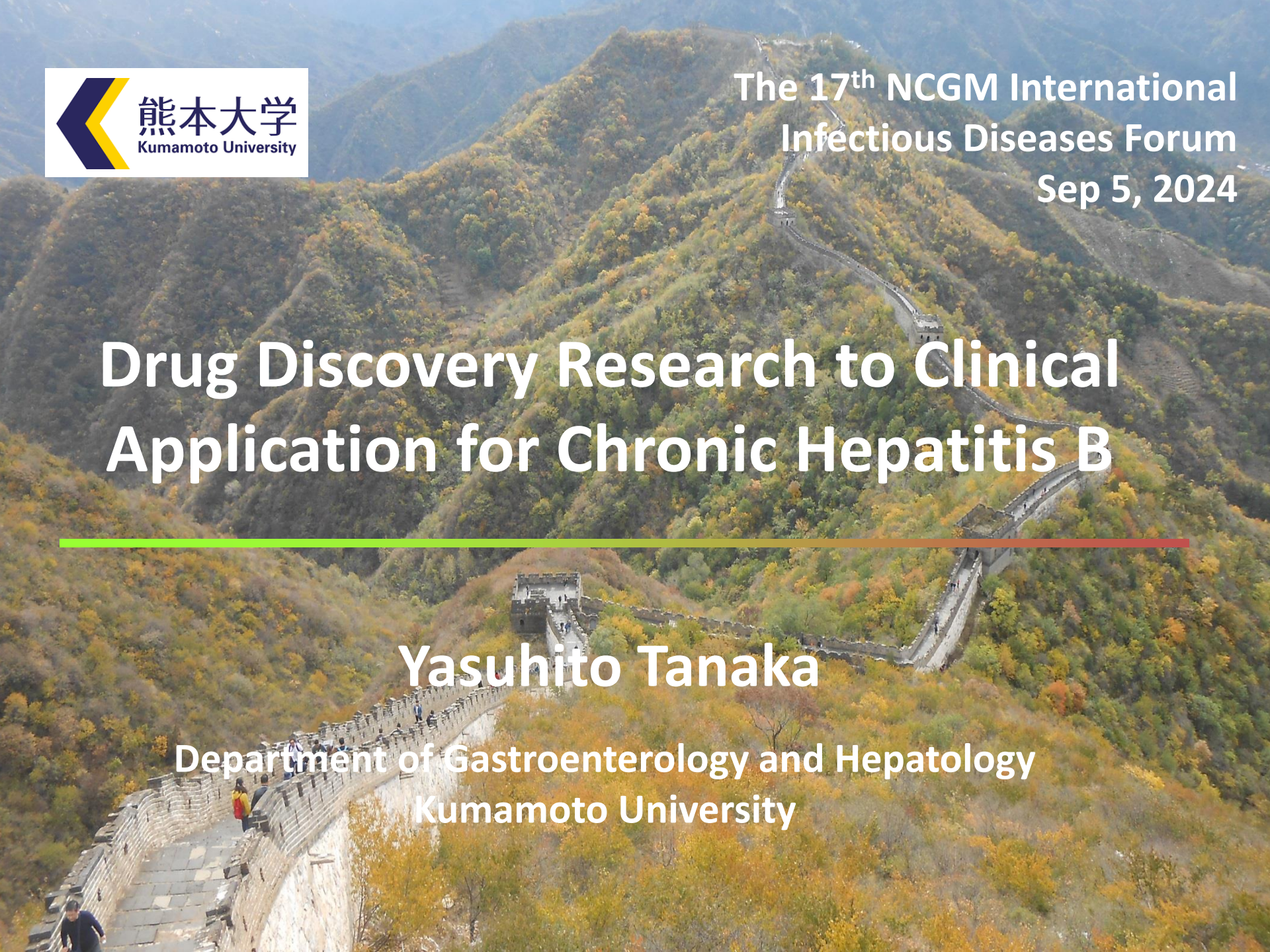


熊本大学
Kumamoto University

The 17th NCGM International Infectious Diseases Forum
Sep 5, 2024

Drug Discovery Research to Clinical Application for Chronic Hepatitis B

Yasuhito Tanaka

Department of Gastroenterology and Hepatology
Kumamoto University

COI Disclosure 2024

All Presenters： Yasuhito Tanaka
Affiliated Organizations： Department of Gastroenterology and Hepatology, Kumamoto University

COI Related to This Presentation

① Advisor： None
② Stock Ownership/Profits： None
③ Royalties： None
④ Lecture Fees：Yes (Fujirebio Inc, Gilead Sciences, AbbVie, ASKA , GlaxoSmithKline PLC, Otsuka, Takeda, AstraZeneca, Eisai)
⑤ Manuscript Fees： None
⑥ Consigned/Joint Research Expenses： Yes (Gilead Sciences, AbbVie, GlaxoSmithKline, Fujirebio Inc, Sysmex, Janssen Pharmaceutical K.K.)
⑦ Scholarship Donations：Yes (AbbVie GK., Otsuka Pharmaceutical Co., Ltd)
⑧ Course Affiliation： None
⑨ Gifts & Other Remuneration： None

Introduction

熊本 くまモン

Nagoya

1991 ~ 1999

2001 ~ 2009

2009 ~ 2020

Professor, Nagoya City University

Kumamoto

2020 ~ present

Director and Professor,

Department of Gastroenterology and Hepatology,

Kumamoto University

Prof. Ueda

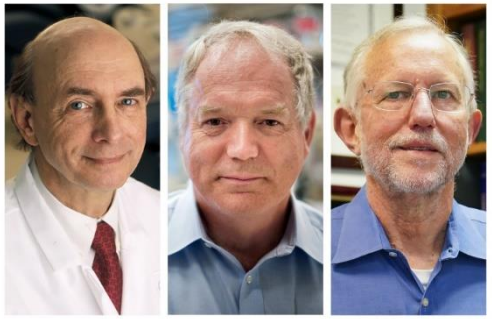
Prof. Mizokami

Study abroad

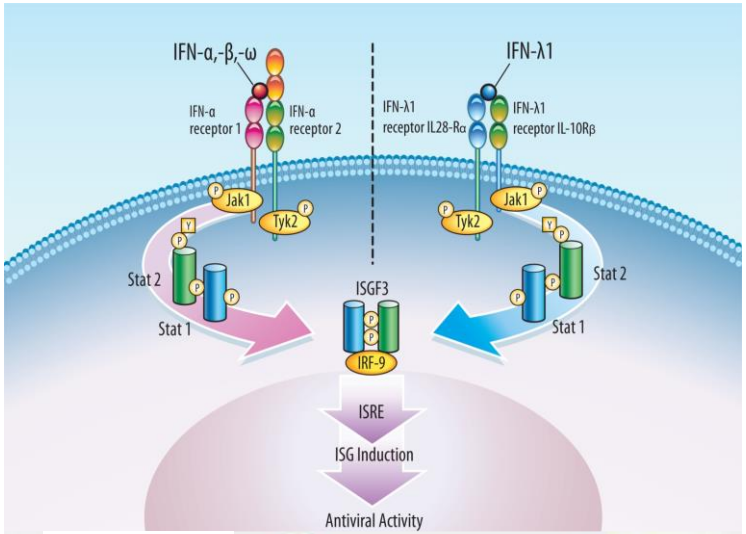
NIH (DC, USA)

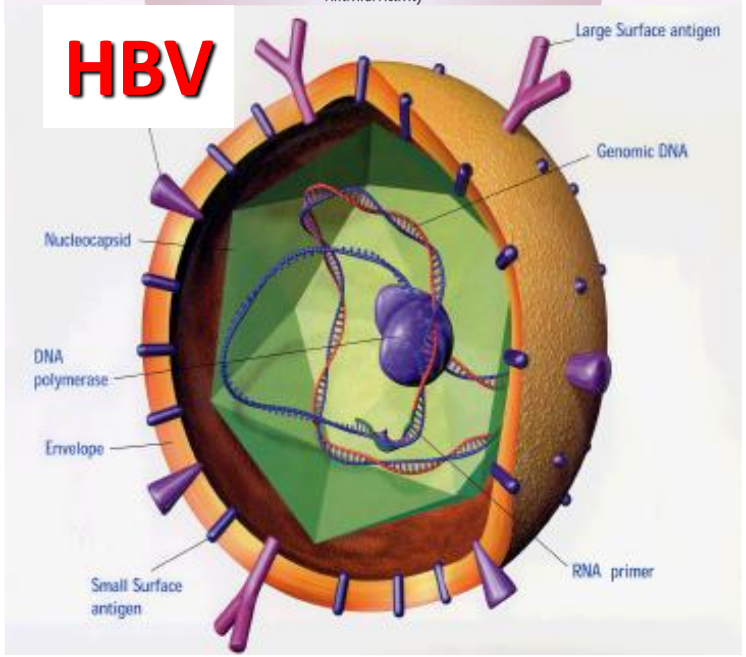
in 1999-2001

Nobel Prize 2020



Today's Topics





1. Current status of HBV infection worldwide
2. Current Guideline for HBV treatments
3. Development of novel drugs for HBV

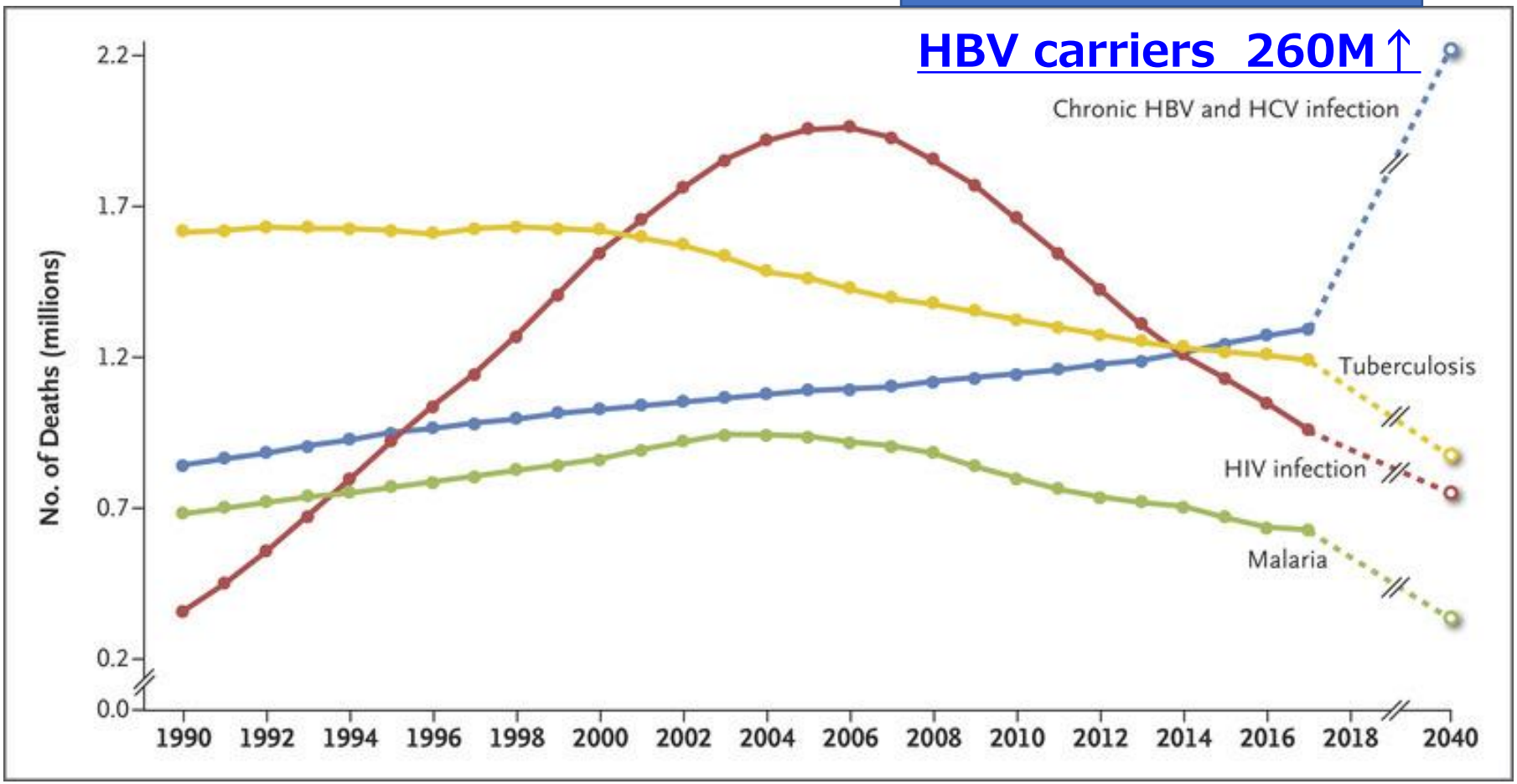
a) Antisense oligo: Bepi

b) HBV destabilizer: SAG-524

c) Inhibition of PD-L1

Worldwide Deaths from Chronic Viral Hepatitis as Compared with Deaths from Tuberculosis, Human Immunodeficiency Virus (HIV) Infection, and Malaria.

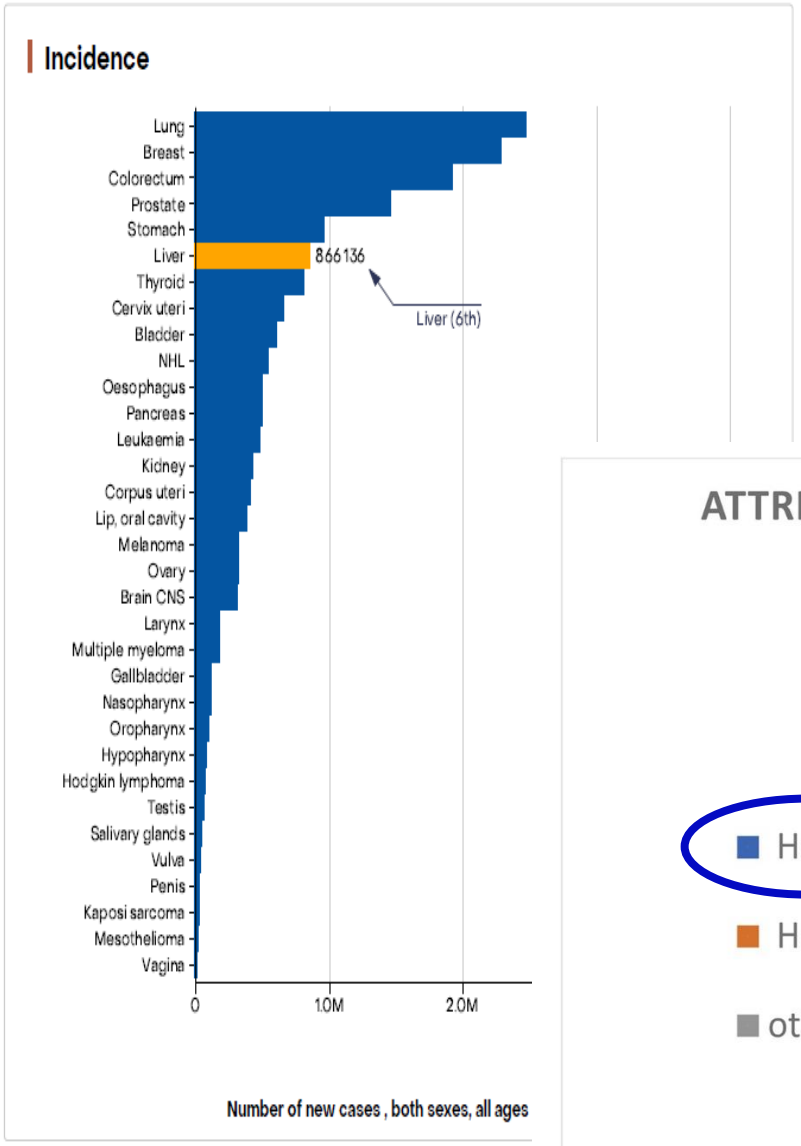
HBV increase

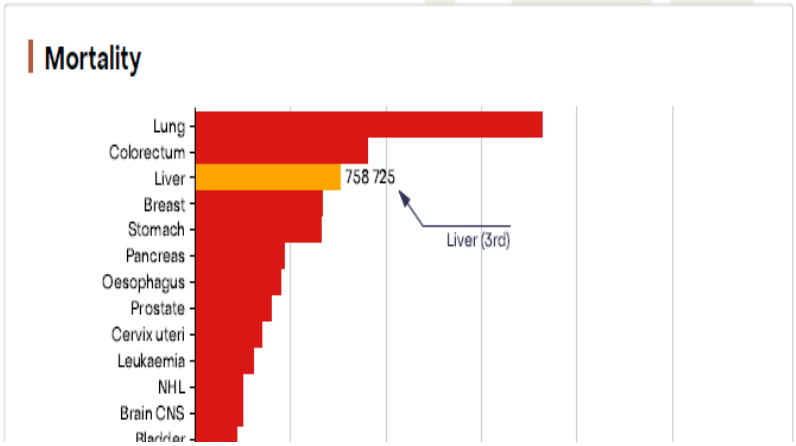


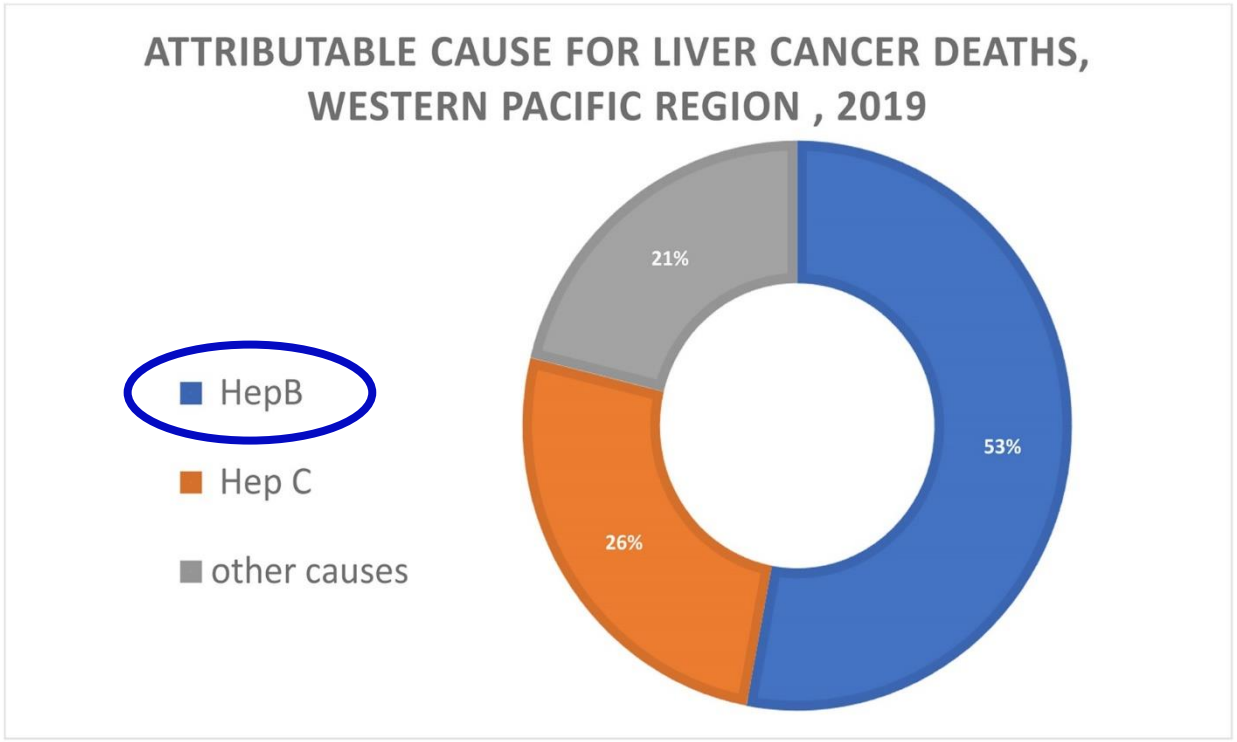
Thomas DL N Engl J Med. 2019

Liver Cancer Mortality: Worst 3

Cancer site ranking

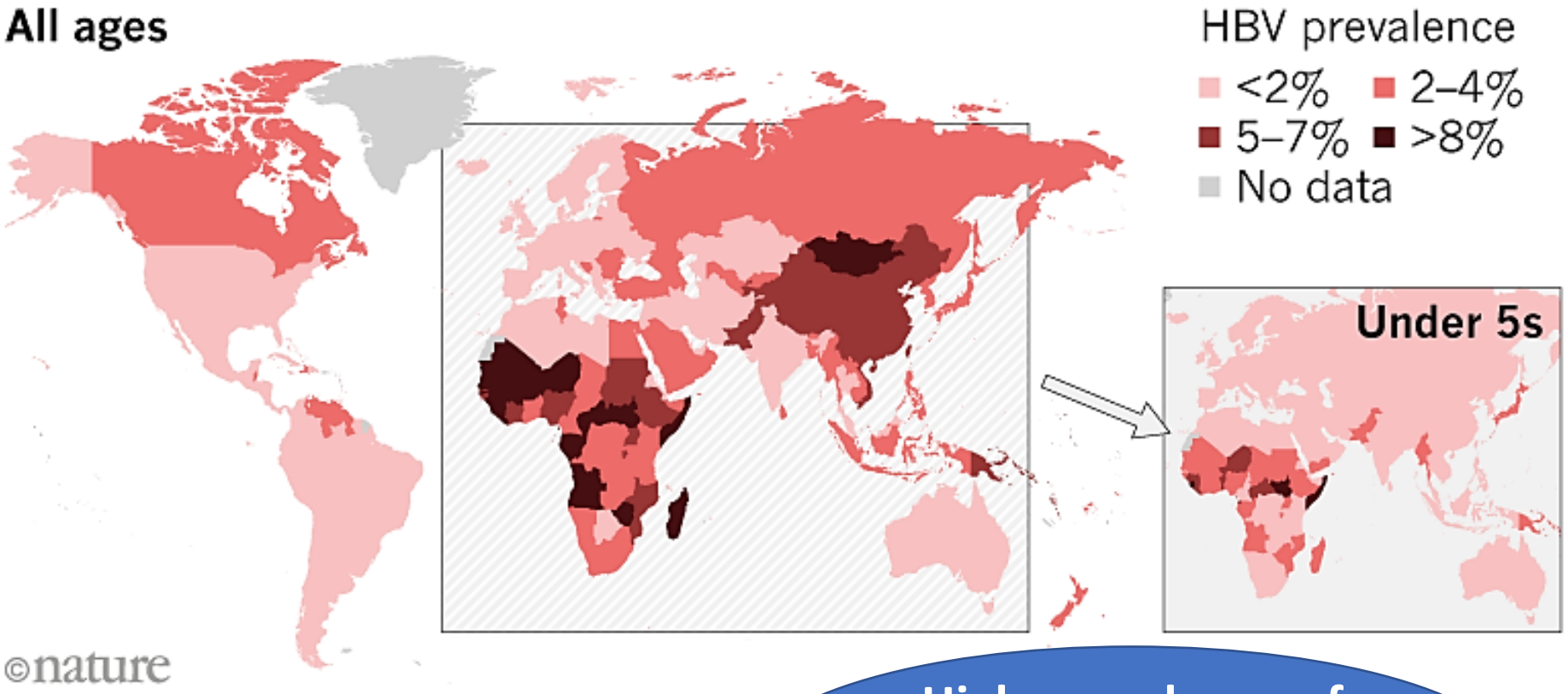






An unequal burden

The hepatitis B virus (HBV) is most prevalent in Africa and the Western Pacific, but in infants (inset), it is found mainly in Africa.



Source: WHO Hepatitis B dashboard
http://whohbsagdashboard.com/

High prevalence of HBV among children under 5 years old

Graber-Stiehl I, Nature. 564(7734):24-26, 2018.

Vaccination lag （海外データ）

Africa is the least-vaccinated region against hepatitis B; the Western Pacific, the most. Only one in ten infants in Africa are vaccinated at birth.

The NEW ENGLAND JOURNAL of MEDICINE

(TDF RCT)

ORIGINAL ARTICLE

Tenofovir to Prevent Hepatitis B Transmission in Mothers with High Viral Load

Calvin Q. Pan, M.D., Zhongping Duan, M.D., Erhei Dai, M.D., Shuqin Zhang, M.D., Guorong Han, M.D., Yuming Wang, M.D., Huaihong Zhang, M.D., Huaibin Zou, M.D., Baoshen Zhu, M.D., Wenjing Zhao, M.D., and Hongxiu Jiang, M.D., for the China Study Group for the Mother-to-Child Transmission of Hepatitis B*

TDF prophylaxis to prevent mother-to-child transmission

Graber-Stiehl I, Nature. 564(7734):24-26, 2018.

Special patient groups: pregnant women

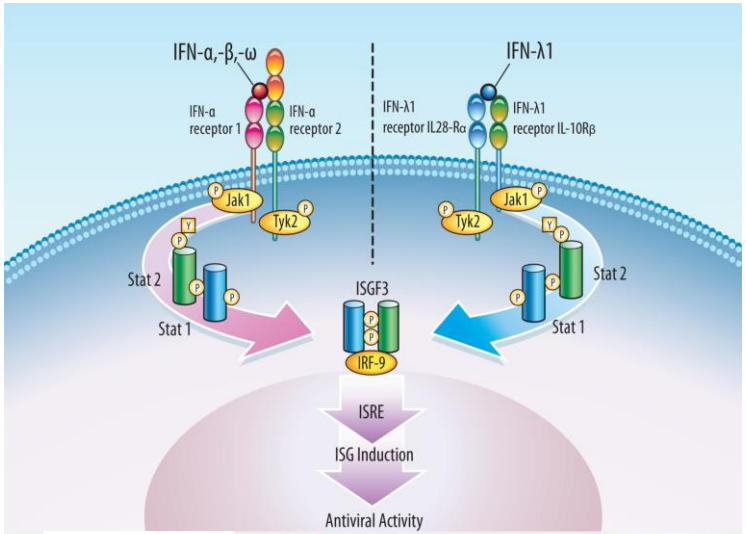
- Management may depend on severity of liver disease and timing of a future pregnancy

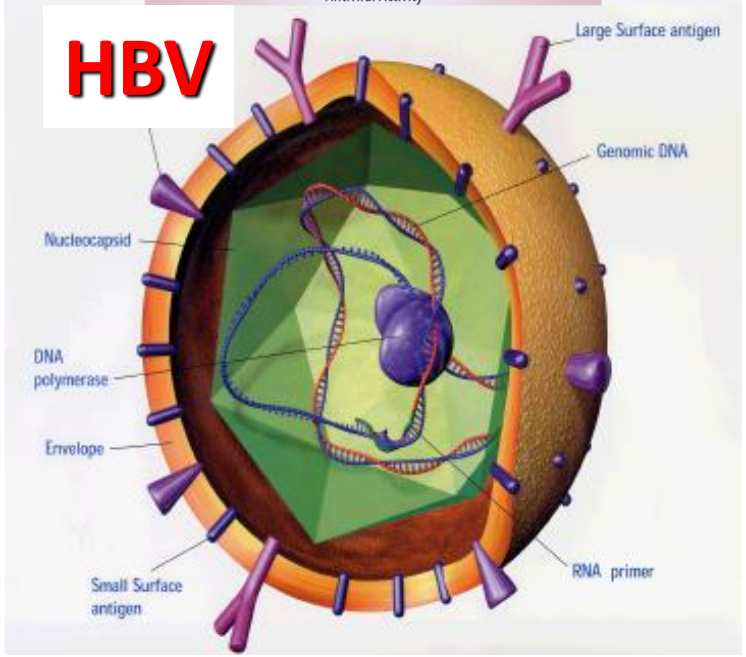
Recommendations	Grade of evidence	Grade of recommendation
Screening for HBsAg in the first trimester is strongly recommended	I	1
In women of childbearing age without advanced fibrosis planning a pregnancy in the near future, it may be prudent to delay therapy until the child is born	II-2	2
In pregnant women with chronic hepatitis B and advanced fibrosis or cirrhosis, therapy with TDF is recommended	II-2	1
In pregnant women already on NA therapy, TDF should be continued while ETV or other NA should be switched to TDF	II-2	1
In all pregnant women with HBV DNA >200,000 IU/ml or HBsAg >4 $\log_{10}$ IU/ml, antiviral prophylaxis with TDF should start at Week 24–28 of gestation and continue for up to 12 weeks after delivery	I	1
Breast feeding is not contraindicated in HBsAg-positive untreated women or those on TDF-based treatment or prophylaxis	III	2

EASL™ The Home of Hepatology

EASL CPG HBV. J Hepatol 2017;67:370–98

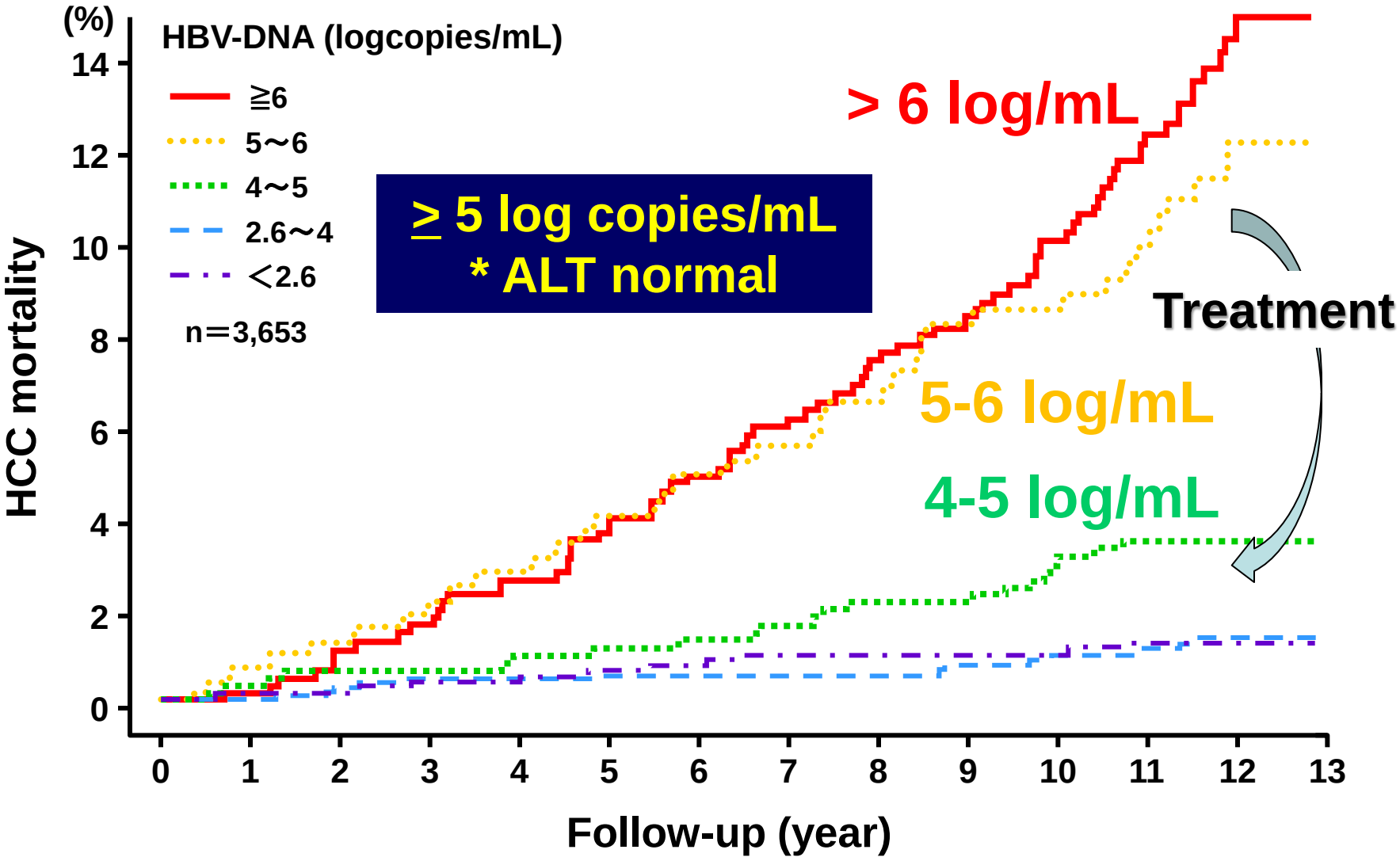
Today's Topics





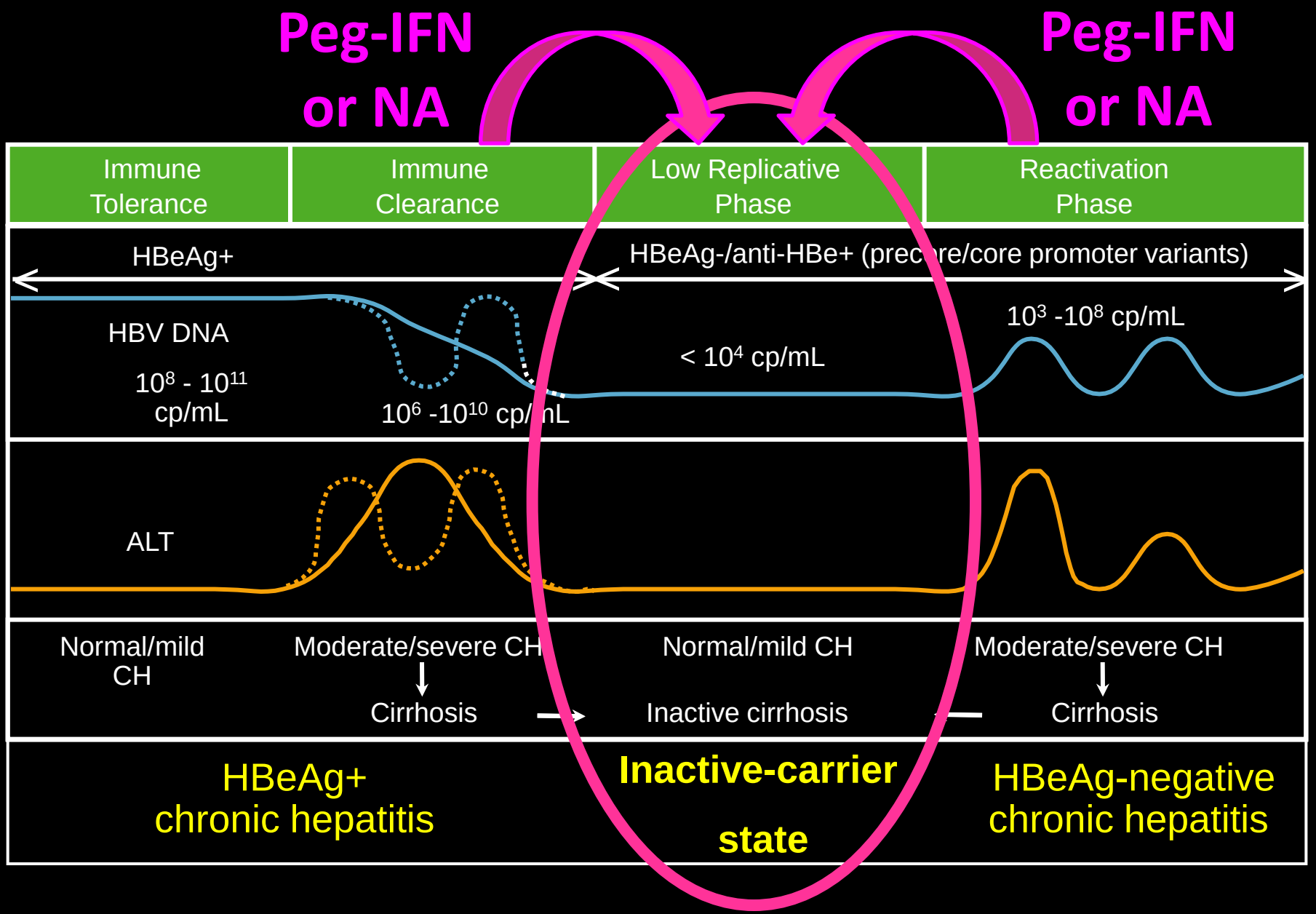
1. HBV markers and disease progression
2. **Current Guideline for HBV treatments**
3. Clinical application of HBcrAg assay
4. Strategy for Grey Zones

Association of HCC mortality with HBV DNA levels at entry (REVEAL)



Chen, CJ et al.: JAMA 295(1): 65-73, 2006

Current indication of anti-HBV therapy?



Recommendations of treatment initiation slightly different on guideline

	APASL 2015 [1]	EASL 2017 [2] JSH	AASLD 2018 [3]
HBV DNA (IU/mL)	>2000 (eAg-) >20,000 (eAg+)	>2000	>2000 (eAg-) >20,000 (eAg+)
ALT	>2 × ULN	>ULN (>30, JSH)	≥2 × ULN
Age	>35	>30	>40

Many grey zones with inexplicit recommendations

- EASL: European Association for the Study of the Liver; APASL: Asian Pacific Association for the Study of the Liver; AASLD: American Association for the Study of Liver Diseases; eAg: hepatitis B e-antigen.
- 1. Sarin SK, et al. *Hepatol Int.* 2016;10:1–98; 2. EASL. *J Hepatol* 2017;67:370-398. 3. Terrault NA, et al. *Hepatology* 2018;67:1560–99.

Received: 18 March 2019 | Revised: 11 September 2019 | Accepted: 31 October 2019

DOI: 10.1111/jvh.13244

NON-COMMISSIONED REVIEW

JVH WILEY

Asian consensus recommendations on optimizing the diagnosis and initiation of treatment of hepatitis B virus infection in resource-limited settings

A panel of 24 experts from 10 Asian countries

Edward John Gane[1] | Michael R. Charlton[2] | Rosmawati Mohamed[3] | Jose Decena Sollano[4] | Kyaw Soe Tun[5] | Thuy Thi Thu Pham[6] | Diana Alcantara Payawal[7] | Rino Alvani Gani[8] | David Handojo Muljono[9,10,11] | Subrat Kumar Acharya[12] | Hui Zhuang[13] | Akash Shukla[14] | Kaushal Madan[15] | Neeraj Saraf[16] | Satyendra Tyagi[17] | Karam Romeo Singh[18] | Ian Homer Yee Cua[19] | Ganbolor Jargalsaikhan[20,21] | Davadoorj Duger[22] | Wattana Sukeepaisarnjaroen[23] | Hery Djagat Purnomo[24] | Irsan Hasan[25] | Laurentius Adrianto Lesmana[26] | Cosmas Rinaldi Adithya Lesmana[27] | Khin Pyone Kyi[28] | Win Naing[29] | Allampura Chandrashekar Ravishankar[30] | Sanjay Hadigal[30]

Recommendation 7: All HBV-infected **compensated or decompensated cirrhotic** individuals with detectable serum HBV DNA levels should be initiated on antiviral therapy, regardless of ALT levels, or HBeAg status.

Recommendation 8: Guidance on treatment initiation in HBeAg-positive or HBeAg-negative treatment-naïve, HBV-infected **non-cirrhotic** patients (**next Figure**).

Algorithm to guide initiation of antiviral therapy in HBeAg-positive and HBeAg-negative treatment-naïve, non-cirrhotic individuals

Asia consensus Recommendation 8

(Gane Ed et al. J Viral Hepat 2019)

HBeAg
Positive or negative

HBV DNA
< 2,000 IU/mL

Presence of any of the following

- First-degree family member with cirrhosis or HCC
- Extrahepatic manifestations

Antiviral treatment required irrespective of ALT levels

HBV DNA
> 2,000 IU/mL

elevated ALT levels greater than upper limit of normal

ALT levels
< ULN

Presence of any of the following

- Age >30y
- **At least moderate fibrosis**
- First-degree family member with cirrhosis or HCC
- Extrahepatic manifestations

(Glomerulonephritis, polyarteritis nodosa, mixed cryo-globulinemia)

Antiviral treatment required

ALT levels
> ULN
(upper limit of normal)
Where ULN is defined by local laboratory

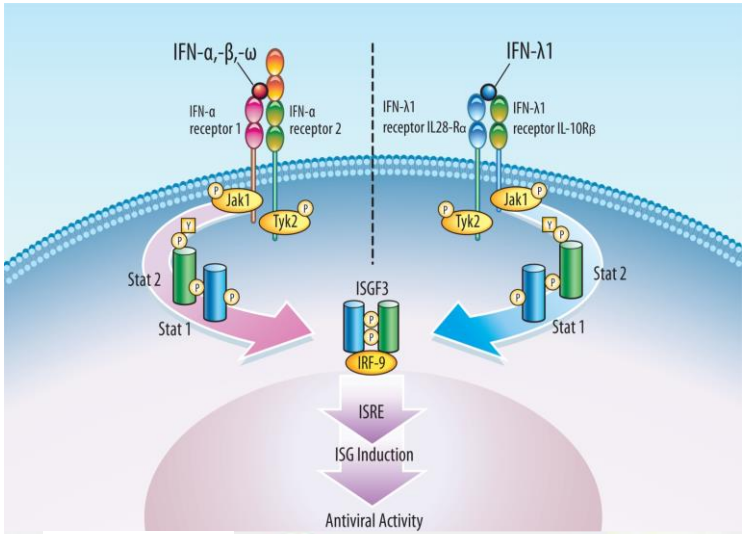
Liver stiffness ≥ 8 kPa (by Fibroscan) or APRI ≥1.5

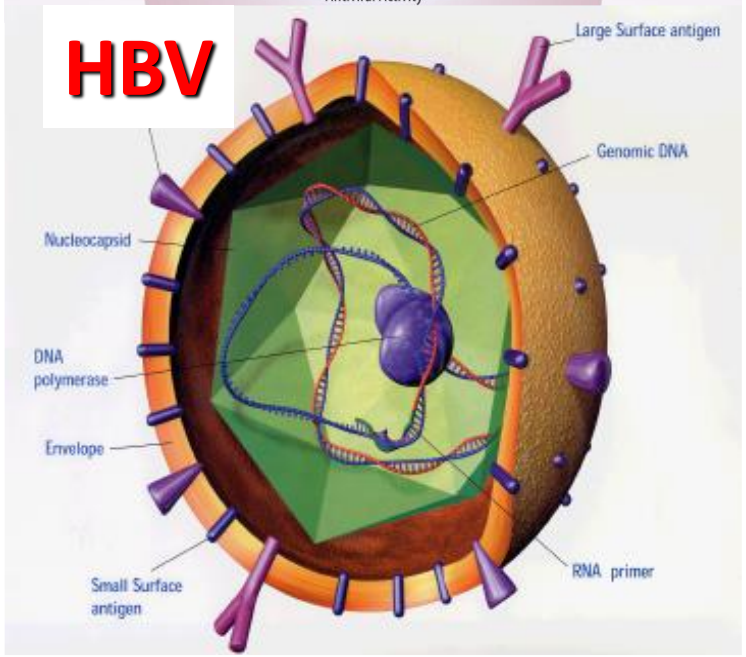
Antiviral treatment required

Summary 1

Recent European and Asian guidelines showed that decision of anti-HBV treatment initiation would depend on HBV-DNA and ALT level, regardless of HBeAg status.

Today's Topics





1. Current status of HBV infection worldwide
2. Current Guideline for HBV treatments
3. **Development of novel drugs for HBV**

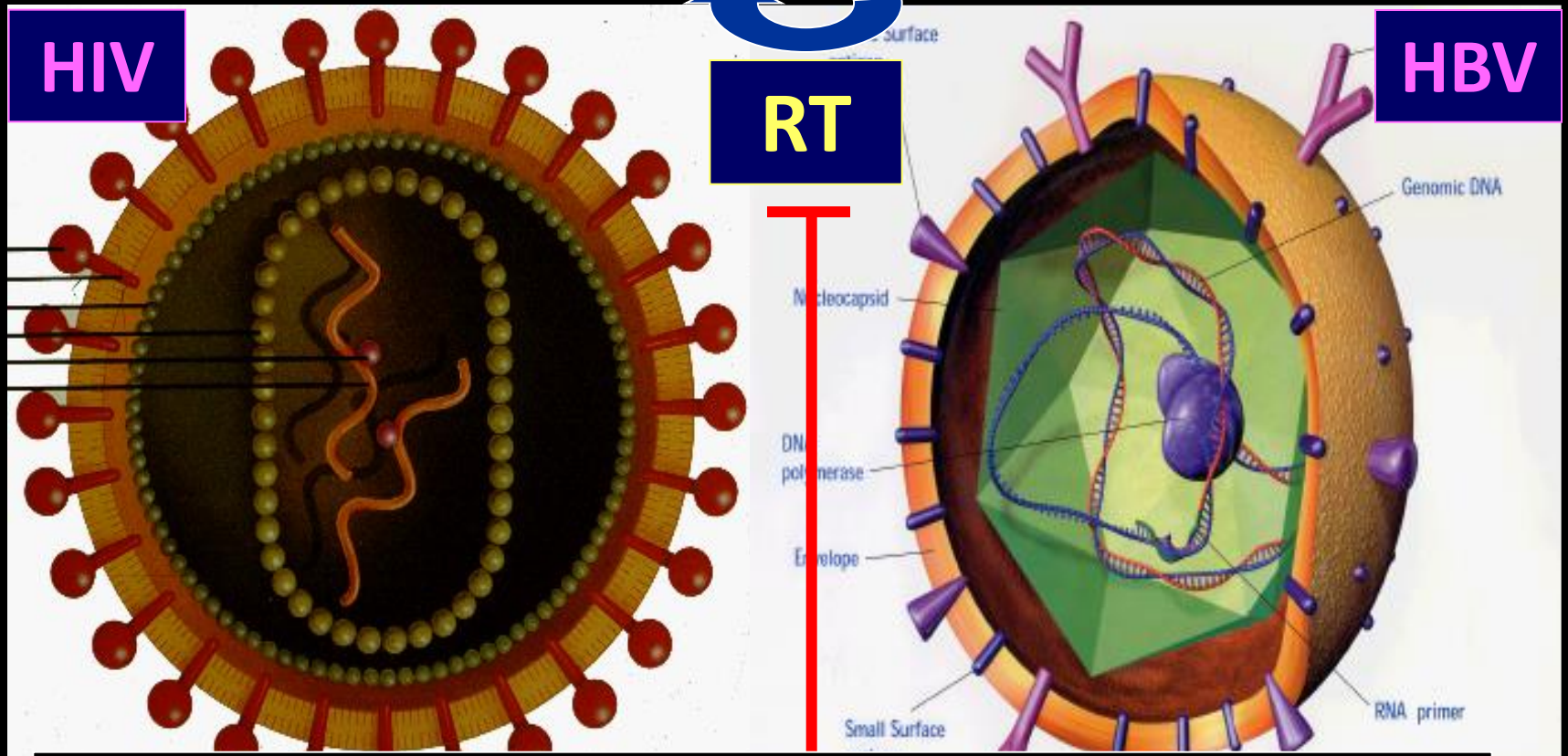
a) **Antisense oligo: Bepi**

b) **HBV destabilizer: SAG-524**

c) **PD-1 mAb**

Reverse transcription (RT) inhibitor

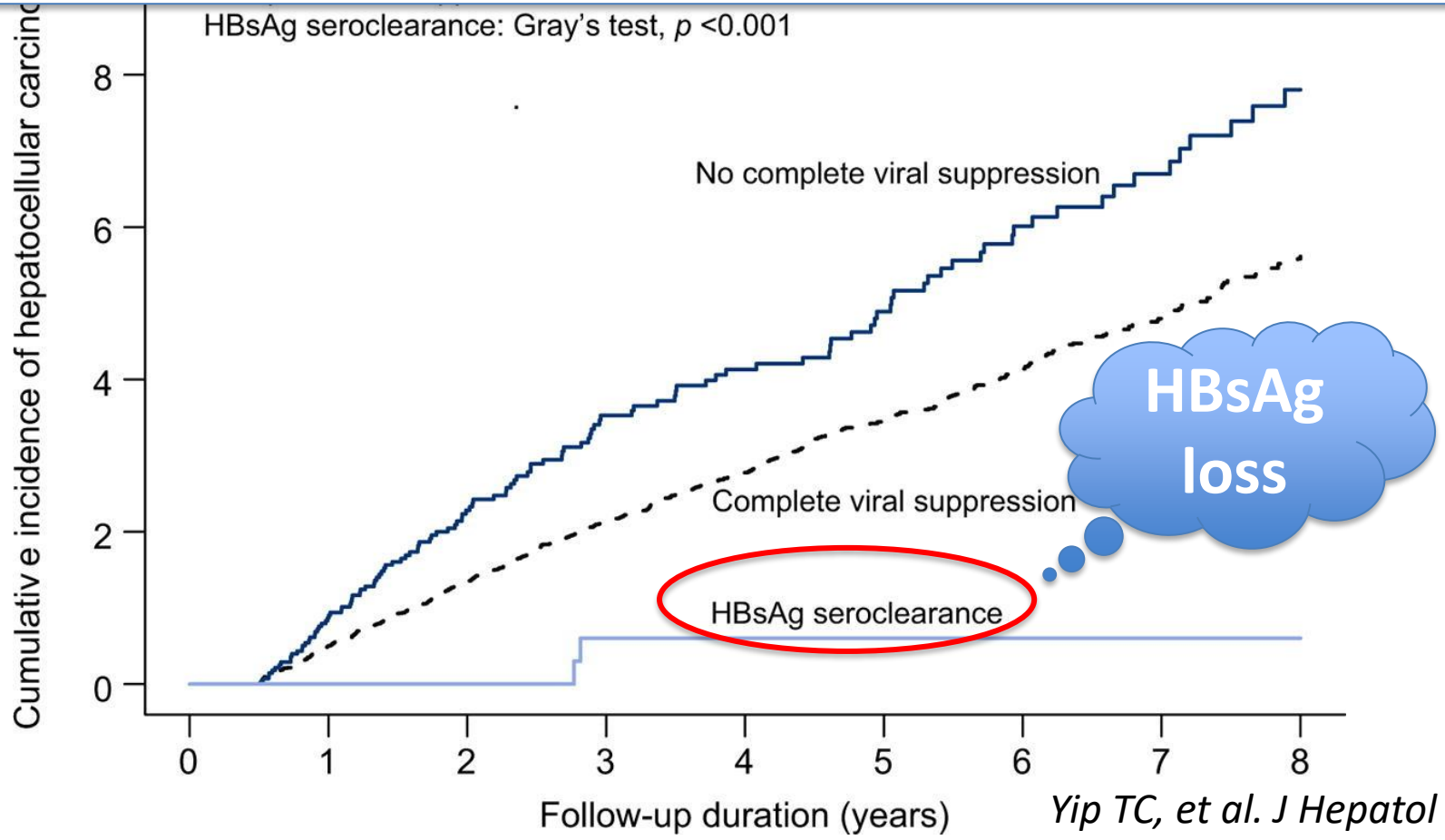
DNA ⇨ RNA ⇨ Protein



RT inhibitors of HIV were also effective in chronic hepatitis B

JOURNAL
OF HEPATOLOGY

Complete viral suppression with nucleoside analogues was not enough to reduce HCC incidence



Yip TC, et al. J Hepatol 2019

Hepatitis B Strategy for Functional Cure

Intensify Antiviral Treatment

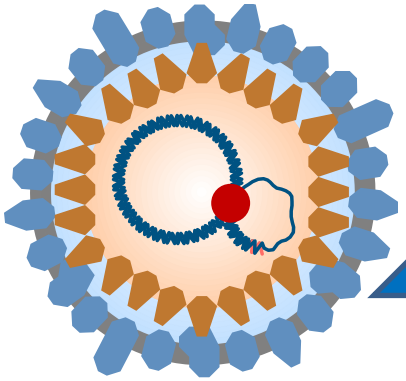
抗ウイルス治療の強化

Reduce cccDNA Formation & Virus Production

Silence/ Eliminate cccDNA

機能的治癒を目指した併用療法

HBV



Launch successive waves of combination treatment to increase rate of functional cure

Boost Immune Response

免疫応答の増強

Boost Effective HBV Specific T-cell Responses

HBV特異的なT細胞の増強作用

Boost Innate Immunity

自然免疫の増強

Overall and Japanese subgroup analyses of global Phase 2b study (B-Clear study) for bepirovirsen in patients with chronic HBV infection

Yasuhito Tanaka[1], Hiroshi Yatsuhashi[2], Hiroshi Ito[3]

[1]Department of Gastroenterology and Hepatology, Kumamoto University, Kumamoto, Japan

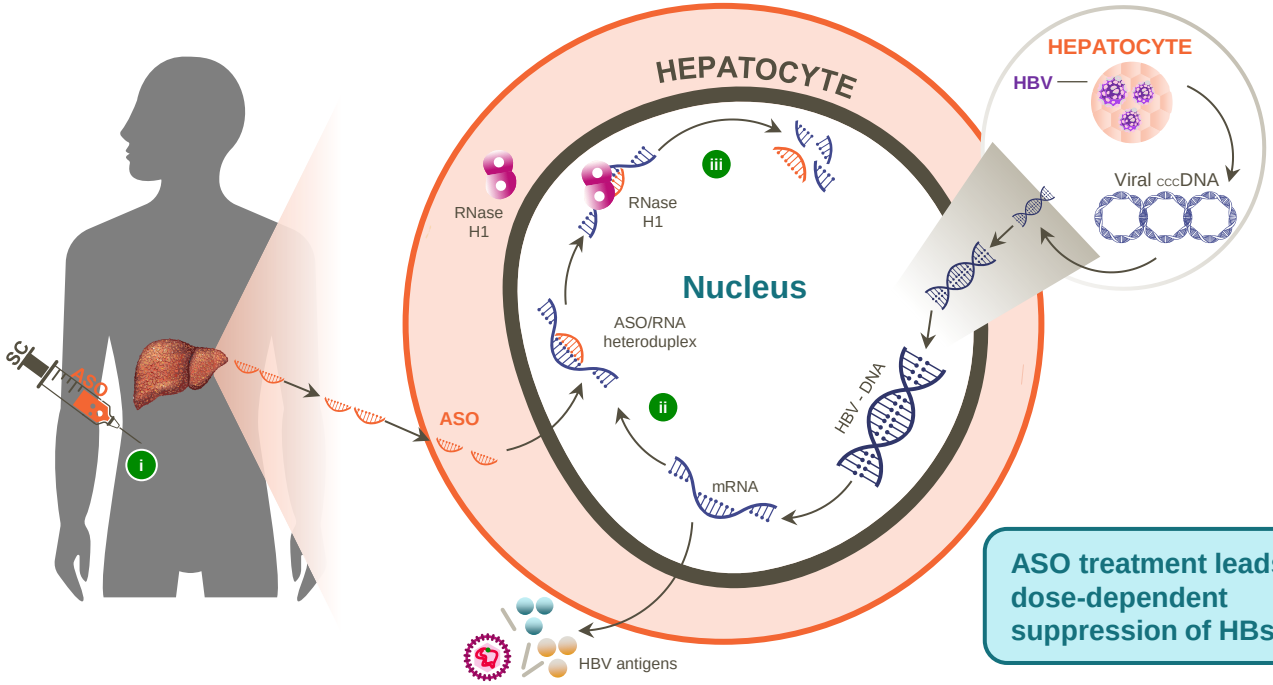
[2]National Hospital Organization, Nagasaki Medical Centre, Nagasaki, Japan

[3]Japan Medical and Development, GlaxoSmithKline K.K., Tokyo, Japan

The Japan Society of Hepatology
59th Annual Meeting
15–16 June 2023

Bepirovirsen: Mode of action
A novel unconjugated antisense oligonucleotide

Subcutaneous injection



ASO treatment leads to dose-dependent suppression of HBsAg[1]

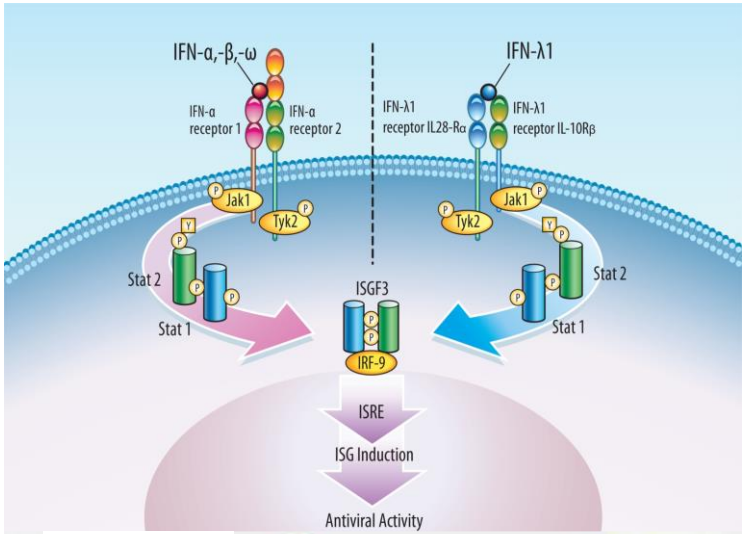
i. Following SC injection, ASO enters the hepatocyte[1]

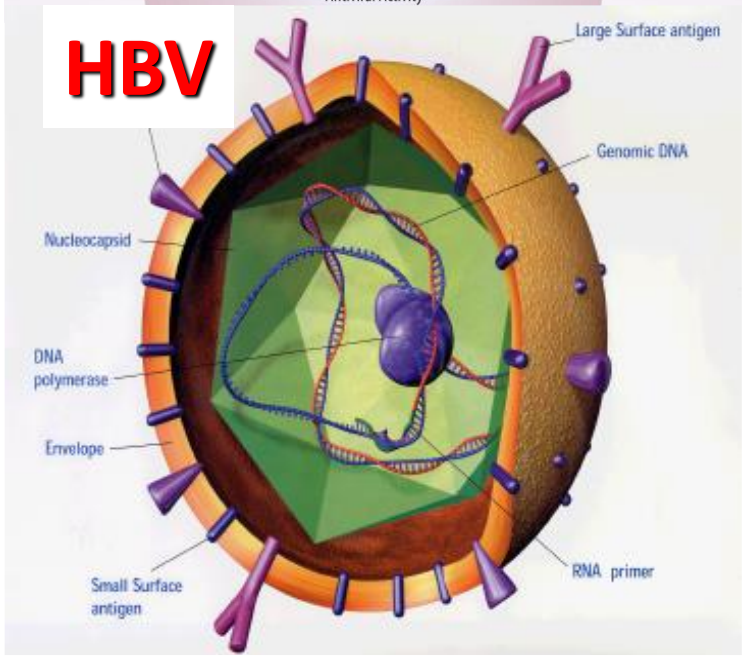
ii. In the nucleus, ASO directly binds to complementary hepatitis B virus mRNA transcripts forming the hybrid ASO/RNA complex[2]

iii. RNase H enzyme attaches to the complex, creating a nick in the HBV mRNA which is susceptible to nucleases for degradation as well as causes premature transcription termination[3]

Image adapted from Han K et al. Clin Pharmacol Drug Dev 2019;8:790–801.
1. You et al. Poster presented at International HBV Meeting 2016, Seoul, Korea; 2. Crooke ST et al. Nat Biotechnol 2017;35:230–237. 3. Lee JS et al. Mol Cell 2020;77:1044–1054.
ASO, antisense oligonucleotide; DNA, deoxyribonucleic acid HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; RNA, ribonucleic acid; RNase H, ribonuclease H; SC, subcutaneous

Today's Topics



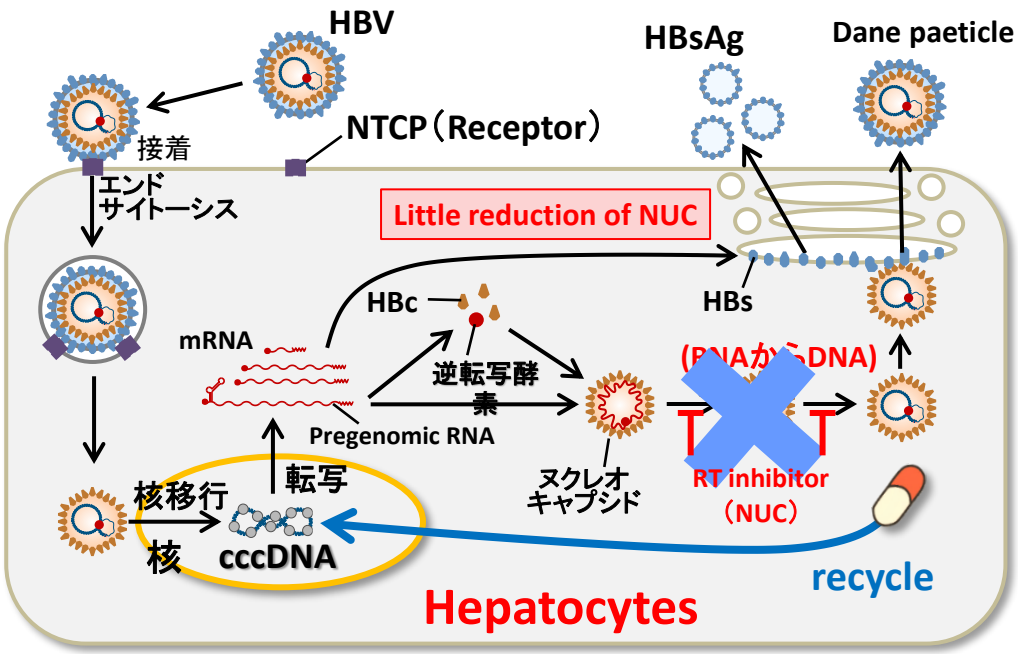


1. Current status of HBV infection worldwide
2. Current Guideline for HBV treatments
3. **Development of novel drugs for HBV**
   a) Antisense oligo: Bepi
   b) **HBV destabilizer: SAG-524**

Orally available small molecule compounds

a) PD-1 mAb

HBV lifecycle and the Target



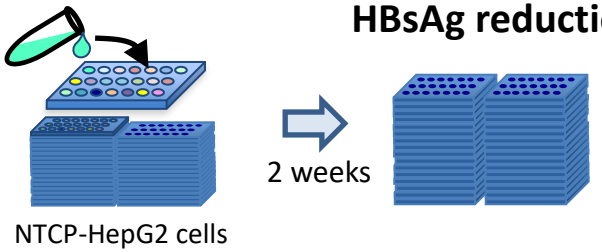
It is difficult to do
"Functional Cure"
(HBsAg loss)
by NUC therapy

Novel dugs required

Drug Screening

In vitro screening

Screening for hit compounds by HBsAg reduction

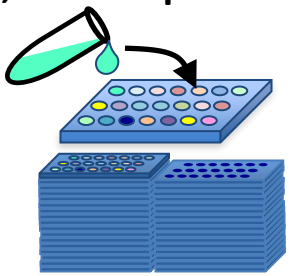


(Watanabe T, Tanaka Y, et al. J Gastroenterol 2024)

Screening

In vitro HBV-infected models

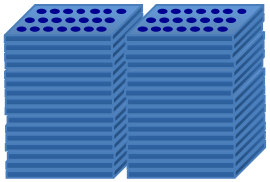
30,000 compound library



NTCP-HepG2 cells
HBV-infected PXB cells

2 weeks

Measurement of HBsAg
in supernatant
to seek hit compounds

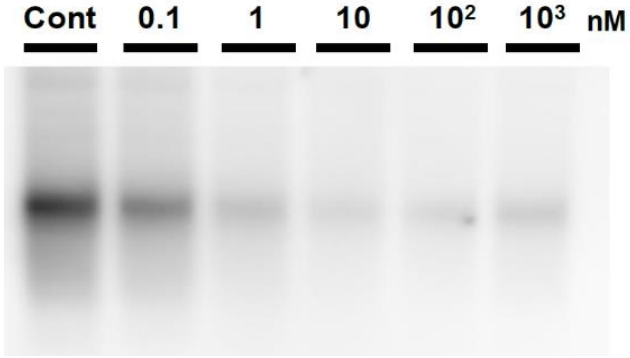


Optimization

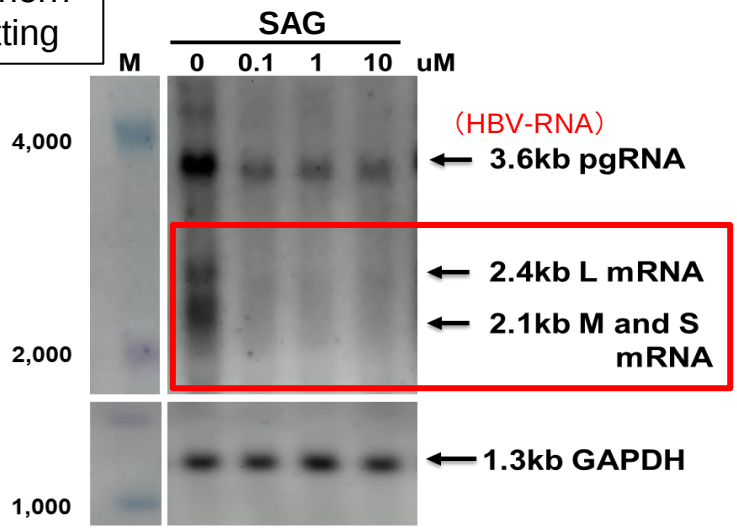
HBV DNA / HBsAg IC_{50} (nM)			
	HepG2.2.15 (Gt D)	PXB (Gt C)	CC_{50} (nM) (HepG2.2.15)
SAG-524	0.89 / 1.4	34 / 8.5	>1,000

SAG-524 decreased HBV DNA and HBsAg with IC_{50} in nano-molar range, and showed anti-HBV activity against genotype C and D.

Southern Blotting



Northern Blotting



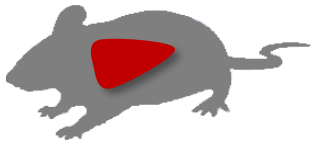
SAG compounds reduce HBV-RNA

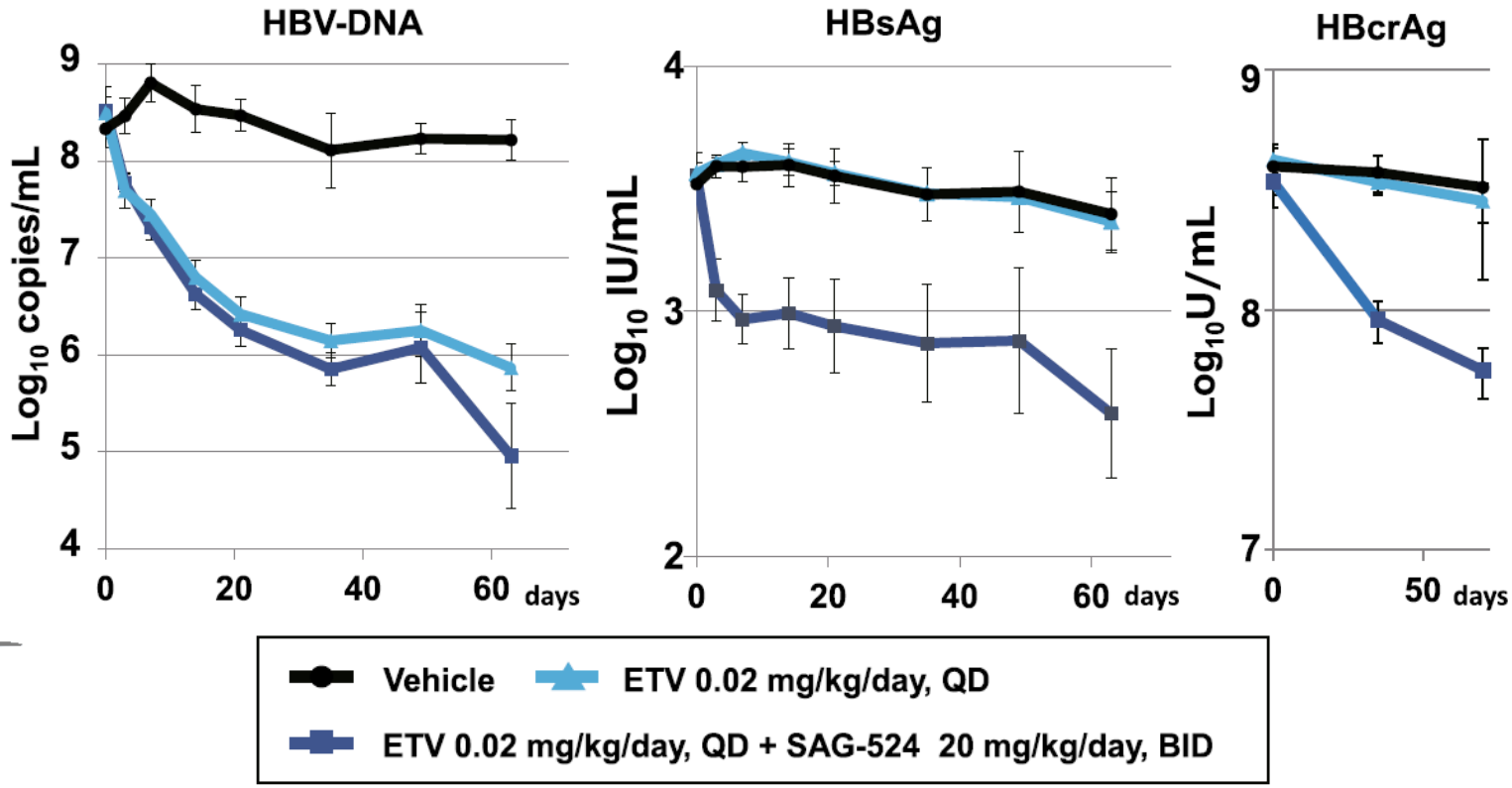
Results of *in vivo* combination study of SAG-524 (oral drug) with entecavir

Oral comb.

HBV-infected PXB mice

ETV p.o.
± SAG p.o.
(oral route)

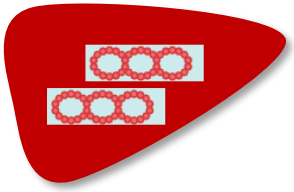


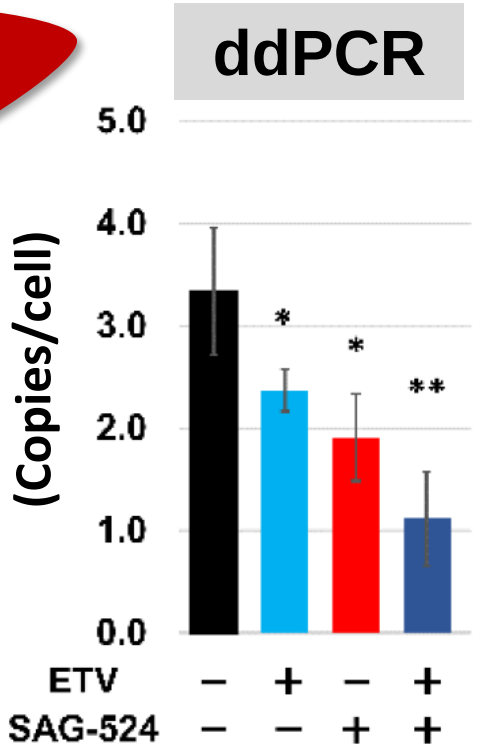


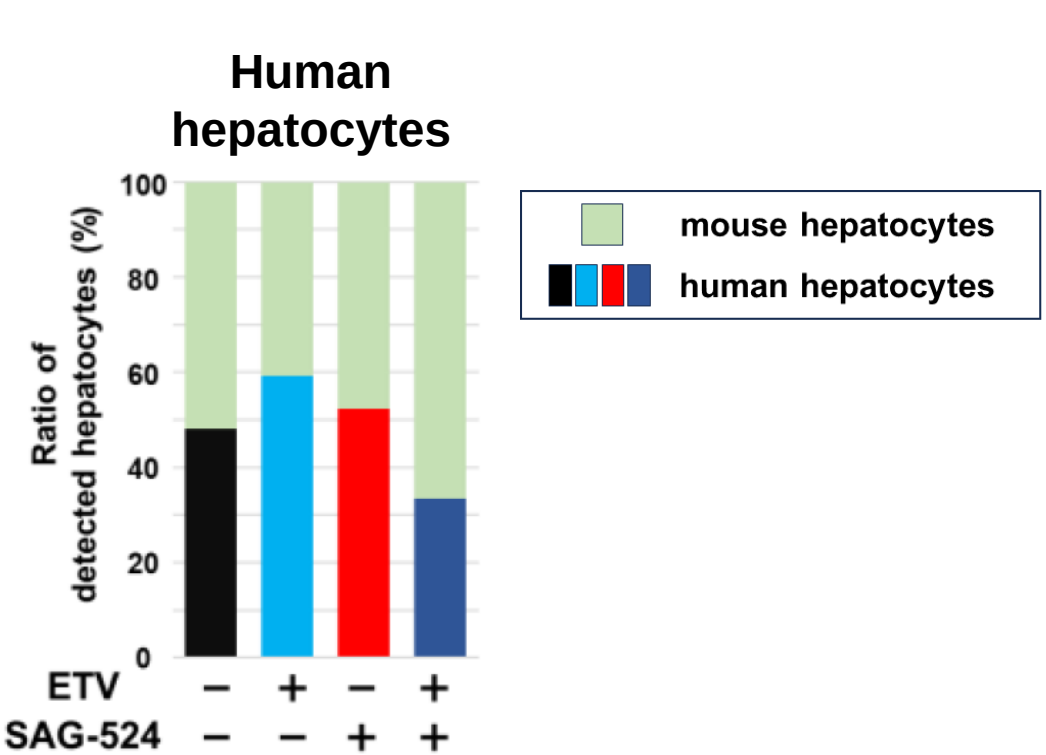
The add-on of SAG to ETV markedly reduced both HBV-DNA and HBsAg in the serum of HBV-infected PXB mice

(Watanabe T, Tanaka Y, et al. J Gastroenterol 2024)

Efficacy evaluation of SAG-524 to intrahepatic cccDNA *in vivo*







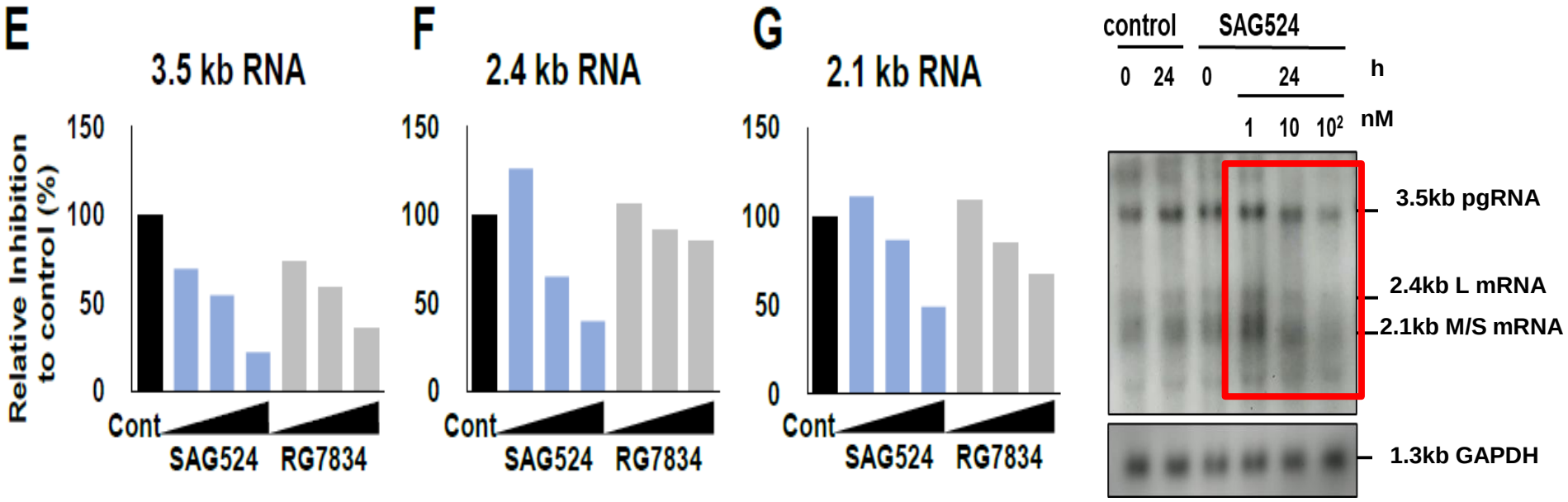
✓**The combination of ETV and SAG decreased intrahepatic cccDNA, possibly reducing *de novo* cccDNA production and eliminating HBV-infected cells.**

Student's t-test; * P<0.05, * P<0.01

※ N=4, ETV 0.02 mg/kg/day, QD ± SAG-524 20 mg/kg/day, BID, using t-test.
(Watanabe T, Tanaka Y, et al. J Gastroenterol 2024)

SAG: Mode of action

Studying RNA-destabilization by BRIC-assay

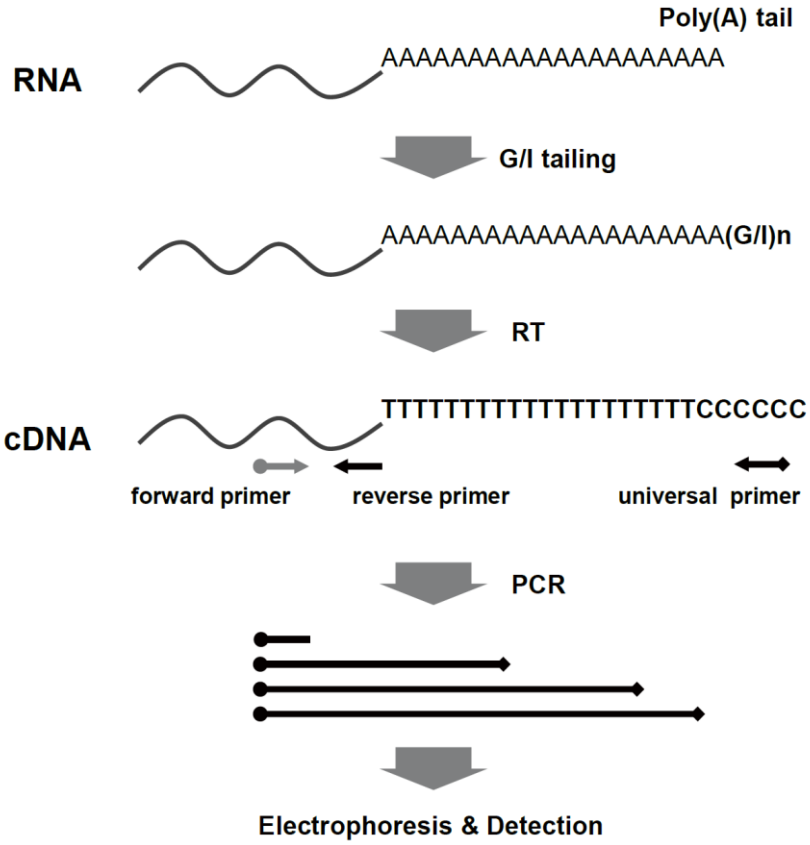


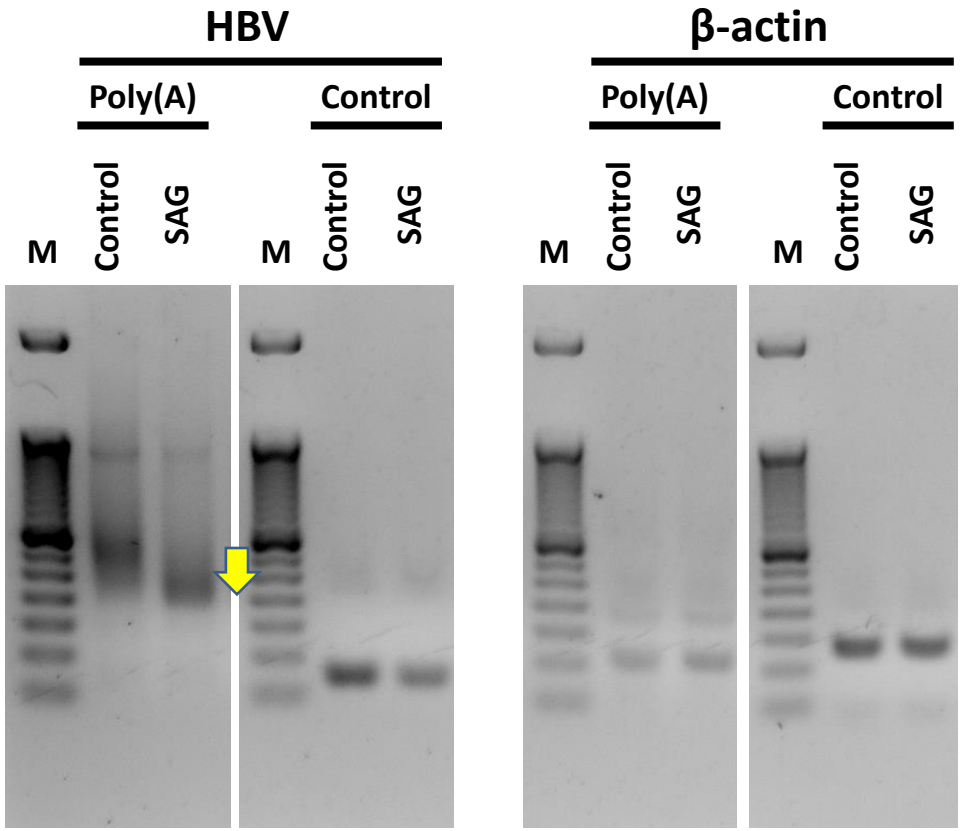
SAG compound destabilized HBV RNA and significantly reduced HBV-RNA, but not degradation of GAPDH and albumin.

(Watanabe T, Tanaka Y, et al. J Gastroenterol 2024)

SAG: Mode of action

Poly(A) tail assay

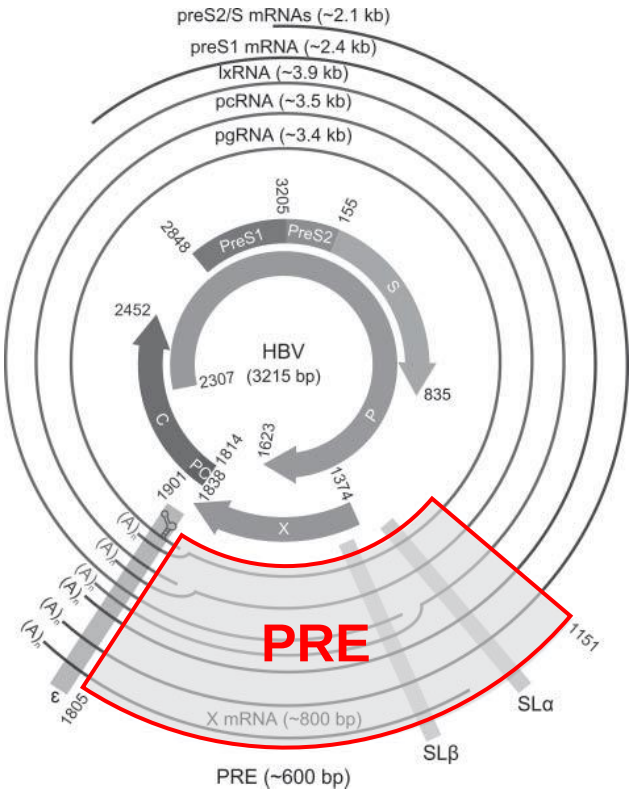




SAG compound destabilized and degraded HBV-RNA

(Watanabe T, Tanaka Y, et al. J Gastroenterol 2024)

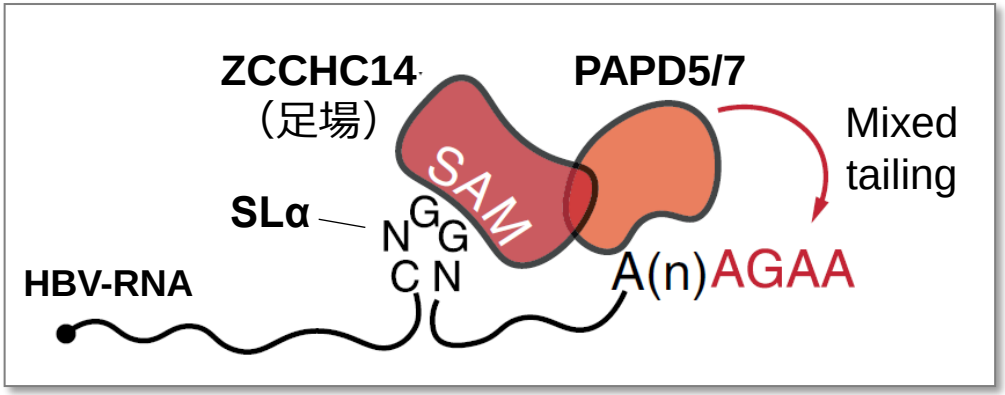
HBV post-transcriptional regulatory element (PRE)



PRE: Nucleotides 1151-1805

HBV RNA stem-loop (SL) α, β

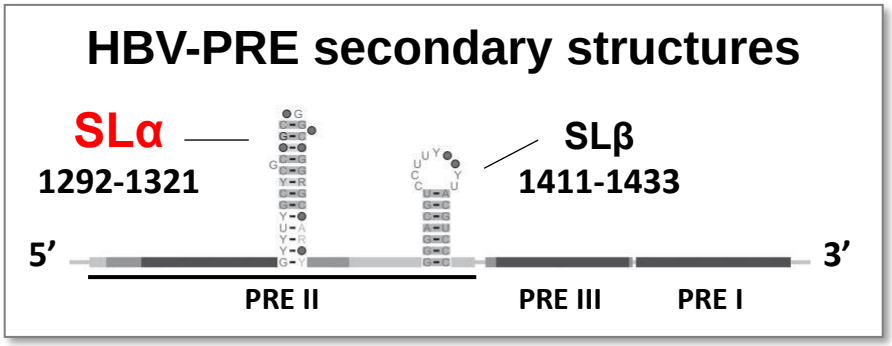
- Nuclear export
- Escape over-splicing the preS2/S mRNAs



(zinc finger CCHC-type containing 14 protein)

The SAM domain of ZCCHC14 binds to the pentaloop of HBV RNA

Stabilize HBV-RNA via PAPD5/7 and ZCCHC14 complex



RNA Biol 2016, 13; 743–747

Cell Rep 2019, 29; 2970–2978
Nat Struct Mol Biol 2020, 27; 581–588

One of SAG's targets is PAPD5

Science PAPD5 (TENT4B)

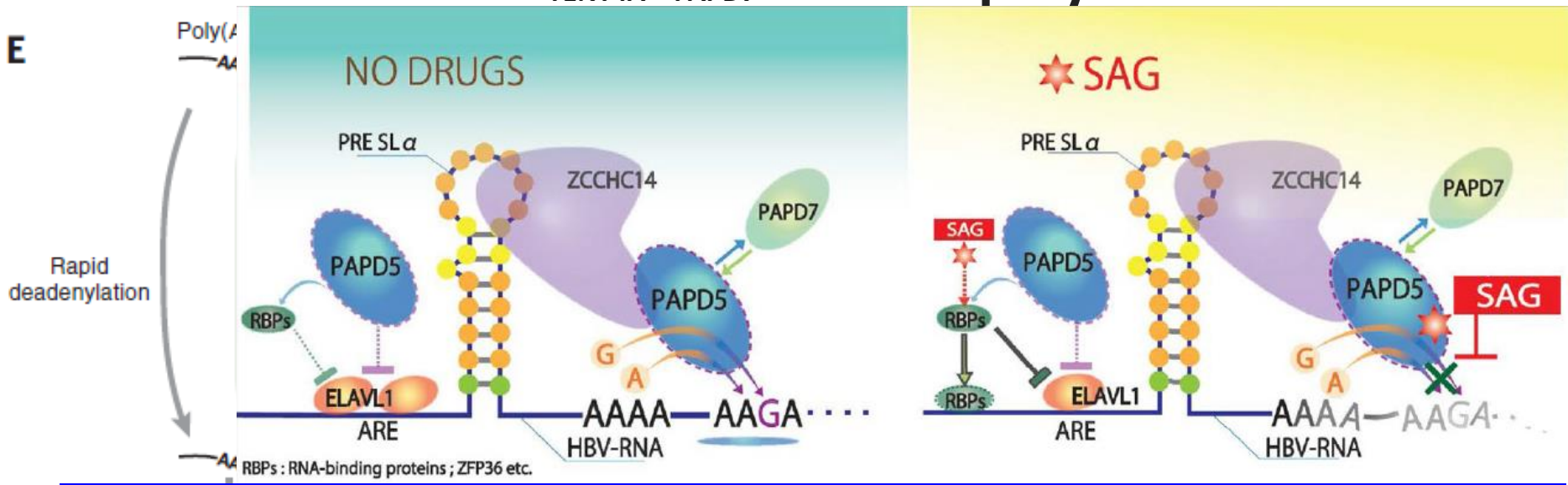
Mixed tailing by TENT4A and TENT4B shields mRNA from rapid deadenylation

PAPD5/7: RNA binding protein

↓

noncanonical poly(A) polymerases

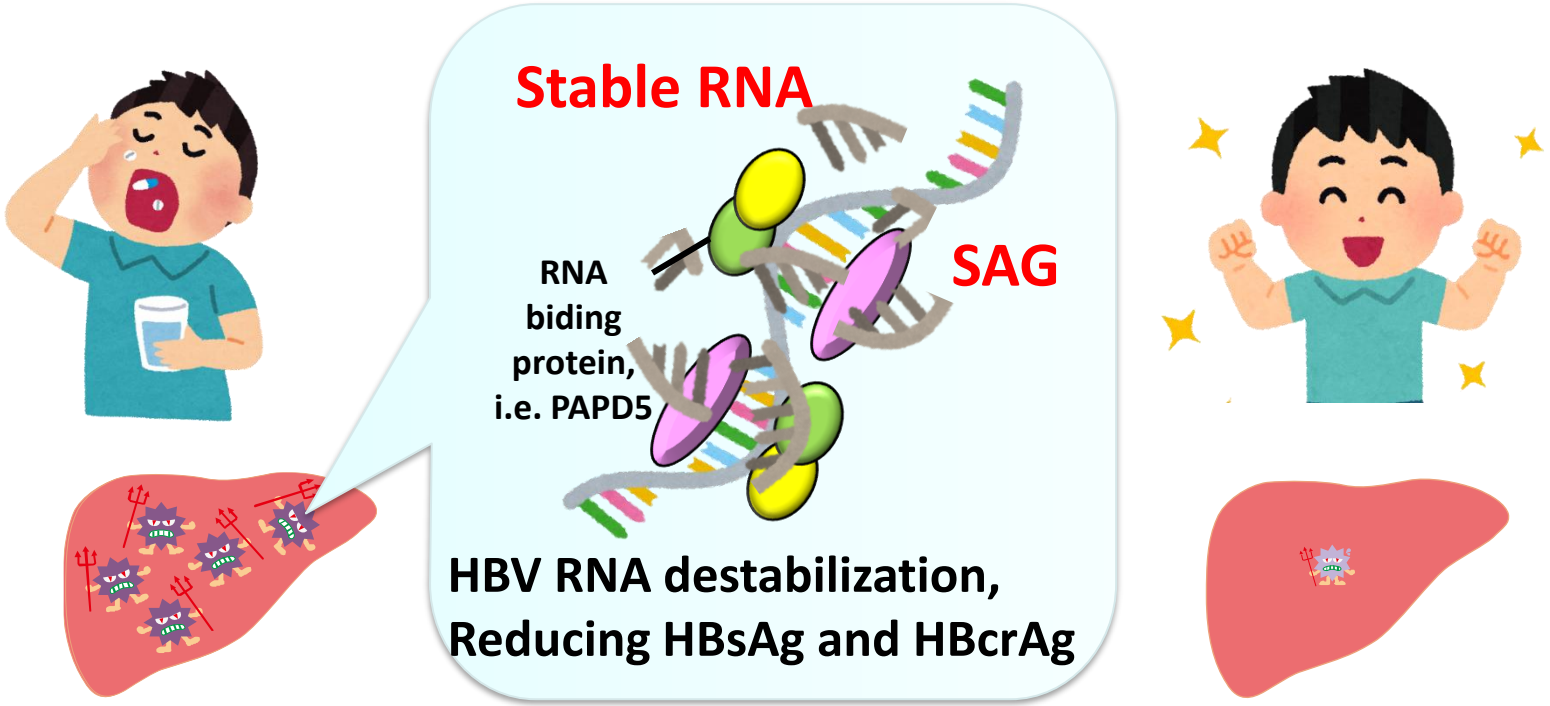
TENT4A = PAPD7



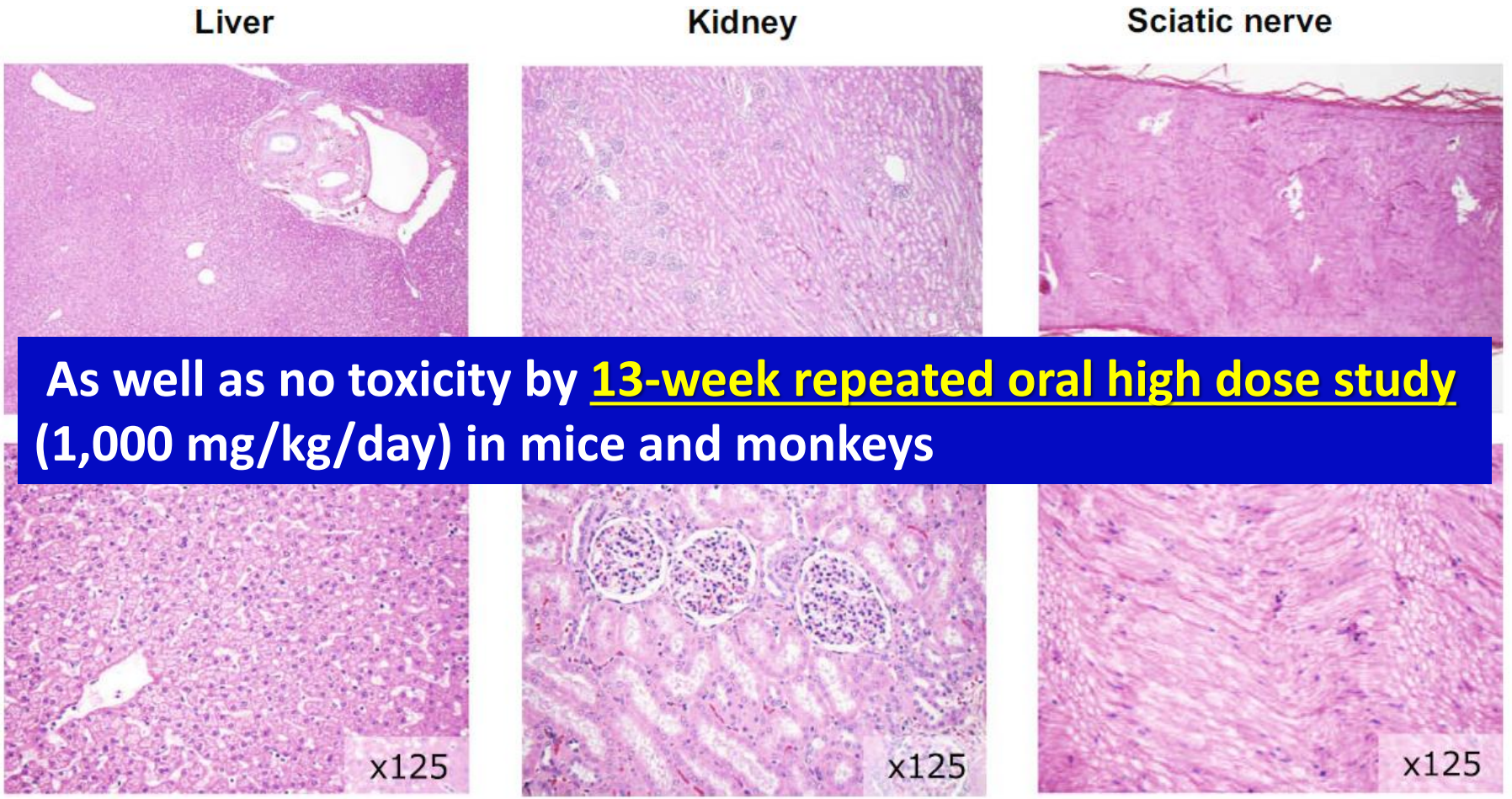
SAG compounds directly bind PAPD5, not PAPD7 and destabilize HBV RNA, thus exerting the anti-HBV effects via PAPD5.

MoA of SAG compound, HBV RNA destabilizer

SAG compound targeting (competitive) RNA binding proteins, i.e. PAPD5 (RNA polymerase)



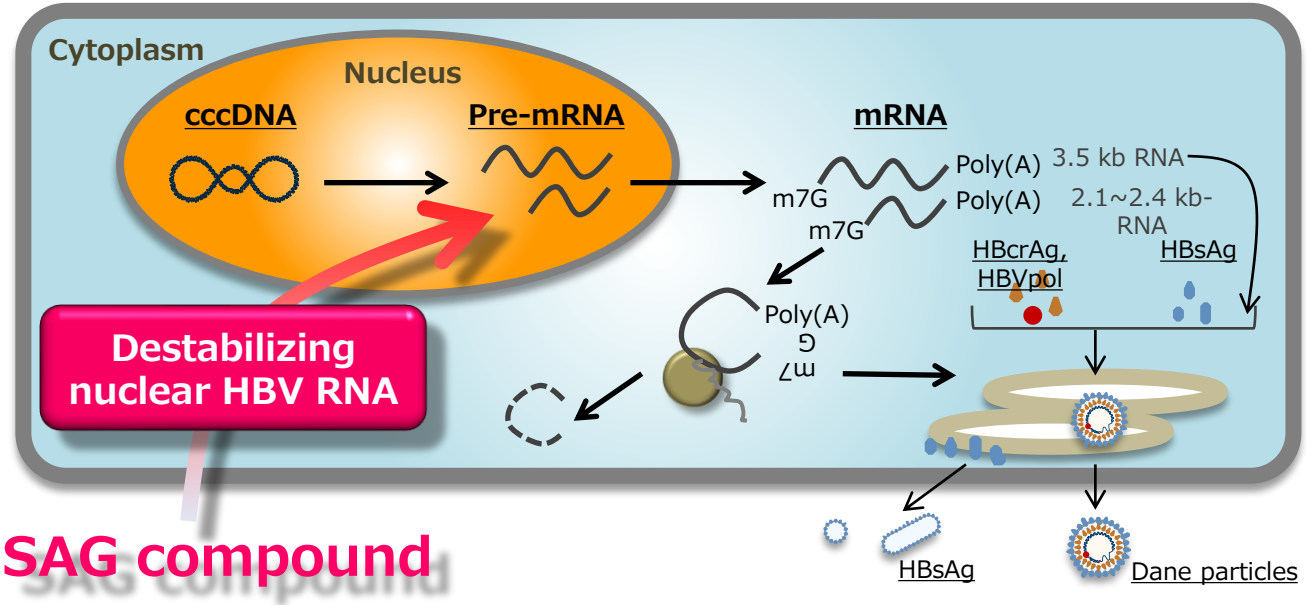
No histopathological changes of a 2-week repeated oral dose toxicity study were observed in (cynomolgus) monkeys.



As well as no toxicity by 13-week repeated oral high dose study (1,000 mg/kg/day) in mice and monkeys

(Watanabe T, Tanaka Y, et al. J Gastroenterol 2024)

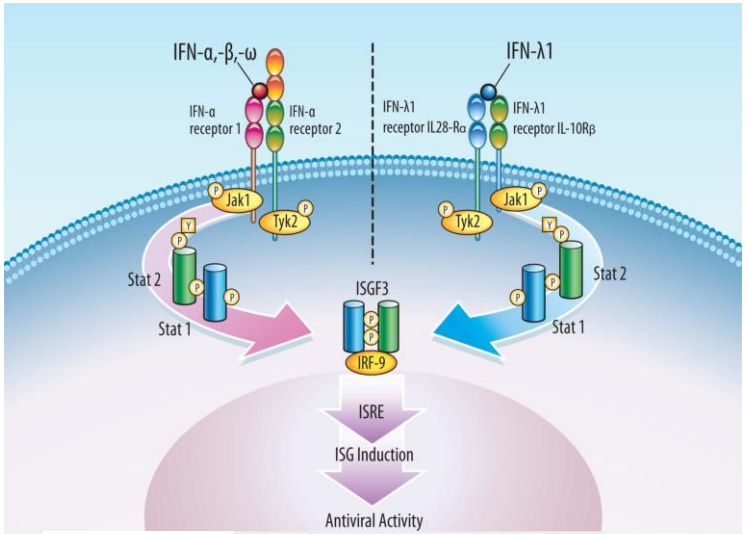
Summary 3

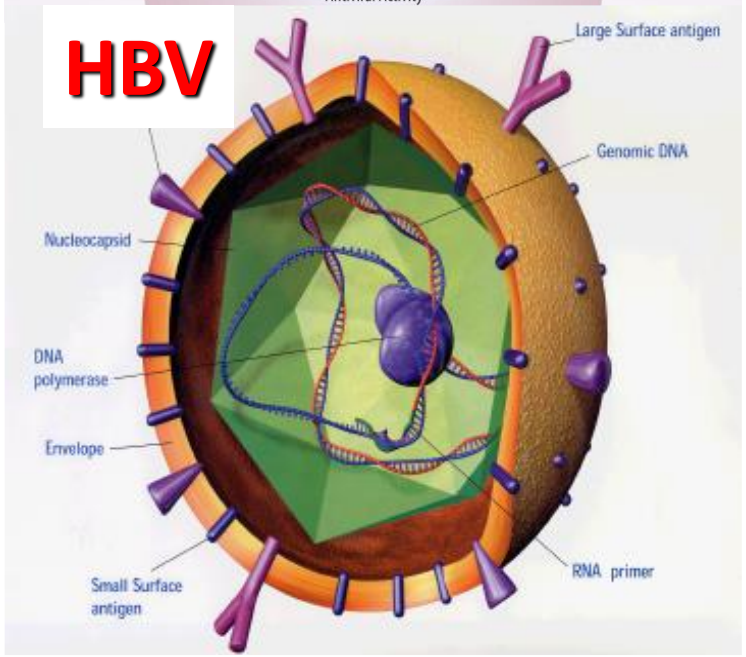


- ✓ **Orally available small molecule compounds.**
- ✓ **destabilizes HBV RNA and reduces HBV markers (HBV-DNA, HBsAg, HBcrAg).**
- ✓ **shows anti-HBV activity against multiple genotypes (gtA, gtC and gtD).**
- ✓ **No obvious toxicity was observed (well tolerated).**
- ✓ **Planning to clinical trial.**

(Watanabe T, Tanaka Y, et al. J Gastroenterol 2024)

Today's Topics





1. Current status of HBV infection worldwide
2. Current Guideline for HBV treatments
3. **Development of novel drugs for HBV**
   a) Antisense oligo: Bepi
   b) HBV destabilizer: SAG-524
   c) **Inhibition of PD-L1**

Proof of concept: T cell activation by inhibition of PD-L1 (PD-1 mAb)

AASLD
AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES

Clinically approved dose 3 mg/kg for Melanoma etc, but 0.3 mg/kg for HBV

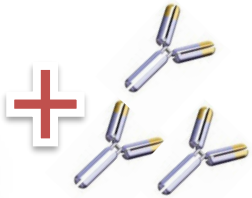
Primary endpoint
(12 weeks post Nivolumab)

Baseline — Week 4 — Week 12 — Week 16

Sentinel A (n=2)
0.1mg/kg Nivolumab

Cohort A (n=12)
0.3mg/kg Nivolumab

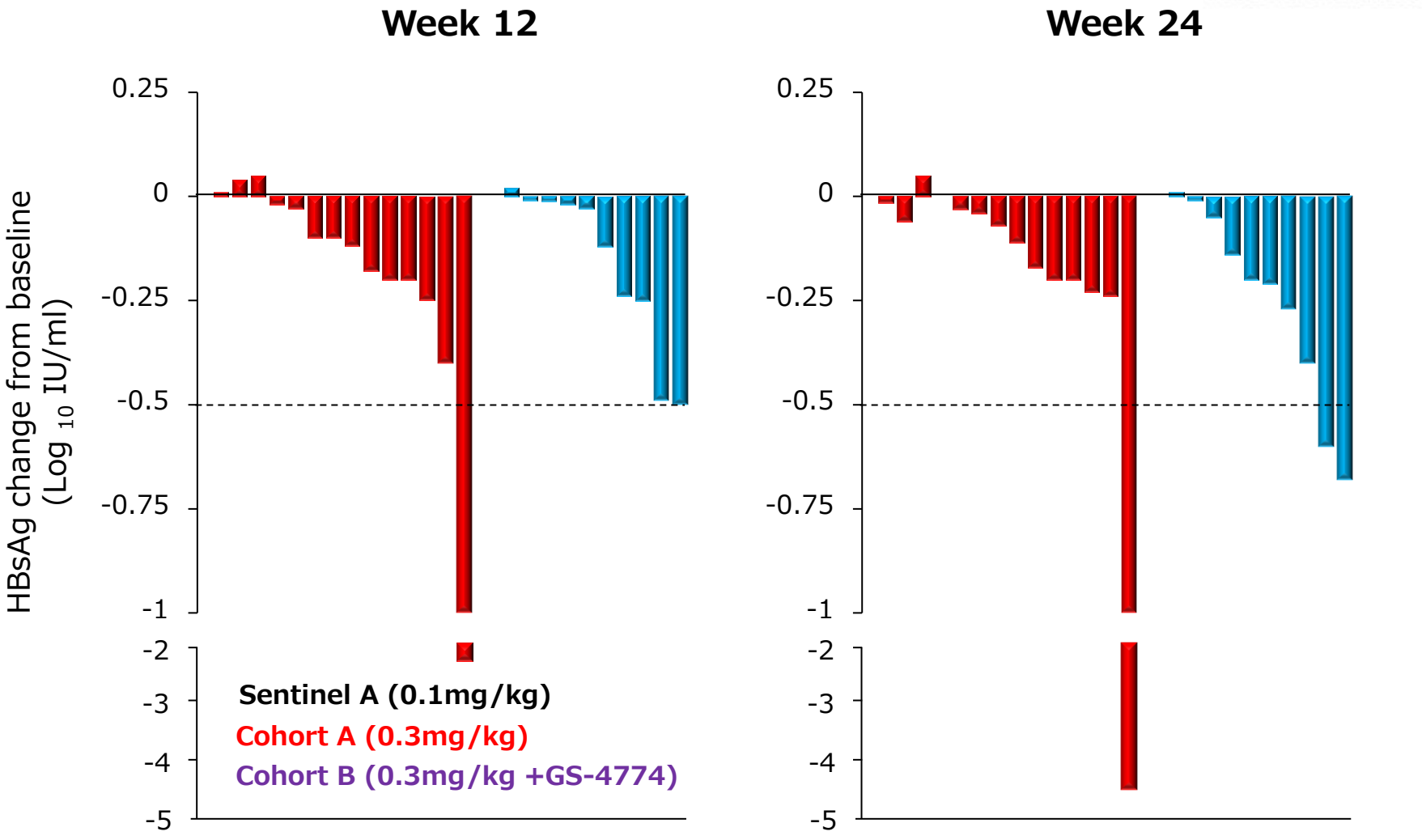
Cohort B (n=10)
0.3mg/kg Nivolumab
40 YU GS-4774



Primary efficacy endpoint: Change in HBsAg $\log_{10}$ IU/mL levels 12 weeks following Nivolumab treatment. GS-4774 is a heat-inactivated, yeast-based, T-cell vaccine designed to elicit hepatitis B virus (HBV)-specific T-cell responses. YU; yeast units

Gane, et al., J. Hepatol 2019; Daniel Verdon et al; AASLD 2017, Washington DC

Results: HBsAg change from baseline



9%(2/22) at Week 12 and 14%(3/22) at Week 24 with a >0.5 log reduction in HBsAg.
Only one patient achieved HBsAg loss.

Gane, et al., J. Hepatol 2019; Daniel Verdon et al; AASLD 2017, Washington DC

Results: Case Study

AASLD
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Clinically approved dose 3 mg/kg
for Melanoma etc,
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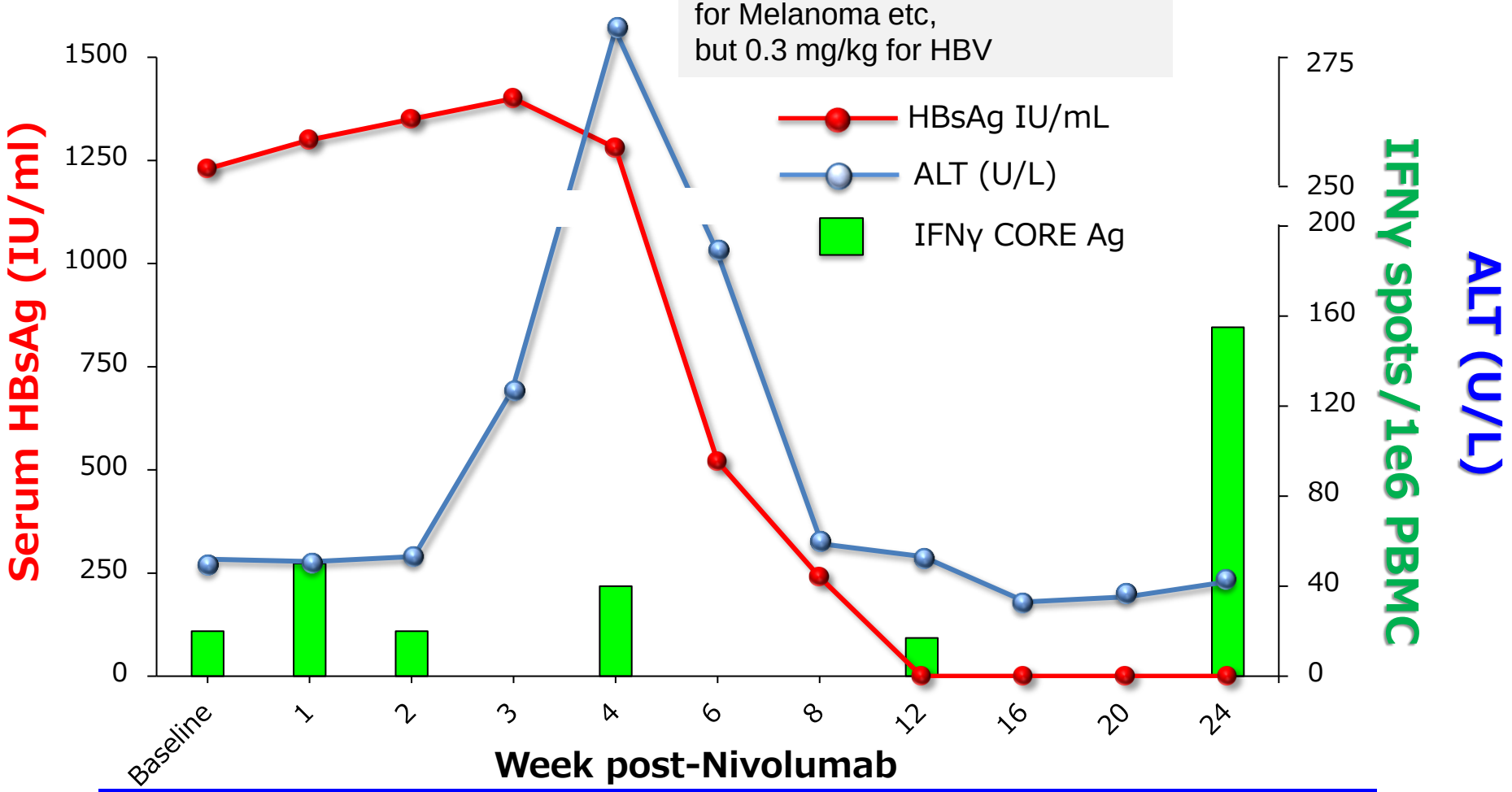

The patients had ALT elevation, followed by HBsAg decline, that was related to HBV core-specific IFN-γ production.

Gane, et al., J. Hepatol 2019; Daniel Verdon et al; AASLD 2017, Washington DC

Future perspectives: Future HBV curative regimen?

Potent NA	Agent to prevent viral spread and cccDNA re-amplification
+	
HBV antigen inhibition	Agents to inhibit other components in the HBV life cycle (i.e. entry, cell-spread, capsid assembly, HBx, HBeAg, HBsAg)
+	
Immune modulator	Agents to activate specific antiviral immunity or relieve repression/exhaustion of the system
+	
cccDNA inhibitor	Safe and selective agent to reduce or silence cccDNA

Take home massages

- ✓ **Both prevention and treatment should be required for HBV elimination.**
- ✓ **HBV RNA destabilizer, SAG-524 will plan to clinical trial for aiming functional cure.**
- ✓ **The combination of HBV RNA inhibitors and immune modulators could be required.**

Acknowledgments

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科研費
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Thank you for your attention