

BRIEF HISTORY OF HEPATITIS MILESTONES

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Stephen Hawking – Brief History of time



MENTORS AND FRIENDS



HEPATOLOGY

Official Journal of the American Association for the Study of Liver Diseases



MASTER'S PERSPECTIVE

The Road Not Taken or How I Learned to Love the Liver: A Personal Perspective on Hepatitis History

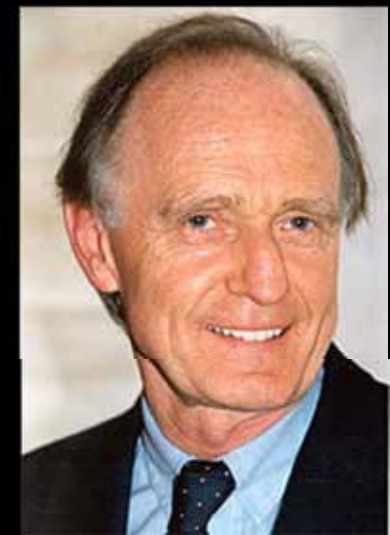
Harvey J. Alter



EASL MONOTHEMATIC LYON 2013

WHAT HAVE WE LEARNED FROM THE HISTORY
OF VIRAL HEPATITIS RESEARCH ?

Hubert Blum



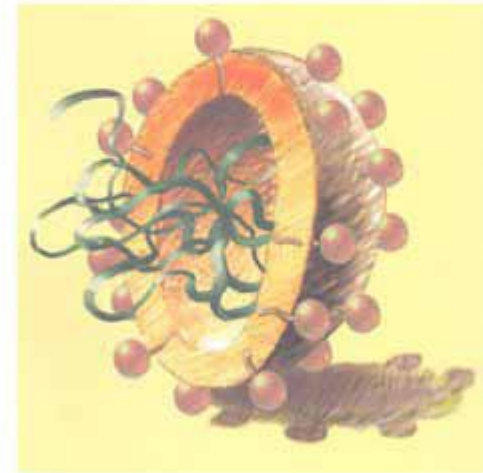
History of hepatitis – From jaundice to HCV



Hepatoscopy

De la jaunisse à l'hépatite C

5 000 ans d'histoire



Jean-Louis Payen



The
hunt
for a
killer

virus
Hepatitis B

Baruch S. Blumberg

Winner of the Nobel Prize in Physiology or Medicine

ALFRED M. PRINCE



THE POETRY OF LIFE

IN SCIENCE
IN AFRICA

I. Epidemic hepatitis (-3000 to 1900)

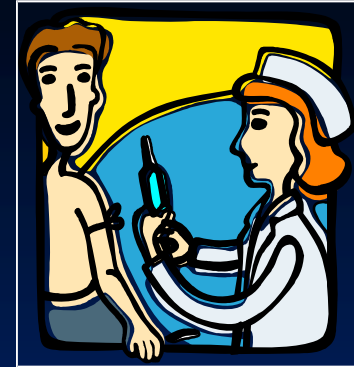
- -3000 Sumerians (Jaundice...)
- -420 Greek Hippocrate → « icterus »
- +750 Middle Ages
Pope Zacharie St Boniface
→ isolation
- 1800 Jaundice of camps

Sieges < St Jean d'Acre (1799)
Paris (1870)

→ prevention



I. Serum hepatitis (1880-1945)



« Syringe » hepatitis
injections for syphilis

Post-vaccine hepatitis

1882 : anti-small pox: Lurman (Breme)

1937 : anti-yellow fever: Findlay

1942 : 28000 cases US navy



Serum and post transfusion hepatitis
(1945-1975)

Duality of hepatitis

1947 Mc Callum

- Hepatitis A epidemic
- Hepatitis B serum

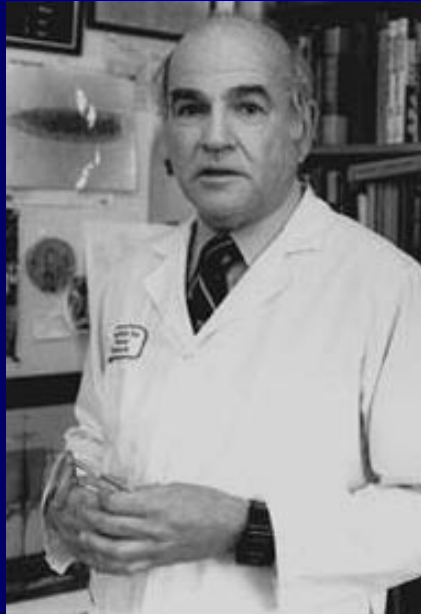
1964 Krugman (Willow Brook School)

- Hepatitis A → oral (30-45d) « MS1 »
- Hepatitis B → parenteral (60-90d) « MS2 »

HBV



Baruch Blumberg



1964



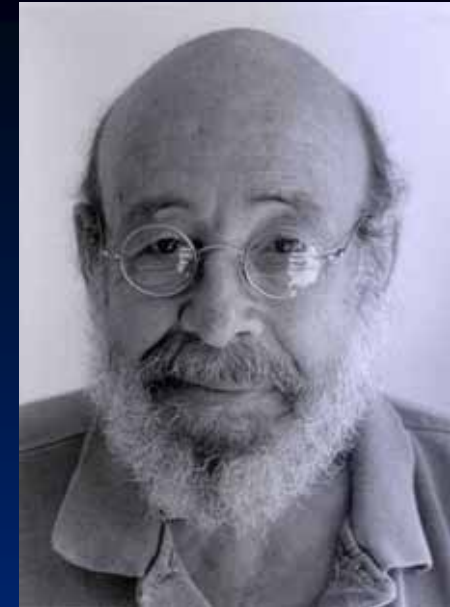
The discovery by Blumberg in 1964 of a new antigen in the serum of an Australian aborigine initiates a new era in the history of hepatitis. Blumberg, an ethnologist, demonstrates that this “Australian antigen” is a marker of hepatitis.

Nobel Prize in 1976 for the discovery of new mechanisms for the dissemination of infectious diseases

Alfred PRINCE

(New York Blood Center)

1968

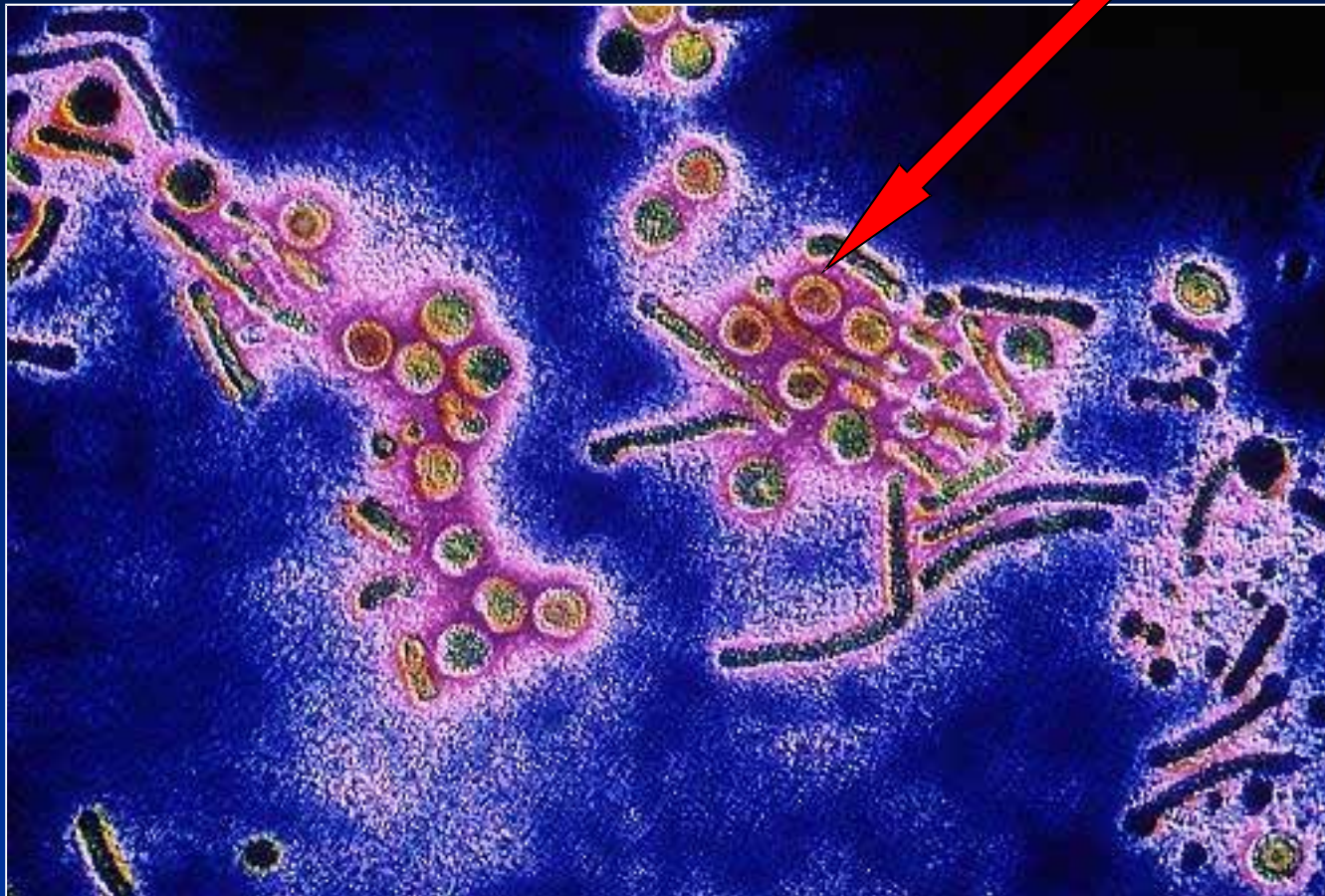


- Described the SH Ag in patients who developed a post-transfusion hepatitis
- Confirmed
 - The specificity of this Ag for hepatitis B
 - The identity with Australia Ag

The virus

1970

DANE



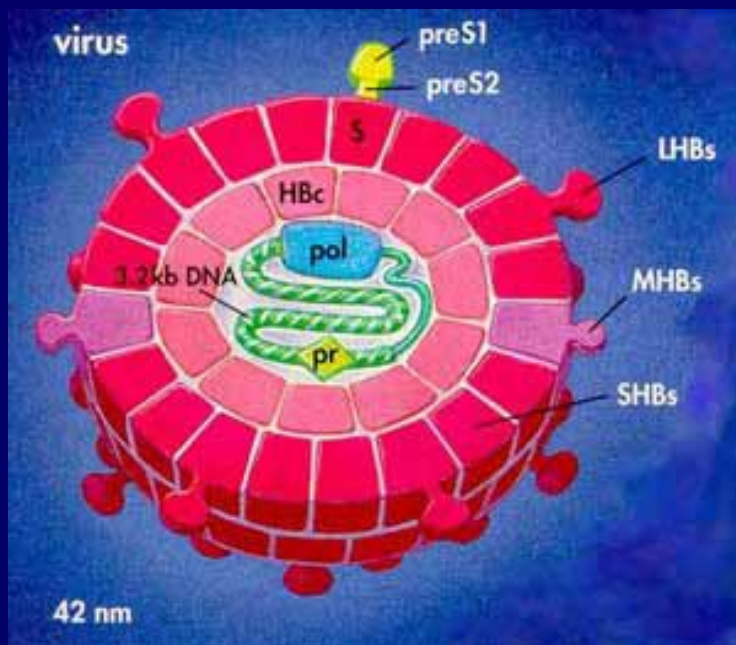


ZENIRAM

HBV Serologic Markers

Antigens

- HBsAg (1965)
- HBeAg (1972)



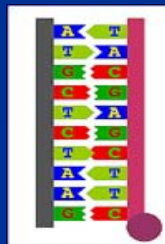
Antibodies

- Anti-HBc total (1971)
- Anti-HBc-IgM
- Anti-HBe
- Anti-HBs
- ADN POL (1972)
- ADN (1974)

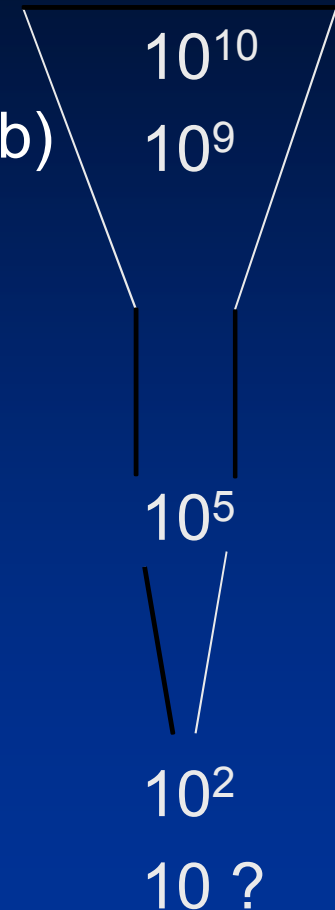
Tests / techniques

- 1965 Immunodiffusion (HBs/e Ag and Ab)
- 1970 Counter-electrophoresis (HBs/e Ag and Ab)
- 1970 RIA Ag/Ab
HBV DNA polymerase
- 1975 ELISA (Ag/Ab)
- 1980 DNA hybridization

PCR Real time

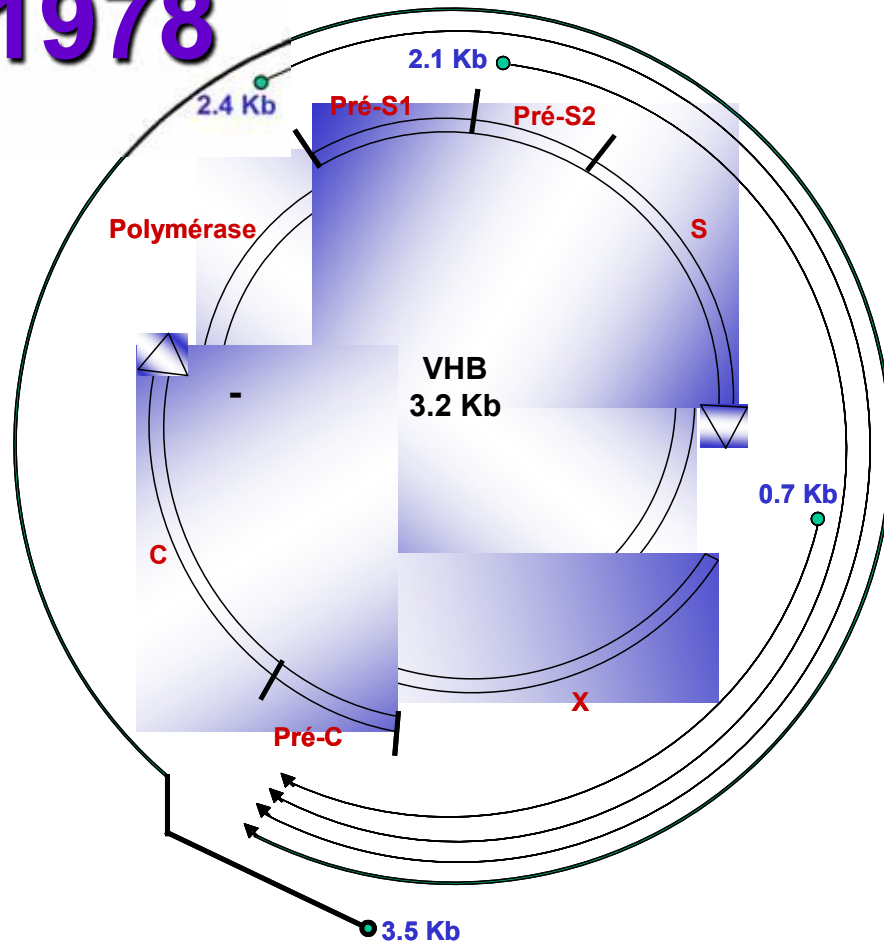


HBV/mL



Cloning of HBV

1978



P. TIOLLAIS/
F. GALIBERT



The Vaccine



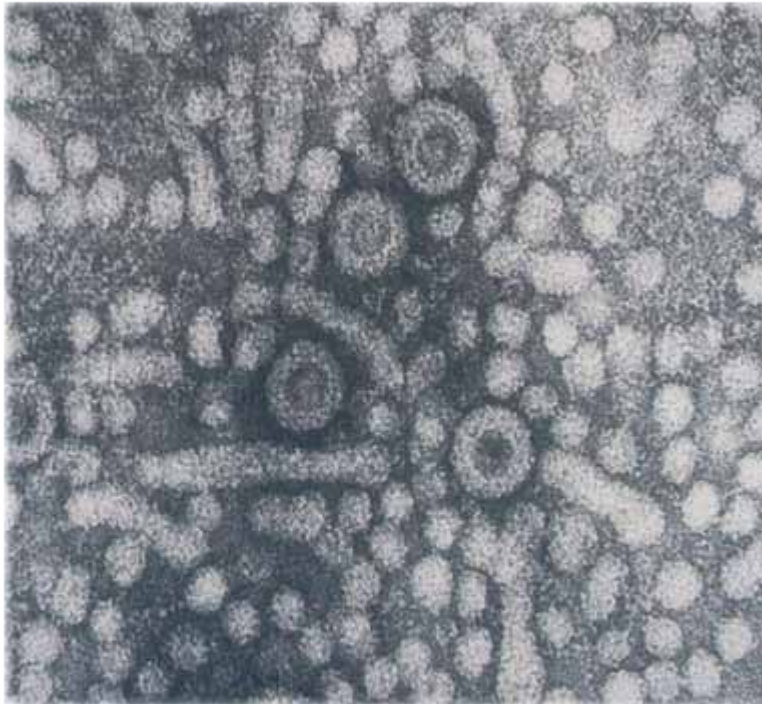
Plasmatic vaccines (1975-76)

- Ph. Maupas / institut Pasteur (F)
- M. Hilleman / MSD (USA)

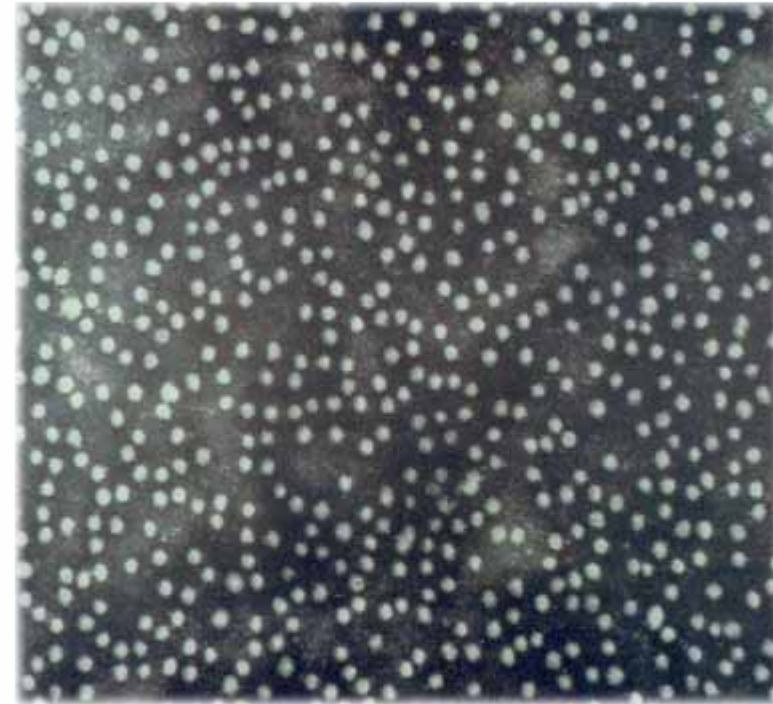
Recombinant vaccines (1981)

- P. Tiollais / institut Pasteur
- W. Rutter/ Chiron/MSD

HBV PARTICLES



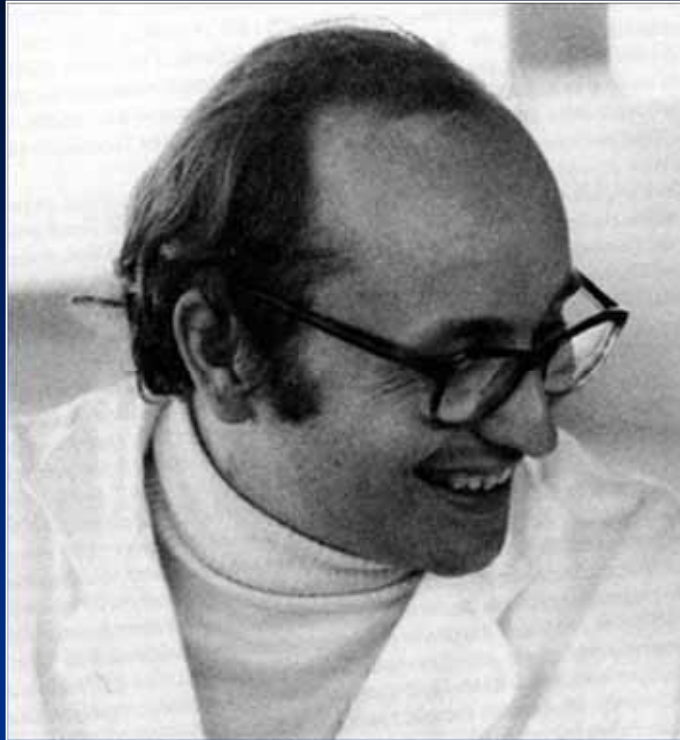
Serum



Serum-derived vaccine: Szmuness W
et al. N Engl J Med 1980; 303: 833-841

Recombinant vaccine: Valenzuela P
et al. Nature 1982; 298:347-350

Pr Ph. Maupas

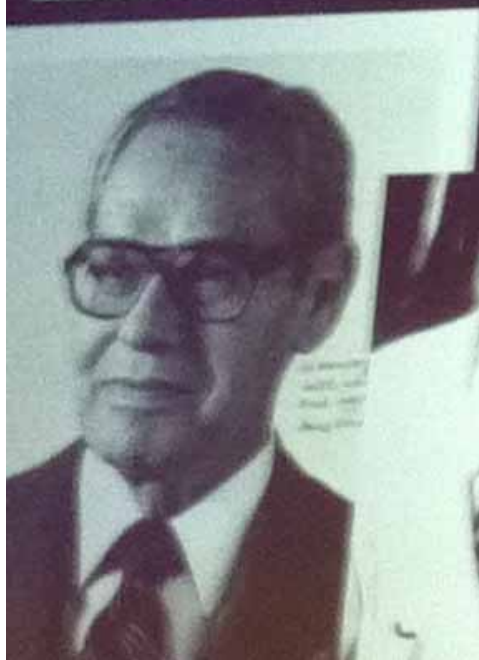
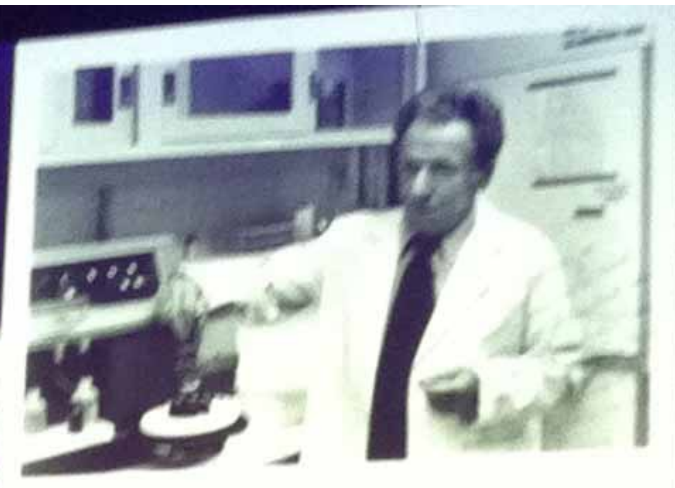
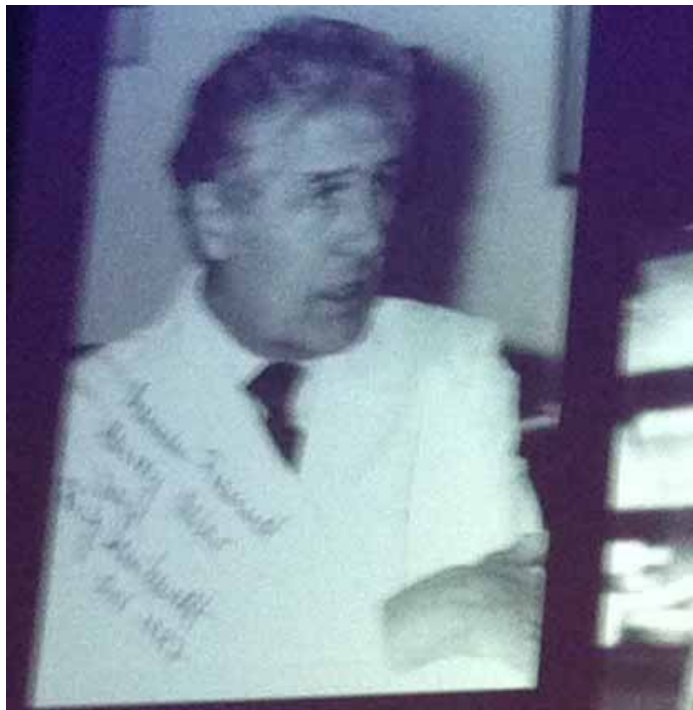


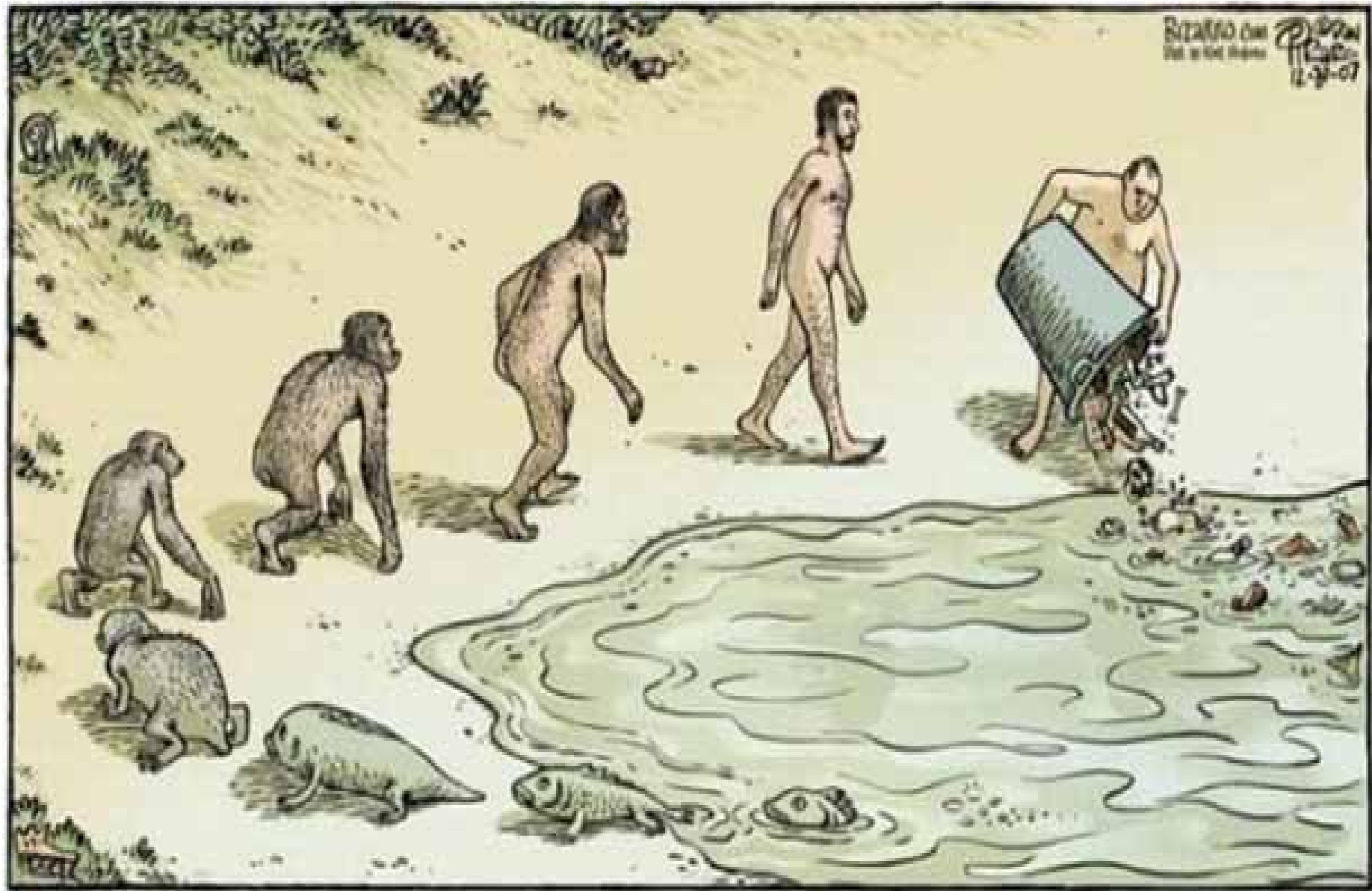
- Discovered the first HBV vaccine in humans (1976)
- Confirmed the association between HBV and primitive liver cancer

IMPACT OF HBV VACCINATION IN TAIWAN

- Incidence of HBs Ag :
 - 1985 : 9 %
 - 1995 : 1 %
- Réduction of HCC incidence and almost disappearance in children

HEPATITIS LEGENDS





STAFFO dal Negro
12-21-07

HEPADNAVIRUSES

Orthohepadnavirus

HBV - Humans
WHV - Woodchucks
GSHV - ground squirrel
TSHV - Tree squirrel
ASHBV - Artic squirrel

Avihepadnavirus

DHBV - Duck
HHBV - Heron
SGHBV - Goose
STHBV - Stork

Old world monkeys :

- Gibbon (GiHBV)
- Gorilla (GoHBV)
- Orang-outang (OuHV)
- Chimpanzee (ChHBV)

New world monkeys :

- Woolly Monkey (WMHBV)

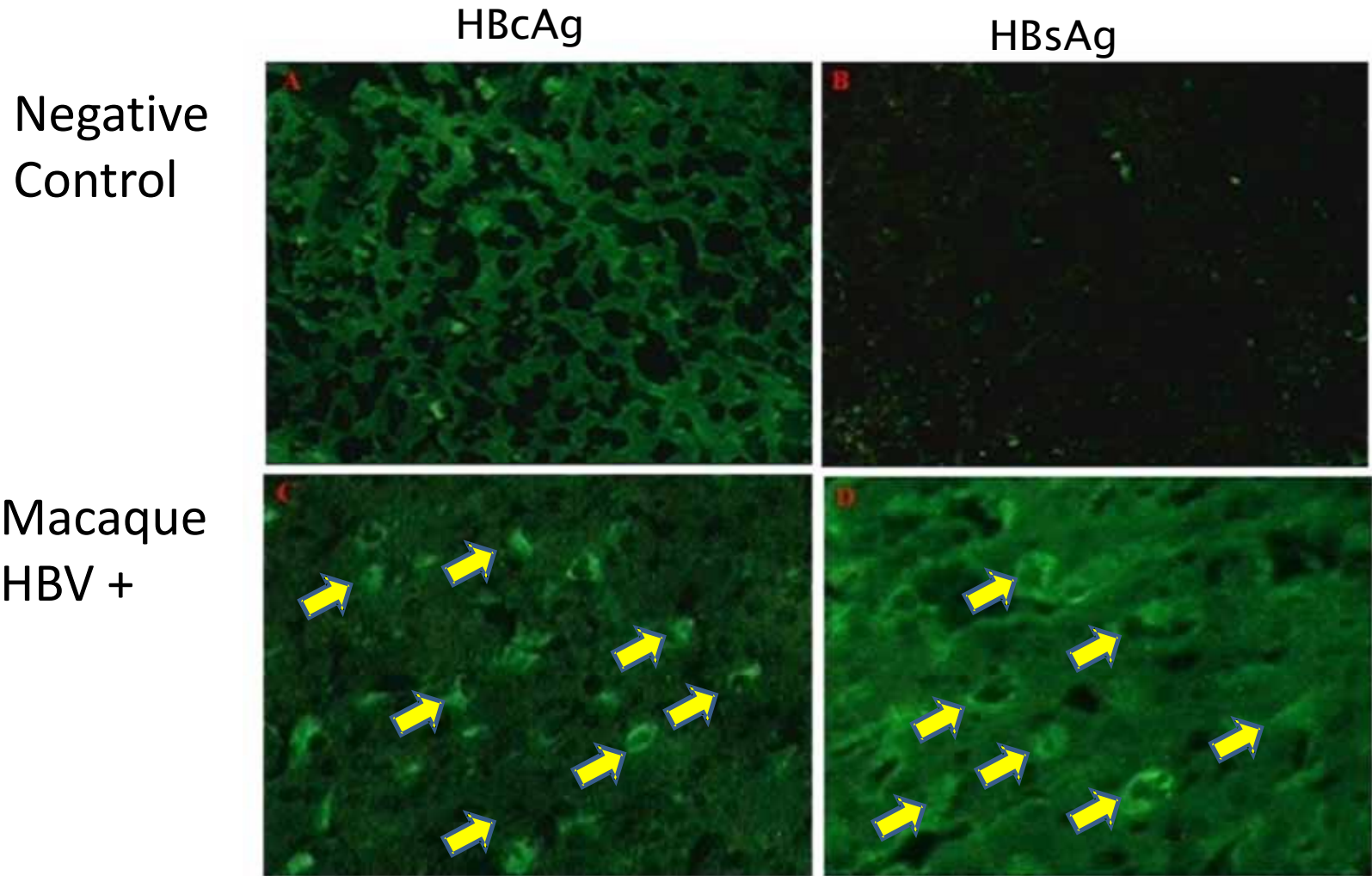
**Missing
small available
monkey**

Discovery of Naturally Occurring Transmissible Chronic Hepatitis B Virus Infection Among *Macaca fascicularis* From Mauritius Island

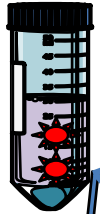
Tatiana Dupinay,^{1,2,3,4*} Tarik Gheit,^{5*} Pierre Roques,^{6,7} Lucyna Cova,^{1,2,3} Philippe Chevallier-Queyron,^{1,2,8}
Shin-i Tasahsu,⁹ Roger Le Grand,^{6,7} François Simon,¹⁰ Geneviève Cordier,¹¹ Lahcen Wakrim,¹²
Soumaya Benjelloun,¹² Christian Trépo,^{1,2,3,8} and Isabelle Chemin^{1,2,3}

- Editorial – Hepatology (November 2013)
Persistent Human Hepatitis B Virus Infection in Cynomolgus Monkeys: A Novel Animal Model in The Search for a Cure?
J Bukh, R.E. Lanford, and R.H. Purcell
- Comment – Nature/middleeast (May 15, 2013)
Macaques-new animal models to test anti-HBV drugs and vaccines. *B. Das*

HBV protein expression in Macaques liver sections



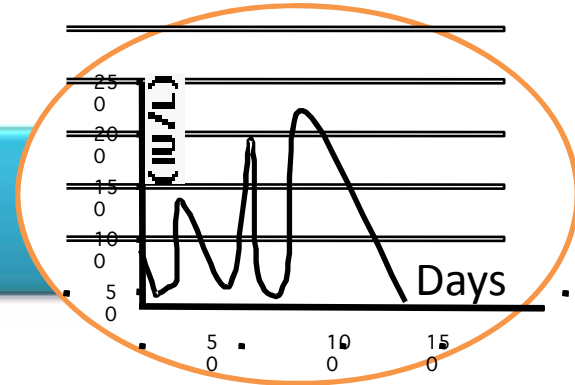
HBV transmission from cynomolgus to sylvanus Macaques



BL14

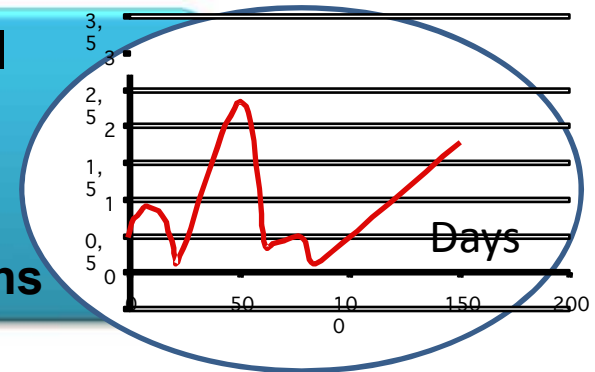
BL13

**ALT peak
3/3 animals**

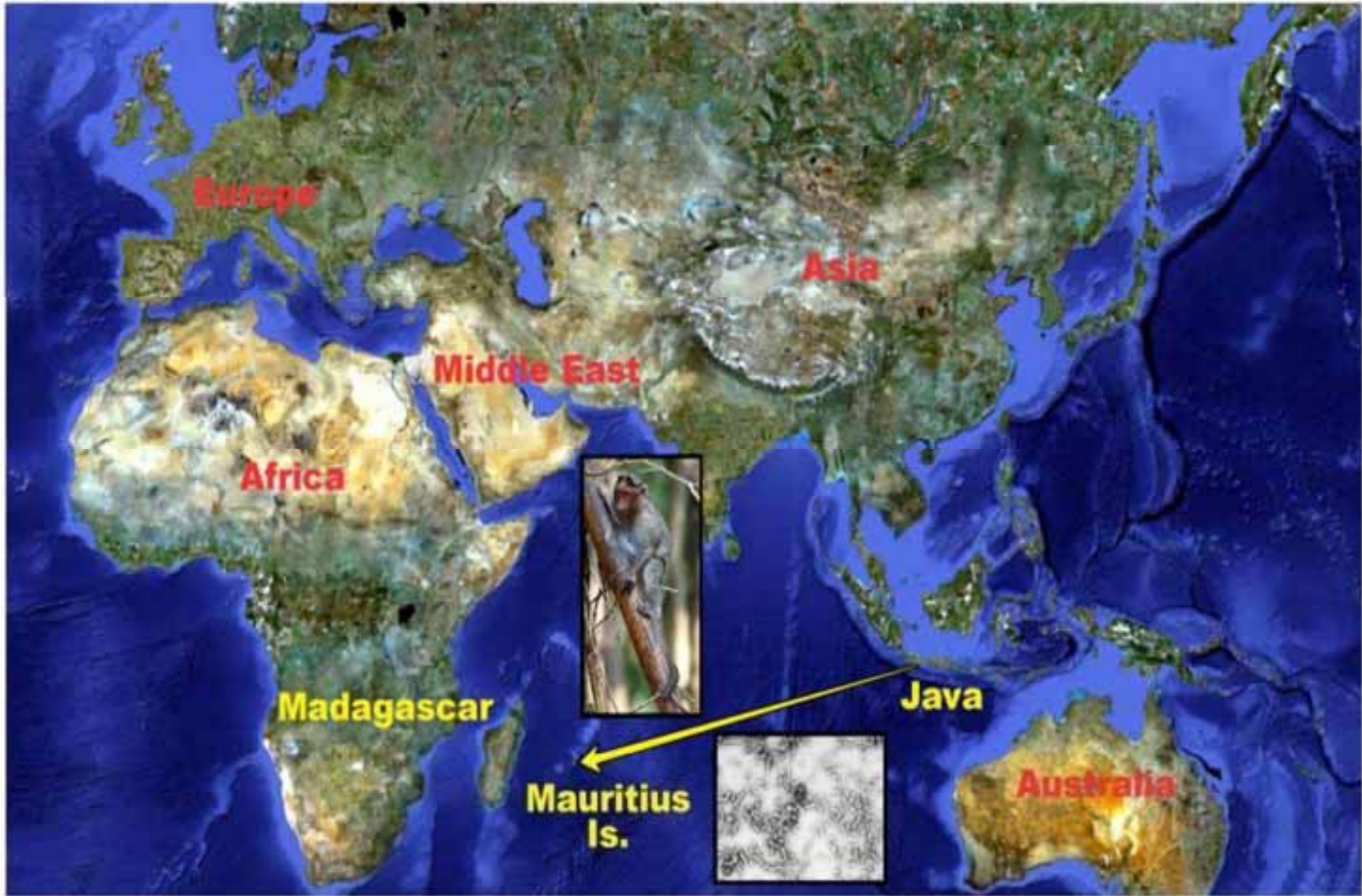


HBsAg+ week 4/7 PI

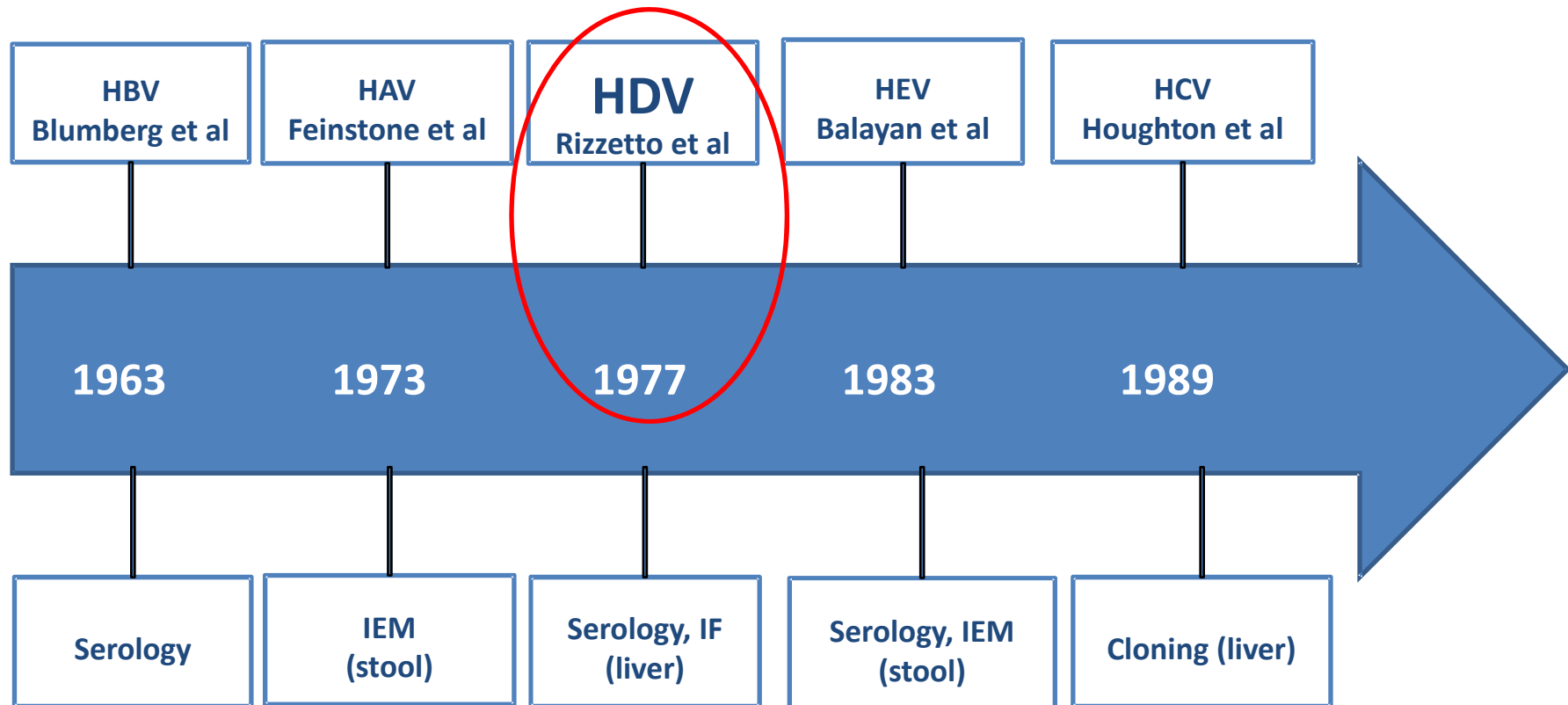
**HBsAg+ /HBcAg
+ by IF on liver sections**



**PCR HBV DNA (+) X 12 weeks
About 10³ copies HBV DNA/ ml
at week 9 pi**



HEPATITIS VIRUSES DISCOVERY



HDV 37th Birthday

Gut, 1977, 18, 997-1003

Immunofluorescence detection of new antigen-antibody system (δ /anti- δ) associated to hepatitis B virus in liver and in serum of HBsAg carriers

M. RIZZETTO,¹ M. G. CANESE, S. ARICÒ, O. CRIVELLI, C. TREPO, F. BONINO, AND G. VERME

From the Department of Gastroenterology, Ospedale Mauriziano Umberto I, Turin, Italy, the Electron Microscopy Centre of the Faculty of Medicine, University of Turin, Italy, and INSERM U45, and Laboratory of Hygiene, University Claude Bernard, Lyon, France

SUMMARY A new antigen-antibody system associated with the hepatitis B virus and immunologically distinct from the HB surface, core, and *e* systems is reported. The new antigen, termed δ , was detected by direct immunofluorescence only in the liver cell nuclei of patients with HBsAg positive chronic liver disease. At present, the intrahepatic expression of HBcAg and δ antigen appears to be mutually exclusive. No ultrastructural aspect corresponding to the δ antigen could be identified under the electron microscope. δ antibody was found in the serum of chronic HBsAg carriers, with a higher prevalence in patients with liver damage. The nuclear fluorescence patterns of HBcAg and δ antigen were similar; it is only possible to discriminate between the two antigens by using the respective specific antisera.

While studying liver biopsies from patients who were seropositive for the hepatitis B surface antigen (HBsAg) in direct immunofluorescence, it was noted that an antiserum against the hepatitis B core antigen (HBcAg), as well as staining specimens in which core particles could be demonstrated by the electron microscope (EM), also reacted with additional biopsies which did not contain core particles (at electron microscopy) and were negative with other reference antisera against HBcAg.

When the EM core positive and core negative specimens were tested with several HBsAg positive sera, it soon became apparent that some sera reacted with either one or the other liver substrate; this suggested that there were two distinct nuclear antigenic specificities.

The identification of this new antigen and of its antibody as an immunological system independent of other known reactions associated with the HB virus is reported in this communication. Provisionally, we propose that it should be called δ .

¹Address for correspondence: Dr M. Rizzetto, Department of Gastroenterology, Ospedale Mauriziano Umberto I, C.s. Turati 46, 10128 Turin, Italy.

Received for publication 30 May 1977

Methods

PREPARATION OF STANDARD FLUORESCENT ANTISERA AGAINST δ ANTIGEN (δ ANTISERUM), AGAINST HBcAg (HBC ANTISERUM), AGAINST HBsAg (HBS ANTISERUM), AND AGAINST *e* ANTIGENS (*e*_s + *e*_p ANTISERUM), STANDARD δ ANTIGEN (δ) AND HBcAg POSITIVE LIVER SUBSTRATES
A fluorescein isothiocyanate (FITC) conjugated antiserum against HBsAg was prepared from Behringwerke rabbit precipitating serum RBBO4 (Rizzetto *et al.*, 1976b). A FITC conjugated antiserum against *e* antigens (*e*_s + *e*_p) was prepared from a human serum as previously described (Trepo *et al.*, 1976).

A FITC antiserum monospecific against HBcAg and one monospecific against δ were prepared from the blood of two apparently healthy HBsAg carriers; both sera were negative when tested by the Reuma and Waaler-Rose techniques. The gamma globulin fractions, isolated after precipitation with (NH₄)₂SO₄, did not contain autoantibodies (in indirect Immunofluorescence (IFL)), antibodies against HBsAg, *e* antigens, or *e* antibodies.

After conjugation with FITC, the HBc antiserum



Hepatitis D (Delta) Virus

The diagram shows a circular virus particle with a yellow outer boundary labeled "HBsAg" and an orange inner boundary labeled "delta antigen". Inside the particle is a tangled orange line representing the "RNA". To the right is an electron micrograph showing several spherical virus particles with a distinct outer shell and a darker core.

delta antigen

HBsAg

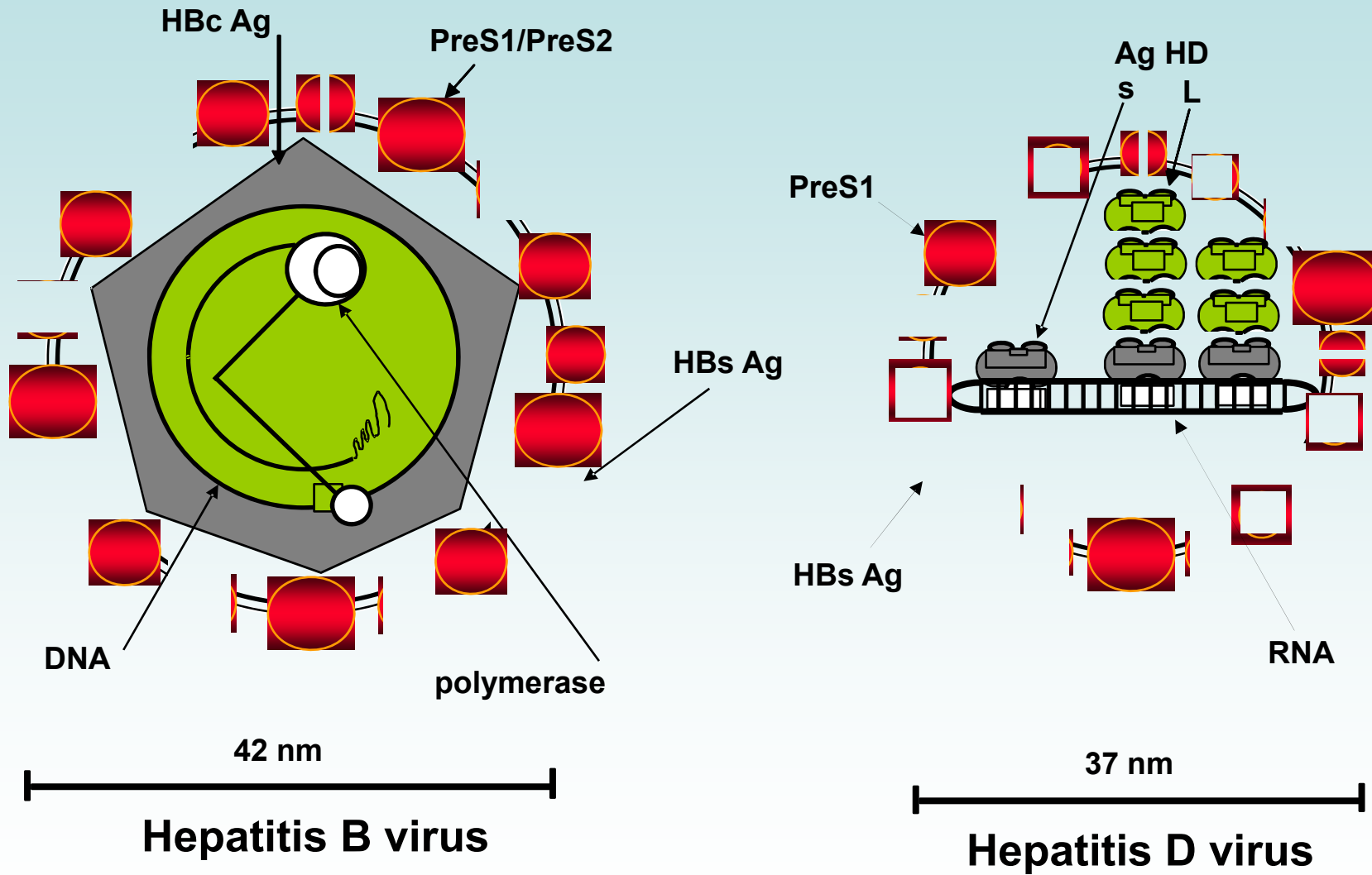
RNA

CDC
U.S. DEPARTMENT OF HEALTH & HUMAN SERVICES

MAJOR CHARACTERISTICS

- Unique agent
- Defective virus
- Highly pathogenic
- Reemerging
- Most challenging therapy

HDV – the virion



HDV

HBV



HDV INHIBITS HBV REPLICATION

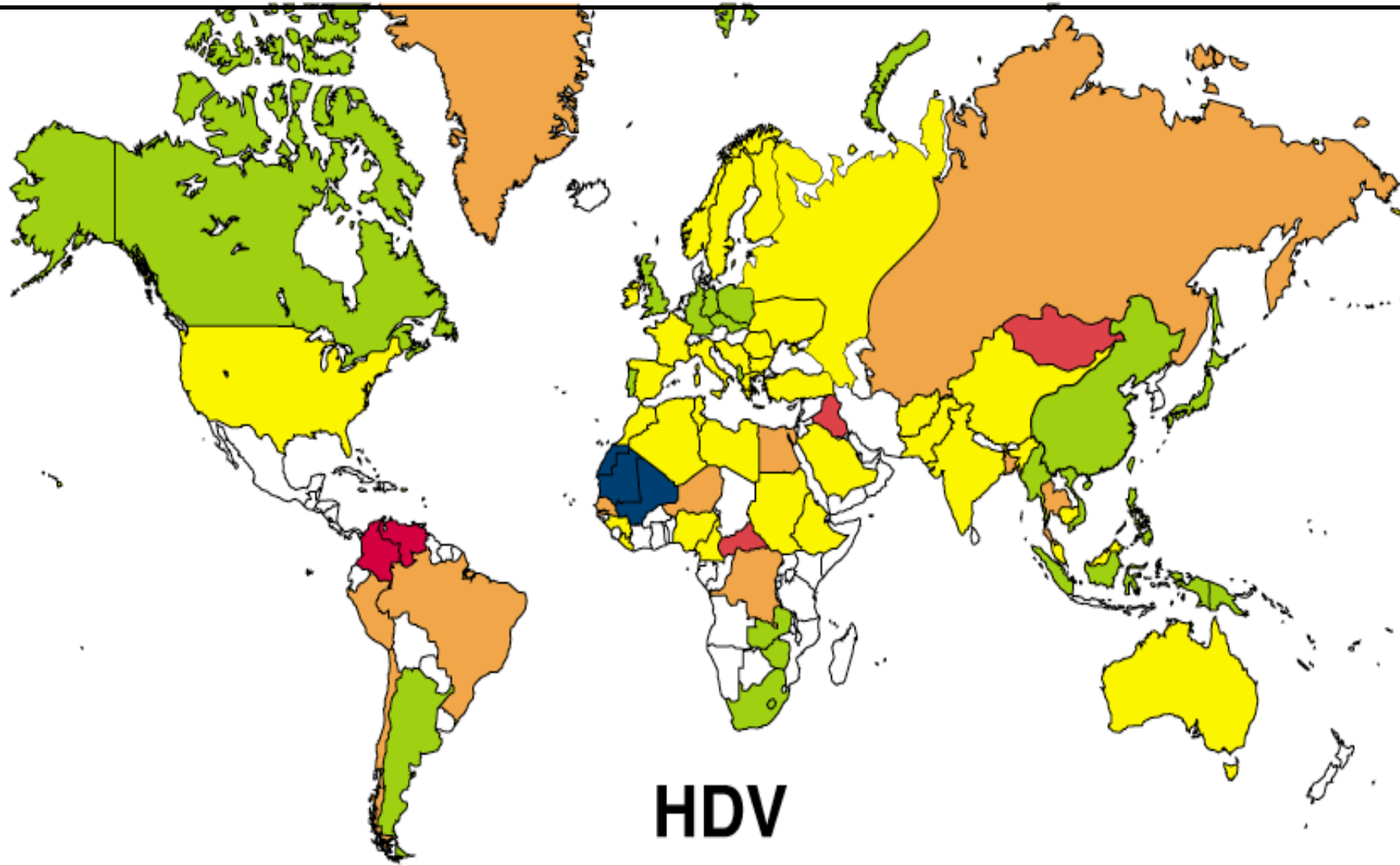


Anti-transcriptional effect

Competition envelope

Cytokines (MxA ?)

1980s: Global Anti-HDV Prevalence in HBsAg Carriers (15,000,000 Positive)

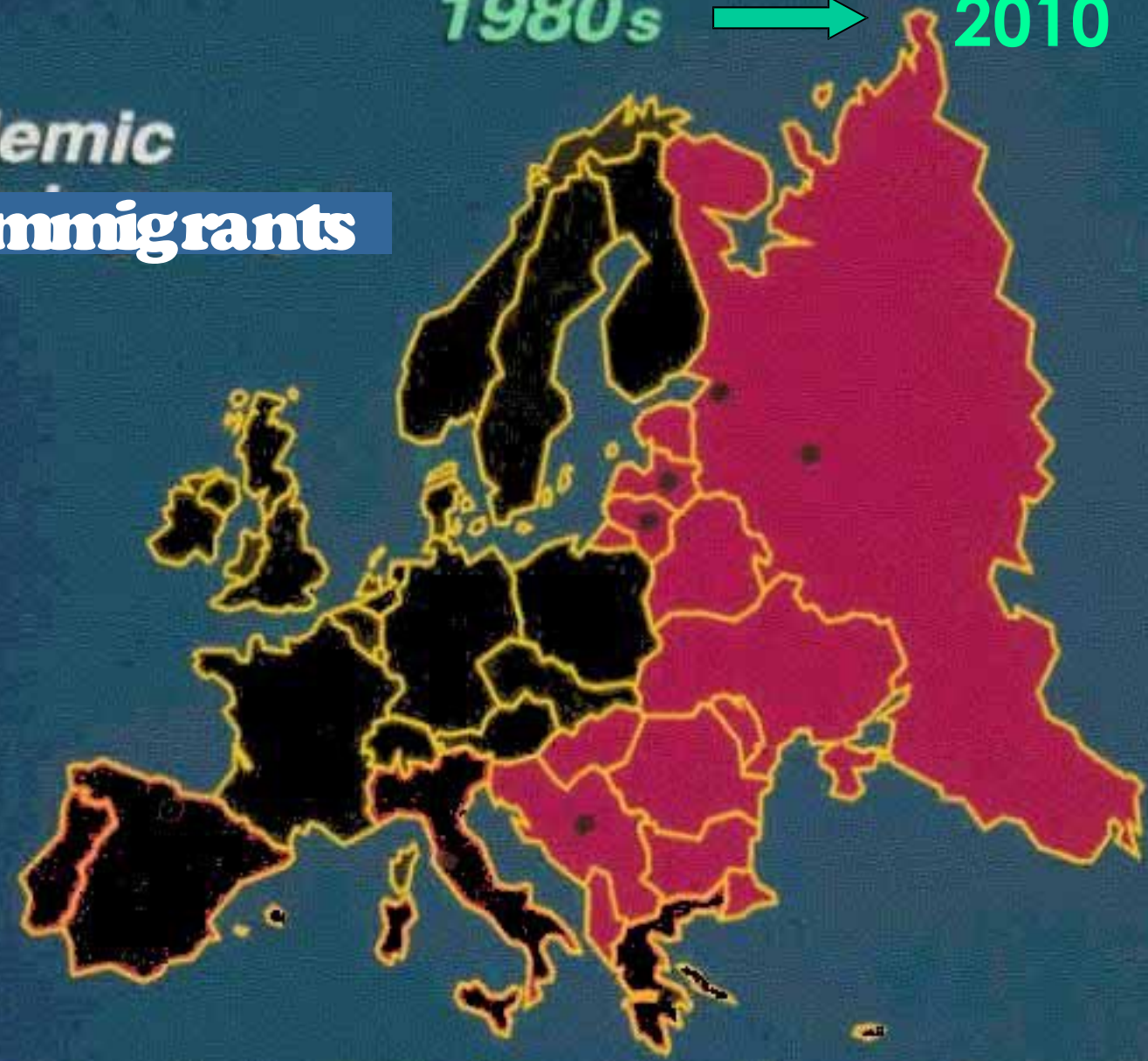


Anti-HD(HBsAg (+)) ? 0-5% 6-20% 21-60% >60%

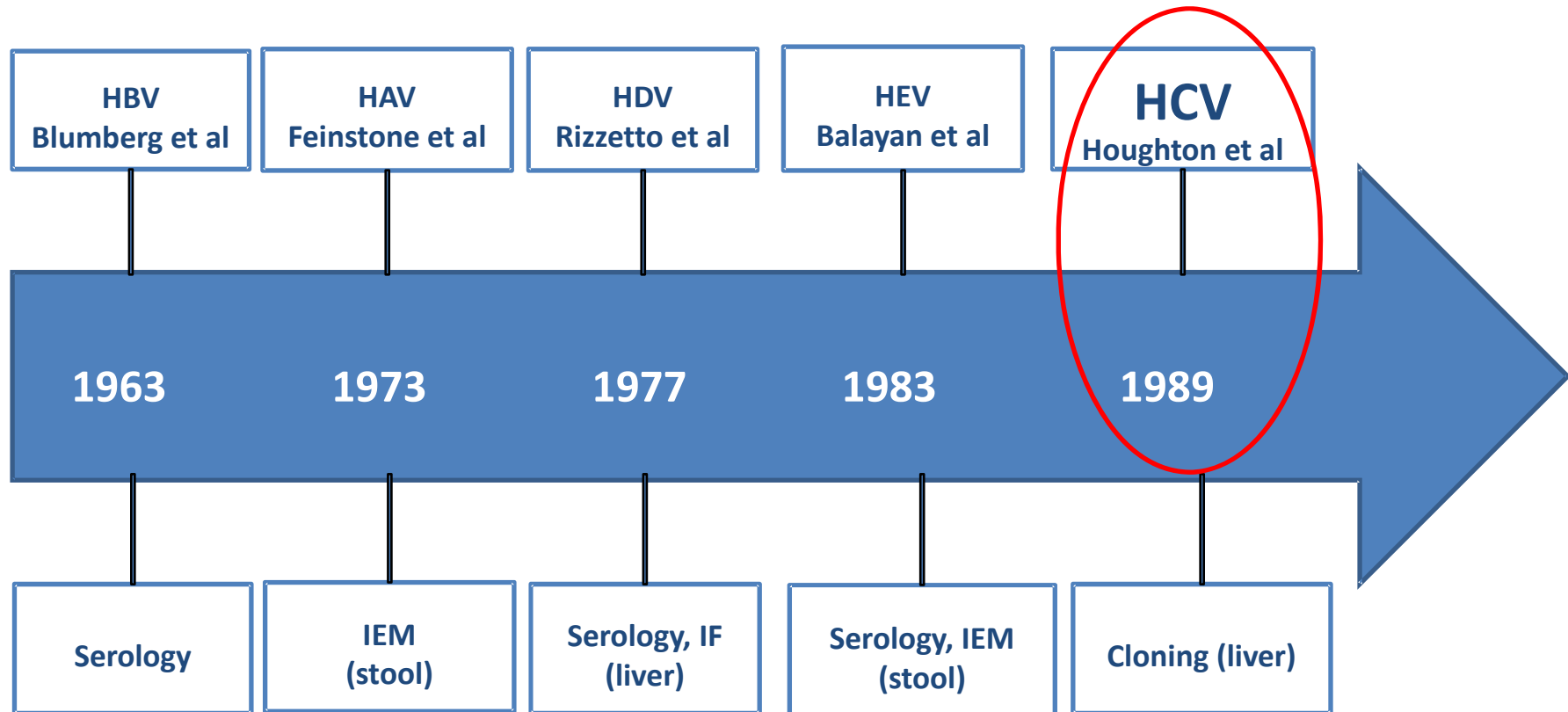
Epidemiology of HDV in Europe

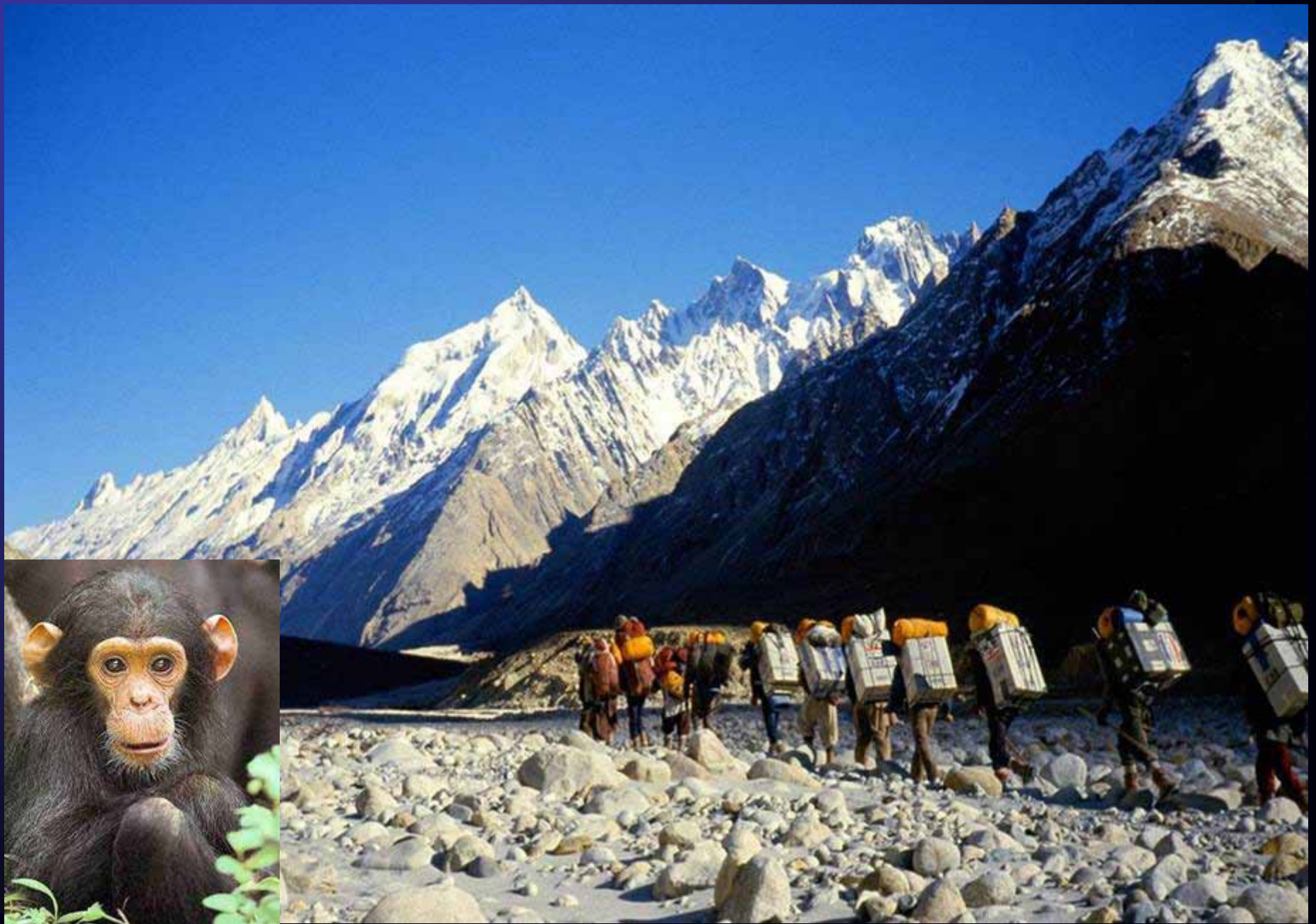
1980s → 2010

- Endemic
- In immigrants



HEPATITIS VIRUSES DISCOVERY





The approach

1. **1979** Tubule Forming Agent → Shimizu (NIH)
2. **1985** D. Bradley (CDC)
 - Togavirus
 - Flavivirus
 - HDV
3. **1987** <50 nm filter
4. Post-transfusion NANB hepatitis 1980-
incidence 7% in France → 10% USA



Dead End

- 1983 HDV like → Kamimura/Purcell
- 1984 Retrovirus → Seto/Gerety
- 1984 Spumavirus → Prince
- 1985-9 Non-A, non-B Ag/Ab systems
Shimizu & many others



The ascent

- **1st viral isolation**

- without culture
- without electronic microscopy
- without serology



Direct molecular approach

Since then:



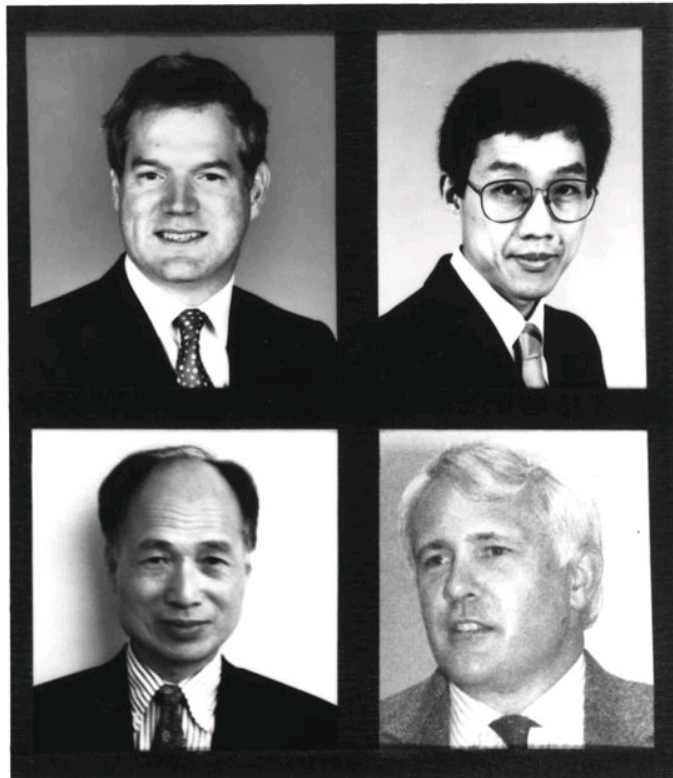
- HEV, HHV8
- TTV
- HGV

Discovery of the hepatitis C virus

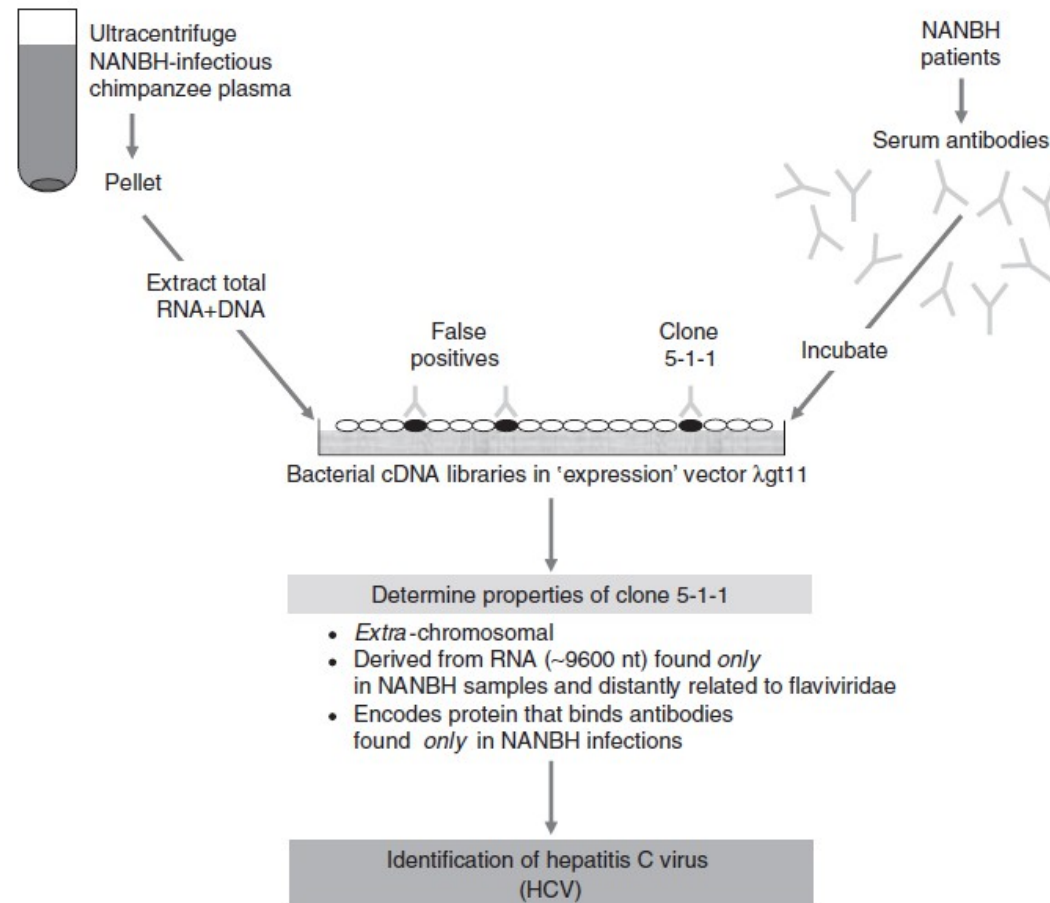


Isolation of a cDNA Clone Derived from a Blood-Borne Non-A, Non-B Viral Hepatitis Genome

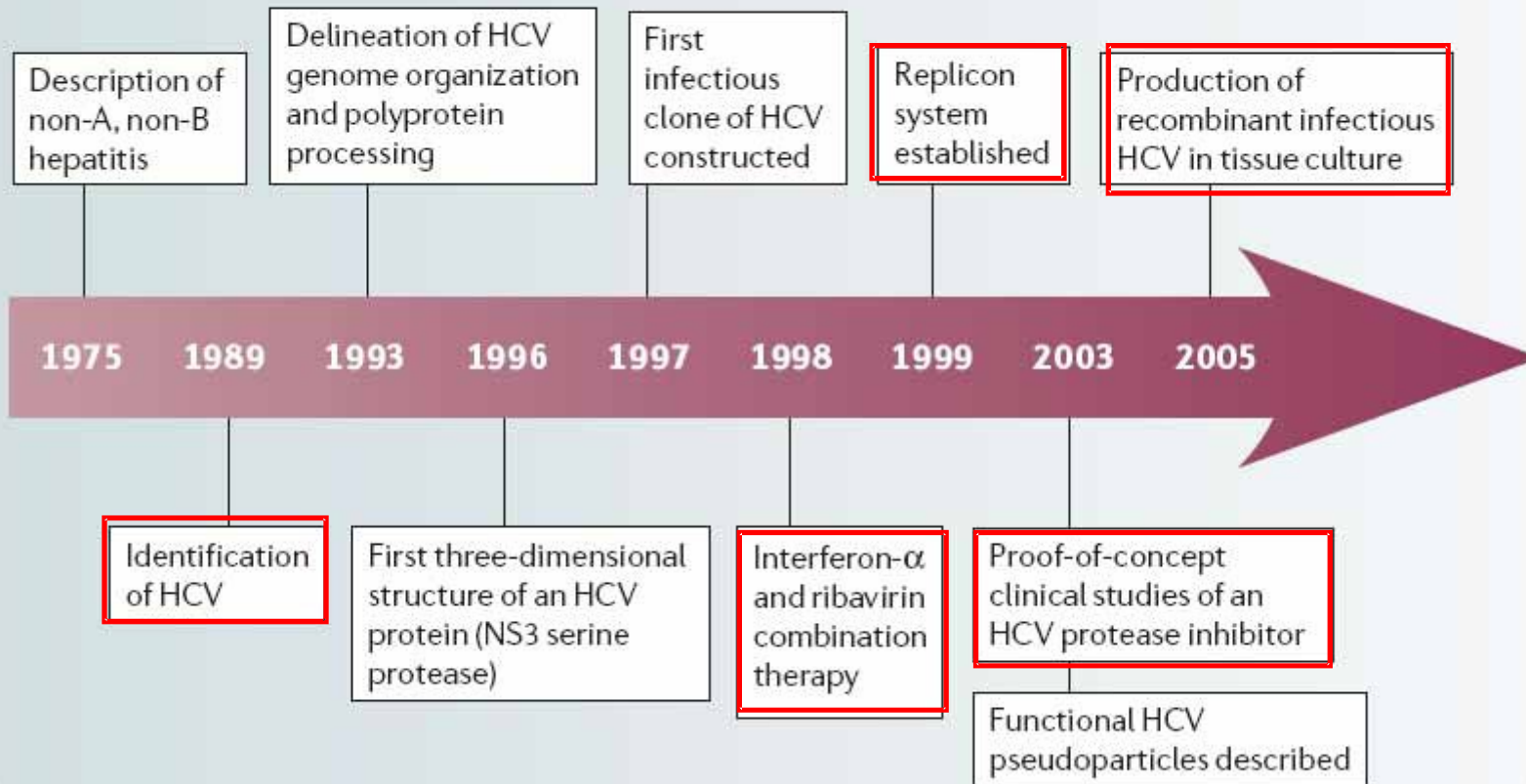
QUI-LIM CHOO, GEORGE KUO, AMY J. WEINER, LACY R. OVERBY,
DANIEL W. BRADLEY, MICHAEL HOUGHTON



The HCV discovery team
(from left to right; M. Houghton,
Q-L Choo, G. Kuo and D. Bradley)



Timeline | Milestones in hepatitis C virus (HCV) research





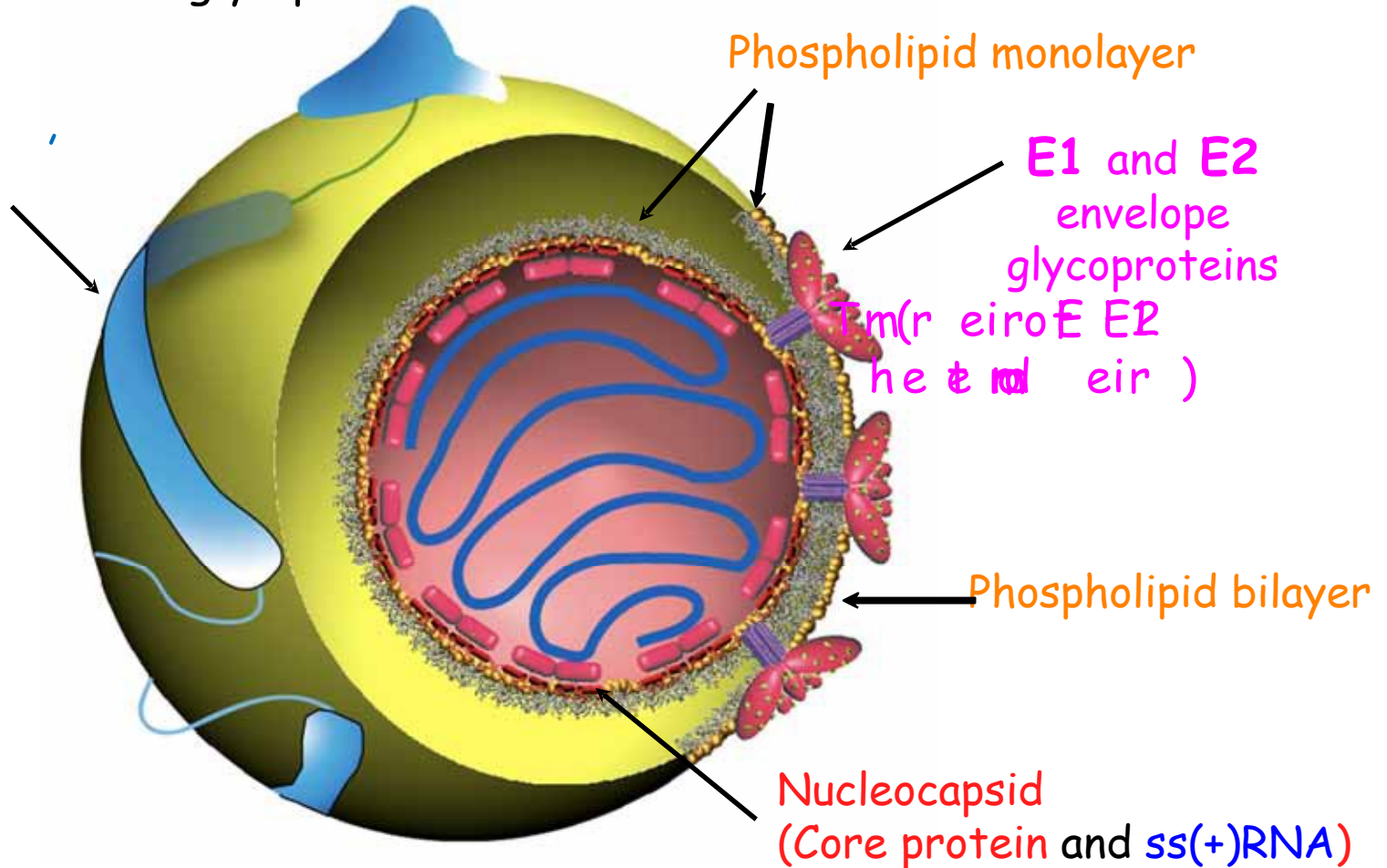
Temptative model of HCV "Lipo-Viro-Particle"



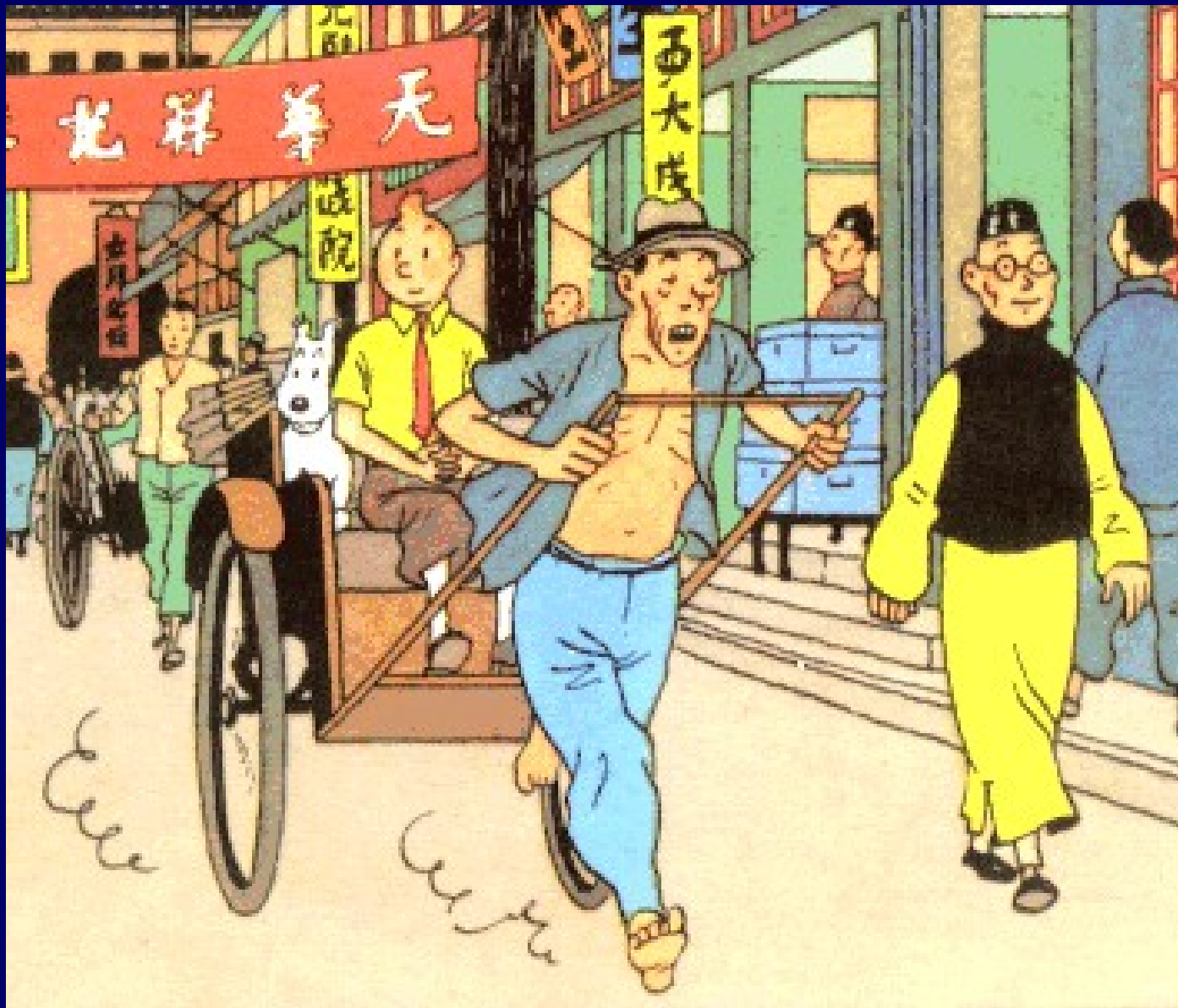
Francois PENIN & Patrice ANDRE

A tentative model of HCV assembly
VLP: HCV nucleocapsid and ER envelope
glycoproteins

A phospholipid bilayer
and other proteins
(e.g. pB)



HISTORY OF HEPATITIS THERAPY



1981

DELETERIOUS EFFECT OF PREDNISOLONE IN HBsAg-POSITIVE CHRONIC ACTIVE HEPATITIS

KUI CHUN LAM, M.B., B.S., CHING LUNG LAI, M.B., B.S., R. P. NG, M.B., B.S., CHRISTIAN TREPO, M.D.,
AND P. C. WU, M.B., B.S.

380

THE NEW ENGLAND JOURNAL OF MEDICINE

Feb. 12, 1981

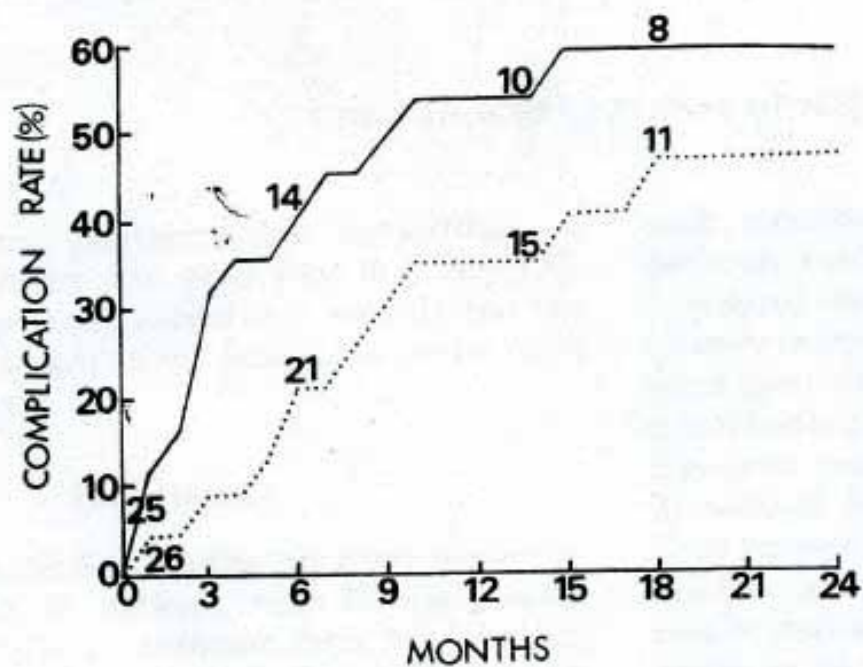


Figure 5. Cumulative Rate of Complications in 51 Patients with HBsAg-Positive Chronic Active Hepatitis Who Were Receiving Prednisolone (Solid Line) or Placebo (Dotted Line). Figures above each line denote the numbers of patients remaining in the study. Prednisolone increased the rate of complications ($z = 1.6709$, $P < 0.0001$).

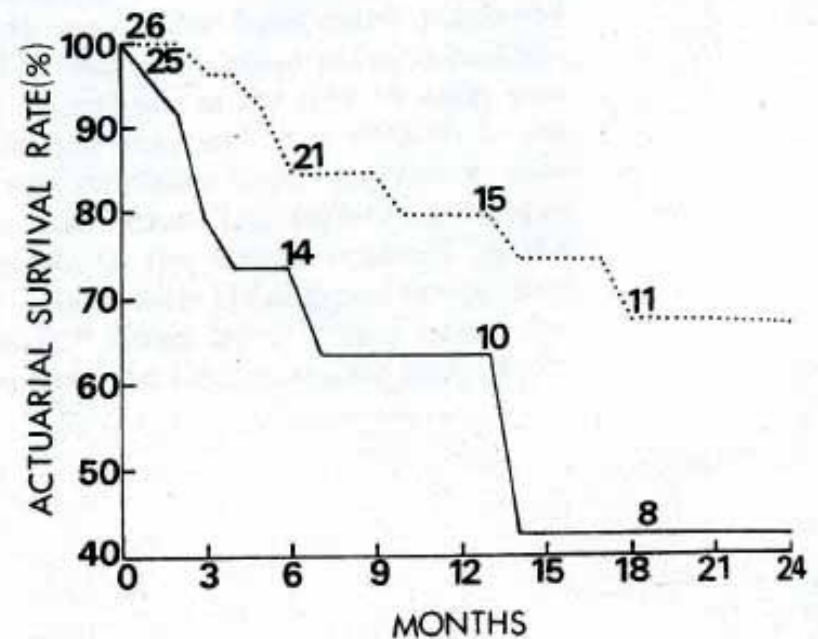


Figure 6. Actuarial Survival Rate in 51 Patients with HBsAg-Positive Chronic Active Hepatitis Who Were Receiving Prednisolone (Solid Line) or Placebo (Dotted Line). Figures above each line represent numbers of patients remaining in the study. The survival rate was decreased by prednisolone ($z = 0.5171$, $P < 0.01$).

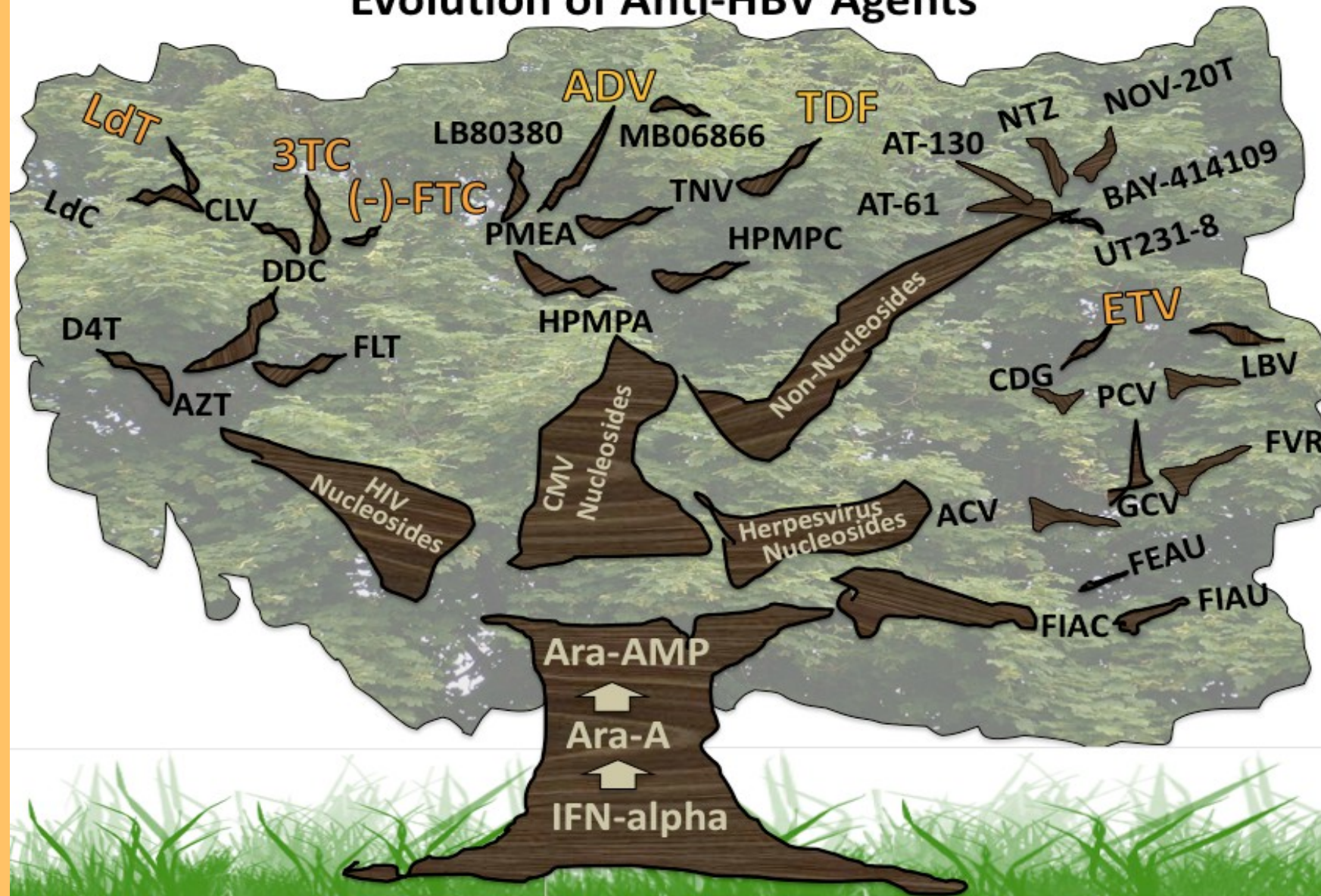
Chronic Hepatitis

- **1955** - From steroids to abstinence
- **1976** – The Anti-Viral Era: “The Prophets”
 - Aciclovir and herpes
- **1981** - Interferons
 - Alpha
 - Beta
- **2004** - PEG IFNs

Treatment with Oral Nucleos(t)ides

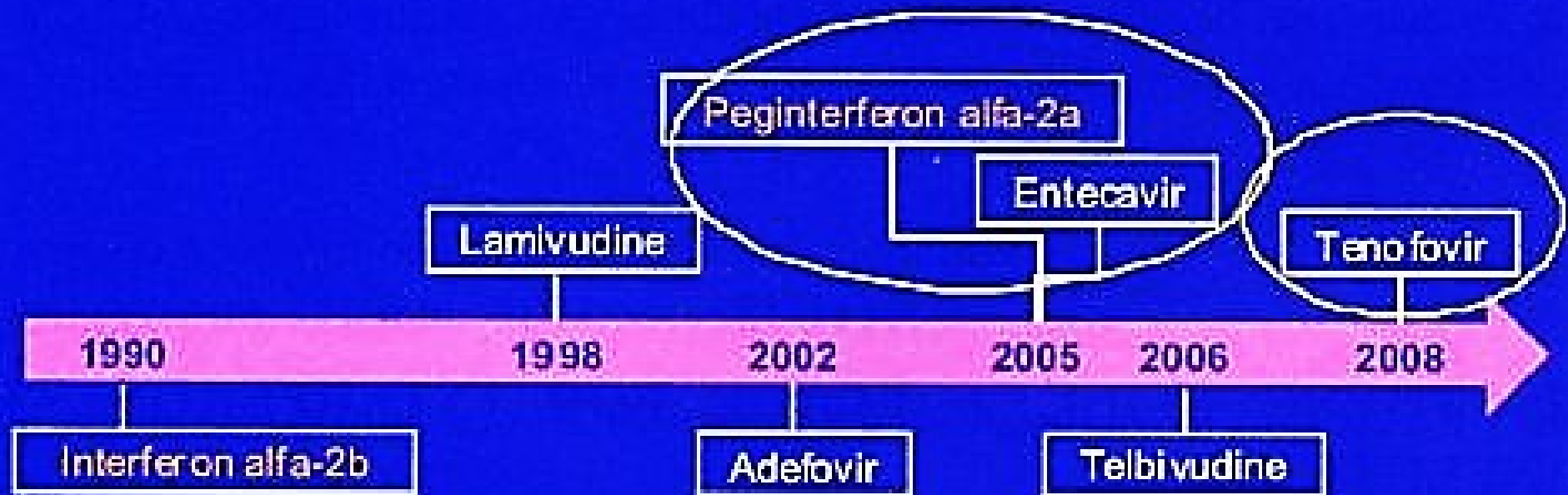
- 1978 Vidarabine
 - 1979 Foscarnet
 - 1985 Ganciclovir
 - -----12 years-----
 - 1997 Lamivudine
 - 2003 Adefovir
 - 2006 Entecavir/Telbivudine
 - 2008 Tenofovir
-

Evolution of Anti-HBV Agents



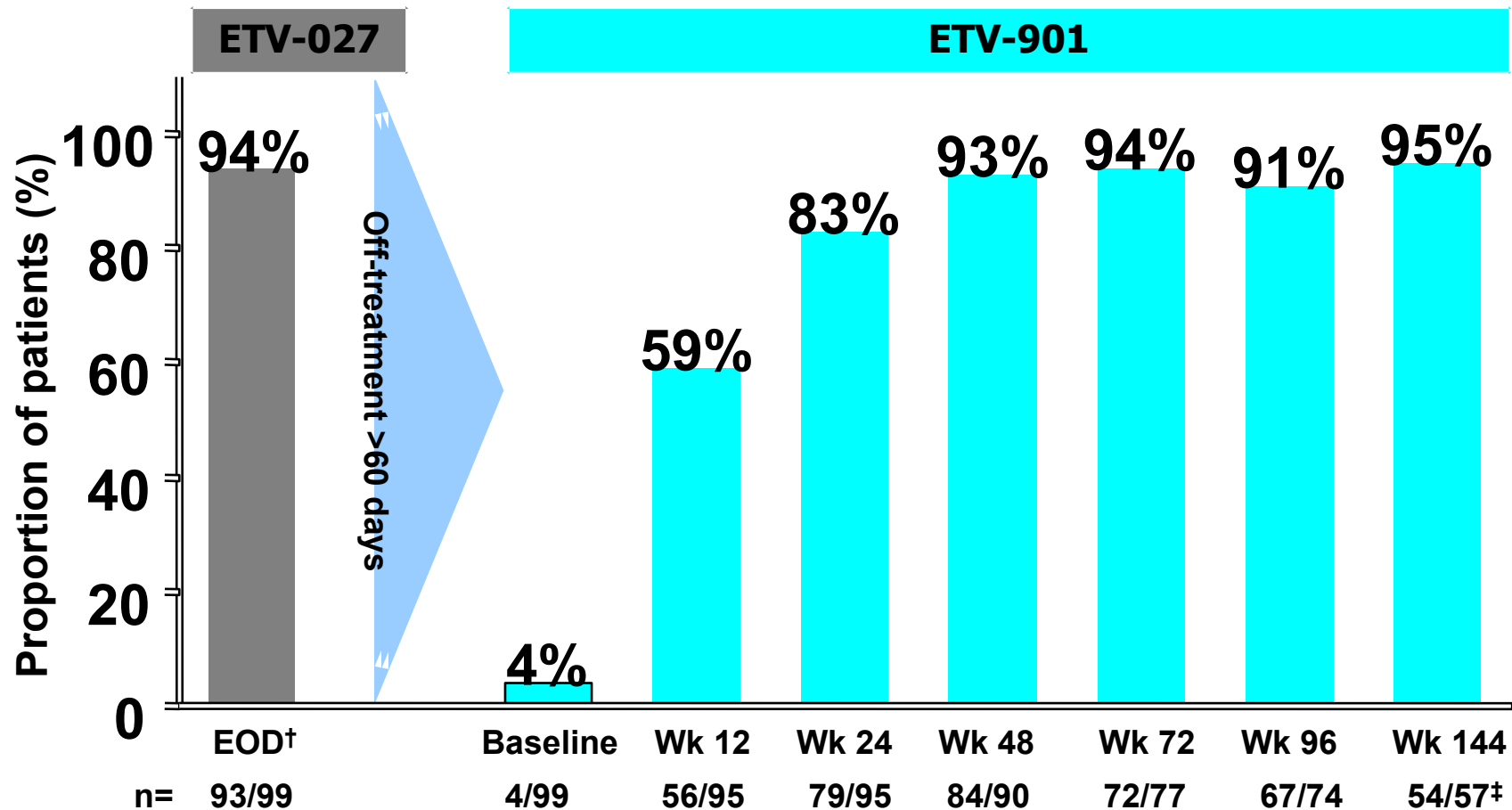
Raymond SCHINAZI

HBV Treatment Landscape in 2012



SUSTAINED BENEFIT

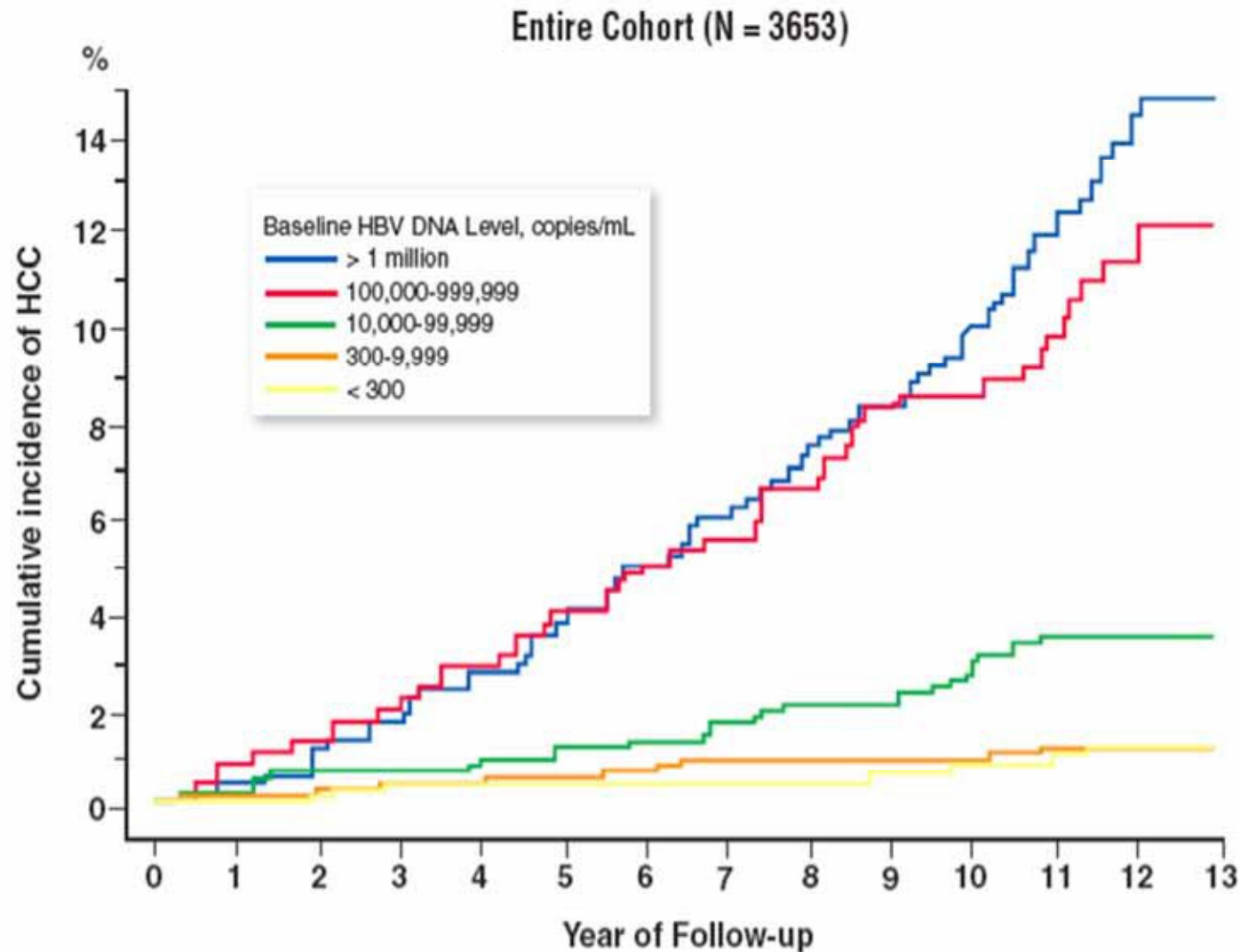
Entecavir in HBeAg negative patients: Proportion of Patients with HBV DNA <300 copies/mL



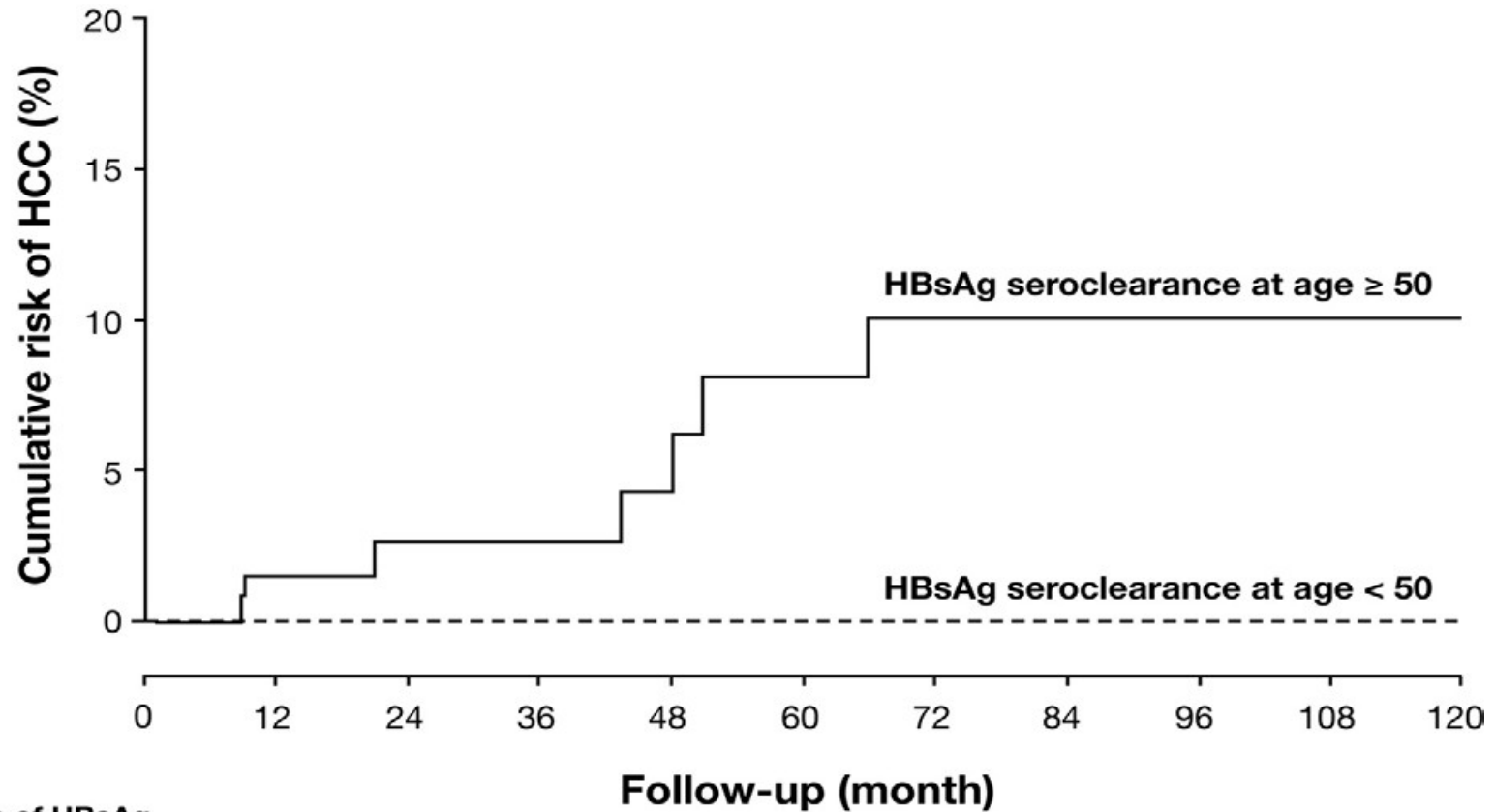
† EOD= end-of-dosing

‡ 10 patients who remained on treatment at the Week 144 of ETV-901 visit had missing PCR samples

VIRAL LOAD PREDICTS OUTCOME



Cumulative Risk for HCC



		Age of HBsAg seroclearance											
		< 50	12	24	36	48	60	72	84	96	108	120	
No. of patients at risk	< 50	151	124	102	87	71	56	47	37	21	15	10	
	≥ 50	147	120	86	63	51	46	38	31	24	18	12	

Paradigm shift

1) Break of tolerance

- Disease



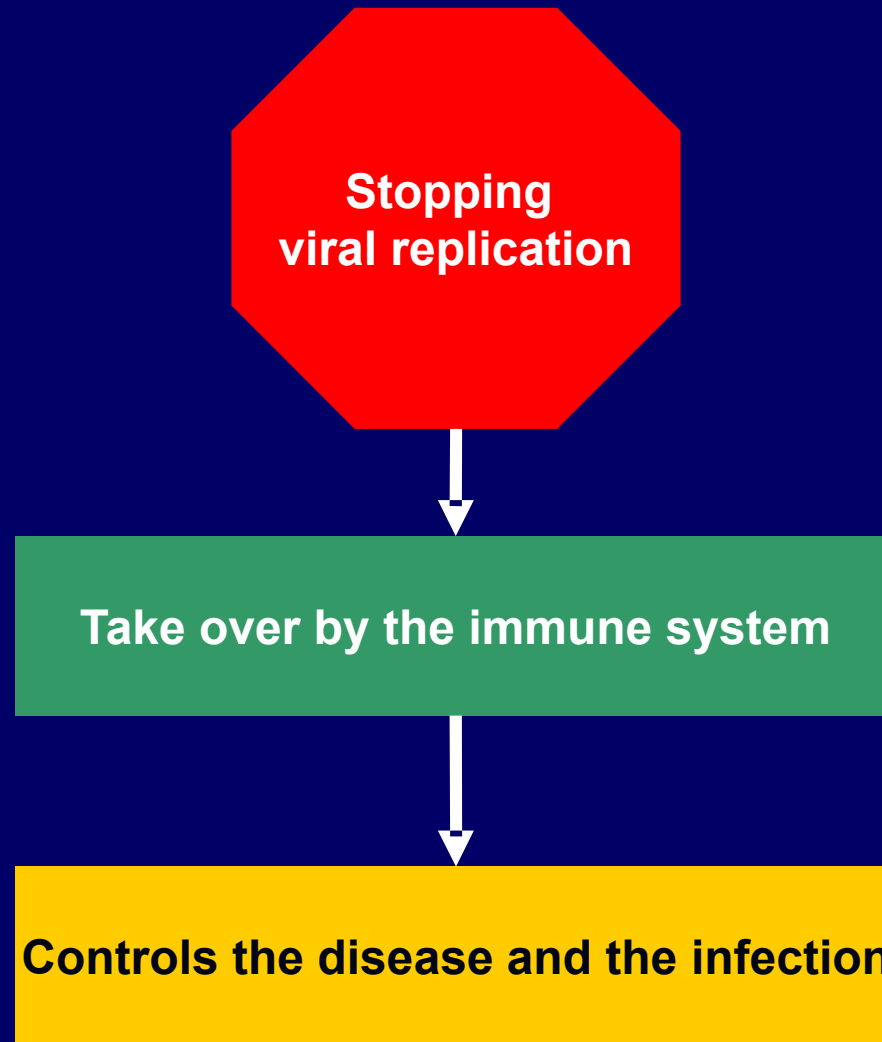
2) Replication is the driving force of complications

- Anticipate
- Prevention >> cure

BEYOND VIROSUPPRESSION :

**Can we achieve HBsAg clearance,
thereby preventing disease
progression and resistance?**

PAN – Proof of Concept for Cure

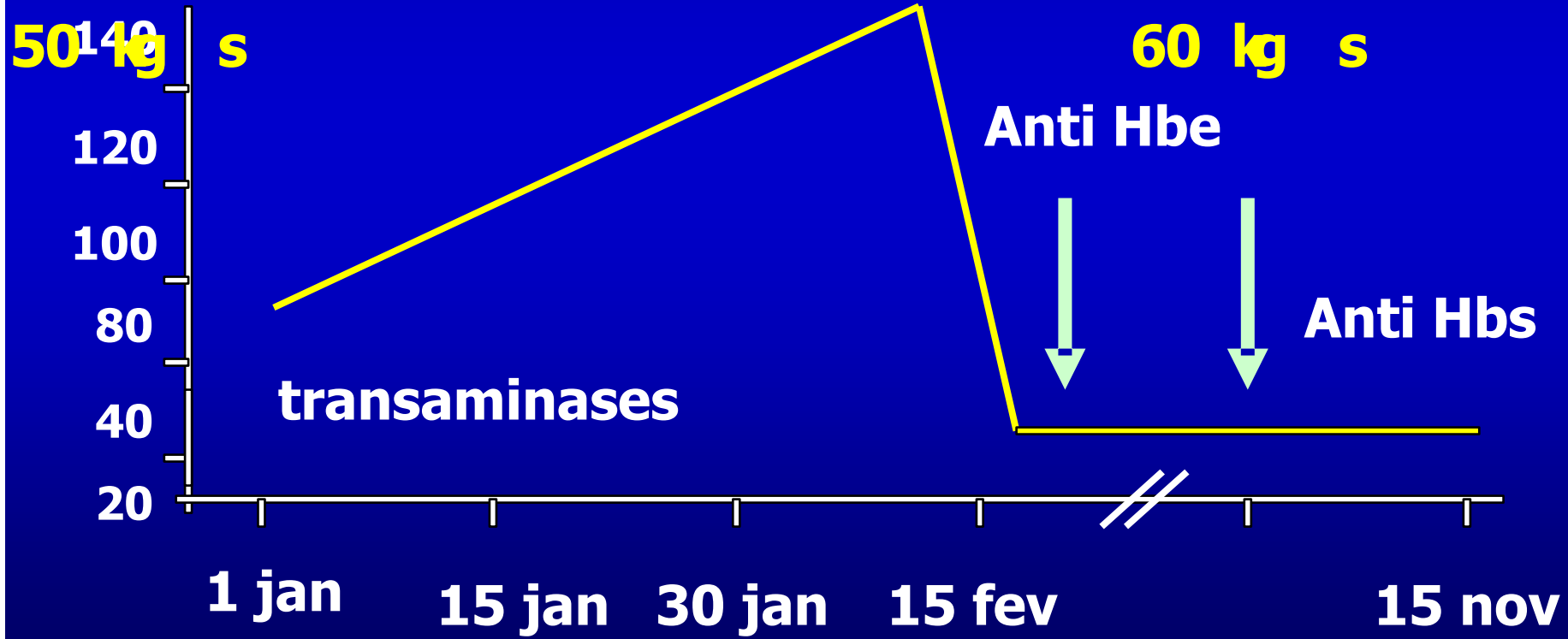
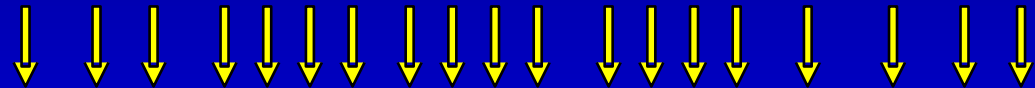


HBV-RELATED POLYARTERITIS NODOSA

Mr Gu.,
19 ans

CS

vidarabine



Results from the PAN Treatment According to Antiviral Protocol

Protocol	Cure	Anti HBe	Anti HBs
Vidarabine - 1978 (3 weeks) +PE-cortic/sev	75% 25/3	45%	19%
Interferon - 1976 (6 months) +PE+cortic/sev	80% 11/14	64%	50%
Lamivudine - 1997	90%	80%	60%
Tenofovir - 2010	9/10	90%	80%

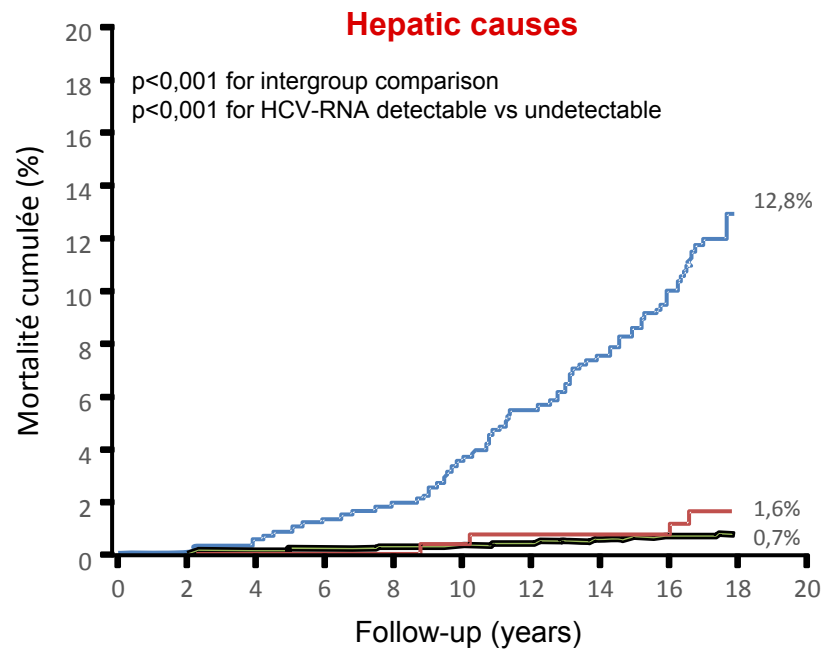
CHRONIC HEPATITIS C THERAPY

HCV infection and global mortality risk

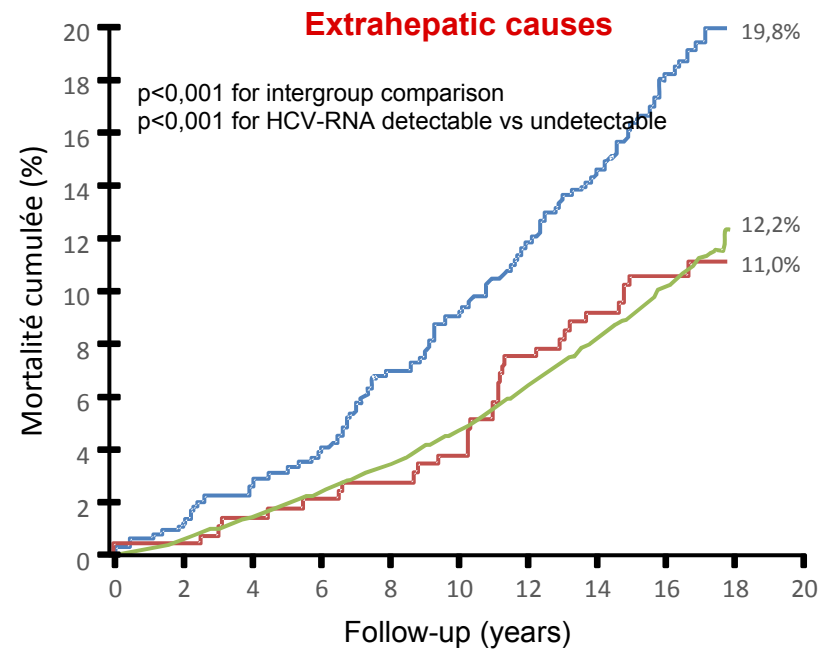
The REVEAL HCV Cohort Study

- 23 820 adults, Taiwan
- 1095 anti-HCV positive ; 69.4% HCV-RNA detectable

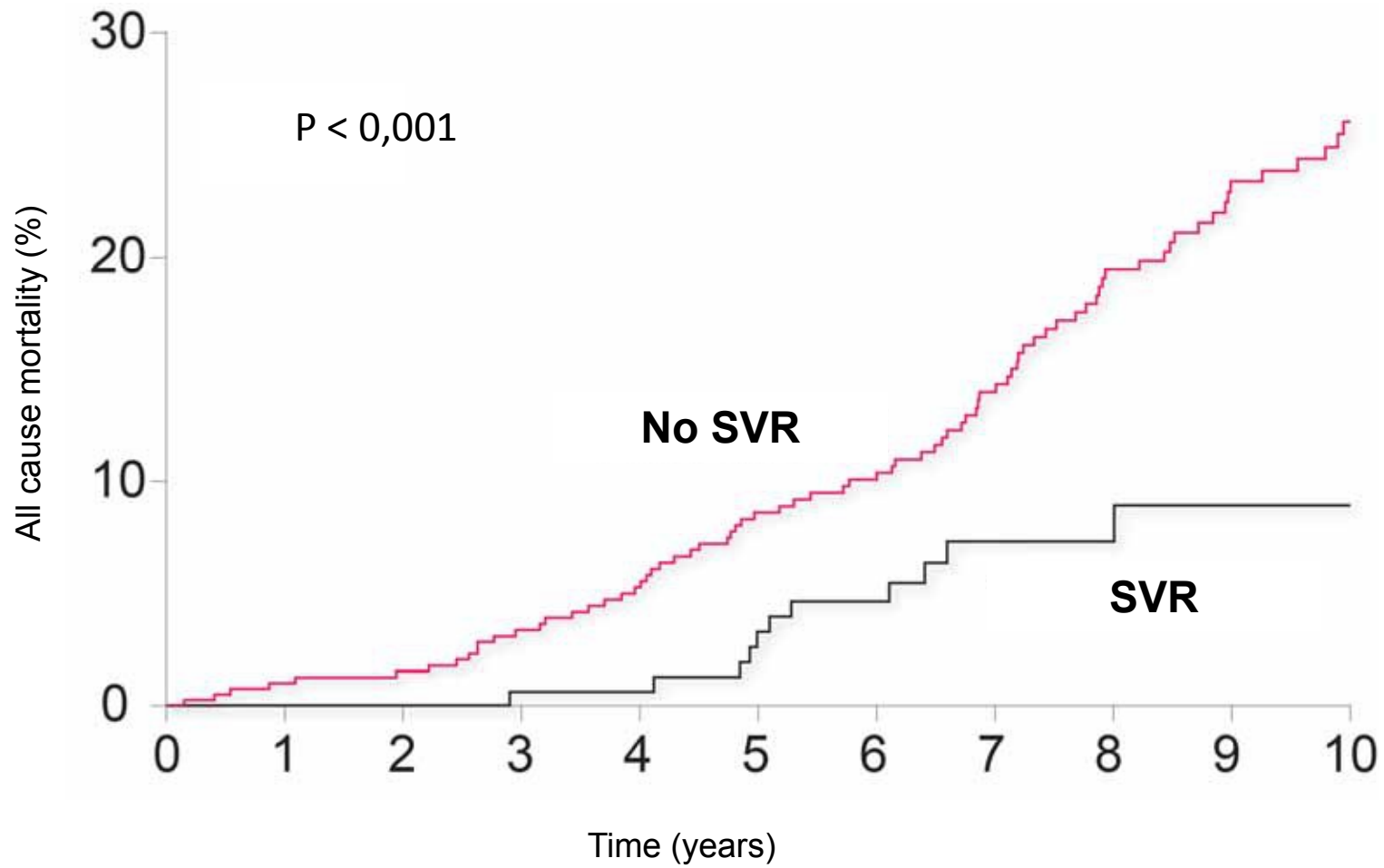
- Anti-HCV Ab positive, HCV-RNA detectable
- Anti-HCV Ab positive, HCV-RNA undetectable
- Anti-HCV Ab negative



Lee MH et al. *J Infect Dis* 2012;206:469-477



SVR improves global survival

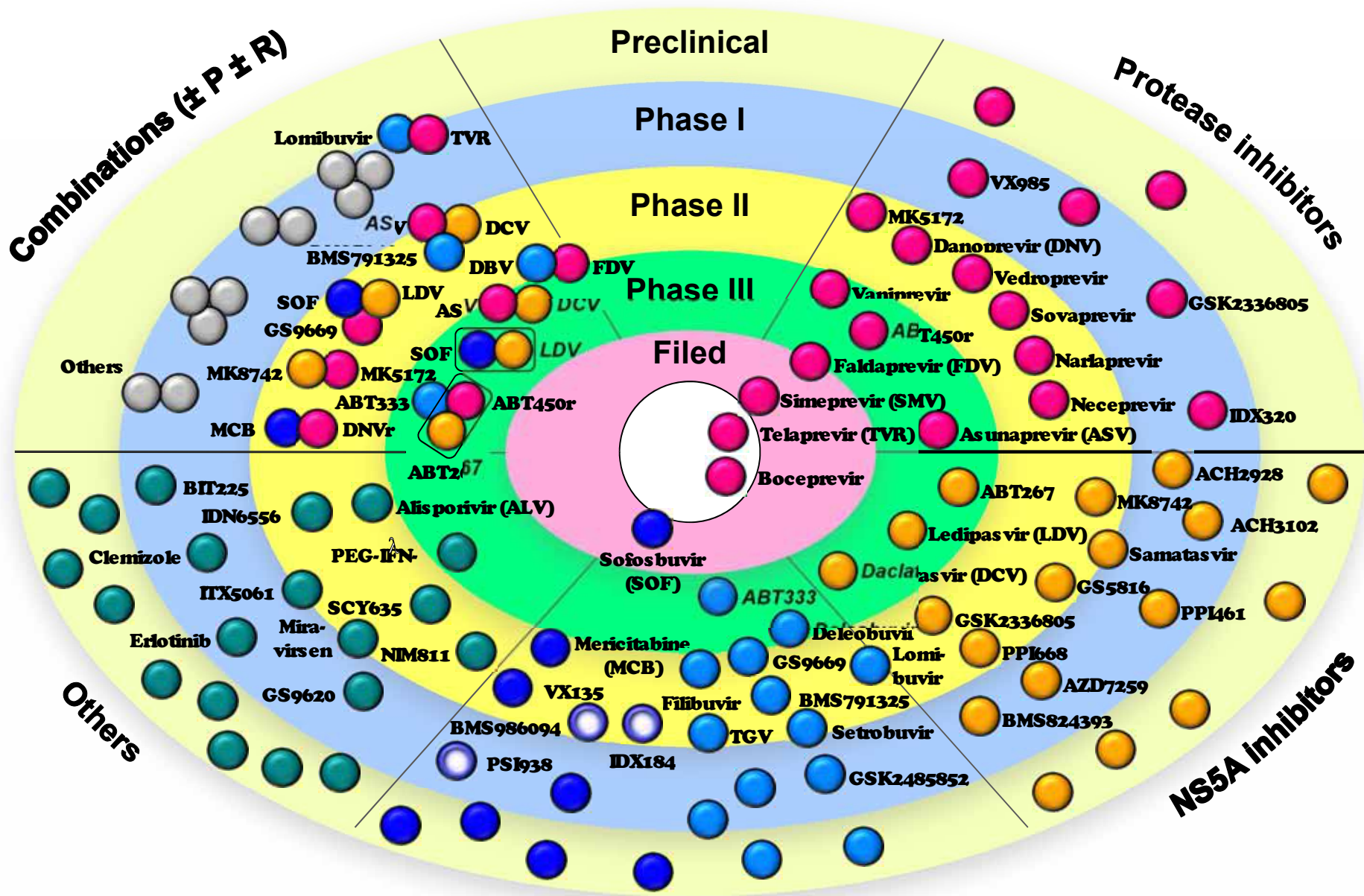


What is the Goal?

- Interferon-free combination therapy
- High barrier to antiviral resistance
- Once daily oral therapy
- Pan-genotypic antiviral activity
- Reasonable safety and minimal drug-drug interactions
- Short duration (12 weeks)
- SVR rates > 90%
- ... and affordable



The Hurricane of HCV Drug Development



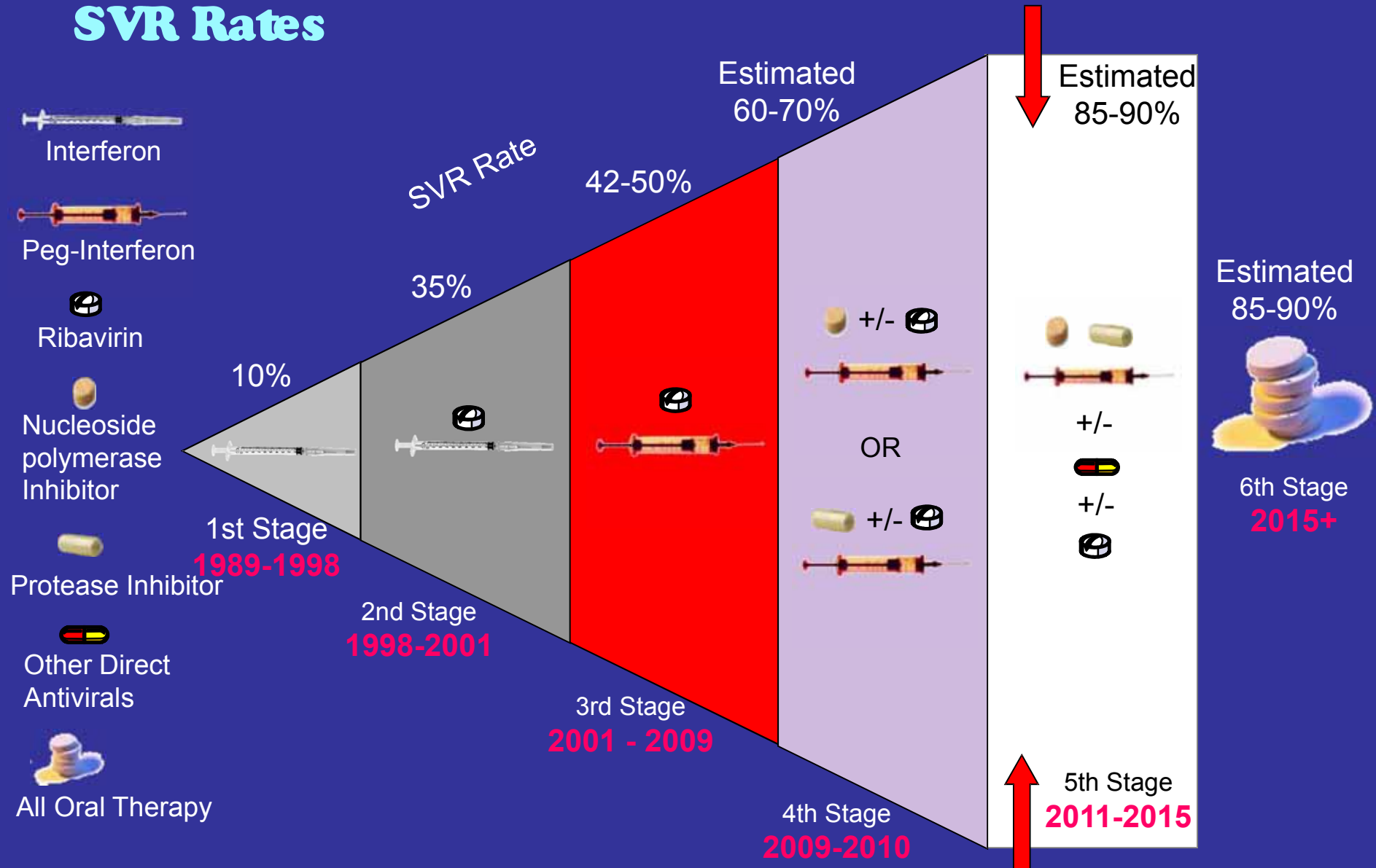
 Coformulation

Polymerase inhibitors

Status 11/2013 (selection)

Potential Evolution of HCV Therapy for GT 1

Small Molecules will be Added in an Effort to Improve SVR Rates



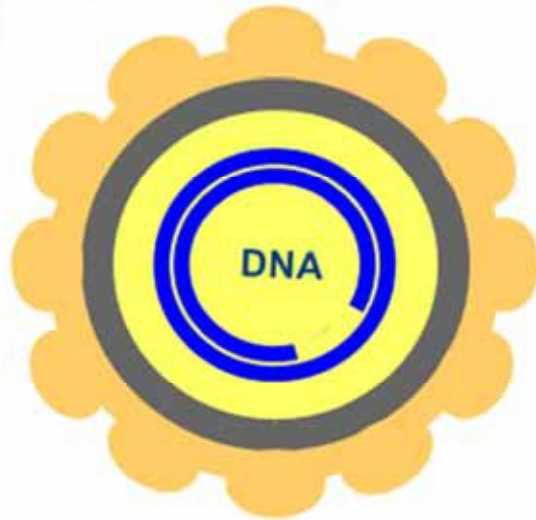


*One of the most beautiful
histories of medicine since
small pox, polio and...
tuberculosis*

**CONCLUSIONS
AND
PERSPECTIVES**

HISTORY OF CHRONIC VIRAL HEPATITIS

Major Advances in 50 Years: 1960s - 2013



HBV



HCV

- **Analytical tools**
- **Clinical aspects**
 - Epidemiology
 - Natural Course
 - Pathogenesis
 - Diagnosis
 - Therapy
 - Prevention
- **Virological aspects**
 - Structure and genetic organization
 - Life cycle, incl. receptors
- **Individualized hepatology**

« I Have a dream »

HCV

1. Predictive genetic tests

- fibrosis
- IFN response
- efficacy/tolerance of DAAs

2. Specific anti-HCV Ig

3. Prevention of HCC

- primary
- secondary

« I have a dream »

HBV

- 1) Chronic hepatitis B must become **priority** research
- 2) We should aim at **HBV CURE**
- 3) Since most HBV infected people live in resource limited countries new immunotherapeutic approaches including **therapeutic vaccine** are needed
- 4) Availability of a small primate macaque model may be most helpful.

Yes we can !

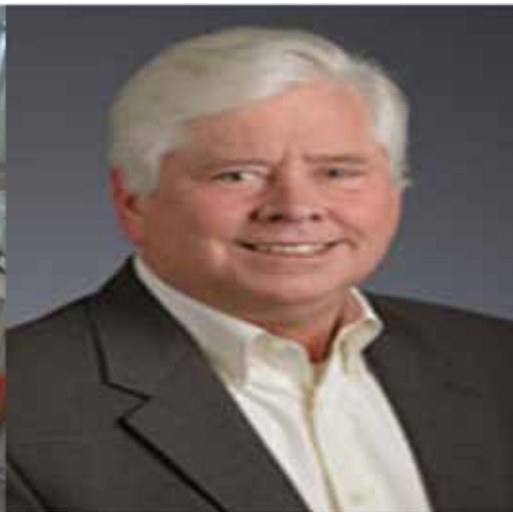
We should have a dream



Worldwide famous wizard

Marcellin P et al. Adefovir dipivoxil for the treatment of B e antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808-16

They paved the way



Acknowledgements



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Congress President



- Public Health
- Pathogenesis
- Prevention
- Diagnosis
- Treatment

The Global Viral Hepatitis Summit

15th International
Symposium on Viral Hepatitis
and Liver Disease