An 18-Gene Signature Predicting Treatment Response to Interferon in Patients Chronically Infected with Hepatitis C Virus

Dr. Limin Chen
Institute of Blood Transfusion, Chinese Academy of Medical Sciences/Peking Union Medical College, China & University of Toronto, Canada
HCV infection: a serious liver disease

Normal liver → HCV → Cirrhosis & HCC

PegIFN/Rib

50% effective
50% failure

Predict treatment response
Mechanism of IFN resistance
Presentation outline

• **Introduction to HCV**: - milestones in HCV research
- diagnosis and treatment

• **Identification of HCV response signature** by microarray
gene expression profiling
Part I: Introduction to HCV infection
Hepatitis C Around the World

Hepatitis C, 2007

Europe 8.9 million (1.03%)
Eastern Mediterranean 21.3 million (4.6%)
Westem Pacific 62.2 million (3.9%)
Southeast Asia 32.3 million (2.15%)
Africa 31.9 million (5.3%)
Americas 13.1 million (1.7%)

World: 169.7 million (3.1%) prevalent cases

Source: ©WHO, 2008. All rights reserved.
HCV in China

40 million people infected

¼ of all infected worldwide

Genotype 1b most common
Milestones for HCV research


NANB   Chimps  cDNA  synthetic HCV RNA  replicon  JFH1  Innate immunity  DAA

Michael Houghton and Harvey Alter (Lasker Award 2002)

Isolated Filtratable Agent and Sequenced Genome of Hepatitis C Virus
Injected synthetic HCV RNA genome into chimps to cause hepatitis C (1998)
Ralf Bartenschlager (left) Developed Replicon System

Allows HCV RNA replication in cell culture
Dr. Takaji Wakita, National Institute of Infectious Diseases, Tokyo

Discoverer of the JFH-1 strain of HCV which grows in culture
HCV Affects Pathways Controlling Innate Immunity

Michael Gale Jr.
University Washington, Seattle

Stanley Lemon
UNC Chapel Hill
Adaptive Immunity and CTL Escape Mutations

Chris Walker, Columbus Children’s Hospital, Ohio State University
Diagnosis of HCV infection: Antibody detection by ELISA

Identification, Cloning & Expression of Non-A, Non-B Virus Proteins - HCV

Wall Street Journal, May 11, 1988

- Identification & characterization without visualization
- First generation anti-HCV enzyme immunoassay (EIA)
Increased sensitivity

Peptide Mapping

capsid  | envelope protein | protease/helicase | RNA-dependent RNA polymerase

core  | E1  | E2  | NS2  | NS3  | NS4  | NS5  

5'  | c22 | 33c | c-100 |  |  | 3'  

optimize the anti- HCV EIA

Peptides  | Enzymes

Optimize the Anti-HCV EIA
HCV nucleic acid testing (NAT)

HCV Virus Replication

Antibody

Antigen

proteins

$10^{10-13}$ Virions per day

Nucleic Acid Testing

Neuman et al. Science 1998
Most commonly used NAT for HCV

COBAS TaqMan™ HCV-RNA
Quantification Range
43 to 69,000,000 IU/mL

Test Signal

LOD 15 IU/mL

Patient’s Viral Load log_{10} IU/mL

Almost entire quantification range is covered by the assay
Early detection of HCV RNA by NAT

*Improves EIA Sensitivity & Predictive Values*

1st → 3rd generation anti-HCV EIA
- Sensitivity 70% → > 99%
- ↑ predictive value
- ↓ time between acute infection and detection

Gretch 97, Schiff 99, Pawlotsky 99, Krajden 2000

<table>
<thead>
<tr>
<th>NAT</th>
<th>HCV Antigen</th>
<th>EIA 3.0</th>
<th>EIA 2.0</th>
<th>EIA 1.0</th>
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<tbody>
<tr>
<td>0</td>
<td>~13 ~14</td>
<td>~70</td>
<td>~80</td>
<td>~150</td>
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</table>

Time in Days From Infection
HCV life cycle

- a. Interaction with host cell
- b. Receptor mediated endocytosis
- c. Fusion/uncoating
- d. Translation & Processing
- e. Membrane associated RNA replication
- f. Virion morphogenesis
- g. Virion maturation
- h. Virion release

No RT process!
HCV treatment: IFN-based Standard of Care (SOC)
Pegylated Interferon/Ribavirin

- Individualized therapy
  - viral/host factors
- Viral genotype → 6 major types
  - G1=61%; G2=14%; G3=23% & G 4, 5, 6 = 1%
  - Genotype
    - 1, 4, 5, 6 → cure rates of ~45% with 48 wks of Rx
    - 2 → cure rates of 80% to 90% with 24 wks of Rx
    - 3 → cure rates of ~75% with 24 wks of Rx
High through-put gene expression profiling

- Gene chip = Microarray
- Detect tens of thousands of mRNA at the same time
- Traditional/classical method: one or a few genes at a time
- Post-genomic HTP tools: study gene expression
cDNA microarray

A

Normal        Cancer

mRNA

Gene X

cDNA

DNA microarray

B

Cy 3

Cy 5

Cy 5

Cy 3

200

10000

50.00

4800

4800

1.00

9000

300

0.03

Over-expressed

Under-expressed
Data analysis is the key ...
Part I: Introduction to HCV
summary

- HCV is a (+) strand RNA virus, hypervariable
- 170 million infected individuals (3%) worldwide
- No DNA phase, no genomic integration-curable disease
- 6 major genotypes: 1-6
- Diagnosis: ELISA for Antibody, NAT for RNA
- Treatment: IFN based+ DAA (from 2011)
- No vaccine- antibody not protective
Part II: Identification of HCV response signature by microarray gene expression profiling
Screen for gene signature for predicting treatment response in HepC

- 15 Non-responders and 16 Responders
- Biopsies prior to treatment
- Gene expression profiling done (19,000)
- Compare the difference between NR and SVR
- Generate gene signature
18 Genes Whose Expression Levels Are Statistically And consistently Different Between Responders (R) and Nonresponders (NR)

An 18-gene signature differentiates Responders and Non-responders

An 8-gene Subset Accurately Classifies 30 out of 31 patients

A.

RESPONDERS

NONRESPONDERS

B.

KNN
Nearest neighboring

LDA
Linear Discriminatory Analysis

PCA
Principal component analysis

Prospective Validation

Validate the trends in gene expression (the original “signature”) in a prospective cohort of CHC patients

78 patients with CHC, all treated in the same center (U of T/ Toronto Western Hospital) from 2004-2006.
Hierachial clustering analysis of 78 new samples based on 18 signature gene expression

## Prediction accuracy (78 patients)

<table>
<thead>
<tr>
<th>Methods</th>
<th>sensitivity</th>
<th>specificity</th>
<th>PPV</th>
<th>NPV</th>
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<tbody>
<tr>
<td>KNN</td>
<td>0.80 +/- 0.07</td>
<td>0.86 +/- 0.15</td>
<td>0.96 +/- 0.04</td>
<td>0.46 +/- 0.09</td>
</tr>
<tr>
<td>DQDA</td>
<td>0.73 +/- 0.07</td>
<td>0.86 +/- 0.12</td>
<td>0.96 +/- 0.03</td>
<td>0.45 +/- 0.07</td>
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<tr>
<td>DLDA</td>
<td>0.73 +/- 0.07</td>
<td>0.86 +/- 0.13</td>
<td>0.96 +/- 0.04</td>
<td>0.43 +/- 0.08</td>
</tr>
<tr>
<td>CART</td>
<td>0.80 +/- 0.07</td>
<td>0.86 +/- 0.21</td>
<td>0.96 +/- 0.05</td>
<td>0.46 +/- 0.11</td>
</tr>
</tbody>
</table>

KNN: k-nearest neighbor  
DQDA: diagonal quadratic analysis  
DLDA: linear discriminant analysis  
CART: classification and regression trees  
“Predict: responders”  

Chen, et al. AASLD 2007 Boston  
Chen, et al. Gastroenterology 2010
Genetic Markers Can Predict Response to Hep C Treatment

BY KATE JOHNSON
Elsevier Global Medical News

Genetic signature involving 18 genes can reliably predict response to treatment with pegylated interferon α plus ribavirin, according to researchers at the AGA Institute.

Dr. Shuai Chen, lead author of the study, explained: "We were able to show that if we silence this USP18 gene, the virus actually gets more sensitive to interferon. In other words, we can reduce the amount of interferon needed to kill the virus," Dr. Chen said.

The group's latest work validates the findings from a prospective cohort study of 78 HCV patients (mean age, 51 years; 23 nonresponders and 55 responders. Using pretreatment liver biopsies, "we confirmed that USP18 is more highly expressed in nonresponders," Dr. Chen explained.

The study determined that the genetic evaluation of pretreatment liver biopsies with regard to this gene signature can predict treatment response with a positive predictive value of 96%. However, the negative predictive value was only 50%, he said.

"In other words, if you use this gene signature, your prediction that someone would respond to treatment would be 96% accurate, but if you predicted non-response, you would have only a 50% chance of accuracy," he said. "Therefore, you cannot use it to exclude patients from treatment."

Dr. Robert S. Brown Jr. commented, "Better predictors of response are needed, but perhaps more importantly we need predictors of non-response. Data, if verified, could provide motivation to those patients who are predicted to have a high likelihood of success, but unfortunately will not spare patients who have a low likelihood of success from side effects." Dr. Brown is the Frank Cardile associate professor of medicine and surgery and chief of the division of abdominal organ transplantation at Columbia University College of Physicians and Surgeons, New York.

Current combination treatment with pegylated interferon α plus ribavirin has only a 50% success rate, and patient compliance with therapy is frequently jeopardized by the treatment's significant side effects and expense, according to Dr. Chen.

Generic markers such as USP18 that predict good treatment response might help physicians encourage compliance in certain patients, he said at the meeting, which was sponsored by the Canadian Association of Gastroenterology.
Genomic Arrays Can Help Predict HCV Patients Who Will Likely Respond to PegIFNα Plus Ribavirin Therapy

PegIFNα plus ribavirin (PegIFN/rb) treatment is effective for treatment of chronic hepatitis C (HCV), but a significant number of patients do not respond to therapy for reasons that are unclear. Because of the substantial cost and side effects of this treatment, the ability to predict nonresponding (NR) or responding (R) patients would be clinically useful. The study by Chen et al attempts to develop a genomic analysis of liver biopsy samples to distinguish between the two groups. The investigators identified 18 genes whose expression differed significantly between all responders and all nonresponders, with \( P < .005 \). Several of these genes were interferon-responsive, reinforcing a paradigm relevant to treatment responses. On further analysis, an 8 gene subset was found that accurately predicted treatment response for most of the patients, applicable to genotype 1 patients, but not correlated with viral load, disease activity, or fibrosis (Figure 5). In conclusion, NR and R patients differ fundamentally in their innate interferon response to HCV infection. These differences likely reflect aspects of HCV pathogenesis and form the basis for a predictive subset of genes that can predict treatment responses prior to initiation of PegIFN/rb therapy.

See page 1437

Ubiquitin-Specific Protease 18 Expression Inhibits Interferon-Induced Hepatitis C Virus (HCV) Reduction in an In Vitro HCV Replication System

Interferon α (IFN), usually in combination with ribavirin, is a major component of the treatment regimen for controlling hepatitis C (HCV) viral infection in humans. Although some patients respond, more than half treated with IFN do not effectively clear the virus, which may be due to many factors including viral genotype and host response. Of factors identified that attenuate the host response to IFN for HCV infection is the up-regulated expression of ubiquitin-specific protease 18 (USP18), a protease that cleaves ubiquitin like (and IFN-induced) pro-
22 April 2013

Lumin Chen
Chinese Academy of Medical Sciences
Chengdu, China

Subject: Presidential Awards
APASL Liver Week 2013
6 – 10 June 2013, Singapore

Dear Lumin Chen,

On behalf of the Local Organising Committee of the APASL Liver Week 2013, we are pleased to inform you that you have been selected as one of the recipients of the APASL Liver Week Presidential Awards.

Congratulations and we look forward to welcoming you to APASL Liver Week 2013 in Singapore.

Yours sincerely,

Seng Gee LIM
Congress Chairman
APASL Liver Week 2013, Singapore

Yock Young DAN
Chairman, Scientific Committee
APASL Liver Week 2013, Singapore
Have been invited to give talks...

- International conference on HCV (2005, Montreal, Canada; 2007, Glasgow, UK; 2009, Nice, France)
- International conference on interferon and cytokines (2006, Shanghai)
- International conference on infectious diseases (2009, Beijing- sole Gold winner!)
Invited as key-note speakers & as session chairs...
### Day 1: Wednesday, Nov 13, 2013

**Ball Room**

Hainan International Convention & Exhibition Center, Hainan, China

**Event Title:**

**Keynote Forum**

<table>
<thead>
<tr>
<th>Time</th>
<th>FaceOn</th>
<th>Title</th>
<th>Speeches and Speakers</th>
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<tbody>
<tr>
<td>13:30-14:00</td>
<td></td>
<td><strong>Title:</strong> Metagenomics Reveals the Molecular Mechanisms Driving Chronic Disease</td>
<td>Dr. Trevor G Marshall, Professor and Director, Autoimmunity Research Foundation, USA</td>
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<tr>
<td>14:00-14:30</td>
<td></td>
<td><strong>Title:</strong> Early Diagnosis of Cancer: HAAH-A Predictive Biomarker, A Serum Immunoassay And Personalised Medicine</td>
<td>Dr. Mahmood Moshiri, President and CEO, Panacea Global Inc., Canada</td>
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<tr>
<td>14:30-15:00</td>
<td></td>
<td><strong>Title:</strong> An 18-gene signature predicting treatment response to interferon in patients chronically infected with hepatitis C virus</td>
<td>Dr. Limin Chen, Professor and Director/Chief Scientific Officer, Institute of Blood Transfusion &amp; University of Toronto, China &amp; Canada</td>
</tr>
<tr>
<td>15:00-15:30</td>
<td></td>
<td><strong>Title:</strong> Application of Translational Sciences to Precision Medicine, the Ipsen experience</td>
<td>Dr. Patrice P. Denéffe, Senior Vice President, Translational Sciences, Ipsen, France</td>
</tr>
<tr>
<td>15:30-16:00</td>
<td></td>
<td><strong>Title:</strong> Under Proposal</td>
<td>Dr. Norbert W. Paul, Professor and Vice Dean, Universitätsmedizin Mainz, Germany</td>
</tr>
</tbody>
</table>
Invited to contribute review papers for various journals
IL28B SNP plays an important role in HCV clearance (spontaneous and treatment-induced)

4 papers published on nature or nature genetics

   PMID: 19759533 [PubMed - indexed for MEDLINE]
   Related articles

2. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy.
   PMID: 19749758 [PubMed - indexed for MEDLINE]
   Related articles

3. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C.
   PMID: 19749757 [PubMed - indexed for MEDLINE]
   Related articles

   PMID: 19884573 [PubMed - indexed for MEDLINE]
   Related articles
GWAS: IL28B SNP affects treatment response

Mutations (SNPs) in the IL28B promoter region are strongly associated with response to IFN-based treatment. ... What is NOT clear is why???

**Figure 1 | Percentage of SVR by genotypes of rs12979860.** Data are percentages + s.e.m.
Which type of cells express these 18 genes?

Different cell types in the liver
- Hepatocytes (majority)
- Kupffer cells (macrophages)
- Stellate cells
- Endothelial cells
- Other cells: inflammatory cells, etc…
The cellular basis of the ISG HIGH “nonresponder phenotype” – ISG15 immunohistochemistry

Chen et al, Gastroenterology 2010
Hepatic Cell-Type Specific Gene Expression Better Predicts HCV Treatment Outcome Than IL28B Genotype

IAN McGILVRAY,*,‡ JORDAN J. FELD,*,§ LIMIN CHEN,‡ VENESSA PATTULLO,‖ MAHA QUINDI,*,‡ SANDRA FISCHER,*,‡ IVAN BOROZAN,‡ GANG XIE,‡ NAZIA SELZNER,*,‡ E. JENNY HEATHCOTE,‡,§ and KATHERINE SIMINOVITCH‡,*

*Toronto Western Hospital Liver Centre, †University Health Network, Toronto, Ontario, Canada; ‡University of Toronto, Toronto, Ontario, Canada; ‖Faculty of Medicine, University of Sydney, Sydney, Australia; ‡Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, California, and §Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

Keywords: Treatment Outcome; Prognostic Factor; Liver Disease; SVR.

The clearance of hepatitis C virus (HCV) requires a robust response to either endogenous or therapeutic interferon. HCV has evolved numerous strategies to circumvent interferon responses, leading to a high rate of chronicity after acute infection and sustained virologic response (SVR) rates of only 50% with peginterferon alpha-2b plus ribavirin. Although direct-acting antivirals (DAA)...

BACKGROUND & AIMS: Cell-type specific expression patterns of hepatic interferon-stimulated genes (ISGs) and single nucleotide polymorphisms (SNPs) near the IL28B gene are associated with response to interferon-based therapy in patients with chronic hepatitis C virus (HCV) infection.
Potential market

- **China**: 40 million HCV, if 10% use this test, potential market valued **8 billion** Chinese Yuan. More infected patients due to no vaccine, bigger market up to **20-30 billions**.

- **US**: 75,000-112,500 HCV genotype I infected (2015 increased by 5-fold) **$200 million/year ($ 1 billion/2015)** (Oncotype Dx $3820/test)
Huge demand: Oncotype Dx predicts whether chemotherapy is necessary following breast cancer surgery: Increased by 7-fold in 2 years

2008 income: 100,000x $3,820/test = $382 million USD
Acknowledgements

University of Toronto:

Dr. Ian McGilvray
Dr. Aled Edwards
Dr. Jenny Heathcote
Dr. Ivan Borozan
Jing Sun
Larry Meng
Catalina Coltescu

Rockefeller University:

Dr. Charles Rice
Dr. Maryline Panis
Dr. Brett Lindenbach

Granting agencies:

CIHR-CGS
NCRTP-HepC
Institute of Blood transfusion
Chinese Academy of Medical Sciences

Dr. Shilin Li
Dr. Peibin Zeng
Xiaoqiong Duan (PhD candidate)
Yujia Li (PhD candidate)
Chunhui yang