mm^3 *(23)*. Early therapy consisted of 250 mg ZDV every 4 h, whereas late therapy was placebo until the CD4 cell count dropped below 200 cells/mm^3 or until an AIDS-defining event occurred, at which time the same 1500 mg daily dose of ZDV was initiated. During a mean follow-up of more than 2 yr, there were 23 deaths in the early therapy group and 20 deaths in the late-therapy group. Twenty-eight patients in the early therapy group and 48 patients in the late-therapy group progressed to AIDS. There was an increased time to reach a CD4 cell count of fewer than 200 cells/mm^{3}and an increased incidence of side effects in the early treatment group.

Asymptomatic HIV Disease

The first large study of the efficacy of ZDV in asymptomatic HIV-infected patients was ACTG 019. This was a three-arm study to determine the safety and efficacy of ZDV at two different daily doses (100 mg or 300 mg every 4 h, 5 doses/d) compared with placebo in patients with CD4 cell counts either above or below 500 cells/mm^3. Volberding et al. reported the initial results in asymptomatic patients with CD4 cell counts fewer than 500 cells/mm^3, after a mean follow-up of 55 wk *(24)*. Thirty-three of 428 patients in the placebo group progressed to AIDS, as compared with 11 of 453 patients in the 500 mg ZDV daily group and 14 of 457 patients in the 1500 mg ZDV daily group. The ZDV treatment groups had significant increases in their CD4 cell counts and significant declines in their HIV p24 antigen levels compared with placebo recipients. Higher-dose therapy recipients had more adverse effects than those in the lower-dose group. In August 1989, on the basis of evidence that treatment at

Table 2
Major Studies of Zidovudine Monotherapy[a]

Trial (ref.)	Design	Dosage	No. of subjects	Entry criteria	Entry CD4 cell count (cells/mm^3)
BW 002 *(21)*	Placebo-controlled, randomized, double-blind	250 mg ZDV q4 h	282	AIDS or ARC; CD4 < 100 cells/mm^3; CD4 101–499 cells/mm^3	49 and 54 (median); 128 and 190 (median)
ACTG 016 *(22)*	Placebo-controlled, randomized, double-blind	200 mg ZDV q4 h	711	Mildly symptomatic; CD4 200–800 cells/mm^3	225 (median)
ACTG 019 *(24)*	Placebo-controlled, randomized, double-blind	300 mg or 100 mg ZDV 5 times daily	1338	Asymptomatic; CD4 < 500	350 (median)
VA Study *(23)*	Placebo-controlled, randomized, double-blind	250 mg ZDV q4 h Open label ZDV when CD4 < 200	338	Mildly symptomatic; CD4 200–500 cells/mm^3	355 (mean)
Concorde *(26)*	Placebo-controlled, randomized, double-blind	250 mg ZDV QD	1749	Asymptomatic	≤200, 6%; 201–500, 52%; >500, 42%
ACTG 019 *(27)*	Placebo-controlled, randomized, double-blind	300 mg or 100 mg ZDV 5 times daily; Open label after 1989 for CD4 < 500 cells/mm^3	1637	Asymptomatic CD4 > 500 cells/mm^3	655 (median)

Table 2 *(Continued)*

CD4 cell count response (cells/mm^3)	Antiviral response	Clinical outcome	Comments
Increased CD4 count in ZDV group	P24 antigenemia decreased in ZDV group	ZDV improved survival and decreased number of opportunistic infections in 24-wk period	
Increased +44 in patients with entry counts 200–500 cells/mm^3; no significant changes for those with entry counts >500 cells/mm^3	Serum p24 antigen levels decreased significantly for ZDV group	At CD4 200–500 cells/mm^3, ZDV delays progression to AIDS but no delay with CD4 > 500 cells/mm^3; no survival benefit seen	Little toxicity in these mildly symptomatic subjects
Median change +39 in 500 mg ZDV; +26 in 1500 mg ZDV; and –16 in placebo group	Significant improvements in p24 antigenemia in both ZDV groups	Lower rate of progression to AIDS in ZDV groups; no survival benefit of ZDV	Higher dose ZDV had more severe hematological toxicity
At 20 mo, mean changes = –35.5 early therapy; –83.6 late therapy	Conversion to p24 antigen seronegative: 79% early therapy; 35% late therapy	ZDV slowed progression; no survival benefit	More side effects in early therapy group
Increase of 20 in immediate therapy group c/w decrease of 9 in deferred therapy group at 3 mo	Not reported	No significant benefit in progression or mortality with early ZDV	Time-limited benefit of ZDV monotherapy shown
Median interval to decline of CD4 to <500 significantly longer in immediate therapy group c/w deferred group	Not reported	No difference in duration of overall or AIDS-free survival between early and deferred-therapy groups	Toxicity greater with higher dose ZDV

[a]q4 h, every 4 hours; QD, once daily; c/w, compared with

the lower dosage delayed the progression of disease in patients with initial CD4 cell counts below 500 cells/mm^3, the placebo arm of the substudy was terminated and all subject were offered open-label ZDV at 500 mg/d. In a subsequent evaluation of the cohort with CD4 cell counts of fewer than 500 cells/mm^3, after a mean follow-up of 2.6 yr, ZDV at 500 mg daily was still found to result in a significant delay in progression to AIDS or death, but there was no survival benefit associated with earlier use compared with delayed ZDV initiation *(25)*.

The Concorde Study conducted by the European Collaborative Group was the second large-scale clinical trial to address the efficacy of early ZDV therapy for HIV infection *(26)*. One thousand seven hundred forty-nine asymptomatic patients were randomized to either ZDV (immediate-treatment group) or placebo (delayed-treatment group) and followed for a mean of 3.3 yr. There was no significant benefit in the immediate-treatment group regarding mortality or progression to AIDS or ARC, with 29% of patients in the immediate-treatment group vs 32% of patients in the delayed-treatment group reaching one of these endpoints. However, there were differences in the CD4 cell counts between the two groups over time, with median CD4 cell count changes from baseline to 3 mo of +20 cells for the immediate-treatment group and –9 cells for the delayed-treatment group.

Finally, the second substudy of ACTG 019, in the cohort of patients with CD4 cell counts greater than 500 cells/mm^3 was reported in 1995 *(27)*. Volberding et al. described no difference in duration of overall or AIDS-free survival between early and deferred-treatment groups. The results of these ZDV monotherapy studies suggested that the efficacy of ZDV used alone in the treatment of patients with either mildly symptomatic or asymptomatic HIV infection was of time-limited benefit and provided minimal survival advantage.

ZDV for Prevention of Mother-to-Child Transmission

ZDV is approved for use in HIV-infected women and their infants for the prevention of perinatal transmission of HIV. In the landmark study, Pediatric AIDS Clinical Trials Group (PACTG) 076, ZDV was administered to pregnant women antepartum starting at 14 to 34 wk, intrapartum at 2 mg/kg during 1 h, then at 1 mg/kg/hr infusion; and administered to the newborn at 2 mg/kg orally, four times daily, for 6 wk. There was an 8.3% risk of HIV infection in the ZDV-treated group compared with a 25.5% risk in the placebo group, a 67.5% reduction in relative risk of HIV transmission *(28)*. A detailed discussion of prevention of vertical transmission may be found in Chapter 15.

ZDV Nucleoside Combination Therapy

Several large studies have demonstrated that ZDV combined with other nucleoside agents provide improved and more durable clinical and survival

benefits compared with ZDV alone (Table 3). The Delta trial evaluated ZDV monotherapy vs combination therapies of ZDV plus zalcitabine or ZDV plus didanosine in both ZDV-naive and ZDV-experienced patients *(29)*. At a median follow-up of 30 mo, ZDV-naive patients had a 42% relative reduction in mortality with the ZDV plus didanosine combination and a 32% relative reduction in mortality with the ZDV plus zalcitabine combination compared with ZDV monotherapy. In ZDV-experienced patients, the addition of didanosine improved survival, with a relative reduction in mortality of 23%, but there was no direct evidence of benefit with the addition of zalcitabine (relative reduction, 9%).

Similarly, ACTG 175 evaluated treatment with ZDV alone, didanosine alone, ZDV plus didanosine, or ZDV plus zalcitabine in ZDV-naive and ZDV-experienced patients *(30)*. The progression to a primary endpoint of either at least a 50% decline in CD4 cell count, development of AIDS, or death was more frequent with ZDV alone (32%) than with ZDV plus didanosine (18%), ZDV plus zalcitabine (20%), or didanosine alone (22%) in both ZDV-naive and ZDV-experienced patients. For ZDV plus zalcitabine, the benefits were limited to patients without previous ZDV treatment. The combination of ZDV and 3TC has also proven to be a potent nucleoside combination (*see* "Lamivudine" section). In several large trials, benefits were demonstrated in CD4 and HIV RNA responses, disease-free survival, and overall survival.

ZDV in Triple-Combination Therapy

It soon became apparent that dual nucleoside analog combination therapy also had limited durability, attributed in part to the same factors that made ZDV monotherapy of limited use, incomplete suppression of viral replication and development of resistant virus. Focus turned toward development of agents with different mechanisms of action than the NRTIs. With the discovery and development of NNRTIs and PIs came knowledge that potent combination regimens, particularly three-drug regimens containing these new agents, provided greater virological and immunological benefits than were seen with nucleoside analogs alone.

The Italy, the Netherlands, Canada, and Australia Study (INCAS) compared the virological effects of ZDV plus nevirapine, ZDV plus didanosine, and ZDV plus didanosine plus nevirapine in antiretroviral-naive patients *(31)*. The triple-therapy group had the greatest virological effect. ACTG 229 evaluated ZDV in combination with the PI, SQV; ZDV plus SQV plus 2′,3′-dideoxycytidine (ddC); and ZDV plus ddC in patients with 4 mo of previous ZDV use *(32)*. Studies combining ZDV and 3TC with IDV, NFV, or EFV also showed improved virological and immunological benefits and are detailed in the "Lamivudine" section of this chapter. The results of these triple-combination studies provided data to support the use of ZDV and a second NRTI in combination with a third

Table 3
Clinical Trials of Zidovudine Combination Therapy[a]

Trial (ref.)	Design	Dosage	No. of subjects	Entry criteria	CD4 cell count at entry (cells/mm^3)
Delta *(29)*	Randomized, double-blind; ZDV vs ZDV +ddC vs ZDV +ddI	ZDV 600 mg QD; ddI 400 mg QD; ddC 2.25 mg QD	3207 ZDV naive, *n* = 2124; Previous ZDV, *n* = 1083	AIDS and CD4 >50 cells/mm^3; no or minimal symptoms and CD4 <350 cells/mm^3; Delta 1: ZDV-naive; Delta 2: ZDV experienced	205 (mean)
ACTG 175 *(30)*	Randomized, double-blind; ddI vs ZDV vs ddI+ZDV vs ddC+ZDV	Same doses as Delta	2467 ZDV naive, *n* = 1060 Previous ZDV, *n* = 1407	No AIDS-defining illness; CD4 200–500 cells/mm^3; antiretroviral naive or experienced	352 (mean)
INCAS *(31)*	Randomized, double-blind placebo-controlled; ZDV+NVP vs ZDV+ddI vs ZDV+ddI+NVP	ZDV 200 mg TID; ddI 125 or 200 mg BID based on weight; NVP 200 mg QD for 2 wk, then 200 mg BID	Treatment naive *n* = 151	Antiretroviral naive; CD4 200–600 cells/mm^3	346–387 (mean)
ACTG 229 *(32)*	Randomized, double-blind placebo-controlled; SQV+HGC +ddC+ZDV vs SQV+HGC+ZDV vs ZDV+ddC	SQV 600 mg TID; ddC 0.75 mg TID; ZDV 200 mg TID	Previous ZDV *n* = 302	CD4 50–300 cells/mm^3	SQV+ddC +ZDV 145; SQV+ZDV 156; ddC +ZDV 171

Table 3 *(Continued)*

CD4 response	Antiviral response	Clinical outcome	Comments
Improved with both combinations c/w ZDV alone	Not stated	Decrease in mortality, 33% for ddI+ZDV and 21% for ddC+ZDV; Decrease in disease progression, 36% for ddI+ZDV and 17% for ddC+ZDV (for Delta 1 and 2 c/w ZDV alone)	For ZDV-naive pts either combination had significant decrease in progression or death; for ZDV-experienced pts, no significant increase in CD4 count or clinical benefit with combination
Significant improvement in combination or ddI alone groups for both ZDV naive and experienced	Not stated	Progression of AIDS-defining event or death or ≥50% decline in CD4 count: ZDV 32%, ZDV+ddI 18%, ZDV +ddC 20%, ddI alone 22%	Survival benefit for all combinations and ddI alone c/w ZDV monotherapy; ZDV+ddC benefits limited to ZDV-naive pts
Triple therapy group with sustained increases at week 52	HIV RNA <20 for triple therapy 51%, ZDV+ddI 21%, ZDV+NVP 0% at wk 52	Rates of disease progression or death: ZDV+NVP 23%, ZDV+ddI 25%, triple therapy 12%	Triple drug therapy with greater and sustained decrease in HIV viral load
Significant improvements over baseline for triple therapy group at 24 wk	Mean HIV RNA decrease 0.4 $\log_{10}$ copies/mL for triple therapy; 0.1 $\log_{10}$ copies/mL for SQV +ZDV and ZDV+ddC groups over 24 wk	Not stated	Support for triple therapy to achieve CD4 cell count and viral load improvements

[a]QD, once daily; BID, twice daily; TID, three times daily; c/w, compared with; pts, patients

agent from either the NNRTI or PI class to create a potent and durable combination regimen.

ZDV in the Pediatric Population

ZDV was approved for use in HIV-infected children aged 3 mo to 12 yr in May 1990. It has good central nervous system penetration and is the NRTI of choice when treating children with HIV-related central nervous system disease. Pizzo et al. reported the results of continuous iv ZDV administration in 21 children *(33)*. Thirteen children had neurodevelopmental abnormalities and 11 children had CD4 cell counts fewer than 200 cells/mm^3. ZDV was administered at four dose levels: 0.5, 0.9, 1.4, and 1.8 mg/kg/h. Improvement in neurodevelopmental abnormalities occurred in all 13 children who had presented with encephalopathy before treatment. IQ scores increased in these 13 children and in 5 other children who had no detectable evidence of encephalopathy before treatment. Most patients also had increased appetite and weight, decreased lymphadenopathy and hepatosplenomegaly, and increased numbers of CD4 cells. Bone marrow suppression was the only evident toxicity, with dose-limiting neutropenia occurring in most patients who received doses of 1.4 mg/kg/h or more.

ZDV Monotherapy

Eighty-eight children with advanced HIV disease and a mean age of 3.9 yr received oral 180 mg/m^2 ZDV four times daily for 24 wk *(34)*. After a median follow-up of 56 wk, one or more episodes of hematological toxicity occurred in 61% of children. Kaplan-Meier analysis demonstrated that the probability of survival was 0.89 after 24 wk and 0.79 after 52 wk. There was marked improvement in weight, cognitive function, and serum and CSF concentrations of HIV-1 p24 antigen. The authors concluded that this dose of ZDV can be safely administered to children with advanced HIV disease and that the immunological and virological improvements in children are similar to those seen in adults.

ZDV Combination Therapy

The PACTG Study 152 was a multicenter, double-blind study of 831 symptomatic HIV-infected children ages 3 mo through 18 yr. Ninety-two percent of children were antiretroviral-naive *(35)*. Patients were randomized to receive either 180 mg/m^2 ZDV four times daily, 120 mg/m^2 2′,3′-dideoxyinosine (ddI) twice daily, or ZDV plus ddI. The primary endpoint was length of time to death or to progression of HIV disease. An interim analysis at a median follow-up of 23 mo showed a significantly higher risk of HIV-disease progression or death in patients receiving ZDV alone than in those receiving combination therapy. The study arm with ZDV alone was stopped and unblinded. At the end of the study, ddI alone had an efficacy similar to ZDV plus ddI. The authors concluded that

in symptomatic children with HIV, treatment with either ddI alone or ZDV and ddI was more effective than treatment with ZDV alone.

ZDV Resistance

HIV resistance to ZDV is associated with the accumulation of specific mutations sites on the HIV *pol* gene that encode for the reverse transcriptase. The mutations associated with decreased ZDV susceptibility occur at amino acid sites 41, 67, 70, 215, and 219; sites 41, 70, and 215 are the most important sites of mutation *(36,37)*. Cross-resistance to multiple nucleoside analogs, including ZDV, didanosine, zalcitabine, and d4T, has been demonstrated with mutations at sites 62, 75, 77, 116, and, most notably, 151. The presence of the M184V mutation, induced by 3TC and ABC, has been shown to induce ZDV hypersusceptibility of the virus and a resensitization of ZDV-resistant virus to ZDV *(38,39)*.

Drug Interactions

Coadministration of ZDV with phenytoin may result in decreased levels of phenytoin. However, a pharmacokinetic study of a single 300-mg dose of phenytoin during steady-state ZDV administration showed no change in phenytoin kinetics *(40)*. ZDV administration seems to have a negligible effect on methadone kinetics *(41)*. Probenicid may increase ZDV levels through inhibition of glucuronidation and reduced renal excretion of ZDV *(42)*. Rifampin coadministration resulted in an increase in ZDV clearance and a decrease in AUC; however, an increase in AUC and peak plasma concentrations were seen for the active metabolite, zidovudine triphosphate (ZDV-TP). Fluconazole dosed with ZDV showed a 74% increase in the AUC of ZDV and a 128% increase in its half-life. Atovaquone coadministration resulted in a 24% decrease in ZDV oral clearance and a 35% increase in AUC.

Current Clinical Use

ZDV Dosing and Formulations

The recommended daily dose of ZDV in adults is 600 mg daily, in two or three divided doses. This recommendation is based on studies of early and advanced HIV disease, which compared the initial 1200 mg/d dose with 100 mg five or six times daily and demonstrated equivalent efficacy and less hematological toxicity *(43)*. In addition, in one study, ZDV administered for 48 wk at 100 mg every 4 h or at 300 mg every 12 h showed no significant difference in adverse events *(44)*. ZDV dosing for pediatric patients 3 mo to 12 yr of age is 180 mg/m^2 every 6 h, not to exceed 200 mg every 6 h. ZDV is available in four formulations: a 300 mg tablet, a 100 mg capsule, a 50 mg/5 mL syrup, and a 10 mg/mL infusate.

Toxicity

Toxicities associated with ZDV include headache, myalgias, malaise, fatigue, nausea, anorexia, anemia, and neutropenia *(45)*. Constitutional symptoms occurring with initiation of therapy can generally be managed symptomatically. Anemia and neutropenia are more common in patients with advanced HIV disease and, if severe, may necessitate discontinuation of ZDV, with substitution with a different antiretroviral agent. Use of other agents for treatment or prophylaxis of HIV-associated infections, such as trimethoprim/sulfamethoxazole and ganciclovir, may exacerbate the hematological perturbations. Long-term use of ZDV may be associated with muscle toxicity, hepatic toxicity, and nail hyperpigmentation.

Current Uses

Current clinical indications for the use of ZDV include HIV infection in which treatment is indicated, prevention of maternal–fetal transmission, and occupational postexposure prophylaxis. ZDV is currently recommended as part of an initial treatment regimen that includes a second NRTI plus an NNRTI or one or two PIs. The use of ZDV combined with a second NRTI and ABC as an initial therapy is recommended only in patients who cannot take a NNRTI or PI.

LAMIVUDINE

Introduction

3TC is the negative or *cis* enantiomer of 2′-dideoxy-3′-thiacytidine and has activity against HIV-1, HIV-2, and hepatitis B. This agent is a pyrimidine nucleoside analog in which the 3′ carbon of the ribose ring of 2′-deoxycytidine has been replaced by a sulfur atom (Fig. 1). The drug was approved for use in adults in February 1995 and for children at least 3 mo of age in November 1996. 3TC is indicated for treatment of HIV infection only in combination with other antiretroviral agents.

Mechanism of Action and In Vitro Studies

3TC requires intracellular phosphorylation to become active and is preferentially active in resting cells. The active compound, 3TC-triphosphate, is a reverse transcriptase inhibitor that competes with deoxycytidine triphosphate, an endogenous nucleotide, for binding in the HIV reverse transcriptase-binding site. Insertion of 3TC-triphosphate into the proviral DNA leads to chain termination because 3TC lacks the 3′ hydroxyl group necessary for the 5′ to 3′ linkage required for DNA synthesis *(47)*. The compound was initially synthesized as a racemic mixture (BCH-189), and this mixture has potent activity in vitro against HIV-1, with a mean 50% inhibitory dose (IC_{50}) of 0.73 μM in an MT4 cell line assay *(46)*. BCH-189 was also shown to have activity against

ZDV-resistant isolates and caused less cytotoxicity than ZDV *(48)*. Subsequent analysis revealed that both the positive and negative enantiomers of BCH-189 had in vitro activity against HIV *(49,50)*. The negative enantiomer (3TC) demonstrated greater anti-HIV activity, which is attributed to the compound σ relative resistance to deoxycytidine deaminase, thus, preventing cleavage of the compound from the HIV RNA/DNA complex. In addition, the negative enantiomer has less in vitro bone marrow toxicity, relatively little activity against mammalian DNA polymerase-γ and, thus, little host mitochondrial toxicity in vitro *(51,52)*. 3TC is highly active against HIV-1 in lymphoid cell assays and peripheral mononuclear cell lines, with an IC_{50} from 4 to 670 n*M* and 2.5 to 90 n*M*, respectively *(51)*. 3TC has been shown to be synergistic or additive in vitro with NRTIs (ZDV, d4T, and didanosine), NNRTIs (nevirapine and delavirdine), and PIs (SQV and IDV) *(53,54)*. 3TC interferes with the phosphorylation of zalcitabine, and the combination of these two agents may be antagonistic against HIV replication *(55)*.

3TC Pharmacokinetics

3TC is rapidly absorbed after oral administration, with an absolute bioavailability of approx 86% in adults and 66% in children. Food has no significant effect on absorption. After oral administration of 2 mg/kg twice daily to nine adults with HIV, the peak serum 3TC concentration was 1.5 ± 0.5 (mean ± SD) and well above the in vitro IC_{90} of HIV-1 *(56)*. The CSF-to-plasma ratio of 3TC is 0.11. The serum half-life of 3TC is 2 h and its intracellular half-life is 10 to 15 h, allowing for twice-daily or once-daily dosing. Binding of 3TC to human plasma proteins is low, and 3TC freely crosses the placenta and crosses into breast milk *(57)*. 3TC shows marked concentration in the male genital tract and the semen-to-blood concentration ratios for 3TC are higher than for other NRTIs, NNRTIs, or single PIs *(58)*. The majority of the drug is eliminated unchanged in the urine *(59)*. Dose adjustment is required if there is significant renal impairment. 3TC is cleared by hemodialysis but no dose adjustments are necessary because of its large volume of distribution *(60)*.

Clinical Development-Phase I/II Studies

Early studies of 3TC as monotherapy were primarily small trials designed to study the drug's safety and pharmacokinetic parameters. In a multicenter, open-label, dose-escalating study, 97 patients with AIDS or ARC, and a CD4 cell count of fewer than 300 cells/mm^3 were administered doses of 3TC ranging from 0.5 to 20 mg/kg/d for 24 wk *(56)*. At the higher doses of 8 mg/kg/d and 12 mg/kg/d, transient decreases in p24 antigen and increases in CD4 cell counts were seen, but the CD4 counts subsequently decreased to baseline after 20 wk. Neutropenia was observed only at the 20 mg/kg/d dose. In a second dose-esca-

lating study, 104 asymptomatic or mildly symptomatic HIV-infected patients with CD4 cell counts of at most 400 cells/mm^3 were also administered doses of 3TC ranging from 0.5 to 20 mg/kg/d *(61)*. Sustained decreases in p24 antigenemia independent of dose were seen over the 52-wk study. Small and transient increases in CD4 cell counts were detected during the first 4 wk of treatment. No dose-limiting toxicities were observed. Schuurman et al. demonstrated an initial decline in HIV-1 p24 antigenemia and RNA viral load in the first 2 wk of 3TC monotherapy, followed by a rise in both values that coincided with the appearance of 3TC-resistant viruses in plasma *(62)*. This study was one of the initial trials that demonstrated emergence of resistant virus with 3TC monotherapy.

Double-Combination Therapy

Protocol NUCA 3001 was a North American, randomized, double-blind trial comparing the safety and efficacy of 200 mg ZDV three times daily, 300 mg 3TC twice daily, 150 mg 3TC twice daily plus ZDV (low-dose combination), and 300 mg 3TC twice daily plus ZDV (high-dose combination) (Table 4) *(63)*. Three hundred sixty-six patients were enrolled, of which 87% were men and 61% were white, with a median age of 34 yr. Eligible patients had received ZDV for 4 wk or less and had CD4 cell counts of 200 to 500 cells/mm^3. The median CD4 cell count was 352 cells/mm^3 and the mean baseline plasma HIV RNA level was 4.47 $\log_{10}$ copies/mL. At 24 wk, the median change in $\log_{10}$ HIV RNA was –0.31, –0.60, –1.20, and –1.10 for the ZDV only, 3TC only, low-dose combination therapy, and high-dose combination therapy groups, respectively. There was no difference in CD4 cell count or viral load changes within monotherapy or combination therapy groups. Protocol NUCB 3001 was a randomized, double-blind trial in Europe comparing ZDV monotherapy at 200 mg three times daily and 300 mg 3TC twice daily plus ZDV in 129 antiretroviral-naive adult patients with CD4 cell counts of 100 to 400 cells/mm^3 *(64)*. Seventy-four percent of the patients were men, 82% were white, the median age was 33 yr, and the median baseline CD4 cell count was 260 cells/mm^3. CD4 cell count changes at 24 wk were –9 cells/mm^3 for ZDV monotherapy and +78 cells/mm^3 for the ZDV plus 3TC combination. Viral load decreases at 24 wk were –0.3 $\log_{10}$ copies/mL for the ZDV group and –1.2 $\log_{10}$ copies/mL for the ZDV plus 3TC group.

In protocol NUCA 3002, conducted in North America, 254 patients with previous ZDV monotherapy for at least 24 wk (83% men, 63% white; median age, 37 yr) were randomized to one of three treatment arms: 300 mg 3TC twice daily plus 200 mg ZDV three times daily, 150 mg 3TC twice daily plus 200 mg ZDV three times daily, or 200 mg ZDV three times daily plus 0.75 mg ddC three times daily *(65)*. The use of ZDV was open label, whereas ddC and 3TC were double blind. Eligible patients had CD4 cell counts between 100 and 300

cells/mm^3 and had been on ZDV monotherapy at least 24 wk. The baseline median CD4 cell count was 211 cells/mm^3 and the mean baseline plasma HIV RNA was 4.60 $\log_{10}$ copies/mL. At 24 wk, the CD4 cell count change was –16 cells/mm^3 for the ZDV plus ddC group, and +31 cells/mm^3 and +15 cells/mm^3 for the low- and high-dose 3TC plus ZDV combination groups, respectively. Significant decreases in HIV RNA were found in the ZDV plus 3TC groups compared with the ZDV plus ddC group. There was no clear benefit of the higher 3TC dose. Protocol NUCB 3002 was a randomized, controlled trial performed in Europe, comparing ZDV monotherapy and 3TC plus ZDV in patients with CD4 cell counts between 100 and 400 cells/mm^3 and at least 24 wk of ZDV *(66)*. Two hundred twenty-three patients (83% men; 96% white; median age, 36 yr) were randomized to receive one of three regimens: 200 mg ZDV three times daily, 200 mg ZDV three times daily plus 150 mg 3TC twice daily, or 200 mg ZDV three times daily plus 300 mg 3TC twice daily. The absolute change in CD4 cell counts at 24 wk favored either combination therapy, with no difference noted between the two 3TC dose-containing regimens. Median reduction of HIV RNA at 24 wk was –0.9 $\log_{10}$ copies/mL and –0.7 $\log_{10}$ copies/mL for low- and high-dose 3TC plus ZDV combinations, respectively, whereas patients receiving ZDV monotherapy had an increase in HIV RNA of 0.2 $\log_{10}$ copies/mL.

3TC in Triple-Combination Therapy

Multiple trials have investigated the combination of 3TC with ZDV or d4T and a PI or an NNRTI and demonstrated the potency of these combinations (Table 4). AVANTI 2 was a trial of antiretroviral-naive patients to evaluate the efficacy of ZDV plus 3TC combination therapy compared with ZDV plus 3TC plus IDV *(67)*. At week 52, the proportions of patients with a plasma HIV-1 RNA level less than 500 copies/mL were 75% and 23% in the triple- and double-therapy groups, respectively. The median CD4 cell count increase at week 52 was 177 cells/mm^3 in the triple-therapy group and 91 cells/mm^3 in the ZDV plus 3TC group. Similarly, ACTG 320 evaluated the combination of ZDV plus 3TC vs ZDV plus 3TC plus IDV in patients with at least 3 mo of previous ZDV therapy *(68)*. Clinical endpoints were progression of disease, survival, CD4 cell count change, and plasma HIV-1 RNA change. Patients were followed for a median of 38 wk. Six percent of patients in the triple-therapy group had AIDS-defining events or died, compared with 11% in the double-therapy group. At week 24, the proportions of patients with plasma HIV-1 RNA levels less than 400 copies/mL were 60% and 9% for the triple- and double-therapy groups, respectively. The mean CD4 cell count increases were 121 cells/mm^3 in the triple-therapy group compared with 40 cells/mm^3 in the double-therapy group.

Table 4
Clinical Trials of 3TC Combination Therapy

Trial (ref.)	Design	Dosage	No. of subjects	Entry criteria	Entry CD4 cell Trial counts (cells/mm^3)
NUCA 3001 *(63)*	Randomized, double-blind; 3TC vs ZDV vs ZDV+3TC (low dose) vs ZDV + 3TC (high dose)	ZDV 200 mg TID; 3TC 150 mg BID (low dose); 300 mg BID (alone and high dose)	366	ZDV <4 wk; CD4 200–500 cells/mm^3	352
NUCB 3001 *(64)*	Randomized, double-blind; ZDV vs 3TC+ZDV	ZDV 200 mg TID; 3TC 300 mg BID	129	ZDV <4 wk; CD4 100–400 cells/mm^3	ZDV, 250; ZDV +3TC, 260 (median)
NUCA 3002 *(65)*	Randomized, double-blind; ZDV+ddC vs ZDV+3TC (2 doses)	ZDV 200 mg TID; 3TC 150 mg BID or 300 mg BID; ddC 0.75 mg TID	254	ZDV >24 wk; CD4 100–300 cells/mm^3	211 (median)
NUCB 3002 *(66)*	Randomized, double-blind ZDV vs ZDV 3TC (low dose or high dose)	ZDV 200 mg TID; 3TC 150 mg BID or 300 mg BID	223	ZDV >24 wk; CD4 100–400 cells/mm^3	ZDV, 250; ZDV+3TC low dose, 250; ZDV+3TC high dose, 230 (medians)

AVANTI 2 *(67)*	Randomized, double-blind, ZDV +3TC vs ZDV+3TC+IDV	ZDV 200 mg TID; 3TC 150 mg BID; IDV 800 mg TID	103	Treatment naive	2-Drug group, 270; 3-drug group, 280 (median)
ACTG 320 *(68)*	Randomized, double-blind; ZDV +3TC vs ZDV+3TC+IDV	ZDV 200 mg TID, 3TC 150 mg BID, IDV 800 mg TID	1156	ZDV ≥ 3 mo	87
AVANTI 3 *(69)*	Randomized, double-blind; ZDV +3TC vs ZDV+3TC+NFV	ZDV 200 mg TID; 3TC 150 mg BID; NFV 750 mg TID	102	Treatment naive	2-Drug group, 279; 3-drug group, 287 (median)
Murphy, et al. *(70)*	Randomized, comparison of doses of lopinavir (LPV) and ritonavir (RTV) with d4T and 3TC added at 3 wk (group 1) or at day 0 (group 2)	LPV plus RTV (400 mg/100 mg or 200 mg/100 mg); ZDV 300 mg BID; d4T 40 mg BID	100	Treatment naive	Group 1, 398 Group 2, 310 (median)
Staszewski, et al. *(71)*	Open label; ZDV+3TC+EFV; ZDV+3TC+IDV; EFV+IDV (low dose)	ZDV 300 mg BID; 3TC 150 mg BID; EFV 600 mg QD; IDV 800 mg TID or 100 mg TID	450	No previous 3TC, NNRTI, or PI	345 (median)

(Continued)

Table 4 *(Continued)*

Duration of follow-up	CD4 response (cells/mm^3)	Antiviral response	Comment
24 wk (28-wk extension phase)	ZDV, +17; 3TC, +24; low-dose 3TC+ZDV, +55; high-dose 3TC+ZDV, +45	ZDV, –0.31 log_{10} copies/mL; 3TC, –0.60 log_{10} copies/mL; Low dose 3TC, –1.20 log_{10} copies/mL; high dose 3TC, –1.10 log_{10} copies/mL	No difference in CD4 cell count or viral load changes within monotherapy or combination therapy groups
24 wk (24-wk extension phase)	ZDV –9; ZDV+3TC, +78	ZDV, –0.3 log_{10} copies/mL; ZDV +3TC, –1.2 log_{10} copies/mL	Combination ZDV plus 3TC with larger CD4 and viral load changes than ZDV alone
24 wk	ZDV+ddC –16; ZDV+3TC (low dose) +31;ZDV+3TC (high dose) +15	Significant decreases for ZDV+3TC vs ZDV+ddC	No clear benefit of higher dose 3TC
24 wk	ZDV, –28; 3TC (low dose), +40; 3TC (high dose), +35	ZDV, +0.7 log_{10} copies/mL; 3TC, –0.9–0.65 log_{10} copies/mL	Combination ZDV +3TC with larger CD4 and viral load changes than ZDV alone

52 wk	Dual therapy, +91; triple therapy, +177	Proportion with HIV-RNA <500: 75% for triple therapy vs 23% for dual therapy	Triple combination with larger VL and CD4 changes than dual therapy but VL ≤ 20 seen in only 46% of patients with IDV
38 wk (median)	Dual therapy, +40; triple therapy, +121 (24 wk)	Proportion with HIV-RNA < 400: 60% for triple therapy vs 9% for dual therapy (24 wk)	6% in triple therapy vs 11% in dual therapy developed AIDS defining event or died
52 wk	At week 28, triple therapy, +101.5; dual therapy, +47	Proportion with HIV-RNA <500: 83% for triple therapy vs 18% for dual therapy	Demonstrated virological superiority of combination ART regimens including PI's
48 wk	Group I +244; Group II +213 (mean)	75–79% with HIV RNA <50 copies/mL	Lpt plus rtv mean trough plasma concentrations 50-100 fold higher than protein-binding corrected EC50 for wild type HIV-1
48 wk	Range increase, 180–200	HIV RNA <400: ZDV+3TC+EFV, 70%;ZDV+3TC+IDV, 48%; EFV+IDV, 53%	More treatment discontinuation in IDV groups

[a]QD, once daily; BID, twice daily; TID, three times daily

AVANTI 3 evaluated the use of ZDV plus 3TC and ZDV plus 3TC plus NFV in antiretroviral-naive patients *(69)*. At week 28, the proportions of patients with HIV-1 RNA levels less than 500 copies/mL were 83% and 18% for the triple- and double-therapy groups, respectively. Murphy et al. reported the results of a randomized, double-blind, multicenter trial of the combination of 3TC plus d4T plus the PI lopinavir (also known as ABT-378) combined with low-dose RTV in antiretroviral-naive patients *(70)*. Patients either began lopinavir/RTV alone with addition of 3TC plus d4T at week 3 (group 1) or received all agents together at day 0 (group 2). In an intent-to-treat analysis at 48 wk, HIV-1 RNA was less than 400 copies/mL for 91% (<50 copies/mL, 75%) and 82% (<50 copies/mL, 79%) of patients in groups 1 and 2, respectively. Staszewski et al. investigated the combination of 3TC paired with ZDV and combined with the NNRTI, EFV. In this study, the EFV group demonstrated greater virological suppression compared with an IDV-based regimen *(71)*. At 48 wk, a significantly larger proportion of patients treated with 3TC plus ZDV plus EFV had HIV-1 RNA levels below 400 copies/mL than those treated with 3TC plus ZDV plus IDV or with IDV plus EFV (70% vs 48% vs 53%, respectively).

Pediatric Trials—3TC Monotherapy

In a phase I/II study, 90 children ages 3 mo to 17 yr were stratified into two arms based on previous antiretroviral use. Subjects received dosages of 3TC ranging from 1 to 20 mg/kg daily *(72)*. CD4 and CD8 cell counts remained stable during 24 wk in therapy-naive children and decreased slightly in previously treated children. Viral burden decreased by 0.43 $\log_{10}$ copies/mL in both groups combined and by 0.68 $\log_{10}$ copies/mL in the naive group after 24 wk. Grouping patients into subsets of the lower (1 and 2 mg/kg/d), middle (4 and 8 mg/kg/d), and higher (12 and 20 mg/kg/d) dosage levels revealed significantly lower HIV RNA in children receiving at least 4 mg/kg/d 3TC. In vitro resistance to 3TC (M184V) was documented in sequential virus isolates from 20 of 26 patients after 8 to 48 wk of therapy. Increases in hepatic transaminases and development of pancreatitis were the most serious side effects.

Pediatric Combination Therapy—3TC and ZDV vs Didanosine

PACTG 300 was a multicenter, randomized, double-blind study that compared 4 mg/kg 3TC twice daily plus 160 mg/m^2 ZDV three times daily with 120 mg/m^2 didanosine monotherapy twice daily or a combination of ZDV plus 90 mg/m^2 ddI twice daily *(73)*. A total of 471 symptomatic, antiretroviral therapy-naive pediatric patients were enrolled. The median age was 2.7 yr, 58% were girls, and 86% were nonwhite. The mean baseline CD4 cell count was 868 cells/mm^3 and the mean baseline plasma HIV RNA was 5.0 $\log_{10}$ copies/mL. The median duration of follow-up was 10.1 mo for the ZDV plus

3TC arm and 9.2 mo for the ddI arm. Primary clinical endpoints were disease progression, including physical growth failure, central nervous system deterioration and Centers for Disease Control Clinical Category C, and death. In the 3TC plus ZDV arm, 6.4% of patients reached a clinical endpoint, as did 15.7% patients in the ddI arm. Both ZDV plus 3TC and ZDV plus ddI recipients had a lower risk of HIV disease progression than patients who received ddI alone ($p = 0.0026$ and $p = 0.0045$, respectively).

Pediatric Double Combination Therapy—Addition of 3TC to Current NRTI

The Pediatric European Network for Treatment of AIDS (PENTA)-4 study was a double-blind randomized trial of the addition of 3TC (4 mg/kg twice daily) or placebo to stable NRTI therapy in 162 pediatric patients with a median age of 6.5 yr, a median CD4 cell count of 328 cells/mm^3, and a median HIV viral load of 4.9 $\log_{10}$ copies/mL *(74)*. Background therapy included ZDV in 52 patients, ddI in 39 patients, ZDV and ddI in 54 patients, and ZDV and ddC in 17 patients. At week 24, the addition of 3TC resulted in a median change in CD4 cell count of +47 cells/mm^3 and an HIV viral load change of –0.3 $\log_{10}$ copies/mL compared with placebo. The decrease in viral load was 0.38 $\log_{10}$ copies/mL greater in the ZDV vs the ddI background therapy groups.

Pediatric Combination Therapy—3TC, ZDV, and RTV

As demonstrated in adults, the combination of 3TC with a second nucleoside analog and a PI seems to have an enhanced antiviral effect. In PACTG 338, an interim analysis demonstrated that children receiving RTV and one or two NRTIs had a mean decrease of greater than 1.5 $\log_{10}$ copies/mL in viral RNA levels after 12 wk of therapy *(75)*. After 48 wk, 42% of children receiving the triple combination of ZDV plus 3TC plus RTV had an undetectable viral load, as compared with 27% receiving a single NRTI plus RTV.

3TC Resistance

3TC monotherapy results in high-level HIV resistance, which is caused by a single mutation in codon 184 of the HIV-1 reverse transcriptase gene, in which methionine is replaced by either isoleucine or valine. In vitro experiments with 3TC demonstrated the IC_{50} of these variants to 3TC is 500- to 1000-fold greater than that of wild-type virus *(76)*. In vivo studies demonstrated an initial decline in HIV-1 p24 antigenemia and RNA viral load in the first 2 wk of 3TC therapy, followed by a rise in both values that coincided with the appearance of 3TC-resistant viruses in plasma *(77)*. ZDV-resistant HIV isolates that acquire the M184V mutation have been found to regain their antiretroviral activity *(78)*. The M184V mutation reverses the selective advantage ZDV-resistant strains

attain in continuation of HIV DNA chain elongation and, thus, leads to increased ZDV phenotypic susceptibility in the setting of genotypic resistance *(79,80)*. In addition, HIV with the M184V mutation induced by either 3TC or ABC treatment results in increased tenofovir susceptibility for HIV in the presence or absence of ZDV-associated reverse transcriptase mutations. Other important mutations that confer 3TC resistance include groups of mutations based at codons 69 or 151.

Drug Interactions

Very few drug interactions between 3TC and other antiretroviral agents or other medications exist. 3TC coadministration with trimethoprim/sulfamethoxazole resulted in a 43% increase in AUC and a 35% decrease in renal clearance of 3TC *(81)*. No changes in the pharmacokinetics of trimethoprim/sulfamethoxazole were seen.

Current Clinical Uses

Dosing and Formulations

The recommended daily dose of 3TC for adults is oral administration of either 150 mg twice daily or 300 mg daily, and for pediatric patients, 3 mo to 16 yr of age, is 4 mg/kg daily (up to a maximum daily dose of 300 mg). Dosage adjustment is recommended for creatinine clearance less than 50 mL/min. The drug is supplied in 150 mg and 300 mg tablets and as an oral solution of 10 mg/mL.

3TC dosed as a 300 mg tablet once daily was approved for use in June 2002. In a nonblind, sequential, pharmacokinetic study, 13 patients with HIV-1 infection received 150 mg 3TC twice daily and then switched to 300 mg once daily in randomized order *(82)*. The plasma pharmacokinetic profile of 3TC was determined over a 12-h period on day 7 after twice-daily dosing and over 24 h on day 7 after once-daily dosing. Statistical analysis did not show a significant difference regarding the mean values of half-life, average steady-state concentration, or AUC between the two dosing regimens.

COLA4005 was a prospective, randomized, multicenter trial comparing the efficacy and safety of a switch to 3TC once-daily dosing vs continued standard dosing of 150 mg 3TC twice daily in subjects on a stable regimen with an HIV RNA level less than 400 copies/mL and CD4 cell counts greater than 50 cells/mm^3 *(83)*. At week 24, 82% of subjects on the once-daily regimen had HIV viral loads less than 50 copies/mL vs 81% on the twice-daily regimen. Both dosing regimens were well-tolerated with comparable safety profiles.

3TC is also available combined with ZDV alone and with ZDV plus ABC in a fixed-dose combination (FDC) tablet. The first coformulation of antiretroviral therapy, Combivir™ (COM) tablets contain 150 mg of 3TC and 300 mg of ZDV. COM was approved by the FDA in September 1997 for use in

HIV-infected adults and children greater than 12 yr of age. This drug is indicated for treatment of HIV infection in combination with other antiretroviral agents. Pharmacokinetic studies in adults revealed COM to be bioequivalent to one 150-mg 3TC tablet and one 300-mg ZDV tablet after single-dose administration to fasting healthy subjects *(84)*. A randomized, open-label study in antiretroviral-experienced patients was performed to establish the clinical equivalence of COM plus a marketed PI, compared with a conventional regimen of 150 mg 3TC twice daily plus 300 mg ZDV twice daily plus a PI *(85)*. In the 223 patients that were followed for 16 wk, the two regimens were shown to have equal efficacy. The FDC, 3TC plus ZDV plus ABC, known as Trizivir, is also available for treatment of HIV infection. A detailed discussion of Trizivir is presented in the "Abacavir" section of this chapter.

Toxicity

Significant toxicity related to 3TC is uncommon. The most common adverse effects described are headache, nausea, and neutropenia. The relative lack of neutropenia associated with 3TC use as compared with ZDV is likely related to decreased affinity of 3TC to human DNA polymerase. Pancreatitis has been reported in pediatric patients receiving 3TC, however, advanced HIV disease and concomitant medications may have contributed to these episodes *(86)*. In adult clinical trials of 3TC, increased incidence of pancreatitis has not been demonstrated.

Current Use

Current indications for 3TC include HIV infection in which treatment is indicated, prevention of vertical transmission of HIV, occupational postexposure prophylaxis for HIV, and treatment of chronic Hepatitis B. 3TC is currently recommended as the nucleoside analog of choice combined with a second nucleoside analog (ZDV or d4T) or the nucleotide analog, tenofovir, to form the backbone of combination antiretroviral therapy in antiretroviral-naive patients *(87)*.

ABACAVIR

ABC (also known as Ziagen™, formerly 1592U89) is a synthetic carbocyclic nucleoside analog with potent and selective inhibitory activity against HIV-1. This agent was approved by the FDA for use in combination therapy of HIV-1 infection in adults and children age 3 mo or older in December 1998. ABC is currently recommended as an alternative agent to PIs or EFV in combination with two other NRTIs for initial treatment of established HIV infection *(87)*.

Mechanism of Action and In Vitro Studies

Vince et al. reported the antiretroviral effects of the carbocyclic analog of 2′,3′-dehydro-2′,3′-dideoxyguanosine, a compound known as carbovir (Fig. 1)

(88). Initial data were based on analysis of the racemic mixture. Subsequent investigation revealed the negative enantiomer to be the biologically active isomer, and this compound became known as 1592U89 *(89)*. ABC is anabolized intracellularly to its active triphosphate form via a unique metabolic pathway using enzymes that do not phosphorylate other NRTIs *(90)*. Carbovir triphosphate is an analog of deoxy-guanosine-5′-triphosphate and inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate, deoxy-guanosine-5′-triphosphate, and by incorporation into viral DNA. The lack of a 3′-OH group in the incorporated nucleoside analog prevents the formation of the 5′ to 3′ phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated.

The in vitro anti-HIV activity of ABC was demonstrated in a HIV-1 IIIB strain cultured in MT-4 cells, peripheral blood mononuclear cells, and macrophages, with IC_{50} values ranging from 4.0 to 0.65 μ*M* *(89)*. Eight clinical isolates of HIV-1 from ZDV-naive patients amplified in peripheral blood mononuclear cells showed a mean IC_{50} of 0.26 μ*M* *(89,91)*. ABC demonstrated synergistic activity in vitro against HIV-1 when used in combination with ZDV, 3TC, didanosine, nevirapine, or amprenavir in MT4 cells *(92–94)*. Additive effects with the other nucleoside analogs, such as d4T, were also noted.

Pharmacokinetics

ABC crosses the blood-brain barrier, with CSF-to-plasma concentration ratios of 18 to 25%. Bioavailabilty is 83% and serum half-life is 1.5 h. After oral administration of 300 mg ABC twice daily in 20 patients, the steady-state peak serum ABC concentration was 3.0 ± 0.89 μg/mL *(95)*. Binding of ABC to human plasma proteins is approx 50%, however, physiological concentrations of albumin or α-1-glycoprotein do not markedly alter ABC activity *(96)*. Food intake does not affect the bioavailability of ABC. In humans, cytochrome P450 enzymes do not significantly metabolize ABC and it, in turn, does not inhibit human CYP3A4, CYP2C, or CYP2D6 activity at clinically relevant concentrations. The primary routes of elimination are metabolism by alcohol dehydrogenase and glucuronyl transferase. The pharmacokinetic properties of ABC have not been determined in patients with impaired renal function. In the CNAB1006 study, patients with mild liver impairment had a 1.9-fold increase in AUC and a 1.6-fold increase in ABC half-life, suggesting that patients with mild hepatic failure may need lower ABC doses to achieve similar AUCs to patients without liver disease *(97)*.

Phase I/II Studies

Wang et al. reported results of 15 HIV-1 infected adults with a median CD4 cell count of 347 cells/mm^3 who were enrolled in a randomized, seven-period crossover study *(98)*. The pharmacokinetics and safety of single doses of ABC,

ZDV, and 3TC were evaluated when each drug was administered alone or when any two or three drugs were administered concurrently. No clinically significant pharmacokinetic interactions occurred between ABC, ZDV, and 3TC. No increase in adverse events with the three-drug combination were seen.

CNAA2001 was a multicenter trial comparing the safety and efficacy of four doses of ABC alone and in combination with ZDV (Table 5) *(99)*. Patients were randomized to four different ABC doses for the first 4 wk, and, thereafter, to combination therapy with 300 mg ABC twice daily and ZDV or placebo for 8 wk. At week 12, the percentages of patients with plasma HIV-1 RNA levels less than 400 and less than 40 copies/mL for ABC monotherapy were 28% and 11%, respectively, vs 69% and 22% for ABC plus ZDV. Median CD4 cell counts increased by 79 to 195 cells/mm^3 and 93 to 142 cells/mm^3 for ABC monotherapy and ABC plus ZDV, respectively, but these differences were not significant. Eight subjects (10%) discontinued the study prematurely because of adverse events, three with hypersensitivity reactions. After 12 wk on study, 72 of 79 patients were required to interrupt ABC treatment until essential preclinical studies were completed. In the extension phase of the trial, 43 of 72 subjects elected to restart open-label ABC therapy in combination with either an NNRTI or a PI after up to 1 yr of interruption *(100)*. After 48 wk of therapy, more than 50% of patients receiving either nucleoside-only therapy or PI-containing therapy with ABC had an HIV RNA level less than 400 copies/mL.

Staszewski et al. reported results of a dose-ranging trial to evaluate the safety and efficacy of ABC alone or in combination with ZDV and 3TC in antiretroviral-naive subjects *(101)*. Patients were randomized to three ABC doses for 24 wk, after which subjects could switch to open-label 300 mg ABC twice daily, with ZDV and 3TC or other antiretrovirals as determined by their physician for an additional 24 wk. At week 4, the subjects in the 300 or 600 mg ABC groups had greater reductions in plasma HIV-1 RNA (median changes –1.55 and –1.61 $\log_{10}$ copies/mL, respectively) than patients in the 100 mg ABC twice-daily group (median change, –0.63 $\log_{10}$ copies/mL). Differences between the 300 and 600 mg twice-daily ABC groups were not significant. At week 48, a median reduction in plasma HIV-1 RNA of 2.8 $\log_{10}$ copies/mL from the baseline of pooled ABC-treated subjects was seen. Sixty-five percent and 43% of patients had less than 400 and less than 50 HIV-1 RNA copies/mL, respectively, after 48 wk of ABC-containing therapy. A hypersensitivity reaction attributable to ABC was seen in 3.3% of patients.

ZDV Plus 3TC Plus ABC Vs ZDV Plus 3TC Alone in Antiretroviral-Naive Adults

CNAA3003 investigated the safety, tolerance, and antiviral activity of ZDV plus 3TC plus ABC at 16 and 48 wk. This multicenter trial of therapy-naive

Table 5
Clinical Trials of Abacavir Combination Therapy

Trial (ref.)	Design	Dosage	No. of subjects	Entry criteria
CNAA2001 *(99)*	Randomized, double-blind; ABC alone for 1st 4 wk, then ABC+ZDV or placebo for 8 wk	ABC 200 mg, 400 mg, or 600 mg TID; 300 mg BID; ZDV 300 mg BID	79	<12 wk ZDV
CNAB2002 *(101)*	Randomized, double-blind; ABC alone for 24 wk, then open-label ABC+ZDV+3TC or other NRTIs per physician	ABC 100 mg, 300 mg, or 600 mg BID; ABC 300 mg BID + ZDV 300 mg BID + 3TC 150 mg BID	60	VL ≥30,000 copies/mL; CD4 ≥ 100 cells/mm^3
CNAA3003 *(102)*	Randomized, double-blind; ZDV+3TC vs ZDV+3TC+ABC for 16 wk, then open-label ZDV+3TC+ABC	ZDV 300 mg BID; 3TC 150 mg BID; ABC 300 mg BID	173	Treatment naïve; CD4 ≥100 cells/mm^3
CNA3014 *(103)*	Randomized, double-blind; ABC+COM vs IDV+COM	ABC 300 mg BID, IDV 800 mg TID	342	Treatment naive; HIV RNA 5-100K
CNAF3007 *(104)*	Randomized, open-label; COM+ABC vs COM+NFV for 48 wk	ABC 300 mg BID, NFV 1250 mg TID	196	Treatment naive: HIV RNA 1000–500,000 copies/mL
ACTG 5095 *(105)*	Randomized, double-blind ZDV+3TC+ABC vs ZDV+3TC+EFV vs ZDV+3TC+ABC+EFV	ZDV+3TC/FDC (Combivir); ZDV+3TC+ABC/FDC (Trizivir); EFV 600 mg QD	167	Treatment naïve
CNA3002 *(107)*	Randomized, double-blind; addition of ABC vs placebo to stable regimen	ABC 300 mg BID	185	CD4 >100 cells/mm^3; VL 400–500,000 copies/mL
CNAA3017 *(108)*	Open-label switch study; continue PI-based regimen vs switch to ABC-based regimen	ABC 300 mg BID	211	Stable PI-based regimen; HIV RNA <50 copies/mL

Table 5 *(Continued)*

Duration of follow-up	CD4 response (cells/mm^3)	Antiviral response	Comments
12 wk	ABC alone, 79–195; ABC+ZDV, 93–145	VL < 400 copies/mL: ABC, 28%; ABC+ZDV, 69%; VL < 40: ABC, 11%; ABC+ZDV, 22%	10% of subjects discontinued study prematurely for adverse events
48 wk	At week 24: ABC 100 mg, +26; ABC 300 mg, +97; ABC 600 mg, +40 At wk 48: median +111 pooled	At week 24, mean $\log_{10}$ copies/mL change: ABC 100 mg, –0.63 ABC, 300 mg/600 mg: –1.55–1.61 At wk 48, pooled ABC median VL reduction, 2.8	All ABC doses well-tolerated, 2 subjects with hypersensitivity reaction
48 wk	Median ZDV+ 3TC +150 ZDV+3TC+ABC +152	VL < 400: ZDV+3TC, 35%; ZDV+3TC+ABC, 75%	Loss of virological response and difficulty with regimen more common in IDV group
48 wk	Not reported	VL < 400: ABC plus COM 64%; IDV plus COM 50%	Increased continuation of regimen and self-reported adherence in ABC group
48 wk	Mean increase: COM+ABC, 109; COM+NFV, 120	VL < 50: COM+ABC, 64%; COM+NFV, 61%	ABC+COM comparable antiviral activity to IDV+COM
Median 32 wk	Mean increase: ABC arm, +174; pooled EFV, +173	Virological failure: ABC arm 21% vs pooled EFV 11% ($p < 0.001$)	Interim results resulted in triple-NRTI arm termination
16 wk	ABC +30; stable regimen + 1 (intent to treat)	VL < 400: ABC 39%; stable regimen 8%	Antiviral response seen despite M184V mutation
48 wk	Cont PI arm +13; ABC arm +26 (intent to treat)	Virological failure continued PI 23%; ABC 12%	Significant reductions in cholesterol and triglyceride in ABC arm

[a]QD, once daily; BID, twice daily; TID, three times daily; VL, viral load; Cont, continued

patients randomized subjects to ZDV plus 3TC plus ABC vs ZDV plus 3TC *(102)*. Subjects were stratified by baseline HIV-1 RNA level: less than 10,000 copies/mL; 10,000 to 100,000 copies/mL; or more than 100,000 copies/mL. At week 16, all patients had the option to switch to open-label ABC plus ZDV plus 3TC. Patients with confirmed plasma HIV-1 RNA levels greater than 400 copies/mL were permitted to switch to a new regimen to include ABC and other available antiretroviral agents. In an intent-to-treat analysis at week 16, 75% and 35% of subjects in the ABC plus ZDV plus 3TC and ZDV plus 3TC groups, respectively, had an HIV-1 RNA level less than 400 copies/mL. The triple combination was effective at all HIV-RNA strata, whereas the dual-therapy group had a diminished virological response with a higher baseline HIV-1 RNA level. The mean increase in CD4 counts was similar between the treatment groups at 16 wk. There was no difference in tolerance between the two groups.

Triple-Combination Therapy: ABC Vs PIs

CNA3014 compared the efficacy, safety, and adherence of ABC plus COM (300 mg ZDV and 150 mg 3TC twice daily) vs IDV plus COM in 342 antiretroviral therapy-naive patients *(103)*. Subjects were stratified based on screening HIV-1 RNA levels (stratum 1, 5000–100,000 copies/mL; stratum 2, >100,000 copies/mL). At week 48, by intent-to-treat analysis, 105 of 164 (64%) ABC plus COM subjects vs 82 of 165 (50%) IDV plus COM subjects had an HIV-1 RNA level less than 400 copies/mL. Time-to-treatment failure during 48 wk was significantly longer for the COM plus ABC group than for the COM plus IDV group. For stratum 1 and 2, the values were 73 of 106 (69%) and 32 of 58 (55%), respectively, for ABC plus COM subjects vs 49 of 100 (49%) and 33 of 65 (51%) for IDV plus COM subjects. Eleven percent of ABC plus COM subjects vs 13% of IDV plus COM subjects discontinued randomized study therapy because of an adverse event. Ten (6%) ABC plus COM subjects reported possible hypersensitivity to ABC. Self-reported adherence to randomized treatment was significantly higher in the ABC plus COM group.

The CNAF3007 study evaluated the antiviral activity of COM plus ABC vs COM plus NFV in antiretroviral-naive adults *(104)*. In this randomized, open-label study, 196 patients with HIV-1 RNA levels of 1000 to 500,000 copies/mL were followed for 48 wk. Baseline viral loads were comparable in the two treatment groups. In the intent-to-treat analysis at 48 wk, 64% and 61% of subjects had HIV-1 RNA levels of less than 50 copies/mL in the COM plus ABC and COM plus NFV arms, respectively. The COM plus ABC group had median CD4 cell count increases of 109 cells/mm^3, as compared with 120 cells/mm^3 in the COM plus NFV group. Possible hypersensitivity reactions to ABC were seen in 4% of subjects.

Triple-Combination Therapy—ABC Vs EFV

ACTG 5095 was a comparative study of three PI-sparing antiretroviral regimens in treatment-naive patients *(105)*. Subjects were randomized 1:1:1 to ZDV plus 3TC plus ABC (FDC); ZDV plus 3TC (FDC) plus EFV; or ZDV plus 3TC plus ABC (FDC) plus EFV to assess safety and virological responses. Virological failure was defined as a confirmed HIV-1 RNA levels greater than 200 copies/mL more than 16 wk after randomization. Based on a planned interim review, the Data and Safety Monitoring Board recommended termination of the ZDV plus 3TC plus ABC arm. One hundred sixty-seven patients reached protocol-defined virological failure: 82 (21%) on ZDV plus 3TC plus ABC and 85 (10%) on pooled EFV arms. Time-to-virological failure was shorter with ZDV plus 3TC plus ABC compared with pooled EFV arms ($p < 0.001$). This trial suggested that ZDV plus 3TC plus ABC was inferior to EFV-containing regimens regarding virological failure in treatment-naive patients.

ABC Expanded Access Program

The ABC Expanded Access Program was an international, multicenter, nonrandomized, open-label study *(106)*. In part A of the Expanded Access Program, all 2580 patients had a plasma HIV-1 RNA level greater than 30,000 copies/mL, a CD4 cell count of fewer than 100 cells/mm^3, and virological failure to standard antiretroviral therapy that included at least two NRTIs and a PI. Part B enrolled 11,624 patients and required only that patients have infections that did not respond to standard therapy and that their providers' could not construct a viable treatment regimen without ABC. In both parts A and B, ABC was included as a component in a treatment regimen that contained at least one other antiretroviral drug that the patient had not received in the past. Virological analysis was performed only for Part A patients. By month 2 of the ABC-containing treatment, plasma HIV-1 RNA levels decreased by at least 0.5 $\log_{10}$ copies/mL in 31.4% of patients, and 5.6% of patients had a decrease in HIV-1 RNA levels to less than 400 copies/mL. Drug-related serious adverse events were reported by 7.7% of patients, and 4.6% of patients experienced a hypersensitivity reaction that was possibly drug related.

ABC Addition to Stable Background Therapy

Katlama et al. reported the results of CNA3002, which evaluated the addition of 300 mg ABC twice daily vs placebo to a stable background antiretroviral regimen (SBG) *(107)*. One hundred eighty-five patients with CD4 cell counts greater than 100 cells/mm^3 and an HIV-1 RNA level of 400 to 50,000 copies/mL were randomized. Median plasma HIV-1 RNA level at entry was 3.68 $\log_{10}$ copies/mL and 3.53 $\log_{10}$ copies/mL for the ABC plus SBG and SBG groups, respectively. The proportion of subjects with up to 18 mo of previous

NRTI therapy and previous 3TC usage was similar in both treatment groups. The most frequent background regimens were 3TC plus ZDV (36%), two NRTIs plus a PI or NNRTI (21%), d4T plus 3TC (19%), and ZDV plus ddI (10%). At week 16, 36 of 92 (39%) vs 7 of 93 (8%) patients in the ABC plus SBG and SBG groups had an HIV-1 RNA level less than 400 copies/mL. A similar response was observed in both 3TC-naive and 3TC-experienced subjects. Seventy-three percent of patients with the M184V mutation alone had a greater than 1 $\log_{10}$ copies/mL reduction in plasma HIV-1 RNA level or had less than 400 copies/mL by week 16. The presence of three or more thymidine analog mutations with or without the M184V mutation was associated with reduced activity of ABC plus SBG.

Simplification With ABC-Based Triple Nucleoside Regimen Vs Continued PI Therapy

CNA30017 was an open-label, multicenter study in which 211 patients who had been on a stable highly active antiretroviral therapy regimen with two NRTIs plus one PI for at least 6 mo and had a plasma HIV-1 RNA level less than 50 copies/mL were randomized to replace the PI with ABC or to continue the same regimen *(108)*. A significantly longer time-to-treatment failure was demonstrated in the ABC arm compared with patients who continued the same regimen, and more treatment failures were seen in the PI arm (23%) than the ABC arm (12%). A significant reduction in cholesterol and nonfasting triglyceride was demonstrated in the ABC arm. The incidence of treatment-related adverse events was not significantly different between treatment groups, although the number of adverse events resulting in discontinuation of randomized medication was higher in patients remaining on a PI (14% vs 8%).

ABC Pediatric Trials—Phase I

In ACTG 330, 47 HIV-infected children discontinued previous antiretroviral therapy and were orally administered 4 mg/kg ABC every 12 h for 6 wk, followed by 8 mg/kg ABC every 12 h for 6 or 12 wk *(109)*. At a dose of 8 mg/kg every 12 h, the AUC for plasma concentration vs time and the plasma half-life values were comparable to those reported for adults receiving ABC at a dose of 300 mg twice daily. One case each of hypersensitivity reaction and peripheral neuropathy occurred during ABC monotherapy. Three children developed neutropenia while receiving ABC in combination with another antiretroviral agent. Mean CD4 cell count and plasma HIV-1 RNA level did not change when previous antiretroviral therapy was changed to ABC monotherapy.

Pediatric ABC Combination Therapy

CNAA3006 was a randomized, double-blind trial of ABC plus 3TC plus ZDV vs 3TC plus ZDV in antiretroviral-experienced HIV-infected children *(110)*.

Two hundred five children with CD4 cell counts of at least 100 cells/mm^3 were randomized to receive 8 mg/kg ABC twice daily plus 4 mg/kg 3TC twice daily plus 180 mg/m^2 ZDV twice daily; or only 3TC plus ZDV. In an intent-to-treat analysis at week 48, the proportion of patients with plasma HIV-1 RNA levels less than 10,000 copies/mL were 36% and 26% for the ABC plus 3TC plus ZDV and 3TC plus ZDV groups, respectively. Three percent of children experienced ABC-related hypersensitivity reactions.

In PENTA-5, antiretroviral-experienced children were randomized to three NRTI regimes with or without NFV *(111)*. At 48 wk, the ABC-containing regimens with or without NFV resulted in the greatest HIV-1 viral load reduction.

ABC Resistance

ABC selects for several mutations on the reverse transcriptase gene, including M184V, K65R, L74V, and Y115F. The M184V mutation alone does not lead to significant ABC resistance. Clinical trials indicate that resistance to ABC is associated with the presence of the M184V mutation in combination with at least three thymidine analog mutations *(112)*. Mutations at codons 65, 74, and, possibly, 184 lead to cross-resistance to ddI and ddC. Each of these mutations results in a twofold to fourfold decrease in susceptibility to ABC.

Drug Interactions

Very few drug interactions with ABC and other medications exist. The coadministration of ABC and ethanol increases the ABC AUC by 41% and the ABC half-life by 26% *(113)*. Ethanol pharmacokinetics were unchanged.

Current Clinical Uses

Dosing and Formulations

ABC is supplied as 300 mg tablets and as an 20-mg/mL oral solution. The recommended daily dosage is 600 mg either once daily or in two divided doses for adults and 8 mg/kg twice daily (up to a maximum dose of 600 mg daily) for adolescent and pediatric patients 3 mo to 16 yr of age.

Trizivir™ is the only three-drug fixed-dose coformulation of antiretroviral medications and was approved for use in adults and adolescents weighing more than 40 kg in November 2000. Each Trizivir tablet contains 300 mg ABC, 150 mg 3TC, and 300 mg ZDV. One Trizivir tablet was bioequivalent to one 300-mg Ziagen tablet, one 150-mg Epivir tablet and 300-mg Retrovir tablet after single-dose administration to 24 fasting healthy subjects *(115)*. The recommended oral dosage of Trizivir is one tablet twice daily. Because it is a fixed-dose tablet, Trizivir should not be prescribed for patients requiring dosage adjustment, such as those with creatinine clearances less than 50 mL/min or those experiencing dose-limiting adverse events.

Fischl et al. reported results of an open-label, randomized study of the efficacy of COM plus ABC compared with Trizivir *(116)*. One hundred eighty-six subjects were on previous therapy with COM and ABC twice daily with or without a PI or an NNRTI and had an HIV-1 RNA level less than 400 copies/mL and a CD4 cell count greater than 200 cells/mm^3. Patients were randomized to continue COM plus ABC or switch to Trizivir. Prestudy PI or NNRTI was continued if applicable. At 24 wk, 30 of 34 (88%) and 27 of 34 (79%) subjects had an HIV-1 RNA level less than 400 copies/mL and less than 50 copies/mL, respectively, in the COM plus ABC group as compared with 33 of 34 (97%) and 28 of 34 (82%) in the Trizivir group.

Toxicity

The most common side effects seen during ABC therapy are gastrointestinal and neurological side effects. The gastrointestinal side effects include nausea, vomiting, and diarrhea, and tend to abate after the first few weeks of ABC therapy. Dizziness, headache, malaise, and insomnia are the most common neurological side effects.

A hypersensitivity reaction to ABC has been reported in 3 to 7% of patients and is characterized by multisystem involvement. Symptoms usually appear within the first 6 wk of treatment, with a median time to onset of 11 d. Manifestations include fever, rash, gastrointestinal symptoms, myalgias, and lethargy. Less common symptoms include cough, dyspnea, and arthralgias. Symptoms worsen with continued therapy and usually improve within 24 h of ABC discontinuation. Use of prednisolone does not prevent ABC hypersensitivity and may increase the risk of this reaction *(117)*. Rechallenge with ABC after development of hypersensitivity-related symptoms typically results in recurrence of symptoms within hours, with the potential to induce a more severe clinical syndrome, with increased risk of life-threatening hypotension and death. The mechanism of the ABC hypersensitivity reaction is not known, but clinical symptoms suggest an immunological reaction influenced by genetic factors. An association between development of ABC hypersensitivity and certain human leukocyte antigen haplotypes has been reported, although results are conflicting *(118,119)*.

Current Uses

ABC is indicated for treatment of adults and children with HIV-1 infection in which treatment is indicated. The use of ABC as part of an initial therapy for antiretroviral-naive patients is attractive from the standpoint of pill burden and potency when combined with a second NRTI and an NNRTI. ABC is currently recommended as part of an alternative initial regimen containing 3TC and EFV *(87)*. The use of the three-NRTI regimen of ABC plus ZDV plus 3TC as an initial therapy has been associated with virological failure and, as such, is recommended for use only in patients in whom an NNRTI or a PI cannot be used. In addition,

the combination of ABC plus tenofovir plus 3TC should not be used as the sole combination at any time, based on data showing early virological nonresponse *(120)*. ABC is a useful component of salvage therapy in patients without HIV isolates resistant to multiple nucleoside compounds.

SUMMARY

The discovery and development of the antiretroviral agents ZDV, 3TC, and ABC has lead to widespread reductions in morbidity and mortality for persons infected with HIV. From the use of these agents as monotherapy, followed by their combination together and with other nucleoside analogs to form the "nucleoside backbone" of triple-combination therapy, these agents have become some of the most commonly prescribed antiretroviral medications. The development of the coformulation of ZDV plus 3TC as COM, and of ZDV plus 3TC plus ABC as Trizivir, along with the once-daily dosing formulation of 3TC have been exciting additions to the armamentarium of antiretroviral medications, with obvious adherence implications. The capacity for use of 3TC for treatment of HIV-1 and hepatitis B, along with its exceptional toxicity profile and ease of dosing, have made 3TC particularly attractive for initial regimens. Finally, in a once-daily dose, the coformulation of 3TC plus ABC represents a future direction for these agents.

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3

Stavudine, Didanosine, and Zalcitabine

Gail Skowron, Sapna Chowdhry, and Michael R. Stevens

INTRODUCTION

After the development and commercialization of zidovudine (as Retrovir®, also known as 3′-azido-3′-deoxythymidine or AZT) as the first anti-HIV compound in 1987, researchers logically turned to other nucleoside analogs in an attempt to expand the armamentarium against the disease. The next three compounds in this class to become commercially available were didanosine (as Videx®, Bristol-Myers Squibb) in 1991, zalcitabine (as Hivid®, F. Hoffmann-La Roche) in 1992, and stavudine (as Zerit®, Bristol-Myers Squibb) in 1994 (Fig. 1).

Dideoxynucleoside Mechanism of Action

As is the case with zidovudine, each of these compounds must be triphosphorylated by intracellular kinases before they can compete with the natural nucleoside substrates to bring about viral DNA chain termination. Once triphosphorylated, stavudine, similar to zidovudine, competes with deoxythymidine triphosphate for binding to reverse transcriptase. Likewise, zalcitabine competes with deoxycytidine triphosphate, whereas didanosine undergoes conversion to an analog of deoxyadenosine triphosphate. Through using the viral reverse transcriptase to integrate themselves into the growing DNA strand, these agents also prevent the next endogenous nucleoside triphosphate from continuing to build the chain. Successful inhibition and/or termination of DNA reverse transcription prevent the host cell from becoming infected with HIV *(1)*.

Nucleoside analog chain termination is accomplished by the removal or replacement of the natural substrate's 3′ hydroxyl group, where successive nucleosides must attach to the growing chain. In the case of zalcitabine and didanosine, hydrogen replaces the 3′ hydroxyl group, resulting in a dideoxy nucleoside (2′,3′-dideoxycytidine [ddC] and 2′,3′-dideoxyinosine [ddI], respectively). The stavudine molecule has a double bond inserted between carbons 2 and 3, resulting in 2′,3′-didehydro-3′-deoxythymidine (d4T) (Fig. 1). Based on this nomenclature, these three compounds are sometimes collectively referred to as the "d" drugs.

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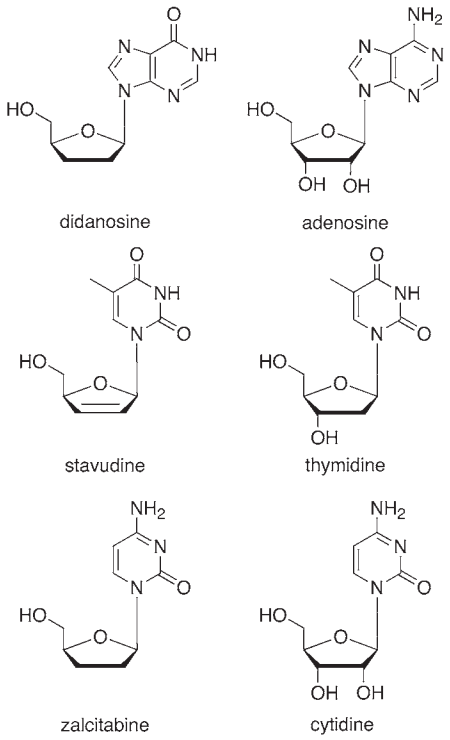

Fig. 1. Chemical structures of stavudine, didanosine, and zalcitabine with their natural nucleoside analogs.

STAVUDINE

Stavudine Overview

Stavudine (also known as d4T) is available as an immediate-release product (Zerit) and has been investigated as a once-daily extended-release formulation (Zerit XR). The absorption of stavudine is not significantly affected by food. Although no clinically significant drug–drug interactions occur between stavudine and didanosine, lamivudine, or nelfinavir, concurrent use of zidovudine and stavudine is not recommended in clinical practice, because of intracellular antagonism. Because of the potential for overlapping toxicities, the use of the combination of stavudine plus didanosine in therapy-naive patients is decreasing and is not recommended.

Extensive data on the efficacy of stavudine-containing triple-therapy regimens in patients with HIV infection show that stavudine in combination with another nucleoside reverse transcriptase inhibitor (NRTI) and a protease inhibitor (PI) (with or without low-dose ritonavir) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) reduced plasma HIV RNA levels to fewer than

500 copies/mL in 53 to 100% of antiretroviral-naive patients and to fewer than 50 copies/mL in 41 to 100% of antiretroviral-naive patients after 48 to 52 wk of therapy (ITT M=F) *(2–4,29–32)*.

Adverse events associated with stavudine-containing regimens include peripheral sensory neuropathy and, rarely, lactic acidosis. Although stavudine and other nucleoside analogs have also been associated with lipoatrophy, clear answers regarding any link between individual nucleosides, lipoatrophy, and potential mechanisms of action are beginning to emerge as randomized, controlled studies are performed.

Stavudine was the fourth nucleoside analog to become commercially available in the United States. More than 12,500 patients participated in the stavudine Parallel Track Program (Study AI455-900), a phase III multicenter, randomized trial comparing two doses of stavudine (20 and 40 mg, twice daily) in patients who were intolerant of or had failed previous therapy with zidovudine and didanosine *(5)*. Stavudine is indicated for use in combination with other antiretroviral agents for the treatment of HIV-1 infection *(6)*. The current US Department of Health and Human Services (DHHS) guidelines recommend that stavudine may be used as an alternative two-NRTI backbone, in combination with lamivudine or emtricitabine, as part of an initial combination therapy regimen *(7)*.

Stavudine Dosage Administration and Adjustment

Stavudine is available as 15-, 20-, 30-, and 40-mg capsules or as a dye-free, fruit-flavored powder that provides 1 mg/mL of stavudine after reconstitution with water. For adults, stavudine is administered at a dosage of approx 1 mg/kg/d divided into two doses. The recommended dose is 40 mg twice daily for patients weighing ±60 kg (132 lb) and 30 mg twice daily for patients weighing less than 60 kg. For pediatric patients, the recommended dose for those weighing less than 30 kg is 1 mg/kg per dose administered every 12 h. Pediatric patients weighing at least 30 kg (66 lb) should receive the recommended adult dosage based on their weight.

With appropriate dosage adjustment, stavudine may be administered to patients with renal impairment, those undergoing hemodialysis (Table 1), as well as those patients who develop peripheral neuropathy (*see* "Stavudine Tolerability and Management of Adverse Events" section for details).

Stavudine is absorbed rapidly and achieves peak plasma concentrations (C_{max}) 1 h after oral administration *(8)*. The drug is acid-stable and has an oral bioavailability of 86.4 ± 18.2% *(9)*. The bioavailability of stavudine is unaffected by food and stavudine can be administered without regard to meals *(10)*. Stavudine does not accumulate appreciably in plasma after extended dosing *(6)*. Approximately 40% of an administered dose of stavudine is excreted unchanged in the urine *(9)*. The remaining proportion is thought to be metabolized, although the route of elimination is unclear *(11)*. The elimination half-life of stavudine after an oral dose is 1.44 h *(6)*.

Table 1
Recommended Dosage Adjustment for Stavudine in Patients With Renal Impairment *(6)*

Creatinine clearance (mL/min)	Recommended dosage by patient weight	
	≥60 kg	<60 kg
>50	40 mg every 12 h	30 mg every 12 h
26–50	20 mg every 12 h	15 mg every 12 h
10–25	20 mg every 24 h	15 mg every 24 h
Hemodialysis	20 mg every 24 h[a]	15 mg every 24 h[a]

[a]Administered after completion of dialysis and at the same time on nondialysis days

Stavudine Drug Interactions

Stavudine, like zidovudine, undergoes intracellular triphosphorylation to compete with naturally occurring deoxythymidine triphosphate. Because both drugs are thymidine analogues, they use the same intracellular thymidine kinases, but zidovudine is preferentially phosphorylated over stavudine *(12,13)*. As a result, stavudine is not fully phosphorylated in the presence of zidovudine.

The clinical significance of the stavudine–zidovudine interaction has been demonstrated in clinical trials that showed inferior antiviral efficacy with the stavudine and zidovudine combination vs other antiretroviral regimens *(14)*. Havlir et al. further demonstrated that intracellular concentrations of stavudine triphosphate were sixfold lower in patients receiving stavudine plus zidovudine vs stavudine alone. Therefore, the concurrent use of zidovudine and stavudine is not recommended in clinical practice.

Regarding other antiretroviral agents, drug interaction studies show that there are no clinically significant interactions between stavudine and didanosine *(15)*, lamivudine, or nelfinavir *(6,16,17)*. Further, an in vitro study suggested that the PIs, indinavir, ritonavir, and saquinavir, have no effect on the intracellular phosphorylation of NRTIs and that intracellular interactions in vivo are unlikely *(18)*. Interactions with NNRTIs, such as efavirenz or nevirapine, would not be expected because the agents are metabolized via different routes *(6)*.

Because stavudine does not inhibit the major cytochrome P450 isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) and is not protein bound, clinically significant drug interactions are not expected with drugs metabolized through these pathways or with drugs that are protein bound. The area under the plasma concentration vs time curve (AUC) of stavudine was not significantly altered by concomitant fluconazole with or without rifabutin and/or clarithromycin administration, suggesting that interaction between stavudine and these anti-infective agents is unlikely *(19)*. However, methadone decreased the AUC of stavudine by approximately one-fourth and decreased

peak drug concentrations by 44% *(20)*. The clinical implications of this interaction are presently unknown.

Resistance to Stavudine

HIV isolates with a reduced susceptibility to stavudine have been selected in vitro *(21)* and also have been obtained from patients treated with stavudine *(22–24)*. Phenotypic analysis of 61 HIV isolates from patients receiving prolonged stavudine monotherapy showed a moderate decrease (mean twofold change) in stavudine sensitivity after 6 to 29 mo of treatment *(22)*. Posttreatment isolates from 15 of the 61 patients exhibited a greater than fourfold reduction in sensitivity vs pretreatment isolates, a change that was deemed significant based on the variability of the assay *(22)*. However, to date, high-level, stavudine-specific resistance in clinical isolates has not been observed *(22)*.

Changes in stavudine sensitivity are usually associated with the presence of multiresistant phenotypes *(22)*. Several studies have shown that prolonged stavudine treatment can select and/or maintain mutations associated with zidovudine resistance *(23,25)*. Isolates containing these mutations remain sensitive to stavudine *(26)*, although the clinical relevance of these findings is unknown *(27)* and complicated by the use of differing definitions of clinical or biological cutoffs.

Stavudine Clinical Studies

There are now extensive data available on the efficacy of stavudine in the treatment of patients with HIV infection. Although the initial regulatory trials of stavudine investigated its use as monotherapy, the primary focus of this section concerns its use as part of triple-therapy regimens in line with current treatment guidelines *(7)*. If possible, ITT data, the most conservative assessment of drug efficacy, are presented for each of the trials reviewed.

Stavudine Monotherapy

The efficacy of stavudine monotherapy was confirmed in a large, randomized, double-blind, multicenter comparison with zidovudine conducted in 1992 to 1994 (AI455-019), which showed similar outcomes in terms of disease progression and mortality in both treatment groups *(28)*. A total of 822 HIV-infected adult patients were recruited to the trial. Although the risk of death was 26% lower with stavudine, the difference between treatment groups was not statistically significant ($p = 0.066$) *(28)*.

Stavudine-Containing Triple-Therapy Regimens

Stavudine in combination with another NRTI and a PI (with or without low-dose ritonavir) *(2,3,29–31)* or another NRTI and a NNRTI *(4,31,32)* reduced plasma HIV RNA levels to fewer than 500 copies/mL in 53 to 100% and to fewer than 50 copies/mL in 41 to 100% of antiretroviral-naive patients after 48 to 52 wk (Table 2).

Table 2
Summary of Selected Randomized, Multicenter, Long-Term (i.e., 48 to 52 Wk) Trials Investigating the Efficacy of Triple Stavudine-Containing Regimens in the Treatment of Antiretroviral-Naive Patients With HIV Infection[a]

			Mean baseline values		Median or mean change from baseline [wk]		Patients with plasma HIV RNA levels (%) [wk]	
Study	No. of patients	Regimen	CD4 count ($\times 10^6$ cells/L)	vRNA ($\log_{10}$ copies/mL)	CD4 count ($\times 10^6$ cells/L)	vRNA ($\log_{10}$ copies/mL)	<500 copies/mL	≤50 copies/mL
OzCombo	34	d4T + 3TC + IDV	313	5.21	237 [52]	–3.28 [52]	NR	59% [52]
(29)	37	d4T + ddI + IDV	277	5.00	176 [52]	–2.68 [52]	NR	48% [52]
	35	ZDV + 3TC + IDV	267	5.01	175 [52]	–2.72 [52]	NR	66% [52]
OzCombo	22	d4T + 3TC + NVP	398	4.62	201 [52]	–1.10 [52]	68% [52]	68% [52]
2 *(32)*	23	d4T + ddI + NVP	357	4.74	190 [52]	–1.97 [52]	87% [52]	805 [52]
	20	ZDV + 3TC + NVP	448	4.52	172 [52]	–1.64 [52]	70% [52]	73% [52]
Murphy	16	LOP + RTV + d4T + 3TC[b]	471	4.88	NR	NR	100%[f] [48]	100% [48]
et al. *(30)*	16	LOP + RTV + d4T + 3TC[c]	330	4.96	NR	NR	81%[f] [48]	50% [48]
	35	LOP + RTV + d4T + 3TC[d]	343	4.78	NR	NR	91%[f] [48]	86% [48]
	33	LOP + RTV + d4T + 3TC[e]	275	4.97	NR	NR	73%[f] [48]	73% [48]
Start I *(2)*	101	d4T + 3TC + IDV	424	4.57	227 [48]	–2.89 [48]	62% [40–48]	49% [48]
	103	ZDV + 3TC + IDV	422	4.46	198 [48]	–2.60 [48]	54% [40–48]	47% [48]

Start II *(3)*	102	d4T + ddI + IDV	NR	NR	214 [48]	–2.50 [48]	53% [40–48]	41% [48]
	103	ZDV + 3TC + IDV	NR	NR	142 [48]	–2.60 [48]	41% [40–48]	35% [48]
ACTG 384	155	d4T + ddI + EFV	273	5.0	NR	NR	ZDV/3TC/EFV significantly	
(31)	155	d4T + ddI + NFV	264	5.0	NR	NR	delayed time to first virological	
	155	ZDV + 3TC + EFV	272	4.9	NR	NR	failure (HR = 0.39) and failure	
	155	ZDV + 3TC + NFV	307	4.9	NR	NR	of first regimen (HR = 0.35)	
							compared with d4T plus ddI	
FTC 301A	232	FTC + ddI + EFV	312	4.8	168 [48]		81%[f,g] [48]	78% [48][g]
(33)	193	d4T + ddI + EFV	324	4.8	134 [48]		68%[f,g] [48]	59% [48][g]
Gilead 903	299	TDF + 3TC + EFV	276	4.9	263 [144]	–3.1 [48 & 144]	80%[f] [48]	76% [48]
(4)	301	d4T + 3TC + EFV	283	4.9	283 [144]	–3.1 [48 & 144]	84%[f] [48]	80% [48]

3TC, lamivudine; d4T, stavudine; ddI, didanosine; IDV, indinavir; LOP, lopinavir; NVP, nevirapine; NR, not reported; RTV, ritonavir; vRNA, plasma HIV RNA; ZDV, zidovudine; FTC, emtricitabin

[a]In all trials, the dosages of antiretrovirals used were in accordance with manufacturers' recommendations, with the exception of the trial by Murphy et al. *(30)*, which was a dose-finding study

[b]200 mg LOP plus 100 mg RTV every 12 h

[c]400 mg LOP plus 100 mg RTV every 12 h

[d]400 mg LOP plus 100 mg RTV every 12 h

[e]400 mg LOP plus 200 mg RTV every 12 h

[f]Fewer than 400 copies/mL

[g]$p < 0.001$ for comparison between arms

In the two trials that permitted a direct comparison between stavudine- and zidovudine-containing regimens *(2,29)*, similar reductions in viral load were observed. However, the stavudine-containing regimen was associated with a significantly greater increase in median time-weighted CD4 cell count at both 24 and 48 wk in one trial *(2)*. Of interest also are the results of Murphy et al., who reported favorable results compared with those achieved in other trials with a ritonavir-boosted regimen of lopinavir in combination with stavudine plus lamivudine *(30)*.

Gilead 903, a prospective, randomized, double-blind study in 753 antiretroviral-naive patients, compared stavudine with tenofovir, both in combination with lamivudine and efavirenz *(4)*. The primary analysis at 48 wk evaluated the proportion of patients with viral suppression to fewer than 400 copies/mL in an ITT M=F. In this analysis, the stavudine arm was marginally superior to the tenofovir arm (84% vs 80%, confidence interval, –10.4 to 1.5%). However, using a cutoff of 50 copies/mL, the two regimens were equivalent at week 48 and through week 144.

In two recent, large multicenter trials, the combination of stavudine plus didanosine as a two-NRTI backbone with efavirenz or nelfinavir was associated with comparatively less efficacy than comparator arms. The AIDS Clinical Trials Group (ACTG) 384 study compared sequential three- and four-drug regimens for initial therapy for HIV infection in 620 therapy-naive individuals *(31)*. The time-to-first virological failure was significantly delayed with the combination of zidovudine plus lamivudine plus efavirenz, compared with three other regimens of stavudine plus didanosine plus efavirenz, stavudine plus didanosine plus nelfinavir, or zidovudine plus lamivudine plus nelfinavir (hazard ratio, 0.34). A second trial, FTC-301A, compared stavudine plus didanosine plus efavirenz with emtricitabine plus didanosine plus efavirenz in 571 antiretroviral-naive adults *(33)*. At the 24-wk interim analysis, the data safety monitoring board recommended offering open-label emtricitabine to stavudine recipients, based on a higher probability of a persistent virological response to fewer than 50 copies/mL in the emtricitabine group vs the stavudine group (85% vs 76%, $p = 0.005$).

Stavudine Studies in Children

Current treatment guidelines for the use of antiretroviral regimens in children recommend the use of a PI in combination with two NRTIs *(34)*. Although data with stavudine remain limited, several small studies have shown the efficacy of stavudine-containing triple regimens in both antiretroviral-naive *(35)* and antiretroviral-experienced populations of children *(36–41)*. In the latter patient group, average viral load was reduced by at least 2 $\log_{10}$ copies/mL from baseline after 4 to 24 mo *(36)*. A recent trial also showed a

satisfactory viral outcome after 1 yr of dual therapy with stavudine and didanosine, which was associated with better adherence than is often observed with triple therapy *(42)*.

Stavudine Tolerability and Management of Adverse Events

Stavudine Mitochondrial Toxicity

The tolerability profile of all NRTIs is influenced, to some degree, by the occurrence of mitochondrial toxicity. This is a result of inhibition of human DNA polymerase-γ, which is involved in the replication of mitochondrial DNA (mtDNA). Adverse events thought to occur because of mitochondrial toxicity are myopathy, neuropathy, cardiomyopathy, lactic acidosis, and pancreatic and/or hepatic failure, although the range of events associated with individual agents differs *(43)*.

Peripheral Neuropathy With Stavudine

For stavudine, the major dose-limiting toxicity is peripheral sensory neuropathy. The event is dose related and symptoms include pain, tingling, or numbness of the hands and feet, distal sensory loss, and mild muscle weakness. Stavudine-related peripheral neuropathy is observed more frequently in patients with advanced HIV, a history of neuropathy, low baseline CD4 counts, or hemoglobin levels of less than 110 g/L *(28,44)*.

In early controlled clinical trials, the incidence of peripheral neuropathy associated with 80 mg/d stavudine ranged from 8 to 21% *(2,44)*. Patients undergoing treatment with stavudine should be monitored for the development of neuropathy. If detected, stavudine-related peripheral neuropathy may resolve if therapy is withdrawn promptly. If symptoms resolve completely, patients may tolerate resumption of treatment at one-half the dose (i.e., 20 or 15 mg stavudine twice daily for patients weighing at least 60 kg or less than 60 kg, respectively). If peripheral neuropathy recurs, permanent discontinuation of stavudine should be considered *(6)*.

Lactic Acidosis With Stavudine

A rare and potentially fatal syndrome of lactic acidosis and severe hepatomegaly with steatosis can occur with nucleoside analogs, including stavudine. Some longitudinal and retrospective studies suggest that the incidence of lactic acidosis may be more commonly associated with antiretroviral combinations containing stavudine *(6)*. In the Gilead 903 randomized comparison of stavudine vs tenofovir, in combination with lamivudine and efavirenz, investigator-defined lactic acidosis occurred in three patients, all of whom were in the stavudine group *(4)*.

Lactic acidosis occurs infrequently at an estimated rate of 1.2 to 3.9 events per 1000 person years with all nucleotide and nucleoside analogs *(45)*. Notably,

fatal lactic acidosis has been reported in pregnant women who received the combination of stavudine and didanosine with other antiretroviral agents *(46)*; therefore, combined stavudine and didanosine should be used with caution during pregnancy.

Early recognition of the signs and symptoms of lactic acidosis is important. These include generalized fatigue, digestive symptoms (nausea, vomiting, abdominal pain, and sudden weight loss), respiratory symptoms (tachypnea and dyspnea), and neurological symptoms (motor weakness). However, prospective monitoring of lactate levels is not recommended at this time *(6)*. If symptoms suggestive of lactic acidosis are identified, antiretroviral treatment should be suspended promptly and a full medical work-up performed.

Lipodystrophy With Stavudine

Lipodystrophy (or fat redistribution syndrome) is a complication of prolonged antiretroviral therapy. It is characterized by the wasting of peripheral fat of the distal extremities, buttocks, and face (lipoatrophy), and central fat accumulation, notably in the dorsocervical region, breasts, and abdomen (lipohypertrophy). It can also be accompanied by metabolic abnormalities, such as hypertriglyceridemia, hypercholesterolemia, insulin resistance, and type 2 diabetes mellitus. It is unclear whether lipodystrophy represents a single syndrome or several related syndromes *(47)*.

Despite the clear temporal association between the use of antiretroviral therapy and the onset of lipodystrophy, researchers have yet to clearly differentiate between changes in lipid metabolism and distribution that can be attributed to individual pharmacological agents and changes that are a result of the natural course of HIV infection. A substudy of the large HIV Outpatient Study found that HIV-associated lipodystrophy was associated with several host, disease, and drug-related factors *(48)*. They observed that the prevalence of lipoatrophy increased substantially with the number of nondrug risk factors, suggesting that lipoatrophy is related to the effective control of HIV infection or drug use in patients with more advanced disease *(48)*. Importantly, lipoatrophy did not occur with stavudine unless other nondrug risk factors were present *(48)*. From the results of their analyses, Lichtenstein et al. suggested that lipodystrophy was more than a drug-related adverse event and more than one syndrome.

The relationship between antiretroviral agents and the development of lipodystrophy remains controversial and continues to be investigated. Although several observational studies have reported an increased risk of lipodystrophy with stavudine vs zidovudine *(49)*, this has not been a consistent finding *(50,51)*. Further, another study found no correlation between the use of stavudine and lipodystrophy *(52)*.

A clearer association between stavudine and lipodystrophy was suggested by the results of the Gilead 903 study, comparing stavudine plus lamivudine plus efavirenz to tenofovir plus lamivudine plus efavirenz in antiretroviral-naive patients. The tenofovir arm demonstrated more favorable changes in triglyceride ($p < 0.001$), total cholesterol ($p < 0.001$), low-density lipoprotein ($p < 0.001$) and high-density lipoprotein ($p = 0.003$) levels compared with the stavudine arm, and a lower frequency of investigator-reported lipodystrophy (3% vs 19%, respectively, $p < 0.001$) *(4)*. In a substudy of 262 patients, whole-body dual-energy X-ray absorptiometry scans showed significantly more limb fat in the tenofovir arm than the stavudine arm at 96 wk (7.9 kg vs 5.0 kg, respectively, $p < 0.001$). Both arms experienced an increase in weight from baseline through week 144, which was more pronounced in the group receiving tenofovir (2.9 kg) than stavudine (0.6 kg) ($p = 0.001$) *(4)*. Although the incidence of nucleoside-associated toxicities in tenofovir subjects was 3% compared with 10% in the stavudine arm ($p < 0.001$), a 48-wk substudy of 277 subjects demonstrated a median increase from baseline in mtDNA copies/cell in both groups (82 mtDNA copies/cell in the tenofovir arm vs 18 mtDNA copies/cell in the stavudine arm [$p = 0.001$]) *(53)*.

Other suggestive evidence for the association between stavudine use and lipoatrophy was seen in the results of the TARHEEL study *(54)*. This 48-wk, open-label study in 118 virologically suppressed patients with lipoatrophy evaluated changes in lipoatrophy after a switch from stavudine to either abacavir (86 patients) or zidovudine (32 patients). At 48 wk, full-body dual-energy X-ray absorptiometry scans showed a median increase in arm fat of 35%, in leg fat of 12%, and in trunk fat of 18% compared with baseline *(54)*. In a subset of 16 patients, mtDNA content in skeletal muscle, adipose tissue, and peripheral blood mononuclear cells were measured at study entry and week 48. MtDNA levels were low at baseline, but rebounded by week 48 in skeletal muscle (mean 141% increase), adipose tissue (mean 146% increase), and peripheral blood mononuclear cells (369% increase) *(55)*. Improvements in quantitative adipocyte apoptosis and in the function of seven mitochondrial enzymes were also seen by week 48 *(55)*.

Although a clear mechanism for the pathogenesis of PI-related lipodystrophy has been suggested *(56)*, less is known about the pathogenesis of NRTI-related events. Recently, it has been proposed that, similar to several other adverse events related to NRTIs, lipodystrophy may be a function of mitochondrial toxicity *(57,58)*. At present, there are only limited observations in support of this theory. Those observations include a strong association between the onset of lipodystrophy and the time of exposure to NRTIs, and the fact that lipodystrophy has been observed in PI-naive patients *(57)*. However, several lines of evidence suggest that mitochondrial toxicity is not the cause of lipodystrophy in patients receiving NRTIs *(59)*.

Table 3
Clinical Adverse Events[a] of Moderate or Severe Intensity in at Least 1% of Patients *(6)*[b]

Adverse events	Stavudine + lamivudine + efavirenz $n = 467$ (% of patients)[c]
Body as a whole	
Headache	2%
Fatigue	1%
Digestive system	
Diarrhea	2%
Nausea	2%
Vomiting	<1%
Dyspepsia	1%
Metabolic and nutritional system	
Lipodystrophy	3%
Nervous system	
Dizziness	5%
Abnormal dreams	2%
Insomnia	1%
PNS/neuropathy	6%
Somnolence	1%
Abnormal thinking	1%
Depression	1%
Skin and appendages	
Rash	5%
Pruritus	1%

PNS, peripheral neurological symptoms (includes neuropathy, paresthesia, and peripheral neuritis)
[a]Considered by the investigator to be of possible, probable, or unknown relationship to any component of the drug regimen
[b]Data pooled from stavudine combination studies
[c]Patients received 40 mg stavudine twice daily each in combination with 150 mg lamivudine twice daily and 600 mg efavirenz once daily. Median duration of treatment was 56 wk

Other Adverse Events With Stavudine

Other adverse events reported in association with stavudine as part of a triple-therapy regimen with lamivudine and efavirenz are shown in Table 3. Laboratory abnormalities were infrequent, as shown in Table 4.

DIDANOSINE

Didanosine Overview

Didanosine (also known as ddI) is available in its original buffered formulations (Videx) and as a delayed-release formulation with enteric-coated beadlets

Table 4
Selected Laboratory and Hematological Abnormalities From Combination Studies of Stavudine (Pooled Data) *(6)*[a]

Parameter	Stavudine + lamivudine + efavirenz $n = 467$ (% of patients)[c]
AST (>5 × ULN)	3%
ALT (>5 × ULN)	3%
Lipase (≥2.1 × ULN)	3%
Total bilirubin (≥2.6 × ULN)	0%
Neutropenia (ANC <750/mm^3)	5%
Anemia (hemoglobin <8 g/dL)	<1%
Thrombocytopenia (platelets <50,000 cells/mm^3)	2%

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ULN, upper limit of normal; ANC, absolute neutrophil count

[a]Patients received 40 mg stavudine twice daily, in combination with 150 mg lamivudine twice daily and 600 mg efavirenz once daily. Median duration of treatment was 56 wk

of the drug (Videx EC). Because of its unique acid lability, didanosine must be protected from stomach acids either by the enteric coating of the delayed-release formulation, or by the antacid component of the buffered formulations. Additionally, didanosine should be taken on an empty stomach, 30 min before or 2 h after eating. Most drugs that interact with didanosine actually interact with the buffer component of the earlier formulations rather than with didanosine itself. Diarrhea, peripheral neuropathy, and nausea are the most commonly experienced side effects of concomitant use of didanosine with stavudine plus nelfinavir or lamivudine plus nelfinavir.

Data from studies of didanosine-containing triple-therapy regimens in HIV-infected, antiretroviral-naive patients show that didanosine with another NRTI and an NNRTI reduced plasma HIV RNA levels to less than 500 copies/mL in 68 to 98% of patients and to less than 50 copies/mL in 50 to 93% of patients after 6 to 15 mo of therapy (ITT M=F).

Adverse events experienced with didanosine-containing regimens include pancreatitis, peripheral neuropathy, retinal changes and optic neuritis, and gastrointestinal (GI) disturbances.

Didanosine is a member of the nucleoside class of HIV-1 NRTIs. When it was commercially introduced in 1991, didanosine (as Videx) was the second antiretroviral compound to be licensed. This followed a large, expanded access program in which more than 21,000 patients received didanosine either under a treatment Investigational New Drug protocol designed for patients who were intolerant to zidovudine, or through an open-label study designed for patients whose conditions were deteriorating clinically despite continued zidovudine

therapy. The trials opened for accrual in October 1989 and continued until the drug received Food and Drug Administration (FDA) approval in October 1991. Originally introduced for patients failing or intolerant of zidovudine, didanosine is now indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection *(60)*. The current US DHHS guidelines recommend that didanosine may be used as an alternative two-NRTI backbone in combination with lamivudine or emtricitabine, as part of an initial combination-therapy regimen *(7)*.

In vitro activity of didanosine against HIV-1 was evaluated in HIV-1-infected lymphoblastic cell lines and in monocyte/macrophage cell cultures. The didanosine concentration required to inhibit viral replication by 50% (IC_{50}) ranged from 2.5 to 10 μ*M* in lymphoblastic cell lines and 0.01 to 0.1 μ*M* in monocyte/macrophage cell cultures *(61)*. Didanosine has high in vitro antiviral selectivity. Its in vitro selectivity ratio (IC_{50}/ median effective concentration [EC_{50}]) was 2.5 times greater than that of zidovudine *(62)*.

Didanosine Clinical Pharmacology

Didanosine is commercially available in a number of formulations, including Videx (didanosine) EC Delayed-Release Capsules containing enteric-coated beadlets, Videx (didanosine) Chewable/Dispersible Buffered Tablets, Videx (didanosine) Buffered Powder for Oral Solution, and Videx (didanosine) Pediatric Powder for Oral Solution. This diverse group of formulations is necessitated by didanosine's unique acid lability. Unlike other nucleoside analogs, didanosine is highly acid labile and quickly undergoes hydrolysis in the GI system to hypoxanthine, an inactive form *(60)*. To prevent this rapid degradation, didanosine must be protected from the acidic environment of the stomach. This is accomplished in two ways.

With Videx EC, the active ingredient is sheltered from stomach acid degradation by enclosing didanosine within individual enteric-coated beadlets within the capsule. The enteric coating dissolves, releasing the didanosine, when the beadlets encounter the higher pH of the small intestine, where absorption of the drug occurs.

With buffered formulations of didanosine, administration with antacid raises gastric pH, providing protection from degradation by stomach acid. The antacid is either provided in the dosage form itself (buffered with calcium carbonate and magnesium hydroxide or dibasic sodium phosphate, sodium citrate, and citric acid), or, in the case of Videx Buffered Powder for Oral Solution, must be admixed by the pharmacist at the time the drug is dispensed. All dosage forms of didanosine are to be administered on an empty stomach, but the number of tablets/capsules, preferred dosing interval, and even the dose administered varies depending on the dosage form administered.

Table 5
Mean (±SD) Pharmacokinetic Parameters for Buffered Didanosine in Adult and Pediatric Patients *(60)*[a]

		Pediatric patients	
Parameter	Adult patients	8 mo to 19 yr	2 wk to 4 mo
Oral bioavailability (%)	42 ± 12	25 ± 20	ND
Apparent Vd (L/m^2)	43.70 ± 8.90	28 ± 15	ND
CSF:plasma ratio	21 ± 0.03%	46% (range, 12–85%)	ND
Systemic clearance (mL/min/m^2)	526 ± 64.7	516 ± 184	ND
Renal clearance (mL/min/m^2)	223 ± 85.0	240 ± 90	ND
Apparent oral clearance (mL/min/m^2)	1252 ± 154	2064 ± 736	1353 ± 759
Elimination half-life (h)	1.5 ± 0.4	0.8 ± 0.3	1.2 ± 0.321
Urinary recovery of didanosine (%)	18 ± 8	18 ± 10	ND

Vd, volume of distribution; CSF, cerebrospinal fluid; ND, not determined
[a]Pediatric patients (8-mo to 19-yr old) demonstrate a lower oral bioavailability (25%), smaller Vd (28 L/m^2), and shorter half-life (0.8 h) *(60,65)*

Didanosine Pharmacokinetics

Didanosine Buffered Formulations

Buffered didanosine, the first available formulation of didanosine, is rapidly absorbed, with C_{max} occurring 0.25 to 1.5 h after oral dosing. Increases in plasma didanosine concentrations have been shown to be dose proportional between 50 and 400 mg. Steady-state pharmacokinetic parameters do not differ significantly from values obtained after a single dose. Binding of didanosine to plasma proteins in vitro is low (<5%) *(60)*. It is thought that the metabolism of didanosine in humans occurs by the same pathways responsible for the elimination of endogenous purines, with 18% of an orally administered dose recoverable in the urine *(60)*.

Buffered didanosine is 42% bioavailable orally with an apparent volume of distribution (Vd) of 1.08 L/kg in adults *(60)*. Plasma half-life is approx 1.5 h (*see* Table 5). Despite its short plasma half-life, didanosine can be administered once daily in adults. The relatively long intracellular half-life (25 to 40 h) of its active moiety, dideoxyadenosine triphosphate, allows this type of dosing *(60,63)*. Didanosine C_{max} and AUC are decreased by approx 55% when buffered tablets are administered up to 2 h after a meal *(64)*. Administration of buffered tablets up to 30 min before a meal does not result in any significant changes in bioavailability. Buffered didanosine should be taken on an empty stomach, at least 30 min before or 2 h after eating *(60)*.

Didanosine Enteric-Coated Formulation

The intrinsic pharmacokinetic properties of didanosine (e.g., volume of distribution and half-life) are unchanged when using the enteric-coated beadlet formulation. In healthy volunteers, as well as subjects infected with HIV, the AUC is equivalent for didanosine administered as Videx EC relative to the buffered-tablet formulation. However, the C_{max} of didanosine, administered as enteric-coated beadlets, is reduced approx 40% relative to didanosine buffered tablets. Because the beadlets must move from the gastric environment of the stomach into the small intestine before absorption can occur, the time to the peak concentration increases from approx 0.67 h for buffered didanosine formulations to 2.33 h in healthy subjects and 2.0 h in HIV-infected subjects for the enteric-coated formulation *(66,67)*. The mean half-life of didanosine was similar between treatment groups, ranging from 1.6 to 1.7 h in healthy and infected subjects. As a result, patients experience equivalent didanosine exposure (AUC) with delayed and lower C_{max} *(60,66)*.

Effect of Food on Absorption of Didanosine

The administration of didanosine with food results in decreased exposure to the agent, regardless of dosage form. Didanosine C_{max} and AUC were decreased by approx 55% when didanosine tablets were administered up to 2 h after a meal. Administration of didanosine tablets up to 30 min before a meal did not result in any significant changes in bioavailability. All buffered didanosine formulations should be taken on an empty stomach, at least 30 min before or 2 h after eating.

In the presence of food, the C_{max} and AUC for Videx EC were reduced by approx 46% and 19%, respectively, compared with the fasting state *(67)*. Videx EC should be taken on an empty stomach *(60)*.

Didanosine Drug Interactions

Drug–drug interactions involving didanosine can be divided into two groups: those unique to the antacid buffer, and those caused by didanosine itself. This distinction is important, because interactions caused by the antacid buffer are not encountered when using Videx EC.

Buffer-Specific Interactions

Buffered didanosine can reduce the AUC and C_{max} of ciprofloxacin by 26% and 16%, respectively *(68,69)*. It also reduces the AUC and C_{max} of indinavir by 84% and 82%, respectively, when the two drugs are administered concurrently, or by 11% and 4%, respectively, when indinavir is administered 1 h before buffered didanosine *(70)*. Buffered didanosine also reduces the AUC and C_{max} of ketoconazole by 14% and 20%, respectively *(71)*. Because administration of the buffered tablets in the setting of chronic methadone maintenance reduces the AUC of didanosine by 41% and the C_{max} by 59%, increased

buffered didanosine doses may be needed *(20)*. Interactions such as these, which are dependent solely on the presence of the buffers used to protect didanosine from inactivation in the acid environment of the stomach, do not occur with Videx EC, the enteric-coated formulation of didanosine *(72)*.

Administration of buffered didanosine also affects delaviridine (decreases the AUC 32% if simultaneous, increases the AUC 20% if administered 1 h before didanosine), ganciclovir (decreases the AUC 21% when administered 2 h after didanosine), and nelfinavir (increases the AUC 12% when administered 1 h after didanosine) concentrations *(60)*. Any other drugs that list drug interactions with antacids would be expected to interact with all buffered didanosine formulations *(60)*.

Interactions Increasing Didanosine Exposure

TENOFOVIR DISOPROXIL FUMARATE

The coadminstration of tenofovir disoproxil fumarate and didanosine results in significantly elevated didanosine exposure without significant increases in tenofovir exposure. This interaction has been demonstrated with both buffered and enterically coated didanosine. The administration of 300 mg of tenofovir disoproxil fumarate 1 h after either 250 mg or 400 mg of didanosine buffered tablets resulted in a 44% increase in AUC for didanosine and a 28% increase in C_{max}. Likewise, when 400 mg of Videx EC was administered to healthy, fasting subjects 2 h before 300 mg of tenofovir disoproxil fumarate administered with a light meal (373 kcal, 8.2 g fat), both the AUC and C_{max} of didanosine increased by 48 percent. The values were somewhat higher when the two drugs were administered together with a light meal; the AUC for didanosine was increased by 60%, whereas the C_{max} increased by 64%. When 250 mg didanosine enteric-coated capsules were administered with tenofovir disoproxil fumarate, systemic exposures to didanosine were similar to those seen with the 400-mg enteric-coated capsules alone under fasted conditions *(73)*.

A mechanism whereby systemic exposure to didanosine is increased by coadministration of tenofovir has recently been proposed *(74)*. Didanosine is degraded by the enzyme, purine nucleoside phosphorylase (PNP), in the purine nucleoside salvage pathway. Tenofovir monophosphate and diphosphate, phosphorylated ganciclovir metabolites, and allopurinol all inhibit PNP in cell culture *(74)*. The resultant inhibition of didanosine phosphorolysis may explain the increase in didanosine exposure seen with coadministration of these drugs.

A dose reduction of buffered Videx tablets to 250 mg (adults weighing ≥60 kg with creatinine clearance ≥60 mL/min) or 200 mg (adults weighing <60 kg with creatinine clearance ≥60 mL/min) once daily is recommended. The appropriate dose of VIDEX coadministered with tenofovir in patients with creatinine clearance less than 60 mL/min has not been established *(60)*.

Buffered Videx tablets and tenofovir disoproxil fumarate may be taken together in the fasted state. Alternatively, if tenofovir is taken with food, buffered Videx tablets should be taken on an empty stomach (at least 30 min before food or 2 h after food) *(60)*. Because increased exposure may cause or worsen didanosine-related clinical toxicities, coadministration of tenofovir with Videx EC should be undertaken with caution, and patients should be monitored closely for didanosine-related toxicities.

ALLOPURINOL

Administration of allopurinol significantly increases didanosine exposure (AUC increases 113%, C_{max} increases 69%) *(75)*. This effect is further magnified in patients experiencing renal impairment (exposure AUC increases 312%, C_{max} increases 232%) *(60)*.

GANCICLOVIR

Ganciclovir increases the didanosine AUC (by 111%) but not C_{max} *(60,76)*.

RIBAVIRIN

An important interaction that takes place at the molecular level is the increased activation of didanosine (and abacavir) by ribavirin *(77)*. By inhibiting inosine monophosphate dehydrogenase, ribavirin produces an increase in inosine monophosphate, the molecule thought to be the phosphate donor. Thus, ribavirin promotes the activation of didanosine to its active triphosphate moiety, dideoxyadenosine 5′-triphosphate, which may increase the risk of didanosine toxicity *(78,79)*. In an analysis of adverse events reported to the US FDA, patients coinfected with HIV and hepatitis C who received a regimen of ribavirin and didanosine, with or without stavudine, were at increased risk for events associated with mitochondrial toxicity, including fatal hepatic failure, peripheral neuropathy, pancreatitis, and symptomatic hyperlactatemia/lactic acidosis *(80)*. Therefore, the coadministration of ribavirin and any didanosine dosage form (buffered or enterically coated) is not recommended.

Didanosine Interactions With Other Antiretroviral Agents

Most of the drug interactions traditionally seen with didanosine result from the presence of the buffer. These interactions are not seen with such severity with the enteric-coated formulation, Videx EC.

Didanosine Dosing

Didanosine dosing is based on the patient's weight. A daily dose of 400 mg is administered for adults weighing at least 60 kg (132 lb), whereas 250 mg is administered daily for those weighing less than 60 kg *(60)*.

Videx EC is administered once daily as a single capsule (125, 200, 250, or 400 mg). The capsules are swallowed whole on an empty stomach because the individual enteric-coated beadlets must remain intact until a higher pH is

Table 6
Recommended Dosage of Videx EC in Renal Impairment by Body Weight[a] *(60)*

	Dosage	
Creatinine clearance (mL/min)	≥60 kg	<60 kg
≥60	400 mg once daily	250 mg once daily
30–59	200 mg once daily	125 mg once daily
10–29	125 mg once daily	125 mg once daily
<10	125 mg once daily	—[b]

[a]Based on studies using a buffered formulation of didanosine
[b]Not suitable for use in patients weighing less than 60 kg with creatinine clearance less than 10 mL/min. An alternate formulation of didanosine should be used

encountered as the agent passes from the stomach. Dosing adjustments are required for patients with renal insufficiency and those receiving maintenance hemodialysis (*see* Table 6) *(60)*.

Chewable/dispersible buffered didanosine tablets contain adequate antacid buffer only when two or more tablets are administered. Each tablet size (25, 50, 100, 150, or 200 mg) contains only one-half of the amount of buffer needed to prevent the degradation of didanosine; therefore, each dose must consist of at least two tablets. To reduce the risk of GI side effects, patients should take no more than four tablets at each dose *(60)*.

Didanosine Clinical Studies

The course of HIV-1 infection has been changed by the introduction of multidrug highly active antiretroviral therapy (HAART) regimens. The strategy is built around the use of two NRTIs with either a PI or an NNRTI. Because even these regimens do not totally eliminate the HIV-1 virus, the therapies must be continued indefinitely. Because of the lifetime nature of this therapy, patient compliance (affected by number of pills taken and dosing frequency), drug interactions, and long-term adverse effects *(81)* become issues for successful therapy. Antiviral potency and adherence to the regimen by patients are crucial to the usefulness of HAART *(81)*.

Didanosine Plus Stavudine in Triple-Drug, Twice-Daily Regimens

As outlined in the toxicity sections for stavudine and didanosine, this combination has the potential for greater mitochondrial toxicity and related side effects, such as peripheral neuropathy, pancreatitis, and lactic acidosis, than comparator NRTI backbones. In addition, in three large randomized studies, treatment arms containing stavudine plus didanosine demonstrated lower virological efficacy than comparator arms *(31,33,60)*. Table 7 summarizes treatment arms containing stavudine plus didanosine as the nucleoside backbone, with comparative adverse event dropout rates and virological efficacy

Table 7
Stavudine Plus Didanosine Backbone in Twice-Daily Regimens[a]

Study name	Regimen	Sample size	AE dropout rate in d4T + ddI arm	AE dropout rate in NRTI comparator arm (regimen, sample size)	Difference in virological efficacy between NRTI backbones?
Start II *(3)*	d4T/ddI/IDV	102	16%	16% (AZT/3TC/IDV, 103)	No
Atlantic *(82)*	d4T/ddI/IDV	100	5%	N/A	N/A
	d4T/ddI/NVP	89	7%		
	d4T/ddI/3TC	109	6%		
AI424-007 *(83)*	d4T/ddI/ATV	103	6%	N/A	N/A
	d4T/ddI/NFV	103	7%		
AI454-148 *(60)*	d4T/ddI tablets/NFV	503	4%	2% (AZT/3TC/NFV, 327)	Yes, favors AZT/3TC
AI454-152 *(84)*	d4T/ddI EC/NFV	258	6%	7% (AZT/3TC/NFV, 253)	No
ACTG 384 *(31)*	d4T/ddI/EFV	155	13%[a]	7%[a] (AZT/3TC/EFV, 155)[b]	Yes, favors AZT/3TC
	d4T/ddI/NFV	155	12%[a]	2%[a] (AZT/3TC/NFV, 155)[b]	No
FTC-301A *(33)*	d4T/ddI/EFV	285	15% through wk 60	7% (FTC/ddI/EFV, 286) through week 60	Yes, favors FTC/ddI

d4T, stavudine; ddI, didanosine; IDV, indinavir; NVP, nevirapine; 3TC, lamivudine; ATV, atazanavir; NFV, nelfinavir; EFV, efavirenz; AZT, zidovudine

[a]Proportion of subjects with toxicity-related failure of first regimen

[b]Use of AZT/3TC as backbone with EFV or NFV delayed ocurrence of first serious toxic side effect ($p < 0.001$), delayed occurrence of first symptom or diagnosis of peripheral neuropathy ($p < 0.001$) compared with d4T/ddI

(3,31,33,60,82–84). Current US DHHS guidelines recommend against the use of a stavudine plus didanosine NRTI backbone, because of high incidences of peripheral neuropathy, pancreatitis, and lactic acidosis, except when no other antiretroviral options exist and the potential benefits outweigh the risks *(7)*.

Didanosine in Triple-Drug, Once-Daily Regimens

With the goal of simplifying regimens, particularly through the use of low pill burden, once-daily antiretrovirals, didanosine has been studied in triple-drug regimens in which all drugs are administered once a day. Candidate once-daily nucleoside/nucleotide analogs for use in combination with didanosine include lamivudine, emtricitabine, abacavir, and tenofovir, in combination with once-daily efavirenz or nevirapine. Four studies of didanosine in triple-drug once-daily regimens are discussed next and depicted in Table 8.

Lamivudine Plus Didanosine Plus Efavirenz Once Daily

Landman et al. *(85)* conducted a prospective, open-label study in which 40 HIV-1-positive patients who were naive to antiretroviral therapy were administered the combination of 200 or 400 mg didanosine (patients weighing <60 kg or >60 kg, respectively) plus 300 mg lamivudine plus 600 mg efavirenz at bedtime. The safety and efficacy of this regimen was studied for 15 mo. Ninety-five percent of patients reached plasma HIV-1 RNA levels of less than 500 copies/mL at 6 mo, and 78% of patients had plasma HIV-1 RNA levels of less than 50 copies/mL at that time. By the end of the study, 69% of patient HIV-1 RNA levels were less than 50 copies/mL. The median decrease of plasma HIV-1 RNA levels was greater than 3.4 $\log_{10}$ copies/mL at 15 mo. Patient compliance was high, and there were no treatment-limiting toxicities, although six patients had efavirenz-related central nervous system symptoms at the beginning of the study, and patients experienced some adverse events expected of didanosine, including three episodes of diarrhea at week 2, two episodes of epigastralgia, and six peripheral grade 1 neuropathies *(85)*.

In another 48-wk open-label trial, Maggiolo et al. *(86)* studied the safety and efficacy of the combination of 300 mg didanosine plus 300 mg lamivudine plus 600 mg efavirenz administered once daily (usually at bedtime) to 75 antiretroviral-naive, HIV-infected patients. The ITT analysis showed that 77% of patients had plasma HIV-RNA levels less than 50 copies/mL at week 48. The median decrease of plasma HIV-RNA levels was greater than 3.4 $\log_{10}$ copies/mL at 48 wk. Most patients reported fewer than one missed dose during the study. Seven patients withdrew because of adverse events—two from rashes, and one each from dizziness, hallucinations, gastric discomfort, gastric intolerance to didanosine, and increased alanine aminotransferase and aspartate aminotransferase *(86)*.

Table 8
Didanosine Plus Other Nucleosides in Backbone of Once-Daily Regimens

	Regimen	Study design	Sample size	Virological outcome	Median CD4 rise	Discontinuation because of AE (%)
Landman, 2002 *(85)*	3TC/ddI/EFV	Antiretroviral naive, prospective open label	40	78% VL <50 at 6 mo	Month 6: 142 cell/μL	0 of 40 (0%)
Maggiolo, 2001 *(86)*	3TC/ddI/EFV	Antiretroviral naive, prospective open label	75	77% VL <50 at week 48 (ITT analysis)	Mean baseline: 251 cell/μL Mean week 48: 459 cells/μL	7 of 75 (9.3%); rashes (2%); dizziness (1%); hallucinations (1%); GI side effects (2%); increased transaminases (1%)
ALIZE/ANRS 099, 2005 *(87)*	FTC/ddI/EFV	Antireroviral naive, prospective open label	40	93% VL<50 at week 24	Week 24: 159 cells/μL	1 of 40 (2.5%)
FTC 301A *(33)*	FTC/ddI/EFV	Antiretroviral naive	286	85% VL < 50 at week 24	Week 24: 156 cells/μL	7%[a] increased AST (2%), increased ALT (2%), increased amylase level (<1%) and neuropathy (<1%)

3TC, lamivudine; ddI, didanosine; EFV, efavirenz; VL, viral load; FTC, emtricitabin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; VL, viral load; GI, gastrointestinal

[a]Probability of developing a treatment-limiting adverse event through week 60

Emtricitabine Plus Didanosine Plus Efavirenz Once Daily

Molina et al. performed a 24-wk prospective, open-label study of 40 adults with HIV-1 infection who had no previous antiretroviral therapy (ALIZE/ANRS 099) *(87)*. The study objective was to assess safety and antiviral and immunological effects of 200 mg emtricitabine plus 400 or 250 mg didanosine (patients weighing ≥60 kg or <60 kg, respectively) plus 600 mg efavirenz in combination as a once-daily regimen administered at bedtime. Plasma HIV-1 RNA levels declined with a median decrease at the end of the study of 3.5 $\log_{10}$ copies/mL. At 12 wk, 39 of 40 (98%) patients had plasma HIV-1 RNA levels less than 400 copies/mL. This proportion was sustained at 24 wk. The percentage of patients with HIV-1 RNA levels less than 50 copies/mL increased over time to 37 of 40 patients (93%) at week 24. The median CD4 cell count increase at the end of the study period was 159 cells/μL. The regimen was well-tolerated; only one patient discontinued the study because of adverse events. Most adverse events were mild to moderate and included sleep disturbances, dizziness, asthenia, headaches, maculopapular rash, diarrhea, and abdominal pain. Six patients experienced serious adverse events, but only two of them (both cases of hypertriglyceridemia) were possibly related to study medication. The two patients who failed to achieve virological success were patients who discontinued therapy for more than 28 study days. The once-daily combination regimen of emtricitabine plus didanosine plus efavirenz seems to be a safe and effective alternative to PI-containing regimens *(81)*.

FTC-301A was a randomized, double-blind, placebo-controlled comparison of twice-daily stavudine vs once-daily emtricitabine, both in combination with open-label, once-daily didanosine plus efavirenz *(33)*. The study enrolled 571 antiretroviral-naive subjects with plasma HIV-1 viral loads of at least 5000 copies/mL. When all subjects had completed 24 wk in the study, an interim analysis was conducted (the median follow-up was 42 wk). In this analysis, subjects in the emtricitabine-treatment arm had a significantly greater probability of a sustained virological response (85%) than subjects in the stavudine group (76%) ($p = 0.005$). The emtricitabine group also had a higher mean CD4 cell count change from baseline of 156 cells/μL, compared with 119 cells/μL in the stavudine group. Based on this analysis, the Data Safety Monitoring Board recommended offering open-label emtricitabine to all subjects. The analysis also revealed a higher probability of an adverse event leading to study discontinuation in the stavudine group (15%) than in the emtricitabine group (7%) ($p = 0.005$) *(33)*.

Didanosine in Triple-Nucleoside/Nucleotide Regimens

In 2003 and 2004, several reports indicated that regimens containing triple NRTIs or two NRTIs plus tenofovir demonstrated early virological failure and early emergence of NRTI-associated resistance mutations, including studies of

zidovudine plus abacavir plus lamivudine *(88)*, abacavir plus didanosine plus stavudine *(89)*, abacavir plus lamivudine plus tenofovir *(90,91)*, and lamivudine plus didanosine plus tenofovir *(92)*. Details of those studies using didanosine are discussed next.

Gerstoft et al. randomized 180 antiretroviral-naive patients to abacavir plus stavudine plus didanosine (A/S/D, each at standard doses), 400 mg ritonavir plus 400 mg saquinavir (both twice daily) or 200 mg nevirapine plus 1250 mg nelfinavir (both twice daily). Median baseline CD4 count was 161 and median baseline viral load was 5.0 $\log_{10}$ copies/mL. At 48 wk of therapy, the proportion of patients achieving viral load suppression to fewer than 20 copies/mL in the 3 arms was 43% (A/S/D), 62% (ritonavir plus saquinavir) and 69% (nevirapine plus nelfinavir). Poorer virological outcome in the A/S/D arm was seen in the subgroup of patients with AIDS at baseline, those with baseline CD4 count fewer than or equal to 50 cells/μL, and those whose baseline viral load was greater than 20,000 copies/mL. Sixty-three percent of patients receiving A/S/D had to change at least one drug during the study; peripheral neuropathy was diagnosed in 27% and abacavir hypersensitivity reaction in 12% *(89)*.

Jemsek et al. treated 24 patients with the once-daily combination of 250 mg didanosine (Videx EC), 300 mg lamivudine, and 300 mg tenofovir *(92)*. All patients had an entry viral load of greater than 20,000 copies/mL; the mean viral load was approx 80,000 copies/mL, and the mean entry CD4 count was 133, with a range of 4 to 475. At week 12, 20% of patients demonstrated a rise in viral load and only 25% had a decline in viral load of 1 $\log_{10}$ copies/mL or greater. By week 24, the mean decline in viral load was 0.49 $\log_{10}$ copies/mL, and no patient achieved a viral load reduction to fewer than 50 copies/mL. Twenty-two subjects (92%) had virological failure, defined as less than a 2 $\log_{10}$ copies/mL decline in viral load by week 24. Twenty subjects had genotyping and phenotyping of resistant virus; all contained M184V, and 10 samples contained the K65R mutation. Nineteen samples were lamivudine (3TC) resistant, six were resistant to didanosine, and three were resistant to abacavir. No virus resistant to tenofovir, zidovudine, or stavudine were detected *(92)*.

Didanosine Plus Tenofovir in Once-Daily Triple-Combination Regimens With NNRTIs

Additional studies of two NRTI plus NNRTI regimens have been evaluated, in which all agents were administered once daily. Didanosine was included in many of these studies in combination with tenofovir and either efavirenz or nevirapine.

Preliminary data have been published or presented by two groups in Spain. One group used didanosine plus tenofovir with either efavirenz or nevirapine in nonrandomized switch/simplification studies in patients who were virologically suppressed on a twice-daily regimen. In the tenofovir plus didanosine plus efavirenz study reported by Barrios et al., only 64% of 127 once-daily regimen

patients maintained a viral load of fewer than 50 copies/mL at 6 mo, compared with 91% of those continuing a twice-daily regimen *(93)*. In another report from this group, 85 patients switched from their current twice-daily regimen to once-daily didanosine plus tenofovir plus nevirapine and were followed concurrently with 84 patients who continued on their twice-daily regimen. In an ITT analysis at 48 wk, 76% of the once-daily regimen patients were virologically suppressed to fewer than 50 copies/mL, compared with 86% of the twice-daily regimen patients. Of note, the authors demonstrated a mean 95 cell/μL decline in the once-daily regimen group by week 48 *(94)*. In an further analysis, the authors analyzed a total of 302 patients who maintained virological suppression while receiving didanosine plus tenofovir alone or in combination with nevirapine, efavirenz, or lopinavir/ritonavir; groups of patients who received either didanosine or tenofovir but not both served as controls. They noted that only those patients receiving a standard dose of didanosine plus tenofovir, and in this group, more than 50% of patients demonstrated a decline in CD4 count of more than 100 cell/μL." Plasma levels of didanosine were elevated in all patients in this group, and CD4 counts improved after didanosine dose reduction *(95)*. A subsequent larger analysis of 570 patients confirmed these results and identified that CD4 cell declines were more common in patients who simplified to a didanosine plus tenofovir regimen, particularly to an NRTI-only regimen, and were more common in patients on full-dose didanosine *(96)*.

Preliminary evidence of a similar suboptimal response to didanosine plus tenofovir plus NNRTI as an initial antiretroviral regimen have been recently been reported. Based on small numbers of patients in each report, it seems that patients with early virological failure on these regimens were more likely to have a low baseline CD4 count (<200 cells/mm^3) and a high baseline viral load (>100,000 copies/mL) *(97–99)*. In response to these data, the DHHS guidelines issued an update on July 15, 2005, recommending that regimens of didanosine plus tenofovir plus NNRTI *not* be used as an initial regimen in treatment-naive patients. Insufficient data were available to recommend for or against the use of didanosine plus tenofovir plus PI in treatment naive patients *(7)*.

One hypothesis to explain the high rate of virological failure in patients receiving tenofovir plus another purine nucleoside analog (didanosine or abacavir) and the decline in CD4 count in patients receiving didanosine plus tenofovir was put forward by Kakuda et al. *(100)*. In the presence of didanosine, tenofovir anabolites potently inhibit the action of PNP, an enzyme involved in didanosine catabolism *(74)*. The authors propose two effects of this PNP inhibition. First, intracellular levels of deoxyadenosine triphosphate and deoxyguanine triphosphate would increase and out-compete the active triphosphate anabolites of abacavir, didanosine, and tenofovir for binding to the HIV reverse transcriptase, leading to a decrease in virological inhibition. Second, the build-up of deoxyadenosine triphosphate and deoxyguanine triphosphate would lead

Table 9
Selected Clinical Adverse Events, Study AI454-152 *(60)*[a]

Adverse events	Percent of patients[b]	
	Videx EC+ stavudine + nelfinavir $N = 258$, n (%)	Zidovudine/lamivudine[c] + nelfinavir $N = 253$, n (%)
Diarrhea	57 (22.1)	58 (22.9)
Peripheral neurological symptoms/neuropathy	25 (9.7)	11 (4.3)
Nausea	24 (9.3)	36 (14.2)
Headache	22 (8.5)	17 (6.7)
Rash	14 (5.4)	12 (4.7)
Vomiting	14 (5.4)	19 (7.5)
Pancreatitis	<1 (<0.4)	—[d]

[a]Median duration of treatment was 62 wk in the Videx EC plus stavudine plus nelfinavir group and 61 wk in the zidovudine plus lamivudine plus nelfinavir group
[b]Percentages based on treated patients
[c]Zidovudine/lamivudine combination tablet
[d]This event was not observed in this study arm

to direct lymphocyte toxicity, analogous to congenital PNP deficiency, which accounts for some cases of cellular immunodeficiency *(100)*. Additional studies of intracellular nucleotide pools in relation to combination therapy with purine NRTIs are needed to confirm this hypothesis.

Studies Comparing Buffered and EC Formulations

GI side effects have been associated with the buffer used in the earlier didanosine preparations, but are encountered much less in patients using the Videx EC preparation. Kunches et al. performed a study in 42 HIV-infected men and women who had taken didanosine tablets either once or twice daily for at least 4 wk. Patients were switched to the Videx EC formulation of didanosine for 4 wk of treatment. Patients experienced significant declines in severity of diarrhea, gas, bloating, GI upset, and nausea, 2 and 4 wk after switching to the enteric-coated formulation ($p < 0.01$). Abdominal cramps improved significantly after 4 wk of Videx EC. While taking buffered didanosine, 40% of patients had side effects that were severe enough to alter their daily activities. Only 7% of those taking Videx EC reported this problem. After 4 wk of therapy with the enteric-coated formulation, 100% of patients in the study stated they preferred Videx EC to buffered didanosine *(101)*.

Didanosine Tolerability and Management of Adverse Events

Selected clinical and laboratory adverse events that occurred in a study of Videx EC in combination with other antiretroviral agents are provided in Tables 9 and 10 *(60)*.

Table 10
Selected Laboratory Abnormalities, Study AI454-152 *(60)*[a]

	Percent of patients[b]			
	Videx EC + stavudine + nelfinavir $N = 258$		Zidovudine/lamivudine[c] + nelfinavir $N = 253$	
Parameter	Grades 3, 4[d]	All grades	Grades 3, 4[d]	All Grades
AST	5	46	5	19
ALT	6	44	5	22
Lipase	5	23	2	13
Bilirubin	<1	9	<1	3

AST, aspartate aminotransferase; ALT, alanine aminotransferase
[a]Median duration of treatment was 62 wk in the Videx EC plus stavudine plus nelfinavir group and 61 wk in the zidovudine plus lamivudine plus nelfinavir group
[b]Percentages based on treated patients
[c]Zidovudine plus lamivudine combination tablet
[d]Greater than 5 × upper limit of normal (ULN) for AST and ALT; at least 2.1 × ULN for lipase; and at least 2.6 × ULN for bilirubin

Didanosine Mitochondrial Toxicity

As discussed in the "Stavudine Mitochondrial Toxicity" section, nucleoside analogs, including didanosine, inhibit mtDNA replication and each analog targets specific organs for toxicity. Electron microscopy of cells treated with didanosine and tests performed with other agents confirm that nucleoside analogs damage mtDNA *(102)*.

Peripheral Neuropathy With Didanosine

Peripheral neuropathy is a toxicity of didanosine when used alone or in combination with other antiretroviral agents *(103)* (*see* the "Peripheral Neuropathy With Stavudine" section). Several nucleoside analogs are neurotoxic, to varying degrees, and their effect is dose dependent *(103)*. Peripheral neuropathy has occurred more frequently in patients with advanced HIV disease, those with a history of neuropathy, or in patients being treated with neurotoxic drug therapy *(103,104)*. The incidence of didanosine-induced peripheral neuropathy varies with dose and schedule, but a reversible neuropathy occurs in up to 23% of patients after 10 mo of treatment *(103)*. Neuropathy associated with didanosine occurs less frequently than does neuropathy associated with zalcitabine *(103)*. In one early study, 7 of 59 patients (11.8%) experienced grade 2–3 neuropathy that started within weeks 10 and 26 of therapy. Patients had resolution of the neuropathy after stopping stavudine but not didanosine treatment *(105)*. Even without the effect of nucleoside analogs, peripheral neuropathy can occur because of the HIV-1 virus itself. Therefore, it can be difficult to determine the cause of the neuropathy *(44,104,106)*.

Table 11
Treatment of Peripheral Neuropathy

1. Exclude or eliminate other neuropathy-associated factors, such as immune (i.e., vasculitis), nutritional (i.e., B12), viral (CMV, DSPAN), other drugs (INH), or multisystem failure.
2. Lower the dose of the offending NRTI, or rechallenge with a lower dose after 8 to 10 wk of discontinuation. Partial or complete resolution of neuropathic symptoms may be evident by reducing the dose of the drug.
3. Analgesics, such as nonsteroidal anti-inflammatory agents, topical capsaicin, Ultram, or narcotics can be used.
4. Antidepressants (e.g., amitryptiline) can be used.
5. Anticonvulsants (e.g., phenytoin, Neurontin, Tegretol, or lamotrigine).
6. Other treatments, such as Mexilitine and nerve growth factor.

CMV, cytomegalovirus; DSPAN, distal sensory painful axonal neuropathy; INH, isoniazid. (Adapted from ref. *103*.)

Didanosine dosing can be adjusted in response to peripheral neuropathy. Symptoms of peripheral neuropathy may resolve if therapy is withdrawn promptly. If symptoms resolve completely, patients may tolerate resumption of didanosine treatment at a reduced dose. If neuropathy recurs after resumption of didanosine, permanent discontinuation of didanosine should be considered (*see* Table 11, adapted from ref. *103* for suggested treatment of peripheral neuropathy).

Lactic Acidosis With Didanosine

Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogs alone or in combination, including didanosine *(107–109)*. In one analysis of cross-sectional and longitudinal data from a prospectively collected clinical database, female gender and and use of didanosine were associated with an increased risk of hyperlactatemia; didanosine-containing regimens doubled the relative hazard of hyperlactatemia compared with those sparing didanosine *(109)*. Further analysis of this cohort determined that, after controlling for didanosine use, current use of stavudine was an additional risk factor for hyperlactatemia *(110)*. Fatal lactic acidosis has been reported in pregnant women who received the combination of didanosine and stavudine with other antiretroviral agents *(46)*. The combination of didanosine and stavudine should be used with caution during pregnancy and is recommended only if the potential benefit clearly outweighs the potential risk. Treatment with didanosine should be suspended in any patient who develops clinical or laboratory findings suggestive of lactic acidosis or pronounced hepatotoxicity (which may include hepatomegaly and steatosis even in the absence of marked transaminase elevations).

Symptoms of lactic acidosis include nausea, vomiting, and abdominal pain. Alternatively, fatigue and weight loss may be the overriding symptoms. An enlarged liver may be palpable and accompanied by shortness of breath, tachypnea, hyperventilation, liver and/or renal failure, clotting abnormalities, seizures, and cardiac arrhythmias, followed by death *(111)*. Laboratory abnormalities include increased lactate and lactate-to-pyruvate ratio, acidosis with low bicarbonate, anion gap widening, increased lactate dehydrogenase, and increased hepatic transaminase and creatinine kinase *(111)*.

Pancreatitis With Didanosine

Pancreatitis is a dose-dependent adverse event of didanosine. It occurs in 0.7 to 5.5% of patients taking 200 to 750 mg/d and usually appears within the first 9 mo of treatment *(112)*. Both fatal and nonfatal pancreatitis has occurred during therapy with didanosine used alone or in combination regimens in both treatment-naive and treatment-experienced patients, regardless of degree of immunosuppression. Didanosine should be suspended in patients with signs or symptoms of pancreatitis and discontinued in patients with confirmed pancreatitis *(60)*. Patients treated with didanosine in combination with stavudine, with or without hydroxyurea, may be at increased risk for pancreatitis. Hydroxyurea potentiates the action of nucleoside analogs, increasing viral uptake of the drugs. One patient reportedly developed pancreatitis after hydroxyurea was added to a regimen of stavudine, didanosine, and nevirapine, after the patient had been on the regimen for approx 18 mo *(112)*.

In cases in which treatment with life-sustaining drugs known to cause pancreatic toxicity is required, suspension of didanosine therapy is recommended *(60)*. In patients with preexisting risk factors for pancreatitis (including obesity and prolonged nucleoside exposure), didanosine should be used with caution and only if clearly indicated. Patients with advanced HIV infection, especially the elderly, are at increased risk of pancreatitis and should be observed closely. Patients with renal impairment or lower body weight (<60 kg) may be at greater risk for pancreatitis if treated without dose adjustment *(60)*.

The coadministration of ribavirin and didanosine can result in increased dideoxyadenosine 5′-triphosphate, increasing the risk of didanosine-associated toxicities *(78–80)*. The coadminstration of didanosine with agents known to be associated with increased risks of pancreatitis (including ethanol), lactic acidosis, or peripheral neuropathy should be undertaken with care and proper monitoring.

Lipodystrophy With Didanosine

The features that characterize lipoatrophy and were presented earlier (*see* the “Lipodystrophy With Stavudine” section). The relationship between nucleoside analogs and the development of lipodystrophy remains controversial and

continues to be a subject of investigation. However, long-term exposure (i.e., 30 mo) *(111)* to the combination of stavudine and didanosine has been associated with lipodystrophy, particularly lipoatrophy, with morphological changes mild in severity in more than 40% of patients *(113)*. Many clinical studies have evaluated didanosine in combination with stavudine, making it difficult to ascribe lipoatrophy to the didanosine component *(31,114)*.

Other Adverse Events With Didanosine

Retinal Changes and Optic Neuritis

Retinal changes and optic neuritis have been reported in adult and pediatric patients. Retinal lesions found in a 6-yr-old female patient taking didanosine revealed areas of retinal pigment epithelial (RPE) loss with some RPE hypertrophy and/or hypopigmentation at the margins of RPE loss. The findings in this patient confirm previous studies suggesting that retinal lesions seen with didanosine therapy primarily affect the RPE *(115)*. Periodic retinal examinations should be considered for patients receiving didanosine.

GI Side Effects

The tablet formulations of didanosine are well-known for GI disturbances, including diarrhea and nausea. These effects are believed to be caused by the buffer component of the compound. Patients who switched from buffered didanosine to Videx EC in studies have experienced significant decreases in severity of GI symptoms, including diarrhea, gas, and bloating within 2 to 4 wk of changing formulations. Patients experienced improved quality of life and decreased side effects severe enough to alter their activities of daily living. After the studies, 100% of patients indicated the desire to remain on the Videx EC formulation *(101)* (unpublished data on file, Bristol-Myers Squibb, Princeton, NJ).

Mitochondrial Toxicity Associated With Stavudine Plus Didanosine

Mitochondrial toxicity is particularly noted when stavudine is combined with didanosine. Large comparative trials have confirmed a higher frequency of toxicity, particularly of peripheral neuropathy, using the combination of stavudine plus didanosine, compared with other two-NRTI backbones. In ACTG 384, the occurrence of the first serious toxic effect or the occurrence of peripheral neuropathy were significantly delayed in regimens containing the zidovudine plus lamivudine backbone compared with regimens using the stavudine plus didanosine NRTI backbone (both $p < 0.0001$) *(31)*. In FTC-301A, subjects in the stavudine plus didanosine plus efavirenz treatment arm (n, 285) had a greater probability of an adverse event leading to drug discontinuation than subjects in the didanosine plus emtricitabine plus efavirenz group (n = 286) (15% vs 7%, respectively, $p = 0.005$). Pancreatitis and symptomatic hyperlactatemia plus lactic acidosis were observed only in the stavudine group.

Investigator-reported lipodystrophy occurred in 0.4% of emtricitabine plus didanosine recipients vs in 6% of stavudine plus didanosine recipients ($p < 0.05$) *(33)*.

Current US DHHS guidelines recommend against the use of a stavudine plus didanosine NRTI backbone, because of the high incidences of peripheral neuropathy, pancreatitis, and lactic acidosis, except when no other antiretroviral options exist and the potential benefits outweigh the risks *(7)*.

ZALCITABINE

Zalcitabine Overview

Zalcitabine (also known as ddC), a cytidine analog, is a potent inhibitor of HIV-1 reverse transcriptase. It was the second antiretroviral nucleoside analog to undergo clinical testing and the third agent licensed by the FDA for use in HIV-infected patients in 1992. The results of large clinical trials in the early 1990s suggest improved clinical and immunological outcomes when zalcitabine was used in combination with zidovudine in treatment-naive patients compared with zidovudine alone. In zidovudine-experienced patients, however, CD4 cell increases were more modest, and survival differences between zalcitabine plus zidovudine and zidovudine-alone arms were not demonstrated in most clinical studies.

The major dose-limiting side effect of zalcitabine is a dose-related distal sensory peripheral neuropathy. The incidence of peripheral neuropathy is higher with zalcitabine than with the other licensed dideoxynucleoside analogs, didanosine and stavudine. Together with the required thrice-daily dosing, these characteristics of zalcitabine have resulted in its decreasing popularity as a component of a HAART regimen. Zidovudine plus zalcitabine is not recommended in the current US DHHS guidelines for use as a two-NRTI backbone for initial therapy, because of the inferior virological efficacy and the higher rate of adverse effects compared with the other two-NRTI alternatives *(7)*.

Zalcitabine Chemistry and Mode of Action

Zalcitabine (also known as ddC) is a synthetic pyrimidine nucleoside analog of the natural nucleoside deoxycytidine, with a substitution of hydrogen for the hydroxyl group at the 3′ position of the ribose sugar (Fig. 1). In vitro, zalcitabine is one of the most potent of the nucleoside analogs, completely inhibiting HIV replication in T-cell lines at concentrations of 0.5 to 1 μ*M* *(1)*. IC_{50} and IC_{95} values are in the range of 2 to 500 n*M* and 100 to 1000 n*M*, respectively *(116–118)*. Zalcitabine is active in vitro against both HIV-1 and HIV-2 *(119)*.

Zalcitabine enters the cell by facilitated diffusion *(120)*. It is then converted to its active metabolite ddC 5′-triphosphate (ddCTP) via sequential phosphorylation to the monophosphate, diphosphate, and triphosphate by cellular

enzymes *(121)*. The active triphosphate, ddCTP, inhibits HIV replication by both competing with the natural dCTP substrate and by acting as a chain terminator after incorporation into the growing viral DNA strand. DNA polymerase-α, which is important in DNA synthesis, is relatively resistant to the effects of ddCTP *(122)*. DNA polymerase-β, which is involved in DNA repair, is intermediate in sensitivity to ddCTP and mtDNA polymerase-γ is most sensitive *(122)*. Inhibition of mtDNA synthesis by zalcitabine has been demonstrated in cultured cell lines in vitro *(123–125)*. In addition, clinical adverse events seen with zalcitabine therapy, particularly peripheral neuropathy, have been associated with histological evidence of mitochondrial dysfunction *(126)*.

The ratio of the active metabolite, ddCTP, to the natural nucleoside, dCTP, and, as a result, the antiviral activity, of zalcitabine is greater in resting than activated cells *(127,128)*. In vitro, zalcitabine inhibits bone marrow CD34+ cells *(123)* and erythrocytic and granulocytic progenitor cells *(129)* to a similar extent as zidovudine; the much lower plasma concentrations of zalcitabine may account for the infrequent reports of anemia or granulocytopenia as an adverse effect of zalcitabine administration. In contrast, zalcitabine strongly inhibits a megakaryocyte progenitor cell line *(130)*, which correlates with the reports of thrombocytopenia in early, high-dose clinical studies of zalcitabine. Zalcitabine is also more active in monocyte/macrophage cells than in lymphocytes *(116,131)*, and its activity is greater in acutely infected cell cultures than in chronically infected cells *(118,132)*. Initial studies documented the in vitro antiretroviral activity of zalcitabine against zidovudine-resistant *(133–135)* and didanosine-resistant virus *(136)*, with small increases in IC_{50} that remained less than 1 μ*M* (*see* additional discussion in the "Resistance to Zalcitabine" section).

Zalcitabine exhibits in vitro synergy with zidovudine *(137)*, stavudine *(136,138)*, nelfinavir *(17)*, recombinant interferon-α or human leukocyte interferon *(139,140)*, non-nucleoside inhibitor derivatives of tetrahydroimidazo [4,5,1-*jk*][1,4]-benzodiazepin-2(1*H*)-thione (TIBO) *(141)*, *bis*(heteroaryl)piperazine (BHAP) *(142)*, and with the binding inhibitors, dextran sulfate and recombinant, soluble CD4 *(143)*. Additive-to-synergistic effects are seen with zalcitabine in combination with saquinavir *(144)*, *N*-butyl deoxynojirimycin *(145)*, or abacavir *(146)*. Granulocyte/macrophage colony-stimulating factor *(147,148)*, macrophage colony-stimulating factor *(149)*, tumor necrosis factor *(150)*, and ribavirin *(151)* all reduce the in vitro antiretroviral activity of zalcitabine. Lamivudine inhibits zalcitabine phosphorylation, through competition with the shared intracellular enzyme (deoxycytidine kinase) that both drugs use for phosphorylation *(152)*.

Zalcitabine Pharmacokinetics in Adults

The mean absolute bioavailability of zalcitabine is greater than 80% after oral administration without food *(153)*. Initial studies using 0.09 to 0.25 mg/kg

of zalcitabine established a mean plasma half-life of 1.2 h, with a range of 1 to 3 h in individual patients *(153–155)*. A subsequent study using a single 1.5-mg (~0.02 mg/kg) dose in 40 adults demonstrated a mean C_{max} of 21.2 ng/mL *(156)*. A longer mean half-life of 2.7 h was established in 25 patients receiving the standard dose of 0.75 mg zalcitabine (thrice daily) in combination with zidovudine and saquinavir *(157)*. A 39% decrease in mean C_{max}, a twofold increase in time to C_{max} (from 0.8 h to 1.6 h) and a 14% reduction in AUC were noted in 20 patients administered zalcitabine with food *(158)*. Binding to serum proteins is less than 4% (data on file, Hoffmann-LaRoche, Nutley, NJ). Intracellular concentrations of the active metabolite, zalcitabine triphosphate, are too low to measure *(118)*. In one early study of nine patients administered a single high dose of zalcitabine intravenously (0.06 or 0.09 mg/kg), CSF-to-plasma concentration ratios ranged from 9 to 37% (mean 20%) *(154)*.

The major route of elimination of zalcitabine is via the renal route, such that 80% of an iv dose and 60% of an orally administered dose is detectable as the unchanged parent drug within 24 h after dosing *(118,153)*. Renal tubular secretion may contribute to glomerular filtration in clearance of zalcitabine by the kidney *(153)*. In a study of 10 patients with creatinine clearances between 11 and 50 mL/min (mean 33.5 mL/min), the mean half-life of zalcitabine was 6.7 h, leading to the recommendation of dose adjustments in patients with diminished creatinine clearance (*see* "Zalcitabine Dosage") *(159)*. Zalcitabine is not significantly metabolized by the liver.

Zalcitabine Dosage

The recommended dosage for adults is 0.75 mg orally every 8 h *(118)*. With impaired renal function, adjustments should be made. For creatinine clearances between 10 and 40 mL/min, the dosing interval should be increased to 0.75 mg every 12 h. For a creatinine clearance less than 10 mL/min, zalcitabine should be administered as one 0.75-mg tablet every 24 h *(118)*. Zalcitabine is dispensed as 0.375-mg and 0.75-mg tablets.

Zalcitabine is classified as a Pregnancy Category C drug, because of the demonstration of teratogenic effects of supratherapeutic dosing in mice and rats (data on file, Hoffmann-LaRoche). Safety in human pregnancy has not been established; zalcitabine should be used during pregnancy only when then potential benefit outweighs the potential risk to the fetus. Women of childbearing potential should practice effective contraception during zalcitabine use *(118)*.

Zalcitabine Drug Interactions

Concurrent administration of medications that share the toxicity profile of zalcitabine should be avoided if possible. Therapeutic agents causing peripheral neuropathy, such as dapsone, disulfiram, isoniazid, lithium, pentamidine,

ribavirin, metronidazole, phenytoin, and vincristine should be used with caution *(118)*. The combination of didanosine plus zalcitabine or stavudine plus zalcitabine is not recommended because of the increased risk of peripheral neuropathy *(7)*. Treatment with zalcitabine should be interrupted during iv pentamidine therapy, because of an increased risk of pancreatitis. Because zalcitabine is excreted renally, additional monitoring is necessary when using nephrotoxic agents, such as aminoglycosides, foscarnet, and amphotericin B *(118)*. Probenecid and cimetidine both decrease renal clearance of zalcitabine, most likely by inhibition of tubular secretion, resulting in an increase in exposure (AUC) of 50% and 36%, respectively *(160)*. Magnesium/aluminum-containing antacid products decrease bioavailability of zalcitabine by 25%; simultaneous coadministration of these agents with zalcitabine is not recommended *(161)*. Lamivudine inhibits zalcitabine phosphorylation in vitro, and this combination is not recommended *(7)*.

Zalcitabine Efficacy, Alone and in Combination

Zalcitabine was the second antiretroviral nucleoside analog to undergo extensive clinical evaluation in adults with HIV infection. Early phase I studies evaluated doses ranging from 0.01 to 0.25 mg/kg every 4 h and delineated the pharmacokinetic and toxicity profiles for zalcitabine monotherapy *(154,162,163)*. Despite dose-limiting toxicity, an antiretroviral effect was suggested by rises in the CD4 cell count and by suppression of serum HIV p24 antigen *(154,162,163)*.

Subsequent studies directly compared zalcitabine monotherapy (at the dose of 0.75 mg thrice daily) with zidovudine monotherapy *(164,165)* or didanosine monotherapy *(166)*. In treatment-naive patients (ACTG 114, 635 patients), zidovudine was significantly superior to zalcitabine in reducing the rate of progression to AIDS or death *(164)*. In ACTG 155, patients with symptomatic HIV disease and CD4 counts of at most 300 cell/mm^3 or asymptomatic HIV disease and CD4 counts of at most 200 cells/mm^3 who had tolerated zidovudine for 6 mo or more were randomized to continue zidovudine monotherapy (283 patients), switch to zalcitabine monotherapy (285 patients), or add zalcitabine to their zidovudine regimen (423 patients). No significant reduction in risk of disease progression or death was seen in the group of patients who switched to zalcitabine monotherapy, compared with the group that continued zidovudine monotherapy *(165)*. In contrast, the Community Programs for Clinical Research on AIDS (CPCRA) 002 study in 467 zidovudine-experienced or -intolerant patients with advanced HIV disease (median CD4 count, 37 cells/mm^3 at entry) demonstrated that switching to zalcitabine conferred a possible survival advantage over switching to didanosine *(166)*. However, the majority of patients in either treatment group had disease progression (66%) and at least one adverse experience (66%) *(166)*.

The combination of zalcitabine and zidovudine was initially studied in alternating regimens, designed to maximize antiretroviral effect and limit toxicity *(154,167)*. In the larger of these studies (ACTG 047), weekly or monthly alternating zalcitabine and zidovudine regimens demonstrated less toxicity and greater CD4 cell count, p24 antigen suppression, and weight gain than continuous zidovudine, intermittent zidovudine, or intermitted zalcitabine regimens *(167)*.

Based on favorable data from an initial pilot study of zalcitabine and zidovudine administered concurrently *(168)*, subsequent large, randomized trials evaluated this combination in comparison with zidovudine monotherapy. In 1067 antiretroviral therapy-naive patients with CD4 counts between 200 and 500 cells/mm^3 (ACTG 175), the combination of zalcitabine and zidovudine significantly reduced the risk of progression to an AIDS-defining event or death compared with zidovudine monotherapy *(169)*. Similar benefits of zalcitabine plus zidovudine combination therapy were seen in 2124 therapy-naive patients with lower CD4 cell counts (mean baseline, 213–215 cells/mm^3) in the Delta study *(170)*. In the CPCRA 007 study, a subgroup analysis demonstrated a clinical benefit from combination zalcitabine plus zidovudine or didanosine plus zidovudine therapy in patients with no previous use or less than 12 mo of previous zidovudine use, but not in patients with more than 12 mo of previous use *(171)*.

In zidovudine-experienced patients, superiority of zalcitabine plus zidovudine over zidovudine monotherapy was suggested in a subgroup of patients with CD4 cell counts of 150 cells/mm^3 or greater at baseline *(165)*. This was not confirmed in the zidovudine-experienced subgroups of the ACTG 175 (1400 patients) or Delta (1083 patients) trials *(169,170)*.

In randomized clinical trials, the combination of zalcitabine plus zidovudine has been compared with didanosine plus zidovudine and with lamivudine plus zidovudine. In ACTG 175, there was no difference in rate of progression of disease or death between treatment arms receiving zalcitabine plus zidovudine and those either receiving didanosine plus zidovudine or didanosine alone *(169)*. Similarly, the Delta and CPCRA 007 trials demonstrated no difference in clinical outcomes between zalcitabine plus zidovudine and didanosine plus zidovudine treatment groups *(170,171)*. In the ACTG 193A study of 1313 patients with CD4 cell counts of fewer than 50 cells/mm^3, zalcitabine plus zidovudine was not superior to didanosine plus zidovudine or to monthly alternating monotherapy with didanosine and zidovudine *(172)*. In the NUCA 3002 study, which enrolled 254 zidovudine-experienced patients and assessed nonclinical endpoints, 300 mg/d or 600 mg/d of lamivudine plus zidovudine therapy was associated with significantly greater rises in CD4 count than zalcitabine plus zidovudine therapy; suppression of plasma RNA was similar among the three treatment groups *(173)*.

A meta-analysis was performed by the HIV Trialists' Collaborative Group, using individual patient data from 7700 participants in six randomized trials comparing zalcitabine plus zidovudine, didanosine plus zidovudine, vs zidovudine alone *(174)*. Five of the six trials involved randomized comparisons of didanosine plus zidovudine vs zalcitabine plus zidovudine; in these trials, didanosine plus zidovudine had greater effects on disease progression (p = 0.004) and death (p = 0.009) than zalcitabine plus zidovudine *(174)*.

Subsequent studies with zalcitabine and other nucleoside analogs clearly demonstrated the superiority of three-drug therapy over dual nucleoside therapy *(172,175,176)*. With the evaluation of additional nucleoside analogs (didanosine, stavudine, and lamivudine), enthusiasm waned for the use of zalcitabine, primarily because of the higher incidence of peripheral neuropathy with zalcitabine and the lack of clear clinical, immunological, or virological superiority over the other agents in this class. As new agents were approved for twice-daily administration, the popularity of thrice-daily zalcitabine declined further.

Zalcitabine Pediatric Studies: Pharmacokinetics, Efficacy, and Toxicity

Few data exist on the pharmacokinetic profile of zalcitabine in children. In an early pilot study of zalcitabine monotherapy, drug was not detectable in plasma in two children who received 0.02 mg/kg as a single dose *(177)*. At doses of 0.03 and 0.04 mg/kg, mean bioavailability was 54% and mean half-life was 0.8 h *(177)*. Chadwick et al. *(156)* used a pediatric syrup formulation and a more sensitive analytic method to determine drug concentration in 23 mildly symptomatic children each receiving a single dose of 0.02 mg/kg of zalcitabine. This study demonstrated a C_{max} of 9.3 ng/mL and an AUC of 25 ng·h/mL, values 44% and 42%, respectively, of those seen in adults administered comparable dosages, suggesting more rapid clearance in children *(156)*. Another study found that pharmacokinetic parameters were not altered by age (after adjusting for weight or body surface area) and were similar after the first and multiple doses *(178)*.

Safety and effectiveness of zalcitabine in children under 13 yr of age has not been established; zalcitabine is not licensed by the FDA for use in children. However, several studies of zalcitabine monotherapy and combination zalcitabine plus zidovudine therapy have been performed in children under 13 yr of age.

In a phase I study, Pizzo et al. evaluated four dosages of zalcitabine monotherapy (0.015, 0.02, 0.03, or 0.04 mg/kg, every 6 h) for 8 wk in 15 children. Thirteen of these children were antiretroviral naive. Serum p24 antigen declined in 6 of 9 children, and 8 of 15 children had increases in CD4 cell count. Mild mouth sores occurred in 9 of 15 children and rash occurred in 3 of 6 children at the 0.04 mg/kg dose. No children developed evidence of peripheral neuropathy,

neutropenia, or anemia *(177)*. After the 8-wk period of zalcitabine monotherapy, 13 of the children were treated for 12 to 18 mo with 1 wk of zalcitabine alternating with 3 wk of zidovudine. Of these 13, 11 children gained weight and 54% showed an increase of more than 10% in CD4 cell counts. No neuropathy was seen during the course of follow-up *(177)*.

ACTG 138 randomized 170 children between the ages of 3 and 18 yr of age with zidovudine intolerance or progression of disease after 6 mo of zidovudine therapy to two dosages of zalcitabine (0.005 or 0.01 mg/kg, every 8 h) *(179)*. The risk of adverse events was higher in the 0.01 mg/kg dose group. Peripheral neuropathy occurred at the same frequency (5%) at both dose levels; no child had severe symptoms and none had permanent sequelae. The study was not powered to detect differences in clinical progression between dosage arms and no significant differences were seen in progression to AIDS-defining opportunistic infection, neoplasm, serious bacterial infection, or death. Serum p24 antigen declined significantly in both groups (no dosage difference) at week 12, but not at weeks 24 or 36. A significant difference in the rate of CD4 decline was seen, favoring the lower dose *(179)*.

The combination of zalcitabine plus zidovudine treatment in children was studied in ACTG 190 *(178)*. This clinical trial randomized 250 clinically stable, zidovudine-experienced children to 0.01 mg/kg zalcitabine plus 240 mg/m^2 zidovudine (every 8 h) or 240 mg/m^2 zidovudine alone (every 8 h). More children on combination therapy developed neutropenia (18 vs 5 children), with a shorter time-to-onset of neutropenia in this group. The trial was not powered to detect significant clinical differences between the two treatment groups; however, trends were seen in favor of the combination arm in the number of deaths (4 vs 10, $p = 0.083$) and the number of opportunistic infections (3 vs 6). The rate of CD4 cell count decline was significantly slower in the combination arm (13% vs 25% decline per year, $p = 0.03$). There was no difference in neuropsychological testing results or weight change between the arms. Skin rashes and stomatitis were not seen in this study. Two children in the zalcitabine plus zidovudine arm (1.6%) developed dose-limiting peripheral neuropathies, compared with none in the zidovudine alone arm *(178)*.

Zalcitabine Toxicity

In early clinical trials, zalcitabine was administered at doses between 0.01 and 0.09 mg/kg every 4 h, a total daily dose approx 2 to 18 times the currently approved daily dose. At these doses, a syndrome of fever, rash, and aphthous stomatitis appeared shortly after therapy was begun but generally resolved without discontinuation of therapy *(154,162,180)*.

The major dose-limiting toxicity of zalcitabine was a distal sensory peripheral neuropathy of the feet and lower legs, occurring in more than 70% of

patients receiving doses equal to or greater than 0.01 mg/kg every 4 h *(162)*. Neuropathic symptoms included pain, paresthesias, or numbness, and occasionally weakness; an early sign of neuropathy was the loss of ankle deep tendon reflexes *(154,162,181)*. Electrophysiological studies were consistent with axonal neuropathy *(154)*. After discontinuation, symptoms worsened for up to 5 wk after onset, a phenomenon known as coasting *(154)*. At lower doses (0.005 mg/kg zalcitabine every 4 h), mucocutaneous complications were very mild, and peripheral neuropathy was milder, had a later onset, and was fully reversible *(162,163)*.

Large, phase III clinical trials monitored the safety of the currently approved dose of 0.75 mg zalcitabine orally thrice daily. Of 635 antiretroviral-naive patients randomized to receive either zalcitabine or zidovudine, 23.1% of the zalcitabine group developed a moderate or severe peripheral neuropathy compared with 6.0% of the zidovudine group *(164)*. In one study in patients with previous zidovudine experience or intolerance and CD4 count of at most 300 cells/mm^3, peripheral neuropathy occurred at grade 2 or greater severity in 28.3% of the patients *(166)*. However, in a study of patients with higher CD4 counts (200–500 cells/mm^3), the incidence of grade 3 or greater peripheral neuropathy was similar in patients receiving the combination of zalcitabine plus zidovudine (3.3%) to those receiving zidovudine alone (3.1%) *(118,169)*.

Risk of developing peripheral neuropathy with zalcitabine use increases in patients with low CD4 counts (<50 cells/mm^3), diabetes, weight loss, vitamin B12 deficiency, preexisting peripheral neuropathy, or heavy ethanol consumption, or with concomitant use of drugs with the potential to cause peripheral neuropathy *(118,182,183)*. Zalcitabine should be used with extreme caution in patients with preexisting neuropathy *(118)*. In addition, the subsequent use of drugs with the potential to cause neuropathy, such as didanosine, may exacerbate a preexisting zalcitabine-associated neuropathy *(184)*.

In large phase III studies at the current dose of 0.75 mg zalcitabine thrice daily, rash associated with zalcitabine occurred in 1.6 to 3.4% of patients, similar to the rates of rash for zidovudine and didanosine *(166,169)*. Oral lesions or stomatitis occurred in 1.5 to 3.0% of subjects, compared with 0% for didanosine and 0.6% for zidovudine *(166,169)*. There is one case of esophageal ulceration linked to zalcitabine therapy *(185)*.

Pancreatitis is a rare but serious complication of zalcitabine therapy, occurring in up to 1.1% of patients *(118,186)*. In the zalcitabine expanded access program, patients with a history of previous pancreatitis or increased amylase had a higher incidence of pancreatitis (5.3%) or increased amylase (4.4%). Therefore, patients with previous pancreatitis, elevated amylase, or risk factors for pancreatitis (such as alcohol abuse) should be monitored closely during zalcitabine therapy *(118)*. Zalcitabine use should be interrupted if treatment with iv pentamidine is necessary.

A syndrome of lactic acidosis and severe hepatomegaly with steatosis, which may be fatal, has been reported with the use of nucleoside analogs *(118)*. Zalcitabine should be used with caution in patients with known risk factors for liver disease. In addition, rare cases of hepatic failure and death associated with zalcitabine therapy in the setting of underlying hepatitis B have been reported *(118)*.

One case of reversible ototoxicity *(187)* and one case of irreversible ototoxicity *(188)* have been reported after zalcitabine use. No consistent differences in the rate of lymphoma was seen between zidovudine monotherapy, zalcitabine monotherapy, and zidovudine plus zalcitabine combination therapy in one analysis of several large clinical endpoint studies *(189)*.

A syndrome of cardiac dysfunction, temporally improving after withdrawal and worsening after rechallenge, has been described in a group of six patients who received nucleoside analogs, including zidovudine, didanosine, or didanosine plus zalcitabine *(190)*.

Resistance to Zalcitabine

Clinical isolates of HIV with reduced in vitro susceptibility to zalcitabine have been infrequently described, in contrast to high rates of development of resistance to zidovudine or lamivudine. Initially, in vitro selection of resistance by sequential passage of virus in the presence of increasing concentrations of zalcitabine identified the key resistance mutation, Lys65Arg, associated with increases in IC_{50} to zalcitabine, didanosine, and lamivudine *(191)*. Viruses containing the K65R mutation, or a second mutation at codon 69 associated with low-level zalcitabine resistance, have been isolated from patients receiving zalcitabine monotherapy or alternating zalcitabine and zidovudine therapy *(192–194)*. A third mutation, Met184Val, conferring low-level resistances to zalcitabine and didanosine, and high-level resistance to lamivudine, has also been identified from patients on prolonged zalcitabine monotherapy, either alone or with K65R *(195)*. The Gln151Met mutation, most often accompanied by additional mutations at codons 62, 75, 77, and 116, has been identified in viral isolates resistant to zalcitabine, didanosine, and zidovudine *(196)*. Two additional mutations, one at codon 74 (seen in patients treated with didanosine or didanosine plus zidovudine therapy) and a second at codon 75 (identified by in vitro selection using stavudine) confer cross-resistance to zalcitabine *(197–199)*. Further complicating this issue is some level of nucleoside cross-resistance. Nucleoside cross-resistance was first noted in a study of 128 patients who had received AZT monotherapy, in which each 10-fold decrease in zidovudine susceptibility was associated with a 2.0-fold decrease in zalcitabine sensitivity and a 2.2-fold decrease in didanosine susceptibility *(200)*. This has been confirmed and more clearly delineated in a recent, large phenotypic resistance study *(201)*.

The emergence of resistance to zalcitabine occurs infrequently during combination therapy *(202)*. Several early studies of zalcitabine plus zidovudine therapy in treatment-naive adult patients found no phenotypic resistance to zalcitabine after 24 to 112 wk of dual therapy *(176,203,204)*. However, in both adults and children, combination therapy did not prevent the emergence of resistance to zidovudine *(176)*. In a zalcitabine monotherapy study in children, only one child developed zalcitabine resistance, an overall incidence of 3% *(205)*. A LiPA hybridization analysis of rebounding plasma virus from eight patients receiving zalcitabine plus zidovudine therapy, demonstrated the T69D mutation in one-third of the patients, and detected no L74V or M184V mutations *(206)*.

Clinical Use of Zalcitabine in the Developed World

Zalcitabine (as Hivid) is approved by the FDA in the United States for use in combination with antiretroviral agents for the treatment of HIV infections. Zalcitabine was used commonly in the early 1990s, either as an alternative to, or in combination with, zidovudine. However, with the introduction of other more effective and less toxic alternative nucleoside analogs, the use of zalcitabine has fallen off sharply. Current United States Public Health Service guidelines do not recommend use of zalcitabine in standard HIV therapy *(7)*.

Use of Zalcitabine Therapy in the Developing World

Although use of zalcitabine in the United States, Europe, and Australia declined sharply because of concerns about toxicity, efficacy, and thrice-daily dosing, more recent clinical studies have been performed in resource-poor countries in the developing world. Carey noted that the perception of zalcitabine's potential to cause peripheral neuropathy is a result of its early use at high doses, and that current doses of zalcitabine in combination therapy infrequently results in either peripheral neuropathy or resistance *(207)*. Accordingly, given the global epidemic of HIV infection, recent studies of zalcitabine have been conducted in the developing world. In the **HIV N**etherlands **A**ustralia **T**hailand Research Collaboration Thai Red Cross AIDS Research Centre (HIVNAT) 001 trial in Thailand, 111 antiretroviral-naive patients with CD4 counts between 100 and 500 cells/mm^3 were randomly assigned to receive zalcitabine and zidovudine, either at the standard, full doses or each drug at half-dose *(208)*. HIV RNA was suppressed below 400 copies/mL in 52% of patients in the full-dose arm and in 20% of patients in the half-dose arm by week 48. Peripheral neuropathy occurred in seven participants, at grade 1 or 2 severity, with no difference in adverse events between the two treatment arms *(208)*.

Two additional studies were conducted using zalcitabine in Nigeria. The first trial treated 24 patients with zalcitabine plus saquinavir therapy for 6 mo. Clinical improvement was seen in 79% of patients; however, 40% had adverse

events, and a minimal increase in CD4 was seen *(209)*. A second trial in Nigeria treated 40 patients who had CD4 cell counts between 100 and 500 cells/mm^3 with open label zalcitabine plus zidovudine plus nelfinavir. Although only 8% of patients had a viral load suppression below the limit of detection by week 24, mean CD4 cell counts increased in 85% of patients, from a mean of 273 cells/mm^3 to 414 cells/mm^3 *(210)*. Seventy-seven percent of patients had weight gain, ranging from 1.5 to 32 kg. Two patients were withdrawn from treatment, one for severe peripheral neuropathy and one for severe diarrhea.

Additional data relevant to the developing world is a multicenter study published in 2004 that evaluated zalcitabine administered twice daily—the HIVBID study *(211)*. The study was based on preliminary data from a retrospective study in London in a cohort of 17 patients who switched from thrice-daily to twice-daily (1.125 mg twice daily) dosing of zalcitabine, and saw no loss of therapeutic effect or unexpected adverse events over a median of 130 d of follow-up *(212)*. The HIVBID study was a randomized, open-label, 24-wk pilot study of 1.125 mg zalcitabine twice daily vs lamivudine twice daily, both combined with zidovudine and a PI (either 1250 mg nelfinavir twice daily or 1600 mg saquinavir soft-gel twice daily). A total of 47 patients were included in the ITT analysis, distributed across 12 centers in five countries. At 24 wk, 42% of the zalcitabine group and 52% of the lamivudine group had HIV RNA viral load suppression to fewer than 400 copies/mL; 21% and 44%, respectively, had suppression to fewer than 50 copies/mL. At 48 wk, 38% of the zalcitabine group and 26% of the lamivudine group had HIV RNA suppression to fewer than 400 copies/mL; 17% and 22%, respectively, had suppression to fewer than 50 copies/mL. At week 48, CD4 cell count increases from baseline were 128 and 115 cell/mm^3 for the zalcitabine and lamivudine groups, respectively. Most adverse events were attributable to zidovudine or to the PI. No serious adverse events and no cases of peripheral neuropathy were reported in the zalcitabine group *(212)*. These data, although preliminary, are interesting regarding the potential use of zalcitabine in a twice-daily regimen in the developing world.

Zalcitabine Conclusion

Zalcitabine's early clinical development used high doses and delineated the toxicity profile of the drug. Peripheral neuropathy is the major dose-limiting toxicity, however, this occurs with lower frequency when zalcitabine is used in patients with earlier-stage HIV disease. Large clinical trials failed to show superiority of zalcitabine over other available nucleoside analogs with more favorable toxicity profiles. In addition, zalcitabine is licensed for thrice-daily administration, whereas other available nucleoside and nucleotide analogs are available for once- or twice-daily administration. The use of zalcitabine in the

developed world has declined sharply with the licensure of more-effective, less-toxic, and more-convenient agents; its distribution is anticipated to be halted in 2006. Zalcitabine may find additional clinical use in resource-poor settings in the developing world.

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4
Emtricitabine

Gail Skowron, Jeffrey Bratberg, and Rudi Pauwels

INTRODUCTION

Emtricitabine (FTC, Coviracil®) is a synthetic nucleoside analog of cytosine with activity against HIV-1. It is chemically similar to lamivudine, differing only in having a fluoride at the 5-position of the cytosine ring. Emtricitabine is the (-) enantiomer, which is more active than the (+) enantiomer. Emtricitabine has a long plasma half-life (10 h), and even longer intracellular half-life of the active triphosphate (39 h), supporting once-daily dosing. Short-term monotherapy studies have demonstrated that emtricitabine 200 mg QD reduces HIV RNA levels by 1.7 to 1.92 $\log_{10}$ copies/mL. Large, multicenter clinical trials have demonstrated emtricitabine's efficacy, in combination with efavirenz and either didanosine or tenofovir. Similar to lamivudine, emtricitabine is generally well tolerated. One notable side effect attributable to emtricitabine is skin discoloration of the palms and soles, particularly in African American and Hispanic patients. Preliminary data on emtricitabine in the pediatric population have also demonstrated safety and efficacy. Emtricitabine has not been studied in pregnant women. Resistance to emtricitabine is correlated with the emergence of the same Met184Val mutation in reverse transcriptase that mediates resistance to lamivudine. The emergence of M184V appears to be less frequent in patients failing a regimen containing emtricitabine than in patients failing a lamivudine-containing regimen. Current US DHHS and IAS-USA guidelines support emtricitabine as a recommended component of an initial antiretroviral regimen *(1,2)*.

EMTRICITABINE CHEMISTRY AND PRECLINICAL ACTIVITY

Emtricitabine (also known as FTC, Coviracil®) is a synthetic nucleoside analog of cytosine with activity against human immunodeficiency type 1 (HIV-1). Emtricitabine is the (–) enantiomer of 2′,3′-dideoxy-5-fluoro-3′-thiacytidine(*cis*-5-fluoro-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl(cytosine) *(3)*; it differs from lamivudine only by the presence of a fluoro atom at the 5-position

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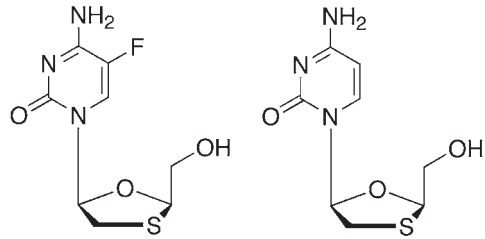

Fig. 1. Chemical structure of emtricitabine in comparison with lamivudine.

of the cytosine ring (Fig. 1). Similar to other nucleoside analogs, emtricitabine is phosphorylated by cellular enzymes to the active triphosphate (TP). Emtricitabine is initially phosphorylated by 2′-deoxycytidine kinase *(4)*, and competition for this enzyme is expected with concomitant cellular exposure of the cell to lamivudine and deoxycytidine. The active anabolite, emtricitabine 5′-TP, competes with the natural substrate, deoxycytidine 5′-TP, for insertion into the growing viral DNA strand, exerting its antiviral activity via competitive inhibition and chain termination. Emtricitabine 5′-TP is a weak inhibitor of mammalian DNA polymerase-α, -β, and -ε and mitochondrial DNA polymerase-γ *(3)*.

Emtricitabine was first reported to show anti-HIV activity comparable to lamivudine (also known as 3TC) by researchers at Emory University and the University of Georgia *(5)* who synthesized and tested a series of enantiomerically pure L-oxathiolanyl pyrimidine and purine nucleosides. The (–) enantiomer was 20-fold more active and significantly less toxic than the (+) enantiomer *(6)* and was chosen for subsequent development. In one in vitro clinical isolate assay system, emtricitabine was the most potent of the nucleoside analogs that were tested (emtricitabine > 2′,3′-dideoxycytidine > lamivudine > 3′-azido-3′-deoxythymidine > 2′,3′-didanosine) *(7)*. (–) emtricitabine-TP is incorporated approx 10-fold more efficiently than lamivudine-TP during RNA-dependent DNA synthesis, because of a higher rate of incorporation and a higher binding affinity *(8)*. The TP of emtricitabine has a high relative substrate specificity for HIV-1 reverse transcriptase and a low substrate specificity for mitochondrial DNA polymerase-γ. In contrast, the TP of zalcitabine (also known as ddCTP) has a high substrate specificity for both enzymes *(9)*, providing a mechanistic explanation for the clinical manifestation of mitochondrial toxicity during therapy with zalcitabine, but not with emtricitabine. In vitro, emtricitabine demonstrates antiviral activity against clinical and laboratory strains of HIV-1 in peripheral blood mononuclear cells (PBMC), monocytes, and macrophages *(10,11)*. Emtricitabine is more potent in PBMC than is lamivudine, with median 50%-effective concentration values of 0.0014 to

0.14 μ*M* and 0.002 to 2.5 μ*M*, respectively *(11)*. In contrast, Hazen and Lanier demonstrated that emtricitabine, lamivudine, and zidovudine were equally active in vitro against single-passage primary HIV-1 isolates from antiretroviral-naive subjects in PBMCs *(10)*. These conflicting data remain to be clarified.

In vitro drug combination studies demonstrated additive-to-synergistic effects of emtricitabine with nucleoside reverse transcriptase inhibitors (NRTIs; abacavir, lamivudine, stavudine, tenofovir, zalcitabine, and zidovudine), non-nucleoside reverse transcriptase inhibitors (NNRTIs; delavirdine, efavirenz, and nevirapine), and protease inhibitors (PIs; amprenavir, nelfinavir, ritonavir, and saquinavir) *(3)*.

In preclinical pharmacokinetics and safety studies in monkeys, emtricitabine showed favorable profiles *(12)*. Emtricitabine also demonstrates in vitro activity against hepatitis B virus (HBV) *(13–15)*.

EMTRICITABINE PHARMACOKINETICS

Overall, the pharmacokinetics of emtricitabine are favorable for this drug to be part of once-daily (QD) and/or directly observed therapy in highly active antiretroviral therapy regimens *(16)*.

Absorption of oral emtricitabine occurs rapidly, peaking in the plasma between 1 and 2 h after a single daily dose *(3)*. On an empty stomach, the mean absolute bioavailability of emtricitabine is quite high, at 93%, with less than 4% of the dose binding to plasma proteins in a concentration-independent fashion *(3)*. At peak concentrations, the mean emtricitabine plasma-to-blood drug concentration ratio is approx 1.0, and the mean plasma-to-semen ratio is approx 4.0 *(3)*. Cerebrospinal fluid concentrations were measured at 4% of the plasma concentration in cynomolgus monkey animal studies *(14)*. In two studies of HIV-infected study volunteers taking multiple doses of 200 mg emtricitabine, the mean plasma peak concentration was 1.7 μg/mL, the time-to-peak concentration (T_{max})was 2 h, the area under the plasma concentration–time curve (AUC) during a 24-h dosing interval was 10 ± 3.1 h·μg/mL, and the plasma half-life was nearly 10 h. Twenty-four hours after a dose, mean steady-state trough levels drop to a mean of 0.09 μg/mL (*3;* Fig. 2). Interestingly, the intracellular half-life of emtricitabine-TP in PBMCs is reported at 39 h *(16,17)*. Some debate exists, however, regarding the applicability of this value to a more diverse population of HIV-infected patients *(18)*. The clinical impact of this extended intracellular activity remains unknown.

Food Effects

Emtricitabine can be taken without regard to meals *(3)*. When emtricitabine was coadministered with a high fat meal (1000 kcal of fat), the emtricitabine

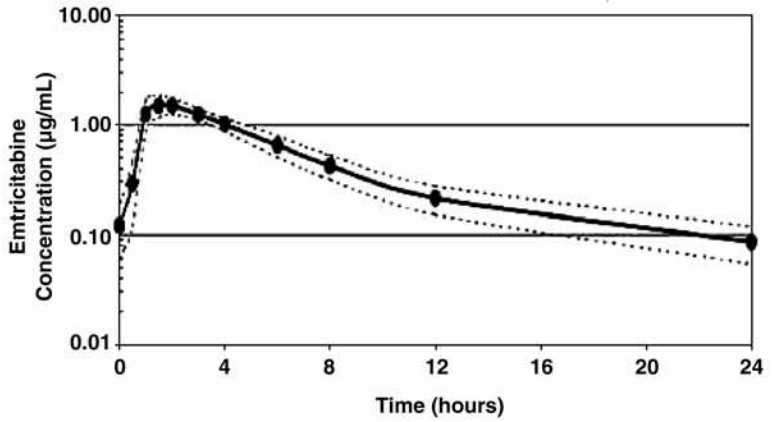

Fig. 2. Mean (±95% CI) steady-state plasma emtricitabine concentrations in HIV-infected adults ($n = 20$) *(3)*.

peak concentration (C_{max}) was decreased by 23%, the time-to-peak concentration (T_{max}) was prolonged to 2.8 h, yet systemic exposure (AUC) remained unchanged *(3,19)*.

Metabolism/Elimination

The chief metabolic advantages of emtricitabine are a complete lack of cytochrome P450 enzyme inhibition (isoforms 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, and 3A4) at supratherapeutic doses, and a lack of uridine-5′-disphosphoglucuronyl transferase inhibition (the enzyme responsible for glucuronidation) *(3)*. Radiolabeled emtricitabine studies revealed complete dose elimination in the urine (~75% unchanged, ~9% oxidated to 3′-sulfoxide diastereomers, and ~4% conjugated to 2′-*O*-glucuronide) and the feces (~14% unchanged) *(3)*.

Emtricitabine clearance occurs primarily through the kidney, at a rate greater than the estimated creatinine clearance (CLcr), suggesting active tubular secretion as well as glomerular filtration (Table 1) *(3)*. The safety and efficacy of emtricitabine in patients with a CLcr less than 50 mL/min have not been studied. Approximately 30% of the emtricitabine dose is removed by hemodialysis over 3 h (blood flow rate 400 mL/min, and dialysate flow rate 600 mL/min), when the treatment is begun within 1.5 h of the administered dose *(3)*. The clearance of emtricitabine by peritoneal dialysis is unknown *(3)*.

Special Populations

Emtricitabine pharmacokinetics have been studied in males and females, in both HIV-infected and healthy volunteers, with similar results among these groups *(3)*. No pharmacokinetic differences because of race have been identified *(3)*. Limited data exists for the use of emtricitabine in the elderly, in children, and

Table 1
Emtricitabine Pharmacokinetic Parameters (Mean ± SD) in Patients With Varying Degrees of Renal Function After a Single-Dose Administration of 200 mg[a]

CL_{cr} (mL/min)	>80 (n = 6)	50–80 (n = 6)	30–49 (n = 6)	<30 (n = 5)	ESRD[b] < 30 (n = 5)
Baseline CLcr (mL/min)	107 ± 21	59.8 ± 6.5	40.9 ± 5.1	22.9 ± 5.3	8.8 ± 1.4
C_{max} (μg/mL)	2.2 ± 0.6	3.8 ± 0.9	3.2 ± 0.6	2.8 ± 0.7	2.8 ± 0.5
AUC (h·μg/mL)	11.8 ± 2.9	19.9 ± 1.1	25 ± 5.7	34 ± 2.1	53.2 ± 9.9
CL/F (mL/min)	302 ± 94	168 ± 10	138 ± 28	99 ± 6	64 ± 12
CL_r (mL/min)	213.3 ± 89	121.4 ± 39	68.6 ± 32.1	29.5 ± 11.4	NA

n, number of patients; CLcr, creatinine clearance; C_{max}, maximum plasma concentration; AUC, area under the curve; CL/F, apparent clearance; CLr, renal clearance; NA, not applicable
[a]Emtriva US Prescribing Information. ESRD, end-stage renal disease
[b]ESRD patients requiring dialysis

in patients with hepatic impairment *(3)*. However, because emtricitabine is eliminated primarily through the kidney, hepatic impairment is thought to have little effect on its pharmacokinetics *(3)*.

Emtricitabine is rated as Pregnancy Category B. The incidence of fetal variations and malformations was not increased in embryofetal toxicity studies performed with emtricitabine in mice and rabbits at exposures (AUC) approx 60-fold and approx 120-fold, respectively, higher than human exposures at the recommended daily dose. There are, however, no adequate and well-controlled studies in pregnant women *(20)*. Because animal reproduction studies are not always predictive of human response, emtricitabine should be used during pregnancy only if clearly needed *(3)*.

EMTRICITABINE DOSAGE

The dosage of emtricitabine (as Emtriva™) for adults is 200 mg/d orally *(3)*. Because of its renal clearance, the dosage must be adjusted for renal insufficiency (Table 2). Emtricitabine is dispensed as 200 mg tablets. An oral liquid solution (10 mg/mL) has been studied in children; although it is approved for use in Europe, it has not been approved by the US Food and Drug Administration and is not commercially available in the United States at this time.

Emtricitabine is also available in a fixed-dose combination pill with tenofovir disoproxil fumarate (as Truvada™), which contains 200 mg of emtricitabine and 300 mg of tenofovir disoproxil fumarate. It is administered as one pill QD for patients with a calculated CLcr (calcCLcr) of 50 mL/min or greater. The dosing interval should be lengthened to every 48 h in patients with a

Table 2
Dosing Interval Adjustment in Patients With Renal Impairment[a]

CLcr (mL/min)	Recommended dose and dosing interval
≥50	200 mg every 24 h
30–49	200 mg every 48 h
15–29	200 mg every 72 h
<15 (including patients requiring hemodialysis)[b]	200 mg every 96 h

CLcr, creatinine clearance
[a]Emtriva US Prescribing Information
[b]If dosing on day of dialysis, give dose after dialysis

calcCLcr between 30 and 49 mL/min. Truvada is not recommended for use in patients with a calcClcr of less than 30 mL/min or in patients on hemodialysis.

EMTRICITABINE DRUG INTERACTIONS

The likelihood of drug–drug interactions with emtricitabine is quite low. In vitro data have shown emtricitabine's benign effect on many clinically relevant cytochrome P450 isoforms *(3)*. In several single-dose healthy volunteer studies combining tenofovir disoproxil fumarate, indinavir, famciclovir, stavudine, or zidovudine with emtricitabine, negligible effects were observed, except for a 13% and 17% increase in zidovudine's AUC and C_{max}, respectively *(3,19)*. Although statistically insignificant differences may be caused by small sample sizes, the differences are thought to be clinically unimportant (Gilead Sciences, Foster City, CA, data on file). A regimen of didanosine and efavirenz at typical doses coadministered with emtricitabine to treatment-naive patients did not change the pharmacokinetic profile of emtricitabine after 4 wk of therapy *(19)*.

EMTRICITABINE ACTIVITY AND EFFICACY CLINICAL TRIALS

The antiretroviral activity of emtricitabine was demonstrated in two short-term monotherapy trials in HIV-1-infected adults. Study 101 was a phase I/II, open-label, nonrandomized, dose-ranging study in 41 lamivudine- and abacavir-naive patients. Emtricitabine was administered at 25 mg twice daily (BID), 100 mg QD, 100 mg BID, 200 mg QD, or 200 mg BID for 14 d *(3)*. At the 200-mg QD dose level, emtricitabine reduced HIV RNA levels by a mean of 1.93 $\log_{10}$ copies/mL *(21)*. Study 102 was a phase I/II, open-label, randomized, dose-ranging study in 81 HIV-infected adults. Three dose levels of emtricitabine (25 mg BID, 100 mg QD, and 200 mg QD) were compared with lamivudine at 150 mg BID. After 10 d, the mean change in the emtricitabine

200 mg QD group was −1.7 $\log_{10}$ copies/mL, compared with −1.5 $\log_{10}$ copies/mL for the lamivudine group. A further analysis of average AUC-minus-baseline from baseline to day 12 demonstrated a significantly greater reduction in viral load over time with emtricitabine (−1.14 $\log_{10}$ copies/mL), compared with lamivudine (−1.01 $\log_{10}$ copies/mL) ($p = 0.04$) *(22)*.

Treatment-Naive Patients

Phase II and III studies have evaluated the safety and efficacy emtricitabine in comparison with other nucleoside backbones, predominantly administered BID. In the open-label, phase II, Montana/ANRS 091 study, 40 treatment-naive adults were administered a QD regimen of emtricitabine (200 mg), didanosine (400 mg, weight-adjusted) and efavirenz (600 mg). At 24 wk, 39 of 40 patients (98%) had viral load suppression to fewer than 400 copies/mL, and 37 of 40 patients (93%) achieved viral load suppression to fewer than 50 copies/mL *(23)*. Using a missing = failure, intent-to-treat analysis, 85% and 80% of patients had an HIV RNA level of fewer than 400 copies/mL and fewer than 50 copies/mL, respectively, at 96 wk *(24)*. CD4 counts increased from baseline by a mean of 259 cells/mm^3 at 96 wk. In a 3-yr analysis, CD4 counts had increased from baseline by 304 cells/mm^3, and 75% of patients maintained viral load suppression to fewer than 400 copies/mL *(25)*.

Another small pilot study (ODECTA) compared emtricitabine (200 mg QD) with abacavir (300 mg BID), both in combination with stavudine plus efavirenz *(26)*. Thirty-seven patients were evaluable at the 24-wk primary endpoint intent-to-treat (missing = failure) analysis. At 24 wk, a greater proportion of patients in the emtricitabine arm achieved an HIV RNA level of fewer than 400 copies/mL (83% vs 74% in the abacavir arm) and fewer than 50 copies/mL (83% vs 63% in the abacavir arm). On the other hand, CD4 rises were greater in the abacavir arm (340 cells/mm^3) than in the emtricitabine arm (329 cell/mm^3). Because of the small sample size of the study, however, none of these differences were statistically significant.

FTC-302 compared the combinations of either emtricitabine or lamivudine, with stavudine plus either efavirenz or nevirapine in 468 treatment-naive patients in South Africa *(27)*. A 48-wk intent-to-treat analysis of FTC-302 showed similar proportions of patients with viral loads of fewer than 50 copies/mL in both groups (61% with emtricitabine and 65% with lamivudine) *(21)*. There was a higher incidence of virological failure in the emtricitabine arm. However, this may have been the result of poor compliance, because 60% of those who experienced viral load rebound receiving emtricitabine had wild-type virus, compared with 23% of those in the lamivudine group, and only 15% had the M184V mutation in the emtricitabine group compared with 54% of the lamivudine group ($p = 0.03$) *(28)*.

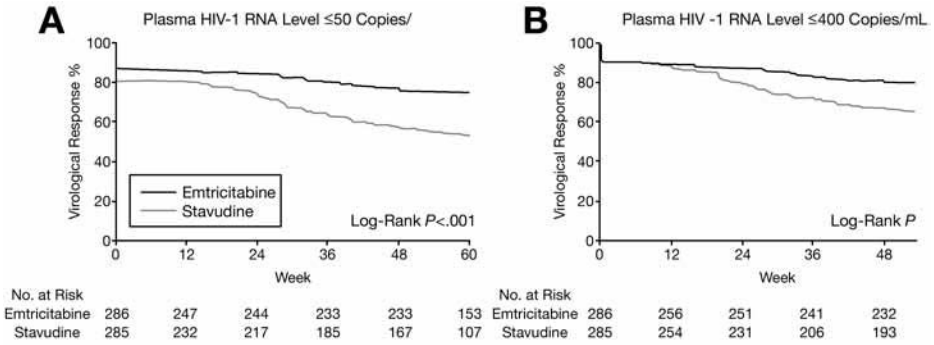

Fig. 3. Proportion of patients achieving a virological response defined as suppression of plasma HIV-1 RNA levels to at most 50 copies/mL **(A)** or at most 400 copies/mL **(B)** in FTC-301A. (From ref. *29*, with permission. Copyrighted © 2004 American Medical Association.)

FTC-301A was a 48-wk phase III, double-blind, randomized study, comparing 200 mg emtricitabine QD vs 40 mg stavudine BID (30 mg stavudine BID if patient weight was <60 kg) in combination with 400 mg didanosine QD (250 mg didanosine QD if patient weight was <60 kg) and 600 mg efavirenz QD *(29)*. Five hundred and seventy-one patients included in the analysis were randomized to receive either emtricitabine QD or stavudine BID in combination with didanosine QD plus efavirenz QD. At baseline, the median HIV RNA level was 4.9 $\log_{10}$ copies/mL (range 2.6–7.0 $\log_{10}$ copies/mL) and the mean CD4 cell count was 3.18 cell/mm^3 (range 5–1317 cell/mm^3). After the last patient had completed week 24, blinded data were reviewed by the Data and Safety Monitoring Board. This analysis showed that patients in the emtricitabine arm were significantly more likely to have a viral load of fewer than 50 copies/mL (85%, vs 76% in the stavudine arm), and all patients were offered the option to switch to open-label emtricitabine *(29)*. Only four patients (three randomized to emtricitabine and one randomized to stavudine) opted to switch to open-label emtricitabine, at weeks 44 (one patient), 46 (two patients) and 47 (one patient). At week 48, 78% of emtricitabine recipients and 56% of stavudine recipients maintained a viral load suppression of fewer than 50 copies/mL ($p < 0.001$) (Fig. 3) *(29)*.

Of patients completing 48 wk of their assigned treatment (as-treated analysis), 91% of the patients in the emtricitabine arm and 84% in the stavudine arm achieved viral load suppression to fewer than 50 copies/mL ($p = 0.004$). Statistically significant differences in virological response were maintained out to 60 wk of follow-up; the probability of virological failure through week 60 was 4% in the emtricitabine group vs 12% in the stavudine group

($p < 0.001$). At week 48, mean CD4 increases were not statistically different between the two treatment arms (emtricitabine, 168 cell/mm^3; stavudine, 134 cells/mm^3; $p = 0.15$).

Genotypic analysis was performed on 13 patients with virological failure in the emtricitabine group and 35 patients with virological failure in the stavudine group. At least one new genotypic mutation developed in 11% of patients with virological failure in the stavudine vs 4% in the emtricitabine group ($p = 0.005$); failure with wild-type virus was more frequent in the emtricitabine arm (4/285 patients, 1.4%) than in the stavudine arm (1/286 patients, 0.3%). Of the 13 patients experiencing virological failure in the emtricitabine group, 85% (11 patients) developed an NNRTI-associated mutation; 45% (5/11 patients) also developed an M184V/I mutation and 9% (1/11 patients) developed a K65R mutation. Of the 35 patients experiencing virological failure in the stavudine arm, 89% (31/35 patients) developed an NNRTI-associated mutation; 20% (7/35 patients) also developed a thymidine analog mutation, and 9% (3/35 patients) developed the L74V mutation.

Adverse event frequencies that were statistically significantly different between arms were diarrhea, nausea, lactic acidosis, lipodystrophy, abnormal dreams, neuropathy, paresthesia, skin discoloration, and increased cough. Cough and skin discoloration were more frequent in the emtricitabine arm. Pancreatitis and symptomatic lactic acidosis were observed only in the stavudine arm. Lipodystrophy was reported by the investigator more frequently in the stavudine arm (6%) than in the emtricitabine arm (0.4%). Ten patients in the emtricitabine arm (3%) and one patient in the stavudine arm (0.3%) had skin discoloration, manifested by hyperpigmentation of the palms and/or soles. The relative risk of peripheral neuropathy was 2.7-fold higher in the stavudine group than in the emtricitabine group (95% confidence interval [CI], +1.7 to +4.4). The number of patients with serious adverse events did not differ significantly between the two treatment arms. Grade 3 and 4 laboratory abnormalities were similar between the two treatment arms, with the exception of elevation of serum amylase (10% in the stavudine group vs 5% in the emtricitabine group; $p = 0.02$). The probability of developing a new treatment-limiting adverse event was higher in the stavudine (15%) than in the emtricitabine (7%) group ($p = 0.005$) *(29)*.

Preliminary data are available from an ongoing, multicenter, phase III, randomized, open-label 96-wk clinical trial, comparing the nucleoside/nucleotide backbone, emtricitabine plus tenofovir, with the dual nucleoside backbone, zidovudine plus lamivudine, both backbones in combination with efavirenz *(30,31)*. Gilead 934 enrolled 517 patients in the United States and Europe. Subjects received 600 mg efavirenz QD and either Combivir (300 mg zidovudine plus 300 mg lamivudine, as a fixed-dose combination pill) BID or 200 mg

emtricitabine QD plus 300 mg tenofovir QD (not as a fixed-dose combination pill). All subjects were antiretroviral naive and had plasma HIV RNA levels greater than 10,000 copies/mL at baseline. In the 48-wk analysis of 509 patients, 81% of patients in the emtricitabine plus tenofovir arm, compared with 70% of patients in the Combivir arm, achieved and maintained an HIV RNA level of fewer than 400 copies/mL, using the time-to-loss of virological response algorithm ($p = 0.005$; 95% CI, +3.4% to +18.1%). Similarly, 77% of emtricitabine plus tenofovir patients and 68% of Combivir patients achieved and maintained an HIV RNA level of fewer than 50 copies/mL ($p = 0.034$; 95% CI, +0.9% to +16.2%). In addition, at week 48, patients receiving emtricitabine plus tenofovir had a greater CD4 cell response than patients receiving Combivir (190 cells/mm^3 vs 158 cells/mm^3; $p = 0.002$). Adverse events leading to permanent discontinuation were more frequent in the Combivir (9%) arm than in the emtricitabine plus tenofovir (4%) arm ($p = 0.016$). The most common of these adverse events were anemia (6% vs 0%), nausea (2% vs 1%), vomiting (1% vs 0%), fatigue (1% vs 0%), NNRTI-associated rash (0% vs 1%) and neutropenia (1% vs 0%) in the Combivir and emtricitabine plus tenofovir arms, respectively *(31)*.

Treatment-Experienced Patients

Emtricitabine has also been studied in antiretroviral-experienced patients, either as part of a simplification regimen after PI-based therapy or as a replacement for lamivudine in virologically suppressed patients.

The ALIZE/ANRS 099 study was a randomized, phase III, open-label, multicenter study, in which 355 patients were randomized to continue their PI-based regimen (two NRTIs plus one PI) or to switch to a QD regimen of 200 mg emtricitabine QD plus 400 mg didanosine QD (as Videx EC; 250 mg didanosine QD if patient weight was <60 kg) plus 600 mg efavirenz QD *(32)*. All enrolled subjects were naive to NNRTI and had HIV RNA suppression to fewer than 400 copies/mL for at least 6 mo and a CD4 cell count of at least 100 cells/mm^3 at the time of screening.

In the primary endpoint analysis, 87.6% of the PI group and 90.5% of the QD group sustained virological suppression to fewer than 400 copies/mL (lack of two consecutive viral load measurements ≥400 copies/mL between week 0 and week 48, by intent-to-treat, missing = failure analysis). The treatment difference was −2.9%, less than the predefined noninferiority threshold. The intent-to-treat analysis and a receiving-study-medication analysis both indicated no significant difference in the probability of virological failure (HIV RNA level ≥400 copies/mL on two consecutive measurements) between the PI and QD study groups. In a secondary analysis, the probability of follow-up without virological failure, using a 50 copies/mL threshold, was greater in the

Table 3
FTC-303 Results at Week 48 *(33)*[a]

Parameters	Emtricitabine (n = 294), % of patients	Lamivudine (n = 146), % of patients
Responder[b]	77% (67%)	82% (72%)
Virological failure (>400 copies/mL)	7%	8%
Rebound	5%	5%
Never suppressed through week 48	2%	3%
Study discontinuation		
Because of adverse events	4%	0%
For other reasons[c]	12%	10%
Death	0%	<1%

n, Number of patients
[a]Gilead Sciences, data on file
[b]Percentage of patients who achieved and maintained confirmed HIV RNA levels of fewer than 400 copies/mL (<50 copies/mL) through week 48
[c]Includes lost to follow-up, patient's withdrawal, noncompliance, protocol violation, and other reasons

QD (87%) group than in the PI (79%) group ($p < 0.05$). Median increases in CD4 cell count between week 0 and week 48 were similar in the PI (15 cells/mm^3) and QD (16 cells/mm^3) arms. Genotypic analysis was available in five patients in the QD arm, and all samples had detectable mutations associated with resistance to emtricitabine (M184V) and NNRTIs (K103N in four patients and L100I in two patients). Adverse events in the QD arm were predominantly neurosensorial (efavirenz related) and increases in hepatic aminotransferases *(32)*.

FTC-303 was a phase III, 48-wk, open-label switch study comparing continued lamivudine therapy with a switch to emtricitabine in 440 HIV-infected individuals who had been on a stable NNRTI or PI regimen containing lamivudine and either stavudine or zidovudine for at least 12 wk *(33)*. Patients were randomized in a 2:1 fashion to switch to emtricitabine or remain on lamivudine, with maintenance of their thymidine NRTI and either NNRTI or PI. Median viral load on entry was 1.7 $\log_{10}$ copies/mL and the mean CD4 cell count was 527 cells/mm^3.

The probability of sustained viral suppression at week 48 was equivalent between treatment arms at both the 50 and 400 copies/mL threshold, with a probability of virological failure of 7% in the emtricitabine arm and 8% in the lamivudine arm (Table 3). Increases in CD4 cell counts at week 48 were not different between the emtricitabine and lamivudine arms (29 and 61 cells/mm^3, respectively), however, a statistically greater increase in CD4 cell percentages

was noted in the emtricitabine arm (2.5% vs 1.7%; $p = 0.038$). At the time of virological failure, the M184V mutation associated with resistance to emtricitabine and lamivudine was detected in 20 of 21 samples from patients receiving emtricitabine and 9 of 10 patients receiving lamivudine. Of note, baseline genotyping detected M184V in 16 of 18 patients failing emtricitabine treatment and in 3 of 4 patients failing lamivudine treatment *(33)*.

No significant differences in adverse event frequencies were seen between treatment arms. Emtricitabine and lamivudine were well-tolerated by the majority of patients, and side effects were predominantly mild to moderate in both treatment arms. Skin discoloration, predominantly a mild and asymptomatic hyperpigmentation of the palms and/or soles, occurred in 1.7% of emtricitabine recipients and 1.4% of lamivudine recipients.

Participants in FTC-303 who were stably suppressed to an HIV RNA level of at most 400 copies/mL at week 48 continued in an open-label extension protocol, Study 350 *(33)*. Two hundred fifteen patients continued emtricitabine and 74 patients switched from lamivudine to emtricitabine (Gilead Sciences, data on file). Among emtricitabine-treated patients continuing in Study 350, the probability of virological failure at 4 yr was 11%. During 4 yr of treatment, emtricitabine was well tolerated, with a probability of treatment-limiting adverse events requiring discontinuation of emtricitabine of 13% *(33)*. In the subset of patients receiving zidovudine, the probability of virological failure (6.5% overall) and the time-to-loss of virological response, were low through 228 wk of follow-up *(34)*.

EMTRICITABINE TOXICITY

More than 2000 adult patients have received emtricitabine, either alone or in combination with other antiretroviral agents, for periods of 10 d to 200 wk in phase I to III clinical trials (Gilead Sciences, data on file). Short- and long-term clinical trials have demonstrated a low incidence of emtricitabine-related adverse events.

Safety data through 48 wk of treatment with emtricitabine are available from two large randomized clinical trials, Study FTC-303 (571 treatment-naive patients) and Study FTC-301A (440 treatment-experienced patients) (Table 4) *(29,33)*. The most common clinical adverse events were headache, diarrhea, nausea, and rash, which were generally of mild-to-moderate severity. Approximately 1% of study participants discontinued treatment because of these events. Of note, all adverse events were reported with similar frequency in the emtricitabine and control groups, with the exception of skin discoloration, which was reported with higher frequency in emtricitabine recipients. Skin discoloration was manifested by hyperpigmentation on the palms and/or soles and was generally mild and asymptomatic. Laboratory abnormalities in

Table 4
Selected Treatment-Emergent Adverse Events (All Grades, Regardless of Causality) Reported in at Least 3% of Emtricitabine-Treated Patients in Either Study FTC-301A or FTC-303 (0–48 Wk)[a]

	Study FTC-303 (% of patients)		Study FTC-301A (% of patients)	
Adverse event	Emtricitabine + ZDV/d4T + NNRTI/PI (n = 294)	3TC + ZDV/d4T + NNRTI/PI (n = 146)	Emtricitabine + d4T + ddI + EFV (n = 286)	ddI + EFV (n = 285)
Body as a whole				
Abdominal pain	8%	11%	14%	17%
Asthenia	16%	10%	12%	17%
Headache	13%	6%	22%	25%
Digestive system				
Diarrhea	23%	18%	23%	32%
Dyspepsia	4%	5%	8%	12%
Nausea	18%	12%	13%	23%
Vomiting	9%	7%	9%	12%
Musculoskeletal				
Arthralgia	3%	4%	5%	6%
Myalgia	4%	4%	6%	3%
Nervous system				
Abnormal dreams	2%	<1%	11%	19%
Depressive disorders	6%	10%	9%	13%
Dizziness	4%	5%	25%	26%
Insomnia	7%	3%	16%	21%
Neuropathy/peripheral	4%	3%	4%	13%
Neuritis				
Paresthesia	5%	7%	6%	12%
Respiratory				
Increased cough	14%	11%	14%	8%
Rhinitis	18%	12%	12%	10%
Skin				
Rash event[b]	17%	14%	30%	33%

ZDV, zidovudine; d4T, stavudine; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; 3TC, lamivudine; ddI, didanosine; EFV, efavirenz; *n*, number of patients

[a]Emtriva US Prescribing Information *(3)*

[b]Rash event includes rash, pruritus, maculopapular rash, urticaria, vesiculobullous rash, pustular rash, and allergic reaction

Table 5
Treatment-Emergent Grade 3/4 Laboratory Abnormalities Reported in at Least 1% of Emtricitabine-Treated Patients in Either Study FTC-301A or FTC-303 (0–48 Wk)[a]

	Study FTC-303 (% of patients)		Study FTC-301A (% of patients)	
Laboratory abnormalities	Emtricitabine + ZDV/d4T + NNRTI/PI (*n* = 294)	3TC + ZDV/d4T + NNRTI/PI (*n* = 146)	Emtricitabine + ddI + EFV (*n* = 286)	d4T + ddI + EFV (*n* = 285)
Total	31%	28%	34%	38%
ALT (>5 × ULN)	2%	1%	5%	6%
AST (>5 × ULN)	3%	<1%	6%	9%
Bilirubin (>2.5 × ULN)	1%	2%	<1%	<1%
Creatine kinase (>4 × ULN)	11%	14%	12%	11%
Neutrophils (<750 mm^3)	5%	3%	5%	7%
Pancreatic amylase (>2 × ULN)	2%	2%	<1%	1%
Serum amylase (>2 × ULN)	2%	2%	5%	10%
Serum glucose (<40 or >250 mg/dL)	3%	3%	2%	3%
Serum lipase (>2 × ULN)	<1%	<1%	1%	2%
Triglycerides (>750 mg/dL)	10%	8%	9%	6%

ULN, upper limit of normal; AST, alanine aminoaspartate; ALT, alanine aminotransferase; ZDV, zidovudine; d4T, stavudine; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; 3TC, lamivudine; ddI, didanosijne; EFV, efavirenz; *n*, number of patients
[a]Emtriva US Prescribing Information *(3)*

these studies occurred with similar frequency in the emtricitabine and comparator groups (Table 5).

The favorable safety profile of emtricitabine was confirmed in a 48-wk study in HIV-seronegative patients with chronic HBV infection *(35)*. In this study, 167 subjects received 200 mg emtricitabine QD. Serious adverse events were similar between the emtricitabine (8%) and placebo (9%) groups (13/167 patients vs 7/81 patients, respectively). Five emtricitabine recipients discontinued the study drug; two patients because of mild skin hyperpigmentation, two because of elevated creatinine kinase, and one because of pregnancy *(35)*.

During 4 yr of emtricitabine treatment in Study 350, the probability of treatment-limiting adverse events requiring discontinuation of emtricitabine was

13%. In this population, grade 3 or 4 laboratory abnormalities occurred at the following annualized incidence: creatine kinase (7%), hypertriglyceridemia (5%), aspartate aminotransferase (2%), neutropenia (2%), alanine aminotransferase (2%), glucose (2%), and amylase (1%) *(33)*. In the subset of patients in Study 350 who were receiving zidovudine (123 patients initially randomized to switch to emtricitabine and 68 patients randomized to continue with lamivudine), tolerability failure (death or adverse event leading to permanent discontinuation of emtricitabine) was 8.9% overall. With a mean follow-up of 143 wk, 4 patients (3%) developed an adverse event leading to discontinuation of emtricitabine and 26 patients (22%) developed a serious adverse event. An additional 53 patients (45%) had a grade 3 or 4 laboratory abnormality; of these, 27 patients (23%) because of elevation in creatine kinase and 15 patients (13%) because of elevated triglycerides *(34)*.

In a recent analysis of 814 patients enrolled in phase III clinical trials of emtricitabine, 29 (4%) developed discoloration of their skin, nails, or tongue. This was a slightly higher percentage than seen in control populations. Higher rates were reported in black patients. None of the affected patients discontinued emtricitabine because of this adverse event. In 17% of patients (5/29 patients), the discoloration resolved *(36)*.

Earlier concerns had been expressed regarding a high incidence of liver toxicity in study FTC-302, but this was later found to be caused by the use of nevirapine in combination with stavudine *(27,37,38)*. Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogs alone or in combination, including emtricitabine and other antiretroviral treatments (ARTs). A majority of these cases have been in women. Obesity and prolonged nucleoside exposure may be risk factors, however, cases have been reported in patients with no known risk factors. Treatment with emtricitabine should be suspended in any patient who develops clinical or laboratory findings suggestive of lactic acidosis or pronounced hepatotoxicity (which may include hepatomegaly and steatosis even in the absence of marked transaminase elevations) *(3)*.

Emtricitabine is active against HBV in vitro and in vivo, and severe acute exacerbations of HBV have been reported in patients after discontinuation of emtricitabine. It is recommended that all patients with HIV be tested for the presence of chronic HBV infection before initiating antiretroviral therapy. Liver function should be closely monitored with both clinical and laboratory follow-up for at least several months in patients who discontinue emtricitabine and are coinfected with HIV and HBV. Emtricitabine is not indicated for the treatment of chronic HBV infection, and the safety and efficacy of emtricitabine have not been established in patients coinfected with HBV and HIV *(3)*.

EMTRICITABINE IN PEDIATRIC PATIENTS: PHARMACOKINETICS, EFFICACY, AND SAFETY

Pharmacokinetics

The pharmacokinetic profile of emtricitabine was established in children in a phase I, multicenter, open-label trial (FTC-105) *(39)*. Two dose levels of emtricitabine were studied in children younger than 18 yr of age: 60 mg/m^2 and 120 mg/m^2, up to a maximum of 200 mg, in an oral solution formulation (10 mg/mL). Children 6 yr of age and older who could swallow the solid oral dosage formulations also received a third dose of 120 mg/m^2 of emtricitabine (to the nearest 25 mg) in a capsule formulation. The 25 patients studied ranged in age from 1.8 to 17.8 yr (median 7.6 yr) and ranged in weight from 10.2 to 76 kg (median 24.5 kg). The resultant data demonstrated mean concentration-vs-time profiles that were similar across age groups, and apparent total body clearance (CL/F) values that normalized to the body surface area. The capsule formulation provided approx 20% higher plasma exposure than the solution formulation; accordingly, the maximum dose of the oral solution was 240 mg. Based on these data, it was projected that a 6-mg/kg dose (up to a maximum of 240 mg) of emtricitabine oral solution would produce a plasma AUC of emtricitabine in children comparable to that of adults administered a 200-mg dose *(39)*.

Clinical Efficacy in Pediatric Patients

Emtricitabine is currently under study in the pediatric population in two multicenter clinical trials, the phase I/II Pediatric AIDS Clinical Trials Group (PACTG) 1021/FTC-202 study and the large phase II international study, FTC-203.

FTC-203 enrolled 116 patients between the ages of 3 mo and 17 yr of age, at 12 sites in the United States, Mexico, Panama, and South Africa *(40)*. Seventy-one patients were naive to ART and were initiated on emtricitabine (6 mg/kg of the oral solution, up to 240 mg; or a 200-mg capsule) plus stavudine (at standard pediatric doses) plus lopinavir/ritonavir (at standard pediatric doses). Forty-five patients were switched from a stable, lamivudine-containing regimen (stable regimen for ≥3 mo and HIV RNA level of ≤400 copies/mL) to an emtricitabine-containing regimen, with change in background regimen at the investigator's discretion. The median time on study was 96 wk (range 6–172 wk). At week 48, 93% of the ART-naive patients and 87% of the ART-experienced patients achieved and/or maintained durable suppression of plasma HIV-1 RNA of at most 400 copies/mL; 78% of both groups had suppression to 50 copies/mL or fewer. Confirmed virological failures at week 48 were similar in both groups (7% of ART-naive and 4% of ART-experienced patients). Four of five treatment-naive patients with paired baseline and time-of-failure genotypic analysis demonstrated emergence of the M184V mutation associated with emtricitabine resistance. No

treatment-emergent mutations were detected in three ART-experienced patients who had genotyping at baseline and time-of-failure. Overall, 12% of patients experienced at least one severe (grade 3 or greater) adverse event. Adverse events of moderate or greater (≥grade 2) severity that were judged possibly or probably related to emtricitabine included leukopenia (two patients), anemia (one patient), pancreatitis/pleural effusion (one patient), Herpes zoster (one patient), vomiting (one patient), and skin discoloration (one patient).

An ongoing, phase I/II open-label study was conducted by the PACTG. PACTG P1021/FTC-202 evaluated the pharmacokinetics, safety, and efficacy of a QD regimen of 6 mg/kg emtricitabine daily (maximum of 200 mg/d) plus 240 mg/m^2 didanosine daily (maximum of 400 mg) plus efavirenz (adjusted by body weight to a maximum of 600 mg daily as capsules or 720 mg daily as the oral liquid) *(41,42)*. A total of 37 children between 3 and 21 yr of age (median 10.5 yr) were enrolled. At baseline, median HIV RNA levels were 47,775 copies/mL and the median CD4 count was 310 cells/mm^3. Similar to the results of FTC-203, described above, 79% of patients achieved virological suppression at 48 wk, both to fewer than 400 copies/mL and to fewer than 50 copies/mL (refs. *41* and *42*, and Gilead Sciences, Foster City, CA, data on file).

Safety in Pediatric Patients

In the phase I study reported by Wang et al. (FTC-105), 13 of 29 adverse events (45%) were considered drug-related. The most frequently reported adverse events were vomiting, diarrhea, abdominal pain, and headache. All drug-related adverse events were mild, and no subject experienced a treatment-emergence event of grade 3 or greater intensity. None of the abnormal test results was felt to be clinically significant *(39)*.

In the other two clinical studies described above, emtricitabine was administered together with stavudine plus lopinavir/ritonavir (FTC-203, treatment-naive patients), with other background antiretroviral treatments (FTC-203, treatment-experienced patients), or with didanosine plus efavirenz (PACTG P1021/FTC-202). In study FTC-203, seven patients experienced an adverse event that was at lead moderate in severity and possibly or probably related to emtricitabine, including one patient who experienced unresolved grade-2 skin discoloration. Seven percent of treatment-naive and 11% of treatment-experienced patients experienced a grade 3/4 laboratory abnormality. One treatment-naive and one treatment-experienced patient discontinued the study before week 48 because of adverse events (anemia and pancreatitis). In PACTG P1021/FTC-202, emtricitabine was administered with efavirenz, and two patients discontinued treatment because of a grade 2 or 3 rash, two developed a grade 3 creatine phosphokinase elevation, and one patient developed a low serum glucose, all judged possibly related to emtricitabine *(41,42)*.

RESISTANCE TO EMTRICITABINE

Resistance to emtricitabine is associated with a mutation in codon 184 of the HIV-1 reverse transcriptase, resulting in the substitution of the methionine by valine or isoleucine (M184V/I) *(3,43)*. These isolates have been selected by in vitro passage studies, and the same M184V/I mutation is selected by lamivudine. Emtricitabine-resistant isolates have been detected from patients with virological failure on emtricitabine alone or in combination. However, in vitro studies suggest a slower emergence of this mutation than for lamivudine *(43)*, and, in clinical trials, M184V is observed much less frequently during emtricitabine treatment than during lamivudine treatment *(44–46)*.

The FTC-301A study analyzed the emergence of new mutations at the time of virological failure in two randomized treatment arms, which compared emtricitabine with stavudine, both in combination with didanosine and efavirenz. Of 57 subjects who experienced virological failure, the incidence of new mutations was significantly lower in the emtricitabine arm than in the stavudine arm. For patients who entered the study with wild-type virus, the incidence of new mutations was significantly greater in the stavudine arm than in the emtricitabine arm ($p = 0.012$). The M184V/I mutations occurred significantly more frequently in the emtricitabine arm than in the stavudine arm ($p < 0.001$), with 37.5% of patients developing this mutation at the time of virological failure vs 0% of patients, respectively (Table 6). In this analysis, the presence of an NNRTI mutation at baseline was predictive of virological failure in both stavudine and emtricitabine arms ($p = 0.003$ for stavudine and $p = 0.026$ for emtricitabine); an even stronger association was seen between a baseline K103N mutation and virological failure ($p < 0.001$). The presence of NRTI mutations, however, was only associated with virological failure in the stavudine arm ($p = 0.055$) *(45)*.

In the FTC-303 study, patients on a lamivudine-containing regimen were randomized 1:2 to continue lamivudine or switch to emtricitabine. Failure rates were similar between the emtricitabine and lamivudine arms at week 48 (7% and 8%, respectively). Baseline genotyping for codon 184 was successful in 23 of these 32 patients; 20 of 23 sequences revealed the M184V mutation at baseline. Patients with at least 50 copies/mL of HIV RNA at baseline were more likely to fail treatment (39% compared with 5% of those with a baseline HIV RNA level of <50 copies; $p < 0.0001$). Among these higher baseline viral load patients, those with the M184V mutation at baseline were significantly more likely to fail than those without the mutation (50% vs 9%, respectively; $p = 0.03$).

Emtricitabine-resistant viruses containing the M184V/I mutations are cross-resistant to lamivudine and didanosine, but retain sensitivity to tenofovir, zidovudine, stavudine, abacavir, didanosine, and NNRTIs. Viruses containing the K65R mutation, selected for in vitro by abacavir, didanosine, tenofovir, and

Table 6
Incidence of New Mutations in Patients With Virological Failure in FTC-301A[a] *(45)*

Mutation	Emtricitabine arm (n = 16), no. of patients (%)	Stavudine arm (n = 41), no. of patients (%)
Any	11 (69%)	35 (85%)
NNRTI	10 (63%)	35 (85%)
Any TAMs	0	6 (15%)
L74V, K65N	1 (6%)	3 (7%)
M184V/I	6 (37.5%)	0
No change	5 (31%)	6 (15%)

n, Number of patients; NNRTI mutations, K103N, L100I, G190A/E/S, Y188C/Y, K101E/N, V106I/M, A98G, V108I, and P225H; TAM mutations, T215F/I/S/Y, D67G, and K219N; ddI mutations, L74V and K65N; NNRTI, non-nucleoside reverse transcriptase inhibitor; TAM, thymidine analog mutations; ddI, didanosine

[a]Failure defined as never achieving fewer than 400 copies/mL or rebound greater than 400 copies/mL on two consecutive measurements

zalcitabine, demonstrate reduced susceptibility to emtricitabine. Viruses containing mutations conferring reduced susceptibility to zidovudine and stavudine (thymidine analog mutations M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E) or didanosine (L74V) remain sensitive to emtricitabine.

In combination with other antiretroviral agents, distinct patterns of emergence of resistance mutations are seen *(47)*. M184V is the most common mutation seen in viruses from patients failing a regimen with emtricitabine plus tenofovir; K65R is also seen regularly, whereas thymidine analog mutations and L74V are rare. Patients who fail a regimen containing emtricitabine plus abacavir may harbor resistant viruses containing the L74V mutation; K65R is rarely seen in these patients *(47)*.

USE OF EMTRICITABINE IN THE DEVELOPED WORLD

Emtricitabine has been prescribed with increasing frequency since licensure in July 2003. It shares an excellent safety profile with lamivudine and has a longer half-life, which has prompted some physicians to preferentially use emtricitabine over lamivudine in initial and subsequent treatment regimens. Emtricitabine is recommended as a preferred NRTI for initial therapy by the US Department of Health and Human Services Guidelines *(1)*.

USE OF EMTRICITABINE IN THE DEVELOPING WORLD

Recent studies of new antiretroviral agents have included clinical sites in the developing world. These studies allow an evaluation of tolerability and efficacy in patients with a variety of concomitant conditions and infectious diseases,

such as poor nutrition, tuberculosis, and parasitic disease, in addition to their HIV infection. The importance of extending life-sustaining ART to the millions of infected individuals cannot be overstated, and this has increasingly been brought to the attention of the public and the governmental agencies who serve them. In addition to the minimum provision of any antiretroviral therapy, there is mounting concern regarding long-term side effects, such as lipoatrophy, which occur at different frequencies with different antiretroviral agents *(48)*. Emtricitabine, either alone, or in the fixed-dose combination pill, Truvada, has an excellent safety profile and efficacy. Truvada will be part of the global access program at Gilead Sciences, which provides antiretroviral agents at cost to developing nations (Gilead Sciences, personal communication).

EMTRICITABINE CONCLUSION

Although the cross-resistance between emtricitabine and lamivudine means that emtricitabine has limited usefulness as part of a salvage regimen, excellent efficacy and tolerability data suggest that it will play an important role in highly potent combinations for first-line treatment. The added convenience of the fixed-dose combination with tenofovir (Truvada), providing a one-pill-a-day nucleoside backbone for triple therapy, suggests that use of emtricitabine will continue to expand. In December 2004, Gilead Sciences and Bristol-Myers Squibb announced the establishment of a joint venture to develop and commercialize a fixed-dose combination pill containing efavirenz (Sustiva®, Bristol-Myers Squibb, Princeton, NJ) and Truvada (Gilead Sciences). The resultant single-pill daily regimen would provide a regimen (emtricitabine plus tenofovir plus efavirenz) that is one of the preferred NNRTI-based treatments for use in appropriate patients who are antiretroviral-therapy naive *(1)*.

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5
Nucleotide Analogs

Craig J. Hoesley

INTRODUCTION

The nucleotide analogs are agents with proven in vitro and in vivo efficacy against a wide variety of DNA viruses and retroviruses. Structurally, nucleotide analogs are acyclic nucleoside phosphonates (nucleoside monophosphates) that are designed to circumvent the first phosphorylation step necessary for the activation of nucleoside analogs, such as zidovudine, stavudine, didanosine, lamivudine, and abacavir *(1)*.

To date, three nucleotide analogs have received Food and Drug Administration (FDA) approval in the United Sates: cidofovir, adefovir dipivoxil, and tenofovir disoproxil fumarate (tenofovir DF). Cidofovir ([S]-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine; Vistide™) is an intravenous drug approved for treatment of retinitis caused by cytomegalovirus infection. Adefovir dipivoxil (9-[2-{*bis*pivaloylmethoxymethyl}phosphonylmethoxyethyl]adenine; Hepsera™) is an oral prodrug of adefovir (phosphonylmethoxyethyl adenine [PMEA]) and is approved for the treatment of chronic active hepatitis B virus (HBV) infection in adults. Tenofovir DF (Viread™) is the oral prodrug of tenofovir (9-[(R)-(2-phosphonylmethoxy)propyl]adenine [PMPA]) and is approved for the treatment of HIV infection in combination with other antiretroviral infections. The chemical formulae of adefovir dipivoxil and tenofovir DF are shown in Figs. 1 and 2, respectively.

This chapter will provide an overview of the in vitro activity, pharmacological properties, clinical efficacy data, and toxicity profiles of tenofovir DF, and, to a lesser extent, adefovir dipivoxil. Although both of these drugs are HIV reverse transcriptase inhibitors, only tenofovir DF has received FDA approval for the treatment of HIV-1 infection. Conversely, the clinical development of adefovir dipivoxil for the treatment of HIV infection was limited by adverse events (specifically, reversible proximal renal tubular dysfunction) and is not approved for use in HIV infection, but, at lower doses than previously used in

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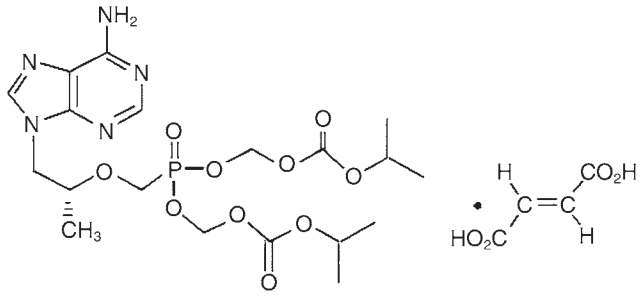

Fig. 1. Structure of tenofovir DF, 9-[(R)-(2-phosphonylmethoxy)propyl]adenine.

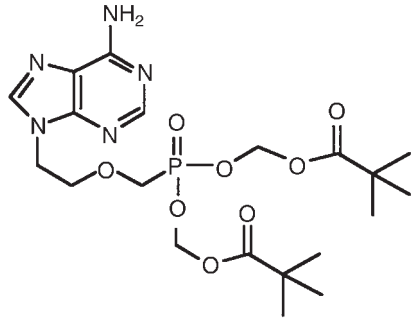

Fig. 2. Structure of adefovir dipivoxil, 9-[2-(*bis*pivaloylmethoxymethyl)phosphonylmethoxyethyl]adenine.

HIV clinical trials, adefovir dipivoxil is efficacious and safe in the treatment of chronic HBV infection.

MECHANISM OF ACTION AND IN VITRO ACTIVITY

Adefovir dipivoxil and tenofovir DF are lipophilic ester derivatives of adefovir and tenofovir, respectively, and were designed as prodrugs in effort to improve oral bioavailability. After oral administration and adsorption, adefovir dipivoxil and tenofovir DF are rapidly cleaved by nonspecific carboxylesterases into adefovir and tenofovir, respectively. Once inside cells, these compounds are metabolized by adenylate cyclase to adefovir monophosphate and tenofovir monophosphate, and, subsequently, by nucleoside diphosphate kinase to adefovir diphosphate (PMEApp) and tenofovir diphosphate (PMPApp), the active moieties. The antiviral effect of the drugs is the result of selective interaction of the diphosphate metabolite (PMEApp and PMPApp) with the viral DNA polymerase. Based on the structural resemblance to natural deoxyadenine triphosphates (dATP), PMEApp and PMPApp may act as both a competitive inhibitor and an

alternative substrate during the DNA polymerase chain reaction, resulting in DNA chain termination *(1)*. The antiviral activity of unphosphorylated nucleoside analogs (e.g., zidovudine, stavudine, didanosine, lamivudine, and abacavir) may be impeded by their low affinity for cellular nucleoside kinases. The nucleotide analogs differ from these "classic" nucleoside analogs in that they may be less dependent on intracellular enzymes for activation *(2)*.

Adefovir and tenofovir are both active in vitro and in vivo against hepadnaviruses (such as HBV) and retroviruses *(3–5)*. The spectrum of in vitro antiretroviral activity is similar for both compounds, and includes HIV-1 and HIV-2, simian immunodeficiency virus, feline immunodeficiency virus, visnamaedi virus of sheep, and murine leukemia and sarcoma viruses *(6–9)*. Of note, tenofovir has demonstrated activity against non-B HIV-1 subtypes. The mean 50% inhibitory concentration (IC_{50}) values for tenofovir against HIV-1 subtypes A, C, D, E, F, G, and O in primary peripheral blood mononuclear cell cultures were all within twofold of the HIV subtype B IC_{50} value (range, 0.55–0.22 μ*M*) *(10)*. Although cidofovir is not active against retroviruses, it possesses in vitro activity against a variety of DNA viruses, including herpes simplex viruses 1 and 2, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, papillomavirus, polyomavirus, adenovirus, and poxvirus *(6)*.

Tenofovir has demonstrated an anti-HIV effect in both lymphocytes and macrophages *(11)*. The K_i (inhibition constant) value of tenofovir diphosphate (PMPApp) against HIV-1 reverse transcriptase is 0.022 μ*M*, which is approx 200-, 3700-, and 2700-fold lower than the K_i values against human DNA polymerase-α, -β, and -γ, respectively. The K_i value of adefovir diphosphate (PMEApp) is 0.012 μ*M* and is comparable to tenofovir diphosphate *(12)*. Moreover, tenofovir and tenofovir DF were evaluated in vitro for antiviral activity (IC_{50}) and cytotoxicity (50% cytotoxicity concentration) using both laboratory and clinical HIV-1 strains, and both compounds had favorable selectivity indices (50% cytotoxicity concentration-to-IC_{50} ratios greater than 2000) *(13)*.

Several studies have compared the in vitro potency of tenofovir with other anti-HIV agents. In general, the antiviral activity of tenofovir against HIV-1 wild-type laboratory strains ranges from 0.2 to 6.0 μ*M* *(13)*. In vitro, PMPApp (K_i = 0.022 μ*M*) and PMEApp (K_i = 0.012 μ*M*) are slightly less potent than the active phosphorylated metabolite of zidovudine (K_i = 0.008 μ*M*) *(12)*. The potency of tenofovir DF monotherapy (one 300-mg tablet daily) was assessed in 10 antiretroviral-naive HIV-1-infected individuals who underwent frequent monitoring of plasma HIV-1 RNA levels during 3 wk. The individual first-phase decay slopes of the plasma HIV-1 RNA levels ranged from –0.24 to –0.59 $\log_{10}$ copies/mL (median, 0.40 $\log_{10}$ copies/mL) which is similar to values previously described with ritonavir monotherapy, indicating comparable potency between the two drugs *(14)*.

In vitro analysis of MT2 cells infected with the HIV-IIIb strain were used to assess the activity of tenofovir in combination with a variety of other antiretroviral agents. The combination of tenofovir plus zidovudine, amprenavir, or non-nucleoside reverse transcriptase inhibitors (NNRTIs) demonstrated strong synergistic anti-HIV activity, whereas combinations with didanosine, adefovir, or nelfinavir resulted in resulted in minor synergistic inhibition of in vitro HIV-1 replication. Additive HIV inhibition was noted with tenofovir in combination with abacavir, stavudine, lamivudine, zalcitabine, ritonavir, saquinavir, or indinavir *(15,16)*. Using similar techniques, the combinations of adefovir with zidovudine, stavudine, zalcitabine, saquinavir, ritonavir, or nelfinavir demonstrated modest synergy. Additive inhibition was seen when adefovir was combined didanosine, lamivudine, or indinavir *(15,16)*. No tenofovir- or adefovir-containing combination regimen demonstrated antagonism.

PHARMACOKINETICS

The formulation of tenofovir DF and adefovir dipivoxil as oral prodrugs for tenofovir and adefovir, respectively, has allowed for improved oral bioavailability. In vitro metabolic studies using radiolabeled tenofovir and tenofovir DF in resting and activated peripheral blood lymphocytes demonstrated a more than 1000-fold higher intracellular concentration of the active diphosphorylated metabolite (PMPApp) after incubation with the prodrug compared with tenofovir alone *(13)*. Owing to its increased cellular permeability, the anti-HIV activity of tenofovir DF is increased by 17- to 90-fold over tenofovir. The diphosphorylation of both tenofovir and adefovir in cells seems to be independent of viral infection, and there is evidence that these active intracellular metabolites persist in cells after the drugs are removed, suggesting an antiviral effect may continue in absence of repeated tenofovir or adefovir exposure. Moreover, the long intracellular half-life of both PMPApp (~10–50 h) and PMEApp (~7 h) allows for once-daily dosing. Animal models have demonstrated that tenofovir is eliminated unchanged in the urine 24 h after intravenous dosing *(13)*. Tenofovir is not a substrate nor inhibitor of the cytochrome P450 enzyme system and does not have significant binding to plasma proteins *(17)*.

The pharmacokinetics of tenofovir and tenofovir DF have been studied in HIV-1-infected subjects. An aqueous suspension of tenofovir was administered intravenously in single doses of 1 mg/kg and 3 mg/kg, resulting in mean peak serum concentrations of 2.7 and 9.1 μg/mL, respectively *(18)*. Serum tenofovir concentrations declined in a biexponential manner, with a terminal half-life of 7.1 h. All subjects in the higher dose (3 mg/kg) group had quantifiable serum levels for up to 24 h after the initial dose.

In a randomized, double-blind, placebo-controlled phase I/II study (GS-97-901) in HIV-infected individuals, the pharmacokinetics of tenofovir DF were stud-

ied at doses of 75, 150, 300, and 600 mg administered in a tablet form. For each dose cohort, fasting patients received a single oral dose, and, after a 1-wk observation period, the same patients received a single oral dose after ingestion of a high-fat meal for 28 consecutive days. After fasting, the median peak plasma concentrations (C_{max}) were 68.6, 111, 240, and 618 ng/mL in the 75, 150, 300, and 600 mg groups, respectively. Tenofovir has a long terminal half-life in serum, of approx 17 h. At steady state (day 15), median C_{max} values were 80.8, 163, 303, and 633 ng/mL and area under the curve (AUC) serum concentration values were 717, 1613, 2937, and 6073 ng·hr/mL in the same dosing groups when the drug was administered in a fed state *(19)*. The oral bioavailability of tenofovir DF when administered with food was enhanced (~40%) and was not affected by repeated dosing. In general, tenofovir DF displayed linear pharmacokinetics through the 75 to 300 mg/d dose range and was rapidly converted to the active form (tenofovir) after oral absorption; this process was facilitated by administration in the fed state. In a recent study, single-dose and steady-state pharmacokinetics of 300 mg tenofovir DF daily were evaluated in HIV-infected children, and the AUC and C_{max} were comparable to those values seen in HIV-infected adults *(20)*.

TOXICITY

Tenofovir DF

The preclinical toxicology studies in animals (rats, dogs, and monkeys) administered tenofovir during a minimum of 11 months and identified the kidney and bone as potential target organs. Specifically, urinary excretion of calcium and phosphorus was increased in rats and dogs when tenofovir exposure was 11- and 9-fold higher than standard human exposure, respectively; these findings were reversible and not seen when exposure was increased 3- to 5-fold over standard human exposure. Moreover, glycosuria and proteinuria were described in dogs and monkeys at 9- and 12-fold increased exposure, but, again, was reversible and not described when dosing was 3- to 4-fold higher than standard human exposure. Decreased bone marrow density and increased parathyroid hormone secretion were noted in animals when dosing was increased 9- to 50-fold over standard human exposure. These bone toxicities were felt to be secondary to renal toxicity and were reversible with discontinuation of the drug or not seen at all when exposure was only twofold to fourfold higher than standard human exposure. Other adverse effects noted in animal studies were gastritis, duodenitis, and slight elevations in liver transaminases; these findings were limited to rats in the higher dosing groups (ref. *17*; and Gilead Sciences, Foster City, CA, personal communication).

More than 1600 HIV-infected individuals have received tenofovir DF alone or in combination with other antiretroviral agents in clinical trials, including a

proportion of study participants who have been treated for at least 3 yr. In general, tenofovir DF has been well-tolerated. In a randomized, double-blind, placebo-controlled phase II study (GS-98-902), HIV-infected patients received 75, 150, or 300 mg tenofovir DF, or placebo once daily for 24 wk in addition to the patient's existing stable antiretroviral regimen at the time of enrollment. At week 24, placebo patients received 300 mg tenofovir DF once daily and all treatment groups were followed for a total of 48 wk (*see* "Efficacy" section). At week 24, the number of grade 3 or 4 adverse events seen in subjects receiving tenofovir DF was similar to those receiving placebo (14–19%), and the percentage of grade 3 or 4 laboratory abnormalities was also similar among the treatment and placebo groups *(21)*. There was no evidence of dose-related increase in adverse events. In a separate randomized, double-blind, placebo-controlled phase III study (GS-99-907), HIV-infected patients received 300 mg tenofovir DF or placebo once daily for 24 wk in addition to their existing antiretroviral regimen. At week 24, placebo recipients received 300 mg tenofovir DF once daily and were followed for a total 48 wk (*see* "Efficacy" section). As noted in the phase II study, the number of grade 3 or 4 adverse events and laboratory abnormalities were not substantially different between the placebo and treatment groups *(22)*. A combined list of reported grade 3 or 4 laboratory abnormalities in these studies is presented in Table 1.

Interestingly, there have been no grades 2, 3, or 4 serum creatinine elevations associated with tenofovir DF administration in phase II or phase III clinical trials *(23)*. These findings are particularly significant given the high rate of proximal renal tubular dysfunction described with the structurally similar compound, adefovir dipivoxil. The frequency of grade 3 or 4 serum hypophosphatemia in clinical trials was low (~1%) and reversible with discontinuation of treatment *(23)*. In addition, bone mineral density testing was performed in a small proportion of subjects participating in the phase II study (GS-98-902), and no clinically significant decrease in bone mineral density from baseline was demonstrated in patients receiving tenofovir DF through 48 wk *(21)*. In a combined analysis of the phase II and phase III studies (GS-98-902 and GS-99-907), no increase in bone fracture rate was seen with tenofovir DF treatment (687 subjects with 778 patient-years of exposure; fracture rate 1.7/100 patient-years) compared with placebo recipients (210 subjects, 99 patient-years of follow-up; fracture rate 3.0/100 patient-years) *(24)*. In a safety study of patients with advanced HIV disease (mean absolute CD4 cell count, 36 cells/mm^3) who received tenofovir DF 300 mg daily in addition to their existing antiretroviral therapy, severe renal insufficiency and serum hypophosphatemia remained uncommon. A grade 3 or 4 serum creatinine elevation or serum hypophosphatemia were noted in 1% and 3% of participants, respectively *(25)*. In this study, previous adefovir dipivoxil therapy did not seem to be

Table 1
Combined Grade 3 or 4 Laboratory Abnormalities Reported in GS-99-907, GS-98-902 *(21–23)*

Placebo vs 300 mg tenofovir DF daily (0–24 wk)			All tenofovir DF recipients (mean 58 wk)[a]	
	Placebo, no. (%)	300 mg, no. (%)		No. patients (%)
Total patients	210	443	Total patients	687
Patients with abnormality[b]	99 (47)	146 (33)	Patients with abnormality[b]	377 (55)
Creatinine kinase elevation	30 (14)	36 (8)	Creatinine kinase elevation	103 (15)
Triglyceride elevation	28 (13)	37 (8)	Triglyceride elevation	89 (13)
Amylase elevation	14 (7)	21 (5)	Amylase elevation	47 (7)
AST elevation	6 (3)	16 (4)	AST elevation	40 (3)
Glycosuria	6 (3)	12 (3)	Glycosuria	27 (4)
Serum glucose elevation	8 (4)	8 (2)	Serum glucose elevation	24 (3)
ALT elevation	4 (2)	10 (2)	ALT elevation	30 (4)
Neutropenia	3 (1)	6 (1)	Neutropenia	17 (2)

AST, aspartate aminotransferase; ALT, alanine aminotransferase
[a]"All tenofovir DF recipients" group includes all patients in the 300 mg tenofovir DF group, those placebo recipients who later received 300 mg tenofovir DF in studies 902 and 907, and those subjects who initially received 75 mg or 150 mg tenofovir DF for 24 wk in study 902 who later received 300 mg tenofovir DF daily
[b]Abnormalities occurring in at least 2% of patients in any group

associated with an increased risk of nephrotoxicity. However, since completion of these clinical trials, and with expanded use of tenofovir DF in clinical practice, there have been several small case series or case reports describing proximal renal tubular dysfunction and/or hypophosphatemia in HIV-infected patients *(26,27)*.

In contrast to adefovir dipivoxil, serum carnitine deficiency has not been observed with tenofovir DF administration and is not expected, because pivalic acid, a component of adefovir dipivoxil, is not present in tenofovir DF.

To date, adverse events associated with mitochondrial dysfunction have not been common. Lactic acidosis was reported in 7 (1%) patients receiving 300 mg tenofovir DF daily in clinical trials, and all of these subjects were receiving stavudine or didanosine concomitantly *(24)*. These data correlate with the in vitro studies suggesting that tenofovir DF is less toxic to mitochondria and has less effect on mitochondrial DNA polymerase than stavudine, zalcitabine, or didanosine *(12,28)*.

Adefovir Dipivoxil

As stated previously, proximal renal tubular dysfunction was observed in a significant number of HIV-infected patients receiving adefovir dipivoxil in HIV efficacy studies. Proximal renal tubular dysfunction has been characterized as having three of the following abnormalities:

1. Serum creatinine at least 0.5 mg/dL over baseline.
2. Serum phosphate less than 2.0 mg/dL.
3. Serum bicarbonate less than 17 mEq/L.
4. Proteinuria (2+ or greater).
5. Glycosuria (1+ or greater).

In a phase III, placebo-controlled trial of 442 HIV-infected patients in which either 120 mg adefovir dipivoxil daily or placebo was added to a stable antiretroviral regimen, the cumulative percent of patients with proximal renal tubular dysfunction at 12 months was 17% in the adefovir group and 0.4% in the placebo group. Median time to resolution of proximal renal tubular dysfunction was 15 weeks among patients assigned adefovir, and 16% of patients did not resolve completely 41 weeks after onset. More drug discontinuations occurred in the adefovir group than in the placebo group *(28a)*.

Currently, adefovir dipivoxil is approved for use in the treatment of chronic HBV infection in adults. At the reduced dose of 10 mg daily, proximal renal tubular dysfunction has not been observed in this setting. The safety of 10 mg adefovir dipivoxil daily added to existing antiretroviral therapy (including lamivudine) has been studied in a small number (n = 92) of individuals coinfected with HBV and HIV. At 92 wk, only two patients experienced a rise in the serum creatinine of at least 0.5 mg/dL over baseline and no patients experienced serum phosphate abnormalities *(29)*.

EFFICACY

The clinical efficacies of tenofovir DF as measured by absolute CD4 cell count and/or plasma HIV-1 RNA levels are listed in Table 2.

Tenofovir DF Monotherapy

In a phase I/II randomized, double-blind, placebo-controlled, dose-escalation study (GS-97-901), antiretroviral-naive and -experienced adults with absolute CD4 cell counts of at least 200 cells/mm^3 and plasma HIV-1 RNA levels of at least 10,000 copies/mL were administered placebo or tenofovir DF at once-daily doses of 75, 150, 300, or 600 mg for 4 wk *(19)*. Antiretroviral-naive patients receiving tenofovir DF had mean HIV-1 RNA-level decreases of 0.45, 0.60, 1.57, and 1.40 $\log_{10}$ copies/mL in the 75-, 150-, 300-, and 600-mg groups, respectively. Antiretroviral-experienced patients receiving tenofovir

DF had mean HIV-1 RNA-level decreases of 0.27, 0.49, 1.06, and 0.66 $\log_{10}$ copies/mL in the 75-, 150-, 300-, and 600-mg groups, respectively. Overall, a dose-related treatment effect was observed, and individuals receiving 300 or 600 mg tenofovir DF once daily had an overall mean HIV-1 RNA-level reduction of 1.20 and 0.84 $\log_{10}$ copies/mL, respectively, when compared with baseline, whereas placebo recipients experienced a 0.03 $\log_{10}$ copies/mL increase during the same time period.

Combination Therapy With Tenofovir DF

In a randomized, double-blind, placebo-controlled phase II study (GS-98-902), 189 HIV-infected patients received placebo or 75, 150, or 300 mg tenofovir DF once daily for 24 wk in addition to the patient's existing stable antiretroviral regimen (four drugs at most) at the time of enrollment. At week 24, patients who had been administered placebo received 300 mg tenofovir DF once daily, and all treatment groups were followed for a total of 48 wk *(21,30)*. Mean baseline plasma HIV RNA levels were 3.8, 3.6, 3.6, and 3.7 $\log_{10}$ copies/mL in the placebo, 75-, 150-, and 300-mg treatment groups, respectively. Study participants were highly treatment experienced, with a mean of 4.6 yr on antiretroviral therapy and a baseline mean absolute CD4 cell count of 374 cells/mm^3. In addition, baseline genotyping demonstrated that 97% of patients had nucleoside-associated reverse transcriptase mutations, 57% had protease inhibitor-associated resistant mutations, and 32% had non-nucleoside-associated reverse transcriptase mutations.

At week 24, the mean change in plasma HIV RNA levels from baseline were –0.1, –0.4, –0.4, and –0.7 $\log_{10}$ copies/mL in the placebo, 75-, 150-, and 300-mg groups, respectively. At week 48, the mean change in HIV RNA levels from baseline were –0.4, –0.6, and –0.6 $\log_{10}$ copies/mL in the 75-, 150-, and 300-mg groups, respectively. Placebo recipients were administered 300 mg tenofovir DF at week 24, resulting in a mean plasma HIV RNA reduction from baseline of 0.6 $\log_{10}$ copies/mL at week 48. In summary, this phase II study demonstrated dose-related reductions in HIV RNA levels in a highly treatment-experienced population with extensive baseline genotypic nucleoside reverse transcriptase mutations, and this antiviral efficacy was sustained through the 48-wk study period *(21,30)*.

In a randomized, double-blind, placebo-controlled phase III study (GS-99-903), 600 antiretroviral-treated subjects were randomized to efavirenz (an NNRTI) plus lamivudine with either 40 mg stavudine twice daily plus tenofovir DF placebo or 300 mg tenofovir DF once daily plus stavudine placebo *(31)*. The participants' baseline plasma HIV RNA levels and absolute CD4 cell counts were 81,300 copies/mL and 279 cells/μL, respectively. At week 48, the intent-to-treat analysis demonstrated equivalence, with 87% of subjects in each

Table 2
Clinical Trials Evaluating Activity and Efficacy of Tenofovir DF

Trial (Ref.)	Design	Dose	No. of subjects	Entry criteria	Duration (wk)	CD4 cell count (cells/mL^3)[a]	HIV RNA (copies/mL)[b]	
GS-97-901 *(19)*	Randomized, double-blind, placebo-controlled	75 mg 150 mg 300 mg 600 mg	59	CD4 ≥ 200 cells/mm^3; HIV RNA ≥ 10,000 copies/mL; ART naive or experienced	4	Baseline: 356; NA[c]	Naive: 75 mg: –0.45 $\log_{10}$ 150 mg: –0.6 $\log_{10}$ 300 mg –1.57 $\log_{10}$ 600 mg –1.40 $\log_{10}$	Experienced: 75 mg: –0.27 $\log_{10}$ 150 mg: –0.49 $\log_{10}$ 300 mg: –1.06 $\log_{10}$ 600 mg: –0.66 $\log_{10}$
GS-98-902 *(21)*	Randomized, double-blind, placebo-controlled	75 mg 150 mg 300 mg	189	HIV RNA 400–100,000 copies/mL; TDF added to stable existing regimen	48	Baseline: 374; NS[d]	Week 24: placebo: –0.1 $\log_{10}$ 75 mg: –0.4 $\log_{10}$ 150 mg: –0.4 $\log_{10}$ 300 mg: –0.7 $\log_{10}$	Week 48: placebo/300 mg: –0.6 $\log_{10}$ 75 mg: –0.4 $\log_{10}$ 150 mg: –0.6 $\log_{10}$ 300 mg: –0.6 $\log_{10}$
GS-99-903 *(31)*	Randomized, double-blind, placebo-controlled; 3TC + EFV + d4T or TDF	300 mg	600	HIV RNA > 5000 copies/mL; ART naive	48	Baseline: 279; week 48: TDF: +169; d4T: +167		Week 48: <400: TDF 87%; d4T 87% <50: TDF 82%; d4T 81%

GS-00-907 (22)	Randomized, double-blind, placebo-controlled	300 mg	550	HIV RNA 400–100,000 copies/mL; TDF added to stable existing regimen	48	Baseline: 427; week 24: placebo: –10.8; 300 mg: +12.6	Baseline: 3.36 $\log_{10}$ week 24: placebo: –0.03 $\log_{10}$ 300 mg: –0.61 $\log_{10}$	Week 48: placebo-to-TDF crossover: –0.70 $\log_{10}$ TDF: –0.56 $\log_{10}$

ART, antiretroviral therapy; NA, not available; TDF, tenofovir DF; DAVG24, time-weighted average change in HIV-1 RNA level from baseline to week 24; NS, not significant; 3TC, lamivudine; EFV, efavirenz; d4T, stavudine

[a]Mean change in absolute CD4 cell count (cells/mm^3)—DAVG24

[b]Mean reduction in plasma HIV RNA level—DAVG24

[c]The study was not powered to assess changes in absolute CD4 cell counts

[d]No statistically significant difference between placebo and TDF groups (not powered to assess changes in CD4 cell counts)

group achieving plasma HIV RNA levels of fewer than 400 copies/mL. Similarly, 82% and 81% of subjects achieved plasma HIV RNA levels of fewer than 50 copies/mL in the tenofovir DF and stavudine groups, respectively. The absolute CD4 cell count increased by 169 cells/μL in the tenofovir DF group compared with a 167 cells/μL increase in the stavudine group *(31)*.

In a separate randomized, double-blind, placebo-controlled phase III study (GS-00-907), 550 antiretroviral-experienced HIV-infected patients received 300 mg tenofovir DF once daily or placebo for 24 wk in addition to their existing antiretroviral regimen. After week 24, placebo recipients were allowed to receive tenofovir DF for the remainder of the 48-wk study period. At baseline, patients had a mean plasma HIV RNA level of 3.36 $\log_{10}$ copies/mL, a mean absolute CD4 cell count of 427 cells/mm^3, and had received antiretroviral therapy for a mean of 5.4 yr. Baseline genotypic analyses in a subset of patients ($n = 253$) demonstrated that 94% of participants had nucleoside reverse transcriptase-associated mutations, 58% had protease inhibitor-associated resistant mutations, and 48% had non-nucleoside-associated reverse transcriptase mutations *(22)*.

Through week 24, the mean change in plasma HIV RNA levels from baseline were –0.61 and –0.03 $\log_{10}$ copies/mL in the tenofovir DF and placebo groups, respectively ($p < 0.001$). In addition, 45% of tenofovir DF recipients achieved plasma HIV RNA levels of fewer than 400 copies/mL compared with 13% in the placebo group ($p < 0.001$). Reduction in plasma HIV RNA levels to fewer than 50 copies/mL was seen in 22% of those receiving tenofovir DF compared with 1% of placebo recipients ($p < 0.001$). The mean changes in absolute CD4 cell counts from baseline were +12.6 and –10.6 cells/mm^3 in the tenofovir DF and placebo groups, respectively ($p = 0.008$). Through week 48, the prevalences of tenofovir DF recipients with plasma HIV RNA levels of fewer than 400 and fewer than 50 copies/mL were 41% and 18%, respectively, suggesting a sustained response *(22)*.

The role of triple-nucleoside analog reverse transcriptase inhibitor regimens for the treatment of HIV-1 infection is undergoing extensive investigation. The once-daily triple nucleoside/nucleotide analog combination of 600 mg abacavir plus 300 mg lamivudine plus 300 mg tenofovir DF seemed advantageous relative to potency and tolerability, but was associated with an unacceptably high rate of virological failure based on preliminary data from two clinical trials that were subsequently discontinued *(32,33)*. Similarly, a 24-wk pilot study evaluating the efficacy of once-daily 250 mg didanosine EC plus 300 mg lamivudine plus 300 mg tenofovir DF in antiretroviral-naive, HIV-infected individuals demonstrated suboptimal virological responses. At week 12, only one participant demonstrated an optimal response (defined as a ≥2 $\log_{10}$ copies/mL reduction in plasma HIV-1 RNA level); the median change from baseline plasma HIV-1 RNA level was –0.61 $\log_{10}$ copies/mL *(34)*. The reason(s) for the poor

performance of these regimens is unclear, but early investigations point to early development of resistance.

RESISTANCE

The presence and frequency of genotypic mutations related to tenofovir DF exposure has been examined in vitro, and information regarding drug-resistant clinical isolates is accumulating. In the presence of tenofovir or adefovir, a K65R mutation in HIV reverse transcriptase has been detected during in vitro HIV passage experiments *(35,36)*. This single amino acid substitution, shown previously to be selected in vitro in the presence of zalcitabine, results in a 3- to 4-fold increase and a 12- to 15-fold increase in the IC_{50} values for tenofovir and adefovir, respectively, compared with wild-type virus *(37,38)*. Tenofovir exposure resulted in additional HIV reverse transcriptase mutations in these in vitro HIV passage experiments, including L228R, W25R, and P272S mutations. The K70E reverse transcriptase mutation has been linked to adefovir exposure.

Tenofovir DF

Recombinant viruses carrying the T69D reverse transcriptase mutation demonstrated a threefold increased IC_{50} for tenofovir compared with wild-type virus; in contrast, recombinant viruses expressing alternative reverse transcriptase mutations associated with multinucleoside drug resistance (Q151M, A62V, V75I, F77L, and F116Y) showed wild-type virus susceptibility to tenofovir, but reduced susceptibility to zidovudine (38-fold) *(37)*. In addition, recombinant viruses expressing both the M184V (associated with lamivudine resistance) and T215Y (associated with zidovudine resistance) mutations displayed increased susceptibility to tenofovir compared with wild-type virus *(37)*. In summary, in vitro studies suggest tenofovir may be active against HIV strains expressing one or more of the common nucleoside reverse transcriptase mutations. A possible explanation for these findings is that tenofovir may be less efficiently removed through pyrophosphorolysis and dinucleotide synthesis in HIV strains possessing these reverse transcriptase mutations *(39)*.

In a randomized, double-blind, placebo-controlled phase II study (GS-98-902), tenofovir DF was added to background therapy in antiretroviral-experienced, HIV-infected individuals (*see* "Efficacy" section). In 159 participants, baseline and after baseline (week 24, week 48, or early termination) genotypic evaluations were performed. The development of one or more new reverse transcriptase mutations was noted in approx 40% of the study population receiving tenofovir DF at 48 wk, but these mutations were consistent with the patient's background antiretroviral therapy and were not associated with loss of plasma HIV RNA suppression *(30)*. The K65R reverse transcriptase mutation, associated with tenofovir in vitro, developed in only four (2%) patients and was not

linked to increasing plasma HIV RNA levels *(30)*. Baseline and after baseline phenotypic analyses were performed in those subjects randomized to receive 300 mg tenofovir DF once daily ($n = 54$). At baseline, phenotypes revealed a mean reduced susceptibility of 1.9- and 13.8-fold over wild-type virus for tenofovir DF and zidovidine, respectively. In regression analyses, plasma HIV RNA suppression was significantly correlated with baseline susceptibility to tenofovir DF ($p = 0.007$) and zidovudine ($p = 0.035$), but not to other nucleoside agents *(30)*. At week 48, approx 50% of evaluable patients demonstrated increased susceptibility to tenofovir DF, and the remaining half displayed reduced susceptibility (2.7- to 4.3-fold) to the drug. Individuals developing the K65R reverse transcriptase mutation ($n = 4$) showed threefold to fourfold reduction in susceptibility to tenofovir DF, consistent with previous in vitro studies *(30)*. As was noted in the genotypic analysis, low-level reduced susceptibility to tenofovir DF was not associated with loss of HIV RNA suppression at 48 wk.

In a separate randomized, double-blind phase III intensification study (GS-00-907), a more detailed genotypic and phenotypic analysis was performed on a larger subset of patients. In addition to stable background antiretroviral therapy, HIV-infected patients received placebo or 300 mg tenofovir DF once daily for 24 wk, followed by 24 wk of open-label 300 mg tenofovir DF once daily for all patients (*see* "Efficacy" section). Fifty percent ($n = 274$) and 25% ($n = 137$) of the study population were randomly assigned to the genotyping and phenotyping substudies, respectively. In participants with baseline and week 48 genotypic data, the development of mutations associated with tenofovir DF therapy occurred infrequently (3%); specifically, eight patients developed the K65R mutation, but loss of plasma HIV RNA suppression was only noted in three of these patients during the 48-wk study period *(40)*. Moreover, only low-level reduced (less than twofold) tenofovir DF phenotypic susceptibility was associated with the presence of the K65R mutation in the majority of these patients, with only one participant demonstrating a greater than threefold reduced susceptibility to the drug *(40)*. Through week 48, the development of M41L or L210W mutations as part of at least three thymidine analog-associated mutations was equally common in participants receiving tenofovir DF or placebo, suggesting that the background antiretroviral therapy may have been responsible for the presence of these mutations *(39,40)*. In summary, the development of phenotypic or genotypic resistance to tenofovir DF was uncommon in this study of antiretroviral-experienced patients, and loss of plasma HIV RNA suppression was not linked to reduced susceptibility or to mutations associated with tenofovir DF therapy.

The development of resistance mutations was also investigated in a randomized, double-blind, placebo-controlled phase III study (GS-99-903) of 600 antiretroviral subjects randomized to efavirenz (an NNRTI) plus lamivudine with

either 40 mg stavudine twice daily plus tenofovir DF placebo or 300 mg tenofovir DF once daily plus stavudine placebo (*see* "Efficacy" section). At week 48, the K65R mutation developed in seven patients receiving tenofovir DF compared with two stavudine recipients. At week 96, only 2.7% of tenofovir DF recipients and 0.6% of stavudine recipients developed the K65R mutation; this trend was not statistically significant *(41)*. Of note, efavirenz resistance mutations (K103N and others) preceded or accompanied the development of a K65R mutation in all cases, either with or without the M184V mutation. Among HIV-1 isolates from tenofovir DF-treated patients with a K65R mutation ($n = 8$), there were only low-level changes in tenofovir susceptibility in vitro (mean, 1.2 fold), increased susceptibility to zidovudine (mean, 0.5 fold), and no change in susceptibility to stavudine *(41)*.

Adefovir Dipivoxil

To date, previous adefovir dipivoxil exposure has not resulted in documented reduced susceptibility to tenofovir DF *(42)*. In an open-label pilot study of chronic HBV infection treatment in HIV-infected patients, 10 mg adefovir dipivoxil once daily was added to existing lamivudine therapy to assess for safety, efficacy, and the development of reverse transcriptase mutations. Despite uncontrolled HIV-1 replication in many of the subjects ($n = 35$; mean plasma HIV level of 2.88 $\log_{10}$ copies/mL), no adefovir dipivoxil-associated reverse transcriptase mutations (specifically, K65R or K70E) were observed at baseline or throughout the 48-wk study period *(43)*. This study is limited by a relatively small study population, but the results are noteworthy because the adefovir dipivoxil dosing for chronic HBV infection is significantly lower than the dosing previously used in HIV clinical trials (120–250 mg daily).

DRUG INTERACTIONS

Tenofovir DF

A randomized, open-label, multiple-dose, crossover study was performed to assess pharmacological interactions after coadministration of tenofovir DF with lamivudine, indinavir, lopinavir/ritonavir, or efavirenz. Tenofovir steady-state exposure (AUC) was found to be equivalent when coadministered with lamivudine, indinavir, lopinavir/ritonavir, or efavirenz *(44)*. Similarly, indinavir and efavirenz pharmacokinetic parameters were unaffected in the presence of tenofovir DF therapy. Lamivudine plasma levels were lower in the setting of tenofovir DF coadministration, as manifested by a 24% reduction in C_{max} and a 0.9-h time-to-peak concentration delay. These findings are presumably secondary to a slower absorption rate for lamivudine in this setting, but AUC levels were not significantly changed, and this interaction is not thought to be clinically significant *(44)*.

In a separate analysis, coadministration of didanosine (buffered tablet formulation) and tenofovir DF resulted in a 44% increase in didanosine AUC, whereas tenofovir steady-state levels were unchanged *(45)*. Tenofovir DF coadministration with the more commonly used didanosine formulation (enteric-coated capsules) resulted in didanosine increases of 48% and 60% in the unfed and fed state, respectively *(17)*. Based on these data, coadministration of tenofovir DF and didanosine should be undertaken with caution, and patients receiving this combination should be monitored closely for didanosine-associated toxicities, including pancreatitis and neuropathy *(17)*. To date, the manufacturers of these drugs have not provided formal dosing recommendations in this setting, but a reduction of the didanosine dose to 250 mg daily may be an appropriate measure when tenofovir DF is administered concomitantly.

A drug interaction study has also been conducted evaluating the combination of 300 mg tenofovir DF daily with ritonavir-boosted atazanavir (100 mg ritonavir daily plus 300 mg atazanavir daily) in 10 HIV-infected volunteers. The AUC for 300 mg atazanavir daily decreased 25% when atazanavir was administered with ritonavir plus tenofovir DF, but the trend did not reach statistical significance *(46)*.

Tenofovir DF is primarily eliminated through renal excretion, and dosing adjustment are recommended for individuals with a baseline creatinine clearance of less than 50 mL/min. Specifically, individuals with a creatinine clearance of 30 to 49 mL/min should receive 300 mg tenofovir DF every 48 h, whereas those patients with a creatinine clearance of 10 to 20 mL/min should receive 300 mg tenofovir DF twice weekly. In patients receiving hemodialysis, tenofovir DF is dosed once weekly after completion of dialysis *(17)*. Clinicians should monitor the prescription of concomitant drugs with overlapping toxicities and of agents that may reduce renal function. The pharmacological profile of tenofovir DF is not significantly altered in patients with moderate or severe hepatic impairment, and no dose adjustments are currently recommended in the setting of hepatic insufficiency *(47)*.

Adefovir Dipivoxil

To assess potentially significant drug interactions between adefovir dipivoxil and drugs used frequently by patients with chronic HBV infection, a phase I randomized, open-label, multiple-dose, three-period crossover pharmacokinetic drug–drug interaction study was performed in healthy subjects. The findings suggest coadministration of 10 mg adefovir dipivoxil once daily with lamivudine, paracetamol, ibuprofen, or systemic trimethoprim/sulfamethoxazole does not result in clinically significant drug interactions, but systemic adefovir AUC levels were notably higher when coadministered with higher doses of ibuprofen (800 mg three times daily) *(48)*. There were no significant adverse events associated with

these combinations of drugs in healthy subjects, including the administration of adefovir dipivoxil and concomitant higher doses of ibuprofen *(48)*.

Adefovir dipivoxil is also eliminated by renal excretion and studies have been performed to develop dosing guidelines in the setting of impaired renal function. In patients with a creatinine clearance (CrCl) of less than 50 mL/min, a reduction of adefovir renal elimination has been observed, and the following dosing adjustments are recommended: CrCl of 20 to 49 mL/min: 10 mg adefovir dipivoxil every 48 h; CrCl of 10 to 19 mL/min: 10 mg adefovir dipivoxil every 72 h; and CrCl of less than 10 mL/min: 10 mg adefovir dipivoxil every 7 d *(49)*. In hemodialysis patients, adefovir concentrations reached high levels and no extrarenal route of elimination was noted. Hemodialysis will remove approx 36% of an administered adefovir dose, and the dosing recommendation in this setting is 10 mg adefovir dipivoxil every 7 d after hemodialysis *(49)*. Similar analyses in subjects with varying degrees of hepatic insufficiency (Child-Pugh-Turcotte classifications B and C) demonstrated no substantial alterations in adefovir pharmacokinetics compared with normal subjects, and no dosing adjustment is currently recommended for individuals with hepatic dysfunction *(49)*.

SUMMARY

The nucleotide analogs are acyclic nucleoside phosphonates (nucleoside monophosphates), with proven in vitro and in vivo efficacy against a wide variety of DNA viruses and retroviruses. Of the three nucleotide analogs (cidofovir, adefovir dipivoxil, and tenofovir DF) approved for use in the United States and Europe, only tenofovir DF is approved for the treatment of HIV infection. Adefovir dipivoxil and cidofovir are approved for treatment of chronic HBV infection in adults and retinitis caused by cytomegalovirus infection, respectively.

Tenofovir and adefovir have favorable pharmacological properties, allowing for single daily dosing. The oral prodrugs, tenofovir DF and adefovir dipivoxil provide improved oral bioavailability and, in the case of tenofovir DF, further improvement is noted when the agent is administered in the fed state. These compounds are eliminated via renal excretion, and dosing adjustments are necessary in patients with renal insufficiency (specifically, creatinine clearance of <50 mL/min).

At dosing of 300 mg daily, tenofovir DF has anti-HIV activity as documented by suppression of plasma HIV RNA levels in antiretroviral-naive (range, 0.45–1.4 $\log_{10}$ copies/mL) and antiretroviral-experienced patients (range, 0.3–1.1 $\log_{10}$ copies/mL). Sustained antiretroviral activity has been observed up to 48 wk. At doses of 60 to 120 mg daily, adefovir dipivoxil also possesses anti-HIV activity, but, in clinical trials, its usefulness was limited by nephrotoxicity, specifically proximal renal tubular dysfunction. At lower doses than previously used in HIV clinical trials (10 mg once daily), adefovir dipivoxil is efficacious

and safe in the treatment of chronic HBV infection in adults, including both lamivudine-sensitive and lamivudine-resistant HBV strains.

The safety of tenofovir DF has been studied in more than 1600 HIV-infected patients in clinical trials and in many more patients in routine clinical care, and the drug is generally well tolerated. The preclinical toxicology studies of tenofovir in animals identified the kidney and bone as potential target organs. Interestingly, nephrotoxicity and severe serum hypophosphatemia have not been commonly noted in patients receiving tenofovir DF in clinical trials, such as was described in individuals receiving the structurally similar compound, adefovir dipivoxil, but, with the increased use of tenofovir DF in HIV-infected patients, several small case series of proximal renal tubular dysfunction have now been published. Elevations in serum triglyceride levels, liver transaminases, and creatine kinase were observed in clinical trials, but the percentage of study participants with abnormal values was not significantly different than the frequency of these findings observed in patients receiving placebo. To date, adverse events associated with mitochondrial dysfunction (e.g., lactic acidosis) have been rare and only documented when tenofovir DF was coadministered with stavudine or didanosine. In patients with chronic HBV infection (including HIV- and HBV-coinfected patients), proximal renal tubular dysfunction has not been observed with lower-dose adefovir dipivoxil administration.

In vitro genotypic analysis has demonstrated that tenofovir DF maintains activity against most nucleoside-resistant HIV strains, but may select for a K65 HIV reverse transcriptase mutation resulting in a three to fourfold reduction in tenofovir susceptibility. The clinical relevance of the development of genotypic mutations in the setting of tenofovir DF therapy is unclear, but the development of these mutations was rare (fewer than 3% of clinical trials participants) and not linked to loss of HIV RNA suppression in 48-wk clinical trials. To date, previous adefovir dipivoxil exposure has not resulted in documented reduced susceptibility to tenofovir DF.

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6

Resistance to Nucleoside and Nucleotide Reverse Transcriptase Inhibitors

Nancy Shulman and Mark Winters

INTRODUCTION

Long-term virological suppression of HIV replication to restore immune function is the cornerstone of HIV management. Antiretroviral regimens are becoming simpler, more potent, and better tolerated, but the development of drug resistance remains a significant problem. Despite very low rates of virological failure with the current regimens in some of the more recent clinical trials *(1–4)*, virological failure rates are often higher in clinical practice *(5–7)*, especially in patients who are treatment experienced *(7,8)*. Virological failure, defined as detectable plasma HIV RNA levels after initial suppression or the inability to suppress HIV RNA to undetectable levels with treatment, is influenced by many factors, including regimen potency, patient adherence, drug pharmacokinetics, and preexisting drug resistance. However, the development of drug resistance in response to treatment is a major cause and consequence of virological failure *(9)*.

Resistance Evolution

An estimated 10^9 to 10^{10} HIV virions are produced per day in the average infected person *(10)*. Coupled with the high error rate of the HIV reverse transcriptase (RT) *(11,12)*, this creates a large population of genetically distinct HIV quasispecies within infected individuals. This genetic diversity provides a large pool of HIV variants from which subpopulations can emerge in response to selective pressures, such as antiretroviral therapy. If viral replication is not suppressed to minimal levels with combinations of antiretroviral drugs, drug resistant strains will rapidly emerge. The rate at which drug resistance develops depends on a number of factors, including the size and diversity of the viral population in a given person, the magnitude of viral replication that persists on treatment, the complexity of the genetic barrier (number of mutations) that must be overcome to allow viral replication in the presence of a given antiretroviral regimen, and the replicative fitness of the evolving resistant strains.

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When treatment is stopped, resistant strains no longer have a selective advantage and wild-type strains generally become dominant *(9,13)*. Although the resistant strains may become undetectable by conventional assays, they remain at low levels and rapidly resurface if the drugs that favor their growth are reinstituted *(14)*.

EPIDEMIOLOGY

In places where antiviral therapy is readily available, antiretroviral resistance is a significant problem. The prevalence of phenotypic resistance in the United States was evaluated in late 1998 in a study designed to represent all 208,900 HIV-infected adults in the United States who received HIV treatment in urban centers *(15)*. Of all patients whose HIV phenotypic resistance could be measured, 76% of patients had virus with some degree of drug resistance. Resistance to nucleoside and nucleotide analog RT inhibitors (NRTIs) was the most prevalent, found in 71% of the patients. Higher rates of drug resistance were associated with better access to treatment.

Patterns of resistance generally reflect current prescribing patterns. Resistance to the non-nucleoside RT inhibitors (NNRTIs) has steadily increased since their popularity increased in 1999 *(16,17)*. Changes in NRTI prescribing trends have altered the rates of certain NRTI resistance patterns. For example, the increased use of tenofovir and abacavir has increased the prevalence of the K65R mutation selected by both of these drugs *(17,18)*, whereas mutations associated with zidovudine and stavudine have decreased, paralleling their decreased use or the declining proportion of patients in the population who have received single and dual nucleoside therapies containing thymidine analogs *(17,18)*.

The transmission of drug-resistant HIV is also a significant problem. Reported rates of primary HIV resistance (resistance in patients who have never received antiretroviraltherapy) vary depending on how resistance is defined, the geographical area, and the population surveyed. The transmission rates in North America peaked at an overall estimate of 12 to 13% of patients with strains resistant to any class of drugs in 1999–2000, and are currently thought have decreased to approx 8 to 10% *(19–21)*. Certain geographical areas, such as San Francisco, CA, have reported rates of primary resistance as high as 25% *(22)*. Rates of resistance from a large European cohort of treatment-naive patients (excluding the United Kingdom) were stable at an average of 11% during 1996–2002 *(23)*. Rates of primary resistance reported in the United Kingdom are much higher, and climbed to as high as 26% in 2002 *(24)*.

MEASURING RESISTANCE

Resistance to nucleoside analogs results from changes in the nucleotide sequence of the RT gene that cause changes in the amino acid sequence of the

Table 1
Amino Acid Abbreviations[a]

Abbreviation	Amino acid	Abbreviation	Amino acid
A	alanine	M	Methionine
C	cysteine	N	Asparagine
D	aspartate	P	Proline
E	glutamine	Q	Glutamate
F	phenylalanine	R	Arginine
G	glycine	S	Serine
H	histidine	T	Threonine
I	isoleucine	V	Valine
K	lysine	W	Tryptophan
L	leucine	Y	Tyrosine

[a]The amino acid preceding the position number is the consensus wild-type amino acid and the one immediately after the position number is the substituted amino acid, i.e., M184V represents a valine substitution for methionine at amino acid 184

translated enzyme. These amino acid changes alter the structure of the enzyme in regions that affect drug susceptibility, and allow the RT to function in the presence of the particular drug(s).

Phenotypic drug resistance is defined as an increased concentration of drug required to inhibit in vitro growth of a virus or construct derived from a virus compared with a known wild-type (nonmutated) control. Phenotypic resistance is often reported as a fold change in the amount of drug required to inhibit viral replication by 50% compared with the wild-type control. The in vitro fold change in susceptibility correlates with reduced in vivo antiviral activity, but varies widely among the drugs. An increasing number of drugs have fold change cut-off data that are based on clinical trial outcomes, but these cut-off data are still needed for many of the available drugs.

Genotypic resistance is defined by the presence of specific mutations in the HIV RT or protease genes known to be associated with phenotypic and/or in vivo resistance to a given drug or drugs. It is determined by analysis of the nucleotide sequence of the RT and protease genes for the presence of specific genetic mutations. The genotype is typically reported as a list of amino acid mutations of the patient virus compared with the consensus B wild-type HIV sequence. Mutations are described starting with the consensus B wild-type amino acid abbreviation (Table 1), followed by the amino acid residue number, and followed by the abbreviation of the mutant amino acid in the given sequence. For example, a valine substitution for the naturally occurring methionine at amino acid 184 is written as M184V. A predicted susceptibility profile is also reported, which is generated from algorithms that are based on the accumulated knowledge of specific amino acid changes on in vitro susceptibility and/or virological response.

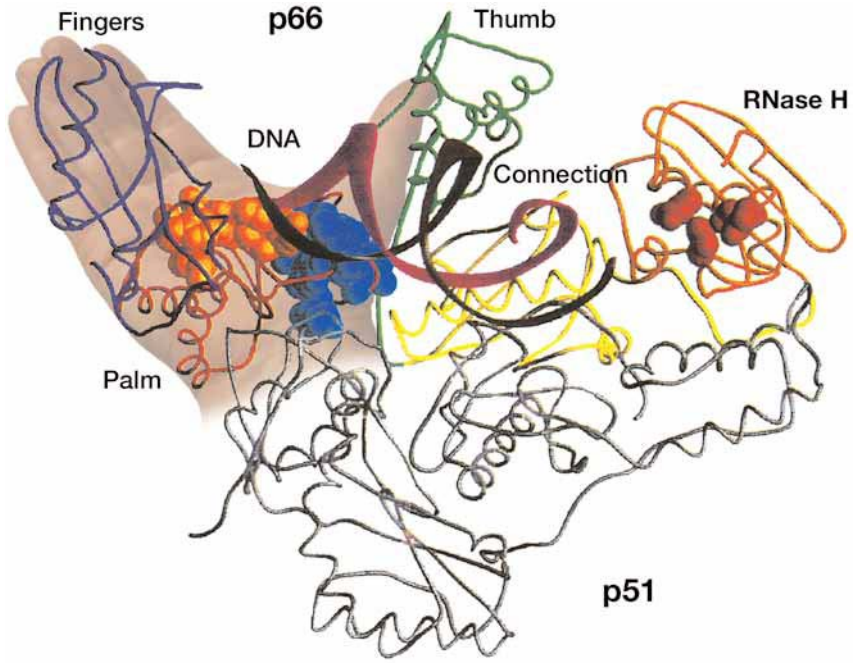

Fig. 1. A ribbon structure of HIV RT with the three-dimensional conformation of p66 resembling a right hand (superimposed in the background). It has a palm where the polymerase active site is contained, fingers, a thumb, a connector region that interacts with the p51 subunit to stabilize the dimer complex, and an RNase H domain. The light spheres represent dNTP bound to the active site. The adjacent darker spheres represent nevirapine bound to the NNRTI-binding pocket. (Adapted from ref. *184*.)

Clinical or in vivo resistance can be defined as reduced antiviral activity despite therapeutic drug levels in the blood and tissues. Phenotypic and genotypic resistance testing are both predictive of in vivo resistance (reviewed in ref. *25*), and choosing regimens based on resistance-testing results improves virological response rates in patients who have failed at least one drug regimen *(26–30)*.

RT STRUCTURE AND LOCATION OF NUCLEOSIDE RESISTANCE MUTATIONS

RT is a heterodimeric protein consisting of 66- and 51-kDa subunits (p66 and p51, respectively). The p66 subunit is composed of all 560 amino acids of the *pol* gene. The p51 subunit is composed of the first 440 amino acids of the *pol* gene. Although p51 contains a polymerase active site, p51 is catalytically inactive and predominantly serves as a structural base for the active p66. The three-dimensional conformation of p66 resembles a right hand (Fig. 1). It has a "palm" containing the active site, "fingers," a "thumb," a connector region that

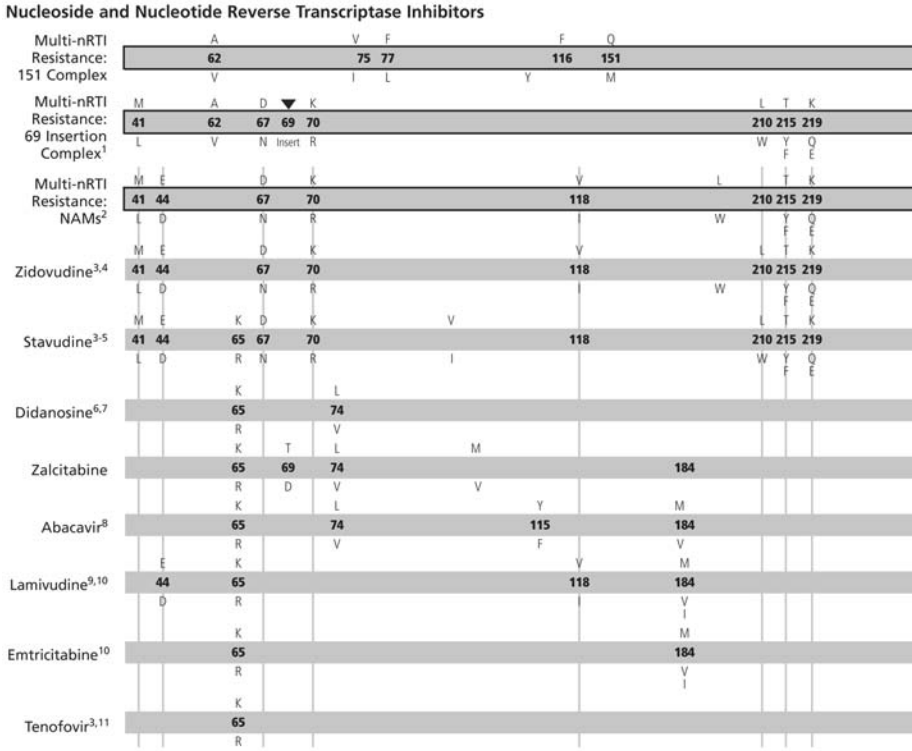

Fig. 2. Chart of mutations associated with nucleoside and nucleotide resistance. (Reprinted from ref. *33* with permission from the International AIDS Society–USA. Updated information and thorough explanatory notes are available at www.iasusa.org.)

interacts with the p51 subunit to stabilize the dimer complex, and an ribonuclease H (RNase H) domain *(31)*. When incoming nucleotides bind to the enzyme, the tips of the fingers "close down" on the palm to trap the template and the developing primer strand in the active site *(32)*. The major resistance mutations that are selected for by NRTIs in vivo (Fig. 2) *(33)* and which have been shown by site-directed mutagenesis studies to confer nucleoside resistance, are distributed in the fingertips and the palm (Fig. 3) *(34)*.

GENERAL MECHANISMS OF HOW THE MUTATIONS CONFER DRUG RESISTANCE

HIV RT converts genomic single-stranded RNA into double-stranded DNA to be integrated in the host DNA. HIV RT has RNA- and DNA-primed DNA polymerase activity as well as RNase H activity that degrade the DNA-RNA intermediates. DNA elongation exists as an equilibrium between a dominant

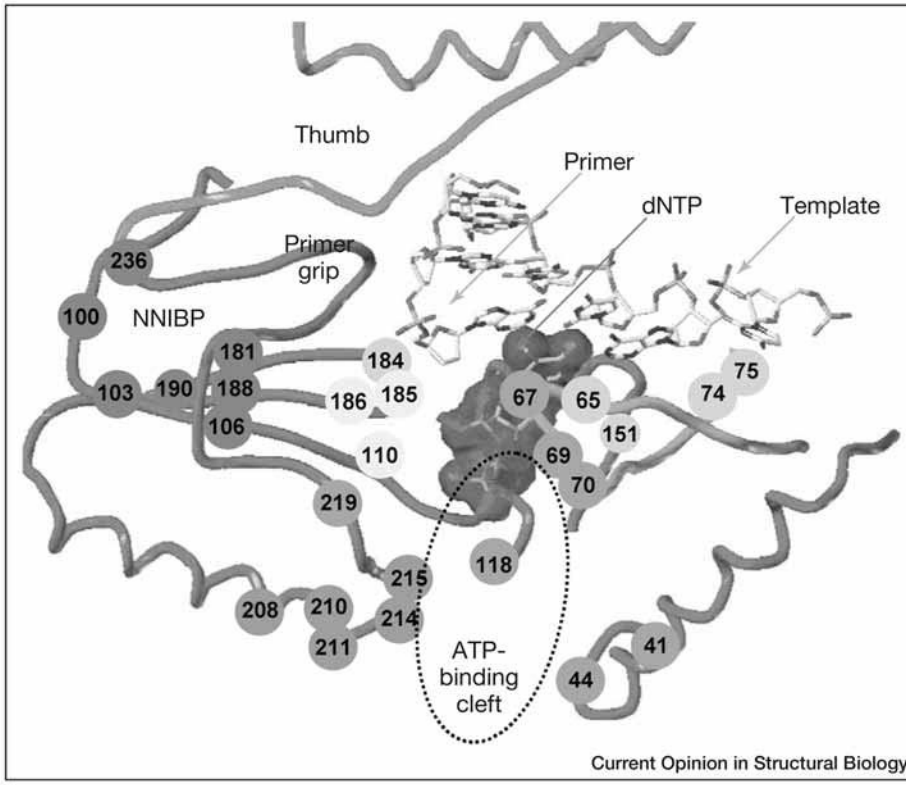

Fig. 3. Sites of drug-resistant mutations in HIV-1 RT. The structure of HIV-1 RT in the region near the polymerase active site is shown. A template–primer is shown. The incoming dNTP (spheres) is bound at the N site. The putative ATP-binding cleft for excision is near amino acid 215. The catalytic aspartates of the active site are D110, D185, and D186. The amino acids involved in NRTI resistance are 41, 44, 67, 69, 70, 74, 75, 118, 151, 210, 215, and 219. Amino acids involved in NNRTI resistance are in clustered in the NNRTI binding pocket (NNIBP) *(34)*.

forward reaction and a minor reverse reaction. The forward reaction is characterized by attack of the 3′-hydroxyl group of the primer on the α-phosphate of the incorporating nucleoside triphosphate (dNTP) to form a phosphodiester bond, releasing pyrophosphate (Fig. 4A). This reaction extends the length of the primer chain by one base. This process continues to extend the primer until natural completion or until an incorporated base is a nucleoside analog, which lacks the 3′-hydroxyl group that forms a bond with the next dNTP, and therefore prematurely terminates the chain. The reverse reaction of the RT enzyme involves excision of the terminal nucleoside monophosphate from the primer by coupling with adenosine triphosphate (ATP) or pyrophosphate (Fig. 4B). If

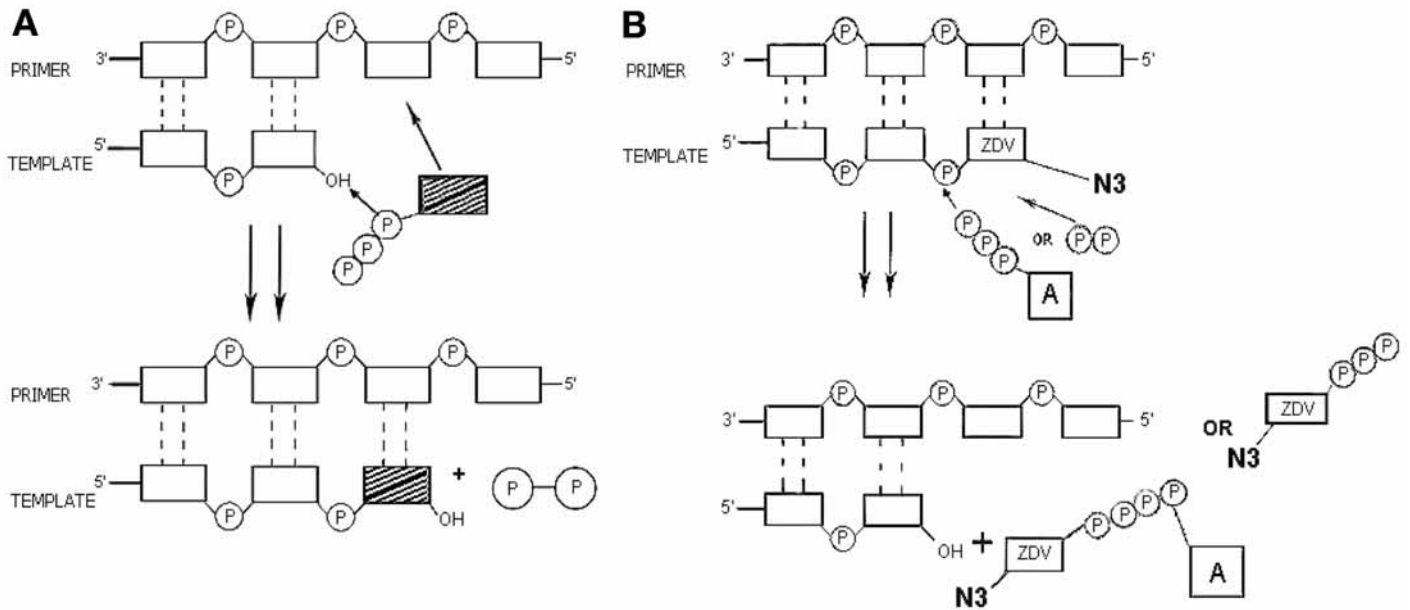

Fig. 4. (A) DNA elongation is characterized by attack of the 3′-hydroxyl group of the primer on the α-phosphate of the incorporating dNTP to form a phosphodiester bond, releasing pyrophosphate. **(B)** Pyrophosphorolysis involves excision of the terminal nucleoside monophosphate from the primer by coupling with ATP or pyrophosphate. If the terminal base is an NRTI, this excision reaction it is termed primer unblocking, because it rescues the terminated chain so it can continue elongation.

the terminal base is a NRTI, its removal from the terminated primer will unblock the chain so it can continue extension. This is referred to as primer unblocking.

In generalNRTI resistance mutations cause resistance either by decreasing the incorporation of the NRTIs compared with the natural dNTPs (the forward reaction) or by increasing the rate of primer unblocking (the reverse reaction). Allosteric interference with the incorporation of the nucleoside analog is the major mechanism by which mutations M184V, Y115F, Q151M, L74V, V75T, V118I, and K65R cause resistance *(32,35–46)*. The thymidine analog mutations (TAMS) at positions M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E/N do not affect incorporation to any significant degree *(47,48)*; instead, they enhance the reverse reaction of nucleotide excision by enhancing the binding of ATP to the terminated primer complexed with RT *(49–51)*. An increase in the number of these mutations proportionally increases the degree of primer unblocking *(52)*, The E44D mutation also increases primer unblocking in combination with the TAMS *(46)*. Some mutations can reduce the rate of primer unblocking, thereby antagonizing this mechanism of resistance, including the NRTI-associated mutations M184V, K65R, and L74V *(45,53–57)*, as well as the NNRTI mutation Y181C *(58)*. Although TAM-mediated excision occurs with all NRTI-terminated primers, the efficiency differs among the individual NRTIs. The efficiency is greatest with the thymidine analogs zidovudine and stavudine, and is much lower for other NRTIs, such as abacavir, didanosine, tenofovir, lamivudine, and emtricitabine *(59)*. The concentration of the next complimentary dNTP in the given environment influences the rate of excision, particularly for dideoxy NRTIs, such as stavudine and didanosine *(42,60–63)*. When RT is bound to the template and the primer, the 3′ end of the primer can be in one of two positions, termed the priming (P) site or the nucleotide-binding (N) site (Fig. 5A). The nucleotide binds at the N site and, after chemical bond formation, it can translocate to the P site. If an NRTI-terminated primer translocates to the P site, the next complimentary dNTP can then occupy the N site. This forms a dead-end complex of variable stability, depending on a several factors, including the specific NRTI and next dNTP. Excision can only occur at the N site, so this dead-end complex prevents excision. Zidovudine has a bulky azido side chain that does not favor the binding of the next complimentary nucleotide (Fig. 5B) *(42,62,63)*. This allows a zidovudine-terminated primer to move back and forth from N to P and from P to N, allowing excision to occur independent of the dNTP concentrations. In contrast, stavudine and other dideoxynucleosides favor the dead-end complex with the next complimentary dNTP, which keeps the 3′ end of the terminated primer in the P site and makes excision less efficient at higher dNTP concentrations (Fig. 5C) *(42,62,63)*. Thus, in in vitro susceptibility assays, in which cellular dNTP concentrations are

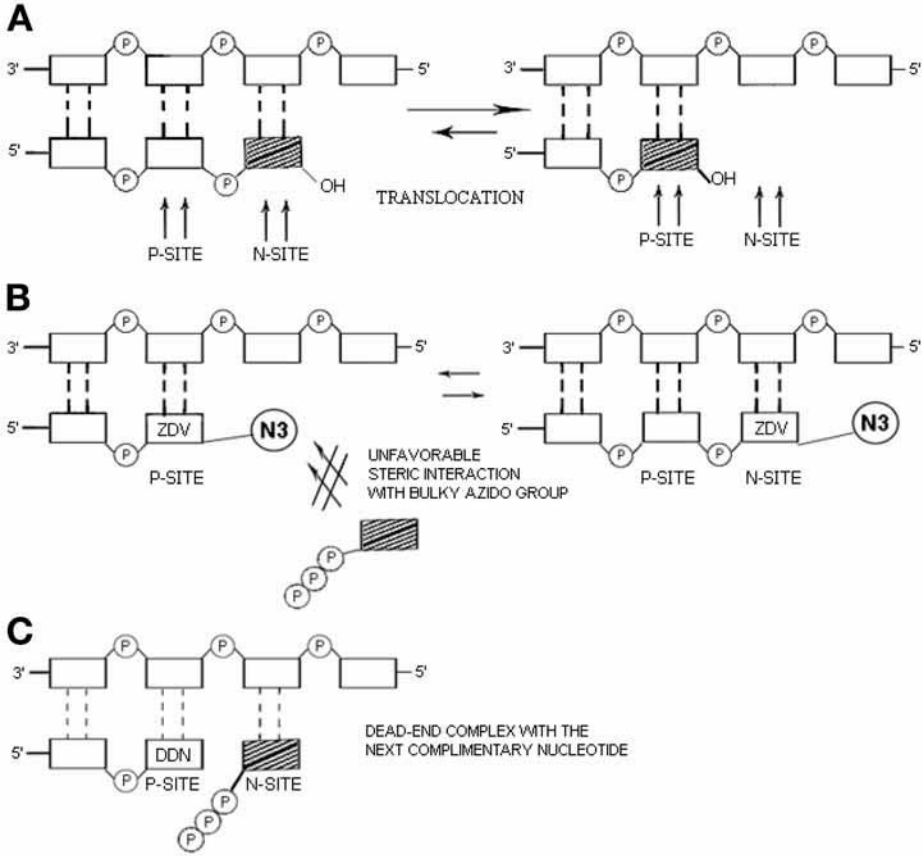

Fig. 5. (A) When RT is bound to the template and the primer, the 3′ end of the primer can be in the P site or the N site. The incoming nucleotide binds at the N site and, after chemical bond formation, it translocates to the P site. **(B)** Zidovudine (ZDV) has a bulky azido side chain that does not favor the binding of the next complimentary nucleotide. This allows a zidovudine-terminated primer to move back and forth from N to P and from P to N. **(C)** Stavudine and other dideoxy nucleotide (DDN)-terminated primers favor a stable dead-end complex with the next complimentary nucleotide, which keeps the 3′ end in the P site.

high, the TAMS confer the highest degrees of resistance to zidovudine and very small degrees of resistance to stavudine *(61)*. In vivo, dNTP concentrations vary, thus, TAMS cause significant clinical resistance to stavudine *(64–66)*.

Insertions in the fingers domain between amino acids 69 and 70 have been shown to destabilize the dead-end complex formed by the next complimentary dNTP *(67)*, enhancing resistance mediated by TAMS and increasing the resistance to all of the NRTIs, including stavudine.

Table 2
Nucleoside Mutations Reported in Antiretroviral-Naive Patients Receiving Specific Single, Dual, and Triple NRTIs With or Without NNRTIs or Protease Inhibitors

Monotherapy	Mutations that arise after therapy in naive patients
Zidovudine	TAMS *(69,70,72,83,84,185,186)*
Didanosine	L74V, M184V, T69D/N, K65R, rarely TAMS *(109,154–156)*
Zalcitabine	T69D *(171)*
Stavudine	TAMS *(87,88)*
Lamivudine	M184V *(84,102,126,187,188)*
Abacavir	M184V, L74V, K65R, Y115F *(105,106)*
Tenofovir	No monotherapy data
Dual-nucleoside therapy	
Zidovudine+didanosine	TAMS, Q151M complex *(82)*
Zidovudine+zalcitabine	TAMS *(83)*
Stavudine+didanosine	TAMS, L74V, Q151M complex *(85,119,135)*
Zidovudine+lamivudine	M184V, TAMS *(84,89,110,135,189)*
Stavudine+lamivudine	M184V, TAMS, recently K65R *(1,86,89,189,190)*
Abacavir+lamivudine	M184V, L74V, K65R, Y115F *(110,147)*
Abacavir+zidovudine	TAMS, M184V *(110)*
Didanosine+lamivudine	M184V, L74V, K65R *(157)*
Didanosine+emtricitabine	M184V, L74V, rarely TAMS *(119,158)*
Tenofovir+lamivudine	M184V, K65R *(1)*
Tenofovir+didanosine	K65R, L74V *(143,144)*
Triple-nucleoside therapy	
Abacavir+lamivudine+zidovudine	M184V, TAMS, rarely L74V and Y115F *(148,159,191)*
Abacavir+didanosine+stavudine	K65R, S68G *(192)*
Tenofovir+lamivudine+abacavir	M184V, K65R *(140,142)*
Tenofovir+lamivudine+didanosine	M184V, K65R *(193)*

PARTICULAR MUTATIONAL PATTERNS

Table 2 describes the specific patterns seen with particular single NRTIs or with combinations with or without NNRTIs or protease inhibitors. The individual mutation(s) are summarized next.

Thymidine Analog Mutations

Shortly after zidovudine was first used to treat HIV, isolates from patients on prolonged zidovudine monotherapy therapy were found to accumulate characteristic RT mutations at RT amino acid positions 41, 67, 70, 210, 215, and 219

(68,69). These mutations were associated with increasing phenotypic resistance to zidovudine *(70)*. Similar mutational patterns were seen from in vitro passage experiments *(71)*. Initial observations on relatively few patient isolates described a particular ordering of mutations that progressively increased the level of resistance. The initial mutation most often was K70R, which confers a fourfold to eightfold increase in resistance to zidovudine. This was typically followed by T215Y, the disappearance of K70R, and the subsequent evolution of M41L. D67N, K219Q/E/N, and L210W were seen, but less frequently *(69,72)*. More-recent observations using much larger data sets have found two different pathways of the evolution among TAMS *(73–76)*. The first and most common pathway clusters M41L, D67N, L210W, and T215Y. The second cluster includes D67N, K70R, T215F, and K219Q/E/N. The second pathway generally displays less phenotypic resistance to all of the nucleosides than the first *(77,78)*, perhaps because the major amino acid 215 mutation occurs later in the order of mutations *(76)*. There is little data on what drives the selection of these different TAM pathways.

The TAMS are selected primarily by the thymidine analogs, stavudine and zidovudine, either alone or in combination with other drugs *(69,79–88)*. Stavudine and zidovudine select for TAMS at similar rates *(89)*. Although TAMS are primarily selected by the thymidine analogs, the presence of increasing numbers of TAMS confers increasing phenotypic cross-resistance to all of the nucleoside and nucleotide analogs available *(90)*. TAMS also decrease in vivo activity of the nucleoside and nucleotide analogs. For this reason, some experts refer to them as nucleoside analog mutations. A 215Y/F present at baseline was predictive of increased progression to AIDS or death in patients treated with zidovudine or didanosine monotherapy *(80,91)*. TAMS substantially reduced antiviral activity to stavudine monotherapy *(92)*, didanosine monotherapy *(93)*, stavudine plus didanosine therapy *(64)*, and stavudine plus lamivudine therapy *(65)*. The presence of TAMS, particularly T215Y/F, was associated with diminished viral load reduction in patients who had tenofovir, abacavir, lamivudine, or didanosine added to existing therapy *(94–97)*. Incremental reductions in response were seen with increasing numbers of TAMS *(94,95)*.

Other mutations at position 215, including T215S, C, and D do not cause measurable reduced drug susceptibility in vitro, but are transitional mutations on the way from wild-type T to the two-position mutations Y or F. They are commonly called T215 revertants, because they are often seen in patients who once had T215Y/F virus, but discontinued therapy, or are more commonly found in strains of treatment-naive patients who were infected with drug-resistant virus *(20,98–100)*. They are more fit in the absence of drug pressure than the T215Y/F mutants *(101)*. Presence of these mutations in antiretroviral-naive patients was associated with an increased risk of virological failure and selection of the T215Y in one recently described cohort *(100)*.

M184V

Codon 184 is a component of the catalytic active site of the RT that directly interacts with the incoming nucleotide *(32)*. An M184I/V mutation causes steric hindrance that selectively reduces the incorporation of nucleosides with β- or L-ring configurations, namely lamivudine, didanosine, zalcitabine, abacavir, and emtricitabine *(35,36)*. Lamivudine in vitro and in vivo rapidly selects for the M184I mutation, which is replaced by the M184V mutation because of a fitness advantage *(102–104)*. The M184V mutation is seen as early as 1 wk after initiation of lamivudine treatment and is present in nearly all patients after 3 mo of lamivudine monotherapy *(102)*. M184V is also selected by abacavir monotherapy *(105,106)*, occasionally by didanosine monotherapy *(107–109)*, by abacavir plus zidovudine *(110)* combination therapy, and by all combination therapies that include either lamivudine or emtricitabine *(1,2,111–119)*.

M184V causes high-level in vitro resistance to lamivudine and emtricitabine *(90,120)*, and although M184V confers some degree of resistance to abacavir and didanosine *(90)*, this mutation alone is not sufficient to significantly decrease the response to abacavir or didanosine *(121,122)*. A recent small study also revealed that despite high levels of in vitro resistance, lamivudine may retain some in vivo activity against viruses with the M184V mutation *(123)*. As mentioned in the "General Mechanisms of How the Mutations Confer Drug Resistance" section, M184V reverses TAM-associated resistance to zidovudine, stavudine, and tenofovir *(90,124,125)* by decreasing the rate of primer unblocking *(53,54)*.The M184V mutation has been the subject of intense basic and clinical investigation. Initial studies of lamivudine monotherapy found that HIV RNA levels remain approx 0.5 $\log_{10}$ copies/mL below the pretreatment levels for 6 to 12 mo, despite the presence of high-level lamivudine resistance caused by the M184V mutation *(102,126,127)*. Laboratory studies have found that M184V reduces the viral replication capacity compared with wild-type virus in the absence of drug by decreasing the RT processivity (the relative efficiency of completing successful transcription) *(128–131)*. In addition to decreased processivity, RTs with M184V have up to a 50-fold increase in the RT fidelity (the ability to select the correct nucleotide) compared with wild-type RT, theoretically reducing mutation rates *(132,133)*. Clinical studies have also documented a diminished rate of acquiring TAMS when lamivudine is administered with thymidine analogs, perhaps because of the presence of the M184V mutation *(134–136)*.

K65R

Codon 65 is in the fingers region of the RT and interacts with the γ-phosphate of the incoming dNTP *(32)*. Mutations at this site confer resistance by

decreasing nucleoside analog incorporation rates compared with the natural dNTPs *(43,44)*.

As mentioned in the "Epidemiology" section, the K65R mutation is increasing in prevalence, correlating with the increased use of tenofovir and abacavir *(16,137)*. K65R was initially found to be selected by zalcitabine in vitro *(107,138)*. It occasionally occurred in patients treated receiving didanosine *(109)* or abacavir monotherapy *(105,106)*. K65R is the first mutation selected by tenofovir in vitro *(139)*, and is selected in antiretroviral-naive patients who receive tenofovir in any dual or triple combination with lamivudine, didanosine, abacavir, or emtricitabine *(1,140–144)*. K65R rarely occurs when tenofovir is added to failing regimens in patients with preexisting TAMS *(145,146)*. Combination therapy with abacavir plus lamivudine can select for a K65R *(110,147)*, but usually not when zidovudine is included as a third agent *(148)*. In contrast to zidovudine, regimens containing stavudine have been reported to rarely select for K65R. In a recent study of stavudine plus lamivudine plus efavirenz in treatment-naive patients, 2 of 25 patients with virological failure developed a K65R mutation *(1)*. Another study that included a combination of abacavir plus didanosine plus stavudine found that five of eight failures contained the K65R mutation, along with the unusual mutation, S68G *(149)*.

K65R confers partial resistance to zalcitabine, didanosine, abacavir, tenofovir, lamivudine, emtricitabine, and possibly stavudine *(99,125,139,150,151)*. A K65R mutation at baseline was associated with a poor response in patients who had tenofovir added to an existing regimen *(94)*, although the few patients who developed a K65R mutation after therapy in the same cohort did not have a corresponding increase in viral load toward baseline *(146)*. This may result from multiple factors. Similar to M184V, K65R reduces TAM-mediated primer unblocking *(45,55)*, resulting in a net hypersusceptibility to zidovudine. In addition, K65R reduces RT processivity and viral replicative capacity, comparable to the M184V mutation *(125,152,153)*.

L74V

Codon 74 interacts directly with the template and the incorporating dNTP *(32)*. The mutation L74V confers resistance by selectively reducing incorporation rates of specific nucleoside analogs *(40)*. L74V is the most common mutation that arises in patients receiving didanosine monotherapy *(109,154–156)* and is often seen in patients receiving abacavir monotherapy *(105,106)*. Combinations of didanosine plus lamivudine *(157)*, didanosine plus emtricitabine *(158)*, and abacavir plus lamivudine *(110,147)* can select for L74V. Coadministration of zidovudine with abacavir or didanosine significantly decreases the selection of L74V *(110,135,148,156,159,160)*, probably because L74V causes hypersusceptibility to zidovudine *(154,161)* through diminished

primer unblocking *(57)*. L74V has occasionally been seen in patients receiving stavudine plus didanosine *(85,86)*.

L74V confers resistance or partial resistance to didanosine, zalcitabine, lamivudine, emtricitabine, and abacavir *(109,151,154)*. L74V at baseline prevented a virological response to didanosine intensification *(162)*. L74V viruses are hypersusceptible to zidovudine and to tenofovir but not to stavudine *(154,161,163)*. Similar to M184V, enzymes possessing the L74V mutation have less processivity than wild-type virus, conferring a replication defect in the absence of drug *(130,152)*.

Q151M Complex

Codon 151 is in a conserved region that interacts with the incoming dNTP *(32,164)*. Q151M interferes with NRTI binding and incorporation *(38)*. Q151M develops in 5 to 10% of patients treated with thymidine analog plus didanosine combinations, and causes moderate resistance to all of the nucleoside analogs *(82,85,86,135,165,166)*. The Q151M mutation is generally associated with mutations at positions A62V, V75I/F, F77L, and F116Y. Each of these mutations substantially increases the degree of resistance and/or the fitness in the presence of drug of isolates containing the Q151M mutation *(167,168)*. Tenofovir retains some activity against HIV with this mutational complex *(169)* and lamivudine may also have activity *(170)*.

Codon 69 Mutants and Insertions

Codon 69 is in the fingertip region that makes contacts with the incoming dNTP *(32)*. Initially, T69D mutation was associated with zalcitabine therapy *(171)*, but a variety of changes at this site develop with most of the available nucleosides *(172)*. T69D/N/S/A have all been shown to cause some degree of resistance to zidovudine, didanosine, zalcitabine, and stavudine in site-directed mutagenesis studies *(172)*. T69D/N developed with didanosine monotherapy and was associated with clinical progression and CD4 decline in a group of 30 HIV-infected children, whereas other mutations, such as L74V and M184V, were not associated with CD4 decline *(173)*.

Insertions at the fingers region between codons 67 and 70 (most commonly two amino acids at position 69) occur in 1 to 2% of patients extensively treated with nucleoside analogs *(174,175)*. These inserts are typically found in viruses that also contain TAMS. As described in more detail in the “General Mechanisms of How the Mutations Confer Drug Resistance” section, these inserts enhance primer unblocking of TAMS by destabilizing the dead-end complex formation with the next complimentary nucleotide *(67)*. Insertions by themselves confer minor degrees of resistance to the nucleosides, but in the presence of TAMS, resistance is markedly increased *(176)*. Isolates with inser-

tions at codon 69 and TAMS are highly cross-resistant to all of the currently available NRTIs *(166,169,176,177)*.

E44D and V118I

Mutations E44D/A and V118I both occur in untreated patients, but their prevalence increases in subjects who have received extensive NRTI treatment *(178,179)*. They have generally been observed in patients receiving multiple nucleoside regimens who also have TAMS *(178)*. V118I causes resistance by decreasing the NRTI incorporation, whereas E44D enhances ATP-mediated NRTI excision *(46)*. These mutations were initially presented as alternative pathway lamivudine-resistance mutations because they confer approx 10- to 15-fold resistance to lamivudine *(180)*, as well as lower-level resistance to other antiretroviral agents *(181)*. However, these mutations are not selected by lamivudine *(179)* and did not impair the antiviral activity of lamivudine-containing combination regimens in one study *(182)*.

Y115F

Codon 115 directly interacts with the incorporating nucleotide *(32)*. A mutation Y115F was selected in vitro and in vivo by abacavir monotherapy *(105,106,151)*, but not by other NRTIs. Few data are available on the impact of Y115F on resistance to other NRTIs.

Other Mutations

An exploratory analysis from a large database of sequences from both antiretroviral-naive and antiretroviral-experienced patients found that nine other mutations were definitely associated with NRTI therapy. These were mutations at codons 20, 39, 43, 203, 208, 218, 221, 221, 223, and 228. Nine additional mutations were associated with NRTI therapy but did not achieve statistical power after correcting for multiple comparisons. These were mutations at codons 60, 64, 104, 122, 135, 196, 200, 207, and 211 *(183)*.

SUMMARY

NRTIs were the first class of drugs used to treat HIV and are still included in most antiretroviral regimens. Despite the increasing number of different NRTIs available, substantial cross-class resistance exists. Further investigations are needed to determine the optimal sequencing strategies for nucleosides to maximize their use before extensive cross-resistance develops. Advancing knowledge of the biochemical and structural basis of resistance will aid in the design of newer compounds that are active against HIV resistant to the currently available drugs, ultimately prolonging virological suppression and life in the millions of people who are infected with HIV.

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7

Pharmacokinetics of Reverse Transcriptase Inhibitors

Patrick Hoggard, Stephen Kewn, Saye Khoo, and David Back

INTRODUCTION

The nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of compounds discovered to be potent inhibitors of HIV replication *(1)* and, to date, these drugs remain the backbone of antiretroviral therapy. NRTIs are essentially prodrugs, inactive in their parent form and requiring activation to exert their antiviral effects *(2,3)*.

This chapter describes the activation of NRTIs, focusing on their intracellular kinetics, and explains how antiviral activity of NRTIs may potentially be enhanced through manipulation of activation pathways.

Reverse Transcription

The nucleic acid of HIV is RNA. To replicate, the virus must reverse transcribe its RNA into DNA (Fig. 1). HIV reverse transcriptase (RT) catalyses the production of a DNA copy of the viral RNA using the host's endogenous deoxynucleoside triphosphates (dNTPs) as substrates *(4)*. Host dNTPs within the cell are formed through either the salvage or the *de novo* pathways (Fig. 2). The proviral DNA then enters the nucleus, where it is randomly incorporated into host chromosomal DNA by viral integrase *(5)*.

Reverse Transcriptase Inhibitors

To block the reverse transcription process, a number of drugs that inhibit the enzyme RT have been developed. The NRTIs are structurally similar to the endogenous deoxynucleosides, with structural substitutions of the OH group on the deoxyribose sugar (Fig. 3).

After entry into host target cells, NRTIs are phosphorylated by intracellular kinase enzymes to their active triphosphate derivatives (ddNTPs) (Fig. 4). NRTIs use the host's intracellular kinase enzymes, which are normally respon-

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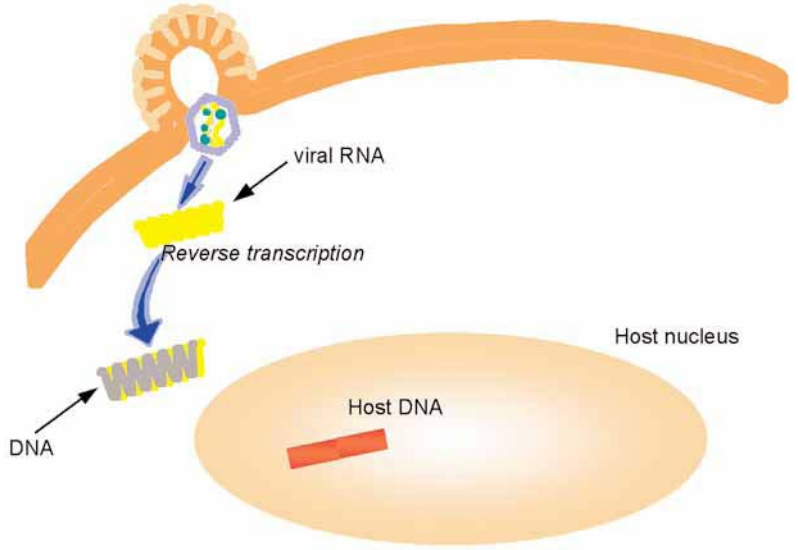

Fig. 1. HIV reverse transcription. The conversion of viral RNA to DNA.

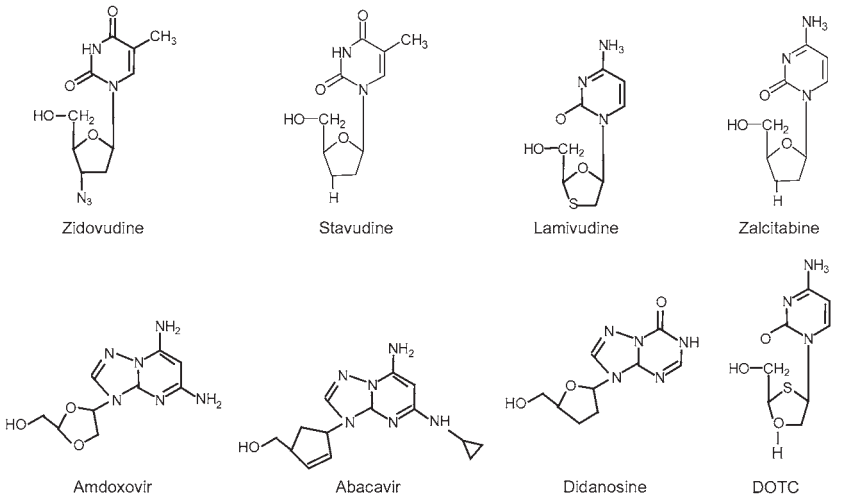

Fig. 2. The source of nucleotides from the *de novo* and salvage pathways.

sible for the activation of endogenous deoxynucleosides to their dNTPs as part of the replicative process of the cell's DNA.

The drug triphosphates inhibit HIV replication by two mechanisms *(1,6)*. First, they compete with the host's endogenous dNTPs for incorporation into viral DNA, thus, inhibiting viral DNA synthesis (Fig. 5). Therefore, although

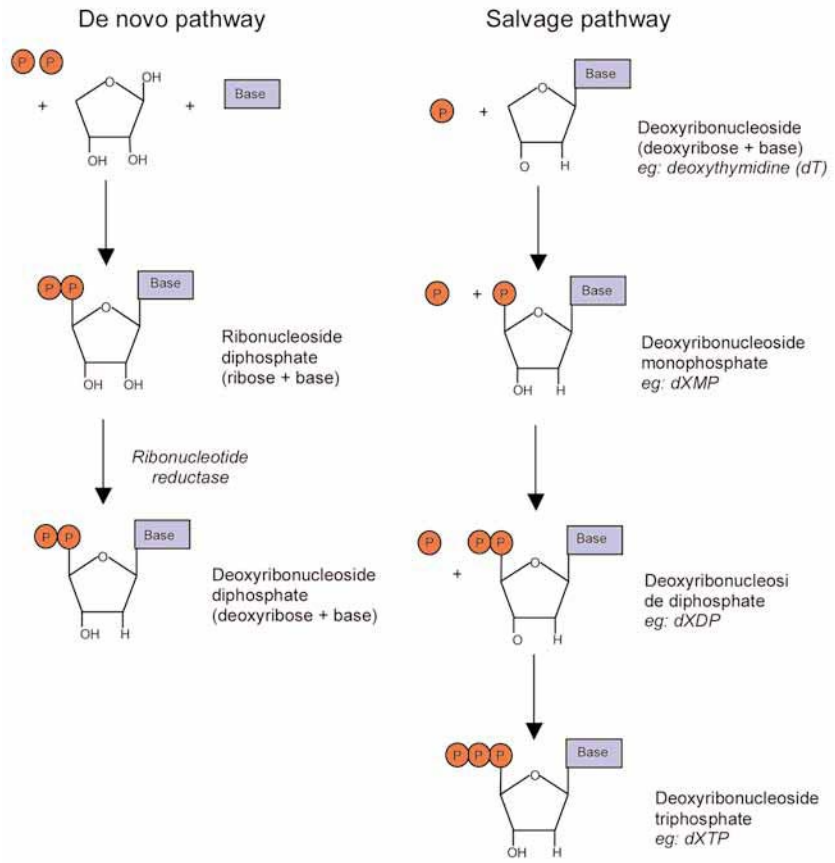

Fig. 3. Structure of the NRTIs showing the structural substitutions of the 3′ hydroxy group.

the phosphorylation of the NRTIs is essential, it is the ratio of the exogenous ddNTPs to the endogenous dNTPs that ultimately determines antiviral activity *(7–9)*. To gain an understanding of the intracellular pharmacology of NRTIs involves studies of both endogenous and exogenous triphosphates.

A second mechanism of inhibition of HIV replication is chain termination. The lack of a 3′-hydroxy group required to form 3′,5′-phosphodiester linkages means that chain termination results after incorporation of NRTIs into the viral DNA (Fig. 5) *(1,6)*.

Binding of ddNTPs as chain terminators to the RT is reversible *(10)*. That is, it is thought that, through mechanisms such as pyrophosphorolysis, the bound NRTIs can be removed from the proviral DNA chain. This will limit the activity and/or potency of these drugs.

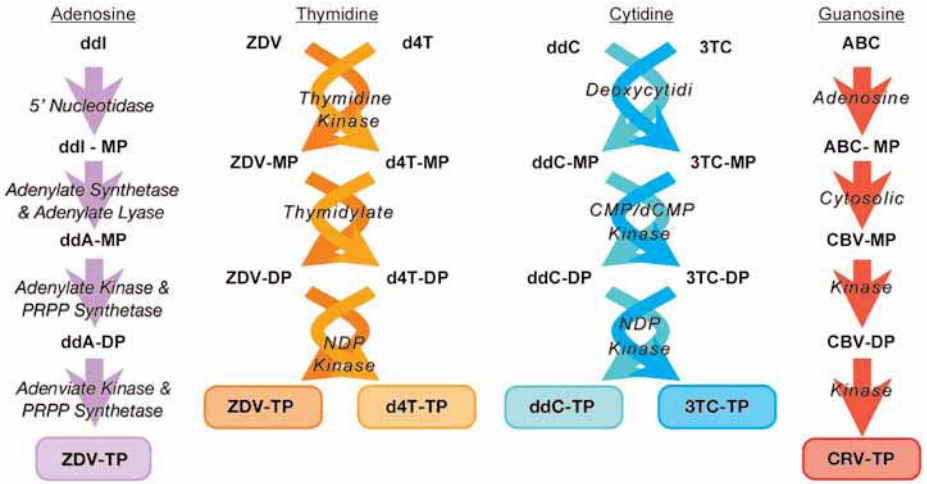

Fig. 4. Activation of the nucleoside analogs. NDP, nucleoside diphosphate; MP, monophosphate; and TP, triphosphate.

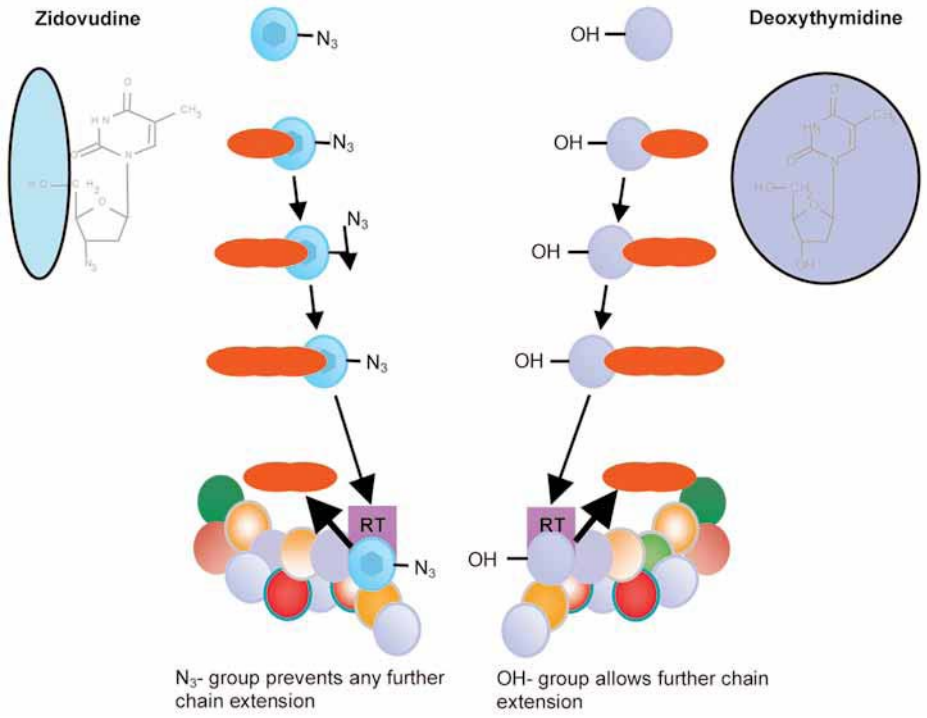

Fig. 5. The activation of ZDV showing the competition for endogenous kinases with endogenous deoxythymidine.

INTRACELLULAR PHARMACOKINETICS OF THE NRTIs

Determining NRTI plasma concentrations is of limited benefit when attempting to relate drug concentrations to antiviral effect *(11)*. Of greater significance are the studies that have measured active triphosphate in peripheral blood mononuclear cells (PBMCs) from HIV-infected patients or from cells in culture. Table 1 gives a compilation of data for all NRTIs and indicates that the triphosphate anabolites have a much longer half-life than the parent drug in plasma.

Considerable research effort has been put into methodologies for determining intracellular triphosphate metabolites to optimize dosing regimens. The techniques involved are time consuming and difficult but essential to investigate pharmacokinetics at the active site. Several different approaches to triphosphate determination have been published and all have highlighted the large variability in triphosphate levels between patients.

In Vitro Assays

Until recently, most data regarding the intracellular activation of the NRTIs have been obtained in vitro. The majority of in vitro studies have been performed using radiolabeled NRTIs in either immortal cell lines or isolated PBMCs. Because HIV infects both resting and activated cells, antiviral activity may not correspond to the in vitro data. Estimations of in vitro anti-HIV activity should only be used as a guide for potential clinical efficacy. Therefore, it is imperative that intracellular measurements of the NRTIs should be performed in vivo.

In Vivo Studies—HPLC Coupled to Tandem Mass Spectrometry

One approach to measuring intracellular levels of NRTIs involves separation of intracellular phosphates by high-performance liquid chromatography (HPLC) and enzyme digestion of these metabolites before analysis of the parent drug by radioimmunoassay *(24)*. Another approach, allowing quantification of zidovudine (ZDV) triphosphate (ZDVTP) and lamivudine (3TC) triphosphate (3TCTP) in HIV-infected patients, is solid phase extraction combined with mass spectrometry *(25)*. A promising direct method involving immunoaffinity and liquid chromatography coupled to tandem mass spectrometry has been recently developed for measurement of ZDVTP *(26)*, allowing measurement of femtomole concentrations in PBMCs from 7 mL of blood. In addition, the latest technology, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, has been described by van Kampen et al. for analysis of ZDVTP *(27)*. Methods have also been developed for determination of stavudine (d4T; 2′,3′-didehydro-3′-deoxythymidine) and didanosine (ddI; 2′,3′-dideoxyinosine) phosphorylated metabolites, which can be used to routinely determine both drug and endogenous triphosphate levels. Again, the method involves liquid chromatography coupled to tandem mass spectrometry (electrospray ionization; refs. *28* and *29*).

Table 1
Half-Lives of the NRTIs (in Plasma) and Their Triphosphates (in Cells)[a]

Drug	Parent drug plasma half-life (h)	Drug triphosphate intracellular half-life (h)	Ref.
ZDV	1.5	3.5	*(12)*
d4T	1	7.0	*(13)*
3TC	5	12	*(14)*
ddC	1.5	2.6	*(15,16)*
ddI	1	36	*(17,18)*
ABC	1.5	12	*(19,20)*
DAPD	1	27	*(21)*
dOTC	1	2.5	*(22,23)*
TDF	16 h	>60 h	*(65)*
FTC	10 h	39 h	*(55,62)*

[a]Data collaged from a number of studies

Template–Primer Extension Assays

Because inhibition by the NRTIs is a competition between drug and endogenous triphosphates, it is clearly preferable to also determine the endogenous dNTP levels. Both drug and endogenous triphosphates have recently been determined by template–primer extension assays. The basis of the method is similar to the mechanism of action of the NRTIs, i.e., there is competition for RT between drug triphosphate and endogenous triphosphate. Using isolated HIV RT, a synthetic DNA primer, and radiolabeled dNTPs, triphosphates of 3TC, ZDV, abacavir (ABC), and (–)-β-D-2,6-dioxalane guanosine (DXG) have been determined *(21,30,31)*. Because the endogenous dNTPs are also measured, the drug-to-endogenous nucleoside triphosphates ratio can be calculated.

HPLC Methods

Early studies separated ZDV phosphates by HPLC, after enzymic removal of the phosphates. ZDV was subsequently measured by radioimmunoassay *(32)*. Quantification of all three phosphates has more recently been performed with a solid-phase extraction method. After resolution of 3TC nucleotides on an anion exchange cartridge, the phosphates are removed by enzyme digestion, and the 3TC product quantified by HPLC with ultraviolet detection *(33)*.

NRTIs IN CLINICAL USE

Zidovudine

ZDV is a thymidine analog (Fig. 3) that enters the cell by passive diffusion *(33)*. After entry into the cell, ZDV is converted to monophosphate (*34*; Fig. 4).

The diphosphorylation step (catalyzed by thymidylate kinase) is rate limiting and results in the intracellular accumulation of ZDV monophosphate *(35,36)*.

In vitro studies have shown that ZDVTP formation is saturated at ZDV concentrations higher than 2 μ*M*, but ZDVMP formation is not saturated at concentrations up to 10 μ*M*. Clinical studies have shown that the triphosphate metabolite of ZDV, in common with the other NRTIs, has a much longer half-life within PBMCs than the plasma half-life of the parent drug (Fig. 6) *(32,37,38)*.

In HIV-infected patients administered ZDV, the main metabolite in PBMCs was monophosphate, illustrating that conversion to diphosphate is rate limiting both in vivo and in vitro. Studies have shown antiviral effect to be correlated with the intracellular level of ZDVTP and the ZDVTP-to-deoxythymidine ratio in vitro. The intracellular concentration of ZDVTP was positively correlated to both the increase in CD4 cells and the decrease in plasma viral load during therapy *(39)*. On the other hand, there is evidence that cytotoxicity has a better relationship with ZDV monophosphate *(40)*. Interestingly, ZDV monophosphate accumulation is greater in HIV patients than in seronegative volunteers *(32)*.

Stavudine

After entering the cell by passive diffusion *(41)*, d4T initial phosphorylation is catalyzed by thymidine kinase (Fig. 4). In vitro studies have shown that the majority of intracellular d4T is unphosphorylated parent drug, thereby indicating that this first enzyme conversion is rate limiting for d4T activation to its 5′-triphosphate metabolite (Fig. 4).

The intracellular concentration of d4T triphosphate provides a better measure of in vivo antiviral activity than plasma d4T levels *(42)*. d4T has increased antiviral activity in replicating cells compared with quiescent cells. After mitogen stimulation there is a 17-fold increase in the activity of thymidine kinase. This shows that thymidine analogs are preferentially phosphorylated in activated cells and have greater activity in activated rather than quiescent cells.

Recently, the first in vivo data on d4T triphosphate were obtained in a clinical trail involving 28 antiretroviral-naive HIV-infected patients who received d4T (40 or 30 mg twice daily) in combination with ddI and efavirenz. Median d4T-TP concentrations were 31 fmol/10^6 cells (range 0–99 fmol/10^6 cells) *(43)*.

Lamivudine

3TC is a cytidine analog (Fig. 3) that enters the cell either by passive diffusion or by the facilitated diffusion nucleoside transport process *(44,45)*. The initial activation is by deoxycytidine kinase, followed by further phosphorylation to 3TCTP (Fig. 4) *(44)*. In vitro studies have shown the predominant anabolite to be 3TC diphosphate, indicating that the rate-limiting step in 3TC

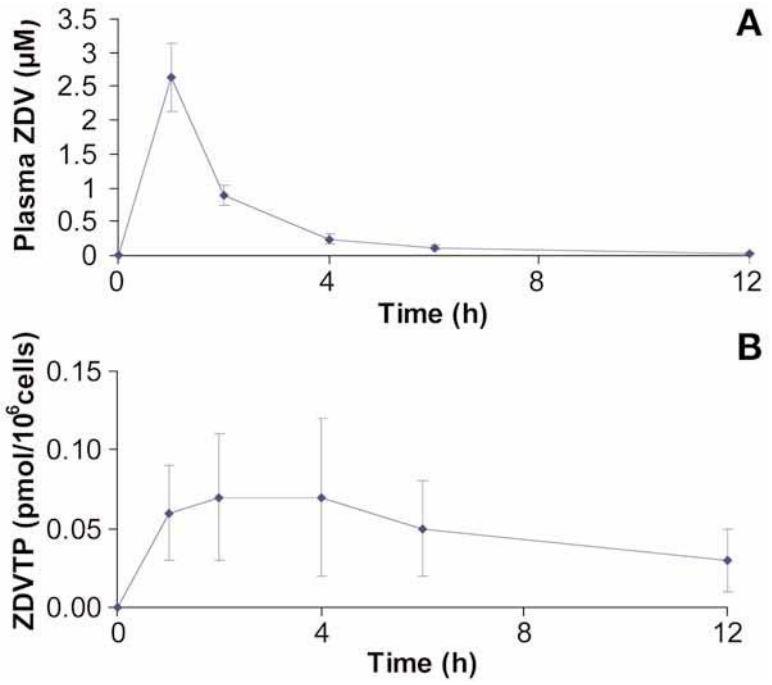

Fig. 6. The pharmacokinetic profile of ZDV in **(A)** plasma and **(B)** PBMCs from HIV-infected patients after oral administration of 300 mg ZDV. Data are expressed as mean ± SEM; $n = 10$ *(36)*.

activation is conversion to the active triphosphate (Fig. 4) *(46)*. The phosphorylation of this drug has shown no saturation in vitro with proportional increases in intracellular 3TC activation up to concentrations of 10 μ*M*.

A pharmacokinetic study determining the intracellular phosphorylation of 3TC in asymptomatic HIV-infected patients administered doses of 150 or 300 mg 3TC twice daily showed a less than twofold difference in the intracellular concentrations of the phosphate anabolite, despite a doubling of plasma 3TC concentrations *(14)*.

Recently, Yuen et al. compared the steady-state pharmacokinetics of 3TC in plasma and 3TCTP in PBMCs in healthy subjects after 7-d courses of treatment with 300 mg 3TC once daily and 150 mg 3TC twice daily *(47)*. Steady-state intracellular 3TC-TP levels were bioequivalent with respect to the area under the curve after once- and twice-daily regimens.

Zalcitabine

Zalcitabine (ddC; 2′,3′-dideoxycytidine) is also a cytidine analog (Fig. 3) and enters the cell by either nucleoside carrier-mediated or noncarrier-mediated

transporters *(48,49)*. Activation of ddC uses the same intracellular enzymes responsible for 3TC phosphorylation (Fig. 4) *(50)*. No individual metabolite predominates, indicating that the initial conversion of ddC to its monophosphate is the rate-limiting step in its activation.

On mitogen stimulation, a relatively small (twofold) increase in deoxycytidine kinase is seen, indicating that cytidine analogs are preferentially phosphorylated, and that they exert anti-HIV activity that is more potent in resting cells than in activated cells. That is, although more active ddC triphosphate is formed in the stimulated cells, the actual ratio of ddC triphosphate to endogenous deoxycytidine triphosphate is decreased *(51)*.

The intracellular concentrations of ddC in in vitro experiments are considerably smaller than those of 3TC when studied at the same concentrations. In addition, in contrast to 3TC, the activation of this drug is not saturated. There are no data available on ddC triphosphate levels in vivo.

Abacavir

After entry by nonfacilitated diffusion, ABC undergoes conversion by adenosine phosphotransferase to its monophosphate before catalysis to carbovir monophosphate, a guanosine analog (Figs. 3 and 4). Further activation yields carbovir triphosphate (CBVTP), which competes with endogenous deoxyguanosine triphosphate (dGTP) *(52,53)*. Although carbovir shows potent antiviral activity, it is not directly administered because of toxicity and low bioavailability. In vitro studies have shown a linear relationship between the concentration of ABC and its active intracellular metabolite. Preliminary pharmacokinetic studies with 600 mg ABC suggest a relatively long half-life of CBVTP (Fig. 7), and support once-daily dosing *(54,55)*.

Didanosine

ddI is an inosine analog (Fig. 3). The mechanism of cell entry has to be fully elucidated. It is known that active transport is not involved, but the role of passive carriers is unclear *(17)*. It has an unusual activation pathway (Fig. 8), in that the enzyme primarily responsible for the first phosphorylation step is not a kinase but a phosphotransferase, with the major phosphate donor being inosine monophosphate (IMP) *(56)*. After the initial phosphorylation, ddI is converted to a dideoxyadenosine analog before undergoing further activation to dideoxyadenosine triphosphate (ddATP), which competes with endogenous deoxyadenosine triphosphate (dATP) for incorporation into the proviral DNA *(17,57)*. ddI is also metabolized by purine nucleoside phosphorylase, resulting in catabolism to hypoxanthine and a low conversion to the active ddATP. The active triphosphate anabolite has recently been determined in vivo, with median ddATP levels of 8 fmol/10^6 cells (range 0–23 fmol/10^6 cells) recorded *(29,43)*.

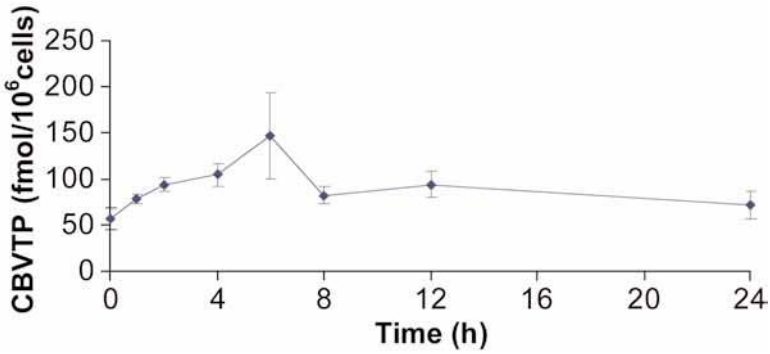

Fig. 7. The intracellular CBVTP pharmacokinetic profiles in HIV-infected patients during 24 h after a dose of 600 mg ABC *(30)*.

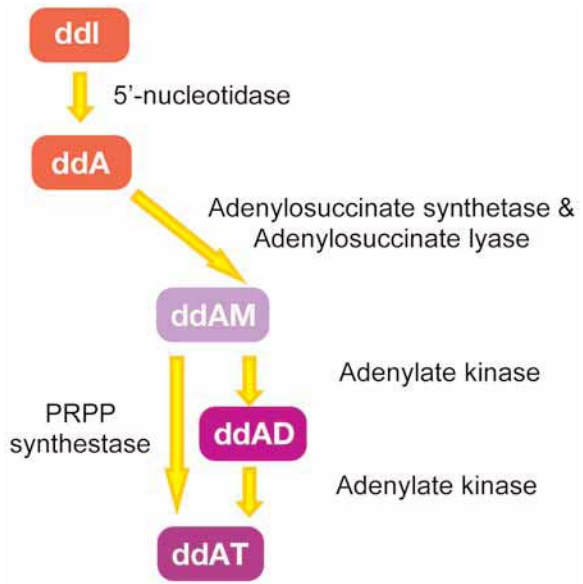

Fig. 8. The intracellular metabolism of didanosine. PRPP, 5′-phosphoribosyl-1-pyrophosphate.

Amdoxovir

Amdoxovir (DAPD; [–]-β-D-2,6-diaminopurine dioxalane) is a guanosine analog prodrug of DXG ([2R-*cis*]-2-amino-1,9-dihydro-9-[2-hydroxymethyl]-1,3-dioxolan-4-yl-6H-purin-6-one). Both DAPD and DXG are novel nucleosides displaying activity against HIV. DAPD is deaminated in vivo by adenosine deaminase to DXG *(58)*. DXG is then sequentially phosphorylated to its active

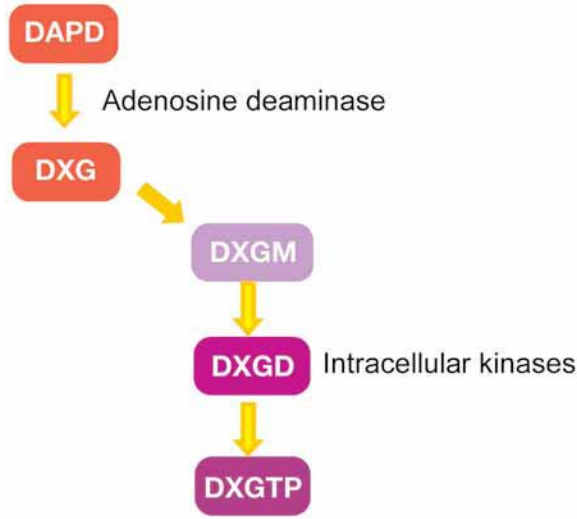

Fig. 9. The activation of DAPD. DXGMP, DXG monophosphate; DXGDP, DXG diphosphate; DXGTP, DXG triphosphate.

metabolite, DXG triphosphate (Fig. 9) *(59)*, which competes with its endogenous substrate, dGTP for binding to HIV RT. Lesser amounts of DAPD triphosphate are also formed, but in vitro studies have shown that DXG triphosphate is much more potent than DAPD triphosphate against HIV-1 RT *(60)*.

dOTC (SPD754)

The novel nucleoside analog 2′deoxy-3′-oxa-4′-thiocytidine (dOTC) is a racemic mixture of two enantiomers. Both enantiomers show some activity but the (–) isomer (SPD754) shows the greater activity against HIV-1 *(23)*. dOTC is structurally related to 3TC (Fig. 3). Intracellular metabolism studies showed that dOTC is phosphorylated within cells by the deoxycytidine kinase pathway and that approx 2 to 5% of dOTC is converted to triphosphate derivatives, which had measurable half-lives (2–3 h) within cells *(23)*. Adams et al. *(61)* have described the pharmacokinetics of plasma SPD754 and intracellular SPD754 triphosphate. The intracellular half-life of the triphosphate was approx 6 to 7 h.

Emtricitabine

Emtricitabine (FTC) is an analog of deoxycytidine that undergoes activation through the deoxycytidine kinase pathway. β-L-2′,3′-dideoxy-5-fluoro-3′-thiacytidine ([–]FTC) is in phase III trials for HIV treatment; it is being considered for use in multidrug combination therapy of HIV-1. The half-life of FTC in plasma and cells is reported to be 7.4 h and 39 h respectively *(62)*.

Nucleotide Analogs—Tenofovir

Nucleotide analogs are custom-designed drugs with a phosphate group present so that only two activation steps are necessary. Nucleoside monophosphates are not stable in vivo and, consequently, more-stable nucleotide analogs with phosphonate bonds have been designed. These phosphonate nucleotides, such as tenofovir, have poor bioavailability because of the high polarity of the phosphonate group *(63)*. The attachment of labile lipophilic groups to these analogs is able to mask the polarity and allow cellular entry. Cleavage of the masking groups regenerates the monophosphate group, which is then metabolized to the active triphosphate metabolite.

Tenofovir is an acyclic adenine derivative that is administered as the prodrug, tenofovir disoproxil fumarate. After cell entry has occurred, unmasking occurs, followed by conversion to the active tenofovir diphosphate *(64)*. The incorporated tenofovir anabolite acts as a chain terminator because of the lack of a 3-hydroxy moiety in the molecule. Because tenofovir diphosphate is the active form, measurement of the intracellular anabolite is necessary to fully elucidate the pharmacokinetics of the drug. The half-life of the intracellular diphosphate in vivo has recently been determined to be approx 60 h *(65)*.

It has also been suggested that nucleotide analogs may play an important role against HIV replication in macrophages *(66)* and other nondividing cells, a unique and potentially important property that is not found with many members of the nucleoside analog class, particularly with thymidine analogs, such as ZDV and d4T. Tenofovir is a highly potent antiretroviral drug *(67)* and an important addition to the armamentarium.

FACTORS THAT MAY INFLUENCE INTRACELLULAR PHOSPHORYLATION

Cell Activation

Because the NRTIs require intracellular activation for antiretroviral activity, influences on the enzymes responsible for activation can modulate drug activity. In vitro, cellular factors have been shown to alter antiretroviral activity. The activation state of the cell may change the relationship between drug triphosphate and endogenous triphosphate. For instance, in activated cells, the ratios of ZDVTP to deoxythymidine triphosphate (dTTP) and of d4T triphosphate to dTTP are greater than in resting cells *(51,68)*. This is thought to be a result of changes in the cell cycle, in which increased expression of thymidine kinase is seen during S-phase. Conversely, ddC and 3TC have been shown to have a more favorable drug-to-endogenous triphosphate ratio in resting cells. Resting cells, such as monocytes/macrophages have low endogenous dNTP levels, which results in low catalytic activity of HIV RT *(66)*. Consequently, because

the ratio of competition between drug and endogenous triphosphates determine activity, less drug phosphorylation may be required for the same antiviral effect.

HIV Infection

Cell culture studies have shown the intracellular phosphorylation of the NRTIs to be similar in control and HIV-infected cells. However, in vivo studies showed total intracellular phosphorylation of ZDV in PBMCs isolated from healthy volunteers to be less than that in PBMCs isolated from HIV-infected patients. Furthermore, in patients with decreased CD4 cell counts, ZDV phosphorylation was greater in resting PBMCs, suggesting that HIV infection results in an increase in activation state and in increased drug phosphorylation.

Ex vivo studies in PBMCs show that ZDV phosphorylation is greater in cells from HIV-infected patients than healthy volunteers, but in cells from HIV-infected patients there is a reduced capacity for activation by in vitro stimuli.

Long-Term Changes in Intracellular Activation—Cellular Resistance

Drug regimens fail for many reasons, including virological resistance, poor tolerability, lack of adherence, and pharmacological resistance. Possible mechanisms of pharmacological resistance to antiretroviral agents include decreased influx of drug into cells or increased efflux of drug from cells. However, a decrease in the activity of kinase enzymes responsible for the formation of NRTI triphosphates may result in subtherapeutic levels of the active metabolite and, thus, decreased efficacy of antiretroviral therapy. Determination of drug levels in patients receiving combination therapy is required to evaluate the extent (if any) of intracellular kinase downregulation and, thus, the ability to assess whether altered phosphorylation occurs in these patients.

Early studies indicated that ZDV may downregulate its own metabolism during long-term therapy *(69)*. A modest inverse association between length of time on therapy and the formation of total ZDV phosphates was seen *(69)*. Subsequently, the ALTIS trial suggested a difference in virological response between ZDV-naive and ZDV-experienced patients after antiretroviral therapy with d4T plus 3TC. In patients who started therapy on d4T plus 3TC, a greater decrease in plasma HIV RNA level (1.66 $\log_{10}$ copies/mL) was seen in patients who had not previously received ZDV alone or as part of a combination (0.66 $\log_{10}$ copies/mL) than in those who were ZDV experienced. The ALTIPHAR study showed reduced d4T triphosphate and 3TCTP levels in a small number of ZDV-experienced patients who had not responded to the dual combination therapy, compared with ZDV-naive patients *(70)*. This suggested that previous therapy with ZDV reduced the subsequent phosphorylation of d4T and 3TC. The decreased activation to d4T triphosphate was thought to be a result of decreased thymidine kinase activity in vivo.

However, other studies failed to confirm the findings of the ALTIPHAR study. A cross-sectional study investigating ZDV phosphorylation showed no difference between patients receiving short- and long-term therapy *(71)*. A 12-mo longitudinal study investigating ZDV activation in a heterogeneous population of HIV patients observed no difference in total intracellular ZDV phosphates over time *(72)*. Second, there was no decrease between activation in ZDV-naive and -experienced patients. Studies also investigated whether long-term administration of ZDV altered the activation of d4T *(73)*. Using ex vivo measurements, no significant differences in the activation of d4T was seen in PBMCs isolated from HIV patients with or without previous exposure to d4T. These results suggest that if a reduction in the expression of thymidine kinase occurs in vivo during long-term therapy, d4T phosphorylation is unaltered.

The CHARM trial phosphorylation substudy measured triphosphates of ZDV, 3TC, and ABC to 48 wk. The results showed no changes in either ZDV or ABC phosphorylation over time. However, 3TC phosphates at 48 wk were decreased to less than 50% of baseline (week 0) *(74)*. Further studies are required to investigate the consequences of this reduction.

However, because drug efficacy is determined by the competition between drug triphosphate and endogenous triphosphate, it should be remembered that the efficacy of the NRTIs will be dependent on the triphosphate ratio. Determination of ratios in cohorts of patients receiving combination therapy is required to evaluate the extent of intracellular kinase downregulation and, thus, assess whether altered phosphorylation occurs in these patients.

Modulation of Intracellular Phosphorylation

As previously mentioned, ddNTPs compete with their corresponding dNTP for HIV RT and subsequent incorporation into the proviral DNA chain. Therefore, the activity of existing NRTI-containing combination therapy regimens may be improved by the inclusion of compounds that can modulate phosphorylation *(75)*.

Hydroxyurea

Hydroxyurea (HU) inhibits the enzyme ribonucleotide reductase, which is responsible for the reduction of ribonucleotides to deoxyribonucleotides *(76)*. Therefore, inhibition of this enzyme potentially results in decreased levels of dNTPs formed using the *de novo* pathways (Fig. 10). However, some dNTPs are also formed using salvage pathways, which, in turn, can be affected (through feedback mechanisms) by any alteration in the *de novo* synthesis.

In vitro studies have investigated the effects of HU in combination with several NRTIs. The mechanisms behind the potential synergism of HU with these agents have been postulated. First, dATP is preferentially depleted by HU

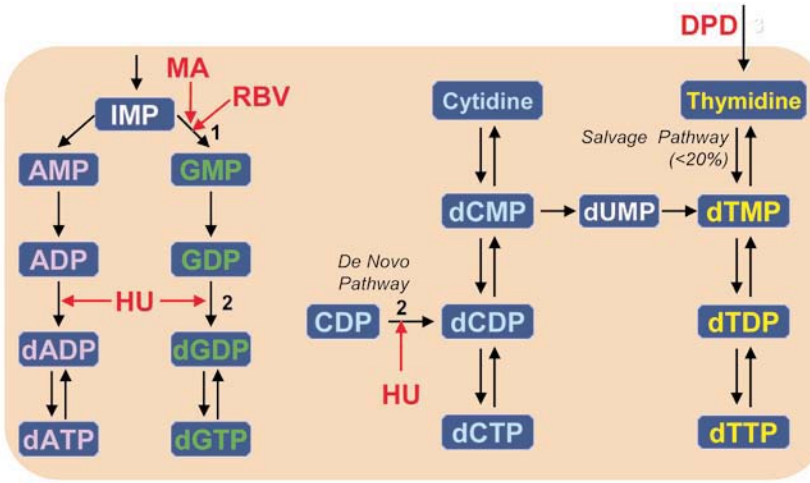

Fig. 10. The metabolic pathways of cellular nucleotides, showing the sites of action of mycophenolate (MA) and RBV at (1) IMP dehydrogenase; HU at (2) ribonucleotide reductase; and dipyridamole (DPD) at (3) deoxythymidine uptake.

(77–79). This depletion favors the incorporation of ddATP, the active anabolite of ddI, into the growing viral DNA strand. An enhancement of the anti-HIV activity of ddI, when combined with HU, has been noted in vitro *(7,78,80)*, presumably because of the increased ddATP-to-dATP ratio. Similarly, the activity of other purine analogs, such as ABC and DAPD, may be enhanced when combined with HU. However, HU does not enhance the anti-HIV activity of low-dose tenofovir disoproxil fumarate *(81)*.

In vitro studies of HU with pyrimidine analogs (e.g., ZDV and 3TC) have demonstrated less favorable enhancements of anti-HIV activities of these agents *(78,82)*. Increases in pyrimidine ddNTP levels have been observed, but the effects on the corresponding levels of dNTP are varied *(7,55,77,79,83)*. The differences in the effects of HU on purine and pyrimidine NRTIs may involve the reliance of purine dNTP production on *de novo* synthesis, whereas pyrimidine dNTPs are also produced using salvage pathways (Fig. 10).

To date, the majority of clinical studies investigating the effects of HU in combination with NRTIs have centered on the interaction with ddI. Some studies show a clinical benefit by adding HU to ddI-containing regimens *(76,84,85)*. However, other studies have been associated with a less favorable outcome. For example, a randomized trial comparing ddI plus d4T plus efavirenz with and without HU was discontinued because of excess drug toxicity in the HU-containing arm *(86)*.

Results from the pharmacology substudy of the CHARM trial, a study in which patients received ZDV plus ABC plus 3TC with and without HU, demonstrated

that intracellular dNTP levels were unaltered by HU *(74)*. Similarly, Hamzeh et al. *(87)* observed that dATP levels were unaffected in patients receiving HU monotherapy. The discordance in data from in vitro and in vivo studies stresses the importance of determining ddNTP and dNTP levels in clinical trials.

Ribavirin

Ribavirin (RBV) is a broad-spectrum antiviral agent that inhibits IMP dehydrogenase (IMPD) *(88)*. This enzyme is responsible for the conversion of IMP to xanthosine monophosphate, thus, leading to a reduction in the levels of dGTP, a purine dNTP. Because dGTP is an inhibitory regulator of the reduction of pyrimidine nucleotides, the reduced levels of dGTP result in an increased pyrimidine dNTP production (Fig. 10). In vitro studies have demonstrated that RBV reduces dGTP levels, whereas it increases the activation of purine NRTIs (e.g., ddI) *(79,89)*. Furthermore, the antiviral activity of purine NRTIs is also enhanced by RBV *(90,91)*. Therefore, theoretically, the combination of RBV plus ABC or DAPD could be advantageous.

Conversely, RBV has been shown to increase the levels of pyrimidine dNTPs (dTTP and dCTP), whereas, in vitro, it inhibits the activation of pyrimidine NRTIs, such as ZDV *(89,92)*. The effect of RBV on these pyrimidine metabolites is supported by RBV being shown to antagonize the anti-HIV activities of pyrimidine NRTIs *(90,92)*. Clinical use of the combination of RBV with interferon or pegylated interferon is the standard of care to treat hepatitis C virus coinfection in HIV-infected patients. However, the latest clinical data indicate that RBV does not adversely affect HIV disease control in patients receiving ZDV- and d4T-containing regimens (despite the in vitro interaction data) *(93–95)*, and does not alter intracellular concentrations of ZDVTP and d4T triphosphate *(96)*, at least in the short term. There is some evidence of an effect of high-dose RBV on ZDV phosphorylation in the short term *(97)*. In addition, because RBV is an IMPD inhibitor, and because it augments the inhibitory effects of the purine analog, ddI, there have been a number of reports citing clinically important ddI-related toxicities, including pancreatitis and lactic acidosis in patients coinfected with HIV and hepatitis C virus, and the recommendation is not to combine these agents *(98,99)*.

Mycophenolate Mofetil

Mycophenolate mofetil is hydrolyzed to its active metabolite, mycophenolic acid (MPA) in vivo. Similar to RBV, MPA also inhibits IMPD (Fig. 10), thus having similar effects on purine and pyrimidine metabolites in vitro *(79,100)*.

In vitro studies have also demonstrated that MPA can augment the anti-HIV activity of purine-based NRTIs and display activity against drug-resistant strains of HIV *(101–104)*. For example, ABC and MPA have been observed to

have a profound and synergistic anti-HIV activity in combination *(102)*. However, the same study also reported no synergy or even antagonism when MPA was combined with pyrimidine NRTIs against HIV.

A number of clinical studies have been undertaken, or are ongoing, to investigate the effects of MPA when used in combination with NRTIs, specifically ABC. Margolis et al. *(105)* concluded that in vivo modulation of the CBVTP-to-dGTP ratio may be achievable with doses of mycophenolate mofetil 50% lower than those used in organ transplantation. Additional studies are planned to examine the effects of MPA when administered in combination with other NRTIs (e.g., DAPD or tenofovir disoproxil fumarate), on anti-HIV activity in vivo.

Other Modulators of Phosphorylation

The following compounds may also be viable candidates for use in modulating the phosphorylation of both dNTPs and ddNTPs.

Azodicarbonamide

Azodicarbonamide has been previously tested as an anti-HIV agent through its ability to expulse zinc from viral-cysteine factors and to interfere with calcium mobilization machinery. However, it shares structural analogy with HU and has been observed to inhibit ribonucleotide reductase. Unlike HU, azodicarbonamide has also been observed to inhibit thymidine phosphorylation *(75)*.

Methotrexate

Methotrexate is thought to inhibit cell growth through an inactivation of dihydrofolate reductase, resulting in a disturbance of the *de novo* thymidylate and/or purine biosynthesis. Methotrexate has previously been shown to reduce intracellular dNTP levels *(79,106)*, whereas enhancement of the phosphorylation of both d4T *(79)* and 3TC *(107)* has also been observed in vitro.

Fludarabine

Fludarabine (FLU) has been shown to increase the phosphorylation of 3TC as well as to reduce dCTP levels, at high concentrations, resulting in an increased 3TCTP-to-dCTP ratio *(107)*. Similarly, studies by Rahn et al. *(45)* demonstrated modest increases in 3TC activation, using lower concentration of FLU. The activity of FLU has been attributed to direct and indirect effects of the active moiety of FLU, FaraATP, on deoxycytidine kinase *(108)*.

Modulation of Nucleoside Transport

The sites of cellular entry of nucleosides may also act as targets for the modulation of NRTI activity. The cellular uptake of endogenous nucleosides and NRTIs is mediated by a variety of nucleoside-specific membrane transporters. These include facilitated diffusion and sodium-dependent processes *(109)*.

Knowledge of the abilities of the different nucleoside transporters to transport both endogenous nucleosides and NRTIs across the cell membrane may have a bearing on modulating NRTI activity. For example, passive diffusion is sufficient for ZDV to reach therapeutically relevant intracellular levels without the need for membrane transporters *(34)*. Therefore, by inhibiting transporters used in the uptake of endogenous nucleosides, levels of dTTP can theoretically be reduced, whereas the levels of ZDVTP will be unchanged. Dipyridamole has the ability to inhibit the transport of endogenous nucleosides into the cell *(110,111)*. This may be linked to the ability of dipyridamole to potentiate the activity of ddC in cells infected with HIV-1 *(112)*. However, the subject of nucleoside transporter modulation is complicated, because the expression of these transporters may be altered by the influence of exogenous compounds.

The complex interactions between modulators and endogenous nucleoside and NRTI phosphorylation again highlight the need to determine both dNTP and ddNTP levels. By combining a modulator with an NRTI, it may be possible to reduce the dose, or even the frequency of dosing, of the NRTI and retain efficacy. This may cause a reduction in the associated toxicity of the NRTI in question, and also have economic advantages. However, in practice, the toxicity associated with the administration of the modulator can be a major issue.

THE ROLE OF NRTI ACTIVATION IN DRUG-COMBINATION SELECTION

Fletcher et al. *(39)* demonstrated a positive correlation between the intracellular concentration of ZDVTP and 3TCTP, which was related to the baseline CD4 count (i.e., lower CD4 count with higher triphosphates). These data, which show a correlation in the ability of HIV patients to phosphorylate NRTIs with different activation pathways, suggest that if individuals have reduced phosphorylation of one NRTI it may impact on the phosphorylation of a subsequent NRTI. Although it may be argued that this may solely reflect the activation state of the cells, other studies have shown the activity of deoxycytidine kinase to be less affected than thymidine kinase during cell activation. Because endogenous triphosphates are formed to replicate DNA, synchronization may result in similar regulation with all the nucleoside analogs. Further studies are required in this area.

In a small group of patients receiving combinations including 3TC and ABC, a weak relationship was seen between the ratios of 3TCTP to dCTP and of CBVTP to dGTP. These findings may partially explain how some patients who change from one NRTI to another have a reduced response on a subsequent NRTI.

Modulation Through Drug–Drug Interactions

Phosphorylation and clinical data clearly indicate that it is essential to coadminister two nucleoside analogs with different activation pathways, such as

3TC and d4T, rather than those with shared enzymes, e.g., d4T and ZDV, to ensure maximal anti-HIV activity.

ZDV and d4T are two thymidine analogs competing directly for the same cellular kinases (Fig. 4). Incubation of these two drugs together in vitro at equal concentrations results in a decrease in activation by d4T triphosphate but activation by ZDVTP remains unaltered. This is because the first enzyme in the cascade (thymidine kinase) has a 600-fold greater affinity for ZDV than d4T. This interaction has been shown to decrease antiviral activity both in vitro and in vivo *(113,114)*. Patients receiving ZDV plus d4T had a significantly worse response to this combination compared with patients receiving ZDV plus ddI or ZDV plus ddC.

Similarly, studies investigating ddC phosphorylation found that 3TC inhibited ddC phosphorylation in a concentration-dependent manner at identical concentration ratios (10 and 100) *(46)*. 3TC also inhibits the phosphorylation of SPD754 *(61)*.

Interconversion of Nucleoside Triphosphates

A recent study has shown that ZDV administration in vivo results in the formation of some d4T triphosphate; intracellular concentrations of d4T triphosphate represented approx 5% of the amount of ZDVTP *(115)*. The interaction between ZDV and d4T at the thymidylate kinase step suggests that the d4T metabolites are formed after this activation step, but more studies are required to investigate whether this anabolite contributes to toxicity and/or efficacy. However, in a further study, no evidence has been found for this interconversion *(116)*.

Failure of Triple-Nucleoside Regimens

Recently, several triple-nucleoside regimens (ABC plus 3TC plus TDF; or ddI plus 3TC plus TDF) have been well-documented to have surprisingly high rates of virological failure. The reason for the failure of these regimens is currently unknown, but one appealing hypothesis is that there is an, as yet, unidentified intracellular interaction between two of the drugs in the regimens. However, the present data does not support this. Hawkins et al. *(65)*, have presented data from a small (therefore, should be interpreted with caution) but well-designed clinical study in which intracellular CBVTP and tenofovir diphosphate levels were determined in 15 patients. Intracellular CBVTP and TFVDP were not altered during a period of 28 d after discontinuation of either ABC or TDF. This lack of change in intracellular phosphate anabolite was also shown in vitro when incubating cells with the drugs either alone or in combination *(117)*. Considering also the data from the TONUS study *(118)*, it is likely that a low genetic barrier to resistance is the major cause of failure of the triple NRTIs.

SUMMARY

This chapter focused on the intracellular pharmacology of antiretroviral drugs. Methodological advances have enabled us to determine low concentrations of nucleoside analog triphosphates within cells and to determine transport molecules on the surface of cells. The challenge is now to use analytical, cellular, and molecular techniques to explore how best to ensure adequate drug concentrations at the relevant target site.

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8

Peripheral Neuropathy Associated With Nucleoside Reverse Transcriptase Inhibitor Therapy

Bruce A. Cohen and Russell Bartt

Peripheral neuropathies are the most common neurological syndromes associated with HIV infection. The spectrum of neuropathic syndromes is broad (Table 1), and neuropathies may be encountered during any stage of the infection. Some disorders of the peripheral nerves in patients with HIV infection are presumed to be caused by pathological factors resulting from the virus itself, whereas others may result from responses of a competent immune system to viral antigens, opportunistic processes unleashed when immune deficiency has occurred, or as adverse effects of therapies for AIDS-related diseases. To provide a context for understanding the toxic neuropathies, these other HIV-related neuropathies are briefly described in this first section.

Inflammatory demyelinating polyneuropathies (IDP) are immune-mediated reactions directed at myelin peptides, which often present in association with a viral infection. They may occur acutely (Guillain-Barré syndrome or acute inflammatory demyelinating polyneuropathy [AIDP]) or follow a chronic fluctuating or progressive course (chronic inflammatory demyelinating polyneuropathy [CIDP]). Because they result from competent immune responses directed at antigens that are either derived from an invading pathogen or exposed by damage induced by the resulting inflammatory response, these syndromes tend to occur earlier in the course of HIV infection, even at the time of seroconversion. IDP is notable for a predominant picture of motor weakness and reflex loss, which can progress rapidly, compromising respiratory muscles and cranial nerve function as well as limb strength. Autonomic features may be present at onset, including tachycardia, blood pressure elevation, diaphoresis, and sphincter disturbances. In HIV infection, the clinical picture is indistinguishable from other settings, except for the presence of a moderate lymphocytic pleocytosis accompanying the elevation of protein levels in cerebrospinal fluid, in contrast to the usual increased protein in the absence of cells (albumino–cytological dissociation).

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Table 1
Neuropathies Associated With HIV Infection

IDPs
Acute IDP (Guillain-Barré syndrome)
Chronic IDP
Mononeuritis multiplex
Inflammatory—vasculitic
Opportunistic (cytomegalovirus)
Polyradiculitis
Cytomegalovirus, Varicella-zoster, lymphomatous meningitis, syphilis
Dermatomal zoster radiculitis—may affect multiple dermatomes
DSP
Toxic neuropathies
Dideoxynucleosides (clinically DSP)
Acute neuromuscular weakness syndrome associated with hyperlactatemia and d4T
Other toxic neuropathies caused by treatment agents
Hydroxyurea, vincristine, INH, dapsone, thalidomide, etc
Diffuse infiltrative lymphocytosis syndrome

Electrophysiological studies are indicative of a demyelinating process, demonstrating severe slowing and blockage of nerve conduction velocities (NCV) and dispersion of compound action potentials. Variable degrees of axonal involvement may be seen and may have prognostic significance when present. Fortunately, the response to conventional therapy with plasmapheresis or pooled human immune globulin transfusions is usually successful *(1–3)*.

Mononeuritis multiplex is a multifocal process affecting multiple nerves. Inflammatory cells may be found in the epineurial and endoneurial perivascular spaces, however, vascular destruction is not seen. The distribution of clinical features reflects the specific cranial and/or peripheral nerves involved. This type of neuropathy is characteristically asymmetric; affecting both motor and sensory functions, and may occur during the period of symptomatic HIV disease *(3,4)*. It may occur because of vasculitis as part of a multiorgan systemic syndrome *(1)*. In patients with AIDS, multiple mononeuropathy has been found in association with cytomegalovirus infection *(5)*.

An acute polyradiculopathy syndrome may be encountered in patients with AIDS, most commonly resulting from cytomegalovirus infection, although cases caused by varicella-zoster virus, lymphoma, and syphilis have also been reported. Typical features include an ascending motor weakness with reflex loss and sphincter disturbances, usually urinary retention. Painful paresthesias in the perineum and lower limbs are common, and sensory levels or Babinski signs may indicate involvement of the spinal cord in addition to the nerve roots. Diagnosis is usually made by cerebrospinal fluid analysis, which commonly

reveals a polymorphonuclear pleocytosis and evidence of specific viral DNA when tested with polymerase chain reaction amplification techniques. Although the clinical picture superficially resembles an IDP, electrophysiological testing demonstrates features of an axonal process, including diminished action potential amplitudes and increased recruitment patterns. Treatment with specific antiviral agents may be successful when initiated early in the course of the opportunistic infection *(6–9)*. Additional HIV-associated neuropathies include varicella-zoster virus ganglio-neuritis, which presents with pain in a dermatomal distribution, similar to that affecting patients without HIV. In some patients, multiple segments will be affected, and occasionally the syndrome can be seen in the absence of the characteristic vesicular rash. Among the rarer neuropathic entities is the diffuse infiltrative lymphocytosis syndrome, which is a painful axonal sensory–motor neuropathy that may be either symmetric, or multifocal and asymmetric. Nerve biopsies reveal an angiocentric infiltration of CD8 lymphocytes in the epineurium. Peripheral blood CD8 lymphocytosis and infiltration of salivary glands are characteristic *(10,11)*. Dorsal root ganglionopathies producing sensory ataxia, acute motor–sensory axonal neuropathies, and neuropathies related to nutritional deficits are also reported in HIV-infected patients *(1,12)*.

The most common of the HIV-associated neuropathies, however, is the distal sensory polyneuropathy (DSP), which is seen most often in patients with HIV infection that is more advanced, and causes neurological symptoms in up to one-third of such individuals *(13–15)*. Typical clinical symptoms include numbness beginning in the soles of the feet followed by burning or shooting pains, pin and needle-like paresthesias, and variable aching and cramping pains. Symptoms occur symmetrically, gradually ascend into the legs, and may result in sufficient loss of sensation to cause impairment of balance (sensory ataxia). Eventually, numbness and paresthesias involve the distal upper extremities as well. Ankle reflexes are typically reduced or lost early, and reduction of other reflexes may follow. Characteristically, motor weakness is not a prominent complaint, although examination may disclose modest weakness in the small muscles of the feet and hands, especially the toe extensors. Pathologically, DSP is a dying-back axonopathy, affecting both small and large nerve fibers. Because conventional NCV and EMG studies are relatively insensitive to pathology in the smallest nerve fibers, they may be unrevealing in early stages of symptomatic neuropathy, despite severe pain and evidence of distal sensory loss. Alternatively, some individuals without neurological complaints or findings may be found to have evidence of neuropathy using neurophysiological testing *(1,14–18)*. Quantitative testing of sensory thresholds (QST), a computer-assisted determination of perception dependent on patient response to repeated stimulus presentation at various intensities, may

be more sensitive to small fiber involvement. Such testing may reveal abnormalities in individuals without clinical findings or complaints, sometimes foreshadowing the evolution of symptomatic DSP *(19)*. In a study using QST, the pain was associated with diminished thresholds to mechanical noxious stimulation and increased responses to supramaximal mechanical stimuli. In contrast, although thermal detection thresholds were also increased in subjects with neuropathy, the levels were similar in subjects with HIV-associated neuropathy, regardless of whether the neuropathy was painful or not. This selective relationship of pain to mechanical receptors was interpreted as indicative of a peripheral pain mechanism selectively involving a class of nociceptors responsive to pressure *(20)*.

Assessment of intraepidermal nerve fiber density in skin biopsy specimens has recently been used in clinical trials as a means of quantifying loss of small nerve fibers in patients with polyneuropathies *(21,22)*. The extent of epidermal small fiber loss was shown to correlate with measures of pain intensity in a recent clinical therapeutic trial of nerve growth factor for HIV-associated polyneuropathy *(23)*.

The pathological process causing DSP is not completely understood at present. Direct HIV infection of nerves is not seen, although occasional macrophages containing HIV genetic material are found in biopsies of affected nerves and some biopsies also show lymphocyte infiltration in the perineurium *(3,24)*. A current theory is that the neuropathy results from local secretion of proinflammatory cytokines by activated macrophages, resulting in cytokine-mediated neurotoxicity *(1)*. Recent studies have suggested that the prevalence of DSP is related to both CD4 count and plasma viral load *(14,25,26)*, although epidemiological studies comparing periods before and after the common use of highly active antiretroviral therapy (HAART) have shown conflicting effects on the prevalence of DSP, possibly in relation to an increasing frequency of adverse effects of components of that therapy *(27,28)*.

TOXIC NEUROPATHIES ASSOCIATED WITH NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

Early clinical studies of dideoxynucleoside reverse transcriptase inhibitors were complicated by a painful neuropathy, which proved to be a dose-limiting toxicity. It has been estimated that approx 10% of patients receiving zalcitabine (ddC) or stavudine (d4T) and 1 to 2% of those receiving didanosine (ddI) will have to discontinue therapy because of a painful neuropathy *(29)*. Affected individuals developed burning or shooting pains, or pins and needles paresthesias in the soles of the feet and toes associated with numbness and sometimes with aching and cramping pains in the feet and calves. The pain and paresthesias worsened over time, gradually ascending up the legs. On detailed neuro-

logical examination, impairments of distal sensation to pain, temperature, light touch and vibration, variable impairment of balance as a result of the sensory loss, and allodynia (hypersensitivity to normally nonpainful stimuli), were accompanied by loss or reduction of ankle reflexes, and variable weakness of the small muscles in the feet. Symptoms could precede abnormal examination findings, and were remarkable for their resemblance to the HIV-associated DSP. The initial symptoms might be aching or neuralgic pain or paresthesias, depending on the dosage of the neurotoxic agent *(18,30–33)*. When the dideoxynucleoside treatment was discontinued, the majority of individuals experienced gradual improvement in the neuropathic symptoms, however, this improvement was often preceded by a period of up to 3 months following cessation of the medication during which symptoms persisted or might even worsen, a phenomenon referred to as coasting *(32,34)*. EMG and NCV could be normal, depending on the severity of the neuropathy, but demonstrated features of an axonal neuropathy when abnormalities were present *(30–32)*. QST was more sensitive than nerve conduction velocities in one study evaluating both methods *(32)*.

Clinical trials have identified a number of factors associated with increased risk for the development of painful neuropathies in association with dideoxynucleoside therapy, in addition to the parameters of drug dose and administration (Table 2). These include lower CD4 lymphocyte levels in some but not all studies, previous history of neuropathy from other cause, excessive use of alcohol, exposure to potentially neurotoxic medications such as isoniazid or vincristine, neuromuscular or neuropathic symptoms at onset of therapy, diabetes mellitus, or weight loss before entry *(35–37)*. Most of these factors are either causes of neuropathy in their own right, or are associated with increased risk of HIV DSP. Not all individuals who develop paresthesias or pain go on to develop clinical neuropathy. In a retrospective study of subjects at one center participating in a large clinical trial, 28% of individuals receiving either ddI or ddC experienced transient numbness, paresthesias, or pain, but did not develop signs of toxic neuropathy *(36)*.

Criteria have been suggested for attribution of a compatible clinical syndrome to a toxic neuropathy (Table 3). These criteria emphasize the occurrence of symptoms after exposure to the agent and their improvement after its discontinuation. A subacute progression is also considered suggestive of a toxic neuropathy, because HIV-associated DSP is typically slowly progressive. Involvement of the upper extremities is more common in DSP than in toxic neuropathy, but has been associated with nucleoside exposure as well *(18,37)*.

The descriptions of toxic DSP were derived from experience in early clinical trials of dideoxynucleoside agents, and reports of clinical anecdotes. It is immediately evident from these descriptions that HIV-associated DSP and painful toxic neuropathies commonly associated with exposure to dideoxynucleoside

Table 2
Reported Risk Factors for Toxic Neuropathy With Dideoxynucleoside Therapy

Dose and regimen of dideoxynucleoside
Combination therapy with multiple dideoxynucleosides or with hydroxyurea
Lower CD4 levels
Hyperlactatemia
History of peripheral neuropathy before dideoxynucleoside exposure
Exposure to other potentially neurotoxic drugs
Heavy alcohol use
Diabetes mellitus
Diminished creatinine clearance
Vitamin B_{12} deficiency
Weight loss during dideoxynucleoside therapy

Table 3
Criteria for Diagnosis of Dideoxynucleoside-Associated Toxic Neuropathy

Symmetrical pain and/or paresthesias in distal lower extremities—may ascend over time
Diminished distal sensation to vibration, pain, and/or light touch on neurological examination—may have distal contact hypersensitivity (allodynia)
Normal motor tone and normal strength, except for mild weakness in small distal muscles[a]
Absent or diminished ankle reflexes
Onset of symptoms associated with exposure to dideoxynucleoside agents and improvement associated with their discontinuation or dose reduction, allowing for a period of persistence or intensification within the first 3 mo after the change in regimen
Elevated serum lactic acid levels[b]

[a]The recently described acute neuromuscular syndrome associated with hyperlactatemia and d4T therapy is characterized by acutely or subacutely progressive motor weakness and areflexia. The clinical pattern superficially resembles Guillain-Barré syndrome rather than the more commonly seen distal sensory polyneuropathy (*see* text).

[b]Elevated serum lactic acid levels have recently been found to discriminate distal sensory polyneuropathy caused by dideoxynucleosides from that caused by HIV (*see* ref. *88*)

reverse transcriptase inhibitors are remarkably similar in their clinical symptoms and examination findings. This leads to the obvious question of whether evolution of such symptoms during treatment with one of these agents represents unmasking or aggravation of an occult DSP, or represents a primary effect of the potentially neurotoxic agent itself. In many of these early trials, subjects either did not undergo detailed screening for prevalent polyneuropathy at entry or were entered with symptoms suggestive of preexisting neuropathy. In the next section, we review this data for each of the relevant nucleoside analogs.

TOXIC NEUROPATHY WITH DIDEOXYNUCLEOSIDE THERAPY

Zalcitabine

Early phase I trials encountered painful neuropathy as a major dose-limiting toxicity in ddC therapy. Yarchoan and colleagues administered doses of ddC ranging from 0.03 mg/kg to 0.25 mg/kg, every 8 h, to 20 subjects with AIDS or AIDS-related complex (ARC) and CD4 lymphocyte levels fewer than 350 cells/mm^3. A reversible painful neuropathy syndrome occurred in 10 of the participants, beginning between 6 and 14 wk after onset of therapy. This syndrome was characterized by aching pain in the feet followed by burning paresthesias 2 to 3 wk later, with impairment of light touch, temperature, vibration, and position senses, and diminished ankle reflexes on neurological examination. Three subjects also developed mild weakness of the toe and foot extensor muscles. After discontinuation of ddC, worsening of neuropathic symptoms continued for up to 5 wk before improvement. Four weeks after discontinuing continuous ddC, one patient with toxic neuropathy who was treated with an alternating regimen of 200 mg zidovudine (ZDV) every 4 h for 1 wk and 0.03 mg/kg ddC every 4 h for the next week, experienced improvement in neuropathic symptoms that continued during the subsequent 5 wk *(30,34)*.

Electromyography (EMG) and nerve conduction velocity (NCV) studies were obtained in seven of the ten subjects who developed neuropathy at baseline, or within the first 2 wk of therapy. Five of these patients had normal test results and two patients showed a preexisting neuropathy, one axonal and slowly progressive (HIV-associated DSP?), the other a polyneuropathy attributed to diabetes mellitus. Ten additional studies were performed after the onset of neuropathic symptoms, which began concurrently with treatment in nine subjects, and 4 wk after discontinuation of ddC in the 10th patient. NCVs revealed markedly decreased or absent sural nerve action potentials in the lower extremities, and, in addition, diminished sensory action potentials from ulnar nerves in the upper extremities, with preserved distal latencies, of seven subjects in whom these tests were performed. EMGs demonstrated abnormal spontaneous activity, increased polyphasic potentials, and abnormal interference patterns *(30)*. These features are consistant with an axonal neuropathy.

Merigan and colleagues conducted a dose-finding study in 61 subjects with AIDS or ARC, excluding individuals with “significant neurological disease.” All subjects administered 0.06 or 0.03 mg/kg ddC every 4 h developed painful neuropathy. The time-to-onset was dose related, with the higher-dose group developing neuropathic symptoms in a mean of 7.4 wk, and the 0.03-mg/kg group developing neuropathic symptoms in a mean of 8.2 wk. Individuals receiving 0.01 mg/kg ddC every 4 h also developed painful neuropathy, which occurred in 80% of these subjects in a mean of 11.8 wk. Symptoms escalated

with onset over weeks and involved the distal upper extremities in half of the cases. QST revealed abnormalities of vibratory perception coincident with the earliest symptoms. Even the group receiving the lowest dose of 0.005 mg/kg ddC every 4 h had a small incidence of neuropathy, which occurred in 17% of the patients, evolving more insidiously during a mean of 17.5 wk. Improvement on discontinuation of ddC was dose related, with the two lower-dose groups experiencing complete recovery within 6 mo. Improvement was slower in the higher-dose arms (requiring up to 1 yr), and incomplete in several subjects receiving the highest dose who continued to experience pain and sensory loss after 1 yr *(38)*. Fifty-two of these subjects receiving ddC every 4 h at 0.06 mg/kg, 0.03 mg/kg, 0.01 mg/kg, or 0.005 mg/kg, were evaluated for toxic neuropathy using serial clinical and electrophysiological monitoring combined with symptom questionnaires. Criteria for neuropathy were specified as at least two symptoms of moderate intensity on the questionnaire, or one symptom of severe or moderate intensity plus an objective abnormality on either the neurological examination or the electrophysiological testing. In the two higher-dose arms, all subjects developed neuropathy with a mean onset of 7.7 wk. QST for vibration became abnormal before the appearance of clinical symptoms, with a mean onset of 7.3 wk. In the intermediate 0.01 mg/kg-dose arm, all subjects developed similar but less intensely painful symptoms, evolving more gradually with a mean onset at 9.3 wk. Vibratory QSTs became abnormal in 70% of this group, coincident with the clinical symptoms. NCVs were obtained in only 20% of the total cohort. They revealed abnormalities in all subjects from the higher-dose arms after the development of clinical dysfunction. NCVs were mildly abnormal in 40% of the intermediate-dose cohort. In the low-dose group, one subject developed mild but transient neuropathic symptoms 26 wk after onset of therapy. Another developed asymptomatic abnormalities on vibratory QST and a loss of ankle reflexes, however, no further change occurred despite continued therapy with ddC. One might reasonably question whether this subject developed HIV-associated DSP rather than a toxic neuropathy. In contrast, however, when ddC was discontinued in symptomatic participants, improvement occurred in 83% of patients, with a mean time-to-onset of improvement of 19 wk, 15 wk, and 11 wk for the high-dose, intermediate-dose, and low-dose groups, respectively, consistent with a toxic etiology *(32)*.

In an open-label study conducted in individuals taking ZDV for at least 48 wk, ddC administered in a dosage of 0.75 mg every 8 h was associated with symptoms of painful neuropathy in 25% of 59 subjects. The pain was characterized as moderate or severe in 17% of patients, and 10% of patients withdrew from the study because of the neuropathic symptoms. Subjects entering the trial had CD4 counts of fewer than 200 cells/mm^3 and no known peripheral neuropathy, although the screening methodology is not elaborated in the report.

A regression analysis found that neuropathic symptoms occurred more frequently in subjects with CD4 levels of fewer than 50 cells/mm^3 and in subjects with diminished creatinine clearances of less than 110 mL/min *(39)*.

Another open-label trial compared courses of 200 mg ZDV plus ddC at either 0.01 mg/kg or 0.03 mg/kg every 4 h alternating either weekly or monthly, 0.03 mg/kg ddC at administered on alternate weeks, and 200 mg ZDV every 4 h administered either on alternate weeks or continuously, in a cohort with a median CD4 level of 142 cells/mm^3 (mean, 164 ± 139 cells/mm^3) and no known previous neuropathy. Neurological screening methods included a baseline evaluation for neuropathy, a neuropathic symptom questionnaire administered every 2 wk, a focused examination for neuropathy every 4 wk, and a vibratory QST every 8 wk. The frequency of painful neuropathy in subjects on ddC was related to the dose. Among individuals in the three 0.03 mg/kg-dose arms combined, neuropathy occurred in 34% of patients compared with 15% of patients in the two 0.01 mg/kg-dose arms. Of patients on ZDV alone taken either continuously or on alternate weeks, 17% of patients developed neuropathy, which was comparable to the results for those taking lower-dose ddC on a weekly (10%) or monthly (21%) alternating regimen, and those taking higher-dose ddC on the monthly alternating regimen (14%). In subjects alternating ddC and ZDV weekly, 46% developed neuropathy, compared with 14% of those alternating ddC and ZDV monthly. In those who developed neuropathy, 68% experienced the onset of symptoms within 24 wk of starting therapy. This cohort had relatively low CD4 levels at entry and it is not noted whether the time-to-onset of neurological symptoms differed between those on ddC and those taking only ZDV, or whether any increase in CD4 level in response to therapy had an independent relationship with the occurrence of neuropathy *(40)*.

A large community-based, open-label clinical trial compared 500 mg/d ddI with 2.25 mg/d ddC in subjects with up to 300 CD4 cells/mm^3 who had failed therapy with ZDV. Peripheral neuropathy was an exclusion criterion for entry, and occurred during the study at a rate of 22.1 per 100 patient-years with ddI compared with 45.1 per 100 patient-years for ddC, a statistically significant twofold increased risk in the ddC arm *(41)*.

A subsequent study evaluated the frequency of toxic neuropathy in a clinical trial using ddC dosed at 2.25 mg/d (~0.03 mg/kg) alone, or in combination with ZDV, in subjects with CD4 counts of up to 200 cells/mm^3 or up to 300 cells/mm^3 with concurrent systemic symptomas of HIV. At one site, 76 subjects entered into this multicenter clinical trial were evaluated with serial neurological examinations, neuropathy symptom questionnaires, and vibratory QST. Individuals who reported moderate or severe symptoms of peripheral neuropathy on the initial questionnaire, or who had moderate deficits on examination (undefined) at screening were excluded. Operational criteria for toxic neuropa-

thy included at least one distal sensory symptom and at least one objective sign on examination, persisting for at least 72 h, and appearing while the subject was receiving ddC. During a follow-up period of up to 73 wk, the incidence of neuropathy was 34% in the two groups receiving ddC compared with 4.3% in the group receiving ZDV alone, an increased risk of 7.9 times for the ddC groups. The mean time-to-onset of neuropathy was 16 wk, but varied widely, from 1 to 51 wk *(37)*. In the entire multicenter cohort, the rate of moderate or worse neuropathy was 22% in the ddC group and 23% in the combination arm, compared with 13% for the ZDV group alone. In addition, in the multicenter cohort, for those entering with CD4 lymphocyte counts greater than 150 cells/mm^3, moderate or worse neuropathy was significantly associated with ddC therapy alone (16%) or in combination (21%), compared with ZDV alone (9%). However, there was no significant association with toxic neuropathy for ddC therapy, alone or combined, compared with ZDV alone, for patients with CD4 cell levels of fewer than 150 cells/mm^3 at entry. Although there seemed to be a trend favoring a relationship to ddC, this result could also reflect a greater risk of HIV associated DSP in patients with reduced CD4 levels. Interestingly, the rate for neuropathies characterized as severe in the parent study was similar in all groups, at 4% for ddC alone and 6% for combination therapy or ZDV alone *(42)*. In the smaller single-center cohort, a case–control study comparing baseline characteristics found only diabetes mellitus to be a significant risk factor for neuropathy, whereas weight loss during the study showed a trend suggesting a relationship but did not reach statistical significance. No effect of CD4 level was demonstrated in this smaller sample in contrast to the total cohort. Development of moderate or worse neuropathic symptoms was managed by dose interruption until improvement occurred, followed by resumption of treatment at half of the original dose. Blum and colleagues observed subjective symptomatic improvement within an average of 10 wk in 67% of their subjects after dose reduction, but no reversal of abnormal findings on neurological examination. The persistent neurological abnormalities could represent either residual damage from a toxic neuropathy or emergence of HIV-associated DSP during the study.

Didanosine

Some early dose-escalation studies with ddI were limited by a painful neuropathy similar to that seen with ddC. Lambert and colleagues encountered symptoms of neuropathy in almost one-third of a cohort of 37 subjects with CD4 levels of fewer than 400 cells/mm^3, who received doses of ddI greater than 12 mg/kg/d, but in only 1 of 15 subjects who received smaller doses. Subjects were not screened for preexisting neuropathy at entry. The investigators also noted an increased risk of neuropathy with total cumulative dose greater than 2 g/kg. The

time-to-onset of symptoms was dose related and ranged from 55 to 201 d after initiation of therapy. Withdrawal of ddI resulted in resolution or improvement of pain and paresthesias during 4 to 8 wk in seven of eight affected individuals, three of whom tolerated rechallenge with ddI at 10 mg/kg/d for 4 to 12 wk without recurrence *(43)*. A subsequent follow-up, of these subjects and several additional individuals taking 250 to 750 mg/day of ddI, for a mean of 34 wk (2–72 wk) revealed neuropathy in two additional subjects, both taking ddI at 750 mg/d. One developed symptoms at 30 wk. The other, who entered with what was described as mild peripheral neuropathy symptoms (HIV-associated DSP?), developed intensified neuropathic discomfort at 18 wk. After an 8-wk suspension of ddI, however, the patient was able to continue treatment at a reduced dose of 500 mg/d *(44)*.

In contrast, a large multicenter trial comparing ddI with continued ZDV in individuals previously taking ZDV for at least 16 wk and having either AIDS, AIDS related complex (ARC), and fewer than 300 CD4 cells/mm^3 or fewer than 200 CD4 cells/mm^3without systemic symptoms, found no difference in the rates of symptomatic neuropathy between the groups. Neuropathy at entry was not noted to be an exclusion criterion, and it appears that only symptomatic neuropathic complaints were evaluated. Perhaps of some importance, CD4 counts rose in the two ddI-treatment arms but not in the continued-ZDV arm *(45)*.

In another dose-escalation study, a cohort of 58 subjects with AIDS or ARC and a median CD4 count of 47 cells/mm^3 (range, 4–267 cells/mm^3) was followed for a median of 8.9 mo (with 28 subjects followed for longer than 1 yr). Yarchoan and coworkers encountered painful neuropathy in 15 subjects. Twelve of these individuals received doses greater than 9.6 mg/kg/d. Preexisting neuropathy was not evaluated at entry *(46)*. A subsequent report from this group described a 5-yr follow-up of 72 subjects, including individuals from this study and others from phase I studies who were subsequently switched to doses of 200 or 250 mg ddI twice daily. The authors reported 11 cases of painful neuropathy during this period, affecting approx 15% of the cohort. Of these, four patients continued treatment without dose change, two were successfully rechallenged at the same dose after temporary suspension of therapy, two tolerated rechallenge at a lower dose, and three discontinued ddI and switched to ZDV. No evaluations for persistent neurological abnormalities in those who experienced pain were described *(47)*.

In an open-label, dose-escalation study of ddI administered once daily to 34 individuals with CD4 cell levels fewer than 400 cells/mm^3 (mean, 125 ± 110 cells/mm^3 at entry), followed for an average of 15.8 wk (2–56 wk; median, 12 wk), severe painful neuropathy was reported in only one of six individuals receiving the highest dose, of 30.4 mg/kg ddI. After suspension of ddI, partial resolution was noted during the next 8 wk. An interesting aspect of this study is

the observation of 11 subjects entering with grade 1 neuropathy (paresthesias not requiring treatment), 8 had resolution of their symptoms during the trial, whereas the other 3 were unchanged *(48)*. During the trial, CD4 levels rose to greater than 200 cells/mm^3 in the majority of the subjects, suggesting that neurological improvement of the baseline neuropathy (HIV-associated DSP?) might have occurred because of a therapeutic response induced by the ddI regimen.

Evaluation of subjects from a single site participating in another multicenter ddI dose-escalation study identified toxic neuropathy, confirmed by a neurologist, in 10 (23%) of 44 subjects who complained of pain or numbness or were noted to have abnormalities on study examinations. Neuropathic symptoms improved or resolved after suspension of ddI. Toxic neuropathy was defined as the occurrence of distal sensory symptoms in the lower extremities with reduced or absent ankle reflexes and distal sensory impairments on examination, appearing in relation to ddI therapy and improving on its withdrawal. One subject maintained at half of the original dose had resolution of the neuropathic symptoms during 6 mo follow up, whereas four others tolerated rechallenge after suspension of therapy either without recurrence or with mild paresthesias. In these 10 subjects, the mean CD4 level at entry was 114 cells/mm^3 (0–212 cells/mm^3) and improved by an average of 49 cells/mm^3 (range, 0–170 cells/mm^3) in seven patients, whereas it worsened by a mean of 38 cells/mm^3 (range, 11–75 cells/mm^3) in three other patients *(31)*.

The Alpha trial, a large, double-blind European study, used two dose regimens of ddI, 10.5 mg/kg/d and 3.3 mg/kg/d. Peripheral neuropathy at entry was not an exclusion criterion, except in French centers. Only neuropathy leading to study discontinuation was reported, with rates of 7.8% in the higher-dose group and 5.9% in the lower-dose arm *(49)*. An open-label, community-based study comparing ddI with ddC in 467 subjects with CD4 levels of up to 300 cells/mm^3 reported the development of neuropathy in 21.6% of subjects taking ddI at 500 mg/d *(41)*.

Stavudine

Painful neuropathy was a dose-limiting side effect in early studies of d4T, as with the other dideoxynucleosides. In a series of 41 subjects with CD4 cell levels of fewer than 400 cells/mm^3, 55% of patients developed symptoms of painful neuropathy. The time-to-onset ranged from 5 to 40 wk. Painful neuropathy was related to both dose and dosing frequency. Each increase of 1 mg in the dose unit was associated with a twofold increased risk of neuropathy. Dosing twice daily was associated with a fourfold increased risk of neuropathy compared with administering the same total dose as a thrice daily regimen. It appears from the report that no screening for neuropathy at baseline was incorporated into the entry criteria. Of 20 subjects developing painful neuropathies,

4 were noted to have used alcohol excessively, 1 was taking INH, and 1 was taking amphotericin. Five additional subjects reported myalgias at baseline. Some subjects tolerated rechallenge at reduced dose. The maximally tolerated dose was determined to be 2 mg/kg/d *(50)*.

Skowron reviewed early trials of d4T and found painful neuropathy to be the principal dose-limiting side effect. In a combined analysis, the rate of neuropathy at doses of 0.5 and 1.0 mg/kg/d was 21 per 100 person-years compared with 66 per 100 person-years for doses of 2 mg/kg/d. In addition to the factors noted in the Browne study *(50)*, existing neuropathy at entry was also found to be a risk factor for subsequent neuropathic adverse effects. In a phase II study of subjects with CD4 levels of up to 250 cells/mm^3, painful neuropathy was dose- and treatment duration-dependent and occurred up to 48 wk after initiation at 2.0 mg/kg/d. The rate of painful neuropathy was 17 per 100 person-years at a dose of 0.5 mg/kg/d compared with 41 per 100 person-years at a dose of 2 mg/kg/d. Resolution of pain and tolerance to rechallenge with a lower dose of d4T was noted in 55% of subjects who were followed for a median of 32 wk *(35)*.

A large, double-blind, multicenter trial compared 40 mg d4T twice daily with 200 mg ZDV thrice daily in a cohort of 882 subjects with CD4 levels from 50 to 500 cells/mm^3, and previous exposure to ZDV for at least 6 mo. Individuals with a history of peripheral neuropathy were excluded. The rate of painful neuropathy requiring dose modification in the d4T group was 12%, compared with a rate of 4% in patients taking ZDV. Neuropathy was more common in those with AIDS at baseline, regardless of treatment assignment, with rates of 25% in the d4T group and 12% in the ZDV group, compared with 10% and 3%, respectively, for those without AIDS at entry. Of those receiving d4T who developed neuropathy, 63% resolved symptomatically within a median of 17 d after suspension of the medication (range, 4–57 d) and tolerated resumption of therapy at reduced dose for a median of 23 wk (range, 1–120 wk) *(51)*. Differences in neuropathy rates for those with AIDS, in addition to toxic neuropathy associated with the antiretroviral therapy may affect these results.

A different pattern of neurotoxicity has recently been described in association with d4T therapy, which superficially resembles the acute inflammatory polyneuropathy, or Guillain-Barré syndrome, described in the first section of this chapter. This new neuromuscular syndrome has been attributed to hyperlactatemia related to dideoxynucleoside reverse transcriptase inhibitor therapy, particularly with d4T. Individuals with this syndrome develop profound progressive motor weakness during several weeks, which may be associated with ataxia and numbness. The cranial nerves may be involved, resulting in facial weakness, and involvement of the respiratory muscles may lead to ventilatory failure. In contrast to typical Guillain-Barré syndrome, in which the primary pathology is demyelinating, electrophysiological studies in this syndrome

reveal features of an acute axonal neuropathy. In some instances, neurological symptoms were preceded by symptoms of lactic acidosis syndrome, including malaise, nausea, emesis, and abdominal cramping. Elevated plasma lactate levels have been documented in some of the relatively few case reports thus far reported. Recovery in those individuals who do improve may be prolonged up to 1 yr or longer *(52–54)*. Cases have been described in pediatric patients *(85)*. Recently, a patient presenting with the Miller-Fisher variant of Guillain-Barré syndrome consisting of the clinical triad of ophthalmoplegia, ataxia, and areflexia evolving in association with hyperlactatemia and after discontinuation of antiretroviral therapy including d4T was reported *(86)*.

As this chapter was being finalized, a report appeared describing a retrospective collection of 69 cases, including some from previous reports, meeting criteria of new acute or subacute neuromuscular limb weakness associated with lactic acidosis. Cases were classified for diagnostic certainty based on the presence of confirmatory diagnostic studies and the exclusion of confounding causes of neuromuscular weakness. In 88% of the cohort, the dideoxynucleoside used was d4T, with a median duration of 10.5 mo of therapy (range, 1–71 mo). No association between serum lactate level and duration of d4T use was found. Of interest is the finding that cases were almost equally likely to occur in women as in men. Approximately one-third of the cases were associated with symptoms of lactic acidosis syndrome. An important finding was that the neuromuscular weakness syndrome occurred despite previous discontinuation of the antiretroviral therapy in 36% of the cases (median, 14 d). Respiratory failure requiring ventilatory support occurred in 23% of the cohort. Mortality was notable at 16% of the patients and was marginally associated with levels of serum lactate greater than 6 mmol/L, although levels greater than 10.08 mmol/L showed a strong trend to associate with death. Most cases evaluated more extensively were sensorimotor polyneuropathies, although both axonal and demyelinating pathologies were seen in the limited nerve biopsies obtained. Four of 15 muscle biopsies revealed evidence of mitochondrial dysfunction, and 3 revealed inflammatory infiltrates *(87)*.

The full spectrum of this syndrome, its underlying pathophysiology, and its relationship to nucleoside therapy are only beginning to be understood as this is written. Similarly, although isolated case reports have described improvement in association with various treatments *(53,87)*, no consistently effective therapy has yet been identified.

TOXIC NEUROPATHY WITH COMBINED AGENTS

Several reports have evaluated cohorts or described neuropathy in individuals receiving combinations of dideoxynucleoside agents, or combination dideoxynucleoside agents with hydroxyurea (HU). An early case report concerned an individual participating in a clinical trial alternating ddC and ZDV

monthly. Neurological findings at the onset of therapy were not described. After 16 wk of treatment, the patient developed paresthesias, followed by burning pain 4 wk later, at which time the ddC was discontinued. Two weeks later, he reported the neurological symptoms to be the same. The following week, another physician initiated ddI treatment, and 1 wk after that, the neurological symptoms worsened with ascending sensory impairments in the legs and increased pain. The ddI was stopped, and the symptoms resolved 1 mo later. The authors suggested that ddI could potentiate ddC neurotoxicity *(55)*.

The combination of ddI and d4T was evaluated in a cohort of 52 treatment-naive subjects with a mean baseline CD4 count of 330 cells/mm^3. During 24 wk, nine subjects developed neuropathic symptoms (17%). Two of these withdrew and the other seven continued therapy. In a 24-wk extension completed by 30 subjects, 1 additional subject withdrew because of painful neuropathy and 2 other subjects developed minor paresthesias. The authors noted that toxic neuropathy was the most common adverse event in the trial, but that only three subjects discontinued therapy because of it, and concluded that the combination was safe and well-tolerated *(56)*.

In a dose-finding, double-blind study comparing the effect of combinations of ddI and d4T on plasma viral load, there were 13 adverse events in 86 subjects followed for a median of 349 d (1–728 d). Patients with peripheral neuropathy and previous treatment with neurotoxic drugs were excluded, and most subjects were treatment naive, with CD4 cell counts greater than 200 cells/mm^3 (mean, 343 cells/mm^3). It seems, from the report, that determination of neurotoxicity was based solely on subject complaints and only two subjects were reported to develop neuropathy as a serious adverse event (2.3%) *(57)*.

The addition of HU to dideoxynucleosides has been shown to increase neurotoxicity. A cohort of 144 subjects with CD4 levels of 200 to 500 cells/mm^3 were randomized to receive ddI and d4T with or without HU. Seventy-two subjects were initially randomized to the three-drug arm and 12 additional subjects switched to that arm after 12 wk. After 2 yr, only 25% of subjects remained in the trial, most withdrawing because of lack of efficacy or adverse events. Neuropathy occurred in 35% of the group taking all three drugs, compared with 15% of those taking only the ddI and d4T, a statistically significant difference *(58)*.

In a retrospective database review of 1116 patients treated with various regimens containing ddI, d4T, and HU in one large center, the risk of neuropathy was calculated for the agents alone and in combination. Inclusion criteria for neuropathy required sensory symptoms in the lower extremities or all four extremities, beginning, recurring after resolution, or (if preexisting) worsening after the onset of therapy with the agents. The mean CD4 count was 270 cells/mm^3. The risk of developing neuropathy was related to lower CD4 count, history of non-drug-associated neuropathy, and, weakly, with age greater than 40 yr. Adjusting for these

factors yielded a relative risk compared with ddI alone of 1.39 for d4T, 2.35 for ddI plus HU, 3.5 for ddI plus d4T, and 7.8 for ddI plus d4T plus HU. The crude incidence rate for ddI alone was 6.8 per 100 person-years of treatment *(59)*.

Another retrospective review found a frequency of peripheral neuropathy in 27% of a small group of subjects administered ddI plus d4T in conjunction with HU, compared with 10% in a group administered ddI plus d4T *(60)*. Although the trend did not reach statistical significance, this report is consistent with the findings in the two studies described above *(58,59)*.

A single case report describes a patient with neuropathic toxicity attributed to lamivudine (3TC). This individual experienced onset of paresthesias while taking ddC plus ZDV. His symptoms improved after discontinuing the ddC, however, distal sensory impairment and mild distal weakness persisted, with electrophysiological findings consistent with a polyneuropathy. Eighteen months later, he started taking 3TC, and, within 3 wk, he experienced recurrent painful dysesthesias. These symptoms improved within 4 wk after the 3TC was discontinued, but recurred on rechallenge *(61)*, consistent with a toxic exposure aggravating the preexistent neuropathy.

PATHOPHYSIOLOGY OF NUCLEOSIDE TOXIC NEUROPATHY

The mechanism of nucleoside-induced neurotoxicity is not completely understood at the present time. Current hypotheses emphasize toxic effects of nucleoside reverse transcriptase inhibitors on mitochondria, and tissue-specific vulnerabilities based on energy requirements. Nucleoside analogs can replace nucleotide bases and can be incorporated into newly synthesized DNA. In contrast to other forms of DNA synthesized from nuclear chromosomal genetic material, mitochondrial DNA (mtDNA) is inherited from the cytoplasm of the oocyte and replicates by random segregation rather than recombination. Replication of mtDNA requires nuclear-coded DNA polymerase-γ. In mtDNA, there are few introns and limited self-repair mechanisms, thus, mutations are more likely to affect coding sequences altering protein production, and dysfunctional mutations are more likely to persist with subsequent replication. This results in mixtures of mitochondria containing wild-type and mutant DNA in the same cells in varying proportion, referred to as heteroplasmy. The impact of heteroplasmy on a particular tissue may be a threshold effect, dependent on the severity of the resulting defect in oxidative phosphorylation in the abnormal mitochondria and the relative metabolic demands of the tissue for energy production *(62–64)*. The hypothesis, as articulated by Lewis and Dalakas, suggests that manifestations of nucleoside toxicity reflect:

1. Subcellular availability and abundance of nucleoside analog in the target tissue.
2. The ability of the cellular thymidine kinases to use the nucleoside analog as a competitive alternative substrate for monophosphorylation and, subsequently, triphosphorylation.

3. The ability of the triphosphate nucleoside analog to inhibit DNA polymerase-γ either by acting as an alternative substrate for incorporation into mtDNA via competitive inhibition resulting in aberrant chain extension, or by noncompetitive incorporation and premature chain termination.
4. The relative metabolic requirements of the tissue for oxidative phosphorylation *(62)*.

It has been suggested that depletion of the mtDNA induced by nucleoside analogs results in the depletion of enzymes encoded by the mtDNA, leading to impaired oxidative phosphorylation. An analogy has been drawn to multiple symmetric lipomatosis, a hereditary condition in which mtDNA mutations and deletions impairing oxidative phosphorylation complex IV have been identified. This condition results in hypertriglyceridemia, insulin resistance, and fat deposition abnormalities similar to those associated with HAART, and is associated with peripheral neuropathy in almost all cases *(65)*.

In vitro studies have demonstrated the relative potency of nucleoside agents to inhibit mtDNA in a T-lymphoblastoid cell line, CEM, to be ddC greater than d4T greater than ZDV greater than ddI. The concentration of nucleoside analog required to inhibit mtDNA replication was less than that required to inhibit cell growth at 4 d for ddC, ddI, and d4T, but not for ZDV *(66)*. In a neuronal cell model, using PC12 cells derived from rat pheochromocytoma and stimulated to differentiate into neurons by incubation with nerve growth factor, ddC and ddI inhibited production of mtDNA and neurite generation at pharmacological concentrations, whereas ZDV and 3TC did not. In contrast, d4T inhibited neurite generation at concentrations that did not affect mtDNA synthesis, despite the ability of d4T to impede activity of DNA polymerase-γ, and despite the presence of d4T triphosphate in the mitochondria, suggesting a different mechanism of neurotoxicity. The authors suggested that a repair mechanism, perhaps the 3′5′ exonuclease present in DNA polymerase-γ, might offset toxicity of d4T to a degree. Absence of phosphorylated 3TC in the isolated mitochondria was consistent with the hypothesis that the inability to transport phosphorylated 3TC accounted for its lack of toxic effect on mtDNA *(67)*. The lack of neurotoxicity of 3TC may also lie in its ability to act as a substrate for the integral 3′5′ exonuclease activity of DNA polymerase-γ, making incorporation less likely *(63)*.

A consequence of mtDNA polymerase-γ dysfunction is a reduced capacity of the mitochondria to synthesize uridine nucleotides, which are integral to the synthesis of oligosaccharides on membrane glycoproteins and glycolipids. Cytidine nucleotides, derived from uridine nucleotides, are involved in phospholipid synthesis. In PC12 cultures, the addition of uridine and pyruvate can prevent ddC-induced reduction of mtDNA replication *(68)*.

Nucleoside analog neurotoxicity has been demonstrated in animal models. Axonal degeneration and reduction in fiber size, myelin degeneration manifested by myelin splitting and edema, and abnormal mitochondria in Schwann

cells were seen in rabbits treated with ddC. It was not possible to determine from the study whether the axonal changes were a primary toxic effect or were secondary to the myelin damage *(69)*. In rats treated with ddI, sections of sciatic nerve taken at various durations after onset of exposure demonstrated myelin toxicity manifested by edema and myelin splitting at 15 wk. Concentrations of ddI used resulted in peak plasma levels five to six times those in humans administered therapeutic doses *(70)*.

A recent study analyzed morphological changes in sural nerve biopsies taken from patients with ddC-induced toxic neuropathy, in comparison with biopsies from patients with HIV-associated distal sensory neuropathy, who either never received any antiretroviral therapy or received only ZDV, and from individuals with non-HIV-associated axonal neuropathies. Dalakas and colleagues found abnormalities in mitochondria in axons and Schwann cells of subjects receiving ddC more frequently than in controls. No unique features were noted on light microscopy; however, using electron microscopy, abnormal mitochondria with interrupted inner morphology, enlarged size, osmophilic deposits between the inner and outer limiting membranes, and diminished cristae were noted in all three groups, with different degrees of severity. In the ddC-associated neuropathies, 55% of axonal mitochondria were abnormal, compared with 28% in HIV-associated DSP and 9% of the other axonal neuropathies. In Schwann cells, 47% of the mitochondria were abnormal in the ddC-associated neuropathies, compared with 5% in the HIV-associated DSP and 14% in the other neuropathic controls. Competitive polymerase chain reaction amplification was used to quantify mtDNA in the nerves, and demonstrated significant depletion of mtDNA in the ddC-treated group compared with the neuropathic controls. Results were considered to be supportive of the ability of ddC to induce a toxic mitochondrial neuropathy by depletion of neural mtDNA, consistent with the selective inhibition of DNA polymerase-γ demonstrated in neural cell lines *(71)*.

The potential role of acetyl-carnitine deficiency as a result of mitochondrial damage in toxic neuropathy was suggested by Famularo and colleagues, after a study in which they demonstrated diminished serum levels of acetyl-carnitine but not total carnitine, in 12 subjects with symptoms of painful neuropathy while taking ddI, ddC, or d4T in comparison with 10 subjects taking ddI and 11 subjects taking ZDV, who had no symptoms of neuropathy. Acetyl-L-carnitine is a transport molecule for long-chain fatty acids into the mitochondria, where they undergo β-oxidation, generating adenosine triphosphate, and donate the acetyl group to acetyl coenzyme A for subsequent participation in Krebs cycle reactions, generating additional adenosine triphosphate. The authors hypothesized that acetylation of carnitine is important to normal function of peripheral nerve, facilitating binding of nerve growth factor and enhancing neural repair, and that lower levels in serum reflected defects in carnitine metabolic pathways caused by mitochondrial injury *(72)*.

In contrast to Famularo's findings, in a sample of 232 subjects drawn from a clinical treatment trial of nerve growth factor for HIV-associated neuropathy, Simpson and colleagues found an increased proportion of subjects with abnormally low levels of total and free carnitine among the study subjects compared with normal HIV-negative controls (no HIV controls without neuropathy were assessed), but found no association between the levels of total or free carnitine, or acetyl-carnitine levels and the severity of the peripheral neuropathy as measured by clinical symptoms, QST, or nerve fiber density in skin biopsy specimens. No relationship of carnitine levels to current or recent use of dideoxynucleoside agents, as compared with subjects with no use or remote use (6 mo or longer before entry), was found. There was no difference in any of the neuropathy measures studied between subjects with decreased levels compared with subjects having normal levels of carnitine *(73)*. A possible explanation for the discrepancies betweem these studies could lie in differences between tissue and plasma levels of carnitine compounds.

A recent report describes results of open label acetyl-carnitine treatment in 21 subjects with nucleoside reverse transcriptase inhibitor associated toxic neuropathies. Subjects were treated with 1500 mg acetyl-L-carnitine twice daily for a median of 14 mo (range, 5–33 mo) and followed both clinically and by serial skin biopsies taken from the distal leg. At baseline, biopsies in patients with DSP showed reduced total innervation of epidermis, dermis, and sweat glands in comparison with five healthy controls. Selective staining for small sensory (type C and Aδ) fibers revealed an 80% reduction in epidermis and a 25% reduction in the dermis. After 6 mo of therapy with acetyl-L-carnitine, the total epidermal and dermal nerve fiber innervations reached or exceeded normal levels, whereas the fibers around sweat glands increased by 75%. Selective small fiber innervation reached 40% of control in the epidermis and 175% in the dermis. Further increases were seen on subsequent biopsies in those continuing therapy for longer periods. Clinical neuropathic symptoms improved in 76% of the subjects and did not change in the others. No significant changes in CD4 or CD8 lymphocyte counts or HIV plasma viral load occurred during the study period and, thus, did not affect results *(74)*.

In a brief trial, 10 of 16 patients with painful DSP receiving 500 to 1000 mg daily of acetyl-L-carnitine intramuscularly or intravenously reported improvement in pain symptoms after 3 wk. Five subjects reported no change in pain and one reported worsened pain *(75)*. No large controlled clinical trial of acetyl-carnitine therapy in HIV-associated or nucleoside-associated neuropathies has been published, to date.

MANAGEMENT OF NEUROPATHY ASSOCIATED WITH NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR THERAPY

Complaints of pain or paresthesias in the lower extremities of a patient taking ddI, ddC, or d4T suggests the possibility of a toxic neuropathy and warrants

detailed evaluation. Compared with HIV-associated DSP, toxic neuropathies appear more abruptly and progress more quickly, thus, an individual who develops such progressive symptoms within several months after the introduction of a potentially neurotoxic antinucleoside agent is more likely to have a toxic neuropathy. The diagnosis, however, can be confirmed only if symptoms resolve on discontinuation of the agent *(18,29)*. Because some subjects in clinical trials experience only transient paresthesias *(36)*, minor symptoms may not necessarily require immediate change in therapy. Furthermore, in some instances, it may be preferable to continue the agents despite minor neuropathic symptoms because of a robust antiretroviral response. A detailed neurological examination to assess the extent of sensory and reflex impairment, and a detailed neurological history to pursue other causes of neuropathy is an important measure, which should be obtained at the onset of symptoms and is also a reasonable screening measure before institution of dideoxynucleoside therapy. Other potential treatable causes for neuropathy, such as vitamin B_{12} deficiency, diabetes mellitus, thyroid disease, syphilis, Lyme disease, or exposure to other neurotoxic substances or medications should be sought. Electrophysiological studies may be helpful in centers with the capability to do both QST and conventional EMG–NCV studies, however, routine EMG–NCV studies may be unrevealing until after clinical signs appear.

Serial monitoring of the neurological symptoms and examination is indicated in any individual experiencing incident neuropathic symptoms or abnormal findings on examination while taking dideoxynucleosides. In the event the symptoms progress or are severe enough to limit function, and if other antiretroviral treatment options are available, consideration should be given to suspending therapy with the nucleoside analog to allow the neuropathy to improve. The patient should be counseled about the possibility of symptoms worsening in the first months after discontinuation of the presumed offending agent (coasting). If substantial improvement occurs, rechallenge with the agent at a lower dose is reasonable after 2 to 3 mo if no alternative therapy is available.

Neuropathic pain may be intermittent and tolerable for some patients and require only nonsteroidal anti-inflammatory agents for relief. For many individuals, the pain is both continuous and severe enough to limit function. The World Health Organization has published a three-step analgesic ladder to provide a systematic and rational approach to pain management. Although developed to treat cancer-related pain, the same approach is useful for addressing neuropathic pain associated with HIV and dideoxynucleoside therapy. Initially, adjuvants such as tricyclic agents (amitriptyline and others) or anticonvulsants (gabapentin, lamotrigine, carbamazepine, and others) are used in combination with nonopioid analgesics. In the second step, a second adjuvant with a differing mechanism of action is added. Many patients with moderate neuropathic

pain can be controlled at this level; however, for those who prove refractory to these measures, step three adds opioid analgesics, which should be used in long-acting formulations (fentanyl, oxycodone, and others) *(76)*. A recent study comparing the analgesic efficacy of lamotrigine to placebo for HIV-associated neuropathic pain found self-reported benefit in subjects taking dideoxynucleoside agents, but not in those who were not using neurotoxic treatment regimens *(77)*. A randomized trial of amitriptyline and mexiletine failed to show benefit for either drug compared with placebo in subjects with painful HIV-associated neuropathies and varying exposure to dideoxynucleosides *(78)*.

Individuals with sensory ataxia caused by loss of postural sensation may require support for safe ambulation. Such patients are at increased risk of falling in the dark, when changing posture or direction, or when descending stairs.

In contrast to the patient complaining of distal paresthesias, urgent evaluation is required for the individual who complains of acute motor weakness. These types of neuropathies can progress rapidly to paralysis and, potentially, to respiratory failure. Plasma lactate levels should be drawn, and dideoxynucleoside agents discontinued, particularly if the neurological symptoms are associated with gastrointestinal symptoms. EMG–NCV studies may help to differentiate IDPs from axonal or mixed neuropathies that are more characteristic of toxic syndromes or opportunistic disease. In a patient with progressive weakness, hospitalization and serial monitoring of neurological and respiratory function is indicated until stability or sufficient recovery occurs to allow independent function. Cerebrospinal fluid samples should be obtained to exclude opportunistic infections or lymphoma. Ventilatory support should be initiated for subjects with deteriorating respiratory status when vital capacity falls to 1 L, and prophylaxis for venous thrombosis should be initiated. Physical and occupational therapy to maintain range of motion and prevent contracture formation should be started early in individuals anticipated to have prolonged weakness. No specific therapy for the acute neuromuscular syndrome associated with nucleoside analogs and lactic acidosis has been shown to be effective. In one review of reported cases of pathological syndromes attributed to nucleoside-associated lactic acidosis, some individuals who improved were treated with cofactors such as thiamine, riboflavin, L-carnitine, prostaglandin E, or coenzyme Q. When neuropathic weakness improved, it did so very slowly, taking up to 1 yr after discontinuation of the nucleoside analog agent *(53)*.

ISSUES IN THE CURRENT DATA ON TOXIC NEUROPATHIES: IMPLICATIONS FOR FUTURE CLINICAL TRIALS

Current data on the toxicity of dideoxynucleoside analogs in humans has been drawn largely from subject complaints during clinical treatment trials and from case reports. No large carefully controlled prospective neurological trial

focused specifically on nucleoside-associated neuropathy in HIV has been performed, to date. In many clinical treatment trials, previous neuropathy was not an exclusion factor at entry. In others, detailed neurological examination to determine the prevalence of asymptomatic neuropathy at entry, and to confirm the origin of symptomatic complaints during the study, were not incorporated into the protocols. As a result, errors in attribution of symptoms or in the recognition of occult neuropathy may have occurred. Simpson and colleagues reanalyzed data from a large clinical trial of ZDV, ddI, ddC, and placebo in four combinations. Using criteria for DSP as described above, they reevaluated diagnoses made at the treatment sites. In 63% of reported cases of neuropathy, the site investigators were unable to determine the cause of the symptoms. On review, Simpson and colleagues were able to classify approximately half of these as DSP, and, therefore, potentially treatment related. Of subjects classified as having treatment related neuropathy, 27% did not have typical features of DSP as reflected in the criteria used by the reviewers, and were, therefore, considered unlikely to have medication-related toxic neuropathy *(79)*. These findings suggest that data from studies not incorporating detailed baseline and serial neurological assessments may both underestimate or overestimate true treatment-related neurotoxicity. Furthermore, in addition to modifying the frequency of DSP, successful HAART might also modify the time-to-onset and rate of progression of neuropathies induced by nucleoside analog therapy, confounding the temporal characteristics currently used to discriminate HIV-associated DSP from toxic neuropathy.

The question of whether painful neuropathies appearing in association with nucleoside therapy represent exacerbation of a previously occult HIV-associated distal sensory neuropathy, or whether they arise *de novo* as a result of nucleoside toxicity is of significant clinical importance. Neuropathy in advanced HIV infection seems to be common, and not infrequently asymptomatic. Marra and colleagues examined 226 subjects drawn from a large AIDS clinical trials unit and found DSP in 21%. Of the subjects with DSP, 71% had signs of neuropathy on examination but no symptoms. Individuals with DSP were more likely to have received dideoxynucleosides, they had more advanced HIV infection by Centers for Disease Control classification, and they had a trend toward lower CD4 counts *(80)*.

Schiffito and associates determined the frequency of DSP in a cohort selected for cognitive symptoms and CD4 counts of fewer than 300 cells/mm^3 or fewer than 200 cells/mm^3. They found symptomatic DSP in 35% of subjects, and asymptomatic DSP in an additional 20% of subjects. In this cohort undergoing serial neurological evaluations, 72% of subjects without neuropathy at entry developed incident DSP during a period of 30 mo, with a 1 yr incidence of 52%. Among those with no neuropathy or asymptomatic neuropathy

at entry, the 1-yr incidence of symptomatic DSP was 36%. Only 23% of the subjects were taking dideoxynucleosides and, interestingly, neither these subjects nor those with asymptomatic neuropathy were more likely to develop symptomatic DSP during the study *(81)*.

In a series of 251 HIV-infected subjects undergoing clinical and neurophysiological assessments, Tagliati and coworkers found evidence of DSP in 38% of the entire cohort, and in 48% of subjects with AIDS. Of subjects with clinical features of DSP, 19% had normal electrodiagnostic studies, whereas, of those without clinical findings, 28% had abnormalities on the neurophysiological assessments suggestive of DSP *(14)*.

The limitations of previous data and the frequency of asymptomatic polyneuropathy demonstrated in these studies underscore the need for any future clinical trial using potentially neurotoxic therapies, including nucleoside analogs, to incorporate a sufficiently detailed neurological screening at baseline and during the observation period to accurately assess and attribute incident neuropathy, and to determine risk factors for the emergence of incident neuropathy.

In some recent clinical trials incorporating dideoxynucleoside treatment regimens, fewer subjects have been noted to develop symptomatic painful neuropathy. Additionally, some individuals seem to experience improvement of neuropathic symptoms in association with dideoxynucleoside therapy, further confounding the determination of frequency and etiology of painful neuropathies in patients with advanced HIV infection who are treated with potentially neurotoxic therapies. Factors contributing to the emergence of treatment-related neuropathic toxicity need to be elucidated. Do host factors, such as genetic susceptibilities, predispose to the development of toxic neuropathy? Are there particular interactions among the multiplicity of drugs taken by HIV-infected patients and the multiorgan pathologies, particularly those affecting the liver or kidneys, which combine to increase the toxicity of a standard therapeutic dose of a dideoxynucleoside in a given individual? Are the beneficial effects of successful therapy on DSP partially masking the neurotoxicity of dideoxynucleoside therapy?

The risk of acquiring HIV-associated DSP s appears to be related to both increased plasma HIV viral load and decreased CD4 count *(25,26,81)*. Successful treatment with current HAART may have the capacity to improve peripheral nerve function, as demonstrated in a study using serial QST assessments *(82)*, and as reported in an earlier study of ddI, described above *(48)*. Thus, HIV-associated DSP may become less prevalent with successful retroviral suppression and control of other, as yet unidentified factors, offsetting an increased frequency of neuropathy induced by dideoxynucleoside components of HAART regimens. This offsetting effect was suggested in a retrospective study of a German cohort, in which the prevalence of DSP in a 2-yr period

before the introduction of HAART was 42.5%, with a suspected rate of toxic neuropathy of 20.4%. In the following 2-yr period, during which subjects were placed on HAART in large numbers, the prevalence of DSP was reduced to 34.4%; however, the proportion of neuropathies attributed to nucleoside analog toxicity increased to 31.2% *(28)*.

Recently, a small prospective study assessed serum lactate levels in patients with DSP, comparing those taking d4T to those without exposure to dideoxynucleosides, as well as to HIV-infected individuals without neuropathy taking d4T and non-HIV-infected controls. An elevated serum lactate level was found to be 90% sensitive and 90% specific in discriminating d4T-related toxic DSP from HIV-associated DSP *(88)*. If results of this study are confirmed, the presence of a surrogate marker should contribute to the understanding of the pathogenesis of toxic DSP, and perhaps clarify the potential contributions of both the dideoxynucleoside- and HIV-related pathologies to the natural history of DSP.

Future controlled clinical trials which include longitudinal assessments both of symptomatic and asymptomatic neuropathy from onset of antiretroviral therapy, and epidemiological studies of the natural history of HIV-associated neuropathies in relation to HAART therapy to identify factors associated with their occurrence, may help to clarify which subjects are at risk of severe toxic neuropathies and provide prognostic indicators to facilitate modification of therapeutic regimens. Perhaps serum lactate levels will prove useful in this regard. It may be possible, with better understanding of the pathophysiology of these syndromes, to identify adjunctive therapeutic agents which protect against the emergence of toxic neuropathies, as has recently been suggested for acetyl-L-carnitine. In addition to detailed serial clinical assessments, better measurement tools may be required, including electrophysiological tests that are more sensitive, such as QST assessments *(82)*, and pathological measurements, such as quantification of intraepidermal and, perhaps, dermal nerve fiber density obtained by skin biopsy *(21–23,74)*. Treatments relieving neuropathic pain may not result in improvement of clinical, electrophysiological, or pathological measures of neuropathy, as was seen in the recent clinical trial of nerve growth factor *(83,84)*. The natural history of such painful neuropathies relieved, but not resolved, after withdrawal of dideoxynucleosides, awaits elucidation in future studies. Will these prove to be aborted toxic neuropathies with incomplete resolution, or incident HIV-associated DSP exacerbated by a superimposed agent with some neurotoxicity, perhaps synergistic with primary neuropathological processes? Thus, most importantly, studies to improve our understanding of the mechanisms of toxicity in both HIV-associated DSP and nucleoside-associated toxic neuropathies are essential to provide the basis for future therapies that specifically target the pathophysiological mechanisms of these syndromes.

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9

Mitochondrial Dysfunction and Nucleoside Reverse Transcriptase Inhibitor Therapy

A Pathophysiological Perspective

William Lewis

INTRODUCTION

New, more effective antiretroviral therapeutic agents *(1)* and promise from HIV vaccine studies *(2)* have not yet prevented the AIDS epidemic's global spread. Nucleoside reverse transcriptase inhibitors (NRTIs), in combinations termed highly active antiretroviral therapy (HAART), are cornerstones of AIDS therapy in the developed world. Biochemical, cell biological, and clinical features intertwine to amplify the DNA polymerase-γ hypothesis *(3)* into a mitochondrial dysfunction hypothesis *(4)* that focuses on the effects on mitochondrial DNA (mtDNA) replication and intramitochondrial and intracellular processing of NRTIs *(5)*. During the past decade or longer, it has become increasingly reasonable to link organelle (i.e., mitochondrial) toxicity to NRTI therapy and to consider that the mechanisms operative in the pathogenesis of NRTI toxicity are interrelated with the mitochondria. Early observations were based on in vitro experiments *(6)*, animal experiments confirmed those findings in vivo *(7–9)*, and clinical correlations were found in AIDS patients *(4,10)*.

ENERGY DEPLETION AND MITOCHONDRIAL DYSFUNCTION CAUSED BY NRTIs

During the years that mitochondrial toxicity has been recognized, three cornerstones of the pathogenesis appear fairly consistently. First is energy deprivation secondary to mtDNA depletion. Overall, mtDNA depletion seems to be a consistent, if not causative, event in the pathophysiological phenotype in human and animal studies *(9,11)*, but an unambiguous causation is not confirmed. Energy deprivation, possibly the initiating step of NRTI toxicity based on mtDNA depletion, relates decreased energy abundance in tissues (e.g., heart) to decreased abundance of normal, functional mitochondria.

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Wallace's oxidative phosphorylation paradigm *(12)* indicates that tissue requirements for oxidative phosphorylation and threshold effects of dysfunction seem to be integral to the development of symptoms in genetic illnesses of mtDNA, and similar events may occur in acquired defects of mtDNA that relate to NRTIs.

OXIDATIVE STRESS AND MITOCHONDRIAL TOXICITY CAUSED BY NRTIs

The second key event concomitant with or resulting from mitochondrial energy deprivation is mitochondrial oxidative stress. As yet, this point has been demonstrated in few studies clinically and experimentally *(13,14)*, but acceptance is growing for the pathogenetic role of oxidative stress in NRTI-induced mitochondrial toxicity. Our group *(15)* and others *(16)* have demonstrated oxidative stress arising from NRTI administration in experimental systems.

As mentioned above, energy depletion from altered mtDNA replication in NRTI toxicity is a logical consequence *(3,4,8,9,17–21)*. Furthermore, it follows that related events of oxidative stress also impact on energetics and mtDNA replication. Oxidative stress is defined as an imbalance between the production of reactive oxygen species (i.e., superoxides, hydrogen peroxide, lipid peroxides, hydroxyl radicals, and peroxynitrite) and the cellular antioxidant defenses that prevent damage from those moieties *(22)*. Mitochondria are a logical target for oxidative stress, based on their ability to generate reactive oxygen species, and mitochondria may be primarily involved in oxidative stress associated with HIV therapy. Chronic zidovudine (ZDV) treatment induces oxidative damage of skeletal muscle in mice *(13)*.

The pathophysiological importance of oxidative stress is apparent from work in other systems. mtDNA from rat liver has more than 100 times the level of oxidative DNA damage than nuclear DNA (nDNA). Differences in oxidative damage between nDNA and mtDNA may relate to:

1. Lack of known repair enzymes for mtDNA error excision.
2. A lack of histones protecting mtDNA.
3. A subcellular proximity of mtDNA to these oxidants.

Exposure of DNA to superoxide-generating systems causes extensive strand breakage and degradation of deoxyribose *(23)*. On a mass-action basis, random mtDNA mutations would likely inactivate complex I, because of the significant contribution from mtDNA-encoded elements. Moreover, deficiency of complex I proteins could amplify superoxide formation and increase oxidative stress *(24)*.

Oxidation of mtDNA by hydroxyl radicals results in the formation of the oxidized base 8-hydroxydeoxyguanosine (8-OHdG); 8-OHdG is present in hepatic mtDNA at 16-fold higher levels than corresponding nDNA *(25)*. It

follows stochastically that, during any given oxidative event, mtDNA will sustain more damage than nDNA *(26)*. The number of oxidative hits in rat DNA is estimated at approx 100,000 hits/cell/d. Although most of the components of a mitochondrial base-excision repair system have been identified, it is unclear how efficiently this repair removes the wide spectrum of adducts that may occur from oxidative damage. Mitochondrial oxidative damage was supported indirectly by the coexistence of malondialdehyde on (or near) the inner mitochondrial membrane *(27)*. Its interaction with mtDNA could lead to crosslinking, deletion errors in transcription, or mtDNA polymerization. In oxidative stress, abundance of 8-OHdG is higher in mtDNA than in nDNA *(28)*. Pathophysiological events would not occur until the thresholds of damage were severe enough to impact on organ function *(12)*.

MUTATIONS OF mtDNA

In addition to NRTI-induced energy deprivation, oxidative damage to mtDNA by respiration-linked reactive oxygen species may relate to damage of cardiac myocytes and development of cardiomyopathy. Because reactive oxygen species, such as hydrogen peroxide, hydroxyl radicals, and others, are generated close to the inner membrane of the mitochondria and can react with and oxidize mtDNA, the mtDNA is a likely target for oxidative stress. mtDNA mutations may result from oxidative mtDNA damage, aberrant mtDNA replication, and altered mtRNA transcription *(29)*. mtDNA mutations may have important impacts on HIV-negative patients who received antiretroviral therapies with NRTIs antenatally and postpartum. This important area is being explored aggressively in other laboratories *(29–32)*.

CURRENT NRTI THERAPY

NRTIs currently used to treat HIV infection *(33)* include ZDV (3′-azido-2′,3′-deoxythymidine; also known as AZT), zalcitabine (ddC; 2′,3′-dideoxycytidine), didanosine (ddI; 2′,3′-dideoxyinosine), stavudine (d4T; 2′,3′-didehydro-3′-dideoxythymidine), lamivudine (3TC; 3-thiacytidine; *cis*-1-[2′-hydroxymethyl-5′-{1,3oxathiolanyl}]cytosine), emtricitabine (–FTC), tenofovir, and abacavir. At present, a relatively large number of NRTIs are available. Most NRTIs are administered in HAART combinations that vary widely in their constituents. This compounds the complexity and interpretation of data obtained.

MECHANISMS OF TOXICITY

Overview

A prevailing theory suggests that ZDV-induced mitochondrial toxicity involves defective mtDNA replication (reviewed in refs. *4* and *34–36*), but this hypothesis is not universally accepted *(37)*. Decreased mtDNA, mtRNA, mitochondrial

polypeptides, and defective mitochondrial ultrastructure correlate with micromolar, mixed inhibition constants for dideoxy-NRTI triphosphates in various experimental systems *(6–9,15,17,18,21,38–54)*. Some other explanations for mitochondrial toxicity from ZDV include inhibition of: adenylate kinase *(55)*, adenine nucleotide translocator *(56,57)*, nicotinamide adenine dinucleotide oxidase *(58)*, protein glycosylation *(59)*, and a bystander effect *(60)*. Other mechanisms unrelated to mtDNA replication also have been suggested (including glutathione depletion; *see* ref. *61*, for example).

Zidovudine

ZDV was the first NRTI antiretroviral drug used in the treatment of AIDS and affords the greatest toxicological experience. Both in clinical *(10,11,62–68)* and in experimental studies *(4,34,69,70)*, ZDV has been implicated in the development of mitochondrial diseases with features of myopathy, ragged red fibers, decreased mtDNA, and defective mtDNA replication. ZDV has worsened mitochondrial genetic illnesses, been implicated in the genesis of lactic acidosis *(71–77)*, and has caused mtDNA mutations *(78)*.

With respect to toxicity in various target tissues, observationally based clinical correlates were made in some of the early studies in which ZDV liver toxicity was associated with obesity and female gender *(42)*. Refined genetic correlates were lacking. NRTI toxicity presents a variable and complex diagnostic phenotype in the treated population and mimics key features of mitochondrial diseases. NRTI toxicity may serve as an important model system for relevant pharmacogenetic studies. Such a pharmacologically based review is beyond the scope of this work, but was addressed elsewhere *(3–5,69)*.

One important toxic target is the liver. In ways that resemble fialuridine (FIAU; described following), hepatomegaly, steatosis, and mitochondrial ultrastructural changes *(64,79)* have been documented with ZDV treatment. Hepatic toxicities from ddI and ddC were also reported *(62)*. The toxic mechanism is presumed to relate to liver mitochondria. Fatal hepatomegaly with severe steatosis *(64)*, severe lactic acidosis, and adult Reye's syndrome *(62)* in ZDV-treated, HIV-seropositive patients were all pathogenetically linked to ZDV-induced hepatotoxicity. Clinical features resembled some of those seen in FIAU toxicity (reviewed in ref. *3*).

The cardiovascular system effects of ZDV include cardiomyopathy with cardiac dilation and failure; mitochondrial cristae dissolution; and elevated serum lactate *(21,80)*. Some reports have suggested resistance to toxicity from ZDV *(81)* or susceptibility to toxicity from ZDV *(82)*. Mitochondrial toxicity to cardiovascular tissues was documented *in utero (29,32,83–89)*. More recently, ZDV therapy has been implicated in lipodystrophy in AIDS patients *(90,91)*.

Stavudine

d4T emerged as a first-line HAART component. Similar to ddC (*see* "Zalcitabine" section), a painful peripheral neuropathy *(92)* was described with d4T treatment; however, one study presented evidence to contest that relationship. A clinical proof-of-principle used acetyl-L-carnitine successfully to treat d4T-induced mitochondrial neuropathy *(93)*.

Preclinical and basic studies further support the mitochondrial toxicity of d4T. Distorted cristae and decreased mtDNA levels in cultured cells occurred after d4T exposure, and a d4T-induced mitochondrial neuropathy was generated in vivo *(94)*. Kinetics of inhibition with d4T and DNA polymerase-γ *(95)* resulted in an inhibition constant in the nanomolar range *(15)*. Clinical treatment with certain NRTIs (d4T or 3TC) results in anion gap acidosis. Moreover, the lactic acidosis–hepatic steatosis syndrome may be more common than previously appreciated in adults and children treated with NRTIs. d4T treatment was also associated with lipodystrophy *(96)*.

Patients treated with various NRTIs experienced lactic acidemia *(84,97–99)*, and a phenotype of mtDNA depletion *(100)*. Depending on the specifics of the system used in the study *(101)*, deleterious effects on mitochondrial structure and function in selected targets have been documented *(3,8,19,102)*.

Specificity of blood cell mtDNA depletion as a surrogate marker for NRTI toxicity has been documented in some studies *(82,90,103–105)*; however, the impact of the studies was confounded by the control groups used *(106)*. In principle, mtDNA depletion is mechanistically consistent with NRTI toxicity. However, the impact of mitochondrial dysfunction in surrogate tissues remains unclear, even in face of a logical working hypothesis. Methods for diagnosis usually include examination of plasma lactate levels or lactate-to-pyruvate ratios, but require careful sample preparation and handling to assure meaningful results and interpretations. Overall, depletion of mtDNA seems to be accepted as an important marker of the toxic process, and may even serve as a diagnostic hallmark *(9,11)* to monitor successful HAART therapy *(100)*. It should be emphasized that the ideal surrogate tissue to monitor mtDNA depletion from NRTIs remains to be determined, and is an active focus of clinical research.

Zalcitabine

ddC is less frequently administered today. Nonetheless, painful peripheral neuropathy attributed to mitochondrial dysfunction has been associated with clinical ddC toxicity *(107,108)*, and inhibition of mtDNA replication has been observed in vitro *(6,40,109,110)*.

3TC and –FTC

3TC is widely used in HAART regimens, and –FTC is becoming an important therapeutic tool since its recent addition to the armamentarium of

antiretroviral treatments. Of the commonly used NRTIs, 3TC seems to have a favorable safety profile, but similar to the other NRTIs, requires coadministration in a HAART regimen, particularly because of induction of HIV-resistance mutations. Toxicity to muscle is reported clinically with 3TC *(111)*, but basic evidence for toxicity of 3TC monotherapy is lacking in our in vivo systems.

More-recently approved –FTC exhibited a relatively favorable safety profile *(112)*, and kinetics with DNA polymerase-β favored efficacy *(113)*. Long-term experience with –FTC is not available yet.

Didanosine

ddI remains an important element in HAART regimens. Two principal toxicities are recognized from a clinical perspective. As with ddC, a painful peripheral neuropathy is documented with ddI therapy in humans. Early in the development of ddI, severe pancreatitis was identified as an important side effect *(114)*. Experimental work documented pancreatic changes by flow cytometry *(115)*. Fatal hepatotoxicity was described, and lactic acidosis has occurred with coadministration of tenofovir.

SUMMARY

NRTI toxicity now is an important clinical problem with long-term significance to AIDS patients. Mechanisms likely relate to energy depletion, oxidative stress, and mtDNA mutations. Analogous to treatment of other serious infectious agents, combinations of multiple anti-HIV-1 drugs are used to target different viral proteins or points in virus–host life cycle *(116)*, and may create combined toxicities to mitochondria. Because current clinical guidelines recommend combined therapy, usually containing NRTIs, such regimens may be important to the development of mitochondrial toxicity in new tissue targets.

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10
Mitochondrial Toxicity and Lipodystrophy

Grace McComsey

The advent of potent antiretroviral drugs (ARV) in recent years has had an impressive impact on mortality and disease progression in HIV-infected patients *(1)*, therefore, issues related to long-term effects of ARV are of growing importance. Perhaps the most serious and distressing toxicities are mitochondrial toxicity and lipodystrophy, a syndrome of fat redistribution recently linked to mitochondrial dysfunction. Lipodystrophy includes peripheral fat wasting (referred to as lipoatrophy), with or without fat accumulation (abdominal visceral fat accumulation, breast enlargement, and/or buffalo hump). It is commonly associated with metabolic abnormalities—dyslipidemias and/or insulin resistance *(2,3)*. The lipodystrophy syndrome was initially believed to be associated with use of protease inhibitor (PI) agents *(3,4)*, but recent studies called into question this association *(5–7)*. Lipodystrophy is not only disfiguring, but is also associated with decreased adherence to ARV *(8)*, hypertension *(9)*, as well as decreased quality of life, self-esteem, and sexual difficulties *(10)*. The lipodystrophy syndrome will be discussed here, mainly as it relates to nucleoside reverse transcriptase inhibitor (NRTI) therapy and mitochondrial toxicity.

BASIC UNDERSTANDING OF MITOCHONDRIA

The most important and critical mitochondrial function is oxidative phosphorylation, a mechanism by which the energy derived from metabolism of nutrients in the presence of oxygen is transformed into adenosine triphosphate (ATP). Figure 1 describes the mitochondrial oxidative energy metabolism. The system of oxidative phosphorylation includes five multienzyme complexes, designated as electron-transport chain complexes I through V. These enzymes are encoded by either mitochondrial or nuclear DNA (nDNA) *(11)*. Therefore, a deficit of either mitochondrial or nDNA may lead to malfunction of oxidative phosphorylation. Mitochondrial DNA (mtDNA) is a double-stranded, circular molecule that encodes for 2 ribosomal RNAs, 22 transfers RNAs, and 13 polypeptides of complexes I, III, IV, and V of the respiratory chain. mtDNA

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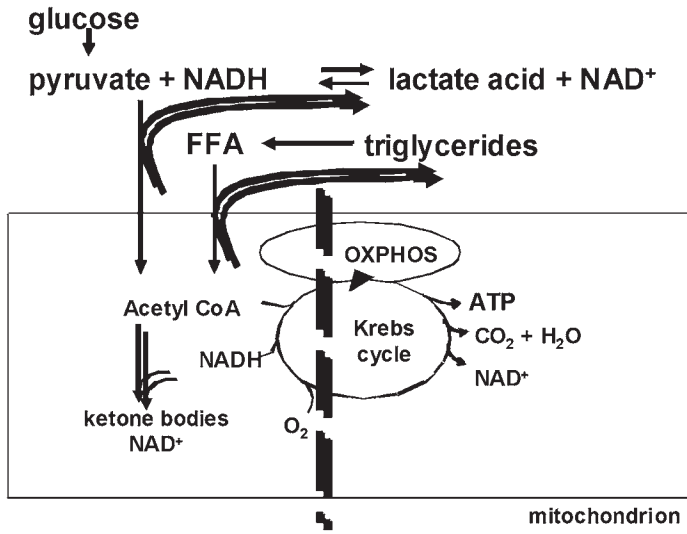

Fig. 1. Schematic representation of the steps of oxidative ATP energy production in the mitochondria. After formation by glycolysis, pyruvate is metabolized by pyruvate dehydrogenase to acetyl coenzyme A (acetyl CoA). Acetyl CoA donates its acetyl group to the first compound of the Krebs cycle, oxaloacetate, to form citrate, beginning a turn of the oxidation cascade of the Krebs cycle. These reactions yield CO_2 and H^+, the latter of which is trapped in the form of reduced coenzymes, including nicotinamide adenine dinucleotide (NADH). NADH transfers its reducing equivalents to the first complex of the respiratory chain, with subsequent flow of reducing equivalents, electrons, in the respiratory chain. This will reduce oxygen to water; the resultant proton gradient is sufficient for synthesis of ATP.

Inhibition of mtDNA would cause a malfunction of the mitochondrial respiratory chain, which would be unable to break down acetyl CoA. This would shift the pyruvate metabolism toward an alternate pathway, with reduction of ATP production, rise in NADH-to-NAD+ ratio, followed by channeling of acetyl CoA toward ketogenesis, and increased lactate production. The increased production of lactate exceeds the capacity of the liver to clear the lactate from the circulation, leading to its accumulation and to the resultant acidosis. OXPHOS, oxidative phosphorylation; FFA, free fatty acids.

replication depends on nDNA-encoded-specific polymerase, DNA polymerase-γ, for its replication. mtDNA is inherited maternally. Each cell contains hundreds to thousands of mitochondria, with each mitochondrion containing 2 to 10 mtDNA molecules *(12)*. mtDNA is prone to mutations at a 5- to 10-fold higher rate than nDNA. A mixture of normal (wild-type) and mutant mtDNA can coexist within the same cell; this condition is known as heteroplasmy. The consequences of such mutations will depend on the proportion of normally functioning and abnormally functioning mtDNA in a particular cell. Once the proportion of abnormal

mtDNA exceeds a certain threshold level, cellular function is impaired. The threshold of mutated genome needed to produce a deleterious phenotype varies among persons, among organ systems, and within a given tissue. The threshold effect depends on the balance between oxidative supply and demand. In addition, mtDNA lacks protective histones or an effective repair system, rendering it more prone to somatic mutations and oxidative injury. It is exposed continuously to oxygen free radicals generated by oxidative phosphorylation.

POTENTIAL MECHANISMS OF NRTI-INDUCED MITOCHONDRIAL TOXICITY

Inherited disorders of mitochondrial function or biogenesis exhibit several of the features of the syndromes that have been associated with NRTI therapy, including liver dysfunction, lactic acidosis, and even lipodystrophy *(13)*. Therefore, mitochondrial dysfunction is postulated link to most of the NRTI-induced toxicities. The exact molecular pathogenesis of the mitochondrial dysfunction remains under investigation, and perhaps NRTIs can induce mitochondrial dysfunction in several possible ways. Most believe that the most important mechanism is through inhibition of mtDNA synthesis *(14)*, and subsequent depletion of mtDNA. In fact, it is well established that NRTIs in vitro can inhibit the activity of DNA polymerase-γ, the primitive DNA polymerase found in mitochondria but not in nuclei of eukaryotic cells *(15–19)*. This mtDNA depletion may worsen after long exposure to NRTIs, to a point at which a threshold of energy depletion may be reached and symptoms become manifested. In addition, the degree of DNA polymerase-γ inhibition, and subsequently of mtDNA depletion, depends on the type of nucleosides used (zalcitabine [ddC] > stavudine [d4T] > didanosine [ddI] = zidovudine [ZDV] > lamivudine [3TC] ≈ abacavir) *(15,19)*. Similarly to abacavir and 3TC, tenofovir and emtricitabine have low affinity for polymerase-γ and are less likely to cause mtDNA depletion *(20,21)*. Furthermore, there is in vitro and in vivo evidence of additive or synergistic mitochondrial toxicity when two NRTIs are used in combination *(22)*. There are other potential mtDNA-independent mechanisms for NRTI-induced mitochondrial dysfunction. For instance, ZDV triphosphate binds to adenylate kinase, an enzyme involved in ATP formation, and also inhibits the mitochondrial adenosine diphosphate/ATP translocator *(23,24)*.

Lastly, a new plausible mechanism for NRTI-induced mitochondrial dysfunction is under investigation. An intact respiratory chain function is necessary for the *de novo* synthesis of all cellular pyrimidines, because an efficient electron flux is essential for the activity of dihydroorotate dehydrogenase *(25)*. Decreased natural pyrimidines as a result of the inhibition of dihydroorotate dehydrogenase may lead to worsened mtDNA depletion by pyrimidine NRTIs, with which they compete at polymerase-γ, closing a vicious cycle (Fig. 2).

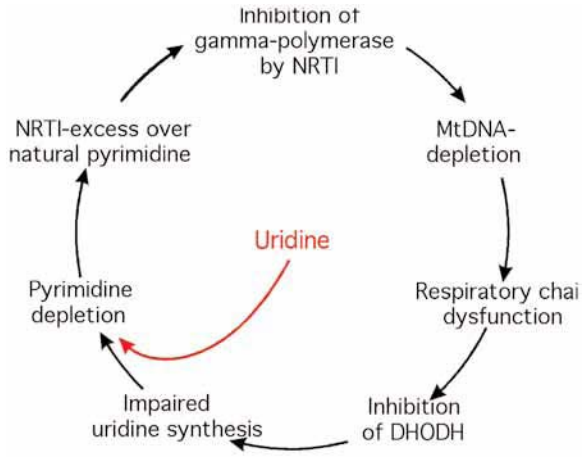

Fig. 2. Suggested mechanism of the beneficial effect of uridine supplementation on mitochondrial toxicity. DHODH, dihydroorotate dehydrogenase. (Adapted from ref. *26*.)

Indeed, supplying uridine as an exogenous source of pyrimidine precursors attenuated mtDNA depletion and fully abrogated mitochondrial toxicity in hepatocyte and adipocyte models *(26,27)*.

Evidence has been mounting that the pathology of NRTI-induced defects of electron transport may, to some degree, be mediated by reactive oxygen species, which damage cellular proteins, lipids, and mtDNA itself, possibly causing a vicious cycle leading to progressive worsening of mitochondrial function. ZDV has been shown to increase mtDNA oxidized guanosine levels *(28,29)*. Two recent studies supported a potential role for oxidative stress in NRTI-induced mitochondrial dysfunction *(30,31)*. The first study showed that supplementation with the antioxidants, vitamins C and E, prevented the ZDV-induced increase in the level of urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine, a good marker for oxidative damage to mtDNA *(30)*. In addition, in ZDV-treated animals, vitamins E and C were able to prevent ZDV-induced histological mitochondrial damage. The second study demonstrated that these antioxidants were able to reverse d4T-induced oxidative stress in mice *(31)*. These studies provide an incentive for future clinical trials evaluating the usefulness of antioxidants as therapeutic and preventive agents for NRTI-induced mitochondrial toxicity. Indeed, in one clinical trial, a strong association was found between the oxidative marker, F2 isoprostanes, and lipoatrophy *(32)*.

Another possible mechanism of NRTI-induced mitochondrial toxicity remains, with only weak evidence supporting it. Deletion in mtDNA has been shown in liver *(33)*, muscle *(34)*, fat *(34)*, and sperm *(35)* of NRTI-treated subjects, the majority of whom had some presumed NRTI-related toxicities. In

two separate studies, we were unable to detect point mutations or deletions in any of the specimens taken from two separate groups of NRTI-treated subjects, with or without clinical mitochondrial toxicities *(36,37)*.

CLINICAL MANIFESTATIONS OF MITOCHONDRIAL TOXICITY

Symptomatic Forms of Hyperlactatemia

Lactic Acidosis

Lactic acidosis is the most dramatic manifestation of NRTI-associated mitochondrial dysfunction. Lactic acidosis has been reported in HIV-infected patients since the early 1990s, but recent clusters of reports increased physicians' awareness about this potentially fatal syndrome. Fortunately, severe lactic acidosis remains a rare occurrence. The typical clinical presentation is one of precipitous occurrence of nausea, vomiting, abdominal pain, severe fatigue, malaise, tachycardia, dyspnea on exertion, and rapid weight loss. It is almost always accompanied by hepatic steatosis, and, at times, by hepatic failure *(38–40)*. Prospective data on the incidence of lactic acidosis are not available, but observational cohort studies suggest a likely incidence of 1.3 to 1.7 cases per 1000 person-years of NRTI exposure *(41–43)*.

Symptomatic Hyperlactatemia

More common than the severe lactic acidosis, is a milder form of mitochondrial dysfunction termed symptomatic hyperlactatemia. Its incidence has been reported at 25.6 cases per 1000 person-years among patients taking d4T, compared with 1.9 cases per 1000 person-years among patients taking other NRTIs *(44)*. In a recent report, Brinkman estimated the incidence of clinically significant hyperlactatemia at 11 per 1000 person-years of ARV*(45)*. d4T has been strikingly involved in these cases *(44,46–48)*. Symptomatic hyperlactatemia or lactic acidosis have been diagnosed as early as 1 mo after initiation of therapy, but more commonly occur 6 mo after initiation of therapy *(49)*. Female gender and obesity are risk factors for both lactic acidosis and the milder symptomatic hyperlactatemia *(49,50)*. In addition, HIV-infected pregnant women treated with the combination of d4T plus ddI seem to be at particular risk of lactic acidosis with or without pancreatitis *(51,52)*. The risk seems to be greatest in the third trimester and with longer duration of ddI plus d4T therapy. Therefore, the use of d4T with or without ddI should be avoided in HIV-infected pregnant women, except in cases of very limited treatment options, in whom the potential benefits clearly outweigh the risks of these drugs *(52)*. In addition, pregnant women treated with any NRTI-containing regimen should be closely followed throughout pregnancy, and even more closely during the last trimester; lactate testing should be promptly performed in case of any symptom(s) consistent with mitochondrial toxicity. There have been no demonstrated correlations

between these cases and CD4 cell count, duration of HIV infection, clinical stage of disease, HIV-1 RNA levels, and use of PI or non-NRTI therapy *(49)*. Clinical symptoms associated with symptomatic hyperlactatemia include fatigue, weakness, nausea, or abdominal discomfort. Hepatic steatosis, myopathy, pancreatitis, and vacuolar, noninflammatory cardiomyopathy can occur in cases of symptomatic hyperlactatemia or lactic acidosis. The Food and Drug Administration recently reported on 25 cases of profound motor weakness suggestive of Guillain-Barré syndrome, all associated with hyperlactatemia *(52)*. Twelve of the cases occurred in women, and six of the seven fatalities occurred in women. This is in keeping with previous reports of lactic acidosis, in which the female sex was overrepresented *(49,50)*.

Hepatitis C itself is associated with lipoperoxidation that can lead to mitochondrial dysfunction and depletion in mtDNA *(53)*. A recent study found significant mtDNA depletion as well as electron microscopic alterations of mitochondria in 92% of patients with hepatitis C, genotype 1 *(54)*. In addition, ribavirin may enhance the risk of NRTI-associated mitochondrial dysfunction *(55)*. Therefore, in subjects coinfected with HIV and hepatitis C, the potential for mitochondrial damage is significant; these subjects deserve close observation and a low threshold to undergo lactate testing in case of any suggestive symptom(s).

Diagnosis of Lactic Acidosis/Symptomatic Hyperlactatemia

There is ample evidence that these symptomatic forms of hyperlactatemia (lactic acidosis and symptomatic hyperlactatemia) are secondary to NRTI-induced mitochondrial dysfunction. Both ultrastructural and functional defects in liver and muscle mitochondrial have been demonstrated in NRTI-treated patients with symptomatic hyperlactatemia or lactic acidosis *(34,37,56)*. In addition to elevated serum lactate levels, other laboratory abnormalities reported in these patients included elevated aminotransferases, elevated lactate-to-pyruvate ratios, elevated pancreatic enzymes, and elevations in muscle enzymes. In some cases, lactate levels may be initially normal, but the lactate-to-pyruvate ratio may be elevated, thus providing an earlier marker of mitochondrial damage. It is established that, in inherited mitochondrial diseases, exercise may help in diagnosing early mitochondrial dysfunction, by enhancing the sensitivity of the lactate-to-pyruvate ratio. In the HIV-infected population, the use of the lactate-to-pyruvate ratio has been sparse, mostly because of the significant technical requirements in obtaining pyruvate testing. Therefore, there is limited data on the predictive value of the lactate-to-pyruvate ratio in this population. Nonetheless, if measured, this ratio should be determined at rest and then after exercise, in cases in which the lactate level is normal, in the setting of a clinical suspicion of mitochondrial toxicity. Blood mtDNA level is

another noninvasive marker currently under investigation. A significant decrease in blood mtDNA was found in a small group of subjects with symptomatic hyperlactatemia *(57)*, whereas other studies found no mtDNA depletion in this population, including in one subject with fulminant lactic acidosis *(36)*. Therefore, more data are needed on the reproducibility of this assay and its sensitivity in diagnosing early mitochondrial toxicity.

Management of Lactic Acidosis/Symptomatic Hyperlactatemia

Subjects with symptomatic hyperlactatemia should have their therapy modified or even temporarily interrupted. In fact, because of the concern regarding progression to lactic acidosis, most experts would recommend a temporary interruption of all ARV until patients are asymptomatic and lactate levels are back to normal levels. This may take several weeks to months. Management of such patients after the resolution of the serious hyperlactatemia is controversial. Two strategies have been adopted in this setting: switch to potentially less mitochondrial-damaging NRTIs (such as replacing d4T with either abacavir or ZDV) or switch to an NRTI-sparing regimen. The latter option is probably safer but, unfortunately, a growing proportion of the HIV-infected population has already been exposed to all available classes of ARV and, therefore, lacks this option. Available data demonstrate a normalization of serum levels with substitution of d4T with either abacavir or ZDV *(44,49,58)*. There is not enough data to reach a conclusion regarding the efficacy of antioxidants, vitamin B complex vitamins, carnitine, and other cofactors in mitochondrial and anaerobic metabolism. The rationale for the investigation of antioxidants was mentioned previously in "Potential Mechanisms of NRTI-Induced Mitochondrial Toxicity." A pilot trial of antioxidants in HIV-infected subjects with lipoatrophy was performed and showed a significant decrease in the waist-to-hip ratio, but without changes in other anthropometric measurements *(59)*; larger investigations are needed.

Both thiamine (vitamin B_1) and riboflavin (vitamin B_2) are important for intact mitochondrial function. Thiamine is a coenzyme of pyruvate dehydrogenase, therefore, thiamine deficiency can lead to defective pyruvate metabolism and accumulation of lactate *(60,61)*.

Riboflavin (vitamin B_2) is also important in mitochondrial energy metabolism; it is converted to flavin mononucleotide and dinucleotide; these flavins are coenzymes and necessary cofactors for the electron-transport chain. Thus, deficiency of either or both B vitamins can potentially play a role in NRTI-associated mitochondrial dysfunction, as previously suggested *(62)*. The administration of such agents may potentially activate residual mitochondrial oxidative enzyme activity. These vitamins have been reported to be successful in the treatment of lactic acidosis *(63–65)*, although no randomized studies

have been performed. Our group reported the successful use of these agents in the secondary prevention of symptomatic hyperlactatemia *(66)*.

Carnitine is necessary for transporting long-chain fatty acids across the inner mitochondrial membrane for the process of β-oxidation, a process that occurs mainly in skeletal muscle, heart, and liver. Treatment with L-carnitine has also been considered in inherited mitochondrial diseases, because secondary carnitine deficiency frequently exists in this setting. The frequency of carnitine deficiency in HIV infection remains unclear. The typical dose of levocarnitine used for inherited mitochondrial diseases is 100 mg/kg/d for children and 2 to 4 g/d for adults, in three divided doses *(67)*. Anecdotal reports exist of the use of L-carnitine for treatment of lactic acidosis in HIV-infected patients *(63,68)*.

Uridine supplementation is perhaps the most promising therapy for mitochondrial toxicities of NRTI therapies. In hepatocytes and adipocytes culture, the addition of uridine to pyrimidine NRTIs (including zalcitabine, d4T, and ZDV) led to a prompt improvement in lactate production and in mtDNA levels *(26,27)*. There are anecdotal reports of its successful use in HIV-infected patients with NRTI-induced myopathy and hepatic steatosis (personal communication, Ulrich Walker). One of these cases was recently published *(69)*. Currently, several clinical trials are investigating the use of uridine in HIV-infected subjects with clinical mitochondrial toxicities.

Significance of Asymptomatic Hyperlactatemia

Clinicians are currently relying on measurements of venous lactate levels as a marker of mitochondrial toxicity, but, unfortunately, lactate levels are neither specific nor sensitive for the detection of early mitochondrial dysfunction. The use of elevated serum lactic acid levels as a marker of mitochondrial dysfunction is based on the fact that oxidation of lactate/pyruvate is one of the prime functions of the mitochondrial oxidative phosphorylation system. However, lactic acidosis is not pathognomonic of impaired mitochondrial function *(70)*. Lactate/pyruvate can also be disposed of through hepatic gluconeogenesis, therefore, impairment of gluconeogenesis can result in lactic acidemia. Overproduction of serum lactate may also occur if, for example, glycolysis is disinhibited (as occurs in some tumors). Finally, physiological modulators can interfere with mitochondrial pyruvate oxidation (such as excessive oxidation of fatty acids), without implying that dysfunction of the organelle is present.

The significance and reproducibility of lactate levels in the HIV-infected population are also questionable. Several studies found a prevalence of hyperlactatemia of up to 36% of largely asymptomatic NRTI-treated subjects *(34–36,71)*. In our cohort, when strict criteria for collection of venous lactate were adopted, only 4% of heavily NRTI-treated HIV-infected subjects had an elevated lactate

Table 1
Guidelines for Lactate Level Specimen Collection From the AIDS Clinical Trials Group Mitochondrial Focus Group[a]

1. Have subject sit relaxed for 5 min before venipuncture.
2. Instruct subject to not clench the fist before or during the procedure and to relax the hand as much as possible.
3. If possible, do not use a tourniquet. If a tourniquet is necessary, then apply tourniquet lightly and draw lactate first, before the other samples, with the tourniquet still in place.
4. Collect the blood in a chilled gray-top (sodium fluoride–potassium oxalate) tube.
5. Place the specimen immediately on ice and send to the laboratory for immediate processing, preferably within 30 min of collection.
6. If random lactate is elevated, then repeat steps 1–5, with the following additional patient instructions: no alcohol within 24 h, no exercise within 8 h, and no food or drink except water within 4 h of the blood draw.

[a]Venous lactate levels are highly dependent on collection techniques. Therefore, it is recommended that these instructions be closely followed. If carefully collected, venous lactate level is equivalent to an arterial collection in most clinical situations

level *(72)*, again supporting the evidence of a high rate of false elevation of lactate levels if strict collection methods are not adopted. Longitudinal studies have so far shown that even patients with elevated lactate levels have not consistently progressed, and some subjects with sustained elevated lactate levels (up to 6.6 mmol/L) remained asymptomatic after a median follow-up of 210 d (range, 30–585 d) *(73)*. Thus, the significance of these elevations of lactate in asymptomatic subjects has been questioned *(73,74)*. Routine screening of asymptomatic NRTI-treated subjects for lactate levels is not warranted.

Additional noninvasive screening methods for mitochondrial toxicity are urgently needed for HIV-infected subjects. As mentioned above, blood testing for mtDNA depletion is under investigation as a noninvasive and easily obtainable marker, but its usefulness needs to be consistently demonstrated in larger studies before any recommendation for its routine use could be ascertained. There should be a low threshold to promptly check lactate levels in subjects who experience symptoms consistent with mitochondrial toxicity, in the absence of other plausible etiologies, such as ischemia, sepsis, or malignancies. When the clinical situation dictates testing for lactate levels, then venous lactate should be drawn as recommended by experts, without a tourniquet or fist clenching, and strictly using a chilled sodium fluoride–potassium (gray top) tube, with prompt transport on ice and prompt processing of the specimen. Table 1 shows the recommendations of the AIDS Clinical Trials Group Mitochondrial Focus Group for optimal collection of venous lactate.

Lipoatrophy and Its Link to Mitochondrial Toxicity

Athough identified in the medical literature since 1997, HIV-associated fat abnormalities still remains controversial in its definition and mysterious in its etiology. Thus, estimates of its prevalence among adult HIV-infected patients ranged from 7 to 84% *(75,76)* and from 1 to 43% among pediatric HIV-infected patients *(77)*. Subcutaneous fat wasting (or lipoatrophy) of the face, arms, buttocks, and/or legs has been described in HIV-infected persons, occurring with or without fat accumulation in central areas of the body, dyslipidemias, and insulin resistance. The term "lipodystrophy" generated significant confusion because it was used to describe either subcutaneous fat loss (lipoatrophy) and/or lipohypertrophy (fat accumulation in the abdomen and/or neck). The results of several large cohorts indicated that lipoatrophy is the distinctive feature of fat abnormalities in HIV. In these cohorts, after adjustment for age, HIV-infected subjects were not more likely than HIV-uninfected controls to have central fat accumulation *(78,79)*. Therefore, lipoatrophy and lipohypertrophy should be regarded as two separate entities, which could coexist in the same HIV-infected individuals.

The exact molecular or metabolic mechanisms behind lipoatrophy remain unclear, and a number of causes have been proposed implicating HIV disease itself, immune restoration after effective highly active antiretroviral therapy (HAART), and several metabolic dysregulations; to date, none has been confirmed. The hypothesis that has gained the most significant ground is NRTI-induced mitochondrial dysfunction. Indeed, recent clinical reports highlight the fact that lipoatrophy may be linked primarily to NRTI therapy, whereas the metabolic abnormalities, dyslipidemias and insulin resistance, are more readily associated with PI therapy. A short course of PIs administered to HIV-uninfected healthy subjects was able to cause significant dyslipidemias and insulin resistance *(80,81)*. Recent data indicate that lipoatrophy is very uncommon in subjects who are treated with an NRTI-sparing regimen *(82,83)*. Cohen et al. reported a minimal prevalence of lipoatrophy in subjects treated exclusively with ritonavir plus saquinavir, compared with subjects treated with NRTIs in addition to the same duration of ritonavir plus saquinavir *(82)*. In addition, in multivariate analysis, the use of NRTIs was the most significant risk factor *(82)*. Another study showed a similar low rate of lipodystrophy in subjects on exclusive PI therapy *(83)*, and interestingly, no significant lipoatrophy has been detected in a trial using NRTIs, which do have only a low potency of polymerase-γ inhibition *(84)*. Although these studies do not definitively implicate NRTI-induced mitochondrial dysfunction as the sole cause of lipodystrophy, they do demonstrate a powerful association with the use of NRTIs. They also suggest that PIs may not be as important in the development of lipodystrophy.

Observational studies revealed a significant rate of lipoatrophy in NRTI-treated PI-naive subjects *(5–7,85)*. In addition, therapy with d4T has been

associated with a higher rate of lipoatrophy compared with other NRTIs *(6,7,86)*.

The recent association of NRTI-associated hyperlactatemia and lipoatrophy led to the attractive hypothesis that mitochondria may be playing a key role in these body-fat changes, possibly through release of apoptosis mediators that in turn lead to peripheral fat loss *(87–89)*. In an effort to validate this hypothesis, several investigators have examined tissue biopsies in patients with lipodystrophy. They found evidence of significant ultrastructural abnormalities in the adipocytes of these subjects, the most common being disturbed architecture of the cristae, inclusion bodies, and increased size and number of mitochondria *(90,91)*. Such ultrastructural changes are very similar to the ones described in the muscle and liver of subjects with NRTI-induced lactic acidosis or symptomatic hyperlactatemia *(37,44,48,92)*. Several groups have shown a significant decrease of mtDNA content in the fat of subjects with lipodystrophy *(37,90,91,93)*. In contrast to the consistent mtDNA depletion found in subjects with lipoatrophy, several investigations revealed the absence of depletion in blood mtDNA of these subjects *(36,93,94)*. Both respiratory chain dysfunction *(34,37,95)* and mtDNA deletions *(34,95)* have been reported in the muscle and fat of subjects with lipoatrophy.

Further support of the role of NRTIs in lipodystrophy comes from the numerous switch studies in which the substitution of a non-NRTI or abacavir to the PI of a virologically successful regimen did not lead to any improvement in the fat abnormalities, despite significant improvement of dyslipidemias and insulin resistance *(96,97)*. On the contrary, recent experience from our group *(98)* and others *(99,100)* indicates that changing NRTIs, from d4T to either abacavir or ZDV, does lead to at least a partial resolution of hyperlactatemia and lipoatrophy, implying a differential action of NRTIs. This does support the in vitro observations mentioned earlier, that d4T is a more potent inhibitor of mtDNA than other currently used NRTIs, and supports the observational studies, which revealed a higher rate of lipodystrophy in subjects treated with d4T- vs other NRTI-containing regimens.

EVIDENCE OF EFFECT OF HIV ITSELF ON MITOCHONDRIA

Several observations link HIV itself to mitochondrial dysfunction. Histological evidence of mitochondrial abnormalities, including red-ragged fibers and abnormal mitochondria with paracrystalline inclusions have been seen in ARV-naive HIV-infected subjects with clinical myopathy *(101,102)*. Further evidence of HIV-related mitochondrial toxicity comes from recent reports of significant decrease in mtDNA, as assessed by the mtDNA-to-nDNA ratio in blood of HIV-infected, ARV-naive subjects compared with HIV-uninfected controls *(57,84)*. In addition to these clinical observations, several in vitro data highlighted the effect of viral protein R (vpr) on mitochondria *(103)*.

MITOCHONDRIAL TOXICITY IN CHILDREN

Hyperlactatemia does occur in the pediatric population (reviewed in ref. *104*). There are several concerning reports regarding HIV-exposed but uninfected infants who developed hyperlactatemia from *in utero* exposure to HAART. One prospective study examined serial lactate levels in 25 infants born to HAART-treated mothers and reported that 92% of infants had elevated lactate levels, with 35% of the cohort demonstrating levels greater than 5 mmol/L *(105)*. Only one infant was symptomatic. All of the infants were exposed to NRTIs. There was no correlation with duration of *in utero* exposure to NRTIs or to other classes of ARVs. Lactate levels were normal in the few mothers who were tested before delivery. The hyperlactatemia resolved in all of the infants by 6 mo of age *(105)*. Another similar study of lactate levels in a group of 20 NRTI-exposed neonates also showed elevated lactate levels in 85% of these infants, none of whom was symptomatic *(106)*. Similar to the first study, lactate levels returned to normal after the first few weeks of life, without apparent consequence on the infants. Although infrequent, as in adult patients, severe mitochondrial dysfunction can occur. The central nervous system seems to be particularly vulnerable. Blanche et al. reported eight cases of hyperlactatemia with neurological and developmental sequelae in perinatally NRTI-exposed, HIV-negative children *(107)*. Similar cases were more recently described from the same French cohort *(108)*. Foster et al. reported three full-term infants who were exposed to NRTIs perinatally and who experienced lactic acidosis and hypoglycemia *(109)*. All three infants recovered. Another unique aspect of lactic acidemia in the pediatric population is its likely effect on growth. Chronic acidemia is well-known to inhibit linear growth in children and adolescents. As in adults, lactate levels should be monitored and therapy adjusted as needed. We should recognize the severe technical difficulties associated with obtaining venous lactate levels in children; the use of a tourniquet and fist clenching are unavoidable in the pediatric population and may lead to false elevations of lactate levels. Therefore, the studies showing asymptomatic transient elevations of venous lactate in newborns should be considered with caution *(105,106)*. Despite the frightening reports of rare mitochondrial dysfunction in NRTI-exposed infants, this risk is still outweighed by the remarkable success in preventing vertical transmission of HIV.

In HIV-infected NRTI-treated children, approx 30% are found to have asymptomatic hyperlactatemia *(110,111)*. Similar to adults, elevated venous lactate levels in HIV-infected children should be interpreted with caution, because of the technical difficulties in adequately collecting venous blood for lactate testing. Longitudinal studies are necessary to determine the prevalence and long-term effects of chronic hyperlactatemia in children.

BONE DISEASE: ANY LINK TO MITOCHONDRIAL TOXICITY?

The prevalence and etiology of decreased bone mineral density (BMD) in HIV-infected patients are not completely known. The advent of decreased BMD in these patients seems to correlate with the introduction of HAART therapy. In a frequently cited study, Patton and colleagues describe a cohort of HIV-infected males with normal BMD that was stable over time before the HAART era *(112)*. The reported prevalence of decreased BMD in HIV-infected adults has been in the range of 22 to 50% of adult patients with osteopenia and 3 to 21% with osteoporosis *(113,114)*. As is true for patients with hyperlactatemia, most HIV-infected patients with decreased BMD are asymptomatic. Fragility fractures in these patients are limited to a few case reports in the literature *(115,116)*, although recent case series of a large number of fragility fractures are a reason for heightened concern regarding the eventual development of these fractures as HIV-1-associated mortality falls *(117)*. The etiology of decreased BMD is unclear and could be a side effect of HAART therapy, although an effect of HIV itself, either directly, or indirectly through cytokines, cannot be ruled out. Indeed, the relationship of specific ARVs, such as PIs, to the bone loss is only speculative and has not been clearly demonstrated *(118,119)*. Some available observations seem to hint at a possible role of NRTIs in bone disease. In a cohort of 221 HIV-infected subjects from Australia, Carr reported on their multivariable analysis that only lower before antiretroviral therapy weight and higher lactate levels were independently linked with decreased BMD *(114)*. For every 1 mmol/L increase in lactate, he reported an odds ratio of 2.39 for developing osteopenia or osteoporosis ($p = 0.002$). This association of hyperlactatemia and bone disease suggests, but does not prove, a role for NRTIs in bone disease. The absence of improvement of BMD after PI withdrawal *(120)* and the significant association found between osteopenia and lipoatrophy (which had been previously linked to mitochondrial dysfunction) support this hypothesis *(121)*. NRTIs can induce bone disease by several possible mechanisms. The chronic acid state created by NRTI-induced hyperlactatemia may lead the bone to act as a buffer and release hydroxyapatite. Osteoporosis has been described as the only clinical manifestation of mtDNA deletions in HIV-uninfected males with osteoporosis, who were otherwise asymptomatic *(122,123)*. These hypotheses are currently awaiting investigations. To date, tenofovir is the only NRTI agent with a proven worse effect on bone than the head-to-head comparator NRTI in a controlled clinical trial *(84)*. In the Gilead 903 trial, bone loss in the lumbar spine and hip was greater in subjects receiving tenofovir plus 3TC plus efavirenz compared with subjects receiving d4T plus 3TC plus efavirenz *(84)*.

Until further investigations clarify the role of NRTI-induced mitochondrial dysfunction on bone density, these remain hypotheses. For now, we have to

deal with a growing concern regarding long-term effects of ARV therapy on bone. Pediatricians, in particular, are very concerned about these bone abnormalities, because children are likely the most vulnerable because of their status as growing organisms and because of their likely longer-term exposure to HAART. We recently reported that approx 74% of our HIV-infected children had decreased BMD *(124)*. Supplementation with calcium and vitamin D failed to improve the bone loss *(124)*.

CONCLUSIONS

NRTIs remain the cornerstone of ARV. It is unclear why some HIV-infected patients had tolerated these medications for more than a decade without any noticeable side effects, whereas others are experiencing distressing and severe progressing toxicities. The future of ARV development should focus on new drugs with more acceptable short- and long-term toxicity profiles. For now, we should focus on early diagnosis and management of the known toxicities, so we may improve the quality of life and long-term outcome of HIV-infected subjects.

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11
Nevirapine

David Asmuth and Richard Pollard

Nevirapine is the first example of a non-nucleoside reverse transcriptase inhibitor (NNRTI) approved for use in HIV-infected patients. Information about efficacy and toxicity of nevirapine in various patient populations has continued to appear in presentations and publications. This has assisted in understanding which patients might benefit and which patients might require frequent monitoring to limit toxicity. Nevirapine is a highly active antiviral compound that is used commonly in various patient populations.

STRUCTURE AND MECHANISM OF ACTION

Nevirapine is a dipyridodiazepinone inhibitor of the HIV-1 reverse transcriptase polymerase and is remarkably specific for the HIV-1 reverse transcriptase enzyme (RT). Nevirapine is synthesized in four steps, starting from 2-chloro-4methyl-3-nitropyridine. This is reduced to 3-amino-2-chloro-4-methylpyridine, which is then condensed with 2-chloronicotinic acid chloride to form the amide. Reaction with cyclopropylamine, followed by cyclization, results in the chemical structure for nevirapine represented in Fig. 1. At high concentrations, nevirapine fails to inhibit the RT of HIV-2, simian immunodeficiency virus, or feline immunodeficiency virus, nor does it inhibit human DNA polymerase-α, -β, -δ, or -γ *(1)*. As a prototypic member of the NNRTI class of antiretroviral agents, nevirapine demonstrates noncompetitive inhibition with respect to deoxyguanosine triphosphate either through allosteric binding to the binary (RT:template–primer) or ternary (RT:template–primer:deoxyguanosine triphosphate) enzyme complex *(2)*, and does not cause chain termination *(3)*. It exhibits a 50% inhibitory concentration (IC_{50}) of 0.084 μ*M* (0.023 μg/mL) in enzyme assays, and an IC_{50} of 0.040 μ*M* (0.011 μg/mL) against wild-type HIV-1 replication in cell culture, where one molecule of inhibitor is sufficient to inactivate one molecule of the RT enzyme, binding at the tyrosine 181 and tyrosine 188 sites of the enzyme *(4)*. These conserved tyrosine residues of the HIV-1 RT subunit lie in a pocket that is defined by two β-sheets composed of amino acid

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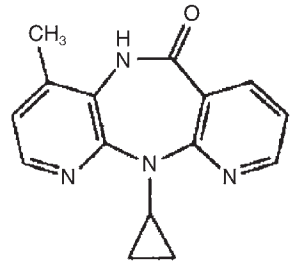

Fig. 1. Chemical structure of nevirapine.

residues 100 to 110 and 180 to 190 *(5,6)*. By binding to this hydrophobic pocket close to the polymerase catalytic site of RT, nevirapine slows the rate of polymerization catalyzed by the enzyme *(7)*; this binding also predicts where RT mutations leading to resistance would occur.

RESISTANCE

Selection for HIV-1 mutants that are resistant to nevirapine occurs rapidly in vitro. After a single passage in the presence of low concentrations of drug, several strains of HIV-1 developed a mutation at tyrosine 181 to cysteine (Y181C) that significantly reduced sensitivity to nevirapine *(8)*. Emergence of resistance mutations selected during therapy with nevirapine alone or in combination with other antiretroviral agents in HIV-1-infected patients can also occur very rapidly, suggesting that nevirapine has a very low genetic barrier to resistance *(9)*. Several studies have evaluated the emergence of RT resistance mutations in the setting of monotherapy *(10–12)*. The HIV Network for Prevention Trials (HIVNET) 012 clinical trial, conducted in Uganda, treated women with a single 200-mg dose of nevirapine at the onset of labor and treated their newborn babies with 2 mg/kg within 72 h of birth to prevent maternal-to-child transmission of HIV *(11)*. RT resistance mutations to nevirapine were found in 18 of 102 women analyzed 8 wk after delivery, despite the fact that they received no subsequent antiretroviral therapy (ART); the mutations were predominantly the K103N mutation (16/18 mutations) *(13)*. In contrast, virus isolated from infants in whom prophylaxis failed contained the Y181C mutation in five of nine RT sequences tested. The extraordinary high viral replication rate in neonates might have accounted for the discordant resistance patterns. These results in HIV-infected pregnant women are contrasted with an analysis of RT gene mutations in 167 virus isolates from 38 subjects treated with nevirapine monotherapy or in combination with zidovudine (ZDV) in ZDV-experienced subjects as part of AIDS Clinical Trials Group (ACTG) protocols 164 and 168 *(14)*. HIV-1 isolates with reduced sensitivities to nevirapine were

detected as early as 1 wk after initiating therapy. In subjects taking monotherapy, initially depressed plasma levels of HIV-1 returned to baseline within 8 wk. Coincident with a rebound in HIV-1 viral load, was the isolation of mutant virus with, preferentially, an RT Y181C mutation. This RT mutation conferred a greater than 100-fold reduction in nevirapine susceptibility. Of the 96 resistant isolates, all but four had an IC_{50} of greater than 0.5 μ*M*, and all but nine had an IC_{50} of greater than 1.0 μ*M* *(14)*. The K103N mutation has been seen more frequently in subjects taking combination ART with nevirapine and ZDV *(15)*. This is believed to be caused by an increased susceptibility to ZDV in viruses carrying the Y181C mutation that are otherwise resistant to ZDV when the T215Y mutation is present *(16)*.

The pattern of resistance mutations that emerge after initiation of nevirapine in ART-experienced (but NNRTI-naive) subjects was studied in ACTG 241 *(17)*. This clinical trial compared response to therapy in severe-to-moderate HIV-advanced subjects (CD4 cell count, ≤350 cells/mm^3) treated with ZDV plus didanosine with and without nevirapine for 48 wk *(18)*. In HIV isolates obtained after 8 wk of therapy ($n = 30$), the most frequent resistance mutations were RT G190A (27%), Y181C (23%), V106A (23%), K103N (20%), and V108I (10%). In isolates obtained at the end of the study ($n = 30$), the most common mutations were different: RT Y181C (50%) and G190A (50%) were followed by K101E (30%), K103N (20%), and Y188L (13%) *(17)*. The first report of interclass cross-resistance has been documented by Baldanti et al., who confirmed a previously unnoticed role of Y181I/C RT changes selected by nevirapine or other NNRTI in determining stavudine resistance *(19)*. To assess the implications of this pattern of resistance mutations seen in ACTG 241 for future salvage regimen options, Gilbert et al. developed a mathematical model to quantitate resistance cost *(20)*. They concluded that the addition of nevirapine in the setting of partially suppressive ART attenuates future effectiveness of NNRTI treatment choices. The principle of assessing the impact of progressive resistance mutations on future treatment choices was also investigated in the setting of nevirapine combined with a protease inhibitor (PI), after failure of a previous PI-containing regimen *(21)*. The authors drew several important conclusions from this extensive genotypic and phenotypic resistance analysis related to nevirapine use. Although most subjects had a baseline nevirapine-susceptible virus, nearly every virus isolated from subjects who failed therapy after 24 wk demonstrated nevirapine resistance. Also consistent with the studies discussed above *(18,19,21)*, the most common mutation after nevirapine plus PI failure was the Y181C substitution. Although there was a close correlation between genotypic and phenotypic resistance with respect to nevirapine, cross-resistance to other NNRTIs was not absolute. In addition, although the presence of the K103N mutation was associated with high-level resistance to

all NNRTIs, the single Y181C mutation did not always translate into phenotypic resistance to efavirenz. Nearly one-third of the nevirapine-resistant isolates carrying this single mutation in the RT gene remained susceptible to efavirenz, and 14% of those viral isolates showed only an intermediate level of resistance to efavirenz and delavirdine. This led the authors, as well as others *(22)*, to propose identifying factors that would favor a response to efavirenz after nevirapine failure. Similarly, mutations at the G190 position have been observed to increase sensitivity to delavirdine by 3- to 300-fold *(23)*. Initiation of nevirapine in the absence of ZDV has favored the emergence of the Y181C mutation at the time of failure, which may preserve efavirenz as a potential component in subsequent regimens *(24)*. Findings such as these have important implications regarding strategies of sequencing antiretroviral regimens, which should be exploited in the design of future clinical trials, despite limited success in exploiting these principles at the present time *(25,26)*.

PHARMACOKINETICS

Nevirapine has excellent bioavailability (>90%) after oral administration in both tablet and liquid form *(27)*. From 24 to 168 h, in healthy volunteers receiving a single oral 200-mg dose, concentrations of nevirapine decline monoexponentially, with an apparent terminal phase half-life of 28.4 ± 8.1 h, an area under the curve (AUC) of 123 μg·h/mL, a peak concentration of 1.8 ± 0.4 μg/mL, and a time-to-peak concentration of 2.7 ± 2.0 h for the tablet form. These pharmacokinetic parameters for the tablet form were not significantly different than for the oral solution *(27)*.

The metabolism and routes of excretion were studied in healthy volunteers who received a 50-mg dose containing 100 μCi of [^{14}C]nevirapine after 4 wk of nevirapine (2 wk at 200 mg nevirapine daily, then 2 wk at 200 mg nevirapine twice daily) *(28)*. A one-compartment model with zero-order input and first-order elimination best fits the nevirapine data. Greater than 90% of the reactivity was recovered from the urine and feces during a 10-d period, with renal excretion accounting for approx 80% and feces for approx 10%. At the time of peak concentration (C_{max}), parent compound represented 75% of the radioactivity in plasma. Excretion of the parent compound in urine represented approx 3% of the dose. The remainder of the radioactivity in the urine was accounted for by the major metabolites of nevirapine; 2-hydroxynevirapine glucuronide (23%), 3-hydroxynevirapine glucuronide (32%), and 12-hydroxynevirapine glucuronide (29%) appeared in the highest quantities *(28)*.

Nevirapine is principally metabolized by members of the cytochrome (CYP) P450 system, CYP3A4 and CYP2B6 *(29)*, but has a low potential to be involved in inhibitory drug interactions (*see* Drug–Drug Interactions, p. 324). The autoinduction of the CYP450 enzyme system, particularly those isoforms

responsible for nevirapine metabolism, is the principle rationale for initiating therapy at a lower dose (200 mg nevirapine daily) for 2 wk followed by increasing the dose to a total of 400 mg nevirapine daily thereafter.

To investigate the bioequivalence of 200 mg nevirapine twice-daily dosing to 400 mg nevirapine once-daily dosing, 21 HIV-1-infected subjects on a long-term 200 mg nevirapine twice-daily dosing schedule were randomized to either continue that schedule or to change to a 400 mg nevirapine once-daily schedule *(30)*. Pharmacokinetic parameters were measured during a 24-h period 2 wk after the change in dosing. The important findings of this study relate first to comparison of the results between the morning and evening dose in the twice-daily schedule and second, to the comparison between the once-daily and twice-daily schedules. The measured parameters were not statistically different for the twice-daily dosing schedule between the morning and evening doses, suggesting that circadian variations in gastric acid secretion or emptying time, regional blood flow, or urinary pH were unlikely to have a significant impact on nevirapine pharmacokinetics. Second, the AUC (101.8 μg·h/mL), time-to-peak concentration (1.54 h), and half-life (21.5 h) were not statistically different between the two dosing schedules. However, the C_{max} (6.69 μg/mL vs 5.74 μg/mL) and the minimum concentration (C_{min}) (2.88 μg/mL vs 3.73 μg/mL) were significantly higher and lower in the cohort of subjects receiving a single-daily vs a twice-daily dose of nevirapine, respectively. These results are consistent with those reported by other investigations of subjects receiving the twice-daily dosing schedule of nevirapine (C_{min}, 3.8 μg/mL; C_{max}, 6.2 μg/mL; and AUC, 109.1 μg·h/mL) *(31)*. Because the C_{min} is well above the in vitro IC_{50} for viral replication (the IC_{50} for nevirapine is 10.6 ng/mL) *(4)*, the lower C_{min} seen in once-daily dosing would not be expected to have a clinically significant impact. Indeed, a clinical trial comparing twice-daily stavudine plus either twice- or once-daily nevirapine plus didanosine *(32)* and a second trial comparing once- or twice-daily nevirapine plus daily didanosine plus twice-daily stavudine *(33)* were equally effective in reducing viral loads and in increasing CD4 T-cell counts.

Several investigators have sought to correlate virological outcomes to measured pharmacokinetic parameters measured in subjects participating in clinical trials. These studies provide a context that gives the information described in the previous paragraph a clinically relevant perspective. In a substudy of ACTG 164, an open-label phase I/II study of 400 mg nevirapine monotherapy once daily, plasma trough levels of nevirapine were correlated with virological response at week 8 of the study *(34)*. Steady-state trough drug levels in responders were significantly higher than those in nonresponders (4.7 μg/mL vs 3.1 μg/mL, respectively; $p = 0.02$). In a substudy of the INCAS trial, random nevirapine levels taken from subjects throughout the study (after the day 14 escalation to

200 mg nevirapine twice daily) were correlated with the decay constant calculated from plasma HIV RNA viral load determinations *(35)*. Only subjects from the triple-combination therapy cohort (didanosine plus ZDV plus nevirapine) were included in this analysis, because very few subjects in the ZDV plus nevirapine cohort experienced a virological response. A statistically significant and positive relationship was found between the decay constant and the median nevirapine concentration (although the Pearson correlation coefficient was only 0.28). Others, however, have observed similar relationships *(36)*. The results of the 2NN study, which will be described subsequently (p. 319), compared once-daily to twice-daily dosing of nevirapine in two of the four cohorts, provide important insights into the clinical and virological implications of dosing strategies.

Penetration of ART into compartments other than plasma has obvious implications for durability of viral suppression, emergence of resistance, and controlling localized effects of HIV infection, such as AIDS-related dementia and HIV encephalopathy in the central nervous system (CNS). An in vitro model using bovine brain microvessel endothelial cells has successfully reproduced distinct morphological and enzymatic properties of the mammalian blood–brain barrier. The blood–brain barrier limits perfusion of macromolecules and cells to the CNS through the formation of continuous tight junctions with minimal endocytotic activity. In a study comparing the permeability of various antiretroviral agents in this model, nevirapine was the most permeable of all of the agents tested, with delavirdine and saquinavir demonstrating extremely low permeability indices and the nucleoside reverse transcriptase inhibitors (NRTIs)—didanosine, ZDV, and stavudine—scoring intermediate *(37)*. In vivo measurements of nevirapine drug levels in the cerebrospinal fluid (CSF) have also been reported *(31,38,39)*. The pharmacokinetic characteristics in serum and CSF for subjects on a multidrug regimen was most extensively studied by van Praag et al., who followed 15 HIV-1-infected men treated with ZDV, lamivudine (3TC), abacavir, nevirapine, and indinavir during a 96-wk period *(31)*. Serial lumbar punctures performed on nine of the subjects revealed CSF median nevirapine levels of 0.9 μg/mL (range, 0.2 – 1.8 μg/mL) at week 8 of therapy that changed minimally over the course of the study. This value represents a CSF-to-serum ratio of 0.24 at the C_{min} that is significantly lower than what would have been predicted based on the 40% unbound serum fraction for nevirapine. Others have reported similar *(38)* or higher fractions, closer to the expected 40% *(40)*. The clinical correlation of these measurements is the change of CSF HIV viral load *(41)* and whether the neurological sequelae attributed to direct HIV-1 infection of the CNS are attenuated in the setting of nevirapine therapy *(42)*. In a study that monitored psychomotor slowing as the parameter for HIV-related CNS disease in 39 subjects receiving nevirapine and 65 subjects receiving efavirenz in combination with two NRTIs, significant

improvements were noted in both groups compared with a control group that received dual nucleoside therapy only ($n = 66$) *(43)*. Although the results did not reach significance, the nevirapine combinations seemed to be more effective than the efavirenz combinations in subjects who were naive to ART.

Antiretroviral penetration into seminal fluid has important implications both from the perspective of risk of transmission to uninfected partners and viral control in sequestered reservoirs that may serve as the source of virus during treatment interruption *(44)*. The pharmacokinetics of nevirapine in seminal fluid has been studied in the setting of HIV-infected subjects participating in clinical trials *(45,46)*. Although the seminal-to-blood plasma drug ratios of approx 0.5 for nevirapine were lower than the other antiretroviral agents assayed (3TC and stavudine, both approx 1.0), concentrations were well above the IC_{50} at all time-points assayed.

TOXICITY

The spectrum and frequency of adverse events associated with nevirapine therapy have been extensively reviewed *(47,48)*. Data from long-term clinical trials evaluated by Pollard et al. indicated that the safety profiles changed in comparison with short-term studies *(47)*. Among adult subjects in long-term studies (time on therapy >6 mo; $n = 487$), 15% of the subjects experienced nevirapine-related adverse events. However, among all subjects taking nevirapine in the pooled data set of the 22 short-term phase I through III clinical trials evaluated ($n = 906$), 52% of the subjects experienced nevirapine-related adverse events.

Rash

Rash, the most frequently observed nevirapine-related adverse event, was reported in 19.9% of the 906-subject database and in 3.7% of adult subjects in long-term studies *(47)*. In long-term trials, the majority of rashes in adults were of mild severity, with an incidence of severe rash, such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), in 0.3% of subjects taking nevirapine. The highest incidence of rash occurs in the first 4 wk of therapy, particularly for the more severe episodes, as demonstrated in Fig. 2 *(47)*. With the exception of abnormal liver function tests (LFTs) (2.7%), the most frequently reported nevirapine-related adverse events among adults in the long-term studies occurred with an incidence of less than 2%, consistently lower than the incidences reported for the phase I to III short-term clinical trials data set. These consisted of nausea, fatigue, fever, headache, and somnolence *(47)*.

The incidence of rash reported by Pollard et al. is similar or slightly lower than other long-term clinical trials that included nevirapine. In a retrospective analysis of the nevirapine compassionate use program in 13 HIV outpatient clinics in the Netherlands, the total incidence of rash was 13.9%, with 6.4%

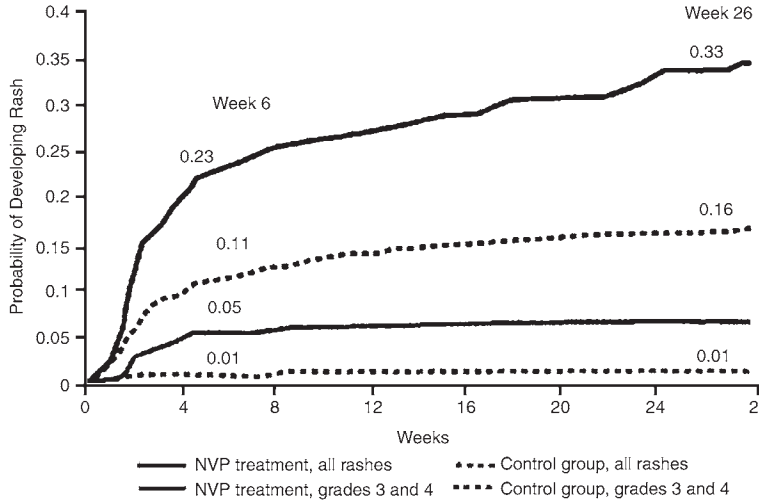

Fig. 2. Time-to-onset of first rash in comparative trials. Patients treated with nevirapine (NVP; $n = 350$) vs controls ($n = 308$). Figures at weeks 6 and 26 represent the probability of experiencing rash at these time-points. (Adapted from ref. *47* with permission from *Excerpta Medica*.)

(12/187) of episodes leading to discontinuation of nevirapine *(49)*. Higher rates of discontinuation of nevirapine in retrospective analysis of nevirapine use in the community have been reported *(50)*. Rash attributable to nevirapine cannot be determined in this highly ART-pretreated population that received other novel antiretroviral agents simultaneously in a salvage regimen. The INCAS trial randomized 151 treatment-naive adults with CD4 T-lymphocyte counts between 200 and 600 cells/mm^3 to receive either ZDV plus nevirapine, ZDV plus didanosine, or all three in combination for 52 wk *(51)*. Treatment-related rash occurred in 22% (22/98) of subjects in the nevirapine-treatment arms vs 6% (3/53) of subjects in the ZDV plus didanosine treatment arm. Rash was described as severe in four (8%) and one (2%) of the cases, respectively. Of note, gastrointestinal complaints were the most frequently identified adverse events for all treatment groups in this study, leading to the discontinuation of ZDV in 20 (13%) subjects, of didanosine in 23 (22%) subjects, and of nevirapine in 10 (10%) of subjects *(51)*. In ACTG 241, 398 nucleoside therapy-experienced adults with CD4 T-lymphocyte counts of at most 350 cells/mm^3 received ZDV and didanosine with or without nevirapine (200 mg nevirapine daily for 2 wk, then 200 mg nevirapine twice daily) for 48 wk *(18)*. Severe rash was reported in 17 (9%) subjects assigned to receive the triple combination and in 3 (2%) subjects assigned to receive the double combination ($p = 0.002$). Rashes graded as severe or potentially life-threatening occurred early in the course of

treatment (median, 4 wk). Of the seven cases of rash considered life-threatening, six occurred in the triple-combination group and all resolved on withdrawal of therapy. The VIRGO study was an open-label study in treatment-naive adults with CD4 T-cell counts of at least 200 cells/mm^3 who received triple-combination ZDV plus didanosine plus nevirapine either as a twice-daily (200 mg) or once-daily (400 mg) dosage for 48 wk *(33)*. Treatment-related rash occurred in 23% (14/60) and 25% (10/40) of subjects receiving twice- and once-daily nevirapine, respectively. The median time-to-rash onset was 12 d (range, 3–26) and only required treatment modification in 9 of the 24 subjects. There were no episodes of TEN or SJS.

Attempts to understand the risk factors for development of rash and strategies for reducing the incidence of this complication have yielded mixed results. In an extensive retrospective cohort study of all patients taking nevirapine during a 5-yr period, women had a sevenfold increase in risk for severe rash and were 3.5 times more likely to discontinue nevirapine therapy as a result *(52)*. Other studies have supported these observations *(53,54)*. The frequency and severity of rash may be related to nevirapine plasma concentrations *(55)*. The need for a dose escalation was recognized early and, when not adhered to, increases the risk of rash. For instance, in the VIRGO study, rash occurrence was significantly increased in subjects who did not adhere to the lead-in dose of nevirapine (200 mg/d nevirapine for 2 wk): 78% (7/9) vs 19% (17/90) of subjects who adhered to the lead-in dose ($p = 0.0001$) *(33)*. Other attempts at reducing the risk of rash with corticosteroid therapy have resulted in conflicting results. A four-arm study comparing standard 2-wk lead-in of nevirapine to a slow dose escalation (100 mg/d increment increases per week during 4 wk), 50 mg prednisone every other day during the first 2 wk, or a combination of the latter two strategies, resulted in diminished rash, from 18.7% using the standard schedule to 11.2%, 8.6%, and 7.7% in the slow-escalation, prednisone, or combination group *(56)*. Conversely, a retrospective study *(54)* and two prospective studies of low-dose prednisone daily during the 2-wk lead-in time for nevirapine *(57,58)* demonstrated that steroid therapy did not decrease and perhaps increased the rates of rash during initial nevirapine therapy. Desensitization algorithms have been applied to patients who developed rash, with promising results *(59)*.

SJS is a life-threatening complication of nevirapine that has been reported sporadically *(60–62)*. Although some had argued that these complications seem to be related to nonadherence to the lead-in dosing and/or failure to discontinue medication with the onset of symptoms *(63)*, a careful analysis of all cases of SJS and TEN reported in a European registry found 18 cases to have occurred in HIV-positive patients (7.3% of the total cases), and 15 of those cases had a recent exposure to nevirapine. All patients had received escalating doses, with 10 cases occurring during the initial 2 wk. A related hypersensitivity condition known as

drug rash with eosinophilia and systemic symptoms, initially associated with various drugs (such as allopurinol, sulfonamides, and aromatic anticonvulsants) has also been described with nevirapine use *(64,65)*. The constellation of findings include a generalized maculopapular rash without mucosal involvement that reveals a superficial dermal leukocytoclastic vasculitis and lichenoid reaction on pathological examination, enlarged lymph nodes, and hepatosplenomegaly with fever. Laboratory evaluations reveal eosinophilia (18%) and marked elevation of LFTs and creatinine kinase determinations (>20 times the upper limit of normal levels [ULN]) *(64)*. The recommendations for management of rash in the setting of nevirapine administration are reproduced in Table 1 *(47)*.

Hepatotoxicity

Hepatic injury during treatment with nevirapine has been more difficult to characterize than rash. Medication-induced transaminase elevations during ART are difficult to distinguish from

1. Fluctuations in underlying or new hepatic disease (infectious or not).
2. Interaction with or uniquely resulting from other medications, especially PIs.
3. Other comorbid conditions, such as alcoholism.

Nevirapine-related hepatitis was infrequently reported during the initial phase I, II, and III trials. Up to the end of 1996, nine cases were reported in 906 subjects enrolled in the trials and taking study medication, most occurring within the first 5 wk after initiation of therapy *(47)*. After 1996, case reports of severe liver damage began to emerge *(66–71)*, culminating in a series of severe adverse events in previously healthy patients receiving nevirapine in the setting of postexposure prophylaxis *(72–74)*.

Several recently conducted cohort studies have delineated the risk factors for hepatotoxicity associated with ART *(48,75–83)*. Martínez et al. followed 610 subjects initiated on a nevirapine-containing regimen for a median duration of 8.7 mo *(77)*. Eighty-two (13.4%) were ART naive when starting therapy, and 46.2 and 8.9% were hepatitis C virus (HCV) and hepatitis B virus coinfected, respectively. Hepatotoxicity was defined as a threefold elevation of transaminase levels above the baseline determination. The Kaplan-Meier estimated incidence of hepatotoxicity at 3, 6, and 12 mo was 3.7%, 9.7%, and 20.1%, respectively. Clinical hepatitis developed in seven (1.1%) subjects, which was reversible with discontinuation of nevirapine. Multivariate analysis identified the duration of previous exposure to ART, HCV coinfection, and higher baseline levels of alanine aminotransferase (ALT) as independent risk factors for hepatotoxicity. Although one-third of subjects were also receiving a PI as a component of their ART regimen, controlling for PI use in the multivariate analysis failed to detect PI as a significant risk factor for hepatotoxicity

Table 1
Rash Management Guidelines[a]

Description	Action with nevirapine
Mild/moderate rash (may include pruritus) Erythema Diffuse erythematous macular or maculopapular cutaneous eruption	Can continue dosing without interruption. If rash occurs during lead-in period, dose should not be escalated until rash resolves. If nevirapine is interrupted for >7 d, reintroduce with 200 mg/d lead-in
Urticaria	As above; however, if nevirapine is interrupted, it should not be reintroduced
Any rash with associated constitutional findings, such as: Fever >39°C Blistering Oral lesions Conjunctivitis Facial edema Myalgia/arthralgia General malaise Severe elevation of LFT levels Evidence of acute organ dysfunction, such as hepatitis, granulocytopenia, eosinophilia, or renal dysfunction	Permanent discontinuation; no reintroduction
Severe rash Extensive erythematous or maculopapular rash or moist desquamation Angioedema Serum sickness-like reactions SJS TEN	Immediate discontinuation; no reintroduction

[a]Adapted from ref. *47* with permission from *Excerpta Medica*

(77). Sulkowski et al. observed similar rates of hepatotoxicity with nevirapine use *(78)*. During a 5-yr period from 1996 to 2001, 568 subjects who were prescribed new NNRTI-containing ARTs (nevirapine, 256 subjects; and efavirenz, 312 subjects) were studied to determine the risk factors for severe hepatotoxicity associated with NNRTI use. To avoid selection bias favoring the inclusion of hepatitis virus coinfected subjects in the definition of treatment-induced hepatitis, subjects with normal transaminases at baseline were graded by eleva-

tions above the ULN, and subjects with elevated transaminases at baseline were graded by fold changes above the baseline determination. After initiation of nevirapine, transaminase levels remained less than 1.25 times ULN, or the same as their pretreatment level in only 20% of subjects. Severe (>2.5 × ULN or baseline levels) hepatotoxicity was observed in 15.6% (40/256) of subjects receiving nevirapine. Dividing those cases by PI use reveals a frequency of 16.9% (34/201) in subjects also treated with a PI, vs 10.9% (6/55) in those who received only NRTIs in combination with the nevirapine. Severe hepatotoxicity was observed in 19.3% (23/119) of HCV-infected and in 12.4% (17/137) of HCV-uninfected subjects. The highest incidence of severe hepatotoxicity was observed among HCV-infected subjects receiving nevirapine in combination with a PI (19.6 cases per 100 persons exposed), similar to the rate in HCV-infected subjects receiving efavirenz in combination with a PI (20.9 cases per 100 persons exposed), even though the duration of nevirapine therapy was somewhat longer than efavirenz therapy. The median duration of treatment before the detection of severe hepatotoxicity was 137 d (interquartile range, 49–305 d), with less than one-third of episodes detected within the first 12 wk. Nevirapine-related hypersensitivity reaction, including rash, fever, and eosinophilia, was observed in one male patient with severe hepatotoxicity 2 wk after initiating therapy. In multivariate analysis, hepatitis virus coinfection (relative risk [RR] = 2.1), nevirapine use (RR = 1.92), concurrent PI therapy (RR = 2.19), and an increase in CD4 T-cell counts greater than 50 cells/mm^3 during therapy (RR = 1.95) were independently associated with the development of severe hepatotoxicity. The authors concluded that because 68% of nevirapine-associated severe hepatotoxicity in their data set were observed after the first 12 wk of therapy, safety monitoring of liver enzymes should continue throughout the treatment period *(78)*. However, a significant criticism of this report notes that hepatotoxicity was characterized as severe without reference to clinical symptoms. In addition, when others have conducted similar retrospective surveys of hepatic injury in the setting of NNRTI therapy, no differences are seen between cohorts *(80)*. Stern et al. reviewed a larger data set consisting of all Boehringer Ingelheim-conducted trials plus other large cohort trials to better understand the relationship between nevirapine and hepatotoxicity *(48)*. The two baseline characteristics associated with a fivefold elevation in transaminase levels on nevirapine therapy were a pretreatment ALT or aspartate aminotransferase (AST) level greater than 2.5 times the ULN (RR = 3.2; $p < 0.01$) and hepatitis B virus coinfection (RR = 3.9; $p < 0.01$). Baseline CD4 T-cell counts, female sex, race, and HCV coinfection were not found to be consistent risk factors for subsequent severe hepatitis. Also, in contrast to other studies cited above *(77–78)*, the authors observed that after the initial 12-wk period, incidence of fivefold elevations in transaminase levels were common to all

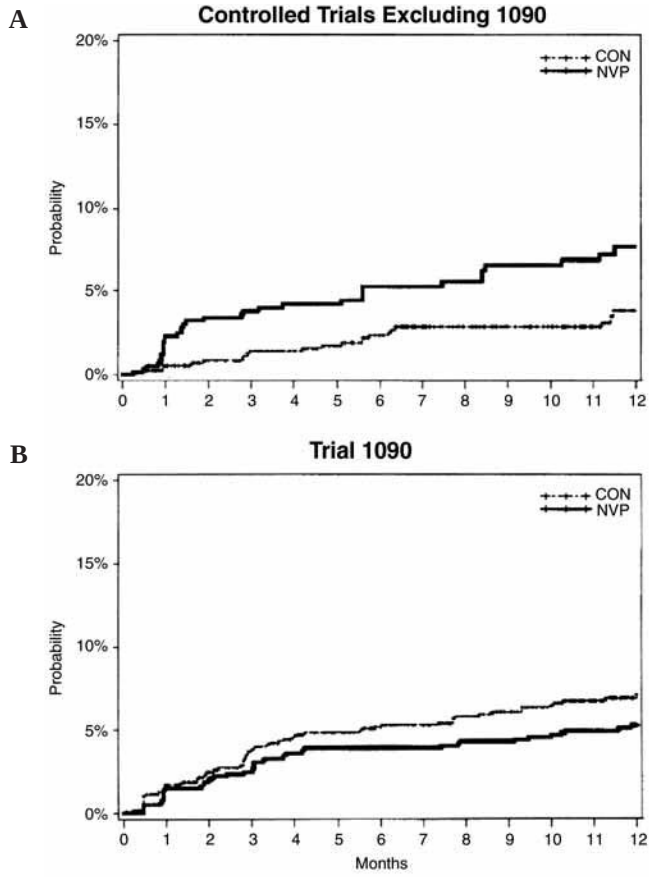

Fig. 3. Development of ALT/AST greater than five times ULN in nevirapine and placebo controls. (**A**) is a Kaplan-Meier curve from initiation of therapy, and (**B**) reflects only those events occurring after 12 wk of therapy. CON, control; NVP, nevirapine. (Adapted with permission from ref. *48*.)

ARV regimens and were not nevirapine related (Fig. 3) *(48)*. Conclusions drawn from this analysis include:

1. The risk of nevirapine-associated hepatic events is greatest during the first 6 wk of treatment.
2. Increased clinical and laboratory surveillance for patients with hepatitis virus coinfection and/or elevated transaminase measurements at baseline is warranted.
3. Patients with symptomatic hepatic events should be permanently discontinued from nevirapine therapy.
4. Nevirapine therapy should be interrupted for asymptomatic transaminase elevations, evaluated for new or underlying liver diseases and reintroduced to nevirapine on a case-by-case basis, with close clinical and laboratory monitoring.

At least two possible mechanisms for nevirapine-related hepatoxicity are consistent with the findings of these studies. The first is an immune-mediated mechanism that occurs in conjunction with dermatological manifestations a few days to weeks after beginning nevirapine-containing regimens. This hypersensitivity reaction seems to occur more frequently in patients with healthy immune systems or in HIV-1-infected patients with high CD4 T-lymphocyte counts. The occurrence of fulminant hepatitis in patients receiving nevirapine in the setting of postexposure prophylaxis is likely mediated by this mechanism *(74)*. A second mechanism has a delayed onset and may represent an intrinsic toxic effect on the hepatocytes. Adverse events by this pathway would be expected to occur in patients with underlying liver disease who perhaps are initiating therapy with baseline abnormal transaminase levels. Hepatitis may occur in relation to accumulating drug plasma levels by this explanation, as some authors have suggested *(84,85)*. However, metabolism of the parent compound itself was not found to be affected by progressive levels of hepatic insufficiency *(86)*.

CLINICAL TRIALS

Several reviews have summarized the clinical experience and application of nevirapine for the treatment of HIV infection *(9,87,88)*. Nevirapine has been tested in clinical trials in three different settings:

1. As first-line therapy.
2. As salvage therapy.
3. As an alternative to PI in virologically suppressed patients experiencing PI-related side effects.

First-Line Therapy

Nevirapine-containing trials conducted in patients who were naive to ART can be further divided into three categories:

1. Early trials that compared dual nucleoside regimens with and without nevirapine *(18,51,89)* (ACTG 241 trial is unique to the trials reported in this section in that subjects were not ART naive at baseline).
2. PI-sparing regimens *(32,33,90–92)*.
3. Trials that compared PI-sparing with PI-containing regimens *(93–95)*.

Several reports describing virological and immunological results of the use of nevirapine in nonrandomized clinical trials have also been instructive *(49,96–100)*.

The meta-analysis of the INCAS and the ISS047 trials performed by Raboud et al. provides an extension of the benefit of nevirapine when added to a dual nucleoside regimen in ART-naive subjects over a wide range of CD4 T-lymphocyte counts at baseline *(101)*. Combining the studies, there were 83 subjects randomized to triple ART. However, the proportions of subjects in each of the

two arms of the analysis (baseline HIV RNA plasma viral load [pVL] ≤ 100,000 vs ≥ 100,000 copies/mL) were not reported. Subjects with a higher baseline pVL were less likely to achieve pVL of at most 20 copies/mL during the trials ($p = 0.02$, $p = 0.01$, and $p < 0.01$ at weeks 8, 40, and 48, respectively) and were less likely to achieve pVL of at most 400 copies/mL at week 48 ($p = 0.01$).

Attempts by others to correlate the likelihood of achieving an undetectable pVL based on baseline high or low pVL while treated with nevirapine triple-combination therapy do not support these findings *(93,97,102–104)*. Interestingly, in a separate analysis of the INCAS data set, the same authors noted that (after controlling for treatment assignment and whether or not a participant's pVL nadir was above or below 20 copies/mL) baseline pVL, baseline CD4 T-cell count, and compliance were not associated with virological failure *(105)*. Further, when subjects in the ISS047 trial randomized to triple-combination therapy were dichotomized according to baseline pVL with a break point of 250,000 copies/mL, no significant differences were observed between the two viral load groups in terms of proportion of subjects with HIV RNA levels of fewer than 400 copies/mL, both at 24 and at 48 wk *(89)*. Raffi et al. assessed the efficacy of six nevirapine-containing, PI-sparing studies in ART-naive subjects with respect to baseline pVL *(102)*. Prospective and retrospective studies presented at international conferences between 1998 and October 2000 were included in the analysis sample. Their composite analysis, encompassing a total of 416 participants, concluded that baseline viral load does not affect virological outcome. In a meta-analysis of 30 published and presented studies to investigate the relationship between viral load suppression and baseline pVL, as well as between pVL suppression and baseline CD4 T-cell count, Skowron et al. concluded that baseline CD4 T-cell count was a better predictor of virological suppression induced by triple-combination therapy than was baseline pVL *(104)*. The same pattern was seen in a subanalysis of trials of nevirapine-containing therapy that identified the baseline factors associated with successful virological suppression (baseline CD4 T-cell count, $p = 0.014$ at 6 mo; baseline viral load, $p = 0.415$) *(104)*. Ultimately, only prospective clinical trials designed to compare nevirapine- vs PI-containing triple-combination therapy regimens enrolling subjects with a range of baseline viral load measurements, such as the Atlantic and Guardiola et al. studies described next, can address the important clinical question broached by these retrospective, *ad hoc*, and meta-analysis investigations. In addition, studies involving efavirenz have shown equal to superior activity to protease-containing regimens and in subjects with higher viral loads suggesting that active NNRTI therapy can be successful in this setting *(106)*.

The Atlantic study was a large, open-label trial comparing nevirapine, indinavir, and 3TC, each combined with didanosine plus d4T in 298 ART-naive subjects with CD4 T-lymphocyte counts of at least 200 cells/mm^3 at entry *(94)*.

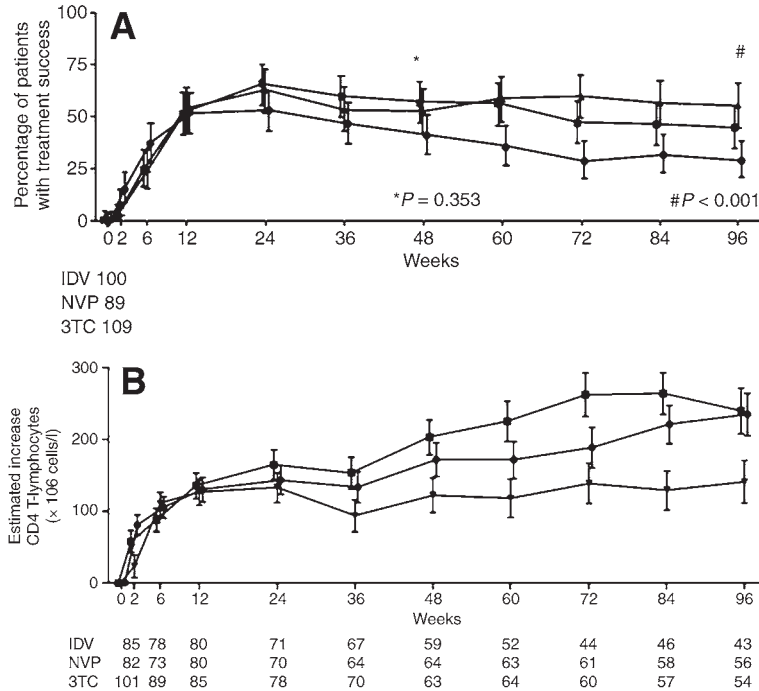

Fig. 4. The results of the Atlantic study. Percentage of subjects with a treatment success. (**A**) HIV RNA level of fewer than 50 copies/mL, intention-to-treat population. Treatment regimens: didanosine plus stavudine plus indinavir (IND) (■); didanosine plus stavudine plus nevirapine (NVP) (▲); didanosine plus stavudine plus 3TC (●); results given as mean ± 95% confidence interval. (**B**) Estimated mean increase (SE) in CD4 T lymphocytes. Treatment regimens: didanosine plus stavudine plus IDV (■); didanosine plus stavudine plus NVP (▼); didanosine plus stavudine plus 3TC (●); $p = 0.103$ for effect of study arm; $p = 0.012$ for interaction of study arm and time. (Adapted with permission from ref. *94*.)

All three arms were well-tolerated. Median baseline CD4 T-cell count was 408 cells/mm^3 (interquartile range, 322–536 cells/mm^3) and pVL was 4.36 $\log_{10}$ copies/mL for all subjects, with no differences between cohorts. The fraction of subjects achieving an undetectable pVL, to at most 50 copies/mL, in an intention-to-treat analysis was 0.55, 0.44, and 0.28 for the nevirapine-, indinavir-, and 3TC-containing arms, respectively ($p \leq 0.001$ for the 3TC arm) (Fig. 4A). By an unexplained mechanism, the nevirapine arm experienced a statistically significant inferior rise in CD4 T-cell counts by study's end of approx 100 cells/mm^3 (Fig. 4B). Of note, there were no significant differences in rate of serious adverse events between the groups. Similar virological results were obtained in a trial comparing indinavir vs nevirapine, each in combina-

tion with d4T and didanosine in an open-label trial of ARV-naive subjects *(93)*. Median entry HIV RNA pVL and CD4 T-cell count were 5.2 $\log_{10}$ copies/mL and 5.4 $\log_{10}$ copies/mL; and 370 cells/mm^3 and 337 cells/mm^3 for the nevirapine ($n = 26$) and the indinavir ($n = 24$) arms, respectively. Based on an intention-to-treat analysis, the fraction of subjects achieving an undetectable pVL, to at most 50 copies/mL, was 0.50 for both groups. Treatment discontinuations because of medication toxicities were similarly low in both groups. These studies taken in the context of the results of the clinical trials just discussed support the continued investigation of PI-sparing regimens as first-line therapies for the treatment of HIV infection.

Before the 2NN study, there were limited data regarding the use of dual NNRTIs *(107,108)*. These nonrandomized studies suggest that nevirapine and efavirenz in combination with an NRTI is safe, well-tolerated, and effective in subjects who are naive or experienced to ART. The 2NN study was a four-arm randomized trial that included a dual NNRTI cohort with efavirenz dosing at 800 mg/d *(109)*. Results of the 1216 ART-naive subjects randomized to either 400 mg nevirapine once daily, 200 mg nevirapine twice daily, 600 mg efavirenz once daily, or once daily combination of 400 mg nevirapine plus 800 mg efavirenz, with a backbone regimen of d4T plus 3TC were recently published *(110)*. The four cohorts were well-matched, with a mean CD4 T-cell count of 190 cells/mm^3 (range, 70 – 330 cells/mm^3) and plasma HIV RNA of 4.7 $\log_{10}$ copies/mL (range, 4.4 to 5.5 $\log_{10}$ copies/mL) at baseline. The rates of having a previous AIDS-defining illness (Centers for Disease Control Class C), a positive hepatitis B surface antigen result, and hepatitis C seropositive rates were 21%, 5.3%, and 9.5%, respectively, and were evenly matched across groups. All analyses were performed after 48 wk of therapy on the intention-to-treat population. The percentage of subjects achieving undetectable pVL (<50 copies/mL HIV RNA) was 70% in both daily NNRTI arms, and was slightly lower in the other two arms ($p > 0.05$ in pair-wise analysis) (Table 2) *(110)*. Overall, treatment failure was similar among the single NNRTI arms, but was higher in the combination nevirapine plus efavirenz arm, mainly because of more treatment discontinuations in this arm. The incidence of clinical adverse events did not differ significantly between the single NNRTI arms. Only the incidence in the liver-associated laboratory adverse events was significantly different between the arms, with the highest incidence in the once-daily nevirapine arm. The virological and immunological efficacy was comparable among all four arms. The authors concluded that nevirapine and efavirenz demonstrated equivalent potency, as did the once-daily vs twice-daily administration of nevirapine. Combination NNRTI therapy was inferior to the other strategies because of increased toxicity in this treatment-naive cohort of subjects.

Table 2
2NN Treatment Results After 48 Wk on Study by Intention-to-Treat Analysis

Treatment arm	NVP (od) A $n = 220$	NVP (bd) B $n = 387$	EFV C $n = 400$	NVP+EFV D $n = 209$	A vs C	B vs C	A vs D	C vs D
Treatment failure (%)[a]	43.6	43.7	38.3	53.1	0.19	0.12	0.05	<0.001
pVL < 50 copies/mL (%)	70	65.4	70	62.7	1	0.17	0.11	0.07
CD4 T-cell increase (cells/mm^3)	170	160	160	150	0.49	0.74	0.91	0.71
Clinical AE (%)[b]	27.7	27.1	22.3	35.4	0.13	0.11	0.09	<0.001
Liver-associated lab AE (%)[c]	13.2	7.8	4.5	8.6	<0.001	0.06	0.13	0.04
Other lab AE (%)[d]	8.2	12.9	8.8	9.6	0.81	0.06	0.61	0.74

NVP, nevirapine; od, once daily; bd, twice daily; EFV, efavirenz; lab, laboratory value
[a]Treatment failure defined as a less than 1 $\log_{10}$ copies/mL decline in HIV RNA pVL in first 12 wk, two consecutive pVL levels greater than 50 copies/mL from week 24 onwards, a new AIDS-defining illness or death, or a change of allocated treatment
[b]Percentage of patients with at least one grade 3 or 4 adverse events (AE)
[c]Grade 3 or 4 elevations in AST, ALT, or total bilirubin levels
[d]Excluding isolated (-glutamic transferase elevations
Adapted from ref. *151*

Salvage Therapy

The role of nevirapine in salvage therapy has been investigated in the setting of randomized *(111–115)* and nonrandomized *(116–118)* clinical trials and in retrospective chart reviews *(49,96,99,119)*. Nevirapine in combination with nelfinavir and two NRTIs provided a significant advantage over nelfinavir plus two NRTIs alone in subjects failing previous PI-containing regimens (pVL < 200 copies/mL in 52% vs 22% of subjects, respectively), without any difference between the two groups in terms of immunological response at the end of the 36-wk study *(113)*. ACTG 373 was designed to determine the efficacy and safety of nevirapine, indinavir, d4T, and 3TC as a salvage regimen in the setting of amprenavir failure *(118)*. Intention-to-treat analysis at week 48 demonstrated 59% of subjects with a pVL of at most 500 copies/mL. The 56 subjects in this study were highly selected for minimal previous ART exposure, with many involved in an amprenavir monotherapy trial. Deeks et al. randomized 20 subjects failing an indinavir- or ritonavir-containing treatment regimen to receive nelfinavir, saquinavir, abacavir, and either another NRTI or nevirapine

(111). The median reduction in pVL was 0.39 $\log_{10}$ copies/mL vs 2.67 $\log_{10}$ copies/mL in the NRTI vs the nevirapine group, respectively, at week 24. By week 36 of therapy, the six subjects with undetectable pVL were all in the nevirapine group. Baseline phenotypic drug susceptibility was strongly correlated with outcome in both treatment arms. These findings underscore the hypothesis that successful salvage therapy depends on limited evolution of resistant strains and the need for at least two new agents to which the subjects' HIV strain are sensitive as part of the salvage regimen *(120,121)*. Benson et al. reported 70 subjects failing their first triple-combination ART regimen (pVL > 1000 and < 100,000 copies/mL) with minimal previous NRTI exposure in a 48-wk study *(115)*. Subjects were randomized to substitute only the PI with two dosing levels of lopinavir/ritonavir at study entry. NRTIs were switched and nevirapine was added 2 wk later. At week 48, 60% of subjects receiving treatment had pVL levels of fewer than 50 copies/mL by intent-to-treat analysis. Mean CD4 T-cell counts increased by 125 cells/μL. Baseline phenotypic susceptibility assay results demonstrated sensitivity to only one of the new agents in one-third of the subjects, using an assay which was likely to underestimate the in vivo activity of lopinavir/ritonavir. These studies suggest that the use of nevirapine in the setting of salvage HIV treatment is successful when patients are naive to NNRTIs and at least one other agent to which the patients' isolate is sensitive is contained in the new regimen. Potential pharmacokinetic interactions between nevirapine and the other ART medications must also be taken into consideration beyond baseline HIV-1 isolate sensitivity to each of the agents when devising a new regimen in this setting. Pharmacokinetic interactions may be particularly important in the setting of reduced susceptibility. This issue will be pursued in more detail below *see* Drug–Drug Interactions, p.324.

Switch From PIs

Carr and colleagues were among the first to report changes in fat distribution and metabolism in patients receiving PI ART *(122)*. They described a syndrome of peripheral lipodystrophy, hyperlipidemia, and insulin resistance that seemed to be associated with duration of PI therapy but was of uncertain etiology. Extensive efforts, summarized in several reviews *(123,124)* and in Chapters 12 and 14, have revealed a strong correlation with PI therapy, NRTI therapy (especially d4T; refs. *125* and *126*), and other unknown risk factors related to HIV infection itself. Much of the difficulty studying this syndrome and, more importantly, devising strategies to correct the consequent abnormalities, has been in developing a case definition for what now seems to be a mixture of several distinct, and potentially overlapping syndromes *(127)*. Several approaches have been used to correct these metabolic abnormalities associated with long-term therapy using antiretroviral agents, including glucose- or lipid-lowering

agents, diet modification, hormonal therapy, and PI-switch strategies, wherein the potentially offending PI is substituted with an NNRTI. The latter has met with the most, albeit occasionally mixed, success *(128–138)*.

Despite subjects receiving d4T in all three arms, analysis of the lipid profiles of subjects participating on the Atlantic study provides evidence that nevirapine is unlikely to contribute significantly to the lipid abnormalities seen with other antiretroviral agents *(139)*. At week 24, increases in high-density lipoprotein (HDL) cholesterol (HDL-c) (49%), apolipoprotein A1 (19%), lipoprotein A1 (38%), and HDL particle size (3%) were observed in the nevirapine arm. Low-density lipoprotein cholesterol increased significantly both in the nevirapine and indinavir arms, but only in the nevirapine arm was this offset by a significant reduction (14%) in total cholesterol (TC)-to-HDL-c ratio. In a multivariate linear regression model adjusting for baseline and on-treatment CD4 T-cell and HIV RNA levels, randomization to the nevirapine arm was significantly correlated with favorable changes in HDL-c and other HDL-related parameters *(94)*. These differences persisted through 96 wk of follow-up. Similarly, a subanalysis of the recently completed 2NN study previously described (p. 319) demonstrated the favorable impact on the lipid profile in subjects receiving nevirapine in comparison with those receiving efavirenz, again with all subjects receiving stavudine in the backbone regimen (Table 3) *(140)*. Subjects receiving nevirapine, compared with efavirenz, had significantly larger increases in HDL-c ($p < 0.001$), a larger decrease in TC:HDL-c ($p < 0.001$) and a smaller increase in triglycerides ($p = 0.010$). Men had a smaller decrease in the TC-to-HDL-c ratio because of a smaller increase in HDL-c compared with women, and subjects with a body mass index greater than 25 had a significantly smaller decrease in TC-to-HDL-c ratio compared with patients with a body mass index less than 25, because of a larger increase in TC. The changes in HDL-c and the TC-to-HDL-c ratio were not associated with elevations in liver transaminase levels or virological outcome at week 48. This antiatherogenic lipid profile is in sharp contrast to that reported for patients receiving PI-containing triple-combination ART and experiencing the dyslipidemia syndrome *(127)*.

Ruiz et al. randomized 106 subjects maximally suppressed on a PI-containing regimen and diagnosed with lipodystrophy as initially defined by Carr et al. *(122)* either to replace the PI with nevirapine or to continue the PI in a 48-wk open-label trial *(132)*. Overall, a significant decrease in fasting TC (mean, 228 ± 48 mg/dL to 207 ± 40 mg/dL) and triglyceride (mean, 270 ± 180 mg/dL to 217 ± 137 mg/dL) levels occurred in the nevirapine substitution arm at week 48 compared with baseline ($p < 0.05$), whereas no changes were seen in the continued PI therapy group (mean, 222 ± 50 mg/dL to 220 ± 50 mg/dL; and mean, 285 ± 198 mg/dL to 270 ± 121 mg/dL, respectively). No significant changes in anthropometric or body-shape measurements were found after

Table 3
Change in Plasma Lipid Concentrations During 48 Wk in 2NN Study

	Nevirapine		Efavirenz		Nevirapine + Efavirenz	
	Baseline	Change[b]	Baseline	Change[b]	Baseline	Change[b]
Triglycerides (mg/dL)[a]	134.5	10.6[c]	126.5	32.7	125.7	38.9
TC (mg/dL)	154.4	37.8	154.4	43.2	156.8	53.7
LDL-c (mg/dL)	90.7	22.0	91.5	27.0	94.6	29.3
HDL-c (mg/dL)	38.6	14.3	38.6	9.3	37.5	15.8
TC:HDL-c ratio	4.29	–0.36	4.37	0.04[NS]	4.53	–0.17[c]

N.B., significant rises from baseline in total TC, low-density lipoprotein cholesterol (LDL-c), and HDL-c were observed within each of the treatment arms

[a]Measurements in mmol/L were converted to mg/dL

[b]Absolute change, adjusted for baseline

[c]Not significant

Adapted from ref. *151*

48 wk, with the exception of a significant decrease in abdominal skinfold in the nevirapine substitution arm ($p = 0.004$). Virological and immunological parameters were similar between the two groups at the end of the study. In an observation noted by authors of each study using this strategy, subjects in the nevirapine substitution arm reported a better quality of life index than those continuing the PI, largely because of simplicity of the new drug regimen ($p < 0.001$). This finding of significantly improved quality of life indices is consistent in other studies reporting this parameter *(141,142)*.

The risk of losing virological suppression after substituting nevirapine for the PI in a triple-combination therapy regimen is low *(143)*. For instance, among 138 subjects randomized to substitute nevirapine or continue the PI in a 3:1 ratio, a rebound in pVL occurred in 11% of subjects during the first 6 mo after replacing the PI by nevirapine, whereas it appeared in 29% of those who remained on PI therapy *(129)*. Treatment failure was related to lack of adherence in 90% of subjects on PI, but only in 22% of those receiving nevirapine. The PIILR study group substituted nevirapine, abacavir, hydroxyurea, and adefovir for the PI in maximally suppressed subjects who were naive to all four agents ($n = 49$) or continuing previous therapy ($n = 32$) in a 3:2 randomization ratio *(131)*. The majority of subjects discontinued the adefovir (96%) and hydroxyurea (59%) by week 48. The proportions of subjects with pVL of at most 50 copies/mL at 24 wk in the switch and continue groups were 98% and 83%, respectively ($p = 0.061$). Masquelier et al. prospectively followed 34 subjects who were virologically maximally suppressed on a triple-ART regimen including a PI, and substituted nevirapine for the PI *(133)*. Previous exposure to single- or dual-nucleoside

therapy was present in 12 subjects, whereas 22 subjects were naive to all ART at the time the triple-combination regimen was initiated. After a median follow-up of 40 wk, no subject in the naive group, vs 41% of the experienced group, developed virological failure after the substitution of nevirapine ($p = 0.003$). These findings both support the safety of nevirapine switch studies and add an important caveat of caution that must be observed when substituting nevirapine alone in heavily ART-experienced patients.

DRUG–DRUG INTERACTIONS

Because nevirapine is metabolized by the CYP450 system, and activates both the CYP2B6 and the CYP3A4 isoforms *(29)*, interactions with other antiretroviral agents or medications commonly used in HIV-1-seropositive patients that are also metabolized by the CYP450 system could have important implications for treatment efficacy and adverse drug reactions. The antiretroviral agents that have been studied for their interactions with nevirapine are saquinavir *(144)*, nelfinavir *(145,146)*, indinavir *(147)*, lopiramide *(148)*, and efavirenz *(109)*. Recommendations regarding dosing modifications summarized in Table 4 are extracted from the in-text references and the Centers for Disease Control HIV Treatment Guidelines *(149)*.

Nelfinavir is metabolized by the CYP3A4 isoform and acts as an inhibitor of this enzyme. Merry et al. performed pharmacokinetic measurements on seven subjects with advanced HIV disease receiving nelfinavir who had nevirapine added to their regimen to determine the interaction between these two agents *(145)*. In the presence of nevirapine, there was a significant decrease in the AUC from 0 to 8 h ($AUC_{0–8h}$) of nelfinavir, from 23.4 to 11.6 μg·h/mL ($p = 0.016$). This represented a greater than 50% reduction in plasma levels. C_{max} was similarly reduced during treatment with nevirapine, from 4.4 to 2.5 μg/mL ($p = 0.047$). The plasma levels of nevirapine reported in this study were similar to those reported in previous studies. The authors expressed concern that the dosage of nelfinavir may need to be increased when used in combination with nevirapine. This position has been challenged by others, and is not currently part of routine practice. Because both nelfinavir and nevirapine are able to induce their own metabolism, pharmacokinetic determinations performed in 23 subjects on day 36, after both agents had reached steady-state levels, did not demonstrate a significant difference in the nelfinavir plasma $AUC_{0–8h}$ in the absence vs presence of nevirapine (19.7 ± 7.5 vs 19.1 ± 8.2 μg·h/mL, respectively) *(146)*. These findings are consistent with others in which the interaction of nevirapine with a PI was examined. Whereas reductions in $AUC_{0–8h}$ and C_{max} were measured for the PI in the presence of nevirapine, these reductions were not thought to be clinically significant and, therefore, no dose adjustments were recommended *(144,147,148)*.

Considerable interest has emerged for the combination of nevirapine with efavirenz as the backbone for a salvage regimen after nonrandomized studies, with promising results *(107,108,150)*. Because both are metabolized by, and influence, the activity of CYP450 isoenzymes as an autoinducible enzymatic process, it is not possible to predict how the steady-state pharmacokinetics will be affected when both agents are used together. Veldkamp et al. *(109)* determined the pharmacokinetics of efavirenz in 14 subjects on a stable triple-combination regimen that included efavirenz before and 4 wk after nevirapine was added. PI use was an exclusion for participation in the trial. Exposure to efavirenz, as measured by the $AUC_{0–24\ h}$, was decreased by 22% when nevirapine was added (p = 0.001). The median decreases in C_{max} and C_{min} were 17% (p = 0.048) and 36% (p = 0.001), respectively. Exposure to nevirapine seemed unchanged when used in combination with efavirenz, based on comparison to historical controls. No rash or significant elevations in transaminases were observed during the 4-wk study. Randomized trials would be needed to determine which dose of efavirenz would be needed to result in the same exposure in the absence of nevirapine. As noted previously (p. 319), the 2NN study used an increased dose of efavirenz (800 mg daily) when it was combined with nevirapine *(151)*.

Methadone is metabolized by the CYP3A4 isoform of CYP450, and withdrawal symptoms (i.e., perspiration, agitation, sneezing, diarrhea, leg cramps, dilatation or constriction of pupils, rhinorrhea, and yawning) have been described for patients taking methadone who initiate nevirapine therapy *(152,153)*. To more carefully characterize the pharmacokinetic effect of nevirapine on methadone in patients receiving methadone maintenance, Clarke and colleagues measured methadone pharmacokinetic parameters on eight subjects initiating therapy on day 0 and day 14 of 200 mg nevirapine daily *(154)*. All subjects were HCV-antibody positive, four were men, and none of the subjects was receiving a PI or other agents that use the CYP450 enzymes for metabolism. Of the eight subjects, six complained of symptoms of methadone withdrawal from days 8 to 10 onward, and required a mean increase in methadone dose of 16%. The mean $AUC_{0–24h}$ for methadone was decreased from 12,024 ng·h/mL to 5713 ng·h/mL in the presence of nevirapine (p = 0.0052). A 36% reduction in C_{max} was also seen in the presence of nevirapine from 676 to 435 ng/mL (p = 0.066) (Fig. 5). The authors recommend frequent monitoring of patients participating in methadone maintenance programs initiated on nevirapine, with the expectation that methadone doses may need to be increased.

Other medications whose interaction with nevirapine has been studied are oral contraceptive therapy *(155)*, rifampin *(156)*, rifabutin *(157,158)*, ketoconazole *(159)*, clarithromycin *(160)*, warfarin *(161)*, and St. John's Wort *(162)*. Other herbal remedies that significantly affect CYP450 activity and, thus, would be expected to result in interactions with nevirapine include milk thistle,

Table 4
Drug–Drug Interactions Between Nevirapine and Common Agents Used in the Treatment of HIV-Infected Patients

Medication	Interaction with nevirapine	Recommendations for dosage modification[a]	Ref.
Saquinavir	Saquinavir $AUC_{12–24h}$ and C_{max} reduction by 27% and 29%, respectively	No recommendations available at present	*144*
Nelfinavir	No significant changes in PK for either agent	Standard doses	*146*
Indinavir	Indinavir AUC, C_{max}, and C_{min} reduced by 27.4%, 11%, and 47.5%, respectively	Increase dose of indinavir to 1000 mg every 8 h	*147*
Lopinavir	Lopinavir C_{min} reduction by 55%	Consider increasing lopinavir to 533/133 mg every 12 h	*148*
Efavirenz	Efavirenz $AUC_{0–24h}$, C_{max}, and C_{min} reduced by 22%, 17%, and 36%, respectively	No recommendations at present; consider increasing efavirenz to 800 mg daily	*109*
Methadone	Methadone $AUC_{0–24\ hr}$ reduction >50%	Monitor closely and increase methadone as indicated	*154*
OCP	Ethinyl estradiol/norethindrone median reduction of AUC_{∞} of 29%	OCP should not be the primary method of birth control	*155*
Rifampine	Nevirapine $AUC_{0–12h}$ and C_{max} reductions of 31% and 21%, respectively No significant change in C_{min}	Because C_{min} levels are $40 \times IC_{50}$, no need to increase nevirapine dosage	*156*

Rifabutin	Slight enhancement of NVP clearance	No need for dose adjustment	*157*
Ketoconazole	Ketoconazole reduction in AUC of 62.8% and nevirapine reduction of C_{max} of ≈20% compared with historical controls	Alternative antifungal agent should be considered when nevirapine is part of ART regimen	*159*
Clarithromycin	Counter-balanced reduction in parent compound and increase in primary metabolite of clarithromycin	Close monitoring of patients, with no indication for dose adjustment	*160*
Warfarin	Case report of extreme difficulty to achieve therapeutic anticoagulation in patients on nevirapine	No formal recommendation; monitor patients closely and consider alternative ART	*161*
St. John's Wort	Increased oral clearance of nevirapine by 35%	St. John's Wort should be avoided in patients receiving nevirapine	*162*

PK, pharmacokinetics; OCP, oral contraception pills; AUC, area under curve

[a]Recommendations taken from the cited references and the Department of Health and Human Services Guidelines for HIV/AIDS Treatment *(149)*

Angelica dahurica, ginseng, garlic preparations, Danshen, and licorice *(163)*. Some statins (lipid-lowering agents) and glitazones (insulin sensitizers and possible peripheral adipocyte growth factors) are metabolized by CYP3A (which is induced by nevirapine), therefore, coadministration with nevirapine could alter their effectiveness *(164)*. The statin least likely to interact adversely with nevirapine is probably pravastatin, which is metabolized by sulfation. Table 5 is adapted from Piscitelli *(165)* and lists the other medications commonly used in HIV-1-infected patients who are effected by CYP3A4 and, therefore, could be affected by nevirapine administration.

PEDIATRIC ISSUES

The use of highly active ART in children has recently been reviewed *(166)*. Several open-label phase I/II trials and randomized clinical trials, particularly from the Pediatric AIDS Clinical Trials Group (PACTG), report a broad range of pharmacokinetic, resistance patterns, virological, immunological, and safety data that provide valuable information regarding the use of nevirapine in children.

Phase I trials with nevirapine were conducted in children at least 2 mo of age in PACTG 165 (single-dose trial, $n = 9$) and PACTG 180 (multidose trial, $n = 21$) *(167)*. At the highest single dose tested, 120 μg/m^2, C_{max} was attained within 4 h with a median of 2.9 μg/mL (263 × IC_{50} for wild-type HIV-1). As would be expected from the autoinduction of CYP450 enzymes that metabolize nevirapine, plasma concentrations after multiple doses were not as high as previously measured in single-dose pharmacokinetic experiments. Multiple dose oral clearance was correlated with the child's age, prompting the investigators to increase the dose to 200 μg/m^2 twice daily for children younger than the age of 9 yr, from 120 μg/m^2 twice daily for children older than 9 yr. This resulted in attaining target steady-state nevirapine trough concentrations of approx 3 to 5 μg/mL. Others have confirmed the importance of dose adjustment for age *(168)*. Rash was the only adverse event related to study drug and occurred in 1 (5%) of 21 subjects treated. It recurred after rechallenge and led to discontinuation of therapy. After 24 wk of nevirapine monotherapy, high-level resistant virus was isolated from all the subjects.

Luzuriaga and colleagues reported their experience treating maternally acquired HIV-1 infection in eight infants aged 2 to 16 mo, with ZDV plus didanosine plus nevirapine in an open-label, 6-mo study *(169)*. At the end of the study, only two subjects had undetectable pVL (≤20 copies/mL), whereas pVL were reduced by 0.5 to 1.5 $\log_{10}$ copies/mL in five of the remaining six subjects. The median trough plasma concentrations of nevirapine ranged from 2.7 to 8.6 μg/mL. There was no association between the reduction in pVL and nevirapine plasma trough concentrations in this small study. No clinically significant adverse events to the study medications were observed.

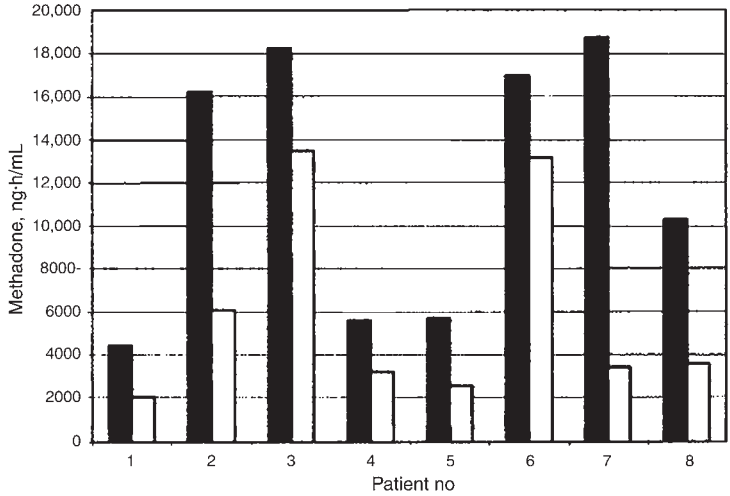

Fig. 5. Methadone time–concentration $AUC_{0–24\ h}$ before and after nevirapine therapy. ■, methadone alone; ❑, methadone plus nevirapine. (Adapted from ref. *154* with permission.)

Table 5
CYP-450 Isoform CYP3A4 Substrates, Inhibitors, and Inducers With Possible Interaction With Nevirapine

Substrates	Inhibitors	Inducers
Astemizole	Amprenavir	Carbamazepine
Clarithromycin	Clarithromycin	Efavirenz
Cyclosporine	Delavirdine	Nevirapine
Dapsone	Efavirenz	Phenytoin
Efavirenz	Erythromycin	Phenobarbital
Erythromycine	Fluconazole	Rifampin
Estrogens	Fluoxetine	Rifabutin
Etoposide	Grapefruit juice	Ritonavir
Fentanyl	Indinavir	Troglitazone
Midazolam	Itraconazole	
Nefazodone	Ketoconazole	
Prednisone	Lopinavir	
PIs	Nelfinavir	
Sertraline	Saquinavir	
Testosterone		
Triazolam		

Adapted from ref. *165*, with permission

PACTG 338 was originally designed to compare ZDV plus 3TC, stavudine plus ritonavir, and ZDV plus 3TC plus ritonavir in treatment-experienced children aged 2 to 17 yr *(170)*. An interim analysis showed that the virological response in the dual nucleoside arm was suboptimal, prompting a protocol modification that provided for subjects in the ZDV plus 3TC arm to receive stavudine, nevirapine, or ritonavir if their plasma HIV RNA level was at least 10,000 copies/mL (Step 2) *(171)*. All subjects were ritonavir- and nevirapine-naive at the time of study entry. Nevirapine was dosed at 120 mg/m^2 twice daily. After 24 wk of treatment in step 2, 48% (23/48) of children had undetectable pVL (≤400 copies/mL) compared with 34% (31/92) and 47% (44/93) of children receiving stavudine plus ritonavir and ZDV plus 3TC plus ritonavir, respectively, for 24 wk of step 1. After 48 wk of treatment in step 2, 44% of children had undetectable pVL compared with 27% and 42% of children receiving dual and triple therapy, respectively, for 48 wk of step 1 ($p = 0.04$). A logistical model was used to compare the pVL response of step 2 therapy to step 1 ritonavir-containing treatments, controlling for differences in baseline CD4 T-cell count and pVL. Baseline pVL, but not baseline CD4 T-cell count, was a significant predictor of pVL response when all of the factors were included in a multivariate analysis ($p = 0.03$; odds ratio, 0.65). There was no difference among the treatment groups with respect to trends over time of the median CD4 T-cell count. The CD4 T-cell counts of the step 2 subjects increased a median 174 cells/mm^3 from a baseline median of 644 cells/mm^3 (interquartile range, 442–918) at 48 wk. At week 48, there were no significant differences between the step 1 or step 2 treatment groups with respect to the rate of severe adverse events. The most commonly observed severe adverse events among step 2 subjects were rash (6%), gastrointestinal symptoms (4%), and fever (4%), with 10 subjects (21% of all subjects with rash) experiencing grade 2 rash. None of the subjects experiencing any grade of rash discontinued therapy secondary to this toxicity.

Preliminary results from the PACTG 377 study have been published, reporting 24-wk *(172)*, and 48-wk *(173)* virological, immunological, and safety data. A total of 193 subjects between the ages of 4 mo and 17 yr, who were stavudine-, 3TC-, PI-, and NNRTI-naive, were randomized into one of five arms:

1. Stavudine plus nevirapine plus ritonavir.
2. Stavudine plus 3TC plus nelfinavir (thrice daily).
3. Stavudine plus nelfinavir (thrice daily) plus nevirapine.
4. Stavudine plus 3TC plus nevirapine plus nelfinavir (thrice daily).
5. An additional 11 subjects received Group C medications, with nelfinavir dosed twice daily.

The nevirapine was dosed at 120 mg/m^2 daily for 14 d, followed by 120 mg/m^2 twice daily thereafter. At week 48, the proportion of subjects with an undetectable pVL (≤400 copies/mL) in an on-treatment analysis was 41%,

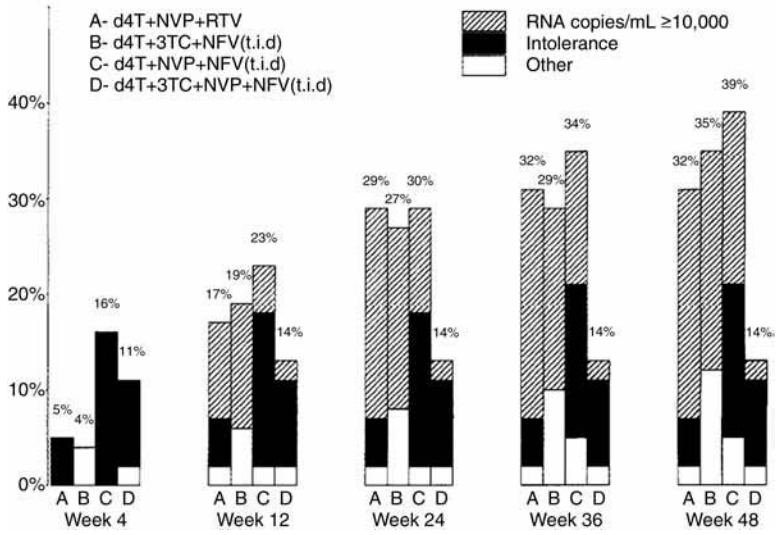

Fig. 6. Distribution of reasons for permanent cessation of initial treatment, by treatment group in the PACTG 377 clinical trial. 3TC, lamivudine; d4T, stavudine; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir; t.i.d., thrice daily. (Adapted from ref. *173* with permission.)

42%, 30%, and 52% for groups 1 to 4, respectively (for groups 3 and 4, Fisher's exact *p* value for pair-wise comparison was $p = 0.048$) *(173)*. The regimens were similar in their drug activity, but viral suppression for subjects receiving the four-drug regimen was slightly more durable. Overall, 78% of the subjects experienced grade 2 or higher toxicity while receiving randomized therapy, and 23% experienced grade 3 or worse toxicity, resulting in discontinuation of assigned treatment in 7% of subjects. Figure 6 depicts the distribution of reasons for discontinuation of assigned treatment by group. Virological failure (pVL ≥ 10,000 copies/mL) was the most frequent cause for treatment discontinuation (17%), but only occurred in two subjects (5%) assigned to the four-drug regimen. There were no significant differences between treatment groups with respect to overall rates of toxicity. Skin rash (28%, grade ≥ 2 overall) occurred more frequently among subjects randomized to nevirapine-containing regimens (33%) than among those of the other treatment group (16%; $p = 0.02$) and led to treatment discontinuation in 10% of subjects randomized to those arms. The reasons for sustained viral suppression in only approx 50% of participants remain unknown. As expected, emergence of resistance mutations partially accounted for the high failure rate *(174)*. Mutations to nevirapine and 3TC were detected more frequently at the time of virological failure, and nevirapine mutations were more frequent in the three-drug vs the four-drug regi-

men. Interestingly, subjects with baseline resistance, primarily at codons conferring ZDV resistance, had consistently greater reductions in virus loads during the course of the 48-wk study than did subjects without these mutations. This effect was seen only in subjects assigned to the nevirapine-containing regimens suggesting a possible hypersusceptibility to nevirapine in this setting or reduced viral fitness of resistant HIV-1 species (*see* Chapter 14). As in the adult population, adherence to the treatment regimen is critically important *(175)*. Further pharmacokinetic studies underway may provide relevant insights into the results of these important studies and further define the role of nevirapine and combination therapy for the treatment of HIV-1 infection in children. For children older than the age of 3 yr, nevirapine is recommended as an alternative NNTRI for initial therapy by the Working Group of Antiretroviral Therapy and Medical Management of HIV-Infected Children, convened September, 2003 by the National Pediatric and Family HIV Resource Center, the Health Resources and Services Administration, and the National Institutes of Health *(176)*.

CONCLUSIONS

Nevirapine, the first NNRTI, has potent activity against HIV-1, and provides durable plasma HIV RNA viral load suppression when used in combination with other antiretroviral agents. It is currently recommended as an alternative for first-line therapy in the treatment of established HIV infection *(149)*. In particular, patients with baseline lipid profile abnormalities in whom a PI-sparing regimen is desired and who have a risk for the CNS side effects of efavirenz, would be expected to respond well to a nevirapine-containing regimen. Nevirapine has an excellent toxicity profile. Management of the two principle adverse events, rash and hepatotoxicity, have been described. Nevirapine should be used with caution in patients with preexisting liver enzyme level elevations, and close monitoring for further increases in LFT abnormalities is indicated.

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Efavirenz

Graeme J. Moyle and Brian Conway

INTRODUCTION

The virological goal of therapy in the era of highly active antiviral therapy (HAART) is to achieve both substantial and sustained suppression of viral replication in all cellular and body compartments *(*1,2). This response seems to be associated with at least partial immune reconstitution and a marked reduction in the risk of clinical events or death. Additionally, reducing the rate of viral replication to very low levels also seems to delay the emergence of drug-resistant viruses, one of the principal reasons for therapeutic failure *(3)*.

The clinical value of three-drug combination antiretroviral therapy has been established by a number of large randomized, controlled trials showing improved survival and a reduction in the risk of disease progression compared with single- and double-agent therapy. As a result, initiating treatment with a combination of three antiretroviral agents (a protease inhibitor [PI] or non-nucleoside reverse transcriptase inhibitor [NNRTI] plus two nucleoside analog reverse transcriptase inhibitors [NRTI]) is now considered the standard of care for the clinical management of HIV infection. However, a significant proportion of treatment-naive patients fail to achieve optimal treatment responses with triple therapy. Many physicians consider, therefore, that initial treatment should be planned so that it does not compromise on initial activity but also maintains second-line or salvage options for patients who fail to achieve or maintain optimal virological responses. Additionally, metabolic toxicities associated with prolonged use of PIs have been recognized *(4)*, and this has caused a reevaluation of the risk of using these drugs, particularly in early disease *(5)*. These concerns have led to a preference, by some practitioners, for protease-sparing regimens to provide equipotent but better tolerated therapy. Concern about toxicities and the pursuit of more conveniently dosed regimens has also led to the investigation of NNRTIs as substitutes for the PI component of an ongoing successful therapy regimen, although newer studies have demonstrated that a number of PIs can be effectively administered once daily, with as few as two pills *(6,7)*.

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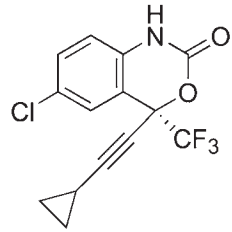

Fig. 1. Structural formula of efavirenz.

A broad range of agents from four distinct therapeutic classes is now approved, with many new agents in advanced clinical development. The challenge remains regarding how to use these drugs in combination most effectively in each individual requiring treatment. Until recently, PIs have been the preferred third agent to combine with two NRTIs because of the established antiviral potency of these agents in both treatment-naive and treatment-experienced patients. However, equipotent NNRTI-based regimens may have several advantages over many PI-based therapies for initial or prolonged therapy, including:

- More convenient, patient-friendly dosage regimens (once or twice daily).
- Lower number of tablets per day.
- No established long-term metabolic disturbances (e.g., lipodystrophy, diabetes, and renal dysfunction).
- Transient initial toxicities that do not generally overlap with or potentiate those associated with NRTI use.
- Maintenance of PIs as an option for second-line therapy.
- Fewer potentially serious drug interactions, as occur with ritonavir boosting.

Data on the durability of the response, the virological benefits in nonplasma or so-called sanctuary sites, such as lymph nodes, and changes in immune function suggest that NNRTIs, particularly efavirenz (EFV), produce similar (or better) benefits to those observed with PIs. Systematic evaluation of metabolic and body shape changes over prolonged follow-up periods are required to confirm the current perception that NNRTIs are not associated with these problems. Studies specifically addressing the potential for PI-based regimens to reestablish virological control in individuals after failure of an initial PI-sparing regimen support the notion of commencing therapy with NNRTIs.

EFV (also known as DMP-266; Sustiva®; Stocrin®) is a member of the NNRTI class of antiretroviral agents. NNRTIs are a chemically diverse group of compounds that are potent inhibitors of HIV-1 reverse transcriptase (RT) in vitro. The first two agents in the class to become available were the *bis*(heteroaryl)piperazine, delavirdine, and the dipyridodiazepinone derivative, nevirapine. EFV is a member of the benzoxazinone chemical class (Fig. 1) and was

synthesized as part of a joint anti-HIV collaborative research program between DuPont Pharma (now Bristol-Myers Squibb) and Merck Pharmaceuticals.

This chapter discusses the available data on EFV, both clinical and preclinical, and examines the current and potential future roles of this agent in antiretroviral therapy.

CHEMISTRY AND MODE OF ACTION OF EFV

EFV, or (*S*)-(−)-6-chloro-4-(cyclopropylethynyl)-4-(triflouromethyl)-2,4-dihydro-1*H*-3, 1-benzoxazin-2-one, has the chemical formula $C_{14}H_9C_{l3}NO_2$ and a molecular weight of 315.68. Only the S-enantiomer is active; the R-enantiomer has been found to have no activity. EFV is poorly water soluble (<10 μg/mL) *(8)*. It is formulated as capsules of 50, 100, and 200 mg, as tablets of 600 mg, and is also available as a liquid preparation *(9)*. The recommended total daily dosage in adults is 600 mg.

EFV is a specific inhibitor of the HIV-1 RT, a viral enzyme that is crucial for HIV replication. Like all NNRTIs, it is not active against HIV-2, and activity against other viruses pathogenic to humans has not been demonstrated. Unlike NRTIs, which require intracellular activation to exert their activity, EFV is active in the administered form.

Despite their diversity of chemical structures, all NNRTIs act through a common pathway. They attach to HIV-1 RT in the NNRTI-binding pocket, a hydrophobic nonsubstrate-binding region of the 66-kDa subunit of HIV-1 RT. Inhibition of RT activity occurs by either interference with mobility of the "thumb" subdomain, or, most likely, by disrupting the orientation of the conserved aspartic acid side chains that are essential for catalytic activity *(10–12)*. Significant resistance to individual compounds seems to develop rapidly, often within a few weeks of initiating monotherapy; it frequently involves only single point mutations, and, in many cases, leads to considerable cross-resistance to other NNRTIs. Most mutations occur in the codon groups 98–108 and 181–190 that encode for the two β-sheets adjacent to the catalytic site of the RT enzyme *(13)*, which form the binding region.

EFV is active against HIV-1 at nanomolar concentrations. The K_i (inhibition constant) for wild-type HIV-1 RT is 2.93 nmol/L, and the in vitro IC_{95} (concentration producing 95% inhibition) is at most 1.5 μmol/L against both wild-type virus and viruses possessing a range of single NNRTI-type mutations other than the K103N mutation *(14)*. The activity of EFV relative to other NNRTIs against the NL4-3 wild-type strain of HIV-1 and multiple single-point mutations is shown in Table 1 *(15)*.

The penetration of EFV into the cerebrospinal fluid (CSF) was established in toxicological studies of cynomolgus monkeys *(16)*. Although most mutagenic studies with EFV have been negative, central nervous system (CNS) abnormalities were observed in several cynomolgus monkey offspring after

Table 1
IC_{90} Values (nmol/L) for EFV, Nevirapine, and Delavirdine Against the NL4-3 Wild-Type Strain of HIV-1 and Multiple Single-Point Mutations

Mutation	EFV	Nevirapine	Delavirdine
NL4-3 (wild type)	3.5	130	38
L100I	77	630	1300
K101E	24	1600	190
K103N	64	5100	1000
V106A	11	14000	580
V108I	3.8	330	39
Y181C	4.1	12000	980
Y188C	13	5300	100
G190A	14	4700	6.3
P236L	1.9	260	2400

(Adapted from ref. *15*.)

treatment of the mother during pregnancy with high dosages of EFV *(17)*. The abnormalities included microphthalmia and anencephaly. Some congenital defects have been observed in children born to women who took EFV during pregnancy *(18)*, and the drug has recently been reclassified as Category D for purposes of its use during pregnancy *(19)*.

CLINICAL PHARMACOKINETICS OF EFV

Peak plasma concentrations (C_{max}) of EFV occur 3 to 5 h after oral administration, and its half-life at steady-state is 40 to 55 h *(17)*. Thus, EFV can be administered once daily.

EFV is highly protein-bound, with 99.5 to 99.75% of the drug bound to plasma proteins, predominantly albumin *(17)*. The estimated CSF concentration in humans is 0.61% of the total plasma concentration. After administration of 600 mg EFV once daily (with other antiretroviral agents) in 10 HIV-infected patients, the mean CSF concentration of the drug was 35.1 nmol/L, which was above the IC_{95} for wild-type HIV-1 *(20)*.

Steady-state concentrations of EFV are established within 2 wk of initiating therapy. Metabolism occurs via the cytochrome P (CYP)-450 system, predominantly by CYP3A4 and CYP2B6, and results in inactive hydroxylated metabolites that are then glucuronidated to 8-OH-EFV before being renally excreted *(21)*. Less than 1% of the drug seems unchanged in the urine. EFV induces the enzymes involved in its own metabolism, but also inhibits several CYP450 isozymes, including CYP3A4, CYP2C9, and CYP2C19. Consequently, it may affect the pharmacokinetics of a range of other drugs that are metabolized by these enzymes (*see* Drug–Drug and Drug–Food Interactions following).

Table 2
Steady-State Pharmacokinetic Parameters of EFV After Dosages of 600 mg EFV Once Daily in Adults

Parameter	Value
C_{max} (μmol/L)	12.9 ± 3.7
C_{min} (μmol/L)	5.6 ± 3.2
$AUC_{0–24h}$ (μmol/L·h)	184 ± 73

[a]$AUC_{0–24h}$ = area under the 24-h plasma concentration–time curve; C_{max} = peak plasma concentration; C_{min} = minimum (trough) plasma concentration. (Adapted from ref. *17*.)

Steady-state pharmacokinetic parameters of EFV in adults after dosages of 600 mg once daily are shown in Table 2 *(17)*. Trough concentrations of EFV are substantially above in vitro IC_{90} values, even taking into account in vivo protein binding. A minimum (trough) plasma concentration of greater than 3.5 μmol/L, which is the estimated trough concentration necessary for activity against the K103N resistant variant (based on the in vitro IC_{90} value and the in vivo protein binding), is achieved by 80% of adults *(17)*, although this does not translate into therapeutic benefit in the presence of this mutation.

DRUG–DRUG AND DRUG–FOOD INTERACTIONS

As indicated in the previous section, EFV is metabolized via the CYP450 system, predominantly by the CYP3A4 isoenzyme. EFV acts as both an inhibitor and inducer of this enzyme, and induces its own metabolism. Inhibitory effects may predominate during the first weeks of EFV therapy, after which, induction may predominate. Consequently, interpretation of pharmacokinetic interaction studies requires consideration of the timing of coadministration, whether the other drugs were started concomitantly or once an EFV steady-state concentration was established, and whether single doses or multiple doses were administered.

The results of drug interaction studies with EFV are shown in Tables 3 and 4 *(17,21–30)*. Relatively few of these pharmacokinetic interactions seem to require dosage adjustments. There may be an interaction with ritonavir that may potentiate the toxicity of ritonavir. There are no specific guidelines for dosage adjustment, but there is a need for vigilance regarding any possible drug-associated toxicity. Administration of EFV with saquinavir as the sole PI is not recommended because plasma concentrations of saquinavir are markedly decreased; however, a triple-drug combination of EFV plus saquinavir plus ritonavir was not associated with any significant changes in steady-state concentrations of either saquinavir or ritonavir, and dosage modifications with this triple combination seem unnecessary *(31)*. When EFV is administered with the combination of lopinavir plus ritonavir, an increase in the dosage of the PI

Table 3
Effect of EFV on the Pharmacokinetics of Antiviral and Other Drugs *(30)*

Drug	Effect of EFV on pharmacokinetic parameters of coadministered drug	
	C_{max}	AUC
Antiviral drugs		
IDV	–16%	–31%
Nelfinavir	+21%	+20%
Nelfinavir metabolite (Ag1402)	–40%	–37%
Ritonavir		
Morning dose	+24%	+18%
Evening dose	NC	NC
Saquinavir (soft-gel capsule)	–50%	–62%
3TC	NC	NC
ZDV	NC	NC
Other drugs		
Azithromycin	+22%	NC
Clarithromycin	–26%	–39%
14-OH metabolite of clarithromycin	+49%	+34%
Fluconazole	NC	NC
Rifabutin	–32%	–38%
Cetirizine	–24%	NC
Ethinyl estradiol	NC	+37%
Lorazepam	+16%	+7%
Methadone	–45%	–52%

+, increased; –, decreased; NC, no change

combination (from 400/100 mg twice daily to 533/133 mg twice daily) should be considered when reduced susceptibility to lopinavir is clinically suspected, because EFV may decrease the plasma concentrations of this agent *(32)*.

The dosage of rifabutin may need to be increased (by 50%) when used with EFV, whereas clarithromycin may be best avoided and alternative antibiotics, such as azithromycin, considered. Other agents for which coadministration with EFV is not recommended include astemizole, cisapride, midazolam, triazolam, and ergot derivatives. Patients receiving methadone maintenance therapy should be monitored for signs of withdrawal symptoms, and the methadone dosage increased as required.

Nevirapine modestly reduces steady-state plasma concentrations of EFV, whereas EFV does not seem to affect the pharmacokinetics of nevirapine *(33)*. An early observational study *(34)* suggested that the combination might be

Table 4
Effect of Antiviral and Other Drugs on the Pharmacokinetics of EFV *(30)*

	Effect of coadministered drug on pharmacokinetic parameters of EFV	
Drug	C_{max}	AUC
Antiviral drugs		
IDV	NC	NC
Nelfinavir	NC	NC
Ritonavir	+14%	+21%
Saquinavir (soft gel capsule)	–13%	–12%
Other drugs		
Azithromycin	NC	NC
Clarithromycin	+11%	NC
Rifabutin	NC	NC
Rifampin	–20%	–26%
Aluminium hydroxide/magnesium hydroxide/ simethicone	NC	NC
Cetirizine	NC	–8%
Ethinyl estradiol	NC	NC
Famotidine	NC	NC

+, increased; –, decreased; NC, no change

beneficial in some settings. However, when it was evaluated in a randomized clinical trial of drug-naive patients, it was not shown to be superior to either agent used alone *(35)*.

Because administration of EFV with food may lead to increased plasma concentrations of the drug, it is generally recommended that it be taken on an empty stomach. After a single 600 mg dose, a high-calorie/high-fat meal (894 kcal; 54 g fat) and a normal calorie/reduced fat meal (440 kcal; 2 g fat) were found to increase EFV C_{max} concentrations by 39% and 51%, respectively, and area under the curve ($AUC_{0–\infty}$) values by 22% and 17%, respectively, relative to administration under fasting conditions *(30)*. No effect on the pharmacokinetics of EFV was observed when it was administered with antiulcer agents (famotidine) and antacids (aluminium hydroxide/magnesium hydroxide/simethicone).

A possible laboratory test interaction to be aware of in patients receiving EFV is a false-positive urinary cannabinoid test with at least one commercially available assay (Microgenics CEDIA DAU Multi-Level THC assay) *(30)*.

RESISTANCE TO EFV

Because many patients treated with HAART regimens fail to achieve or sustain virological control, decisions regarding choice of initial treatment should

include consideration of subsequent or salvage regimens. Specifically, knowledge of resistance patterns may be essential to guiding clinical decision making. Resistance to all antiretroviral agents has been extensively reviewed. Clearly, one attraction of using NNRTIs with an NRTI in initial regimens is that this saves the PI class for future use, and this sequence has been shown to be effective in clinical practice, as well as in clinical trials *(36)*.

The most common mechanism by which resistance develops is by a mutation in the *pol* gene, leading to an amino acid substitution affecting the site at which the drug binds to the target enzyme. This type of mutation is only beneficial to the virus if the normal function (fitness) of the enzyme is not adversely affected to a point at which continued replication is not possible. Thus, the evolution of resistance becomes a balance between reduced drug sensitivity and the partial loss of viral replication efficiency. This process may initially involve selection of a highly fitness-compromised virus which, however, is able to replicate in the face of drug pressure. This is followed by the accumulation of further mutations, which may contribute to further drug resistance but also may improve viral fitness. These events can only occur with ongoing viral replication while drug pressure is being applied. The best way to avoid this outcome is to select a regimen to ensure that maximal virological suppression is occurring, preventing the random generation of potentially resistant mutants *(3)*.

The key resistance mutation selected by EFV in vitro and subsequently observed with EFV in vivo is Lys103Asn (K103N) *(17)*. This mutation reduces in vitro sensitivity to EFV approx 18-fold. The mutations L100I, Y188L, and G190S may confer higher levels of EFV resistance in laboratory viral constructs, but are observed less frequently in vivo than K103N *(15)*. In vitro, resistant viruses with L100I mutations are most commonly selected *(37)*; however, in vivo, they are only seen in combination with K103N mutations *(38,39)*. Other mutations associated with resistance to NNRTIs, generally in the *pol* gene codons 98–108 and 181–190, may further contribute to higher-level resistance to EFV in the presence of the K103N mutation. In isolation, many of the other mutations that commonly arise during therapy with other available NNRTIs confer less than fourfold changes of in vitro sensitivity to EFV; that is, viruses with these mutations seem phenotypically sensitive (*see* Table 1) *(15)*. In this regard, an in vitro study has shown that, whereas viruses with K103N or Y188L mutations isolated from patients failing on NNRTI therapy exhibit cross-resistance to all of the presently available NNRTI analogs (EFV, nevirapine, and delavirdine), regardless of the initial selecting agent, some virus isolates from nevirapine- or delavirdine-treatment failures that lacked K103N or Y188L mutations remained sensitive to EFV *(40)*. A study in NNRTI therapy-naive patients has indicated that preexisting low-level phenotypic resistance to NNRTIs does not negatively affect virological responses to EFV-containing

regimens, because similar proportions of patients exhibiting viral load suppression and virological failure had demonstrated initial low-level resistance *(41)*.

As more patients with nonsubtype B viruses begin to receive treatment, the patterns of emergence of drug resistance may be different. In those with subtype C, for example, the preferred pathway seems to be V106M, which confers high-grade cross-resistance to all currently available NNRTIs *(42)*.

In early clinical studies with EFV, the K103N mutation was found to be present in more than 90% of patients experiencing virological rebound. Concomitantly administered therapies, including NRTI, such as zidovudine (ZDV) and lamivudine (3TC) and PIs, such as indinavir (IDV), have not been observed to influence the resistance pattern with EFV, although more potent antiviral effects are generally associated with a delay in resistance development. Additional mutations observed with K103N, and generally associated with higher-level resistance, include L100I, V108I, and P225H. The appearance of K103N coincides with viral rebound and is likely to be the key contributor to antiviral failure in EFV-treated patients *(38,39)*. In an early monotherapy clinical study, viruses with resistance mutations were detected as early as 2 wk into therapy, a finding similar to that reported during viral dynamics studies with nevirapine. This underlines the fact that monotherapy with antiretroviral drugs should be avoided at all times.

In a study in patients who stopped EFV-containing therapy in the setting of full virological suppression, NNRTI resistance mutations were not detected after viral load rebound in the majority (6/7) of cases. However, in patients with virological failures on EFV-containing therapy, viruses with the K103N resistance mutation were present in all (10/10) patients at termination of treatment, and these mutations persisted at detectable levels in the plasma for 1 to greater than 6 mo *(43)*. In discontinuing EFV-containing regimens, a potential problem in terms of selection of resistance can arise because its longer plasma half-life vs other components of the regimen may result in the occurrence of a period of functional monotherapy. Detectable levels of EFV may, in fact, persist for many weeks after withdrawal *(44)*. Consequently, discontinuation of otherwise successful EFV therapy (e.g., in the event of major surgery or as a result of intolerance of EFV or other components of the regimen) can be challenging. Currently, no recommendations exist for patients needing to stop or interrupt successful EFV therapy, although substitution of an alternative but more rapidly excreted NNRTI or PI up to 1 wk before the planned discontinuation of EFV may represent an option.

A study of patients enrolled in study 006 who temporarily interrupted EFV-containing therapy because of adverse events has, however, shown that the long-term virological response was not compromised in those patients who were virologically suppressed at the time *(45)*. The proportions of patients

achieving viral loads of less than 50 copies/mL in this 48-wk study were similar in those who did not need to interrupt EFV plus ZDV plus 3TC therapy and in those who interrupted it for a median of 13 d when virologically suppressed (<400 copies/mL); i.e., 72% and 73%, respectively. In comparison, patients whose viral load was not suppressed when the EFV-containing regimen was interrupted had a lower long-term virological response rate (45%), and it would be difficult to determine whether resistance developed before or after treatment interruption.

The observation that the K103N mutation is associated with cross-resistance to other available NNRTIs, such as nevirapine and delavirdine *(3,15)*, indicates that these agents are unlikely to be of value after EFV failure. X-ray crystallographic studies suggest that K103N seems to alter the structure of RT at the entrance to the NNRTI-binding pocket. The mutation seems to act by slowing the binding rate of all inhibitors of the enzyme *(46)*. However, several new NNRTI compounds undergoing clinical development have favorable pharmacological characteristics and in vitro activity against the K103N mutant virus *(47)*.

Another interesting aspect of resistance is the possibility that viral isolates may actually become more susceptible to EFV than wild-type strains. This phenomenon, known as hypersusceptibility, was identified in 10.8% of greater than 17,000 consecutive plasma samples submitted for phenotypic susceptibility testing. This phenomenon was observed more often in NRTI-experienced/NNRTI-naive individuals, compared with those naive to both classes of drugs. The genotypic correlates are complex, but largely relate to the number of mutations in the RT gene that resulted form ongoing viral replication in the presence of NRTI drug pressure. Data that is more recent, looking at 444 NRTI-experienced/NNRTI-naive patients, showed that mutations at positions 215, 208 and 1118 were independently associated with NNRTI hypersusceptibility *(48)*. In a small number (n = 11) of isolates hypersusceptible to EFV, there was an association with virological response to EFV *(49)*, suggesting that this phenomenon may well be of clinical significance, and may help us maximize the benefit of EFV in salvage or second-line therapy.

IMPORTANCE OF THERAPY ADHERENCE

To provide clinical activity, an antiretroviral agent must be reliably taken, absorbed from the gastrointestinal tract (avoiding gut wall and hepatic metabolism), and should ultimately achieve and sustain sufficient intracellular concentrations of the active form to inhibit HIV-1. Failure at any of these steps may cause suboptimal concentrations of the drug, with consequent persistent viral replication—the ideal circumstances to select for drug resistance. In particular, persistently missed or delayed doses may lead to multiple opportunities for viral replication in the presence of drug selection pressure.

The success of any therapeutic intervention is dependent on the patient adhering to the prescribed therapy. Nonadherence among patients is more prevalent when the illness is chronic and in the absence of clinical symptoms, when treatment may be considered (by the patient) to be prophylactic. Experiences with other chronic diseases, such as diabetes, renal failure, tuberculosis, and hypertension, indicate that patient adherence has a direct effect on the clinical outcome. In these illnesses, adherence to treatment averages only 40 to 60%. Factors found to be consistently associated with poor adherence include lack of knowledge about the disease and its treatment, anxiety about taking the medication, concerns about adverse effects, health beliefs, the complexity of the dosage regimens, and poor clinician–patient relationships *(50–52)*. Additionally, patients may choose not to adhere to treatment as a way of retaining control or of coping with their illness *(53,54)*. In HIV-infected patients taking ZDV, factors reported to influence treatment adherence include substance abuse, drug toxicity, the complexity and frequency of dosing, education regarding treatment, expectations regarding the likely efficacy of the drug, individuals' perceptions about the severity of HIV disease, ethnicity, and social and economic circumstances *(55,56)*. In one study, patients taking medications dosed more than three times daily or taking more than 4.5 medications were the least adherent. Importantly, knowledge about medication was associated with improved adherence in this study *(57)*. Others have suggested that treatment adherence by individuals with HIV infection may also be influenced by the fact that the early stages of the disease are relatively symptom-free, and the results of nonadherence are not evident until much later in the course of the disease *(58)*. Additionally, nonadherence has been found to be associated with psychological distress, emotional disturbances, depression, and poor adaptive and coping mechanisms, but those individuals who had experienced opportunistic infection were more likely to adhere to therapy *(56)*. Types of nonadherence include reducing the frequency of doses or the number of medications taken, as well as mistiming doses or failing to follow administration instructions.

A factor that seems critical to adherence is the frequency of dosing. With antihypertensive medication, compliance improved from 59% on thrice-daily dosing to 84% on once-daily dosing *(59)*. Although overall compliance (i.e., proportion of doses actually taken) with once-daily and twice-daily dosing did not differ, less than half of the doses were found to be taken within 12 ± 2 h of the previous dose *(60)*. With the treatment of HIV infection, the timing of doses may be particularly important in persons receiving PIs, which have a close relationship between circulating and intracellular concentrations and relatively short plasma half-lives. For example, rapid rises in plasma viral loads were observed in patients who missed only a few days of dosing with saquinavir, and the poor adherence may have been associated with subsequent emergence

of resistance to this drug *(61)*. The once-daily dosing schedule of EFV and its long plasma half-life suggest that it is an easy-to-take and relatively forgiving medication and, as such, is less likely to disrupt a patient's way of life, particularly in patients for whom adherence may be expected to be problematic.

CLINICAL STUDIES WITH EFV

The results of clinical studies with EFV support the use of EFV in combination with other antiviral agents for initial therapy of HIV infection. Additional data suggest that it may also contribute to the activity of second-line or salvage therapy regimens, and that it may be used to improve the convenience of treatment regimens or after the occurrence of toxicity by substituting it for PIs, especially in the setting of maximal virological suppression.

Analyses of clinical data with EFV have included three main statistical techniques: intent-to-treat (ITT); ITT, last observation carried forward; and observed or on-treatment analysis. These analyses provide different information regarding the clinical outcome. ITT analysis is the most conservative type because it includes all patients who entered the study and missing data are treated as representing failure. ITT analysis will tend to underestimate the treatment effect in practice. With ITT, last observation carried forward analysis, missing data are handled by assigning the value recorded at the last patient visit. Observed or on-treatment analysis, which is the most common way of presenting clinical data, provides results only for patients in whom follow-up data are available; missing values are disregarded. Thus, the denominator for this type of analysis may not be the same as the number of patients who originally entered the study. Observed or on-treatment analysis tends to overestimate the treatment effect in practice by ignoring patients who are unable to take therapy or who change therapy because of an insufficient response, but is a good reflection of the efficacy of a particular approach in those who actually take it.

Data from phase II, III, and IV studies with EFV have been reported and are reviewed in the following section. The early phase II studies were initiated before the establishment of the triple-therapy approach to antiretroviral therapy and, therefore, had designs that are no longer relevant to current clinical practice.

Early Clinical Studies

In early, short-term (12–16 wk) clinical investigations with EFV in HIV-1 infected patients, EFV doses of 600 mg once daily produced superior reductions in viral load than lower doses of 200 mg or 400 mg EFV once daily, both when initiated with ZDV and 3TC in antiretroviral-naive patients and when added to ZDV and 3TC in patients established on this dual-nucleoside combination *(62,63)*. Additionally, the combination of EFV and IDV was superior to IDV monotherapy in achieving a viral load of fewer than 400 copies/mL *(64)*.

Studies of Triple-Therapy EFV Regimens

First-Line Therapy: Study 006

This study, which was a key investigation for regulatory approval of EFV, compared EFV-containing regimens with a standard-of-care PI-based triple-therapy regimen *(65)*. The study allowed an analysis of time-to-failure to be made and was performed on 450 3TC-, PI-, and NNRTI-naive patients during a period of 48 wk. The patients were randomized to one of three treatment arms in an open-label manner:

- 600 mg EFV once daily plus 300 mg ZDV twice daily plus 150 mg 3TC twice daily ($n = 154$).
- 600 mg EFV once daily plus 1000 mg IDV thrice daily ($n = 148$).
- 800 mg IDV thrice daily plus 300 mg ZDV twice daily plus 150 mg 3TC twice daily ($n = 148$).

The mean baseline viral load and CD4 cell count were 4.77 $\log_{10}$ copies/mL and 345 cells/mm^3, respectively, with no significant differences between the groups. When considering the ITT vs observed or on-treatment data analyses, it is important to note that a substantially higher proportion of patients randomized to IDV plus ZDV plus 3TC discontinued treatment (43%) compared with the EFV plus ZDV plus 3TC group (27%; $p = 0.005$). The difference in discontinuations was largely because of a higher rate of adverse events, particularly nausea, in the IDV plus ZDV plus 3TC group. This discontinuation rate was higher than that observed when 3TC and IDV were added to established ZDV therapy *(66,67)*, but was similar to that reported in a previous study of this combination in treatment-naive patients *(68)*.

The findings of this study indicated that EFV-based triple therapy performs at least similarly in terms of its antiviral efficacy, but is better tolerated than IDV-based triple therapy. The proportions of patients achieving a viral load of fewer than 400 copies/mL at 48 wk in the EFV-based triple-therapy and IDV-based triple-therapy groups (ITT analysis) were 70% and 48%, respectively ($p < 0.05$). The observed or on-treatment analysis also demonstrated the superiority of the EFV-based regimen at 48 wk. When a more-sensitive viral load assay (limit of detection, 50 copies/mL) was used, similar outcomes were observed: 64% of patients receiving EFV-based triple therapy had a viral load of fewer than 50 copies/mL at 48 wk compared with 43% receiving IDV-based triple therapy (ITT analysis; $p < 0.05$). The dual-therapy regimen (EFV plus IDV) also performed at least as well as the IDV plus ZDV plus 3TC regimen, in that 47% of patients in this group had a viral load of fewer than 50 copies/mL at 48 wk. CD4 responses were similar across the three treatment groups *(65)*.

Although concerns have been raised regarding the efficacy of some NNRTI-based regimens in achieving optimal virological responses in persons with high

viral loads *(69)*, the responses at week 48 to EFV plus ZDV plus 3TC therapy were similar in patients with baseline viral loads greater than and fewer than 100,000 copies/mL, and were significantly superior to the IDV plus ZDV plus 3TC group in those with baseline viral loads of at least 100,000 copies/mL (observed or on-treatment analysis) *(65)*. Longer-term follow-up of the participants in this study has shown the response to EFV plus ZDV plus 3TC to be quite durable. After 168 wk, 43% of patients continued to have maximal virological suppression by the strictest ITT analysis *(70)*. If one limits the evaluation to those remaining on study treatment, 91% of patients had plasma viral load measures of fewer than 50 copies/mL. Response rates were well-preserved, even in those with the highest baseline viral loads (>300,000 copies/mL). Overall, there were only 12% of true virological failures. These data are extremely encouraging, and attest to the true efficacy of EFV-based HAART.

A drawback of this study was its open-label design. Compared with the IDV plus ZDV plus 3TC group, patients randomized to EFV plus ZDV plus 3TC received a lower number of tablets or capsules per day (11 vs 20) and a lower frequency of dosing (twice daily vs thrice daily). Thus, some of the observed differences in outcomes in this study may have been related to therapy adherence rather than to true differences in activity, although this was not evaluated in this protocol. However, the study may reflect actual clinical practice, in which aspects of a regimen that enable improved adherence and more convenient dosing are associated with a better outcome.

This study has been viewed by the scientific and patient communities as an important landmark in the evolution of antiretroviral therapy, legitimizing the use of NNRTIs as part of HAART. Concerns regarding long-term PI safety also contributed to the shift in approach *(71)*. These two factors have meant that the established HAART regimen paradigm of two NRTIs plus a PI has been amended, and recent treatment guidelines in the United States and the United Kingdom now suggest that EFV may be used as an alternative to a PI or a combination of PIs in initial treatment regimens *(1,2,72)*. Small observational studies of patients initiating therapy with either EFV ($n = 46$) or lopinavir/ritonavir ($n = 51$) do not suggest that there is any significant difference in efficacy or toxicity between these two approaches *(73)*. This issue may need to be addressed in a randomized, controlled study to be definitively answered. However, the difference may not be clinically significant enough to warrant such a large and expensive effort.

Multiple cohort studies have yielded evidence that, in clinical practice, EFV-based regimens are superior to nevirapine in treatment-experienced and treatment-naive individuals *(74–77)*. Similarly, a retrospective data analysis of 1078 antiretroviral-naive patients treated at three regional US centers found that EFV-based regimens were associated with a significantly better clinical outcome than nevirapine-based regimens *(78)*.

More recently, there have been a number of comparisons of EFV with nevirapine as part of initial therapy. The largest of these was the 2NN Study, enrolling 1216 patients, randomized to receive stavudine with 3TC as a backbone along with nevirapine (once or twice daily), EFV, or both *(35)*. Treatment failure (because of disease progression, toxicity, or virological failure) occurred in 43.7% patients taking nevirapine, as compared with 37.8% of patients taking EFV. Further, there was no difference in the proportion of subjects with plasma viral load of fewer than 50 copies/mL between any of the study arms. The combination of nevirapine and EFV was significantly more toxic, without added benefit, to be a feasible alternative in this setting. Further evidence of the relative equivalence of EFV and nevirapine, in terms of antiviral potency, is that the viral decay in the first 2 wk of therapy was equivalent, at 0.30 $\log_{10}$ copies/d *(79,79a)*. More recent data further suggest that the efficacy of EFV and nevirapine may be quite similar. A prospective, nonrandomized multicenter trial evaluated treatment-naive and treatment-experienced patients receiving nevirapine-based ($n = 337$) or EFV-based ($n = 325$) HAART *(80)*. By ITT analysis, there was only a 4.5% difference in the likelihood of achieving virological suppression (<50 copies/mL) at 48 wk. In nontreatment-naive patients, there was a 10.1% benefit for EFV treatment ($p = 0.06$), suggest an advantage over nevirapine in this setting.

NRTI-Experienced Patients: Study ACTG 364

In this study, coordinated by the US AIDS Clinical Trials Group (ACTG), 195 NRTI-experienced patients were randomly assigned to receive one or two new NRTIs plus either nelfinavir ($n = 66$), EFV ($n = 65$), or both nelfinavir and EFV ($n = 64$) *(81)*. The mean baseline viral load and CD4 cell count were 7776 copies/mL and 350 cells/mm^3, respectively. Using a standard viral-load assay with a cut-off of 500 copies/mL, the proportions of patients with a viral load below this level after 40 to 48 wk were 35% (nelfinavir), 60% (EFV), and 74% (nelfinavir plus EFV). Using a more sensitive viral load assay, the proportions of patients with a viral load below 50 copies/mL at 40 to 48 wk were 22%, 44%, and 67%, respectively. Both the nelfinavir plus EFV quadruple-therapy regimen and the EFV triple-therapy regimen were statistically superior to the nelfinavir triple-therapy regimen with both viral load assays. Although the difference between the nelfinavir plus EFV quadruple-therapy and EFV triple-therapy groups was not statistically significant with the standard assay ($p = 0.09$), the quadruple-therapy regimen was statistically superior to the EFV triple-therapy regimen when the ultrasensitive assay was used ($p = 0.008$).

These data support a role for EFV in second-line regimens, although they suggest that use of EFV plus a PI may be the best approach in NRTI-pretreated patients *(81)*.

Substitution of EFV for PIs

A small pilot study reported that 26 patients with viral loads of fewer than 400 copies/mL taking PI-based regimens who were switched to EFV maintained virological control during 48 wk and exhibited improvements in the clinical appearance of lipodystrophy, as measured by weight gain and reductions in abdominal circumference *(82)*. Preliminary data from this study prompted the investigation of PI substitution with EFV in two large randomized studies.

Study 027 and Study 049

These open-label, 48-wk studies evaluated the substitution of EFV for a PI in patients who had achieved viral load suppression to fewer than 50 copies/mL on a combination of a PI and two NRTIs administered for mean periods of 23 to 26 mo. Study 027 enrolled 134 patients and study 049 enrolled 346 patients; 69 patients in study 027 and 226 patients in study 049 were randomized to receive 600 mg EFV once daily instead of the PI agent, whereas the remainder continued on their previous PI-based triple-therapy regimen. ITT analysis at 48 wk indicated that viral suppression was maintained in a significantly higher proportion of patients on the EFV-containing regimen vs the PI-containing regimen in both studies: 94% vs 74% in study 027 ($p = 0.002$), and 84% vs 73% in study 049 ($p = 0.03$). In both studies, CD4 counts were increased to a similar extent with both treatment regimens. Adherence to therapy favored the EFV-containing regimen in study 049; 29% of patients were found to have missed doses on multiple clinic visits with the PI-containing regimen as compared with 12% of patients with the EFV-containing regimen ($p < 0.001$). In both studies, more patients receiving the PI-containing regimen exhibited triglyceride levels greater than 750 mg/dL in comparison with patients receiving the EFV-containing regimen (11% vs 4% in study 027; 11% vs 7% in study 049). However, in both studies, there were no statistically significant differences between the two groups in mean triglyceride levels at week 48 *(83)*.

Swiss HIV Cohort Study

Similar findings were reported in this matched case–control study in virologically suppressed patients (HIV-1 RNA levels <400 copies/mL) who were switched from a PI-containing regimen to an EFV-containing regimen for reasons of tolerance, toxicity, or convenience. After 1 yr, the probability of virological failure was less in patients who switched to EFV ($n = 184$) in comparison with a matched, nonswitched group ($n = 368$) who remained on their PI-containing regimen; i.e., 9.4% (95% confidence interval, 5.5–15.9) vs 27.2% (95% confidence interval, 21.5–34.1), respectively. However, there were no significant differences between the two groups in CD4 cell counts *(84)*.

Use in Children

Evaluation of HAART regimens in children with HIV-1 infection have generally yielded less impressive results than in adults. In a study in 57 NRTI-experienced children aged 3.8 to 16.8 yr with a median viral load and CD4 count of 10,000 copies/mL and 699 cells/mm^3, respectively, a combination regimen of EFV (initial daily dosage, [body weight in kg ÷ 70]$^{0.7}$ × 600 mg) plus nelfinavir (initial dosage, 20–30 mg/kg thrice daily) plus one or more NRTIs achieved viral loads of fewer than 400 copies/mL and fewer than 50 copies/mL in 76% and 63% of patients, respectively, after 48 wk of therapy (ITT analysis) *(85)*. The CD4 count increased by a median of 74 cells/mm^3 during this period. The treatment regimen was well-tolerated by the children, and only five (9%) children required discontinuation of therapy, in each case because of the occurrence of a rash.

In a study in which EFV was substituted for a PI in the HAART regimens administered to 13 children aged 2 to 13.6 yr, viral suppression (HIV-1 RNA < 50 copies/mL) was maintained in all patients during a median follow-up period of 32 wk, and CD4 levels remained stable. Triglyceride and total cholesterol levels fell slightly but significantly at week 24 *(86)*.

Other Studies

Studies involving the use of EFV in triple-therapy regimens with newer NRTIs have produced encouraging results. In one such protocol, 299 drug-naive patients received a combination of 3TC, tenofovir, and EFV *(87)*. At 48 wk, 80% had achieved maximal virological suppression by ITT analysis, a figure that increased to a staggering 99% if patients on therapy were considered. At 144 wk, the benefit was largely maintained, with 73% of patients having viral load measures of fewer than 50 copies/mL (by ITT).

In 285 drug-naive patients administered the combination of emtricitabine plus didanosine plus EFV, fully 85% of patients had plasma viral load measures of fewer than 50 copies/mL at 24 wk, and 76% of patients had plasma viral load measures of fewer than 50 copies/mL at 60 wk *(87)*.

However, not all novel combinations are showing equal efficacy. A small study was undertaken to compare the efficacy of EFV (n = 15) and lopinavir/ritonavir (n =14) administered in combination with didanosine and tenofovir in drug-naive patients *(88)*. The study was discontinued prematurely, when during only 3 mo of follow-up there were seven therapeutic failures in patients taking EFV (associated with significant levels of drug resistance), and only two failures in patients taking lopinavir/ritonavir. These findings were largely confirmed by those of another study comparing 3TC (n = 36) and tenofovir (n = 41), both administered as initial combination therapies along with didanosine

and EFV. By week 12, 5 of 41 patients taking tenofovir had drug resistance, as compared with no patients in the other group. Thus, it seems that in our drive to design simpler regimens, some nucleoside backbones (such as didanosine plus tenofovir) are convenient to administer but offer too low of a barrier to resistance to be combined with EFV, to which resistance is also conferred by a single point mutation.

TOLERABILITY OF EFV AND MANAGEMENT OF ADVERSE EFFECTS

The emergence of concerns regarding the long-term safety of PIs, particularly elevations in plasma lipids and fat redistribution (lipodystrophy syndrome), and their propensity to cause drug interactions have led to a reevaluation of the role of these agents as life-long therapies for persons with HIV-1 infection. This has heralded much debate in the HIV community regarding the choice of initial therapy, between PI-containing and PI-sparing regimens, and the relative tolerability of the various therapies has become an important focus in this debate.

Safety data have indicated that the most commonly reported adverse effects with EFV-containing regimens are CNS disturbances and skin rash, both of which generally occur and resolve during the first month of EFV therapy, and only infrequently lead to withdrawal of therapy.

CNS Symptoms

In clinical trials, CNS symptoms, such as dizziness, sleep disturbances, impaired concentration, vivid dreams, and agitation, have been reported to occur in approx 50% of patients (as compared with 25% in control groups in comparative clinical trials) and have led to treatment discontinuation in 2.1% of cases *(30)*. Although CNS symptoms have mostly been mild or moderate in severity, more severe symptoms that interrupt usual daily activities have occurred in approx 2% of patients. Psychiatric symptoms, including severe depression (1.6% of patients), suicidal ideation (0.6%), aggressive behavior (0.4%), paranoid reactions (0.4%), and manic reactions (0.1%) have also been reported rarely.

CNS symptoms generally begin within the first few days of therapy and have a median duration of 2 to 3 wk. After the first month, however, their incidence is low and their prevalence decreases steadily. In study 006, the incidences of CNS symptoms with the EFV plus ZDV plus 3TC and the EFV plus IDV regimens after the first month of therapy were similar to those occurring with the IDV plus ZDV plus 3TC regimen, although their cumulative prevalence was approx 10% higher with the EFV regimens in the case of CNS symptoms and 5% higher in the case of psychiatric symptoms *(89)*.

Although the mechanism of the adverse CNS symptoms is not established, EFV is not thought to interact with any known neurotransmitter receptor. Plasma concentrations of EFV do not seem to be correlated with the occurrence of the adverse CNS symptoms *(90)*. Recent studies done by the ACTG (5097s) suggest that a CYP2B6 variant more common in blacks is associated both with significantly greater EFV plasma exposure and with CNS symptoms after the first week of therapy *(91)*. At some future time, this type of pharmacogenomic work may help us to predict who might be at highest risk of developing EFV-associated toxicity and to avoid the use of the drug in this population. Until then, it is advisable to monitor patients with a history of psychological or psychiatric disturbances very carefully during the first weeks of EFV therapy. In addition, education regarding the possible occurrence of CNS symptoms should be provided (including a warning to avoid driving or operating machinery if symptoms are significant), along with support systems established to ensure that any problems experienced are promptly addressed. Recommendations for the management of EFV-induced CNS symptoms include adjusting the timing of EFV administration for dizziness, impaired concentration or abnormal dreams; use of mild sedative or hypnotic agents (e.g., 1.5 mg haloperidol or 0.5–2 mg lorazepam) for dream disturbances, mild agitation, or persistent insomnia; and an antidepressant (e.g., a selective serotonin reuptake inhibitor) for moderate to severe depression *(92)*.

Overall, it is unclear whether these symptoms actually affect the quality of life of the individuals taking EFV, as compared with what might be expected to occur if they were taking an alternate therapy. In an attempt to address this, a cross-sectional study was undertaken to compare 60 patients taking EFV with 60 other patients taking PI-based therapies for a mean of 90 to 120 wk *(93)*. Mild dizziness, sadness, mood changes, irritability, light-headedness, nervousness, impaired concentration, abnormal dreams, and somnolence were all more frequent in patients taking EFV ($p < 0.05$). However, this did not affect adherence, efficacy, or quality of life. In one study comparing NNRTI ($n = 55$) and PI ($n = 52$) regimens in salvage therapy, there was actually a better quality of life in patients taking EFV, despite an emphasis on the evaluation of CNS toxicity *(94)*.

Skin Rashes

Rashes associated with EFV are usually mild-to-moderate maculopapular eruptions. In most patients, the rash resolves with continuing therapy, but 1.7% of patients have required discontinuation of treatment as a result of rashes in clinical trials *(30)*. In those who interrupt therapy for this reason, treatment can be restarted, and the use of an antihistamine or other symptomatic intervention may be helpful. Rashes (of any grade) have occurred in approx 26% of adult

patients; however, severe (grade 4) rashes, such as erythema multiforme and Stevens-Johnson syndrome have only been reported in 0.1% of patients *(30)*. Worsening of preexistent skin conditions sometimes occurs on initiation of therapy, possibly as part of an immune reconstitution phenomenon.

Laboratory Abnormalities

Laboratory abnormalities (grade 3 or 4) in patients receiving EFV-containing regimens have occurred at a similar frequency to those in control arms, with elevated hepatic transaminases occurring in 2 to 3% of patients in both EFV and control groups. Because substitution with EFV may be useful to manage some patients experiencing severe or significant metabolic disturbances or lipodystrophy on PI-based therapy *(95)*, it is important to consider changes in lipids during EFV therapy. In study 006, rises in total cholesterol were observed in all treatment groups, but were greater in patients receiving EFV plus IDV. Rises in total cholesterol that plateaued by week 8 were observed in the EFV-based triple-therapy group. This increase was partially accounted for by a rise in the cardioprotective high-density lipoprotein cholesterol level, which continued to rise through week 24 *(96)*. In comparison, rises in total cholesterol with PI-based therapy were primarily related to the more deleterious low-density lipoprotein cholesterol.

Comparative Tolerability vs PI-Based Regimens

Direct comparison of the tolerability of EFV-based regimens with standard-of-care (IDV or nelfinavir based) regimens is available from studies 006 and ACTG 364 (Table 5). Although CNS symptoms were more common with the EFV-based regimens, adverse gastrointestinal events were more evident with the PI-based regimens. Overall, EFV-based triple therapy seems better tolerated in initial therapy than the established standard-of-care regimens.

CURRENT PLACE OF EFV IN THERAPY

Clinical trials of EFV administered in combination with two NRTIs have established that this approach results in similar or greater proportions of patients who were either PI and NNRTI naive or were NRTI experienced, achieving optimal antiviral responses during periods of 168 wk, in comparison with the standard-of-care approach of a PI plus two NRTIs. These data have led to EFV plus one of several double-agent NRTI regimens being afforded a “strongly recommended” rating in the US Department of Health and Human Services guidelines for initial treatment of established HIV infection in adults and adolescents *(72)*. In addition, studies in which EFV was substituted for a PI (e.g., for reasons of tolerance, toxicity, or convenience) in patients who had achieved viral load suppression on a PI-containing regimen have shown that a

Table 5
Percentages of Patients With Adverse Events of Moderate or Severe Intensity Occurring in at Least 2% of Patients in Studies 006 and ACTG 364 Comparing EFV-Based Regimens With Other (PI-Based) Antiretroviral Regimens (30)[a]

	Study 006[b]			Study ACTG 364[c]		
Adverse events	EFV +ZDV +3TC (n = 412)	EFV +IDV (n = 415)	IDV +ZDV +3TC (n = 401)	EFV +NRTIs (n = 65)	EFV +NFV +NRTIs (n = 64)	NFV +NRTIs (n = 66)
CNS symptoms						
Dizziness	8	8	3	6	2	6
Headache	7	4	4	2	5	3
Impaired concentration	5	2	0	0	0	0
Insomnia	6	7	3	0	0	2
Abnormal dreams	3	1	0	…	…	…
Somnolence	3	2	2	0	0	0
Anorexia	1	0	1	2	0	2
Psychiatric symptoms						
Anxiety	1	3	0	…	…	…
Depression	2	1	0	0	3	5
Nervousness	2	2	0	0	2	2
Skin manifestations						
Rash	13	20	7	5	9	9
Pruritus	0	1	1	5	9	9
Increased sweating	2	1	0	0	0	0
Gastrointestinal symptoms						
Nausea	12	7	25	2	3	2
Vomiting	7	6	14	…	…	…
Diarrhea	6	8	6	3	14	9
Dyspepsia	3	3	5	0	0	2
Abdominal pain	1	2	4	3	3	3
Body as a whole symptoms						
Fatigue	7	5	8	2	0	3
Pain	1	1	5	6	13	17

[a]NFV, nelfinavir; …, not specified
[b]Includes adverse events possibly related to the study medication or of unknown relationship
[c]Includes all adverse events regardless of relationship to the study medication

greater proportion of patients maintain viral suppression on the EFV-containing regimen in comparison with the previous PI regimen. Although decreases in plasma lipid levels were recorded in some of these studies, it has not yet been established whether switching patients taking PIs to EFV will result in a decreased risk of lipodystrophy syndrome or, perhaps, reverse established morphological changes.

EFV may well have a role in salvage therapy. Its use in salvage therapy (n = 52) was compared with that in first-line therapy (n = 27) or in those receiving EFV in the setting of previous NRTI therapy (n = 28). In an ITT analysis, only one patient receiving EFV in salvage therapy had maximal virological suppression at 15 mo, as compared with 31 of 79 other patients *(94)*. Thus, the maximal benefit of EFV will be achieved in the earlier courses of therapy.

Although greater than 50% of patients experience mild-to-moderate adverse events, notably CNS symptoms and skin rashes during the first weeks of EFV therapy, few patients discontinue therapy because of adverse events. Based on more recent data, this holds true for long-term therapy (3 yr or longer). Thus, once the initial weeks of therapy are complete, EFV, as with other NNRTIs, seems remarkably well-tolerated. EFV has the additional advantage of a once-daily dosing regimen that involves taking only a single capsule or tablet per day. Its long plasma half-life (40–55 h) provides the additional advantage of reducing the likelihood that subtherapeutic trough concentrations will occur because of occasional missed doses or pharmacokinetic variability. This may decrease the risk of emergence of resistance, although we need to be cautious regarding such risk at the time of treatment discontinuation, when a period of EFV monotherapy may last a few weeks. Resistance may well occur if viral replication resumes to a high level before EFV has been cleared from the blood.

Other clinical scenarios in which emerging data suggest a role for EFV include its use in combinations with PIs, both in treatment-naive patients who are unwilling or unable to receive NRTIs and in patients who have failed on previous nucleoside therapy, as well as in children previously treated with NRTIs. In the latter, a combined regimen of EFV plus nelfinavir plus one or more NRTIs seemed to provide a sustained antiviral effect and was generally well-tolerated.

In summary, EFV may be considered an equipotent alternative agent to PIs for initial treatment regimens in HIV-infected patients, and potentially for other regimens, offering the benefits of once-daily administration, a low pill count, and relatively benign side-effect profile.

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Delavirdine

Brian Conway

INTRODUCTION

During the past decade, the use of highly active antiretroviral therapy (HAART) has led to a significant decrease in morbidity and mortality associated with HIV infection. In one US study of 1255 patients in the early era of HAART, mortality declined from 29.4 per 100 person-years in the first quarter of 1995 to 8.8 per 100 person-years in the second quarter of 1997 *(1)*. A Canadian population-based cohort study also confirmed that patients initially treated with a triple-drug antiretroviral regimen had a 2.37-fold lower risk of morbidity and death after 12 mo than patients receiving double nucleoside analog therapy in the pre-HAART era *(2)*. Beginning in 1997, expert panels began recommending aggressive, early initiation of antiretroviral therapy, consisting of combinations of three drugs, with a goal of suppressing plasma viral load to levels below those accurately quantitated using our most sensitive assays *(3)*. It was initially proposed that the maintenance of plasma viral load at such levels for as little as 3 yr may actually "cure" HIV infection *(4)*. Shortly after this hypothesis was generated, it was clearly shown that, in patients in whom eradication of HIV would have been expected to be imminent, live virus was readily cultured from circulating mononuclear cells *(5,6)*. More recent studies have documented that the half-life of the latent reservoir that contains replicant-competent virus (that would begin replicating after HAART was discontinued) approaches 44 mo; at this rate, its eradication would require longer than 60 yr of treatment *(7)*. Thus, the model-of-care returns to long-term (perhaps lifelong) use of antiretroviral therapy, with careful monitoring of CD4 cell counts and plasma viral load to evaluate the efficacy of treatment.

More recently, we have become aware of the potential for long-term toxicity with HAART *(8,9)*, a consideration that may outweigh any benefit treatment may confer in certain populations. Indeed, outside of certain groups (such as pregnant women and selected individuals with acute HIV infection), it is now recommended that the initiation of therapy be delayed until there is clear evidence of

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progression of immune disease, as ascertained by a CD4 count of fewer than 350 cells/mm^3 and a plasma viral load greater than 30,000 copies/mL *(10)*. It has also been suggested that disease progression and death are mostly limited to individuals who wait until their CD4 counts are fewer than 200 cells/mm^3 before starting treatment *(11)*.

As increasing numbers of compounds are available in all drug classes (nucleoside reverse transcriptase inhibitors [NRTIs], non-nucleoside reverse transcriptase inhibitors [NNRTIs], protease inhibitors [PIs], and fusion inhibitors), there is a need to review all possible options to ensure an appropriate selection of initial and follow-up regimens for HIV-infected patients throughout the long course of their disease. Because therapy may be started at a time when the patient is at real risk of short-term disease progression, it is important that we select a combination that will be maximally effective, with other characteristics (pill count, short and long-term toxicity, and so on) considered to facilitate long-term adherence.

With this in mind, the current chapter focuses on one of the NNRTIs, delavirdine. Its preclinical and clinical development will be reviewed, with a view to establishing its role in the current, complex environment of antiretroviral therapy.

MECHANISM OF ACTION AND IN VITRO ACTIVITY

The process of reverse transcription is an essential step in the life cycle of HIV, necessary for viral replication *(12)*. The first reverse transcriptase inhibitors shown to be effective in vivo were NRTIs, particularly zidovudine *(13,14)*. However, the toxicity of these compounds was readily apparent *(15)*, and the emergence of drug-resistant strains was also problematic *(16)*. It was further reasoned by many researchers that the successful treatment of HIV infection would require reverse transcriptase inhibitors with different mechanisms of action, possibly to be administered in combination with one another. The *bis*(heteroaryl)piperazine (BHAP) class of reverse transcriptase inhibitors were discovered by a computer-directed, broad-based screening of the Upjohn chemical repository *(17)*. To discover the initial lead compound, approx 1500 compounds were screened, and 100 of these were found to inhibit recombinant HIV-1 reverse transcriptase enzyme in vitro *(18)*. Additional screening was then undertaken based on the following criteria:

- Low level of inhibition of cellular DNA polymerases-α and -δ.
- Low level of cytotoxicity to human lymphocytic cell lines.
- Antiretroviral activity as measured by the inhibition of the formation of syncytia (HIV-1_{IIIB} in MT-2 cells).

From this work, the arylpiperazine, U-80493, was identified as a lead compound, having the following characteristics:

- 50% inhibitory concentration (IC_{50}), DNA polymerase-α: 600 μ*M*.
- IC_{50}, DNA polymerase-δ: greater than 1250 μ*M*.

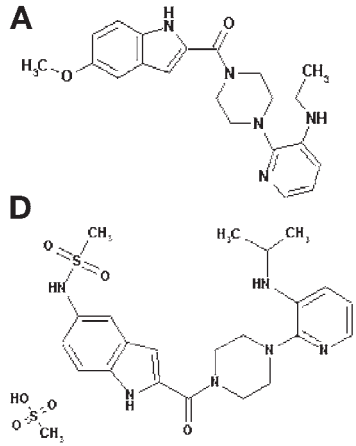

Fig. 1. Chemical structures of atevirdine **(A)** and delavirdine **(D)**.

- 50% cytotoxic concentration (CC_{50}), MT-2 cells: 15 μ*M*.
- IC_{50}, HIV-1_{IIIB} in MT-2 cells: 2 μ*M*.

A number of other derivatives were then synthesized, to enhance both selectivity and activity. Indole substitution for the aryl group resulted in greatly enhanced activity, leading to the identification of atevirdine (also known as U-87201; Fig. 1), having the following characteristics:

- IC_{50}, DNA polymerase-α: 260 μ*M*.
- IC_{50}, DNA polymerase-δ: 1976 μ*M*.
- CC_{50}, MT-2 cells: greater than 20 μ*M*.
- IC_{50}, HIV-1_{IIIB} in MT-2 cells: less than 0.2 μ*M*.

Detailed analyses revealed that the BHAP compounds specifically inhibit HIV-1 reverse transcriptase. No inhibition of HIV-2 is observed at concentrations up to 10 μ*M* *(19)*, nor is there any inhibition of HIV-1 ribonuclease H activity *(20)*. Further studies showed that BHAP compounds do not bind directly with the nucleic acid binding site of the reverse transcriptase enzyme, but rather inhibit its function in an allosteric manner *(17)*. This allosteric binding results in a stable conformational change in the polymerase site of the 66-kDa molecular weight subunit of the enzyme, restricting its flexibility and rendering it inactive *(21)*. In contradistinction with other NNRTIs, the binding of delavirdine to the enzyme is stabilized through a number of hydrophobic interactions with the proline residue at codon 236, perhaps explaining its slightly unique resistance pattern *(22)*. Careful kinetic studies showed that BHAP compounds seem to impair an event occurring after the formation of the enzyme–substrate complex, which involves either inhibition of phosphoester bond formation or translocation of the enzyme after the formation of the ester

bond *(23)*. Consistent with these different modes of action, in vitro evaluations consistently show synergistic activity with zidovudine *(17,24)*. Further in vitro experiments using peripheral blood mononuclear cells (PBMCs) infected with clinical isolates of HIV-1 showed effective dose causing 50% inhibition (ED 50) values of 10 n*M* or less, comparable to that of zidovudine in similar systems *(17)*. In general, drug concentrations that were cytotoxic to 50% of a given culture of cells in which the virus was grown (CC_{50}) were 10^3- to 10^4-fold higher. Preliminary experiments with atevirdine in a SCID-hu mouse model showed that administration of the drug before inoculation of the mice with HIV-1 prevented infection in three of eight animals *(17)*, which represented hopeful early data, but suggested that further work was needed to enhance antiretroviral potency. Indeed, using the same experimental model, zidovudine prevented infection in five of five animals.

Following the principles of rational chemical design, delavirdine (also known as U-90152S; Fig. 1) was synthesized. The IC_{50} for recombinant HIV-1 reverse transcriptase was 0.26 μ*M*, as compared with greater than 440 μ*M* for DNA polymerase-α and -δ. Delavirdine does not inhibit mitochondrial DNA polymerase-γ *(25)*. The IC_{50} for inhibition of syncytium formation in MT-2 cells was 10 n*M*, approx 10-fold or lower than measured for atevirdine *(25)*. In human PBMC cultures acutely infected with HIV-1_{JRCSF}, the IC_{50} was 8 n*M* *(26)*. The IC_{50} against a panel of 25 primary HIV-1 isolates was 66 (range, 5–690) n*M* *(24)*, with clear synergy demonstrated with NRTIs, including zidovudine, didanosine, and lamivudine *(27,28)*. Activity was retained against isolates that were resistant to zidovudine and didanosine. Regarding atevirdine, the CC_{50} of delavirdine in PBMCs was quite high (>100 μ*M*). In experiments assessing inhibition of the spread of HIV-1_{IIIB} in cell cultures, 3 μ*M* delavirdine was completely effective in blocking transmission of viral infection (500 infected MT-4 cells incubated with uninfected cells at a 1:1000 ratio). When the drug was removed from the culture after 24 d, viral regrowth did not occur *(24)*. In the same model, 3 μ*M* zidovudine only delayed the emergence of infection. It should be noted that in vitro selection experiments led to the identification of a novel mutation (P236L) that conferred resistance to delavirdine, but increased susceptibility to other agents, such as nevirapine *(29)*. Taken together with the promising in vitro efficacy and preclinical data, this suggested that further development of delavirdine in the clinic was warranted.

PHARMACOKINETIC PROFILE

The pharmacokinetic profile of delavirdine, which exhibits significant interindividual variability, has been well studied in adults (age 16–65 yr), but, unfortunately, little or no data exist in younger or older patients, or in patients with renal or hepatic dysfunction *(30)*. Delavirdine is rapidly absorbed after

oral administration, with 85% bioavailability, at least in normal volunteers *(30)*. Food does not significantly modify the steady-state pharmacokinetics of delavirdine at therapeutic dosages, but coadministration of antacids reduces most pharmacokinetic parameters by 50% or more *(31)*. Once absorbed, delavirdine is 98% protein bound, mainly to albumin in a nondose-dependent manner *(32)*. As a result of this, central nervous system penetration is quite poor, with concentrations less than 0.5% of those achieved in the plasma *(33)*. The drug is metabolized by desalkylation and 6´-hydroxylation by the hepatic cytochrome P (CYP) 450 enzyme, CYP3A4, and, to a lesser extent, by desalkylation by CYP2D6 *(34)*. It is a moderate inhibitor of CYP3A4, with an IC_{50} of 0.9 μ*M* *(34)*. Interestingly, it is also an inhibitor of CYP2D6, CYP2C9, and CYP2D19 *(35)*. Because these isoforms are subject to significant polymorphic expression (with CYP2C19 being absent in up to 5% of white subjects), this may help explain the great variability in delavirdine pharmacokinetics observed in clinical trials. The steady-state pharmacokinetic profile of delavirdine is nonlinear up to the current daily dose of 1200 mg, with a half-life after multiple 400-mg doses of 4.12 h *(36)*. The mean peak drug concentration at steady state (achieved 1–2 h after administration with dosing at 400 mg delavirdine thrice daily) is approx 35 μ*M* *(37)*. Trough levels exceed 10 μ*M*, a concentration 100-fold greater than the IC_{50} (the drug concentration required to inhibit replication of drug-sensitive isolates by 50%). This is not affected by race, but some data suggest that there may be slower clearances in women *(38)*. In a study of 18 normal volunteers, administration of 600 mg delavirdine twice daily produced steady-state peak and trough concentrations of 33 and 13 μ*M* *(39)*, comparable to levels achieved with more frequent dosing.

More recent data have been generated from a detailed retrospective analysis of two large AIDS Clinical Trials Group (ACTG) clinical trials *(40)*. Race, sex, age, and weight were all associated with variability in drug levels and volumes of distribution, with the coefficient of variability for the volume of distribution reaching 100%. No single parameter was predictive of drug levels, therefore intrinsic interindividual variability seems to be the rule rather than the exception. If this agent is used more widely, it may be an ideal candidate for studies of therapeutic drug monitoring because of this fact.

A summary of drug interactions with delavirdine has been published elsewhere *(41)*, and is nicely presented in a continuously updated format at www.hiv-druginteractions.org. As would be expected, coadministration of delavirdine increases circulating levels of amprenavir, indinavir, nelfinavir, ritonavir, and saquinavir *(39)* (Table 1). However, nelfinavir causes a significant reduction in peak delavirdine levels as well as a less-significant reduction in total drug exposure *(42)*, and the efficacy of this combination would have to be carefully evaluated in clinical trials before its use could be recommended in

Table 1
Expected Effect of Delavirdine on Selected Pharmacokinetic Parameters of PIs When Used at Recommended Doses in Clinical Practice[a]

Drug	Trough concentration	Peak concentration	Drug exposure (AUC)
Amprenavir	↑ 500%	↑ 30%	↑ 300%
Indinavir	No change	↑ 20–50%	↑ 44–70%
Nelfinavir	↑ 80%	↑ 66%	↑ 100%
Ritonavir	↑ 113%	↑ 50%	↑ 60–80%
Saquinavir	↑ 20%	↑ 115%	↑ 114–121%

AUC, area under the curve. ↑, increased
[a]Only single-dose studies are available for amprenavir
(Data adapted from refs. *38*, *39*, *43–45*, and *49*.)

practice. The interaction with indinavir is most attractive from a clinical perspective. In a single-dose crossover study in normal volunteers taking 400 mg delavirdine thrice daily for 10 d, indinavir drug exposure was measured after a single indinavir dose of 800 mg administered on day 1 (before the administration of delavirdine), with a 600 mg dose administered on day 10 *(43)*. Drug exposure to indinavir was 44% higher on day 10, despite the lower dose. In another study (again in normal volunteers), the combination of 800 mg indinavir twice daily with 600 mg delavirdine twice daily (administered with or without food) produced similar pharmacokinetic characteristics (including trough indinavir concentrations) to those achieved with the standard thrice-daily dosing of indinavir *(44)*. This has since been confirmed in a small group of HIV-infected individuals receiving zidovudine plus delavirdine plus indinavir *(45)*. It can, thus, be stated with some confidence that the combination of 600 mg delavirdine twice daily with 800 mg indinavir twice daily (with or without food) could be appropriately used in clinical practice, although newer PIs with more favorable efficacy and toxicity profiles have largely supplanted the previously widespread use of indinavir in clinical practice.

The combination of delavirdine and saquinavir has been formally evaluated within a clinical trial. In a group of 11 patients receiving the soft-gel formulation of saquinavir (Fortovase®), 1400 mg twice daily along with 600 mg delavirdine twice daily, the saquinavir peak and trough levels were increased 115% and 20%, respectively, as compared with saquinavir levels measured in 10 patients receiving Fortovase along with two nucleoside analogs within the same study *(46)*. In a similar vein, in a nonblind, crossover study of 12 normal volunteers, administration of 600 mg delavirdine twice daily for 7 d led to a sixfold increase in trough levels and a fourfold increase in drug exposure to amprenavir, after administration of a single 1200-mg dose of amprenavir *(41)*. Similar results have been observed in six children receiving these two agents

along with two nucleoside analogs *(47)*. However, the benefit of the interaction may be short-lived, because some data suggest that amprenavir levels may be decreased by as much as 70% during 12 mo *(48)*, likely caused by ongoing changes in drug metabolism over time. The benefit of the interaction between delavirdine and amprenavir may be of less clinical significance.

The interaction of ritonavir with delavirdine was initially studied in normal volunteers, and no effects on ritonavir concentrations were reported *(49)*. However, it should be noted that the maximal tolerated dose of ritonavir was 300 mg twice daily. More recently, an elegant crossover study was conducted in normal volunteers to determine the precise nature and magnitude of the interaction between delavirdine and ritonavir *(50)*. Subjects were assigned to receive either 600 mg delavirdine twice daily or 100 mg ritonavir twice daily for 10 d, and then were placed on a combination of the two drugs for an additional 10 d, at which time, formal pharmacokinetic studies were undertaken in the 19 of 22 subjects that completed the study. Delavirdine increased ritonavir peak and trough levels by 50% and 113%, respectively, and increased drug exposure by 80%. In a study of 14 HIV-infected individuals taking full doses of both ritonavir and delavirdine, exposure to ritonavir was increased by 60% *(51)*. Therefore, if the two drugs are to be used together, care should be taken to monitor for ritonavir toxicity. Alternatively, it may be that this strategy will lead to synergistic enhancement of drug levels of other agents that would be used as part of a combination regimen, such as with saquinavir, especially in more advanced courses of therapy, in which pharmacokinetic advantages may help overcome partial levels of resistance that have developed in isolates exposed to many agents in all three classes of drugs. This issue requires further study within clinical trials.

The studies of the interaction of delavirdine with lopinavir (coformulated with ritonavir) are, unfortunately, limited. In three patients, there were increases in drug exposure of 8.2 to 134% *(52)*. Because of this great interindividual variability (albeit in very few patients), no conclusions can be made about the potential clinical usefulness of this interaction.

Although no specific interaction exists with nucleoside analogs, delavirdine cannot be administered simultaneously with the older formulation of didanosine, because the latter agent is formulated with a buffer that would raise gastric pH and delay absorption of delavirdine *(53)*. This being said, if 1 h or longer separates dosing, there is no interaction. The effect of the enteric-coated didanosine on delavirdine absorption has not been studied. However, its lack of interaction with indinavir and ketoconazole *(37)* suggests that the levels of delavirdine are unlikely to be affected by the simultaneous administration of enteric-coated didanosine. One group described a significant effect of the nucleotide analog adefovir (related to the currently available tenofovir) on

Table 2
Expected Effect of Selected Drugs on Pharmacokinetic Parameters of Delavirdine

Drug	Effect on delavirdine levels	Recommended action
Carbamazepine	Significant decrease	Combination contraindicated
Clarithromycin	No effect	None
Fluconazole	No effect	None
Methadone	No effect	None
Phenobarbital	Significant decrease	Combination contraindicated
Phenytoin	Significant decrease	Combination contraindicated
Rifabutin	Significant decrease	Combination contraindicated
Rifampin	Significant decrease	Combination contraindicated

(Data adapted from refs. *39* and *54–58*)

delavirdine drug concentrations, lowering them by 50% *(54)*. The clinical significance of this finding and its applicability to tenofovir remain uncertain.

Although one could expect an interaction between delavirdine and the other NNRTIs, this has never been evaluated, because the role (if any) of the combination of delavirdine with either efavirenz or nevirapine has never been defined.

Additional pharmacokinetic interactions between delavirdine and compounds other than antiretroviral agents are worthy of mention (Table 2). Methadone coadministration does not seem to alter delavirdine levels in any way *(55)*, although delavirdine could be expected to increase methadone levels if the two agents were administered together. This latter interaction would have to be better understood if delavirdine were to be used in patients in methadone programs. Administration of rifampin or rifabutin increases delavirdine clearance 27- and 5-fold, respectively *(56,57)*. These combinations should not be used in clinical practice. In a similar way, because of the risk of diminished delavirdine trough levels (based on limited population pharmacokinetic data), delavirdine should not be administered along with phenobarbital, phenytoin, or carbamazepine *(40)*. Interactions with clarithromycin *(58)* and fluconazole *(59)* are not significant.

ANTIVIRAL EFFICACY: EARLY CLINICAL TRIALS (TABLE 3)

As with all NNRTIs, delavirdine rapidly selects for drug-resistant isolates when used as monotherapy. In ACTG 260, a trial in which delavirdine monotherapy was administered to 115 patients, high-grade resistance emerged in 93% of patients within 8 wk *(60)*. Interestingly, the P236L mutation (that would confer enhanced susceptibility to other NNRTIs) occurred in less than 10% of patients. In a small study of 34 patients performed at Upjohn Laboratories, patients received delavirdine at doses of 400 to 1200 mg/d. These

Table 3
Selected Results of Early Clinical Trials of Delavirdine

Regimen	Sample size (*n*)	Principal finding
DLV	92	93% of patients had high-grade resistance within 8 wk
ZDV+DLV	34	↓P24 antigen levels in 25% of patients
ZDV+DDI+DLV[a]	32	HIV RNA levels ↓ by 0.5 $\log_{10}$ copies/mL

DLV, delavirdine; ZDV, zidovudine; DDI, didanosine; ↓, decreased
[a]Significant previous exposure to NRTIs
(Data adapted from refs. *24*, *32*, and *59*.)

doses were added to a background of stable zidovudine monotherapy. Despite the fact that only 10 patients received the eventual therapeutic dose of delavirdine (1200 mg/d), 25% of the total study group experienced a significant decrease in serum p24 antigen levels during the period of observation *(25)*. In the final pre-HAART study, conducted in 1993, a total of 85 patients were evaluated in a protocol designed to evaluate the potency of delavirdine and its maximal tolerated dose *(33)*. These individuals, most of whom had received previous therapy with one or more NRTIs, were variously assigned to receive delavirdine alone; delavirdine plus either zidovudine or didanosine; or the triple combination of zidovudine plus didanosine plus delavirdine. There were 32 individuals in the latter group (mean baseline CD4 count, 205 cells/mm^3; plasma viral load, 41,773 copies/mL), with 30 patients having previously received zidovudine and 19 patients having received didanosine. In addition, the dose of delavirdine varied from 400 to 1200 mg/d, depending on the timing of study entry. Despite all of these limitations, the patients in this group were the only ones to demonstrate any virological benefit, with a transient decrease in plasma viral load of 0.5 $\log_{10}$ copies/mL during the first weeks of treatment.

ANTIVIRAL EFFICACY: CLINICAL TRIALS IN THE HAART ERA

Initial trials combining delavirdine with NRTIs were conducted (Table 4). ACTG 261 was a phase II, randomized, double-blind, multicenter trial that compared three-drug combinations consisting of delavirdine plus zidovudine plus didanosine with two-drug combinations of these drugs *(61)*. Patients could have received up to 6 mo of previous therapy with either zidovudine or didanosine. Overall, the median baseline CD4 count was 295 cells/mm^3 and plasma viral load level was 28,000 copies/mL. In the triple-drug arm, a transient 1.25 $\log_{10}$ copies/mL decrease in plasma viral load was observed at week 4, with 26% of patients having plasma viral load measures of fewer than 200 copies/mL at week 12, a proportion that was relatively well-maintained to week

Table 4
Selected Results of Trials of Delavirdine in Combination With NRTIs

Study	Regimen	Sample size (*n*)	Principal finding (HIV RNA level)
ACTG 261[a]	ZDV+DDI+DLV	157	26% of patients had <200 copies/mL at week 48
0021 Part 2[a]	ZDV+DDI+DLV	34	59% of patients had <50 copies/mL at week 52
13C (Drug-naive substudy)	ZDV+3TC[b]+DLV	73	72% of patients had <500 copies/mL at week 54

ZDV, zidovudine; DDI, didanosine; DLV, delavirdine; 3TC, lamivudine; DDC, zalcitabine
[a]A minority of patients had previous exposure to NRTIs
[b]A minority of patients received DDI or DDC instead of 3TC
(Data adapted from refs. *60–63*)

48. There were no differences in the results if patients having received any previous therapy were excluded.

Protocol 0021 Part II was a double-blind, placebo-controlled, randomized trial conducted in the United States and Canada from 1996 to 1998 *(62)*. Treatment-naive patients (up to 6 mo of previous zidovudine therapy was allowed) were randomized to receive zidovudine plus delavirdine, zidovudine plus lamivudine, or the combination of all three agents (zidovudine plus lamivudine plus delavirdine). A total of 373 patients were enrolled (mean baseline CD4 count, 355–362 cells/mm^3; plasma viral load, 21,877–31,622 copies/mL; depending on the group). Previous zidovudine usage was noted in 58 (15.5%) study subjects, including 25 (20.2%) of those randomized to triple-combination therapy. At week 52, the mean decrease in plasma viral load in the triple-combination group was 2.1 $\log_{10}$ copies/mL, with 20 (59%) of the 34 patients having values of fewer than 50 copies/mL at this time-point. The results did not differ if the nonnaive patients were removed from the analysis. Longer-term data are available for 16 patients, 9 (56%) of whom retained virological suppression well beyond 52 wk of observation. These results parallel those of the Italy, the Netherlands, Canada, and Australia Study (INCAS), a protocol evaluating nevirapine in a similar setting *(63)*.

Study 13C was a double-blind, placebo-controlled, randomized trial conducted in Europe and South Africa. Study subjects (60% of whom were naive to antiretroviral therapy) were assigned to receive zidovudine and a second NRTI (didanosine, zalcitabine, or lamivudine), in combination with either delavirdine or a matching placebo *(64)*. A total of 345 patients were enrolled (median baseline CD4 count, 221–241 cells/mm^3; and plasma viral load, 91,200–97,700 copies/mL, depending on the group). The second NRTI was

Table 5
Selected Results of Trials of Delavirdine in Combination With NRTIs and PIs

Regimen	Sample size (*n*)	Principal finding (HIV RNA level)	Study reference
(ZDV+DLV+IDV	45	60% of patients had <50 copies/mL at week 48	*65*
3TC+DLV+IDV	57	54% of patients had <50 copies/mL at week 48	*66*
(ZDV or D4T)+ DLV+IDV	47	33% of patients had <500 copies/mL at week 26	*67*[a]
ZDV+DLV+IDV	31	83% of patients had <200 copies/mL at week 48	*68*
NRTI+DLV+FTV	70	57% of patients had <50 copies/mL at week 24	*69*
NRTI+DLV+NFV	130	84% of patients had <400 copies/mL at week 24	*71*
DLV+PI(s)±ADV	185	40% of patients had <500 copies/mL at week 16	*72*[b]
NRTIs+DLV+IDV	26	35% of patients had <200 copies/mL at week 12	*73*[c]
NRTI(s)+NNRTI +NFV	92	54% of patients had <50 copies/mL at week 60	*74*[d]

ZDV, zidovudine; DLV, delavirdine; IDV, indinavir; 3TC, lamivudine; D4T, stavudine; FTV, Fortovase; NFV, nelfinavir; ADV, adefovir

[a]DLV as add-on monotherapy to failing IDV-based regimen

[b]Virological breakthrough in patients taking an IDV-based regimen

[c]Previous exposure to multiple NRTI/PI-based regimens

[d]DLV or EFV administered in patients with previous exposure to multiple NRTI/PI-based regimens

(Data adapted from refs. *64–73*)

lamivudine in 64% cases, with zalcitabine (19%) and didanosine (17%) being used less frequently. At 1 yr, the mean decrease in plasma viral load was 1.8 $\log_{10}$ copies/mL in the patients taking triple therapy, with 40% of patients having values of fewer than 50 copies/mL. A subanalysis was performed, focusing on the patients that were naive to therapy before entering the study. There were 73 patients assigned to triple therapy (median baseline CD4 count, 211 cells/mm^3; plasma viral load, 200,000 copies/mL). At week 54, the median decrease in plasma viral load was 2.16 -$\log_{10}$ copies/mL, with 72% of patients having measures of fewer than 500 copies/mL.

To evaluate the clinical benefit of the positive effect of delavirdine on circulating levels of various PIs, a number of controlled trials were undertaken (Table 5). Protocol 63 was a randomized controlled pilot study of 45 patients

with limited (<1 mo) previous exposure to zidovudine who were randomized to receive various combinations of zidovudine, delavirdine, and indinavir, the latter being administered thrice daily at doses of 400, 600, and 800 mg *(65)*. Baseline CD4 counts were 305 to 391 cells/mm^3 and plasma viral loads were 83,000 to 96,000 copies/mL, depending on the group. After 48 wk, 27 (60%) of the 45 patients achieved virological suppression (<50 copies/mL), with equivalent virological suppression at all indinavir doses.

In a larger study of 225 patients with limited previous exposure to NRTIs, combinations of zidovudine, lamivudine, delavirdine, and indinavir were evaluated *(66)*, with the indinavir dosed thrice daily at 600 mg when administered in combination with delavirdine. A control arm of zidovudine plus lamivudine plus indinavir was included. Baseline CD4 counts were 229 to 299 cells/mm^3 and plasma viral loads were 79,000 to 126,000 copies/mL, according to the group. The best virological response (54% of patients having values of fewer than 50 copies/mL at 48 wk, intent-to-treat analysis) was observed in patients receiving lamivudine plus delavirdine plus indinavir, compared with 46% of subjects enrolled in the control arm, receiving full-dose indinavir.

In an interesting observational study, delavirdine was added to a treatment regimen that included 800 mg indinavir thrice daily plus NRTIs in patients experiencing a virological breakthrough while taking combination therapy *(67)*. A total of 47 patients were enrolled (mean baseline CD4 count, 127 cells/mm^3; plasma viral load, 100,000 copies/mL). In 21 patients, delavirdine was simply added to a regimen of indinavir plus lamivudine plus another NRTI (zidovudine or stavudine). In the 26 other patients, zidovudine was changed to stavudine at the same time. On average, the patients had been taking indinavir for 6 mo, usually showing an initial response to therapy (six patients even achieving levels of fewer than 500 copies/mL) before virological breakthrough was documented. After 6 mo, 12 of the 36 patients (33%) had plasma viral load measures of fewer than 500 copies/mL, with 18 (50%) patients experiencing a greater than 1.0 $\log_{10}$ copies/mL decrease during the period of observation. This response is out of proportion for what would be expected from the addition of a single agent to which resistance readily develops in vivo. It was hypothesized (although not proven) that the observation was caused by the enhancement of indinavir potency through increased blood levels.

ACTG 370 was a randomized, open-label, multicenter study designed to compare the virological activity of continued lamivudine vs a switch to delavirdine when initiating PI therapy in NRTI-experienced patients *(68)*. Individuals who had received lamivudine along with another NRTI (either zidovudine, didanosine, or stavudine) and had a detectable plasma viral load were eligible for inclusion in the study. All study participants were naive to both NNRTIs and PIs, and were randomized to receive zidovudine plus indinavir plus either

lamivudine ($n = 33$) or delavirdine ($n = 31$). Median baseline CD4 counts were 497 to 528 cells/mm^3 and plasma viral loads were 3300 to 3500 copies/mL, depending on the group. At week 48, 83% of patients taking delavirdine had plasma viral load measures of fewer than 200 copies/mL, as compared with only 48% in the other group ($p = 0.007$). Steady-state plasma indinavir levels were higher in patients taking delavirdine, and this boosting effect could have explained at least some of the results. The patients taking delavirdine were also receiving a drug from a new class, a beneficial intervention in itself, especially in patients with such modest plasma viral load measures.

The combination of delavirdine and saquinavir was studied in a prospective, randomized, clinical trial that included 97 drug-naive patients *(69)*. Subjects received saquinavir (1400 mg Fortovase twice daily) plus 600 mg delavirdine twice daily and 150 mg lamivudine twice daily, or a quadruple-drug combination that also included 600 mg zidovudine twice daily, or a control regimen consisting of zidovudine plus lamivudine plus saquinavir (1200 mg Fortovase thrice daily). Mean baseline CD4 counts were 224 to 280 cells/mm^3 and plasma viral loads were 134,000 to 353,000 copies/mL, depending on the group. After 24 wk, 43 to 64% of patients had plasma viral load levels of fewer than 50 copies/mL (intent-to-treat analysis), with no significant differences between the study groups. It would be interesting to see an additional evaluation of the combination of 1400 mg saquinavir twice daily and 600 mg delavirdine twice daily in different settings to further validate the effectiveness of this combination. This might be particularly interesting with double-boosted PIs, in which saquinavir seems to present certain advantages *(70)*, that could be further enhanced in a delavirdine-containing regimen.

In continuing to evaluate the use of delavirdine with PIs in clinical practice, protocol 73 was initiated *(71)*. A total of 173 patients (with limited exposure to NRTIs) were enrolled in this randomized, controlled study and were assigned to receive combinations of 1250 mg nelfinavir plus 600 mg delavirdine twice daily with either didanosine, stavudine, or both (three study arms); a fourth arm was a comparator regimen of the two NRTIs plus nelfinavir. Mean baseline CD4 counts were 275 to 341 cells/mm^3 and plasma viral loads were 64,000 to 89,000 copies/mL, depending on the group. After 24 wk, 81 to 89% of study subjects had plasma viral load levels of fewer than 400 copies/mL. This very encouraging result does not suggest that the negative pharmacokinetic effect of nelfinavir on delavirdine peak levels and drug exposure are necessarily of clinical significance in all patients.

In a more extensive evaluation of the combination of delavirdine plus nelfinavir, protocol ACTG 359, a prospective, randomized, 2×3 factorial, multicenter study, was initiated *(72)*. A total of 277 patients were enrolled (median baseline CD4 count, 229 cells/mm^3; plasma viral load, 31,746 copies/mL). All

subjects had been taking an indinavir-containing regimen for at least 6 mo and were naive to NNRTIs. Treatment consisted of saquinavir plus either ritonavir or nelfinavir, with the two PIs taken with delavirdine, adefovir, or both (a total of six treatment groups). After 16 wk, 30% (77/254) of patients had plasma viral load measures of fewer than 500 copies/mL, which was the primary endpoint of the study. Virological response was greater in the pooled delavirdine groups (40% vs 18%; $p = 0.02$), indicating the benefit of adding the NNRTI in this setting. It may be that the positive pharmacokinetic interaction of delavirdine with PIs would give it an advantage over efavirenz and nevirapine in this setting, but this issue should be addressed in a randomized clinical trial.

There has been increasing attention to the use of delavirdine in the salvage therapy setting. In one study, 26 patients were identified who had experienced a virological breakthrough taking a PI-based regimen, but were naive to NNRTIs *(73)*. The mean baseline CD4 count was 149 cells/mm^3 and the plasma viral load was 76,025 copies/mL. Study therapy consisted of two NRTIs plus 600 mg delavirdine twice daily plus 800 mg indinavir twice daily, taken with food. Treatment-limiting toxicity was significant, with 43% (11/26) of subjects discontinuing therapy within the first 2 wk, largely because of gastrointestinal intolerance. Of the 15 remaining subjects, 9 (60%) had plasma viral load measures of fewer than 200 copies/mL. Even if one considers an intent-to-treat analysis, 9 (35%) of the 26 patients initially starting the regimen showed a maximal response, a result that is comparable to that of ACTG 359, in which a much more complex regimen was administered.

Along the same lines, a group of 92 patients having previously received an average of four NRTIs and two PIs (but naive to nelfinavir and NNRTIs) received nelfinavir plus NRTIs (according to the preference of the prescribing physician) plus either efavirenz or delavirdine *(74)*. The mean baseline CD4 count was 187 cells/mm^3 and the plasma viral load was 311,000 copies/mL. With a mean follow-up of longer than 60 wk, 50 (54%) patients achieved virological suppression (with no benefit of efavirenz over delavirdine), a figure that rises to 73.5% of patients if the 34 individuals with baseline CD4 counts higher than 200 cells/mm^3 and plasma viral load measures of fewer than 50,000 copies/mL are considered separately.

In more advanced disease, it may be that delavirdine will be useful to enhance the activity of PIs in more complex regimens. A small study of stavudine plus saquinavir plus either delavirdine or ritonavir as an agent to increase saquinavir blood levels (with a control arm in which nelfinavir was used as the third drug) was undertaken in patients with a median baseline CD4 count of 370 cells/mm^3 and plasma viral load measures of 4000 copies/mL *(75)*. After 6 mo, virological suppression was superior in the patients taking ritonavir (–0.71 $\log_{10}$ copies/mL), compared with those taking delavirdine (–0.29 $\log_{10}$

copies/mL). In fact, there did not seem to be any benefit of delavirdine over nelfinavir. In a study of 10 patients having experienced virological breakthrough taking at least three previous treatment regimens, subjects were administered two NRTIs plus a combination of delavirdine plus ritonavir plus indinavir *(76)*. Beginning with a baseline plasma viral load of greater than 359,300 copies/mL, 8 of 10 subjects experienced some virological suppression, with a decrease exceeding 1.0 $\log_{10}$ copies/mL in 4 cases. Data in children are limited. In one study of 14 patients (aged 5 mo–15 yr) receiving delavirdine (12–28 mg/kg) plus two unspecified NRTIs, 9 subjects experienced an increase in CD4 cell counts during the 8 wk of observation *(77)*.

TOXICITY AND ADVERSE EVENTS

The most frequent adverse events reported in patients enrolled in clinical trials of delavirdine were headache, fatigue, gastrointestinal complaints, and rash. Slightly more than one-third of patients receiving delavirdine had a rash. In North American and European trials, rash was the only side effect found to be associated with delavirdine by the various safety monitoring boards *(25)*. This rash usually occurs 1 to 2 wk after therapy is started, is maculopapular in nature, and is more common in patients with more advanced immune disease. In 85% of cases, treatment can be continued, and the rash usually resolves during 2 wk. In the first 1000 patients, only one case of mild Steven's Johnson syndrome (with no long-term sequelae) was noted *(25)*.

According to the manufacturer, skin rash occurred in 18 to 50% of patients in phase II/III clinical trials, with 4.3% of patients discontinuing therapy for this reason *(30)*. Severe or life-threatening rashes were only rarely reported, and usually resolved after discontinuation of the drug.

An interesting question to address is whether patients who develop a rash after therapy with one NNRTI can be successfully treated with another drug in this class. A study of 89 patients receiving either delavirdine or nevirapine was conducted *(78)*. Overall, 24 patients (27%) developed a rash that required interruption of therapy, with 5 patients (all taking nevirapine) requiring hospitalization. Rash recurred in 6 of 8 patients reinitiating the same agent, as compared with 7 of 10 patients who crossed over to the other drug. This suggests there is probably little value in attempting to retreat patients with other drugs in the class as compared with reinitiating the same drug if there is an absolute need to use the NNRTI in the regimen.

Pooled data from eight clinical trials has failed to demonstrate an association between delavirdine and any form of hepatic toxicity *(79)*. According to the American Rescriptor® package insert, grade 3 to 4 hepatic toxicity was observed in 5.8% (51/881) of patients receiving delavirdine in two large controlled trials of delavirdine in combination with NRTIs. This compared with a rate of 4.3%

Table 6
The Role of Delavirdine in Clinical Practice

1.	First-line therapy: selected role in patients with a high risk of hepatic toxicity wishing to avoid PI-based regimens.
2.	Second-line therapy: potentially important role in triple-class regimens after virological breakthrough in patients taking a PI-based regimen.
3.	Salvage therapy: potentially important role to enhance the efficacy of double PI-based regimens in patients with limited therapeutic options.

in the control arms, for a delavirdine-attributable rate of less than 2%. In a review of 21 adult antiretroviral therapy trials conducted by the ACTG, grade 3 to 4 hepatic toxicity was observed frequently among patients receiving NNRTIs *(80)*. However, those receiving nevirapine or efavirenz were significantly more likely to experience this level of toxicity than individuals receiving delavirdine, with odds ratios of 2.5 to 2.7 ($p < 0.01$). In a recent study of 272 patients receiving NNRTIs in the New York City, NY area, 84 (31%) patients experienced hepatic toxicity of any grade *(81)*, equally distributed among patients receiving any of the three drugs in this class. However, every case of grade 3 to 4 toxicity was seen in patients taking efavirenz or nevirapine.

The incidence of metabolic disorders (including lipodystrophy) in patients receiving delavirdine has been assessed in a retrospective review of eight controlled trials *(82)*. In all cases, there was no risk of such disorders associated with the addition of delavirdine to any regimen. Further, in a small study of 8 patients receiving delavirdine, significant increases in high-density lipoprotein cholesterol levels were observed during the first 2 mo of therapy, as compared with levels measured in 10 patients starting a new regimen (without delavirdine) at the same time *(83)*.

Data in children are limited. In one study of 14 patients *(67)*, the only side effects that were reported were rash (40%, grade 1–2 only) and vomiting (40%).

THE ROLE OF DELAVIRDINE IN CLINICAL PRACTICE (TABLE 6)

According to its package insert, delavirdine is indicated for the treatment of HIV infection in combination with at least two other agents when antiretroviral therapy is warranted from a clinical perspective. Of seven women who became pregnant while receiving delavirdine as part of a clinical trial, three had ectopic pregnancies, one delivered an infant with a small muscular ventricular septal defect, and three had normal deliveries. In light of this (along with limited animal data suggesting teratogenicity), delavirdine should be avoided in pregnancy *(30)*.

Although the established dose is 400 mg delavirdine thrice daily, a number of studies have supported the administration of 600 mg delavirdine twice daily, both from a clinical and pharmacokinetic perspective.

Delavirdine is very well-tolerated, with an extremely low incidence of severe skin rashes, and, in some studies, the lowest rate of hepatic toxicity of all of the NNRTIs. In addition, because of pharmacokinetic interactions, it may allow for the administration of certain PIs (especially saquinavir and indinavir) at lower doses, with a longer dosing interval and without regard to food.

A number of options are currently available for the use of delavirdine in initial HAART regimens. It is unfortunate that limited data are available on the short- and long-term potency of delavirdine-based regimens in this setting. However, even if this information were available, it is unclear how much delavirdine would be used, because the other drugs in this class have a lower pill count and can be administered once a day. One could argue that delavirdine could be used in patients who cannot take either efavirenz or nevirapine for reasons of toxicity, and in whom there is a desire to avoid the use of PIs. This may include intravenous drug users who could be at increased risk of hepatic toxicity (because of intercurrent hepatitis C virus infection) and who may also experience drug interactions when receiving methadone with efavirenz and/or nevirapine. This may represent an area for future research, especially if additional data can be generated on the efficacy of delavirdine as part of initial HAART regimens.

In second-line therapy, the use of delavirdine is easier to rationalize, and is based on the clinical trial data that are available to us, such as ACTG 359. In patients having experienced a virological breakthrough on an initial NNRTI, it is likely that delavirdine will not be effective because of issues of cross-resistance. If a PI was used, it could be argued that an NNRTI should be used in the design of a triple-class regimen. If this is the case, the pharmacokinetic interactions of delavirdine with the PIs can be used to create a more effective regimen than could be put forward using the other NNRTIs. This would have to be tested in a clinical trial.

The greatest benefit of delavirdine may be in the setting of salvage therapy. Here, the viral isolates will often carry multiple resistance mutations, and the added advantage of boosting the circulating levels of PIs may make the difference between a borderline success and a borderline failure. It would be difficult to generate proper clinical trial data in this population because of its great heterogeneity. However, this does not preclude the systematic collection of clinical data to demonstrate the correlates of efficacy (such as therapeutic drug-level monitoring) that could help refine the role of delavirdine in these patients.

Although the subject of resistance to NNRTIs is discussed in more detail in Chapter 14, it is worthy of mention at this point for a number of reasons.

High-grade resistance to delavirdine develops as a result of one of several point mutations, most commonly K103N or Y181C/I *(84)*. In addition, it seems that these mutations do not lead to a significant decrease in viral replication capacity or "fitness" *(85)*, such that, once they are established, they are more likely than other genetic changes to persist in the absence of drug pressure. In this context, if delavirdine (as for other drugs in this class) were used within regimens that may not confer profound levels of virological suppression, resistance (and loss of drug effect) may occur quickly, and new genetic changes may persist for the lifetime of the patient. This should be borne in mind if the use of delavirdine is being considered, especially in heavily pretreated patients. In contrast, it may be that certain mutations at codon 190 selected by other NNRTIs may confer increased (or at least preserved) susceptibility to delavirdine because they decrease viral fitness *(86)*, possibly making delavirdine an important part of the subsequent regimen, as long as it can be paired with at least two other active agents.

The interesting phenomenon of NNRTI hypersusceptibility has been described, occurring in patients whose isolates carry a number of NRTI-resistance mutations *(85)*. In a retrospective review of more than 17,000 banked isolates, a greater than fourfold reduction in delavirdine susceptibility was identified in 10.8% of cases, significantly more frequently among viruses from NRTI-experienced/NNRTI-naive patients compared with viruses from NRTI-naive/NNRTI-naive patients *(87)*. In a study of 30 such patients, 11 patients had such hypersusceptibility to the non-nucleoside drugs, including delavirdine *(85)*. All patients were then placed on new regimens that included efavirenz, and those with hypersusceptible isolates showed an enhanced virological response to therapy. A subsequent study of 30 individuals taking efavirenz-based salvage therapy showed that the 11 patients with hypersusceptibility to efavirenz had a better virological response during 24 wk than the 19 other members of the group *(88)*. Such patients may well have benefited from the use of delavirdine (10/11 patients also showing increased susceptibility to delavirdine in vitro), both because of its enhanced efficacy and because of its beneficial effect on the blood levels of a PI that would likely be included in the regimen.

FURTHER RESEARCH (TABLE 7)

It is quite clear that the availability of a 200-mg tablet has helped address the issue of pill count that has often limited the use of delavirdine in clinical practice. A more compact tablet (perhaps 400 mg) would be of even greater benefit, and efforts should be made to develop this. However, although logical, the generation of a 300-mg tablet would be more difficult, because delavirdine does not have regulatory approval for twice-daily administration, and this would be the only rationale to create this formulation.

Table 7
Further Research

1.	First-line therapy: collection of observational, long-term data to document efficacy of delavirdine-based regimens in clinical practice.
2.	Second-line therapy: randomized, controlled clinical trial to compare delavirdine with efavirenz and/or nevirapine in NNRTI-naive individuals, especially those with increased susceptibility to NNRTIs as a result of NRTI-resistance mutations.
3.	Salvage therapy: collection of observational data in preparation for more extensive studies, which may include controlled trials.

It would be useful to generate additional data regarding the potency of delavirdine in first-line therapy, but the financial and logistical cost of such an effort would be difficult to justify. If delavirdine is to be used in this setting, it will be on a limited basis in selected populations that could be better identified in consultation with appropriate physician groups and through observational data sets.

In second-line therapy in patients naive to NNRTIs, it could be interesting to compare delavirdine with efavirenz and/or nevirapine, including a specific evaluation of isolates that have increased susceptibility to NNRTIs. It could have a definite advantage and the effort is fully justified. In salvage therapy, delavirdine could be most useful, because clinicians struggle to design regimens that could produce maximal virological suppression. It could be argued that the additional PI-boosting effect of delavirdine could be clinically significant. A more recent study on the effect of delavirdine on lipid profiles in 10 patients taking delavirdine in combination with nelfinavir showed a 35.2% increase in high-density lipoprotein cholesterol levels during 8 wk *(89)*. It may be that this benefit will be observed when delavirdine is combined with other PIs, further enhancing its attractiveness as a component of salvage therapy.

CONCLUSION

Delavirdine has now been used in clinical studies and clinical practice for almost 15 yr. Its development has been marked by a number of strategic errors and simple bad luck. However, in the absence of a cure for HIV infection, we need all of the tools at our disposal to optimize the long-term management of this disease. Delavirdine is just such a tool, with a number of characteristics that differentiate it from all other antiretroviral agents, including those currently available for use in its own class. If we are careful in reviewing these unique characteristics (and, if necessary, designing additional studies to better understand them), we will be in a better position to use this agent to improve the care we provide to those living with HIV infection.

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14

Resistance to Non-Nucleoside Reverse Transcriptase Inhibitors

F. Ramzi Asfour and Richard Haubrich

INTRODUCTION

The prevalence of non-nucleoside reverse transcriptase (RT) inhibitor (NNRTI) resistance is increasing, especially as the number of patients who have been treated with this class of agents has grown. The three Food and Drug Administration-approved NNRTIs, efavirenz, nevirapine, and delaviridine, have been shown to be potent additions to the antiretroviral (ARV) armamentarium. In many studies, these compounds have been shown to be effective components of highly active ARV therapeutic (HAART) regimens; results have been similar to or better than the comparator protease inhibitors (PIs). Resistance to the NNRTIs occurs more frequently than resistance to other classes of ARVs and is a serious limitation to their use. The use of these agents in monotherapy and in partially suppressive regimens rapidly selects for resistance, and this resistance precludes the use of the other agents in this class. When considering these agents in newly infected or ARV-naive patients, the rate of transmitted resistance, especially in areas in which NNRTIs have been used extensively, may ultimately limit the usefulness of this class of therapeutics. As the incidence of transmission of NNRTI resistance increases, resistance testing for recently infected patients should be strongly considered. The potency of these agents must be balanced against the rapid development of resistance.

RESISTANCE ASSAYS

Defining resistance to NNRTIs is straightforward with both genotype and phenotype assays. A number of genotypic changes have been associated with resistance to NNRTIs. Primary resistance mutations often confer high-level (>30-fold) phenotypic resistance, so that genotype and phenotype assays yield concordant resistance interpretations. Occasional viral isolates are encountered in NNRTI-naive patients that have low-level phenotypic resistance (<10-fold

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change [FC] in 50% inhibitory concentration [IC_{50}]). These isolates do not have the primary resistance mutations often found with higher levels of resistance (i.e., K103N). These low level phenotypic shifts are caused by polymorphisms in the genotype and not by primary resistance mutations. Clinical response to treatment with NNRTI-based regimens in patients with low-level phenotypic resistance has not been reduced (*see* section "Low-Level NNRTI Resistance" following). Occasional mutations associated with NNRTI resistance do not confer phenotypic resistance *(1)*, suggesting that some issues in resistance testing and interpretation remain unresolved.

RESISTANCE PROFILES OF NNRTIs (FIG. 1)

NNRTIs bind noncompetitively to the p66 subunit of RT in a hydrophobic pocket near the active site. Nevirapine has been studied with RT in crystal form. It lies on top of a hairpin motif that contains aspartic acid at positions 185 and 186. Tyrosine at positions 181 and 188 are in contact with nevirapine. This partially explains why the Y181C mutation confers resistance to nevirapine. Nevirapine binding may indirectly affect the conformation of aspartic acid residues at the active site, or binding may prohibit movement of different domains of the protein in relation to each other, thus inhibiting the function of the RT enzyme *(2)*.

There are several single point mutations that are associated with significant decreases in susceptibility to NNRTIs, and combinations of mutations may further reduce susceptibility. The most widely recognized NNRTI-resistance mutation is K103N. This mutation confers resistance to all three NNRTIs. NNRTIs bind the RT enzyme at a site distinct from the active site, generally between codons 100 to 110 and 180 to 190 *(2)*. Nucleoside RT inhibitors (NRTIs) bind at the active site, and because of the different binding sites of the two drug classes, there is little overlap in the mutations that confer NRTI and NNRTI resistance. One exception may be the Q145M mutation. This mutation may be responsible for drug recognition and processing, and has been shown to confer resistance to both NNRTIs and NRTIs *(3)*. Although one study suggested that zidovudine augmented the development of K103N, other data found no association between the use of AZT or the presence of AZT mutations on the emergence of K103N *(4)*. In fact, NRTI-resistance mutations have been associated with NNRTI hypersusceptibility *(5)*.

Mutations at other sites may confer resistance to NNRTIs. An example is the Y318F mutation. When site-directed mutants containing only the Y318F mutation were constructed, significant resistance to delavirdine (41-fold decrease in susceptibility) was seen, whereas a less than threefold decrease in susceptibility to nevirapine or efavirenz was seen. However, in clinical isolates, the presence of Y318F when Y181C was also present conferred an additional 3.3-fold

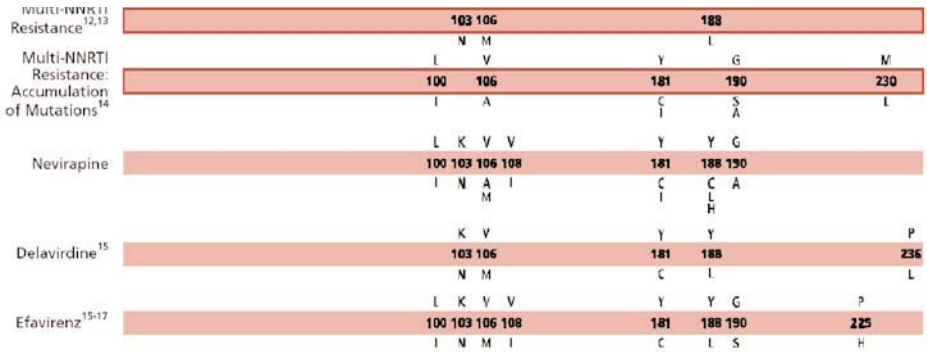

Fig. 1. Mutations associated with resistance to NNRTIs (http://www.iasusa.org/resistance_mutations_figures.pdf). Reprinted with permission from the International AIDS Society-USA *(67)*. Updated information (and thorough explanatory notes) is available at www.iasusa.org.

increase in resistance to efavirenz; when Y318F was present with K103N, there was a significant increase in resistance (from 15-fold for K103N alone to 43-fold when both mutations were present) *(6,7)*.

Detecting which mutations confer resistance to specific drugs is made more difficult because mutations selected by serial passage of virus with subinhibitory drug concentrations in vitro may be different from those seen in vivo. There are several hypotheses for this; among them is viral fitness. The mutations selected for in vitro may confer significant fitness disadvantages, limiting their emergence in vivo. Certain mechanisms of resistance may provide a marked fitness disadvantage in vivo that is not seen in vitro because of the absence of host interactions in the in vitro model.

Efavirenz

In vitro studies with efavirenz have demonstrated that resistance occurs rapidly. Passage of virus in MT-2 cells resulted in mutations V179D, L1991, and Y181C. After 24 passages, there was greater than 1000-fold reduced susceptibility to the drug *(8)*. In another study, highly resistant virus containing the double mutations L100I and K103N was selected for in several passages *(9)*. The rapid development of resistance in vitro correlates with the rapid development of resistance seen clinically, especially when efavirenz is administered alone or in suboptimal regimens.

When efavirenz was administered as monotherapy, an initial decline in HIV RNA was followed by virological rebound to baseline within 6 wk, and rebound was accompanied by resistance. Of the overall study of 135 patients, a subgroup of 19 patients were randomly selected to evaluate drug resistance. All isolates were found to be sensitive to efavirenz before the start of therapy. All five patients

receiving the high dose (1000 mg/d) had resistance (defined as greater than eight-fold change in susceptibility in this study) to efavirenz; four of five patients receiving 300 mg/d had resistance; and two of six patients receiving 50 mg/d had resistance *(10)*. This suggests that exposure to therapeutic levels of efavirenz as monotherapy induces resistance, whereas ineffective doses do not.

In a study of patients failing an efavirenz-containing regimen, either efavirenz plus indinavir or efavirenz plus zidovudine plus lamivudine, K103N (which causes resistance to all three available NNRTIs) was the most common NNRTI-resistance mutation, occurring in 90% of patients. V108I and P225H were also frequently observed, primarily in viruses that also contained other mutations that conferred resistance to NNRTIs. Mutations that often emerge during treatment with nevirapine or delavirdine, such as V106A, Y181C, and Y188C, were rare in this study *(11)*.

Phenotypic susceptibility to efavirenz may seem to be retained with certain primary NNRTI-resistance mutations (i.e., Y181C). When mutations K103N and Y188L are present in a particular viral isolate, high-level reduced susceptibility to efavirenz is likely. In one study that demonstrated this, viral isolates from patients undergoing dual combination therapy with efavirenz plus indinavir or from patients failing a zidovudine plus lamivudine-containing regimen, to which efavirenz was added, were analyzed. Mutations K103N, Y188L, and G190S/E were associated with reduced susceptibility and failure to respond to efavirenz. Secondary mutations V106I, V108I, Y181C, Y188H, P225H, and F227L caused little resistance to efavirenz alone, although they significantly enhanced in vitro resistance when combined with other mutations. Some isolates from patients who had failed nevirapine or delavirdine therapy, with Y181C, Y188C, or V106A mutations that lacked K103N or Y188L mutations retained phenotypic susceptibility to efavirenz in vitro *(11)*. The clinical relevance of this apparently retained susceptibility is unclear. Continued therapy with an NNRTI could rapidly lead to development of additional mutations, such as K103N and Y188L. Because the clinical response to efavirenz with the Y181C mutation alone is probably suboptimal (*see* "Cross-Resistance"), there is an apparent discordance between the phenotypic profile and genotype pattern for efavirenz. However, recognition that higher-level reduced phenotypic susceptibility (>10-fold) to any NNRTI probably precludes response to any currently available member of the class would improve the interpretation of the phenotype assay for patients with Y181C as the sole mutation.

Nevirapine

In vitro studies with nevirapine demonstrate that resistance occurs rapidly, in as little as one passage. One study found Y181C to be the most common single mutation occurring in vitro *(12)*. Another study demonstrated that, by

the fifth passage of nevirapine, mutations V106A, Y181C, and G190A were present and were associated with a greater than 100-fold reduction in susceptibility. An assay of in vitro fitness demonstrated that, in the presence of nevirapine, resistant virus was more fit than wild type, with Y181C being the most fit. A surprising finding of unclear significance was that in two separate assays conducted by the same group, virus containing the Y181C mutation was more fit than wild-type virus in the absence of drug *(13)*. The clinical relevance of this finding is yet to be established. However, anecdotal evidence suggests that NNRTI resistance, both genotypic and phenotypic, persists long after NNRTI therapy is removed (R. Haubrich, personal observation). This argues in favor of a relative lack-of-fitness impairment of NNRTI resistance-associated mutations.

Resistance to nevirapine has recently been shown to be influenced by perturbations in its interaction (and hence binding energy) with the RT enzyme at positions Y181 and Y188. Efavirenz seems to be less influenced by these interactions. Crystal structures of efavirenz complexed with RT containing the Y181C mutation, and nevirapine complexed with RT containing the Y181C mutation and complexed separately to RT containing the Y188C mutation were recently published. With efavirenz and the experimental agent, UC-781, only small rearrangements were seen in the inhibitor binding pocket when compared with nevirapine *(14)*. This represents a structural mechanism for the observation that Y181C confers significant resistance to nevirapine, whereas it only confers a low-level reduced susceptibility to efavirenz (in the absence of other mutations).

Several clinical studies of nevirapine monotherapy have shown that resistance develops quickly in this setting *(15,16)*. In one study, 20 patients with CD4 counts of greater than 500 cells/mm^3 received oral nevirapine monotherapy at 400 mg once daily. Some patients received a dose-escalating regimen of nevirapine at 200 mg/d for the first 4 wk, followed by 400 mg/d afterwards. Seventeen of the patients completed the study, and 16 of them had samples available for resistance assays using virus grown up from peripheral blood mononuclear cells *(17)*. In 13 of 16 subjects, virus was isolated and phenotypic resistance detected as early as 4 wk in 1 subject, 6 wk in another subject, 8 wk in 3 subjects, and 12 wk in 8 subjects. In the remaining three patients, virus became detectable at 20, 24, and 60 wk. There was no correlation between baseline level of RNA and development of resistance.

In another nevirapine monotherapy study, seven patients received nevirapine (one patient was also receiving zidovudine) as part of a double-blind, placebo-controlled trial. As early as 1 wk after starting nevirapine therapy, the Y181C mutation was detected. It was detected in six of seven patients receiving nevirapine at 2 wk. The detection of Y181C was correlated with a rise in plasma viral load. The extremely rapid emergence of resistance in the ART-naive

patients (except for the one patient who received zidovudine) suggests that point mutations conferring NNRTI resistance preexist in a proportion of virions within a patient, and that exposure to nonsuppressive drug pressure leads to rapid replacement of the wild-type population with a population containing the resistant mutation. In addition to mutations at codon 181, two patients had K103N mutations. Also detected were K101E, G190A, K101R, K102Q, Q182R, Y183D, T107A, K102E, and V108I mutations. The plasma HIV RNA levels returned to baseline values within the 28-d observation period in two of the seven subjects. The remaining five patients had viral loads between 0.7 $\log_{10}$ copies/mL and 1.32 $\log_{10}$ copies/mL below baseline at 28 d. Another study was performed to determine whether there was a difference in the development of resistance in patients with lower viral loads in the presence of nevirapine monotherapy. In 16 of the 17 patients for whom drug-resistance data were available, all isolates were genotypically resistant to nevirapine at 12 wk *(15)*.

Drug susceptibility and RT mutations were analyzed in 167 isolates from 38 patients treated with nevirapine, either alone or with zidovudine *(17)*. Resistance to nevirapine occurred rapidly in all patients, in as little as 1 wk, regardless of whether the patient received nevirapine alone or in combination. The presence of zidovudine altered the pattern of mutations, but did not prevent resistance. Mutations at residues 103, 106, 108, 181, 188, and 190 were found. Individual clones were sequenced from selected patients. The presence of different viral subpopulations was suggested by the presence of mixtures of mutations at several positions. Y181C was the most common mutation seen in the absence of zidovudine. Even though Y181C was prevented with the administration of zidovudine, alternative mutations (such as Y181Y, Y181S, and Y181H), which confer nevirapine resistance, were noted in place of the Y181C mutation *(17)*.

In a retrospective cohort of 88 patients who failed a nevirapine plus PI-containing regimen, resistant isolates to nevirapine were found in 92% of patients; the isolates were cross-resistant to efavirenz (68%) and delavirdine (73%) in most cases *(18)*. The Y181C mutation was seen in 76% of mutants. A combination of the Y181C and the K103N mutations was also common (23%). Of the eight isolates carrying only the single mutation Y181C, 29% retained phenotypic susceptibility to efavirenz. Resistance to NNRTIs in this study was correlated with baseline resistance to PIs *(18)*.

Delavirdine

In vitro, delavirdine selects for P236L *(19)*, which rarely occurs in vivo. In a fitness study, the P236L mutant was shown to be less fit than the K103N mutant, which may partially explain the infrequent occurrence of this mutant in vivo *(20)*.

Delavirdine resistance is also associated with cross-resistance to other NNRTIs. In a study of delavirdine administered as monotherapy, phenotypic

resistance was found in 28 of 30 subjects within 8 wk. K103N and Y181C were the most common mutations. P236L, which confers delavirdine resistance, but hypersusceptibility to nevirapine and efavirenz was seen in less than 10% of the 30 patients studied *(21)*. In another delavirdine monotherapy study, resistance was noted to emerge rapidly and, for this reason, the study was discontinued at 8 wk. K103N and Y181C were the mutations most commonly observed *(22)*.

CROSS-RESISTANCE

Cross-resistance among NNRTIs is broad and largely prevents sequential use of agents of this class. Cross-resistance is associated with the K103N mutation, which is among the most common mutations arising with drugs of this class. Some mutations, such as Y181C, confer resistance to delavirdine and nevirapine, but only low-level reduced phenotypic susceptibility to efavirenz. Whether patients with Y181C, when it is present as the sole RT mutation, can respond to efavirenz is of considerable interest. The degree of cross-resistance among NNRTIs was further illustrated in a study of 5000 clinical samples from a large database. Seventy-nine percent of patients who had phenotypic NNRTI resistance to at least one drug had resistance to all three approved NNRTIs. Twelve percent of patients were resistant to both delavirdine and nevirapine, but sensitive to efavirenz. Six percent of patients were susceptible only to delavirdine, and 3% of patients were resistant to nevirapine but sensitive to delavirdine and efavirenz (genotypes not specified) *(23)*.

Although many patients with previous NNRTI exposure experience broad cross-resistance, some do not, and interest in sequencing the NNRTI-resistant has been evaluated in small studies. One study used genotypic data to determine whether a switch from a failing nevirapine regimen to efavirenz would provide additional activity from the NNRTI class. High-level cross-resistance to efavirenz was seen in 41% of nevirapine-treated patients (38% with K103N mutations, 3% with Y188L mutations) and probable resistance in 17% of patients (a variety of mutations, including Y181C). There was a correlation between shorter treatment durations with nevirapine and response to efavirenz. In this study using genotypic resistance, 2 of 12 patients with a single nevirapine-resistance mutation had a response (defined as an undetectable HIV RNA level for 3 mo) to efavirenz, whereas 35 of 67 patients without any known resistance mutations had a response. None of the patients with the Y181C mutation had a response *(24)*. This study illustrates that NNRTI cross-resistance was common, and after treatment failure of one NNRTI, the likelihood of another one retaining antiviral activity was low.

To evaluate the sequential use of NNRTIs, a prospective study of 47 patients who were failing a nevirapine-containing regimen assessed the impact of changing to a regimen containing efavirenz plus abacavir plus a new PI.

NNRTI-resistance mutations were seen in 79% of patients, mainly at codons 181 (49%), 103 (40%), and 106 (19%). Sixty-two percent of patients had phenotypic resistance to efavirenz (>10-fold decreases in susceptibility). All strains with the K103N mutation demonstrated reduced phenotypic susceptibility to efavirenz, but only 20% of specimens harboring solely the Y181C mutation had phenotypic resistance to efavirenz (>10 FC). By 24 wk, 38% of patients taking the efavirenz-containing regimen had at least a 1 $\log_{10}$ copies/mL decrease in their viral load, with 19% of patients having an undetectable viral load. Most of the responses (1 $\log_{10}$ copies/mL viral load decrease) were seen in the small number of patients with no evidence of genotypic or phenotypic NNRTI resistance (7/10 patients responded). Factors associated with failure of the efavirenz-containing regimen were phenotypic resistance, presence of the K103N mutation, and a previous longer period of nevirapine therapy (mean of 288 vs 170 d) *(25)*. Although this data suggests the activity of efavirenz after nevirapine failure, it is far from conclusive.

Cross-resistance between the three available NNRTIs can be explained by their binding to the same site of the RT. A mutation altering the binding properties of one of these drugs also effects the other members of the class. The sequential use of these agents in salvage therapy is still under study, but does not seem promising. It is not clear what, if any, fitness cost the virus must pay to maintain the resistance to NNRTIs, or whether, in a multidrug salvage regimen, recycling these drugs has a role.

LOW-FREQUENCY NNRTI-RESISTANT MUTATIONS

The assessment of NNRTI resistance can be complicated because of failure to detect NNRTI-resistance mutations. Patients with previous NNRTI therapy who subsequently change to new regimens that do not contain an NNRTI may lose the NNRTI-resistance mutations. If an NNRTI is reintroduced in a subsequent regimen, this absence of NNRTI mutations on the genotype may not predict a good treatment response. In ACTG 398, patients with a history of NNRTI treatment were less likely to achieve an HIV RNA level reduction to fewer than 200 copies/mL than those with no history of NNRTI treatment, even if they did not have evidence of NNRTI resistance *(26,27)*. A potential explanation for the inability of the genotype to predict response in these NNRTI-experienced patients could be the relative insensitivity of standard assays to low-frequency mutations. To explore this hypothesis, Mellors examined virus from 11 NNRTI-experienced and 12 treatment-naive patients using single-genome sequencing and other more-sensitive techniques. Using these methods, patients with a history of NNRTI treatment were more likely to have low-frequency resistant variants detected than treatment-naive patients (6/11 vs 2/12, respectively) and had greater numbers of viral clones with resistant variants *(27)*. In the NNRTI

treatment-experienced patients who ultimately failed the new NNRTI-containing regimen, the viral isolates were genetically related to the baseline minority viruses found by single-genome sequencing. This data suggests that previous treatment (if virological failure occurred on the regimen) with an NNRTI is likely to preclude the salutatory use of this class of agents in a future regimen, even if the resistance assay fails to detect evidence of NNRTI resistance.

STIMULATION OF REPLICATION

A newly characterized mutation found after NNRTI therapy seems to stimulate viral replication (in vitro) in the presence of NNRTIs *(28)*. The M230L mutation was observed in four patients, either alone or in combination with other known NNRTI-resistance mutations. The combination of M230L and other NNRTI-resistance mutations seemed to markedly reduce the susceptibility of all NNRTIs (>250-fold decreased susceptibility). Interestingly, the virus isolated from three of the four patients had 50 to 100% greater levels of replication in the presence of NNRTI than in the absence of drug. Thus, certain NNRTI concentrations seemed to stimulate viral growth. This was not observed in the one patient who had M230L combined with Y181C mutations *(28)*. These data suggest that, for certain patients, continued use of NNRTIs after virological breakthrough could be deleterious. Further work is needed to clarify these findings.

NNRTI-RESISTANT VIRUS IN TREATMENT-NAIVE PATIENTS

Transmission of NNRTI-Resistant Virus in Treatment-Naive Patients

Transmitted NNRTI resistance is well-documented and is becoming a major problem. High-level resistance can be defined as a 10-fold decrease in susceptibility of the virus to a particular drug. The rate of high-level phenotypic NNRTI resistance in patients recently infected with HIV-1 has been estimated at 7.1% in a retrospective cohort in 10 major cities in infections occurring between 1999 and 2000 *(29)*. Although this high prevalence of resistance is not found in every population studied, it is concerning, especially given the broad cross-resistance among the NNRTIs. In an earlier primary infection study by the same team, 3 of 141 total patients had high-level phenotypic resistance (>10-fold reduced susceptibility) to one or more ARVs. Two of these patients had resistance to both PIs and NNRTIs, although the presence of known mutations conferring NNRTI resistance was not documented. Thirty-six patients had intermediate susceptibility to one or more classes of ARVs, including NNRTIs *(30)*. Figure 2 shows the patterns of resistance of the virus from patients in whom resistance was detected. Additionally, the rate of resistance to the different NNRTIs was further broken down: 9 of 24 patients with reduced NNRTI susceptibility had resistance to both nevirapine and delavirdine, and

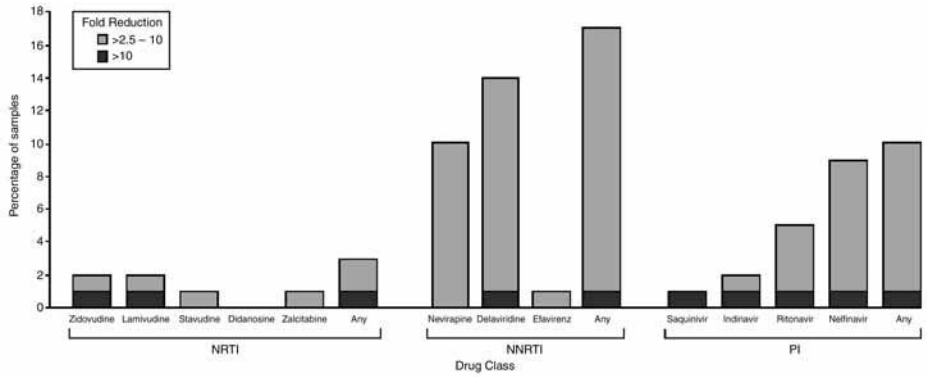

Fig. 2. Prevalence of reduced phenotypic susceptibility from recently infected patients. (From ref. *30*. Copyright © 1999, American Medical Association. All rights reserved.)

only 2 patients had reduced susceptibility to all 3 NNRTIs. In other studies, the rates of transmitted NNRTI resistance were less *(31,32)*.

If the trend of increasing transmission of NNRTI resistance continues, empiric use of NNRTIs may be problematic without resistance testing. Even if resistance is transmitted, the resistance assay may fail to detect the resistance. In untreated, therapy-naive patients, in the absence of selective drug pressure, the virus may revert to wild type, and a routine resistance test may not detect transmitted resistant viral subpopulations. There are emerging data, however, that contradict this conventional wisdom. One study showed that, in four patients with recent infection, mutations conferring resistance to NNRTIs persisted for at least 13 mo *(33)*. A study in five patients with zidovudine resistance in the setting of acute infection showed that resistance persisted for up to 1 yr in three of the five patients, whereas the other two patients had reversion to wild-type virus at 1 yr *(34)*. These data suggest that it may be possible to use resistance assays in treatment-naive patients, even when it is unclear when transmission of resistance occurred. More studies are needed to determine the value of resistance testing in chronically infected ARV-naive patients.

There is also evidence to suggest that drug resistance is detectable in chronically infected patients. In a study of 230 samples of chronically infected, ARV-naive patients, 6% of patients had high-level NNRTI resistance *(35)*. In an observational study, 281 chronically infected ART-naive patients were enrolled. Genotypic resistance to any ARV agent, using the *Visible Genetics* algorithm, was seen in 8.9% of patients, with 5.3% of patients having NNRTI resistance. The K103N and Y181C mutations were the most frequent NNRTI-resistance mutations *(36)*. This finding also argues for considering resistance testing before the initiation of ARV therapy, even in chronically infected patients.

Low-Level NNRTI Resistance

In treatment-naive patients, low-level phenotypic NNRTI resistance (FC between 2.5 and 10) has been noted *(37)*. This low-level resistance does not preclude the use of efavirenz. In one case–control study, baseline resistance was determined for 50 patients who had virological failure on an efavirenz-containing regimen (either with indinavir or zidovudine plus lamivudine) and compared these patients with 50 patients who had long-term suppression on the same regimens. Entry criteria precluded previous use of NNRTIs. Thirty-four percent of baseline samples showed greater than fourfold phenotypic resistance to one or more NNRTIs. Low-level (4- to 10-fold) phenotypic resistance was found in baseline samples of 26% of treatment failures and in 34% of treatment successes. Thus, there was no difference in the rate of low-level NNRTI resistance in failing vs successful treatment, suggesting that low-level NNRTI resistance did not influence virological outcome. High-level (>10-fold) resistance was seen in five of six subjects who subsequently failed their efavirenz-containing regimens. Several of these six patients had the K103N or Y181C mutations at baseline, consistent with undisclosed previous NNRTI therapy or transmitted resistance *(37)*.

Another study demonstrated that low-level reduced susceptibility (4- to 10-fold) to NNRTI was not associated with a significant risk of virological failure *(38)*. In this study of NNRTI-naive patients, a significant level of reduced sensitivity to NNRTIs in the 279 patients was observed. Greater than fourfold reductions in susceptibility to delavirdine were seen in 24% of patients (n = 60), to nevirapine were seen in 6% (n = 15), and to efavirenz were seen in 6% (n =15). Analysis of time-to-viral suppression or time-to-viral rebound for these patients treated with a nevirapine-based regimen were not associated with low-level reduced susceptibility to the NNRTI. In the absence of previous use of NNRTIs, low-level reduced phenotypic susceptibility should not be a contraindication to using an NNRTI.

TREATMENT OF NNRTI-RESISTANT VIRUS

Because the concentration of NNRTI in plasma is many fold greater than the IC_{50}, investigation into whether higher levels of NNRTIs may be able to overcome certain resistance mutations has been considered. One study evaluated 40 patients who had virological breakthrough (HIV RNA levels >50 copies/mL after being <50 copies/mL) while taking a nevirapine plus two-NRTI regimen. After efavirenz was substituted for nevirapine without changing the NRTI in the regimen, 43% (17/40) of the patients had an undetectable viral load or a 1 $\log_{10}$ copies/mL reduction in viral load. For the 31 of 40 patients from whom genotypes were obtainable at baseline, 25 of 31 had NNRTI-resistance mutations (codons 103, 181, and 190). In patients without NNRTI-resistance mutations, five of six regained viral suppression. In those subjects with NNRTI

mutations, viral suppression was seen only in those patients with efavirenz levels greater than 2 μg/mL (therapeutic levels are generally considered to be >1.4 μg/mL). One-third of subjects with efavirenz levels greater than 2 μg/mL had a response (undetectable viral load or >1 $\log_{10}$ copies/mL decrease in viral load) to efavirenz, but details of how many patients had higher levels, which specific mutations correlated with response, or how many had viral loads of fewer than 50 copies/mL after switch were not provided *(39)*. The benefit of this strategy remains speculative at present.

NNRTI RESPONSE WITH NRTI RESISTANCE

The ability of a regimen with two NRTIs plus an NNRTI to achieve and maintain undetectable viral load levels is dependent on the potency of the NRTI component. If one or more of the nucleosides are impaired because of previous treatment and resistance, response to the NNRTI-based regimen may be suboptimal. Switch studies, in which a patient with HIV RNA of fewer than 400 copies/mL taking a current PI-based regimen, has an NNRTI substituted for the PI, have evaluated this concept. In a prospective study involving 34 NNRTI-naive patients with 6 mo of an undetectable viral load on a PI-containing regimen, the PI was changed to nevirapine *(40)*. Before the successful PI-containing regimen, 12 patients had previous treatment with NRTIs, whereas 22 patients were treatment naive. Forty-one percent of the patients with previous NRTI exposure and none of the treatment-naive patients experienced virological failure. All patients with virological rebound had virus with evidence of NNRTI mutations, a K103N mutation in three patients, and Y181C and G190A mutations were seen in one patient each. The K101E mutation was seen in conjunction with other NNRTI-resistance mutations. NRTI- but not PI-resistance mutations were seen in those patients whose treatment failed. The NNRTI mutations occurred in patients who also had NRTI mutations detected during rebound, suggesting that archived resistant virus (NRTI resistance, in this case) played a significant role in treatment failure. Analysis of proviral DNA from archived specimens obtained during virological suppression taking PI therapy in four of the five patients with subsequent failure did not demonstrate NRTI-resistance mutations; however this does not preclude the existence of low levels of archived NRTI-resistance mutations, because the methods used to detect the mutations may not have been sufficiently sensitive. These data suggest that previous NRTI therapy may impair virological response to a regimen containing an NNRTI plus two NRTIs *(40)*.

NEW NNRTIS AND RESISTANCE

Exposure to NNRTIs, in the presence of ongoing viral replication, leads to resistance to the NNRTI class of agents, and this resistance can occur quickly.

New NNRTIs are needed that will circumvent existing patterns of NNRTI resistance and provide additional therapeutic options to patients who have resistance to NNRTIs. Novel NNRTIs are in development, some of which may be active in the presence of the most common, class-limiting NNRTI mutations, especially K103N or Y181C mutations.

The rational approach to designing NNRTIs that retain activity against virus harboring common NNRTI mutations, which will not themselves select for new mutations, is a challenge. One method would be to select a compound that induces mutations that confer a significant decline in viral fitness. W229 is such a site. It resides in the "primer grip" portion of RT. A mutation at this site, such as W229F or W229Y, has been shown to result in a virus with a significantly lowered DNA polymerase activity *(41)*. Although this is a potential target of newer generation NNRTIs, no compounds in development take advantage of this strategy.

There are several new NNRTIs in clinical trials: calanolide A, capravirine, and TMC-125. Calanolide A is a natural product isolated from tropical plants from the genus *Calophyllum (42)*, which has activity against HIV strains with the Y181C mutation. It has enhanced activity against strains with mutations conferring zidovudine resistance. This compound does not have good activity against strains carrying other NNRTI-associated mutations, such as those occurring at sites L100, K103, T139, and Y188 *(43)*.

TMC125 is another NNRTI in human clinical trials. In vitro, it demonstrates potent activity against strains with the K103N or Y181C mutations, but has poor activity against strains with both mutations. It also seems to select for resistance more slowly than the currently available, first-generation NNRTIs *(44)*. In a phase IIa study, 16 patients who had a treatment failure on an efavirenz- or nevirapine-containing regimen had TMC125 substituted for their current NNRTI for 7 d. The median log change in viral load was –0.89 $\log_{10}$ copies/mL *(44)*. Patients in this study had virus with multiple NNRTI mutations, including L100I, K103N, Y181C, Y188L, and G190A/S. At least preliminarily, TMC125 is a promising candidate for those patients who have had treatment failures with the first-generation NNRTIs.

Capravirine is another NNRTI currently in phase I to II clinical trials. In preliminary data, it seems to be a potent inhibitor of RT, and has activity against virus harboring the K103N mutation. A phase I study in which capravirine was administered as monotherapy for 10 d demonstrated 1.2 to 1.7 $\log_{10}$ copies/mL decreases in HIV RNA levels in the monotherapy treatment arms, compared with a 1.7 $\log_{10}$ copies/mL decrease in the nelfinavir plus zidovudine plus lamivudine control arm. This study did not report data regarding resistance *(45)*. In an ongoing phase II study involving PI-naive patients who had previously failed an NNRTI-containing regimen (plasma HIV RNA levels >2000

copies/mL), more than 50% of the 50 patients for whom data was available attained plasma HIV RNA levels of fewer than 400 copies/mL when administered a regimen of capravirine plus nelfinavir plus two NRTIs. Capravirine seemed to be well-tolerated and efficacious *(46)*. Thus, capravirine seems to be an effective and well-tolerated NNRTI, which, similar to TMC-125, has activity against HIV strains that are resistant to currently available NNRTIs.

RESISTANCE AND VIRAL FITNESS

The effect of various NNRTI mutations on viral fitness has been evaluated in several studies. One study used site-directed mutagenesis to construct viruses with NNRTI-resistance mutations and compared replication capacities and phenotypic susceptibilities with wild-type virus. The following commonly occurring mutations had no effect on viral replication capacity: K103N, Y181C/I, Y188C/H/L, and G190A. Several mutations, V106A, G190C/S, P225H, M230L, and P236L, conferred substantial reductions in viral fitness. Double mutants had varying degrees of altered replication capacity, indicating that the replication capacity of virus with more than one NNRTI mutation cannot be predicted based on the replication capacity of single mutants *(47)*. However, the accumulation of multiple mutations has been shown to cause a significant reduction in fitness *(48)*.

Because virus with NNRTI-resistance mutations may persist long after the NNRTI-selective drug pressure is removed, whether these mutations cause fitness impairment remains controversial. Several common mutations (K103N, Y181C, and Y188C/H/L) do not reduce fitness, but other mutations do confer a significant fitness cost. Another study evaluated the replication capacity of the P236L mutation that occurs readily in vitro, but infrequently in vivo in the presence of delavirdine. K103N occurs much more frequently in the setting of delavirdine therapy, and it was hypothesized that virus with the K103N mutation is more fit than virus with the P236L mutation. This was borne out in one in vitro study, which noted a twofold to threefold decreased p24-antigen production in the presence of delavirdine in P236L-mutated virus compared with the K103N mutation *(20)*. The P236L mutant demonstrated decreased rates of ribonuclease H cleavage that were presumed to be the cause of the differential fitness. Differential rates of ribonuclease H cleavage were also noted in another study demonstrating that a virus harboring the V179D mutation was more fit than one with the Y181C mutation, which, in turn, was more fit than one with the V106A mutation *(49)*. Whether the reductions in fitness, caused by NNRTI-resistance mutations, have clinical consequences has not been determined.

HYPERSUSCEPTIBILITY

Recent observations based on phenotypic susceptibility assays have revealed that some virus isolates have increased susceptibility to NNRTIs. For

these isolates, the IC_{50} of the patient virus is lower than the IC_{50} of the reference (wild-type) virus. The FC in IC_{50} would be less than one for these viruses. This phenomenon has been termed hypersusceptibility. The clinical implications and durability of NNRTI hypersusceptibility have been evaluated in clinical trials.

In a large analysis of 17,000 sequentially received plasma samples, specimens were analyzed for phenotypic as well as genotypic resistance. Hypersusceptibility to efavirenz, delavirdine, and nevirapine were detected in 10.8%, 10.7%, and 8% of the specimens, respectively. Hypersusceptibility (defined as FC < 0.4) was most common in NNRTI-naive/NRTI-experienced patients. A subanalysis of NNRTI-naive patients was undertaken to elucidate the effect of NRTI mutations on hypersusceptibility. Among the samples, 331 patients who were NNRTI naive/NRTI naive and 447 who were NNRTI naive/NRTI experienced were identified. The prevalence of hypersusceptibility to delavirdine, efavirenz, and nevirapine was 5%, 9%, and 11%, respectively, in the NRTI-naive group; whereas it was 29%, 26%, and 21%, respectively, in the NRTI-experienced group. Mutations at several sites in RT were significantly associated with NNRTI hypersusceptibility, predominantly at codons 41, 44, 67, 69, 74, 75, 118, 184, 210, 215, and 219 *(5)*.

Another study looked at the clinical relevance of NNRTI hypersusceptibility in a prospective cohort. In this study, hypersusceptibility was defined as a FC in IC_{50} of less than 0.4. NNRTI hypersusceptibility was present in 29% of the cohort of 177 patients. A longer duration of NRTI therapy and a reduced NRTI susceptibility were correlated with efavirenz hypersusceptibility. NNRTI therapy was begun in 106 patients. The mean change in HIV RNA level after 6 mo was –1.2 $\log_{10}$ copies/mL for patients with hypersusceptibility, as compared with –0.8 $\log_{10}$ copies/mL for those without hypersusceptibility ($p = 0.016$). Differences remained statistically significant at 12 mo. Multiple linear regression models confirmed that NNRTI hypersusceptibility was a significant independent predictor of the magnitude of response to NNRTIs ($p < 0.02$) *(50)*.

Efavirenz hypersusceptibility was evaluated in a multidrug salvage protocol. This study treated patients with single vs dual PI plus NRTI plus efavirenz. Phenotype assays were retrospectively performed on a subset of 139 randomly selected patients. Previous NNRTI treatment was positively associated with virological failure (HIV RNA level >200 copies/mL at weeks 24 and 48), whereas NNRTI hypersusceptibility reduced the likelihood of virological failure at week 48 (odds ratio, 0.16; $p < 0.01$) *(51)*. Hypersusceptibility to delavirdine was also shown to be a significant independent predictor of virological response. Several methods to determine a hypersusceptibility cut-point found an FC value near 0.4 to be the best predictor of response *(52)*.

Thus, clinical studies have demonstrated the relevance of NNRTI hypersusceptibility for NNRTI-naive patients. Because hypersusceptibility is more likely to occur in NRTI-experienced patients with the presence of NRTI mutations, whether hypersusceptibility should influence the initial choice of regimens (PI vs NNRTI) remains controversial.

IMPACT OF RESISTANCE ON THE USE OF NNRTIs IN DEVELOPING COUNTRIES

NNRTI resistance in the developing world could be a significant problem in the future. Because HAART is not available to the majority of infected people worldwide, the use of NNRTIs in resource-poor settings has been largely restricted to small cohorts. The ability to obtain resistance assays is also a limiting factor in the effective use of this class of agents. Additionally, especially in parts of West Africa, because of the prevalence of HIV-2, this class of agents has limited usefulness.

Currently, nevirapine has been most widely used in the developing world for the prevention of mother-to-child HIV transmission. Single doses of nevirapine, administered to the mother and newborn at the time of delivery, have been shown to significantly reduce HIV transmission. In this setting, a fully suppressive regimen (i.e., two NRTIs plus one NNRTI) is not provided because of cost and other logistical considerations. Given the efficacy of single-dose nevirapine, the use is justifiable, but resistance after this single dose has been reported. The half-life of the NNRTI drugs could lead to detectable levels of the medications for days to weeks after a single dose is administered *(53)*. This persistent exposure to suboptimal NNRTI concentrations has lead to the development of resistance.

In the HIV Network for Prevention Trials (HIVNET) 012 study in Uganda, 111 treatment-naive pregnant women received a single 200-mg dose of nevirapine, and neonates received a single dose within 72 h after birth. Thirty-three infants were infected despite prophylaxis. Genotypes were performed in 32 of the transmitting mothers and in 72 nontransmitting mothers. Nevirapine-resistance mutations were detected in 7 (22%) of the transmitting women, and 11 (16%) of the nontransmitting women. K103N was the dominant mutation in 90% of samples with a mutation. Y181C was seen in six samples. The women in HIVNET 012 had a follow-up genotype at 12 to 18 mo after therapy, which showed a return to wild-type virus in all of the patients *(54,55)*. Seven of the infected infants also had genotypes performed, the K103N mutation was detected in one and the Y181C mutation was detected in two infants. Interestingly, the infected infants had different mutations than their mothers; this difference suggests that the nevirapine-resistance mutations were caused by the selective pressure of the nevirapine treatment of the infants, rather than

transmission of virus with resistance mutations *(55)*. Additional larger studies have confirmed high rates of development of NNRTI resistance after a single dose of nevirapine administered to prevent HIV transmission from mother to baby *(56)*. The rate of nevirapine resistance was 39% in 456 women who received nevirapine to prevent transmission in a study in South Africa. Nevirapine resistance was more likely in women with a lower baseline CD4 cell count, a higher baseline HIV RNA level, and in women who took more than one dose of nevirapine. In some studies, single-dose nevirapine was administered in addition to background zidovudine; the addition of zidovudine to the prophylactic regimen did not prevent the emergence of nevirapine resistance *(57)*.

The impact of development of NNRTI resistance, after a single dose of nevirapine, on the subsequent response to a nevirapine-containing HAART regimen is a critical concern regarding the use of this strategy to prevent HIV transmission from mother to infant. The potential benefit to the infant could be diminished if maternal NNRTI resistance emerges and reduces the response to a new regimen containing an NNRTI. Even if the NNRTI mutations in the plasma revert to wild type with time, archived virus, present in lymph nodes or proviral DNA, could lead to the rapid reemergence of resistant populations if therapy with an NNRTI is introduced. Because the World Health Organization guidelines and country programs recommend the use of an NNRTI-based regimen for initial therapy in resource-limited settings, data concerning this question are important. A recent study in Thailand suggests that previous exposure to single-dose NNRTI and development of NNRTI mutations can reduce response to an NNRTI-containing regimen *(57)*. In a study of treatment of women who had received single-dose nevirapine for transmission prophylaxis, 18% of 90 tested women had major NNRTI-associated mutations (K103N, G190A, or Y181C). All women subsequently received an NNRTI-based regimen for treatment of their HIV. After 6 mo of dosing, 85% ($n = 27$) of women not exposed to nevirapine had HIV RNA levels of fewer than 400 copies/mL, whereas the response rates for exposed women who did not have mutations was 80% ($n = 97$), and the rate for women with at least one mutation was 68% ($n = 50$; $p = 0.003$ in a test for trend). The time after nevirapine exposure was also a factor in response to treatment, those exposed to nevirapine longer than 6 mo before initiating an NNRTI-based regimen were more likely to respond whether or not they had previously had a NNRTI mutation. Clearly, exposure and resistance from single-dose NNRTI prophylaxis is an important determinant of subsequent response to an NNRTI-containing regimen. Strategies to overcome this limitation of preventing mother-to-child transmission will need to be carefully considered because the role of nevirapine prophylaxis yields such significant benefit in preventing HIV transmission to the baby.

NNRTIs have little activity against the RT of HIV-2. HIV-2 is prevalent in West Africa. It is estimated that the *pol* genes (which contain RT) share a 60% homology between HIV-1 and HIV-2 *(58)*. Despite the difference between these two strains, the RT enzymes seem similar in overall structure. One study compared in vitro susceptibilities of the three available NNRTIs to HIV-1 (III_b) and the two different strains of HIV-2 (ROD and EHO). All three NNRTIs demonstrated significantly reduced activity to HIV-2. For example, delavirdine had an IC_{50} of 0.01 μ*M* against HIV-1, and an IC_{50} of 2.1 to 47.3 to the two strains of HIV-2. To have any significant activity in human serum, the concentrations of the three available NNRTIs will have to be upwards of 50 times greater than those achieved by current dosing regimens *(58)*. One group hypothesized that NNRTI resistance in HIV-2 could be reversed by inducing a mutation at position 188. Indeed, an L188Y mutation led to an HIV-2 that became sensitive to delavirdine and efavirenz *(59)*.

One study evaluated patients with HIV-2 who were treated with a nevirapine-containing regimen. Seven patients were treated with a nevirapine-containing HAART regimen, and had no significant decrease in viral load. Four patients received an indinavir-containing regimen and had undetectable viral loads at 4 mo of therapy *(60)*.

Several studies have looked at *de novo* NNRTI resistance of HIV-1 in different geographic regions, where different HIV clades may predominate. No significant differences in drug susceptibility were noted in HIV-1 isolates across regions or clades *(61–63)*. There are limited data on the pattern of resistance mutations that develop when patients with nonclade B virus are treated and fail a NNRTI-based regimen. As resistance assays become more available, studies can be performed to determine whether patients with non-B virus fail NNRTI regimens with different mutational pathways than patients with clade B virus.

These data indicate that NNRTIs should not be used to treat patients with HIV-2, and that NNRTI susceptibility does not vary significantly among clades. Monitoring HIV resistance will be important in resource-poor countries that have limited access to ARVs, because suboptimal regimens may be the only affordable options *(64)*. Additionally, patients may attempt to share their medications with infected family members, hoping that some benefit may be obtained. As ARVs are introduced in these settings, resistance may become a major problem.

CONCLUSIONS

Resistance to the NNRTIs occurs rapidly in nonsuppressive regimens and results in cross-resistance to all of the currently approved agents in the class. The increased prevalence of NNRTI resistance in newly infected patients provides rationale to obtain a resistance assay before initiating therapy in these patients. Current resistance testing guidelines recommend the use of resistance

testing for ARV-naive patients with recent HIV infection (often defined as infection within the past 12 mo). The routine use of resistance tests in chronic HIV infection has recently been revised to recommend testing in patients who were infected with in the past 2 yr, but testing for those infected for an unknown duration or greater than 2 yr remains controversial and awaits further data *(65,66)*. Newer NNRTIs are targeted to inhibit virus that exhibits current patterns of resistance, and are hoped to benefit patients with multidrug-resistant infections. NNRTIs with differing resistance profiles will be especially useful if they do not induce resistance as rapidly as the currently available agents.

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15

Vertical Transmission of HIV and Therapeutic Interventions

Susan Cu-Uvin, Arlene Bardeguez, and Carla Chibwesha

INTRODUCTION

Epidemiology

More than 20 yr into the HIV/AIDS pandemic, both prevalence and incidence rates continue to climb steadily—in some regions more rapidly than in others. As of the end of 2004, The Joint United Nations Programme on HIV/AIDS reported the total number of adults and children living with HIV/AIDS worldwide was 38 million people; of whom, 17 million were women and 2.1 million were children younger than 15 yr of age. In 2003 alone, there were an estimated 4.8 million new infections, and, of these, 48% were in women and 19% were in children *(1)*. The burden of HIV disease falls disproportionately on the developing world, which is home to more than 95% of individuals infected with HIV. The situation is most bleak in the sub-Saharan African region, where 25 million of the 38 million global HIV infections are estimated to have occurred. In 2003, Africa saw 2.2 million deaths from AIDS *(1)*. Antenatal sentinel data indicate a contiguous belt in sub-Saharan Africa, stretching from Uganda southwards toward Botswana and South Africa, in which HIV prevalence rates are far higher than any other region. In the so-called HIV belt, the proportion of the (urban) population infected with HIV is between 15 and 30%, with significant urban–rural differentials *(2)*.

In 2002, more than 800,000 children younger than 15 yr of age were newly infected with HIV, and more than 90% of these infections were vertically acquired *(3)*. The problem of pediatric HIV/AIDS is a pressing one, one that cannot be answered until there is wide-scale implementation of routine antenatal HIV counseling and testing and antiretroviral treatment for HIV-seropositive women. Without antiretroviral intervention, the rate of transmission from an HIV-infected mother to her child is between 20 and 45% *(4–6)*.

According to United Nations Children's Fund estimates, more than 2.5 million children were at risk for vertically acquired HIV infection in 2002. Of

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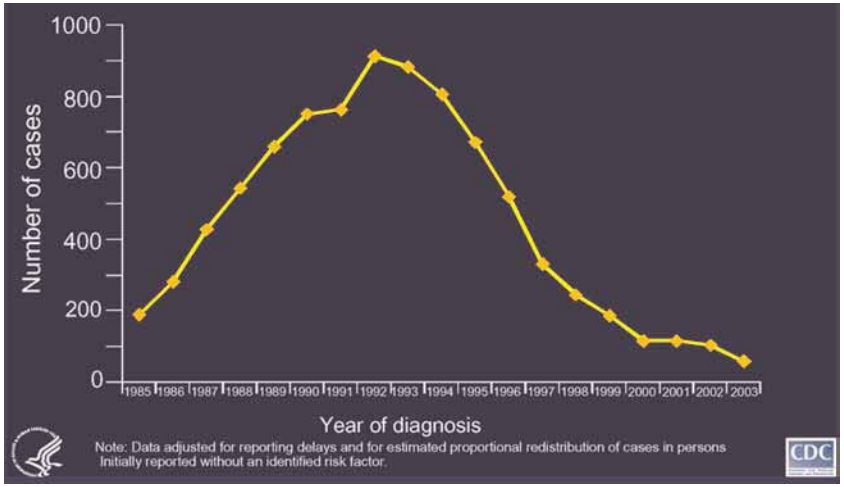

Fig. 1. Neonatal HIV infections, United States.

these children, 2.2 million were born in sub-Saharan Africa; 160,000 were born in South Asia; 68,000 were born in East Asia or the Pacific; 52,000 were born in Latin America or the Caribbean; and 40,000 were born in North Africa or the Middle East *(3)*. By contrast, mother-to-child transmission has been reduced to just several hundred cases in the United States and western Europe, where antiretroviral treatment is readily accessible (Fig. 1).

Early in the epidemic, between 1000 and 2000 infants were infected with HIV in the United States each year. More recently, however, we have seen a dramatic reduction in the number of perinatally associated infections because of increased counseling and testing and the use of antiretroviral therapy during pregnancy. Between 1992 and 2003, cases of perinatally acquired HIV infection decreased by more than 75% in the United States, as antiretroviral use became more widespread (Fig. 2) *(7)*.

Despite tremendous successes in the overall prevention of mother-to-child transmission, in the United States, women of color and their children remain disproportionately affected by the epidemic. Of the 90 reported cases of HIV/AIDS in infants born to HIV-infected mothers in this country in 2003, 77% were in African American and Hispanic children *(7)*. This presents an important challenge in terms of access to healthcare for communities of color in the United States.

Mother-to-Child Transmission of HIV

The primary mode of pediatric HIV infection is vertical transmission from infected mothers to their infants. In the absence of antiretroviral treatment, two

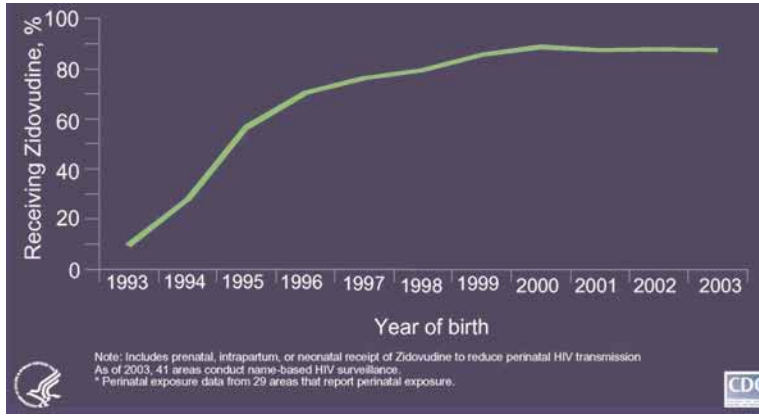

Fig. 2. Antiretroviral uptake, United States.

general patterns of survival are recognized in vertically infected children. Approximately 10 to 20% of these children experience rapid decline in health, dying of AIDS-related causes by age 4 yr. The mean survival time in the remaining 80 to 90% of vertically infected children is 9 to 10 yr. Unlike adults, however, HIV-infected children typically show CD4 T-cell depletion and HIV-related symptoms within the first few years of life *(8)*.

Mother-to-child transmission of HIV may occur during any of three periods: the antepartum period, the intrapartum period, or the postpartum period (mostly via breast-feeding). Although the relative proportions of HIV transmission that occur during the antepartum, intrapartum, or postpartum periods remains controversial *(9,10)*, it has been suggested that at least between 50 and 60% of vertical transmissions occur during labor and delivery *(11–13)*. It is likely that intrapartum infection occurs through fetal exposure to maternal blood and cervicovaginal secretions, with HIV entering through fetal skin, mucous membranes, or the gastrointestinal tract *(4)*. Clinical trials that have demonstrated clear reductions in the rates of vertical transmission either through the administration of antiretroviral agents around the time of delivery and/or through cesarean delivery further corroborate these findings.

In utero (antepartum) exposure is thought to occur through transplacental passage and direct spread of the virus to the amniotic fluid *(5)*. The absence of HIV infection in first- and second-trimester fetuses supports the assumption of low rates of early perinatal transmission *(13,14)*. Postnatal infection of infants is largely through breast-feeding *(15)*. Although the exact risk and timing of postnatal infection though breast milk is still unclear, it is estimated that breast-feeding results in a 15 to 50% increase in the risk of HIV transmission from mother to child *(8,15,16)*.

Various different modalities may be used to decrease the transmission of HIV between mothers and their infants. This chapter focuses on pharmacological agents, specifically, reverse transcriptase inhibitors.

Risk Factors in Vertical Transmission of HIV

Major determinants of vertical transmission relate to a variety of maternal, obstetric, fetal/infant, and viral factors. Several maternal factors are associated with a higher risk of perinatal HIV transmission:

1. High plasma viral load.
2. Low CD4 cell count and later disease stage.
3. Concomitant sexually transmitted disease (STD) infection.
4. Maternal drug use.
5. Poor maternal nutrition and micronutrient deficiencies.
6. Inadequate antenatal and general health care.

Many studies have demonstrated the association between a high plasma viral load and an increased risk of mother-to-child transmission of the HIV virus *(17–23)*. Despite the observation that high maternal viral load is correlated with increased risk of transmission, there is no strict threshold of virus load below which HIV transmission from mother to child cannot occur. Rather, vertical transmission has occurred at all levels of maternal viral load. Furthermore, the role of viral load in the genital tract has yet to be fully elucidated.

A second maternal factor associated with increased risk of perinatal HIV transmission is low maternal CD4 T-cell count, which is considered to be a marker of late disease stage. In the Women and Infant Transmission Study (WITS), Landesman et al. *(24)* demonstrated that low CD4 cell count is independently associated with HIV-1 transmission, and others have also reported this relationship *(25,26)*.

An additional maternal factor associated with vertical HIV transmission is concomitant infection with a classic STD. An important epidemiological synergy exists between STDs and HIV infection, and since the beginning of the HIV/AIDS pandemic, numerous researchers have demonstrated that preexisting STD infection increases the risk of HIV transmission *(27–29)*. STDs increase shedding of HIV in the genital tract *(27,30,31)*, thereby increasing the probability that genital secretions will contain the concentration of HIV required for transmission.

Maternal illicit drug use may also be associated with increased risk of transmission *(24,32,33)*. Additionally, maternal nutritional status, specifically vitamin A deficiency, has been postulated as a risk factor in vertical transmission through observational study *(17,34–36)*. However, the findings of more recent clinical trials suggest that neither vitamin A nor any other vitamin supplementation is effective in reducing the risk of transmission from mothers to infants *(37,38)*.

The risk of mother-to-child transmission is higher in children who have been breast-fed compared with those who have not *(39,40)*. The World Health Organization, United Nations Children's Fund, and The Joint United Nations Programme on HIV/AIDS recommend avoidance of breast-feeding, provided that safe and affordable alternatives are readily available to postnatal mothers.

Obstetric factors, such as vaginal delivery vs caesarean section, long (>4 h) duration of membrane rupture, and invasive fetal monitoring affect the risk of vertical transmission, as do maternal bleeding and chorioamnionitis *(24,41)*. There is also evidence associating caesarean section with lowering the risk of HIV transmission, particularly in women who have received either no therapy or zidovudine (ZDV) monotherapy *(42)*. However, vaginal cleansing with an antiseptic chlorhexidine wash during the intrapartum period does not seem to be significantly associated with decreasing rates of vertical transmission *(43,44)*.

Fetal/infant factors, such as prematurity and/or low birth weight have been found to be associated with differential rates of HIV transmission from mother to child *(45–47)*. However, confounding relationships between both prematurity and low birth weight—and other factors, such as maternal disease stage, nutrition, and drug use—make results difficult to interpret *(48)*. It may, in fact, be impossible to isolate the risk of transmission attributable to these infant factors. Moreover, it is possible that the genetic susceptibility of the infant (specifically homozygosity for the deletion of the *CCR5* gene) accounts, at least in part, for differential rates of HIV transmission to children *(4)*.

Finally, viral characteristics, such as nonsyncytum-inducing type, may also impact the risk of mother-to-child transmission *(49,50)*. In addition, there needs to be further consideration for the possibility of varying transmission rates across strains and clades of the virus *(49)*.

THERAPEUTIC INTERVENTIONS IN THE PREVENTION OF VERTICAL HIV TRANSMISSION

Reverse Transcriptase Inhibitors

The initial reports of HIV in children highlighted the fact that this emerging infection could be transmitted from an asymptomatic mother to her newborn child *(51)*, that HIV-infected infants had faster disease progression, and that pediatric AIDS had a high mortality rate *(52,53)*. The poor prognosis of pediatric AIDS led investigators to design strategies to reduce perinatal HIV-1 transmission. ZDV, the only Food and Drug Administration-approved antiretroviral drug in the mid-1980s, was the obvious choice. The strategy emulated an approach used in the treatment of other perinatal infections, such as syphilis; namely, that *in utero* therapy could be delivered through the mother and a postexposure treatment could further decrease the risk of infection. However,

difficulties in quantifying the short- and long-term risks of the intervention, and barriers to successfully translating research into clinical practice were not fully appreciated at that time; nor did we imagine the impact that this trial would have on the evolution of the HIV epidemic. The success of the Pediatrics AIDS Clinical Trials Group (PACTG) 076 protocol, as unveiled in 1994, was the culmination of the efforts of patients, activists, clinical investigators, basic scientists, pharmaceutical companies, and the health care infrastructure *(54)*. Indeed, worldwide, this groundbreaking trial continues to inspire efforts to improve the delivery methods, therapies, and approaches to eliminating perinatal transmission, and to improving maternal health. This section summarizes what we have learned from the use of nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) as monotherapies or in combination with other agents during pregnancy in industrialized countries, as well as their safety and current controversies surrounding their use. The use of these agents in resource-poor settings is discussed in "Selected International Clinical Trials," following.

ZDV Monotherapy in the Prevention of Mother-to-Child HIV Transmission

At the time the PACTG 076 protocol was being developed, clinical observations and animal models had documented that the virus could be transmitted in early pregnancy, or after birth through breast-feeding *(55–57)*. The natural history of the disease in children suggested that, although some infants with rapid clinical deterioration were infected *in utero*, others seemed to acquire their infection closer to the time of birth. A randomized, double-blind, placebo-controlled, phase III trial was selected as the optimal study design to investigate whether or not perinatal transmission of the HIV virus could be averted with antiretroviral treatment *(54)*. Patients who would potentially benefit from the use of antiretroviral treatment (symptomatic patients or those with AIDS) were excluded from participation, and most women were antiretroviral naive at the time of enrollment. Because of concerns of teratogenicity, drug exposure during the first trimester was avoided, therefore, the earliest exposure to the drug was at 14 wk gestation. Finally, to maximize the effectiveness of this intervention, the study drug or placebo was to be delivered throughout pregnancy, labor, and in the postnatal period.

An open-label, phase I pharmacokinetic study, PACTG 082, was undertaken before initiation of PACTG 076*(58)*. The goal of PACTG 082 was to ascertain the safety of ZDV in pregnant women and their children. Eight HIV-infected women with otherwise uncomplicated pregnancies were orally administered 200 mg ZDV five times daily, starting in the third trimester, followed by an intravenous (iv) 140 mg ZDV dose every 4 h during labor. The pharmacokinetic

evaluations performed at scheduled intervals after both oral or iv dosing showed that the clearance, elimination half-life, and bioavailability of ZDV was not altered by pregnancy. Higher drug levels in umbilical veins than in maternal veins suggested rapid, complete transfer of ZDV across the placental–fetal interphase, and not simple diffusion. Furthermore, the concentrations detected in vaginal secretions and amniotic fluid suggested that viral inhibition could be achieved in these compartments. Thus, ZDV seemed to be safe and well-tolerated among participants, with the exception of a decrease in fetal hemoglobin, with a nadir at 4 wk of life occurring in two infants. This information was invaluable for the subsequent PACTG 076 study design.

PACTG 076 was a randomized, double-blind, placebo-controlled trial. Pregnant women in the intervention group started taking 100 mg ZDV orally five times a day (from 14 to 34 wk of gestation) and continued until labor, at which time intravenous ZDV (loading dose of 2 mg/kg during 1 h followed by continuous infusion of 1 mg/kg/h) was administered. The babies received oral ZDV syrup (2 mg/kg every 6 h) for the first 6 wk of life. Approximately half of the women received placebo.

PACTG 076 initiated enrollment in 1991 and closed to accrual in February 1994, when the Data Safety Monitoring Board observed a dramatic difference in perinatal transmission rates between women who received ZDV vs those who receive placebo (8.6% vs 25%; $p = 0.00006$). The efficacy of the regimen was not affected by the time of enrollment (before or after 26 wk gestation) nor by the baseline CD4 lymphocyte counts (> or <500 cells/mm^3). Severe drug-related toxicities were rare among participants. Hematological (anemia and thrombocytopenia) and hepatic derangements (elevated liver enzymes) were the most common toxicities reported in the mothers. Anemia was the most common adverse event in infants, with spontaneous recovery after discontinuation of the drug 6 wk after birth *(54)*. These results were rapidly translated into clinical practice within industrialized countries, with the development of perinatal guidelines, which were disseminated in August 1994 *(59)*. The impact of such guidelines on clinical practice was evidenced by a decrease in perinatal transmission rates to 6 to 8% *(60,61)*.

Although the PACTG 076 study enrolled only asymptomatic women, the same regimen was recommended for all HIV-infected pregnant women, including those with advanced disease, those who initiated care late in pregnancy, and even those who could only receive newborn therapy *(59)*. Many years later, the foresight of this broad extrapolation of the PACTG 076 study was to be confirmed by PACTG 185 and other observational cohorts *(62,63)*. In the PACTG 185 trial, HIV-infected women with advanced disease (median CD4 cell count, 306 cells/μL; 22% of patients had CD4 counts <200 cells/μL) were randomized to receive γ-globulin or HIV-1 hyperimmune immunoglobulin

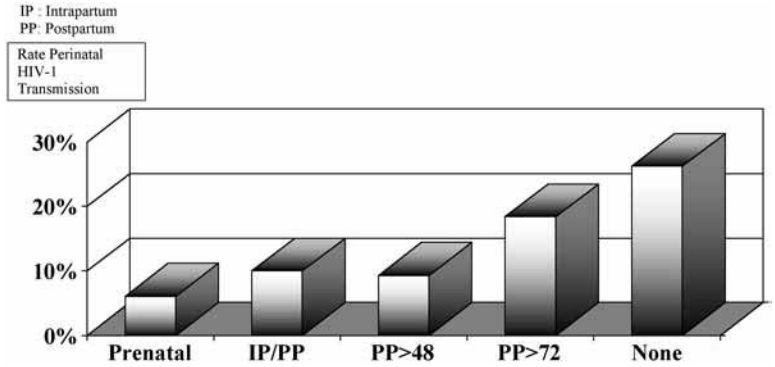

Fig. 3. HIV-1 transmission based on time of ZDV treatment.

(HIVIG) in addition to the PACTG 076 regimen to ascertain the effectiveness of passive immunoprophylaxis in reducing perinatal HIV transmission. However, in March 1997, the study was halted prematurely by the Data Safety Monitoring Board, when the rate of perinatal transmission was found to be unexpectedly low (5.0% overall; γ-globulin, 6.0% vs HIV-1 hyperimmune immunoglobulin, 4.1%; $p = 0.36$), with no difference detected between the treatment arms. This decision was made because the increase in sample size required to test the original hypothesis was deemed too large and the treatment effect seemed to be less than 50%. Nevertheless, this study supported the relevance of incorporating ZDV chemoprophylaxis in the treatment of women with advance disease to reduce perinatal transmission *(62)*.

Using observational data from deliveries of HIV-infected pregnant women in the state of New York, Wade and collaborators showed that the use of abbreviated ZDV regimens, whether by maternal choice or limited prenatal care, were less effective than the complete PACTG 076 regimen but better than no treatment at all. Only 9.3% of the infants who initiated neonatal ZDV treatment within 48 h from birth were HIV infected, compared with 26% of infants with no treatment at all *(63)* (Fig. 3).

The treatment guidelines for adults, adolescents, and pregnant HIV-infected women strongly recommend the use of ZDV as one of the NTRIs taken during pregnancy to achieve optimal chemoprophylaxis. Compliance with such recommendations has been reported by Minkoff et al. using data from the Women's Interagency HIV Study *(64)*. On the contrary, other investigators have reported underuse of this regimen in clinical practice. Wiznia et al. observed that women who reported cocaine or intravenous drug use during pregnancy were more likely to refuse therapy, have inconsistent use of ZDV, or have missed the intrapartum component *(65)*. A study performed using data from New Jersey Medicaid found that use of ZDV chemoprophylaxis was less

likely in those with insufficient or no prenatal care. The authors also noted that inconsistent use was more frequent among women of African American descent *(66)*. Wilson et al., reporting for the Perinatal Evaluation Project, observed that although ZDV was prescribed to most patients, as many as 20% of patients acknowledged inconsistent use of the medication. Concomitant illicit drug use during pregnancy and erratic prenatal vitamin use have also been significantly associated with inconsistent ZDV use *(67)*.

In the same multicenter observational cohort, Ickovics et al. used microprocessor technology (Medication Event Monitoring System) to evaluate adherence patterns to ZDV chemoprophylaxis. These authors confirmed that adherence patterns among participants were very low (mean, 50%) during pregnancy, and that adherence declined significantly 3 wk postpartum *(68)*. Collectively, these studies illustrate the need to evaluate adherence patterns during pregnancy and during the postpartum period, quantifying risk factors associated with nonadherence. Developing strategies to improve adherence in this population is critically important, given the imminent threat of antiretroviral resistance.

Factors in ZDV-Treatment Success

Intracellular phosphorylation of ZDV is necessary for inhibition of viral replication in vitro, and higher ZDV triphosphate levels have been associated with reduction of viral load and improvement in CD4 counts in HIV-infected patients. To determine the pharmacological endpoints associated with therapeutic activity and the prevention of perinatal transmission, Rodman et al. evaluated the extent of intracellular phosphorylation of ZDV in maternal and cord blood peripheral blood monocytes after the standard intravenous infusion of ZDV during labor. Although the authors observed substantial intersubject variability in ZDV pharmacokinetics, the median levels of ZDV monophosphate and triphosphate in maternal circulation and cord blood were similar. Thus, intravenous therapy provided intracellular ZDV triphosphate levels consistent with high antiviral activity. Moreover, the levels found in the cord blood can explain the success of the ZDV regimen in reducing perinatal transmission *(69)*.

Implementation of the guidelines preceded our understanding of the mechanism involved in the efficacy of ZDV chemoprophylaxis. Two alternative mechanisms explaining the efficacy of ZDV were plausible. First, a decrease in maternal viral load by this antiretroviral therapy might result in decreased fetal exposure. Second, postexposure prophylaxis to the infant at the time of greatest risk of transmission may be sufficient to confer protection from infection. Sperling et al. undertook an analysis of HIV-1 viral load among PACTG 076 participants, which shed light on this enigma *(70)*. These investigators observed that in both the placebo and treatment groups, a high HIV-RNA polymerase

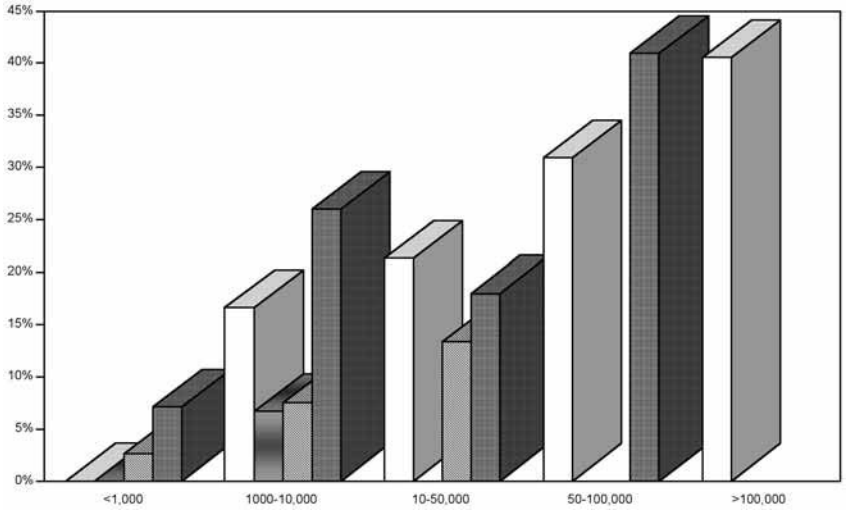

Fig. 4. Perinatal HIV-1 transmission in the HAART era.

chain reaction level at either entry or delivery was associated with an increase risk of transmission. Nonetheless, as noted above, perinatal transmission has occurred through a wide range of viral load levels, implying that there may be no threshold below which transmission will not occur. This data supports the use of ZDV in all pregnant women, irrespective of their viral load or CD4 cell count, and suggests that the efficiency of this modality relies on both a decrease in viral load and on postexposure prophylaxis *(70)*.

The importance of the HIV-1 RNA level before treatment initiation and at delivery as risk factors for perinatal HIV-1 transmission was corroborated by analysis of PACTG 185 and the WITS data *(71,72)*. Mofenson et al. showed that viral load at delivery was the strongest predictor of perinatal transmission in PACTG 185. No transmission was observed among women with viral loads of fewer than 500 copies/mL, suggesting that antiretroviral therapy could reduce maternal viral load below the threshold at which perinatal HIV-1 transmission can be averted *(71)*. Garcia et al. showed that increasing median levels of plasma HIV-1 RNA were associated with increasing rates of transmission. For example, the rate of transmission was 0% for those with viral loads less than 1000 copies/mL, and 40.6% for women with viral loads greater than 100,000 copies/mL (Fig. 4) *(72)*.

Shapiro et al. investigated factors associated with failure of ZDV chemoprophylaxis using data from the PACTG 076 study. Transplacental passage of ZDV occurs by diffusion, therefore, fetal concentrations are highly dependent on the maternal plasma concentration and can be affected by maternal weight. These investigators observed that high maternal weight could decrease the efficacy of

the regimen. Indeed, lower plasma levels of ZDV have been observed among obese subjects administered standard adult doses. The authors also observed that fetal exposure to maternal blood or cervicovaginal secretions could also decrease treatment efficacy *(73)*.

In summary, ZDV efficacy depends on its ability for intracellular phosphorylation leading to a decrease in viral load during pregnancy, delivery, or postpartum. Factors that either alter volume of distribution, such as obesity, or increase viral load may compromise its efficacy.

ZDV Monotherapy: Pharmacokinetics, Safety, Efficacy, and Resistance

In preclinical carcinogenicity studies, animal models showed an association between high-dose ZDV and the development of vaginal epithelial neoplasms in female rodents *(74)*. However, the long-term effect of *in utero* exposure to ZDV in growing children and adults was unknown at the time of PACTG 076 closure. A subsequent trial, PACTG 219, was designed to provide long-term follow-up of all participants exposed to antiretroviral therapies in our efforts to curtail HIV transmission, including those patients enrolled in PACTG 076.

Culane provided the first analysis of the long-term safety of uninfected children (median age, 4.2 yr; range, 3.2–5.6 yr) exposed to ZDV chemoprophylaxis *(75)*. This observational study showed no adverse effects among children exposed to ZDV *in utero* and postnatally. Specifically, there were no differences in weight, height, head circumference, cognitive and developmental function, or sequential lymphocyte subsets *(75)*. Her data were corroborated by the report of Celine and collaborators, regarding the absence of tumors in ZDV-exposed infants by 38 mo of age *(76)*. Despite these reassuring observations, both groups stress the importance of establishing long-term, epidemiological surveillance of these infants to monitor potential carcinogenic effects and other unforeseen, adverse manifestations of ZDV exposure.

Craig and collaborators used a population-based cohort to assess congenital anomalies in children exposed to ZDV *(77)*. Using New Jersey Medicaid claims data from 1993 to 1996, they observed that the prevalence of any anomaly was 2.76 times greater in children exposed to ZDV than in the general pediatric population. The adjusted odds ratios with 95% confidence interval (CI), by trimester of prescription, were 1.20 (95% CI, 0.58–2.51); 1.47 (95% CI, 0.85–2.55) and 1.84 (95% CI, 1.04–3.25) for first, second, and third trimester, respectively. However, these results are difficult to interpret because we typically expect the highest proportion of anomalies to occur in the first trimester, when the developing fetus is most vulnerable to chemical insult. No other risk factors among the mothers were ascertained *(77)*. These data emphasize the importance of ongoing prospective surveillance of exposed populations.

Review of the data (through January 2002) from the Antiretroviral Pregnancy Registry *(78)*, a voluntary, population-based surveillance system, reveals a prevalence of 2.5 birth defects per 100 live births to women with first trimester exposure to any of the antiretroviral agent (95% CI, 1.6–3.7). These results are no different from the Centers for Disease Control population birth surveillance system for births for the period 1991–1995. No increases in overall birth defects or in cardiovascular or genitourinary system malformations have been detected in association with the use of ZDV, lamivudine (3TC), stavudine (d4T), or nelfinavir *(78)*.

Initially, there was some concern that transient use of ZDV would have negative consequences for maternal health. It was thought that after discontinuation of therapy a rebound in viral load or the development of genotypic resistance would lead to faster disease progression and would compromise selection of therapy at the time when the woman required antiretroviral treatment for her own health. PACTG 288 was a 3-yr follow-up study of participants of the PACTG 076 trial. Bardeguez et al. found that after completion of the follow-up period there was no difference in clinical, immunological, or virological parameters between women randomized to the ZDV or the placebo arm *(79)*. Their observations imply that the use of ZDV chemoprophylaxis does not adversely affect women's long-term health. Additionally, detection of genotypic ZDV resistance (at codons 70 and 215) after postpartum exposure to the drug (either as monotherapy or part of a combination regimen) was only 10% and did not differ between treatment groups. These observations led the authors to conclude that ZDV monotherapy could be safely considered for patients with normal CD4 counts and low viral loads who desire reduction in the risk of perinatal transmission of HIV but do not wish to be exposed to complex drug regimens *(79)*.

The widespread use of antiretroviral agents and the increasing occurrence of resistant strains to many of these drugs have raised concerns of:

1. The individual and population implications of development and transmission of resistant strains.
2. The need to monitor the development of antiretroviral resistance among individuals at risk.
3. The urgent need to develop strategies to decrease resistance among patients on treatment as well as newly infected individuals.

The Public Health Service guidelines recommend resistance testing for individuals with poor response to therapy or rising viral loads despite adequate regimen and documented adherence *(80)*. Other groups have advocated more-widespread use of resistance testing, which includes testing of pregnant women before initiation of therapy when there has been previous antiretroviral exposure or if the prevalence of resistance in the community is high *(81)*. More-

recent recommendations by the same panel also suggest that resistance testing may be considered for antiretroviral-naive individuals if infection with resistant strain is suspected *(82)*. Although support for this approach is limited, the increasing numbers of reports documenting perinatal transmission in women with previous ZDV exposure and incomplete viral suppression emphasizes the importance of validating this concept *(83,84)*. The importance of ongoing surveillance of genotypic resistance among pregnant women was recently demonstrated by Palumbo et al. *(85)*. Using observational data from pregnant women enrolled in the Pediatrics AIDS Collaborative Transmission Study, these investigators detected ZDV-associated mutations in 17.3% of the participants between 1991 and 1997. Resistance to NNTRIs and protease inhibitors (PIs), however, was infrequent. Furthermore, there was no association between perinatal transmission and the presence of ZDV-resistance mutations, and, although ZDV resistance was also detected in 8.3% of the neonatal samples, the mutation pattern was different from that seen in the mother.

ZDV and 3TC Combination Therapy: Pharmacokinetics, Safety, Efficacy, and Resistance

As new antiretroviral therapies were approved for the treatment of HIV-infected adults, their use also increased among pregnant women. Combination therapy with two drugs became the strategy in the early 1990s because combination therapy could achieve better suppression of viral load and result in higher CD4 counts than monotherapy. A ZDV and 3TC combination became very popular because its use required only two pills twice daily. The pharmacokinetics of 3TC in pregnant women was first investigated by Moodley et al. Their study protocol involved women receiving therapy from 38 wk gestation until 1 wk postpartum, and the neonates receiving therapy during the first week of life *(86)*. The authors observed that 3TC alone or in combination with ZDV freely crossed the placenta and that 3TC was detected in amniotic fluid and breast milk. The pharmacokinetics of 3TC 1 wk after delivery were not affected by presence or absence of ZDV administration, which suggested that no dose adjustment was necessary for pregnant women. Drug concentrations in the women, cord blood, and neonates near birth were very similar to therapeutic doses, with maternal-to-cord blood-to-neonate ratios near unity. A substantial decline in viral load was observed among participants, with a mean viral load reduction of 1 $\log_{10}$ copies/mL 1 wk after initiation of therapy. However, two patients experienced a rebound in viral load and the M184 mutation was present in both cases. This provided early documentation that resistance to 3TC could develop with short treatment exposure. Very low or no HIV was detected in the neonates at birth, suggesting that combining maternal treatment with postexposure prophylaxis in the infant could decrease perinatal transmission.

This approach was further evaluated by the PETRA study described in detail in "Selected International Clinical Trials," following. Another advantage of the ZDV plus 3TC regimen was that it was well-tolerated by mothers and infants. None of the participants discontinued antiretroviral use because of adverse events, although the authors described two infant deaths at 5 and 6 mo of age.

The transplacental passage of 3TC by a passive diffusion mechanism was corroborated by Mandelbrot et al. *(87)*. These authors also reported amniotic fluid concentrations and found that the median level in this compartment was fivefold higher than the maternal plasma levels, which suggested that accumulation of the drug occurs. Despite the benefit in reduction of viral load that accumulation in this compartment may carry, they warn of an increased risk of *in utero* toxicity.

The efficacy of ZDV plus 3TC was also evaluated by Mandelbrot et al. in an open-label, nonrandomized trial conducted within the context of an ongoing observational cohort in France *(88)*. Four hundred forty-five women were enrolled in the study and received ZDV plus 3TC. The control group consisted of 899 women who received ZDV monotherapy. There was a significant decrease in perinatal HIV transmission in the study group, at 1.6%, a fivefold decrease compared with the control group. The maternal HIV RNA level was fewer than 500 copies/mL at delivery for 74% of participants, with a median decrease of 1.24 $\log_{10}$ copies/mL. However, the M184V mutation was detected in 39% of the women in the 6-wk postpartum specimen. Multivariate analysis showed that low CD4 count and high viral load at enrollment, as well as duration of 3TC treatment longer than 8 wk were all significantly associated with development of the M184V mutation. The most frequent adverse events in children were neutropenia and anemia, however, two of the uninfected children died at 1 yr of age from neurological complications associated with mitochondrial dysfunction. Although the reduction in HIV transmission in the intervention group was marked, the potentially lethal effects of this regimen coupled with the risk of resistance developing among mothers clouded enthusiasm for this approach.

Mitochondrial toxicities ranging from myopathy to lactic acidosis, and death had been observed among individuals receiving NTRIs for treatment of HIV disease *(89–91)*. The report by Blanche et al. of two deaths ascribed to mitochondrial dysfunction among HIV-uninfected children exposed to combination therapy with two NTRIs *(91)* prompted a review of five longitudinal cohorts to ascertain the frequency of this event in the United States *(92)*.

There has been a systematic review of all deaths in HIV-exposed children younger than 60 mo of age (regardless of infection status), which has included assignment of each child to category 1, 2, 3, or 4, indicating whether death was unrelated, unlikely related, possibly related, or highly suggestive of mitochon-

drial dysfunction, respectively. Sudden infant death syndrome (SIDS) was categorized separately. There were 223 deaths (1.1%) among more than 20,000 HIV-exposed children, of whom, half received NTRIs. To date, there has been no infant death in the United States that is highly suggestive of, or with proven relationship to, mitochondrial dysfunction. The SIDS rate for the cohort was 1.8 per 1000 live births, which is comparable to the national statistics. There was no excess of SIDS reported among children exposed to either ZDV monotherapy or ZDV in combination with 3TC.

Although all patients offered antiretroviral therapy during pregnancy should be aware of this potential complication, US data suggest that death from mitochondrial dysfunction is a rare outcome. Nevertheless, because of potentially fatal consequences, prospective surveillance among living children in these cohorts is continuing.

Didanosine: Pharmacokinetics and Safety

Wang and collaborators evaluated the pharmacokinetics of didanosine (ddI) in HIV-infected pregnant women and their neonates. During both pregnancy and the postpartum period, the authors reported no effective change in pharmacokinetics after oral dosing twice daily, and concluded that no adjustment was needed for pregnant women *(93)*. Although this drug could potentially be used in combination regimens for pregnant women, clinicians are strongly cautioned against the use of ddI together with d4T, because metabolic complications and death have been reported in the pregnant population *(94)*.

NNTRIs: Pharmacokinetics, Safety, Efficacy, and Resistance

Nevirapine

Nevirapine (NVP), is a potent NNRTI that is rapidly absorbed after oral administration with a wide and even distribution throughout the body. In addition to its lipophilic properties, this drug can penetrate cell-free virions and inactivate their reverse transcriptase *in situ*, rendering NVP capable of suppressing replication in different compartments. Mirochnick and collaborators evaluated the safety and pharmacokinetics of NVP in pregnant women and their children *(95)*. The drug readily crossed the placenta, and a single dose of 200 mg administered to the mother in labor followed by a 2 mg/kg oral dose to the infant was able to achieve concentrations in the newborn that exceeded the in vitro 50% inhibitory concentration against HIV for up to 1 wk of life. The drug was well tolerated and no adverse effects of NVP were reported among participating mothers or their infants. The simplicity of this regimen made it suitable for efficacy trials in resource-poor countries as an alternative to the ZDV chemoprophylaxis considered the standard of care in developed countries. The HIV Network for Prevention Trials (HIVNET) 012 trial was

one of these attempts and is described in detail in "Selected International Clinical Trials," following.

There has been growing interest in the United States in investigating whether the addition of new antiretroviral treatments to ZDV chemoprophylaxis could further reduce perinatal transmission. To this end, PACTG 316 was designed to evaluate the effectiveness of the addition of a two-dose intrapartum and newborn NVP regimen to the standard antiretroviral therapy *(96)*. This international, randomized, double-blind, placebo-controlled clinical trial was implemented in developed countries among nonbreast-feeding women. Women in the treatment and placebo arm were similar in demographic characteristics, median CD4 cell count, and viral load at entry and at delivery. Thirty-two percent of the participants were delivered by elective cesarean section. Twenty-three percent of participants used ZDV monotherapy, 35% used a non-PI-containing regimen, and 41% used a PI-containing regimen. Overall, the two-dose NVP regimen was well-tolerated based on the few reports of grade 3 or 4 maternal or infant toxicities among participants. Perinatal transmission rate was extremely low and not significantly different between the NVP (1.4%; 95% CI, 0.6–2.7%) and placebo arms (1.5%; 95% CI, 0.8–2.9%) which led to the early closure of this trial in June 2001. Maternal viral load at the time of delivery was the only significant predictor of perinatal HIV transmission *(96)*. Thus, for the nonbreast-feeding woman receiving antiretroviral therapy during pregnancy who can avail herself of elective cesarean section, the additional two-dose NVP regimen does not seem to confer any additional benefit.

Unfortunately, the use of this regimen could potentially compromise future antiretroviral treatment options for women of childbearing age and their children *(97)*. An analysis of a PACTG 316 substudy by Coleen et al. found that new mutations associated with NVP resistance developed in 15% of the women by 6 wk postpartum, after they received a single dose of NVP. The most common mutation was K103N, and mutations were observed in different HIV subtypes. Of interest, CD4 cell count, HIV RNA load at the time of exposure, and the type of antiretroviral regimen used were predictors of the development of resistance. Perinatal transmission was no different among women with or without NVP resistance *(97)*. These observations do not support the use of a two-dose NVP regimen to decrease perinatal HIV transmission among patients already receiving standard antiretroviral therapy, and they emphasized the need to evaluate the long-term implications of these findings for women's health.

Maternal Toxicity With Prolonged NVP Use in Pregnancy

The importance of ongoing research and clinical vigilance surrounding issues of drug safety are highlighted by the ACTG 1022 protocol. Hitti et al. examined the safety of nelfinavir and NVP treatment in HIV-1-infected pregnant women

(98). Thirty-eight antiretroviral-naive women were enrolled between 10 and 30 wk gestation and randomized to either nelfinavir plus ZDV plus 3TC or NVP plus ZDV plus 3TC regimens. The study was suspended because of concerns surrounding the safety of NVP.

Drug toxicity was observed in 29% (5/17) of patients randomized to the NVP arm compared with only 5% (1/21) of patients randomized to the nelfinavir arm ($p = 0.07$). Severe reactions reported in the NVP group included one participant with fulminant hepatic failure who died, and a second participant who developed Stevens-Johnson syndrome. The single adverse reaction reported in the nelfinavir group was hepatitis. The authors caution that continuous NVP use may be associated with increased toxicity among HIV-infected women, particularly those with CD4 lymphocyte counts greater than 250 cells/μL. These findings are corroborated by observations in nonpregnant patients.

However, the authors highlight the fact that their findings cannot be extrapolated to the use of single-dose intrapartum NVP for prevention of HIV transmission from mothers to infants. Moreover, there is no evidence linking NVP toxicity to immune reconstitution. It is not known whether women who tolerate NVP well should discontinue use of this antiretroviral treatment if they experience immune reconstitution, regardless of pregnancy status.

Efavirenz

Efavirenz, another NNRTI approved by the Food and Drug Administration for the treatment of HIV-infected individuals, was readily welcome based on its potency in combination with other antiretroviral therapies, compact once-daily dosing, and tolerability. However, its use during pregnancy and among reproductive-age women not using effective contraception is discouraged because of its teratogenicity. Studies in monkeys and human case reports have documented an association between use of efavirenz and neural tube defects *(99)*. Efavirenz is classified as FDA Pregnancy Category D. Therefore, this antiretroviral should be avoided during pregnancy because it may casue fetal harm when administered during the first trimester. Women who conceive while taking efavirenz should be switched to an alternative antiretroviral therapy.

Newer Agents in the NRTI Arena

The optimal strategy to initiate antiretroviral therapy should consist of simpler regimens that decrease pill burden and dosing schedules, and decrease the frequency of long-term toxicities *(100)*. Some of these strategies use combination therapy with recently approved NTRIs or NNRTIs. Although Public Health Service guidelines for the treatment of HIV-infected pregnant women continue to endorse use of highly active antiretroviral therapy (HAART) during pregnancy, data regarding the pharmacokinetics, safety, and efficacy of many of

these newer antiretroviral treatments during pregnancy are insufficient. Puga et al. evaluated the maternal and infant outcomes among 36 mothers who received abacavir (category C) during pregnancy *(101)*. These authors performed a retrospective chart review of all births to women who received abacavir between 2000 and 2001 at the Children's Diagnostic & Treatment Center in Ft. Lauderdale, Florida. Most of the women were African American (94%), with a mean maternal age of 25 yr. The mean gestational age at the time of initiation of the regimen was 9.5 wk, with 25% of the women taking the regimen at the time of conception.

The majority of the women were taking either 3TC plus ZDV (Combivir) or abacavir plus 3TC plus ZDV (Trizivir). There was an average decline of 1.6 $\log_{10}$ copies/mL in viral load and 58% (21/36) of the women had viral loads that were undetectable at the time of delivery. All women delivered a single live birth, with a mean gestational age of 39 wk (median, 39 wk; range, 33–42), with the exception of one infant born at 33 wk by emergency cesarean section to a mother with advanced AIDS. This child had transient metabolic acidosis, a heart murmur, and was small for gestational age, but at the time of this report the child's growth is within normal limits, the heart murmur had resolved, and she is currently healthy *(102)*.

There were no adverse events in either mothers or infants. There were no reports of hypersensitivity reaction associated with the use of abacavir. Additionally, there were no reports of birth defects in this cohort, and growth parameters at 6 mo of age were normal for all infants. The transmission rate in this group was 2.8%, compared with a 4.2% transmission rate for the overall population cared for at this institution during the study period. The only infected infant in the cohort was born to a woman coinfected with hepatitis C. Thus, in this small series, abacavir was well tolerated by mothers and infants, and resulted in a significant reduction in perinatal transmission. The simplicity of the regimen makes it an excellent option for patients at risk of poor adherence to therapy *(103)*.

HAART During Pregnancy

In the mid-1990s, several investigators reported that patients who received a PI in combination with NRTIs had lower rates of hospital admissions and experienced decreased HIV-related morbidity and mortality *(102–104)*. These observations led to modifications in the treatment guidelines for adults and adolescents, which stipulated that HAART was the optimal approach for treatment, and that such regimens should include a PI and two NRTIs, unless contraindicated *(105)*. The goal of HAART is to decrease HIV viral load, achieve immune reconstitution, and improve the probability of long-term survival *(106)*. Unique to these recommendations was the affirmation that therapy

should not be withheld from pregnant women unless the risk outweighs the benefits *(107)*. This approached was challenged by the report of Lorenzi and collaborators describing the complications experienced by women receiving combination therapy during pregnancy *(108)*. In this small cohort of pregnant women ($N = 37$) there were higher rates of anemia, nephrolithiasis, and diabetes among women with antiretroviral exposure than among historical controls who had not been exposed to antiretroviral therapy. In addition, infants born to women taking HAART experienced a higher rate of prematurity, although most (93%) of those with complete follow-up were uninfected children. This report provided further impetus to accurately ascertain both the risks and benefits of HAART use during pregnancy *(108)*. It also highlighted the importance of evaluating the safety of antiretroviral therapies during pregnancy rather than simply extrapolating data from nonpregnant subjects.

A retrospective survey was conducted in six US centers to evaluate the safety and efficacy of PIs during pregnancy *(109)*. The prematurity rate was 19%, but was significantly associated with cocaine use during pregnancy or premature rupture of membranes. There was no significant association between time of initiation of PI therapy and prematurity. Indeed, adverse maternal or fetal events were rarely associated with use of PIs, and the transmission rate in this series was zero (95% CI, 0–3%). Morris et al. concluded that the use of PIs seems safe, is well-tolerated, leads to low perinatal transmission, and is associated with significant benefit in pregnant women, and prematurity rates were similar to prior data in HIV-positive women not on PIs *(109)*.

Tuomala et al. analyzed data from seven large cohorts of HIV-infected pregnant women to ascertain the risk of adverse pregnancy outcomes associated with the use of antiretroviral therapy *(110)*. This large cohort of 2123 women included data from 1990 through 1998. Contrary to previous observations, these investigators found similar rates of premature delivery, low birth weight, and very low birth weight among patients who received antiretroviral therapy and those who did not, after controlling for CD4 cell count and use of tobacco, alcohol, or illicit drugs. The rate of low Apgar scores and stillbirths was also similar between the groups. The authors also observed an association between use of combination therapy with a PI and a risk of very low birth weight infants, which certainly deserves further evaluation. Based on this large data set, we can conclude that adverse outcomes are rarely associated with the use of antiretroviral therapy during pregnancy. Nonetheless, we should closely monitor patients receiving a regimen that includes a PI, because they could have an increased risk of very low birth weight infants.

Cooper and collaborators evaluated the impact of temporal changes in the use antiretroviral regimens during pregnancy on perinatal transmission using data collected from the WITS trial *(111)*. They observed that use of antiretroviral

therapy and increased complexity of the regimens from ZDV monotherapy to combination therapy with two NRTIs, and, ultimately, HAART regimens, resulted in dramatic decreases in perinatal transmission. HAART use was associated with the lowest perinatal transmission rates. Their study also supported the hypothesis that HIV-1 RNA level at delivery is an independent predictor of perinatal transmission *(112)*. The implications of these observations for clinical practice are important in terms of reducing the need for operative delivery *(113–115)* and its potential adverse outcomes *(116–118)* as the only approach to reduce perinatal transmission.

Selected International Clinical Trials in the Prevention of Mother-to-Child HIV Transmission

Since the PACTG 076 protocol demonstrated that the use of ZDV during the antepartum, intrapartum, and postpartum periods is effective in reducing the rate of mother-to-child transmission by 67% *(54)*, there have been numerous clinical trials of both ZDV alone and ZDV in combination with other antiretroviral treatments to investigate shortened maternal and infant regimens that would be more affordable and better suited to the developing country setting (Table 1). Various studies have been conducted in nonbreast-feeding populations, as well as in breast-feeding women. The treatment effects are explained largely by decreases in maternal viral load at delivery, with absolute HIV viral load being more important than the percentage decrease in viral load *(119)*.

In addition to the trials described in the following sections, there are also several studies currently investigating other drug combinations.

Nucleoside Reverse Transcriptase Inhibitors

ZDV-Only Trials

The Bangkok Trial (Thailand): Nonbreast-Feeding

This was a randomized, double-blind, placebo-controlled, phase III trial conducted in Bangkok, Thailand *(119)*. Between May 1996 and December 1997, 397 women were enrolled in either an intervention arm (n = 194) or a placebo arm (n = 198). The primary endpoint of this antepartum/intrapartum study was infants' HIV-1 status at 2 mo. At enrollment, the median CD4 count was 427 cells/mm^3 for women in the intervention group and 411 cells/mm^3 for women in the placebo group, whereas the median HIV-1 RNA load was 26,952 copies/mL and 33,933 copies/mL, respectively.

Women randomized to the intervention arm received 300 mg ZDV orally, twice daily, beginning at 36 wk of gestation, followed by 300 mg ZDV orally at the onset of labor and every 3 h until delivery. There were no postpartum interventions.

Based on the analysis for data for 395 live births, the incidence of HIV infection in infants whose mothers were randomized to the ZDV arm was 9% (95% CI, 5–14%), whereas the rate of infection in the placebo arm was 19%. The efficacy of this shortened course of ZDV delivered to HIV-infected mothers during the antepartum and the intrapartum period was, therefore, reported as 50%.

This short course of ZDV was safe and well-tolerated by pregnant women. The authors concluded that this regimen reduced the risk of mother-to-child transmission by 50% in HIV-infected women delivering after 36 wk of gestation. ZDV lowered the risk of transmission irrespective of duration of treatment, number of labor doses, duration of labor, duration of ruptured membranes, and type of delivery.

The Perinatal HIV Prevention Trial (Thailand): Nonbreast-feeding

The Perinatal HIV Prevention Trial (PHPT) was a randomized, double-blind, equivalence trial conducted at 27 sites in Thailand between June 1997 and December 1999 *(120).* 1437 women were enrolled in either the long–long (n = 401), long–short (n = 340), short–short (n = 229), or short–long arms (n = 338). The outcomes measured in this antepartum/intrapartum/postpartum study were infants' HIV status at 1, 45, 120, and 180 d. At enrollment, median CD4 count was 370 cells/mm^3 in the long–long arm (for the entire duration of the study), 350 cells/mm^3 in the long–short arm, 360 cells/mm^3 in the short–short arm (preliminary analysis), and 360 cells/mm^3 in the short–long arm. Data analysis is based on a total of 1409 live births.

Women randomized to the long–long arm received 300 mg ZDV orally, twice daily, beginning at 28 wk, followed by 300 mg ZVD orally at the onset of labor and every 3 h until delivery, whereas infants in this trial arm received 2 mg/kg ZDV orally, four times daily for 7 d. Women randomized to the long–short arm received the same antepartum and intrapartum intervention as women in the long maternal arm, but their babies received 2 mg/kg ZDV orally, four times daily for only 3 d. By contrast, women randomized to the short–short arm received 300 mg ZDV orally, twice daily beginning at 35 wk and then 300 mg ZVD orally at the onset of labor and every 3 h until delivery. Their infants received the shortened ZDV course. Women randomized to the short–long arm received the shortened maternal ZDV course and their infants received the lengthened ZDV course.

Interim data analysis revealed HIV infection in 4% of babies in the long–long arm and 11% of babies in the short–short arm. This difference was found to be statistically significant and was further accentuated when the data was stratified by length of gestation at delivery. For women who delivered at less than 36 wk age of gestation, HIV-infection rates were 9% and 44% for the long and short regimens, respectively. However, for women who delivered after

Table 1
Summary of International Clinical Trials in Prevention of Mother-to-Child HIV Transmission

Study		Antepartum intervention	Intrapartum intervention	Postpartum intervention	Results% infected infants (time of testing)
Bangkok Trial NBF, DB n=397	A_1	300 mg ZDV, BID from 36 wks	300 mg ZDV at onset of labor and every 3 hrs until delivery	None	9% (8 wk)
	A_2	Placebo	Placebo	None	19%
PHPT NBF, DB n=1,437	A_{LL}	300 mg ZDV, BID from 28 wk	300 mg ZDV at onset of labor and every 3 hr until delivery	Mothers: none Infants: 2 mg/kg ZDV, QID for 7 d	6%[a] (24 wk)
	A_{LS}	300 mg ZDV, BID from 28 wk	300 mg ZDV at onset of labor and every 3 hr until delivery	Mothers: none Infants: 2 mg/kg ZDV, QID for 3 d	5%
	A_{SS}	300 mg ZDV, BID from 35 wk	300 mg ZDV at onset of labor and every 3 hr until delivery	Mothers: none Infants: 2 mg/kg ZDV, QID for 3 d	11%[b]
	A_{SL}	300 mg ZDV, BID from 35 wk	300 mg ZDV at onset of labor and every 3 hr until delivery	Mothers: none Infants: 2 mg/kg ZDV, QID for 7 d	9%

Table 1 *(Continued)*

RETRO-CI/ DITRAME BF, DB n=701	A_1	250/330 mg ZDV, BID from 36–38 wk	500/600 mg ZDV at the onset of labor OR 300 mg ZDV at the onset of labor and every 3 hr until delivery	Mothers: 250/300 mg ZDV, BID for 7 d (DITRAME only) Infants: none	23% (24 mos)
	A_2	Placebo[c]	Placebo	Placebo	30%
PETRA BF, DB n=1,797	A_A	300 mg ZDV + 150 mg 3TC, BID from 36 wk	300 mg ZDV + 150 mg 3TC at onset of labor and 300 mg ZDV every 3 hr + 150 mg 3TC every 12 hr until delivery	Mothers: 300 mg ZDV + 150 mg 3TC, BID for 7 d Infants: 4 mg/kg ZDV + 2 mg/kg 3TC, BID for 7 d	19% (18 mos)
	A_B	Placebo	600 mg ZDV + 150 mg 3TC at onset of labor and 300 mg ZDV every 3 hr + 150 mg 3TC every 12 hr until delivery	Mothers: 300 mg ZDV + 150 mg 3TC, BID for 7 d Infants: 4 mg/kg ZDV + 2 mg/kg 3TC, BID for 7 d	24%
	A_C	Placebo	600 mg ZDV + 150 mg 3TC at onset of labor and 300 mg ZDV every 3 hr + 150 mg 3TC every 12 hr until delivery	Placebo	25%
	A_D	Placebo	Placebo	Placebo	26%

NBF, non-breastfeeding; BF, breastfeeding; DB, double blind; OL, open label; BID, twice daily; QID, four times daily; SOC, standard of care. Sample size *(n)* listed refers to the total number of women enrolled, and not the mother-infant pairs analysed;

[a]Composite data (24 mo); [b]Interim data; [c]placebo arm stopped early 1998

Table 1 *(Continued)*

Study		Antepartum intervention	Intrapartum intervention	Postpartum intervention	Results% infected infants (time of testing)
BMS NBF, OL n=373	A_1	40 mg d4T, BID from 34–36 wk	40 mg d4T, last dose approx 1 hr prior to delivery	Mothers: none Infants: 1 mg/kg d4T, BID for 6 wk	11% (24 wk)
	A_2	200 mg ddI, BID from 34–36 wk	200 mg ddI, last dose approx 1 hr prior to delivery	Mothers: none Infants: 120 mg/m^2 ddI, BID for 6 wk	11%
	A_3	40 mg d4T + 200 mg ddI, BID from 34–36 wk	40 mg d4T + 200 mg ddI, last dose approx 1 hr prior to delivery	Mothers: none Infants: 1 mg/kg d4T + 120 mg/m^2 ddI, BID for 6 wk	5%
	A_4	300 mg ZDV, BID from 34–36 wk	300 mg ZDV, last dose approx 1 hr prior to delivery	Mothers: none Infants: 4 mg/kg ZDV, BID for 6 wk	6%
HIVNET 012 BF, OL n=626	A_1	None	600 mg ZDV at onset of labor and 300 mg ZDV every 3 hr until delivery	Mothers: none Infants: 4 mg/kg ZDV, BID for 7 d	25% (14–16 wk)
	A_2	None	200 mg NVP at onset of labor	Mothers: none Infants: 2 mg/kg NVP within 72 hrs of birth	13%
	A_3[f]	None	Placebo	Mothers: none Infants: placebo	—

Table 1 *(Continued)*

SAINT BF, OL n=1,306	A_1	None	200 mg NVP at onset of labor	Mothers: 200 mg NVP 24–48 hrs after delivery Infants: 6 mg NVP 24–48 hrs after delivery	12% (8 wk)
	A_2	None	600 mg ZDV + 300 mg 3TC at onset of labor and 300 mg ZDV every 3 hrs + 150 mg 3TC every 12 hrs until delivery	Mothers: 300 mg ZDV + 150 mg 3TC, BID for 7 d Infants: 12 mg/4 mg/kg ZDV + 6 mg/2 mg/kg 3TC, BID for 7 d	9%
PACTG 316 NBF, DB n=1,270	A_1	SOC	200 mg NVP at onset of labor	Mothers: none Infants: 2 mg/kg NVP 48–72 hrs after delivery	1.4% (birth to 6 mos)
	A_2	SOC	Placebo	Mothers: none Infants: placebo	1.6%

[f]Placebo arm stopped early 1998

at least 36 wk of gestation, transmission rates were 3% and 9%, respectively. Because preliminary analysis revealed that the short–short arm was inferior to the long–long arm, the short–short arm was discontinued.

Composite data analysis at 180 d showed 6%, 5%, and 9% transmission rates for the long–long, long–short, and short–long arms, respectively. The efficacy of either maternal or infant shortened regimens was statistically equivalent to that of the long–long regimen.

All regimens were well-tolerated and the adherence reported was high. Because there was a marked differential in the rates of transmission for short–short group infants delivered before 36 wk compared with infants delivered at or after 36 wk (9% vs 44%), the authors suggest that short-course (Bangkok Trial-type) regimens are effective only for women delivering after 36 wk of gestation.

Furthermore, the authors concluded that transmission in short maternal regimens was higher than in longer maternal regimens, and that longer infant regimens cannot substitute for longer maternal regimens. Nevertheless, a lengthened infant regimen may be beneficial if mothers begin ZDV treatment late in pregnancy.

RETRO-CI/DITRAME-ANRS 049A (Ivory Coast and Burkina Faso): Breast-Feeding

These were randomized, double-blind, placebo-controlled, phase II/phase III trials conducted in Abidjan, Ivory Coast (RETRO-CI and DITRAME) and Bobo-Dioulasso, Burkina Faso (DITRAME) (121). Between September 1995 and February 1998, a total of 701 women were enrolled into the RETRO-CI study ($n = 421$) and the DITRAME study ($n = 280$). The primary outcome was HIV infection in infants at 2 wk, 6 mo, and 24 mo. Analysis was based on the pooled data of 662 live births. At enrollment, the median CD4 count was 545 cells/mm^3 in the intervention group and 535 cells/mm^3 in the placebo group.

Women assigned to the intervention arm ($n = 349$) were administered 250/300 mg (phase II/phase III) ZDV orally, twice daily from 36 to 38 wk of gestation. During the intrapartum period, women in the interventional arm received either 500/600 mg ZDV orally at the onset of labor (DITRAME) or 300 mg ZDV orally at the onset of labor and then every 3 h until delivery. DITRAME mothers also received 250/300 mg ZDV orally, twice daily for 7 d after delivery. Neither study protocol included prophylactic antiretroviral treatment for infants.

The authors reported that, at 2 wk and 6 mo, the cumulative rates of mother-to-child transmission in the ZDV arm were 13% and 17%, respectively, whereas incidence of HIV infection in infants born to mothers who had received placebo was 19% and 26%. The cumulative risk of HIV-1 transmission at 24 mo was

23% in the ZDV arm vs 30% in the placebo arm. The cumulative reduction in the risk of mother-to-child transmission associated with ZDV administration was, therefore, 26%. In separate analysis of the DITRAME and RETRO-CI data, the efficacy of ZDV at 24 mo was found to be 28% among DITRAME participants and 23% among RETRO-CI participants.

After stratified analysis, the authors concluded that the efficacy of ZDV was greater in women with higher entry-level CD4 counts. Additionally, they found lower rates of perinatal transmission in mothers with CD4 counts of at least 500 cells/mm^3 than in mothers with CD4 counts of fewer than 500 cells/mm^3, corroborating previous findings of advanced maternal disease as a strong indicator of perinatal transmission.

Finally, following infants to 24 mo demonstrates that ZDV has little or no efficacy in breast-feeding women with advanced HIV disease. In cases in which breast-feeding is an unsafe option, urgent efforts need to be directed toward interventions that will decrease the risk of postpartum transmission without jeopardizing the health of the child.

NRTI-Combination Trials

PETRA (Preventing Early and Late Transmission of HIV-1 From Mother to Child in Tanzania, South Africa, and Uganda): Breast-Feeding

This was a randomized, double-blind, placebo-controlled, phase III trial conducted in Kampala, Uganda; Dar-es-Salaam, Tanzania; and Soweto and Durban, South Africa (122). Between June 1996 and January 2000, 1797 women were enrolled across the four study sites and randomized to either arm A (n = 475), arm B (n = 474), arm C (n = 471), or placebo (377); however, the placebo arm was stopped in February, 1998. Interventional protocols included various combinations of antepartum, intrapartum, and postpartum ZDV and 3TC. The primary endpoints of this study were HIV-1 infection and mortality in infants at 6 wk and 18 mo. At enrollment, the median CD4 counts were 445 cells/mm^3, 475 cells/mm^3, 440 cells/mm^3, and 435 cells/mm^3, in arms A, B, C, and D, respectively.

Women randomized to arm A received 300 mg ZDV orally plus 150 mg 3TC orally twice daily, beginning at 36 wk of gestation. The intrapartum protocol for arm A was 300 mg ZDV orally plus 150 mg 3TC orally at the onset of labor, as well as 300 mg ZDV orally every 3 h plus 150 mg 3TC orally every 12 h until delivery. Postpartum, women in arm A received 300 mg ZDV orally plus 150 mg 3TC orally twice daily for 7 d and infants received 4 mg/kg ZDV orally plus 2 mg/kg 3TC twice daily for 7 d. Women randomized to arm B received 600 mg ZDV orally plus 150 mg 3TC orally at the onset of labor as well as 300 mg ZDV every 3 h plus 150 mg 3TC every 12 h until delivery. The postpartum regimen was identical to that in arm A. Finally,

women in arm C received only an intrapartum intervention according to the same protocol as arm B.

Data analysis for 1501 live births reveals cumulative rates of HIV-1 infection and infant mortality at 6 wk at 7%, 12%, 18%, and 18% in arms A, B, C, and D, respectively. At 18 mo, the cumulative rates of HIV-1 infection and mortality were 19% in arm A, 24% in arm B, 25% in arm C, and 26% in the placebo arm.

Seventy-four percent of women enrolled in the PETRA trial initiated breast-feeding. Stratified analysis of HIV-1 infection and mortality at 18 mo in breast-fed infants alone revealed cumulative rates of 22% in arm A, 24% in arm B, 28% in arm C, and 28% in arm D.

The antiretroviral potency of regimen A is notable because there was 61% reduction in HIV-1 infection in this treatment group compared with the control group at 6 wk. In addition, the intrapartum/postpartum regimen in arm B produced a 36% reduction in the rate of mother-to-child transmission as compared with the control group. However, the intrapartum-only regimen in arm C was not found to be significantly different from the placebo arm. The authors concluded that although regimens A and B were effective in reducing the risk of mother-to-child transmission at 6 wk postpartum, their benefit was considerably diminished with increasing postnatal exposure via breast-feeding.

BMS AI455 094 (South Africa): Nonbreast-feeding

This was a randomized, open-label trial conducted in Soweto, South Africa *(123,124)*. Three hundred seventy-three women were enrolled into one of four interventional arms evaluating the efficacy of d4T and ddI, both alone and in combination, against a ZDV control arm. The primary outcome measure in this antepartum/intrapartum/postpartum study was infants' HIV status at 24 wk after delivery. At entry, the median CD4 count was 392 cells/μL (all study arms).

Women randomized to the d4T arm received 40 mg d4T orally, twice daily from 34 to 36 wk of gestation until delivery. Infants born to mothers in the d4T arm received d4T at 1 mg/kg orally, twice daily for 6 wk. Women randomized to the ddI arm received 200 mg ddI orally, twice daily from 34 to 36 wk until delivery. Infants in the ddI arm received ddI at 120 mg/m^2 orally, twice daily for 6 wk. Women randomized to the combination arm received 40 mg d4T orally plus 200 mg ddI orally, twice daily from enrollment until delivery. Infants in the combination arm received d4T at 1 mg/kg orally in combination with ddI at 120 mg/m^2 orally for 6 wk. In the ZDV control group, women received 300 mg ZDV orally, twice daily from 34 to 36 wk, and their infants received 4 mg/kg ZDV orally, twice daily for 6 wk. There was no postpartum treatment for mothers in any arm of this study.

HIV transmission data were analyzed for 362 infants. At 24 wk, the combined rate of mother-to-child transmission for all study arms was 8.0%. The

HIV transmission rates across the study arms were not significantly different at 11%, 11%, 5%, and 6% in the d4T, ddI, combination d4T plus ddI, and ZDV arms, respectively.

Although the authors concluded that the regimens tested in this study were safe and well tolerated by women, there were several cases of maternal mortality secondary to lactic acidosis in pregnant women with prolonged use of d4T and ddI in combination. Combinations of d4T plus ddI should probably be avoided in pregnant women, because there are other efficacious regimens available.

Combination Antiretroviral Regimens: NRTIs, NNRTIs, and PIs

NRTI and NNRTI Trials

HIVNET 012 (Uganda): Breast-Feeding

This was a randomized, open-label trial conducted in Kampala, Uganda, originally designed as a randomized, double-blind, placebo-controlled phase III trial (125). Between November 1997 and April 1999, 626 women were enrolled in either the ZDV arm ($n = 308$) or the NVP arm ($n = 310$) of the study (18 women were originally enrolled in a placebo arm). The primary outcomes of this intrapartum/postpartum trial were rates of HIV-1 infection at birth, 6 to 8 wk, and 14 to 16 wk. At enrollment, the median CD4 count was 426 cells/mm^3 in the ZDV group and 461 cells/mm^3 in the NVP group.

Women randomly assigned to the ZDV group received 600 mg ZDV orally at the onset of labor and 300 mg ZDV orally, every 3 h until delivery. The postpartum regimen comprised ZDV at 4 mg/kg orally administered to the infant twice daily for 7 d. Women randomized to the NVP arm received 200 mg NVP orally at the onset of labor and their babies received NVP at 2 mg/kg orally within 72 h of birth. Neither study arm included postpartum treatment of mothers.

Data were analyzed for 609 mother–infant pairs, of whom 96% breast-fed. At birth, 10.4% of the infants in the ZDV group and 8.2% of infants in the NVP group were identified as HIV infected ($p = 0.354$). At 6 to 8 wk, 21% of infants in the ZDV group and 12% of infants in the NVP group were infected; whereas, at 14 to 16 wk, 25% of infants in the ZDV group and 13% in the NVP group were HIV infected ($p = 0.0006$). The efficacy for NVP was reported as 47% in breast-fed infants up to age 14 to 16 wk. The authors reported that both regimens were well-tolerated in both pregnant women and their infants.

NVP Resistance in HIVNET 012. Eshleman et al. examined the emergence and fading of NVP resistance mutations in HIV-1-infected Ugandan women and infants who received single-dose NVP to prevent HIV-1 vertical transmission. NVP® mutations were detected in 21 out of 111 (19%) women tested 6–8 wk after delivery *(126)*. The K103 mutation was the most common mutation detected. NVP mutations faded from detection within 12–24 mo in all 11

evaluable women. High-baseline viral load and low-baseline CD4 cell count were associated with development of NVP(R). NVP(R) mutations were detected in 11 out of 24 (46%) evaluable infants had been infected by 6–8 wk of age. The most common NVP(R) mutation detected in the infants was Y181C. Those mutations faded by 12 mo.

On further analysis of the HIVNET 012 data, Eshleman et al. also reported that NVP resistance was found in 70 (25%) of 279 women. NVP resistance was more common in women with subtype D vs A (35.7 vs 19%, $p = 0.0035$) *(127)*. Complex paterns of mutations were detected in both subtypes.

Flys et al. demonstrated that K103-containing variants persist in some women and infants for one year or more after administration of single-dose NVP *(128)*. More sensitive assays (LigAmp) detected the presence of K103N in 3 of 9 women and 1 of 5 infants 12–24 mo after administration of single-dose NVP. The authors concluded that sensitive assays may provide new insight into the impact of antiretroviral drug exposure on HIV-1 evolution.

Conventional sequence analysis detects drug resistance mutations in about 40% of women shortly after they receive intrapartum single-dose NVP. Using sensitive realtime polymerase chain reaction assays, Johnson et al. genotyped 50 South African women before and after single-dose NVP. By sequence analysis, 40 women had no detectable resistance mutations, and an additional 6 women were negative for Y181C after single-dose NVP. They found K103N in 16 (40%) of 40 women and Y181C in 5 (11%) of 46 women at 6–8 wk postpartum. Clonal sequencing confirmed K103N in 5 of 5 representative samples and Y181C in 4 of 4 samples. This findings indicate that resistance mutations emerged in at least 65% of the women after single-dose NVP *(129)*.

The clinical consequences of transient NVP resistance with single-dose NVP prophylaxis are uncertain. However, there remains concern that the presence of transient NVP resistance may compromise the future of NNRTI-based therapy for these women and their children.

SAINT (South African Intrapartum Nevirapine Trial): Breast-Feeding

This was a randomized, open-label trial conducted at various South African sites. Between May 1999 and February 2000 *(130)*, 1317 women were enrolled into either an NVP arm ($n = 652$) or a ZDV plus 3TC combination arm ($n = 654$). The primary endpoints of this intrapartum/postpartum study were intrapartum and early postpartum HIV transmission. At delivery, the median CD4 count for women randomized to the NVP arm was 405 cells/mm^3, whereas the median CD4 count for women randomized to the ZDV plus 3TC arm was 385 cells/mm^3.

Women assigned to the NVP intervention group received 200 mg NVP orally at the onset of labor and 200 mg NVP orally, 24 to 48 h after delivery. Infants

born to women in the NVP group received 6 mg NVP orally, 24 to 48 h after delivery. Women randomized to the ZDV arm received 600 mg ZDV orally plus 300 mg 3TC orally at the onset of labor, followed by 300 mg ZDV orally, every 3 h plus 150 mg 3TC orally, every 12 h until delivery. Mothers in this group also received 300 mg ZDV orally plus 150 mg 3TC orally, twice daily for 1 wk after delivery. Infants with a birth weight greater than 2 kg received 12 mg ZDV orally plus 6 mg 3TC orally, twice daily for 7 d; and infants with a birth weight <2 kg were administered ZDV at 4 mg/kg plus 3TC at 2 mg/kg twice daily for the first week of life.

Data were analyzed for 1307 infants, of whom 60% were breast-fed and the remaining 40% were formula fed. HIV-1 transmission rates were statistically equivalent up to age 8 wk; with 12% of the infants in the NVP arm becoming infected and 9% of the infants in the ZDV plus 3TC combination arm becoming infected. These results are consistent with the finding for corresponding regimens of the PETRA and HIVNET 012 trials. This study confirmed the efficacy and safety of short-course antiretroviral regimens in reducing mother-to-child transmission in developing countries.

NVAZ (Malawi): Breast-Feeding

Despite the relative simplicity and clear efficacy of single-dose, intrapartum NVP demonstrated by the HIVNET 012 trial, there remain multiple constraints to antiretroviral prophylaxis to prevent mother-to-child HIV transmission in resource-poor settings, including accessibility of prenatal HIV counseling and testing services and presentation to the labor ward only shortly before delivery. This open-label trial evaluated the safety and efficacy of combination NVP plus ZDV compared with NVP alone among Malawian babies born to mothers who presented too late to a labor and delivery unit to receive HIV counseling and testing and intrapartum NVP (*131*). Between April 2000 and January 2002, 1119 babies born to women infected with HIV were randomized to receive either NVP alone or combination NVP plus ZDV postexposure prophylaxis. The primary study outcome was HIV infection in infants at 6 to 8 wk, among those not infected at birth.

Infants in the NVP-only arm received NVP at 2 mg/kg orally after delivery ($n = 557$), whereas those in the combination arm received NVP at 2 mg/kg orally after delivery plus ZDV at 4 mg/kg orally, twice daily for 7 d ($n = 562$). At the time of delivery, the mean maternal viral load (±SD) was 4.5 ±0.8 $\log_{10}$ copies/mL in the combination arm and 4.6 ±0.85 $\log_{10}$ copies/mL in the NVP-only arm. Data were analyzed for a total of 1106 babies, the overwhelming majority of whom were breast-fed.

The overall rate of HIV transmission (including infections detected at birth) was 15.3% in babies who received combination NVP plus ZDV, compared with

20.9% in babies who received only NVP ($p = 0.03$). The authors report a 5.6% reduction in HIV-transmission rates among infants receiving combination NVP plus ZDV postexposure prophylaxis, compared with infants receiving NVP alone. This is consistent with the results of various other African trials. Although postexposure prophylaxis should not be considered an alternative to previously established regimens, which include an intrapartum treatment component, it may be useful in risk reduction among late presenters to prenatal care and/or labor and delivery units. Strategies to prevent HIV transmission during breast-feeding also require further investigation.

MALAWI: BREAST-FEEDING

This was a randomized, open-label trial conducted at multiple clinics in Blantyre, Malawi (132). Between April 2000 and March 2003, 894 HIV-infected women were enrolled into either an NVP standard-of-care arm ($n = 448$) or an NVP plus ZVD combination arm ($n = 446$). Study outcomes were HIV infection of infants at birth and at 6 to 8 wk, and adverse events.

Regarding the interventions, women in the standard-of-care arm received a single dose of 200 mg NVP orally during the intrapartum period and their infants received NVP at 2 mg/kg orally after delivery. Women in the combination treatment arm received a single intrapartum dose of oral NVP and their infants received NVP at 2 mg/kg orally after delivery plus ZDV at 4 mg/kg orally, twice daily for a period of 7 d. Women were enrolled after presentation to a labor and delivery unit, and were previously antiretroviral naive.

At the time of delivery, mean HIV viral load (±SD) is reported as 4.4 ±0.77 $\log_{10}$ copies/mL among women who received only NVP and as 4.4 ± 0.76 $\log_{10}$ copies/mL among women who received NVP plus ZVD. Of note, the proportion of caesarean-section deliveries among women in the NVP-only arm was 3.5%, compared with 1.1% in the combination arm ($p = 0.02$). However, the mode of delivery was not associated with the primary outcome at the statistically significant level.

HIV transmissions data were analyzed for a total of 889 infants. At birth, 8.1% of infants in the NVP-only arm and 10.1% of infants in the combination NVP plus ZDV arm were found to be HIV infected ($p = 0.30$). At 6 to 8 wk, the HIV-transmission rate still did not vary significantly between treatment arms. Among infants seronegative at birth, 6.5% (23/353) and 6.9% (25/363) of infants were HIV infected at 6 to 8 wk in the standard-of-care and combination NVP plus ZDV arms, respectively. Using the life-tables approach, by 6 to 8 wk, 14.1% (95% CI, 10.7–17.4) of infants who received NVP, and 16.3% (95% CI, 12.7–19.8) of infants who received combination NVP plus ZDV prophylaxis were estimated to be HIV infected ($p = 0.36$).

Safety analyses showed no significant difference in the proportion of adverse events between trial arms. The authors conclude that although the combination NVP plus ZDV regimen seems safe for infants, the addition of a neonatal ZDV course did not lead to meaningful reductions in the risk of HIV transmission from mothers to infants in this setting.

PHPT, THAILAND: NONBREAST-FEEDING

This was a randomized, double-blind trial conducted at various centres in Thailand. Between January 2001 and February 2003, 1844 women were randomly assigned to one of three arms. In the NVP–NVP group, women received a single dose of 200 mg NVP orally at the onset of labor and neonates received a fixed dose of 6 mg NVP orally, 48 to 72 h after delivery (n = 365) (133). In the NVP–placebo group, only mothers received 200 mg NVP orally in the intrapartum period; their infants received a placebo as opposed to NVP prophylaxis (n = 362). The third arm was a placebo–placebo group. Additionally, all women received ZDV according to the Thai long-course regimen (300 mg ZDV orally, twice daily, beginning at 28 wk gestation, as well as 300 mg every 3 h from the onset of labor) and their infants received ZDV at 2 mg/kg orally, four times daily for 7 d (n = 360). All infants were formula fed. The primary outcome was HIV infection at 1 mo of age.

At the time of first interim analysis, the median baseline CD4 count was 371 cell/mm^3 (interquartile range [IQR], 232–522 cell/mm^3) in the NVP–NVP group, 373 cell/mm^3 (IQR, 256–543 cell/mm^3) in the NVP–placebo group, and 372 cell/mm^3 (IQR, 238–510 cell/mm^3) in the placebo–placebo group. The median plasma viral load was 4.2 $\log_{10}$ copies/mL (IQR, 3.5–4.7 $\log_{10}$ copies/mL), 4.0 $\log_{10}$ copies/mL (IQR, 3.3–4.6 $\log_{10}$ copies/mL), and 4.2 $\log_{10}$ copies/mL (IQR, 3.4–4. $\log_{10}$ copies/mL7) in the NVP–NVP group, NVP–placebo, and placebo–placebo group, respectively. With respect to mode of delivery, 20.3%, 22.8%, and 21.3% of women had caesarean deliveries in the NVP–NVP, NVP–placebo, and placebo–placebo groups, respectively. A total of 1034 deliveries had occurred and could be evaluated at first interim analysis. Of these, 353 were in the NVP–NVP arm, 333 in the NVP–placebo arm, and 348 in the placebo–placebo arm. The overall rate of HIV transmissions (±SE) was 1.1 ±0.6%, 2.1 ±0.8%, and 6.3 ±0.13% in the NVP–NVP, NVP–placebo, and placebo–placebo arms, respectively, by intention-to-treat analysis. It was, thus, decided (by predefined stoppage rules) that the placebo–placebo arm would be discontinued.

Only the NVP–NVP and the NVP–placebo arm were included in the final analysis. Median CD4 counts were 363 cells/mm^3 (IQR, 238–510 cells/mm^3) in the NVP–NVP group and 381 cells/mm^3 (IQR, 249–546 cells/mm^3) in the NVP–placebo group. The median plasma viral loads were 4.1 $\log_{10}$ copies/mL

(IQR, 3.4–4.7 $\log_{10}$ copies/mL) and 3.9 $\log_{10}$ copies/mL (IQR, 3.3–4.5 $\log_{10}$ copies/mL) in the NVP–NVP and NVP–placebo groups, respectively. Nineteen percent of women in the NVP–NVP group were delivered by caesarean section, whereas the caesarean-section rate in the NVP–placebo group was 22.5%.

In the final intention-to-treat analysis, data were available for a total of 1365 mother–infant pairs. Among the 693 deliveries evaluated in the NVP–NVP arm (±SE), 2.0 ±0.5% of infants were found to be HIV infected. Similarly, 2.8 ±0.6% of the 672 infants in the NVP–placebo arm were found to be HIV infected at 1 mo of age. Results from the per-protocol analysis are not meaningfully different from the intention-to-treat results (1.9% HIV transmission rate in the NVP–NVP arm vs 2.8% in the NVP–placebo arm). With respect to safety, rates of serious adverse events were equivalent across groups for both mothers and infants.

The authors conclude that a single dose of NVP added to a standard course of ZDV initiated at 28 wk of gestation is effective in reducing the rate of mother-to-child infection. However, the addition of a prophylactic dose of NVP to infants does not seem to confer additional benefits in reducing HIV transmission from mothers to infants in this nonbreast-feeding population.

NVP Resistance in the PHPT. The Thai PHPT group reports clinically significant consequences in mothers who received single-dose, intrapartum NVP and were subsequently treated with NVP-containing antiretroviral regimens *(134)*. According to the original study protocol, 1844 women were randomized to receive intrapartum NVP or placebo, in addition to ZVD during the third trimester of pregnancy. Of these women, 269 had CD4 lymphocyte counts of fewer than 250 cells/mm^3 and, thus, began NVP-containing antiretroviral regimens.

After 6 mo of therapy, HIV-1 RNA levels were undetectable (<50 copies/mL) in 49% of women who had received intrapartum NVP, compared with 68% of women who were NVP naive ($p = 0.03$). Mutations to non-nucleosides were detected in 32% of women who had received intrapartum NVP; the most frequent resistance mutations being K103N, G109A, and Y181C. Finally, among those who had received intrapartum NVP, only 38% of women with resistance mutations were able to achieve viral suppression, compared with 52% of women without resistance mutations after 6 mo of therapy ($p = 0.08$).

Women who received intrapartum NVP were, thus, less likely to achieve viral suppression after 6 mo of therapy with a NVP-containing regimen. Most notably, even among patients with no apparent NVP resistance, response to therapy is markedly less in women exposed to NVP compared with women never exposed to NVP (52% vs 68% viral suppression). There were no differences in CD4 cell counts between groups at the statistically significant level.

Table 2
Important Landmarks in Perinatal HIV-1 Management

1982	First cases of HIV infection in women were reported *(149)*
1983	Initial reports of perinatal HIV
1987	Changes in the risk exposure for acquisition of HIV infection among women from substance use to heterosexual transmission *(150)*
1994	ZDV can reduce perinatal HIV-1 transmission
1998	Abbreviated regimens can also reduce prenatal transmission?
1999	ZDV chemoprophylaxis works for women with advanced disease
1999	Lethal consequences of metabolic complications after exposure to antiretroviral therapy in children
2001	Lethal consequences of metabolic complications after exposure to antiretroviral therapy for women
2001	Increase trends of detection of genotypic resistance among HIV-infected pregnant women: risk of perinatal HIV transmission and potential for compromising future therapy for maternal heath

SUMMARY

The use of NRTIs and NNRTIs alone or in combination with PIs has dramatically changed both the management of HIV disease during pregnancy and the profile of the HIV/AIDS epidemic *(135–138)*. Since the results of PACTG 076, which showed a 70% decrease in perinatal transmission with ZDV administration, we have come a long way in preventing vertical transmission, and eradication of perinatal HIV in the near future seems feasible (Table 2).

Each patient, clinician, and country must balance the needs, risks, and benefits of available treatment modalities for women and children *(139)*. For many women, pregnancy could mean a new diagnosis and initiation of therapy, which could have short- and long-term implications for her health. For the unborn child and infant, perinatal treatment will have an impact on their HIV status, their risk of congenital anomalies, and their risk of short- and long-term toxicity *(140,141)*. Because the physiological changes of pregnancy can alter drug pharmacokinetics and vulnerability to adverse events, we should not assume that information gathered from nonpregnant subjects is always applicable to pregnant women *(142,143)*. Transplacental passage of each drug and results from animal studies should be evaluated to ascertain risks and benefits before prescribing untested combinations *(144)*. We should also maintain prospective surveillance of cohorts to monitor for unexpected treatment outcomes. In other words, we must always remember the past to effectively address present and future challenges (Table 3).

Several conclusions may be drawn from the findings of clinical trials in the arena of mother-to-child transmission. From reports available to date, it is noted

Table 3
Unresolved Issues

- Which patients are at risk of developing metabolic complications during pregnancy?
- What is the long-term effect of discontinuation of HAART after pregnancy?
- What is the optimal choice to initiate therapy in antiretroviral naive pregnant women during pregnancy: protease sparing or non-sparing regimen?
- What is the role of resistance testing in antiretroviral naive pregnant patients?
- Should women who developed transient NVP resistance receive NNRTIs for reduction of perinatal transmission or as part of treatment regimen for their own health?
- What are correlates for adherence during pregnancy? What are effective strategies to increase adherence during pregnancy and postpartum?
- What are optimal treatment strategies for women with recurrent pregnancies?
- Can an intrapartum strategy reduce HIV-1 transmission to 2% among women with no prenatal care?
- Can we eradicate perinatal HIV-1 in developing countries by 2010?

that the safety and tolerability of drug regimens does not differ significantly between industrialized and developing county settings. With respect to efficacy, it has been demonstrated that both shorter-course antepartum/intrapartum (Bangkok trial) and intrapartum/postpartum (PETRA, HIVNET 012, and SAINT) interventions reduce the risk of mother-to-child transmission by 36 to 50%. It is thought that the mechanism by which these interventions work is via the reduction of HIV viral load. Furthermore, it has been established that, at delivery, women with a lower HIV viral load are less likely to transmit HIV to their infants. Concomitantly, women with higher CD4 cell counts are also less likely to transmit the virus. Intrapartum strategies alone (PETRA) do not seem to be efficacious. An unanticipated finding is that neither the addition of ZDV to a single-dose NVP regimen (Malawi, breast-feeding) nor the addition of NVP to a short-course ZDV regimen (PHPT, nonbreast-feeding) reduces the risk of mother-to-child transmission at a meaningful level.

The PHPT study demonstrates that in cases in which maternal antepartum treatment is short (initiated at ≥36 wk of gestation), the most effective regimens include lengthened protocols for treatment of infants. This highlights the importance of neonatal prophylaxis. The efficacy of postexposure prophylaxis for infants is further demonstrated by the NVAZ study.

Despite potency in reducing transmission rates in the antepartum and intrapartum periods, several of the African studies demonstrate that continued exposure to HIV via breast-feeding leads to marked reductions in the efficacy of antiretroviral regimens for prevention of mother-to-child transmission. Efforts

to develop strategies to reduce the risk of infection during the postpartum period are urgently needed.

The final caveat is that as we begin to move toward wider scale implementation of pharmacological interventions to reduce the risk of vertical HIV transmission, the emergence of drug resistance is likely to become increasingly prominent. Already, there are several reports of development of resistance to nucleoside and non-nucleoside agents, with subsequent failures of mother-to-child transmission prophylaxis *(145–147)*. Resistance has been associated with both abbreviated and longer-term antiretroviral treatments. Nevertheless, it is felt that the potential benefits of antiretroviral therapies for both infected mothers and at risk infants largely outweigh the dangers. In industrialized nations, guidelines suggest that antiretroviral therapy should be the same as for non-pregnant women, with consideration of potential for fetal toxicity within the early stages of pregnancy *(148)*. Indeed, prevention of mother-to-child transmission of HIV should be viewed within the context expanding health care services and antiretroviral access in resource-poor countries, or so-called prevention of mother-to-child transmission (PMTCT)-plus.

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16

New Reverse Transcriptase Inhibitors in Development

Rudi Pauwels

INTRODUCTION

Since the identification of HIV as the etiological agent of AIDS, the HIV reverse transcriptase (RT) enzyme has been considered an ideal selective antiretroviral drug (ARV) target. Although several polymerases are found in human cells, none are comparable to HIV RT in their ability to catalyze the synthesis of new DNA from an RNA template. A selective RT inhibitor should, therefore, exert limited toxicity. The RT enzyme plays a crucial role in the early stages of the viral life cycle and can be competitively inhibited by chain-terminating nucleoside analogs (several of which were synthesized for basic research use well before the discovery of HIV). Therefore, it is not surprising that nucleoside RT inhibitors (NRTIs) were the first ARVs to be made available for the treatment of HIV infection. There now exist two additional classes of RT inhibitors, with independent modes of action; the non-nucleoside RT inhibitors (NNRTIs), first discovered in the early 1990s *(1)*, and the nucleotide RT inhibitors. As described in detail in the earlier chapters of this volume (Chapters 11 to 14), the NNRTIs act by noncompetitive binding to a hydrophobic pocket on the p66 subunit of the RT enzyme. The consequence of NNRTI binding to this unique site within the RT alters the ability of the enzyme to carry out its function. The current generation of NNRTIs selectively inhibits HIV-1 replication, reflecting their very high specificity for RT. They even have no significant activity against HIV-2, simian immunodeficiency virus, or other studied retroviruses. Unlike the NRTIs and the nucleotide RT inhibitors, NNRTIs do not require intracellular activation through phosphorylation. Nucleotide analogs, presented in Chapter 5, inhibit the RT in the same way as nucleoside analogs, but depend on a reduced intracellular activation pathway. These compounds are acyclic nucleoside phosphonates that, once inside the cell, have a very long half-life and require only two (rather than three) phosphorylation steps to be converted to an active form.

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The Need for New RT Inhibitors

The impact of highly active antiretroviral therapy (HAART) on the morbidity and mortality of HIV-infected individuals has been impressive *(2)*. RT inhibitors have remained a cornerstone of today's HAART regimens. However, the growing number of patients experiencing multiple treatment regimens has unearthed new problems to be overcome. The long-term safety of ARV treatment, complicated by drug–drug interactions, is an increasingly important concern because patients are living longer and are exposed to various drug combinations during longer periods. Apart from the extensive short-term side effects of current NRTIs, more recently, new metabolic complications have been described. These complications potentially lead, for example, to abnormal fat distributions and raised cholesterol levels, which have been attributed, in part, to delayed toxicity resulting from long-term NRTI use *(3–5)*. The first generation NNRTIs have been associated with a high incidence of liver damage and several side effects, such as rash, and, for efavirenz, neurological symptoms *(6,7)*.

The combination of a high level of virion production and a particularly error-prone reverse transcription process provides HIV with the capacity to evolve. As a consequence, and because the HIV-1 proteins largely retain their functional capabilities despite these genetic changes, most single and even double mutants are likely to preexist in the viral quasi-species. The presence of these mutants may cause a reduction of the inhibitory effects of antiviral drugs that can lead to drug resistance, and may limit treatment options and eventually lead to HIV-1 disease progression and death. None of the current ARVs has been shown to be resistance proof. Incomplete suppression of virus by suboptimal levels of drug can accelerate the selection of resistant virus, and virological treatment failure and resistance development go hand in hand, each aiding the other. Incomplete suppression and drug resistance are common in the United States and in Europe and there is now widespread resistance to all available classes of ARV. Consequently, transmission of resistant virus is increasing, and a mounting population of individuals is infected *de novo* with multiple drug-resistant strains *(8–11)*. Combinations of RT mutations at codons 41, 67, 70, 210, 215, and 219 that were originally described as associated only with resistance to 3′-azido-3′-deoxythymidine (AZT) also reduce susceptibility to several other NRTIs, such as stavudine (d4T), didanosine, and abacavir *(12,13)*. More than a decade of extensive NRTI use has given the virus ample opportunity to find new mechanisms of evasion, such as the multidrug-resistant Q151M complex *(14)* and the two to three amino acid insertions between codons 66 and 69 of RT *(15)* that render the virus insensitive to all available NRTIs.

Although currently available NNRTIs are highly selective and extremely potent, they rapidly select for resistant virus and for single mutations that lead

to dramatic reductions in susceptibility, often to all available inhibitors within the class of currently available NNRTIs.

There is now an urgent need for potent new ARVs, active against viruses resistant to the first-generation molecules, to continue to provide treatment options for highly treatment-experienced individuals and to prolong the duration of response to HAART in patients beginning treatment.

Challenges and Strategies

Today's ideal new RT inhibitor has numerous challenges to meet before it can be considered for clinical development. First, it must be at least as potent as currently available inhibitors. To achieve effective and prolonged suppression of viral replication, the virus needs to be hit as hard as possible, and it is only by using the most effective combinations available that this will be possible. Insufficiently active compounds run the risk of more rapid selection of resistant virus and resultant clinical failure. Most NNRTIs, such as efavirenz, are extremely potent, with 50% inhibitory concentration (IC_{50}) values in the low nanomolar range. The in vitro potency of NRTIs varies widely, and is not necessarily correlated with the levels of drug that need to be administered in vivo. After entry into the cell, all nucleoside analogs must be first phosphorylated by host-cell kinases to produce the active 5′-triphosphate form of the molecule. The efficacy of the cellular uptake and phosphorylation process varies between compounds and between cell types, and the lack of activity of some nucleoside analogs is caused by their poor phosphorylation under certain conditions. The rate of phosphorylation and the intracellular half-life of the phosphorylated NRTI will be major determinants of the in vivo potency of any new compound in this class. For this reason, it is important to test candidate NRTIs in a variety of cell types and culture conditions to identify potential phosphorylation problems at an early stage.

High antiviral potency (expressed as an IC_{50} value) combined with low toxicity (expressed as a 50% cytotoxic concentration value) yielding a large therapeutic index (i.e., the 50% cytotoxic concentration:IC_{50} ratio) provides more opportunity for increasing the dose to overcome virus with reduced susceptibility. Low toxicity and minimal side effects will also aid in and encourage good adherence to the treatment regimen, which has been shown to be critical in determining the long-term success of HAART *(16,17)*.

Mitochondrial toxicity resulting from the inhibition of mitochondrial DNA polymerase-γ has been proposed as a mechanism for the toxicity associated with long-term NRTI use *(18)* (*see also* Chapter 9). It has been suggested that the extent to which an NRTI displays mitochondrial toxicity depends not only on the inhibition of mitochondrial DNA polymerase-γ, but also on how well

the active metabolites are taken up by the mitochondria *(19)*. Therefore, it is important to assess mitochondrial toxicity in several cell-based assay systems.

Other major determinants of adherence are pill burden and dosing schedule. Good oral bioavailability and long plasma and intracellular half-lives will allow for low doses and infrequent dosing regimens that have less of an impact on the daily lives of treated individuals.

The effects of HAART on viral load in different body fluids and tissues vary greatly between treatment regimens because of differing abilities of compounds to cross barriers to reach tissues such as the lymphatic system, the brain, the testes, the seminal tract, and the cervicovaginal tract. The brain is a particularly important reservoir of HIV infection, because the virus can establish infection in microglial cells, which are very long lived. In addition, HIV in the central nervous system can reinfect lymph nodes through the lymphatic system. All NNRTIs penetrate into the brain, whereas the ability of NRTIs and protease inhibitors to penetrate into the brain varies considerably from drug to drug. More compounds that can effectively reach all potential HIV reservoir sites in the body are needed if the elimination, or at least the life-long control, of HIV in infected individuals is to be realized.

Resistance is clearly a major hurdle for new NRTIs. With the prevalence of viral strains carrying multiple NRTI resistance-associated mutations in treatment-naive patients rising to as high as 23% in some countries *(8–11)*, and with new multi-NRTI resistance mutations emerging, it is imperative that the susceptibility of any new NRTI is not impaired by these mutations.

The broad cross-resistance between current NNRTIs limits their consecutive use. The most common mutations to emerge in clinical practice are those leading to K103N and Y181C amino acid substitutions in the HIV RT—new NNRTIs should ideally retain activity against viruses carrying these substitutions. Some candidate NNRTIs, for example, opraviraline (also known as HBY 1293 or GW 420867), progressed along the clinical development pathway before finally being discontinued because of insufficient activity against resistant isolates.

Several strategies are being used to identify new RT inhibitor candidates for development. Advances in high-throughput screening techniques mean that large compound libraries derived synthetically or from natural sources can be rapidly screened for active molecules. For example, calanolide A, a derivative of the plant *Calophylulum lanigerum*, was identified using a cell-based HIV bioassay to screen extracts from the National Cancer Institute (NCI) Natural Products Repository, one of the world's largest collections of plant extracts for drug discovery *(20)*.

In the early days of anti-HIV drug discovery, the developments of several nucleoside analogs were dropped because they showed no better activity than

the previously licensed compounds. The reevaluation of these compounds may reveal different patterns of resistance development and may identify molecules that retain activity against virus strains resistant to currently available NRTIs. This was the case for alovudine or 3′-fluorodeoxythymidine (FLT; also known as MIV-310), now in phase II trials in treatment-experienced patients. FLT was originally dropped by American Cyanamid in 1994 because it did not show any clinical advantage over AZT *(21)*. The development was reinitiated by Medivir when information on AZT resistance became available, and researchers found that FLT was active against highly AZT-resistant HIV strains *(22)*.

Subtle alterations in the chemical structure of active compounds, or synthesis and use of enantiospecific forms of these compounds, can improve the toxicity profiles, potency, or solubility. In some cases, for example, that of 2′-deoxy-3′-oxa-4′-thiocytidine (dOTC; *see* SPD-754 following), the separation of a racemic mixture can reveal one enantiomer that is less toxic and/or more active than the other *(23)*. In the design of new RT inhibitors, not only activity against resistant virus but also resilience against the selection of resistant virus should be considered. Capravirine was selected for development from a series of derivatives by antiviral profiling against a panel of NNRTI-resistant HIV strains. The selection of virus resistant to this drug, by in vitro passage, proved more difficult than with first-generation NNRTIs *(24)*. The larger size of this molecule and its binding via an extensive network of hydrogen bonds involving the main chain of residues 101, 103, and 236 of RT have been suggested as reasons for its relative insensitivity to mutations *(25)*. Another new NNRTI, TMC125, which exhibits a broad spectrum of activity against a variety of NNRTI-resistant viruses, was the result of a careful drug resistance-guided lead selection and optimization program, whereby potency against wild-type and NNRTI-resistant HIV, as well as increased metabolic stability were optimized in parallel *(26,27)*. The identification of highly conserved or immutable amino acids in the inhibitor-binding sites of RT could provide targets for the rational design of compounds. The mutation of certain amino acids in the NNRTI-binding pocket leads to nonviable virus (e.g., W229 and Y318N), thus, new NNRTIs targeted to interact with the RT at these points may select for resistant virus strains less easily than those currently available *(28)*.

The use of inactive prodrugs that are rapidly metabolized to yield an active product can bypass bioavailability and activation problems. For example, prodrugs that directly deliver the 5′-monophosphate of a nucleoside analog after uptake by cells could be effective under conditions in which nucleoside analog activity is reduced because of inefficient phosphorylation *(29)*. An example of this approach has been the nucleotide analog, tenofovir, that uses a phosphonate linkage rather than a more easily hydrolyzed phosphate group in the 5′-position of the putative sugar moiety. The added advantage of such a linkage is

that it is not cleaved by the cellular enzymes responsible for the conversion of nucleoside monophosphates back to the nucleoside form, therefore, leading to a longer intracellular half-life.

The NRTI, (–)-β-D-2,6- dioxolane guanine (DXG), is highly active but has very limited solubility, making drug delivery problematic. However, the prodrug, (–)-β-D-2,6-diaminopurine dioxolane (DAPD), also known as amdoxovir, is soluble and is very rapidly metabolized in vivo to yield DXG *(30)*.

Clinical Development

Despite technological and efficiency improvements, the costs of developing new drugs are rapidly increasing. Drug developers need to be able to better separate potential failures from sure successes at an early stage and to design clinical trials that yield a maximum of relevant information.

Any new anti-HIV candidate needs to be able to prove enhanced potency compared with available treatments both in vitro and in vivo, although some candidates with equal potency to available treatments may still prove superior in terms of activity against resistant strains or in terms of pharmacokinetic or toxicity profiles. Well-planned in vitro studies will give a good indication regarding the cellular toxicity, activity, and resistance profiles of new compounds, and may also help elucidate the cellular metabolism; this is particularly relevant for NRTIs.

Pharmacokinetics, toxicity, and drug interactions must be assessed in a range of animal models and in phase I clinical trials in humans. Because new agents will only be used in combinations, their effect on the metabolism and levels of other ARVs is of critical relevance. The NNRTI, emivirine, whose original leads were discovered in the early nineties *(31)* and were licensed from Mitsubishi Chemical Corporation to Triangle Pharmaceuticals, progressed a long way down the clinical development pathway, as far as phase III clinical trials. However, this compound was not active against virus resistant to first-generation NNRTIs and was discontinued after showing insufficient potency compared with abacavir in a comparative trial (Triangle Pharmaceuticals press release; January 17, 2002). Before this press release, clinical trials had already been amended because of lack of clinical benefit and increased side effects in combination with nelfinavir in patients with advanced disease (Triangle Pharmaceuticals press release; October 7, 1999). Emivirine was also found to reduce the plasma levels of both efavirenz and nevirapine *(32,33)*.

The intrinsic in vivo potency of a new compound can best be determined in short-term monotherapy trials, in which the effects of other anti-HIV drugs of the regimen are eliminated. Because of legitimate concerns of potential drug resistance development, ethically, such trials can clearly only be of limited

duration. With the tools now available to detect extremely low viral loads and calculate viral decay rates, the relative efficacy of different compounds can be more accurately assessed.

Because the most urgent need for new compounds lies with heavily pretreated patients, well-planned clinical trials are needed to focus on this population, whose treatment options become limited. However, clinical trials in patients who have failed multiple regimens are much more complex to perform and to interpret than trials in treatment-naive individuals, because this patient population is much more heterogeneous in terms of genotypic and phenotypic drug resistance, pharmacokinetic interactions, and so on.

A number of RT inhibitors are currently under investigation as potential ARVs for use in the treatment and prevention of HIV infection. However, the rigorous development pathway means that only a fraction of those compounds that are discovered to show potent anti-HIV activity in the laboratory will actually succeed in being licensed for clinical use. The remainder of this chapter focuses on the new candidate RT inhibitors that are currently in clinical development.

NUCLEOSIDE ANALOG RT INHIBITORS IN DEVELOPMENT

The new NRTIs presented in this section are listed in Table 1, along with their activity against wild-type and resistant viruses. Figure 1 shows the chemical structures of the compounds.

Amdoxovir

DAPD is the water-soluble prodrug of DXG. DAPD is converted intracellularly by adenosine deaminase into D-dioxolane. This metabolite is subsequently further phosphorylated into DXG triphosphate, the active form of this compound. Both DAPD and DXG were originally synthesized by Kim et al. at Emory University *(34)* as part of series of dioxolane nucleosides that were tested for anti-HIV activity. DXG was found to be the most potent, with an IC_{50} of 0.03 μ*M* against HIV in peripheral blood mononuclear cells, and demonstrated no cellular toxicity up to 100 μ*M*. The problem with DXG is that it is highly water insoluble. However, DAPD has much more favorable pharmacokinetic properties and is rapidly metabolized to yield DXG in vivo *(35)*. DAPD has been in development at Triangle Pharmaceuticals and Gilead Sciences under the name of amdoxovir *(36)*. In 2004, the rights returned to Emory University and the University of Georgia.

After in vitro passage in the presence of DXG, variants with either a K65R or a L74V mutation were selected, which demonstrated 7.3- and 12.2-fold reductions in susceptibility, respectively *(37)*. Recombinant viruses and clinical isolates carrying multiple AZT resistance-associated mutations (41L, 70R,

Table 1
New NRTIs in Clinical Development

Company	Compound	Stage of development	In vitro EC_{50} (or EC_{90}[a]) μM against					Mutations selected by in vitro passage	Key refs.
			WT	AZT R[c]	M184V	Q151M	69S ins		
Emory University/ University of Georgia Research Foundation	Amodoxovir DAPD (DXG prodrug)	Phase II	0.03–0.25	0.045–0.24	0.18–1.1	4.6–fold	na	K65R (7-fold) L74V (12-fold)	(34,35, 37,39)
Achillion	Elvucitabine ACH-126,443 β-L-Fd4C	Phase II	0.1–0.3	0.1–0.3	1–4	0.1–0.3	0.1–0.3	M184I	(46,48, 49)
Pharmasset/Incyte	Reverset DPC-817 β-D-Fd4C	Phase I/II	1.1[a]	0.08	0.14	na	na	K65R	(55,56)
Medivir/Boehringer Ingelheim	Alovudine MIV310 FLT	Phase II	0.0007–0.02	0.0014–0.017	0.0014–0.017	0.004–0.05	0.0014–0.017	No mutations selected after 49 passages	(22,60, 63)
Shire Pharmaceuticals/ Avexa	SPD-754 BCH-10652 (–)dOTC	Phase II	0.1–4.8	2.5	2.5	na	na	K65R, V75I, M184V (low-level resistance)	(66,67, 105)

WT, wild type; na, not available
[a]If no EC_{50} is available, the EC_{90} is given. As a very general rule, EC_{90} can be divided by 5 to give an approximate EC_{50}
[b]AZT-resistant virus carrying mutations 41L and 215Y with or without 67, 70, and 219Q

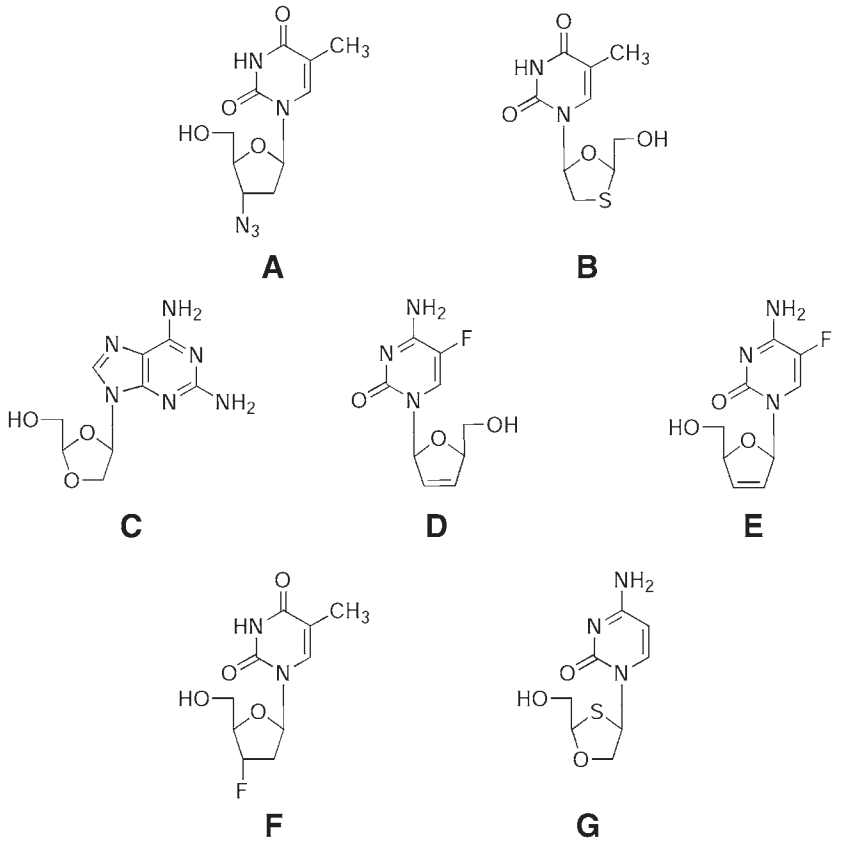

Fig. 1. Structures of NRTIs in development alongside AZT and 3TC. (**A**) AZT; (**B**) 3TC; (**C**) DAPD; (**D**) elvucitabine (also known as ACH-126,443); (**E**) DPC-817 (also known as Reverset); (**F**) FLT; (**G**) SPD-754.

215Y, and 219Q) seem to remain susceptible to DXG, and virus with a 184V mutation shows only limited resistance (twofold to fivefold) *(38)*. DXG also retains some activity against virus with RT insertions at codon 69 or with the 151M mutation (a less than 10-fold reduction in susceptibility) *(39,40)*. High levels of resistance have only been seen with the combination of 65R and 151M *(39)*. Viruses with multiple mutations that included K65R, F116Y, and Q151M had a 40- to 54-fold increase in median effective concentration (EC_{50}) for DAPD (high-level resistance).

A study in 2002 suggested that the coadministration of mycophenolic acid, an inosine monophosphate dehydrogenase inhibitor that blocks the *de novo* synthesis of guanosine nucleotides, could increase the relative intracellular levels

of DXG triphosphate and, hence, the activity of DAPD against multidrug-resistant virus *(41)*. In the presence of mycophenolic acid, virus carrying both the 65R and 151M mutations showed only a fourfold reduction in susceptibility to DAPD. Several phase I/II clinical trials have been completed using DAPD. A 14-d monotherapy study in 29 treatment-naive, HIV-infected individuals tested five doses (from 25 mg to 500 mg DAPD twice daily). All doses were well-tolerated, with no discontinuations caused by toxicity. DAPD was rapidly converted to DXG yielding a mean DXG-to-DAPD ratio of 4 to 12 at all doses. The peak concentration occurred within 1 to 2 h of dosing, and the plasma half-life of DXG was approx 7 to 9 h, supporting twice-daily dosing. Median viral load reductions ranged from 0.5 $\log_{10}$ copies/mL for the lowest dose to 1.5 $\log_{10}$ copies/mL for the 300 mg dose *(42)*. Several studies were conducted in ARV-experienced patients. In the first such study, DAPD was administered at doses ranging from 25 mg to 500 mg twice daily, as a single-drug therapy after a washout period of 7 d. After 15 d of follow-up, HIV RNA levels dropped in a dose-dependent manner, ranging from 0.5 $\log_{10}$ copies/mL in the 25-mg group to 1.6 $\log_{10}$ copies/mL in the 500-mg group. In the second study, the addition of 500 mg DAPD twice daily to the failing treatment regimen of five patients resulted in a median 1.9 $\log_{10}$ copies/mL drop in viral load *(43)*. In a randomized, open-label, phase I/II study, subjects were treated with 300 mg or 500 mg DAPD twice daily combined with an optimized background therapy that was selected based on resistance testing. The subjects had previously received a median of 10 ARVs and demonstrated a median of five NRTI mutations. At week 12, viral load levels had decreased by a mean of 1.5 $\log_{10}$ copies/mL in the 300-mg group and by 0.75 $\log_{10}$ copies/mL in the 500-mg group *(44)*. More recently, a randomized, prospective, double-blind, placebo-controlled study (AIDS Clinical Trials Group [AACTG] 5118) was performed in which the ARV activity and safety of DAPD (300 mg DAPD by mouth, twice a day) was compared with placebo in combination with enfuvirtide (90 mg enfuvirtide subcutaneously, twice a day) plus optimized background therapy in enfuvirtide-naive subjects who had failed two ARV regimens, including at least two NRTIs, two protease inhibitors, and one NNRTI *(44)*. Intention-to-treat analysis performed on data from 24-wk of treatment of 18 patients enrolled in the study showed that the time-averaged area under the curve minus baseline was –1.1 $\log_{10}$ copies/mL in the DAPD arm and –0.8 $\log_{10}$ copies/mL in the placebo arm ($p = 0.69$). Patient enrollment was terminated after an unplanned interim review convened when the long-term development plans for DAPD became uncertain *(44)*.

Elvucitabine

Elvucitabine (2′,3′-dideoxy-2′,3′-didehydro-β-L[–]-5-fluorocytidine [β-L-Fd4C]; also known as ACH-126,443), is one of a series of β-L(–) nucleoside

analogs originally developed at Yale University *(45)* and now being developed by Achillion Pharmaceuticals (Achillion Pharmaceuticals press release; February 8, 2000). These agents, of which lamivudine (3TC) is also an example, seem to reduce the mitochondrial damage caused by β-L(+) nucleoside analogs, such as d4T, by interfering with their uptake *(46)*. The L-nucleoside configuration of elvucitabine may provide protection against mitochondrial toxicity, because this compound is not a substrate for mitochondrial deoxypyrimidine nucleoside kinases. Elvucitabine did not have any inhibitory effect on mitochondrial DNA synthesis at concentration up to 10 m*M* *(47)*. Initial studies focused on the activity of elvucitabine against hepatitis B virus. Elvucitabine proved to be much more active than 3TC in several models *(48–50)* and to retain some activity against 3TC-resistant strains of hepatitis B virus. These studies also demonstrated that the intracellular half-lifes of phosphorylated metabolites of elvucitabine were greater than 20 h, approximately five times longer than that of 3TC *(51)*. Phase I clinical trial data suggested that elvucitabine could be effectively dosed as a once a day regimen with minimal side effects. Studies with HIV have shown elvucitabine to be 10-to-20 times more potent than other nucleosides, and to be active against a wide range of resistant isolates, including those bearing 184V, Q151M and 69SS insertions *(52)*. In vitro selection yielded virus with a 184I mutation *(53)*. Similarly to 3TC, elvucitabine showed synergistic activity in combination with AZT plus d4T *(47)*. A phase II clinical trial (study 443-006) in HIV-infected patients with the fingerprint 3TC mutation (M184V) in HIV RT was initiated in the United States in July 2002 (Achillion Pharmaceuticals press release; July 17, 2002). Fifty-six subjects remained on their 3TC–HAART regimen and then switched their 3TC for elvucitabine dosed at either 50 or 100 mg once daily for up to 28 d. Elvucitabine demonstrated good antiviral activity. Mean HIV RNA levels dropped by 0.67 and 0.78 $\log_{10}$ copies/mL at the 50 and 100 mg doses, respectively *(54)*. However, because bone marrow suppression was seen in several patients, future dosage regimens will likely be lower.

Reverset

Reverset (DPC-817; 2′,3′-dideoxy-2′,3′-dideoxy-5-fluorocytidine [β-D-Fd4C]), the stereoisomer of elvucitabine, is under development by Pharmasset and Incyte under the trade name of Reverset. The preclinical development of this compound was described by Schinazi et al. *(55)*. Similar to elvucitabine, DCP-817 showed good bioavailability and a long intracellular half-life (13–17 h). Although DPC-817 inhibited mitochondrial DNA polymerase-γ in vitro, no mitochondrial toxicity was observed at concentrations of up to 1 m*M* in mouse bone marrow cells. DCP-817 demonstrated similar in vitro potency to 3TC in a range of cell-based assays and maintained activity against mutant virus with

multiple AZT and 3TC resistance-associated mutations, such as 41L, 67N, 70R, 184V, 215Y, and 219Q. The multidrug-resistance mutation, 151M, and insertions at codon 69 were not tested. As seen for DAPD, another D-nucleoside, only the 65R mutation resulted in a significant (sevenfold to ninefold) reduction in DCP-817 susceptibility. This mutation has also been selected by in vitro passage in the presence of DCP-817 *(56)*. Preliminary pharmacokinetic studies performed in rhesus monkeys indicate that DPC-817 has a lower rate of systemic clearance and a longer half-life than AZT or 3TC *(57)*. In a 10-d monotherapy study, drug-naive patients were administered doses of 50, 100, or 200 mg DCP-817 daily, and viral loads were reduced by 1.67, 1.74, and 1.77 $\log_{10}$ copies/mL, respectively *(58)*.

Alovudine

FLT is structurally similar to AZT and was originally tested against HIV in 1988 *(59)*. It was found to be a potent inhibitor with good cellular penetration and high intracellular triphosphate levels *(60)*, and it progressed to phase II trials in untreated AIDS patients in 1991 and 1992. However, because FLT showed no clear advantage over AZT, it was discontinued by Lederle (a division of American Cyanamid) in 1994, until the increased incidence of AZT resistance and the emergence of multidrug-resistance mutations prompted its reevaluation by Medivir. It is currently developed by Medivir/Boehringer Ingelheim.

In monkeys, FLT inhibited simian immunodeficiency virus 10 times more potently than AZT, and its oral bioavailability was excellent *(61)*. However, FLT also showed increased toxicity in animal models *(62)*.

A major advantage of FLT is that it is active against AZT-resistant viruses carrying various combinations of mutations *(22,63)*. Even strains carrying the 151M mutation and insertions at codon 69 show only minimal reductions in susceptibility, and it seems that large numbers of major resistance mutations are required to give significant resistance. Attempts to select FLT-resistant viruses have been fruitless, even after 49 passages *(63)*.

In a phase II pilot study, 15 patients failing treatment on an NRTI-containing regimen, with a median of four thymidine analog-resistance mutations, added 7.5 mg FLT once daily to their failing regimen for 4 wk *(64,65)*. The treatment was generally well-tolerated, with no serious adverse events noted. The overall median reduction in viral load was 1.13 $\log_{10}$ copies/mL. Patients receiving d4T as part of their treatment had a much better reduction in viral load than those with no d4T in their regimen (0.57 $\log_{10}$ copies/mL vs 1.88 $\log_{10}$ copies/mL, respectively), suggesting an interaction between FLT and d4T. The disadvantage of FLT is that its high toxicity leaves a very narrow therapeutic window for optimal dosing. The toxicity of FLT could be problematic in humans and must be carefully evaluated.

SPD-754

SPD-754 (also known as BCH-10618) is the negative enantiomer of dOTC, which is a racemic mixture. dOTC had shown promising potential, with a potency similar to other NRTIs and retaining activity against 3TC- and AZT-resistant virus *(66,67)*. dOTC was originally under development by Biochem Pharma and Shire Pharmaceuticals. Phase I/II trials were completed (BioChem Pharma press release; October 20, 1999; refs. *67–69*) but further development was delayed after long-term primate studies indicated toxicity at high doses. It was subsequently established that (–)dOTC was more active and less toxic than (+)dOTC, and development (now at Avexa) was continued with the (–)dOTC enantiomer, SPD-754 *(23)*.

Like dOTC, SPD-754 is active against AZT- and 3TC-resistant virus and shows intermediate loss of activity against virus with RT codon 69 insertions. Greater than 10-fold resistance is only observed in virus with Q151M mutations *(23)*. In vitro drug resistance emerges slowly and seems to be primarily associated with the selection of 65R and 75I *(70)*. In vitro studies have also shown no mitochondrial toxicity *(66)*. A pharmacological study in 27 healthy volunteers indicated the possibility of once-daily dosing. The antiviral activity of this compound was studied in a 10-d monotherapy study in which 63 treatment-naive subjects were randomized to receive doses of 200 mg, 400 mg, or 600 mg SPD-574 twice daily; 800 mg or 1200 mg SPD-574 once daily; or placebo *(71)*. A dose-dependent reduction in viral load was observed, ranging from –1.16 to –1.44 $\log_{10}$ HIV RNA copies/mL. Because intracellular levels of SPD-574 were found to be reduced when patients were taking 3TC, it is likely that combinations with 3TC or other cytidines will be precluded clinically *(72)*.

NNRTIs IN DEVELOPMENT

Table 2 summarizes the NNRTI presented in this section and Figure 2 shows the structures of the compounds.

Capravirine

Capravirine, formerly known as S-1153 and AG-1549, is an imidazole compound identified by Shionogi Pharmaceuticals through screening of various derivatives against NNRTI-resistant viruses *(24)*. The clinical development of this drug is being continued by Agouron-Pfizer. Capravirine is more potent in vitro than nevirapine and delavirdine, and seems to retain activity against virus bearing various single NNRTI resistance-associated mutations *(24)*. However, dual mutations, such as L100I with K103N, and single mutations at codon 181, often seen in viruses isolated from patients failing nevirapine therapy, can render the virus resistant to capravirine *(24,73)*. In serial

Table 2
New NNRTIs in Clinical Development

Company	Compound	Stage of development	In vitro EC_{50} (or EC_{90}[a]) μ*M* against: WT	K103N	Y181C	K103N +Y181C	Mutations selected by in vitro passage	Key refs.
Pfizer/Agouron	Capravirine AG1549 S1153	Phase II	0.001–0.003	0.001	0.009–0.015	na	K103T+V106A+L234I or V106A+F227L	*(24)*
Tibotec	TMC125	Phase II	0.001	0.001	0.007	0.004	Resistant virus carries multiple mutations including positions 101, 179, 181, 227, and 230	*(26,86,87)*
Tibotec	TMC278	Phase II	0.0004	0.0003	0.001	0.001	na	*(81,92)*
Sarawak/Medichem	(+)-Calanolide A	Phase II	0.1–0.5	na	na	na	T139I, N348K	*(100,101)*

WT, wild type; na, not available
[a]If no EC_{50} is available the EC_{90} is given. As a very general rule, EC_{90} can be divided by 5 to give an approximate EC_{50}

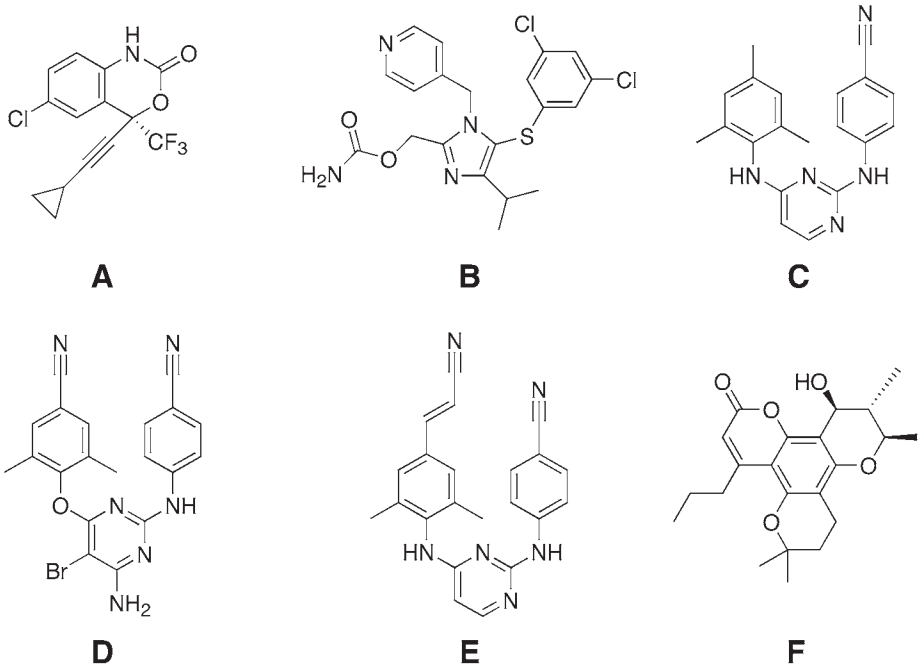

Fig. 2. Structures of NNRTIs in development alongside efavirenz. (**A**) Efavirenz; (**B**) capravirine (AG1549); (**C**) TMC125; (**D**) TMC120; (**E**) TMC278; (**F**) calanolide A.

passage experiments, virus with high-level resistance to capravirine is slow to emerge and contains multiple RT mutations, suggesting a high genetic barrier to resistance *(24)*.

In the phase I studies reported to date, capravirine did not seem to cause rashes (a common side effect of NNRTIs) and was generally well-tolerated, with common side effects including nausea, taste disorders, and headache *(74–76)*. The bioavailability was high and target plasma levels were maintained at 8- to 12-h dosing intervals *(76)*.

Preliminary data from a phase II placebo-controlled study in NNRTI-experienced patients were presented at the Eighth Conference on Retroviruses and Opportunistic Infections, in 2001 *(77)*. Fifty subjects had been randomized to receive either placebo or capravirine at one of two doses (1400 mg or 2100 mg, twice daily) plus nelfinavir (1250 mg twice daily) and two new NRTIs. The low number of patients completing the study did not allow for an intention-to-treat analysis of the data. An on-treatment analysis revealed no difference in viral load decrease between the placebo and treated arms. At 12 wk, 75% of subjects in the 1400-mg group and 50% of subjects in the 2100-mg group had a viral load of fewer than 400 copies/mL. Median viral load reductions were

1.5 $\log_{10}$ copies/mL and 2 $\log_{10}$ copies/mL in the 1200-mg and 2100-mg groups, respectively. A higher incidence of adverse events in the higher-dose group was proposed as an explanation for the reduced response in these patients.

Several phase II/III trials of capravirine were commenced during late 1999 and 2000. In January 2001, these studies were suspended after a report of severe vasculitis in dogs (Agouron press release; January 25, 2001). However, phase II trials have been resumed after a human toxicology study revealed no systemic vasculitis *(78)*. In 2005, data were presented from a 24-wk, phase 2, prospective, randomized, double-blind, dose-ranging study (Study 1002). In this trial, capravirine (700 mg or 1400 mg) or placebo were combined with a standard-of-care regimen of nelfinavir plus two NRTIs chosen based on phenotypic resistance testing *(79)*. The patients were NNRTI experienced and protease inhibitor naive. The percentage of patients achieving a viral load reduction to fewer than 400 copies/mL was 48%, 50%, and 60% for the placebo, 700-mg, and 1400-mg arms, respectively *(79)*. The percentages for the reduction to fewer than 50-copies/mL threshold were 39%, 40%, and 52%, respectively. These favorable trends did not reach statistical significance.

TMC125, TMC278, and TMC120

TMC125 (also known as etravirine or R165335), TMC278 (also known as rilpivirine or R278474), and TMC120 (also known as dapivirine or R147681) are all diarylpyrimidine (DAPY) derivatives that were derived from lead structures discovered with the aim of finding new, highly potent, and broad-spectrum NNRTIs *(26,80,81)*. The strategy used by researchers from Tibotec and Janssen Pharmaceutica/Johnson & Johnson to identify potent agents with high levels of activity against NNRTI-resistant viruses was based on systematic testing against panels of NNRTI-resistant viruses, with a parallel optimization of other factors (such as resilience to the development of drug resistance, drug metabolism, and protein binding) *(26,81,82)*. In addition, molecular modeling was used to evaluate structural determinants of inhibitor potency and to guide further chemical synthesis. The more-flexible molecules were designed to enable extended binding interactions with the HIV-1 RT NNRTI-binding pocket, including backbone interactions *(83–85)*. The currently most advanced compound in clinical development, TMC125, was found to inhibit 98% of greater than 2000 clinical isolates of HIV tested with an EC_{50} of less than 100 n*M* and 77% of NNRTI-resistant strains with an EC_{50} of less than 10 n*M* *(26,27)*. In vitro selection experiments in the presence of TMC125 demonstrated a reduced rate of emergence of resistant virus compared with other NNRTIs *(86)*, even when strains containing the K103N or Y181C mutations were used as the seed virus *(87)*.

In a phase IIa clinical study (TMC125-C208), 12 treatment-naive patients received 900 mg TMC125 twice daily as single-drug therapy for 1 wk. The mean plasma trough drug level was 237 ng/mL. The most common adverse event was mild somnolence. At the end of the monotherapy period, the mean reduction in viral load was 1.99 $\log_{10}$ copies/mL, and the median increase in CD4 T-cell count was 119 cells/μL *(88)*. Within 7 d of taking TMC125, 2 of the 12 patients had viral loads of fewer than 50 copies/mL, and 8 patients had viral loads of fewer than 400 copies/mL. The virus clearance rate for TMC125 monotherapy (0.68 $\log_{10}$ copies/mL viral RNA per day) was comparable to a five-drug, triple-class regimen *(89)*.

TMC125 was selected as a drug candidate because of its unique in vitro profile against NNRTI-resistant HIV. The effects of TMC125 in NNRTI-experienced patients with high levels of resistance to efavirenz (mean, 111-fold) were investigated in another phase IIa trial (TMC125-C207) *(89)*. Sixteen patients substituted their failing NNRTI treatments for 900 mg TMC125 twice daily for 7 d. The median reduction in viral load was 0.9 $\log_{10}$ copies/mL. Mild (grade I) side effects were reported in 11 patients, with diarrhea and headache being the most common. Further clinical trials are in progress to determine the optimal dose and to assess long-term safety and tolerability of TMC125 in HAART regimens.

TMC120 also shows significant activity against NNRTI-resistant virus and has been tested in a phase IIa study in 43 treatment-naive patients *(90)*. A mean decrease in viral load of 1.51 $\log_{10}$ copies/mL was seen after 7 d of monotherapy with 100 mg TMC120 twice daily. TMC120 was superseded by TMC125. The topical application of a gel containing TCM120 was also recently shown to prevent HIV infection of hu–SCID mice by vaginal transmission, providing the first evidence of the in vivo effectiveness of an NNRTI as a potential microbicide *(91)*.

More recently, a third DAPY analog, TMC278, was described that was also taken into clinical development *(81,92,93)*. This newest DAPY analog showed a median EC_{50} against wild-type HIV-1 of 0.5 n*M*, with a selectivity index of 16,000 *(92)*. It has a significantly expanded antiviral spectrum; 89% of 1500 NNRTI-resistant clinical isolates were inhibited at an EC_{50} of less than 10 n*M*, as compared with 33% and 0% for efavirenz and nevirapine, respectively *(92)*. TMC278 was also shown to have an increased genetic barrier. Using high multiplicity-of-infection experiments, no virus breakthrough was observed after 30 d of culture in the presence of at least 40 n*M* TMC278 . In contrast, currently available NNRTIs, such as efavirenz and nevirapine, led to virus breakthrough within 7 d at concentrations of 1 μ*M*.

Initial data on the in vivo activity of TMC278 were obtained in a 7-d, monotherapy trial in 47 drug-naive subjects who were treated with either 25, 50, 100, or 150 mg TMC278 twice daily or placebo *(93)*. At day 8, the median viral load reductions ranged from –1.1 to –1.3 $\log_{10}$ copies/mL, in contrast

with placebo (+0.002 $\log_{10}$ copies/mL) ($p < 0.001$). The overall increase in CD4 cell count in the TMC278 groups was +55 cells/μL. No severe adverse effects were reported.

Calanolide A

Calanolide A is derived from *Calophylulum lanigerum*, which grows in the rainforests of Sarawak, Malaysia. Eight compounds were originally isolated from this plant in 1992 by researchers at the NCI using anti-HIV bioassay-guided fractionation of an extract of the plant. Canalolides A and B were identified as the most active, with IC_{50} values of 0.1 μ*M* and 0.4 μ*M*, respectively *(20)*. Because the compound is only a minor component of the leaves, the structure was elucidated and, in response to an NCI call for proposals, MediChem Research devised a method of chemical synthesis *(94)*. Several structural analogs of calanoloide A have also been synthesized, but none have displayed any increase in anti-HIV activity *(94–96)*. In 1995, MediChem was granted a worldwide exclusive license to the NCI patent and to the rights held by the government of Sarawak, and, in 1997, the company announced the formation of Sarawak MediChem Pharmaceuticals, Inc., a joint venture between MediChem and the government of Sarawak to advance the clinical development of calanolide A (MediChem research press release; April 25, 1997).

Although, similar to other NNRTIs, calanolide A is inactive against HIV-2, it does seem to interact with the HIV-1 RT at a novel binding site *(97)*. Evidence for this comes from the synergistic binding of calanolide A and nevirapine to RT *(98)* and the susceptibility to calanolide A of viral strains carrying Y181C and K103N mutations *(20,99)*. In fact, the Y181C mutation seems to increase susceptibility to calanolide A *(100)*. Resistant viruses with T139I and N348K mutations were selected by eight sequential passages in calanolide A *(101)*.

A phase Ia clinical trial carried out in 47 healthy subjects showed reasonable oral bioavailability of (+)-calanolide A. Only minor side effects (dizziness, taste perversion, headache, eructation, and nausea) were seen, which were not dose-related and were not all judged to be related to study medication *(102)*. In a 14-d, dose-ranging, phase Ib monotherapy trial in 43 HIV-infected ARV-naive subjects, viral load was decreased by a mean of 0.81 $\log_{10}$ copies/mL in the highest dosing group, and no resistant virus was observed *(103)*.

According to Sarawak MediChem, calanolide A shows good penetration of the central nervous and lymph systems (Sarawak MediChem website). Also, because calanolide A is metabolized by cytochrome P450, it has been suggested that coadministration with ritonavir may enhance drug levels *(104)*. In July 2002, Sarawak MediChem announced that a 48-subject phase II clinical trial had begun (Sarawak MediChem press release; July 18, 2002). The continued development of calanolide A will depend on the results of this study.

SUMMARY

The search for new RT inhibitors for the treatment of both treatment-naive and treatment-experienced patients continues to be very active. A number of the drugs under development meet most of the challenges outlined in the introduction to this chapter, offering significant advantages over currently available compounds in terms of potency and tolerability. Several of these candidates are extremely active against drug-resistant virus and may offer new hope to heavily treated patients carrying multidrug-resistant virus. Some of the highly potent new generation NNRTIs also seem to select resistant virus much less rapidly (increased genetic barrier) than the first-generation compounds in this class, and, thus, may also be useful in prolonging the response to first-line combination regimens. Although not all of the drugs listed in this chapter will continue to the market place, we can expect to see at least two or three new products licensed within the next few years.

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17
Antiretroviral Drugs for Millions

Allan Ronald, Elly Katabira, and Merle Sande

INTRODUCTION

Highly active antiretroviral therapies have markedly altered the illness course of approx 1.3 million people since these therapies became widely prescribed in 1997. However, approx 80% of individuals who are on treatment are residents of industrialized countries of the world, where fewer than 10% of the HIV-infected individuals make their home. In contrast, in December 2004, in sub-Saharan Africa, less than 300,000 individuals are taking antiretroviral treatment (ARV) in an HIV-infected population of approx 26 million. This is substantially less than 1 patient out of 50 who meet the "Western" criteria for treatment.

This chapter explores the debate regarding ARV access in Africa, advocates for a rapid introduction of ARVs, and proposes a course of action.

Elsewhere in this book, the remarkable efficacy of ARVs is identified. Numerous drug options are available that can be tailored to the individual's lifestyle, concurrent medication, and side-effect profile. Sophisticated, sensitive laboratory techniques can allow both the patient and the caregivers to track the response to therapy with satisfying precision. New therapeutic targets and modalities are being identified by biotechnology and pharmaceutical companies, and several new agents and new classes are expected to enter clinical trials during the next 5 yr.

However, in the industrialized world, patients still die from AIDS and the consequences of AIDS remain substantive. In a review of 13 prospective studies, Egger et al. reported that 344 patients died during 24,310 person-years of follow-up *(1)*. Almost half of the deaths were caused by neoplasms that continue to occur in patients despite treatment. However, ARVs do have a profound effect on both morbidity and mortality, reducing hospitalizations by greater than 80% in developed countries and, for the individual, who can be treated consistently and continuously, ARVs have reduced mortality by greater than 80% during the past 8 yr *(1)*.

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The nucleoside analog reverse transcriptase inhibitors (NRTIs) offer new exciting options for the treatment of HIV infection. New agents have been introduced during the past 4 yr with improved pharmacokinetics, better patient tolerance, and enhanced effectiveness for resistant isolates. Tenofovir has been studied as the "third agent" along with two of the earlier NRTIs, with treatment results that almost parallel those in which the third agent has been either a protease inhibitor or a non-NRTI *(2)*. Additional options and simpler regimens are making a major difference in the ease of day-to-day care of patients in the industrialized world, as well as substantially prolonging life for patients with HIV infection.

The arguments for a massive scaling-up of ARV therapy for resource-limited countries, particularly Africa, are obvious and valid. However, important African and Western leaders, particularly those committed to HIV prevention and the broader public health agenda, have expressed reservations regarding programs that primarily focus on ARVs and fail to recognize the difficulties inherent in a massive program expansion.

THE "YES" ADVOCATES

The argument rests on first-order principles. Human rights requires that all of us give value to the health and well-being of the individual, regardless of race, religion, or geography. We can predict that at least 12 million of the 26 million currently infected individuals with HIV in sub-Saharan Africa will die by 2012 if ARV treatment is not accessible. The consequences have been dramatically presented *(3)*. These individuals wish to live out their lives, raise their children, and be productive citizens. We should not let the epidemic run its course.

Second, the global consequences of HIV have been well-characterized by various organizations and political leaders who have assessed risks to security, global economic growth, migration, and poverty alleviation. With approx 8000 deaths each day, HIV-infection is having a global impact, and this impact will increase if we fail to address the epidemic at all levels of prevention and care.

Third, we are being challenged by spokespersons who identify our apathy, possible racism, and bureaucracy that prevent access to drugs for the majority of individuals dying of HIV infection *(4)*. All of us in positions of leadership in government, health, and other sectors must identify our role as individuals and as organizational leaders and prevent tragic scenarios.

Fourth, the obstacles to widespread antiretroviral therapy noted in this Chapter (pp. 501–511) have been minimized by the advocates for immediate global access to ARVs. The level of expertise for caregivers may be set too high. Concerns regarding the lack of systems for ARV distribution and the urgent need for more research is downplayed. Issues regarding intellectual property, patents, and profits are deemed inconsequential in light of the need for ARVs.

Access to generic drugs is presented as the immediate savior for the world's infected individuals. These are compelling arguments.

THE "NO, NOT YET" ARGUMENT

Some leaders in public health, some donors, and a few individual scientists have expressed dismay regarding the mobilization of human and fiscal resources as well as the grandiose plans to treat HIV patients throughout the developing world. Their concerns are based on their individual experiences as well as a foreboding concern that failure will be catastrophic and lead to a precipitous increase in the index of global despair for impoverished societies in Africa and elsewhere. Disillusionment will occur not only for HIV prevention and care programs but also for the wider range of health and development initiatives. These forecasters of potential failure have credibility and share our concern regarding suffering and premature death caused by HIV in the developing world. Their angst must be heard and addressed in a shared effort to mitigate any downside potential as we attempt to rapidly expand our commitments to HIV care.

Although the challenges are addressed individually, it should be recognized that these difficulties are often linked to challenging cultural milieus and infrastructure inadequacies. Although dogmatic statements will be made, these statements are intended to further the debate and to move the agenda forward, rather than to be an absolutist approach to HIV care.

IS THERE SUSTAINED COMMITMENT FROM COUNTRY LEADERSHIP TO HIV/AIDS PROGRAMS?

Political and administrative leadership in most of the world, with a few exceptions, have given limited direction to the issues surrounding HIV care and prevention. Brazil is the most notable exception in terms of HIV care programs, whereas President Musevani of Uganda has been exceptionally effective in his leadership of HIV prevention programs. Even in Uganda, AIDS care has received less evident support. However, Stephen Lewis, the United Nations (UN) Special Representative for "AIDS in Africa," has eloquently argued for widespread access to AIDS care and has used every opportunity to convince governments and philanthropists throughout the world that this can be achieved. Otherwise, leadership has tended to use the crisis of HIV as a security issue, or as an issue to confront the wealthy nations regarding their lack of concern. However, there has been limited significant revisions of national budgets or priorities, and a limited amount of serious preparation within most governments, universities, or health care institutions in preparation to address and allot the massive resources necessary for HIV care programs. The Joint Clinical Research Centre in Uganda, established in 1990 by President Musevani, as a

visionary cooperative initiative between several government ministries, is an exception, but its care capacity is limited. In addition, this institution has not been replicated elsewhere in Africa. A commitment is required from leaders in all countries to rank HIV care as a priority.

Although Western countries can be faulted for their apathy toward HIV care, leadership is now occurring, with accompanying resources. President Chirac in 1997, in Abidjan, Côte d'Ivoire, committed France to provide leadership for HIV care programs. More recently, the world has found resources for the Global Fund for AIDS, Tuberculosis, and Malaria. Individual countries are rapidly increasing their long-term commitment to addressing HIV care programs. The announcement in 2003 by President Bush committed the US government to $15 billion during 5 yr, through the President's Emergency Plan for AIDS Relief, which will enable programs to proceed with substantial long-term commitment. Inadequate fiscal resources are frequently identified as the only block to widespread care programming. Although this is politically correct, in most instances lack of money is not the penultimate obstacle. Monies are often not spent because of a lack of adequate human resources, poorly developed business plans, or multitiered bureaucracy. In a sincere effort to avoid misallocation of funds, most countries have established criteria and processes that are challenging to navigate efficiently. As a result, introduction and scaling up of care programs takes years instead of months. These very substantial obstacles to improve care need to be addressed by the individuals in leadership positions throughout the developing world, within national educational and health care structures. Business as usual, demonstrated during the past decade, will not suffice if HIV care is to be offered to even one-third of the individuals who will otherwise die between now and 2012.

Change is difficult in resource-limited countries for many reasons, including the lack of managerial resources to initiate and facilitate change. However, a primary reason why change does not occur may relate to the lack of external review, accreditation processes, and standards. In much of the Western world, change is driven by continuous program review, with ongoing disruption because of program closures, budget revisions, and downsizing, and critical review of programming outcomes and impacts. Peer review and accreditation processes are only now being introduced in many resource-limited societies.

SUMMARY

Governments, both in the West and in resource-constrained societies, need to boldly declare that HIV care has a higher priority than monies for weapons. Leaders within resource-limited countries as well as in the West must seriously address issues regarding HIV care if resources are to be mobilized and made available to the masses of people who will otherwise die. Most of the other strate-

gies to be subsequently discussed in this chapter can only occur within renewed organizational structures and with sustained serious commitment of the national and institutional leadership to rapid deployment of human and fiscal resources.

WHY IS THERE NOT MORE ACTIVISM IN DEVELOPING COUNTRIES?

In most developing countries, patients infected with HIV do not have the skill set to be advocates for care programming or activists within their societies. Outside of South Africa, there is almost no significant activist movement in Africa. Political leaders and health care professionals are placed on pedestals by the wider society and are seldom challenged to change the status quo. The self-interests of health care professionals and individuals within government institutional structures must not be prioritized ahead of the populations they serve.

SUMMARY

HIV activism needs to be organized and supported to challenge the status quo.

WHERE IS THE COMMITMENT TO DEVELOPING THE HUMAN RESOURCES ESSENTIAL FOR THE TASK?

The lack of strategic planning for human resources within resource-constrained societies is alarming. The marketplace cannot function as its sole determinant. There is a serious lack of individuals throughout much of the resource-constrained world who can provide organizational leadership, adequate training, or large-scale implementation of HIV care *(5)*. In a needs analysis in 2002, only 1 of 12 countries had begun human-resource planning for HIV/AIDS, despite an epidemic that was rapidly undermining their country's future. As a result, there is a dependence on expensive, often inappropriately experienced, expatriate personnel, not only in areas of program design and implementation, but also in areas of training, supervision, motivation, and care delivery.

SUMMARY

All educational institutions, including universities, should be collaborating closely with governments to ensure that human resource needs are being adequately met through the scope and size of the educational and training programs within their institutions.

CAN CARE BE SCALED UP WITHOUT ALTERING THE PRIORITY OF PREVENTION?

In all societies, prevention initiatives, including strengthening of public health systems, is continually at odds with monies allocated to care. In most Western societies, a hugely disproportionate amount of resources is provided

for care services that do little more for many patients than delay death by weeks to months. Meanwhile, public health activities are severely underfunded by most governments.

In several countries, notably Thailand and Uganda, prevention has been remarkably successful and enables these countries, at least, to show dramatic reductions in HIV incidence with the longer-term expectation that the burden of disease will be substantially reduced during the next decade *(6)*. This outcome has to be valued above all others. Can it survive with the emphasis shifting from prevention to care in many of these countries during the next 5 yr? The experience in many Western countries is depressing. Prevention efforts have not been sustained within the communities at risk or within governments. As a result, substantive increases in HIV incidence have occurred *(7)*.

Prevention can be relatively inexpensive. Sex-worker interventions cost approx $12 to $50 per case of HIV-infection prevented *(8)*. Voluntary counseling testing will prevent an HIV infection with an investment of $250 to $500 per case averted *(9)*. Sexually transmitted infection control cost effectiveness may also be in this price range. Unfortunately, a sense of accomplishment does not accompany "a case prevented" and prevention must be given the resources and emphasis that ensures its dominance within the health care planning and service activities. Providing public monies for the care of HIV patients should only be allocated as the society commits itself to enhance and sustain its prevention activities.

Nowhere are all prevention activities adequately deployed. Strategically, every country should annually review and evaluate its prevention activities, scale up and improve those that are lagging, introduce new proven prevention interventions, and be accountable to the wider society for the efficiency of the interventions that are underway.

Surveillance programs are inadequate in most societies, particularly in resource-limited countries. Surveillance is the major infrastructure requirement for prevention. This includes an ability to identify prevalence and incidence in selected populations throughout the country, recognize trends, and respond to hot spots and outbreaks. Effective national surveillance has to be a priority for every society, with careful real-time analysis and reporting back through the media, and holding the leadership accountable. Goals and objectives have to be set and achieved for this accountability to occur. Very few countries anywhere have established the priority that surveillance requires if surveillance is to have a significant role in HIV prevention.

All countries should also be able to identify the resources allocated to prevention and ensure that these are continually being increased as they become available. All societies should expect their government to give at least an annual address on the effectiveness of prevention programs within the nation and the

changes undertaken to ensure that their country has measures in place that will reduce HIV incidence and prevalence.

Prevention and care have been theoretically connected and there is some evidence that they can be mutually dependent. However, more research is needed to understand how awareness of HIV status, reduced stigma, and the mainstreaming of HIV through care programs can enhance prevention. This is an area in which there has been substantial claims with little prospective critical evidence. However, some care programs can undoubtedly reduce HIV transmission. If couples are discordant, treatment of the infected spouse will presumably reduce the probability of transmission. Vulnerable individuals with multiple partners, such as sex workers, their clients, and others, may have a reduced likelihood of infecting sex partners if they are receiving treatment. Both NRTIs and non-NRTIs have been shown to significantly reduce the transmission from mothers to their infants. All of these interventions make biological sense. However, their total impact on countrywide HIV incidence and prevalence relies on mathematical models rather than proven effectiveness. Much more research is needed to link care and prevention and to ensure that they strengthen each other within the scientific context of both behavioral-based and biologically based interventions.

SUMMARY

The emphasis on prevention must remain, and evidence to ensure this priority must be gathered prospectively within every society as care programs are introduced.

CAN HEALTH CARE SYSTEMS CURRENTLY UNABLE TO MEET MANY OF THE PRIMARY CARE NEEDS OF ILL INDIVIDUALS BE EXPECTED TO ADDRESS THE NEEDS OF A COMPLEX, CHRONIC, INCURABLE DISEASE?

HIV care has many levels of complexity, from straightforward regimens for prophylaxis and basic care in the context of social support and counseling, through the difficult experience-based decision making that involves drug choices in patients with adverse reactions or virological failure. In resource-limited countries, as elsewhere, levels of care need to be established and expertise needs to be developed appropriate to the care function expected. At present, because of inadequate human and financial resources as well as limited supervision (particularly of nongovernment facilities), care is irregular and not standardized within most major African cities in which it has been examined. The care difficulties at the point of patient entry into the health care system relate to at least the following:

- Patient numbers that overwhelm the available care resources. For instance, in the Mulago Teaching Hospital in Kampala, 40 to 70 patients present in each 24-hr

period to the Department of Medicine for emergent care. Approximately two-thirds of these patients are HIV infected.
- Very limited diagnostic and treatment resources. Secondary and tertiary care have been given a lower priority within many African health care systems, often because of pressure from donors. As a result, resources for acute care institutions are inadequate by any standard.
- Many caregivers are inadequately supported regarding their own needs. Salaries are often late, team building and organizational support are lacking, morale is poor, and many caregivers are chronically discouraged by the unsatisfactory outcomes for many of their patients. The hospital death rate among HIV-infected patients is a daily reminder of the futility of care caused by limited resources and the terrible ravages of the disease.
- Limited time, inadequate salaries, and busy practices prevent many academic clinicians from spending adequate time within the care environment and becoming excited about clinical illness or the science of caring.
- Distribution programs for drugs, diagnostics, and other material to care for patients is often deficient, unreliable, or compromised by product diversion.

These issues are not readily addressed and will require bold new approaches. Some possibilities include the following:

- Repatriation of generations of health professionals that have emigrated to other countries. These individuals will have to be wooed back to their country of origin, where they understand the culture and language, and will have to be given the opportunity to provide leadership and direction. They will also require financial incentive, which will be expensive but necessary to achieve their recruitment.
- Accountability must occur throughout the health care system, with appropriate rewards for performance and penalties for failure to perform to minimum standards.
- The private sector needs to be involved. Distribution systems may not require new technologies. For the past 50 yr, large companies have been getting nonessential products through a complex distribution system from manufacturing sites in other parts of the world, to a point of contact at which the consumer can make purchases. Health care products, including drugs, should be distributed by the most efficient systems possible, not by ineffective bureaucratic government systems. This becomes mandatory if ARVs are to consistently be made available.
- Assistance will be required from the global community, and this will need to include not only dollars but systems experts who can train and advise, to ensure the creation of new efficient public–private partnerships that enable the delivery of services. The ultimate goal is that all services improve. A new, vertical HIV service is not sustainable, and will only divert resources from a system in desperate need of support.

SUMMARY

Health care services and systems need to be extensively revamped to provide adequate care for the large numbers of patients with HIV. This will only

be possible if governments, universities and all educational institutions, and health communities accept the need for a shared effort, with input from individuals with expertise.

WILL PROGRAMS FOR AIDS CARE INCREASE THE INEQUITIES ALREADY PRESENT IN SOCIETY?

Issues around equity must be addressed within programs to deliver care. Wealthy influential men will receive a disproportionate amount of HIV care resources unless measures are taken to ensure that the poor, the rural and disadvantaged, and women and children are given their share of resources. Without protection and oversight, HIV treatment will increase disparities.

SUMMARY

Equity issues must be addressed in planning the introduction of ARVs into all societies.

HOW CAN ARVs AND THE OTHER COSTS OF ENHANCED CARE BE FUNDED IN RESOURCE-CONSTRAINED SOCIETIES?

First, costs can be further reduced. Costs remain substantial despite price reductions achieved through UN ACCESS Programs in several countries and the introduction of generic drugs in others. Treatment regimens that cost more than $6000/yr if prescribed in North America are now available for $200/yr from generic manufacturers in India. However, for a number of reasons, the generic drugs may not be the best or only answer in all instances. The use of generic drugs has avoided the issues of strict standards and good manufacturing practices, patent infringement and intellectual property, and the necessity of ongoing pharmaceutical research to ensure new products.

On the other hand, the argument for brand name companies to develop two-tier differential pricing systems is persuasive. Most brand name companies can efficiently and rapidly scale up their production of additional product. It can be labeled to differentiate it from the product manufactured for wealthy countries. It will require that Western countries accept higher prices, and that resource-constrained countries agree to establish controls to ensure that products sold at lower prices are not diverted to the West.

The Brazil experience is relevant to all countries. Although further economic analysis is required, it seems that the reduction in price of drugs in Brazil of between 70% and 90% enabled more than 105,000 individuals to access therapy, and reduced hospitalization by greater than 70% *(10)*. Some spokespersons have stated that Brazil has saved more than a billion dollars in health care costs and economic productivity through their introduction of ARVs.

Health care funding systems must be flexible. Currently, in most African countries, health care costs have soared and the health care system has been distorted by costs of HIV illness. These expenditures can be markedly reduced. This has been shown in the West and now in Brazil. Systems to ensure that monies can be transferred from acute care budgets to drug budgets need to be developed and introduced.

Second, employers must develop employee programs that address both HIV prevention and care. Fortunately, many companies are now beginning to realize that their employees are their most important resource, and individuals who become ill with HIV need support, including assistance for the purchase of ARVs. These programs should rapidly expand during the next 3 yr, and perhaps 2 to 3 million individuals will be eligible for enhanced care. In particular, governments need to address this issue with health benefits for civil servants that enable stable, productive, and motivated work forces.

Third, philanthropy has been largely been untapped. Many individuals taking ARVs are dependent on family members who provide resources. However, with the reduced cost of ARVs, many individuals from wealthy countries may financially support drug purchase for infected patients. This model could find resources for an additional several hundred thousand HIV-infected patients during the course of the next 3 to 5 yr. Currently, a donation of $250 semiannually would enable a person to be treated with ARVs in Uganda.

Fourth, many individuals can afford to purchase drugs from their income or their assets. In Uganda, the cost of generic drugs are approximately three times the monthly costs of a mobile phone. Although further studies are needed, it seems that individuals earning an equivalent of $150 US/mo may be able to afford as much as 20% of their salary, or $30 US/mo to purchase therapeutic drugs. Between 10 and 15% of infected individuals in urban regions of Africa will be able to purchase therapeutic drugs, and, as a result, most will presumably continue to be healthy and productive.

Finally, bilateral and multilateral donors are allocating resources for ARVs. The World Health Organization intends 3 million individuals to be treated with ARVs in resource-limited countries by December 2005 *(11,12)*.

SUMMARY

Large numbers of individuals are going to be able to afford and presumably access ARVs within most low-income countries during the next 3 to 5 yr, through a variety of funding mechanisms. Less expensive drugs, donations, and cost sharing for populations between government and Western sources, employee plans, philanthropy, and personal purchases will enable millions of HIV-infected people to initiate therapy during the next 4 to 5 yr. Although price is an obstacle, it can be effectively addressed.

WILL ADHERENCE NOT BE A MAJOR PROBLEM WITH RAPID EMERGENCE OF DRUG RESISTANCE?

Few studies have been performed in resource-constrained societies. However, in observational studies, most individuals who purchase their medication seem to have excellent compliance *(12,13)*. Educational support will be necessary, and further research is needed to identify what factors will interfere with the high levels of adherence necessary to ensure optimal laboratory and clinical outcomes.

Individuals who have had a near death experience from their illness and who have a satisfactory response to treatment may be particularly adherent. More than 90% of patients taking fluconazole for suppression of *Cryptococcus neoformans* are compliant with daily fluconazole administration in the Mulago HIV Clinic.

SUMMARY

Although more studies are needed, most individuals provided with ARVs in developing countries may be more compliant than in Western countries. However, sequential monitoring for ARV resistance with well-planned studies is essential.

CAN LARGE ENHANCED CARE PROGRAMS BE INITIATED WITHOUT ADDITIONAL RESEARCH, PARTICULARLY OPERATIONAL RESEARCH AND LARGE CLINICAL TRIALS?

Research is an urgent priority and needs to be quickly scaled up. The traditional approaches to research funding used in the West are not working. Little research is occurring in Africa to improve the care of patients with opportunistic infections (OIs) to determine how ARVs can be optimally deployed. As a result, models of disease prevention and care are being used in the West that may be inappropriate in African society.

The following interventions need to be considered to quickly ensure that research can be performed efficiently in Africa and throughout the developing world:

- Clinician-scientists, particularly individuals with expertise in clinical trials need to be trained and given academic appointments. Critical masses of individuals are needed at a number of academic centers so that most of the training can occur within these countries. At least 300 HIV clinician-scientists are needed for Africa by 2008, with at least 10 centers at which 15 or more individuals work together in a wide range of areas, from pharmacokinetics and pharmogenomics through to bioethics, clinical trials, and operational research. These individuals require long-term commitments for academic positions, with adequate stipends to enable them to pursue their science and academic roles rather than private practice.

- Health research councils with dollars, largely from the West, need to be developed outside of the political process, with excellent peer review to ensure resources, both salaries and grants, to African investigators. At least $300 million for HIV research should be available to fund African science annually within 5 yr.
- Large multisite clinical trials modeled after the AIDS Clinical Trials Groups in the United States need to be urgently developed. These should enter thousands of patients, ask important questions relevant to Africa, and train a range of care providers, including nurses, pharmacists, counselors, laboratory technicians, and physicians, in both the art and the science of clinical trials. At least 10 multisite trials entering 1000 to 5000 individuals should be underway within 5 yr.
- Access to HIV research needs to be provided much more effectively to African caregivers and investigators. Multiple strategies are essential to ensure that information flow occurs as readily as it does in the West.

SUMMARY

Research must inform HIV care in Africa and other countries, as it already increasingly does in the West. The research must ensure evidence-based planning and service delivery, as well as individual clinical decisions. However, widespread access to research still being planned cannot delay initiating individuals on treatment regimens. Research and "enhanced care," including access to ARVs, must proceed in parallel and both must be viewed as urgent priorities.

WHAT LABORATORY STUDIES ARE NEEDED FOR INITIATION IN MONITORING OF THERAPY IN DEVELOPING COUNTRIES?

Laboratory tests in Western countries costing between $1000 and $2000 annually are used to provide serial CD4 counts, viral loads, occasional resistance tests, and other laboratory tests for initiating and monitoring HIV care. In many countries in Africa, few laboratory studies are available and most patients have no resources to pay for laboratory studies. As a result, the majority of patients are treated without Western-type laboratory support.

Several initiatives need to urgently be taken. First, critical studies are needed to identify what clinical parameters can replace the surrogate laboratory studies now used to diagnose and monitor ARV treatment. Can the presence of mild or moderate OIs, such as thrush, be used instead of CD4 counts to initiate therapy? Can these be useful indicators for continuing therapy? What errors are made when clinical markers are used for managing patients, and how can these errors be substantially reduced? Are viral loads measures ever necessary in a setting of limited resources? How should treatment failure be recognized and patients offered a second treatment option? Another strategy currently under investigation attempts to simplify and replace conventional technologies with reliable tests that will predict CD4 cell counts and viral load measures at reduced cost. These tests are currently in review and should become available by 2006. Presumably if a

CD4 cell count can be performed for $5 or less and is readily available, it will be feasible for initiation and monitoring treatment.

SUMMARY

Laboratory support must be available for the care of patients in resource-limited countries. The cost must be realistic in view of annual health costs in these societies, but with planning, research, and external support laboratory support will be possible.

ONE RESPONSE TO THE EPIDEMIC

The Academic Alliance for AIDS Care and Prevention is a private–public partnership launched in June 2001 to build capacity to address HIV/AIDS in Uganda and elsewhere in Africa.

This is a collaboration between nine senior academic physicians in Uganda and five academic physicians in North America. The Academic Alliance was funded by a grant from Pfizer Pharmaceuticals, Ltd. and from the Pfizer Foundation. It is addressing four issues, including:

1. The need for enhanced care, including access to ARVs for HIV-infected individuals.
2. The necessity to build capacity for health care providers.
3. The continuing priority for prevention within a care environment.
4. The intent to strengthen the scientific evidence on which care and prevention activities are based.

During its initial 4 yr, the Academic Alliance has trained more than 400 physicians with a 4-wk course in Enhanced HIV Care and Prevention. The Academic Alliance has also augmented the HIV Clinics at Mulago Hospital and now more than 13,000 patients are receiving care. Randomized controlled trials, as well as observational and operational research activities, are underway. New prevention programs are also being introduced. The codirectors of this program are Professor Nelson Sewankambo, Dean of the Faculty of Medicine, Makere University and Merle Sande, Professor of Medicine, University of Utah. This Alliance seeks to promote change and encourage HIV programming within the context of the University of Makerere medical school, the Mulago Teaching Hospital, and the wider HIV community of government and nongovernment organizations. Networking, continuous promotion of educational activities within all sectors, and critical evaluation of all activities are a part of the mission and mandate. In addition, the Alliance is collaborating with scientists and other organizations internationally to create academic inputs into HIV that will facilitate change and ensure sustainability, both within Africa and globally. Universities and other educational institutions must provide the leadership in African countries, as they do in the Western world, to enable government and the private sector to become

partners in a grand, and ultimately successful, effort to control and reduce the impact of HIV on individuals as well as populations.

CONCLUSIONS

The World Health Organization and other supporting UN agencies have identified the goal to ensure that at least 3 million HIV-infected individuals will be receiving ARVs in developing countries by 2005. This is an immense task and will only be possible if each of us in our individual roles within our societies become committed to the task and engage the process effectively to make it happen. It also requires that research, human resource development, and other enabling strategies occur concurrently, with a rapid increase in drug manufacture, purchase, and distribution throughout these societies. The usual decision-making processes, with sequential obstacles that require months to years to resolve cannot be followed if the world is to become serious about preventing the early death of millions of HIV-infected individuals. Fiscal resources are no longer the constraining problem. Rather, the constraining problem is our ability as societies and individuals to make it happen.

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Postscript

Richard Ogden and Gail Skowron

As this book goes to press, the prospect of treating millions in the developing world living with HIV/AIDS and in urgent need of antiretroviral drugs is being translated into reality. The vast majority of that treatment, and indeed the majority of first-line treatment in the developed world, will be exclusively with reverse transcriptase inhibitors. The problems that occurred with their early use as monotherapy in the developed world, or in less potent combinations, are being avoided, but it will be particularly important to monitor long-term safety and the spread of resistance as their use greatly expands.

Many of the drugs described in the preceding chapters represent milestones in HIV treatment history for reasons other than that they were the first agents to save or prolong lives of adults and children worldwide. There has been an unprecedented effort by the pharmaceutical industry to surmount the hurdles associated with coformulation of these drugs into regimens that have the convenience of a lower daily pill burden. The first agent approved, zidovudine, is now available coformulated with lamivudine, as combivir, with abacavir, as epzicom and with both as trizivir. It has undoubtedly helped up to this point that the commercialization of these coformulated drugs has been driven by common ownership of the component medications. The benefits to patients, in terms of convenience, have been huge. Recent coformulation of emtricitabine and tenofovir as truvada and the ongoing work to formulate and gain approval for a triple combination of truvada with efavirenz has not only brought one of the most potent and popular combinations together in a once-a-day pill but has set the precedent for intercompany research and development in this area that will certainly grow in scope. There is frequently in vitro synergy observed between combinations of these agents, and those approved have shown no unanticipated drug–drug interactions with third agents or any reductions in bioavailability of the coformulation as compared to the constituents.

These innovations have been closely paralleled in the developing world as coformulations manufactured and sold by a growing pharmaceutical manufacturing sector in the developing world are being purchased with global fund money and are being widely used as initial therapy. The challenges associated with bringing affordable, high-quality, and efficacious medicines to those in

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greatest need in the developing world are myriad, but they all, for now, are based on inhibition of the single target protein, reverse transcriptase.

HIV reverse transcriptase continues to be a target for the discovery of antiretroviral drugs with novel mechanisms of action. The hitherto unexploited catalytic activity encoded by reverse transcriptase, the RNase H activity, which degrades the RNA strand in the intermediate RNA:DNA duplex that precedes synthesis of the double-stranded viral DNA, is the target of preclinical and early development drug candidates and may yield viable clinical candidates in the near future.

With basic research into immune-based interventions to prevent infection or alter the course of disease progression still very much in the research arena, attention has turned to the use of reverse transcriptase inhibitors as prophylactic agents to prevent establishment of chronic infection. Recognizing the ethical issues surrounding trial design and long-term safety considerations, initial studies are being conducted by pharmaceutical companies, clinical research consortia, and charitable foundations in certain at-risk populations in both the developed and developing world, in full consultation with all the stakeholders.

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