ABSTRACT 001

Therapeutic Agents and Viral Targets: What is on the Horizon?

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Dramatic reductions in morbidity and mortality have been achieved since the administration of the first antiretroviral nucleoside 15 years ago. Imposing challenges remain. More effective therapy will require new and better drugs. These drugs must address several challenges including toxicity, resistance, and the pharmacologic obstacles of bioavailability, short half lives and drug interactions. New drugs to address these challenges represent members of the current classes directed against reverse transcriptase and protease, as well as compounds directed against new targets. These new targets include gp41 (fusion inhibitors), chemokine receptor inhibitors, integrase inhibitors and zinc finger inhibitors.

ABSTRACT 002

Functional Genomics Reveals Cellular Targets for Antiviral Drug Development

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Target discovery in virology has been limited to the few open reading frames encoded by viral genomes. However, several recent examples show that inhibiting host cell proteins can prevent viral infection. The human genome sequence should therefore harbor many more genes essential for viral propagation than viral genomes. One approach to find such potential cellular antiviral targets is global gene expression analysis using DNA microarrays. Work in progress shows that each virus develops its own unique strategy to manipulate the gene expression profile of the host cell. Moreover, some of the host pathways discovered by expression profiling were shown to be important for viral replication. Such global approaches also revealed the antiviral activity of drugs approved for other applications. Thus, human genomics can deliver novel antiviral drugs.

ABSTRACT 003

Assembly and Viral Core Maturation - Novel Targets for Drug Discovery

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Current therapeutic regimens for HIV infection in the clinic target two virally encoded enzymes that are involved in conversion of the RNA genome into a DNA provirus (reverse transcriptase) and in processing viral proteins during particle maturation (aspartyl proteinase). In addition, promising drug candidates that block fusion of the viral membrane with that of the cell are at an advanced stage of clinical testing. We will discuss here the potential to develop novel therapeutics that block retrovirus replication by targeting the structural proteins at the stages of capsid assembly and core maturation.

A key stage in retrovirus replication is the assembly of viral particles. During this event, the translation products of the *gag* and *pol* genes, along with the genomic RNA of the virus, are transported to the site of assembly and a spherical immature core structure is formed. Recent electron microscopic evidence from *in vitro* assembled capsids of the primate D-type retrovirus, M-PMV, points to a role for localized trimeric/hexameric symmetry in this immature core assembly process, even though strict icosahedral symmetry is not observed. Following release from the cell and cleavage of the Gag precursor protein, a massive reorganization of virion components occurs and the major capsid protein CA assembles into the cylindrical (M-PMV) or cone-shaped (HIV) core shell. Cryo-electron microscopy and three-dimensional reconstructions of assembled HIV CA have identified hexamers of CA molecules linked through CA-CA interactions. The transition from spherical immature core to conical mature core involves a major conformational switch of the CA N-terminus, which could provide an attractive target for drug discovery. The application of a light-scattering methodology for assaying CA assembly, as well as the development of an *in vitro* translation/assembly system for HIV Gag that mimics *in vivo* assembly, opens up the possibility to screen for inhibitors of these processes. The challenges and opportunities for the development of specific therapeutics to target HIV assembly will be discussed.

ABSTRACT 004

Removal of Chain Terminators by HIV-1 Reverse Transcriptase

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It has recently become evident that the ability of HIV reverse transcriptase to remove chain terminators from blocked DNA chains may be an important factor in the effectiveness of nucleoside analogues as inhibitors of HIV-1. The viral reverse transcriptase lacks exonuclease proofreading activity but is capable of removing the 3' terminal nucleotide from a DNA chain through transfer to an acceptor that may be a nucleotide or pyrophosphate (nucleotide- or PPi-dependent pyrophosphorolysis). Although the intracellular acceptor for this reaction is still not known, ATP is an attractive candidate since it is present in most cells at concentrations similar to the K_m for the reaction (1 to 10 mM). The removal reaction occurs much more slowly than the forward polymerization reaction and is likely to be inconsequential except when the chain is blocked, in which case removal of the block is necessary to allow polymerization to occur.

Under physiological conditions, the rate of removal of chain terminators will be controlled by the availability of substrates for the reaction and also by the presence of the next complementary dNTP which is a potent inhibitor of both nucleotide- and PPi-dependent pyrophosphorolysis. Presumably, this inhibition occurs because the incoming dNTP must compete for the same site on the enzyme that is occupied by the primer terminus prior to its removal. The sensitivity to this inhibition depends on the structure of the nucleotide at the primer terminus — for example, removal of d4TMP is very sensitive to this inhibition (IC_{50} ~ 0.5-2 micromolar); whereas, removal of AZTMP is about 100-times less sensitive (IC_{50} > 100 micromolar).

The importance of nucleotide-dependent removal of chain terminators is underscored by studies on AZT-resistant reverse transcriptases. Although resistance to nucleoside reverse transcriptase inhibitors can occur through mutations that decrease incorporation of the drug, most naturally occurring AZT-resistance mutations have little effect on the incorporation of AZTMP. However, several of these mutant enzymes have increased nucleotide-dependent AZTMP removal activity which enables the mutant enzyme to replicate DNA more efficiently than wild type enzyme in the presence of AZTTP, provided that ATP is present in the reaction.

We have studied the removal of various nucleoside reverse transcriptase inhibitors including thymidine and cytidine analogues by wild type and mutant enzymes. The influence of structural features of the terminal nucleotide on the removal reaction will be discussed.

ABSTRACT 005

Mechanisms of Anti-HIV Action of SJ-3366: Effects on Reverse Transcriptase and Cell Surface Events

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BACKGROUND: SJ-3366 is a highly potent inhibitor of both HIV-1 and HIV-2. Against HIV-1, SJ-3366 inhibits both laboratory and clinical strains of virus with IC95 values in the low nanomolar range. HIV-2 strains are inhibited at concentrations approximately 100-fold higher.

METHODS AND RESULTS: We have performed a variety of mechanistic assays to address the means by which SJ-3366 inhibits the replication of these diverse viruses and have demonstrated that the compound acts through at least two distinct antiviral mechanisms of action. Against both HIV-1 and HIV-2, SJ-3366 inhibits the initial events of infection by interfering with a post-attachment, self surface event involved in virus entry and subsequent infection. SJ-3366 does not interfere with the interaction of CD4 and gp120 or virus attachment to the surface of the target cell, but does interfere with the ability of the virus to penetrate the cell membrane. In cell based assays SJ-3366 interferes with the fusion of infected and uninfected cells. We have shown in virus/cell complex assays that SJ-3366 may act similarly to the peptide T20 by binding to a tertiary complex formed upon interaction of the virus and the cell. This self surface mechanism is the primary inhibitory activity of SJ-3366 against HIV-2 and the secondary (in terms of potency) mechanism of action against HIV-1. SJ-3366 acts as a typical nonnucleoside RT inhibitor against HIV-1. The compound acts at low nanomolar concentrations to inhibit HIV-1 RT in biochemical assays, exhibiting Ki values of 1-3 nM, but is totally inactive against HIV-2 RT in the same assays. Evaluation of the activity of SJ-3366 against a variety of NNRTI-resistant strains has demonstrated the specificity of the interaction of SJ-3366 in the hydrophobic NNRTI binding pocket. Resistant strains of both HIV-1 and HIV-2 have been selected in cell culture which provide further evidence of the two mechanisms of antiviral action, with amino acid changes occurring in both env and RT with HIV-1 and only in env with HIV-2. Structure activity assays have

been performed to attempt to assign these two antiviral activities to distinct features of the molecule.

CONCLUSION: These data suggest a complex interaction of molecular features occurs and may hint at the possibility of an additional mechanism of action for SJ-3366 or synergistic interactions between the RT and surface active antiviral activities.

ABSTRACT 006

Long Patch Base Excision Repair as a Model for the Late Stages of Integration of Human Immunodeficiency Virus Type 1 Proviral DNA

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The integration of HIV-1 DNA is believed to proceed via a multi-step mechanism in which the viral integrase plays a central role. Early biochemical reactions in this process have been extensively characterized. HIV-1 integrase removes GT dinucleotides from the 3'-ends of the viral DNA and then catalyzes a DNA strand transfer reaction whereby the processed 3'-ends of the viral DNA are joined to the host cell chromosome. The resulting DNA integration intermediate consists of viral and host DNA segments separated by 5-nucleotide gaps adjacent to 5'-AC unpaired dinucleotides. These pre-repair integration intermediates are structurally similar to DNA loci undergoing long - patch base excision repair (BER) in mammalian cells. Cellular proteins, FEN-1 (flap endonuclease), PCNA (proliferating cell nuclear antigen), replication factor C and DNA polymerase delta, are required for the repair of this type of DNA lesion. The role of FEN-1 in the BER pathway is to cleave 5'-unpaired flaps so that DNA ligase can seal the single stranded breaks that remain following gap repair. We have been attempting to define the biochemical reactions involved in the repair of HIV-1 integration intermediates. The rate of excision by FEN-1 of 5'-flaps from oligonucleotide substrates designed to mimic pre- and post-repair HIV-1 integration intermediates, and the effect of HIV-1 integrase on these reactions, was examined in the present study. The results indicate an interaction between FEN-1 and HIV-1 integrase that may play a role in the completion of HIV-1 integration. Drugs with the ability to alter this regulatory aspect of integrase on the function of FEN-1 are predicted to block DNA repair at sites of HIV-1 integration and thus have lethal consequences for HIV-1 replication.

ABSTRACT 007

DNA Methylation in the Promoter Sequence of Human Thymidine Kinase Gene: Analyses of HIV-Positive Patients Receiving Anti-viral Nucleoside Analog Therapy

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DNA hypermethylation of CpG-rich promoter sequences inactivates gene transcription, which may serve as one mechanism for the development of resistance to anti-HIV therapy. Specifically, the nucleoside analog AZT has been reported to induce DNA hypermethylation *in vitro* in the gene encoding thymidine kinase that is responsible for the phosphorylation process of AZT. We studied the methylation patterns in the thymidine kinase gene promoter in two HIV-positive patient groups. Group 1 patients (n=12) received d4T treatment for up to one year. Group 2 patients (n=17) were first treated with AZT for more than two years, followed by d4T for another three months. Genomic DNA extracted from PBMCs of drug-naive and treated patients was modified by sodium bisulfite. A 500 bp fragment corresponding to thymidine kinase promoter including exon 1 (the sequences corresponded to base numbers 130-630 of the TK promoter gene, 5' flanking region, GenBank M15205) was amplified by PCR and analyzed by sequencing. Six fully methylated and several partially methylated CpG sites in the promoter region were found in all 68 DNA samples from healthy controls and patients. In addition, ten methylated cytosines were observed beyond CpG promoter islands. In contrast, no CpG methylation was observed in the exon 1 sequences. PCR using 3' mismatched primers as well as single-strand conformation polymorphism analyses further suggested that partial methylation existed in both normal and patient DNA samples. The degree and specificity of methylation were comparable in drug-naïve HIV-1+ individuals, group 1 patients treated with d4T only, and group 2 patients receiving AZT and d4T therapy. However, the statistical significance existed between HIV-1-seronegative and HIV-infected subjects ($P < 0.05$).

ABSTRACT 008

Nucleotide-dependent Removal of C- and T-nucleotide Analogues by Wild-type and AZT-resistant HIV-1 Reverse Transcriptase

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BACKGROUND: HIV-1 reverse transcriptase (RT) is capable of removing nucleotide analogues from blocked DNA chains through either nucleotide-dependent removal or through pyrophosphorolysis. Several mutations conferring AZT-resistance are associated with increased nucleotide-dependent removal of nucleotide analogues from blocked DNA chains. For example, HIV-1 RT containing the 67N,70R,215Y,219Q mutations (mutant RT) is about 10 fold more efficient than wild-type (WT) RT in ATP-dependent removal of T-analogues (AZTMP, d4TMP and ddTMP, and A-analogues (e.g., ddAMP); however, ATP-dependent removal of C- and G-analogues has not yet been reported.

OBJECTIVE: To determine ATP-dependent removal of C- analogues (ddCMP, [+] and [-] enantiomers of 3TCMP, and [+] and [-] enantiomers of FTCMP) and T-analogues (AZTMP, d4TMP) in multiple sequence contexts by WT and mutant RTs.

METHODS: Radioactive DNA primer/template was terminated at various positions with each of the C- or T-nucleotide analogues listed above. Purified chain-terminated products were incubated with HIV-1 RT in the absence or presence of ATP followed by heat-inactivation of the RT. Unblocked chains were extended by exonuclease-free Klenow fragment of *E. coli* DNA polymerase 1, and the radioactivity in chain-terminated and fully extended products was determined by phosphorimaging.

RESULTS: ATP-dependent removal of chain-terminators from blocked DNA chains resulted in decreased amounts of blocked products and in increased amounts of fully extended products. ATP-dependent removal was increased by 5-10 fold in the mutant RT compared to WT. Removal of C-analogues was inefficient compared to removal of T-analogs. After 5 min incubation with mutant RT only 0-8% of the C-analogues had been removed from blocked DNA chains, compared to 40-60% of T-analogues. Removal of [+] and [-] enantiomers of 3TCMP was particularly inefficient, with < 5% of chain-terminating residues removed after 40 min incubation with mutant RT. Removal of [+] and [-] enantiomers of FTCMP occurred at about twice that rate. The rate of removal of [-] enantiomers was similar to that of [+] enantiomers.

CONCLUSION: ATP-dependent removal of C-analogues is inefficient compared to removal of T-analogues. Even a mutant RT that has 5-10 fold increased removal activity compared to WT RT removes C-analogues less efficiently than WT RT removes AZTMP. These results suggest that many C-analogues should be efficient inhibitors of AZT-resistant HIV-1.

Session Two

Chemistry and Pre-clinical Development

ABSTRACT 009

Use of Inhibitory Quotient (IQ) to Estimate the Activity of HIV Protease Inhibitors

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The design and development of new protease inhibitors (PIs) with improved properties requires a detailed understanding of the pharmacokinetics and pharmadynamics of this class of antiretroviral agents. Many protease inhibitors are significantly bound to human serum proteins, which attenuate their activity *in vivo*. Furthermore, the trough plasma concentrations of PIs have been shown to correlate with clinical response. We have developed a semiquantitative pharmacodynamic model to estimate PI activity *in vitro* (inhibitory quotient). Defined as the C_{trough}/EC_{50} ratio, the inhibitory quotient (IQ) is a standardized method that correlates with virologic response. Mean IQ for a regimen is calculated using mean PI C_{trough} values from HIV-infected subjects and mean EC_{50} values against three wild-type laboratory strains of HIV in the presence of 50% human serum. For PI regimens with calculated IQ of <4, a 4-fold change in baseline susceptibility is statistically correlated with diminished virologic response. In contrast, for ritonavir-enhanced regimens with higher calculated IQ, phenotypic susceptibility breakpoints are correspondingly higher. The case study of Kaletra (lopinavir/ritonavir) will be discussed. In multiple PI-experienced, NNRTI-naïve subjects, treatment with Kaletra, efavirenz and NRTIs produced a decline in plasma HIV RNA to <400 copies/mL after 24 weeks in 93%, 73% and 50% of subjects with <10-fold, 10- to 40-fold and >40-fold reduced baseline susceptibility to lopinavir, respectively. The IQ for individual subjects was predictive of virologic response, with HIV RNA <400 copies/mL in 70%, 80% and 100% of subjects with individual IQ <4, 4-15 and >15, respectively ($p<0.03$). The inhibitory quotient calculated by this method predicts clinical response and appears to be useful for the estimation of *in vivo* potency of new PI regimens.

ABSTRACT 010

Development of SCH, a Small Molecule Antagonist of CCR5, as a Novel HIV Therapeutic

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The chemokine receptor CCR5 is known to play a critical role in HIV infection and transmission by acting as a coreceptor in concert with CD4 to allow viral attachment and entry into cells. Blockade of this receptor by natural ligands (MIP-1a, MIP-1b and RANTES) or monoclonal antibodies inhibits viral infection, thus demonstrating the validity of this receptor as a novel target for HIV therapy. We have recently developed a small molecule antagonist of the CCR5 receptor, SCH C, which has potent antiviral activity. This compound effectively inhibited replication of a large panel of genotypically and geographically diverse HIV-1 isolates in primary peripheral blood mononuclear cell cultures with mean IC_{50} concentrations ranging between 0.8 – 9 nM. To study viral resistance we generated escape variants to SCH-C by *in vitro* passage in primary PMBC cultures. Viruses with reduced sensitivity to SCH-C arose only after several months in culture and when analyzed for coreceptor use were found to still utilize CCR5 but not CCR3 or CXCR4 for infection. The antagonist activity of this compound was demonstrated in calcium flux, chemotaxis and GTPgS binding assays. In each of these assays, SCH-C inhibited the CCR5-ligand mediated response, but alone did not induce a response. Counterscreen assays performed with, SCH-C, showed no crossreactivity to any of the receptors tested, including other closely related chemokine receptors. Pharmacokinetic and metabolism analysis of SCH-C showed excellent oral bioavailablity of this compound in several species, while no significant inhibition of liver micosomal P450 isoforms 34A and 2D6 was observed. In summary, the antiviral efficacy, favorable pharmacokinetic and safety profiles of SCH-C make this compound an ideal candidate for further development as a novel agent for HIV therapy.

ABSTRACT 011

Strand Transfer Specific Inhibitors of HIV-1 Integrase

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Integrase is the third of the virally encoded enzymes of HIV-1 and the only one for which clinical agents have not been developed. Integrase catalyzes the integration of the HIV-1 DNA into the genome of the host cell. Integration is essential for HIV-1 replication and thus presents a potential opportunity for the development of novel chemotherapeutic agents. Integrase is the only protein known to be required to catalyze each of the specific steps required for integration including 1) assembly of a stable complex with the viral DNA, 2) 3'processing of the viral DNA ends, and 3) strand transfer or joining of the viral and cellular DNAs. We recently demonstrated that specific diketo acid (DKA)

analogs which inhibit integrase activity *in vitro* are effective as inhibitors of integration and HIV-1 replication. Integrase itself was validated as the molecular target responsible for the antiviral effect both by analysis of mechanism of action in infected cells and mapping resistance mutations to the integrase active site. The DKAs exhibit the unique ability to selectively inhibit only one of the catalytic functions of integrase, strand transfer. The DKAs also exert their effect on HIV-1 replication exclusively as a result of inhibiting the strand transfer reaction in the infected cell. The ability of the DKAs to exclusively recognize the active integrase strand transfer complex with the viral DNA is shown to account for this distinct mechanism of action. The recent results of studies using a variety of structural analogs comparing the activities of wild type integrase and DKA resistant variants and the implications for mechanism and future integrase drug discovery efforts will also be presented.

ABSTRACT 012

Study of Mode of Action of Nucleoside RT Inhibitors and Drug Resistance by Molecular Modeling Approach

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L-Nucleosides such as 3TC, FTC, L-FMAU, and L-OddC, etc. have been discovered as potent antiviral/anticancer agents, and 3TC is being used as a combination chemotherapy along with AZT and a protease inhibitor. L-Nucleosides have been known to be phosphorylated to their monophosphates by cellular kinases, including thymidine kinase and deoxycytidine kinase. However, the mechanisms of phosphorylation of these unnatural nucleosides at a molecular level are currently not well understood. In view of the fact that the 3D-structures of these enzymes are not yet known, it has been difficult to explain how the cellular enzyme may be able to phosphorylate the unnatural nucleosides without compromising the stereochemical requirements of the enzymes and/or the nucleosides.

In understanding the molecular level of phosphorylation with respect to the antiviral/anticancer activity of L-nucleosides, we have performed the molecular modeling studies with reported X-ray structures of nucleosides in comparison to their D-counterparts. From these studies, we are able to qualitatively explain how these L-nucleosides can be phosphorylated by the cellular enzymes, which are translated to the antiviral efficacy. Furthermore, we are able to explain how the triphosphates of these L-nucleosides interact with the HIV reverse transcriptase. Additionally, we were able to explain by molecular modeling technique how the M184V mutant virus RT interacts with 3TC triphosphate and causes drug resistance.

(Supported by NIH grants AI 32351 & AI 25899).

ABSTRACT 013

NNRTIs as Candidates to Prevent Mucosal HIV Transmission

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The absence of an HIV vaccine, the lack of access to effective anti-HIV therapy in Third World countries, and the persistence of a high viral load in endocervical fluid and semen even in patients with treatment-suppressed HIV in their blood, has placed new emphasis on the development of topical agents capable of reducing sexual transmission of HIV. At present, all commercially available products have ingredients that disrupt cell membranes because of their structural affinity for membrane lipids. The major problem in their use is the detergent-type effect on epithelial cells and normal vaginal flora, causing increased risk of mucosal infections, irritation or ulceration. Hence the need for new compounds with no detergent-type action. A variety of strategies are being proposed and investigated: (i) reconstitution of the acidic vaginal barrier (buffers or estrogen); (ii) enrichment of the vaginal flora with lactobacilli genetically engeneered to produce a protein capable of preventing HIV from binding to cell surfaces; (iii) topical use of cell-fusion blockers (from sulfated polysaccarides to cyanovirin) or monoclonal antibodies that bind to HIV envelope proteins. Recently, Uckun et al. have reported that the NNRTI 5-isopropyl-2-((methylthiomethyl)thio)-6-benzyl)-pyrimidin-4-one (1H)-one (MeSMe-DABO), possess a spermicidal activity unrelated to cytotoxicity. Since our group has been involved in the development of NNRTIs (and of a variety of DABOs in particular), we deemed interesting to investigate the dual anti-HIV and spermicidal activity of the whole class of DABOs. Data will be presented on the DABOs clinical potential as topical agents to prevent sexual transmission of HIV.

ABSTRACT 014

Development of Novel Anti-HIV Vaginal Microbicides

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BACKGROUND: The development of a safe and effective vaccine to protect against HIV infection is probably the world's most important public health

priority. Unfortunately, this goal may require many more years of research. In the meantime, progress must also be achieved toward development of vaginal microbicides that will protect women against infection.

RATIONALE: Although non-specific approaches such as use of nonoxynol-9 have failed in clinical trials, several categories of drugs have strong rationale as specifically active anti-HIV agents in microbicide development. These include: antagonists of viral entry into cells, e.g. CD4 as well as co-receptor antagonists; 2. Tight-binding non-nucleoside reverse transcriptase inhibitors (NNRTIs); 3. Antagonists of viral nucleocapsid protein (NCp).

RESULTS: We have demonstrated that compounds in each of the above categories are able to prevent HIV infection of target cells in dose-dependent fashion. This work was performed exposing infectious HIV to the drugs in question for a limited period, following which viruses were centrifuged and resuspended in medium that did not contan the inhibitory agent. The compounds studied were the following: 1. MCDS 71 (modified cyclodextrin sulfate) an inhibitor of CD4-gp 120 interactions, active at 10mg/ml; 2. Efavirenz, a tight-binding NNRTI, active at a concentration of 0.1μM ; 3. Azodicarbonamide and 3-nitrosobenzamide, zinc finger oxidants of NCp, both active at 2μM.

CONCLUSION: These findings establish rationale for the development of compounds in each of the above categories as anti-HIV microbicides. Formulation of these and other molecules toward this goal is urgently needed as are inexpensive clinical trials to provide proof-of-concept that these compounds can act as effective vaginal antiviral agents, prior to conduct of large and costly field studies to determine efficacy.

ABSTRACT 015

BCH-10618, a New Potent Anti-HIV-1 NRTI

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BACKGROUND: BCH-10618 is a member of a new class of heterosubstituted nucleoside analogues with activity against HIV-1.

METHODS: To assess anti-HIV-1 potency, BCH-10618 was tested against various wild-type and drug resistant HIV-1 laboratory strains and clinical isolates. Combination effects of BCH-10618 with other antiretrovirals were also evaluated. Cytotoxicity was assessed in primary cells (PBMCs and CBMCs) and several established cell lines. In addition, mitochondrial (DNA content) toxicity was also studied with consecutive treatment of HepG2 and Molt-4 cells with BCH-10618 for 28 days.

RESULTS: The IC50s of BCH-10618 against wild-type viruses ranges from 0.2-4.8 μM in T cell lines and PBMCs. 3TC®-resistant strains had slightly decreased sensitivity (3-5 fold) to BCH-10618, but HIV-1 strains resistant to AZT or to non-nucleoside RT inhibitors remained fully sensitive to BCH-10618. Studies assessing the efficacy of BCH-10618 against genotyped HIV-1 clinical isolates resistant to multiple HIV therapies will be presented. *In vitro* drug combination studies showed that BCH-10618 was slightly synergistic with d4T, nevirapine, and additive with 3TC, ddI, AZT, and saquinavir, when tested in T cell lines. Cytotoxicity studies demonstrated that BCH-10618 had CC50s >200μM in both primary cells and established cell lines, and no mitochondrial toxicity was observed up to 200 μM, the highest concentration tested.

CONCLUSION: We have demonstrated that BCH-10618 has potent *in vitro* activity against wild type and drug resistant HIV-1, and a very good safety profile *in vitro*. These results support the further development of BCH-10618 as an anti-HIV-1 agent.

ABSTRACT 016

Amino-Terminus Modified Rantes Analogues as Topical Virustats

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BACKGROUND: Almost all acute HIV-1 infections are due to isolates that utilize the CCR5 co-receptor. Beta chemokine ligands for this receptor can inhibit HIV propagation and can be chemically modified to increase their antiviral potency.

METHODS: Amino-terminus modifications of RANTES were made by total chemical synthesis and tested for their ability to decrease CCR5 expression, to block envelope/CCR5 fusion events, and to block HIV-1 infection in both peripheral blood cells and Langerhans cells within tissue explants.

RESULTS: The lead molecule, aminooxypentane (AOP)-RANTES, can inhibit propagation of primary HIV-1 isolates representing clades A-F at concentrations ranging from 0.1 – 5 nM in peripheral blood cells. Fusion inhibition can be demonstrated at subnanomolar concentrations while native RANTES is inactive at concentrations up to 500nM. AOP RANTES

can also effectively block HIV-1 replication in epidermal Langerhans cells. Newer analogues, for example QST-DIH RANTES, more effectively blocks HIV replication than AOP-RANTES achieving inhibitory activity at subnanomolar concentrations. Exposure of peripheral blood cells to these compounds can also produce durable downregulation of surface CCR5 expression with 80% surface depletion demonstrable for as long as 16 hrs. In contrast, comparable concentration of native RANTES has minimal activity in these experiments.

CONCLUSION: The durable activity of these potent water soluble inhibitors of HIV-1 co-receptor expression and HIV-1 propagation suggest that these compounds could be useful as topical inhibitors of sexual HIV-1 transmission.

ABSTRACT 017

Potent Antiviral Activity of Racivir™ in the HuPBMC-SCID Mouse Model of HIV Infection

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OBJECTIVE: To determine the antiviral efficacy of Racivir™ (RCV, racemic FTC) relative to emtricitabine [(-)-FTC, Coviracil] in the HuPBMC-SCID mouse model of HIV-1 infection.

METHODS: The plasma pharmacokinetics of the orally administered compounds were first determined in uninfected SCID mice. Then, groups of 6-9 mice were treated with either antiviral agent orally (0.01, 0.1, 0.3, or 1 mg/ml) for one week after infection. The viral load in plasma from treated and untreated mice was quantified by the NASBA assay or by the TaqMan real-time PCR assay. Viral load data were analyzed further in a pharmacodynamic model of HIV infection to compare the antiviral potency of RCV and (-)-FTC.

RESULTS: The pharmacokinetic study in SCID mice indicated that RCV achieved a higher C_{max} and an earlier time to maximum concentration than did (-)-FTC. In HIV-infected mice, both RCV and (-)-FTC, at the doses selected, produced similar levels of potent, dose-dependent antiviral activity, suppressing viral load in plasma to below detectable levels (≤ 500 copies/ml). ED_{50} values, calculated by pharmacodynamic modeling of the viral load data, were 5.6 mg/kg/day and 15 mg/kg/day for RCV and (-)-FTC, respectively, indicating an advantage for RCV over (-)-FTC. Furthermore, RCV was equally effective by the oral or intraperitoneal routes, and it retained its antiviral activity even when the initiation of treatment was delayed until after HIV infection.

CONCLUSION: Both RCV and (-)-FTC had potent, dose-dependent antiviral activity in the HuPBMC-SCID mouse model of HIV-1 infection, and RCV was at least as potent as (-)-FTC even though half of RCV is comprised of the plus-FTC. These studies provide additional support for the clinical development of RCV.

ABSTRACT 018

Structured Treatment Interruptions Induce Control of Viral Replication in SIV Infections

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BACKGROUND: HAART effectively controls HIV replication, however, its toxicity often leads to treatment failure, and uncontrolled interruption of therapy accompanied by rapid rebounds in circulating viral RNA.

METHODS: To determine the feasibility of structured treatment interruptions (STI) a randomized-controlled trial was performed on SIVmac251 infected rhesus macaques 6 weeks after receiving the challenge. Groups of 6 animals per treatment regime were randomized by circulating viral RNA levels (ranging between 10^5 and 10^6 copies/ml) and humoral immune response, one group of 5 infected animals remained untreated. Treatment groups consisted of various combinations and timing schedules using the drugs PMPA, ddI and hydroxyurea (HU). Treatments schedules were as follows: a) Continuous-daily treatment (CT) for 21 weeks; b) STI of all three drugs (3 weeks on/3 weeks off) for a total of 21 weeks; c) STI using PMPA and DDI; d) STI using PMPA and DDI, with HU continuously administered for 21 week; e) Untreated controls.

RESULTS: Drug treatments effectively inhibited virus replication in 22 of 24 animals. However, all 6 CT animals developed drug-related side effects, mainly liver and pancreatic toxicity, after 20 weeks of treatment. Five of these animals develop elevated glucose levels (>700 mg/dl) requiring daily insulin treatments. In striking contrast, no signs or symptoms of toxicity were documented in any of the STI animals. In the CT group, viremia rapidly became undetectable and remained undetectable during the 24 weeks of treatment. However, a rapid rebound in viremia (to

levels similar to pretreatment baseline) occurred 2 to 4 weeks after treatment discontinuation in all CT animals. Similarly, viremia rebounds occurred in all STI animals after the first STI. However, rebound became less frequent during the following interruptions becoming mostly undetectable after 3 cycles. Viremia has remained undetectable in 12/16 successfully treated STI animals 50 weeks after permanent therapy discontinuation.

CONCLUSION: CT potently inhibited SIV, however, it induced toxicity and failed to inhibit rapid return of virus replication after treatment interruption, similar to HAART treated human patients. In contrast, STI induced virus control in the absence of drugs and limited drug induced toxicity. The use of structured treatment interruptions early during the infection represents a novel approach to the treatment of an infectious disease. (This work was funded by NIAID contract N01 AI65312)

Session Three

Viral Resistance

ABSTRACT 019

New Developments in Antiretroviral Drug Resistance

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Retrospective analyses and prospective, randomized clinical trials (e.g., GART, VIRADPAT, VIRA 3001 and HAVANNA) support the role of resistance testing in guiding the choice of antiretroviral treatment regimens for experienced patients. Both the DHHS and International AIDS Society-USA guidelines recommend the use of resistance testing, combined with treatment history and an assessment of medication adherence, in patients who are experiencing antiretroviral treatment failure. Resistance testing should be performed on the failing treatment regimen to maximize the detection of drug resistance, and the results should be used to 1) assess whether drug resistance is contributing to failure of the current regimen, and 2) identify remaining drugs that are most likely to be active against the patients' virus. These recommendations have led to the widespread use of resistance testing in clinical practice, but the optimal use of these tests will require refinement in the interpretation of their results. Specifically, clinically relevant resistance "cut-offs" for both phenotypic (i.e., fold resistance) and genotypic (i.e., mutation score or "virtual phenotype") tests need to be identified for each antiretroviral drug. For example, the standard 2.5 or 4.0 fold resistance "cut-offs" for phenotypic tests, which are based on assay reproducibility compared to a wild-type control, lack clinical relevance in predicting response to ABT-378/ritonavir(r)-based regimens. Clinical trials of ABT-378/ritonavir in multiple PI-experienced, NNRTI patients indicate that the response to ABT-378/r-based regimens with efavirenz is excellent despite 10fold *in vitro* cross-resistance to ABT-378/r, as determined by commercial phenotypic assays. This is probably because the high plasma concentrations of ABT-378 achieved with standard dosing can overcome 10-fold or higher cross-resistance. Similarly, genotypic test results indicate that 7 or more mutations at key protease codons (10, 20, 24, 46, 53, 54, 63, 71, 82, 84, 90) are required to affect response to ABT-378/r-based regimens in PI-experienced patients. These observations underscore the importance of interpreting resistance test results in the context of achievable drug concentrations. Datasets that relate genotype, phenotype, and drug exposure to virologic response will be most helpful in predicting individual patient's responses to antiretroviral therapy.

ABSTRACT 020

Use of Genotypic and Phenotypic Data for Optimal Monitoring of HIV-1 Drug Resistance.

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Since HIV-1 drug resistance is a recognised cause of treatment failure, there is significant interest in the use of resistance testing to enhance patient management. Currently, three different technologies are used to assess resistance; namely, phenotyping, virtual phenotyping and genotyping. However, there has been recent debate regarding the relevance of the cut-off values currently used in phenotyping assays, particularly with respect to the NNRTIs and ddNs. These cut-offs are usually the same value for each drug tested and are determined by the assay variability seen on repetitive testing of a single wild type standard virus. We have re-defined the phenotypic cut off values in the Antivirogram assay based on the natural phenotypic variability in drug susceptibility among >1000 geographically diverse samples from untreated individuals. Mean and standard deviation (SD) values of fold change in susceptibility showed that the patient samples had inherently different susceptibilities to each drug. The 2xSD value above the mean for each drug was used as the cut-off between sensitive (within normal range) and resistant (above normal range). The impact of these changes on the degree of nucleoside analogue resistance was assessed in 5000 clinical samples. There was an increase in the overall prevalence of ddI, ddC and d4T resistance (both by phenotypic assay and as predicted by the virtual phenotype). The mutations responsible for d4T resistance in this sample were deduced by pattern recognition techniques using an extensive database. A neural network model, based on 26 RT mutations and over 2000 phenotyped and genotyped samples, was also used to predict the impact of nucleoside mutations on d4T resistance. These data demonstrated that AZT mutations alone were usually not sufficient to explain nucleoside analog cross-resistance. The combined use of pattern recognition, which utilizes an extensive genotype-phenotype database and neural networks, has enabled us to clarify the RT mutations that are the common cause of nucleoside cross-resistance. The newly defined phenotyping cut-offs are an accurate reflection of natural variation in the population and give a solid foundation for defining reduced drug susceptibility in HIV-1 samples from individual patients.

ABSTRACT 021

Frontiers in Antiretroviral Drug Susceptibility Testing

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Phenotypic drug susceptibility assays are used to guide antiretroviral drug treatment of HIV infection and assess the antiviral activity of new drugs in development. In contrast to genotyping, phenotypic assays directly measure the ability of antiretroviral drugs to inhibit the replication of HIV in cell culture systems. Most phenotypic assays now utilize recombinant viruses that contain PR and RT coding sequences derived from patient virus samples. In PhenoSenseÔ HIV, recombinant viruses carry a firefly luciferase gene and drug susceptibility is rapidly assessed by measuring the amount of luciferase activity produced in infected cells following a single round of infection. The accuracy and reproducibility of this system has facilitated the characterization of many previously-undefined aspects of HIV drug susceptibility that can influence treatment response, including novel resistance-associated mutations, complex interactions of drug mutations, drug hypersusceptibility, , subtle reductions in drug susceptibility, and drug dependent stimulation of viral replication. The genotypic correlates of these novel aspects of drug susceptibility are poorly defined.

In addition to inhibitors of protease and reverse transcriptase, this assay can be easily adapted to measure the activity of drugs that target other steps in HIV replication, such as virus entry, integration, or assembly. PhenoSense HIV can also be used to monitor and evaluate other aspects of HIV replication, such as replication capacity. Recent studies have indicated that drug resistant HIV often does not replicate as well as susceptible virus, i.e. drug resistance mutations impair virus replication. As a result, drug resistant viruses may be less pathogenic than drug susceptible viruses and disease progression may be slowed in patients harboring drug resistant viruses. Using PhenoSense HIV, a rapid assessment of replication capacity can be obtained by comparing the amount of luciferase activity produced by a patient derived virus to that of a well-characterized "wildtype" reference virus.

New applications of phenotypic drug susceptibility testing may provide useful prognostic information for the treatment of newly infected and drug experienced patient populations that cannot be provided by genotypic testing.

Session Four

Pharmacology

ABSTRACT 023

Pharmacologic Considerations for Antiretroviral Drug Development in Children

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Four particular challenges in pediatric antiretroviral drug development are: (1) recognition that children are not "small adults" but rather are pharmacokinetically, and perhaps pharmacodynamically distinct; (2) an understanding that dose recommendations for the "average" child ignore maturational changes in pharmacokinetic behavior and pharmacokinetic variability; (3) an arbitrary choice of whether to administer the drug on a body weight or body surface area basis can lead to differences in the dose administered that may be clinically significant; and (4) the development of acceptable pediatric formulations can present significant difficulties.

The ontogeny of childhood has important implications for drug disposition and therapy. Age related differences in renal and hepatic function result in different pharmacokinetic parameters between children and adults, and among children of various ages. In general, values for oral clearance in children are faster than those in adults on a weight-adjusted basis. For example, the median oral clearance of nelfinavir was 0.63 L/h/kg in adults compared with 1.21 L/h/kg in children. These data explain the need to administer a larger dose on a mg/kg basis to children than to adults.

Adult doses often provide the reference point for pediatric doses. Both body weight and body surface area (BSA) are commonly used to adjust doses. The choice of which to use is often arbitrary; however, these two approaches yield different absolute doses. For the "typical" child 5 years of age, a BSA-adjusted dose will be more than 50% larger than a dose adjusted for body weight. For zidovudine specifically, a "typical" 5 year old would receive a dose of ~80 mg with a mg/kg adjustment, contrasted with a dose of 140 mg with a BSA adjustment. The magnitude of the difference between these two doses has the potential to be clinically significant. An understanding of relationships between body size and pharmacokinetic characteristics can provide a basis for selecting the best approach for dose normalization.

Differences among patients in medication adherence, and the pharmacokinetic processes of absorption, distribution, metabolism, and elimination collectively contribute to different systemic concentrations among children, even when doses are adjusted for body size. The effect of an antiretroviral agent is related to the concentration in the body. Therefore, some children (as well as adults) will not respond to antiretroviral therapy because of subtherapeutic concentrations. An ongoing study conducted by the Pediatric AIDS Clinical Trials Group (PACTG) is investigating a concentration-guided dosing regimen as a strategy to minimize the potential of subtherapeutic concentrations (PACTG 382). In this study, 57 children received efavirenz and nelfinavir in a dosing regimen designed to meet selected target concentrations. In an intent-to-treat analysis, 75.5 percent of subjects had plasma HIV RNA levels of < 400 copies/mL and 63.3 percent had < 50 copies/mL after 48 weeks of therapy. While a strict comparison with other pediatric studies cannot be made the virologic success in this concentration-controlled study of efavirenz and nelfinavir is superior to any other study in children that has used potent combination antiretroviral therapy. These data provide strong support that strategies that minimize subtherapeutic concentrations contribute to a high degree of virologic success.

The role of clinical pharmacology in pediatric drug development is to describe, quantitate, and predict drug effects, and to then translate this information into rational drug dosing guidelines. The improved integration of pharmacokinetic and pharmacodynamic knowledge into the drug development process should accelerate and advance this process and thereby, the pharmacotherapy of pediatric HIV infection.

ABSTRACT 024

Cytochrome P450 (CYP) Isozymes and Drug-Drug Interaction with Antiretroviral Drugs

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CYP enzymes are a group of heme containing enzymes involved in the oxidation of endogenous lipids and lipophilic xenobiotics. Only a handful is involved in drug metabolism. The most prominent in this role, in order of importance as measured by the number of therapeutic drugs metabolized, are CYP3A4, CYP2D6, CYP2C9, CYP2C19, CYP1A2, CYP2E1, CYP2B6, and CYP2A6. HIV protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTI) are lipophilic drugs that are substrates as well as inhibitors and/or inducers of CYPs. In order to predict drug-drug interactions with concomitant use of PIs, NNRTIs and other therapeutic drugs, understanding as to which CYP isozymes are inhibited and/or induced by PIs and NNRTIs, and as to which CYPs are utilized for metabolism by concomitantly used drugs is necessary. PI, Ritonavir (RTV), is a potent inhibitor of CYP3A4, and less potent inhibitor of CYP2D6. In addition, RTV is an inducer of CYP3A4, CYP2C9, CYP2C19, CYP2E1, and CYP1A2. Indinavir, nelfinavir (NFV), amprenavir, and saquinavir (SQV) are inhibitors of

CYP3A4, but to a lesser degree than RTV. In addition, NFV is an inducer of CYP3A4, CYP2C9, and CYP2C19. NNRTIs, nevirapine and efavirenz (EFV), are inducers of CYP3A4 and CYP2B6, and EFV inhibits CYP2C19. Delavirdine inhibits CYP3A4, CYP2C9, and CYP2C19. The above information can be utilized to predict and then confirm drug-drug interactions between PIs, PIs and NNRTIs, and other therapeutic drug used concomitantly with HAART therapy. ACTG A5047 examined the effect of RTV/SQV on the pharmacokinetics of selected HMG CoA reductase inhibitors (statins). In this study lipophilic statins that have a high affinity towards CYP3A4 had major increases drug exposure after the addition of RTV/SQV. In ACTG 401, RTV/SQV administration had a slight inductive effect on methadone exposure which was opposite to what was predicted from *in vitro* data showing that methadone is mainly metabolized by CYP3A4. Subsequent data were generated which indicate that multiple CYP can metabolize methadone, some of which may be induced by RTV. In summary, only by thorough understanding of CYP substrate specificity, drug-drug interactions between antiretroviral drugs and other therapeutic drugs can be correctly predicted.

ABSTRACT 026

Intracellular Studies of Nucleoside Reverse Transcriptase Inhibitor Active Metabolites in HIV-infected Patients

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Plasma concentrations from the majority of the nucleoside reverse transcriptase inhibitors (NRTIs) used against HIV proliferation do not correlate with clinical efficacy or toxicity in HIV-infected patients. These agents need to be phosphorylated to their triphosphate moiety to become active against HIV-infection. Thus, the characterization of the NRTIs intracellular metabolite pharmacological parameters might provide a better understanding that could lead to the development of more rational dose regimens in the HIV-infected population. Furthermore, intracellular measurements of NRTIs could provide a better marker with respect to clinical efficacy, toxicity, and adherence than plasma concentrations. Thus, we will present the latest information regarding the intracellular pharmacological parameters of NRTIs active metabolites in HIV-infected patients. We will discuss the *in vitro* and *in vivo* intracellular studies with particular emphasis in the method development to measure these metabolites and the most current data from clinical trials.

Session Five

Immunotherapy in HIV Infection

ABSTRACT 028

An Adrenal Steroid Derivative is an Immunomodulator in HIV Infected Individuals

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BACKGROUND: As androstene (i.e., dehydroepiandrosterone) levels fall during HIV disease progression, it seems important to determine what happens when it is provided to HIV infected individuals. This class of steroids also has produced useful effects in a variety of animal models – ranging from malaria to SHIV infections.

METHODS: Intramuscular injection of 16α-bromo, 3ß-hydroxy-5α androstan-17-one (α-Epi-Br), a dehydroepiandrosterone analogue, was utilized in 37 South African HIV patients who were otherwise untreated and viral and immune parameters were followed. Dosages of 50, 100 and 200 mg per injection were evaluated in different patient groups with CD4+ T cell levels of ≥ 200 per microliter and plasma RNA levels of 5,000 to 1 million copies per ml. After 1 test injection, 5 successive daily injections were given every 6 weeks and the cycle repeated for up to 8 cycles. The only drug-related adverse event was transient, dose independent, injection site irritation in three patients in their first treatment course. One patient discontinued treatment due to injection site irritation. It cleared in the other two allowing for further injections.

RESULTS: By area under the curve statistical analysis, CD4+T cells and viral RNA in plasma did not change significantly over the study period. The most significant findings were in immunologic monitoring. The number of CD11c+ and CD123+ dendritic cells per ml increased in the 30 studied patients after each of three courses, rising to 30 and 26% respectively, above baseline values after the first course. The ratio of DC1 (CD11c+) to DC2 (CD123+) dendritic cells increased by 33% in the same time frame. DC1 and DC2 subpopulations relate to the initiation of Th1 and Th2 T cell responses. Activated T cells (CD3+CD8+CD69+) NK (CD16+ and LAK (CD16+CD38+) cells also increased significantly over the same time in the treated patients. All these effects were generally maximal in the 50-100 mg treated cohorts.

CONCLUSION: Thus, the most critical finding in this study is that both dendritic cell and lymphocyte subset markers suggest that this compound is causing a significant flux of mature dendritic cells and activated cytotoxic T cells into the blood circulation. Enhancement of antigen specific cellular immunity and a shift in the T_2 to T_1 lymphocyte balance could also be a result of this approach. Our hope is that the changes we are reporting are sufficient to significantly reconstitute the immunosuppression of HIV infected patients but whether this drug represents a useful therapy can only be seen by following treated HIV infected patients for opportunistic infections and cancers. Studies with different schedules and routes may further optimize immunological changes prior to testing in large randomized, placebo-controlled trials.

ABSTRACT 029

Current Status of Immunotherapy for HIV Infection

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Despite dramatic advances in antiretrovirals there continues to be increased interest in the use of immunotherapy to augment immune responses of HIV-infected individuals. Earlier attempts in patients with poorly controlled HIV replication were mostly disappointing, but the availability of newer reagents and the fact that many patients have their viral replication suppressed has created the opportunity to re-explore the activity of immunologic interventions. One approach to heighten immunity is to use substances that act through different mechanisms to enhance general immunity. A recently completed study utilizing cyclosporin suggested that markers of immune activation were decreased but there were no changes in virus replication. A study utilizing IL-12 in patients with advanced as well as those with higher CD_4 cells has recently been completed. Because of some pilot data suggesting that GM-CSF had some influence on HIV replication a controlled trial of GM CSF plus antiretrovirals has been enrolled and results should be available soon. The compound studied for the longest time and in the most patients is IL-2. A recent report suggests that IL-2, when combined with antiretrovirals, increased CD_4 counts and even significantly lowered HIV levels as compared to placebo. A larger randomized trial failed to show a change in viral load due to IL-2; however, increases in CD_4 counts were observed. A newly proposed study will attempt to show that IL-2 can increase the time off antiretrovirals in patients who will have these drugs discontinued until they read a CD_4 count of 350/mm^3.

Attempts to assist in immunologic control of HIV infection by stimulation with exogenous virus stimulation (therapeutic vaccines) or endogenous exposure (strategic treatment interruptions) are actively being performed in patients who have virologic suppression by antiretrovirals. The ability of IL-12 to enhance HIV specific immunity to a peptide vaccine is currently being evaluated. Responses to an ALVAC

vaccine with additional stimulation using IL-2 will be measured by the time to virologic relapse after treatment discontinuation. The immunologic response to two HIV specific vaccines as compared to one and the influence of these on virologic success is being studied. Strategic treatment interruptions have resulted in some prolonged virologic suppression and enhanced HIV specific responses in patients treated early in primary HIV infection. Several groups are evaluating treatment interruptions. A study about to get underway will evaluate whether enhanced responses will be detected when HIV vaccination is combined with treatment interruption. These and other studies underway will improve our understanding of the possibility to enhance the immune system in HIV-infected patients.

ABSTRACT 031

Structured Treatment Interruptions

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The introduction of Highly Active Antiretroviral Treatment (HAART) represented a milestone in the treatment of HIV infection, and has been associated with a significant decline in mortality among AIDS patients. However, virus suppression by HAART is not associated with the appearance of HIV-specific immune responses, and withdrawal of HAART is usually followed by a rapid viral rebound and loss of CD4 T lymphocytes. Further, the long-term use of HAART is prohibitively expensive for many patients, and has been associated with toxicity and adherence problems.

STI-HAART, structured treatment interruptions involving repetitive on-and-off cycles of HAART, are an attractive alternative to continuous treatment because they might be used to enhance the utility of HAART. The initial excitement began with the description of the Berlin patient, who was able to control HIV after cycling on and off therapy twice. There is some evidence that STI-HAART can be used shortly after infection, to induce immune control of viral replication, or during established infection, to reduce drug-related toxicity or to favor the re-appearance of the wild-type virus. Two potential strategies might be followed with STI-HAART: cycle HAART according to a fixed schedule, or resume drug treatment after the virus reappears in the plasma. The first randomized, controlled trial comparing HAART vs. STI-HAART has been performed on acutely SIV-infected macaques. Fixed-schedule STI-HAART (3 weeks on - 3 weeks off therapy) suppressed viral load as efficiently as continuous HAART. In the STI-HAART group, T cell Virus-specific Immune Response (VIR) and control of viral rebound increased concurrently during subsequent interruptions. In contrast, VIR did not increase and SIV rebounded after permanent treatment withdrawal in all animals on continuous HAART. Fixed-schedule STI-HAART appear a valuable alternative to continuous HAART for the early treatment of retroviral infection.

ABSTRACT 032

Practical Curtailment of the HIV Epidemic by Adapting the Potent Oral Defense to Topically Prevent Vaginal and Rectal Transmission

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BACKGROUND: The majority of the 6.4 million transmissions of HIV are sexual. Condoms are highly effective but the fact that there are millions of sexual transmissions each year indicates that many people do not use condoms. Thus, additional preventives are needed. The objective of our study was to adapt the potent salivary defense (*Arch. Int. Med.* **158**:303-310, 1999; *JID* **181**:498-504, 2000) to reduce the sexual transmission of HIV by chemically targeting the transmitting infected leukocytes and any cell-free HIV in seminal fluid. The previously recommended anti-HIV topical microbicide, nonoxynol-9, has not prevented HIV transmission in humans, probably because it causes mucosal irritation which attracts susceptible CD4+ cells. To identify effective preparations that are established to be non-irritating, we studied the anti-HIV activity of commercially available, over-the-counter (OTC) lubricants and vaginal preparations that are judged safest by government regulatory agencies and are non-irritating.

METHODS: The effect of OTC preparations on both the production of HIV by infected leukocytes and on cell-free HIV suspended in seminal fluid was measured under simulated *in vivo* conditions.

RESULTS: We found four (Astroglide, KY Liquid, Vagisil and viAmor) of the 22 OTC preparations to be highly active against HIV-infected leukocytes suspended in seminal fluid and active against cell-free HIV. Two of the components of the OTC preparations have the anti-HIV activity of inhibiting monocyte production of HIV by >1,000-fold as well as inhibiting cell-free HIV.

CONCLUSION: The anti-HIV OTC preparations identified here have the advantages of being widely available, inexpensive, acceptable, and may be used by recipient women or men. Clinical trials should be done to evaluate prevention of sexual transmission of HIV by these OTC lubricants. Medical application of such FDA-approved OTC products or their individual components may be an immediate practical way to curtail the epidemic of HIV.

ABSTRACT 033

High Prevalence of Protease Inhibitor Resistance in Brazilian Compared To US HIV-1 Samples Genotyped by North American Reference Laboratory

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BACKGROUND: HIV-1 infection in Brazil is reported as the highest in South America, with over 110,000 incident cases through 1996. The Brazilian HIV-1 viral reservoir is genetically diverse and includes non-Clade B viruses including Clades F, C and D and recombinants. This study describes and contrasts the frequency of HIV-1 Protease (Pr) and Reverse Transcriptase (RT) mutations and their associated predicted resistance to antivirals in samples drawn from Brazilian patients with access to viral genotyping.

METHODS: Among 21,234 HIV-1 clinical samples submitted for HIV genotype testing to Quest Diagnostics between 1998 and 2000 were 311 Brazilian samples. The occurrence of mutations and their associated resistances for RT inhibitors (RTI) and Pr inhibitors (PrI) were compared between the Brazilian samples and the larger North American (US) data set.

RESULTS: PrI resistance was more common in the Brazilian samples: 50.5% showed resistance to indinavir and ritonavir whereas such resistance was found in only 29.6% of the U.S. samples. Nelfinavir and saquinavir resistance was identified in 59.2% and 42.4% of Brazilian samples vs. 45.3% and 31.1% respectively of US samples. 59.2% of Brazilian samples were resistant to at least one PrI, whereas only 45.5% of US samples showed PrI resistance. The median number of PrI resistances in the Brazilian samples was 3.3, while the U.S. median was only 2.8 ($p < 0.001$). Brazilian Pr sequences had on average 9.5 +/- 4.2 amino acid substitutions vs. 7.3 +/- 3.9 for the U.S. ($p < 0.001$). Substitutions of Pr codons 10, 48, 82, 84 and 90, frequently associated with PrI resistance, were 1.2 to 3-times more common in Brazilian samples, but nelfinavir resistance substitution at codon 30 was found only in 6/311 samples, 3.8-times lower than in US samples.

Resistance mutations to the nucleoside RT inhibitors zidovudine and abacavir were also common. 61.7% showed zidovudine resistance and 59.2% had abacavir resistance vs. 38.4% and 36.3% respectively for the US. Resistance mutations for non-nucleoside RT inhibitors were fewer: nevirapine: 30.4% (Brazil) vs. 38% (US); delavirdine: 29.6% (Brazil) vs. 39.4% (US); efavirenz: 26.4% (Brazil) vs. 37.9% (US) This decrease in predicted resistance was due to a 1.7-times lower frequency of the K103N RT mutation.

CONCLUSION: We have identified patterns of antiretroviral resistance in a Brazilian population with access to HIV viral genotyping that are significantly different from those found in the US. Resistance mutations arise in response to selective pressure from drug therapy. Our results support observations made by others contrasting Brazilian and US differences in treatment and patient adherence. These findings have important implications for empiric treatment selection and the need for resistance testing.

ABSTRACT 034

Structural Basis for Activation of Alpha-Boranophosphate Nucleotide Analogues Targeting Drug Resistant Reverse Transcriptase

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The efficiency of AIDS chemotherapy is limited by inadequate intracellular concentrations of the active triphosphate form of nucleoside analogues, leading to incomplete inhibition of viral replication and the appearance of drug-resistant virus. Recent results on phenotype/genotype correlations of RT-mediated drug-resistance suggest that two critical factors govern RT-mediated drug resistance: the loss of affinity of RT for the nucleotide analogue, and/or post-replicative repair through ATP-mediated or pyrophosphate-mediated unblocking chain-terminated viral DNA.

We reasoned that a chemical modification onto the a-phosphate of a nucleotide analogue might address some of these limitations by inhibiting the RT-mediated repair reaction. However, such a modified nucleotide analogue must first be a substrate for nucleotide kinases responsible for the building of nucleoside 5'-triphosphate pools. Therefore, drug activation by nucleoside diphosphate kinase (NDPK) and the inhibition of HIV-1 reverse transcriptase were studied comparatively.

We synthesized analogues with a borano (BH3-) group on the alpha-phosphate, and found that they are substrates for both enzymes. X-ray structures of complexes with NDPK gave a structural basis to their activation. We found that d4T-DP is 10-fold better activated to the triphosphate than AZT-DP, and that the presence of the BH3- increases 10-fold further this

phosphorylation efficiency. The complex with d4T-TP displayed an intramolecular CH...O bond which gave a molecular basis to these observations. The Rp diastereoisomer of thymidine alpha-borano-triphosphate bound like a normal substrate. Using alpha-(Rp)-boranophosphate derivatives of the clinically relevant compounds AZT and d4T, the presence of the alpha-borano group improved several fold both the phosphorylation by NDPK and the inhibition of reverse transcription. Moreover, repair of blocked DNA chains by either ATP or pyrophosphorolysis was significantly inhibited in variant reverse transcriptases bearing substitutions found in drug-resistant viruses such as, D67N/K70R/T215F/K219Q, L74V, K65R, and V75T. Thus, an alpha-borano modification of vectorized nucleotide analogues may be of generic value in fighting viral drug resistance.

ABSTRACT 035

Design of Anti-resistance HIV-1 Protease Inhibitors

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BACKGROUND: One of the most serious obstacles to drug therapy of HIV infection is viral resistance. The resistance against all HIV protease (P) drugs starts with the appearance of HIVP species with single substitutions in the HIVP gene (single mutants) and ultimately selects for highly resistant proteases with multiple substitutions. The viruses which harbor single mutant proteases, who sacrifice catalytic efficiency for less inhibitor sensitivity, are obligatory intermediate strains and are thus targets for the design of anti-resistance inhibitors.

OBJECTIVES: To design anti-resistance HIVP inhibitors. The idea is to design inhibitors that bind HIVP in two independent modes. To resist against this type of inhibitor, instead of the obligatory single mutants, HIV is forced to mutate more sites in order to reduce sensitivity to both binding modes, thus sacrificing more catalytic efficiency to be incompetent for survival.

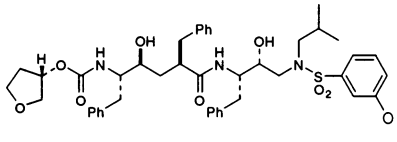

Fig. 1

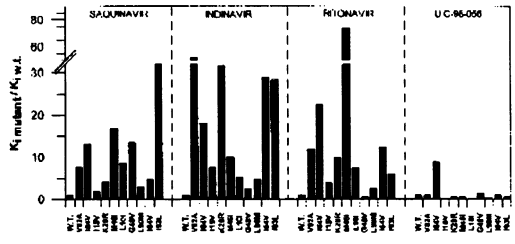

Fig. 2

RESULTS: We designed UIC-98-056 (Fig. 1), which contains two transition-state isosteres to bind in two modes. The K_i of UIC-98-056 is 6.2 nM vs. wt protease. A crystal structure of UIC-98-056 bound to HIVP shows two binding modes of the inhibitor. Analyzed *vs.* 10 single mutants, the 'anti-resistance pattern' (K_i of mutant/K_i of wt protease) of UIC-98-056 is superior than those generated for saquinavir, indinavir and ritonavir (Fig. 2). Substitution of the left isostere with a peptide bond in UIC-98-056 produced a far less effective anti-resistance pattern.

CONCLUSION: Two-isostere inhibitors can bind HIVP in two modes and produce superior anti-resistance properties. This principle may be generally applicable in HIVP inhibitor design.

ABSTRACT 036

Safety and Efficacy of Lodenosine (FddA) in Combination with Stavudine and Indinavir in Antiretroviral Naive, HIV-Infected Adults: Association with Severe Hepatotoxicity

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BACKGROUND: Lodenosine (FddA) is a fluorinated dideoxypurine nucleoside that demonstrated promising preclinical and Phase I clinical antiretroviral activity.

OBJECTIVES: To select a dose of FddA for further clinical evaluation based on (1) antiviral activity; (2) durability of viral suppression at 48 weeks and (3) safety and tolerabilty of three different doses of FddA (100 mg, 200 mg or 300 mg bid) in combination with stavudine (d4T; 30 or 40 mg bid) and indinavir (IDV; 800 mg q8h) compared to lamivudine (3TC; 150 mg bid) in combination with d4T/IDV.

METHODS: Phase II randomized, parallel-group open label trial conducted in the United States, Puerto Rico, United Kingdom and Brazil. The study was initiated in Nov 1998 with a total of 209 patients randomized in a 2:2:2:1 fashion.

RESULTS: The study was suspended on Oct 13, 1999 following the receipt of a report of liver toxicity that resulted in fatality. At the time of study suspension, the majority of patients had completed 24 weeks of therapy; only 2 had completed 48 weeks. Subsequently, three other patients with noted drug-related liver toxicity expired. Of the four fatalities, one patient was randomized to the 200 mg FddA treatment group; three were randomized to the 300 mg treatment group. Liver function test abnormalities varied from patient to patient, and may have been influenced by a number of other factors (viral hepatitis, excessive alcohol use, excessive re-dosing of study medications). Including the four fatal cases, thirteen subjects experienced serious adverse events associated with study drug-related hepatotoxicity. Liver biopsies were obtained from 11 patients with non-fatal hepatotoxicity. The predominant histopathologic pattern was a combined cholestatic and hepatocellular injury. Elements suggestive of mitochondrial involvement (oncocytic changes) were observed in two liver cases. Serious hepatocellular damage (elevated bilirubin and liver function test (LFT) abnormalities, occasionally with lactic acidosis) were reported in 1 (1.7%), 4 (6.7%), and 5 (8.6%) of subjects receiving 100 mg, 200 mg and 300 mg, respectively and none among patients receiving 3TC. The majority of LFT abnormalities were relatively mild and transient. All significant LFT abnormalities in non-fatal cases have resolved. Early termination of the study limits the interpretability of efficacy data. By week 24, the percentages of patients with viral loads < 50 copies were 56%, 65% and 70% in the three lodenosine study groups and 82% in the 3TC study group; no statistically significant differences were seen. The mean increase in CD4 cells was similar for all treatment groups at week 12, and was 10%-15% higher in FddA-treated patients compared with 3TC-treated patients at week 24. There were no significant differences in the percentage of patients who experienced virologic failure at either 12 or 24 weeks of study.

CONCLUSION: Premature study termination limits the interpretation of efficacy data. Virologic and immunologic effects of FddA/d4T/IDV were similar to 3TC/d4T/IDV. The most common adverse event was hepatotoxicity. The majority of LFT abnormalities were relatively mild and transient. Severe hepatotoxicity was observed in 13 patients on study many of whom had predisposing factors. Available liver biopsy data suggests that cholestatic and hepatocellular injury with possible mitochondrial involvement may play a pathogenetic role in this toxicity.

ABSTRACT 037

Therapeutic Drug Monitoring - A Survey of the Use in Clinical Practice in the UK and Ireland

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INTRODUCTION: The role of TDM for antiretrovirals is a topic of increasing interest in Europe and the USA. In order to allay some of the concerns about the clinical utility of yet another diagnostic test it is important that a number of key issues are addressed. Clearly we need data from randomized controlled trials that demonstrate that TDM is of clinical benefit to the patient and is also cost effective. There are currently a number of such studies in progress (e.g. ATHENA, VIRADAPT II, GREAT). However we also need some clear assurance of validated assays (QA schemes) and consensus on interpretation of results. There is also the need for education on the importance of understanding the pharmacokinetics of antiretroviral drugs.

METHODS: We have been involved in PK studies of ART for more than 6 years using initially HPLC but more latterly LC/MS/MS technology. During that time we have performed more than 300 full AUC profiles for PIs and NNRTIs and have used the results to modify dosing regimens in order to maximize efficacy and minimize toxicity. More recently we commenced a TDM service for the UK and have requested that physicians send a trough and peak plasma sample for analysis.

RESULTS: Since the commencement of the TDM service in February 1999, over 1200 requests for individual drug assays (PIs and NNRTIs) have been received (data to end of October 2000) from main teaching hospitals and smaller district general hospitals. The rank order of requests by drug was nelfinavir (32.5%), saquinavir (25.8%), indinavir (14.7%), nevirapine (10.2%), ritonavir (9.7%), efavirenz (5.1%), amprenavir (1.5%) and delavirdine (0.5%).

Reasons for requesting TDM included use of a non-standard dose (40.7%), suspected failure (24.0%), pharmacoenhancement by ritonavir (17.0%), drug interaction (15.3%), and suspected toxicity, change in dose, clinical indication or paediatric patients (all <10% each). Approximately 20% of trough samples analyzed were below the MEC used by this laboratory during that period (e.g. SQV and IND 100 ng/ml; NLF 400 ng/ml; RIT in dual PI 2100 ng/ml). A consensus view of laboratories involved with TDM would now be that the

MEC values of some of the PIs should be higher (e.g., SQV 200 ng/ml; IND 120 ng/ml; NLF at least 700 ng/ml – but note there is diurnal variability) and therefore the percentage of patients below the MEC will increase. Repeat TDM or dose modification was recommended if good adherence was reported. When either trough or peak levels were considered to be high, dose reductions were recommended if there were suspicions of toxicity. For a small percentage of samples received, a clinical interpretation was not possible due to inadequate information or inappropriate samples.

CONCLUSION: Although we strongly advocate the need for well-designed, randomized, controlled clinical trials to assess the widespread utility of TDM, our experience encourages us to believe that there is an important role for TDM in clinical practice.

ABSTRACT 038

HE2000 Stimulates Immunity in Patients with HIV

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BACKGROUND: Androstanes and androstenes have been shown to correct immunodeficiency in aged mice. This effect has been linked to repair of dendritic cell maturation defects. These hormones have been shown to modulate cellular cytokine production *in vitro*. An increased survival in lethal viral infections in mice and primates has also been shown. The dendritic cell (DC1) presents antigen to the T helper cell (Th1) and stimulates cell-mediated immunity in the host. The concentration of these cells in circulation has been reported to decline in patients with progressive HIV disease.

METHODS: A phase I/II study was conducted in treatment naïve HIV infected patients that were given three courses of HE2000 therapy. Each course consisted of 5 daily injections followed by a six week observation period. Patients returned to the clinic periodically and samples were taken for 4-color flow cytometric analysis, CD4 concentration and viral loads.

RESULTS: In these studies we observed a stimulation of DC1 and DC2 dendritic cells as well as other immune cell subtypes important to host protection. In an area-under-the-curve analysis (AUC) we found an overall increase in lineage negative HLA-DR+, CD11c+ (DC1) cells AUC=+30% (n=30, $p<0.001$) while the lineage negative HLA-DR+CD123+ (DC2) cells showed an AUC=+26% change (n=30, p=0.001). Calculating the change in DC1/DC2 ratio showed an overall increase indicating a preferential stimulation of cell mediated immunity AUC=+32% (n=30, $p<0.001$). The CD3+CD8+CD69+ activated cytotoxic T cell concentration was observed to increase AUC=+66% (n=36, $p<0.001$). The CD8-CD16+ NK cell concentration increased AUC=+10% (n=35, p=0.043) and the CD8+CD16+ LAK cells showed an AUC=+16% increase (n=35, p=0.001). There was a small decline in the concentration of CD4 cells -7% (n=37, p=0.002) possibly reflecting increased cellular activation and trafficking of CD4+ T-helper cells to the tissues. There was no significant difference in log viral RNA concentration from baseline in AUC analysis -0.03 log (n=37, NS).

CONCLUSION: These data indicate an overall increase in immune cell phenotypes suggesting that HE2000 may improve immunity to HIV and opportunistic infections.

Session Six

First Line Antiretroviral Therapy/Salvage Therapy/Pediatrics

ABSTRACT 039

Novel Strategies for the Treatment of HIV-1 Infections

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Dramatic reductions in HIV-1 morbidity and mortality have resulted from the widespread use of combination antiretroviral therapy in developed countries. Various combination regimens can profoundly diminish HIV-1 replication for prolonged periods, although current therapies are associated with substantial toxicity, emerging resistance, and the inability to eliminate replication-competent virus from latent reservoirs. New strategies involve the targeting of alternative sites in the HIV replication cycle and stimulating host immune responses through the use of structured treatment interruptions.

In vitro studies have contributed substantially to the current combination strategies used to control HIV replication. Viral attachment/entry has not yet been attacked in a comprehensive combination approach. The attachment/entry pathway includes initial binding of HIV-1 gp120 to the CD4 receptor, conformational changes that allow gp120 to bind to co-receptors (CXCR4 or CCR5), and still further changes allowing gp41 to fuse with cell membranes. We have evaluated agents that act at each of these sequential steps, singly or in combination. *In vitro* antiviral interactions observed ranged from strong synergy to antagonism, depending on the specific agents studied and the clinical virus isolates utilized. Data regarding these interactions will be discussed.

An alternative approach to controlling virus replication is to stimulate host immune responses. One approach utilized by our group (Rosenberg and Walker) is to use structured treatment interruptions to augment host responses to endogenous virus.

This has been successful in the setting of patients initially treated during acute HIV syndromes, and then maintained on suppressive therapy for prolonged periods. Following one or more structured treatment interruptions, virus-specific CD4 proliferative and CD8 cytotoxic lymphocyte responses could be stimulated, leading to sustained reductions in virus replication. An updated review of these studies will be presented.

ABSTRACT 040

Ritonavir (Rtv)/Indinavir (Idv) (100/400 Mg Bid): A Simple and Well Tolerated Pi Containing Regimen

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BACKGROUND: RTV improves pharmacokinetics of IDV allowing bid regimen. However RTV/IDV (100/800 mg) has been associated to a high rate of side effects.

OBJECTIVE: To describe pharmacokinetics of a RTV/IDV (100/400 mg) bid regimen in patients switching form a standard IDV (800 mg tid) regimen.

METHODS: Prospective open label study where 20 patients with a stable 2 NRTI + IDV (800 mg tid) regimen, plasma HIV RNA < 200 cp/ml (day 0) were switched to RTV-IDV (100-400 mg). Plasma Cmin, Cmax, HIV RNA were determined at day 0, day 15, day 30.

RESULTS:

	IDV 800 mg tid		RTV-IDV 100/400 mg bid			
Concentration ng/ml	Cmin D0	Cmax D0	Cmin D15	Cmax D15	Cmin D30	Cmax D30
Mean	**277**	**8 432**	**508**	**3 853**	**659**	**4 087**
SD	+ 254	+ 3 728	+ 267	+ 2 985	+ 661	+ 2 695
Median	**194**	**8 449**	**536**	**2 983**	**475**	**2 997**
Min	35	407	121	2 047	7*	1 927
Max	922	18 148	1 071	14 434	2 462	13 606
IDV<120ng/ml	**7/20**		**0/17**		**1*/20**	

*Non compliant

In all patients pVL remained <200 copies/ml. No major side effects were observed.

CONCLUSION: Significantly higher Cmin and lower Cmax (2.5 fold) obtained with RTV/IDV (100/400) were associated with a sustained efficacy and good tolerance. Reduction in cost of such combination is of major interest in countries with poor resources.

ABSTRACT 041

Selected Topics on Antiretroviral Associated Toxicity Derived from a Cohort Study (BCN-Clinic Cohort)

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The BCN-Clinic cohort contains all HIV-1 infected patients diagnosed in a single institution from the beginning of the epidemy in Spain (1986) up to now and followed since the moment of the diagnosis until last medical control, dead or loss to follow-up. The total number of patients is near 5,000 representing near

10,000 person-years of follow-up. In addition of demographic, clinical and laboratory variables, data on therapeutic regimens and side effects are routinely collected.

Moderate or severe body fat changes were clinically assessed and categorised as subcutaneous lipoatrophy, central obesity, or both in all consecutive antiretroviral naive HIV-1-infected adults who initiated HAART with 2 nucleoside reverse transcriptase inhibitors plus at least 1 PI from October 1996 to September 1999. A person-years analysis was used to calculate the incidence of types of lipodystrophy and Cox proportional hazards models were used to describe the univariate and multivariate factors associated with progression to any lipodystrophy. After a follow-up of 18 months 85 (17.2%) of the 494 patients developed any lipodystrophy. The incidences of any lipodystrophy, lipodystrophy with subcutaneous lipoatrophy, and lipodystrophy with central obesity were 11.7 (95% CI 9.2 – 14.2), 9.2 (95% CI 7.0 –11.4), and 7.7 (95% CI 5.7 – 9.7) per 100 patient-years, respectively. An increased risk for any lipodystrophy was found among females as compared with males (RH 1.70; 95% CI 1.08 – 2.66), heterosexuals (RH 3.48; 95% CI 1.91 – 6.34) and homosexuals as compared with iv drug users (RH 2.20; 95% CI 1.17 – 4.12), with increasing age (RH 1.51 per 10 years older; 95% CI 1.24 – 1.83) and with the duration of exposure to antiretroviral therapy (RH 1.45 per 6 months extra; 95% CI 1.22 – 1.72) but not with any individual antiretroviral agent. The factors associated with an increased risk for lipodystrophy with subcutaneous lipoatrophy or lipodystrophy with central obesity were very similar to those associated with any lipodystrophy. The duration of indinavir use may represent an additional contribution for the development of lipodystrophy with central obesity (RH 1.26 per 6 months extra; 95% CI 0.99 – 1.60, p = 0.064). Likewise, the duration of stavudine use additionally contributed for the development of lipodystrophy with subcutaneous lipoatrophy (RH 1.16 per 6 months extra; 95% CI 1.02 – 2.04, p = 0.023). The risk factors associated with development of any lipodystrophy, in HIV-1-infected patients receiving PI-containing HAART are multifactorial and overlapping, and cannot be exclusively ascribed to the duration of exposure to any particular antiretroviral agent.

Nevirapine (NVP) is a potent and reasonably well-tolerated NNRTI. Recently, a warning has been added to the product information advising to monitor liver function tests (LFT) during first weeks of therapy. To assess the incidence of LFT abnormalities and clinical hepatitis associated with NVP-containing HAART within a prospectively followed cohort of HIV-infected patients. All consecutive patients who initiated a NVP-containing HAART from 9/97 to 5/00. The databases with clinical data and laboratory results (ASAT, ALAT, GGT, alkaline phosphatase (AP), bilirubin, serology for HBV and HCV) were matched. NVP was prescribed to 706 of the 4352 patients of the clinical database. 610 had baseline information, underwent at least one further clinical laboratory evaluation and were included in the study. Median follow-up and exposure to NVP were 12 and 9 months respectively. 12 patients (2%) died, 32 (5.2%) were lost to follow-up while on NVP and 239 (39%) stopped NVP before the end of the study. 13/610 patients (2%) stopped NVP for liver toxicity and the remaining for other reasons, mainly virological failure (13%) or skin toxicity (9%). Patients with a 3-fold increase in ALAT or ASAT and GGT were 76 (15%) and 177 (35%), respectively. There were 7 cases of clinical hepatitis (incidence rate: 1.2 cases per 100 person-years), 6 of them in patients with chronic liver disease due to HCV, developing from 20 to 270 days (median 60) after initiating NVP. There were no cases of fulminant hepatitis or deaths due to liver failure. Independent risk factors for 3-fold increase of ALAT or ASAT were elevated baseline ALAT (p=0.013), seropositivity for HCV (p=0.0024) and duration of exposure to antiretrovirals (p=0.022). NVP was generally well tolerated. Clinical hepatitis seldom appeared and other underlying factors might be related. Abnormalities in ALAT or ASAT increased steadily along first year of therapy, but they were mainly asymptomatic. LFT monitoring during first 1-2 months of therapy does not seem to be justified.

(Data presented in part at the 2nd International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV –Toronto September 2000- and in Fifth International Congress on Drug Therapy in HIV Infection – Glasgow October 2000)

ABSTRACT 042

Efficacy and Safety of Lopinavir (ABT-378)

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BACKGROUND: ABT-378 (lopinavir) is a new peptidomimetic HIV-protease inhibitor that is characterized by its pharmcokinetic enhancement and co-formulation with ritonavir (ABT-378/r, lopinavir/r); trough concentrations of ABT-378/r are estimated to be 75 times greater than the EC_{50} of wild type virus.

METHODS: 4 pivotal clinical studies with ABT-378/r have been performed in treatment naïve and experienced subjects. Study 720 (naïve; N=100) compared combinations of ABT-378 200 mg and 400 mg with ritonavir 100 mg or 200 mg bid. Study 863 (naïve; N=653) compared ABT-378/r or nelfinavir plus 2 NRTIs. Study 765 (single PI-experienced; N=70) compared ABT-378 400 mg with ritonavir 100 or 200 mg bid plus nevirapine and NRTIs. Study 957 (multiple PI-experienced; N=57) compared ABT-378/r 400/100 or 533/133 with efavirenz plus NRTIs.

RESULTS: In the treatment-naïve trials, there was no difference in antiviral activity between any of the doses tested, however the 200 mg ritonavir dose was not as well tolerated as the 100 mg dose, hence the licensed co-formulation is 400 mg/100 mg or ABT-378/ritonavir. In the comparative trial, 70% in the ABT-378/r arm vs 54% in the nelfinavir arm had HIV RNA <50 c/ml (P<0.001). No virologic failures were reported in the naïve patients. In the treatment-experienced trials, 60-62% of subjects had HIV RNA <50 c/ml at 48 wks; more subjects on the higher dose ABT-378/r (533/133) had <400 c/ml, 82% vs 69% respectively. Virologic success was associated with fewer codon mutations at baseline: 0-5 (88%), 6-7 (57%), 8-10 (17%), and baseline fold change in susceptibility compared to wildtype: <10 fold (83%), 10-20 fold (67%), 20-30 fold (67%), >40 fold (13%). Hyperlipidemia and elevation in hepatic transaminases (particularly in hepatitis co-infected subjects) were the most commonly reported lab abnormalities.

CONCLUSIONS: ABT-378/r is a potent PI with favorable pharmacokinetic properties and safety profile.

ABSTRACT 043

Survival Benefit of Antiretroviral Therapy is compromised by Baseline CD4+ Counts Below 200 Cells/mm3 and not by Plasma HIV-1 RNA Levels

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OBJECTIVE: To characterize the effectiveness of antiretroviral therapy initiated at various CD4 and plasma HIV-1 RNA thresholds.

METHODS: We conducted a population-based analysis of antiretroviral therapy naïve HIV-positive men and women 18 years or older in British Columbia, Canada. Eligible patients initiated triple combination therapy between August 1, 1996 and September 30, 1999. Rates of progression from the initiation of antiretroviral therapy to death were determined stratified by CD4 and plasma HIV-1 RNA thresholds. Risk ratios of factors associated with mortality were estimated using Cox-proportional hazard models. An intent to treat principle was used in all analyses.

RESULTS: A total of 1,219 men and women were eligible. As of January 31, 2000, 72 patients had died of AIDS-related causes, for a crude mortality rate of 5.9%. The cumulative mortality rate at 12 months were 3.2% $\pm$ 0.5%. In univariate analyses, a prior AIDS diagnosis, $CD4^+$ cell count, and HIV-1 RNA levels were found to be associated with mortality. There was no difference in mortality by age, gender, or protease-inhibitor use. In multivariate analyses, only $CD4^+$ cell count remained significantly associated with death. After controlling for AIDS diagnosis and baseline plasma HIV-1 RNA levels, the adjusted risk ratio was 7.36 (95% CI: [3.82, 14.21]; $p < 0.001$), and 3.17 (95% CI: 1.69, 5.93; $p < 0.001$) for patients with $CD4^+$ cell counts <50 cells/mm^3, and with $CD4^+$ cell counts =50 to 199 cells/mm^3 respectively, compared to those with $CD4^+$ cell counts =200 cells/mm^3.

CONCLUSION: Our data demonstrates that the effectiveness of antiretroviral therapy is independent of age, gender, AIDS-diagnosis, protease-inhibitor use, and plasma HIV-1 RNA levels, but dependent on CD4 levels. More importantly, our results show that the effectiveness of antiretroviral therapy on survival is compromised in patients initiating therapy with $CD4^+$ cell counts below 200 cells/mm^3. These results not only have important implications regarding the optimal use of therapy, but the allocation of therapy in resource poor settings.

ABSTRACT 044

Antiretroviral Toxicity: an Increasingly Important Issue in the Management of HIV-infected Patients

DA Cooper

Antiretroviral toxicity is an increasingly important issue in the management of HIV-infected patients. With the sustained major declines in opportunistic complications, HIV infection is a more chronic disease, and so more drugs are being used in more patients for longer periods. This presentation focuses on the pathogenesis, clinical features, and management of the principal toxicities of the 15 licensed antiretroviral drugs, including mitochondrial toxicity, hypersensitivity, and lipodystrophy, as well as more drug-specific adverse effects and special clinical settings.

ABSTRACT 045

HAART Treatment Yields No AIDS Deaths in an Inner-City Population Over 3 Years

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BACKGROUND: US AIDS fatalities have plummeted since 1996 with HAART. Yet half of known HIV + in the US are not in treatment, especially those of low socioeconomic status (SES). Many untreated people feel that HAART is not beneficial. We document vastly improved survival with HAART in an inner-city population.

METHODS: Retrospective review at 2 sites, a community health center (CHC) and HIV Clinic (HC). The cohort is primarily Hispanic, African-American and low SES. Number of deaths over 36 months, cause of death and whether patients were on HAART >4 consecutive months were assessed. All pts were offered HAART and intensive case management including: assessment of housing status and social stability; evaluation and treatment of mental illness and substance abuse; consistent, comprehensive, interdisciplinary care; and treatment by physician and nurse HIV specialists.

RESULTS: From 3/31/97-3/31/00, 188 of 318 pts were on HAART >4 months. 93/114 (81.6%) pts at the HC and 95/204 (46.6%) at the CHC were on HAART. Those on HAART were significantly less likely to die than those off HAART: of 28 deaths overall there were, 5/188 (2.7%) on HAART and 23/130 (17.7%) off HAART(p<.001). Deaths at the HC: 2/93 (2.2%) on HAART and 8/21 (38.1%) off HAART(p<.001). Deaths at the CHC: 3/95 (3.2%) on HAART and 15/109 (13.8%) off HAART(p.008).

SITE	HAART	DEATH/100PY
Both sites	Y	2.7
	N	17.7
HC	Y	2.2
	N	38.1
CHC	Y	3.2
	N	13.8

DEATHS ON HAART	#OF DEATHS
Hep C Cirrhosis	3
Pancreatitis	1
Diabetes/CAD	1

DEATHS OFF HAART	# OF DEATHS
AIDS	7
Toxo	4
AIDS&Cirrhosis	4
MAC	2
Drug OD	2
PCP	1
Lymphoma	1
ETOH	1
Unknown	1

CONCLUSION: A cohort of HAART treated people of color and low SES had no AIDS-related deaths over 3 years. Deaths on HAART were 20 Hep C, other underlying illness or Rx side effects. Deaths off HAART were AIDS-related in 20/23 pts. These data are a strong motivator for treatment especially for people of color and low SES.

ABSTRACT 046

Persistent Abnormalities in Lymphoid Tissues of Persons Showing Good Virologic Response to Antiretroviral Therapy in all Treatment Arms of The Atlantic Study

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BACKGROUND: Recently studies have begun to compare efficacy of standard combinations of 2 nucleoside analogues (NA) + 1 protease inhibitor (PI), 2 NA + 1 non-nucleoside analogue (NNA), and 3 NA using changes in peripheral CD4 cell count and plasma HIV-1 RNA as endpoints. However, as the principal site of HIV-1 replication and subsequent damage to immunologic function is in lymphatic tissues (LT), we wanted to examine treatment differences in this compartment. We obtained LT from 22 individuals enrolled into the Atlantic Study, an international comparitive trial of 3 NA vs. 2NA + 1 NNA, vs. 2NA + 1 PI, and used immunocytochemistry (ICC) and in situ hybridization (ISH) to analyze differences in histology, virologic suppression, and immune cell populations between regimens.

METHODS: A combination of histology and ICC staining for CD20 and Ki67 was used to categorize LT as a) normal (nl), b) follicular hyperplasia, c) follicular hypoplasia, or d) follicular depletion (no evidence of secondary follicle formation). Antibodies to P24 antigen and ISH for HIV-1 RNA were used to determine persistence of HIV-1 antigen and presence of replicating HIV in the interfollicular and follicular zones and antibodies to CD4 were used to characterize the quantity and location of these cells in LT.

RESULTS: Of 22 individuals sampled, 10 were randomized to 3 NA, 8 to 2NA + 1 NNA, and 4 to 2 NA + 1 PI. Six samples were inguinal lymph node and 16 were tonsil. The median CD4 cell count and plasma HIV RNA were 548 cells/mm3 (range 291-957) and < 50 copies/ml (range 0-368) respectively. There were no significant differences in peripheral CD4 cell count or plasma HIV-1 RNA in patients grouped by location, tonsil vs. inguinal tissue, or treatment group. All 20/22 LT with sufficient tissue for analysis were judged abnormal by histologic and CD20 and Ki67 ICC analysis. Two samples showed hypoplasia, 7 hyperplasia, and 11 had no evidence of follicle formation. There were no significant differences in CD4 cell count, plasma HIV RNA, or duration of

treatment in these groups. All patients receiving PI therapy and 5/8 receiving 2NA + 1 NNA had no evidence of follicle formation. In contrast, 6/8 receiving 3NA had follicular hyperplasia (5) or hypoplasia (2). Seven of 17 tissues were positive for P24 antigen and there were no obvious differences when grouped by treatment, duration of treatment, CD4 cell count or plasma HIV RNA. Of the 5/7 samples showing evidence of P24 antigen were available for HIV-1 RNA ISH analysis, and only 1 showed evidence of active HIV-1 replication. Quantity and distribution of CD4 T cells was abnormal in 17/20 evaluable tissues with 12 having significant and 5 showing modest decrease in the CD4 T cell population. There were no differences in peripheral CD4 cell count between these groups however the 5 tissues with the most severe depletion were from individuals on therapy for a median of 36 weeks. Most (8/12) tissues showing significant depletion of CD4 T cells were from individuals randomized to receive 3 NA.

CONCLUSION: These preliminary analyses suggest that despite adequate virologic suppression and peripheral CD4 cell count > 500 cells/mm3, there are still significant immunologic abnormalities in lymphoid tissues and persistence of HIV-1 antigen. To more accurately assess the significance of these abnormalities will require longitudinal sampling and addition of functional immunologic assays.

ABSTRACT 047

Nonadherence to Triple Combination Therapy is Predictive of Mortality at Baseline and after One Year of Follow-up

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OBJECTIVE: To characterize the response to antiretroviral (ARV) therapy among participants enrolled in a population-based anti-HIV drug treatment program in British Columbia (BC).

METHODS: In BC antiretroviral therapies are distributed free of charge according to specific therapeutic guidelines. Study subjects were ARV naive, started triple therapy with 2 NRTIs and a PI or a NNRTI between 08/96-12/98, and had a baseline plasma viral load. The primary and secondary outcomes in this analysis were death, and a primary AIDS diagnosis or death respectively. K-M methods were used to estimate the hazard of death and AIDS-free survival from the initiation of antiretroviral therapy and after one-year of follow-up. Adherence was estimated by dividing the number of months of documented prescriptions dispensed by the number of months of follow-up in the first year of ARV therapy.

RESULTS: A total of 950 subjects (815 men/135 women) were studied. The median time on antiretroviral therapy was 13 months (IQR 7-21 months). 807 persons (85%) initiated therapy with a PI-containing regimen and 143 (15%) with a NNRTI-containing regimen. A total of 64 deaths and 11 primary AIDS diagnoses were prospectively observed in this study. The cumulative mortality was 3.6% (+ 0.6) at 12 months. In a multivariate model, mortality was independently associated with being non-adherent to therapy [per 10% decline in adherence] (RR = 1.16; 95% CI: 1.06 – 1.26; $p < 0.001$), and having a lower CD4 cell count [per 100 cell X 106/L] (RR = 1.35; 95% CI: 1.13 - 1.61; $p = 0.001$) at baseline. The results were unchanged when AIDS-free survival was the outcome of interest. In this instance, the likelihood of death and/or AIDS was 1.17 times higher (95% CI: 1.08 – 1.28; $p < 0.001$) per 10% decline in adherence. Finally in order to control for downward drift, adherence was measured over the first year while other prognostic factors were measured at the start of year two. In this analysis of 846 and 727 AIDS-free persons with more than one year follow-up, non-adherent subjects were 1.23 (95% CI: 1.05, 1.43) and 1.36 (95% CI: 1.15, 1.60) times more likely to die or to die and/ or progress to AIDS respectively after controlling for the same prognostic factors.

CONCLUSION: Our study demonstrates that non-adherent participants are more likely to progress than adherent persons. This association remains significant even after adjusting for prognostic clinical markers and physician experience at baseline and one-year.

ABSTRACT 048

Pharmacokinetic and Safety Evaluation of a Once Daily Regimen of Saquinavir Soft Gel Capsules (SQV) in HIV Infected Anti-Retroviral Naïve Patients

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BACKGROUND: SQV 1600 mg/ritonavir (rtv) 100 mg QD has been shown to produce an 800% greater SQV exposure in comparison to SQV 1200mg TID, with a 5.5 fold increase in SQV trough level and a 7.6 fold increase in SQV C_{max} without an associated increase in side effects. An objective of this study is to report on the safety and pharmacokinetics (PK) of once daily SQV 1600mg/rtv 100mg in a cohort of HIV(+) patients.

METHODS: A 44 patient cohort of HIV(+) patients have completed PK analysis and safety. 38 patients had single trough levels drawn at week 4. In addition, full PK profiles at 9 timepoints were obtained on 6 patients receiving SQV/rtv QD and analyzed for C_{min}, AUC_{0-24h} and C_{max}. All patients were clinically followed through week 24 for metabolic changes (fasting triglycerides, total cholesterol, HDL/LDL cholesterol, and glucose/insulin sensitivity), and for safety.

RESULTS: The median SQV trough concentration at week 4 for 18 patients was 0.43 μg/mL. PK profiles in 6 of these patients resulted in a SQV median AUC_{0-24h} of 59.8 μg-h/mL, median C_{max} of 4.78 μg/mL, and median C_{last} of 0.43 μg/mL. Nausea and diarrhea were the most frequently reported adverse events (≥5%) related to study drug and of at least moderate intensity occurring in 12% and 6% of patients, respectively. 5 patients reported 8 serious adverse events, all of which were unrelated to study drug. Marked lab abnormalities included decreased neutrophils (6%), and elevated GGT (2%). The median change from baseline at week 24 for total cholesterol was 20.5 mg/dL, and for triglycerides was -8.0 mg/dL.

CONCLUSION: PK data from HIV (+) patients demonstrates that levels of SQV are 9 times above the *in vivo* EC_{50} (0.05 μg/mL). The SQV/rtv QD regimen is generally well tolerated at 24 weeks.

Session Seven

Regulatory Issues: Viewpoints

ABSTRACT 049

Current Views on Accelerated Approval and Endpoints in Regulatory Trials

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In 1997, FDA began to accept virologic measurements (plasma HIV RNA) as primary study endpoints for both accelerated and traditional approvals of antiretroviral drugs. Prior to this time, changes in CD4, HIV RNA and even p24 Ag had been used for accelerated approval; however, studies with adequate power to detect differences in clinical endpoints (new AIDS defining events or death) were required for traditional approval. Currently, for accelerated approval, changes in HIV RNA over 24 weeks serve as a "surrogate" for more durable (48 week) changes in HIV RNA that are now used to support traditional approval.

The switch to virologic endpoints was based on analyses of data from multiple studies that demonstrated convincing relationships between decreases in HIV RNA levels and decreases in the risk of clinical disease progression. Although most of these analyses evaluated virologic response in terms of mean changes in HIV RNA from baseline, other methods of virologic response, such as suppression below a certain value or assay limit were also explored. Based on these data, it appears that group mean decreases in HIV RNA of approximately 0.5 log over a 16-24 week period reliably predicted clinical benefit. In addition, the largest degree of virologic suppression, specifically a response that maintained levels below assay limits, was considered to be desirable with respect to controlling the emergence of resistance and in line with current goals of therapy. Consequently, the preferred virologic endpoint for accelerated approval is the proportion of patients with HIV RNA levels below 50 or 400 copies at 24 weeks and for traditional approval is the time to loss of virologic response above the assay limit (over 48 weeks). Such endpoints are stringent and appropriate for treatment naïve patients; however, other ways of measuring virologic response may be preferable for patients who are not expected to achieve maximal suppression due to prior exposure to multiple antiretrovirals.

It is imperative that all study participants be offered drug regimens that are predicted to offer a response rate consistent with current standards of clinical care. This often means that studies will need to incorporate an "equivalence" or noninferiority design, in which one drug of a commonly used triple drug regimen is compared with a new drug substituted in its position. In addition studies used for regulatory purposes should be able to clearly discern efficacy and safety for a particular drug of interest. This is often a difficult task for antiretroviral drug development due to: 1) complexity of combination regimens 2) positive and negative drug-drug interactions 3) heterogeneity of response in treatment experienced patients and 4) availability of a control with appropriate assay sensitivity for the patient population studied. Studying the efficacy of a drug in patients with limited remaining treatment options could yield the most important data but at the same time poses the most challenges. FDA is currently considering alternative treatment designs and endpoints for this particular patient population.

Session Eight

HIV/Hepatitis and other Co-infections

ABSTRACT 050

Co-infection with HIV and Hepatitis B Virus (HBV)

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In the wake of recent advances in antiretroviral therapy for human immunodeficiency virus (HIV) infection and the attendant reconstitution of the immune system, survival free from life-limiting opportunistic infections has improved significantly. In place of these opportunists, infections with hepatitis B and C viruses will become increasingly important problems in HIV-infected individuals. Recent data from a European population showed that chronic viral liver disease represented the fifth most common cause of death for HIV-infected patients. In view of the shared routes of transmission between HIV and hepatitis B and C virus infections, morbidity and mortality from hepatitis-related liver failure is expected to become an even more critical problem in the immediate future. This paper will review the pathogenic interactions between HIV and HBV, the natural history of coinfection, and available treatment strategies for the coinfected individual. In addition, a broad overview of newer therapies for HBV will be presented.

ABSTRACT 051

Interferon (IFN) and Ribavirin (RBV) Therapy for Hepatitis C (HCV) Patients who are Co-Infected with HIV: Sustained Virologic Response (SVR)

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BACKGROUND: Morbidity and mortality from HCV in patients co-infected with HIV has become an important issue because patients are now living longer. Progression to AIDS, cirrhosis and end-stage liver disease is accelerated in co-infected patients. Combination IFN/RBV can result in substantial decreases in HCV RNA levels as well as sustained virologic responses similar to HIV seronegative HCV infected patients.

METHODS: This is a retrospective study that evaluated 26 co-infected patients treated with IFN/RBV combination therapy for 6 to 18 months. 22/26 [85%] were dosed with IFN 3mu TIW + RBV 800mg/day and 4/26 [15%] received IFN 3mu QD + RBV 800mg/day. 23/26 [88%] were male, mean baseline CD4 402, mean baseline HIV RNA 39,000 cps/ml (18/26 [69%] had HIV RNA<400cps/ml), 22/26 [85%] were receiving HAART. Mean HCV RNA 2.8 million copies (range1,000 >10 million cps/ml). Mean liver biopsy fibrosis score was 1.8 (2 patients with cirrhosis, 5 patients with transition to cirrhosis, 5 patients had no liver bx), 6/26 [23%] with genotype 1a, 5/26 [19%]1b, 5/26 [19%] 2a, 2b and 3/26 [12%] genotype 3a, 7/26 [27%] were unknown.

RESULTS: 11/26 [42%] had undetectable HCV RNA levels 6 months post-treatment (SVR). Of these, 3/11 [27%] were non 1-genotype with 4 genotypes unknown. Both mean CD4 count and HIV RNA were unchanged from baseline. The main adverse event was noted to be anemia (hemoglobin < 12 gm/dl) and 7/26 [26%] of patients received erythropoietin 40,000 units weekly until the hemoglobin normalized. Only 3 patients discontinued the study because of constitutional symptoms; IFN/RBV was tolerated well by these co-infected patients.

CONCLUSION: 1) IFN/RBV combination therapy has little effect on HIV RNA. 2) HCV RNA <1000 cps/ml at 3 months of treatment can be a positive predictor of SVR. 3) Anemia, a significant side effect of IFN/RBV combination therapy, can be successfully treated with erythropoietin in co-infected patients. 4) SVR is possible in a substantial number of HIV/HCV co-infected patients.

ABSTRACT 052

Treating Co-Infected Patients

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NATURAL HISTORY OF HCV IN CO-INFECTION
More than 30 million people worldwide are living with AIDS, 170 million are infected with HCV. Co-infection is very common and varies significantly between risk groups: uncommon in homosexual man, universal in hemophiliacs, prevalent in up to 95% in IVDUs. Many studies have demonstrated that chronic hepatitis C has an accelerated progression to chronic liver disease in HCV/HIV co-infected patients. Co-infected patients have an increased risk of liver disease, liver failure and liver-related mortality.

RATIONALE FOR TREATMENT
Better HIV therapy has improved outcomes for HIV patients, including life expectancy. HCV-related morbidity and mortality has a higher importance in stable HIV patients. Treatment of HCV in co-infected patients is imperative to improve symptoms, slow disease activity, prevent progression to cirrhosis and end-stage liver disease and to reduce HCV transmission.

TREATMENT OF CO-INFECTED PATIENTS

Limited studies in co-infected patients have shown that HIV-positive patients respond as well as non-HIV patients in monotherapy as well as in combination therapy. There are some indicators of better response, as non 1 HCV genotype, lower HCV viral load, and higher CD4. Treatment of co-infected patients is more difficult because the possibility of hepatic toxicity secondary to the HAART drugs and probably more adverse events as anemia and neutropenia because added toxicity and drug interactions. In Puerto Rico, we have a large experience treating co-infected patients since 1995. Our results in monotherapy and combination trials mirror the published data. We have had more success achieving biochemical and clinical improvement with higher doses of interferon and daily maintenance (unpublished). We also have documented significant histological improvement after treatment in viral non-responders (unpublished). Large global studies in co-infected patients are under way studying the effect of pegylated interferons with and without ribavarin. The results of these studies and maintenance studies in non-responders are extremely important to establish if sustained virological response will be the main aim of treatment in this subpopulation or if histological improvement and decrease of progression of liver disease will be the main goal.

Session Nine

Vaccines/Therapeutic Vaccines

ABSTRACT 053

An Open Label Pilot Study of the Safety and Efficacy of Adefovir Dipivoxil in HIV/HBV Co-Infected Patients with Lamivudine Resistant HBV

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BACKGROUND: Lamivudine resistance to HBV occurs in approximately 15%-32% of both immunocompetent HBV and HIV/HBV co-infected patients after one year of lamivudine therapy. Adefovir dipivoxil (ADV) has potent *in vivo* and *in vitro* activity against both wild-type and lamivudine resistant HBV.

OBJECTIVE: To evaluate the safety and efficacy of ADV 10 mg once daily for the treatment of lamivudine-resistant HBV infection in HIV-infected patients in an open label trial.

METHODS: Thirty-five HIV/HBV co-infected patients receiving lamivudine (150 mg bid) as part of their current anti-retroviral therapy were enrolled in the study. Patients had controlled HIV RNA (≤ 2.60 $\log_{10}$copies/mL) at screening. All patients had detectable serum HBV DNA despite ongoing lamivudine therapy and documented M550V or M5501 mutations in the HBV DNA polymerase gene. ADV 10 mg daily was added to the patients' existing anti-HIV therapy. Patients were seen monthly for safety and efficacy evaluations.

RESULTS: Thirty-five patients, 34 males and 1 female, with a mean age of 41.2±1.6 years were enrolled. The median time on ADV was 28±5.7 weeks. Mean decrease in HBV DNA serum levels from baseline (8.64±0.08 $\log_{10}$ copies/mL) was -2.27±0.10 $\log_{10}$ copies/mL at week 4, -2.61±0.12 $\log_{10}$ copies/mL at week 8, -2.90±0.11 $\log_{10}$ copies/mL at week 12, -3.0±0.12 $\log_{10}$ copies/mL at week 16, -3.16± $\log_{10}$ copies/mL at week 20, and -3.40±0.14 $\log_{10}$ copies/mL at week 24, ($p<0.0001$). There were no significant changes in serum ALT ($p=16$). Adefovir dipivoxil was generally well tolerated. No significant changes in serum electrolytes, renal function, HIV RNA and CD4 cell count were observed.

CONCLUSION: The preliminary results of this ongoing 12 month study, indicate that 7 months of ADV 10 mg once daily added to lamivudine is well tolerated and has significant activity against lamivudine-resistant HBV in HIV/HBV co-infected patients.

ABSTRACT 054

Persistent Immune Activation and Chronic Viral Disease: Diagnostic and Therapeutic Relationship between HIV and HCV Disease

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BACKGROUND: HIV infection has been implicated as a co-factor in Hepatitis C virus (HCV) associated liver disease. Recent studies on blood from HCV infected patients have identified inflammatory gene expression patterns that show a high correlation with degree of liver disease shown by biopsy (McGrath et. al., ICAAC, 2000). Of interest was the disease-associated elevation of IP10, an interferon-g responsive chemokine gene directly implicated in experimental models of liver fibrogenesis. The goal of the current study was to evaluate gene expression patterns in peripheral blood cells from HCV negative, HIV infected patients on HAART with no detectable HIV viral load to evaluate the same immunopathogenic genes implicated in progressive HCV liver disease. The specimens we chose to analyze were obtained from HIV infected patients enrolled in a phase II study of WF10 (OXO Chemie). WF10 is a chlorite-based drug (currently in phase III trials) known to inhibit T cell activation through regulation of macrophage function.

METHODS: Specimens from 7 viral load negative HIV infected patients with a mean CD4 count of 262 who participated in the phase II study of WF10 at SFGH were studied. On days 1-5 the patients received 0.5ml/kg of WF10. Baseline gene expression patterns were compared with those determined at day 7. Genes evaluated (13) included those elevated proportional to degree of liver disease in an earlier HCV specific gene expression study. LightCycler based RT-PCR was performed on RNA extracted from patient blood cells using 2 internal control housekeeping genes for normalization and calculation of inflammatory gene expression.

RESULTS: Baseline expression of IP10 and IFN-g in the 7 patients studied was significantly elevated above control values with levels of IP10 increased up to 25-fold above normal. Expression of IP10 is normally regulated by IFN-g-stimulation suggesting the ongoing elevated production of IFN-g protein in viral load negative HIV infected patients *in vivo*. Expression of the other genes varied, but as a group is similar to levels observed in normal controls. IP10 gene expression decreased significantly ($p=0.016$) after administration of the macrophage-regulating drug WF10.

CONCLUSION: The following points can be derived from this study: (1) HIV infected patients on HAART

with no detectable viral load show gene expression evidence for persistent immune activation particularly associated with up regulation of the fibrogenic/T cell attractant chemokine, IP-10. (2) Previous studies showing a relationship between IP10 expression and HCV liver disease suggest a molecular basis for HIV infection as a pathogenic co-factor. (3) WF10 administration caused down regulation of immunopathogenic IP10 gene expression suggesting that this drug be studied further in patients co-infected with HIV and HCV.

ABSTRACT 055

Induction of Potent HIV-specific T Cell Restricted Immunity by Genetically-modified Dendritic Cells

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BACKGROUND: HIV-specific T cell responses decline with time in patients treated with highly active antiretroviral therapies, and therapeutic immunization has the potential to boost these responses and control HIV.

METHODS: We used a novel replication- and integration-defective HIV-1 vector to genetically modify dendritic cells (GMDC) in order to safely express most HIV antigens and induce T cell immunity. We introduced the vector into DCs as a plasmid DNA using polyethylenimine as gene delivery system, thereby circumventing the problem of obtaining viral vector expression in the absence of integration.

RESULTS: GMDC presented viral epitopes efficiently, secreted IL-12 and primed a high number of naïve T-cells capable of exerting vigorous HIV-specific cytotoxicity *in vitro*. In non-human primates, GMDC migrated into the draining lymph node at an unprecedented high rate and expressed the plasmid DNA. The animals presented a vigorous effector CTL response within 3 weeks, which later developed into a memory CTL response. Interestingly, antibodies did not accompany these CTL responses.

CONCLUSION: These data suggest that GMDC can raise a pure Th1 type of immune response in primates without any toxic side effects. Successful induction of HIV-specific cellular immunity by genetic immunization is expected to contribute to control virus replication in infected individuals.

ABSTRACT 056

HIV Vaccines: Where Are We Now?

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Development of an HIV vaccine is a public health priority. While no correlate of protection has been defined, virological studies of HIV shedding in genital secretions and experimental challenge studies indicate that both extracellular virus and HIV-1infected cells are important means of spread. Primate challenge studies have indicated that vaccine strategies involving both neutralizing antibodies and CTL responses are associated with protection from infection and disease amelioration. Unfortunately, HIV-1 envelope is a poorly immunogenic protein and recent studies of viral host cell interactions indicate that the interaction between gp120 and host cell receptors is complex. Blocking antibodies are quite strain specific and of low avidity. In contrast, CTL responses appear to be broader across strains and clades of HIV-1.

To date, no one candidate vaccine has proven able to elicit both high levels of humoral and cellular responses to HIV-1 proteins. Clinical trials have clearly shown the safety of protein based subunit proteins to viral envelope and gag. Replication competent vectors, such as vaccine recombinants, and replication incompetent vectors, such as canary pox vectors, elicit HIV-1 specific CTL responses. These replicating vectors produce only low levels of antibody responses. As such, combination vaccine strategies containing different types of vaccines have emerged as the best approach to develop a regimen that has both CTL and antibody responses. These responses are capable of eliciting detectable CTL responses in about 50% of vaccines. One of the issues now is what are the standards to move to an efficacy trial. The NIAID supported HVTN has developed a concept phase III efficacy trial designed to define a correlate of protection among CTL vs. non-CTL responses. This design and the community and ethical issues regarding phase III trials will be discussed.

ABSTRACT 057

Viral Vaccines and Immune Memory

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Acute viral infections induce long-term humoral and cellular immunity. However, the nature of T- and B-cell

memory is different. Antiviral B-cell memory is usually manifested by continuous antibody production that lasts for many years after infection or vaccination. In contrast, the effector phase of the T cell response is short-lived (a few weeks), and "memory" in the T-cell compartment results from the presence of memory T cells, which are found at higher frequencies and can respond faster and develop into effector cells (i.e., CTL or cytokine producers) more efficiently than can naïve T cells. In this talk I will discuss the following aspects of immunological memory: (1) The importance of long-lived plasma cells in maintaining humoral immunity. (2) Quantitative and qualitative (TCR usage) analysis of the primary antiviral CD8 T cell response and the relationship between the CD8 T cells responding during the primary infection to the pool of memory CD8 T cells that survive after viral clearance. (3) Functional differences between naive and memory T cells. (4) The role of MHC class I molecules in the maintenance of memory CD8 T cells and (5) Protective immunity by memory CD8 T cells.

ABSTRACT 058

Enhanced DNA, Alphavirus, and Protein Vaccine Approaches Stimulate Potent Immune Responses Against HIV Antigens

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The challenge for developing effective vaccines against HIV infection and disease lies in the generation of potent, broad, and durable immune responses. Toward this end, the HIV Vaccine research group at Chiron has focused on increasing the potency of both DNA and adjuvanted subunit protein vaccine technologies, as stand alone and mixed modality vaccines. High level, Rev-independent gene cassettes that increase the expression efficiency of the HIV-1 gag, pol, and env structural genes 10-1000 fold have been constructed. Delivery methods that improve the *in vivo* transfection efficiency and hence, immunogenicity of HIV DNA vaccines 10-100-fold have been developed. A promising DNA delivery method for future preclinical evaluations utilizes adsorption of DNA onto polylactide co-glycolide (PLG) microparticles. Studies in rodents and primates indicate that PLG-adsorbed DNA vaccines are potent inducers of antibody, cytotoxic T-lymphocyte, and CD4+ T-helper cell responses against HIV antigens. High titer, highly purified stocks of alphavirus replicon particles have been made that target immature human dendritic cells that may be useful to augment immune responses in DNA primed individuals. Furthermore, efforts to induce broadly reactive neutralizing antibody responses against primary HIV-1 isolates have been successful. Employing sequence-modified V2-deleted oligomeric (trimeric; o-gp140) Env from the R5 HIV-1SF162 isolate in a DNA prime-protein boost regimen in rhesus macaques, we were able to induce neutralizing antibodies against diverse primary strains and partial protection against intravenous challenge with the pathogenic SHIVSF162P. These results are encouraging for the future development of prophylactic and therapeutic HIV vaccines.

ABSTRACT 059

Strategies of Immune Evasion Used by the HIV-encoded Envelope Proteins

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It is now clear that HIV has evolved a variety of specific strategies for immune evasion that allow persistent, unrelenting viral replication. These strategies include: i) emergence of genetic variants that are immune escape variants; ii) destruction of CD4+ T-cell helper activity; iii) accessibility of envelope proteins on virions to antibodies; iv) nef-induced downregulation of MHC class I molecules; v) latency and reactivation. My laboratory has become interested over the last few years in how HIV envelope has configured itself to avoid antibody-mediated neutralization. We have studied four types of envelope modifications for their effects on antibody mediated neutralization, ability to elicit antibodies capable of neutralizing viral infectivity, dependence on CD4 for viral infectivity, and ability to be controlled by the host immune response. We have been studying the effects of: i) N-linked carbohydrate attachment mutations in the V1-V2 region of gp120; ii) deletion of the entire V1-V2 region of gp120; iii) N-linked carbohydrate attachment mutations in the ectodomain of gp41; iv) point mutations in gp120 that confer high replicative capacity to tissue macrophages. Our results indicate that a variety of mutational changes impart neutralization sensitivity and that these changes result in more effective immunological control. These results are important at a fundamental level for better understanding of how immune evasion strategies contribute to pathogenesis. They may also be important at a practical level for improving envelope-based vaccine approaches.

Posters

ABSTRACT 060

Synthesis and Antiviral Activities of Enantiomerically Pure Carbocyclic Pyrimidine Nucleosides

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BACKGROUND: Since the discovery of abacavir with potent anti-HIV activity, considerable effort has been devoted to the synthesis and evaluation of carbocyclic nucleosides. In contrast to carbocyclic purine nucleosides, carbocyclic pyrimidine nucleosides, especially chiral analogs, have not been researched extensively. Previously, we reported the synthesis and biological activity of racemic carbocyclic nucleosides. These racemic nucleosides showed low levels of anti-HIV activity. Accordingly, it would be interesting to know if the enantiomers of these nucleosides possess a different antiviral profile.

METHODS: The synthesis of enantiomerically pure carbocyclic pyrimidine nucleosides was based on the formation of chiral p-allylpalladium complexes. From chiral bicyclic lactams, two chiral cyclopentenyl ditosylimides were prepared. Under Trost conditions, the ditosylimides were converted to p-allylpalladium complexes, which were coupled with pyrimidines to yield the desired chiral carbocyclic nucleosides. These nucleosides were evaluated for anti-HIV and anti-HBV activities, and for cytotoxicity in PBM, CEM, and Vero cells.

RESULTS: Four enantiomers of carbocyclic cytosine and 5-fluorocytosine nucleosides were synthesized and evaluated for their antiviral activity. Among the four enantiomers, the two L-nucleosides demonstrated low levels of anti-HIV activity, while the two D-counterparts were inactive. These enantiomers did not show significant anti-HBV activity, and cytotoxicity (up to 100 mM) in PBM, CEM, and Vero cells.

CONCLUSION: 1) The antiviral assays showed that only the L-carbocyclic nucleosides possessed low levels of anti-HIV activity. 2) All the synthesized carbocyclic nucleosides showed no cytotoxicity in various cell lines. 3) An efficient and versatile method for synthesis of enantiomerically pure carbocyclic nucleosides was successfully developed. 4) The synthetic method provides more possibilities for the synthesis of other chiral carbocyclic nucleoside analogs.

ABSTRACT 061

Using Real Time PCR to Determine Anti-HIV-1 Activity and Mitochondrial Toxicity of Nucleoside Analogs

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OBJECTIVE: To evaluate the antiviral efficacy and cytotoxicity of 2',3'-didehydro-2',3'-dideoxy-2'-fluoro-4'-thio-cytidine and compare potencies and selectivity with approved and experimental nucleoside analogues used to treat HIV-1 infected individuals.

METHODS: b-L and b-D analogues of 2',3'-didehydro-2',3'-dideoxy-2'-fluoro-4'-thio-cytidine were compared with AZT, 3TC, d4T, and (-)-FTC against a sensitive (xxBRU) and a 3TC-resistant (184V) viral strain. Human PBMC were PHA stimulated for 2 days, HIV-1 infected, and kept in culture for 5 days in presence of test compounds at different concentrations. Subsequently, culture supernatant was clarified, and tested for reverse transcription activity (incorporation of tritium-labeled TTP) or quantified for HIV-1 viral load (real-time RT-PCR, PE BioSystems). Mitochondrial toxicity (g-DNA polymerase inhibition) was also evaluated by real-time PCR, using the comparative cycle threshold (Ct) method. b-Actin served as an endogenous reference.

RESULTS: The median 50% (EC_{50}) and 90% (EC_{90}) effective antiviral concentrations were in concordance for the two methodologies used. Both the b-L and b-D compound demonstrated potent antiviral activity against the wild-type virus (EC_{90} = 7.14 ± 6.3 mM, and 20.65 ± 3.6 mM, respectively), but a marked increase in EC_{90} value was noted for both compounds when tested against the 184V mutant virus. No effect on mitochondrial DNA synthesis was observed after a 7-day incubation with up to 10 mM of the compounds. However, DDC demonstrated dose-dependent reduction in mitochondrial DNA synthesis. Similarly, in an MTS-dye assay (Promega), no cytotoxicity was observed for these compounds in human PBMC, Vero and CEM cells when evaluated up to 100 μM.

CONCLUSIONS: Real-time PCR assays were designed for viral and human targets and used to evaluate potential antiviral candidates. In this study, a new class of unsaturated nucleoside analogs was evaluated and compared to known antiviral agents. The data indicate that b-L-2',3'-didehydro-2',3'-dideoxy-2'-fluoro-4'-

thio-cytidine is more potent than the corresponding b-D-enantiomer.

ABSTRACT 062

Novel Ribonucleotide Reductase Inhibitors, Didox and Trimidox, Compared to Hydroxyurea to Reverse Established Retroviral Disease in the Murine AIDS (MAIDS) Model

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BACKGROUND: Prevention of deoxynucleotide synthesis by inhibiting ribonucleotide reductase (RR) as a strategy to impair HIV replication has gained acceptance by the success of hydroxyurea (HU) to enhance the deoxynucleoside reverse transcriptase (RT) inhibitor ddI in clinical trials. However HU, as a single agent in HIV therapy, has not demonstrated significant clinical antiviral activity. On the other hand, RR inhibitors Didox (DX) and Trimidox (TX), have shown potent antiviral activity when used alone in murine retroviral models. The antiviral activity was more pronounced with these compounds than HU, especially in the HIV-infected SCID-Hu mouse model where both DX and TX used as monotherapy reduced intracellular HIV RNA copies per ml several logs while hydroxyurea had only a small effect on RNA levels producing a reduction of 1 log or less. In addition, a significant number of infected mice treated with DX and TX reduced viral RNA to undetectable levels. Furthermore, these novel RR inhibitors potentate ddI more effectively than HU most notably in the MAIDS model. This report focuses on comparing DX, TX or HU alone to reverse established disease in the MAIDS model. The purpose of this experiment was to mimic the human situation when a treatment regimen of an RR inhibitor with a RT deoxynucleoside or tide inhibitor such as ddI might be utilized to treat progressing disease when treatment failure has occurred.

METHODS: MAIDS infected animals were not treated until 9 wks post infection. At this stage in this model the infected mice have developed immunodeficiency, large viral load and profound disturbance in splenic architecture and size as well as marked hypergammaglobulinaemia. The mice were treated for 4 wks (wk 9 through wk 13) with once daily drug i.p. injections. Since this treatment strategy targets a cellular reaction, toxicity is an issue. Therefore, the treatment effect on hematological indices (i.e., peripheral blood indices and number of FU-GM and BFU per femur spleen) was also monitored.

RESULTS: All 3 RRI's reduced splenomegaly below levels seen at the 9 wk time point when treatment was initiated. DX and TX were more effective than HU. Most significantly, TX and DX to a lesser extent dramatically restored splenic architecture to nearly normal levels while the HU effect was more modest but did restore the splenic architecture to a state better than the 9 wk infected observation. With regards to hematological toxicity, HU reduced peripheral blood indices, femoral cellularity, femoral CFU-GM and BFU-E progenitor cells more than TX or DX with this late stage treatment regimen.

CONCLUSION: These data support the concept of using RRI for treatment of retrovirus infection as this approach can work in established disease. The RRI's DX and TX were more effective and less hematologically toxic than HU.

ABSTRACT 063

Synthesis and Anti-HIV Activities of Ethidium Arginine Conjugates

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The regulatory protein Tat is essential for viral gene expression and replication of the human immunodeficiency virus type 1 (HIV-1). Tat transactivates the HIV-1 long terminal repeat (LTR) via its binding to the trans-activation responsive element (TAR) and increases the viral transcription. Studies have shown that binding arginine and arginine derivatives induces a conformational change of the TAR RNA at the Tat-binding site. The unpaired A17 residue delimits a small cavity which constitutes a receptor site for small molecules and especially for ethidium. These binding characteristics have prompted us to design a series of ethidium-arginine conjugates capable of interacting with TAR RNA. Six ethidium derivatives linked with arginine side chains were synthesized. These molecules were biologically evaluated and two compounds exhibited an anti-HIV-1 activity at a micromolar concentration *in vitro*, without toxicity (up to 100μM). Melting temperature studies indicate that the most active molecule strongly binds to TAR *in vitro*. RNase protection experiments are consistent with the

molecular model suggesting that the ethidium moiety is inserted next to the A17 residue while the arginine side chain occupies the pyrimidine bulge. With the aim to investigate more precisely the location of the drug-binding site and to improve its antiviral potency and on the basis of molecular modeling studies, we designed and synthesized a compound in which the more active molecule is covalently attached to an artificial site-specific ribonuclease. This compound was evaluated both for its antiviral and nuclease activities.

ABSTRACT 064

New Anti-HIV Prodrugs Based on Nucleoside Phosphonate Derivatives

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BACKGROUND: To inhibit HIV reproduction, nucleoside-based anti-HIV drugs must pass through the intracellular triphosphorylation cascade. Since the effectiveness of the triphosphate formation is very low (for AZT it is only about 0.1%), the design of the compounds bearing one or more modified phosphate residues, which can facilitate or even exclude the phosphorylation process, is actual. The goal of this work was synthesis of novel anti-HIV prodrugs and study their cytotoxicity and anti-HIV activity.

METHODS: Cytotoxicity (CD50) of the tested compounds was measured by determination of cell viability on MT-4 cells by trypan blue exclusion test. Anti-HIV activity of tested compounds (ID50 and ID90) was assessed by means of a cell viability calculation as well as by measurement of p24 antigen by immunoenzyme method. The inhibitory effect on the replication of different HIV strains was evaluated in MT-4 cells. Selectivity index (SI) was determined as the ratio of CD50 to ID50.

RESULTS: We synthesized and evaluated antiviral properties of two groups of modified nucleotides (I, II). The first group includes AZT and D4T derivatives mimicking monophosphates, whereas the second group can be regarded as modified nucleoside triphosphates substituted at the γ-phosphate by a nucleotide residue. The advantage of the compounds synthesized over the parent nucleotides (R = H) is their increased lipophilicity. The key intermediates for the synthesis of compounds of group I were the corresponding nucleoside 5'-hydrogenphosphonates (R = H); in the case of compound II, it was the corresponding monophosphonate (ROH).

All tested compounds I provided high anti-HIV activity. For AZT phosphonate derivatives, 2',3'-dideoxy-3'-azidothymidine 5'-cyclohexylphosphite was the most active (SI > 50000). In contrast, among d4T derivatives the highest SI value was shown for 2',3'-dideoxy-2',3'-didehydrothymidine 5'-isopropylphosphite (SI>2000). Probably, increase in the antiviral activity of the novel compounds appeared to be due to the presence of lipophilic groups. This may result in the higher membrane permeability and the growth of intracellular concentration of the tested derivatives. Compounds II was not active against HIV.

CONCLUSION: The obtained results imply good perspectives for design of new anti-HIV compounds based on phosphonate derivatives of nucleoside analogs.

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ABSTRACT 065

Bypassing the Need for Cellular Activation of Nucleoside Reverse Transcriptase Inhibitors

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BACKGROUND: Nucleoside reverse transcriptase inhibitors (NRTI) such as 3-azido-3-deoxythymidine (AZT; zidovudine) need intracellular conversion to their triphosphate derivatives to have antiviral activity. While the phosphorylation of AZT to AZT-monophosphate by thymidine kinase (TK) is facile, conversion of AZTMP to AZT-diphosphate by thymidylate kinase is very inefficient. This results in low intracellular levels of AZT-triphosphate (AZTTP), the actual antiviral agent. Also, different cell types have different TK levels, leading to variable intracellular levels of AZTTP. Finally, prolonged AZT therapy can lead to "cellular" resistance to AZT, due to diminution of TK activity. All of these factors may impact on the antiviral efficacy of AZT in patients. These uncertainties would be eliminated if AZTTP could be administered directly, but unfortunately cells cannot readily take up nucleotide triphosphates (NTP). SP1008A is an amphiphilic block copolymer composition that enables cell uptake of NTP. We therefore asked whether formulations of SP1008A with AZTTP would provide improved anti-HIV activity compared to AZT.

METHODS: Cell uptake of NTP was determined by FACS analysis of cells exposed to fluorescein-labeled dUTP alone or in formulation with SP1008A. Antiviral experiments involved cells incubated with AZT, AZTTP, SP1008A, or SP1008A-AZTTP for 1h, followed by infection with HIV-1 for 3h. Following removal of exogenous drug and virus, cells were

cultured in the absence of drug and examined for signs of HIV infection.

RESULTS: SP1008A dramatically enhanced NTP uptake by lymphocytic cells in manner dependent both on time and the dose of the carrier. No uptake of NTP was seen in the absence of the carrier. Physico-chemical studies suggest that colloid particles formed by SP1008A are important for the cellular uptake of NTP. Treatment of MT2 cells with AZT or AZTTP alone, as described in Methods, had little or no effect on subsequent HIV-1 infection, even at concentrations as high as 2 uM. In contrast, the same treatment with SP1008A formulations containing as little as 1nM AZTTP reduced HIV infection of the cells by more than 80%. Treatment with SP1008A formulations with 100 nM AZTTP virtually eliminated subsequent HIV-1 infection of these cells. Similar data were obtained with monocyte/macrophage U937 cells, a cell line in which AZT is converted rather poorly to AZTTP.

CONCLUSION: SP1008A dramatically increases cell uptake of NTPs, such as AZTTP. SP1008A-AZTTP formulations provide superior antiviral activity compared to AZT or AZTTP alone. In addition, the anti-HIV protective effect afforded by a single-exposure of cells to SP1008A-AZTTP suggests that this formulation may also be useful in microbicides to prevent HIV transmission.

ABSTRACT 066

ACH-126,443: Designing a New Nucleoside Analog for Improved Safety and Antiviral Efficacy

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BACKGROUND: Extensive work undertaken to elucidate the mechanism of delayed toxicity of existing nucleosides identified the adverse effect of these compounds on mitochondria and their function. This work further indicated that nucleosides of the 'unnatural' L-configuration did not exhibit the same detrimental effects seen with D-nucleosides. ACH-126,443 (ß-L-Fd4C) is an L-nucleoside analog designed to improve on earlier nucleoside RT inhibitors by providing potent anti-HIV activity while avoiding mitochondrial toxicity. Early data demonstrated anti-HIV activity 10-20 fold greater than 3TC, excellent oral bioavailability and an intracellular T1/2 exceeding 24 hours. *In vitro* studies showed no reduction in mitochondrial DNA following cellular exposure to ß-L-Fd4C alone, and amelioration of mitochondrial DNA loss due to d4T when the two drugs were combined, both of which findings support the targeted safety profile of the drug.

METHODS: Confirmation of the antiviral activity was undertaken against wild-type (WT) HIV-I and 65 clinical strains with known nucleoside (NRTI) resistance mutations at M41L, M184V, T215Y, Q151M, 69S insert and NNRTI resistance mutations at K103N, Y181C and G190A. Antiviral activity was tested phenotypically, using a recombinant virus assay approach (Antivirogram®, VIRCO) and genotypically using ABI DNA sequencing. Control drugs included ZDV, ddI, d4T, ddC, 3TC, abacavir, efavirenz, nevirapine and delavirdine. *In vivo* toxicity was explored with 14 day dosing in two species using oral doses up to 200-500 mg/kg/day.

RESULTS: ß-L-Fd4C IC50 against WT was 0.1-0.3 µM and was unchanged against any mutant strains except those harboring M184V. IC50 against M184V mutants was 1-4 µM, equivalent to that of 3TC against WT virus (IC50 of 3TC against the M184V mutants was >30 µM). No change in susceptibility to ß-L-Fd4C was conferred by any other NRTI mutations, including Q151M and 69S insert, or by NNRTI mutations. Toxicology studies showed poor appetite and modest weight loss at highest doses, but no chemical or histopathologic abnormalities, despite plasma concentrations of drug >50 µg/mL (>150 µM).

CONCLUSION: ACH-126,443 (ß-L-Fd4C) is a nucleoside analog with exceptional anti-HIV potency, especially against strains resistant to other nucleosides. The short-term toxicology studies revealed no target organ toxicity. This broad range of potency, combined with very promising safety profiles, both *in vitro* and *in vivo*, suggests that ß-L-Fd4C may provide an important nucleoside component for combination antiretroviral regimens at all stages of disease.

ABSTRACT 067

In Vitro Strategies for the Preclinical Development of Topical Microbicides: Classes of Potential Inhibitors

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BACKGROUND: One of the current challenges in anti-HIV drug discovery is the inhibition of virus transmission between sexual partners.

METHODS: We have developed a series of microtiter-based, high-throughput assays to evaluate the ability of anti-HIV compounds to be used as topical microbicides, including both CD4-independent (ME180) and dependent (GHOST X4/R5) cell-to-cell *in vitro* virus transmission assays. Efficacy and toxicity (especially

against common flora of the vagina, such as Lactobacillus) of candidate compounds is determined in conditions that mimic the type of environment in which the compound will be required to work, including the effects of pH and mucopolysaccharides, as well as other conditions involving time of infection, treatment schedule and multiplicity of infection. An integral part of the assessment of any topical microbicide candidate is demonstration of appropriate range and mechanism of action compatible with a topical microbicide. Range of action assays evaluate the ability of candidate compounds to act against a variety of wild-type, drug-resistant, laboratory-derived and clinical strains of virus, including HIV-2 and SIV. Range of action assays also include supplementary studies for activity against bacterial and fungal pathogens. Mechanism of action assays, encompassing both biochemical/enzymatic and cell-based assays, are employed to further define the activity of the compound in intact cells. For topical microbicide inhibitor candidates, we routinely evaluate the ability of these compounds to inhibit virus-cell attachment (CD4-gp120 interaction) and cell-cell fusion. We have also designed assays which evaluate the ability of compounds to inhibit virus entry after formation of the attachment/fusion complex (virus gp120 and gp41 interaction with cell surface CD4 and chemokine coreceptors). Assays are performed to determine the relative ease of selecting for drug resistant virus strains in culture and to define the interactions of the compounds when used in combination with other active agents. Finally, candidates can be assessed in non-human primate models for *in vivo* efficacy following vaginal or rectal challenge, using RT and Env SHIV viruses.

RESULTS: We have identified a variety of classes of active inhibitors, including polyanionic molecules, surfactants, natural products, peptides, proteins, heterocycles, virucidal agents and other anti-HIV agents.

CONCLUSION: We have developed a comprehensive program to identify potential topical microbicides by both identifying specific activities associated with topical microbicide efficacy and placing these activities in the context of known inhibitors.

ABSTRACT 068

Structure-Activity Relationships of SJ-3366 (2, 4 (1H, 3H)- Pyrimidinediones): Inhibition of Reverse Transcriptase, Virus Attachment and HIV-1 and HIV-2 Replication

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BACKGROUND: We have recently described an N-1 substituted pyrimidinedione, SJ-3366 that inhibits both HIV-1 RT and virus entry. We undertook to specifically examine the structure activity relationships of SJ-3366 for inhibition of HIV-1 and HIV-2 replication as well as virus entry and anti-HIV-1 RT activity by homocyclic substitutions at N-1 of the pyrimidinedione.

METHODS: Seventy three cyclic N-1 substitutions were made, including cyclo-propyl, -butyl, -pentyl and 1, 2 or 3 –cyclopenten–1-yl. The analogs were evaluated for anti-HIV-1 and HIV-2 antiviral activity in a cytoprotection assay with CEM-SS cells. Analogs were also assessed for inhibition of HIV RT in a biochemical assay and virus attachment in HeLa CD4 LTR ß-galactosidase cells.

RESULTS: In general separation of the cyclic moiety from the pyrimidinedione backbone by an ethyl versus a methyl group resulted in less antiviral activity. In analogs employing the methyl spacer, substantially improved 50% inhibitory concentrations over SJ-3366 were identified (IC50 or I50) for HIV-1 (20-fold lower), HIV-2 (160 –fold lower), RT (60-fold lower) and attachment (10-fold lower) inhibition. Several analogs were found to be non-toxic at a high test concentration of 1.1 mM yielding therapeutic indexes (>500,000) equal to or better than SJ-3366. Finally, several of these analogs were assessed against fully resistant SJ-3366 resistant virus and found to retain antiviral activity.

CONCLUSION: Thus, N-1 substituted pyrimidinediones are highly potent and unique inhibitors of HIV-1 and HIV-2 replication.

ABSTRACT 069

Mechanism of Absorption of GW433908, the Phosphate Prodrug of the HIV Protease Inhibitor Amprenavir

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BACKGROUND: The *in vitro* enzyme kinetics of intestinal alkaline phosphatase (AP) from rat, dog, and human were determined with GW433908, the phosphate prodrug of amprenavir (AgeneraseTM), an HIV protease inhibitor. The conversion of GW433908 to amprenavir was investigated with two *in vitro* assays, isolated intestinal AP and intestinal brush border membrane vesicles (BBMV), to better understand the mechanism of absorption of GW433908 after oral administration.

METHODS: Rat and dog intestinal AP (1.25 U/mL and 2.0 U/mL, respectively) were incubated with the sodium salt of GW433908 (1 to 20 mM and 0.5 to 10 mM, respectively) in citrated glycine buffer (pH 10.4) for 20 min at 37°C. Reaction mixtures were quenched with sodium citrate (pH 4.8), and precipitated with methanol. Rat, dog and human intestinal BBMV fractions from duodenum, jejunum, and ileum were prepared and incubated with the sodium salt of GW433908 (0.5 to 10 mM) in mannitol buffer (pH 10.2) for 20 min at 37°C. Reaction mixtures were quenched with 0.02N HCl, and precipitated with methanol. Amprenavir concentrations were determined by HPLC with UV detection at 254 nm. Chromatographic separation was accomplished with a Symmetry C18 column (150 x 3.9 mm, Waters) and a mobile phase of acetonitrile and water delivered at 1.0 mL/min.

RESULTS: GW433908 was converted to amprenavir by incubation with AP isolated from rat and dog, and with rat, dog and human intestinal BBMV. Estimates of Vmax and Km with isolated enzyme preparations were 19.7 nmol/min/U and 8.5 mM with rat AP, and 11.6 nmol/min/U and 4.5 mM with dog AP, respectively. Conversion of GW433908 to amprenavir in BBMV correlated with known expression of alkaline phosphatase (duodenum>jejunum>ileum) in the GI tract. Estimates of Km from the rat and dog BBMV studies were similar to estimates determined with isolated AP.

CONCLUSION: Multiple-dose toxicity studies have shown that systemic exposure to amprenavir and GW433908 increases in a dose-dependent manner in rats and dogs dosed with 116 to 1737 mg/kg/day GW433908 and 58 to 579 mg/kg/day GW433908, respectively. Systemic exposure to GW433908 from these studies correlated well with predicted GW433908 GI concentrations based on GW433908 dose. Estimates of GW433908 AUC were low when the predicted GW433908 intestinal concentrations were less than twice the Km, and increased as predicted GW433908 intestinal concentrations increased from 3 to 26 times the Km. These data confirm that intestinal AP can convert GW433908 to amprenavir at clinically-relevant concentrations, and support the use of the prodrug as a delivery system for amprenavir.

ABSTRACT 070

The Valine-To-Threonine 75 Substitution in Human Immunodeficiency Virus Type 1 Reverse Transcriptase and its Relation with Stavudine Resistance

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In the fight against HIV-1, resistance to 2′3′-didehydro-2′3′-dideoxythymidine (stavudine, d4T) is modest and poorly understood at the molecular level. The amino-acid change V75T in Human Immunodeficiency Virus type 1 reverse transcriptase confers 2- to 5-fold d4T-resistance *in vivo* and *in vitro*. Valine 75 is located at the basis of the fingers subdomain of reverse transcriptase between the template contact point and the nucleotide binding pocket at the polymerase active site. Therefore, we investigated whether resistance could be reflected by discrimination against d4TTP *in vitro*. We found that V75T reverse transcriptase discriminates d4TTP relative to dTTP about 3-fold, as judged by pre-steady state kinetics of incorporation of a single nucleotide into DNA. V75T also increases the DNA polymerization rate up 5-fold by faciliting translocation along DNA or RNA templates. Unlike for thymine-associated mutations found in AZT-resistance (a.a. 41, 67, 70, 210, 215, and 219), V75T increases the reverse transcriptase-mediated repair of the d4TMP-terminated DNA by pyrophosphate but not by ATP. The hydroxyl group of threonine 75 interacts with the hydroxyl group of tyrosine 146 located out of the fingers subdomain. Replacing tyrosine 146 by phenylalanine partially suppressed both increases in rate of polymerization and pyrophosphorolysis, indicating that the novel hydrogen bond introduced between T75 and Y146 is responsible for the observed d4TTP resistance.

A molecular clone of HIV-1 bearing the V75T substitution was constructed. The V75T recombinant virus had no growth advantage relative to wild-type, indicating that its increased pyrophosphorolytic activity might balance its advantage given by an increased DNA polymerization rate. Because pyrophosphorolysis is the reversal of the polymerization reaction, this observation might point to the limited potential of pyrophosphorolysis as a general mechanism of nucleoside resistance. The recombinant V75T virus was 3-fold d4T-resistant and 4-fold PFA-resistant, consistent with the observation that the traffic of pyrophosphate is affected in V75T RT. Although a 3-fold phenotypic resistance is at the limit of significance for a viral isolate, biochemical properties of purified V75T RT are clearly those of a d4TTP-resistant enzyme. Thus, in addition to nucleotide selectivity, V75T defines a type of amino-acid change conferring resistance to nucleoside analogues that links translocation rate to the traffic of pyrophosphate at the reverse transcriptase active site. Comparison of this d4T-resistance mechanism with the ATP-mediated AZT-resistance mechanism points to the intracellular level of triphosphate analogues as one of the driving force towards the selection of drug-resistance substitutions in RT.

ABSTRACT 071

Protease Inhibitor (PI) Resistance Patterns before and after Virologic Failure of Lopinavir/ritonavir (LPV/r)-containing Regimens

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BACKGROUND: Little is known about the emergence of protease mutations following failure of LPV/r-containing regimens, and how these emerging mutations may affect resistance to other PIs. The objective of this study was to compare genotypes obtained from samples prior to LPV/r treatment with those obtained during virologic failure on a LPV/r-based regimen.

METHODS: Patients were identified who received a regimen including LPV/r and NRTIs with or without NNRTIs (but no other PIs) and who either failed to achieve a viral load (VL) <1000 copies/mL or had VL rebound to >1000 after having undetectable VL (<50 copies/mL). Protease and RT sequences were obtained on an ABI 3700 and interpreted using the Virco VirtualPhenotype(TM) from samples obtained before LPV/r and during VL failure while still taking LPV/r. Resistance is expressed as fold change (FC) in IC50 for each PI.

RESULTS: Nine male patients received LPV/r in combination with 2-4 NRTIs and 0-1 NNRTI. Most had extensive prior PI experience (median 4 PIs). Median baseline (pre-LPV/r) VL was 5.8 log10 copies/mL (range 2.3->5.9) and CD4 count was 40 cells/mm3 (range <10-190). Of the first 8 patients for whom VirtualPhenotype(TM) was available, 4 had no change in their resistance profiles: 3 remained sensitive to all PIs (mean FC IC50 <1) and 1 remained resistant to all PIs (mean FC IC50 =18). One individual lost resistance mutations, and MFC for all PIs decreased from 5 to 1; in conjunction with total absence of VL response, this is likely due to nonadherence. For the other 3 individuals, IC50 increased 4, 12 and16-fold for indinavir; 6, 31 and 53-fold for ritonavir; 9, 25 and 26-fold for nelfinavir; 4, 28 and 5-fold for saquinavir; 2, 4 and 3-fold for amprenavir. Increased PI resistance in these patients was associated with appearance of 3 or more of the following new mutations:10I, 46L, 54V, 71V, 73S, 77I, 82A, 84V, and 90M. In most cases these represent archived mutations in these PI-experienced patients.

CONCLUSION: Virologic failure of combination therapy including LPV/r was seen in association with high-level baseline resistance to other PIs (1/8) and with increasing levels of resistance to other PIs (3/8). In half of the cases (4/8), loss of mutations or absence of emerging mutations indicate that other factors, such as adherence or bioavailability issues, may have contributed to their virologic failure. The mutations selected during LPV/r failure do not appear to represent new pathways for the development of PI resistance.

ABSTRACT 072

Antiviral Resistance to SJ-3366, A Novel Dual Mechanism of Action Inhibitor of HIV-1 and HIV-2

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BACKGROUND: The anti-HIV compound SJ-3366 inhibits the replication of both HIV-1 and HIV-2 through specific inhibition of the reverse transcriptase of HIV-1 and inhibition of envelope mediated fusion events with HIV-1 and HIV-2. Against HIV-1, acting as a typical nonnucleoside reverse transcriptase inhibitor, SJ-3366 exhibits IC50 and IC95 concentrations in the low nanomolar to sub-nanomolar range. Inhibition of HIV-1 and HIV-2 entry occurs at concentrations two logs higher.

METHODS: In order to further delineate the role of the viral RT and env as the specific antiviral targets of SJ-3366, drug resistant strains of both HIV-1 and HIV-2 were selected in cell culture and the amino acid changes in RT and env were identified.

RESULTS: With both HIV-1 and HIV-2, the selection of resistant strains in cell culture was rapid and strains of virus which were completely insensitive to SJ-3366 were selected, requiring approximately 20-passages in culture for HIV-1 and 5 passages for HIV-2. With HIV-1, we have shown that the initial passages of virus yielded resistant strains that were no longer susceptible to the attachment inhibition mechanism of action of SJ-3366. This resulted in a strain of virus with approximately 1000-fold loss in sensitivity to SJ-3366. A variety of amino acid changes were detected in the envelope of HIV-1 during this early selection history (passages 1-5). The first RT specific mutation to occur was the typical Y181C amino acid change conferring NNRTI resistance. With increasing passage, additional amino acid changes in the hydrophobic NNRTI binding site were detected. At later passages the overall level of resistance of the virus to SJ-3366 continued to increase in the absence of identifiable mutations in RT. New amino changes did appear, however, in the envelope.

CONCLUSION: We believe these changes may by "fitness" changes that allow the virus to replicate efficiently with the large number of accumulated mutations in RT and env. With HIV-2, resistance also appears rapidly and no amino acid changes are detected

in the viral RT, as would be expected since SJ-3366 is a HIV-1 specific inhibitor of RT. Several amino acid changes do appear in the env. A comparison of the amino acid changes between HIV-1 and HIV-2 should yield information regarding critical changes in the env.

ABSTRACT 073

Antiretroviral Resistance Mutations in Young, HIV-1 Infected Infants and Children

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BACKGROUND: Antiretroviral therapy (ART) has markedly decreased vertical HIV-1 transmission. With the increasing use of ART, an increasing prevalence of resistance mutations has been reported, raising concerns that resistant variants will be transmitted.

METHODS: PACTG 356 is a protocol that evaluated the safety and antiretroviral activity of 3 different ART regimens in treatment-naive, HIV-1 infected infants and children. Plasma samples obtained prior to therapy were evaluated for the presence of genotypic resistance mutations using the TruGeneTM HIV-1 assay and OpenGene system (Visible Genetics, Inc, Toronto, Canada).

RESULTS: Genotyping of plasma viruses was completed on baseline specimens from 45 infants and children aged 15 days to 2 years at study entry (1997-1998). Eighteen mothers had a history of prior reverse transcriptase inhibitor (RTI) therapy and 22 infants had received limited RTI perinatal prophylaxis. None of the mothers or infants had received protease inhibitor (PI) therapy. RT resistance mutations were detected in 3 (6%) infants: K70R/T215Y in 1 infant; M41V/M184V/T215Y in 1 infant; and V108I in 1 infant. All 3 infants had only transient responses to their RTI ART regimens (ZDV/3TC/nevirapine or ZDV/3TC/nevirapine/abacavir). Multiple protease polymorphisms (L63P > V77I/T> L10I/V, M36I, A71T, K20R) were detected singly and in combination; mutations at aa 30, 48, 82,84, and 90 were not observed.

CONCLUSIONS: RTI resistance mutations were detected infrequently in this cohort of young, vertically-infected infants. When detected, however, they were associated with poor control of viral replication. Continued surveillance of early infant viruses for resistance mutations will have important implications for the design of ART regimens for infected infants and children.

ABSTRACT 074

HIV-1 RT Mutations K65R and L74V are Incompatible *in vitro*

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BACKGROUND: Interactions among drug-selected mutations of HIV-1 reverse transcriptase (RT) alter drug susceptibilities and viral replication in cell culture-based assays. In a recent study, HIV-1 isolates resistant to (-)-b-D-dioxolane-guanosine (DXG) were selected by serial passage of HIV-1LAI in increasing DXG concentrations. During two independent experiments, passaging of viruses in the presence of drug yielded resistant isolates that contained either the K65R or L74V mutations (AAC 44:1783,2000). It was hypothesized that the isolation of two distinct resistant mutants resulted from the presence of quasispecies in the starting virus population or variants evolving during drug selection pressure. We sought to determine the interaction of these RT mutations in a homogenous viral population.

METHODS: Point mutants K65R, L74V, and double mutant K65R + L74V were made in a pNL4-3 background by site-directed mutagenesis (SDM). Viruses were produced by transfecting PHA-stimulated human peripheral blood mononuclear cells (PBMC). In order to assess the stability of the mutants, infections were done at different multiplicity of infections (MOI).

RESULTS: Efficient viral replication was achieved for all the SDM mutants post-transfection. To assess the stability of the double mutant 65R + 74V in a homogenous population of viruses, a series of infections using different amount of the viruses were performed. The cultures were grown for 6 days and genomic DNA and HIV RNA from supernatants were PCR amplified and sequenced by automated DNA sequencer. Analysis of sequences from genomic DNA revealed the presence of mixture of provirus with K65 (wild type) and R65 (mutant) in all the infections conducted with $TCID_{50}$s 1666, 333, 166.5, 33.3, and 3.3 per million PBMC. However, viral RNA sequencing showed the presence of 100% wild type sequence at codon 65 for all infections, indicating the rapid reversion of 65R⇒K during the replication of double mutant virus. In contrast to K65R, the second mutation L74V was retained both in the cellular provirus and infectious virus (supernatant) on day 6 at the end of assay.

CONCLUSION: No infectious virus in the supernatant contained both mutations, indicating that mutations K65R and L74V are not compatible in pNL4-3 background. Our results confirm the absence of the

selection of double mutant during passaging of HIVLAI in the presence of DXG in a previous study. Our observations also suggest that during clinical trials with drugs known to select these mutations, the double mutant (65R + 74V) virus will be rarely found in isolates due to this incompatibility.

ABSTRACT 075

Double HIV Mutants with L74V and M184V Mutations Retained their Deleterious Effect on Viral Replication

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BACKGROUND: Drug-selected mutations L74V and M184V lie in a critical region of HIV-1 reverse transcriptase (RT) and have been shown to confer loss of replication fitness to the virus in cell cultures. Recent clinical trials demonstrated that the selection of double mutants with L74V + M184Vgenotype can occur in subjects that are treated with the combination of abacavir, lamivudine and zidovudine. We sought to analyze, if the replication disadvantage due to either L74V or M184V mutations is retained in the double mutant L74V + M184V.

METHODS: Site-directed mutagenesis (SDM) was used to create point mutants L74V, M184V and double mutant L74V + M184V in a pNL4-3 background. Viruses were generated post-transfection of PHA-stimulated human peripheral blood mononuclear cells (PBMC). Replication kinetics of SDM mutants were compared with wild type virus by infecting PBMC with equivalent amount of viruses and quantifying virus production at different time points in culture supernatants.

RESULTS: Efficient viral replication was achieved for all the mutants post-transfection. Comparison of growth kinetics revealed that all the RT mutants replicated less efficiently than the parent wild type virus. The double mutant L74V + M184V produced 33% less virus on day 8 as compared to wild type virus. The presence of mutations was confirmed by sequencing of genomic DNA, as well as by an HIV LiPA.

CONCLUSIONS: All three mutant viruses containing L74V, M184V and L74V + M184V mutations in pNL4-3 background replicated less efficiently compared to wild type virus. The double mutant (74V + 184V) replicated similar to single mutant viruses indicating the absence of any compensatory effect due to the presence of two RT mutations together. These studies will have an impact in optimizing salvage therapy.

ABSTRACT 076

Single-Step Establishment of HIV-Permissive Indicator Cells in the Development of a Sensitive and High-Throughput Phenotypic Assay for Monitoring Antiretroviral Drug Resistance

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Phenotypic antiviral drug resistance assays have been shown to be accurate and increase the efficacy of HAART. However, there has been a need to improve the efficiency and sensitivity of phenotypic assays for monitoring large numbers of patients and for the early detection of drug-resistant minor species. An ideal phenotypic assay should also be applicable to all classes of anti-HIV drugs, including entry-inhibitors, viral protein antogonists, and capsid lockers. We have developed such an assay system using a molecular vector system to produce indicator cells that are permissive to HIV-1 replication. Using these indicator cells, assays can be performed using a direct infection approach with patient sera. To achieve this, we developed a complex adenoviral vector that carries a molecular switch controlling a reporter gene (GFP or lacZ) and genes encoding the HIV-1 receptor and co-receptors (CD4, CXCR4, and CCR5). One-step transduction with the vector introduces the molecular switch-controlled reporter gene into target cells and produces high-level expressions of CD4, CXCR4, and CCR5 on the cell surface. The overexpression of the receptor and co-receptors make the indicator cells susceptible to both T- and M-tropic HIV-1 infection and the molecular switch/reporter gene provides a sensitive mechanism to detect HIV-1 replication. Thus, the vector is capable of transforming a variety of established tumor cells into indicator cells for phenotypic assays. Subsequent HIV-1 infection and replication result in the activation of the molecular switch and high-level expression of the reporter gene. The signal can then be quantitatively measured by standard spectrophotometric or spectrofluorometric microplate readers. The molecular switch was selected from nine engineered promoters that contain basic promoters and multiple TAR sequences arranged in different arrays. This construct, highly sensitive to HIV TAT protein with minimal background activity, was inserted into the complex adenoviral vector. The indicator cells, converted by the vector, can be grown as adherent monolayer cultures in multi-well plates. Infection of these cells with virus-containing sera allows the quantitation of HIV-1 replication in the presence and

absence of antiretroviral drugs by measuring the intensity of the reporter signal. Under experimental conditions, we have shown correlations between signal intensities, viral replication, and drug inhibition. By comparison with an infected control culture that contained no drug, we were able to determine the overall percentage of drug-resistant virus in the population and to calculate the percentage or fold-increase in the IC50. Thus, antiretroviral drug-resistant HIV-1 isolates can be measured directly and quantitatively with this system. Since PCR or cloning steps are not involved, this assay measures directly the intrinsic drug resistance of the original virus population and it can be performed with all classes of antiretroviral drugs. This assay should be capable of readily detecting low levels of drug-resistant virus particles in a mixture with predominantly wild-type virus, since only the resistant virus will replicate in the indicator cells in the presence of drug and trigger the expression of the reporter gene. The new assay promises to be a simple, sensitive, and high-throughput phenotypic test for HIV-1 drug resistance.

ABSTRACT 077

Pharmacokinetics (PK) of Nelfinavir (NFV) in African American and Caucasian HIV Patients

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BACKGROUND: HIV infection is an increasing problem in African Americans. This study was performed to compare the PK of NFV and its active metabolite M8 in African Americans and Caucasians with HIV infection.

METHODS: This was an open-label, parallel-arm study. Thirty-six patients (18 in each group) were enrolled. Patients received NFV 1250 mg BID and two reverse transcriptase inhibitors for at least 7 days. Concentrations of NFV and M8 were measured.

RESULTS: Results of nelfinavir PK (median and range) from 35 patients with evaluable data are presented in the following table.

N	AUC0-12 (mg*h/L)	Cmax (mg/L)	Ctrough (mg/L)	AUC ratio (%M8/NFV)
AA 18	27.9(4.7-88.2)	4.3(0.6-10.7)	2.3(0-8.1)	31(0-69)
C 17	23.5(7.7-54.0)	3.6(1.5-7.0)	1.5(0.3-5.9)	23(0-63)

AA: African American, C: Caucasian

No serious AEs were observed in this study. None of the PK parameters were significantly different between African Americans and Caucasians ($p > 0.05$). Although there were few female patients (3 in each group) on which to perform a formal comparison, PK in these female patients were in the same range of the male patients.

CONCLUSION: These data suggest that NFV PK were similar in African American and Caucasian HIV patients.

ABSTRACT 078

Once-daily Indinavir/ritonavir in Patients who cannot adhere to more Complex Regimens: the PIPO Study

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BACKGROUND: At this moment triple therapy is able to provide long-term suppression of viral replication, but adherence issues (food restrictions, frequent dosing) may hamper long-term treatment with these combinations. Therefore, it is important to explore drug regimens that are easy to take (e.g., no food restrictions, once-daily (OD) dosing) and have acceptable toxicity, while the antiviral activity is preserved.

METHODS: The PIPO study is an open-label pilot phase II study to investigate the pharmacokinetics and safety of once-daily antiretroviral combinations that include indinavir 1200mg + ritonavir 400mg. Antiviral efficacy is a secondary endpoint. HIV-1 infected patients who were not able to adhere to other regimens in the past or prefer a once-daily regimen are eligible for this 6-month period study. Besides indinavir+ritonavir, at least one other antiretroviral agent is administered. Pharmacokinetics of indinavir are measured two weeks after the start. Safety and antiviral parameters are monitored at monthly visits.

RESULTS: Sixteen patients have been included so far, of whom five were treatment-naïve. Eleven patients were (former) IV drug users. Concomitant medications (all once-daily) were: lamivudine 300mg (n=6), stavudine 60-80mg (n=4), stavudine+lamivudine (n=4), nevirapine 400mg (n=1) or efavirenz 600mg (n=1). Indinavir pharmacokinetics were available from seven patients: median AUC0-24h, Cmax, and Cmin were 99 mg/L.h, 13.1 mg/L and 0.16 mg/L, respectively. Only one patient had a Cmin below the proposed minimum effective threshold of 0.1 mg/L. Nine patients have completed the 6 month of follow-up: eight patients were still using the once-daily regimen; one patient switched to a twice-daily regimen because of virological relapse. From the seven patients who have not yet completed the 6 month follow-up period, one patient has stopped the once-daily regimen because of anemia. No other serious adverse events were observed, in particular no nephrolithiasis or hematuria. Serum creatinine increased from a median value of 60 μM at baseline (n=16) to 81

μM at month 3 (n=10), but then stabilized: 83 μM at month 6 (n=9).

Median baseline viral load was 280,000 copies/mL (n=16). After 2, 4, and 6 months of follow-up the percentage of patients with a viral load < 500 copies/mL was 71% (n=14), 80% (n=10), and 89% (n=9), respectively. CD4 counts rose from a median baseline value of 60 cells/mm3 (n=16) to 160 cells/mm^3 after 6 months of treatment (n=9).

CONCLUSIONS: In conclusion, the PIPO study shows that at steady-state the indinavir concentrations are above 0.1 mg/L in all but one patient. The regimen was tolerated well with no signs of nephrological toxicity so far, with the exception of a slight increase in serum creatinine which seemed to stabilize after 3 months. The virological and immunological response was remarkably good for a patient population who in majority had failed previous regimens, including other once-daily regimens.

ABSTRACT 079

Intracellular Carbovir Triphosphate (CBVTP) Levels among Patients Taking Abacavir Once Daily

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BACKGROUND: Relatively long intracellular half-lives of active triphosphate metabolites support once daily dosing of nucleoside analogues such as ddI and 3TC. The standard dose of abacavir is 300 mg twice daily, and previous work has shown a mean CBVTP concentration of 100 fmoles/1 million cells 12 hours after a 300 mg abacavir dose. No data are available for intracellular levels of the active nucleotide CBVTP among patients taking abacavir 600 mg once daily.

METHODS: Six HIV-positive patients received abacavir 600 mg once daily as part of a stable (>30 days) combination regimen. Blood samples were drawn at 0 hours (24 hours after the previous abacavir dose) and 1, 12, 14-16, 18, 20, 22, and 24 hours following an observed 600 mg abacavir dose. After cell extraction, CBVTP and endogenous dGTP levels were determined in the extracts by enzymatic primer extension assays.

RESULTS: Five men and one woman had been taking abacavir 600 mg daily for 5-17 months (median 7). For the first 4 patients, CBVTP levels (fmoles/1 million cells, mean +/- SD) were 172 +/- 47 at 0 hours, 166 +/- 89 at 1 hour, 237 +/- 142 at 14-16 hours, 132 +/- 49 at 20 hours, and 152 +/- 61 at 24 hours. The half-life of CBVTP was estimated to be >12 hours.

CONCLUSION: Preliminary results of 24-hour CBVTP concentrations support the use of once daily abacavir. Further studies are needed.

ABSTRACT 080

Delivery and Transport of Antisense Oligonucleotides Against HIV-1 *in vitro* and *in vivo*

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BACKGROUND: An alternative strategy has been the use of antiviral genes that are delivered to uninfected cells as either RNA or DNA and provide intercellular protection from the human immunodeficiency virus type-1 (HIV-1). Several strategies targeting HIV-1 gene expression have been shown to be effective in inhibiting virus replication, and these include intracellular expression of transdominant proteins, antisense molecules, ribozymes, and intracellular antibodies. Antisense oligonucleotides complementary to specific target genes are made available to suppress gene expression. A variety of techniques are available to enhance the cellular uptake and pharmacological effectiveness of antisense oligonucleotides in the *in vitro* and *in vivo* setting. The selection of technique depends on the situation of investigation, the possibility of cytotoxity due to the delivery agents, and the brief and convenience of the approach. We have investigated intracellular and tissue uptake of antisense oligonucleotide/cationic lipid complex using fluorescent labeled oligonucleotide.

METHODS AND RESULTS: The antisense oligonucleotide was targeted to HIV-1 *gag* gene. We confirmed that the antisense oligonucleotide inhibited the expression of HIV-1 p24 antigen in the T-cell line (MOLT-4 cl.8) and PBMCs. The MOLT-4 cl.8 cells and PHA stimulated PBMCs were infected with HTLV-IIIB at a MOI of 0.01. After an hour infection, the cells were washed and treated with the antisense oligonucleotide at a 1 μM concentration in the culture medium. After 2 days, the medium was changed to a fresh medium containing the same concentration of 1 μM of antisense oligonucleotide on the MOLT-4 cl.8 cells. We investigated the expression of HIV-1 antigen by IF every 4 days. In this result, the antisense oligonucleotide completely suppressed viral antigen within 10 days. Also the antisense oligonucleotide/cationic lipid complex inhibited the amount of p24 antigen at 1/10 in PBMCs after 3 days viral infection. Furthermore, we

confirmed that the antisense oligonucleotide/cationic lipid complex existed intracellulary by fluorescent microscopy. Again, we investigated transporting to tissue in mice by intravenous, intraperitoreal or subcutaneous injection of the antisense oligonucleotide/cationic lipid complex. The antisense oligonucleotide/lipid complex was injected at 300 μg per mouse for 3 days. We observed that the antisense oligonucleotide/cationic lipid complex was transported to the heart, kidney, spleen and liver.

CONCLUSION: We investigated that anti-HIV-1 activity of the antisense oligonucleotide/cationic lipid complex in the T-cell line and PBMCs. We therefore conclude that the antisense oligonucleotide/cationic lipid complex activity was not limited to the T-cell line and PBMCs, but also in many tissues of the mice by intravenous or subcutaneous injection.

ABSTRACT 081

Concurrent Intracellular Pharmacokinetic studies of Zidovudine- and Lamivudine-triphosphates in Hispanic HIV-infected Patients

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Nucleoside reverse transcriptase inhibitors (NRTIs) used against the Human Immunodeficiency Virus (HIV) require intracellular activation to their triphosphate moiety to inactivate HIV replication. Recently, we have used Solid Phase Extraction (SPE) coupled with Tandem Mass Spectrometry (MS/MS) to obtain *in vivo* intracellular concentration measurements of ZDV-TP and 3TC-TP. We have performed an intensive intracellular pharmacokinetic study (pre-dose, 1, 2, 4, 8, 12, 16, and 24 hr post dose) measuring ZDV-TP and 3TC-TP in five HIV-infected patients with a standard dose of 150 mg 3TC (BID) and 300 mg ZDV (BID). Intracellular pharmacology parameters were substantially different between ZDV-TP and 3TC-TP at steady state. The median *in vivo* intracellular half-life for ZDV-TP was estimated to be 9.1 hrs (range 4.7-11.9), while for 3TC-TP was 24.8 hrs (range 14.4-49.5). Furthermore, 3TC-TP concentrations were always higher than ZDV-TP, and significant intracellular levels were maintained for 3TC-TP (>3000 fmol/106 cells) at 24 hrs in all five patients. In only one patient, ZDV-TP concentrations were quantifiable at 24 hrs. These preliminary data suggest that exposure to 3TC-TP is sufficient with only 150 mg of 3TC a day while ZDV should be given twice daily. To the best of our knowledge, this is the first study that concurrently measures both NRTI metabolites *in vivo* in a clinical setting.

ABSTRACT 082

Forgiveness Quotient: Method for Evaluating the Advantage of Ritonavir-Induced Pharmacokinetic Boosting of HIV Protease Inhibitors

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INTRODUCTION: The use of low-dose ritonavir (100 - 200 mg bid) to inhibit metabolism (decrease clearance), thus increasing exposure and trough values (Cmin) of amprenavir, saquinavir, indinavir, and lopinavir has gotten wide-spread use in the treatment of HIV disease. Because antiviral efficacy correlates most closely with trough values, this approach has increased the efficacy and convenience of PI-based regimens — reducing the number of pills and daily doses. Even with improved convenience, patients still miss occasional doses. Decreased adherence has been shown predictive of antiviral effect and durability. Here in we attempt to estimate the relative "forgiveness" of missing a dose in a ritonavir-boosted PI regimen.

METHODS: Inhibitory quotient (IQ or Cmin/IC50) were calculated at 12 and 24 hours post dose for amprenavir/RTV and lopinavir/RTV (600/100 or 400/100 mg, respectively). The forgiveness quotient (FQ) is defined as the IQ after a missed dose (e.g. 24 hours post dose in a BID regimen). IC50 values (mean) were determined in 15% serum against WT laboratory strains of HIV or clinical isolates (n = 28) from PI-experienced subjects, then numerically adjusted for protein binding. The 24-hour post dose drug concentrations were estimated using the Cmin values (mean, reported previously) at 12 hours and the ritonavir-boosted half-lives of amprenavir and lopinavir. This method assumed that the boosted half-life was maintained over the following 24 hours, arguably the best case scenario for both drugs because the ritonavir-effect decreases over time. We also calculated the FQ for a QD amprenavir/RTV regimen (1200/200 mg). Pharmacokinetic data for indinavir and saquinavir in HIV-infected subjects was not available at the time of abstract preparation but an analysis may be presented at the meeting.

RESULTS:

	APV IQ (WT)	LPV IQ (WT)	APV IQ (PI exp)	LPV IQ (PI exp)
IC50 (unadjusted)	80 nM	18 nM	127 nM	226 nM
Protein Binding	90%	98.5%	90%	98.5%
T1/2 apparent (hour)	17	5.4	17	5.4
IQ (Cmin/IC50)	3.6	4.6	2.2	0.4
FQ (BID 1 missed dose)	2.3	1	1.4	0.08
IQ (QD)	3.6	ND	2.2	ND
FQ (QD 48 h post dose)	1.2	ND	0.74	ND

CONCLUSION: Because of amprenavir's long half-life, and distinct resistance profile, it has the highest FQ against both WT HIV and virus from PI-experienced subjects. This suggests that ritonavir-boosted amprenavir regimen may be the most forgiving protease inhibitor.

ABSTRACT 083

A Pilot Trial of Didanosine, Indinavir, Lamivudine, and Ritonavir as a Once-Daily, 4-Drug Regimen for HIV Infection

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BACKGROUND: Adherence to complicated medication dosing regimens remains a major barrier to successful antiretroviral (ARV) therapy. Of the ARV medications currently approved by the FDA, only 2, didanosine and efavirenz, are indicated for once-daily dosing. Recently, pharmacokinetic data presented by two groups support modeling of once-day combinations of ritonavir and indinavir. The following describes tolerance of, pharmacokinetic, and virologic outcomes from a pilot trial of once-daily didanosine, indinavir, lamivudine, and ritonavir through the initial 24 weeks of therapy.

METHODS: Ten HIV seropositive subjects received informed consent and were enrolled under the following Inclusion/Exclusion criteria; ARV naïve, any CD4 lymphocyte count, a plasma HIV viral load > 1,000 copies/ml, absence of multi-drug genotypic resistance, normal renal function and liver enzymes within 5 times upper normal limit. All subjects received didanosine (400mg) 30-60 minutes before a meal followed by indinavir (1,200mg), lamivudine (300mg), and ritonavir (400mg) with a meal. Virologic response was measured with plasma HIV viral load (VLpl,) and CD4+ lymphocyte count. Pharmacokinetic (PK) studies were performed at week 8 for Indinavir and Ritonavir.

RESULTS: Seven of ten subjects have completed at least 16 weeks of therapy with 6 of 10 completing > 28 weeks. Toxicities experienced by subjects were typically mild and consistent with those commonly reported for each of the medications. One subject developed mild hematuria at Week 24 without evidence of a renal stone. Cholesterol and triglycerides levels increased in most subjects with one subject developing a non-fasting cholesterol > 300 mg/dl (baseline cholesterol of 244 mg/dL) and no subjects developing triglycerides > 1,000 mg/dL. Median baseline VLpl and CD-4+ lymphocyte count were 29,292 copies/ml (log10 4.47, range 4,834-356,695) and 222 cells/mm3 (range 36-340), respectively. All ten subjects had VLpl levels < 500 copies/ml by week 12 with 8/10 at < 50 copies/ml. The two remaining subjects with detectable HIV viral load reached < 50 copies/ml at weeks 20 and 28. Median absolute CD4+ lymphocyte counts increased by 223 cells/mm3 for the 7 of 10 subjects with week 24 results. Indinavir and ritonavir plasma concentrations (n=8) remained above IC95 concentrations throughout the 24-hour dosing interval for 4/8 and 7/8 subjects, respectively. All subjects with below IC95 concentrations of either or both PIs reached this threshold between sampling hours 19 and 24. However, this lack of 24 hour coverage above the IC95 for either PI was not associated with virologic failure.

CONCLUSION: The data collected in this pilot study demonstrates that this 4-drug regimen is well tolerated, provides excellent virologic suppression despite 'sub-therapeutic PI concentrations' and is supportive of further study. Such studies should delineate the relationship between IC95 and both PIs relative to virologic success or failure.

ABSTRACT 084

ICC-602: A Pilot Study to Evaluate the Pharmacokinetics, Safety and Efficacy of a Novel BID Dosing Regimen of Nelfinavir, Indinavir, and Efavirenz in Treatment-Naïve Patients

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BACKGROUND: The complex pill-taking requirements of chronic combination therapy regimens compromise patient compliance and antiretroviral efficacy. The objective of the ICC (Inter-Company Collaboration for AIDS Drug Development) 602 Study

is to obtain preliminary data on the pharmacokinetics, safety, tolerability, and efficacy of a novel BID dosing regimen of nelfinavir + indinavir + efavirenz in PI/NNRTI naïve patients.

METHODS: This is an open-label 48 week study where PI and NNRTI treatment naïve patients with plasma RNA > 10,000 copies/mL received treatment with a BID regimen of NFV (1250mg BID) + IDV (1200mg BID) + EFV (600mg QD). Safety, tolerability, and antiretroviral efficacy are assessed every 4-8 weeks. This is the first concomitant use of these three antiretrovirals and a pharmacokinetic substudy was conducted in 6 patients to investigate potential PK interaction among the three study treatments. Plasma samples were obtained 0-24 h for EFV and 0-12 h for IDV and NFV after at least 2 weeks of treatment.

RESULTS: Eight patients (5 M/3 F) were enrolled in the study. Mean and (SD) PK parameters are reported. AUC0-24 for IDV and NFV were calculated from AUC0-12 or AUC0-8.

Drug	Study	N	AUC0-24	Cmax	Ct	t
EFV	ICC 602	6	148 (62)	9.9 (4.0)	4.7 (2.6)	24
EFV	Packg label	35	184 (73)	12.9 (3.7)	5.6 (3.2)	24
IDV	ICC 602	5	105 (27.9)	17.9 (6.2)	0.14 (0.10)	12
IDV	Pkg label (800 q8h)	16	92.1 (34.2)	12.6 (4.0)	0.25 (0.18)	8
NFV	ICC 602	6	66.6 (25.4)	4.8 (1.8)	1.1 (0.6)	12
NFV	AG1343-542	10	52.8 (15.8)	4.0 (0.8)	0.7 (0.4)	12

EFV & IDV units are mM·h and mM; NFV units are mg·h/mL and mg/mL; t is hours. Four patients discontinued the study (adverse event, treatment failure, Non-adherence, patient moved). Three patients completed 48 weeks on study, and two have plasma RNA levels < 50 copies/mL at week 48. Three SAEs were reported. Overall, the regimen was well tolerated.

CONCLUSION: Preliminary data suggest adequate drug concentrations were achieved on a simplified twice-a-day dosing regimen of nelfinavir + indinavir + efavirenz in HIV patients. This dual PI + NNRTI regimen was generally well tolerated and effective in a small number of treatment naïve patients when taken for up to 48 weeks.

ABSTRACT 085

The Effectiveness of Antiretroviral Therapy is diminished among Patients Starting Treatment with CD4+ Cell Counts below 200/mm^3

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OBJECTIVE: To characterize the effectiveness of antiretroviral therapy initiated at various CD4 and plasma HIV-RNA thresholds.

METHODS: This is a population-based analysis of antiretroviral therapy naive HIV+ persons 18 years who initiated triple combination therapy between August 1, 1996 and September 30, 1999. Rates of progression from the initiation of antiretroviral therapy to death were determined stratified using various CD4 and plasma HIV-RNA thresholds. Risk ratios of factors associated with mortality were estimated using Cox-proportional hazard models. An intent to treat principle was used in all analyses.

RESULTS: A total of 1,219 persons (909 receiving PI and 310 receiving NNRTI containing regimens) were eligible. As of January 31, 2000, 72 patients had died of AIDS-related causes, for a crude mortality rate of 5.9%. The cumulative mortality rate at 12 months were 3.2% + 0.5%. In univariate analyses, a prior AIDS diagnosis, CD4+ cell count, and HIV-RNA levels were found to be associated with mortality. There was no difference in mortality by age, gender, or PI use. In multivariate analyses, only CD4+ cell count remained significantly associated with death. After controlling for AIDS diagnosis and baseline plasma HIV-RNA levels, the adjusted risk ratio was 7.36 (95% CI: [3.82, 14.21]; $p < 0.001$), and 3.17 (95% CI: 1.69, 5.93; $p < 0.001$) for patients with CD4+ cell counts <50 cells/mm3, and 50 to 199 cells/mm3 respectively, compared to those with CD4+ cell counts 200 cells/mm^3.

CONCLUSION: Our data demonstrates that the effectiveness of antiretroviral therapy is independent of age, gender, AIDS-diagnosis, protease-inhibitor use, and plasma HIV-RNA levels, but dependent on CD4 levels. More importantly, the effectiveness of antiretroviral therapy on survival is compromised in patients initiating therapy with CD4+ cell counts <200 cells/mm^3.

ABSTRACT 086

Pharmacodynamic Model Development and Clinical Trial Simulations of HIV Treatment with Nucleoside RT and Protease Inhibitors

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There is a need for adequate models that can maximize the information obtained during the drug development process to help predict clinical outcomes for HIV as a function of time and dose for dual or triple combinations of antiviral drugs. A combined *in vitro/in vivo* pharmacodynamic model was used to link concentrations of antiviral drugs to a previously

published predator-prey model of HIV and CD4 dynamics (Stafford M. A., *et al, J. Theoret. Biol. 203*: 235-301; 2000) to predict drug effects as a function of time and dose regimen. We assumed that the primary mechanism of action of the RT inhibitors was due to the additive interaction of the active nucleoside triphosphates (NTP) with the HIV-1 RT. Fractional inhibition of RT was modeled as: $I = (1-(A + B)/(1 + A + B))$ where $A = (CE_1/CE_{51})^{n1}$ and $B = (CE_2)/CE_{52})^{n2}$. Where CE_1 and CE_2 are the respective cellular concentrations of the NTPs; CE_{51} and CE_{52} are NTP concentrations that inhibit RT by 50%, while n1 and n2 are the respective concentration exponents. Cellular accumulation and egress rate constants of the NTPs were estimated using published Phase I clinical trial data, while virus inhibition constants (CE_{51} and CE_{52}; n1 and n2) were obtained from cell-free inhibition studies of the interaction of HIV-1 RT by active NTPs. The fractional inhibition of HIV-1 protease due to Indinavir was estimated as $C/(C+C_{50})$, where C is the plasma concentration of Indinavir and C_{50} is the concentration needed to inhibit the protease *in vitro* by 50%. Average plasma concentrations were calculated using the formula AUC/(dose interval), where AUC is the single dose area under the plasma concentration *versus* time curve obtained from Phase I pharmacokinetic studies. Simulations using simultaneous differential equations involving these parameters produced HIV and CD4 profiles that were in agreement with results from large Phase II and III clinical trials. The possibility now exists to predict the extent of virus depletion in future clinical trials using preclinical data and pharmacokinetic parameters from initial single dose studies.

ABSTRACT 087

Aerosol Interleukin-2 (IL-2) Liposomes: Good Manufacture Practice Synthesis

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BACKGROUND: IL-2 is an attractive therapeutic candidate for immune restoration in HIV infection. Unfortunately, IL-2 is currently administered intravenously or subcutaneously and is commonly associated with significant toxicity.

METHODS: Recombinant non-glycosylated human IL-2 and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) are used for making IL-2 liposomes. One-ml sterile water was added to each vial of IL-2. Then, the contents of eight IL-2 vials were added to a 100-mL vial containing 2.5 gm of sterile DMPC powder. This suspension was vortexed until powder was completely hydrated, bath sonicated to create more uniform particle size and disperse aggregates, frozen in a dry ice /ethanol bath for 5 minutes, then thawed in a 37°C water bath 10 minutes for 3 cycles. To dilute and dispense unit doses, 83.3 sterile 0.9% saline USP was added to one vial of thawed IL-2 liposome bulk concentrate. Diluted IL-2 liposomes were then added in 1.1-ml portions into empty vials. Each unit dose contained 1 mL with 20 mg DMPC and 1 x 106 IU IL-2. The size and uniformity of the liposomes was determined using a FACScan apparatus equipped with an argon laser. Forward light scatter (FSC) was recorded for 10,000 events /sample. Linearity of FSC was established using reference polystyrene beads. A histogram depicting FSC versus the number of evens was generated and compared to the FSC corresponding to the size standards. Bioactivity of the IL-2 liposomes was assessed by a 3H-thymidine incorporation assay with the IL-2-dependent CTLL-2 cell line. The degree of IL-2 incorporation to the liposomes was calculated by comparing the bioactivity of the pelleted liposomes and the supernatant after centrifugation of 1 mL of a 10-fold dilution of IL-2 liposomes for 10 minutes. The percent IL-2 incorporation in the liposomes was calculated by using the dilution of the pellet (P) or the supernatant (S) required to result in 50% CPM in the following formula: $[P / (P + S)] \times 100$.

RESULTS: FACS analysis demonstrated uniform liposome size with >94% of particles being less than 4.2 mm in diameter and >99% less than 21.1 mm, as compared to standard size beads. Size distributions were almost identical for every lot of IL-2 liposomes tested. IL-2 bioactivity assays demonstrated that IL-2 liposomes contained bioactive rIL-2, supporting the growth of the CTLL-2 cell line in a dose dependent manner, comparable to free rIL-2. When bioactivity of the liposome pellet was compared to that of the supernatant, 98-99% of bioactive IL-2 was associated with the liposome pellet.

CONCLUSION: Using novel approaches, such as the aerosolized route and liposomal encapsulation, may enable and facilitate the chronic administration of IL-2 avoiding the toxicities associated with parenteral IL-2. Clinical studies in patients with HIV, hepatitis C, and primary immune deficiencies are underway.

ABSTRACT 088

Autologous Monocyte-Derived Dendritic Cells as a Possible 'Cellular Adjuvant' for a Therapeutic Vaccine in HIV-1+ Patients. *In vitro* Studies

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BACKGROUND: There is evidence suggesting that combining HAART with a therapeutic vaccine able to

induce/boost anti-HIV-1 T-cell responses could be effective to control HIV-1 replication after stopping HAART. Dendritic cells (DC) are the most potent professional antigen-presenting cells, unique to induce primary and secondary responses of CD4+ and CD8+ T lymphocytes. In a clinical setting, monocyte-derived DC (MO-DC) are the most appropriate to use as a 'cellular adjuvant' for a HIV-1 therapeutic vaccine.

OBJECTIVE: To investigate the obtention of MO-DC in conformity with GMP (Good Manufacturing Practice) conditions, and whether they are able to present HIV-1 antigens.

RESULTS: a) free-serum medium (X-VIVO-15) supplemented with 1% of autologous serum are GMP conditions that allow to obtain MO-DC with optimal phenotypic and functional features; b) they can be criopreserved with no loss of their functional properties, provided that antigen pulsing is done after thawing; c) the yield and features of MO-DC in early HIV-1-infected patients receiving HAART were identical to those of normal individuals; d) t MO-DC pulsed with HIV-1 recombinant antigens and heat-inactivated HIV-1 are able to activate CD4 and CD8 T cells from both HIV-1-infected and uninfected individuals. The presence of anti-HIV-1 antibodies in 1% autologous serum favors the antigen capture and activation of MO-DC.

CONCLUSION: Data support the use of autologous MO-DC as a cellular adjuvant for a HIV-1 therapeutic vaccine.

ABSTRACT 089

Pharmacokinetic and Pharmacodynamic Profile of Mycophenolic Acid (MMF) Associated with HAART Chronic HIV-1 Infected Patients (CHI) with CD4+ Count >=500

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OBJECTIVE: To evaluate the pharmacokinetic profile of MMF in early stage of CHI patients receiving low doses (0.25 g bid) plus HAART and to test its efficacy *in vitro* to inhibit spontaneous proliferation of HIV infected cells.

METHOD: 20 CHI patients in very early stage (BVL <5,000 c/ml and CD4+ T cells >500) were treated with abacavir+efavirenz+nelfinavir for 12 months. Thereafter, they were randomized to receive the same HAART vs. the same HAART plus MMF (0.25 g bid) during 4 additional months. The pharmacokinetic profile of MPA (area-under-the-curve, $AUC_{0\text{-}12h}$) was carried out with blood samples (EDTA) collected at 0 (predose), 20, 40 min and at 1, 2, 4, 6, 8, 10 and 12 hours after MMF morning dose. The $AUC_{0\text{-}12h}$ was analyzed at 7, 28 and 120 days post MMF treatment. All specimens were measured by a previous validated HPLC/UV method. The capacity of the patient sera to inhibit the proliferative response of a T cell line (CEM) was also tested.

RESULTS: Data of first 7 out of 10 patients randomized to receive HAART+MMF are presented. The median value obtained for Cmin, Cmax, tmax and AUC0-12h demonstrate a stable PK profile over the time. Cmin (microg/mL) 0.76 at day 7, 0.70 at day 28 and 0.52 at day 120. Cmax (microg/mL): 1.95 at day 7, 2.94 at day 28 and 3.4 at day 120; C2h (hours): 1 at day 7, 1 at day 28 and 1 at day 120; $AUC_{0\text{-}12h}$ (microg x h/mL): 15.03 at day 7, 14.14 at day 28 and 14.7 at day 120. In 3 out of 7 patients (43%) the $AUC_{0\text{-}12h}$ increases about 22% from 7th day (range 10.37-13.14 microg x h/mL) to 28th day (range 14.20-17.00 microg x h/mL). Four patients had $AUC_{0\text{-}12h}$ stable values during the same period. At the end of the first month, 5 patients had $AUC_{0\text{-}12h}$ values $\geq$15 microg x h/mL, and two patients $AUC_{0\text{-}12h}$ values of 13.21 and 13.17 microg x h/mL. The patients sera pre MMF treatment did not modify the spontaneous proliferation of a T cell line; CEM response at day 0 was 88 %. After MMF treatment, CEM response decreases to 41 % and 48 % on days 7 and 28, respectively.

CONCLUSION: These results suggest that there is a strong correlation between MPA $AUC_{0\text{-}12h}$ values (range: 13.2-21.3 microg x h/mL) and its *in vitro* immunosupressive effect (48% inhibition CEM response). A dose of 0.25 g bid of MMF seems adequate to obtain the targeted effect about 50% inhibition of the proliferative response.

ABSTRACT 090

Safety, Efficacy and Resistance Profile under ABT 378/r in the German Expanded Access Program (EAP)

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BACKGROUND: ABT 378/r is a new protease inhibitor (PI). The objective of this study was to examine safety, efficacy and possible development of viral resistance under treatment with ABT 378/r in heavily pretreated HIV-patients within the German EAP.

METHODS: We determined viral load (VL) monthly, CD4 cell count, blood lipids and glucose. In addition

genotypic resistance testing was performed in all patients at baseline and in case of treatment failure (<1,5log decrease in HIV RNA from baseline or rebound >1000cps/ml) at week 12 and/or 24.

RESULTS: 40 patients have been enrolled in the study. 36 (90%) of these patients previously received ART including at least one PI, 31 (77,5%) >2 PIs and 31 (77,5%) had at least one prior NNRTI. The mean number of previously received NUCs was 5.

The median VL at baseline was 32800cps/ml (range 128->500000). The median baseline CD4 cell count was 113/µl (range 0-475).

25/40 patients have already reached week 12, 14/40 week 24, 4/40 patients stopped study treatment, two due to patients wish, two due to adverse events (AE) (1 pancreatitis, 1 died of AIDS progression).

At week 12 9/25 (36%) patients showed a VL below 80cps/ml. The median VL observed was 162cps/ml (range <50-310000). The median decrease of VL was 1,89log. In parallel CD4-cells increased up to median 183/µl (range 21-572). At week 24 5/14 patients (35,7%) had a VL below 80cps/ml.

5/40 patients showed primary treatment failure and 5 patients showed treatment failure within 32 weeks after first having responded virologically.

The median count of PI-mutations at baseline was 4, patients who failed on ABT 378/r showed a median count of 6 baseline PI-mutations. In patients failing virologically an increase of the mean number of PI-mutations was observed up to 7 at week 24. However no specific mutation pattern could be observed. Several PI mutations were detected more frequently in case of failure than in case of response to treatment, e.g. L10(80%vs33%), I54(50%vs5,6%), A71(60%vs33%), I84(40%vs11%). Other commonly seen mutations that showed no relation to patients outcome were M46, L63, V82, L90.

CONCLUSION: ABT 378/r is a highly active antiretroviral drug even in heavily pretreated patients with moderate resistance. The overall rate of AEs is low. Our preliminary data shows no evidence of rapid developing resistances or specific pattern of mutation in patients failing on ABT 378/r. However further data is required to allow reliable conclusions on resistance under ABT 378/r.

ABSTRACT 091

BID First-Line Ritonavir/Indinavir 24 Week Results German Multicenter Study

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BACKGROUND: For treatment of HIV the combination of two protease inhibitors ritonavir (RTV) and indinavir (IDV) (2x100/800mg per day) offers the advantage of bid dosing and no meal restriction. In this study we evaluated efficacy and safety of the RTV/IDV combination in therapy naive patients.

METHODS: 57 patients with high median baseline HIV-RNA-levels of 308.000 copies/ml (range 8100/ml; 3010000/ml) and median CD4-count of 50/µl (range 0/µl; 400/µl) who wished a bid dosed antiretroviral regimen were started on either AZT/3TC (29.8%), D4T/3TC (29.8%), D4T/ddI (8.8%) , d4T/3TC/ABC (10.5%), AZT/3TC/ABC (19.3%), D4T/ABC (1.75%) in combination with ritonavir 2 x 100 mg/d and indinavir 2 x 800 mg/d. CD4-counts and HIV-RNA were determined up to week 24. All adverse events were documented.

RESULTS: Median basline HIV-RNA levels dropped from 5.5 log (range 3.9 log; 6.48 log) to 2.02 log (range 1 log; 4.11 log, n=33) at week 12 and 1.8 log (range 1.3 log, 3.27 log, n=33) at week 24. 79%/94% of the patients (week 12/24) were below 400 copies/ml and 45%/81% (week 12/24) of these below 80 copies/ml respectively. In parallel median CD-4 count increased from baseline (50/µl) to 128.5/µl (range 44/µl; 655/µl) at week 12, 200/µl (range 51/µl; 867/µl) at week 24. Triglyceride levels increased from median 155 mg/dl to 187 mg/dl and cholesterol levels increased from median 166 mg/dl to 195 mg/dl at week 24. Overall the therapy was well tolerated. 17 patients (29.8%) stopped treatment due to adverse events: 3 patients (5.3%) due to severe diarrhea, 11 patients (19.3%) due to kidneystones, 1 patient (1.8%) due to recurrent paronychia, 1 patient (1.8%) due to dry skin and 1 patient (1.8%) due to virological failure.

CONCLUSION: Our preliminary data suggest that even in the presence of high median baseline HIV-RNA levels the protease inhibitor combination RTV/IDV (2x100/2x800mg) plus double/triple nucleoside therapy appears effective and safe based on short-term treatment up to week 24. Nephrolithiasis occurred in 19.3% and therefore patients need extra hydration. The study is ongoing and further clinical and laboratory data will be presented.

ABSTRACT 092

The Potential Cost Savings Associated with Deferring the Start of Antiretroviral Therapy

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OBJECTIVE: To estimate the potential cost savings of deferring triple combination antiretroviral therapy until CD4+ cell counts reach below 200 Cells/mm^3.

METHODS: Population-based analysis of antiretroviral therapy naive HIV+ persons 18 years or older who initiated triple combination therapy between August 1, 1996 and September 30, 1999 in British Columbia, Canada. We modelled the potential pharmaceutical cost savings that could accrue if participants who initiated therapy with CD4+ cell counts greater than or equal to [GTE] 200 cells/mm^3 deferred therapy until their counts were below 200 cells/mm^3. We assumed a fixed rate of CD4+ cell count decline of 60 cells per year. Antiretroviral expenditures were estimated until January 31, 2000 by tallying individual-specific monthly drug costs. All costs are reported in Canadian dollars.

RESULTS: A total of 1,219 men and women (909 receiving PI and 310 receiving NNRTIs containing regimens) were eligible. Of these 1,219 participants, 776 (64%) started therapy when their CD4+ cell counts were GTE 200 cells/mm^3. If we assume a 60 cell decline in CD4+ cell count per year, $9,137,000 could be saved by these 776 persons deferring therapy until their CD4+ cell counts were <200 cells/mm^3. This represents a 50% potential reduction in antiretroviral costs over the study period from $18,393,000 to $9,256,000. We repeated this analysis based on 1,156 (95%) participants who initiated therapy according the most recent IAS - USA guidelines. Of these 1,156 participants, 713 (62%) initiated therapy when their CD4+ cell counts were GTE 200 cells/mm^3. If we assume the same fixed rate of CD4+ cell count decline the cost of therapy drops 48% from $17,762,000 to $9,255,000 over the study period.

CONCLUSION: Our data demonstrate that over 60% of participants could defer the initiation of therapy and this delay would represent a 50% potential reduction in total antiretroviral drug costs. This strategy could also be associated with the potential reduction in drug toxicity or could lead to the possible expansion of existing therapy programs in areas where at this time treatment is unaffordable.

ABSTRACT 093

Poly (I):Poly (C12u) Produces a Sustained Reduction in HIV Load

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BACKGROUND: The emerging resistance of patients to various HAART regimens as well as their associated toxicities has stimulated interest in pharmacological agents with anti-HIV properties that do not target enzymes of the highly mutable HIV genome.

OBJECTIVE: Although abundant *in vitro* data exists for the anti-HIV properties of poly(I):poly(C12U) and its synergistic combination with individual components of HAART regimens, clinical evidence was sought that poly(I):poly(C12U) was capable of reducing HIV load.

METHODS: Nine patients (7 males, 2 females) were treated with poly(I):poly(C12U) in an open label study by twice weekly infusions for 24 weeks as monotherapy. Requirements for study admission were HIV RNA load >4000 copies/ml and a CD4 level >400 cells/ul.

RESULTS: At baseline the mean HIV RNA load was 41,400 copies/ml and the median was 45,100 copies/ml. Following 24 weeks of poly(I):poly(C12U), the mean HIV RNA load was 25,200 copies/ml and the median was 20,800 copies/ml representing a log decrease in HIV load of 0.245 and 0.260, respectively. The p values for the respective observed decreases in HIV load from baseline were 0.045 and 0.039. CD4 levels were stable over the 24-week period despite significant HIV loads. Mean CD4 was 614 cells/ul at baseline and 606 cells/ul following 24 weeks of (p=0.81); median CD4 was 585 cells/ul at baseline and 604 cells/ul at 24 weeks of (p=0.64). The drug was well tolerated with the only significant alteration in either standard blood chemistries or hematological parameters was a decrease in hemoglobin from 15.2 gm/dl to 14.5 gm/dl(p=0.04).

CONCLUSION: Poly(I):poly(C12U) provided significant reduction in HIV load at 24 weeks of while preserving the level of CD4 cells. This study demonstrates that the well documented anti-HIV activity of poly(I):poly(C12U) *in vitro* is reflected by significant sustained anti-HIV activity *in vivo*. By analogy, the recently described anti-HIV synergistic combination demonstrated by poly(I):poly (C12U) with most of the 14 anti-HIV agents currently approved by the FDA for clinical use, suggests that similar synergy may be observed *in vivo* (Robinson, WE, Jr., *et.al., Antiviral Res.* **46**(1):48,2000). A second consideration in HAART regimens is the observed continued suppression of HIV load after cessation of HAART in some patients that has been correlated to restoration, at least in part, of a directed cell mediated immune response to HIV (Rosenberg, ES, *et.al., Nature*

407:523,2000). The reported restoration of a Th1 immune response in HIV-infected subjects treated with poly(I):poly(C12U) provides a strong rationale for the addition of poly(I):poly(C12U) to HAART regimens in a multi-center, double-blind, randomized clinical trial recently approved by the FDA.

ABSTRACT 094

Delavirdine (DLV) is a Potent and Durable Component of Non-Initial HAART Regimens

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BACKGROUND: To profile immunologic and virologic outcomes among patients (Pts) receiving treatment with DLV in a large urban HIV clinic. As a result of continued data DLV may be an effective component for treatment experienced HIV Pts.

METHODS: A retrospective chart review was performed to evaluate the safety and efficacy of DLV in this patient population.

RESULTS: 15 patients were identified in this clinic population. This cohort included the following: 73% men, 27% women, 27% caucasian and 73% African-American. The average age was 30.73 yrs. All Pts were treatment experienced. The mean number of pre-DLV drugs = 3 with a mean duration of 20.27 months. 4/15 (27%) were EFV experienced. 9/15 (60%) were protease inhibitor (PI) experienced. The mean baseline viral load (VL) was 145,488 (range 24,500 – 375,000) and mean baseline CD4 was 222.13 (range 98 – 394). All Pts are currently on DLV. The mean number of months of DLV therapy = 20.67 (range 8-63 months). All Pts are currently undetectable with a VL<50. There was a mean CD4 increase of 144.74. 53% of the Pts are currently on DLV, ddI, d4T and a PI, (50% NFV, 50% IDV). The remaining 47% are on the following: DLV, 3TC, d4T, + IDV (7%), DLV, AZT, 3TC + NFV (7%), DLV, d4T + NFV (7%) and DLV, ddI + d4T (26%). One patient was switched from IDV to NFV due to signs and symptoms of nephrotoxicity. There were no reported laboratory abnormalities after initiation of DLV with respect to the following: T Bili, D. Bili, AST, ALT, GGT, Alk Phos, Platelets, Serum Alb, Hg, WBC and ANC.

CONCLUSIONS: DLV is a safe, efficacious and durable component of a variety of salvage regimens in HIV infected Pts. The results presented here continue to support the use of DLV as part of a HAART regimen in treatment experienced HIV patients.

ABSTRACT 095

Nelfinavir/Nevirapine Containing Salvage Regimens, A Retrospective Study Suggesting No Antagonism And Possible Synergy

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INTRODUCTION: There is a pressing need for tolerable and potent salvage regimens in patients with advanced HIV infection. Regimens containing nelfinavir and nevirapine have been attractive due to non-overlapping toxicities resistance profiles and side effects, separate mechanisms of action, and potentially synergistic pharmacokinetic interactions. There has, however, been some question as to whether nevirapine raises or lowers nelfinavir levels. Several confliciting studies have shown opposite interactions. This study is intended to further study these drugs used in combination.

METHODS: A retrospective chart review was performed in a private practice specializing in the care of HIV infected patients. Charts were reviewed during the period between July 1997 and September 2000.

RESULTS: Thirteen patients treated with HAART regimens containing Nelfinavir and Nevirapine were identified. Viral load was examined before and after the combination therapy was initiated. In ten of thirteen patients, a notable reduction in viral load was seen after the Neviripine/Nelfinavir regimen was started. The remaining three patients were undetectable before and after inititiation of the Nevirapine/Nelfinavir regimen. The mean reduction in viral load in these ten patients was 1.38 logs.

CONCLUSION: Contrary to drug/drug interaction studies presented elsewhere, there did not appear to be any harmful effect in using Neviripine and Nelfinavir together in treatment of HIV in this cohort of salvage patients. Moreover, the substantial decrease in viral load in these patients suggests a possible significant benefit from combination therapy with Neviripine and Nelfinavir, a conclusion supported in other studies suggesting that Nevirapine inhibits the cytochrome p450 metabolism of nelfinavir. We intend to conduct a larger prospective study of this drug combination including serum nelfinavir levels to help clarify the conflicting data for this important drug combination.

ABSTRACT 096

Late Stage AIDS Population treated with WF10 for Salvage Therapy - Phase 3 Study

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BACKGROUND: Despite highly active antiretroviral therapy (HAART) the reemergence of opportunistic infections has occurred in patients with late stage AIDS. The challenge resides in providing this heterogeneous patient population with alternate forms of therapy. WF10 is a macrophage regulator which changes pathogenic inflammatory gene expression. In previous pilot studies, HIV patients who had received WF10 developed fewer opportunistic infections compared to placebo. The ongoing phase 3 trial is designed to evaluate the role of WF10 in delaying disease progression and death in late stage AIDS patients.

METHODS: A double-blind placebo-controlled study to evaluate the effect of WF10 on clinical progression in HIV-patients on stable HIV therapy with CD4 cell count ≤50. Patients receive four 5-day treatment cycles of WF10 / placebo IV (0.5ml/kg) as a 1.5-hour infusion daily (16 days between cycles). Follow-up is up to 96 weeks.

RESULTS: Study enrollment is complete with 245 patients randomized. Among them, 60 percent have completed 48 weeks of follow up. At baseline, the patients data show a mean CD4 cell count of 23/mm^3 and a mean HIV viral load of 5.25 logs. To date 40 clinical endpoints are approved, with a total of 76 reported. The most commonly reported clinical endpoints are: death (22), PCP (16), MAC (9), esophageal candidiasis (6), Cryptococcal infection (4), PML (4). Also, 202 Serious Adverse Events (SAE) are reported for 109 patients, which resulted in 190 hospitalizations representing 1,423 hospitalization-days. In 91 percent of the cases, investigators classify relationship to study drug as "None" or "Remote". The causes of death are AIDS (8), renal failure (4), respiratory failure (3), CNS disease/lesions (2), cryptococcus meningitis (1), PML (1), PCP (1), cardiopulmonary arrest (1) and multiple systems organ failure (1).

CONCLUSION: The number of deaths and opportunistic infections is increasing despite HAART. WF10 represents a novel approach in salvage therapy aimed to help prevent disease progression and improve quality of life. To date, WF10 appears safe and easy to administer. Final analysis will be presented after study completion.

ABSTRACT 097

An Overview in Pediatric HIV Children Management

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OBJECTIVE: First-line and salvage therapy: An overview in paediatric HIV children management. This abstract set to highlight the similarities in outcome of both method of treatment.

DESIGN: Use of AZT/3TC and salvage therapy as first line of management in HIV-patient. Over a certain length of time and comparing their various outcomes in these sets of patient as regard the effective of both methods of treatment. Thirty children were included in these studies; these are children at the Cyrill Ross Nursery, Tunapuna Trinidad and Tobago, over a period of 2 years.

METHOD: Of the thirty children in this study two of them were put on AZT/3TC for 1 ½ years after that period a third patient was put on salvage therapy combination of Viramune, novir, Invrase and Retrovir. The viral loads and clinical symptoms were used to assess their treatment outcome. Over one year, the viral load of the two patients on AZT/3TC combination dropped more than 100% to both undetectable and 3,604 respectively. The 3,604 viral load for 2nd patient one because she develops side effects (severe anemia) to AZT (Retrovir) a change to Zerit/3TC combination was made. And the one on salvage therapy viral load dropped from 52,000 copies to undetectable viral load. But the clinical condition of the first two patient were satisfactory while that on salvage therapy was very poor in spite of the undetectable viral load. After a period of 1 ½ years because of availability of more medication, these 1st two patients were now commenced on salvage therapies of four combination antiretroviral, and at the end of 6 mths period they were all re-evaluated.

RESULTS:

	FIRST LINE THERAPY	SALVAGE THERAPY	TRIPLE PX	OUTCOME
Px A	+	-	-	Satisfactory
B	+	+	-	Satisfactory
C	-	+	-	Unsatisfactory
D	-	-	+	Satisfactory
Others	-	-	+	Satisfactory

The above table of result showed that, of the different sets of children put on first line and salvage therapy, apart from one who later passed away from clinical

symptoms pathognomonic of full blown AIDS, the outcome of both therapies were similar.

CONCLUSION: From the above, it can be concluded that similar outcome can be seen in terms of patient clinical status when put on either first line or salvage therapies simultaneously. These outcomes were measured from their viral loads and clinical status.

ABSTRACT 098

Genotypic Predictors to Response to Abacavir and Efavirenz in Salvage Therapy

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BACKGROUND: Genotypic predictors of successful salvage therapy with abacavir (ABC) and efavirenz (EFV) and are not well defined.

STUDY DESIGN: Prospective, open label study of EFV and ABC plus at least 1 other agent in protease inhibitor (PI) experienced patients. Primary endpoint was time to treatment failure defined as > 0.5 log viral load (VL) increase above nadir or a failure to achieve a 0.5 log VL decrease. Predictors of failure were identified by Kaplan Meier analysis and analyzed by Cox regression. Genotype was performed by Visible Genetics (VGI) technology and interpreted using the VGI rules based system.

RESULTS: 50 patients were followed for a median of 156 days. Median prior anti-retroviral drugs and PIs were 7 & 3 respectively. Efavirenz sensitivity by VGI rules was a strong predictor of prolonged time to failure (141 vs. 38 days, P = 0.005). ABC sensitivity by VGI rules did not predict failure (131 days vs. 81 days, P = 0.6). Analysis of specific mutations demonstrated rapid failure associated with any mutation associated with NNRTI resistance (K103N = 43 days*, V108I = 27 days*, V181C = 43 days, or G190A = 32 days*, *P<0.05). In contrast, the number of TAM and 3TC mutations was associated with time to failure (none = 190 days, 1-2 mutations = 75 days, 3 or more mutations = 28 days, P = 0.006). Best time to failure was seen in subjects with no NNRTI or TAM/3TC mutations (190 days). Significant factors for treatment failure in Cox regression model (adjusted for CD4 count and viral load) were any NNRTI mutation (OR = 3.7, P = 0.001) and the number of TAM/3TC mutation (OR = 3.6, P = 0.001).

CONCLUSION: Therapy with abacavir and efavirenz can be effective in salvage therapy of highly experienced patients. Predictors of failure were any NNRTI mutation and the number of TAM/3TC mutations.

ABSTRACT 099

Nelfinavir(NFV) is a Potent and Durable Component of a Second-line HAART Regimen

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BACKGROUND: NNRTIs are commonly prescribed in initial HAART regimens. The use of NFV as a second line agent in patients that have either failed or who have been intolerant of the NNRTI has not been fully studied.

METHODS: A retrospective chart review was performed to evaluate the efficacy and durability of NFV used as a second line agent in patients treated at an urban nursing home dedicated to HIV care.

RESULTS: 12 patients were identified. All patients were African American, 2/12 (17%) female, 10/12 (83%) male, average age 37.92 years, average number of years since AIDS diagnosis was 1.9 years. All but 1 pt was switched to NFV due to tolerability issues with their initial NNRTI. Patients were on their initial NNRTI regimen for an average of 3.6 months, the 1 patient with virologic failure was on the NNRTI (Nevirapine) regimen for 1 year. The average CD4 nadir prior to starting the NNRTI HAART regimen was 126.08 cells/mm3. 11/12 patients in this cohort were also Hep C positive. The average VL at the time of switch to NFV was 3,586 copies/mL. The average CD4 at the time of switch was 211.83 cells/mm3. All patients are currently undetectable with 12/12 <400 (100%) and 5/12 <50 (42%). The average CD4 increase after switch was +144.83 cells/mm3 (range +68 - +300). All patients are currently on their NFV containing regimen. The average length of time on NFV in this cohort is 13.83 mos (range 3-24mos).

CONCLUSION: In this small cohort, patients tolerated NFV better than the NNRTI. The switch from the NNRTI to NFV led to a successful and durable suppression of viral load to below the limits of detection in all patients, as well as a robust immunologic response.

ABSTRACT 100

Short-Course Maternal and 10 Days Intra-Venous Neonatal Zidovudine Prophylaxis and Elective Caesarean Section: Effective and Safe in Reducing Vertical Transmission of HIV-1 Infection

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BACKGROUND: The objectives of the Berlin prospective HIV perinatal cohort study are to investigate the effectiveness and safety in reducing mother-to-infant HIV transmission of a much reduced three-arm Zidovudine prophylaxis regimen in combination with caesarean section (CS) before labour.

METHODS: The used regimen is Zidovudine (ZDV) given orally five times daily starting at 32-34 weeks of pregnancy, 200 mg ZDV intravenously before elective CS and postnatally ZDV 10 days intravenously 1.3 mg/kg every 6 hours for the children. Between July 1985 and July 1999 179 mother-infant pairs were enrolled. Of these, 75 mothers with 76 infants were exposed to antiretrovirals, 48 mothers and 49 children received the described Berlin prophylaxis regimen, 18 received combination therapy and 9 pairs received only part of the prophylaxis regimen. 104 mothers delivered 104 neonates before the introduction of routine ZDV prophylaxis and represent therefore the control group.

RESULTS: The vertical HIV transmission rate was in the group without antiretrovirals 12.6% (confidence interval 6.2-19%), in contrast there was no transmission among the infants exposed to all or part of the antiretroviral prophylaxis or therapy. Concerning the safety there were significant differences in haemoglobin concentration, haematocrit, white blood count and neutrophils. At birth values were decreased in infants exposed to the Berlin prophylaxis regimen compared to the control group. The differences disappeared within 14-17 days for the white blood parameters, but lasted for 7 weeks for the haemoglobin and haematocrit. The weight and length of both male and female infants born to HIV infected mothers and exposed to Berlin regimen of ZDV prophylaxis, none of whom were HIV infected, was markedly lower than the standard values of infants born to uninfected mothers. The difference was especially pronounced at birth, but returned to the mean standard values after a few weeks.

CONCLUSION: Short-term Zidovudine for the mothers and the neonates in combination with caesarean section before labour virtually eliminates HIV transmission. The regimen is safe and well tolerated.

ABSTRACT 101

Interferon Alfa-2a for Chronic Hepatitis C in HIV-infected Patients: Comparison Between Induction and Non-induction Dosing in a Randomized Trial

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OBJECTIVE: We compared the efficacy of two different dose treatments of interferon alfa-2a in patients co-infected with human immunodeficiency virus (HIV) and hepatitis C virus (HCV).

PATIENTS AND METHODS: Fifty nine patients were randomly assigned to one of the two treatment groups: (i) induction dosing (9 million international units (MIU) of interferon alfa-2a (IFN alfa-2a) daily for two weeks followed by 6 MIU TIW for 46 weeks) and (ii) non-induction dosing (6 MIU of IFN alfa-2a three times a week for 48 weeks). Baseline measurements of HCV RNA, HIV RNA, CD4+ cells, and biochemical assessments (ALT and AST) were compared with measurements at 4, 12, 24, 48, and 72 weeks.

RESULTS: From the 46 evaluable patients, 24 individuals were accrued in the induction dosing group and 22 individuals in the control (non-induction treatment) group. The mean baseline HCV RNA values for the induction and non-induction groups were 662,891 copies/ml and 884,974 copies/ml, respectively. At 24 weeks of treatment, the induction group did not have a statistical difference reduction of HCV RNA (477,886 copies/ml; $p=0.09$), whereas the control group decreased their viral load statistically from baseline (433,613 copies/ml; $p<0.05$). Biochemical responses at 24 weeks (normal ALT serum levels) were better in the induction group 54% than in the control group 32%, but this difference was not significant ($p=0.46$). However, when complete responses (virological and biochemical) was taken into account the control group responded better than the induction group, 18% vs. 8% respectively ($p=0.40$). The end of treatment response (ETR) was also better in the control group than in the induction group, but did not achieve statistically significant differences (14% vs. 0%; $p=0.10$). Sustained responses decreased to 4% (1/22) in the non-induction group at 72 weeks. General malaise was the most frequent adverse effect in both groups, and neutropenia was more frequent in the induction group although it was transitory and resolved with dose reduction.

CONCLUSION: In HCV-HIV co-infected patients, treatment with IFN alfa-2a was well tolerated although the response to treatment was lower than in patients infected only with HCV. No clinical advantages were observed in the induction group when compared to the control group. Other treatment regimens including combination therapy must be considered for this patient population.

ABSTRACT 102

Nevirapine is not Associated with an Increased Risk of Clinically Significant Hepatitis Compared with Other Antiretroviral Medications

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BACKGROUND: Placebo-controlled clinical trials have shown that nevirapine can be associated with clinical hepatitis. To evaluate whether nevirapine is associated with a greater risk of clinically significant hepatic events than other antiretroviral (ARV) medications, we conducted a cohort study using a medical claims database from an insured population in the US comprising about 3 million people from 22 states.

METHODS: We identified individuals who received at least one prescription for an ARV drug from 1/1/96 to 9/30/99. We followed each patient from the date of first prescription for an ARV drug until 9/30/99, termination of plan membership, or date of onset of a case event. We classified treatment days according to nevirapine-containing regimens and non-nevirapine regimens. Clinically significant hepatic cases were hospitalized patients with hepatitis or related hepatic events. Incidence rates were computed as the number of cases divided by the person-years of ARV therapy. The rate ratio was computed as the rate during nevirapine therapy divided by the rate during non-nevirapine therapy.

RESULTS: We identified 1924 patients who filled an ARV prescription. The population was 80% men, with a median age of 39 years. Treatment groups were similar with regard to age, gender, use of concomitant ARV and other hepatotoxic medications, and viral hepatitis. Lab tests that included LFTs were more common among nevirapine treated patients (76%) than among non-nevirapine patients (6%). There were 2724 person-years of ARV treatment, with 252 person-years of exposure to nevirapine regimens. We identified 65 clinically significant hepatic events, 3 of which occurred during treatment with nevirapine, 48 during non-nevirapine treatment, and 14 cases occurred during period of no ARV treatment. Incidence rates of clinically significant hepatic events per 100 person-years were 1.19 during nevirapine therapy and 1.4 during non-nevirapine therapy. The rate ratio estimate was 0.61 (95% confidence interval 0.15, 1.76).

CONCLUSION: Nevirapine was not associated with an increased risk of clinically significant hepatic events compared with other ARV medications.

ABSTRACT 103

Pharmacokinetics of Tipranavir and Nevirapine: A Pharmacokinetic Interaction Study in Healthy Volunteers

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BACKGROUND/METHODS: A randomized, open-label, parallel group, multiple-dose study was conducted in normal male and female adult volunteers (aged 18-55 years) to assess the two-way pharmacokinetic interaction between tipranavir and nevirapine, with and without tipranavir enhancement by ritonavir. Subjects in Group I received tipranavir (soft-elastic capsules) 1250 mg BID from Day 1-7, followed by nevirapine 200 mg QD for 13 days and then 200 mg BID for 14 days, followed by tipranavir 1250 mg BID + nevirapine 200 mg BID for 10 days. Subjects in Group II received the same regimens as above except that ritonavir (oral solution) 200 mg was administered with each tipranavir dose. In Group I the pharmacokinetic interaction was assessed by comparing the pharmacokinetic parameters (Cmax, Cmin, AUC, CLpo) when tipranavir (Day 7) and nevirapine (Day 34) were administered alone to the same pharmacokinetic parameters when the two drugs were administered together (Day 43). In Group II the same assessment was done when ritonavir was coadministered with tipranavir. Of 24 subjects enrolled, 13 subjects discontinued the study because of intolerance to therapy, although there we no serious adverse events reported.

RESULTS: Effects of nevirapine on tipranavir: In Group I (n=7) who received tipranavir without ritonavir, co-administration with nevirapine resulted in a nonsignificant (Wilcoxon Signed-Rank test) increase in tipranavir AUC (+32%), Cmax (+19%) and Cmin (+20%). In Group II (n=4) who received tipranavir with ritonavir, co-administration with nevirapine resulted in a nonsignificant decrease in tipranavir AUC (-15%), Cmax (-19%) and Cmin (-3.4%).

Effects of tipranavir on nevirapine: In Group I (n=7), tipranavir co-administration resulted in a significant

(p=.016) decrease in nevirapine AUC (-37%), Cmax (-34%) and Cmin (-38%). In Group II (n=4), tipranavir/ritonavir co-administration resulted in a nonsignificant decrease in nevirapine AUC (-20%), Cmax (-25%) and Cmin (-14%).

Effects of tipranavir and nevirapine on ritonavir: Ritonavir was co-administered with tipranavir to four subjects in Group II. Tipranavir appeared to increase the total apparent clearance of ritonavir by ~6.5-fold compared to historical values obtained from another study. When nevirapine was included in the regimen, a further 62% increase in total apparent clearance of ritonavir was observed. However, despite the further reduction in plasma concentrations of ritonavir by nevirapine, plasma tipranavir concentrations remained substantially enhanced (>16-fold) in Group II, compared to the respective values for tipranavir in Group I.

ABSTRACT 104

Issues faced in the Usage of Antiretroviral Drugs in India

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A decrease in the number of new AIDS cases and AIDS related deaths was seen in developed countries since 1996 due to the use of new combination of antiretroviral drugs. Between June 1996 and Oct 2000, 2200 persons with HIV were registered and are being followed up for medical and psychosocial care at YRG Centre for AIDS Research and Education, a tertiary HIV referral care centre in Chennai, South India. Oropharyngeal candidiasis (46%), Pulmonary TB (37.6%), Herpeszoster (11.2%), Extrapulmonary TB (8.8%), Dermatophyte infection (8%), Herpes Simplex (7.7%) PCP (5%), Staphylococcal skin infection (2.9%), Cryptococcoal meningitis (2.7%), Toxoplasmosis (2.5%), OHL (2.3%), CMV retinitis (1.9%) and Cryptosporidial diarrhea (1.6%) are the major opportunistic infections diagnosed and treated. Only 9.5% of these individuals could afford antiretroviral drugs due to high cost. Few patients have stopped antiretroviral therapy despite counseling due to expense. Many patients who meet the criteria for initiation of antiretroviral therapy also have multiple opportunistic infections. Many of the protease inhibitors interact with the drugs used for opportunistic infections especially with Rifampicin. Anemia is a common adverse event noticed in our cohort who received antiretroviral drugs. Lymphadenopathy and vitritis were also seen due to immune reconstitution. Several antiretroviral drugs are neither manufactured nor licenced in India which limits the options when switching the combination therapy.

Strict prescribing guidelines must be available for those caring for people with HIV and should be adhered to so that emergence of resistant strains could be prevented. Prescribing physicians should be familiar with the complexities of antiretroviral treatment and should offer extensive counseling on issues of compliance, side effects, maximizing therapeutic levels, drug interactions and financial implications. Generic drugs should be allowed to manufacture locally to reduce the cost and for sustained availability. Ethically approved and culturally acceptable clinical trials should be encouraged in developing countries for access to treatment.

ABSTRACT 105

Some Causes of Treatment Failure and Possible Drug Interactions in HIV/AIDS Children on Antiretroviral Medications

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OBJECTIVE: This abstract set to mention some cases of treatment failure and possible drug interactions in HIV/AIDS children on antiretroviral medications.

DESIGN: Thirty children were included in this study; of these (30) thirty children (14) fourteen were commenced on various combination of antiretroviral. Where the others were not on any antiretroviral except on prophylactic sulphametoxazole-trimetropin.

METHOD: These HIV children were followed for a period of nine months, Feb-Oct, 2000. The (14) fourteen on antiretroviral were either triple therapies or more while the others were not on antiretrovirals. The ages of the children range from 1 yr to 15 yrs.

RESULTS:

NAME	VIRAL LOAD Before Antiretroviral Jan, 2000	VIRAL LOAD After Antiretroviral May, 2000	OUTCOME
1) E.N.	52,108	Undetectable	Dead
2) A.W.	68,550	437	Alive
3) O.D.	Unknown	143,707	Alive
4) S.J.	Unknown	108,156	Dead
5) S.F.	Unknown	Undetectable	Alive

From this study, of (14) children on antiretroviral medication (2) two of them died, one died (5) five months and another (8) months respectively after the commencement of this study; from symptoms described to treatment failure and possible drug toxicities from interaction between the various multiple medication they were on. These could almost be substantiated from patient SJ on the chart who was 3 yrs old and have been apparently well until about 3 weeks before his death, and about 8 weeks after the commencement of his antiretroviral medication. Patient EN also died from

possible treatment failure and drug interaction in spite of her undetectable viral load from 52,100 copies/ml prior to commencement of antiretroviral.

CONCLUSION: From the foregoing it can be concluded that the phenomenon of treatment failure and drugs interaction is still a dilemma in the field of HIV infection and treatment in the overall management of infected persons.

ABSTRACT 106

Liver Damage in Relation to Hepatitis C Virus and HIV Replication in Coinfected Patients after Introduction of HAART: a Long-Term, Prospective Study

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BACKGROUND: Abnormal levels of liver enzymes are common in HIV-HCV coinfected patients and may be due to drug toxicity or reactivation of HCV replication in patients on HAART. In order to determine the relative frequency of either mechanism, we followed prospectively all the subjects in our cohort who started HAART between April 1997 and April 1999 and continued treatment for at least 16 months.

METHODS: Clinical conditions, CD4 cell count, CD4/CD8 ratio, HCV-RNA load, HIV-RNA load and alanine aminotransferase (ALT) levels were determined every 15 days during the first 3 months and monthly thereafter in 124 HBsAg-negative patients with HIV-HCV coinfection, asymptomatic for HIV infection.

RESULTS: A "significant" (at least 5-fold increase in respect of baseline) raise in ALT occurred in 23 patients (18.5%) between 15 and 90 days following introduction of HAART. HCV-RNA (Amplicor Monitor, Roche) increased significantly only in 17 of these 23 patients (mean 2,100,000 copies/ml before starting HAART, peak 4,800,000 during therapy and coincident with peak ALT values). HIV-RNA (Chiron Quantiplex assay) was reduced in 119 out of 124 patients (mean 42,400 copies/ml before HAART, 650 within 3 months of starting triple therapy) and CD4 cells increased in 102 of them (mean 297/microliter prior to HAART, 426 after 3 months). Liver histology during ALT raise (available in 14/23 patients) showed an increase in inflammatory score (mean 6.4 before, 9.5 during HAART) with no signs of drug toxicity. No relations were found between drug combinations used (all including 2 NRTIs and 1 PI), previous antiretroviral experience, HCV genotype, HCV load or Knodell score at liver biopsy (performed in 35 out of 124 patients).

CONCLUSION: HAART did not induce, in our cohort, "significant" drug-related hepatotoxicity, but determined an increase in liver cell necrosis within the first 3 months of therapy in a minority of patients with chronic hepatitis C, leading to decompensation of liver disease in a proportion of those with cirrhosis. The inverse relationship between decrease in HIV-RNA and increase in HCV-RNA levels observed in most patients with "significant" hepatocyte necrosis supports the notion that "viral interference" between HIV and HCV may occur and be responsible for this effect. However HAART must be discontinued only in the few patients with decompensation of chronic liver disease, as continuing treatment is associated with a progressive reduction in aminotransferase values towards pretreatment levels.

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