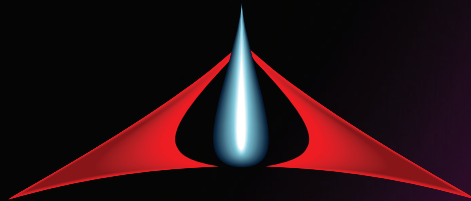




GLOBAL ANTIVIRAL JOURNAL



Bridging the Sciences™

HIV, HBV, HCV and Emerging Viruses

**May 31 - June 2, 2006
Le Palais des Congres de Paris, France**

Final Program and Abstract Book

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GLOBAL ANTIVIRAL JOURNAL

Aims and Scope

Global Antiviral Journal publishes peer-reviewed original works related to international efforts to advance antiviral discovery and development, including full-length articles and short papers, as well as solicited review articles, conference reports, letters and book reviews. Occasional supplements contain conference abstracts presentations and/or posters from international meetings in the fields of virology and antiviral research. The scope of the journal encompasses chemistry and biological advances in the fundamental and clinical study of antiviral diseases and their treatment. Areas covered include HIV, hepatitis B, hepatitis C and emerging viruses, co-infections, vaccines, animal models, pharmacology, microbicides, alternative therapies, viral dynamics and resistance issues.

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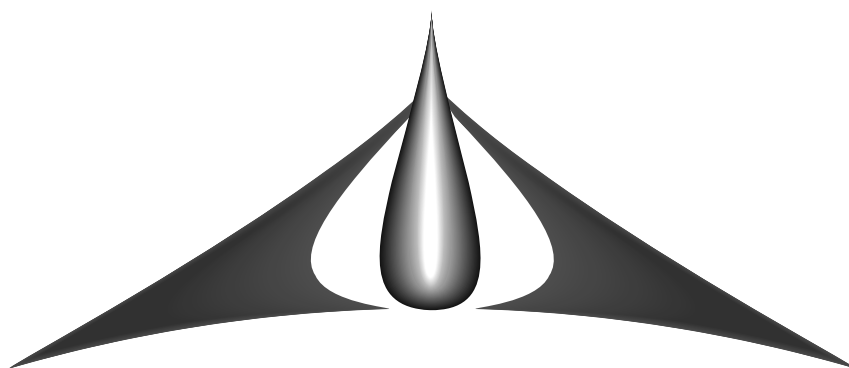
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Conference Objectives

The Scientific Committee has designed the Conference program to ensure that the delegates achieve the following objectives:

- Understand the drug development and discovery process for HIV, HBV, HCV and emerging viruses
- Identify the next generation of inhibitors of HIV and viral hepatitis
- Assess the therapeutic options and outcomes of current and future therapies
- Assess the role of vaccines and therapeutic vaccines

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Bridging the Sciences 2006
HIV, HBV, HCV and Emerging Viruses

Scientific Program

Abstract

Wednesday, May 31, 2006

14:00 Welcome
Raymond Schinazi *Emory University/VA Medical Center, USA*

SESSION 1:

Chairs: Robert Murphy *Northwestern University, USA*
Gilles Gosselin *Laboratoire Cooperatif Idenix-CNRS-UM II, France*

Plenary Lectures

14:10 Bridging chemistry to HIV, HBV, HCV and poxvirus infections: 001
The phosphonate bridge
Erik De Clercq *Rega Institute for Medical Research, Belgium*

14:50 HIV reverse transcriptases: structural basis for inhibition and drug resistance 002
David Stammers *University of Oxford, UK*

15:30 **Break**

Invited Speakers

16:00 The emerging impact of research universities on antiviral drug discovery 003
Dennis Liotta *Emory University, USA*

16:20 The use of animal models to address critical issues in viral therapy 004
Tom North *University of California, USA*

16:40 Overview of new directions in HCV therapy 005
Jean-Michel Pawlotsky *Henri Mondor University Hospital, France*

17:00 Clinical trial design, end points, and regulatory issues
Michael Manns *Medizinische Hochschule Hannover, Germany*

Thursday, June 1, 2006

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Chairs: Stephen Locarnini *VIDRL, Australia*
Dennis Liotta *Emory University, USA*

Plenary Lectures

8:30 Protease inhibitors for chronic hepatitis C 006
Stefan Zeuzem *Saarland University Hospital, Germany*

9:10 Cell culture systems for the hepatitis C virus and their use for antiviral drug 007
development
Ralf Bartenschlager *University of Heidelberg, Germany*

9:50 **Break**

Invited Speakers

10:20 From start to finish: lessons learned in hepatitis therapeutics
Yves Benhamou *Hôpital Pitié-Salpêtrière, France*

10:40 Co-infections with HIV, HBV and HCV 008
Robert Murphy *Northwestern University, USA*

11:00	New drugs in HIV treatment Christine Katlama	<i>Groupe Hospitalier Pitié-Salpêtrière, France</i>	
11:20	Treatment of HIV drug resistance; TMC114 and TMC125 Diego Miralles	<i>Tibotec BVBA, Belgium</i>	009
Oral Abstracts			
11:40	Activation of antiviral nucleotide analogues by human NMP kinases: importance of the alpha-phosphate substitution Dominique Deville-Bonne	<i>Université Paris 6, France</i>	010
11:50	Synthesis of oxazolopyrimidines targeting an anti-HIV activity Luigi Agrofoglio	<i>Université d'Orléans, France</i>	011
12:00	Lunch on your own		
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Chairs:	Yves Benhamou George Lau	<i>Hôpital Pitié-Salpêtrière, Paris, France</i> <i>The University of Hong Kong/Queen Mary Hospital, Hong Kong</i>	
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14:10	VTX-950: A NS3•4a protease inhibitor that specifically targets HCV John Randle	<i>Vertex Pharmaceuticals, Inc., USA</i>	013
14:50	Break		
Invited Speakers			
15:20	Preclinical characteristics of ITMN-191, an orally active macrocyclic inhibitor of the HCV NS3/4A protease Lawrence Blatt	<i>InterMune, Inc., USA</i>	014
15:40	Clinical trial experience of Viramidine: an oral pro-drug of ribavirin for the treatment of hepatitis C Brian Murphy	<i>Valeant Pharmaceuticals International, USA</i>	015
16:00	Interim results of a multiple ascending dose study of R1626, a novel nucleoside analog targeting HCV polymerase in chronic HCV patients George Hill	<i>Roche, USA</i>	016
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16:40	Vicriviroc use in novel antiretroviral regimens Lisa Dunkle	<i>Schering-Plough Research Institute, USA</i>	018
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17:20	Modulation of endoplasmic reticulum (ER) calcium signalling affects hepatitis C virus (HCV) protein expression and replication Patrizia Paterlini-Bréchet	<i>INSERM, France</i>	020

Friday, June 2, 2006

Abstract

SESSION 4:

Chairs: Daria Hazuda *Merck Research Labs, USA*
Françoise Brun-Vézinet *Hôpital Bichat-Claude Bernard, France*

Plenary Lectures

8:30 Hepatitis B virus and the innate immune response: A new paradigm for therapeutic intervention 021
Stephan Locarnini *VIDRL, Australia*

9:10 Suppressing HBV replications with new drugs and combinations 022
George Lau *The University of Hong Kong/Queen Mary Hospital, Hong Kong*

9:50 **Break**

Invited Speakers

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Gilles Gosselin *Laboratoire Cooperatif Idenix-CNRS-UM II, France*

10:40 Prevention of HCC by antiviral drugs 024
Massimo Colombo *IRCCS Maggiore Hospital, Italy*

11:00 HIV, HBV and HCV - different viruses with different kinetics and clinical implications 025
Avidan Neumann *Bar-Ilan University, Israel*

Oral Abstracts

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Gulendam Bozdayi *Gazi University, Turkey*

11:30 Novel peptidomimetic andimers inhibit mutated HIV-1 proteases 027
Michèle Reboud-Ravaux *CNRS-University Paris 6, France*

11:40 Utility and complementarity of replicative phenotyping for drug discovery and in diagnostics 028
Vincent Vidal *InPheno AG, Switzerland*

11:50 High throughput assay screening for inhibitors of ion channels including HCV p7, HIV-1 Vpu and 'flu H5N1 M2 029
Wesley Black *The Macfarlane Burnet Institute for Medical Research and Public Health, Australia*

12:00 **Lunch on your own**

SESSION 5:

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David Stammers *University of Oxford, UK*

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Douglas Richman *University of California, San Diego/VA Medical Center, USA*

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Daria Hazuda *Merck Research Labs, USA*

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15:40	HCV drug development - lessons learned from HIV Doug Mayers <i>Boehringer Ingelheim Pharmaceuticals, Inc., USA</i>	032
16:00	Similarities and differences between HIV-1 and HIV-2 drug resistance Françoise Brun-Vézinet <i>Hôpital Bichat-Claude Bernard, France</i>	033
16:20	Discovery and development of an oral influenza drug: "TAMIFLU" Choung Kim <i>Gilead Sciences, USA</i>	034

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Yves Benhamou *Hôpital Pitié-Salpêtrière, France*

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Session 1

ABSTRACT 001

**Bridging Chemistry to HIV,
HBV, HCV and Poxvirus
Infections: The Phosphonate
Bridge**

E De Clercq

Rega Institute for Medical Research, Leuven,
Belgium

The acyclic nucleoside phosphonates cidofovir, adefovir and tenofovir have acquired an established position in the treatment of, respectively, HIV infections [tenofovir disoproxil fumarate (TDF), orally, 300 mg once daily], HBV infections [adefovir dipivoxil, orally, 10 mg once daily], CMV retinitis in AIDS patients (cidofovir, intravenously, 5 mg/kg once every other week), and, on a compassionate basis, various adeno-, herpes-, pox- and papilloma virus infections (cidofovir, intravenously or topically) [E. De Clercq and A. Holý, Acyclic nucleoside phosphonates: a key class of antiviral drugs, *Nature Reviews Drug Discovery*, 4: 928-940 (2005)]. TDF, in combination with emtricitabine, has also become available as a single oral pill once daily, for the treatment of HIV infections, and TDF, alone or in combination with emtricitabine, may also be advocated for the treatment of HBV infections and HBV/HIV co-infections. The 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidines, *i.e.*, PMEO-DAPy, PMPO-DAPy, and HPMPO-DAPy represent a new class of acyclic nucleoside phosphonates, offering substantial promise for the treatment of a broad range of retro-, hepadna-, adeno-, herpes-, pox- and papillomavirus infections [E. De Clercq, G. Andrei, J. Balzarini, P. Leyssen, L. Naesens, J. Neyts, C. Pannecouque, R. Snoeck, C. Ying, D. Hocková, A. Holý, Antiviral potential of a new generation of acyclic nucleoside phosphonates, the 6-[2-(phosphono-methoxy)alkoxy]-2,4-diaminopyrimidines, *Nucleosides, Nucleotides, and Nucleic Acids*, 24: 331-341 (2005)]. Also, deoxythreosyl phosphonate nucleosides, *i.e.*, PMDTA and PMDTT, have been identified as potent anti-HIV agents [T. Wu, M. Froeyen, V. Kempeneers, C. Pannecouque, J. Wang, R. Busson, E. De Clercq, P. Herdewijn, Deoxythreosyl phosphonate nucleosides as selective anti-HIV agents, *Journal of the American Chemical Society*, 127: 5056-5065 (2005)], for which the potential for the treatment of HIV, HBV and other DNA virus infections still remains to be explored. Phosphonomethoxy-2'-fluoro-2',3'-dideoxydideoxyadenosine represents another cyclic nucleoside phosphonate which has been accredited with potent antiretroviral activity [T. Cihlar, A. Ray,

D. Booramra, L. Zhang, H. Hui, D. Grant, K. White, M. Desai, N. Parkin, R. Mackman, GS9148: a novel nucleotide active against HIV-1 variants with drug resistance mutations in reverse transcriptase, *13th Conference on Retroviruses and Opportunistic Infections (CROI)*, Denver, Colorado, USA, 5-9 February 2006, Oral Presentation no. 45], which may expand to HBV as well. Whether the phosphonate bridge of either acyclic or cyclic nucleoside phosphonates may also span HCV infections, is a very intriguing perspective, which, hopefully, may not be one bridge too far.

ABSTRACT 002

**HIV Reverse Transcriptases:
Structural Basis for Inhibition
and Drug Resistance**

D Stammers

The Wellcome Trust Centre for Human Genetics,
University of Oxford, Oxford, UK

HIV reverse transcriptase (RT) is one of the main target sites for the action of anti-AIDS drugs. Two classes of anti-RT drugs are in clinical use: nucleoside analogues (NRTIs), which bind at the dNTP site and cause DNA chain termination, whilst the non-nucleoside inhibitors (NNRTIs) bind in a pocket distal to the polymerase active site. Extensive crystallographic studies have been used to define the overall architecture of the HIV-1 RT p66/p51 heterodimer, the binding and mode of inhibition for the NNRTIs as well as the binding sites for NRTI drugs. Due to the rapid turnover of HIV and the low fidelity of transcription of RT, drug resistance rapidly emerges which presents a challenge to continued suppression of the virus. Structural studies of many mutant HIV-1 RTs resistant to NNRTIs have shed light on the structural basis for drug resistance and how 'second-generation' compounds are more resilient to the presence of mutations. For RT from a different serotype HIV-2, the crystal structure points to the mechanism of its inherent resistance to NNRTIs. The significant data base of HIV RT structures are being used in structure based design approaches. A number of successful studies have been reported and NNRTIs with greatly improved activity against common drug resistant forms of HIV are now in clinical trials. Thus, although RT was the target for the first anti-HIV drugs, it still has potential for development of new drugs including the targeting of as yet unexploited regions such as the RNaseH active site and tRNA primer binding.

ABSTRACT 003
**The Emerging Impact of
Research Universities on
Antiviral Drug Discovery**

DC Liotta

Emory University, Atlanta, USA

The passage of the Bayh-Dole Act in 1980 enabled US universities to own intellectual property developed from within and thereby catalyzed the creation of an alternative pathway for drug discovery.

This presentation will attempt to define the role of research universities in the drug discovery process and place it in its proper perspective vis a vis the more traditional pathways followed in the pharmaceutical and biotechnology industries. It will also highlight the discovery of several antiviral therapeutics by academic laboratories including our own.

ABSTRACT 004
**The Use of Animal Models to
Address Critical Issues in
Viral Therapy**

TW North

University of California, Davis, USA

The contributions of animal model systems to AIDS therapy have been limited by several factors. Immunodeficient mice with reconstituted human immune components can be infected with HIV and used for *in vivo* evaluation of drug efficacy. However, AIDS pathogenesis is not modeled in these mouse systems and, therefore, they cannot be used to address many key issues and complications in AIDS therapy. Models using feline (FIV) and simian (SIV) immunodeficiency viruses have been useful for studies of nucleoside analog inhibitors of reverse transcriptase (RT). However FIV is not susceptible to protease inhibitors or non-nucleoside inhibitors of RT (NNRTIs), and SIV is not susceptible to NNRTIs. This precludes use of FIV and SIV models for studies of the most potent and durable HAART combinations. We have modeled HAART in rhesus macaques using a chimera of SIVmac239 containing the HIV-1 RT in place of the SIV RT (RT-SHIV). RT-SHIV infection of rhesus macaques results in mean virus loads of 10^6 copies of viral RNA per ml of

plasma, and in pathogenesis similar to that of macaques infected with SIVmac239. We have evaluated the three drug combination of efavirenz + 3TC (or FTC) + PMPA in RT-SHIV-infected macaques. This HAART combination reduced virus loads to undetectable levels (less than 50 copies of viral RNA per ml of plasma) in all animals, with occasional blips of virus in only a few animals. The course of virus load suppression was reproducible and similar to human AIDS patients treated with a potent three-drug combination. Upon cessation of drug therapy virus loads rebounded rapidly in a manner similar to the cessation of therapy in human AIDS patients. RT-SHIV isolated after rebound had no drug-resistance mutations in RT, which is consistent with the reactivation of latent virus from reservoirs. We are currently using this model to study tissue reservoirs of viral latency and to identify sites of residual virus replication. We also plan to study the combination of HAART with agents that may reactivate latent virus. This model can be applied to other studies of HAART that are not feasible in humans. Our model is not ideal for studies of HIV entry inhibitors because RT-SHIV has the SIV *env* gene. We have made replication competent RT-*env*-SHIVs and are working toward a pathogenic variant.

These examples demonstrate that engineered systems and chimeric viruses can be used to address specific issues that may be critical for development of antiviral therapy. Similar approaches may be useful for adapting animal models for therapy of HBV and HCV.

ABSTRACT 005
**Overview of New Directions in
HCV Therapy**

J-M Pawlotsky

Henri Mondor University Hospital, Créteil, France

The goal of hepatitis C virus (HCV) therapy is permanent viral eradication. This requires the use of drug combinations with multiple modes of action. The main target of HCV therapy is steady-state HCV replication kinetics, which can be disrupted by drugs that inhibit virus production (antiviral molecules), inhibit *de novo* cell infection, and/or accelerate the clearance of infected cells. Interferon- α and ribavirin combine all these mechanisms of action when used together, yet fail to clear HCV from a significant number of patients. New therapeutic approaches are needed. The next generation of anti-HCV therapeutic

agents will fall into four main categories: new interferons and interferon inducers that will combine various modes of action in a more potent way than current molecules; alternatives to ribavirin that will have the same efficacy target without significant side effects; specific HCV inhibitors (especially inhibitors of HCV polymerase, protease, internal ribosome entry site, and early steps of the HCV lifecycle); and immune therapies that target *de novo* infection of hepatocytes and accelerated cell clearance. Ideally, these new treatments will increase the rate of sustained viral eradication and improve tolerability and acceptability. Drug combinations will be tailored to the individual patient, based on baseline parameters and viral kinetics during therapy.

Session 2

ABSTRACT 006
**Protease Inhibitors for
Chronic Hepatitis C**

S. Zeuzem

Saarland University Hospital, Homburg/Saar,
Germany

In the first decade after isolation and characterization of the hepatitis C virus many compounds were empirically tested in clinical trials for anti-HCV activity. Only interferons alone and in combination with ribavirin showed significant antiviral efficacy. With the current standard of care - pegylated interferon alfa in combination with ribavirin - excellent sustained virologic response rates of 80-90% are achieved in patients chronically infected with hepatitis C virus (HCV) genotype 2 or 3 isolates. However, virologic response rates in patients infected with the most prevalent genotype HCV-1 are only around 50%. Thus, specifically for HCV-1 infected patients improved therapeutic options are needed.

Basic to the development of new specific anti-HCV drugs is the understanding of the viral life cycle, in particular the genomic organization and the polyprotein processing. Major progress in this field was achieved due to the development of sub-genomic and more recently full-genomic replicon systems. The HCV genome is a single-stranded RNA molecule that contains a single open reading frame encoding a polyprotein of about 3000 amino acids. The polyprotein is subsequently processed at the level of the endoplasmic reticulum (ER) by cellular and viral proteases to yield 4 structural and 6 non-structural proteins. The open reading frame is flanked by 5' and 3' untranslated regions. Each single HCV structure represents a potential antiviral target.

NS3 and NS4A are cleaved by the catalytic activity of the NS3 protease domain. In addition to the protease domain located in the 189 aminoterminal amino acids, NS3 also possesses a helicase domain located in the 442 carboxyterminal amino acids. The NS3 protease domain is responsible to complete the polyprotein processing down to NS4B, NS5A, and NS5B. Despite the fact that the catalytic site is a shallow and largely hydrophobic groove and therefore very difficult to target several compounds have been successfully designed (BILN 2061, VX-950, SCH503034, etc.). BILN 2061 was a very potent compound with a four log decline within two days of monotherapy, however, further clinical development was discontinued due to cardiotoxicity in monkeys.

VX-950 at a dose of 750 mg q8h was well tolerated and showed a mean decline of 4.4 log within 2 weeks of monotherapy and a more than 5 log decline in combination therapy with pegylated interferon alfa-2a. SCH503034 was also well tolerated alone as in combination with peginterferon alfa-2b, however, the viral decline at the doses used was less pronounced than with the other two compounds. Currently, higher doses as well as combination therapies in combination with peginterferon and ribavirin are investigated. Moreover, during treatment with protease inhibitors HCV strains with a lower sensitivity to the respective compounds should emerge. Detailed analyses on the emergence of resistant strains, their kinetics, cross-resistance and replication fitness are currently underway.

ABSTRACT 007
**Cell Culture Systems for the
Hepatitis C Virus and Their
Use for Antiviral Drug
Development**

R. Bartenschlager

University of Heidelberg, Heidelberg, Germany

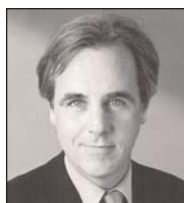
Hepatitis C viruses (HCV) comprise a group of positive-strand RNA viruses belonging to the *Flaviviridae* family. As a major cause of chronic liver disease that currently affects 170 million people worldwide and for which no selective therapy exists HCV has received much attention. In spite of rapid progress in functional and structural characterization of HCV proteins, a major hurdle in HCV research and drug development has been the difficulty to propagate the virus in cell culture. A first step to overcome this block was the establishment of selectable, subgenomic replicons that replicate autonomously to very high levels in the human hepatoma cell line Huh-7. Subsequent studies led to the discovery of more efficient HCV replicons derived from multiple HCV isolates and genotypes, and cell lines other than Huh-7 that also support stable HCV RNA replication. Moreover, replicons that stably express marker genes have been developed that are extensively used both for drug development and for characterization of antiviral drug resistance.

In spite of this remarkable progress, the replicon system is limited because only the intracellular steps of the HCV life cycle can be studied. This limitation has recently been overcome with the molecular cloning of a novel HCV isolate that was found in the serum of a Japanese patient with fulminant hepatitis. For unknown reasons this isolate, designated JFH-1,

replicates to exceptionally high levels in cell culture without requirement for adaptive mutations. Most importantly, upon transfection of Huh-7 cells with the JFH-1 genome virus particles are released from the cells that are infectious for naive Huh-7 cells. Infectivity of these particles can be neutralized by antibodies directed against CD81 as well as immunoglobulins from patient serum demonstrating specificity of the infection process. Cell culture-grown HCV is also infectious *in vivo* and JFH-1 derived particles generated *in vivo* are infectious for Huh-7 cells demonstrating that cell culture grown HCV is authentic.

To expand the scope of this virus culture system, JFH-1 genomes with reporter genes such as luciferase or green fluorescent protein have been developed. These genomes also support production of infectious HCV particles and have the advantage of that infectivity can be quantitated by using simple and reliable reporter gene assays. Furthermore, chimeric genomes carrying the region from core up to NS2 from various HCV isolates fused to the JFH-1 replicase have been generated. These genomes also support production of HCV particles that infect Huh-7 cells in a CD81-dependent manner. Finally, a highly adapted genotype 1a genome was identified that also supports production of infectious HCV, albeit with very low titers. These novel cell culture systems will allow the development of new antiviral concepts targeting the early and late steps of the HCV life cycle.

ABSTRACT 008



Co-Infections with HIV, HBV and HCV

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Both HBV and HCV share many of the modes of transmission as HIV and dual infection, and less frequently triple infections, are common. It is estimated that up to 30% of individuals with HIV are co-infected with HCV and approximately 10% are co-infected with HBV. Co-infected persons are likely to have a more aggressive disease course with regard to their chronic viral hepatitis if they are co-infected with HIV; whereas it is unclear if HIV is so impacted by the presence of active HBV or HCV. With the advent of successful antiretroviral therapy, co-infected individuals have reduced rates of fibrosis progression; however, many are surviving, AIDS-free, for long enough periods of time that they may suffer significant mortality because of their chronic hepatitis virus co-infections, with end-stage liver disease becoming a common cause of mortality in the co-infected. Many of the drugs used to treat chronic HBV infection also have antiviral activity against HIV such as lamivudine, emtricitabine, tenofovir, and adefovir; some do not: telbivudine and entecavir. The rate of resistance development is higher in co-infected patients, particularly with lamivudine and presumably emtricitabine. HBV/HIV co-infected individuals who must change or stop therapy are at significant risk for liver decompensation if the HBV drug(s) are suddenly removed. In the case of HCV treatment, drug tolerability with interferon/ribavirin and the antiretroviral agents is more of the issue. The timing of therapy for HCV is also unknown and whether to treat HCV before HIV, at the same time as HIV or after is not completely understood. Co-infection with these three viral infections remains a significant challenge to the managing clinicians and an active area of research for investigators.

ABSTRACT 009


Treatment of HIV Drug Resistance; TMC114 and TMC125

D Miralles

Tibotec BVBA, Mechelen, Belgium

Darunavir is an inhibitor of the HIV protease selected for clinical development based on its potency, high barrier to resistance, and activity against most protease inhibitor-resistant HIV found in drug-experienced HIV-infected individuals. Two studies (POWER 1 and 2) conducted in treatment experienced patients with evidence of PI resistance demonstrated that TMC114 provided significant improvements in virological and immunological endpoints compared to control PIs. Based on these results, TMC114 has been submitted for regulatory approval worldwide. The clinical development program of TMC114 continues with additional studies in treatment experienced and treatment naïve patients. The presentation will describe the clinical development program for TMC114.

ABSTRACT 010

Activation of Antiviral Nucleotide Analogues by Human NMP Kinases: Importance of the Alpha-Phosphate Substitution

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BACKGROUND: Nucleoside and nucleotide derivatives have become crucial in chemotherapies against cancers and viral infections, in AIDS in particular, targeting HIV-1 reverse transcriptase. Under therapeutic pressure, the viral genes mutate and the modified reverse transcriptases become insensitive to antiviral analogues. We have shown that the presence of a borano group on the alpha-phosphate in the tri-phosphate form of clinically relevant compounds such as 3'-deoxy-3'-azidothymidine (AZT) and 2'3'-dideoxy-2'3'-didehydrothymidine (d4T) leads to recovering the sensibility of mutant HIV-1 reverse transcriptases to the inhibitors.

METHOD: The borano modification has been achieved on acyclic nucleoside phosphonates, designing novel phosphonate nucleosides with a BH₃- group on the phosphorus atom. The first step in their intracellular activation is the addition of a phosphate group by a nucleoside monophosphate (NMP) kinase. By use of recombinant human AMP kinases, we have studied the phosphorylation of 9-[2-(phosphonomethoxy) ethyladenine (PMEA) and (*R*)-9-[2-(phosphono-methoxy)propyl]adenine (PMPA) bearing a borano group on the phosphonate.

RESULTS: Our study confirms that the replacement of deoxyribose by the acyclic moiety results in a strong decrease in kinase catalytic efficiencies. However the presence of a-phosphate without any substitution is essential for an analogue to be substrate of adenylate kinases, at least when the analogue is already a slow substrate. The substitution of a borano group for one oxygen the alpha-phosphate in AZT and d4T monophosphate also resulted in a decrease of the phosphorylation rate by TMP kinase.

CONCLUSION: Vectorisation of antiviral derivatives bearing a alpha-borano-phosphate or phosphonate under their tri-phosphate form will then be necessary to take advantage of their powerful therapeutic potential.

Dedicated to the memory of Dr Simon Robert Sarfati, deceased 12 December 2005.

ABSTRACT 011

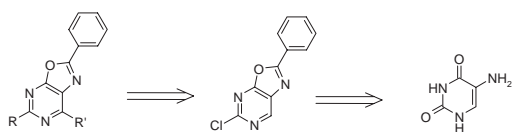
Synthesis of Oxazolopyrimidines Targeting an Anti-HIV Activity

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BACKGROUND: Since the discovery of non-nucleosides, such as Nevirapine, delarvidine or efavirenz, known as anti-HIV drugs, considerable effort have been devoted to the synthesis and evaluation of non-nucleosides. Their structures are often depicted a "butterfly wing". They interfere through an allosteric active site of the HIV-RT. All those compounds have a central heterocycle on which are grafted via a spacer two aryl or heterocyclic functionalized cycles.

METHODS: Thus, as part of our drug discovery program, we decided to develop new compounds having an oxazolopyrimidine moiety. Starting from the commercially available 5-amino-uracil, several R' were introduced through an organolithium chemistry meanwhile the Pd(0)-catalyzed the introduction of various R under Suzuki or Stille conditions.



RESULTS: Around 20 compounds were synthesized through that approach; modifying the order of introduction of R and R' yielded a variation of the angle of the "butterfly wings".

CONCLUSION: The anti-HIV activity and toxicity on different cell lines (PBM, CEM, VERO) of all synthesized compounds have been evaluated. The chemistry and biological data will be detailed. Some of those molecules exhibited significant anti-HIV activity ranging from 1.5 to 30.5 μM .

Session 3

ABSTRACT 012
Subversion of Toll-Like Receptor Functions by Hepatitis C Virus in Dendritic Cells

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The mechanisms whereby Hepatitis C Virus (HCV) evades the host's immune defences and establishes persistent infection remain elusive. With the requirement of functional Toll-like receptor (TLR) signalling pathways for full activation of antigen-presenting dendritic cells (DC) and generation of ensuing CD4⁺ T cell memory responses, this presentation will discuss the concept that specific HCV-DC interactions may exert an inhibitory pressure on innate responses in humans. Analysis of DC function was performed through quantification of cell-associated HCV RNA levels in conjunction with multiparametric flow cytometry analysis of TLR ligand-induced cytokine expression. We show that *ex vivo* FACS-sorted DC carried the highest intracellular viral load (mean log₁₀ 5.07 RNA copies per 10⁶ cells) of all subpopulations analyzed (monocytes, B and T cells) in a cohort of viremic patients. PBMC samples from infected patients and clinical responders were used to survey by intracellular flow cytometry the signal transduction potential of TLR-3 and TLR-4 in CD14⁺CD33⁺ myeloid DC (MDC) and CD14⁺ monocytes. All patients analyzed consistently displayed approximately equivalent levels of IL-6 expression per cell after TLR engagement, suggesting that induction of IL-6 was not markedly inhibited by the presence of HCV. The analysis of the IL-6⁺ CD14⁺ CD33⁺ MDC subset to poly(I):poly(C) and LPS however revealed striking differences in the patterns for expression of IL-12 and TNF- α . We observed a prominent loss of high cytokine-secreting effectors with both TLR ligands, which resulted in a lower expression output at the single-cell level as indicated by the diminished fluorescence intensity shift. In contrast, the CD14⁺IL-6⁺ monocytes responding to LPS had essentially identical expression of TNF- α and IL-12 on a per-cell basis. The defect in cytokine production was confined to MDC of viremic patients since clinical responders showed normal signalling output. An unsupervised hierarchical clustering algorithm was used to investigate whether the patients with TLR signalling anomalies showed mechanistic similarities to each other. This analysis identified two main groups of patients with key differences in their

signalling profiles and revealed a role for a specific virus-host interaction as the cause of TLR desensitization. The group of patients with defective DC contained higher levels of HCV RNA (≥ 5.0 log₁₀ copies/10⁶ cells) than the group of patients with functional DC, suggestive of the ability of HCV to interfere with the function of the signalling pathways of both TLR-3 and TLR-4. This viral-dependent loss-of-function in the TLR-responsiveness of blood DC may represent therefore an unrecognized specific mechanism to possibly account for the failure of chronically infected subjects to generate and maintain long-term HCV-specific CD4⁺ T cell memory responses.

ABSTRACT 013
VX-950: A NS3•4a Protease Inhibitor that Specifically Targets HCV

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BACKGROUND: VX-950 is a potent, selective, peptidomimetic, orally administered inhibitor of the hepatitis C virus (HCV) NS3•4a protease currently in clinical development.

METHODS AND RESULTS: Two Phase 1b studies in patients with HCV genotype-1 infection have demonstrated that VX-950 administered alone is well tolerated and has rapid and substantial antiviral effects. A 750 mg q8h dose induced a rapid initial ~ 3 -log₁₀ reduction of median plasma HCV RNA during the initial 2-3 days of dosing, followed by a second slower phase of decrease to ~ 4 -log₁₀ by day 14.

After the initial decrease, continuous decline of plasma HCV RNA was observed in approximately half of patients studied at this dose, while the others experienced either a plateau or rebound of plasma HCV RNA. VX-950 trough concentrations were higher on average in patients who experienced a continuous decline response compared with those who had viral rebound. Plateau and rebound responses were associated with the emergence of HCV NS3•4a variants with reduced sensitivity to VX-950. The identified variants exhibited various degrees of resistance that were inversely proportional to their replicative fitness. After dosing, wild-type virus replaced resistant virus at a rate dependent on their relative fitness.

VX-950 has also been studied in HCV genotype-1 infection dosed in combination with peginterferon- α -2a (Peg-IFN) \pm ribavirin (RBV), to determine whether these combinations suppress emergence of NS3•4a resistant variants. VX-950 was apparently well tolerated in both the 14-day Ph1b study of VX-950 plus Peg-IFN (in comparison with VX-950 or Peg-IFN alone) and a 28-day Ph2 open-label study of VX-950 plus Peg-IFN+RBV. The initial rapid antiviral effect observed during the first 2-3 dosing days was similar with VX-950 dosed alone or in combination with Peg-IFN. However, VX-950 plus Peg-IFN \pm RBV, caused continuous decline of plasma HCV RNA in all patients in both studies. In the 14-day study, VX-950 plus Peg-IFN induced a decrease in median plasma HCV RNA of 5.5- \log_{10} by the end of dosing, with 6/8 patients below the limit of quantitation (30 IU/mL) and 4/8 below the limit of detection (10 IU/mL) of the Roche Taqman Assay. In the 28-day study of VX-950 plus Peg-IFN+RBV, 2 subjects reached undetectable levels of plasma HCV RNA within 8 days of dosing, as did all 12 subjects by day 28.

CONCLUSIONS: The initial clinical results with VX-950 indicate that it is well tolerated for up to 28 days and has substantial antiviral effects when dosed alone or in combination with Peg-IFN \pm RBV. The combination of VX-950 plus Peg-IFN \pm RBV appears to suppress emergence of NS3•4a protease VX-950-resistant variants. Subsequent studies will evaluate the potential of VX-950, in combination with Peg-IFN \pm RBV, to transform HCV genotype-1 treatment, by allowing elimination of virus in a majority of patients and significantly shortening treatment duration.

ABSTRACT 014



Preclinical Characteristics of ITMN-191, an Orally Active Macrocyclic Inhibitor of the HCV NS3/4A Protease

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Novel therapeutic approaches for the treatment of chronic HCV are needed as current therapies provide sustained virologic response rates of ~50%. Inhibition of the HCV serine protease, NS3/4A, represents a promising new therapeutic strategy. Here we describe the preclinical characterization of ITMN-191, a macrocyclic reversible active site inhibitor that emerged from our discovery program and was nominated as a preclinical candidate.

In biochemical assays using HCV NS3/4A protease domains derived from genotypes 1b, 1a, 2, or 3, the EC₅₀ value of ITMN-191 is < 300 pM, 400 pM, 400 pM and 12.4 nM, respectively. ITMN-191 retains subnanomolar to low-nanomolar potency against the NS3/4A variants at positions A156 and D168 that confer resistance to other experimental NS3/4A inhibitors. In a genotype-1b replicon EC₅₀ = 1.8 nM and EC₉₀ = 15.6 nM using an RT-PCR-based assay. Following a single oral 30 mg/kg dose in rats liver C_{max} was 22.6 μ g/mL (2020-fold EC₉₀) and the concentration in the liver 12 hr post dose was 1.5 μ g/mL (134-fold EC₉₀). An identical dose given to cynomolgus monkeys afforded liver a C_{max} of 1.61 μ g/mL or (144-fold EC₉₀) while the concentration in the liver 12 hr post dose was 0.137 μ g/mL (12-fold the EC₉₀). Similar liver concentrations were observed in multiple dose studies. After a regimen in which a human equivalent dose of 290 mg BID PO was administered for 7 days, compound *trough* liver levels in rats and cynomolgus monkeys were 56-fold and 19-fold the EC₉₀ value, respectively, after the last dose in the study.

In conclusion, ITMN-191 is a highly potent, orally absorbed inhibitor of the NS3/4A protease found in the liver of rats and cynomolgus monkeys at levels predictive of human efficacy. Based partially on these data, ITMN-191 has been nominated for preclinical development and is currently undergoing Phase I-enabling toxicologic assessment.

ABSTRACT 015**Clinical Trial Experience of Viramidine: An Oral Pro-Drug of Ribavirin for the Treatment of Hepatitis C***B Murphy*

Valeant Pharmaceuticals International, Costa Mesa, USA

INTRODUCTION: Viramidine, a liver-targeting oral pro-drug of ribavirin, does not significantly accumulate in the red blood cell (RBC). Data from a phase 2 study revealed that dosing Viramidine 600 mg BID with pegylated-interferon resulted in comparable efficacy but significantly lower rates of anemia compared to ribavirin and pegylated-interferon. This fixed Viramidine dose (600 mg BID) was chosen for phase 3.

METHODS: This multi-center, active-control, randomized, parallel group, double blind study consisted of 970 patients. Patients were randomized 2:1 to receive Viramidine 600mg BID or weight-based dosed ribavirin (1000-1200 mg/day), respectively. Stratification was based on genotype, baseline viral load, and weight. Analyses assessed anemia (Hb < 10 g/dL) for superiority testing and sustained viral response (SVR) based on NGI SuperQuant assay (sensitivity < 100 copies/ml) for non-inferiority testing.

RESULTS: The rate of anemia was 5% in the Viramidine cohort versus 24% in the ribavirin group ($p < 0.001$). The study did not meet the non-inferiority efficacy endpoint on an overall intent-to-treat (ITT) basis: Viramidine 38%, ribavirin 52%, respectively. Viramidine met the non-inferiority criteria in pre-determined per protocol (PP) analyses based on geographic region and weight-based dosing.

CONCLUSIONS: This study confirmed the superior safety profile of Viramidine. Fixed-dose Viramidine

Efficacy: Percent SVR (PP Analysis)

Region	Overall (N=637)		
	Viramidine	Ribavirin	Adjusted difference of proportion and 95% confidence intervals
Overall (N=637)	52%	62%	0.074 -0.002, 0.151
ROW (N=148)	55%	78%	0.209 0.052, 0.365
N.A. & E.U. (N=489)	51%	56%	0.029 -0.060, 0.118
N.A. & E.U. > 75 kg (N=271)	42%	53%	-0.090 -0.034, 0.213
N.A. & E.U. ≤ 75 kg (N=218)	62%	60%	0.049 -0.177, 0.079

Viramidine Weight Based Analysis: Patients with SVR (PP Analysis)

Viramidine Dose	N	SVR	Anemia (Hb < 10 g/dL)
≤18 mg/kg	323	47%	4.3%
19-22 mg/kg	82	66%	2.4%
≥23 mg/kg	16	81%	12.5%

resulted in an average dose of 15 mg/kg which did not maximize the potential efficacy advantages of Viramidine compared to weight-based ribavirin. Analyses of weight-based dosing demonstrated that increasing the mg/kg dose of Viramidine improved the response without a proportionate increase in anemia. Other adverse events did not appreciably increase with higher doses of Viramidine.

ABSTRACT 016**Interim Results of a Multiple Ascending Dose Study of R1626, a Novel Nucleoside Analog Targeting HCV Polymerase in Chronic HCV Patients**

S. Roberts, G. Cooksley, D. Shaw, H. Berns, M. Brandl, S. Fettner, G. Hill, D. Ipe, K. Klumpp, M. Mannino, E. O'Mara, Y. Tu and C. Washington
RDR1021140

BACKGROUND AND AIMS: HCV polymerase is a promising target for the development of compounds for the treatment of HCV infection since it is an essential enzyme for HCV replication. R1626 is a prodrug of the nucleoside analog R1479, a potent inhibitor of HCV replication in vitro. A multiple ascending dose study was designed to evaluate the safety, tolerability, pharmacokinetics, and antiviral activity of R1479 in chronically HCV infected, treatment naïve patients. Preliminary data are reported from this ongoing study.

METHODS: Patients (12 per dose cohort) were randomized 3:1 to oral treatment with R1626 or placebo for 14 days with 14 days of follow up. Two dose cohorts have been completed so far: 500 mg twice daily and 1500 mg twice daily. Assessments included safety, PK, and antiviral activity measured as serum HCV RNA levels.

RESULTS: R1626 at 500 and 1500 mg twice daily was well tolerated in HCV patients with no serious adverse events. No clinically significant changes in

laboratory parameters or vital signs have been observed at the two doses assessed. Following oral administration, R1626 was efficiently converted to R1479 and peak R1479 plasma concentrations were observed in approximately 3 to 4 hours. Exposures following 500 mg and 1500 mg of R1626 were near dose proportional, as reflected by mean AUC_{0-24h} values of 48 and 133 $\mu\text{g}\cdot\text{hr}/\text{mL}$, respectively, and the mean half-life was approximately 24 hours. Dose related antiviral activity was observed with a mean serum HCV RNA reduction of 1.2 \log_{10} from baseline following 14 days of treatment with 1500 mg of R1626.

CONCLUSIONS: Preliminary data from this ongoing multiple ascending dose study suggest that R1626 is well tolerated in treatment naïve chronic HCV patients following twice daily dosing up to 1500 mg for 14 days. R1626 (1500 mg twice daily) was associated with clinically significant reductions in serum HCV RNA and will be further evaluated in combination clinical studies.

ABSTRACT 017

From CCR5 to Maraviroc: The Discovery of a New Investigational Antiretroviral Agent

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Pfizer Global R&D, Sandwich, UK

The binding of HIV to its host cells is one of the latest targets for scientists seeking new therapies for HIV infection and AIDS. Amongst a number of investigational agents, CCR5 antagonists are most likely to be the next new class of antiretrovirals.

CCR5 is a particularly attractive target for the discovery of viral entry inhibitors, as G-protein coupled receptors have traditionally proven tractable targets for the design of selective, low-dose orally bioavailable drugs. Maraviroc (UK-427,857) is the product of a high-throughput screening approach and subsequent medicinal chemistry optimisation. Maraviroc has excellent potency against a range of lab-adapted and primary origin isolates that utilise CCR5 for entry both as a standalone agent and in combination with inhibitors from a number of approved classes of antiretrovirals. The inhibitor blocks viral replication at the point of membrane fusion by preventing the binding of the viral envelope gp120 to the co-receptor CCR5, and does not induce intracellular signalling or trigger receptor internalisation. Maraviroc is non-competitive with regards to chemokine binding, and binds the receptor reversibly albeit with a long half-life. We have generated and analysed viral strains resistant to

maraviroc both *in vitro* and in the clinic, where maraviroc has demonstrated significant reductions in viral load following short-term monotherapy without safety or toleration issues. In conjunction with attractive pharmacokinetics and pharmacodynamics, maraviroc holds much promise for those patients in need of novel efficacious, safe, conveniently administered antiretroviral regimens.

ABSTRACT 018



Vicriviroc Use in Novel Antiretroviral Regimens

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Schering-Plough Research Institute, Kenilworth, USA

The introduction of HIV entry inhibitors as components of antiretroviral regimens has opened opportunities for novel strategies not previously available. Vicriviroc is a potent inhibitor of HIV, acting via antagonism of the CCR5 coreceptor, which demonstrates activity against most strains of CCR5-tropic virus, including strains that are resistant to other existing classes of antiretroviral agents. Synergistic activity has been shown *in vitro* with vicriviroc and other classes of antiretrovirals.

Vicriviroc has also demonstrated predictable, dose-related pharmacokinetics with drug-drug interactions that are easily managed in this clinical setting. As a substrate of CYP3A4, vicriviroc, used in combination with any ritonavir-boosted protease inhibitor-containing regimen, requires no dose adjustment or potential need for therapeutic drug monitoring. Studies with all marketed ritonavir-boosted protease inhibitors (PI), show no interaction with vicriviroc beyond the effect of ritonavir alone.

Vicriviroc has been studied in multiple Phase 1 and two Phase 2 trials - one in heavily treatment-experienced individuals receiving a ritonavir-boosted PI-containing regimen and one in treatment-naïve subjects not receiving ritonavir. The treatment-experienced trial (ACTG 5211) remains on-going evaluating vicriviroc 10 and 15 mg QD versus placebo in combination with an optimized ritonavir-boosted PI-containing regimen; the 5 mg QD dose group was discontinued in October, 2005, because of a high incidence of virologic failure, indicating a dose response in efficacy.

The study in treatment-naïve individuals (P03802, sponsored by Schering-Plough) which evaluated

doses of 25, 50 and 75 mg QD vicriviroc + Combivir® versus efavirenz+Combivir®, was terminated in November, 2005, due to observation of dose-related late viral breakthroughs. While the vicriviroc 75 mg QD dose was not statistically different from efavirenz, an independent DSMB recommended that greater long-term efficacy was expected for patients on their first-line therapy. Preliminary data did not indicate the emergence of resistance to vicriviroc; sequencing of viral envelopes is continuing. Ongoing pharmacokinetic/pharmacodynamic analyses are exploring the likelihood that higher doses of vicriviroc may achieve the desired level of efficacy.

Vicriviroc has been well-tolerated in all clinical trials. Importantly, there has been no evidence of hepatocellular damage or other acute toxicity. Five patients enrolled in the ACTG 5211 study developed malignancies (4 lymphomas, 1 gastric adenocarcinoma), usually after 6 months of treatment, but a causal relationship with vicriviroc could not be established and the trial continues.

The overall safety profile, dose-related efficacy, predictable pharmacokinetics, convenient once daily dosing and manageable drug interactions associated with vicriviroc makes this new drug a promising component of multiple novel regimens. Clinical development in both treatment-experienced and treatment-naïve patients is ongoing.

ABSTRACT 019



Design of Antiviral Agents with Enhanced Pharmacokinetics

J Erickson

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HIV-1 fusion inhibitors (FIs) represent a promising new class of antiretroviral agents (ARVs). The first HIV-1 FI approved for human use, T-20, is a 36-mer peptide derived from the gp41 sequence. T-20 is administered as a twice-daily subcutaneous injection, and is highly efficacious when combined with other ARVs. However, the requirement for frequent dosing combined with painful injection site reactions limit patient acceptance and adherence. Poor adherence to ARV therapy strongly correlates with viral failure due to the emergence of drug resistant viruses. In our effort to discover antiretroviral agents with high barriers to resistance, we have developed a novel technology, termed molecular cloaking (MC), for designing drugs with enhanced pharmacokinetic activity. Our goal is the development of molecularly-

cloaked antiretroviral agents (MC-ARVs) that can be safely and infrequently administered, perhaps once a week or less, without sacrificing efficacy or promoting drug resistance, while maximizing patient tolerability and adherence. The technology entails design and synthesis of a biologically-active pharmacophore ("warhead" moiety), and its covalent coupling to a pharmacologically inert protein ("cloaking" moiety), resulting in novel molecular drug-conjugates. The method is called molecular cloaking because the warhead in the conjugate is preferentially shielded from elimination mechanisms, such as metabolism, by the cloaking moiety, but remains available to interact with its biological target.

We applied this technology to the design of two novel, molecularly-cloaked HIV-1 fusion inhibitors (MC-FIs) using human serum albumin (HSA) as a cloaking protein and chemically-modified peptides as warheads. Both MC-FIs exhibited antiviral IC_{50} s in the 1-10 nM range against a variety of viral strains, and were stable in plasma. A rat PK model was used to evaluate the pharmacokinetics of MC-FIs over 48 hours after a single IV infusion. Both MC-FIs exhibited a well-behaved distribution phase followed by an elimination phase with half-lives of between 12-14 hrs. In comparison, the unconjugated peptides, which were biologically active *in vitro*, were cleared rapidly *in vivo*, and were undetectable after a few hours. Overall, the pharmacokinetic profiles of the two MC-FIs mimicked that of HSA reported in a rat species, while the antiviral activities mimicked those of the warheads. These data are consistent with our hypothesis that pharmacokinetics and antiviral activities of properly designed, molecularly-cloaked inhibitors should be dictated by the carrier and warhead portions of the molecule, respectively.

Extrapolation of the rat PK data to humans, in which the plasma half-life of HSA is around 21-24 days, suggests the possibility of developing an MC-FI that would be effective in a once, or perhaps twice, monthly dosing regimen. Molecular cloaking represents a promising approach to the development of ARVs with enhanced pharmacokinetics, safety, and adherence.

ABSTRACT 020**Modulation of Endoplasmic Reticulum (ER) Calcium Signalling Affects Hepatitis C Virus (HCV) Protein Expression and Replication**

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BACKGROUND: We have previously shown that transient and stable expression of HCV core protein decreases ER calcium concentration and induces apoptotic cell death in a calcium dependent manner (Benali-Furet *et al*, 2005). We thus tested the hypothesis that modulation of calcium concentrations in the ER by specific drugs can have an impact on HCV protein expression and HCV replication.

METHODS: We used the HuH7 cell line stably expressing the full length HCV genome (Pietschmann *et al*, 2002) and non transfected HuH7 cells as controls. Intracellular calcium concentrations were modulated by the following drugs: cyclopiazonic acid (CPA) and 2-AminoethylDiphenylBorate (2-APB). Intracellular calcium concentrations were assessed by specifically targeted recombinant aequorin calcium probes. Viral proteins expression was assessed by Western Blot. The amount of HCV RNA was evaluated by real time quantitative RT-PCR.

RESULTS: In HuH7 cells, concentrations of 2-APB and CPA that do not affect cell viability were shown to induce a significant decrease of ER calcium concentrations ($166 \pm 10 \mu\text{M}$, $n = 5$, $p < 0.05$ and $150 \pm 10 \mu\text{M}$, $n = 5$, $p < 0.01$ respectively, *vs* $199 \pm 20 \mu\text{M}$, $n = 7$) as compared to non treated HuH7 cells. Western blot analyses showed that increasing concentrations of 2-APB and CPA induce a progressive and kinetic decrease of NS3 (from 5% to 95%) and core (from 50 to 100%) protein expression. Real time quantitative RT-PCR targeted to the HCV 5' noncoding region sequence showed that concentrations of 2-APB and CPA that do not modify cell viability induce a significant decrease of HCV RNA level of 65% and 90%, respectively. In contrast, HuH7 replicons transfection with SERCA2 (which increases ER calcium concentration) led to an increase of HCV RNA level of 20%.

CONCLUSION: The endoplasmic reticulum (RE) is known to have a major role both in regulation of calcium signalling and in HCV proteins maturation and genome replication. Our results show that pharmacological decrease of ER calcium concentration affects HCV proteins expression and HCV replication. Our results are consistent with a pivotal role of ER calcium in HCV replication and show a new class of drugs potentially able to control HCV infection.

Session 4

ABSTRACT 021



Hepatitis B Virus and the Innate Immune Response: A New Paradigm for Therapeutic Intervention

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Mechanisms by which hepatitis B virus (HBV) establishes persistent infection remain unclear. In particular, expression of Toll-like receptors (TLRs), increasingly recognised as very important in the innate immune response to bacterial and viral pathogens, has not been widely investigated.

Toll-like receptor (TLR) expression on hepatocytes and Kupffer cells from fresh liver biopsies was measured from twenty patients with untreated hepatitis B e antigen (HBeAg)-positive (8) and HBeAg-negative (12) chronic hepatitis B (CHB). Parallel studies were also undertaken on monocytes from the peripheral blood of these patients. Expression of TLR2 on hepatocytes, Kupffer cells and peripheral monocytes was significantly reduced in patients with HBeAg-positive CHB in comparison to HBeAg-negative CHB and to controls whilst it was significantly increased in HBeAg-negative CHB compared to controls. The level of TLR-4 expression did not differ significantly between the groups. These results were confirmed and extended *in vitro* using hepatic cell lines transduced with recombinant HBV baculovirus expressing wildtype HBV (HBeAg-positive), precore stop codon (G1896A) and mutant HBV (HBeAg-negative). The functional relevance of these findings was established by the demonstration of significantly reduced cytokine production (TNF- α) and phospho-p38 kinase expression in the presence of precore protein. In the absence of HBeAg, HBV replication is associated with upregulation of the TLR2 pathway leading to increased TNF- α production. This study highlights the importance of the precore protein (HBeAg) in the innate immune response to HBV and suggests that this virus specific effect on innate immunity may contribute to the development of persistent infection and provide a new paradigm of pathogenesis and therapeutic intervention for HBV disease.

ABSTRACT 022



Suppressing HBV Replications with New Drugs and Combinations

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In chronic hepatitis B infection, an inadequate host immune response to the virus results in protracted hepatic necroinflammation (1). This will in turn greatly increase the risk of subsequent development of liver cirrhosis and liver cancer (2). In order to achieve disease remission with resolution of hepatic necroinflammation, one need to suppress the HBV DNA viral load to less than 10^{4-5} copies/ml. Currently, we have two different approaches to achieve this objective. We can either aim at restoring the host immune control on the virus with conventional interferon- α /pegylated interferon- α 2a or directly suppress the viral replication with nucleos(t)ide analogues such as lamivudine, adefovir or entecavir (3). Though the use of nucleos(t)ide analogues can result in rapid on-therapy disease remission, there is little improvement on host immune control on the virus, as evidenced by the lack of significant s-seroconversion with such therapy (1). As a result, one need long-term or even life-long treatment with nucleos(t)ide analogues with an increase risk of development of anti-viral resistance (3). On the other hand, not all patients can tolerate or respond to interferon-based therapy. Recently, combination therapy has been examined for an improved therapeutic efficacy but so far, results are disappointing.

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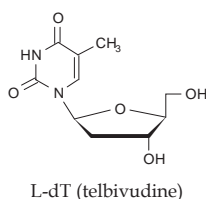
ABSTRACT 023



Discovery of L-dT (telbivudine) as a New Potent Nucleoside Analogue for the Treatment of Chronic Hepatitis B

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BACKGROUND: Recently, a unique series of simple "unnatural" L-enantiomer nucleosides has been discovered to inhibit hepatitis B virus (HBV) replication [1]. Among them, β -L-2'-deoxy-thymidine (L-dT) had the most potent, selective and specific activity against HBV replication, and was selected as an attractive clinical development candidate for the treatment of chronic HBV infection.



METHODS: Novel synthetic routes were developed to produce formerly described [2] L-dT, first on a 20 grams scale, and then on larger scales. The intracellular activation and the metabolism of L-dT were studied in detail, and its pharmacology and pharmacokinetic profiles were assessed in the woodchuck animal model. The data supported the introduction of L-dT in clinical trials.

RESULTS: In a recently completed phase IIb clinical trial, one year of L-dT treatment reduced HBV in the blood to undetectable levels (*i.e.*, less than 200 particles/mL) in 61% of patients, significantly more than the 32% of patients who achieved this result with lamivudine, the current standard of care [3]. An international phase III clinical trial for L-dT, known as the "GLOBE study", is ongoing and fully enrolled, including more than 1,350 patients and approximately 135 clinical centers. The initial phase of this clinical trial was completed in 2005, showing that after one year, L-dT provided significantly greater responses on all direct markers of antiviral efficacy, compared to lamivudine. In these clinical trials, L-dT appeared to be very well tolerated, and to date, there has been no pattern of patient discontinuations due to serious adverse events.

CONCLUSION: L-dT (telbivudine) is developed by Idenix (<http://www.idenix.com>) in collaboration with Novartis (<http://www.novartis.com>) under a development and commercialization agreement. In the beginning of 2006, Idenix and Novartis

announced the submissions of a New Drug Application (NDA) and a Marketing Authorization Application (MAA), respectively to the FDA and the European Medicine Agency (EMA), seeking marketing approval for a 600 mg dose of L-dT as an oral, once-daily drug for the treatment of chronic hepatitis B.

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ABSTRACT 024



Prevention of HCC by Antiviral Drugs

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Prevention is the only realistic approach for reducing mortality rates associated with hepatocellular carcinoma (HCC), worldwide. Vaccination against hepatitis B and screening of blood donations are effective measures of primary prevention which led to a substantial reduction in viral hepatitis transmission among the general population, with a significant reduction in HCC in selected populations. Primary prevention includes also approaches which alter epigenetic and genetic changes in hepatocytes, known to increase susceptibility to HCC, as well as treatments slowing progression to cirrhosis. The only evidence that chemoprevention reduces HCC risk is a multicenter randomized prospective study in Asian patients with advanced hepatitis B who received the oral nucleoside analogue lamivudine. By converse, the risk of HCC is not substantially reduced in patients with hepatitis B treated with interferon. Evidence that HCC risk is reduced in patients with chronic hepatitis C treated with interferon, is controversial. HCC prevention is likely for patients with chronic hepatitis C achieving a sustained virological response whereas it is doubtful for patients who did not respond to interferon. The reanalysis of 2 large databases suggests that HCC still occur in cirrhotics with a sustained virological response. Secondary prevention, *i.e.*, prevention of tumor recurrence after hepatic resection or local ablative therapies, has been pursued with different approaches. Retinoids, hepatic embolization with I¹³¹

lipiodol and adoptive adjuvant immunotherapy have yielded encouraging results. Conversely, approaches based on interferon alfa or beta provided inconclusive evidence for secondary prophylaxis of HCC, mainly due to poor methodologies and scientific background of the studies.

ABSTRACT 025



HIV, HBV and HCV - Different Viruses with Different Kinetics and Clinical Implications

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Although the viral dynamics models for Human Immunodeficiency virus (HIV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV) are rather similar and all of these viruses exhibit very rapid dynamics, still there are some important differences between them that have important clinical implications.

Differences in mechanism of the anti-viral effect of the various therapies for the three viruses, blocking *de-novo* infection versus blocking virion production (Perelson *et al.*, Science 1996; Neumann *et al.*, Science 1998; Tsiang *et al.*, Hepatology 1999) give rise to important differences in the kinetics.

Also, differences in the immune status of patients chronically infected with HIV and HCV versus HBV plays an important role in determining the viral dynamics without treatment, and its correlation with response to treatment.

Furthermore, differences in the various cellular compartments in which the three viruses replicate, as can be analyzed from viral kinetic data, have important implications for the possibility of viral eradication and for maintenance of quasi-species libraries.

Lastly, evolution shows different time scales and/or different mutation rates in the different viruses. That in combination with the different dynamics and the effects of the drugs used against each of them are important to understand the difference we observe between HIV, HBV and HCV in terms of evolution of resistance.

ABSTRACT 026

A Prospective Study on Chemotherapy-induced Hepatitis B Virus Reactivation in Chronic HBsAg Carriers with Hematologic Malignancies and Pre-emptive Therapy with Nucleoside Analogues

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BACKGROUND: Chemotherapy-induced hepatitis B virus (HBV) reactivation is a serious problem in chronic HBV carriers with hematologic malignancies. The present study aimed to determine the effectiveness of nucleoside analogue in the pre-emptive therapy of HBV reactivation.

METHODS: Between 2000 and 2004 HBV carriers with hematologic malignancies had prospectively been screened for chemotherapy-induced HBV reactivation in our department. A total of 12 patients were included in the study and monitored for HBV reactivation during 16 different courses of chemotherapy, including one autologous peripheral blood stem cell transplantation. HBV reactivation was defined as a change in the HBV DNA status from negative to positive in the absence of laboratory evidence of acute infection with hepatitis A, C and delta virus. During the course of chemotherapy ALT/AST levels were performed routinely before each chemotherapy cycle and at day 15. HBV DNA levels were measured bimonthly even in patients with normal ALT/AST levels and monitoring was continued for 4 months after the completion of chemotherapy. When a patient was found to have developed hepatitis B reactivation during the course of chemotherapy lamivudine 100 mg/d or famciclovir 1500 mg/d was commenced immediately.

RESULTS: HBV reactivation occurred in seven patients (58.3%) whereas five of the seven patients (71%) responded to nucleoside analogue therapy. HBV reactivation-related acute liver failure and death was not observed in the present study. All five patients with chronic lymphocytic leukemia (CLL) experienced chemotherapy-induced HBV reactivation regardless of the chemotherapy regimen.

CONCLUSION: We suggest that CLL carries a significant risk of chemotherapy-induced HBV

reactivation. The pre-emptive therapy of chemotherapy-induced HBV reactivation appears to be safe, based on the results of this pilot study. Pre-emptive therapy enables the definition of high-risk patients who cannot be identified by primary prophylaxis.

ABSTRACT 027

Novel Peptidomimetic Andimers Inhibit Mutated HIV-1 Proteases

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4 Macromolecular Biophysics Facility, Institut Pasteur; 5 Rega Institut, Leuven, Belgium.

BACKGROUND: The development of cross resistance to protease inhibitors is a serious limitation in the long-term treatment of AIDS patients. All clinical protease inhibitors are transition state analogues that target the active site. So to overcome increasing cross-resistance, we have used another strategy - we have targeted the dimer interface of the enzyme in a region that is relatively free of mutations. The antiparallel β -sheet formed by interdigitation of N- and C-terminal strands of each protease monomer is highly conserved among HIV-1 isolates and contributes to over 75 % of the dimerization stabilization energy. We demonstrated that lipopeptides^a ($K_{id} = 5$ nM), guanidinium-based molecules^b ($K_{id} = 150$ nM) and constrained molecular hairpins^c ($K_{id} = 80$ nM) act as dimerization inhibitors by preventing the correct assembly of the inactive monomers to the active dimeric enzyme. This presentation is designed to introduce next generations of molecules. We have decreased their peptide characteristics with an emphasis on their activity against mutant proteases found in resistant viruses.

METHODS: In order to improve the metabolic stability of dimerization inhibitors, the peptidic character of the molecular hairpins was decreased by introducing groups that mimic the hydrogen bonding network of a peptide β -strand. D-amino acids were introduced in lipopeptides and their alkyl chains were shortened. The dissociative mechanism was established using complex kinetics and various biophysical methods such as ultracentrifugation. The

stability of andimers have been studied in culture medium containing serum. We produced single, double and multiple-mutated proteases (MDR-HM and ANAM-11) to evaluate their inhibition by andimers. Anti HIV activity and cytotoxicity were determined using HIV-1 (IIIB) and HIV-2 (ROD) in MT4 cells.

RESULTS: Both the type of inhibition and the inhibitory potency depends on the length of the alkyl chain of lipopeptides. Dimerization inhibitors are obtained with C16, C14 and C12 chains but not with shorter chains. Using ultracentrifugation, the formation of a monomer-lipopeptide complex was demonstrated. The dissociative mechanism is maintained when D-amino acids are introduced. The antidimer property of peptidomimetic hairpins depends on the position of the peptidomimetic motif in the hairpin arm. We found that lipopeptides and peptidomimetic hairpins act on multidrug resistant HIV-1 protease mutants. Their inhibitory potency are unchanged or only decreased by factor ranging from 1.2 to 12.

CONCLUSION: 1) Peptidomimetic strands that improve the metabolic stability can be inserted in molecular hairpins and lipopeptides without changing the dissociative mechanism. 2) Our dimerization inhibitors are efficient *in vitro* against mutated proteases. These low-molecular-weight dimerization inhibitors may provide a complementary approach to circumvent the drug resistance observed with active-site antiproteases.

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ABSTRACT 028**Utility and Complementarity of Replicative Phenotyping for Drug Discovery and in Diagnostics**

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BACKGROUND: The advent of highly active antiretroviral therapy (HAART) has dramatically changed the management of HIV disease. Yet the development of viral resistances to current medication has in recent years led to intense research for appropriate, sensitive diagnostic tools.

METHODS: The development of an "integrated resistance platform" combining genotyping, replicative phenotyping, and the assessment of replicative fitness provide a most useful tool, allowing the treating physicians to monitor therapy for their patients as well as the drug developing industry in devising new inhibitor specifications. In the form of "PhenoTect" these tools are today used in Diagnostics to test resistance to Protease Inhibitors (PI), Reverse Transcriptase Inhibitors (RTI) and Fusion Inhibitors (FI) already on the market. This wealth of precious information yet a data explosion with an ever growing level of complexity is handled by "PhenoBase" as integrated search tool. Here we demonstrate its utility in revealing and untangling discordances between Genotyping and Phenotyping, for research on mutations, and for patient's medical follow-up.

RESULTS: Using the information provided by the diagnostic routine we show that replicative phenotyping tends to leave more therapy options for therapy-experienced patients

- by direct determination rather than scoring by rules (in two projects selectively assessing atazanavir and TAMs we show that genotype may over- and underscore certain mutations:)
- by addressing specifically non-B subtypes (with their risk of inappropriate extrapolation from genotyping)
- by not placing all mutations onto only one "virtual genome" (in case of mixed virus populations)
- by detecting minority species (we show superior sensitivity down to 1% in a virus population, e.g. resistant to 3TC and NNRTI) due to the sensitivity of the test.

For the above reasons the unique replicative format of the PhenoTect system has proven excellent

suitability and validity in the profiling of new drugs as well as in evaluating new drug targets (in Gag, integrase, regulatory genes). This tool is in current use for monitoring phase III clinical trials of Capsid Inhibitors as well as for profiling new drugs in pre/early clinical development such as inhibitors of retrovirus integration.

CONCLUSION: The unique features of an enhanced, replication competent phenotyping format may overcome significant shortcomings of single cycle systems and will be of utility for (pre-)clinical profiling of next generation HIV inhibitors. We believe that PhenoTect will represent a valuable complementing tool to genotyping, particularly for therapy-experienced patients.

ABSTRACT 029**High Throughput Assay Screening for Inhibitors of Ion Channels including HCV p7, HIV-1 Vpu and 'flu H5N1 M2**

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BACKGROUND: A number of small virus proteins, viroporins, have been shown to form ion channels in lipid bilayers and are thought to influence virus entry into or exit from cells by directly or indirectly neutralizing acidified cellular compartments. Amantadine is used in the prophylaxis and treatment of influenza A virus infections by blocking the H⁺ ion channel activity of the viroporin M2. Other viroporins are important: HIV-1 Vpu enhances virus budding, and the structurally similar HCV and BVDV p7 proteins are essential for the production of infectious virions. The HCV p7 forms a K⁺/Na⁺ ion channel in vitro that can be inhibited by amantadine and hexamethyl amiloride (HMA). The p7 is predicted to act indirectly to neutralize pH within cellular compartments and may interact with several cellular processes, limiting the utility of in vitro studies. However, in the absence of a robust method to culture the more prevalent HCV genotypes, screening a large number of compounds for the inhibition of p7 ion channel function is more feasible by use of a high throughput cell based assay.

METHODS: We have investigated assays such as HIV-1 Gag virus like particle (VLP) budding and hemadsorption by influenza H5N1 HA for their suitability in a high throughput assay. The gene for the genotype 1a H77 p7 and the upstream signal sequence was synthesized and cloned into a

tetracycline inducible mammalian expression plasmid (pcDNA4), as were pNL4-3 Vpu and amantadine sensitive H5N1 M2 (M2^{AS}) and amantadine resistant M2 (M2^{AR}). BS-C-1, Bt7h, Huh7 and 293T cells were used during transfection studies using FuGene6 reagent.

RESULTS: Huh7 and 293T cells were efficiently transfected (50-90%). Gag was expressed from pGag when co-transfected with viroporin plasmids. While no hemadsorption was observed when pHA was co-expressed with a viroporin, HA was detected immunologically. Effective assays were developed that exhibited significant measurable differences between cells expressing and not expressing functional and non-functional ion channels and were used initially to screen nine compounds for ion channel inhibition at concentrations from 2.5 to 40 μ M. During the validation of the assays, ≤ 10 μ M amantadine did not inhibit M2^{AR} and did measurably inhibit M2^{AS} as expected. At 40 μ M, M2^{AS} was slightly more inhibited than M2^{AR}, but both were significantly inhibited.

CONCLUSION: We intend to screen a large collection of compounds using these assays. The assays could be adapted to examine various viroporins and other ion channels of interest. We will examine the effect of compounds with p7 inhibitory activity in cell culture-derived virus and in animal models.

Session 5

ABSTRACT 030
Drug Resistance, Vaccine Development and Beyond

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With its high replication rate and error prone polymerase, HIV has a tremendous capacity for mutation and genetic diversity. This capacity has major implications both for treatment and vaccine development, but in very different ways.

For treatment, most new drugs are active against the broad range of HIV-1 variants; however, without sustained potent combination therapy any active drug selects for resistant mutants. Drug resistance now plays a major role in drug development and patient management.

In contrast, vaccine design must contend with the same broad range of HIV-1 variants; however, these "wild type" variants have generated much of their diversity in response to the selective pressures of humoral and cell mediated immune responses in infected individuals. The consequences are a major challenge for a broadly reactive vaccine.

These same challenges to both treatment and vaccine development will prove at least as challenging for the emerging initiatives against hepatitis C.

ABSTRACT 031
The Other HIV-1 Enzymes: Integrase and Beyond

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Integrase has long been considered an attractive target for the development of novel antiretroviral agents to treat HIV-1 infection. However, it is only within the past two years that the *in vivo* efficacy of integrase inhibitors has been achieved, with proof of concept demonstrated both in experimental models of retroviral infection and in HIV-infected patients. Several integrase inhibitors are now in clinical development including MK-0518 which is currently in phase 3. While a variety of different chemical structures and mechanisms of action have been demonstrated for inhibitors of integrase *in vitro*, those compounds for which proof of concept has been established to date all belong to a distinct class known as integrase strand transfer inhibitors (InSTIs). InSTIs derive their activity from the ability to bind and sequester divalent metal ion cofactors within the integrase active site. This mechanism of action is consistent with evidence that resistance to these inhibitors can result either as a consequence of mitigating specific interactions with the enzyme or from perturbing the metal binding architecture itself. Understanding of the molecular basis underlying the mechanism of InSTIs and the identification of a wide range of structurally diverse pharmacophores which utilize this mechanism, together with the recent success of such inhibitors in the clinic has provoked an interest in exploiting this approach for other enzyme targets that contain a homologous active site motif. We have now identified structural homologs which specifically inhibit either the polymerase or RNase H functions of HIV-1 reverse transcriptase. Characterization of the respective biochemical and structural basis of activity for these inhibitors supports the general mechanism of this class of pharmacological agents established for HIV-1 integrase and provides the basis for developing novel antiretroviral agents against these additional HIV-1 targets.

ABSTRACT 032
**HCV Drug Development -
Lessons Learned from HIV**

D Mayers

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The Human Immunodeficiency Virus (HIV) and hepatitis C virus (HCV) are both RNA viruses that cause chronic infections in man with significant morbidity and mortality. Both viruses utilize high levels of replication and error-prone viral polymerases to produce "quasispecies" of closely related viruses. These quasispecies allow the virus to chronically escape from the immune system. Antiviral therapy of HIV has demonstrated that this mechanism is readily used to escape drug selection pressure unless fully suppressive combination drug regimens are utilized. It is anticipated that combination regimens of antiviral drugs with complementary mechanisms of action will be needed to provide curative therapy for HCV.

Antiviral drug discovery evolved rapidly after the discovery of HIV as the methods of modern molecular virology were utilized to develop potent and selective drugs to treat HIV disease. High throughput molecular screens with counter screens using related human enzymes have accelerated the discovery of HIV drugs that target viral attachment and the critical HIV enzymes: protease, polymerase and most recently integrase. Crystal structures of viral enzymes with antiviral drugs in the active site have allowed rational drug design of antiviral drugs and improved understanding of the mechanisms of antiviral drug resistance. These techniques were quickly transferred to HCV drug discovery (along with drug discovery programs directed at other human viral pathogens).

HIV drug development and patient management were significantly improved with the development of PCR-based viral load measurements in the peripheral blood. HIV drug resistance testing moved into general clinical use with the development of recombinant virus assays to assess clinical HIV drug resistance in a rapid, cost effective format. HCV has only recently been cultured in vitro but antiviral activity can be assessed using an HCV replicon system

HIV disease has accelerated the development of antiviral drugs and diagnostics to treat antiviral diseases. These technologies have been adapted to HCV drug discovery and development.

Combinations of oral drugs to cure HCV-infected patients are anticipated to become available in the next 5 to 10 years.

ABSTRACT 033
**Similarities and Differences
Between HIV-1 and HIV-2 Drug
Resistance**

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The therapeutic management of HIV-1 infections has dramatically improved with the use of combinations of antiretroviral drugs. Human immunodeficiency viruses type 2 (HIV-2) are naturally resistant to current non-nucleoside reverse transcriptase inhibitors and fusion inhibitor. Limited information is available on the activity of antiretroviral drugs against HIV-2 strains to guide their use in treatment.

Protease Inhibitors (PIs) were designed to fit into the active site of HIV-1 protease. More than 50% of aminoacids of HIV-2 are different from HIV-1 protease with a large polymorphism. These differences may impact on the efficacy of HIV-2 protease inhibition. Preliminary in-vitro phenotypic results showed a reduced susceptibility of HIV-2 clinical isolates to some PIs as compared to HIV-1. In HIV-2 patients failing to PIs the major PI mutations already described for HIV-1 were selected besides other mutations of unknown impact.

As regards nucleoside analogs, there is no in vitro evidence of differences in susceptibility between both viruses. In HIV-2 patients failing to nucleoside analogs the genotypic mutational patterns are different from those described in HIV-1 patients.

In patients enrolled in the French HIV-2 cohort and starting highly active antiretroviral therapy a poor immunological response has been reported raising the hypothesis of a lower activity of PIs in HIV-2 infected patients.

The ongoing implementation of a European collaborative network, aiming at collecting data on all HIV-2-infected patients starting with a first line triple ARV combination, should allow to confirm or not our concern regarding the efficacy of antiretroviral in HIV-2 infected patients.

ABSTRACT 034
**Discovery and Development
of an Oral Influenza Drug:
"TAMIFLU"**

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Influenza continues to be a serious concern with yearly epidemics causing significant morbidity and mortality. Control of highly pathogenic avian H5N1 influenza virus is a major issue of world-wide public-health. Influenza virus neuraminidase catalyses the cleavage of sialic acid residues terminally linked to glycoproteins and glycolipids and plays a key role in the replication of the virus. The rational design of mimicking the transition state of the sialic acid cleavage led to the discovery of a new series of potent influenza neuraminidase inhibitors. In this drug design, the cyclohexene scaffold was used as a transition state analog instead of the sialic acid-based dihydropyran ring. From this series, GS 4071 emerged as one of the most potent NA inhibitors against both influenza A and B. GS 4071 binds into the neuraminidase catalytic pocket different from sialic acid with the strong hydrophobic interaction at the glycerol binding site. This pocket can accommodate both hydrophilic and hydrophobic groups depending on the orientation of Glu 276 as shown by the X-ray crystal structure. Utilizing this hydrophobic interaction, GS 4071 was designed to increase lipophilicity considerably compared to sialic acid analogs for oral absorption. Further prodrug optimization of GS 4071 to GS 4104 (oseltamivir, TAMIFLU) as the ethyl ester was necessary for high oral availability in rats and dogs. Highly efficient, practical synthesis of GS 4104 has been developed using shikimic acid as a starting material. GS 4104 exhibited potent neuraminidase activity against laboratory and clinical isolates of influenza A and B viruses in the low (< 10) nanomolar range. GS 4104 also showed potent effects against influenza A and B viruses in mouse and ferret animal infection models. Dosage of GS 4104 at 1 and 10 mg/kg/day significantly reduced virus titers in the lungs of mice infected with highly pathogenic H5N1 A/VN1203/04 influenza virus. Mechanism of resistance to GS 4104 will also be discussed in this presentation.

ABSTRACT 035
**Emergence of Novel
Retroviruses**

W Heneine

Centers for Disease Control and Prevention, USA

As with many viruses, retroviruses do not always restrict themselves to a single host species. Phylogenetic analyses of human and other primate retroviruses conducted during the past twenty years has revealed that the pandemic human retroviruses, human immunodeficiency viruses (HIV) and human T-lymphotropic viruses (HTLV), were each the result of multiple independent introductions of viruses from nonhuman primates (NHPs) to humans. Nevertheless, whether such transmission of retroviruses was limited to rare historical occurrences or is part of an ongoing process has remained unclear. A series of recent findings by our group, focused on individuals exposed to NHPs has demonstrated that far from being a historical oddity, the transmission of retroviruses from NHPs to humans is a regular and ongoing phenomenon. Recent evidence demonstrate emerging retrovirus zoonosis, both from captive animals to lab workers and primate handlers in North America, as well as from wild animals to Central Africans exposed through hunting, butchering, and keeping of wild animal 'pets'. The results show that three new retroviruses previously undocumented in humans including the simian foamy viruses, HTLV-3, and HTLV-4 have all been identified in persons exposed to the blood and body fluids of NHPs. The regular transmission of primate retroviruses suggests that zoonosis, *per se*, may not be the rate limiting step in pandemic retrovirus emergence, and that other factors such as viral adaptation probably play an important role in successful cross-species transmission and pandemic human retrovirus emergence. The results reinforce the need for defining the public health implications of the emergence of the new retroviruses and for ongoing surveillance efforts aimed at documenting and predicting the retrovirus emergence process.

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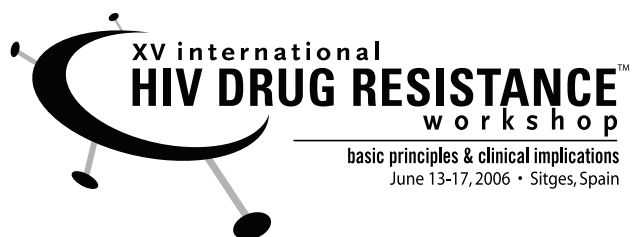
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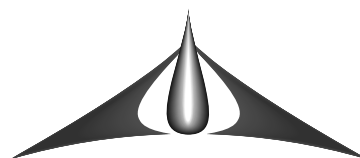
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