Hepatitis C & E virus

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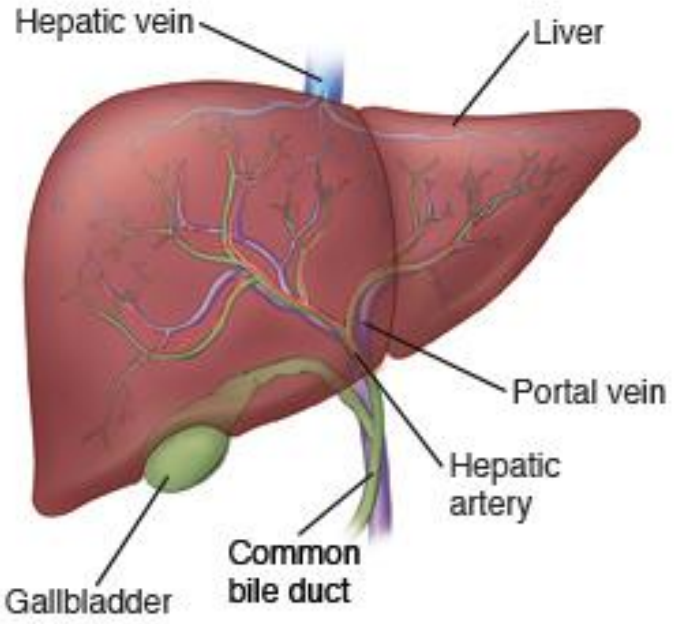
Part 1:

What is hepatitis?

What is hepatitis?

Inflammation of the liver…



Function of the liver:

- Production of **bile** - helps carry away waste and break down fats in the small intestine during digestion
- Production of **hormones**
- Balancing and production of **glucose** as needed

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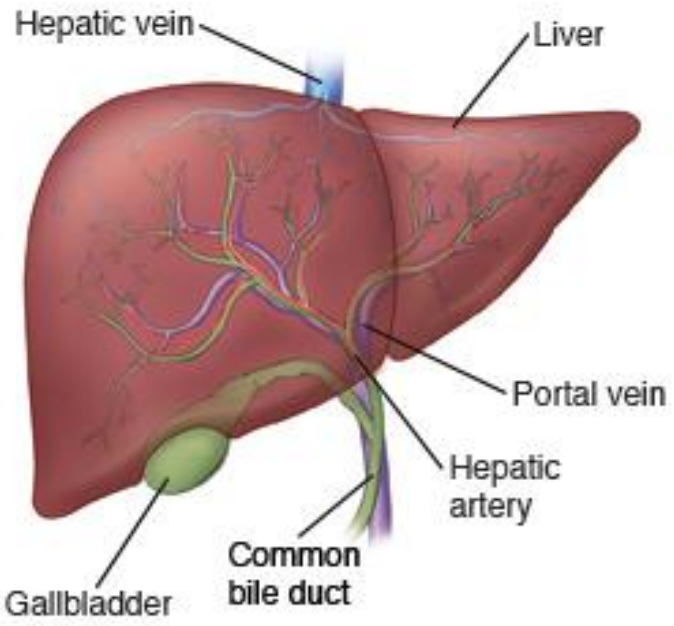

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- Processing of hemoglobin for distribution of its iron content - the liver **stores iron**
- Stores **vitamins –** Liver is nature`s multivitamin

What is hepatitis?

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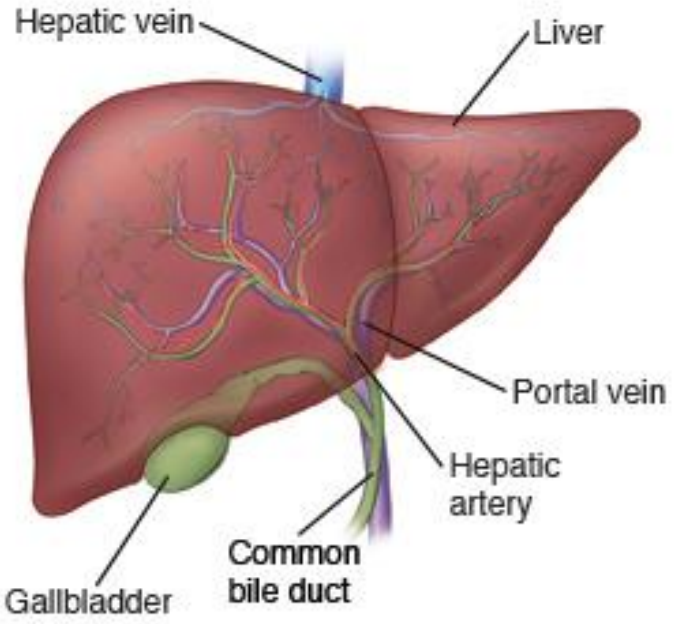

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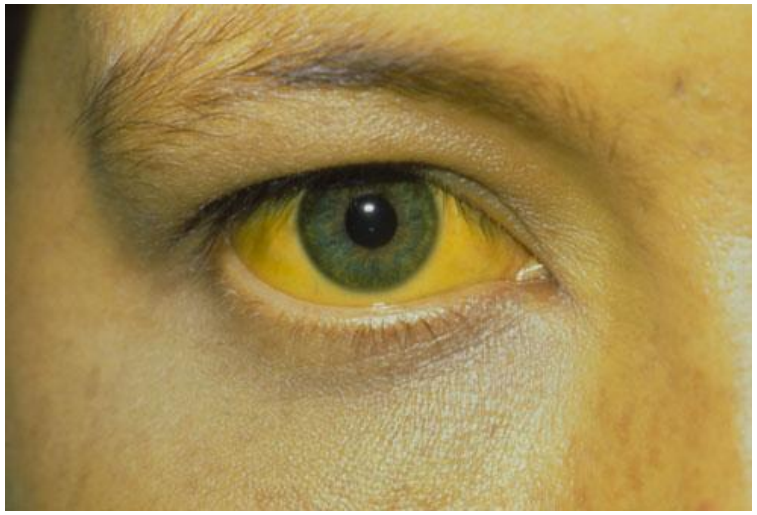
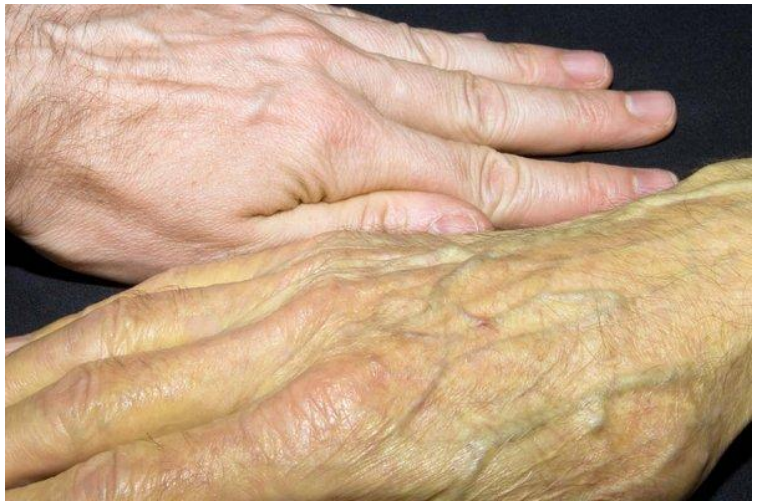
- **Clearing** the blood of **drugs** and other **toxic substances**
- **Decomposition** of **red blood cells**
- **Clearance** of **bilirubin**

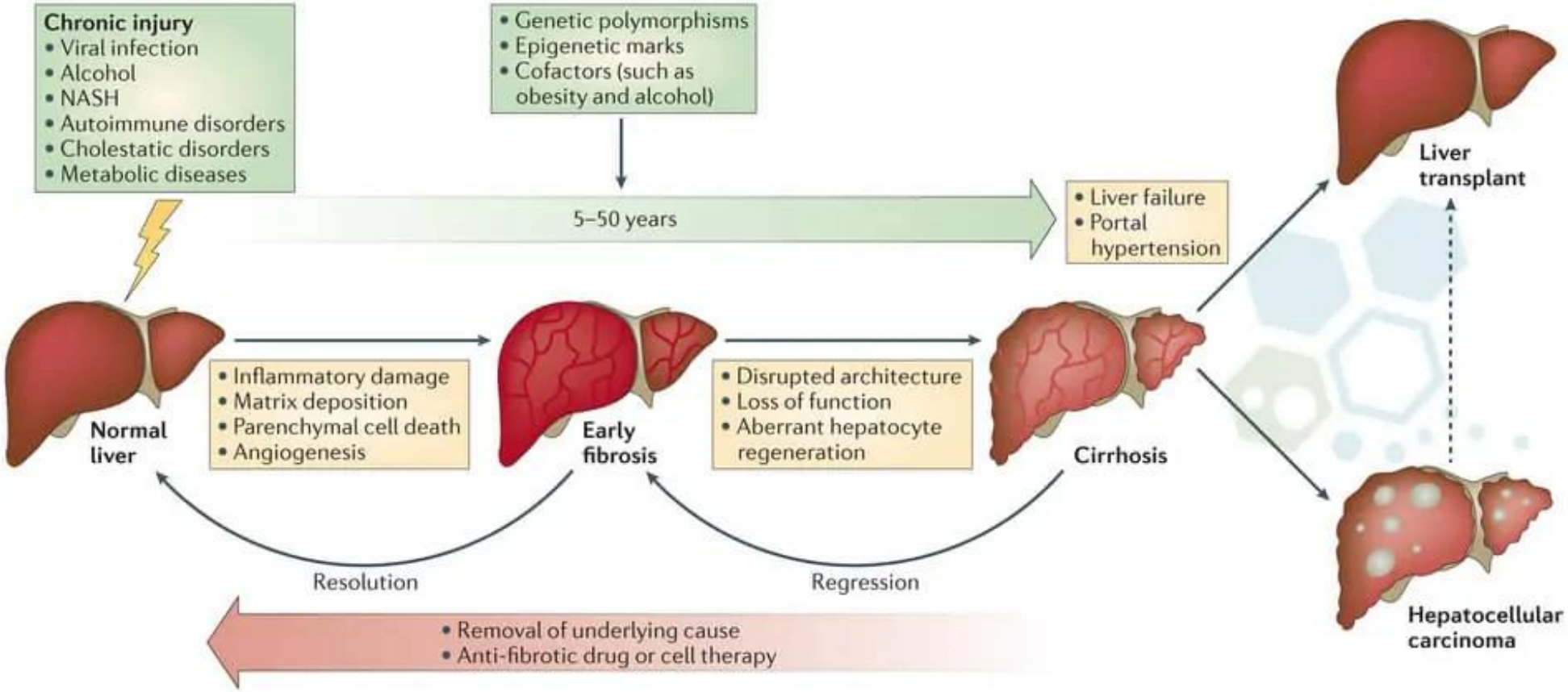
What is hepatitis?

Inflammation of the liver… – often caused by viral infection





What is hepatitis?



→ **Fibrosis** = fibrous connective tissues, that occur as a reparative response to liver injury or damage

→ **Cirrhosis** = scarring that damages the liver and interferes with its functioning; is a result of persistent liver damage over many years

The alphabet of hepatitis viruses

Medizinische Hochschule Hannover

Hepatitis A – „acute“

Hepatitis B – „bad“

Hepatitis C – „chronic or cure“

Hepatitis D – „devil“

Hepatitis E – „emerging“

Types of viral hepatitis?

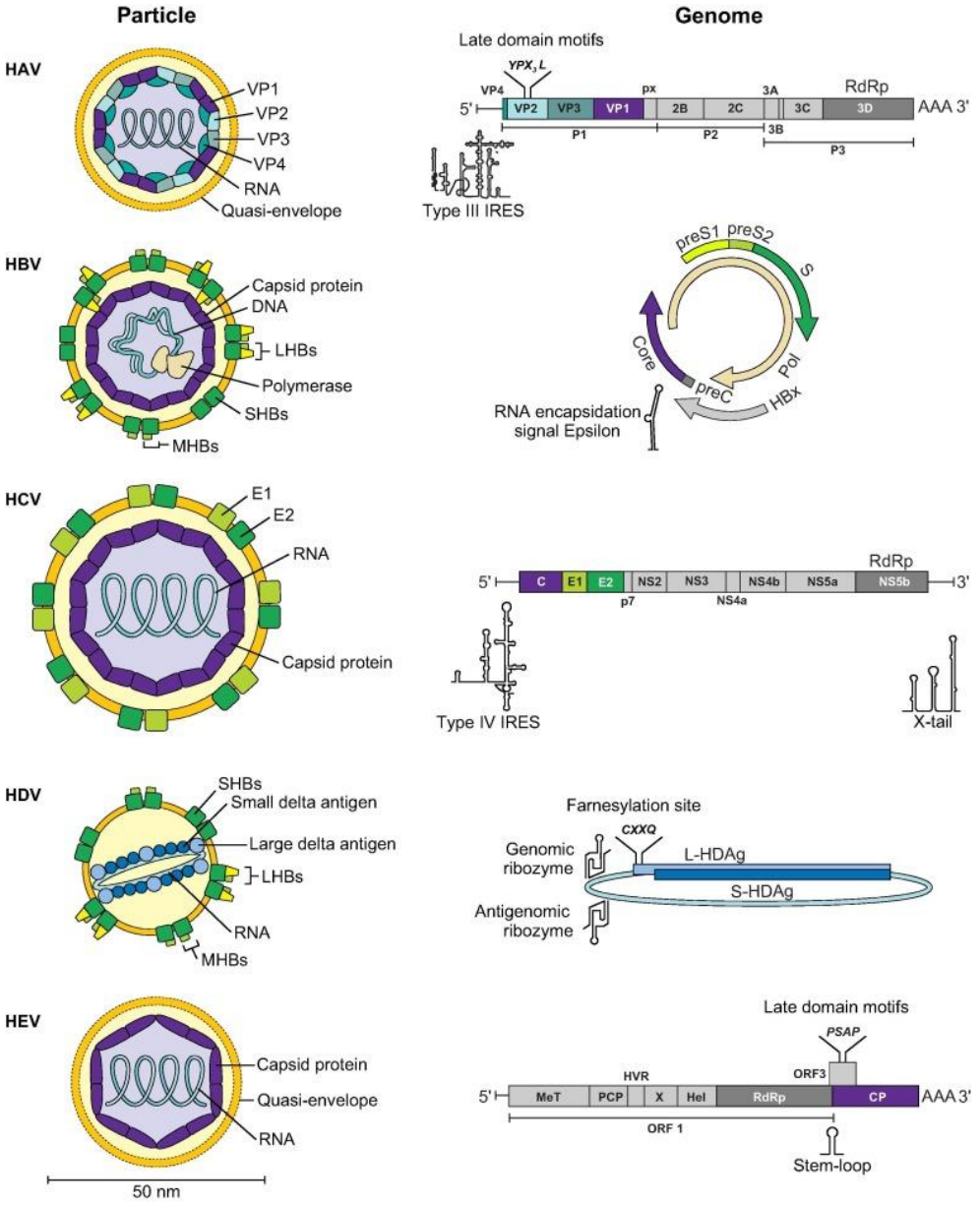
Hepatitis A to E: Are they all relatives? (no…)

Table 1. Properties of human hepatitis viruses.

	HAV	HBV	HCV	HDV	HEV
Virus family, genus	*Picornaviridae, Hepatovirus*	*Hepadnaviridae, Orthohepadnavirus*	*Flaviviridae, Hepacivirus*	Unassigned, *Deltavirus*	*Hepeviridae, Orthohepevirus*
Genome type	Positive-sense linear ssRNA	Circular, partially dsDNA (full length negative-sense, partial positive-sense), replication via reverse transcription	Positive-sense linear ssRNA	Viroid-like, negative-sense circular ssRNA	Positive-sense linear ssRNA
Approx. genome length (nt)	7,500	3,200	9,600	1,700	7,200
Virion diameter (nm)	27–32	42	55–65	36–43	30–34
Envelope	No/quasi-enveloped	Yes	Yes	Yes	No/quasi-enveloped
Course of infection	Acute[61]	Acute/chronic (children 30–90%; adults <5%)[62]	Acute/chronic (80–85%)[63]	Acute/chronic (>80% if superinfection)[64]	Acute/chronic (<1%)[65]
Predominant transmission routes	Mainly faecal-oral, parenteral	Vertical, parenteral, sexual	Parenteral	Parenteral, sexual	Faecal-oral, food-borne, parenteral
Cellular receptor	Unknown[66]	NTCP, heparan sulfate proteoglycans[67,68]	CD81, SR-B1, LDL receptor, claudin-1, occludine[69–71]	NTCP, heparan sulfate proteoglycans[67,72]	Unknown

dsDNA, double-stranded DNA; nt, nucleotide; NTCP, sodium taurocholate co-transporting polypeptide; ssRNA, single-stranded RNA.

Rasche et al. 2019



Question #1

Hepatitis is…

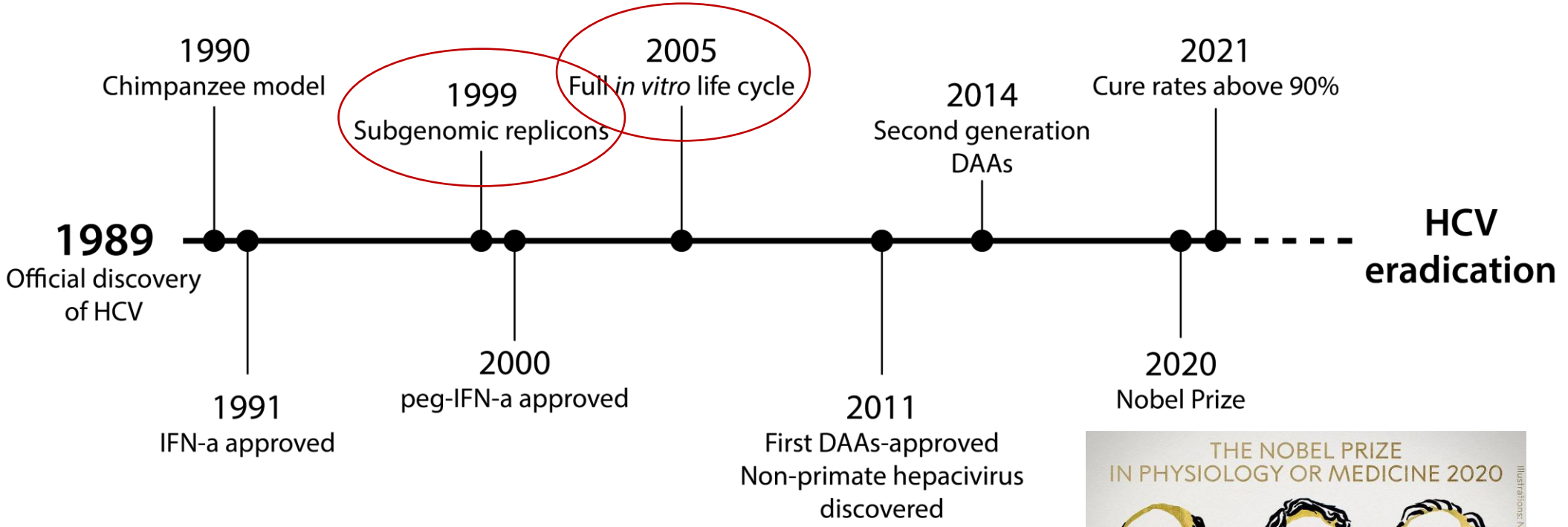
a) metabolic disease
b) inflammation of the liver
c) often caused by viruses
d) Liver cancer

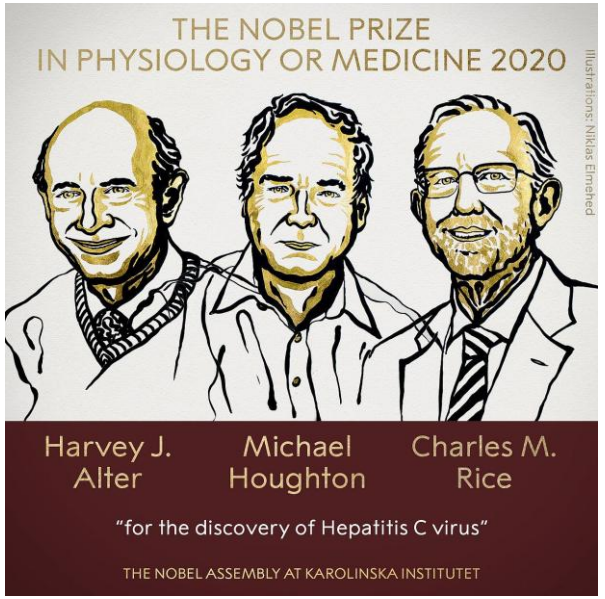
Part 2:

What is hepatitis C?

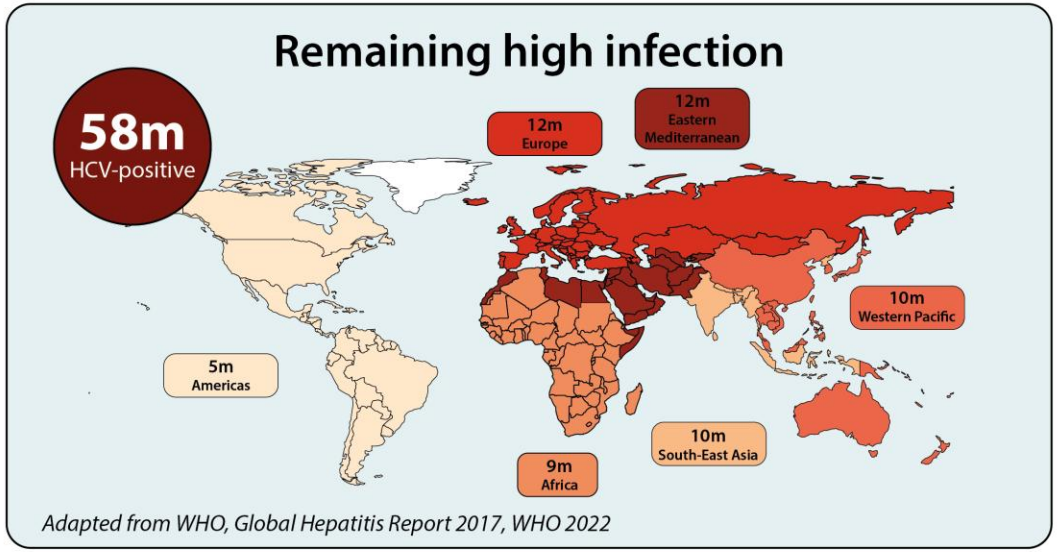
→ Inflammation of the liver caused by the hepatitis C virus

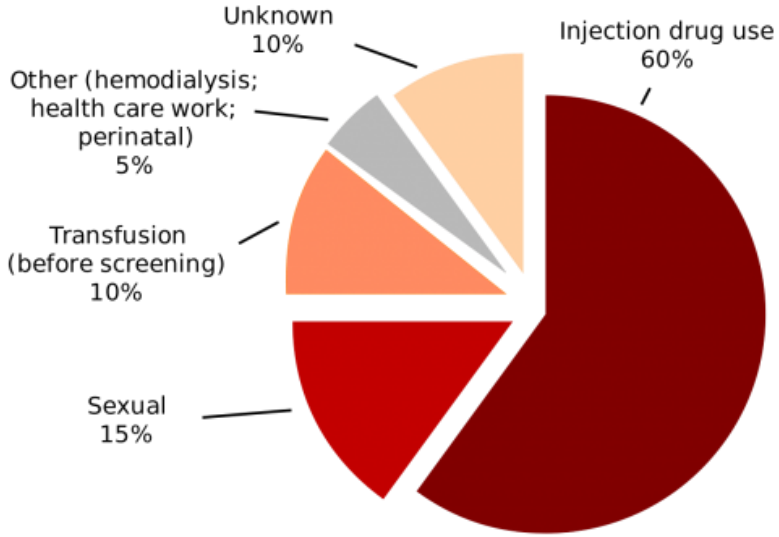
Discovery and History of Hepatitis C Virus



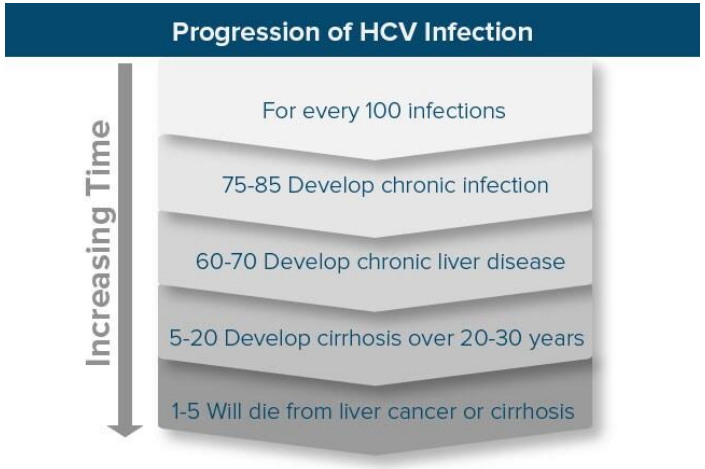


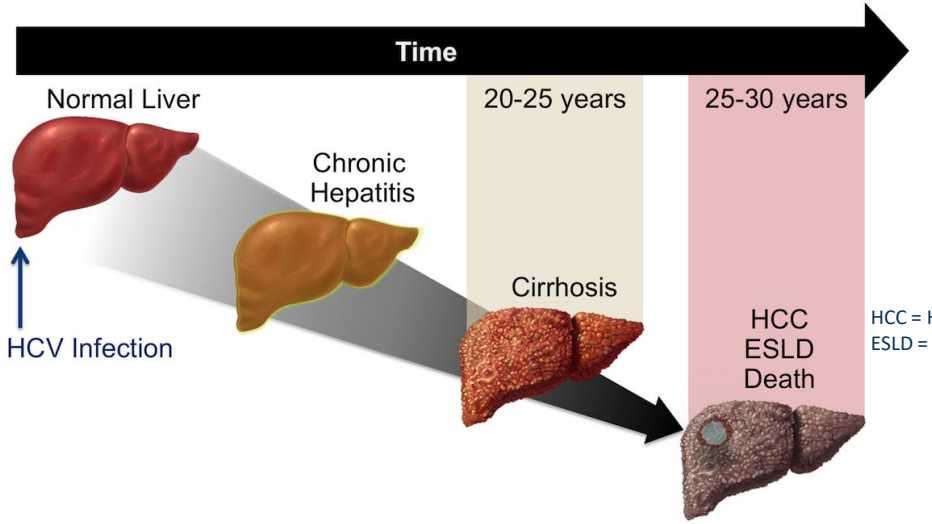
Hepatitis C Virus Prevalence and Transmission





Blood-borne transmission!

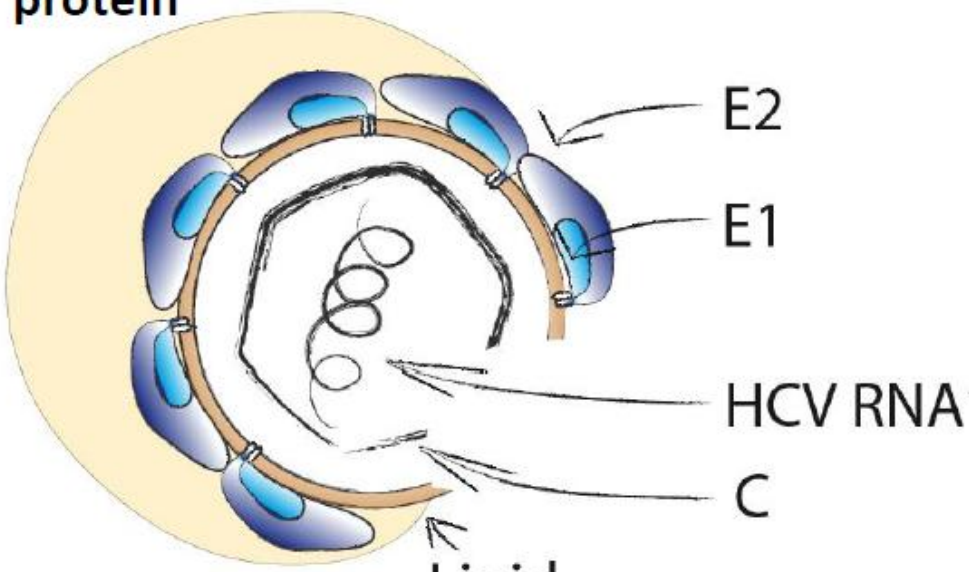




HCC = Hepatocellular calcinoma
ESLD = End-stage liver disease

What is the Hepatitis C Virus?

Family:	*Flaviviridae*
Genus:	*Hepacivirus*
Species:	*Hepatitis C Virus* (7 Genotypen)
Diameter:	50-60 nm
Genome:	(+) RNA, ~9.6 kb



HCV host tropism

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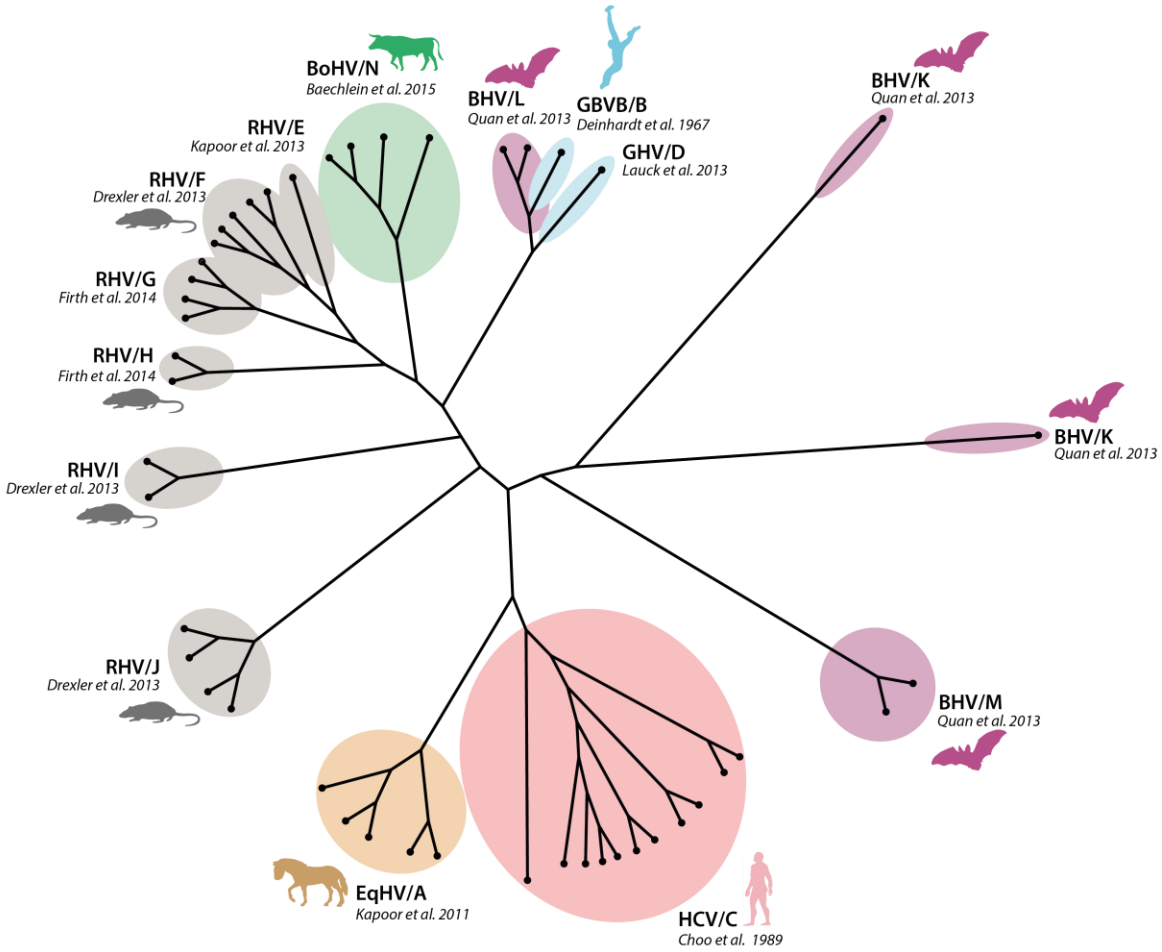
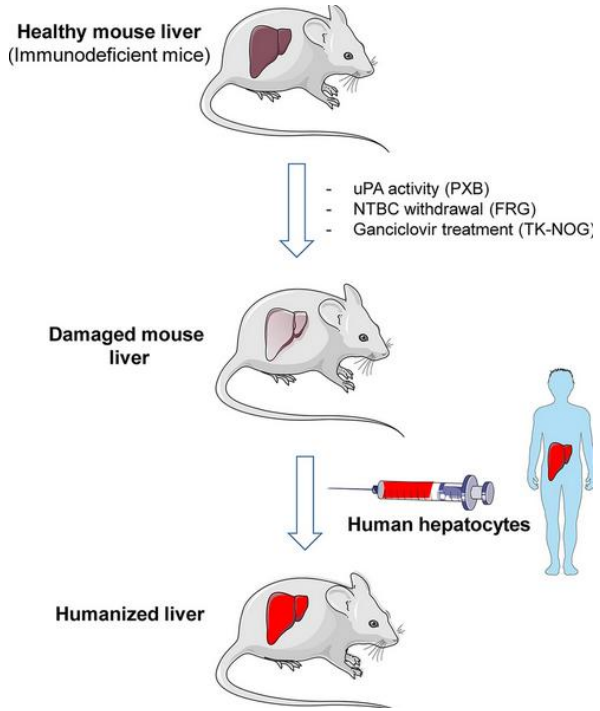
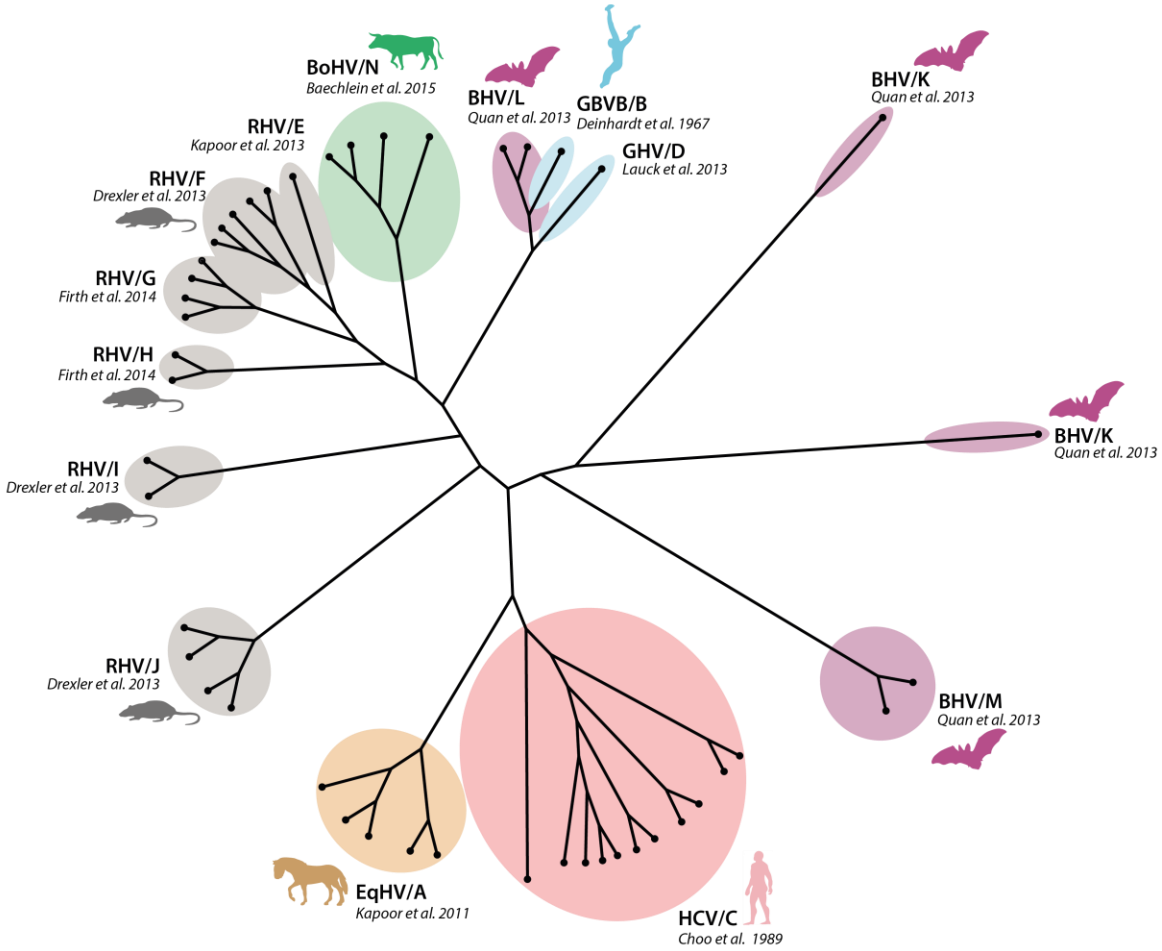


HCV animal models

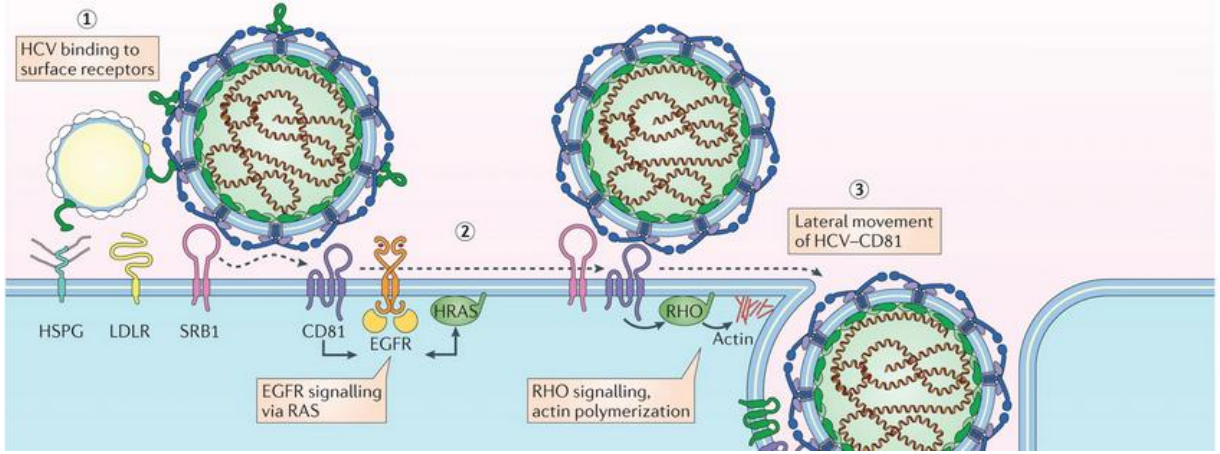
Chimpanzees

Liver chimeric mice





HCV attachment and entry



→ Time and space defined process

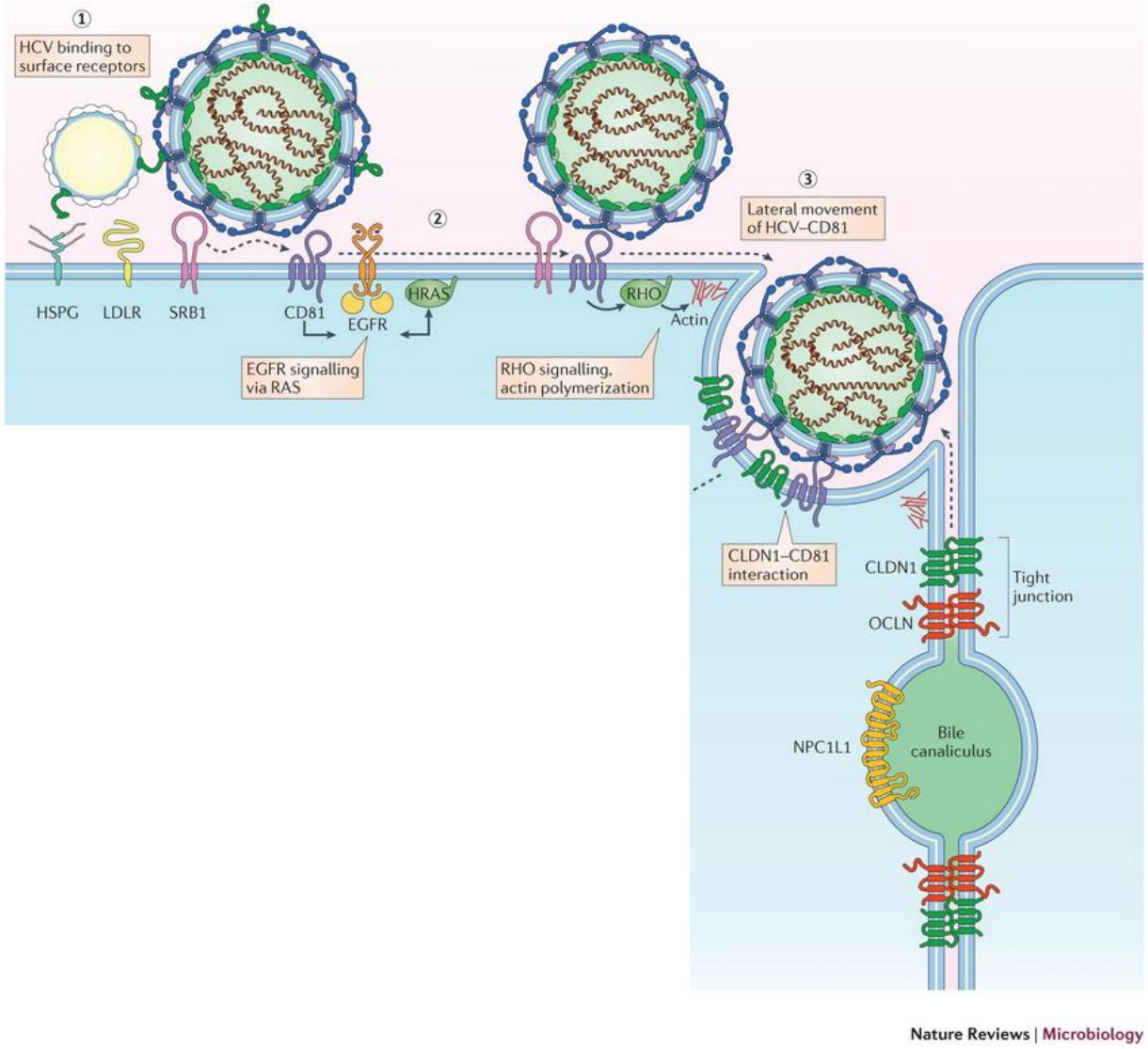
→ Low-affinity binding of HCV to two host receptors (**LDLR** = low density lipoprotein receptors, **HSPG** (heparin sulfate proteoglycans)

→ This triggers the HCV outer **E1/E2 heterodimer** membrane protein to **bind to** the scavenger receptor B1 (**SRB1**) and the tetraspanin protein **CD81**

→ **Interaction between E2 and CD81** then **activates** signal transduction through epidermal growth factor receptor (**EGFR**), **HRAS** and **RHO GTPases**

→ These signaling events create a **wave** in the lipid membrane, propelling the HCV particle to a **tight junction** between hepatocytes

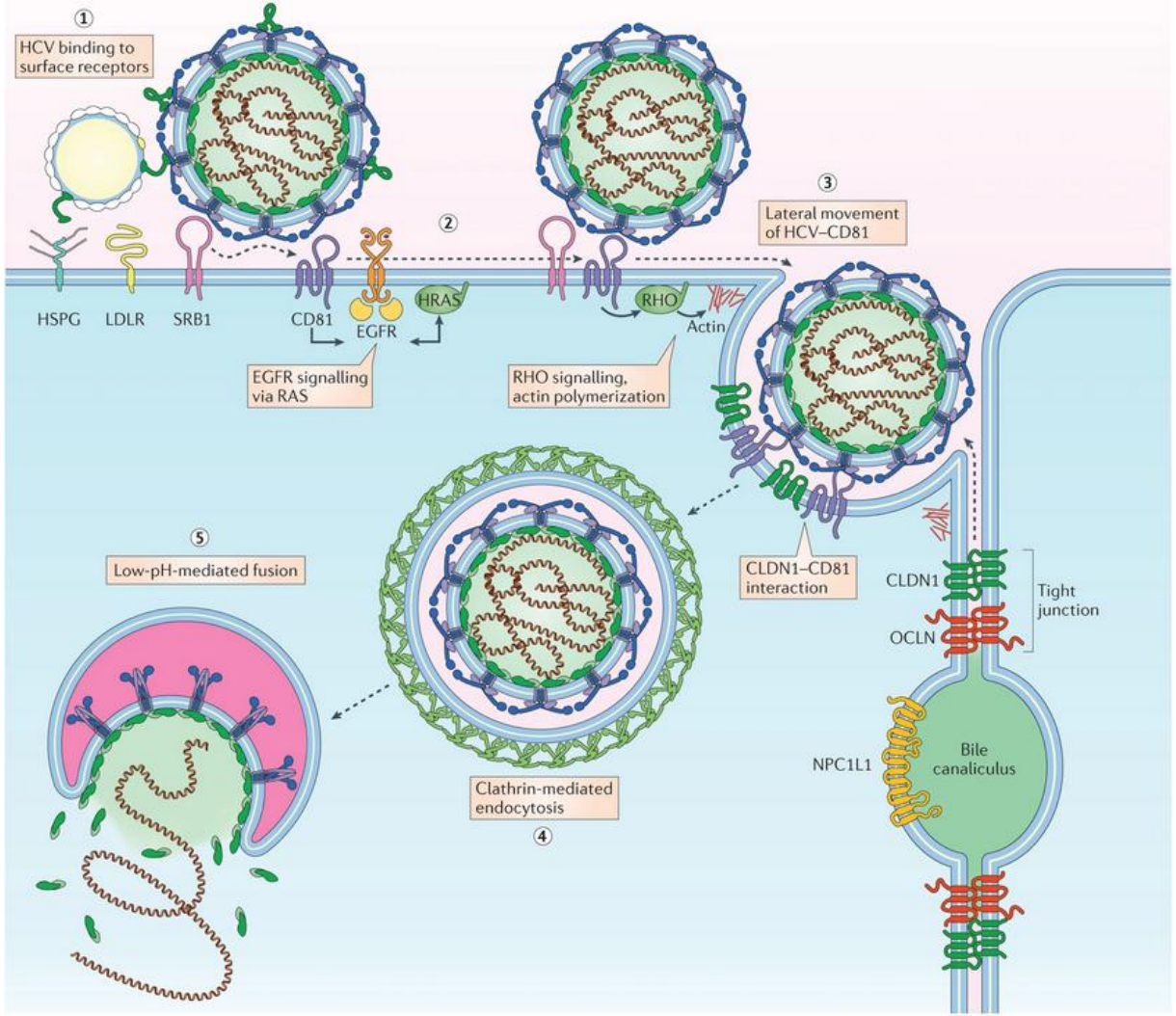
HCV attachment and entry



→ **CD81 interacts with claudin-1 (CLDN1)**, which initiates inward folding of viral particles and hepatocyte cell membrane

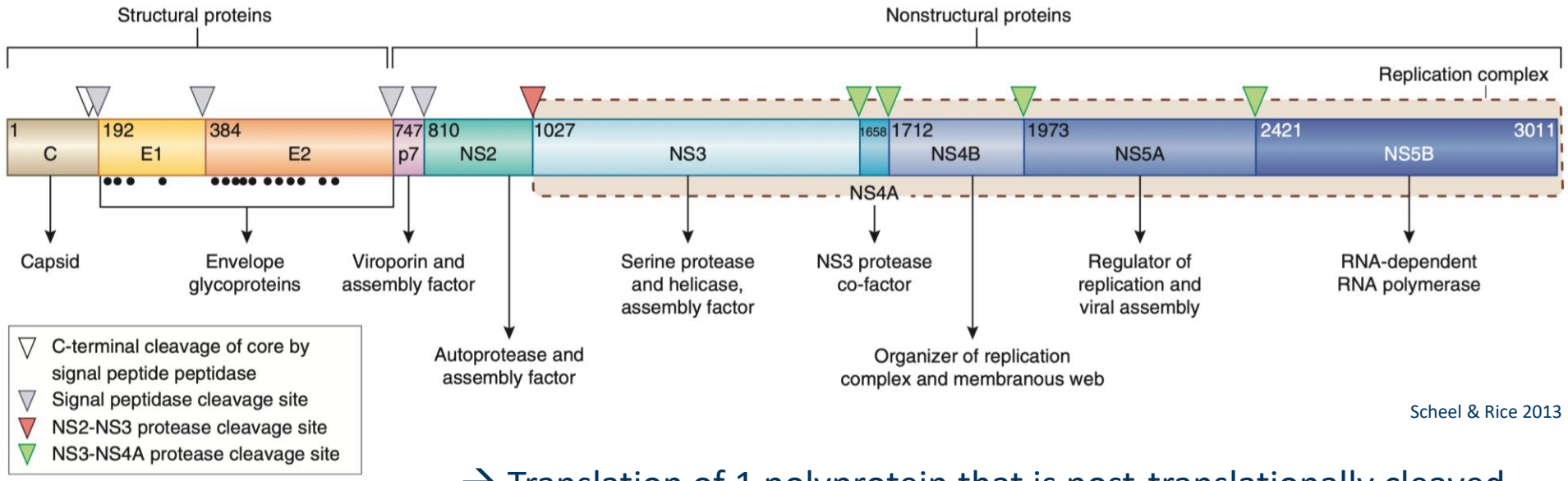
→ HEV gets engulfed into the **endosome**, which is covered by a **clathrin cage**

HCV attachment and entry



- → **CD81 interacts with claudin-1 (CLDN1)**, which initiates inward folding of viral particles and hepatocyte cell membrane
- → HEV gets engulfed into the **endosome**, which is covered by a **clathrin cage**
- → **Clathrin cage disperses** upon entry
- → **Acidic pH** within the endosome triggers **fusion** between viral and host membrane
- → **HCV RNA** is released into the **cytosol** for translation and replication

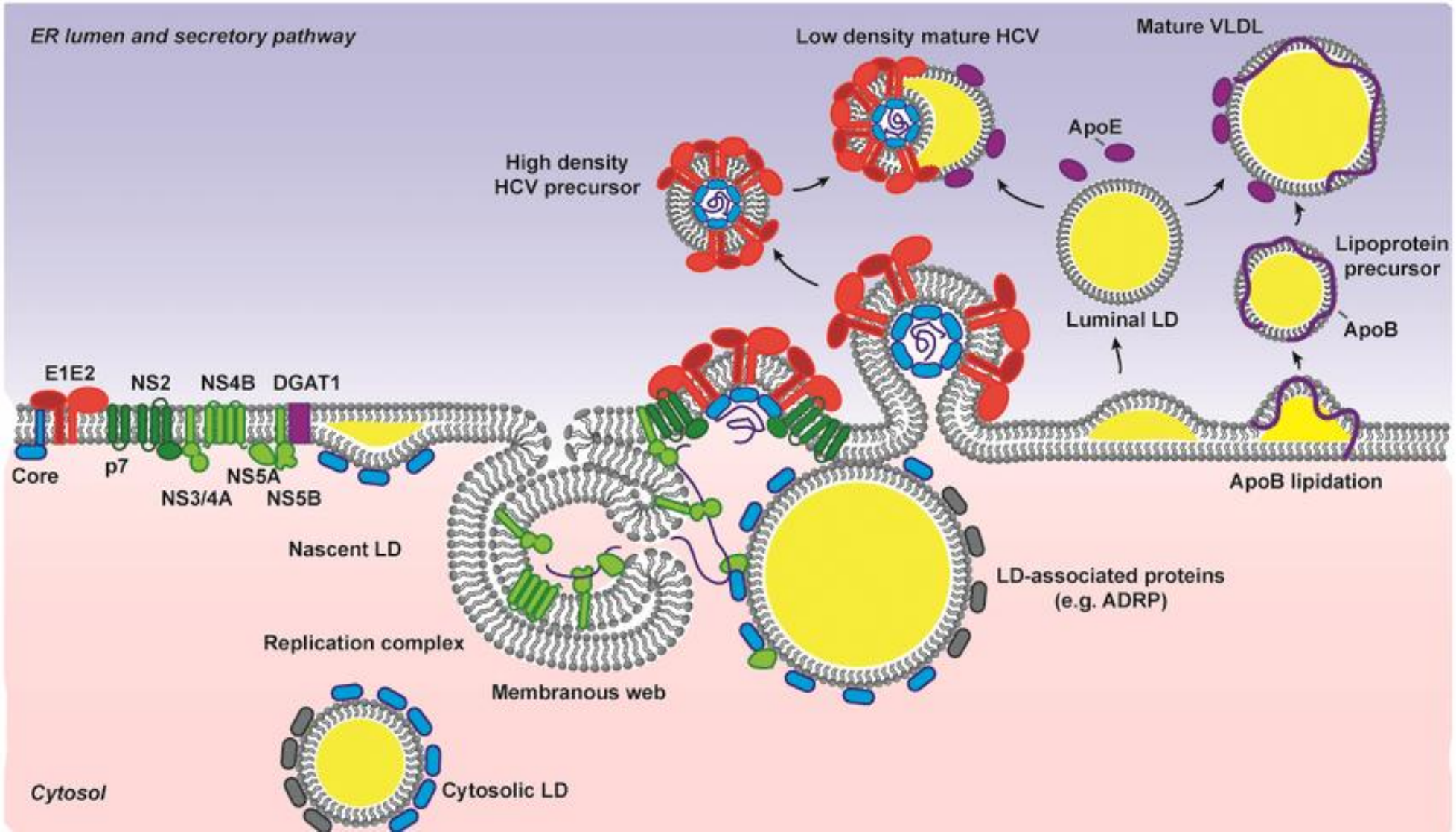
Genome of Hepatitis C Virus



Scheel & Rice 2013

→ Translation of 1 polyprotein that is post-translationally cleaved

HCV replication, assembly and release

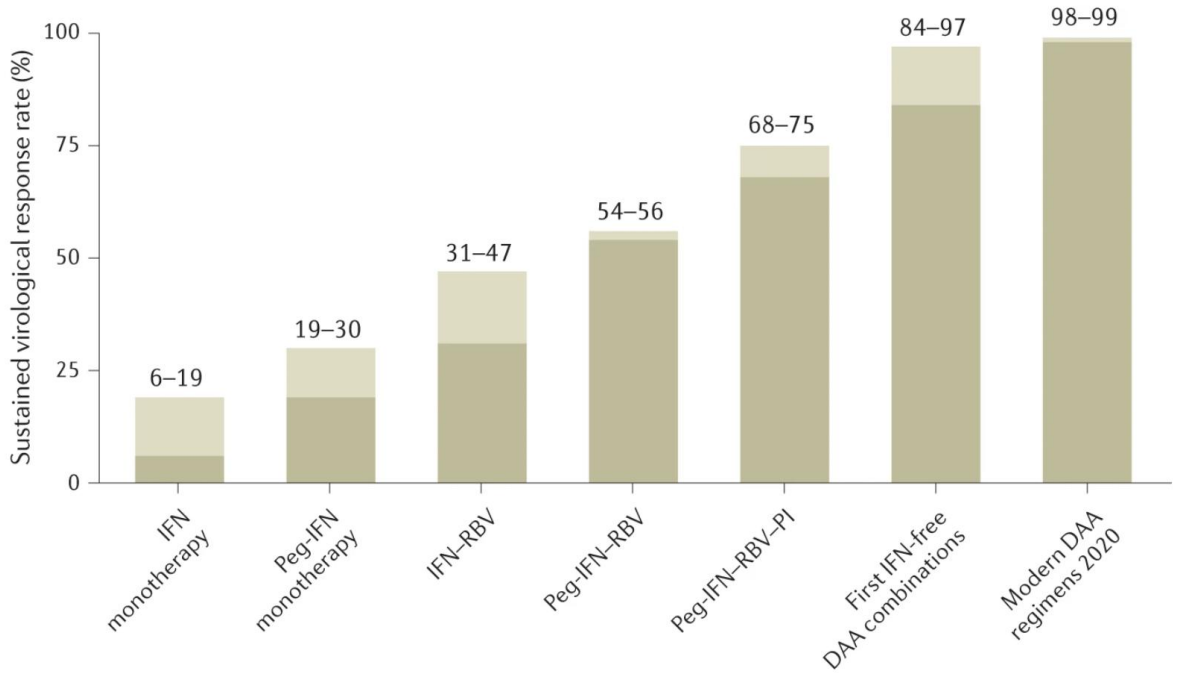


Question #2

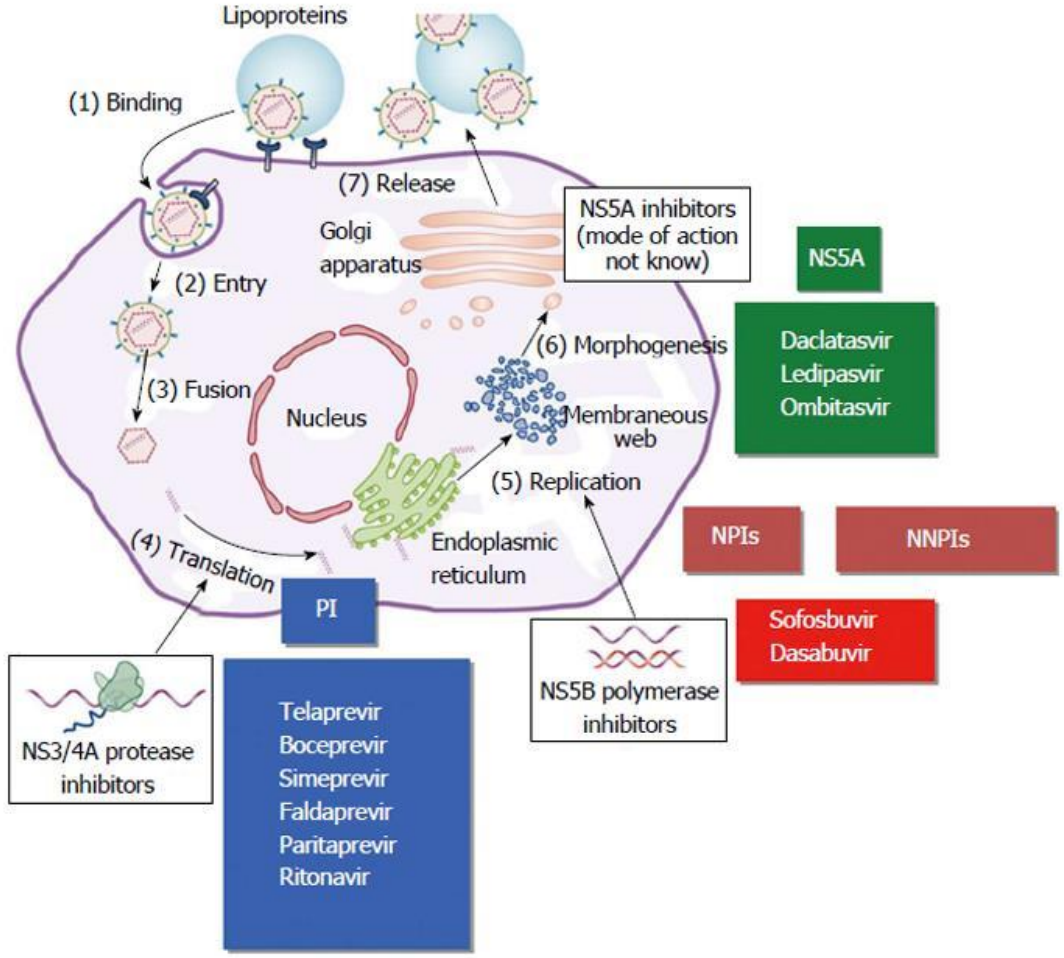
Hepatitis C viruses…

a) Contains a negative sense RNA genome
b) Enter cells through clathrin-mediated endocytosis
c) Encode a RdRp and viral proteases on their genomes
d) Replicate in the nucleus of host cells

How to treat chronic HCV



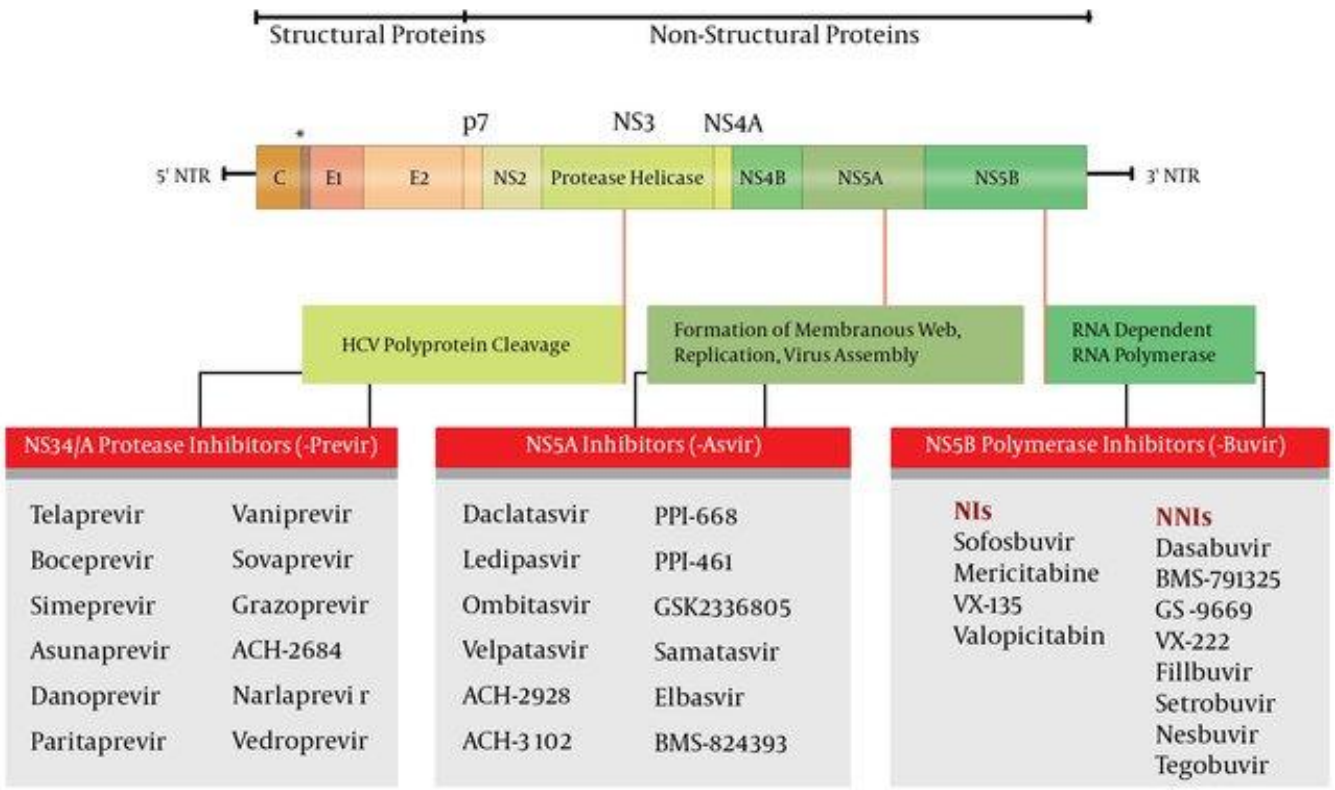
Manns & Maasoumy. *Nat. Rev. Gastro & Hepatol.* 2022



Alavian et al. 2016, Bertino et al. 2015

→ Multiple virus structures can be targeted

How to treat chronic HCV



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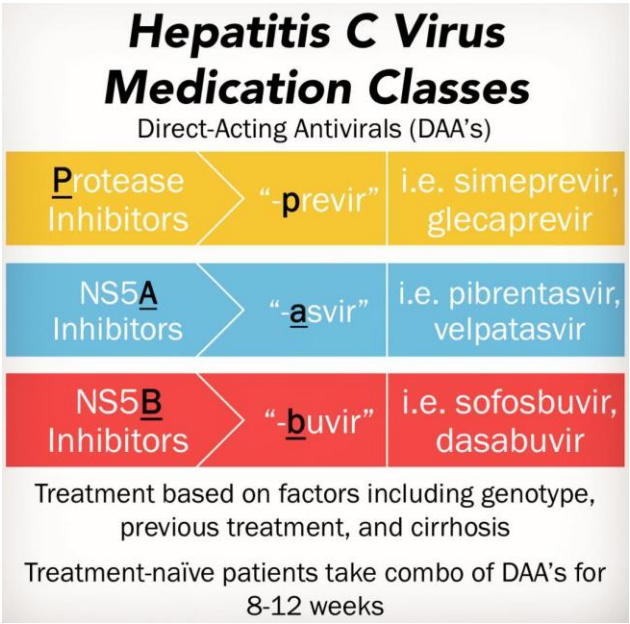
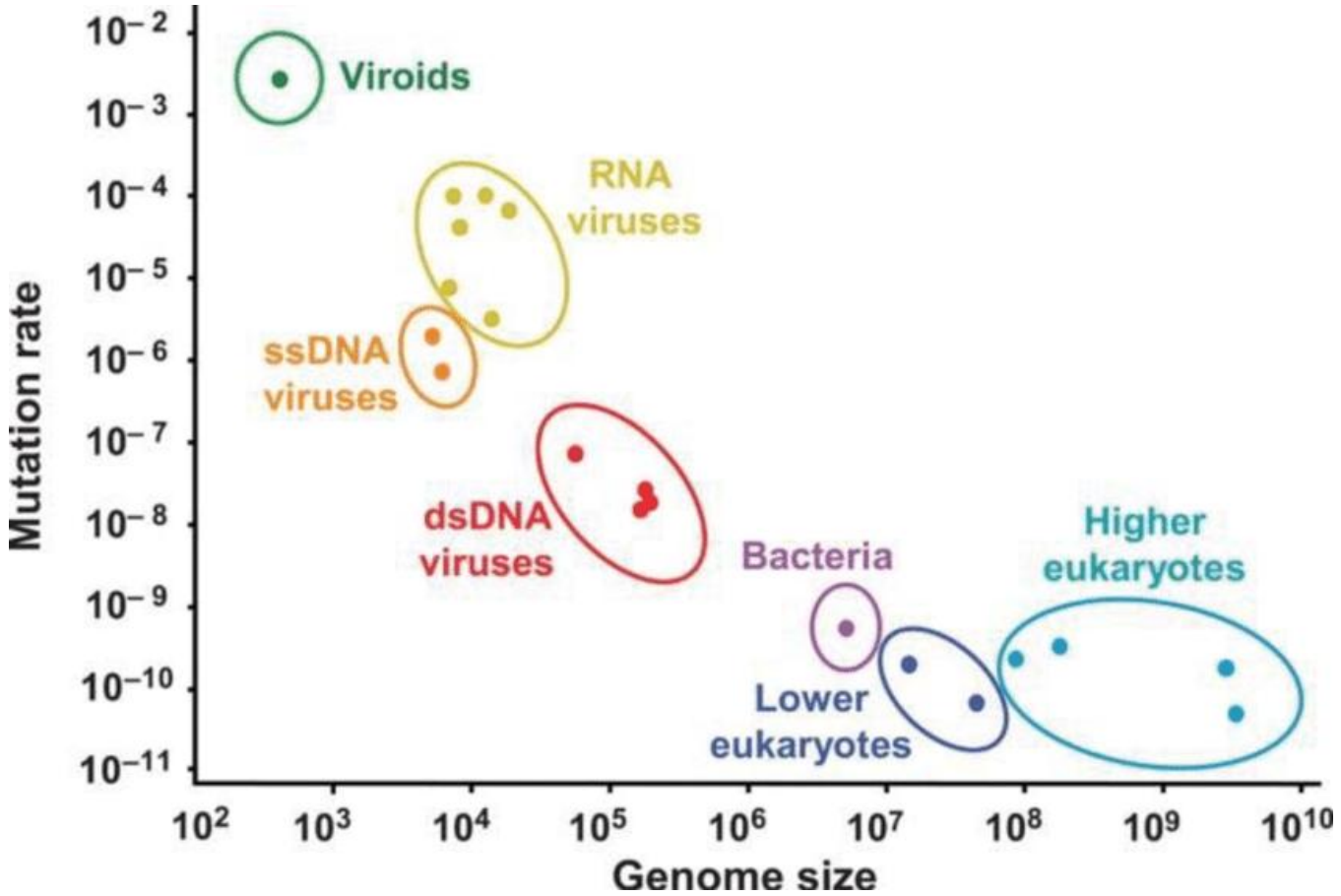


→ Suffix can give a hint on target structure

Viral diversity and its implication for treatment success



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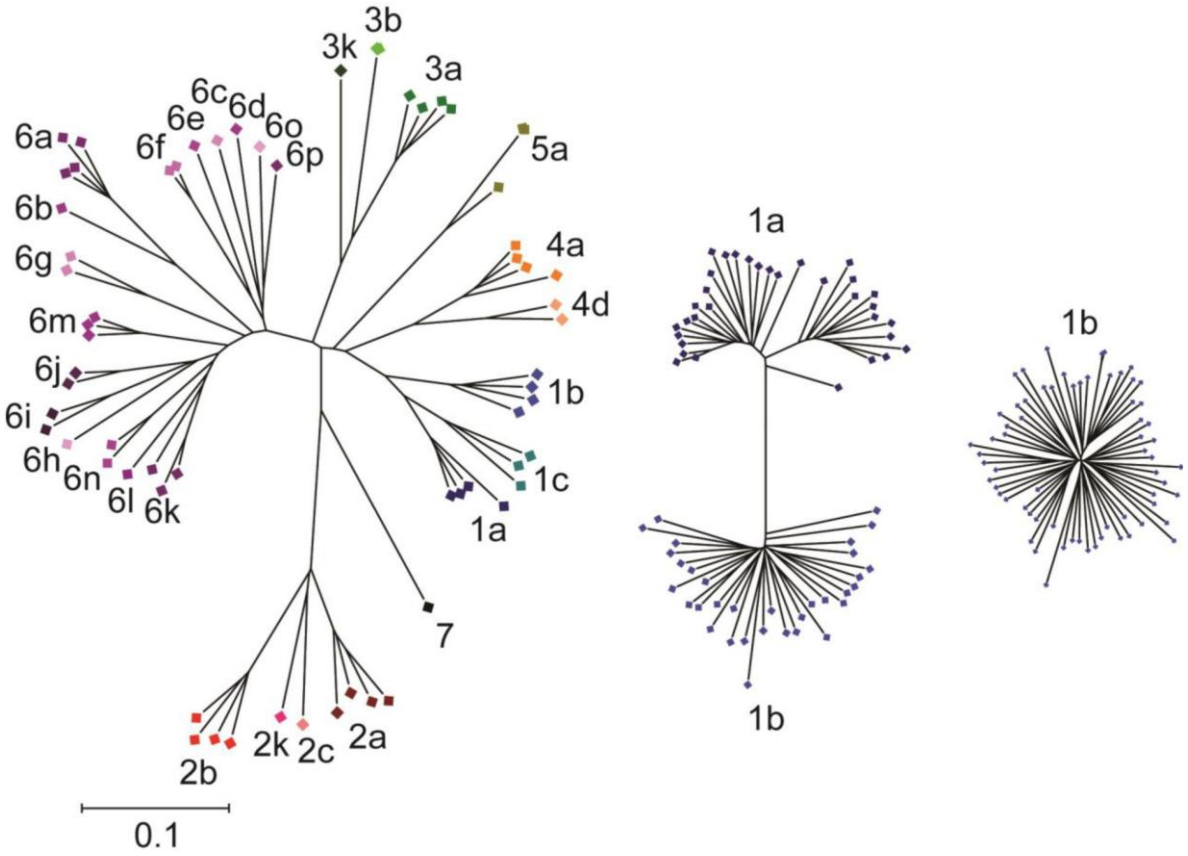


HCV variability

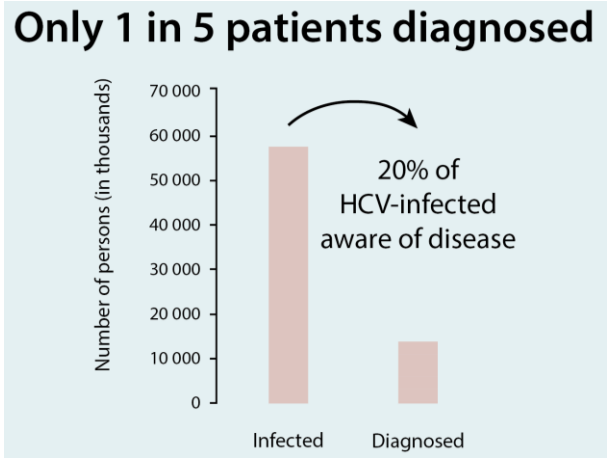
- >30% variability between genotypes
- ~20% variability between subtypes
- error prone RNA replication

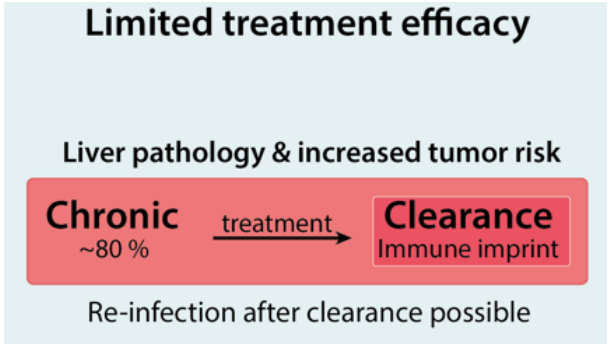
  (1 Mutation per genome)
- high replication rates: 10^{12} new particles/day

→ Increases chance of acquiring resistance mutations or to evade immune responses

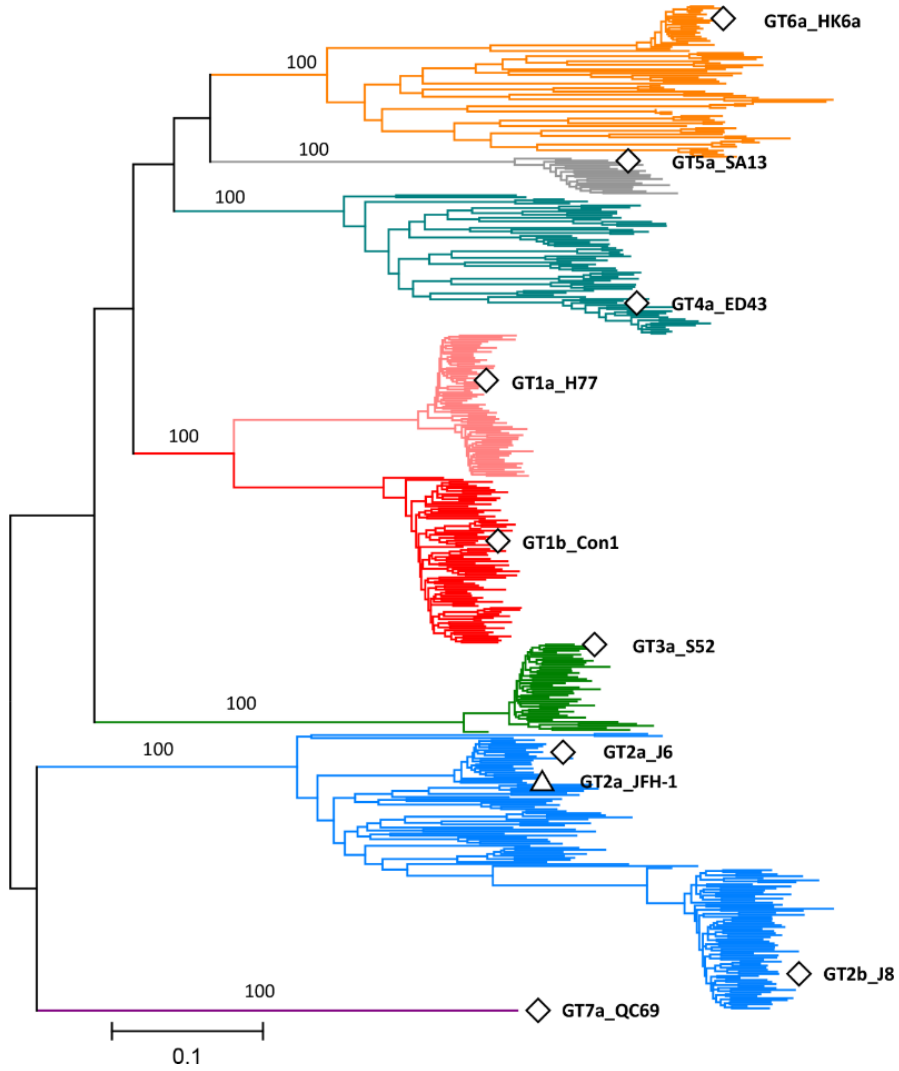
Current HCV challenges

- Limited access to DAAs in developing nations where the disease burden is greatest
- Limited surveillance in developing nations
- Majority of chronically infected patients are undiagnosed
- Re-infection is possible after DAA cure
- No prophylactic vaccine available

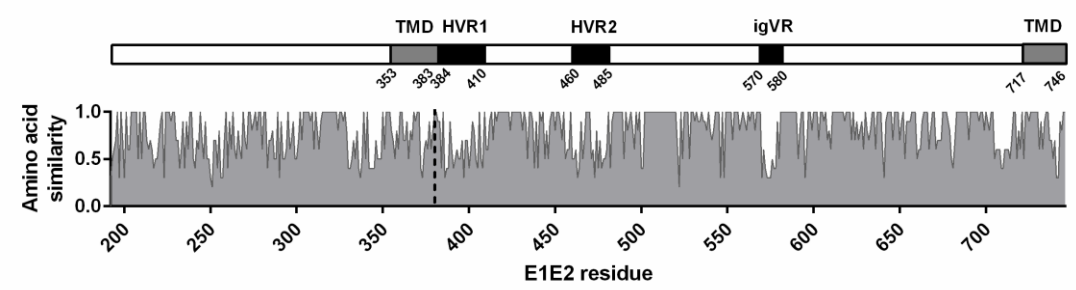


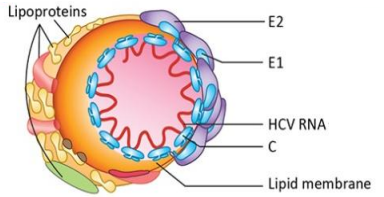


Barriers to an HCV vaccine: viral genetic variability



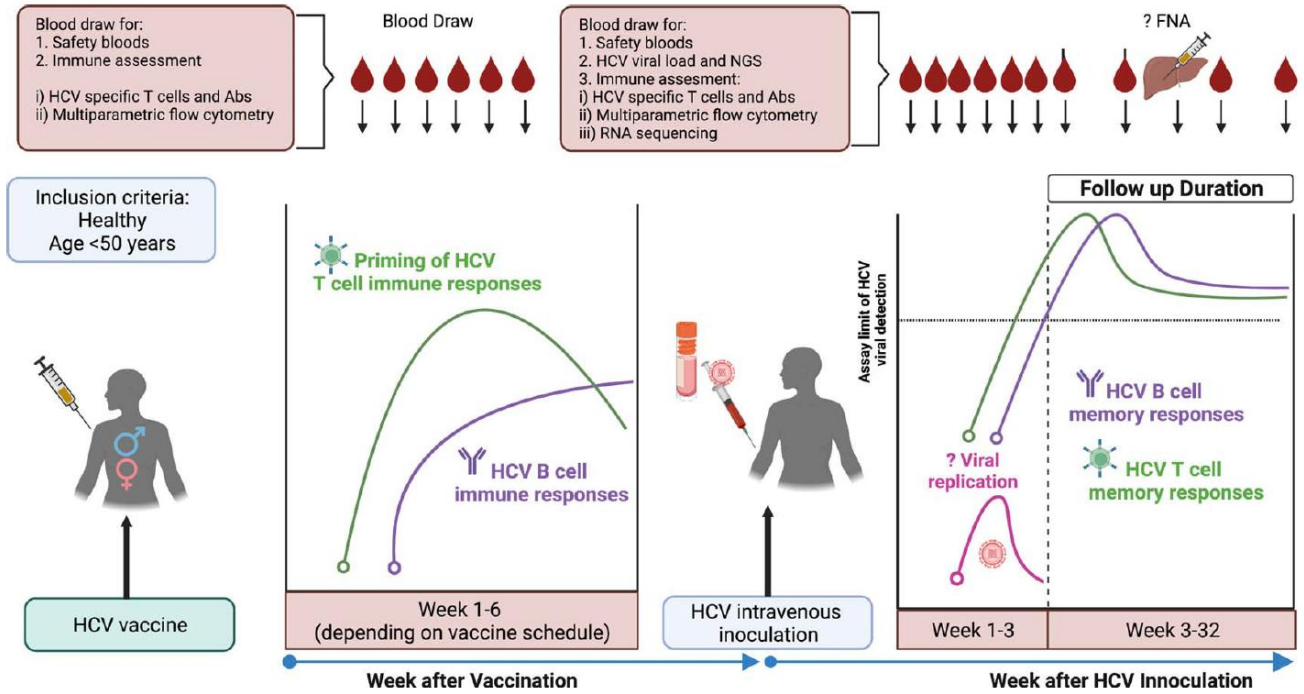
- HCV can be divided into 8 distinct genotypes
- 35% difference between genotypes - High error rate of the viral NS5B polymerase
- HCV evolves with an infected patient as a ´quasispecies‘ – 1-5% variability
- Envelope proteins E1 and E2 are the most variable regions, and heavily glycosylated
- 3 hypervariable regions within E2





- HCV is a lipo-viral particle: a chimera of both viral and host proteins

A human challenge model (CHIM) to assess HCV vaccine candidates?



Barnes et al. *Hepatology.* 2022

Planned human challenge studies

- Leading HCV vaccine candidates will be assessed in human challenge studies
- After confirmation of T-cell and antibody priming, volunteers will be inoculated with HCV
- Protective effects of vaccines assessed
- Any volunteers who develop chronic HCV due to suboptimal immunizations will be cured by DAAs at the end of the study

Ethical & practical concerns

- Informed consent – financial incentive to volunteers
- Uniformity of HCV inoculum across donors
- Epigenetic changes from HCV infection increase chances of developing liver cancer, even after DAA cure

Revision #1

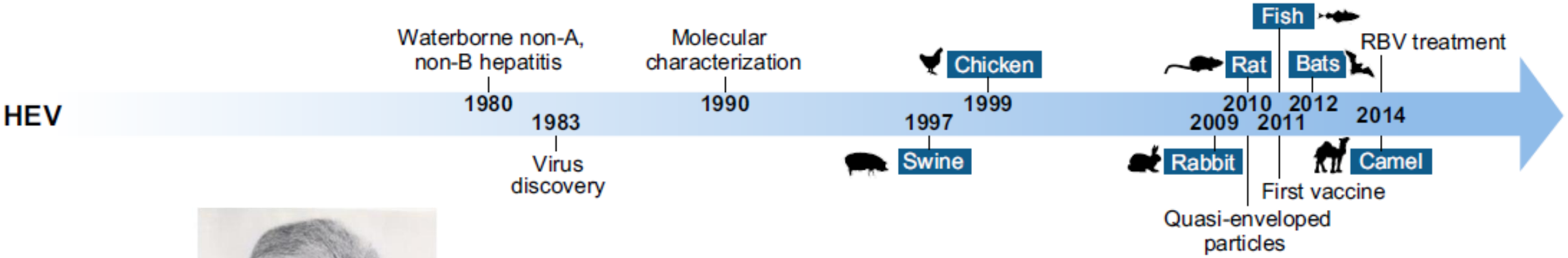
- HCV as an example of Research success
  - But why does it remain a problem?
- How is the particle and the genome organized?
- Replication cycle – focus on entry
- HCV transmission (blood borne, risk groups?)
- Course of infection? Timeframe and damage?

Part 3:

What is hepatitis E?

→ Inflammation of the liver caused by the hepatitis E virus

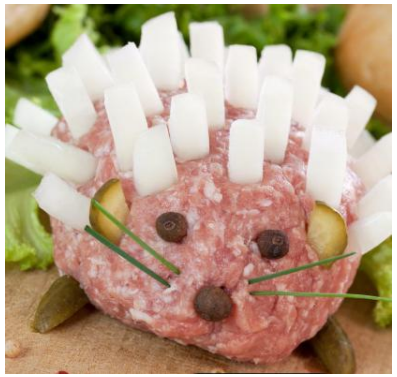
Discovery and History of Hepatitis E Virus

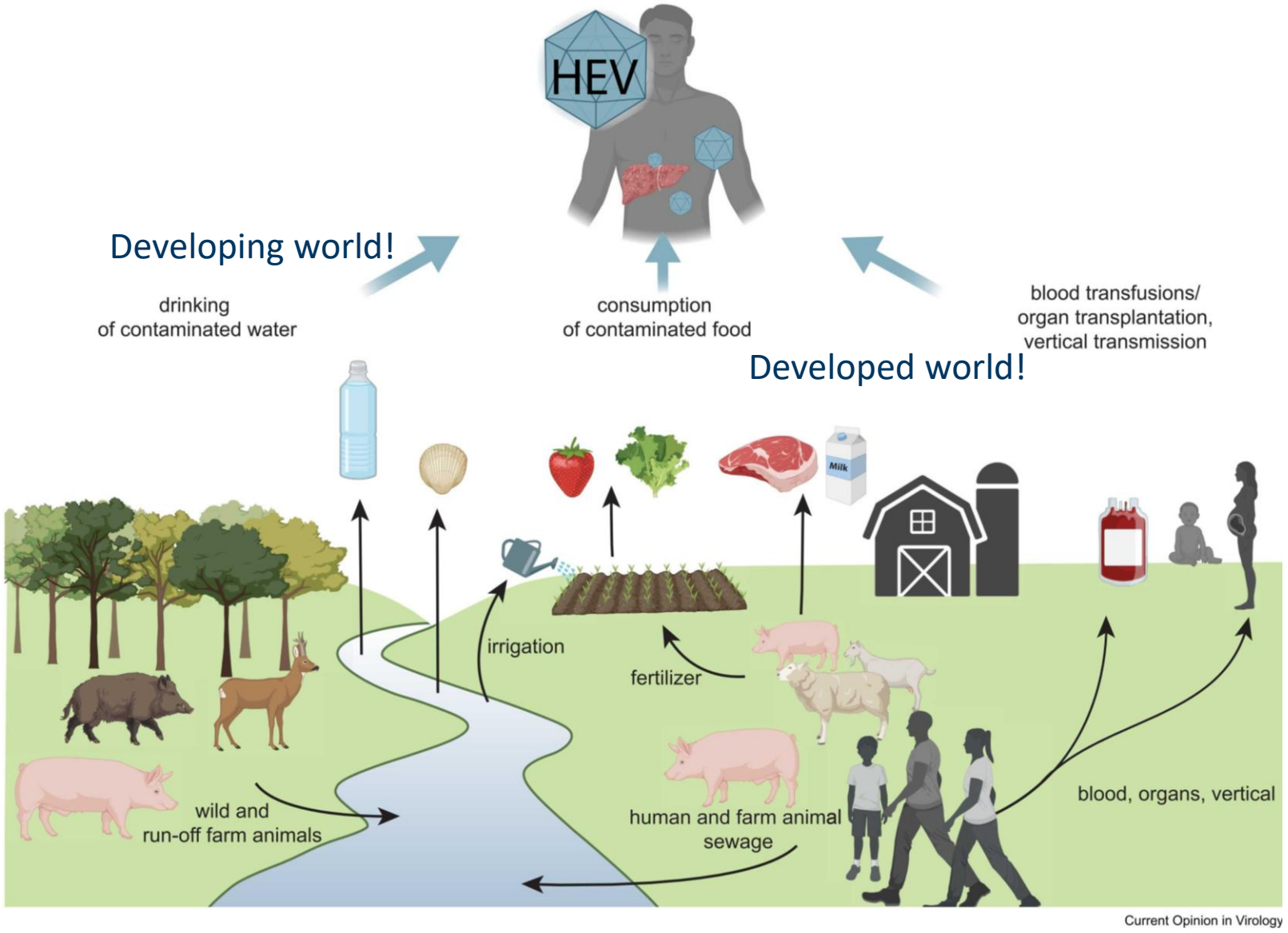


Mikhail Balayan

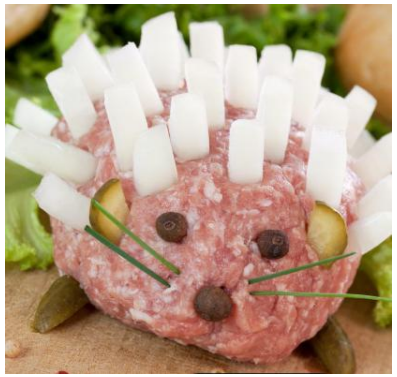
Drank a pooled filtrate of patient stool samples with yoghurt

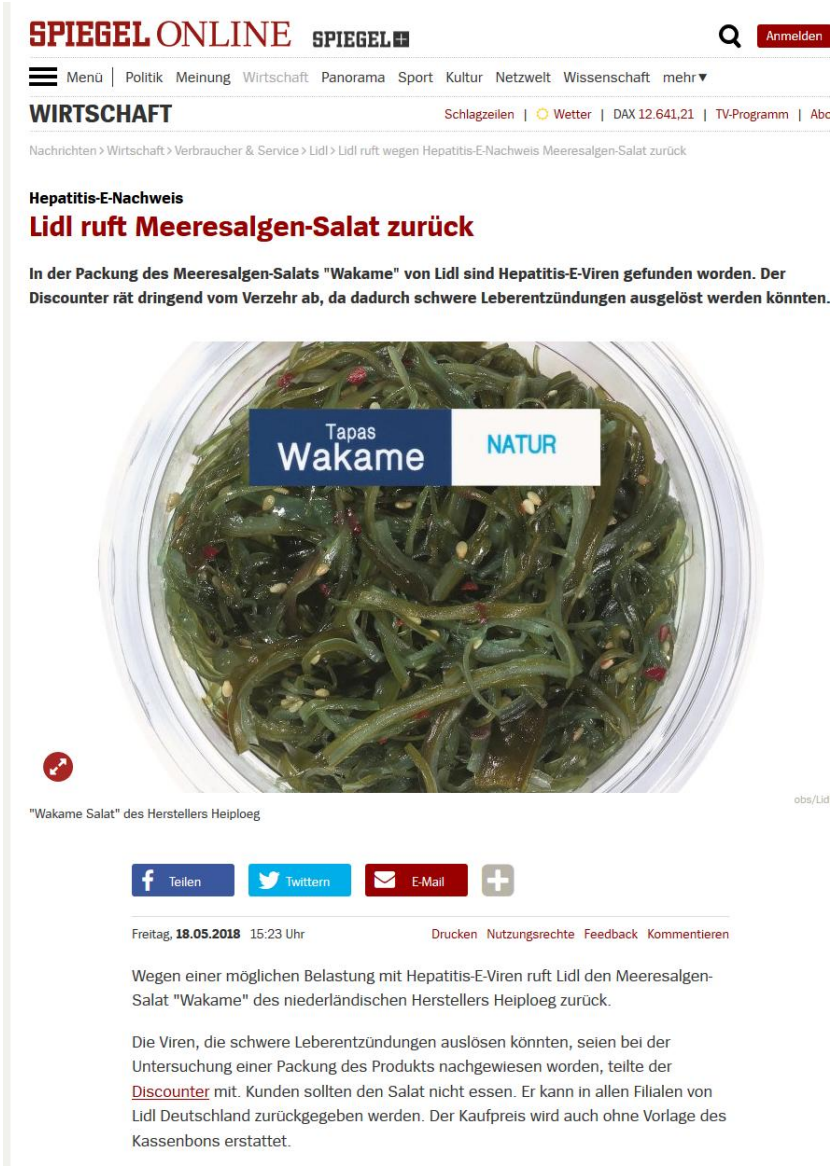
HEV transmission





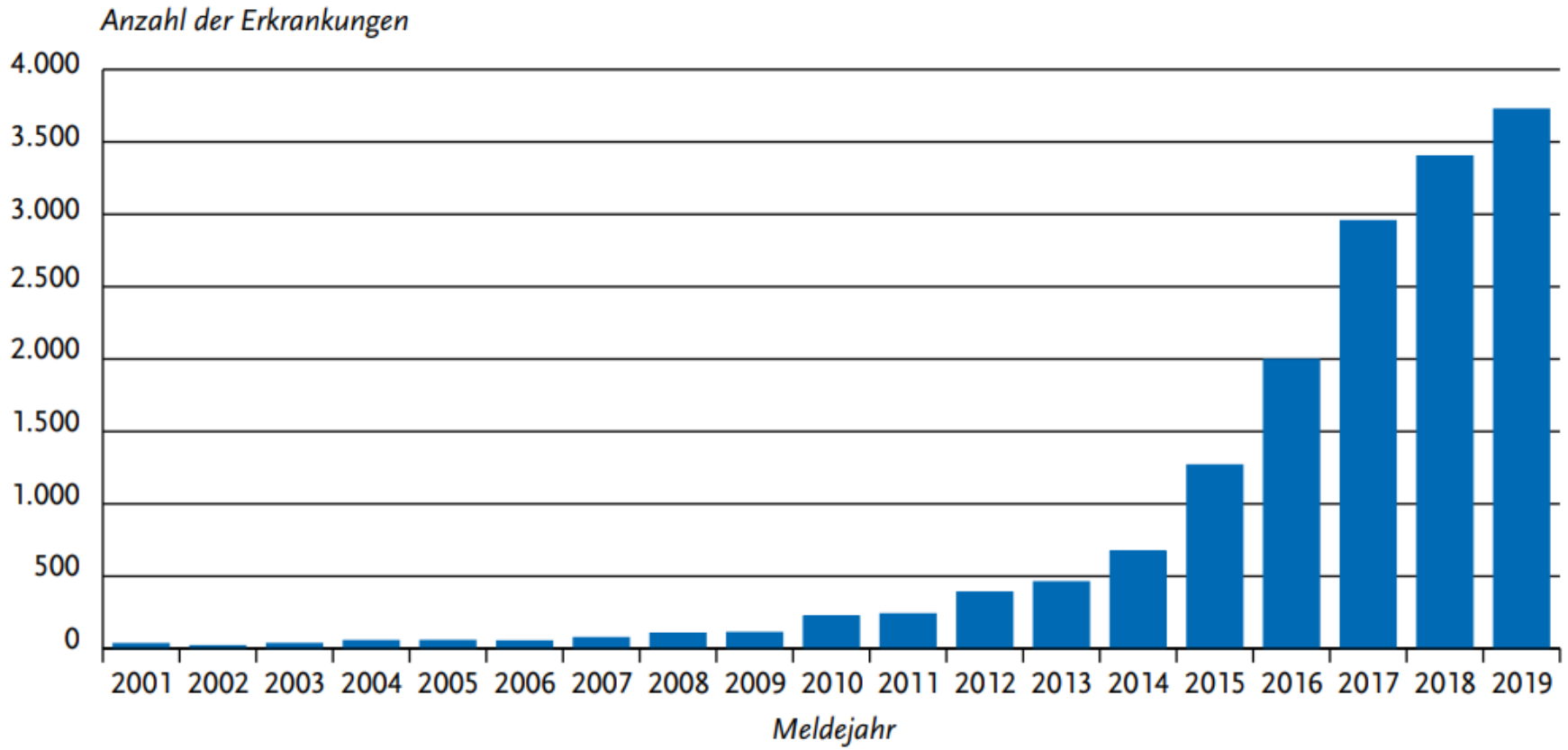
HEV transmission





HEV in Germany

Abb. 6.25.1:
Übermittelte Hepatitis-E-Erkrankungen nach Meldejahr, Deutschland, 2001 bis 2019



HEV in Germany

- almost **420,000** new infections – **most** of them **unnoticed**
- Approx. **4,000** severe HEV infections
- **1:500-1:1,250** blood products HEV RNA positive

Hepatitis E Virus (HEV) – an underestimated infectious disease

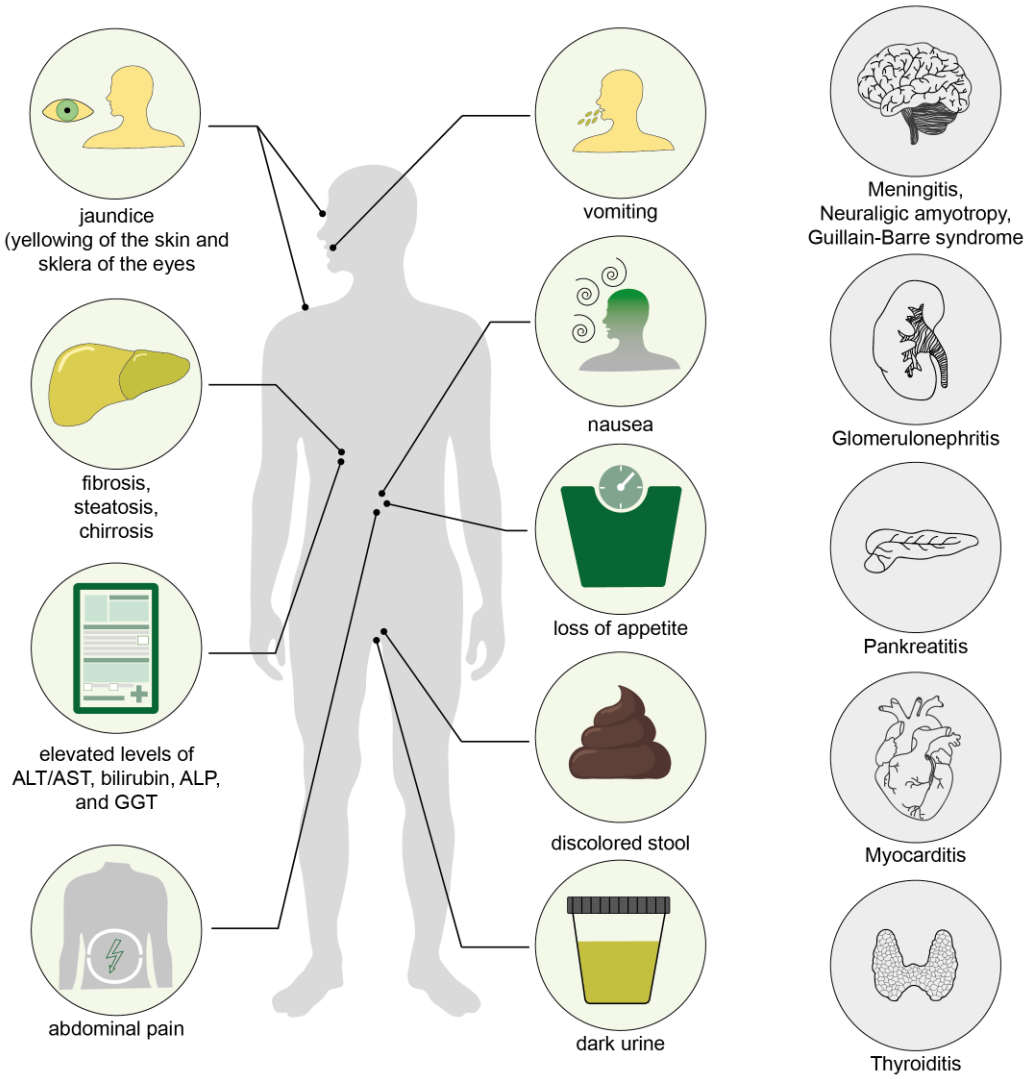
~20 Mio

cases of acute infections/year

~3.5 K

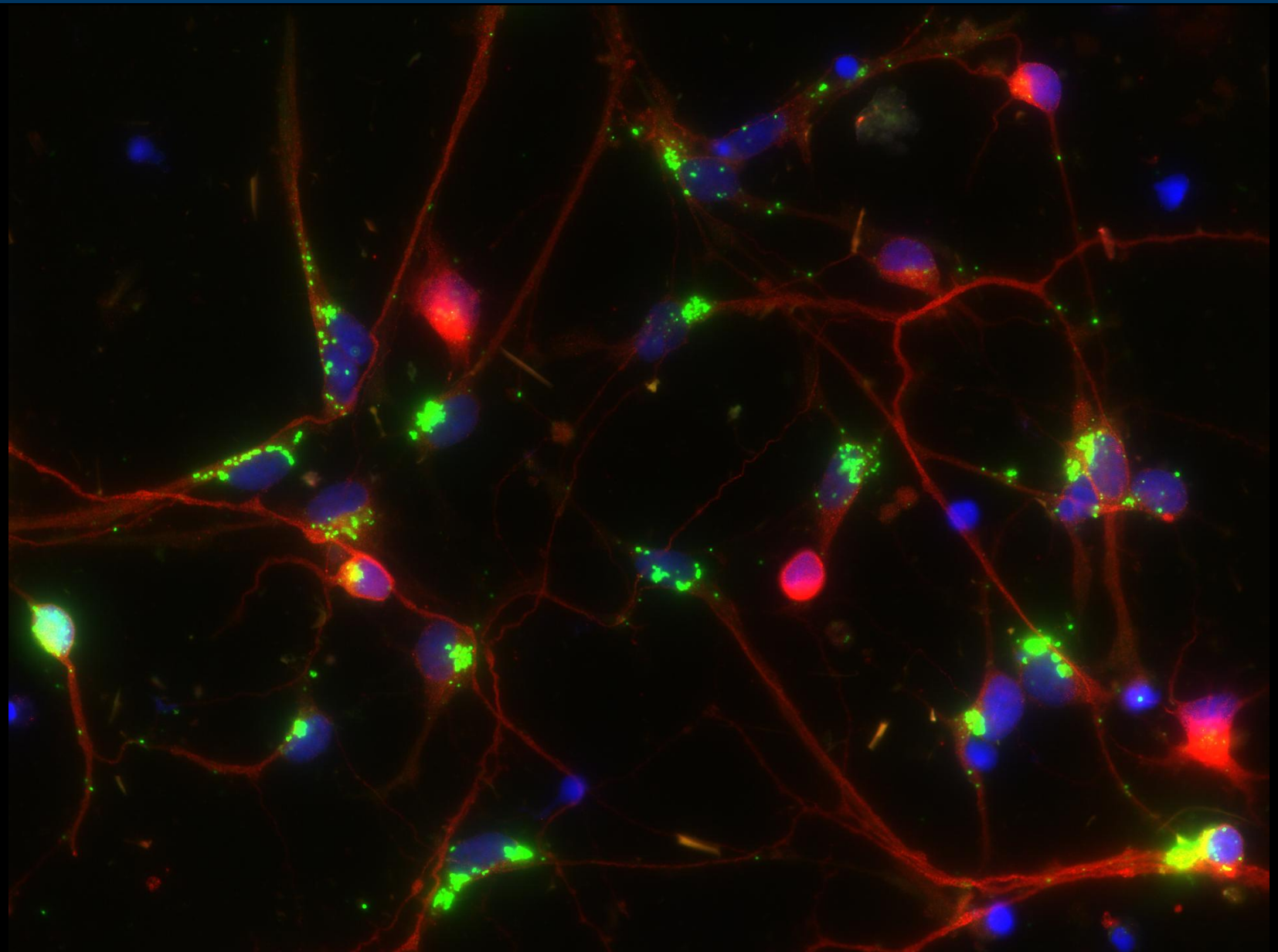
deaths/year

World Health Organization, 2023



No causation so far, but correlation!!!

Extrahepatic manifestations: HEV neurotropism



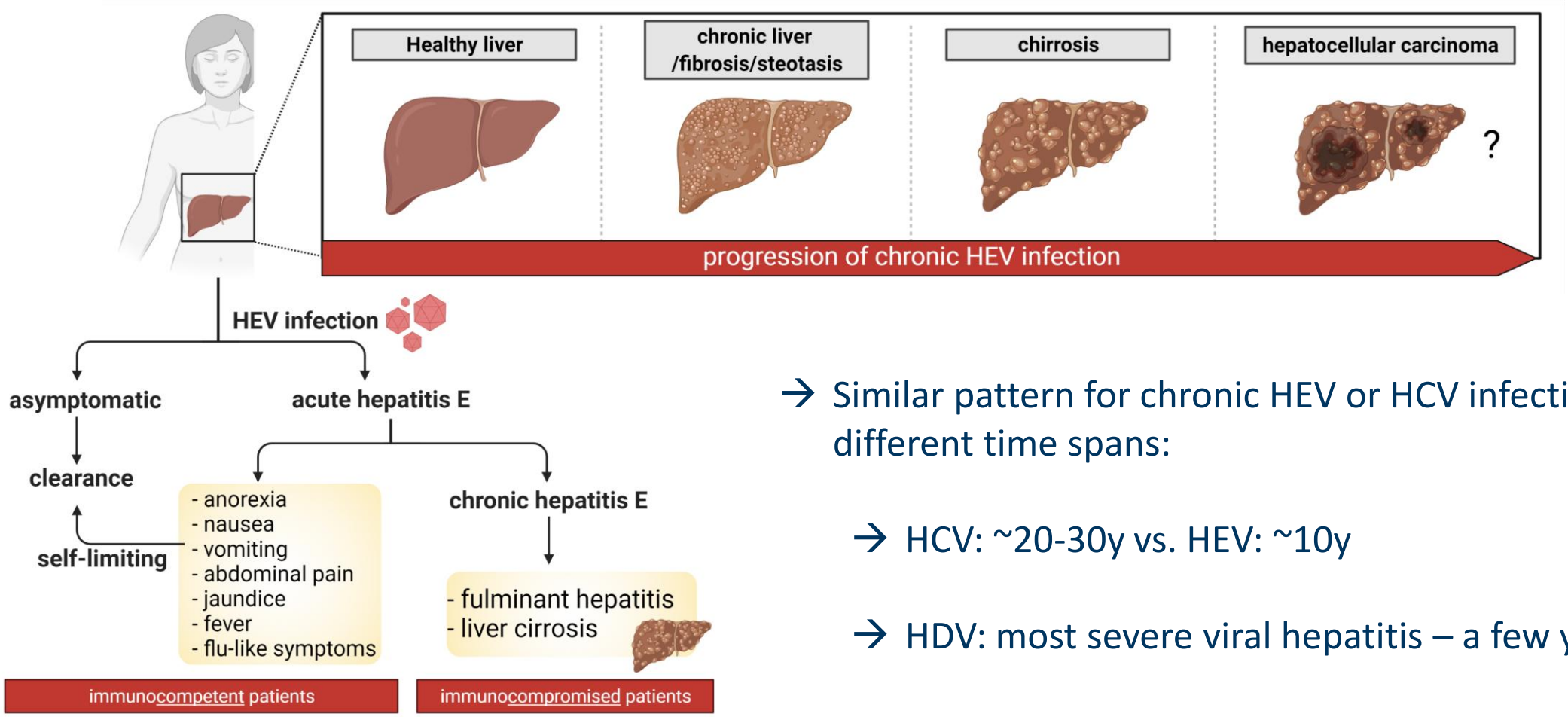
HEV ORF2
Tubulin
DAPI

Question #3

Hepatitis E is transmitted…

a) Via contaminated water in developed countries
b) Via contaminated food products in developed countries
c) Via contaminated water in developing countries
d) Via contaminated food in developing countries

Clinical course of HEV infection



→ Similar pattern for chronic HEV or HCV infection, but different time spans:

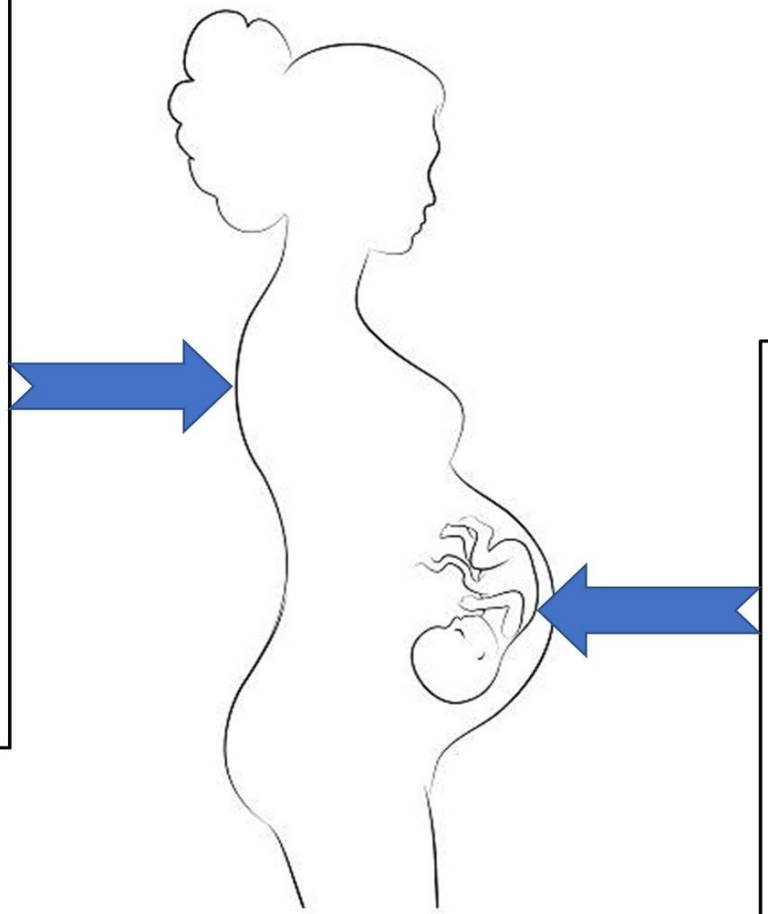
→ HCV: ~20-30y vs. HEV: ~10y

→ HDV: most severe viral hepatitis – a few years

HEV GT1 in pregnant women

Effects on mother

- Higher risk of disease during hepatitis E outbreaks
- Higher risk for developing symptomatic disease with HEV infection
- Higher risk of severe disease and acute liver failure
- These are seen primarily with genotype 1 HEV and not with genotype 3 or 4
- HEV genotype 1 appears to replicate more efficiently in maternal-fetal interface tissue than the genotype 3 virus

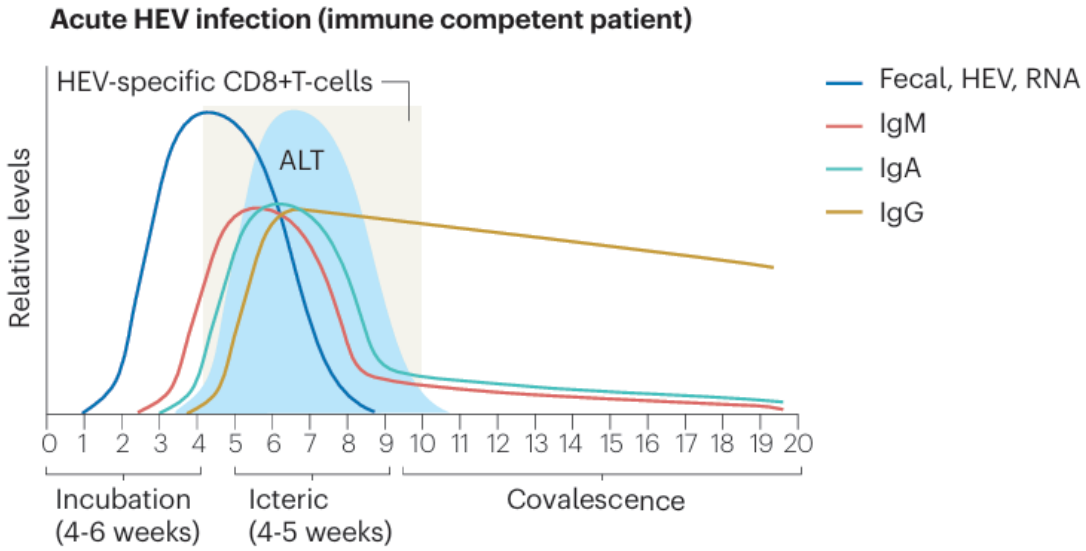


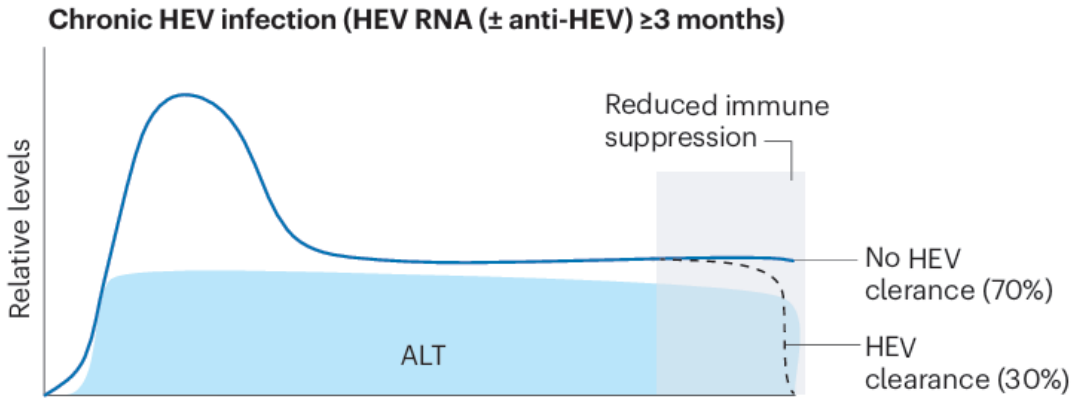
Effects on fetus

- Increased risk of complications during pregnancy
  - Miscarriage
  - Intrauterine death
  - Premature delivery
- Increased risk of neonatal complications
  - Neonatal hepatitis
  - Acute liver failure
  - Neonatal death
  - Low birth weight
  - Small for gestational age
  - Still birth

Aggrawal et al. 2021

Lab parameters during HEV infection



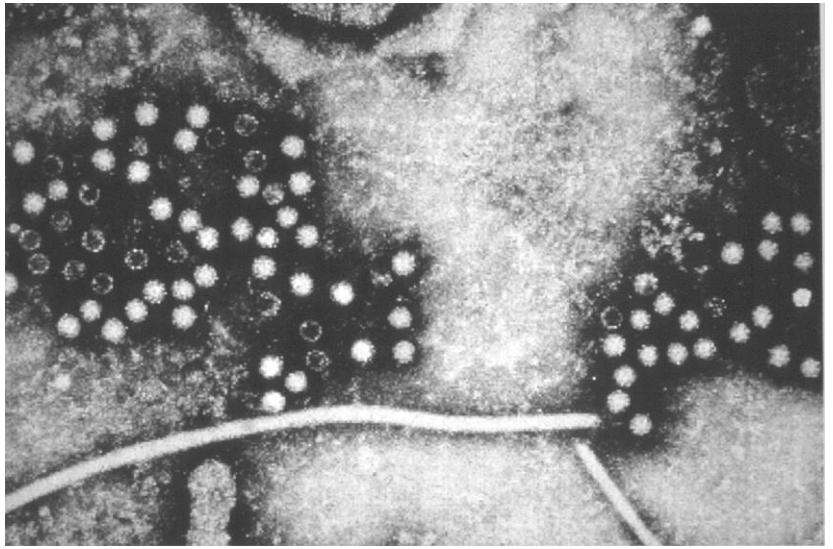


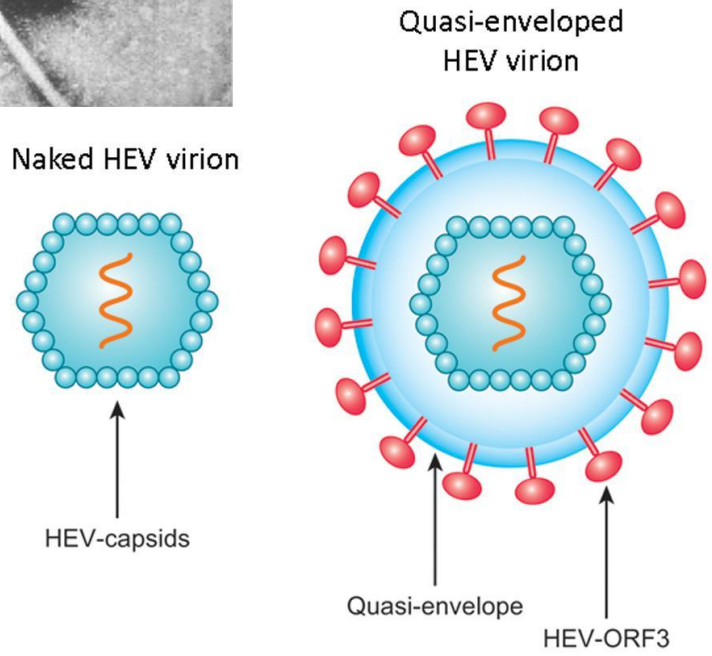
→ Incubation period 15-60 days

→ Increase in ALT (Alanine Aminotransferase) liver enzymes indicate liver inflammation

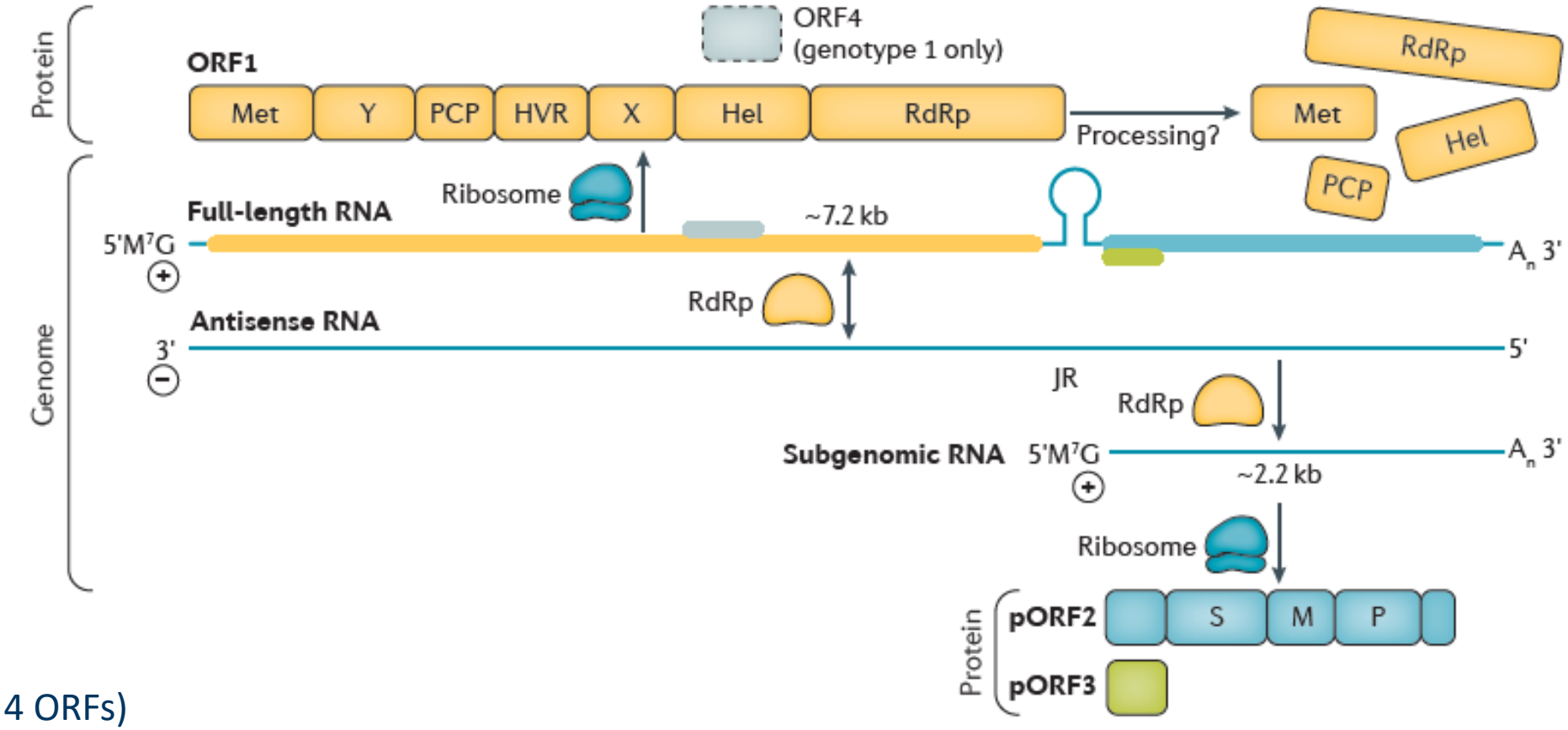
What is HEV?

- Hepeviridae, Orthohepevirus
- **~ 34 nm** in diameter
- **(+) ssRNA** (7,2 kb)
- **Quasienveloped**
- **No HEV-specific** direct acting **antivirals** available
- **vaccine** only in China and Pakistan



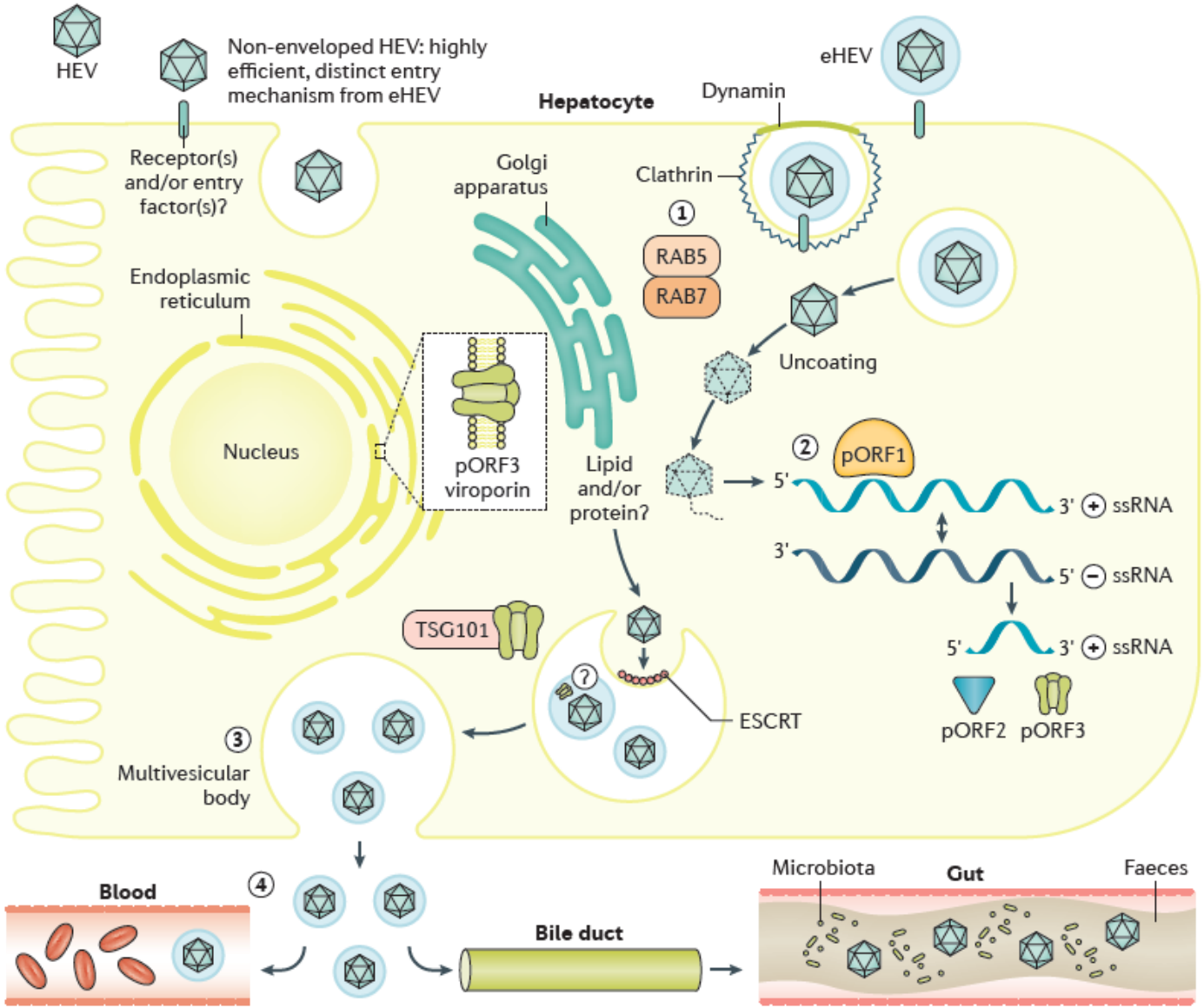


Genomic organization of HEV



3 ORFs (GT1 4 ORFs)
ORF1 replication machinery
ORF2 capsid
ORF3 unknown/viroporin

HEV replication cycle



→ Enveloped in blood
→ promotes immune evasion

→ Naked in stool

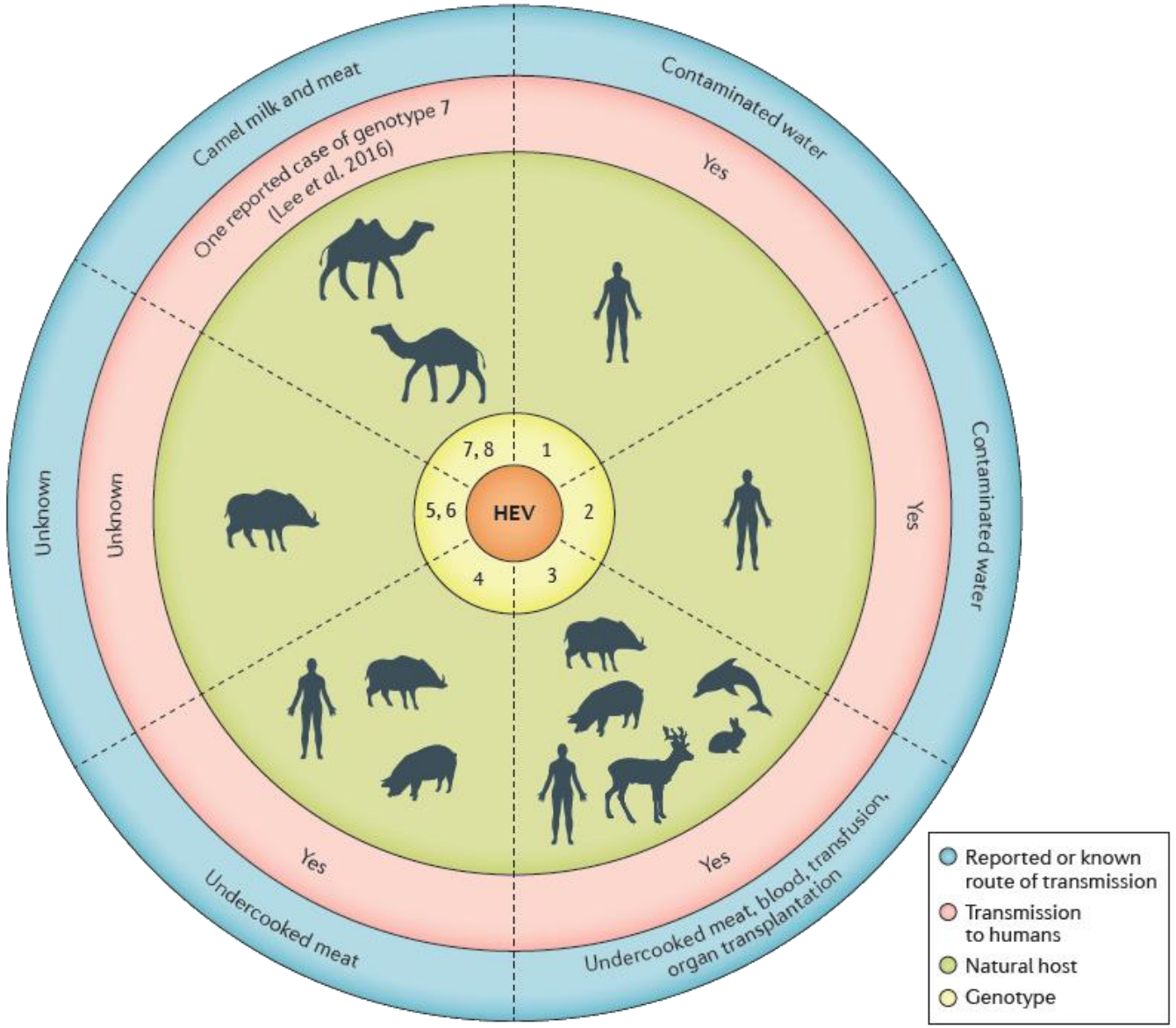
Question #4

Hepatitis E is …

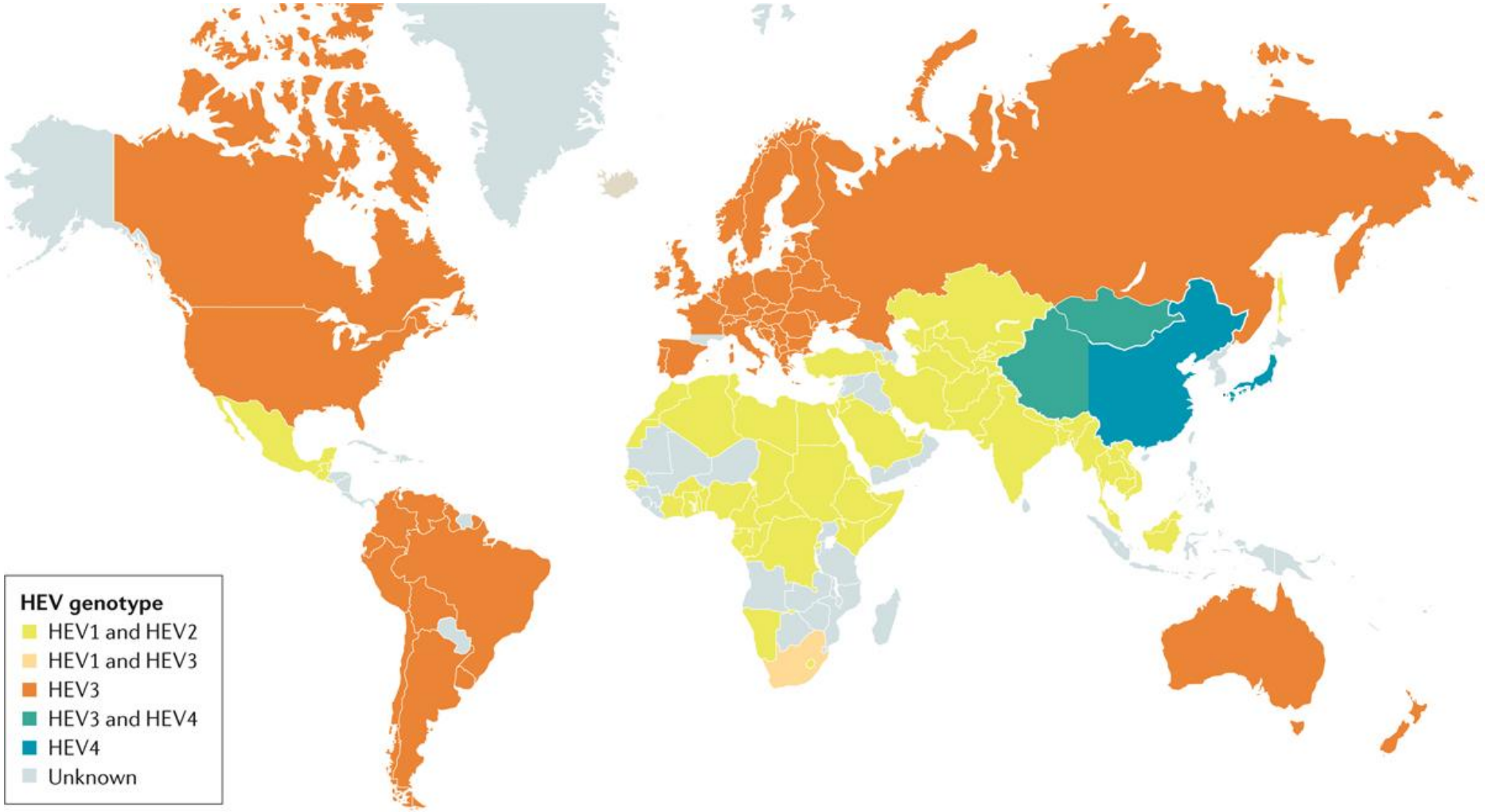
a) A singe-stranded RNA virus with a 7.2kb genome
b) A quasi-enveloped virus
c) Consists mainly of 3 open reading frames (ORFs)
d) enveloped in the bile and non-enveloped in the blood of infected patients

HEV Genotypes

- 8 genotypes
- Genotypes 1 + 2 humane infections
- Genotypes 3 + 4 zoonotic infections



HEV Genotypes - distribution



→ Link between HEV GT distribution and transmission mode

Cell culture models for HEV

Replicon system

Easy reporter read-out
High-throughput
Less authentic – only replication, no virus production

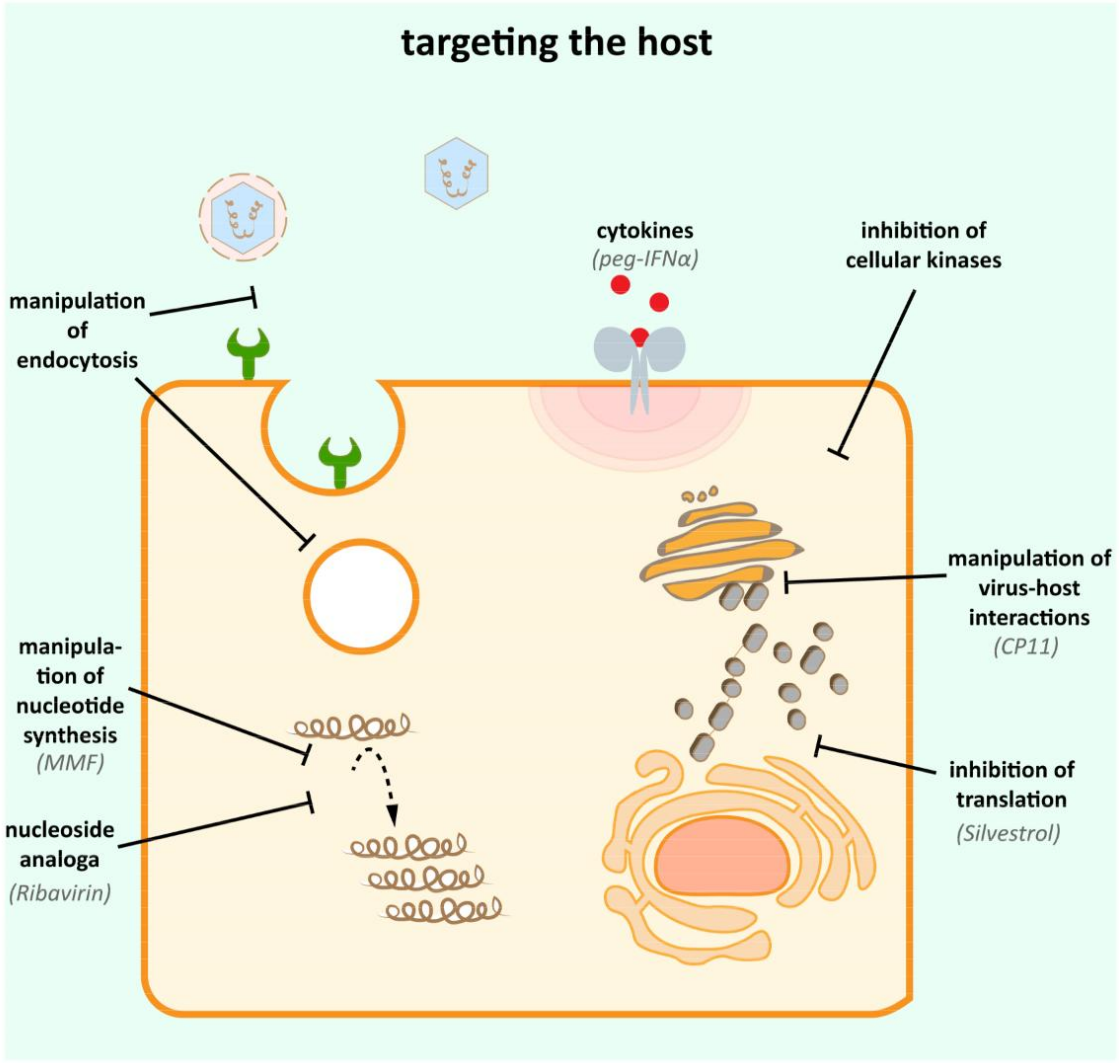
Application:
Drug screenings

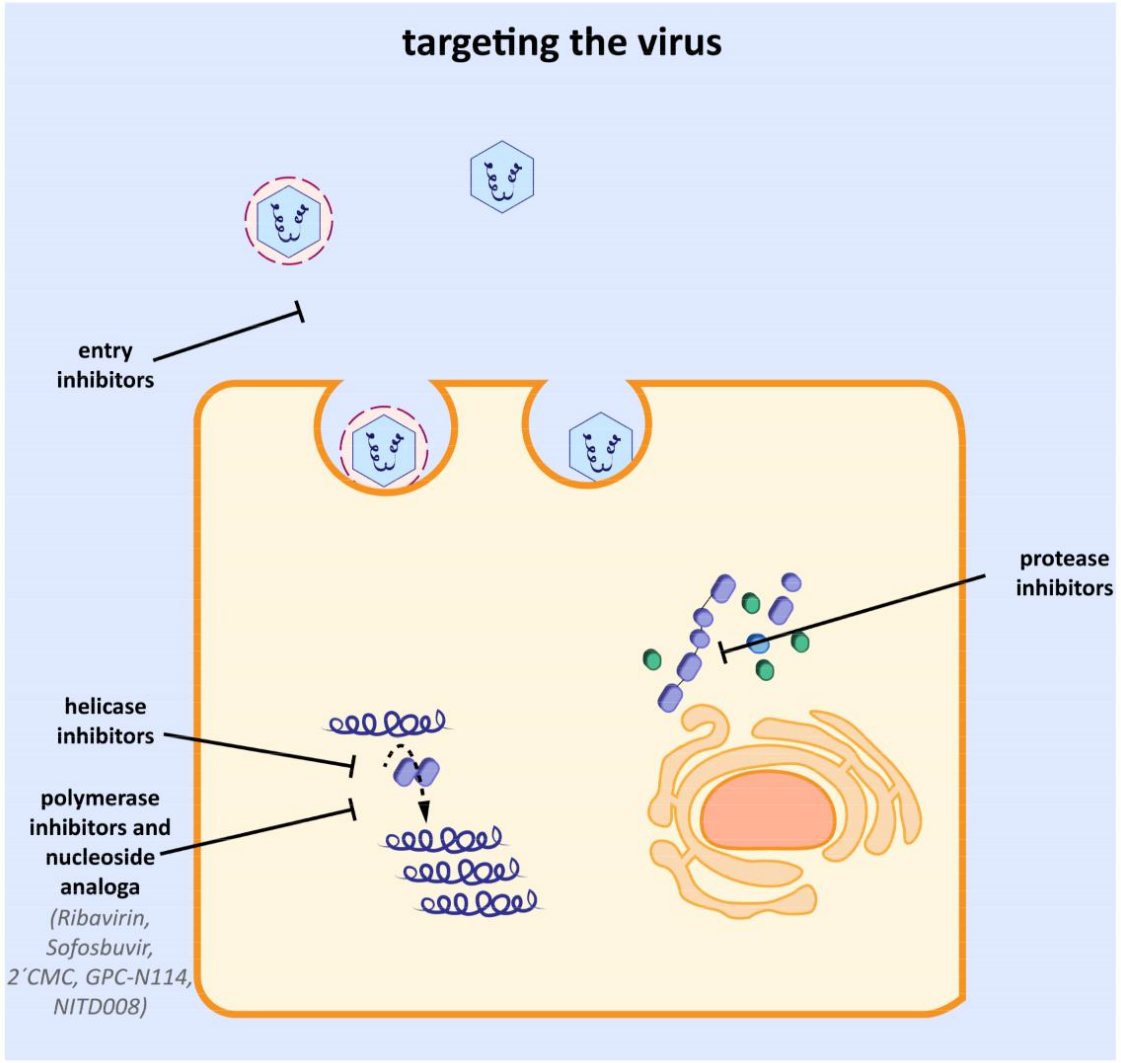
Full length

Immunehisto staining
More labor intensive
Production of authentic virus particles

Application:
Study whole virus replication cycle

Host vs. virus targeting





IFN: Interferon

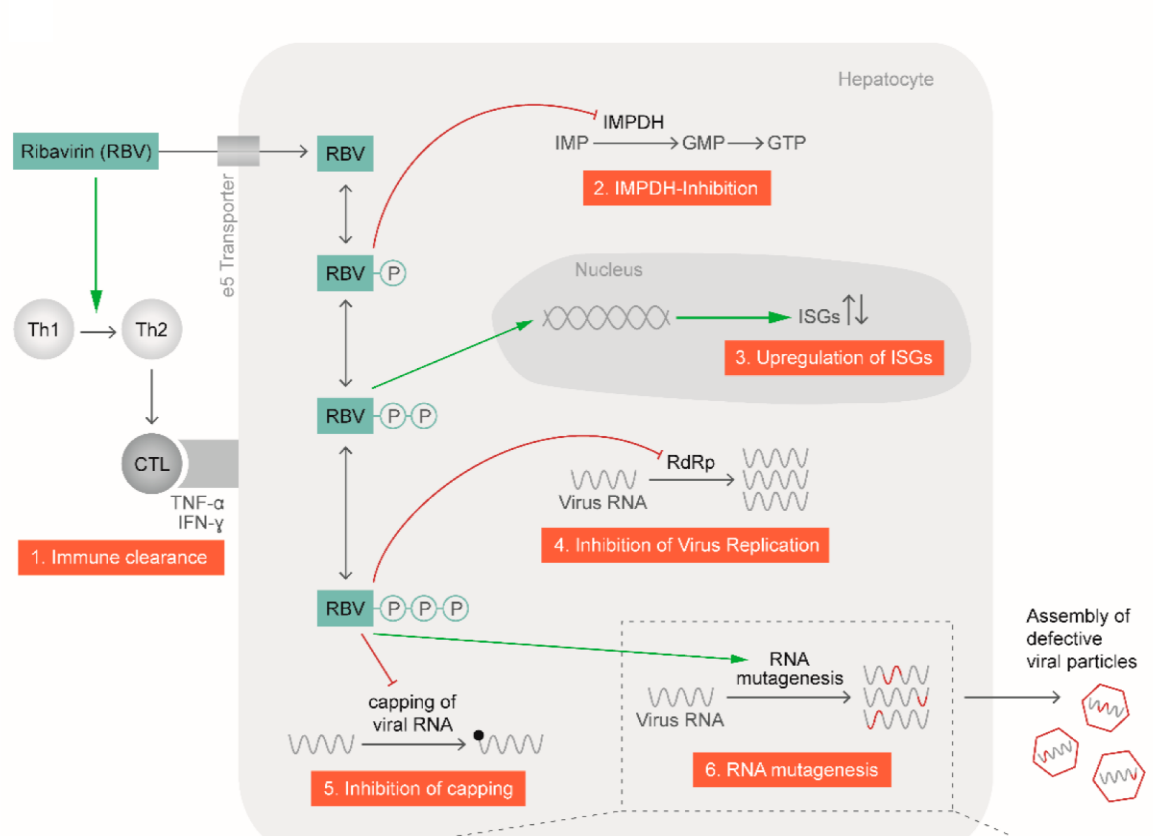
How to treat chronic HEV

No HEV-specific antiviral available

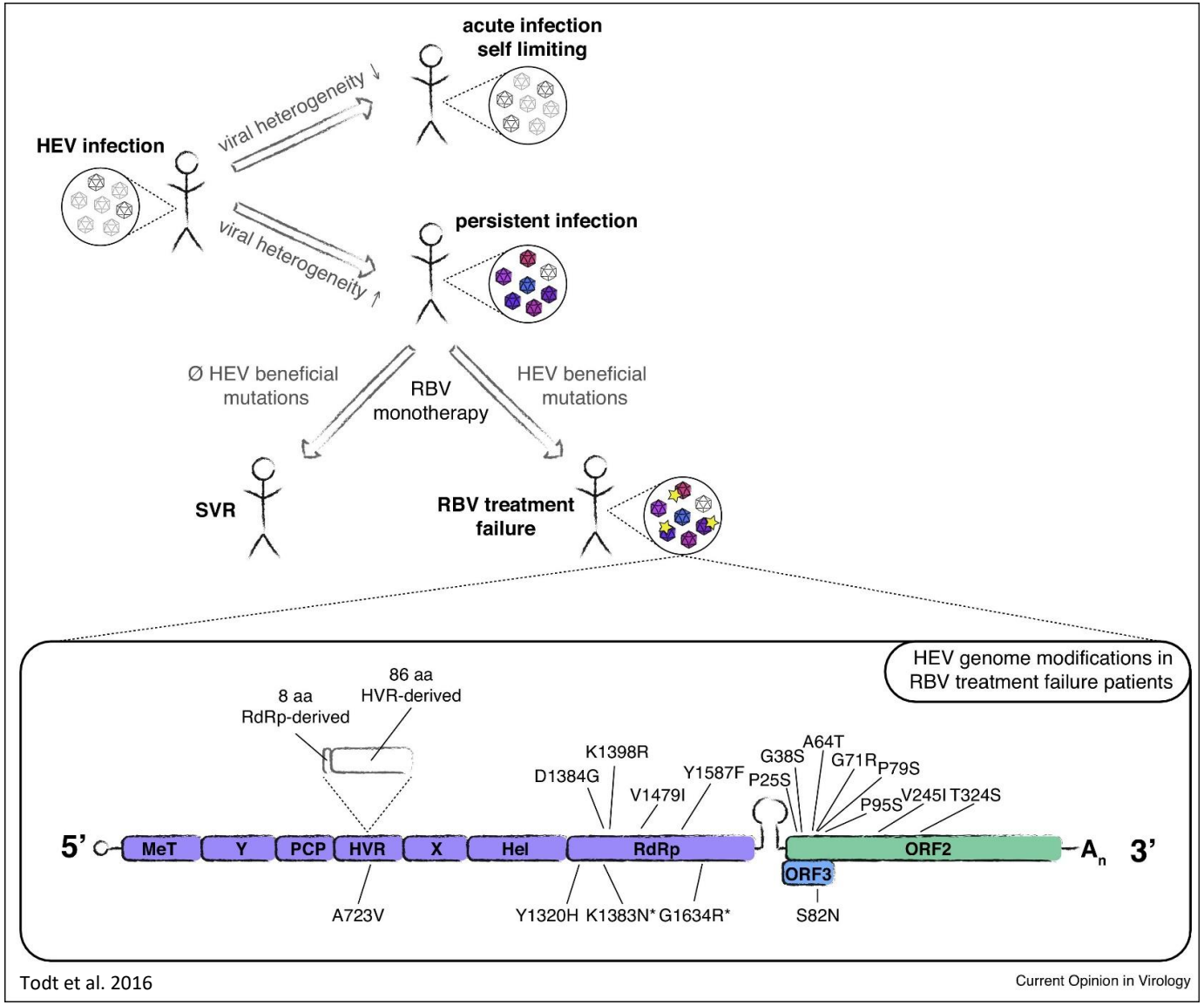
IFN-alpha
Activation of the immune system
Not applicable for transplant patients (graft rejection)
Severe side effects (vomiting, nausea etc.)

Off-label Ribavirin (RBV)
Broad spectrum antiviral
Guanosine analog that causes hypermutation
Not applicable for patients with impaired renal function
teratogenic
80% sustained virological response

Sofosbuvir
Reproposed HCV drug – inhibits viral polymerase
Limited efficacy due to resistance mutations



Viral diversity and its implication for treatment success

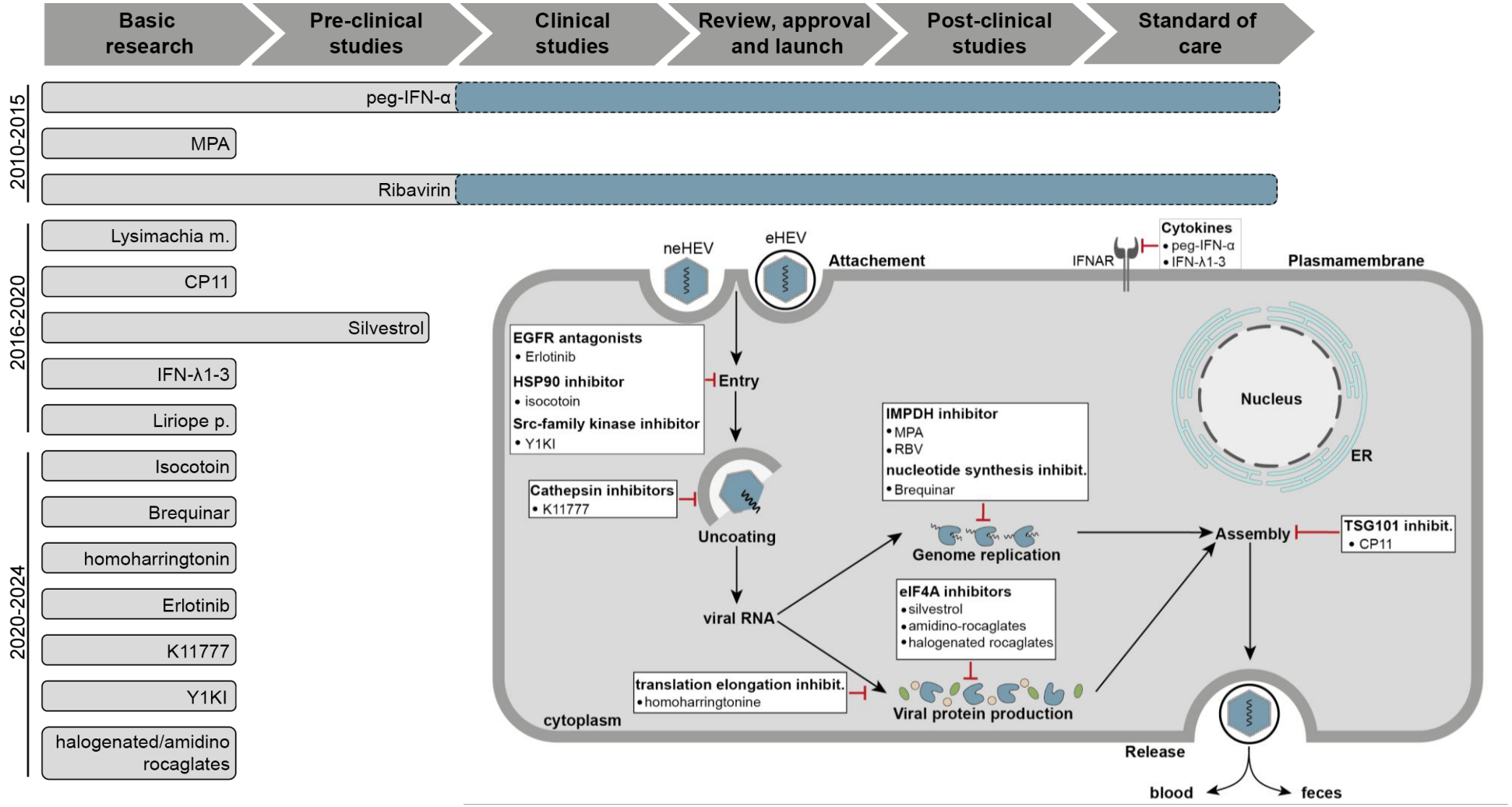


Todt et al. 2016

Current Opinion in Virology

Resistance associated mutations occur in patients with chronic HEV and RBV treatment → often in the polymerase

Antiviral drug discovery



adapted from Frericks N.*, Klöhn M.*, Lange F.* et al., *Antiviral Res.*, 2024

Question #5

Similar to HCV, HEV…

a) Causes acute, but no chronic infections
b) Causes inflammation of the liver
c) Can progress to hepatocellular carcinoma
d) Has a wide host tropism

Revision #2

- Particle and genome structure (translation products, subgenomic RNA etc.)
- Replication cycle: What goes in – what goes out?
- Genotypes, transmission and types of disease / tropism

Summary

1. Hepatitis
    - Function of the liver
    - Causes and symptoms of hepatitis
    - Disease progression
2. Hepatitis C virus
    - Prevalence and Transmission
    - Genome structure, replication and life cycle
    - Antiviral drug development and HCV treatment strategies
    - Remaining challenges
3. Hepatitis E virus
    - Prevalence and Transmission
    - Genome structure, replication and life cycle
    - Antiviral drug development and HEV treatment strategies
    - Remaining challenges