Chairs:
Pietro Lampertico and Heiner Wedemeyer

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Delta Cure
3rd International Meeting

Diagnosis, Staging, HCC risk and antiviral therapy

Peg-IFN+NUC: predictors of response

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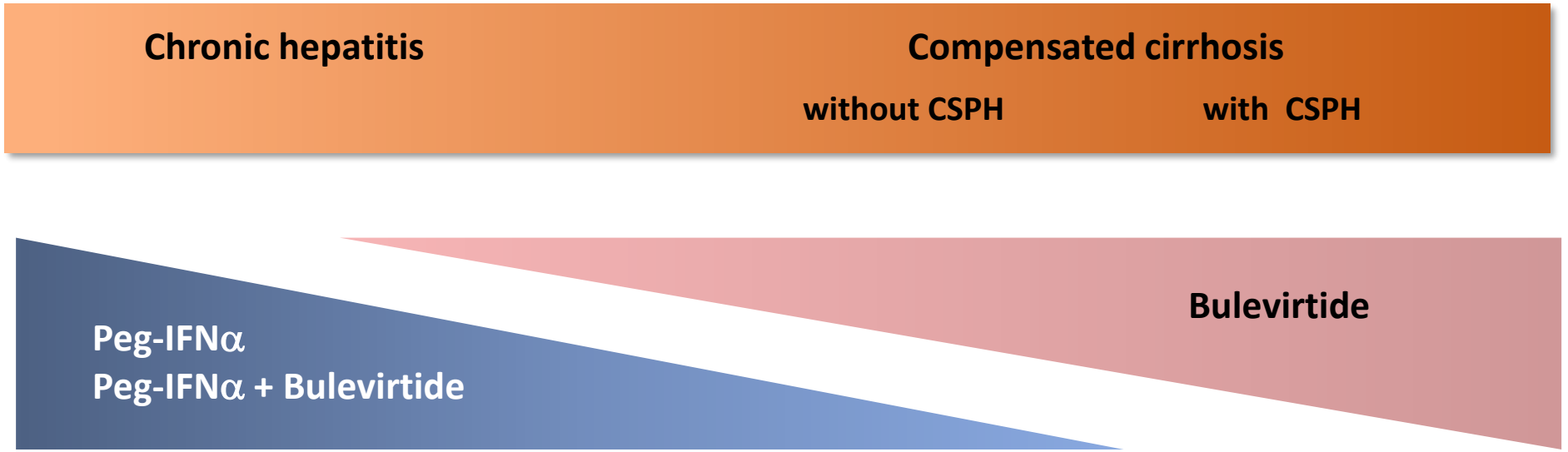
MR Brunetto **Disclosures**

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

Treatment of Chronic Hepatitis Delta



Finite treatment

Prolonged treatment

To cure the infection/disease

To control the infection/disease

Additional factors influencing the treatment schedule

- ✓ Phase of HBV infection (HBeAg/anti-HBe status; HBV-DNA and HBsAg levels)
- ✓ IFNa contraindiciation, tolerability
- ✓ Patient's will and compliance to treatment

Which patients with CHD can be treated with PegIFNa?

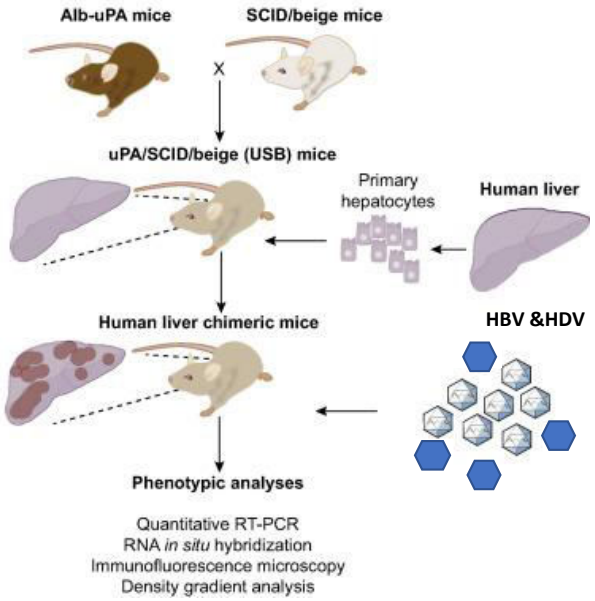
Statement

- IFNa has been used since the ‘90s for the treatment of CHD. Mono- and multicentre studies have been conducted with IFNa, with only two randomised phase II studies published. Nevertheless, long-term data on clinical benefit and safety are available **(LoE 2, strong consensus).**

Recommendations

- All patients with CHD and compensated liver disease, irrespective of whether they have cirrhosis or not, should be considered for treatment with PegIFNa **(LoE 2, strong recommendation, consensus).**
- PegIFNa for 48 weeks should be the preferred treatment schedule **(LoE 3, strong recommendation, consensus).**
- Personalised treatment durations may be considered based on HDV RNA and HBsAg kinetics and treatment tolerability **(LoE 3, weak recommendation, strong consensus).**

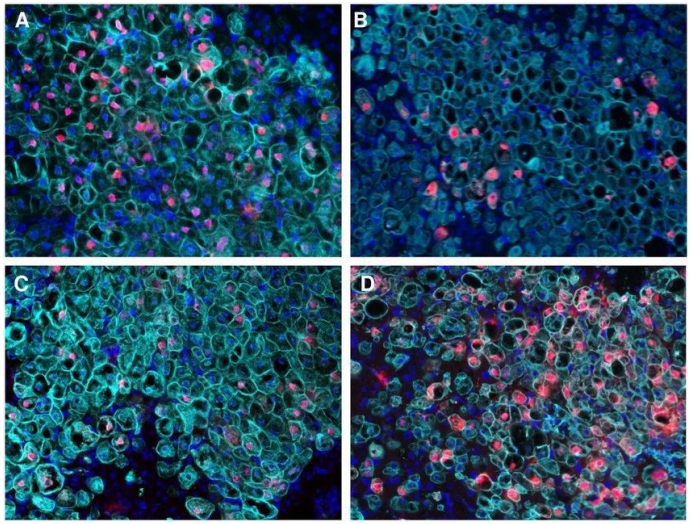
Both interferon alpha and lambda can reduce all intrahepatic HDV infection markers in HBV/HDV infected humanized mice



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 levels determined 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**.

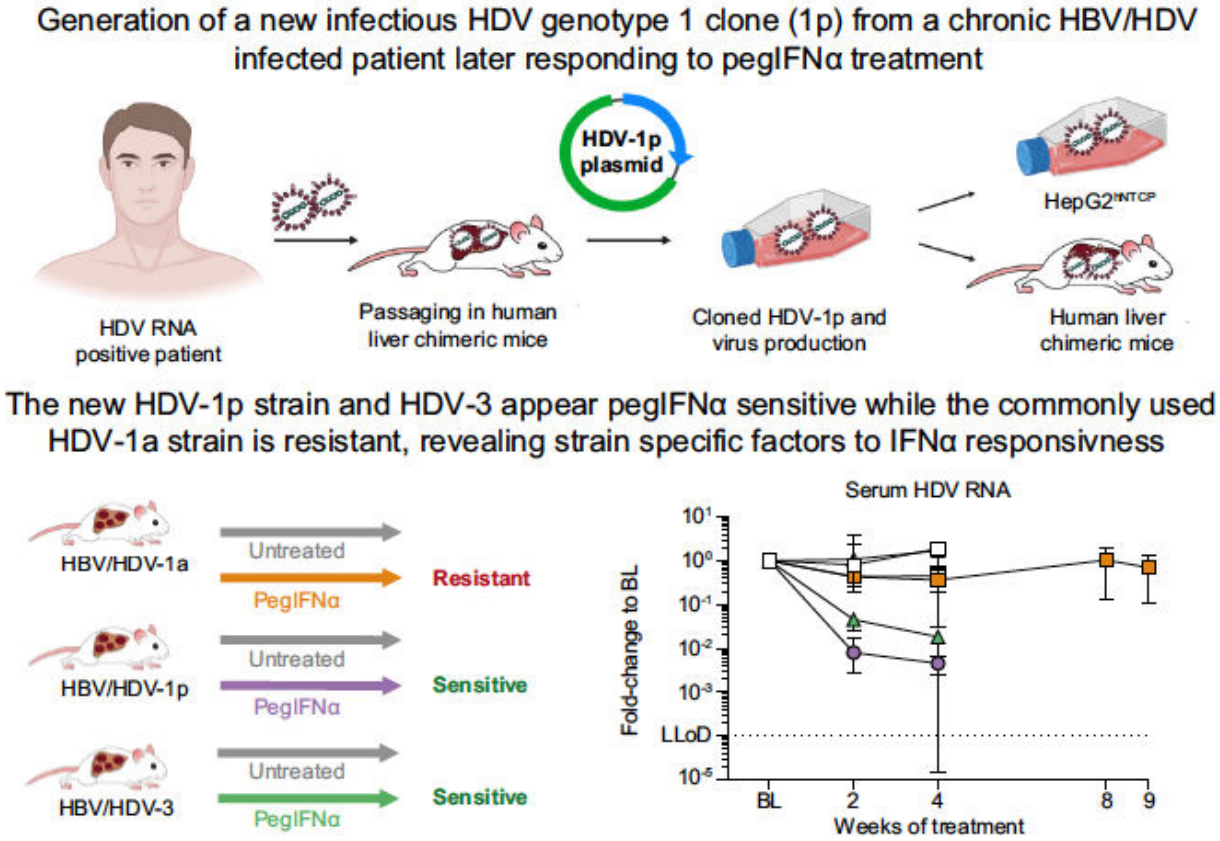
- NA-mediated suppression of HBV replication (2.1-log) did not significantly affect HBsAg levels, HDV productivity and/or release.
- **In humanized mice lacking adaptive immunity, IFNs but not NA suppressed HDV.**
- Viremia decrease reflected the intrahepatic reduction of all HDV markers, including the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



Giersh K et al Scientific report 2017

Strain-specific responsiveness of HDV to interferon-alpha treatment

The responsiveness to Peg-IFNa of 3 different cloned HDV strains was studied in chronically infected mice

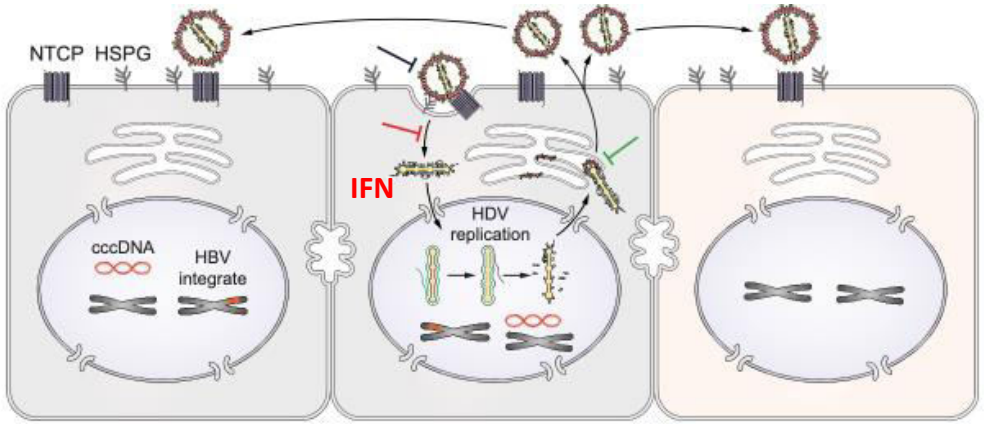


- Peg-IFNa **reduced viremia of ≈ 2 log** and **intrahepatic HDV markers** in mice infected with HBV/**HDV-1p** and HBV/**HDV-3**, but **not with HBV/HDV-1a**
- Primary Human Hepatocytes (**PHHs**) infected with HBV/**HDV-1p** and HBV/**HDV-1a** received Peg-IFNa → **intracellular HDV-RNA decline of 1.6 log after 14 days only in HBV/HDV-1p infected PHHs**
- Human **ISGs**, pattern recognition receptors (**hMDA5**) and **chemokines** were similarly and strongly **upregulated** upon HDV **infection** with all the 3 strains
- **Peg-IFN further enhanced ISGs** (2-29x)
- Genome sequencing showed high identity for the ribozyme site and variability in the large HDAg, but not in known post-translational modification sites

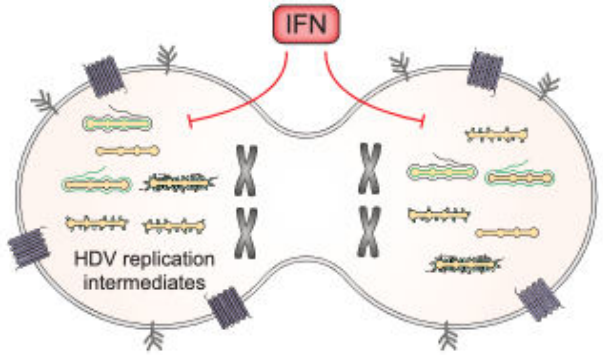
- HDV-1a (obtained from an untreated pt and serially passed in chimps and woodchuck and then cloned) shows intrinsic resistance to Peg-IFN
- **Virus specific determinants may influence the response to IFNa**

Giersch K et al J Hep R 2023

HDV entry, replication and persistence, the importance of cell-to-cell transmission and the role of IFN



- **HDV** depends on **HBsAg for spreading via NTCP** but can also **maintain its genome** in the liver **through cell division**
- **IFN** affects numerous steps of **HDV life cycle** after the canonical **NTCP mediated infection,** including **entry, replication and secretion**

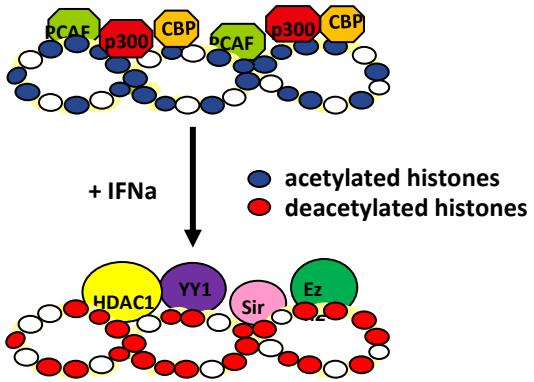


- Both exogenously and endogenously induced **IFNs** (alpha and lambda) responses **restrict HDV persistence during hepatocyte proliferation**.
- The severe loss of HDV replicative intermediates during cell division may be explained by exposure of viral RNA to induced **ISGs**, that may either **cause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

Adapted from Zhang et al, J Hep 2021

Zhang Z et al J Hep 2019 and Zhang Z et al J Hep 2021; Lucifora J et al J Hep 2022

Interferon antiviral activities in CHB



IFNa treatment is accompanied by a decrease in the acetylation of cccDNA bound H4 histones in vitro

Generic antiviral activity

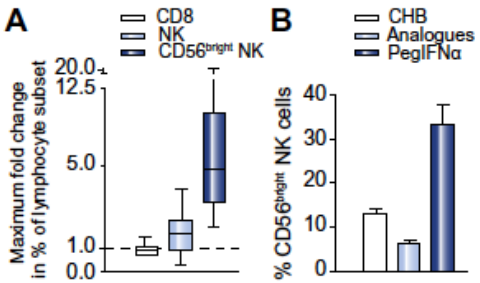
- IFN activates ***multiple genes of the host*** (ISGs), many of which have antiviral activities, interfering viral life cycle.

Specific antiviral activity

- **IFN-α** inhibits HBV transcription and replication by **targeting the epigenetic regulation** of the nuclear **cccDN**A minichromosome
- **cccDNA degradation** induced by **IFN-α** and **lymphotoxin-β-receptor activation** through up-regulation of APOBEC3A and 3B cytidine-deamin

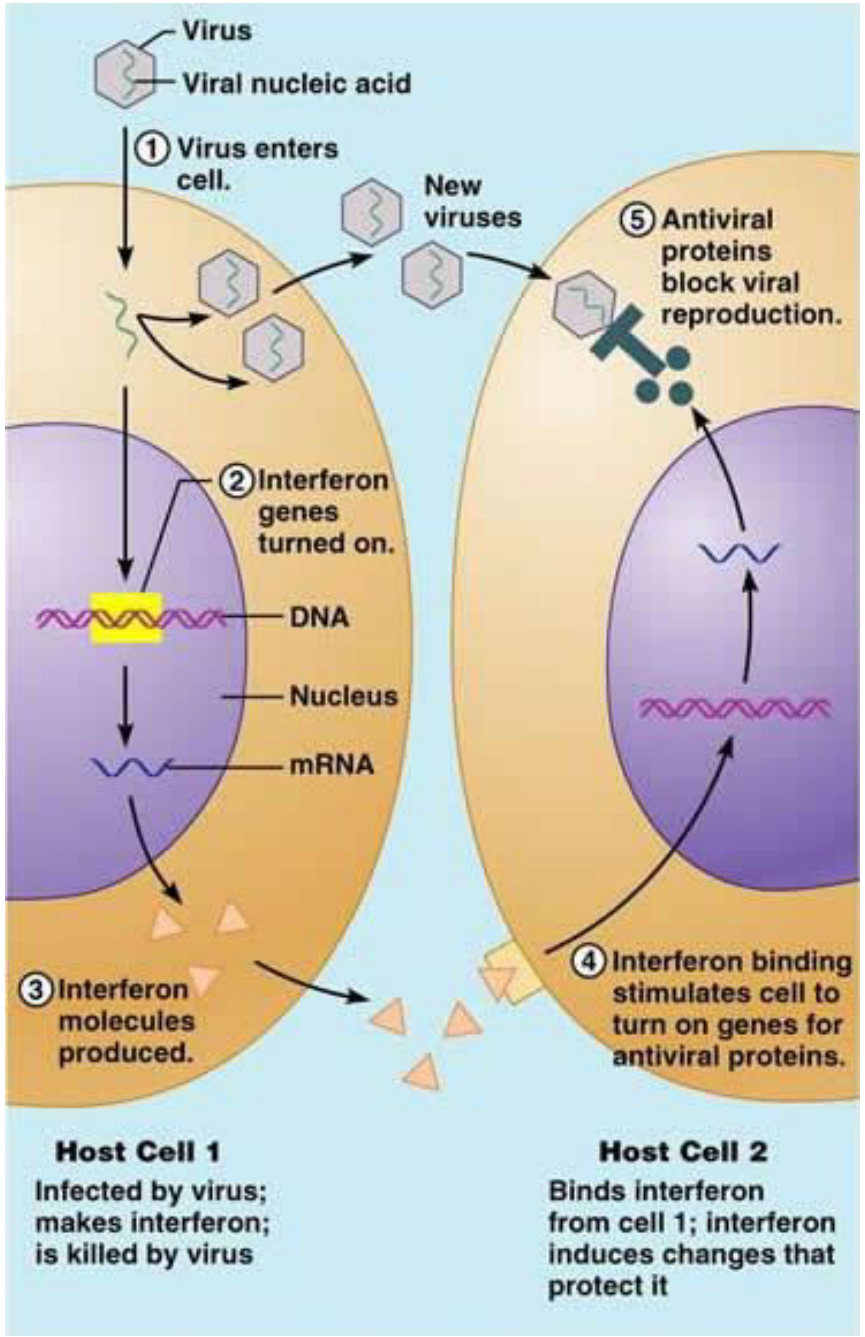
Immunomodulatory Activities

- IFN-α mediates **divergent effects** on the **innate** and **adaptive** arms of the immune system in vivo.
- The efficacy of PegIFNα may be limited by its depleting effect on CD8 T cells; conversely, it can cumulatively drive **proliferation**, **activation** and **antiviral potential** of **CD56(bright) NK cells**.
  - The percentage of CD8 T cells remained stable, whilst NK cells showed a trend to increase.
  - Such boosting of CD56 ^{bright} NK cells was likely to be an immune modulatory effect rather than an indirect effect of viral load reduction



Micco L, J Hepatol 2013, 58: 225-233; Brunetto M et al, Intervirology 2014

Pollicino T, 2006; Belloni et al., J Clin Invest 2012 ; Lucifora J et al Science 2014



The response to IFN implies the activation of multiple genes of the host (ISGs)

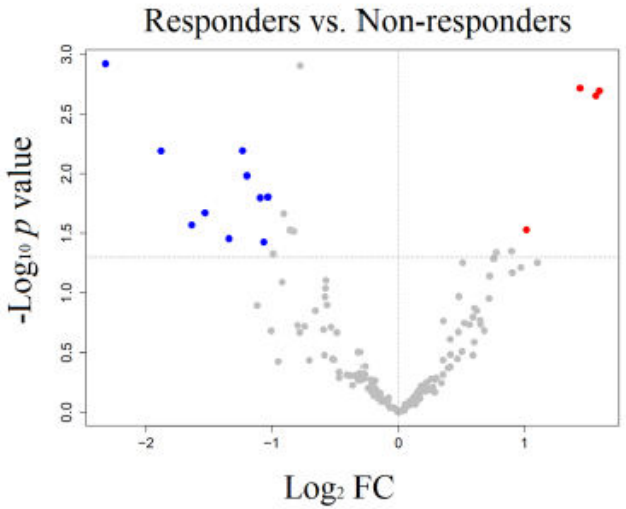
Single nucleotide polymorphisms (SNPs) near the interleukin 28B (*IL-28B*) gene - rs12979860, rs8099917 - are strongly associated with SVR to Peg-IFN +/- RBV in CHC

- 4 studies investigated their role in response to Peg-IFN in CHD: overall 149 CHD patients were stuided with conflicting results
- In 3 studies (112 patients) the response to treatment was not influenced by IL28B polymorphysms, by converse in the remaining study (37 patients) a bordeline association was observed

At present there is no evidence of a role of IL28B polymorphysms in response to Peg-IFN in CHD

Yilmaz b et al Acta Gastroent Belg 2016, Visco-Comandini U et al DLD 2014, Ispiroglu M et al J Infec Dev C 2017; Heller T et al, AP&T 2014

MiRNome Profiling of Circulating Extracellular Vesicles in Patients With CHD undergoing Peg-IFN treatment



- 20 HDV patients Peg-IFN treated: 8 were Responders (HDV-RNA undetectable 6 months after EOT)
- In Responders, at BL 10 miRNA were downregulated and 4 upregulated as compared to Non responders
- After 6 months of Peg-IFN 7 miRNA were de-regulated, with distintc expression profiles according to the response
- **The differential expression of miRNA 155-5p appears to differentiate both at BL and after 6 months of therapy Responders vs Non responders**

- **BL miR-155-5p** expression was inversely correlated with HBsAg (rs = −0.49, 95% CI −0.77 to −0.06; p = 0.028), showing a trend with HDV RNA (rs = −0.39, 95% CI −0.71 to 0.07; p = 0.092).
- **At 6 months of therapy**, **miR-155-5p** showed a **strong inverse correlation** with both **HBsAg** (rs = −0.71, 95% CI −0.88 to −0.39; p < 0.001) and **HDV RNA** (rs = −0.53, 95% CI −0.79 to −0.12; p = 0.016).
- At **logistic regression analysis**, both **miR-155-5p** (at baseline: OR = 4.52, 95% CI 1.25–16.38; p = 0.022; at 6 months: OR = 5.30, 95% CI 1.19–23.65; p = 0.029) and **HDV RNA** (at baseline: OR = 0.19, 95% CI 0.05–0.79; p = 0.022; at 6 months: OR = 0.38, 95% CI0.17–0.84; p = 0.018) resulted **significantly associated to virologic response**.

The assesment of EV miR-155-5p may represent an additional valuable tool for the management of HDV patients undergoing Peg-IFNα treatment

Caviglia GP et al JVHep 2024

To add complexity to the complexity

- ✓ Not all the studies were randomized
- ✓ Not all the patients were treated with NUCs
- ✓ The number of patients was usually small
- ✓ The assays used to quantify HDV-RNA showed different sensitivity and dynamic range
- ✓ The definition of response was not always the same:
  - Cure of both HBV and HDV infection *(achievement and persistence of undetectable HBsAg and HDV-RNA)*
  - Cure of HDV infection *(undetectable HDV-RNA 6 months after EOT)*

Escher MC, Relativity 1953

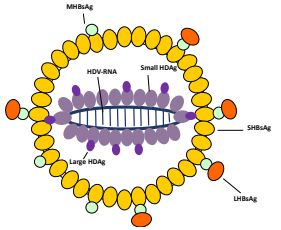
Virologic response to Peg-IFN +/- NUCs

Parameter	Rate of occurrence with IFN treatment	Clinical benefit (improved survival)
HBsAg loss	2.5% (0-25%)*	Yes§
Undetectable HDV-RNA • 24 wPT	29% (24-34%)*	Yes§,**,&
• for 2 years after EOT • ≈ 8.9 years PT	50%** 36.6%***	
2 log HDV-RNA decline at EOT, maintained thereafter	n.a.**** 10/14 pts with normal ALT at EOT, maintained in 7/12 (58.3%) after 12 years	Yes****

*Abdrakhman A et al Ant. Research 2021: Meta-Analysis on 13 and 8 studies using Peg-IFN ;
** Yurdaydin C et al J infct Diseases: 99 pts treated for at least 6 months, cumulative median duration 24 (6-126) months, median 2 courses;
***Wranke A et al JVH 2020: HIDIT I long term follow-up;
**** Farci P et al Gastroenterology 2004: long term follow-up of 36 pts treated with standard IFN;
§ Heidrich B et al Hepatology 2014 and Wranke A et al Hepatology 2017;
&Keskin O et al Clinical Gastroenterology and Hepatology 2015

Baseline predictors of response or non response

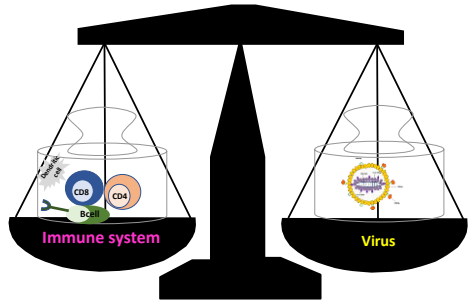
Genetic profile: ***IL28B SNPs***
miRNAs: ***miR-155-5p***



Viral constitutive features: ***genotype 5*** *infection was associated with higher response rates than GT 1*

Roulot D et al, J Hep 2020; Spaan M et al J Hep 2020

Stage of liver disease: ***cirrhosis, portal hypertension***

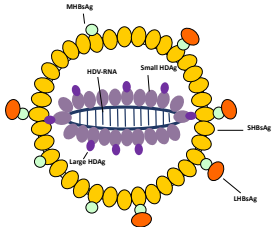


Virus/host interaction: **lower** ***HDV-RNA, HBsAg and HBcrAg levels*** *were associated with higher response rates*

Niro G et al, AP&T 2016, Spaan M et al, J Hep 2020, Yurdaidin C et al, J Infect Dis 2018; Wedemeyer H Lancet Infect Dis 2019; Sandmann L et al Hepatol comm 2022

Baseline predictors of response or non response

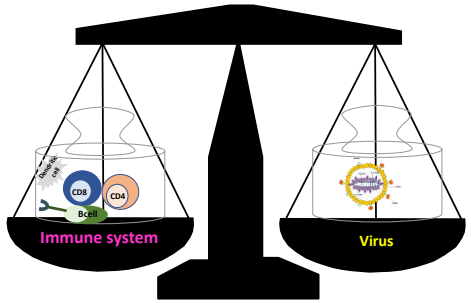
Genetic profile: ***IL28B SNPs***
miRNAs: ***miR-155-5p***



Viral constitutive features: ***genotype 5***

Stage of liver disease:

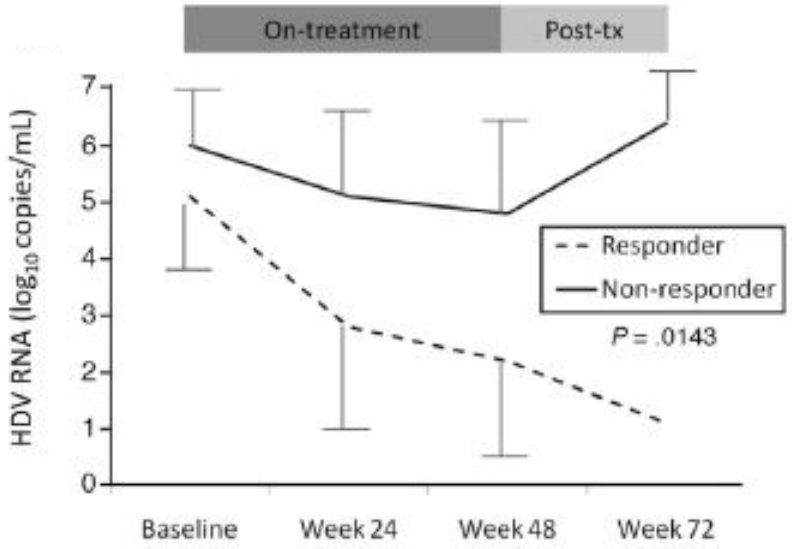
- ✓ ***most of the studies indicate that Peg-IFN is equally effective in patients with or without cirrhosis***
- ✓ ***However, among patients with cirrhosis the chances of response could be lower in those with low PLTS (proxy for portal hypertension)***

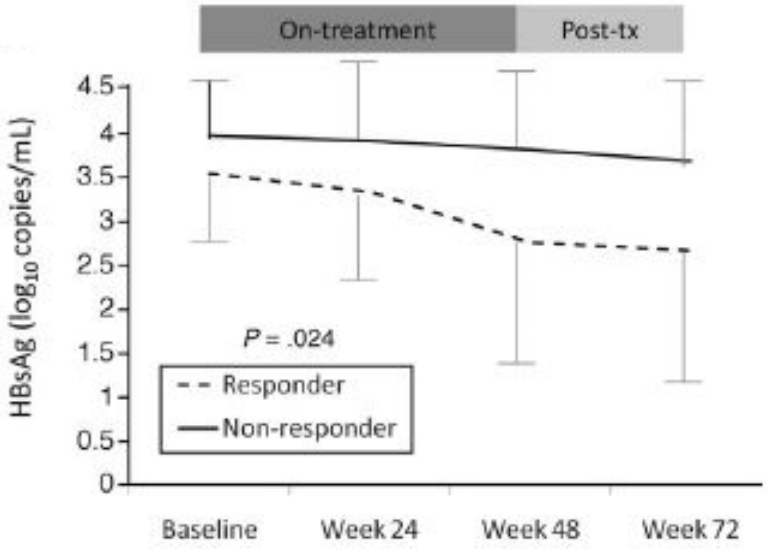


Virus/host interaction: ***HDV-RNA, HBsAg and HBcrAg levels***

Farci P et al Gastro 2004; Wedemeyer H et al Lancet Infect Dis 2019; Kabacam G et al Turk J Gast 2012; Yurdaydin C et al J Infect Dise 2018; Gunsar Antiviral Therapy 2005

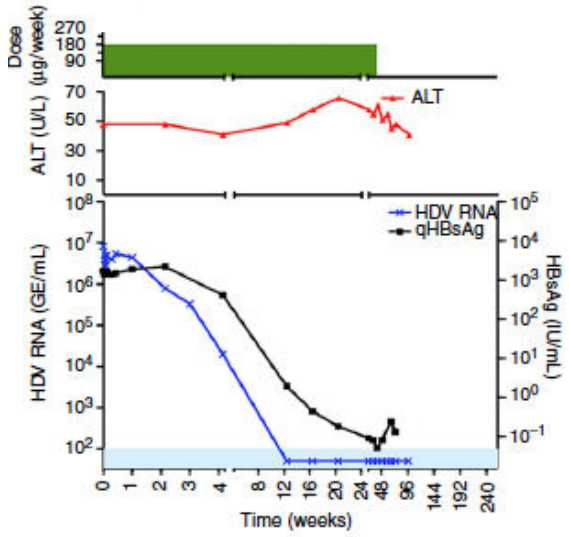
On treatment predictors: HDV-RNA kinetics during and after Peg-IFN treatment

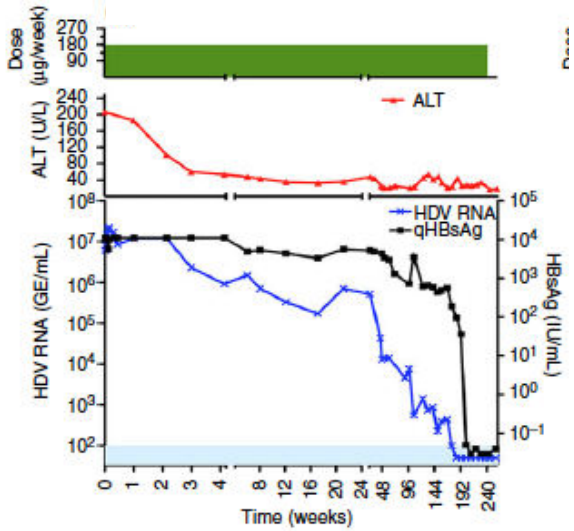




- 50 CHD patients treated with Peg-IFNa +/- ADV for 48 weeks HIDIT-I
- **Significant differences** between **Responders and Non Responders** were observed in the **overall kinetics** for both the viral markers throughout the observation period

However





- Among **Responders at individual** level the HDV-RNA and **HBsAg kinetics may vary significantly**
- **a subset of patients may show a later HDV-RNA decline**: after the first 24 weeks and the clearance of HDV-RNA had been reported after EOT in about 20% of 24 w post-treatment Responders

Keskin O et al Clinical Gastroenterology and Hepatology, 2015; Heller t et al, AP&T 2014; Niro G et al Hepatology 2006; Niro G et al AP&T 2016

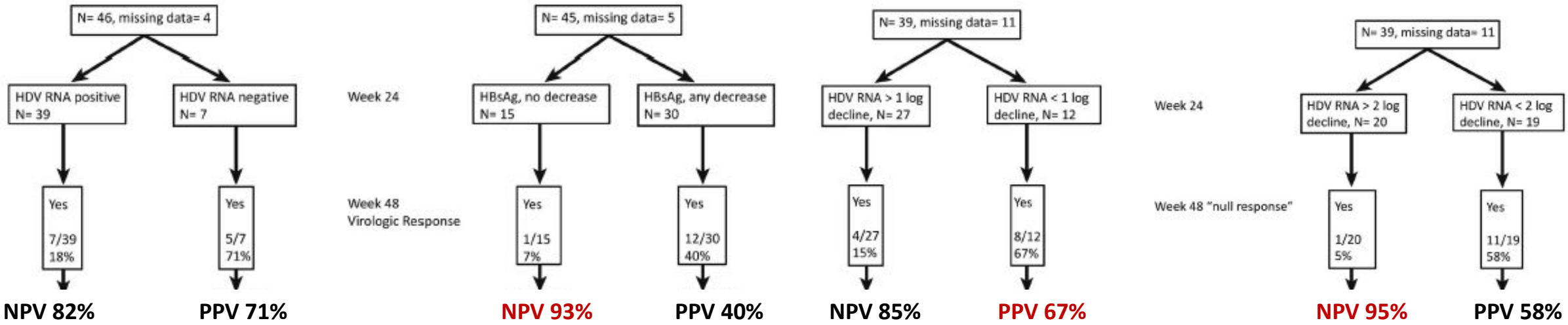
Association Between Level of Hepatitis D Virus RNA at Week 24 of Peg-IFN Therapy and Outcome

- 50 CHD patients from HIDIT-1 trial were studied
- For **41 pts data 24-week post treatment** were available
- **Null response** defined as **< 1 log HDV-RNA decrease at EOT**
- **However** 2 of 11 (**18%**) **NR achieved the virological response at 24 weeks after EOT**, both had BL viremia levels ≤ 4 log

End of treatment response (undetectable HDV-RNA)

EOT Virologic response

EOT Null Virologic response



- In **58% of EOT responders HDV-RNA became undetectable after 24weeks of treatment**
- The **lack of any HBsAg decline** at week 24 was observed only in 1 EOT responder
- Only 8% of Null Responders at EOT had > 2log HDV-RNA decline at week 24

Keskin O et al Clin Gastro and Hep 2015

Association Between Level of Hepatitis D Virus RNA at Week 24 of Peg-IFN Therapy and Outcome

Keskin O et al Clin Gastro and Hep 2015

- 50 CHD patients from HIDIT-1 trial were studied
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- **Null response** defined as **< 1 log HDV-RNA decrease at EOT**
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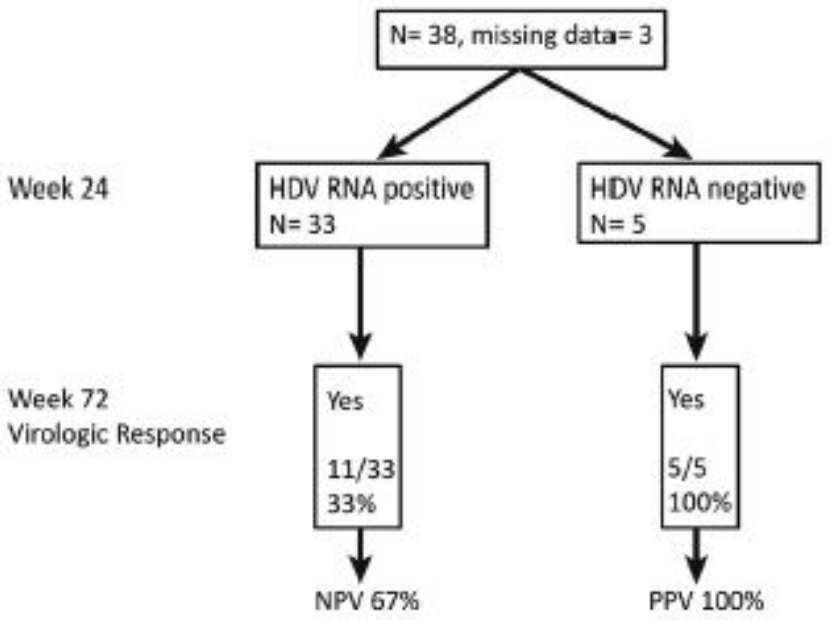
Post treatment week 24 response



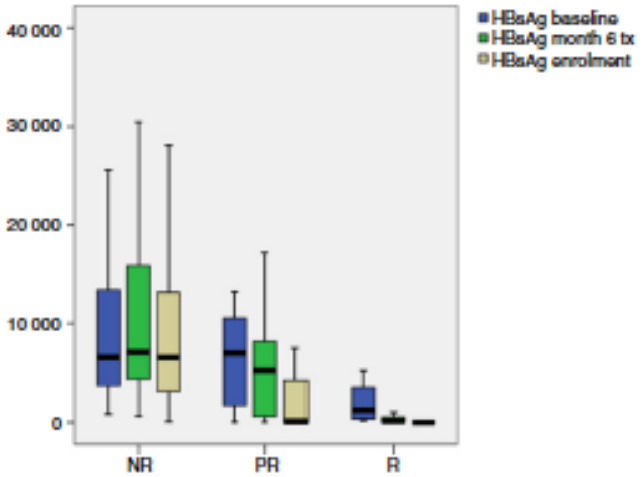
- **All** the patients with **undetectable HDV-RNA at week 24** of treatment achieved **virological response 24 weeks after the EOT** (VR)
- However they were **only** 5 of the 16 (**31%**) **VR**
- Overall pts with **VR** showed a **more significant decrease of HBsAg serum levels** compared to non responders:

Week 24 (39 pts) $\log_{10}$ IU/ml	3.32 ± 0.91	vs	3.93 ± 0.66	p=0.02
Week 48 (37 pts) $\log_{10}$ IU/ml	3.07 ± 1.27	vs	3.80 ± 0.75	p=0.04

- The major limitation of the study is the **small number of cases**, with **data missing** at the different time points
- The definition of **Null Response is questionable** and **consistent only for patients with high BL viremia HDV-RNA > 6 log**

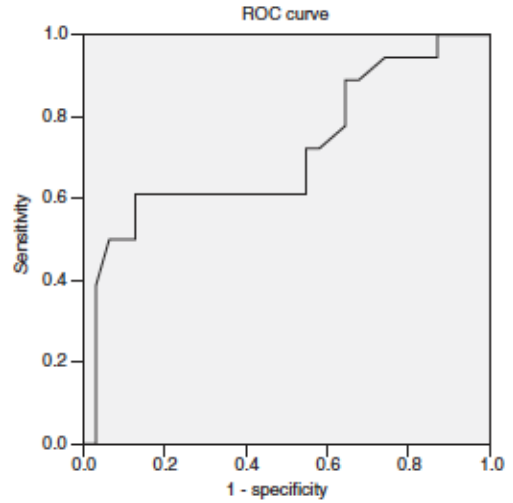
HBsAg kinetics in chronic hepatitis D during interferon therapy: on-treatment prediction of response

- 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 (non responders, NR).
- The mean time from the EOT and the enrollment was 5 +/- 2.9 years



HBsAg IU/ml	NR	PR	R	*P value*
Baseline	6577 (750-42883)	7031 (18-34500)	**1187** (173-8823)	0.020
24 w of therapy	7073	5213	**108**	<0.001
Enrolment	6559	62.5	**0**	<0.001

- ✓ HBsAg < 1000 IU/ml at month 6 discriminated R/PR from NR p<0.001
- ✓ At month 6, in 7/12 (58%) of PR HBsAg was >1000 IU/ml, showing a slow decline
- ✓ By ROC curve, the optimal HBsAg threshold to discriminate R/PR patients from NR was a 0.105 Logarithmic reduction of this antigen from BL to month 6.
  Cut-off value of 0.713, with a sensitivity of 61% and a specificity of 87%.

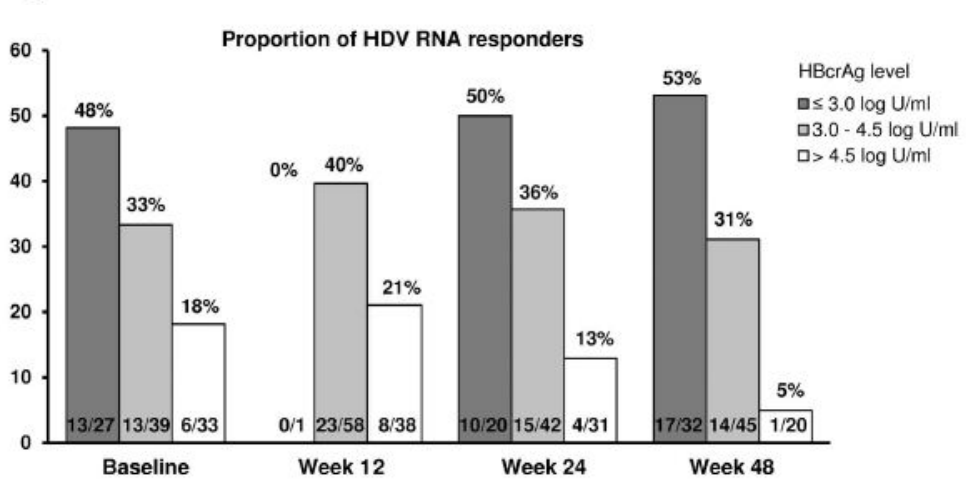


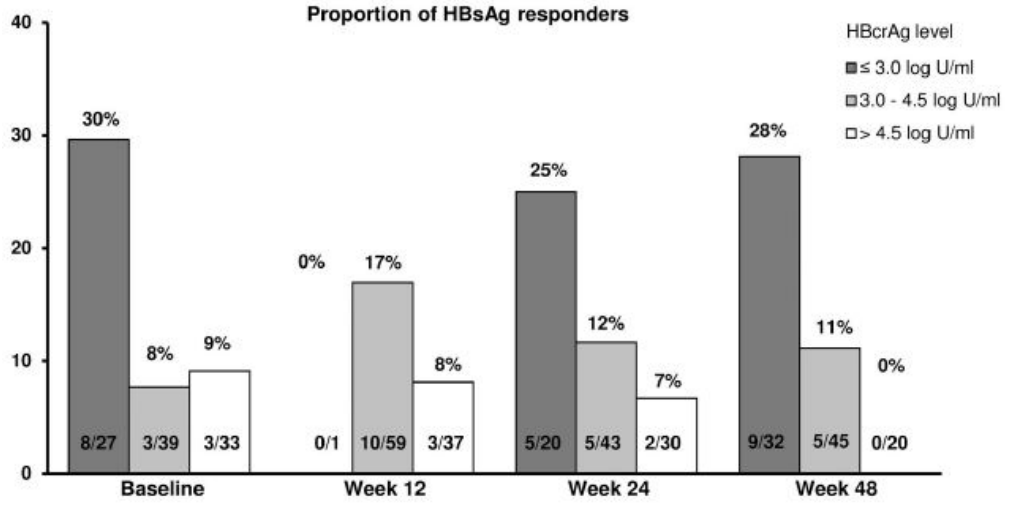
➢ **Quantitative HBsAg may contribute to predict the long-term response to Peg-IFN therapy**

Niro GA et al AP&T 2016

HBcrAg levels are associated with virological response to treatment with IFN in patients with CHD

- 99 or 120 patients enrolled in HIDIT-II trial (peg-IFN + TDF or placebofor 96 w) had results available at w24 post EOT
- 45 pts had undetctable HDV-RNA at EOT, 32 at 24 w post EOT (35.6% relapsed)
- 3 pts with detectable HDV-RNA at w 96 cleared viremia within w24 post EOT





- **HBcrAg serum levels** were significantly **lower at BL, w24 and 48** in pts with **undetectbale HDV-RNA 24 w PT**
- **4.72** and **4.5 log U/ml at BL and w24** were the optimal **cut off to distinguish 24 w PT** responders from non responders

Sandmann L et al, Hepatology Communication 2022

- The **kinetics of HDV-RNA** in Responders to Peg-IFN are **highly variable** and about **20%** of them show a **slow** or **late decline**

- **Undetectable HDV-RNA** at **w. 24 of treatment** had **100% PPV** of virologic response **24 w. PT**, however **it was achieved** in **31%** of VR only (analysis in 41 pts of HIDIT-1 trial)
- **Less than 1 log HDV-RNA decline** at w. 24 of treatment had **67% PPV** in the identification of **Null Responders** (analysis in 41 pts of HIDIT-1 trial)
- On therapy **significant HBsAg declines** were observed **in virological responders** 24 w. after EOT and HBsAg levels **< 1000 IU/ml** were shown to discriminate **virological responders** from **non responders**
- **HBcrAg** (a marker of HBV cccDNAtranscriptional activity) level at BL and during treatment showed **significant correlation** with **HDV-RNA undetectability** and **HBsAg decline < 100 IU/ml 24 w PT**

- **At present consistent futility rules for CHD patients treated with Peg-IFN are lacking**
- **The combined monitoring of HDV-RNA and HBsAg may guide the treatment at the single patient level**

Effects of HDV infection and Peg-IFNa treatment on the NK cell compartment in chronically infected individuals

Lunemann S et al, Gut 2015

- 31 CHD patients were studied
- 16 treated with Peg-IFNa, 7 were responders (HDV-RNA undetectable 6 months after EOT)
- Peripheral blood from treated patients was obtained at baseline and after 12 weeks of treatment

- CHD patients show a **higher than normal frequency of peripheral NK cells,** without detectable change in differetiation status, but with reduced functional capacity in terms of ability to respond to IFNa.
- **IFNα treatment caused a significant change in NK cell differentiation status**, with **selective loss of terminally differentiated NK cells** and a **relative enrichment in immature NK cell subsets**: an increase in $CD56^{bright}$ NK cells and a decrease in $CD56^{dim}$ NK cells with altered expression of activating receptors.

- Overall, **IFNa treatment was associatesd with a marked functional impairement of NK cells and a** polarisation of NK cell IFN-signalling from STAT4 towards STAT1 dependency.
- **High baseline frequency of $CD56^{dim}$ NK cells was associated with a positive outcome of IFNa treatment**
- **Retained high numbers of NK cells is beneficial for the host during Peg-IFNa treatment of chronic HDV infection**.

Frequency of $CD56^{dim}$ NK cells according to Peg-IFNa response