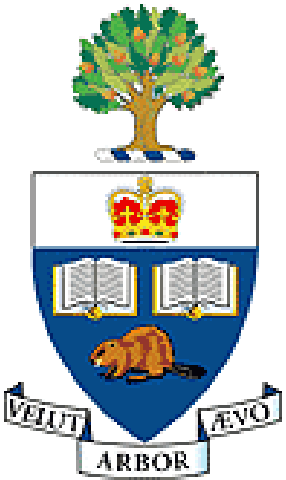
An 18-Gene Signature Predicting Treatment Response to Interferon in Patients Chronically Infected with Hepatitis C Virus

Dr. Limin Chen

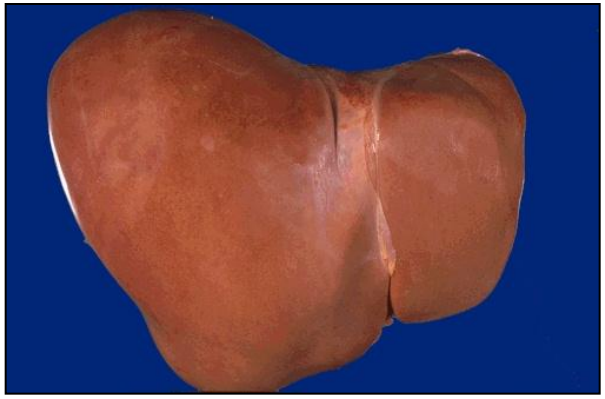
Institute of Blood Transfusion, Chinese Academy of Medical Sciences/Peking Union Medical College, China &

University of Toronto, Canada



HCV infection: a serious liver disease

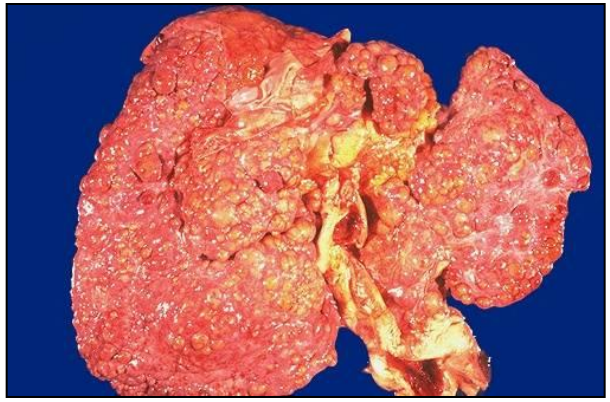
Normal liver



HCV

PegIFN/Rib

Cirrhosis & HCC



Predict treatment response
Mechanism of IFN resistance

50% effective

50% failure

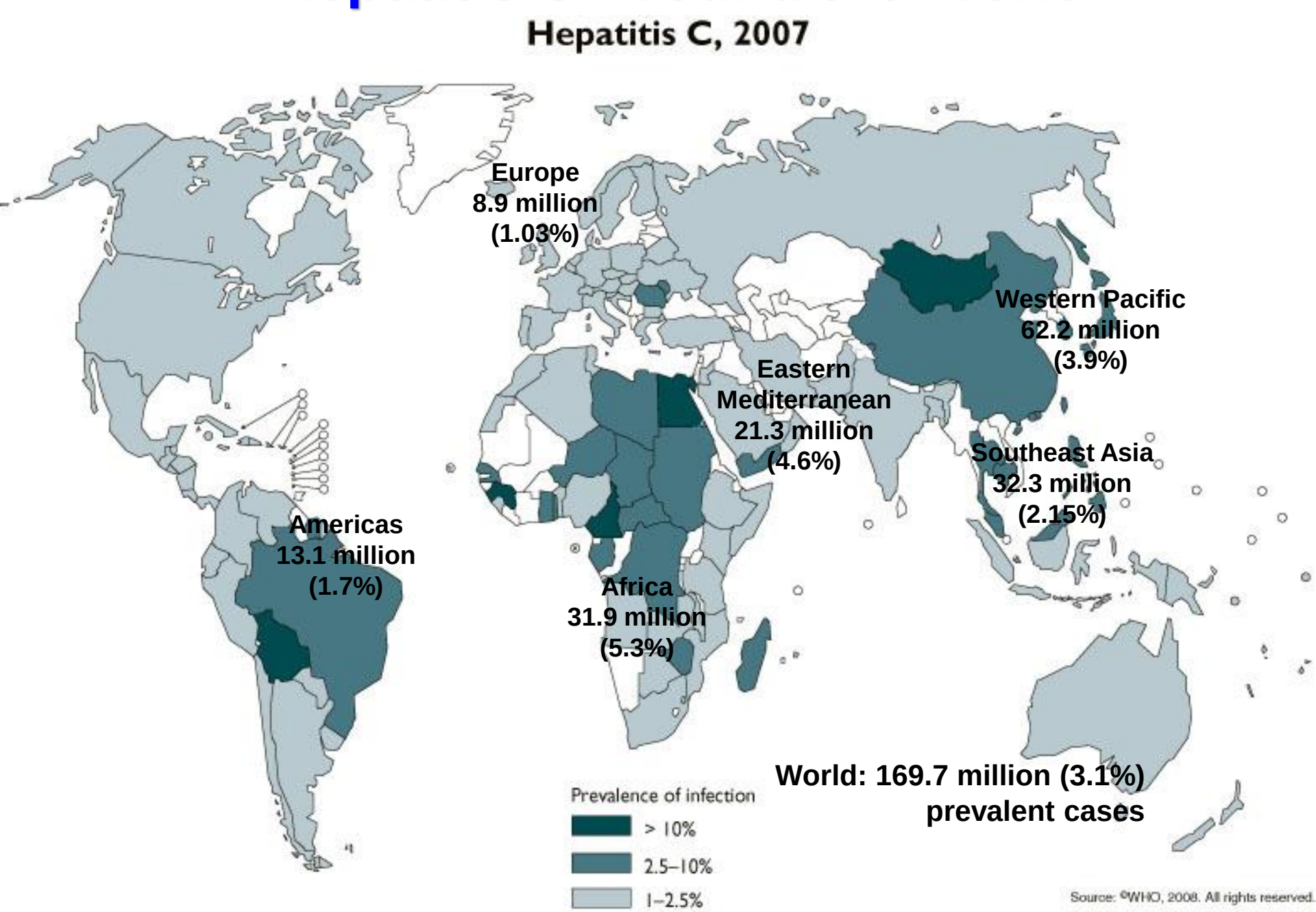
Presentation outline

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

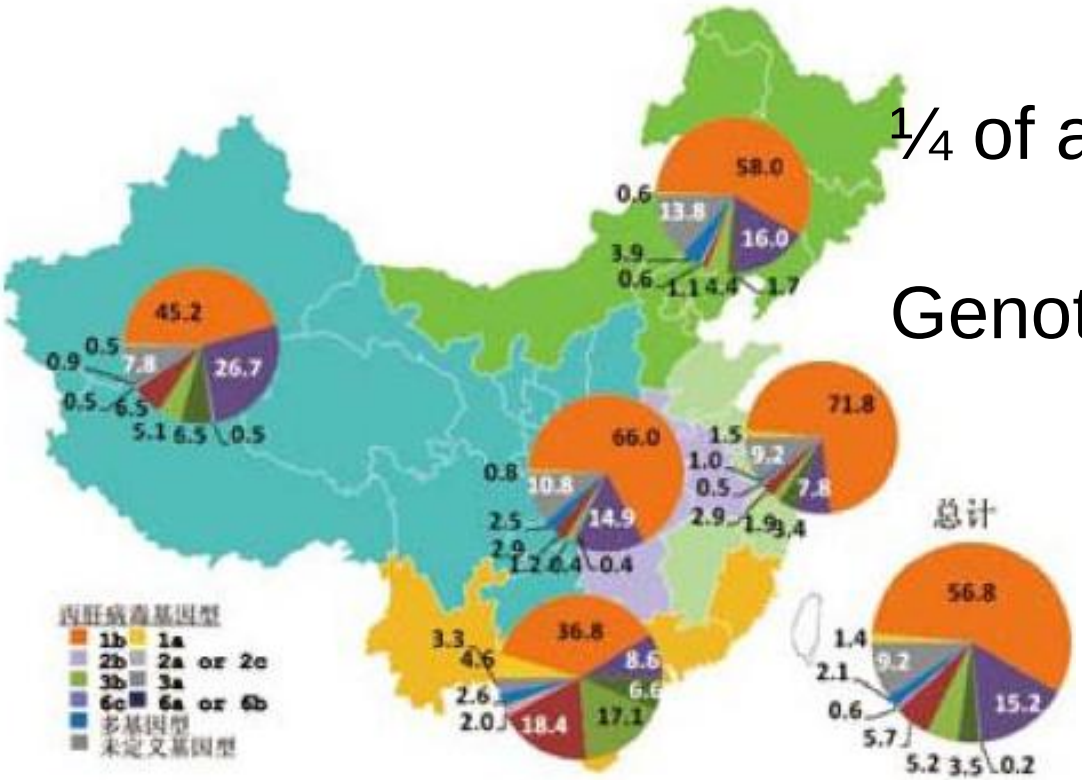
- **Identification of HCV response signature** by microarray gene expression profiling

Part I: Introduction to HCV infection

Hepatitis C Around the World



HCV in China

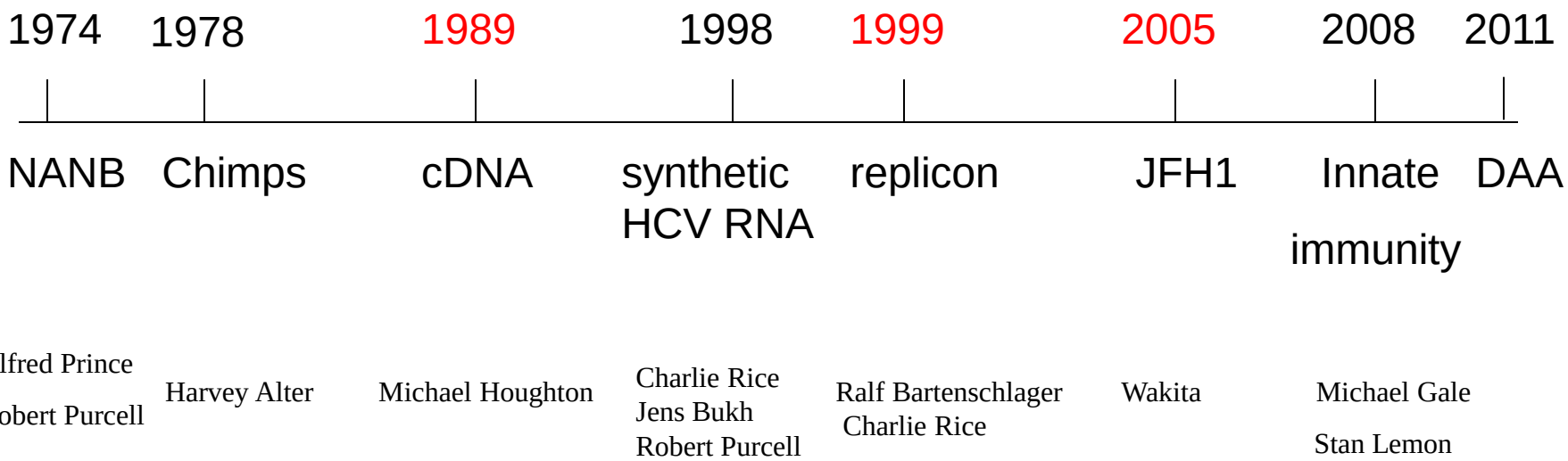


40 million people infected

¼ of all infected worldwide

Genotype 1b most common

Milestones for HCV research



Michael Houghton and Harvey Alter (Lasker Award 2002)

Isolated Filtratable Agent and Sequenced Genome of Hepatitis C Virus

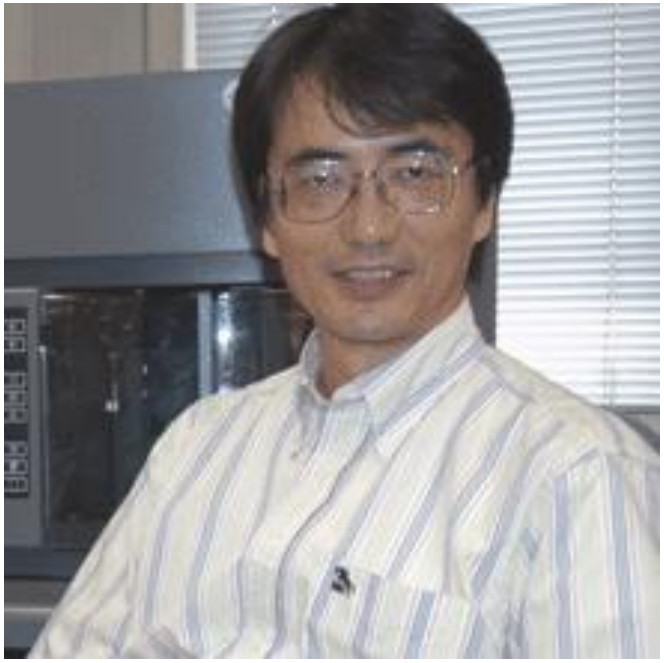
Charlie Rice (Rockefeller University)

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

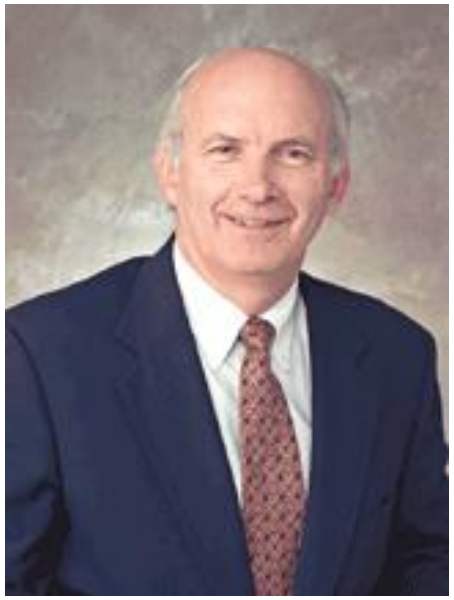
Dr. Takaji Wakita, National Institute of Infectious Diseases, Tokyo



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

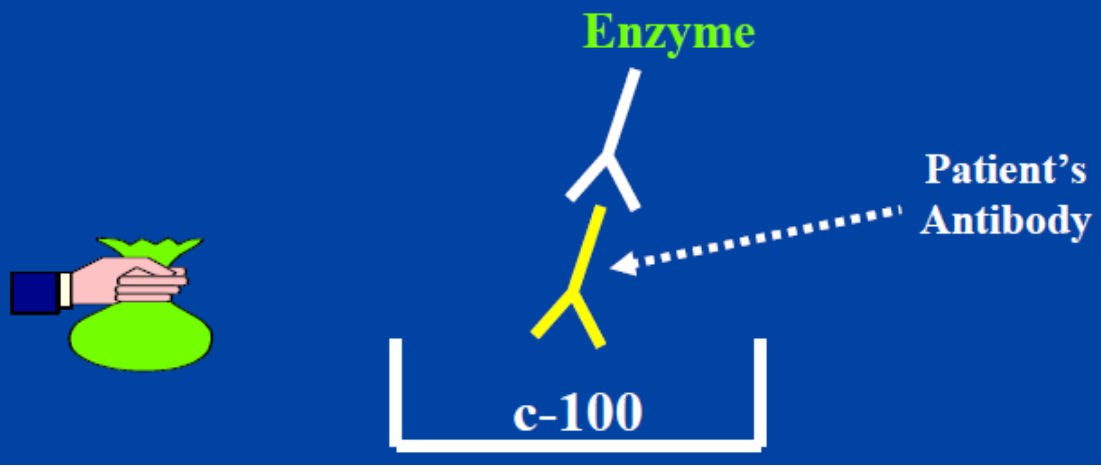
Identification, Cloning & Expression of Non-A, Non-B Virus Proteins - HCV

Wall Street Journal, May 11, 1988

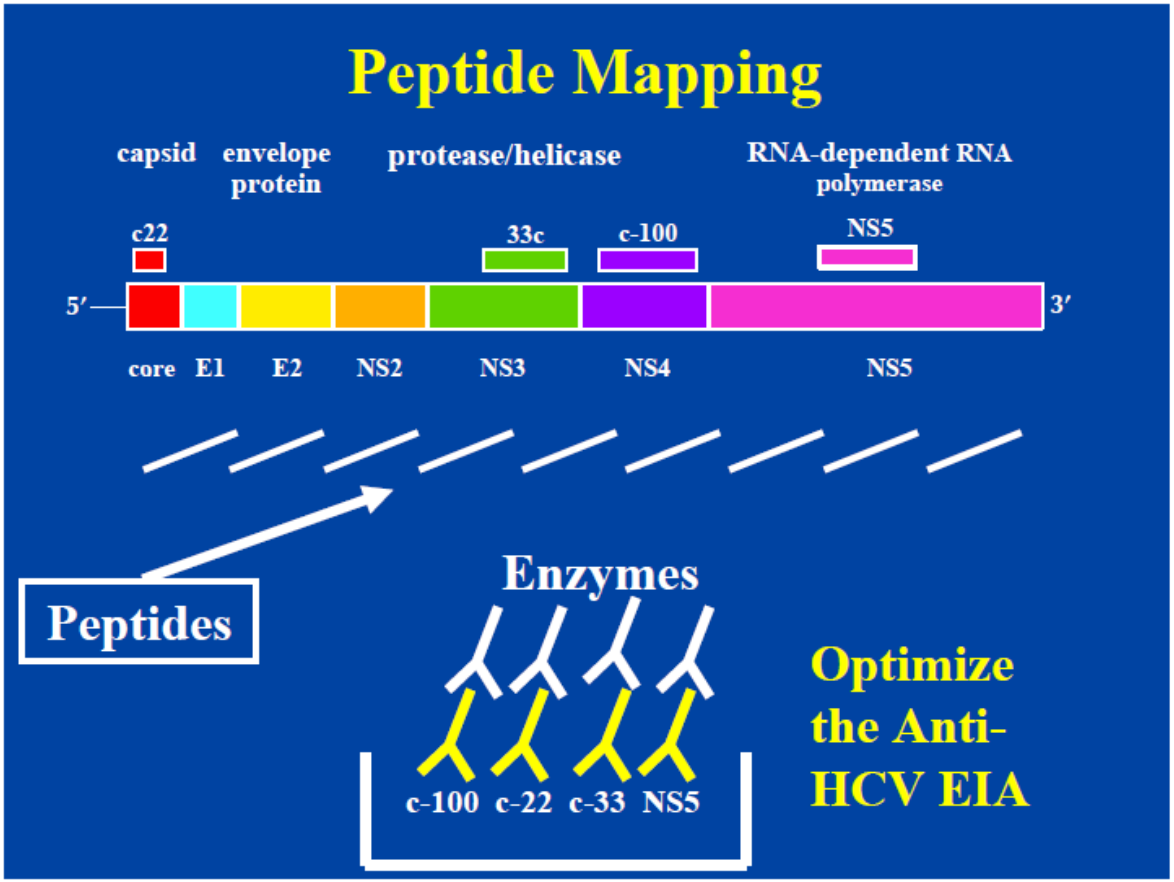
- **Identification & characterization without visualization**

  Choo et al. Science 1989;244:359-362; Kuo et al. Science 1989;244:362-364

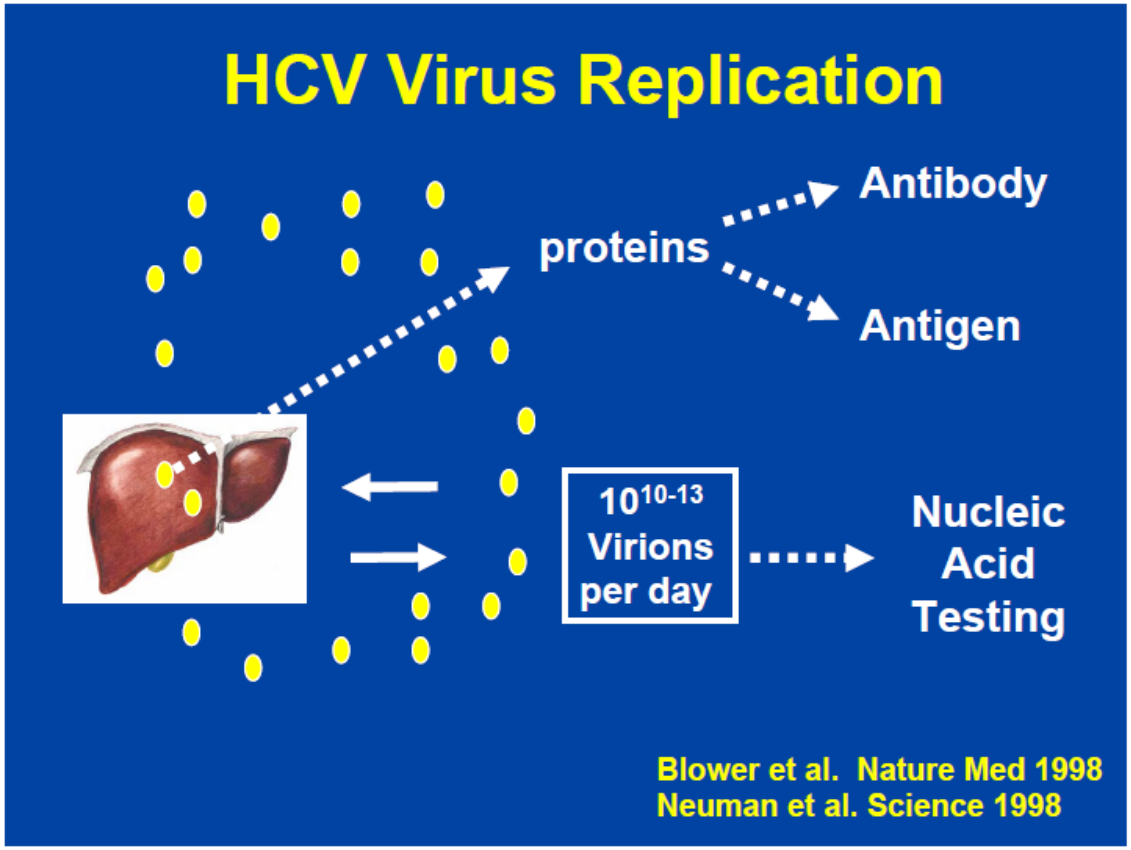
- **First generation anti-HCV enzyme immunoassay (EIA)**



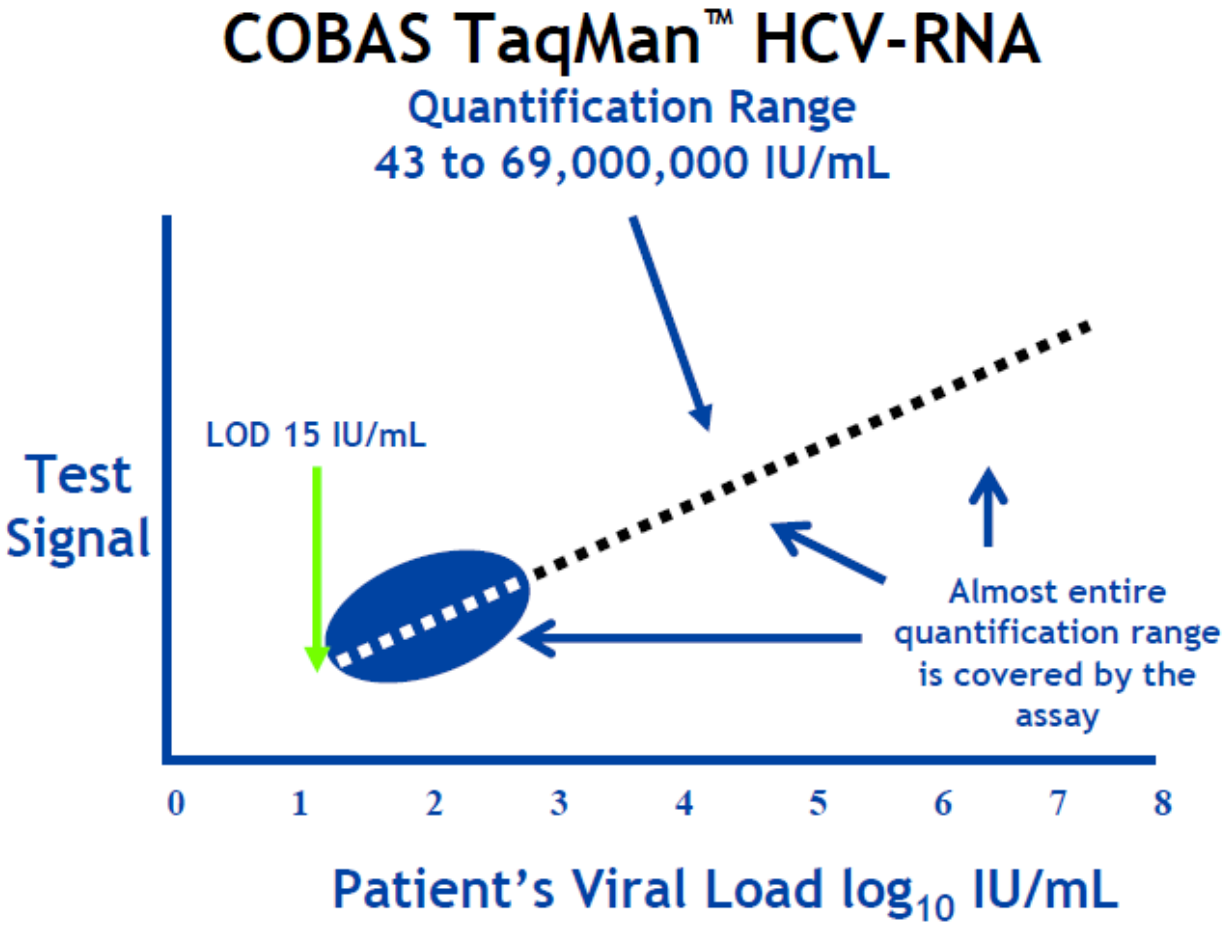
Increased sensitivity



HCV nucleic acid testing (NAT)



Most commonly used NAT for HCV



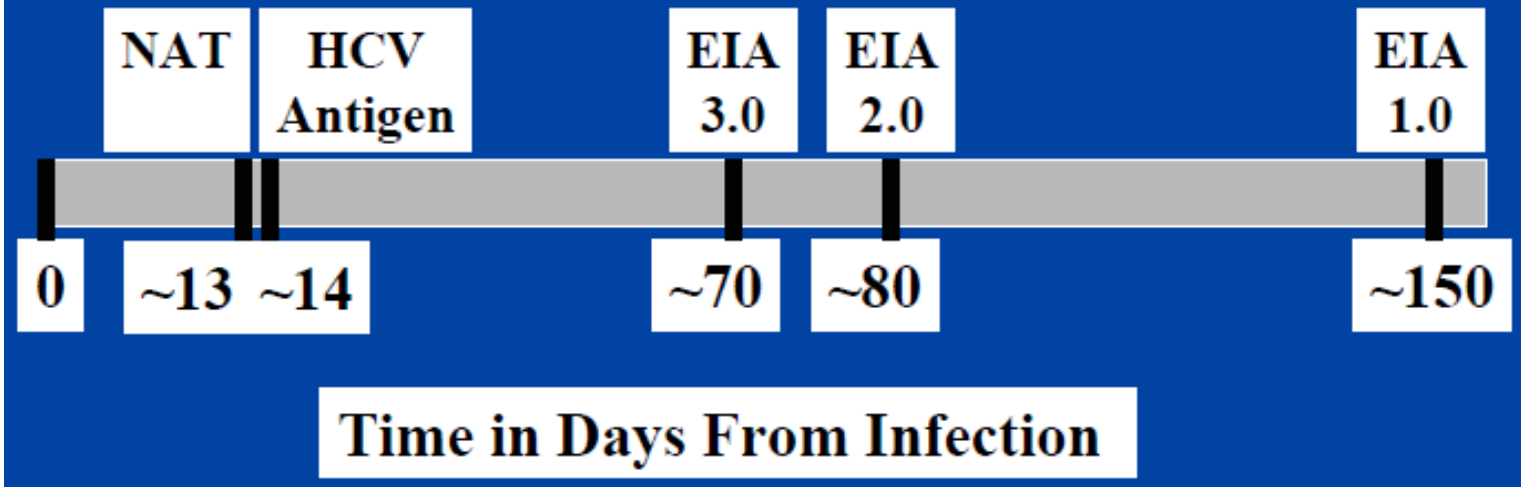
Early detection of HCV RNA by NAT

Improves EIA Sensitivity & Predictive Values

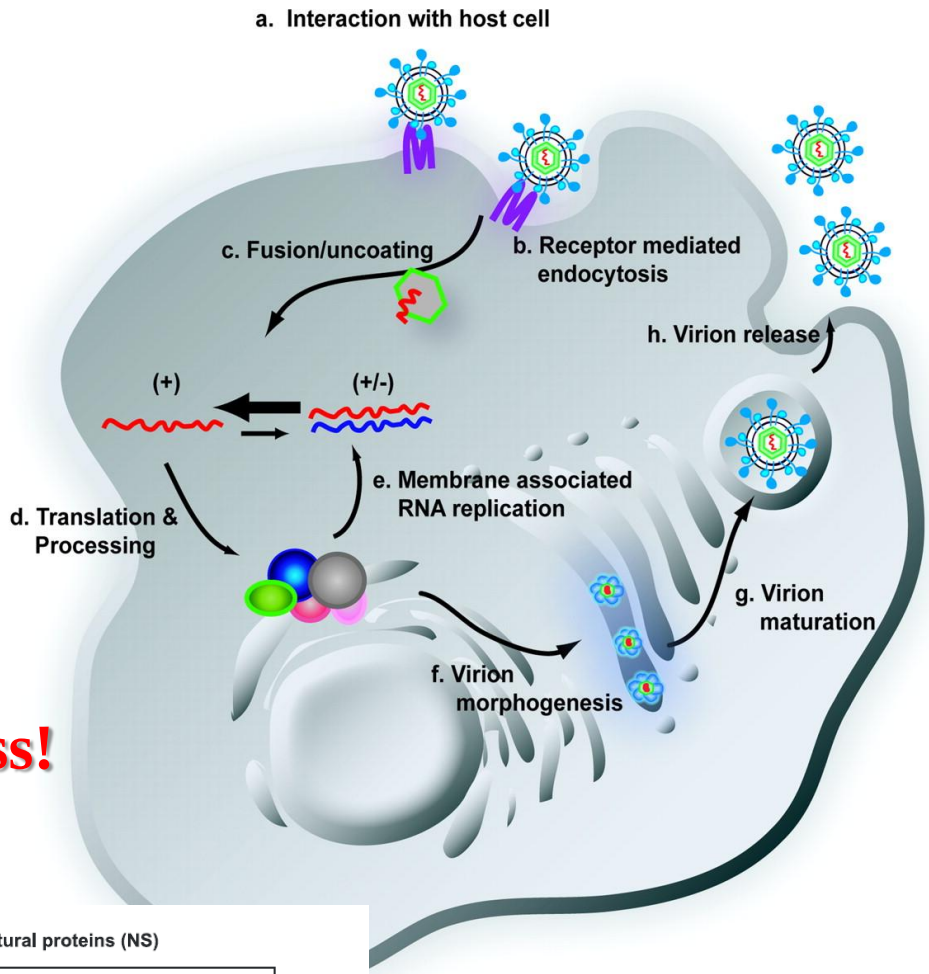
1st → 3rd generation anti-HCV EIA

- Sensitivity 70% → > 99%
- ↑ predictive value
- ↓ time between acute infection and detection

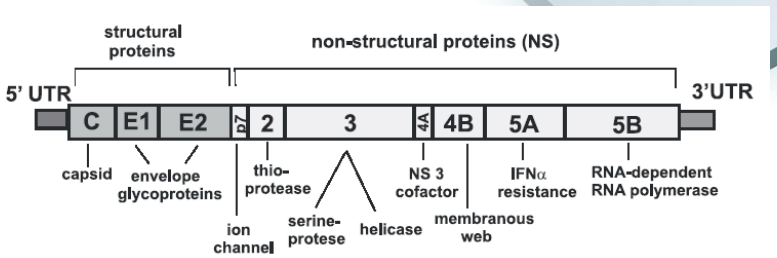
Gretch 97, Schiff 99, Pawlotsky 99, Krajden 2000



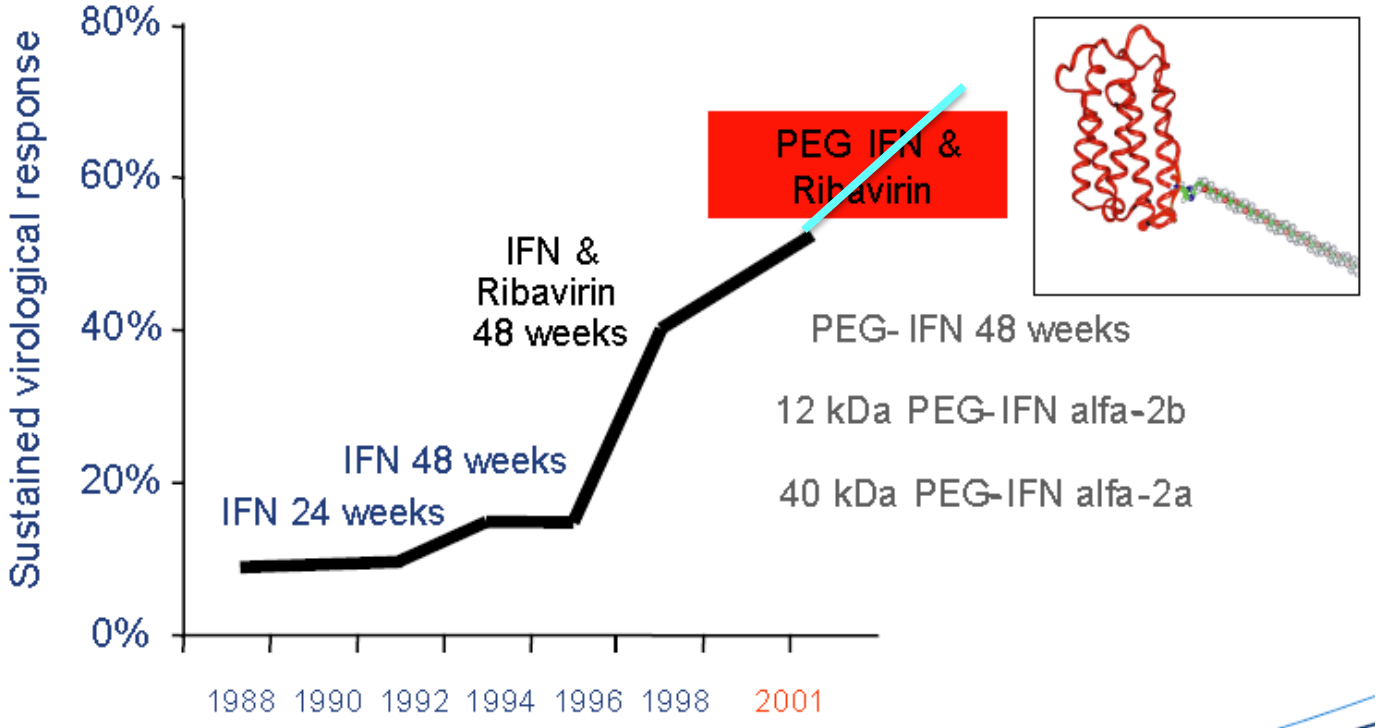
HCV life cycle



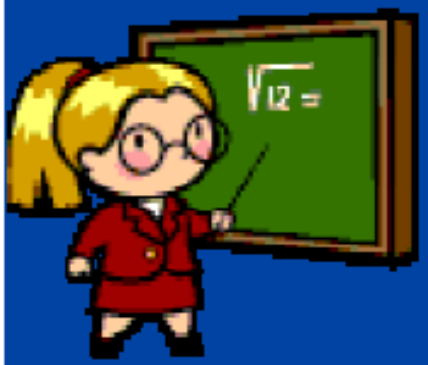
No RT process!



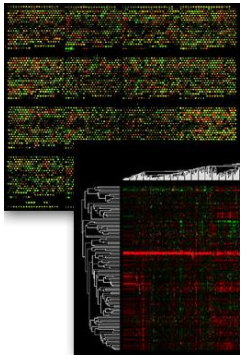
HCV treatment: IFN-based Standard of Care(SOC)



Pegylated Interferon/Ribavirin



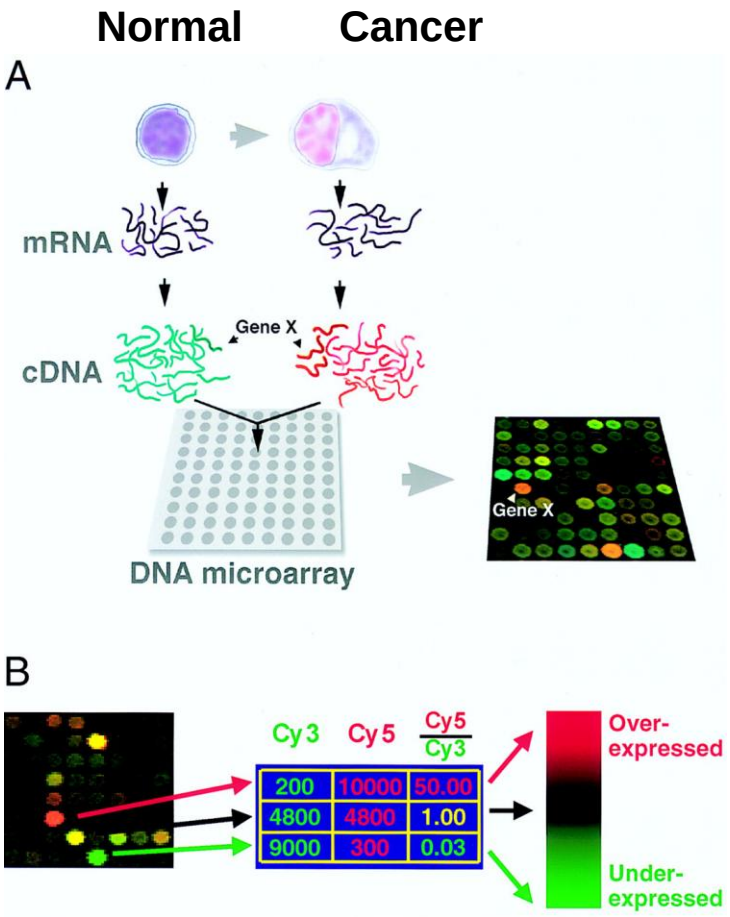
- Individualized therapy
  - viral/host factors
- Viral genotype → 6 major types
  - G1=61%; G2=14%; G3=23% & G 4, 5, 6 = 1%
    - Genotype
      - 1, 4, 5, 6 → cure rates of ~45% with 48 wks of Rx
      - 2 → cure rates of 80% to 90% with 24 wks of Rx
      - 3 → cure rates of ~75% with 24 wks of Rx



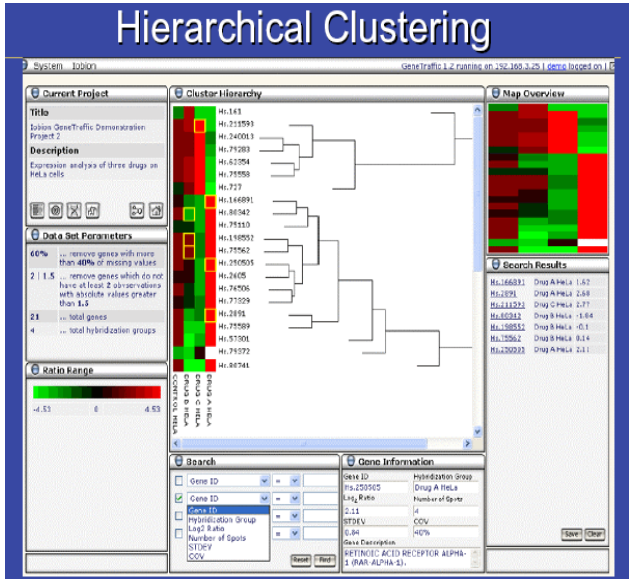
High through-put gene expression profiling

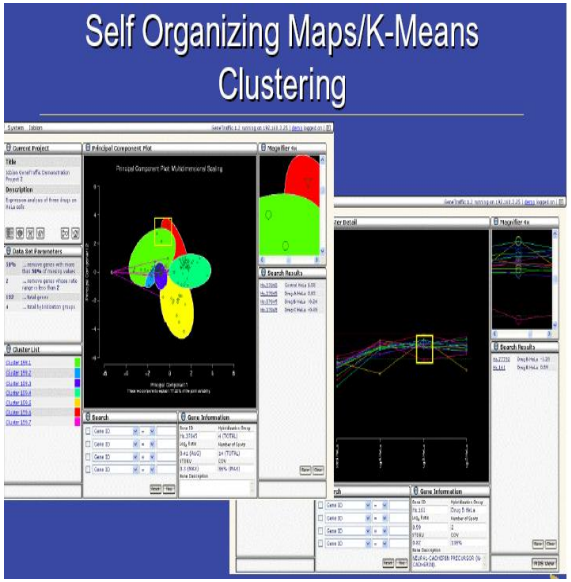
- Gene chip = Microarray
- Detect tens of thousands of mRNA at the same time
- Traditional/classical method: one or a few genes at a time
- Post-genomic HTP tools: study gene expression

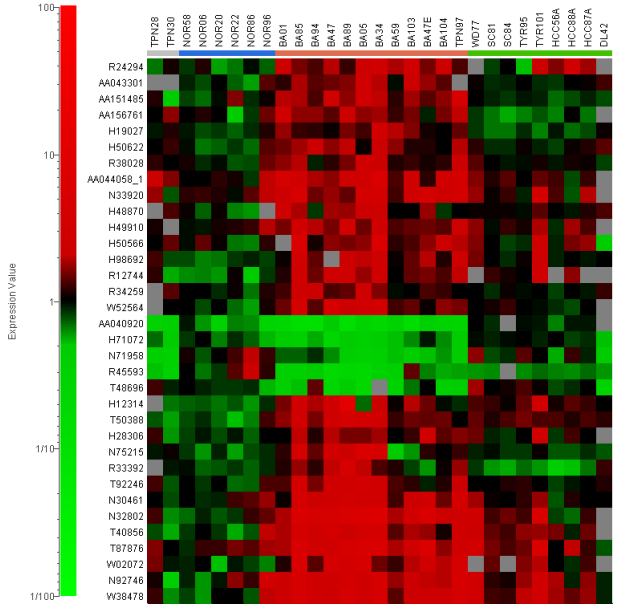
cDNA microarray

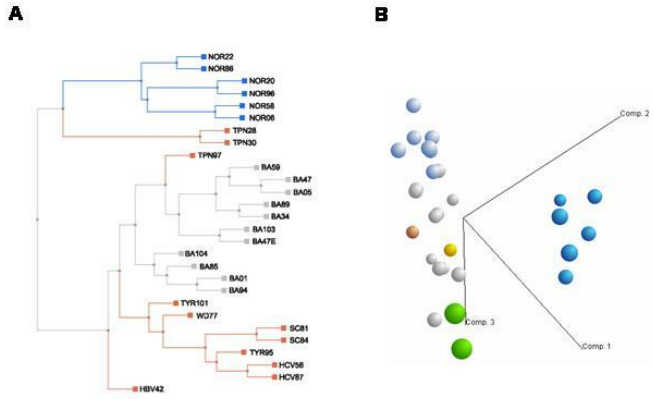


Data analysis is the key …









Part I: Introduction to HCV- summary

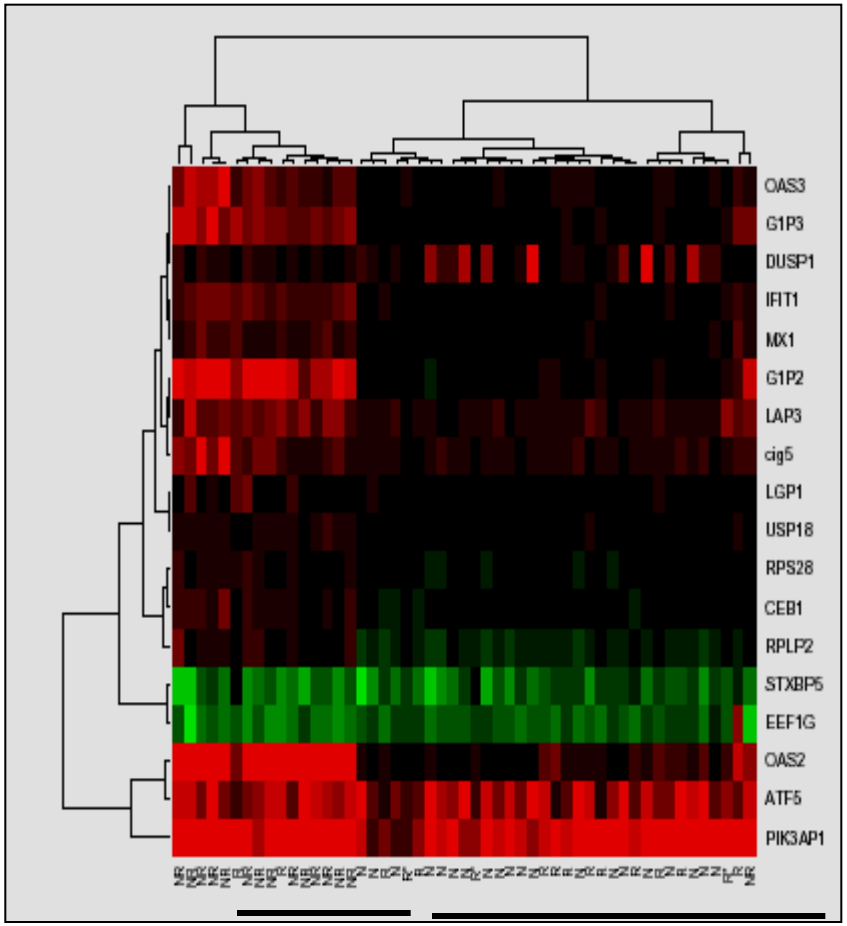
- HCV is a (+) strand RNA virus, hypervariable
- 170 million infected individuals (3%) worldwide
- No DNA phase, no genomic integration-curable disease
- 6 major genotypes: 1-6
- Diagnosis: ELISA for Antibody, NAT for RNA
- Treatment: IFN based+ DAA (from 2011)
- No vaccine- antibody not protective

Part II: Identification of HCV response signature by microarray gene expression profiling

Screen for gene signature for predicting treatment response in HepC

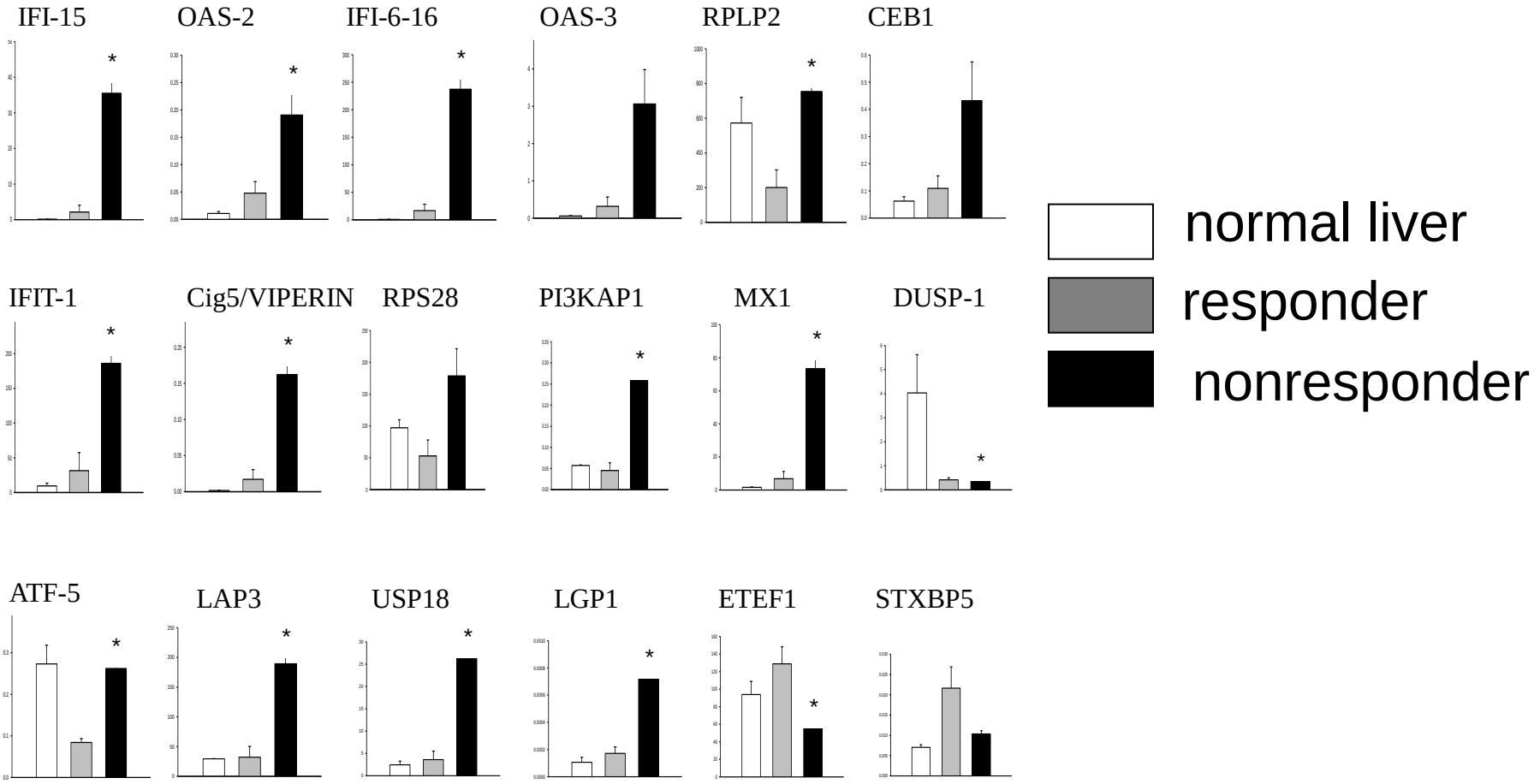
- 15 Non-responders and 16 Responders
- Biopsies prior to treatment
- Gene expression profiling done (19,000)
- Compare the difference between NR and SVR
- Generate gene signature

18 Genes Whose Expression Levels Are Statistically And consistently Different Between Responders (R) and Nonresponders (NR)



Chen, et al. *Gastroenterology* 2005

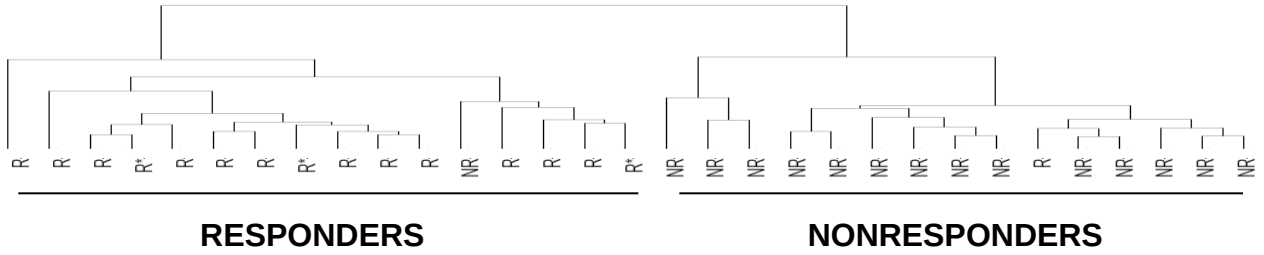
An 18-gene signature differentiates Responders and Non-responders



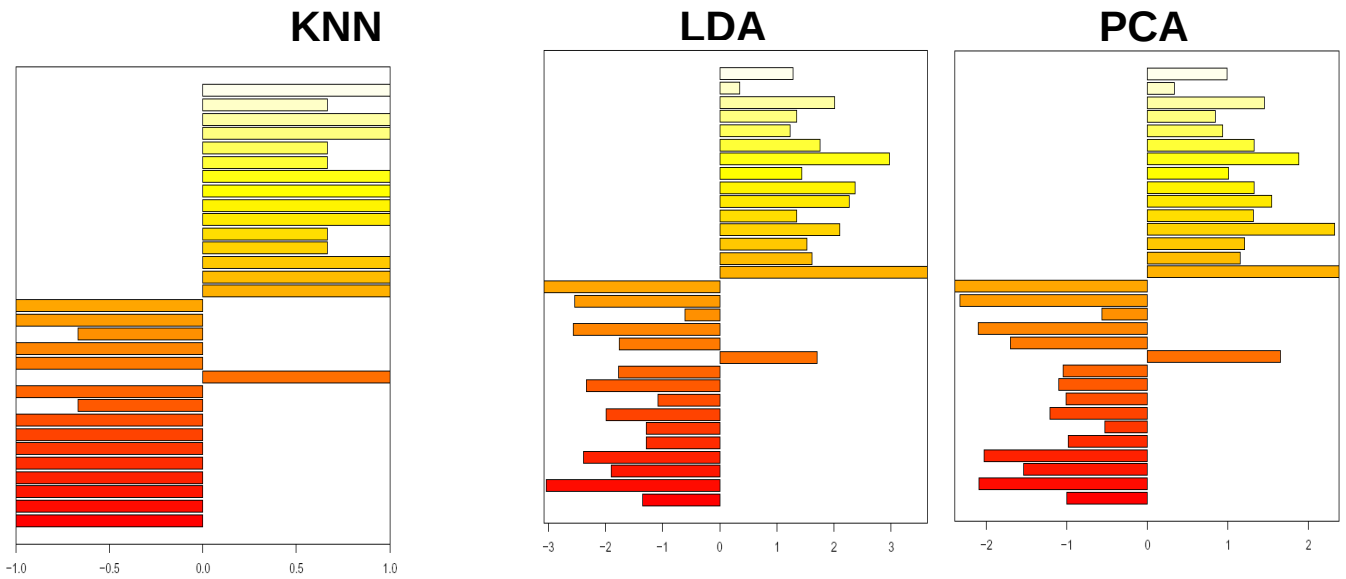
Chen, et al. *Gastroenterology* 2005

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

A.



B.



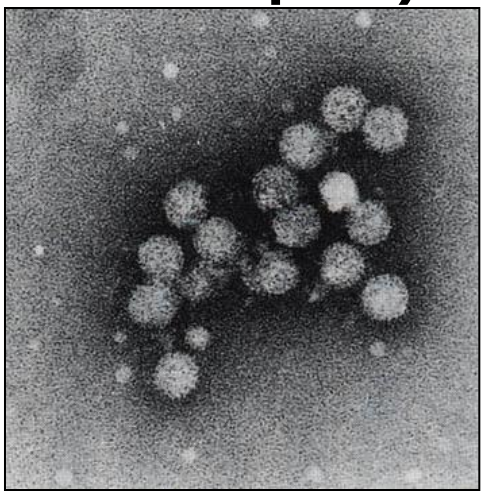
Nearest neighboring | Linear Discriminatory Analysis | Principal component analysis

Chen, et al. *Gastroenterology* 2005

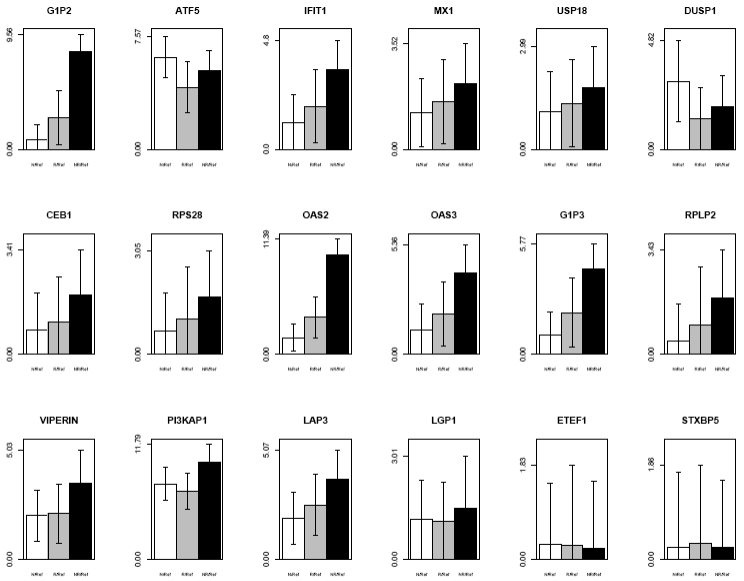
Prospective Validation

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.



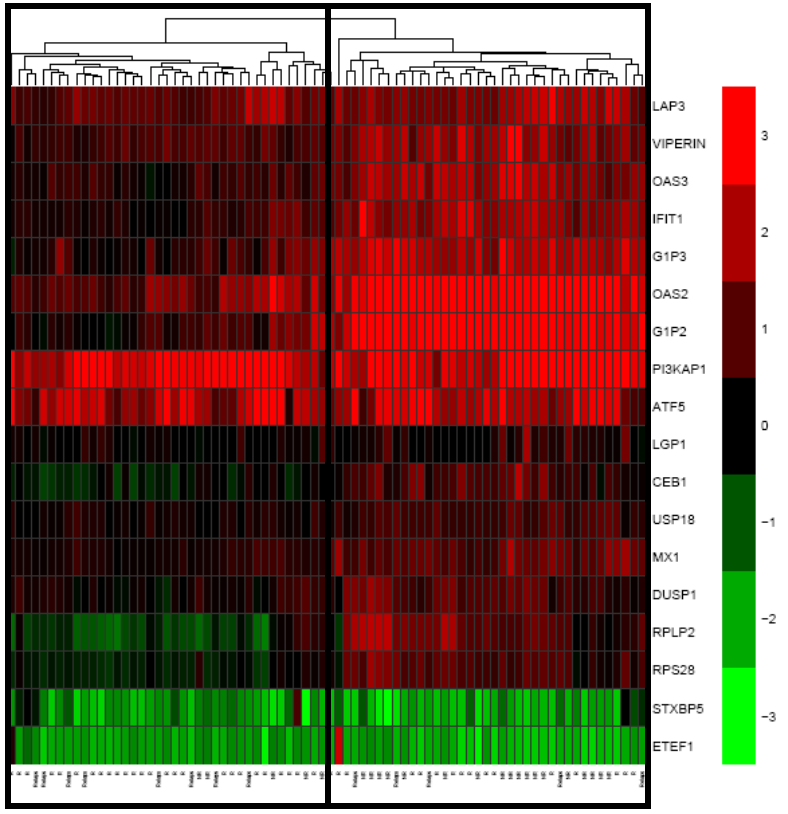
Hierachial clustering analysis of 78 new samples based on 18 signature gene expression



normal liver

responder

nonresponder



R NR

Chen, et al. AASLD 2007 Boston， Chen,et al. Gastroenterology 2010

Chen, et al. International conference on HepC, Glasgow 2007

Prediction accuracy (78 patients)

Methods	*sensitivity*	*specificity*	*PPV*	*NPV*
KNN	0.80 +/- 0.07	0.86 +/-0.15	0.96 +/- 0.04	0.46+/- 0.09
DQDA	0.73+/- 0.07	0.86+/-0.12	0.96+/- 0.03	0.45+/-0.07
DLDA	0.73+/-0.07	0.86+/-0.13	0.96+/-0.04	0.43+/-0.08
CART	0.80+/-0.07	0.86+/-0.21	0.96+/-0.05	0.46+/-0.11

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. International conference on HepC, Glasgow 2007

Chen, et al. Gastroenterology 2010

Reported by AGA Institute

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VOL. 2 • NO. 8 • AUGUST 2008

GI & HEPATOLOGY NEWS

OFFICIAL NEWSPAPER OF THE AGA INSTITUTE

Genetic Markers Can Predict Response to Hep C Treatment

BY KATE JOHNSON
Elsevier Global Medical News

MONTREAL — A genetic signature involving 18 genes can reliably predict response to treatment with pegylated interferon α plus ribavirin in patients infected with hepatitis C, reported Dr. Limin Chen at Canadian Digestive Diseases Week.

This finding "could form the basis for a diagnostic tool to encourage treatment compliance," said Dr. Chen in an interview. The results also confirm the findings of in vitro studies by Dr. Chen's group, led by Dr. Ian McGilvray, Dr. Aled Edwards, and Dr. Jenny Heathcote from the University of Toronto.

His group previously identified gene expression differences in the pretreatment liver biopsies of hepatitis C virus (HCV) patients who subsequently responded, or failed to respond, to pegylated interferon α plus ribavirin, showing that these two groups "differ fundamentally in their innate [interferon] response to HCV infection" (Gastroenterology 2005;128:1437-44).

Upregulation of an 18-gene signature known as USP18 predicted lack of response to treatment, and another study by the same group demonstrated that silencing this particular gene signature in vitro could improve treatment response (Gastroenterology 2006;131:1584-91).

"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 use much less interferon 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 nonresponse, 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. These 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.

Genetic 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. ■

Highlighted in Gastroenterology

This Month in **Gastroenterology**

Genomic Arrays Can Help Predict HCV Patients Who Will Likely Respond to PegIFNα Plus Ribavirin Therapy

PegIFNα plus ribavirin (PegIFN/rib) 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/rib therapy.

See page 1437

(NAG^{Tg+}), which
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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-

APASL : Presidential Award

22 April 2013

Limin Chen
Chinese Academy of Medical Sciences
Chengdu, China

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

Prof. Chen won the APASL presidential award

Dear Limin 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…

- Internatioal conference on HCV(2005,Montreal, Canada; 2007, Glasgow, UK; 2009, Nice,France)
- International conference on interferon and cytokines (2006,Shanghai)
- AASLD(2006, Boston; 2008, San Francisco; 2009, Boston, 2010 Boston， 2011 SF)
- International conference on infectious diseases（ 2009,Beijing- sole Gold winner！）
- Invited to write reviews for J Hepatology,Int J Biochem & Mol Biol , Exp Rev Proteomics

Invited as key-note speakers & as session chairs

Invited as key-note speakers

Day 1: Wednesday, Nov 13, 2013

13:30-20:30 Ball Room

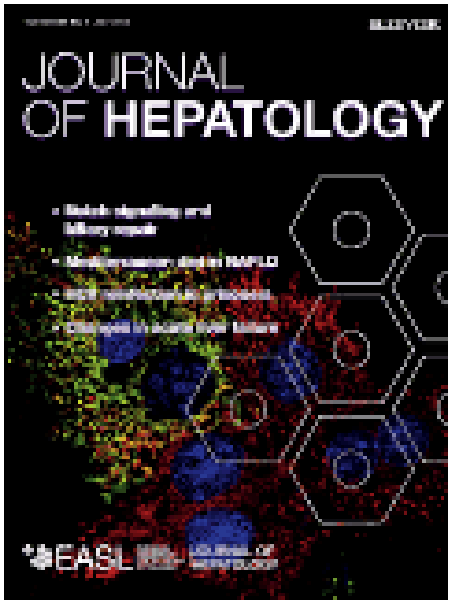
Hainan International Convention & Exhibition Center, Hainan, China

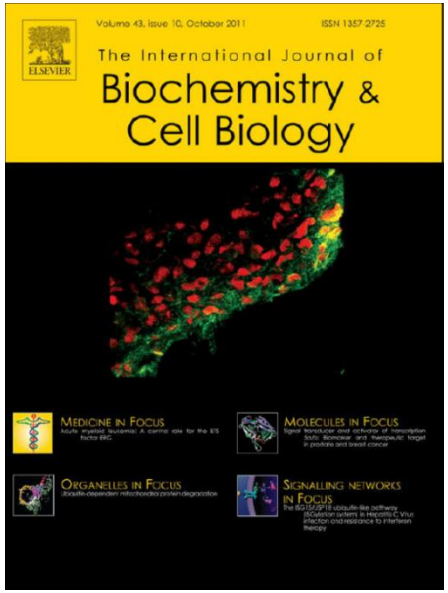
Event Title:

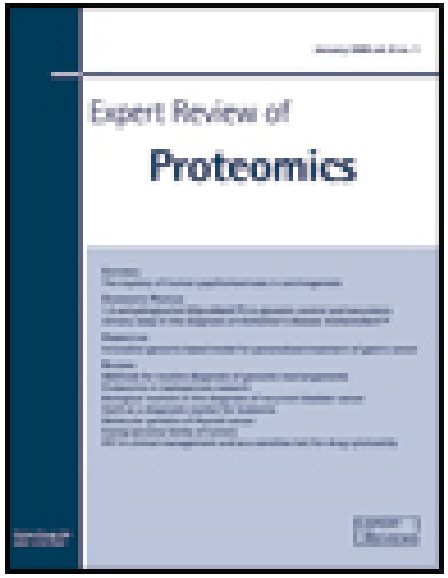
Keynote Forum

Time	FaceOn	Speeches and Speakers	Org. Logo
13:30-14:00		***Title:*** *Metagenomics Reveals the Molecular Mechanisms Driving Chronic Disease* ***Dr. Trevor G Marshall,*** Professor and Director, Autoimmunity Research Foundation, USA	Autoimmunity Research Foundation
14:00-14:30		***Title:*** *Early Diagnosis of Cancer: HAAH-A Predictive Biomarker, A Serum Immunoassay And Personalised Medicine* ***Dr. Mahmood Moshiri,*** President and CEO, Panacea Global Inc., Canada	PANACEA GLOBAL
14:30-15:00		***Title:*** *An 18-gene signature predicting treatment response to interferon in patients chronically infected with hepatitis C virus* ***Dr. Limin Chen,*** Professor and Director/Chief Scientific Officer, Institute of Blood Transfusion & University of Toronto, China & Canada	
15:00-15:30		***Title:*** *Application of Translational Sciences to Precision Medicine, the Ipsen experience* ***Dr. Patrice P. Denèfle,*** Senior Vice President, Translational Sciences, Ipsen, France	IPSEN Innovation for patient care
15:30-16:00		***Title:*** *Under Proposal* ***Dr. Norbert W. Paul,*** Professor and Vice Dean, Universitatsmedizin Mainz, Germany	UNIVERSITÄTSmedizin.

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

☐ Genetic variation in IL28B and spontaneous clearance of hepatitis C virus.

1. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M.
   Nature. 2009 Oct 8;461(7265):798-801.
   PMID: 19759533 [PubMed - indexed for MEDLINE]
   Related articles

☐ IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy.

2. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J.
   Nat Genet. 2009 Oct;41(10):1100-4. Epub 2009 Sep 13.
   PMID: 19749758 [PubMed - indexed for MEDLINE]
   Related articles

☐ Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C.

3. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M.
   Nat Genet. 2009 Oct;41(10):1105-9. Epub 2009 Sep 13.
   PMID: 19749757 [PubMed - indexed for MEDLINE]
   Related articles

☐ Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance.

4. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB.
   Nature. 2009 Sep 17;461(7262):399-401. Epub 2009 Aug 16.
   PMID: 19684573 [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???

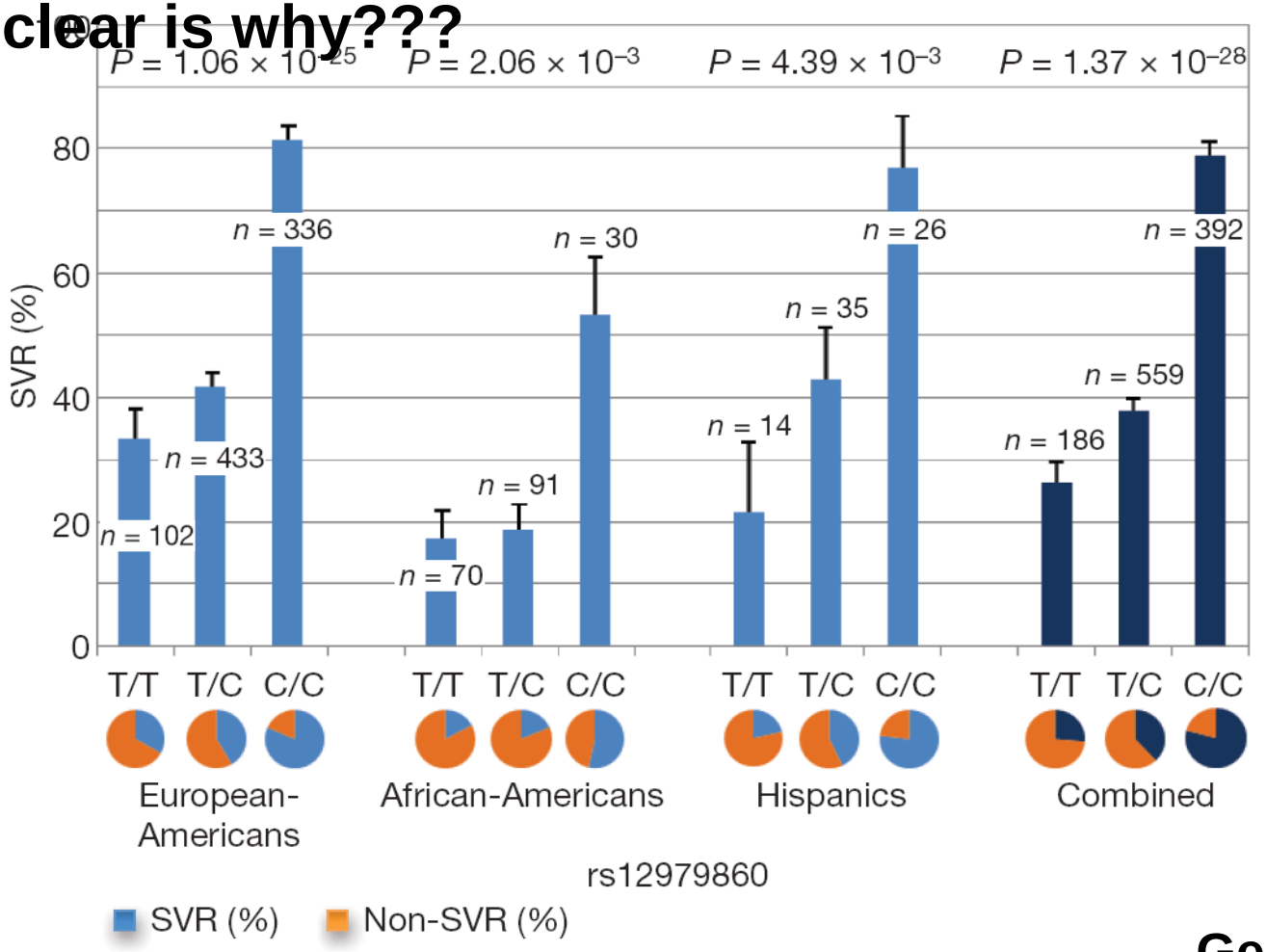


Figure 1 | Percentage of SVR by genotypes of rs12979860. Data are percentages + s.e.m.

Ge et al, Nature, 2009

Which type of cells express these 18 genes?

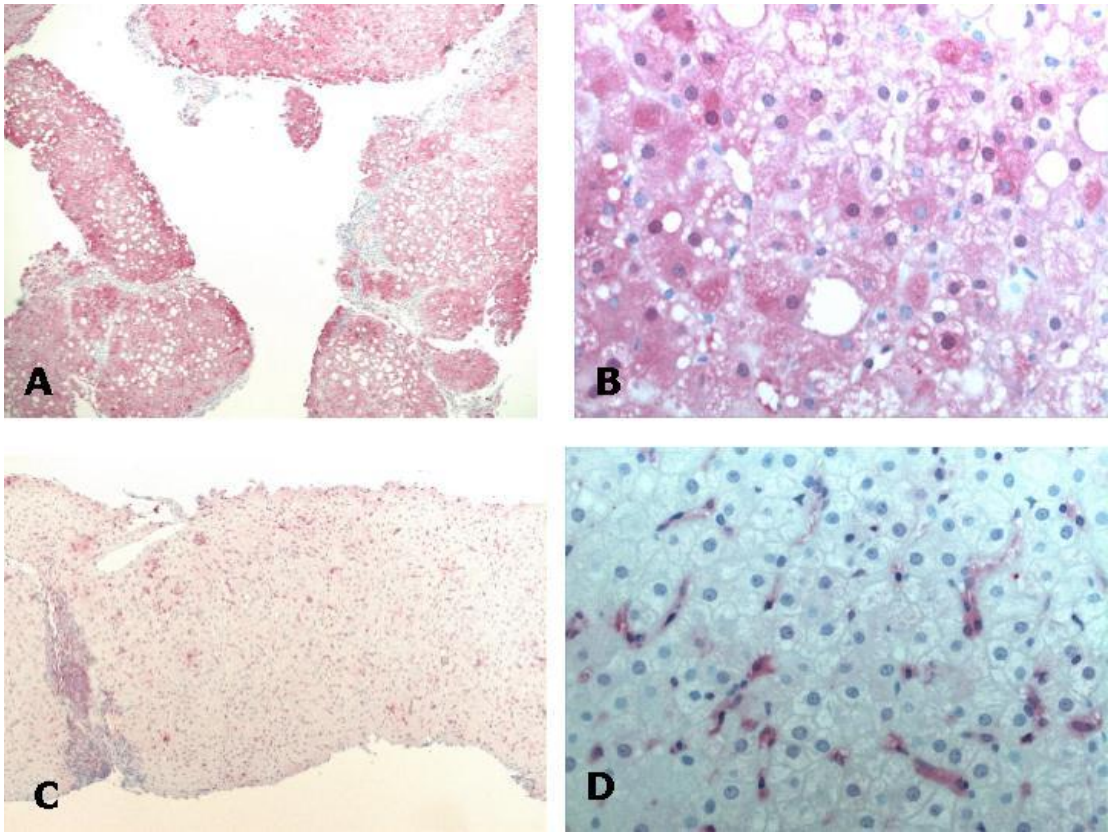
Different cell types in the liver

- -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

NR

R



HEPATOCYTE ISG: Nonresponders

MACROPHAGE ISG: Responders

50X

400X

Chen et al, *Gastroenterology* 2010

CLINICAL—LIVER

Hepatic Cell–Type Specific Gene Expression Better Predicts HCV Treatment Outcome Than *IL28B* Genotype

IAN MCGILVRAY,[*,‡] JORDAN J. FELD,[‡,§] LIMIN CHEN,[‡] VENESSA PATTULLO,[‖] MAHA GUINDI,[‡,¶] 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*

Podcast interview: www.gastro.org/gastropodcast. Also available on iTunes.

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) infec-

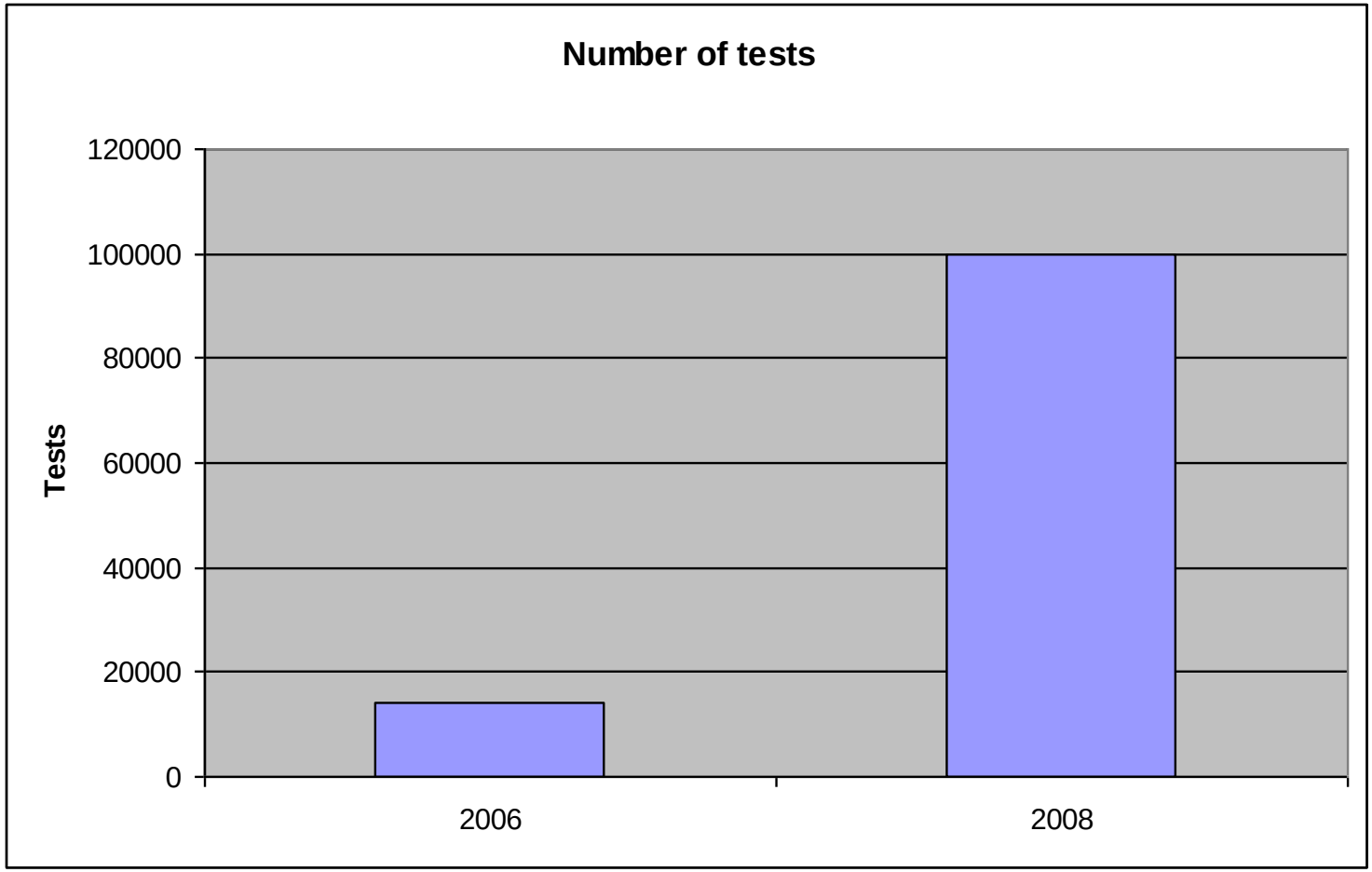
Keywords: Treatment Outcome; Prognostic Factor; Liver Disease; SVR.

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 peginterf
ribavirin.[1,2] Although direct acting antivirals (D

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)**（$2000/test)（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

Dr. Glenn Randall

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