



Trip Report for
**“Strategic Research Institute: 3rd Annual Viral Hepatitis in Drug
Discovery & Development World Summit”**
Boston, MA
March 12-13, 2007

Bruce F. Molino, Ph.D.

Abstract: *Antiviral scientists from industry and academia presented results from their recent research efforts to enhance understanding of hepatitis B (HBV) and hepatitis C viral (HCV) infections, the resulting hepatocellular diseases, and progress towards new antiviral treatments. This report focuses on HCV and status of new antiviral approaches to treat HCV. Scientists from companies with major R&D programs in the HCV antiviral area were in attendance and updated the progress of their antiviral compounds in preclinical and clinical development.*

Background on Hepatitis C Virus (HCV)

Chronic infection with HCV is an epidemic with approximately 170 million infected individuals worldwide. Up to 5 million people are infected in the U.S. and chronic liver diseases resulting from HCV infection are responsible for 13,000 deaths per year in the U.S. alone. HCV is one of the most important causes of chronic liver disease (greater than 50% of infections lead to liver cirrhosis or hepatocellular carcinoma or HCC) and the most common cause of liver transplantation. Because of the high replication rate (10¹⁰-10¹² virions per day) and the lack of proof-reading function of NS5B (which is the RNA-dependent RNA polymerase that catalyses the replication of HCV RNA), the HCV genome has high genetic variability. HCV is classified into 6 main genotypes, which diverge by ~30% at the nucleotide-sequence level, and there are more than 30 subtypes throughout the world. Genotype 1a and 1b are predominant in the United States and Europe and genotype 1b is frequently found in Asian countries. HCV exists in individual patients as quasispecies, which differ mainly in the hypervariable regions of the E2 gene (a heavily glycosylated viral-envelope protein that can interact with plasma membranes of hepatocytes and other cells). The variability of HCV quasispecies seems to correlate with the clinical outcome of the HCV infection.

Current Treatment of Chronic HCV Infection (Standard of Care-SOC)

Treatment options for chronic HCV infection are limited to weekly injections of pegylated alpha interferon (IFN- α) in combination with daily oral doses of the antiviral nucleoside ribavirin, neither of which specifically target HCV. The failure rate for achieving sustained virological response (SVR-undetectable HCV RNA 24 weeks after discontinuation of treatment) using SOC for patients infected with HCV genotype 1 in the United States, Europe and Japan is ~50%. 76-82% of patients with HCV genotypes 2 and 3 disease achieve SVR after 24 weeks of therapy. While the long duration of treatment (48 weeks for HCV genotype 1 disease) is particularly difficult for patients to tolerate given the significant side effects such as fatigue, flu-like symptoms, depression and suicide associated with interferon treatment, and side effects such as hemolytic anemia associated with ribavirin treatment.

Future Treatments

Many of the newer drugs in clinical trials for treatment of chronic HCV infection are classified as *Specifically Targeted Antiviral Therapies for HCV* (STAT-C) directly inhibiting viral replication, or *Non-Specific Targeted Antiviral Therapies for HCV* (non-STAT-C) indirectly affecting viral replication through a cellular target.

Viral targets for STAT-C based therapy that is farthest along in development:

- HCV NS3-4A protease : Is a member of the chymotrypsin serine protease family and is essential for the generation of components of the viral replication process. The enzyme cleaves the viral polyprotein at four junctions with a temporal sequence that is presumably crucial for replication. The NS3-4A protease activity has also been implicated in blocking the host cells ability to mount an innate antiviral response (DeFrancesco and Migliaccio, *Nature* **2005**). Hypothetically, inhibitors of NS3-4A protease combat HCV infection in two different ways, inhibiting viral replication and boosting innate immunity.
- HCV NS5B polymerase: Is the RNA-dependent RNA polymerase (RdRp) contained within the NS5B protein is the catalytic component of the HCV RNA replication machinery. This enzyme synthesizes RNA using an RNA template.

Viral targets for Non-STAT-C based therapy that is farthest along in development:

- Toll-like receptors (TLR) are molecular sentinels that sense the presence of invading microorganisms through the recognition of molecular patterns characteristic of pathogens such as bacteria, viruses and parasites. TLR receptors are expressed by immune cells, which include macrophages, monocytes, dendritic cells and B cells. Signaling by stimulated TLRs initiates acute inflammatory responses by induction of antimicrobial genes and pro-inflammatory cytokines and chemokines. Synthetic agonists of TLR-7 and TLR-9 have progressed through early-phase clinical trials and have begun to show potential in controlling HCV infection.

- Inosine monophosphate dehydrogenase (IMPDH): Is an enzyme that catalyses a rate-limiting step in GTP biosynthesis, which leads to a decreased intracellular pool of GTP levels, and suppression of viral RNA synthesis. Ribavirin action is thought to reside at least in part in IMPDH inhibition.

Host Cell Factors Involved in HCV RNA Replication:

Apart from the viral proteins and *cis*-acting RNA elements, several *host cell factors* play an important role for HCV replication. Four known host cell factors have been identified so far. The first three are listed for completeness, but cyclophilin B was the topic of one of the presentations at the meeting and captures the attention in this report.

- Human vesicle-associated membrane protein-associated protein A (VAP-A) is an interaction partner of NS5A and NS5B.
- VAP-B is an isoform of VAP-A interacting with the same HCV proteins.
- Geranylgeranylated protein FBL-2 interacts with NS5A.
- Cyclophilin B (CypB) is a peptidyl-prolyl *cis-trans* isomerase that may alter the conformation of the RNA polymerase, NS5B. CypB interacts with the C-terminal region of NS5B and appears to stimulate its RNA binding activity. Cyclosporin A and non-immunosuppressive cyclosporin analogues inhibit cyclophilin PPIase activity and inhibit HCV replication. By targeting host factors required for viral replication, such drugs might be less prone to resistance. Later in this report a presentation by Kai Lin of Novartis on the non-immunosuppressive cyclosporin NIM 811 is discussed as a small molecule that affects the host cell factor cyclophilin B.

Presentations

Professor Jack Wands, from Brown Medical School, discussed the intracellular signaling pathways implicated in the progression of HCC. Chronically infected HCV patients show a 30 to 40-increase in the incidence of HCC. Two pathways discussed were the Insulin IRS/IGF pathway and the Wnt- β -catenin pathway. Improved understanding of these pathways may lead to therapeutic intervention points for drug discovery.

Professor Margaret James Koziel discussed the role of the innate and acquired immune systems in combating HCV infection. In addition to elucidating the complex immunological process at play, one of the interesting points taken from the presentation was the paradox that while the immune system is involved in clearance of the virus it also is responsible for some of the liver damage.

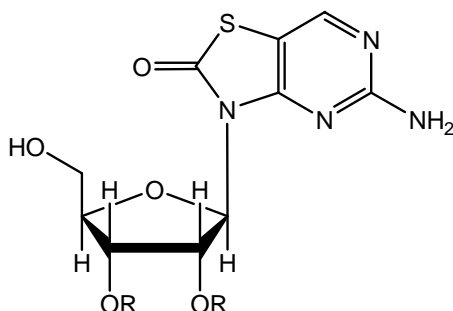
Amy Weiner gave a presentation on an HCV small animal efficacy model that was developed at Chiron (acquired by Novartis). Humans and chimpanzees are the only species susceptible to HCV infection. Over the past few years, *in vivo* models in small animals have been engineered (HCV-Trimera and chimeric scid-Alb/uPA Hepatech mouse models) for studying HCV infection and drug evaluation. These are technically challenging models, low-throughput and very expensive to run. Amy and her team at Novartis developed and validated a simple, reproducible noninfectious HCV mouse

efficacy model for evaluating HCV antiviral compounds (see Zhu et al, *Antimicrob Agents Chemother*, 2006, **50(10)**, 3260-3268 for excellent description of this model and results of testing BILN 2061 and IFN- α).

David Apelian, of GlobeImmune, presented the *Tarmogen* platform technology which is based on recombinant yeast that delivers a broad range of HCV antigens designed to stimulate/couple the host's innate and adaptive immune systems to fight HCV infection. GI-5005 is in development for treatment of HCV.

Devron Averett, CSA of Anadys Pharmaceuticals, spoke about ANA975; a prodrug of Isatoribine, which is a small molecule **Toll-Like Receptor-7 agonist (TLR-7)**. A TLR-7 agonist works by stimulating innate and adaptive immunity against the virus. In contrast to IFN- α treatment, the TLR-7 agonists circulates systemically rather than the drug levels of a cytokine. The small molecule approach should have better pharmacokinetics and improved tolerability relative to IFN- α . Intense proliferation of B-cells was observed in animal toxicology studies after phase Ib studies were initiated. Anadys/Novartis decided to place clinical trial on hold to better understand this observation.

TLR-7 Agonist in Phase 2



R = H, Isatoribine (ANA975)
R = Ac, prodrug of Isatoribine

Anadys/Novartis have since determined that the increase in B-cell proliferation occurs in a dose dependent manner and that this is expected pharmacology of TLR-7 agonists. It has been determined that this was a polyclonal response and not a "rogue cell run amuck". A 13 week animal tox study is in progress and pending outcome. Anadys/Novartis hope to return to the FDA at end of 2007. The prodrug is rapidly absorbed and has 85% or better oral bioavailability. Drug level drops off after 4 hours.

Because of the polyclonal effects for the Tarmogen (recombinant yeast) product and the TLR-7 agonist approach it was predicted that resistance to these treatments is unlikely.

Nucleoside HCV NS5B Polymerase Inhibitors

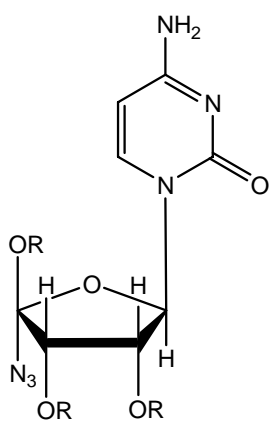
Doug Mayer, of Idenix Pharmaceuticals, presented the preclinical and clinical profile of Valopicitabine (NM283), a nucleoside HCV polymerase inhibitors. Valopicitabine is the

oral prodrug of the nucleoside analog 2'-C-methylcytidine (this is a reference standard that we used in our replicon assay to compare with our novel cyclosporin compounds). Intracellular phosphorylation of the compounds to their 5'-triphosphate analogues results in the formation of the active inhibitor of the viral polymerase. Nucleoside inhibitors function as competitive substrate analogs that prevent RNA chain elongation when incorporated by the viral enzyme, resulting in premature chain termination. Dose escalation studies were performed in clinical trials with good dose-response effects observed. Overall side effects were favorable compared to IFN-ribavirin. Mean HCV RNA reduction of 0.15 to 1.21 log₁₀ IU/ml was observed at doses of 50 to 800 mg/day. Severe dehydration was observed in patients at the 800 mg/kg dose. The 200 mg dose in combination with PEG-IFN was much better tolerated than the 800 mg dose. Dose appears to be limited due to GI-related side effects. By 24 weeks 68% of patients exhibit very low viral loads (HCV RNA < 10 IU). Clinical trials at lower doses will continue in combination with PEG-IFN.

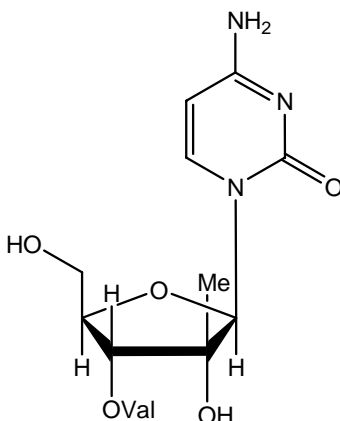
George Hill, of Roche, presented clinical results for R1626 (prodrug of R1479), which is a potent and selective HCV polymerase inhibitor. Good dose-response effects on viral load reduction were observed for doses from 500 mg, 1500 mg, 300 mg and 4500 mg (all doses administered oral bid); this lead to the reduction of HCV RNA from 0.4, 1.2, 2.6, and 3.7 log₁₀ IU respectively. No resistant strains were reported to emerge and tolerability was very good with only non-specific rash reported. Phase 2 studies are underway testing with and w/o ribavirin.

David Olsen, from Merck (West Point), presented results on an HCV NS5B polymerase inhibitor MK-0608. Especially interesting was the new method developed to understand viral resistance for this compound, i.e. Allele specific Ultrasensitive Genotyping assay for Enzyme Resistance (AUGER).

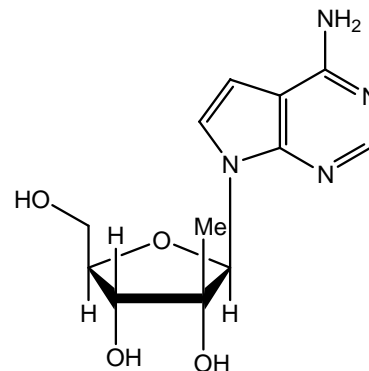
Nucleoside HCV NS5B Polymerase Inhibitors



Roche/Chiron/Novartis
R = tris(2-methylpropanoate), R 1626
Phase 2



Idenix/Novartis
NM283
Phase 2



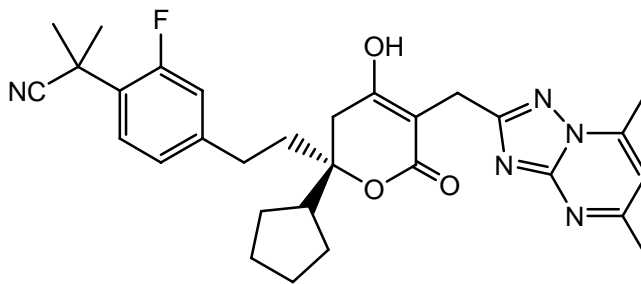
Merck
MK-0608
Preclinical

Non-Nucleoside HCV NS5B Polymerase Inhibitors

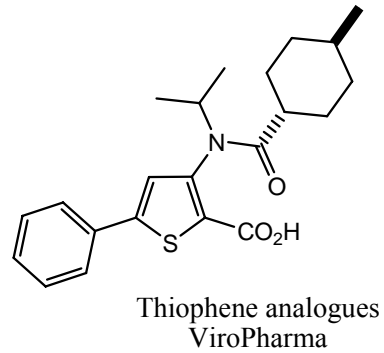
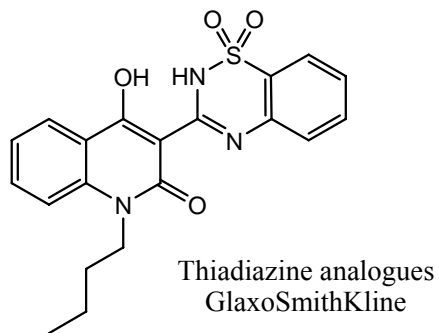
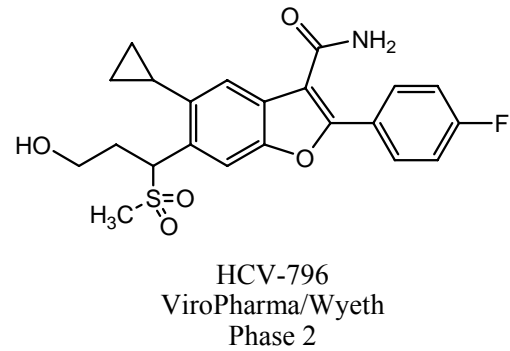
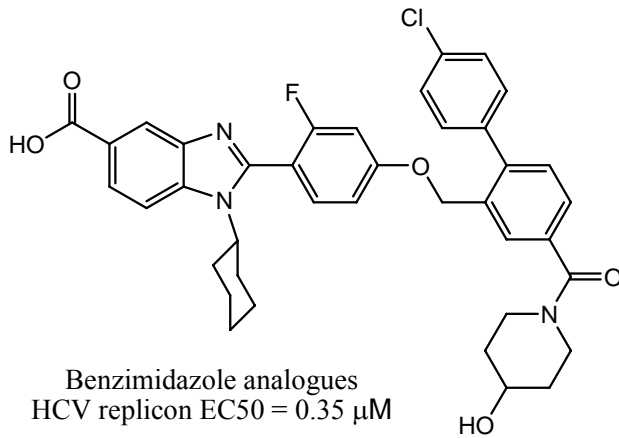
Several putative allosteric binding sites on the surface of HCV NS5B have been suggested based on structural studies, and several chemical classes of NS5B non-nucleoside inhibitors are known. Several examples of non-nucleoside inhibitors illustrate the structural diversity of this class of compounds.

Dr. Hui Lui, from Pfizer LaJolla, presented very elegant medicinal chemistry research that led to an interesting non-nucleoside series of carbon-linked dihydropyrones as potent/orally bioavailable HCV polymerase inhibitors.

Lui, Pfizer, LaJolla
EC₅₀ (replicon) = 0.015 μ M



Non-Nucleoside (HCV NS5B) RNA-dependent RNA Polymerase Inhibitors



HCV NS3-4A Protease Inhibitors

Ann Kwong, of Vertex, presented an overview on HCV protease inhibitors comparing BILN 2061, Intermune's ITMN-191, SCH 503034, and VX-950 (Telaprevir). Protease inhibitors are involved in blocking the processing of the HCV polyprotein which inhibits the production of important non-structural HCV proteins required for viral replication. Another function of the HCV NS3-4A protease is to cleave two important proteins in the viral PAMP signaling pathway known as TRIF and Cardif, which counteract cellular antiviral defense pathways. The protease inhibitors block this action, which provides additional protection, to innate defenses from immune invasion and viral persistence. Therefore the HCV protease inhibitors provide a one-two punch to combat HCV infection.

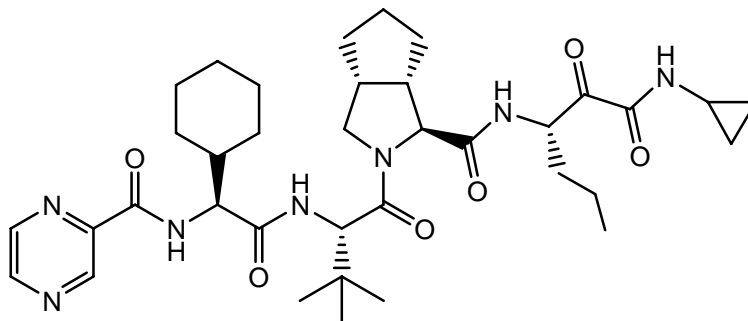
BILN 2061 was the first NS3-4A protease inhibitor in the clinic for treatment of HCV infection. It proved the feasibility of the protease inhibitor approach by knocking down viral load more rapidly than any other known antiviral therapy used before this time. It was well-tolerated in humans in phase 1 clinical trials, however was discontinued in the clinic because of subsequent data from animal toxicity studies showing cardiotoxicity. The rapid knockdown of viral load is characteristic of this class of antiviral agent; however, combination with IFN is essential to prevent viral rebound based on studies run so far. Telaprevir (VX-950) is currently in phase 2b clinical trials. It is effective against HCV genotype 1, and administered (750 mg b.i.d.) in combination with IFN is the most potent protease inhibitor leading to a rapid $4\log_{10}$ reduction in viral load that continues to decline over 15 days. Telaprevir contains an α -ketoamide and is a slow binder ($t_{1/2} \sim 60$ minutes for complex formation) to the protease. This does not seem to pose problems clinically, i.e. adverse effects in patients. Resistant strains of HCV have been characterized and mutations identified. It is hoped that combination therapy with IFN will minimize the resistance to this drug. In another study with VX-950 (750 mg) and PEG IFN 2 α no viral rebound was observed and viral load reduction was $5.5\log_{10}$.

Xiao Tong, of Schering-Plough, presented results of studies on identification and fitness of resistance mutations against HCV protease inhibitor SCH 503034 and other protease inhibitors. Mutations observed in vitro in replicon systems correlate well with mutations observed in the clinic. Three mutations known as T54A, V170A, and A156S conferred low to moderate levels of resistance (<20-fold). Longer exposure (>10 passages) or selection with higher levels of compound led to selection of a more resistant variant, A156T (>100-fold). Although the A156T mutation conferred the highest level of resistance to SCH 503034; it significantly reduced the colony forming efficiency of the mutant replicon RNA, and rendered replicon cells less fit than those bearing wild-type replicons.

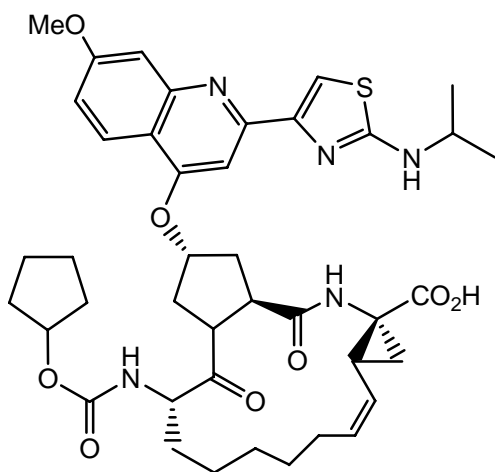
Scott Seinert, of Intermune Inc., discussed results with ITMN-191, which is a novel inhibitor of HCV NS3-4A protease with structural similarity (structure not disclosed) to BILN-2061 in that it possesses a similar macrocyclic framework. ITMN-191 binds to the protease via a two step mechanism and displays a very slow off-rate ($K_i \text{ app} \sim 800 \text{ pM}$). It was stated that no covalent attachment occurs between inhibitor and the enzyme. The

$t_{1/2}$ of the complex is > 5 hours. Aspartic acid (D168) mutation appears to be fundamental to ITMN-191 resistance.

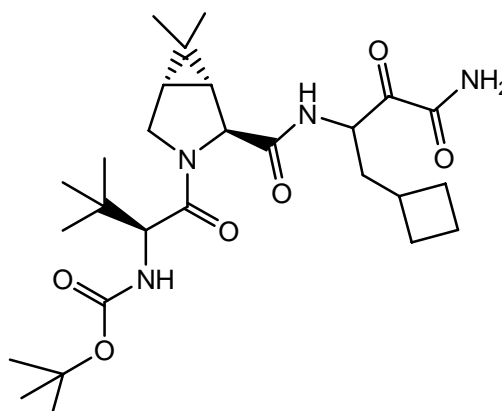
HCV N3-4A Protease Inhibitors



VX-950 Phase 2b



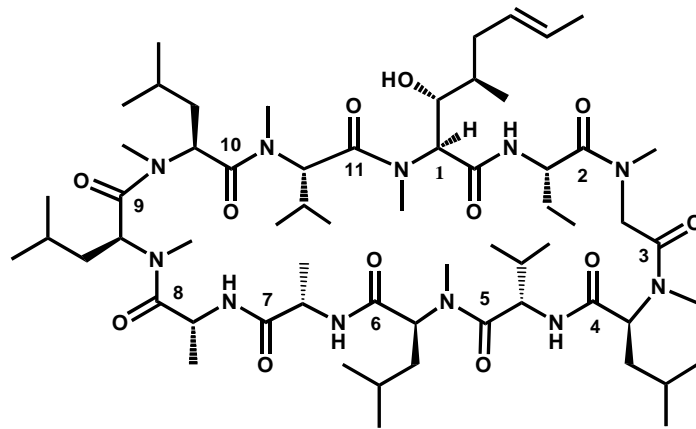
BILN-2061 discontinued



SCH 503034 Phase 2

Kai Lin of Novartis presented a talk entitled “*Cyclophilin Inhibitors as Novel Agents for HCV*”. Two non-immunosuppressive cyclosporin compounds currently in phase 1 clinical trials are NIM 811 (Novartis) and DEBIO-025 (DebioPharm). A recent report by Watashi et al (2005) in *Molecular Cell* reveals a role for the host cell prolyl isomerase cyclophilin B (CypB) in the replication of the hepatitis C viral genome, opening potential avenues for antiviral therapeutic intervention. It was demonstrated that cyclophilin B bound to HCV NS5B polymerase directly and increased its RNA-binding activity, the functions of which were blocked in the presence of cyclosporin A. It was previously reported that cyclophilins play a role in HIV viral infection and that the potent immunosuppressant, cyclosporin A and non-immunosuppressant NIM 811, exhibited antiviral activities against HIV, hepatitis B virus (HBV), and vesicular stomatitis virus. Dr. Lin presented the anti-HCV effects of NIM 811 alone and in combination with IFN- α in vitro using the HCV replicon system.

NIM 811 is a potent inhibitor of HCV RNA replication in the replicon cells (genotype 1-con1-HCV replicon). Treatment of these cells with NIM 811 resulted in a concentration dependent reduction of HCV RNA in the replicon cells. The mean IC₅₀ of NIM 811 was determined to be 0.66 μM. No significant toxicity was observed with NIM 811 at concentrations below 30 μM. NIM 811 was equally effective when tested in several other HCV replicon systems (genotype 1a and genotype 1b). Anti-HCV activity of NIM811 and other cyclosporin derivatives correlated with cyclophilin binding activity. Serum protein-binding effects on NIM 811 showed that at highest human serum levels (40%) there was only a modest effect on anti-HCV activity (5.3-fold increase in IC₅₀), while BILN2061 anti-HCV activity demonstrated more significant effect (54.5-fold increase in IC₅₀).



	<u>Residue</u>	<u>MLR</u>	<u>CYP Binding</u>
CsA	N-Me-Leu ⁴	100	100
NIM 811	N-Me-Ile ⁴	0.1	100
DEBIO-025	N-Et-D-Me-Ala ³ -Val ⁴	-	-

NIM 811 and DEBIO-025 are in Phase 1 clinical trials, both are better tolerated than CsA in man: NIM 811 up to 1600 mg.

In experiments where HCV replicon cells were treated with various concentrations of NIM 811 for three, six, or nine consecutive days a concentration- and time-dependent reduction of HCV RNA was observed. Importantly 1 μM NIM 811 was able to reduce HCV RNA in replicon cells by more than 3-log after 10 days which was comparable to what has been reported with HCV protease inhibitors under similar conditions. In studies of NIM 811 (0.5 μM) with IFN-α (20 U/mL) a remarkable synergism occurred in reducing viral HCV levels (similar synergism has also been shown between DEBIO-025 and IFN-α).

Because the non-immunosuppressants, NIM 811 and DEBIO-025, do not directly target the HCV virus, it is believed that resistance to these drugs will occur more slowly. NIM

811 and DEBIO-025 complement interferon activity and in combination therapy resistance is minimized (because IFN kills resistance strains) and sustained virological response can occur. Since these drugs target host cell factors, it is not known whether side effects may result from blocking cyclophilin B's normal biological function. In consideration of the HCV genotypes treatable with non-immunosuppressive cyclosporins, a recent publication that studied HCV replication in a replicon of genotype 2a HCV (JFH1 strain) was shown not to be mediated by cyclophilin B and was shown to be less sensitive to CsA or NIM 811 treatment than genotype 1b HCV (N. Ishii et al *J. Virol.* 80, 4510-4520).

In concluding this report, several selected points taken from the 3rd Annual Viral Hepatitis meeting are as follows:

- HCV NS5A protease inhibitors have dramatic effects in lowering HCV RNA viral load it relatively short period of time relative to SOC therapy in human clinical trials. Continued development of several protease inhibitors is ongoing.
- HCV viral resistant strains have been identified and characterized, and structural studies show the amino acid point mutations occur in vicinity of the HCV NS5A protease inhibitor binding sites which cause decreased inhibitor potency.
- It is becoming clear that resistant forms of HCV pose a serious threat to effective monotherapy with most if not all known HCV anti-viral agents in human clinical trials. Combination therapy will be required for the foreseeable future.
- It is possible to achieve sustained virological response (SVR) in treatment of HCV, but only with combination therapy; improvements in percentages of patients achieving SVR relative to SOC therapy are desired.
- HCV treatment will consist of combination therapy, new drug plus IFN (and possibly with ribavirin added as well) for some time.
- Other combinations are being explored to replace IFN and Ribavirin.
- It is highly desirable to identify new antiviral agents that work at different targets and by different mechanisms, for example *cell host factors* like cyclophilin B.
- NIM 811, DEBIO-025, and cyclosporin have validated anti-HCV activity in the clinical trials used in combination therapy with IFN.
- Further clinical studies with NIM 811 and DEBIO-025 are required to better define efficacy and safety in combination therapy with IFN or other mechanistically unrelated antiviral agents.
- Human hepatoma HCV Replicon cell culture assays are predictive of efficacy in treatment of HCV in humans and are useful to predict effective combination therapy (remarkable synergism between NIM 811/DEBIO & IFN).
- Drug discovery activity in the antiviral HCV therapeutic area is at a very high level in the pharmaceutical and Biotech industries; however, good opportunities for finding new anti-HCV drugs still exist.