Antivirals for the treatment of chronic HCV infection: Bench to Bedside and beyond

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Disclosures for Mark Sulkowski

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Hepatitis C: Successful translational research

http://catalyst.harvard.edu/pathfinder/
From Non-A, Non-B hepatitis (1972) to Hepatitis C Virus (1989)

- Genus Hepacivirus, Family Flaviviridae
- HCV genome
  - ~9400 nucleotides
  - Positive sense ssRNA
- Rate of replication, $10^{11}$ to $10^{12}$ virions
- Mutations during replication are not corrected which generates diversity
  - 6 genotypes worldwide, many subtypes
  - Quasispecies within individual
- Humans are the natural host

Isolation of a cDNA Clone Derived from a Blood-Borne Non-A, Non-B Viral Hepatitis Genome

QUI-LIM CHOO, GEORGE KUO, AMY J. WEINER, LACY R. OVERBY, DANIEL W. BRADLEY, MICHAEL HOUGHTON

A random-primed complementary DNA library was constructed from plasma containing the uncharacterized non-A, non-B hepatitis (NANBH) agent and screened with serum from a patient diagnosed with NANBH. A complementary DNA clone was isolated that was shown to encode an antigen associated specifically with NANBH infections. This clone is not derived from host DNA but from an RNA molecule present in NANBH infections that consists of at least 10,000 nucleotides and that is positive-stranded with respect to the encoded NANBH antigen. These data indicate that this clone is derived from the genome of the NANBH agent and are consistent with the agent being similar to the togaviridae or flaviviridae. This molecular approach should be of great value in the isolation and characterization of other unidentified infectious agents.

Choo QL et al. Science. 1989 Apr 21;244(4902):359-62
HCV Diversity among patients enrolled in the global trials

Subtypes represented by:
- Color lines were treated with SOF/VEL in the ASTRAL studies
- Black lines are documented subtypes

Genotype 7: Initially identified as GT2b
Globally, 150 million people are infected with hepatitis C and 350,000 to 500,000 people die each year.
In 2010, Global number of deaths due to hepatitis B and C ≈ HIV
In the United States, HCV-related deaths exceed HIV-related deaths

Incidence of HCV infections in young adults in the US

Hepatitis Awareness Month and National Hepatitis Testing Day — May 2015

This month marks the 20th anniversary of Hepatitis Awareness Month and the 6th National Hepatitis Testing Day.

Increases in Hepatitis C Virus Infection Related to Injection Drug Use Among Persons Aged ≤30 Years — Kentucky, Tennessee, Virginia, and West Virginia, 2006–2012

Incidence of HCV infections in young adults in the US
Despite High Prevalence and Increasing Disease Burden, Most Americans With Chronic HCV Have Not Been Diagnosed and Few Have Been Treated

Overall: 3.2 million of U.S. population with chronic HCV

- **Diagnosed**: 50% (1.6M)
- **Referred to Care**: 32-38% (1.0-1.2M)
- **Treated**: 7-11% (220,000-360,000)
- **Successfully Treated**: 5-6% (170,000-200,000)

From interferon for non-A, non-B hepatitis (1986) to oral direct acting antiviral therapies (2014)

Interferon alfa was the treatment of choice prior to the discovery of hepatitis C

TREATMENT OF CHRONIC NON-A, NON-B HEPATITIS WITH RECOMBINANT HUMAN ALPHA INTERFERON

A Preliminary Report

JAY H. HOOFNAGLE, M.D., KEVIN D. MULLEN, M.D., D. BRIAN JONES, M.D., VINOD RUSTGI, M.D., ADRIAN DI BISEGGLIE, M.D., MARION PETERS, M.D., JEANNE G. WAGGONER, B.A., YOON PARK, R.N., AND E. ANTHONY JONES, M.D.

Abstract We treated 10 patients who had chronic non-A, non-B hepatitis with recombinant human alpha interferon in varying doses (0.5 to 5 million units) daily, every other day, or three times weekly for up to 12 months.

In 8 of the 10 patients, elevated serum aminotransferase levels decreased rapidly during therapy and eventually fell into the normal or nearly normal range. In two of these patients, the interferon therapy was stopped after four months, and in both cases, a prompt return of aminotransferase activities to pretreatment values occurred. Prolonged treatment was associated with a sustained improvement in aminotransferase levels; in three cases, biopsy specimens obtained after one year of therapy showed marked improvement in hepatic histology, even though low doses of alpha interferon had been used.

These preliminary findings, although not adequately controlled, suggest that long-term, low-dose alpha interferon therapy may be effective in controlling the disease activity in some patients with chronic non-A, non-B hepatitis. A prospective controlled trial is now needed to assess the role of interferon therapy in this disease. (N Engl J Med 1986; 315:1575-8.)
Interferon was limited by heterogeneous antiviral activity and homogeneous toxicity

Patient genetics (IL28B SNP) determine their likelihood of response to interferon

Ge et al.  Nature 2009
1. Entry
2. Endosomal release and IRES dependent translation
3. Protease cleavages
4. Membranous web formation
5. NS5B RNA dependent polymerase (RdRp)
6. Lipoprotein assembly linked to NS5A
7. Cellular targets
HCV genome organization and potential antiviral targets

Bartensclager R et al. Nature Reviews 2013
Antiviral drug targets were readily identified

Crystal Structure of the Hepatitis C Virus NS3 Protease Domain Complexed with a Synthetic NS4A Cofactor Peptide

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Summary
An estimated 1% of the global human population is infected by hepatitis C viruses (HCVs), and there are no broadly effective treatments for the debilitating progression of chronic hepatitis C. A serine protease located within the HCV NS3 protein processes the viral polyprotein at four specific sites and is considered essential for replication. Thus, it emerges as an attractive target for drug design. We report here the 2.5 Å resolution X-ray crystal structure of the NS3 protease domain complexed with a synthetic NS4A activator peptide. The protease has a chymotrypsin-like fold and features a tetrahedrally coordinated metal ion distal to the active site. The NS4A peptide interleaves within a β sheet of the enzyme core.

The genome indicated a relationship to the flaviviruses and pestiviruses (Miller and Purcell, 1990; Choo et al., 1991), and HCV was assigned a new genus in the family Flaviviridae (Francki et al., 1991). More recently it has become clear that there are multiple HCV genotypes (Bukh et al., 1993; Simmonds, 1994) that share the same essential features. The HCV genome is about 9.4 kb in length and consists of a highly conserved 5’ untranslated region followed by a single open reading frame that encodes a polyprotein of 3010 to 3033 amino acids (Kato et al., 1990; Choo et al., 1991; Takamizawa et al., 1991). All known HCV polyprotein sequences share at least 71% identity. The structural proteins (envelope and core components) are clustered in the N-terminal portion of the polyprotein, followed by the nonstructural (NS) proteins, which represent the essential catalytic machinery for viral replication (Hijikata et al., 1991; Bertenschlager et al., 1993; Grakoui et al., 1993a, 1993c; Tomsley et al., 1993). The polyprotein architecture is shown in Figure 1, together with a summary of the proteolytic processing events that generate the mature viral proteins.

In vivo processing of the HCV structural proteins is probably facilitated by host cell signal peptidases associated with the lumen of the endoplasmic reticulum (Hijikata et al., 1991; Lin et al., 1994a; Mizushima et al., 1994). In contrast, processing of the nonstructural proteins seems to be orchestrated by two viral gene products. The NS2/NS3 junction is cleaved by a zinc-dependent protease associated with NS2 and the N-terminus

Stereo Ribbon Diagram of the tNS3:NS4A Complex

Kim JL. Cell 1996
Lack of models hindered early HCV drug development

• Chimpanzee found to be the only non-human animal to be susceptible to HCV
  – Limited utility for drug development

• Replicon cell culture system (1999)
  – Major driver of drug discovery

• HCVcc cell culture system (2005)
  – Genotype 2a and then JFH-1 derived chimeric genome
  – Complete replication

Subgenomic replicon cell cultures system led to the identification of oral inhibitors of HCV non-structural proteins

- 5’ non-translated region, gene-encoding selection marker (G418 resistance), internal ribosome entry site (IRES) from another virus to allow translation of the region encoding the replicase module, and 3’NTR
- Replication enhancing mutations were identified
- Construction of functional replicons encoding report genes – high throughput screening
HCV suppression replication by NS3 protease inhibitor

Rapid and potent HCV suppression with BILN2061 (2003)

Selection of telaprevir resistant variants with monotherapy x 14 days

Lamarre Nature 2003
Kieffer Hepatology 2007
HCV Drug Development Advisory Group
HCV suppression of HCV replication by NS5A inhibitors

Rapid and potent antiviral activity with a single dose of daclatasvir (2010)

Selection of daclatasvir resistant variants with monotherapy x 14 days

Gao Nature 2010
Nettles Hepatology 2011
HCV Drug Development Advisory Group;
Mechanism of NS5A elucidated by inhibition in HCVcc cell culture system

- Rapidly inhibit intracellular assembly of virions
- Block formation of the “membranous web” that houses HCV RNA replication (replicase complexes)
- No activity against preformed replicase, thereby resulting in slow shut-off of viral RNA synthesis
HCV eradication with Daclatasvir + Asunaprevir (NS5A + NS3)

*First report of interferon-free cure (April 2011)*

Lok NEJM 2012
McPhee Hepatology 2013
HCV suppression replication by inhibition of NS5B polymerase with non-nucleosides (non-nuc)

Binding sites for non-nucleoside HCV NS5B polymerase inhibitors

HCV RNA suppression over 3 days with dasabuvir (ABT-333) monotherapy (~ 1 log$_{10}$)

Poordad EASL 2012
HCV cure with Ombitasvir + Paritaprevir/r + Dasabuvir + Ribavirin in some but not all patients (NS5A + NS3 + non-nuc NS5B)

Patient with prior null-response (< 2 log_{10} decline) during treatment with peginterferon/ribavirin

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U = undetectable (HCV RNA not detected)

Poordad NEJM 2013
HCV eradication with variable combinations of Paritaprevir/r + Ombitasvir + Dasabuvir + Ribavirin (NS3 ± NS5A ± non-nuc NS5B ± Ribavirin)

- No Ombitasvir (NS5A inhibitor) - SVR 83%, Viral Failure 12.2%
- No Dasabuvir (Non-nucleoside NS5B inhibitor) - SVR 89%, Viral Failure 10.1%
- No Ribavirin - SVR 89%, Viral Failure 7.6%
- Three DAAs + Ribavirin - SVR 96%, Viral Failure 1.2%

Kowdley NJEM 2014
Ribavirin prevents the emergence of resistance associated variants during antiviral therapy

HCV breakthrough with or without ribavirin during telaprevir/peginterferon

Zeuzem Hepatology 2012
Hezode NEJM 2009
Suppression of HCV replication by nucleos(t)ide analogue NS5B polymerase inhibitors

Sofosbuvir, β-d-2'-deoxy-2'-α-fluoro-2'-β-C-methyluridine nucleotide prodrug (2010)

Potent HCV suppression with sofosbuvir alone, with ribavirin or with both interferon/ribavirin (2010)

Sofia J Med Chem 2010
Gane NEJM 2013
HCV Drug Development Advisory Group
Development of additional nucleoside analogue NS5B polymerase inhibitors has been arduous

HCV suppression following 7 days of treatment with an uridine nucleotide polymerase inhibitor prodrug, MK-3682 (formerly IDX21437)

Cardiac dysfunction associated BMS-986094, guanosine nucleotide polymerase inhibitor

Dose
- 0 mg
- 50 mg
- 150 mg
- 300 mg

Gane AASLD 2014
Ahmad Hepatology 2014
Suppression of HCV replication with the combination of inhibitors of NS5A and NS5B polymerase (nucleotide analogue)

Daclatasvir plus Sofosbuvir ± Ribavirin

Ledipasvir/Sofosbuvir FDC ± Ribavirin

Sustained Virologic Response

In pursuit of the optimal HCV combination therapy

- High rate of HCV cure (> 95%)
- Effective re-treatment strategies in persons with virologic failure
- Active against all HCV genotypes (pangenotypic)
  - Genotype 1, 2, 3, 4, 5 and 6
- Favors dosing adherence
  - Once daily pills
  - Long-acting injectable nanoformulations
- Favors treatment persistence (duration)
  - Few side effects
  - Short duration
Sofosbuvir/Velpatasvir one tablet daily for 12 weeks: HCV cure rate by genotype

Error bars represent 95% confidence intervals.

Jacoson et al. HEPDART 2015
Translation from in vitro models to phase 3 clinical trials to global control and elimination

Summary of New England Journal of Medicine studies published in 2014

HCV control and elimination programs are underway in Georgia, Iceland and Australia
