Disclosures

• Consultant - Antios, Arbutus, Assembly Bio, Precision Bio, Abbott Labs, GSK.
• Consultant - Gilead, Abbvie, and Viiv.
• (Data and Safety Monitoring Boards) member with Gilead
• Primary Investigator with Janssen
Learning Objectives

• Outline the natural history of hepatitis B virus infection and the implications for clinical disease and treatment
• Describe the current strategy for the treatment of people with chronic hepatitis B virus infection
• Discuss the definition of hepatitis B functional cure and the barriers to eradication of hepatitis B virus (sterilizing cure)
In 2019/2020:

**58 million persons living with HCV**
- HIV, 2.3 million
- PWID, 1.4 million
- New infections, 1.5 million
- Deaths: 0.3 million

**296 million people living with HBV**
- HIV, 2.7 million
- Living in Africa and West Pacific, 67%
- 5 years old or less, < 1%
- New infections, 1.5 million
- Deaths, 0.8 million

**Hepatocellular carcinoma, 0.5 million**
Hepatitis B Was Discovered In 1965

A “New” Antigen in Leukemia Sera

The “Australia antigen” is found in the sera of some normal individuals from foreign populations. The total absence of the antigen from the sera of normal United States subjects and its relatively high frequency in acute leukemia suggests that the presence of the antigen may be of value in the diagnosis of early acute leukemia. Whether the antigen results from or precedes the leukemia process remains to be seen.

Baruch S. Blumberg, MD, Harvey J. Alter, MD, and Sam Visnich

Hepatitis B surface antigen

The Nobel Prize in Physiology or Medicine 1976

Hepatitis B: Hepadnaviridae Family, Double-Stranded DNA Virus
Hepatitis B Global Incidence And Prevalence

**Incidence:**
Chronic HBV infection in children under 5 reduced from 4.7% to 1.3% (immunization)

**Prevalence:**
296 million people living with HBV
68% in Africa / Western Pacific
Hepatitis B Virus Serology

Interpretation of hepatitis B serology

<table>
<thead>
<tr>
<th></th>
<th>HBsAg</th>
<th>Anti-HBc total</th>
<th>Anti-HBc, IgM</th>
<th>Anti-HBs</th>
<th>HBV DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Acute</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Resolved</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>Chronic*</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*HBeAg +/- and Anti-HBe +/-
Acute Hepatitis B and Clinical Features

- Transmission by parenteral or mucosal exposure
- **Incubation period**, 60 to 90 days
- **Prodromal phase**, 3 to 10 days; abrupt onset of fever, malaise, anorexia, nausea, abdominal discomfort, and dark urine before jaundice
- **Icteric phase**, 1 to 3 weeks; jaundice, light or gray stools, hepatic tenderness, hepatomegaly
- **Convalescent phase** lasts weeks to months; malaise and fatigue persist while jaundice, anorexia, and other symptoms disappear
- Most adults recover (95%) while most infants progress to chronic infection (90%).
Risk of Progression to Chronic Infection is Inversely Related to Age at Infection

HBV Mother-to-Child Transmission Was Common Prior to Interventions

- To determine the frequency of vertical transmission of HBsAg from asymptomatic carrier mothers in Taiwan to their offspring, HBsAg was sought by radioimmunoassay and complement fixation.
- Of 158 babies born to carrier mothers, antigenemia developed in 63; 51 of these antigenemic babies had become antigen positive within the first six months of life.

Table 1. Factors Related to the Babies’ Antigenemia (HBsAg).

<table>
<thead>
<tr>
<th>Factor</th>
<th>No. of Babies HBsAg Positive/Total</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal complement-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fixation titer*1:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 1:4</td>
<td>1/66</td>
<td>1.5</td>
</tr>
<tr>
<td>≥ 1:8</td>
<td>61/91</td>
<td>67.0</td>
</tr>
<tr>
<td>Cord blood*:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBs Ag negative</td>
<td>29/82</td>
<td>35.4</td>
</tr>
<tr>
<td>HBs Ag positive</td>
<td>16/21</td>
<td>76.2</td>
</tr>
<tr>
<td>Siblings*:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All HBs Ag negative</td>
<td>4/39</td>
<td>10.3</td>
</tr>
<tr>
<td>Any HBs Ag positive</td>
<td>26/36</td>
<td>72.2</td>
</tr>
</tbody>
</table>

*1mother’s serum anti-complementary. *p < 0.01 by chi-square.

Hepatitis B Vaccine

- Hepatitis B vaccines (first recombinant, 1986)
  - DTaP-HepB-IPV (Pediarix)
  - DTaP-IPV-Hib-HepB (Vaxelis)
  - HepA-HepB (Twinrix)
  - HepB (Engerix-B, Heplisav-B, Recombivax HB)
- All infants, children or adolescent, adults age 19 through 59 years
- Adults age 60 years or older with risk factors for hepatitis B infection.

**Taiwan: HCC incidence rates before vs after the start of the universal HBV vaccination program**

*Rate ratio of vaccinated/unvaccinated birth cohort:

Chang et al. Gastroenterology 2016
Prevention of Mother-to-Child Transmission

- Infant birth dose HBV vaccine + HBIG is 94% effective in preventing perinatal HBV transmission
- Maternal antiviral therapy started at 28–32 weeks’ gestation when maternal HBV DNA is >200,000 IU/mL

Maternal antiviral prophylaxis if high maternal HBV DNA viral load or HBeAg positive

HBsAg testing, linkage to care and follow up of infants. When available, HBIG for infants born to HBsAg+ and HBeAg+ mothers

At least 3 doses of hepatitis B vaccine, including a timely birth dose within 24 hours

HBeAg: hepatitis B e antigen; HBIG: hepatitis B immune globulin; HBsAg: hepatitis B surface antigen

https://www.who.int/publications/i/item/978-92-4-000270-8
Chronic Hepatitis B Virus Infection

- Patients with perinatally acquired infection
- Minimal/no inflammation
- May last 1-4 decades
- EASL: chronic infection

- High or fluctuating HBV DNA
- Persistent or intermittent fluctuation in ALT
- Active inflammation and liver damage
- EASL: chronic hepatitis

- Low/undetectable HBV DNA
- Normal ALT
- Mild hepatitis/minimal fibrosis, but cirrhosis may be present from previous liver damage
- EASL: chronic infection

- Usually, older patients with more advanced liver disease
- Fluctuating levels of ALT and HBV DNA
- EASL: chronic hepatitis

- After many years of infection in some patients
- Not considered a "cure" because intracellular HBV DNA is still present

Clinical Consequences of Chronic Hepatitis B

REVEAL-HBV Study
- 3653 adults with HBsAg+ recruited in Taiwan 1991
- HCC risk: Older age, alcohol, HBeAg+, cirrhosis (aHR 9.1) and HBV DNA at entry (not ALT)

Chen et al. JAMA 2006
HBV Integration is Associated With Hepatocellular Carcinoma

Sequencing of 81 HBV-positive and 7 HBV-negative HCCs and adjacent normal tissues.

- HBV integration is more frequent in HCC (86.4%) than in adjacent liver tissues (30.7%).
- Copy-number variations were increased at HBV breakpoint locations where chromosomal instability was likely induced.
- 40% of HBV breakpoints within the HBV genome were near the viral enhancer, X gene, and core gene

58 Year-Old Man With CLL Initiating Chemotherapy Including Rituximab

- 58 year-old man born in China who presents to the oncology center to discuss treatment options for newly diagnosed chronic lymphocytic leukemia (CLL)
- No history of hepatitis B
- Family history: Born in Shanghai (1963). His father died of liver cancer
HBV Persists After Spontaneous Recovery

The hepatitis B virus persists for decades after patients’ recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response

Chazouilleres et al 1994
- Post-transplant serum from 207 patients who had been HBsAg negative and found 20 to be HBsAg positive.
- The origin of infection was identified in 7 patients: Occult pre-transplant infection in 5 and occult infection in the donor in 2

Dickson et al 1997
- HBV infection after liver transplant in 18 of 23 recipients of livers from anti-HBc-positive donors (78%) compared with only 3 of 651 recipients of anti-HBc-negative donor livers (0.5%) (P < 0.0001)

HBV Reactivation: All Patients Undergoing Immunosuppressive, or HCV Therapy Must Be Tested For Active/Resolved HBV

- HBV reactivation can cause fulminant hepatitis, leading to liver transplantation or death
- Test for HBsAg, anti-HBs and anti-HBc (total or immunoglobulin G)
- HBsAg+ patients need prophylactic antivirals
- Resolved HBV (HBsAg-/anti-HBc+) can be monitored with ALT, HBV DNA, and HBsAg (every 3 months)
  - Exception: Anti-viral prophylaxis for patient treated with anti-CD20 antibody therapy (e.g., rituximab) or undergoing stem cell transplantation
  - If evidence of reactivation, urgent initiations of antivirals

Current HBV Treatment Approach

- Treatment is not recommended for all persons with active HBV
  - All persons with cirrhosis or HIV infection and those with “high” HBV DNA and ALT levels (both)
  - Guidelines outline complicated algorithms for testing and treatment
- Recommended therapies
  - Peginterferon alfa by SC injections weekly for 48 weeks
  - Nucleos(t)ide analog entecavir or tenofovir by mouth daily
- Duration: Indefinite, until HBsAg loss

- In RCTs comparing entecavir, tenofovir disoproxil, and tenofovir alafenamide, there was no difference in outcomes
  - HBeAg loss, 14 to 30% (higher with IFN)
  - HBsAg loss <1%
  - HBV DNA suppression, 65 to 90% (higher with HBeAg negative)
  - Histologic improvement, 70% (biopsy)

Terrault et al. Hepatology 2018; Tang et al. JAMA 2018
HBV Guidelines For Antiviral Treatment Are Complicated

**HBeAg positive**

**HBeAg negative**

*Upper limit of normal for ALT is 35 U/L for men and 25 U/L for women

26-Year-Old Woman With Untreated HBV

- Born in Vietnam and moved to the United States at age 5 following adoption
  - Diagnosed at age 6
  - No prior HBV treatment
- She recently graduated medical school and engaged to be married
- Her laboratory work-up:
  - HBV DNA 170,000,000 IU/mL
  - HBsAg+, anti-HBs-
  - HBeAg+, anti-HBe-
  - Serum ALT = 19 U/L

Questions
- What is the risk of transmission to others?
  - Her male partner?
  - Her patients?
  - Her future children?
- Should she start treatment?
- How often does she need laboratory tests and monitoring?
- Does this impact her medical career?
- Disclosure of her HBV status?
- Can she be cured?
72-Year-Old Man With Treated Hepatitis B

• 72-year-old partially retired physician with chronic HBV infection since his birth in Taiwan
• Treated with tenofovir for more than 10 years
  – No cirrhosis
  – HBV DNA target not detected
  – HBeAg negative
  – HBsAg +, level = 2456 IU/mL
  – ALT =23 U/L
  – Serum creatinine, 1.5 mg/dL

Questions
• Is the antiviral therapy causing kidney toxicity?
• Must he continue surveillance for hepatocellular carcinoma with ultrasound every 6 months?
• Will he lose HBsAg?
• When can he stop therapy?
• Can he be cured?
No Novel Hepatitis B Therapy Since 1998 (Lamivudine)

- Discovering whole hepatitis B virus particles in blood samples examined
- HBX was firstly reported in HBV
- The recombinant vaccine was licensed
- The widespread application of vaccine in children
- The license of Adefovir
- The license of Tenvudine
- Genome-wide survey of recurrent HBV integration in HCC

- Aa, the Australian antigen was discovered in 1963
- Aa was first reported to involve in the development of hepatitis B in 1975
- Beginning tests of the hepatitis B vaccine in 1981
- IFN-α was a mainstay in treatment of HBV in 1994
- The license of Lamivudine in 1998
- The license of Tenofovir in 2002
- The finding of sodium taurocholate cotransporting polypeptide as a functional receptor in 2006

Liu et al; https://doi.org/10.1016/j.biocel.2013.06.017
### Definitions of Cure of Chronic HBV Infection

<table>
<thead>
<tr>
<th>Type of cure</th>
<th>HBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilizing cure</td>
<td>Eliminate cccDNA from all infected hepatocytes: Not achieved in natural recovery</td>
</tr>
<tr>
<td>Functional cure</td>
<td>Mimic naturally acquired immunity, off antiviral therapy</td>
</tr>
<tr>
<td></td>
<td>• Anti-HBs+ and undetectable HBV DNA</td>
</tr>
<tr>
<td></td>
<td>• Residual cccDNA present</td>
</tr>
</tbody>
</table>

- **Sterilizing cure**
  - Eliminate cccDNA from all infected hepatocytes: Not achieved in natural recovery

- **Functional cure**
  - Mimic naturally acquired immunity, off antiviral therapy
  - Anti-HBs+ and undetectable HBV DNA
  - Residual cccDNA present

- **Sustained Virological Response**
  - (sAg +ve, DNA negative, off therapy)
  - An advance but not enough of one

- **Functional Cure**
  - (sAg loss with undetectable DNA & Normal ALT)
  - Challenging but achievable goal

- **Sterilizing cure**
  - (cccDNA loss)
  - Too hard to achieve

88% of attendees at EASL/ASSLD HBV Endpoints conference chose Functional Cure as the preferred goal for future therapies.
Potential Targets In the HBV Lifecycle

- 5' Cap (A)n 3'
- Translocation
- dAdAdG
- New (-) strand DNA synthesis
- pgRNA
- DNA Synthesis
- Encapsidation of pgRNA
- Golgi complex
- Release
- CCC DNA
- DNA repair
- HBV RNA
- Transcripts
- Pregenomic RNA
- Attachment and Penetration
- S Ag
- e Ag
- HBV Virion
- Envelope Proteins S, M, L
- Polymerase Protein
- Core Protein
- uncoating
- transport to cell nucleus
- Block Entry
- Stimulation of innate and/or adaptive immunity
- Target cccDNA - Destruction - Inactivation
- Target HBV RNA
- Target packaging
- Core Protein
- DNA Synthesis
- Target DNA synthesis
- Nucleic Acid Polymers
- Nucleos(t)ide Analogues
- Myrcludex B (late stage, HDV)
- Innate
- Interferon TLR7/8 agonist
- Adaptive
- Vaccines Checkpoint inhibitors
- RNAi/ASO (phase 2)
- CRISPR/CAS 9 (pre-clinical)
- Capsid Assembly Modulators (phase 2)
- Stimulation of innate and/or adaptive immunity
- Block Entry
- Target cccDNA - Destruction - Inactivation
- Target HBV RNA
- Target packaging
- Core Protein
- DNA Synthesis
- Target DNA synthesis
- Nucleic Acid Polymers
- Nucleos(t)ide Analogues
- Myrcludex B (late stage, HDV)
- Innate
- Interferon TLR7/8 agonist
- Adaptive
- Vaccines Checkpoint inhibitors
- RNAi/ASO (phase 2)
- CRISPR/CAS 9 (pre-clinical)
- Capsid Assembly Modulators (phase 2)

Slide courtesy of Jorden Feld, MD
First Combination Trial: CAM + siRNA

Core Assembly Modulators

2 mechanisms:
1. Block encapsidation – decrease HBV DNA & RNA
2. Block cccDNA formation

siRNA

2 mechanisms:
1. Block viral replication – pgRNA
2. Block Ag production – restore immunity
REEF-1: siRNA + CAM

- siRNA (3989) (40, 100 or 200) vs (6379) vs combination (siRNA + CAM) x 48 wks + 24 wks f/U
- Non-cirrhotic, nuc suppressed or naïve, HBsAg>100 IU/mL – stratified by HBeAg & treatment

- Combination therapy did worse
- siRNA alone at 200 mg was most effective

**Patients (%) meeting NUC stopping criteria**

**Yuen MF AASLD 2021**
Combination Therapies Are Under Investigation, Including Immune Active Agents

**HBV DNA suppression** + **Viral Protein Depletion (s, x, core)** + **Immune Target**

- Nucleos(t)ide analogue
- +/- CAM
- +/- RNAi
- +/- cccDNAi
- RNAi
- Nucleic Acid Polymers
- cccDNAi
- Interferon
- Anti-PD1/PDL1
- Therapeutic vaccine
- TLR agonist

**FDA guidelines:** Safety is paramount “in the context of the underlying disease and current treatment options available for the indication”
Targeting the Hepatitis B Covalently Closed Circular (ccc) and Integrated DNA With Gene Editing to Achieve Sterilizing Cure and Reduce Residual HCC Risk

Liver HBsAg staining at 4 weeks after dosing with LNP-containing Gen5 ARCUS-POL nuclease mRNA in AAV mouse model

Gorsuch et al. Targeting the hepatitis cccDNA with a sequence-specific ARCUS nuclease to eliminate hepatitis B virus in vivo. Molecular Therapy (2022)
2016 WHO Called For Hepatitis Elimination as a Public Health Threat By 2030 Compared to 2015 Baseline

“A world where viral hepatitis transmission is halted and everyone living with hepatitis has access to safe, affordable and effective care and treatment services”

90% reduction in new chronic infections

<table>
<thead>
<tr>
<th></th>
<th>2015</th>
<th>2020</th>
<th>2030</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>4.7 million</td>
<td>3.3 million</td>
<td>470,000</td>
</tr>
<tr>
<td>HCV</td>
<td>1.75 million</td>
<td>1.23 million</td>
<td>175,000</td>
</tr>
</tbody>
</table>

65% reduction in mortality rates

<table>
<thead>
<tr>
<th></th>
<th>2015</th>
<th>2020</th>
<th>2030</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>884,000</td>
<td>796,000</td>
<td>309,000</td>
</tr>
<tr>
<td>HCV</td>
<td>400,000</td>
<td>360,000</td>
<td>140,000</td>
</tr>
</tbody>
</table>

HBV Elimination As Public Health Threat Is Not On Track

HBV care continuum

Polaris Observatory: HBV progress elimination targets 2020 data
This day was chosen to commemorate the birthday of Nobel Laureate Professor Baruch Samuel Blumberg, who discovered the hepatitis B virus and developed the first hepatitis B vaccine (HBV).
Hepatitis B Can’t Wait

• Chronic hepatitis B is a major cause of mortality globally
  – 296 million people with HBV; 68% in Africa and Western Pacific
• Current vaccine and treatments (NrtIs) can prevent infection and disease
• We are not on track to meet WHO targets for the elimination of HBV as public health threat by 2030
  – Increase access to birth-dose vaccine
  – Increase access to current treatments
• No novel therapies since 1996
• The global momentum to promote the fight against viral hepatitis and the effective curative treatment of hepatitis C virus (HCV) creates a fertile ground for a global push for an HBV cure
  – International Coalition to Eliminate HBV (ICE-HBV)