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HDV: le nuove opzioni terapeutiche e lo scenario italiano

Maurizia Rossana Brunetto

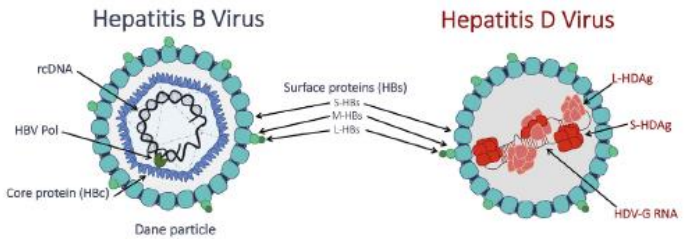
Professore Straordinario di Medicina Interna- Dipartimento di Medicina Clinica e Sperimentale -Università di Pisa

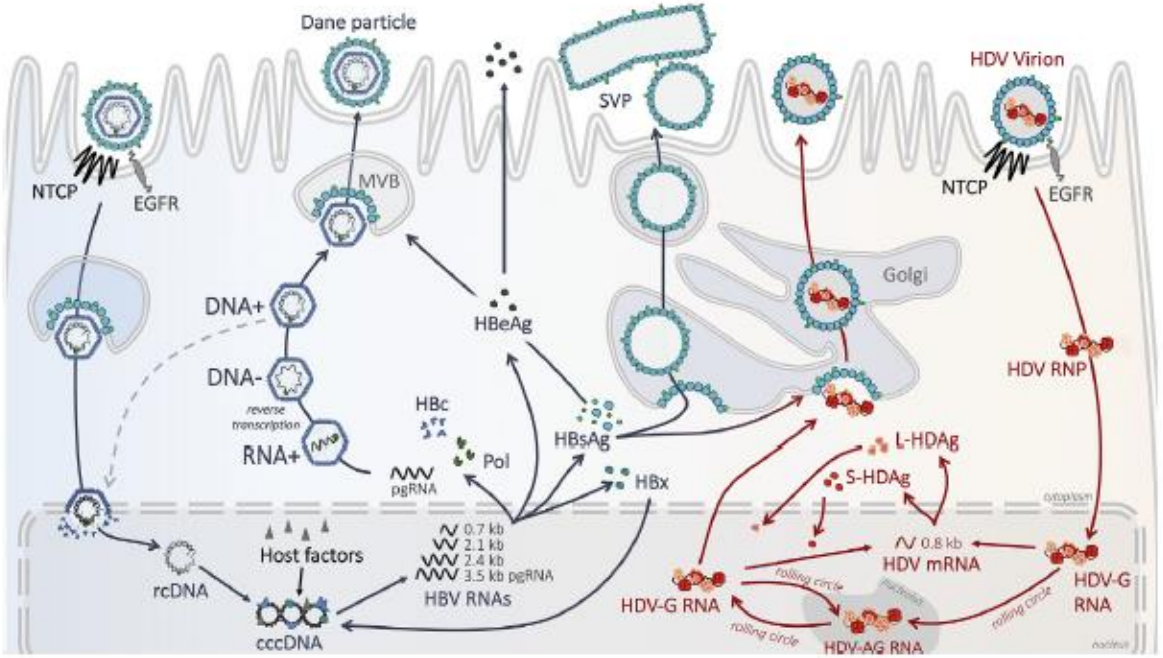
Direttore DAI Specialità Mediche e UO Epatologia – Azienda Ospedaliero Universitaria Pisana

MR Brunetto **Disclosures**

Speakers Bureau: AbbVie, Gilead
Advisory: AbbVie, Gilead, Janssen, Roche, EISAI-MSD

HDV and HBV



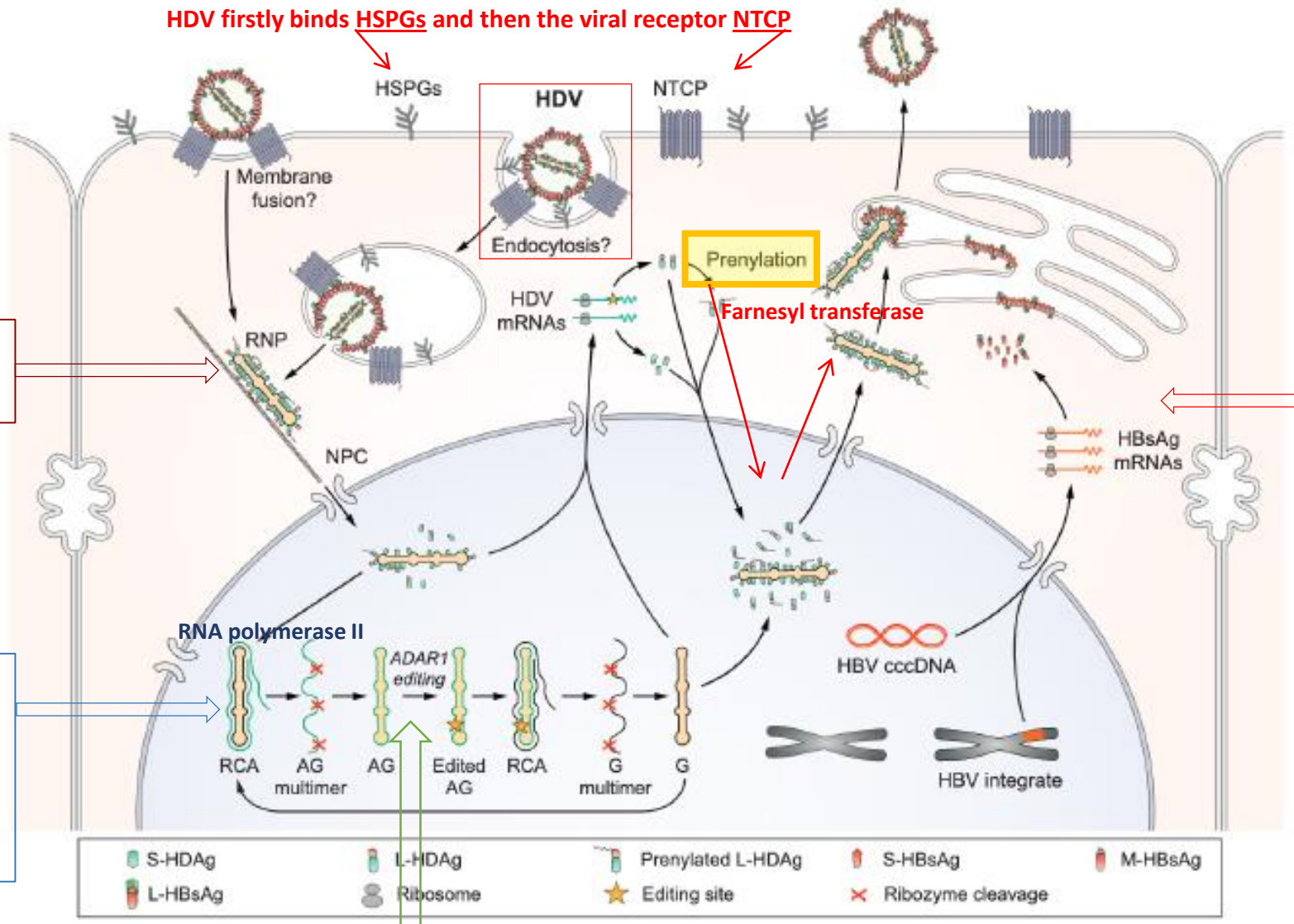


- **HDV** uses the **envelope of HBV** to **egress from** and **to re-enter** into hepatocytes
- Within the hepatocytes the **replicative circles** of **HBV** and **HDV** run completely **separate pathways** even if, in spite of being different for their genome (relaxed circular partially double stranded DNA for HBV and circular RNA for HDV), HBV and HDV are **both maintained** as **episomes** in the **nucleus** of infected cells and **use** the **cellular machinery** for **the transcription of their viral RNAs**
- The establishment of **HBV/HDV co-infecti**on in humanized uPA/SCID/beige (USB) **mice** was associated by the **induction of an antiviral state** in the hepatocytes, by enhancement of many genes involved in innate defense mechanisms (e.g.ISGs genes) .
- The strong **antiviral response** caused **by HDV** infection could also **affect HBV replication** and in part expalin the lower levels of HBV replication observed in HBV/HDV coinfected patients

Lucifora et al, Antivial Research 2020; Giersch K et al J Hep 2015

HDV replication cycle

HDV firstly binds HSPGs and then the viral receptor NTCP



Release of the RNP complex which is transported into the nucleus to initiate RNA replication

L-HDAg is responsible for RNP's interaction with the **HBV pre-S1 envelope proteins** which are expressed by cccDNA or integrated HBV DNA

- Replication proceeds via a double rolling circle amplification mechanism, generating linear multimeric anti genomis and genomic RNAs, which are cleaved by 2 intrinsic rybozime
- The genomic RNA with negative polarity serves as the template for the first RCA step.

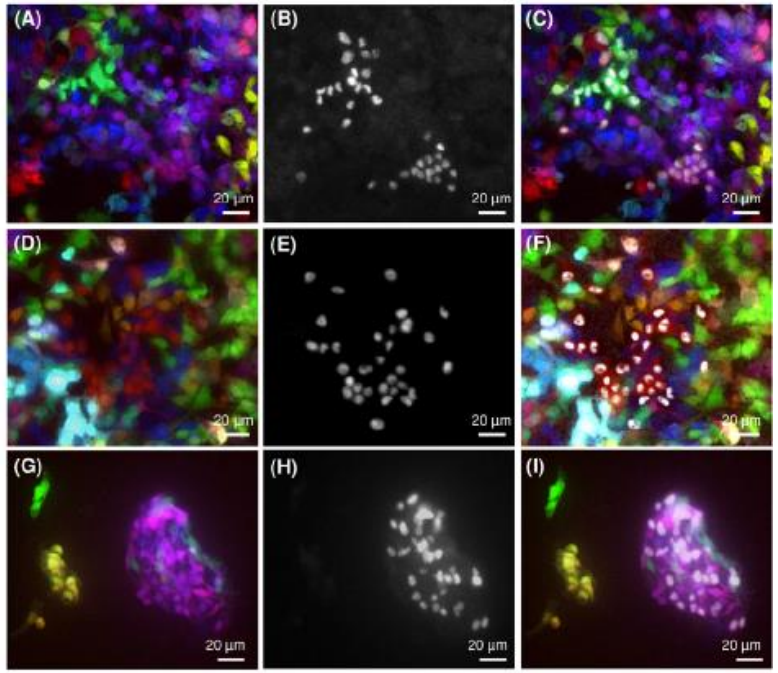
During replication antigenomic is edited by adenosine deaminase acting of RNA1 (ADAR1)

Lucifora et al, Antivial Research 2020; Zhang Z et al J Hep 2021

Hepatitis delta virus persists during liver regeneration and is amplified through cell division both in vitro and in vivo.

- ✓ While **HBV markers rapidly dropped** in proliferating PHHs, **HDAg-positive hepatocytes were observed among dividing cells** at all time points.
- ✓ **HDAg-positive cells appeared in clusters**, indicating that HDV was transmitted to daughter cells during liver regeneration even in the absence of de novo infection.

HepG2-hNTCP-RGB cells infected with HDV, passaged once, visualised in a fluorescence microscope after 7 d.



Cell clusters with the same colour are daughter cells originating from the same parental cell

HDAg was stained using an anti-HDAg-pos, human serum

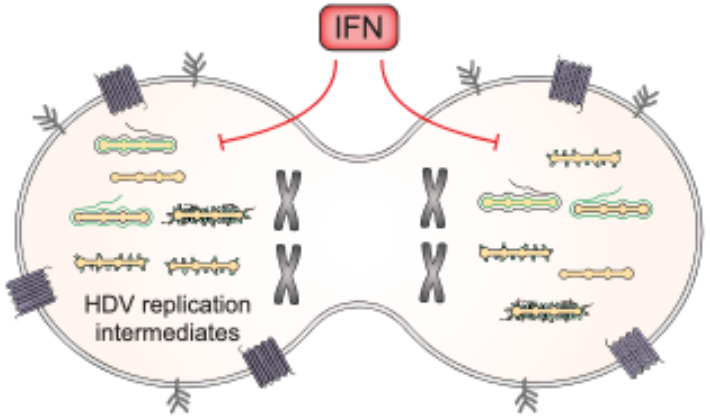
HDV persists during liver regeneration by transmitting HDVRNA to dividing cells even in the absence of HBV coinfection

This may explain why HDV clearance is difficult to achieve in HBV/HDV patients

Giersch K , Gut 2019

Endogenous and exogenous IFN responses suppress HDV persistence during proliferation of hepatocytes in vitro

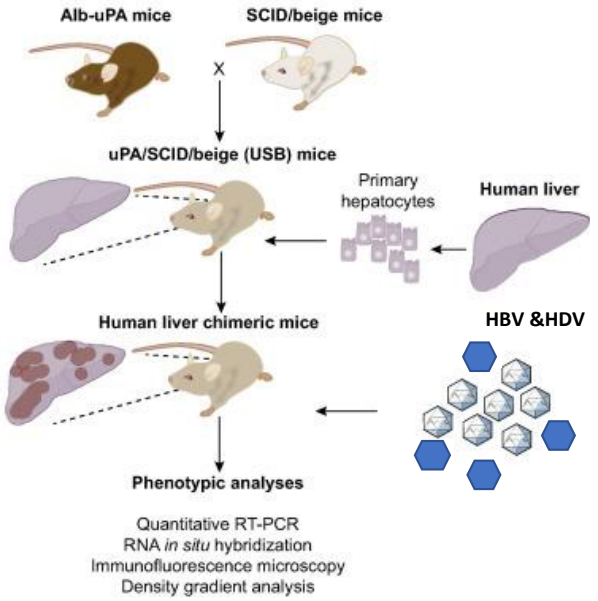
In immune deficient cell lines infected with HDV and splitted (1:6) at day 5 post infection and further splitted every 5 days, different IFNs were applied during cell proliferation.



- Both exogenously and endogenously induced **IFNs** (alpha and lambda) responses **restrict HDV persistence during hepatocyte proliferation**.
- The severe loss od HDV replicative intermediates during cell division may be explained by exposure of viral RNA to induced ISGs, that may either cause direct degradation of HDV-RNA or inhibit the restablishment of replication in the nuclei of daughter cells

Zhang Z et al J Hep 2019 and Zhang Z et al J Hep 2021

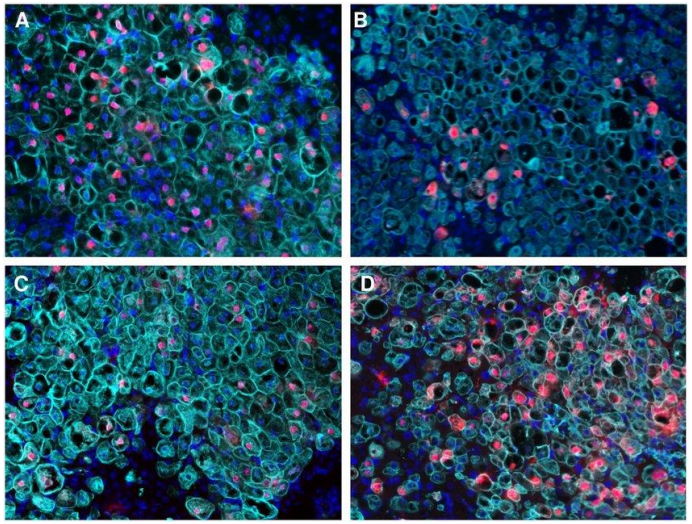
Both interferon alpha and lambda can reduce all intrahepatic HDV infection markers in HBV/HDV infected humanized mice



The effect of **peg-IFNα** and **peg-IFNλ**, compared to a HBV-polymerase inhibitor (NA) on all HDV infection markers was studied using **human liver chimeric mice**

- Peg-IFNα and peg-IFNλ **reduced HDV viremia** (1.4 log and 1.2 log, respectively) and **serum HBsAg levels** (0.9-log and 0.4-log, respectively). Intrahepatic quantification of genomic and antigenomic HDV RNAs revealed a median ratio of 22:1 in untreated mice, resembling levelsdetermined in HBV/HDV infected patients.
- Both IFNs greatly **reduced intrahepatic levels of genomic and antigenomic HDV RNA**, **increasing** the amounts of **HDAg- and antigenomic RNA-negative hepatocytes**.

- NA-mediated suppression of HBV replication (2.1-log) did not significantly affect HBsAg levels, HDV productivity and/or release.
- In humanized mice lacking adaptive immunity, IFNs but not NA suppressed HDV.
- Viremia decrease reflected the intrahepatic reduction of all HDV markers, including the antigenomic template, suggesting that intracellular HDV clearance is achievable.



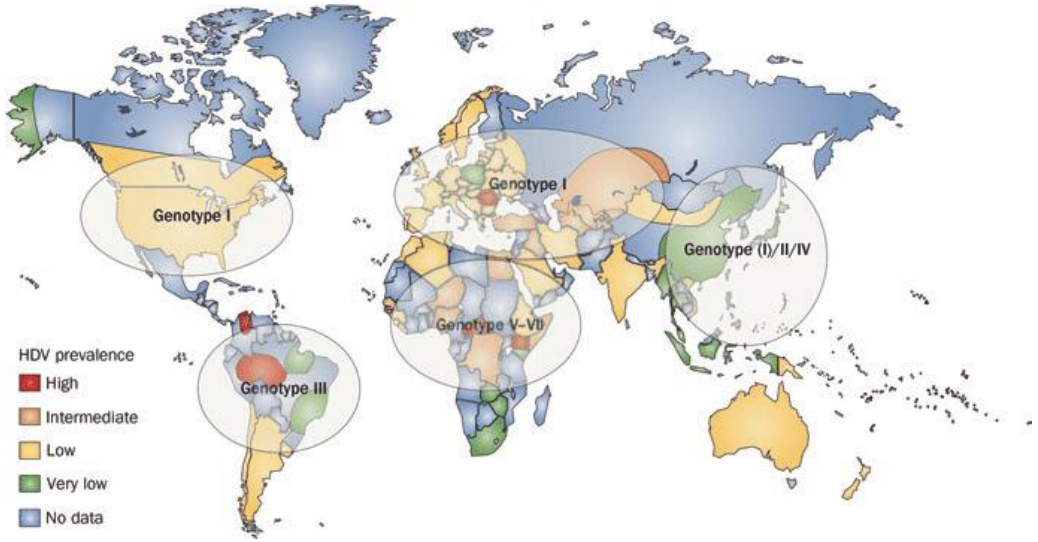
Giersh K et al Scientific report 2017

Chronic Hepatitis D

- **CHD** is considered the **most severe form of viral he**patitis, because HBV/**HDV** infection is **constantly associated** with **liver damage**
- It has been estimated that the **risk of cirrhosis** and **HCC development** in case of **CHD** is **3** and **2 fold increased** as compared to chronic **hepatitis B** and **C**
- Furthermore, the **time to develpment of cirrhosis** in CHD patients is significantly **shorter** as compared to CHB: **5** to **10 years** in **70%** of cases, but **1** to **2 years** in **about 15%** of the patients
- **Virologic** factors (both HDV and HBV), modality of **HDV acquisition**, **phase of HBV infection** at the time of HDV infection and **co-factors** of liver disease **significantly influnce the clinical course of the disease**

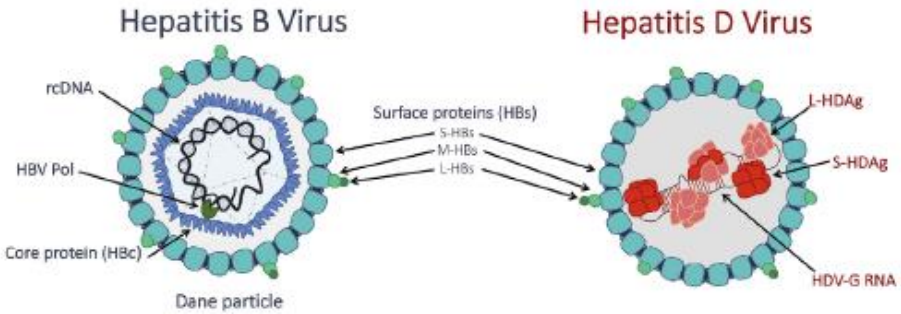
Mentha N e al J Adv Res 2019; Farci P et al Sem Liv Dis 2012; Rosina F et al Gastroenterology 1999

HDV and HBV virologic features and CHD



- ✓ **GT 1** infection is associated with higher pathogenicity and lower response to IFN
- ✓ **GT 2 and 4** infections had been reported in milder forms of liver disease
- ✓ **GT3** infection is associated with an increased risk of fulminant hepatitis

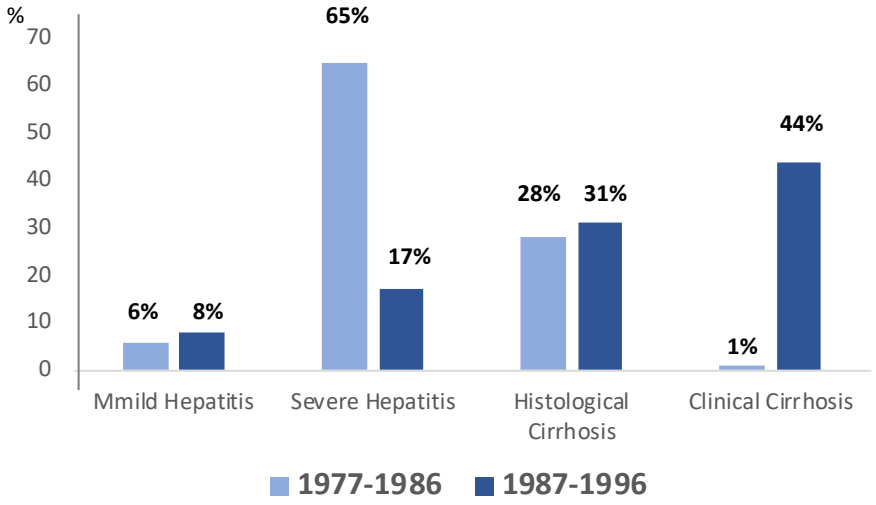
- ✓ **Persistence of HDV replication** had been associated with worst prognosis
- ✓ Preliminary reports suggest that **HDV-RNA serum levels** correlates with disease activity and progression
- ✓ Active **HBV replication** is associated with worst outcome of CHD and the persistence of a transcriptional active HBV infection supports HDV pathogenicity



Su CW et al Gastroenterology 2006, Farci P et al Sem Liv Dis 2012; Kamal H et al Hepatology 2020; Romeo et al PloS one 2014, Ricco G et al, J Vir Hep 2018; Smedile A et al Hepatology 1991

Changing Pattern of CHD in Southern Europe

- 284 patients with CHD consecutively seen in 2 Italian regions (1 center in Piedmont, 2 centers Apulia) from 1977 to 1996



- ✓ In the **second decade** the **prevalence** of patients with clinical **cirrhosis increased** significantly
- ✓ In **10%** of the patients with **mild histology**, liver function tests remained normal or borderline for up to **8 years** of follow-up and **none** of the patients **developed cirrhosis** or **died**
- ✓ In over **90%** of cases baseline histology showed **severe activity** or **cirrhosis**: the estimated time from primary infection to severe liver disease was of about 9 years, with additional 13 years before decompensation

Survival Free of OLT according to baseline liver histology or Clinical Characteristics of the patients

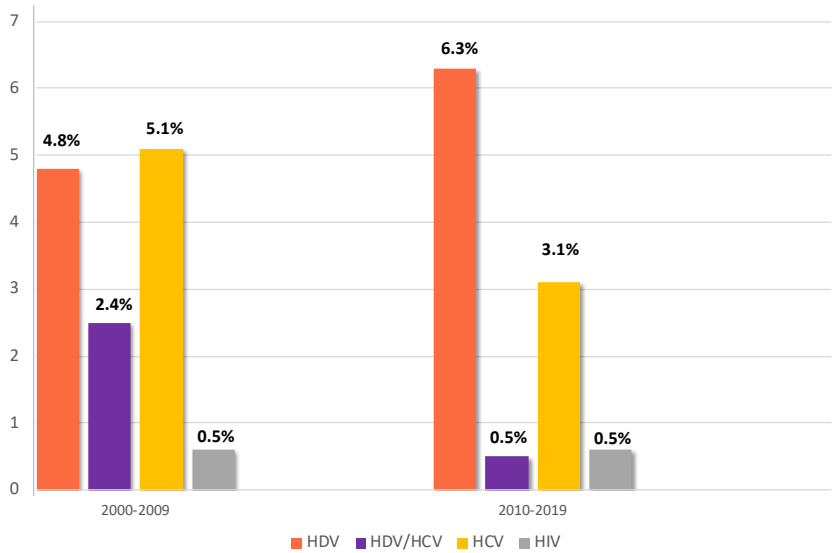
	Total	Mild hepatitis	Severe hepatitis	Histological cirrhosis	Clinical cirrhosis
No. of patients	159	13	73	45	28
Follow-up (*mo*)[a]	78 ± 59	57 ± 33	98 ± 67	72 ± 52	47 ± 35
Survival free of OLT	112 (70%)	13 (100%)	58 (79%)	26 (58%)	15 (54%)
OLT	9	0	1	6	2
Liver-related deaths	32	0	10 **15.1%**	11 **37.8%**	11 **46.4%**
Non–liver-related deaths	6	0	4	2	0

OLT or liver related death

Rosina F et al, Gastroenterology 1999

HDV coinfection over a 20 year observation period

1798 chronic HBsAg positive individuals (828 first decade, 970 second decade)



- 126 (**7%**) patients were **anti-HDV positive**, of whom 25 were also anti-HCV positive.
- **46.8%** of the **HBV/HDV** coinfected patients were **non-Italian natives,** their **prevalence** increased significantly in the second decade, from **31.7% to 60.6% (**P=0.002)
- **Non-Italian natives** were **younger** (34.6 vs 50.8 years, P<0.001) and with a **higher prevalence of female gender** (49.2% vs 26.9%, P=0.017)

- **13.5%** of anti-HDV patients were **HDV-RNA negative** (35.3% previous or ongoing IFN treatment).
- **76.1%** and **71.2% of Italian** and **non-Italian** HBV/HDV coinfected patients **had cirrhosis** (P=0.671).
- **Non-Italian** HBV/HDV **cirrhotic** patients were **younger** than Italian HBV/HDV cirrhotics [37.8 *vs* 51.8 years (P<0.001)].

Ricco G et al, AISF 2021

HDV infection in Tuscany

Preliminary Report from 8 Centers

Arezzo

Empoli

Grosseto

Firenze

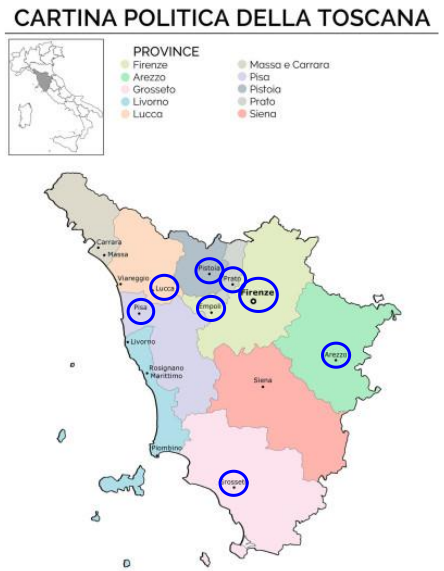
Lucca

Pisa

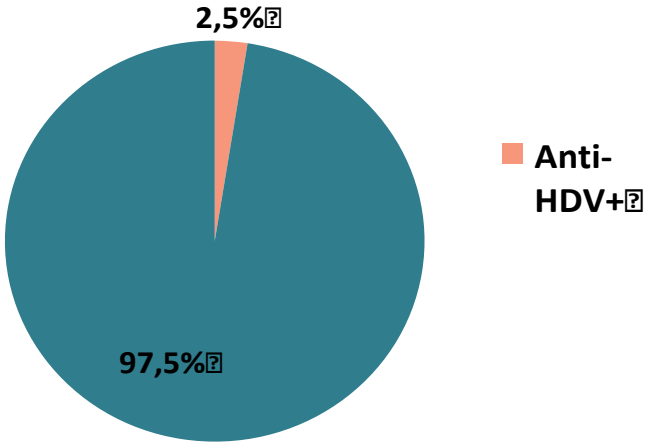
Pistoia

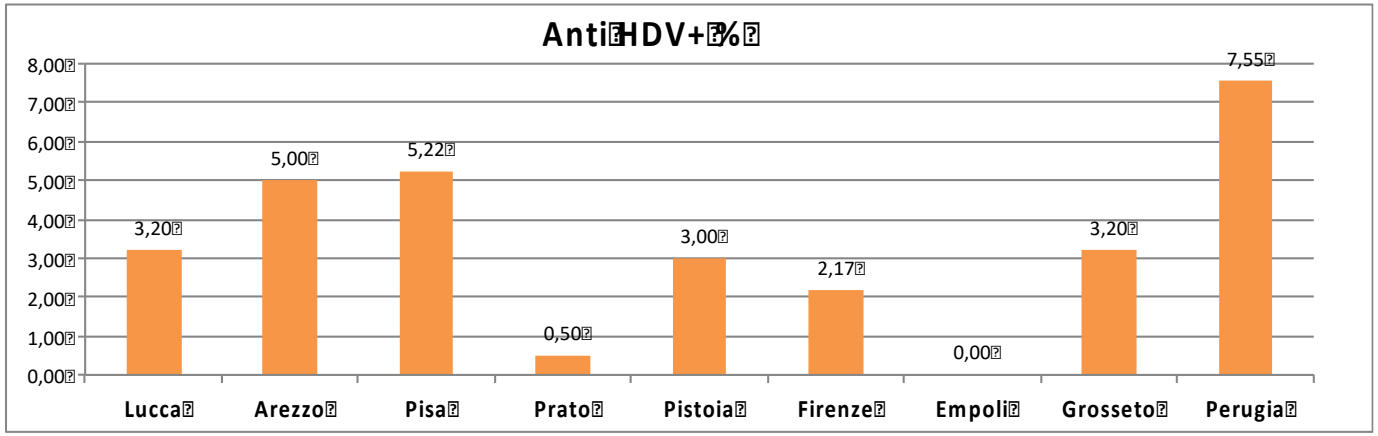
Prato

+ Perugia

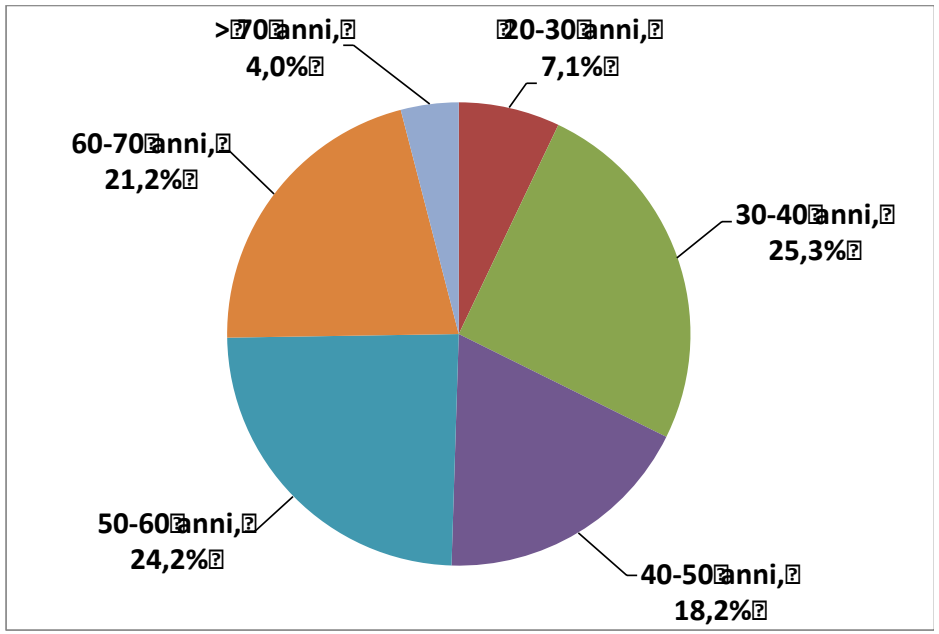


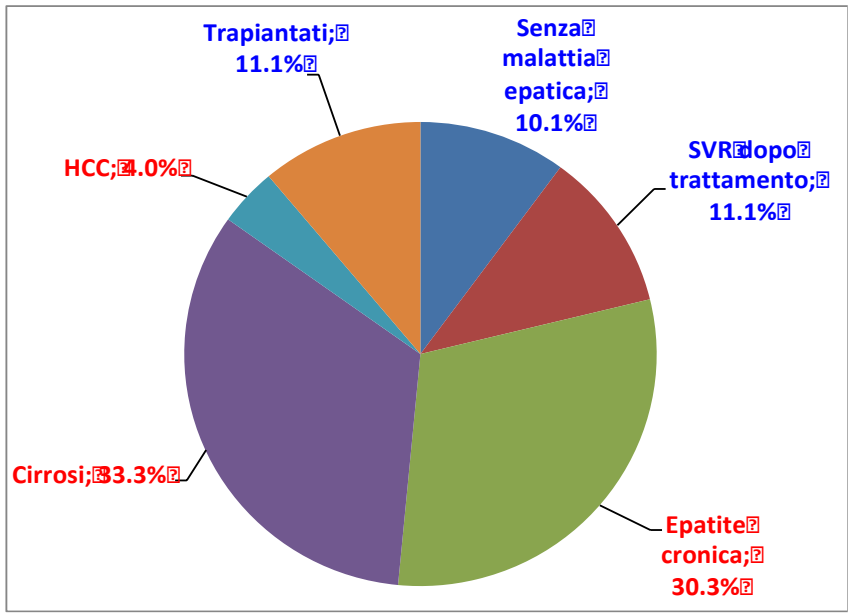
Tutti i Centri: 99 anti-HDV positivi su 3848 HBsAg positivi

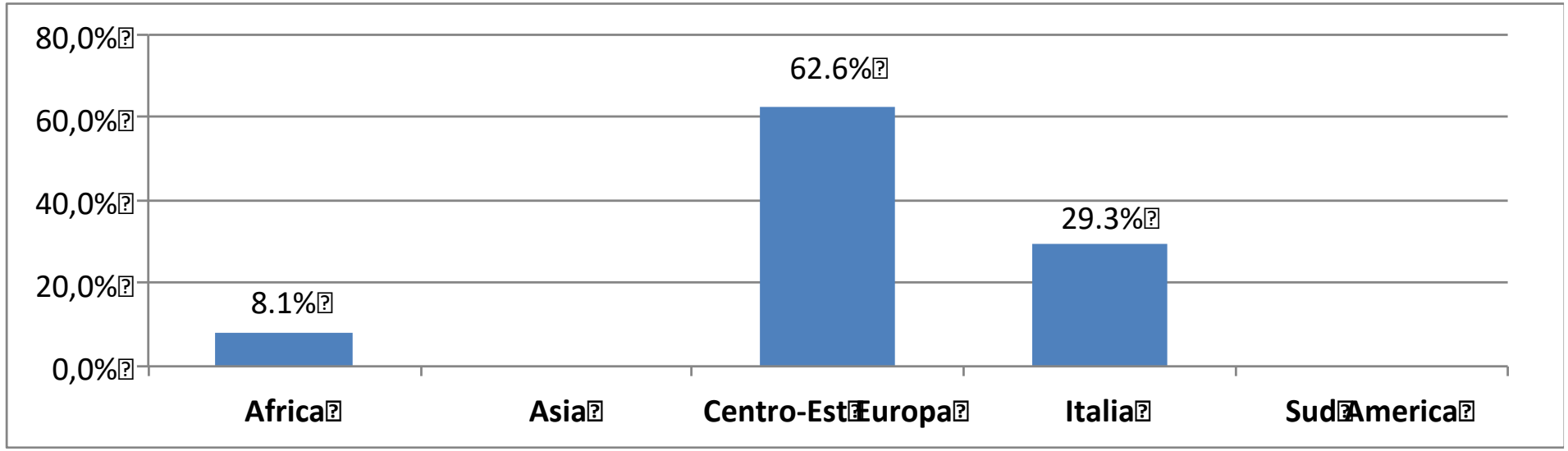




Caratteristiche demografiche anti-HD positivi (99 pz)

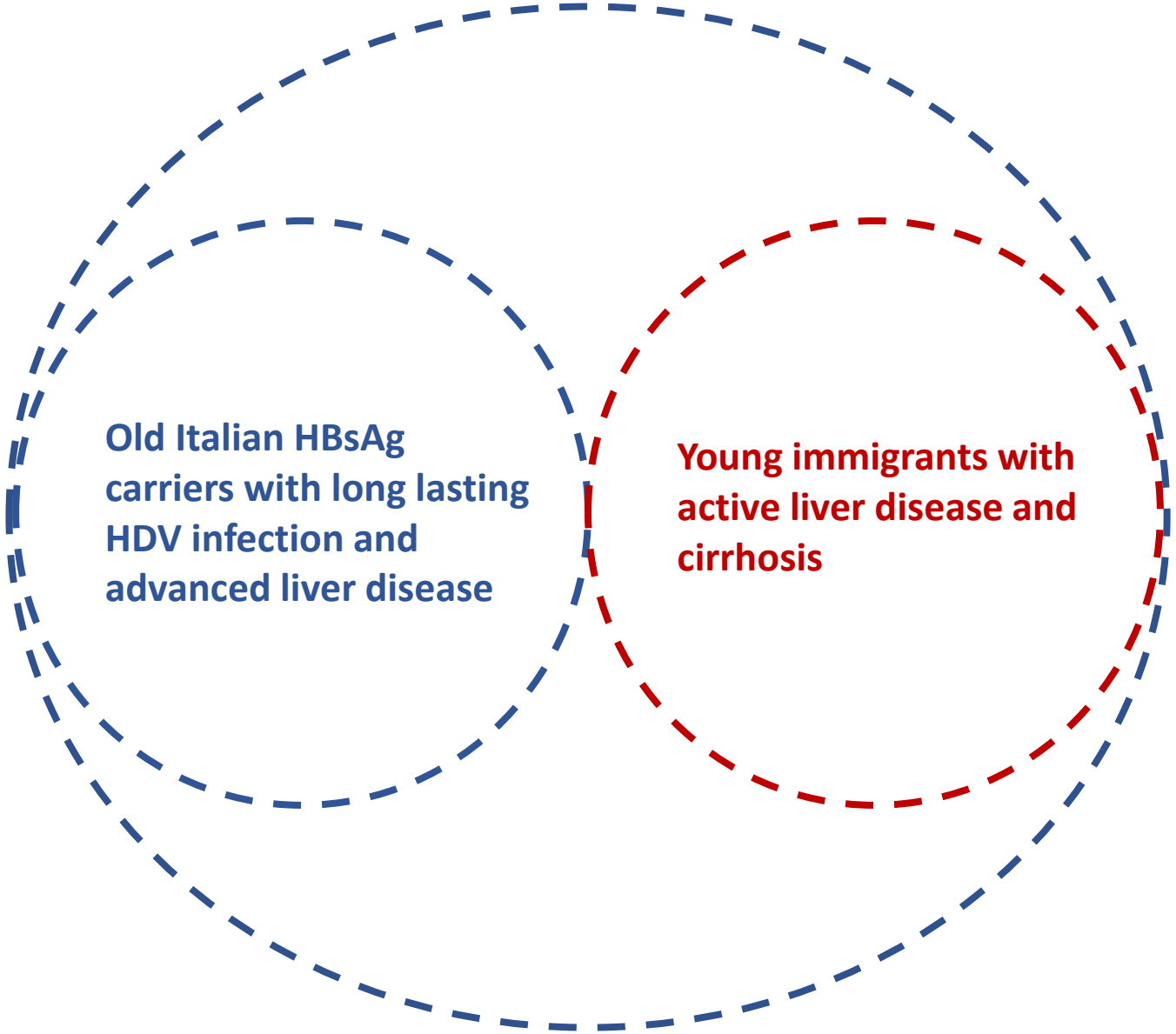






HDV infection in Italy

9% of Italian patients had HCC at admission vs 0% of non Italian natives



Ricco G et al, AISF 2021

The past and the present

Clinical Practice Guidelines

EASL JOURNAL OF HEPATOLOGY

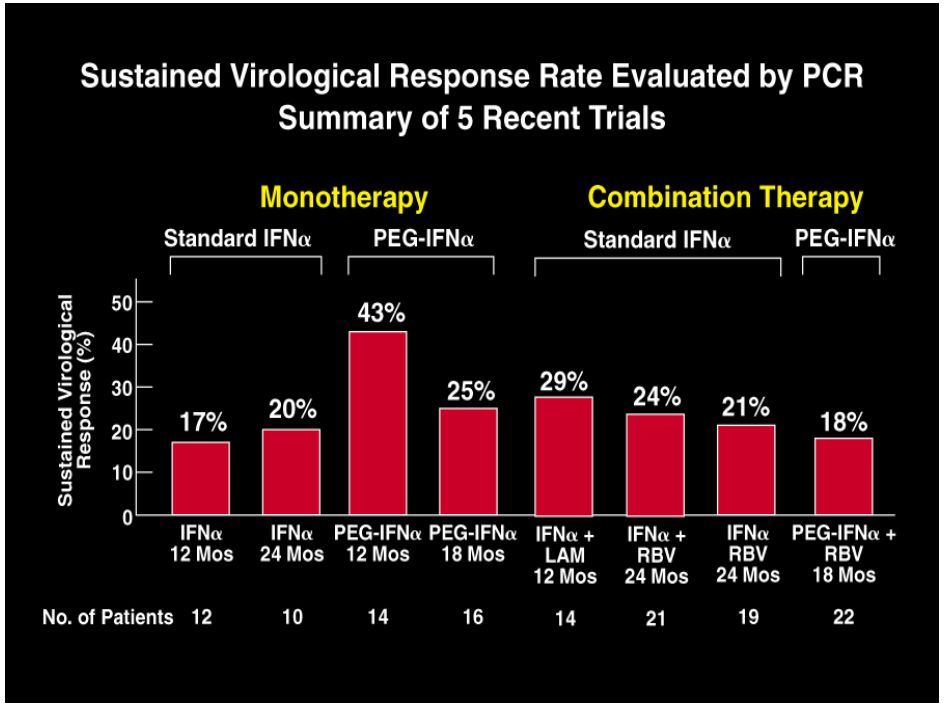
EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection☆

European Association for the Study of the Liver *

- PegIFNα for at least 48 weeks is the current treatment of choice in HDV-HBV co-infected patients with compensated liver disease (Evidence level I, grade of recommendation 1).
- In HDV-HBV co-infected patients with ongoing HBV DNA replication, NA therapy should be considered (Evidence level II-2, grade of recommendation 1).
- PegIFNα treatment can be continued until week 48 irrespective of on-treatment response pattern if well tolerated (Evidence level II-2, grade of recommendation 2).

Interferon and Peg-Interferon in CHD

- ✓ Interferon was **introduced** for the treatment of CHD in the ‘80s
- ✓ A **major limitation** for an accurate evaluation of the virological efficacy of treatment was the **lack of standardized assays to detect HDV-RNA** and the high variability of their sensitivity
- ✓ Therefore, the results of the different studies, where small number of patients were enrolled, were poorly comparable



- ✓ Usually, **SVR** was defined by **undetectable HDV-RNA 6 months** after the **end of therapy**
- ✓ However, **high relapse rates** were reported by long term follow-up studies
- ✓ Nevertheless, **long term follow-up** demonstrated **better clinical outcomes** in patients who maintained SVR
- ✓ **Prolonged admistration of Peg-IFN** does not reduce the relapse rate, but it is associated with **histological improvement**

Barbara L et al J Hep 1986; Farci et al Gastroenterology 2006; Farci P et al, Hepatology 2006; Yurdaydin G et al J Hep 2019

Peg-IFN in monotherapy or with NA in CHD

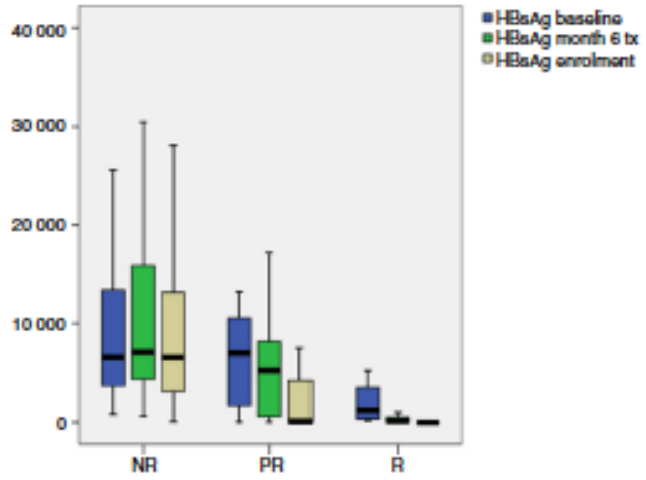
(HIDIT-1 and 2 trials)

- On treatment virologic response (**undetectable HDV-RNA**) in **17-48%** of the patients
- At **24 weeks post treatment** discontinuation persistence of virologic response (undetectable HDV-RNA) in **25%** of the patients, with **later relapses** in over **50%** of responders
- **HBsAg loss** in about **10%** of the patients → the hallmark of **HDV infection cure**
- **Peg-IFN** combination **with TDF does not** improve the **EOT** HDV response rates
- **Long-term follow-up** [mean time of follow-up was 8.9 (1.6 - 13.4) years] of HIDIT-1 study suggests that **off-treatment HDV RNA response** to PEG-IFNα leads to **improved clinical long-term outcome.**

Heidrich B et al Hepatology 2014;Wedemeyer H et al , Lancet gastro& Hepat 2019; Wranke A et al JVH 2020

HBsAg kinetics in chronic hepatitis D during interferon therapy: on-treatment prediction of response

- 62 patients with CHD, treated with Peg-IFN, were considered: 14 patients cleared the HBsAg and HDV-RNA (responders, R), 12 cleared the HDV-RNA remaining positive for HBsAg (partial responders, PR) and 36 cleared neither the HBsAg nor the HDV-RNA (nonresponders, NR).
- The mean time from the EOT and the enrollment was 5 +/- 2.9 years



	NR	PR	R	*P* value
HBsAg baseline mv IU/mL	6577	7031	1187	0.020
HBsAg month 6 mv IU/mL	7073	5213	108	<0.001
HBsAg enrolment mv IU/mL	6559	62.5	0	<0.001

- ✓ HBsAg < 1000 IU/ml at month 6 discriminated R from PR/NR p<0.001
- ✓ PR showed different HBsAg kinetics during treatment, with a trend to a further decline after the EOT

- ➢ A **reduction of serum HBsAg** is **mandatory** for the **definitive clearance of the HDV-RNA**.
- ➢ Quantitative HBsAg may predict the long-term response to Peg-IFN therapy and provide a guide to prolong or stop treatment.

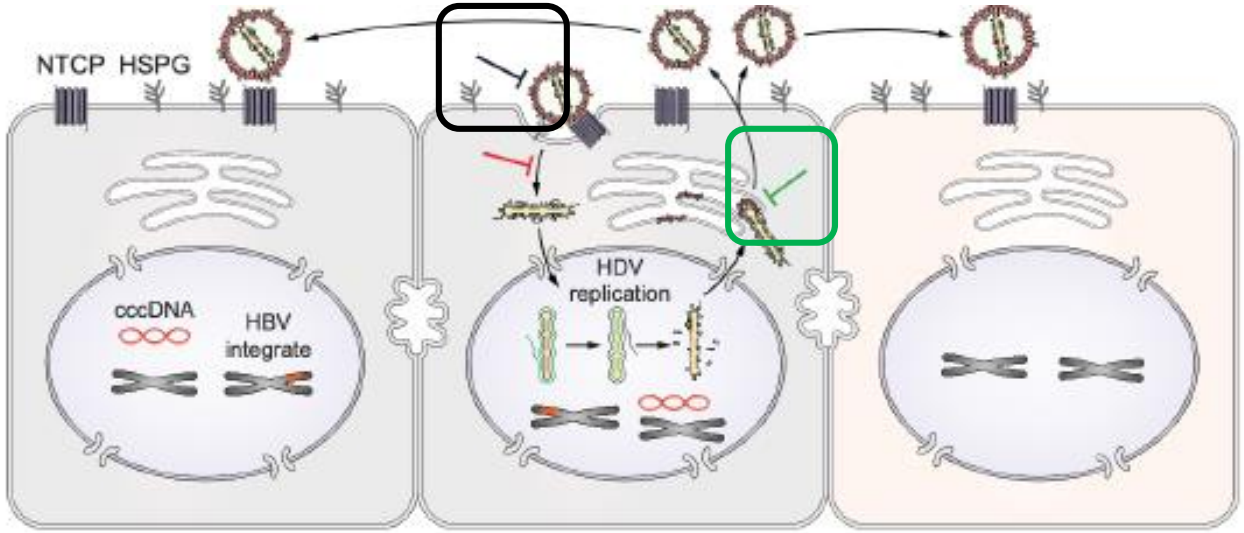
Niro GA et al AP&T 2016

Old and new targets for HDV infection control and CHD cure

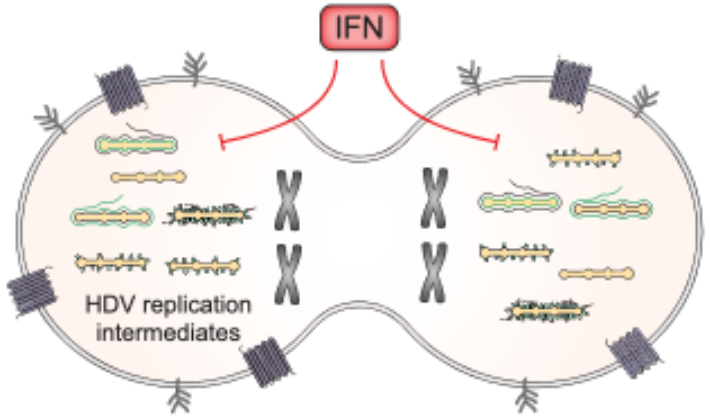
Entry inhibitors

Prenilation inhibitors

IFNs



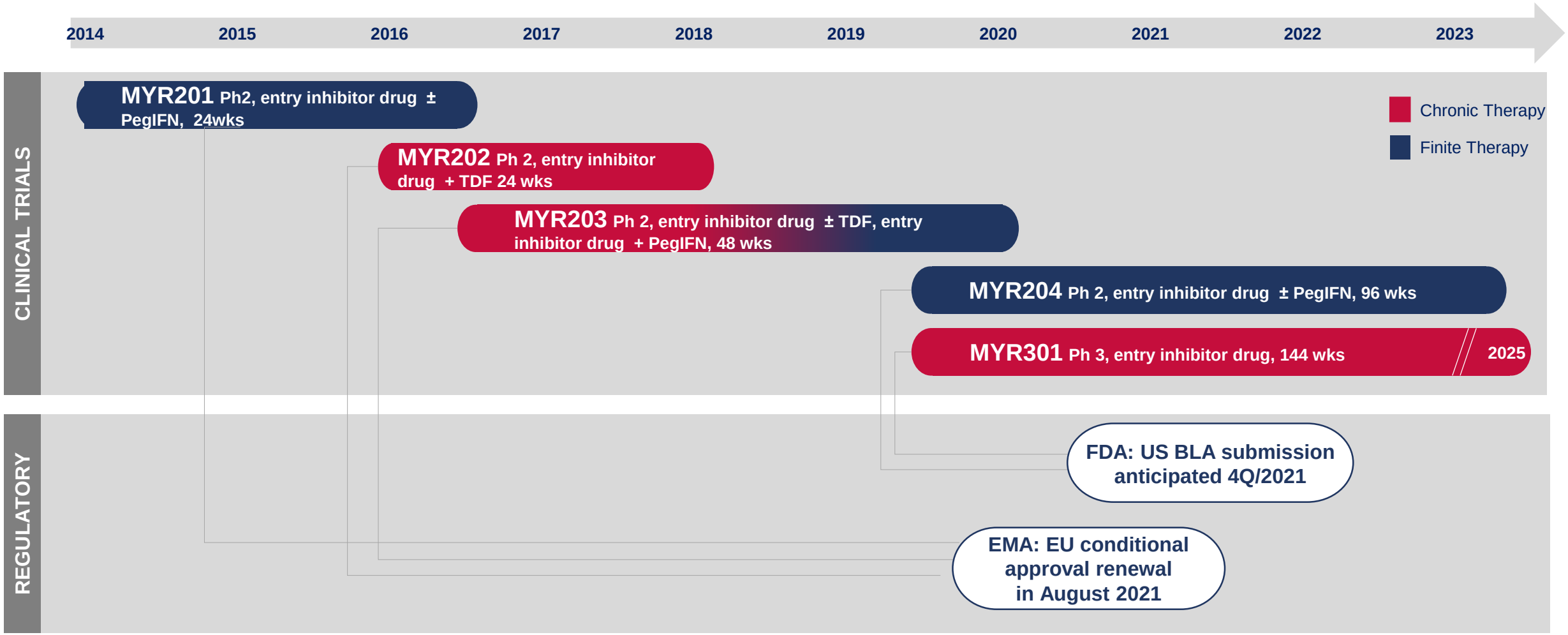
To block *de novo* infection



To block cell division mediated spread

Zhang Z et al J Hep 2021

Entry inhibitor - Bulevirtide: Phase 2/3 Clinical Trial Program

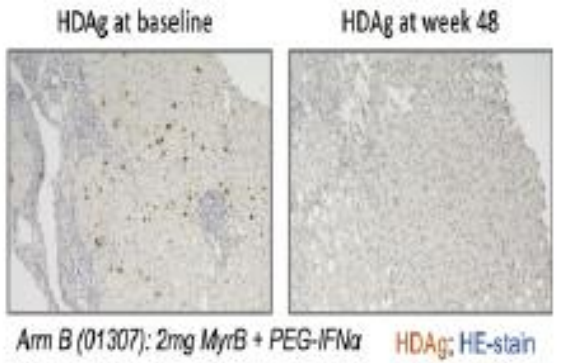


FDA, Food and Drug Administration; PegIFN, pegylated interferon; TDF, tenofovir disoproxil fumarate; US, United States.

References: ClinicalTrials.gov. Study MYR201 (NCT02637999). Study MYR202 (NCT03546621). Study MYR203 (NCT02888106). Study MYR204 (NCT03852433). Study MYR301 (NCT03852719).

Buleviritide: Phase 2/3 Clinical Trial Program

- Interim (6 months of treatment) analysis of Phase 2b entry inhibitor ± Peg-IFN (**MYR 204**):
  - **HDV-RNA** decline ≥ 2 log in **72-92%** of pts
  - **ALT** normalization in **24-64%** of pts
- Interim (6 months of treatment) analysis of Phase 2b (**MYR 301**), entry inhibitor (2 or 10mg) monotherapy:
  - **HDV-RNA** decline ≥ 2 log in **55-68%** of pts
  - **ALT** normalization in **53-38%** of pts
  - Rapid ALT reduction and normalization were observed in >50% of patients in the entry inhibitor 2-mg arm after 24 weeks of treatment
- **Plasma HDV-RNA decline** correlates with **intrahepatic decrease of HDV-RNA** and **HDAg** in **MYR 203** study
- Entry Inhibitor treatment was also associated with **reduced expression** of **interferon-stimulated genes** and **inflammatory chemokines** and cytokines **in MYR 203** study
- **HBsAg response** (≥ 1 log decline) 24 week after EOT in **13 to 40%** of patients receiving entry inhibitor + Peg-IFN in **MYR203**

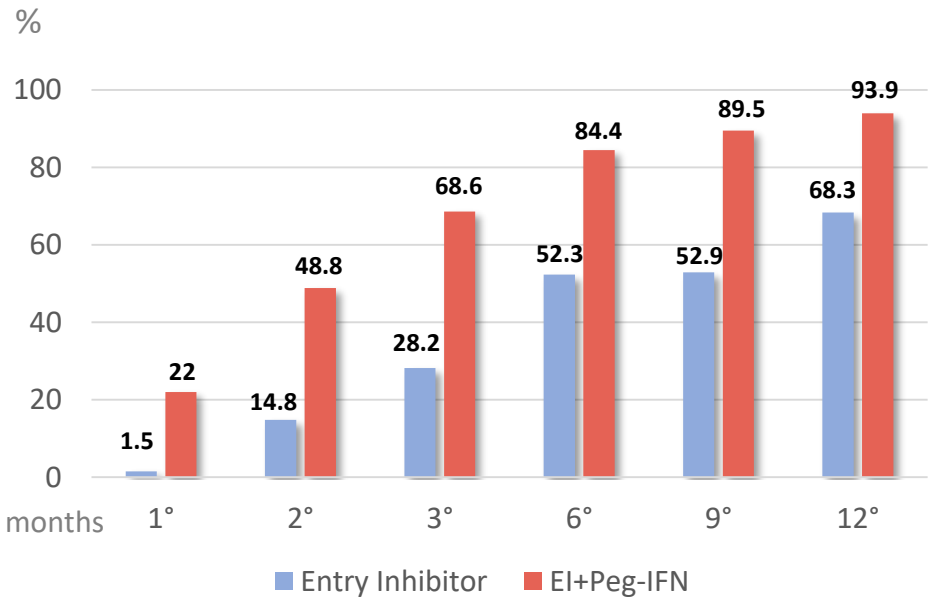


Asselah T, et al. EASL 2021; Wedemeyer H, et al. AASLD 2018; Allweiss. AASLD 2021;Wedemeyer H, et al. EASL 2020 Wedemeyer H, et al. EASL 2021.

Safety and Efficacy of 2 mg Bulevirtide in Chronic HBV/HDV Co-Infection: the French Early Access Program

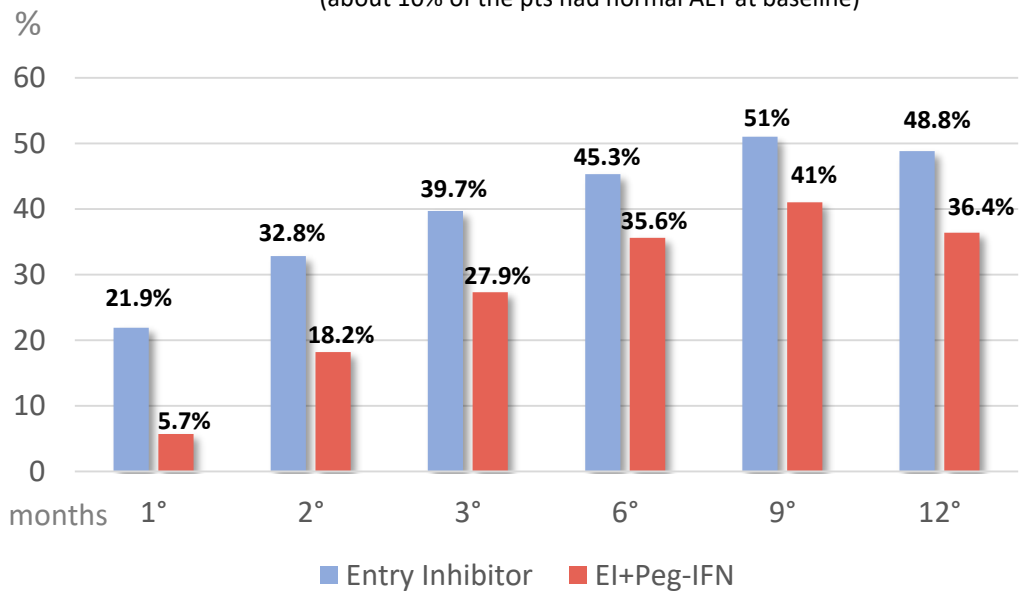
- 145 adults with compensated cirrhosis or severe liver fibrosis (F3) or with F2 fibrosis with persistent ALT >2 x ULN for ≥6 mo (N = 145) were enrolled in a multicenter, observational study;
- 77 received entry inhibitor (2 mg) monotherapy and 68 entry inhibitor (2 mg) + Peg-IFN

HDV-RNA decline ≥ 2log or undetectable



ALT < 40 IU/l

(about 10% of the pts had normal ALT at baseline)



Entry inhibitor monotherapy was associated with a slower HDV-RNA decline as compared to Combo therapy, as expected the opposite occurred for ALT

De Ledinghen. AASLD 2021. Abstr OA21.

Lonafarnib (LNF)

- LNF is an **oral inhibitor of Farnesyl transferase**, an enzyme involved in the **modification of proteins** through a process called **prenylation**
- Originally, LNF was developed as **inhibitor of farnesylation of RAS proteins**, that mediates oncogenic transformation of cells
- Development abandoned due to low efficacy (evasion by oncogenic RAS)
- Prenylation of HDAg promotes its association with HBsAg and is essential for initiating the HDV particle formation process

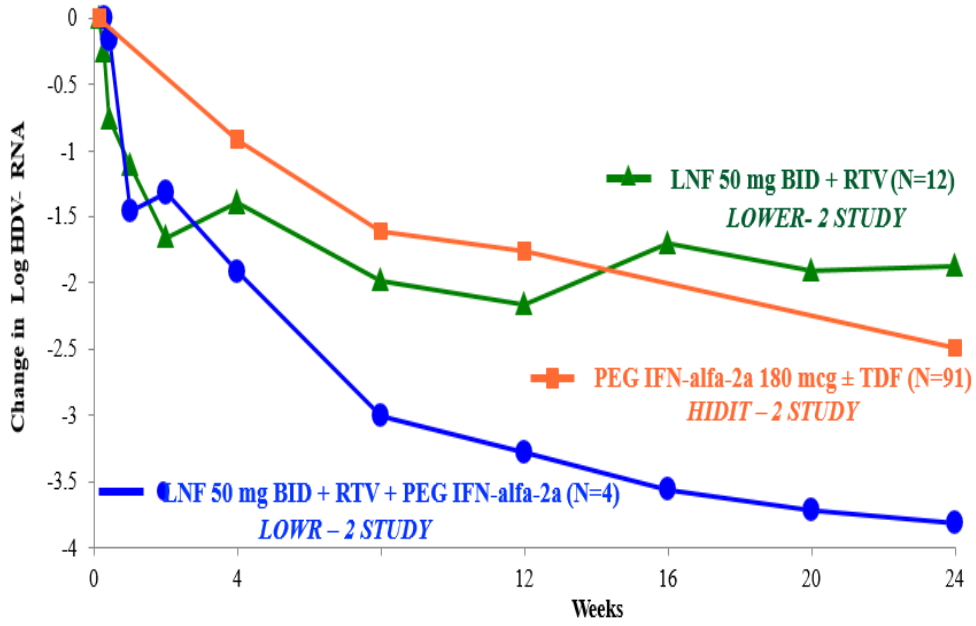
Over 120 HDV patients have been treated in Phase 2 clinical studies evaluating the tolerability and efficacy of LNF, alone and in combinations with other agents, currently a Phase 3 clinical study is planned to enroll 400 pts.

Dose dependent HDV-RNA decline were observed in proof of concept studies: 2 log for 300 mg, 1.54 log for 200 mg and 0.73 for 100 mg at w 4 → but major GI side effects for higher doses

Addition of **Ritonavir** –an inhibitor of CYP3A4, the predominant mediator of Lonafarnib metabolism – achieves greater serum concentrations with less drug to the GI tract (LOWR-HDV studies 1 -4 --LOnafarnib With and without Ritonavir in HDV--)

Elazar M et al in *Hepatitis D, by Rizzetto M 2019*

Lonafarnib + RTV with or without PEG IFNalpha for HDV

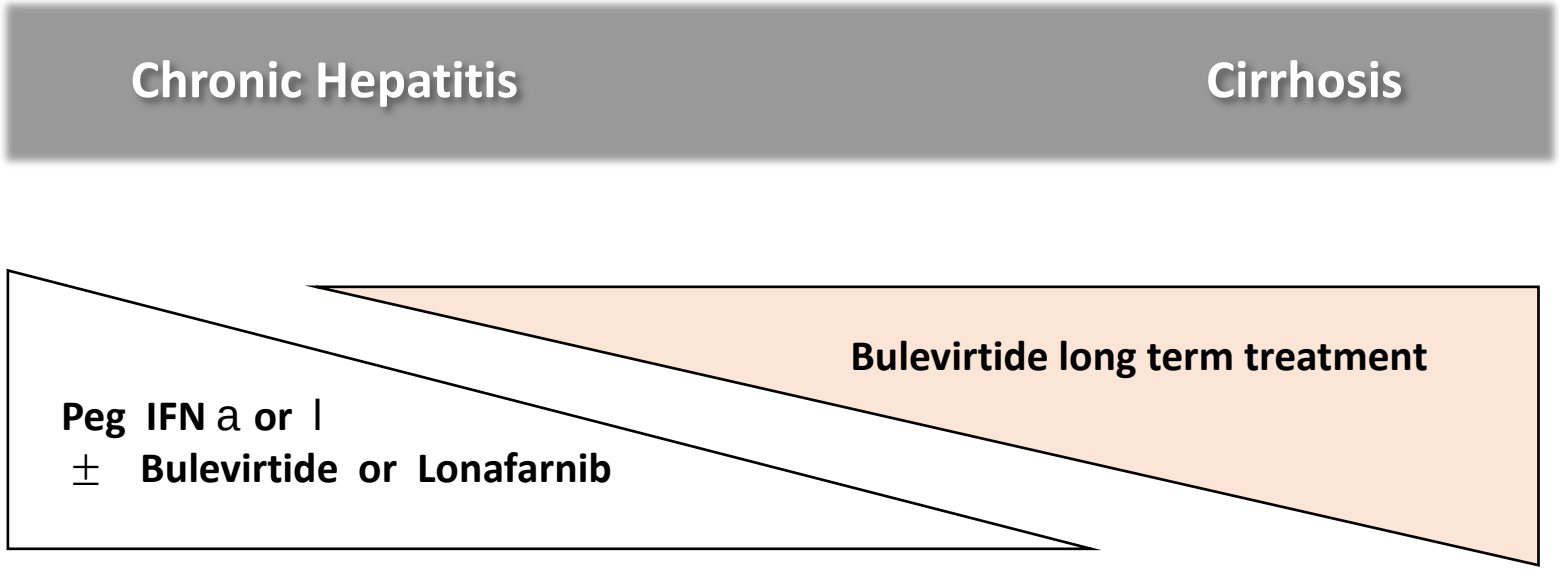


- **All-oral:** Lonafarnib boosted with Ritonavir
  - 39% (7 of 18) patients ≥ 2 log decline or BLQ at Week 24
  - 60% patients normalized ALT at Week 24
- **Combination:** Lonafarnib boosted RTV+PEG IFN-alfa2a
  - 89% (8 of 9) patients ≥ 2 log decline or BLQ at Week 24
  - 78% patients normalized ALT at Week 24
- **Safety:** Predominant AEs for LNF were GI-related (mild/mod.)

- Combination of **LNF+Peg-IFN** achieves the **greatest antiviral responses**
- The **antiviral response** is observed **early,** during treatment, therefore **repeated short** (3 months) courses could be also attempted
- A sub analysis of the study suggests that **LNF 50 mg + RTV 100 mg BID** appeared to be a particularly effective option for patients with **low baseline viral load**, with a **100%** (7/7) response.
- LNF+RTV treatment can result in post-treatment flares, evenutally resulting in viral RNA negativity and ALT normalization in selected patients

Yurdaydin et al, J Hepatol 2018, Abstract #PS-161

Chronic Hepatitis D



To cure the infection/disease **To control the infection/disease**

Additional factors influencing the treatment schedule:

- ✓ Extent of HDV replication
- ✓ Phase of HBV infection (HBeAg/anti-HBe status; HBV-DNA and HBsAg levels)

Brunetto MR, Personal Opinion 2022

New, promising therapeutic options for Chronic Hepatitis D are being tested in clinical trials

However, only a better understanding of HBV and HDV interaction will foster more targeted therapetic approaches

A stratification of the patients according to the status of HDV/HBV interplay could foster a more personalized approach to Chronic Hepatitis D treatment and possibly the achievement of a higher therapeutic index

Magritte R, Les Memoires d'un Saint 1960