

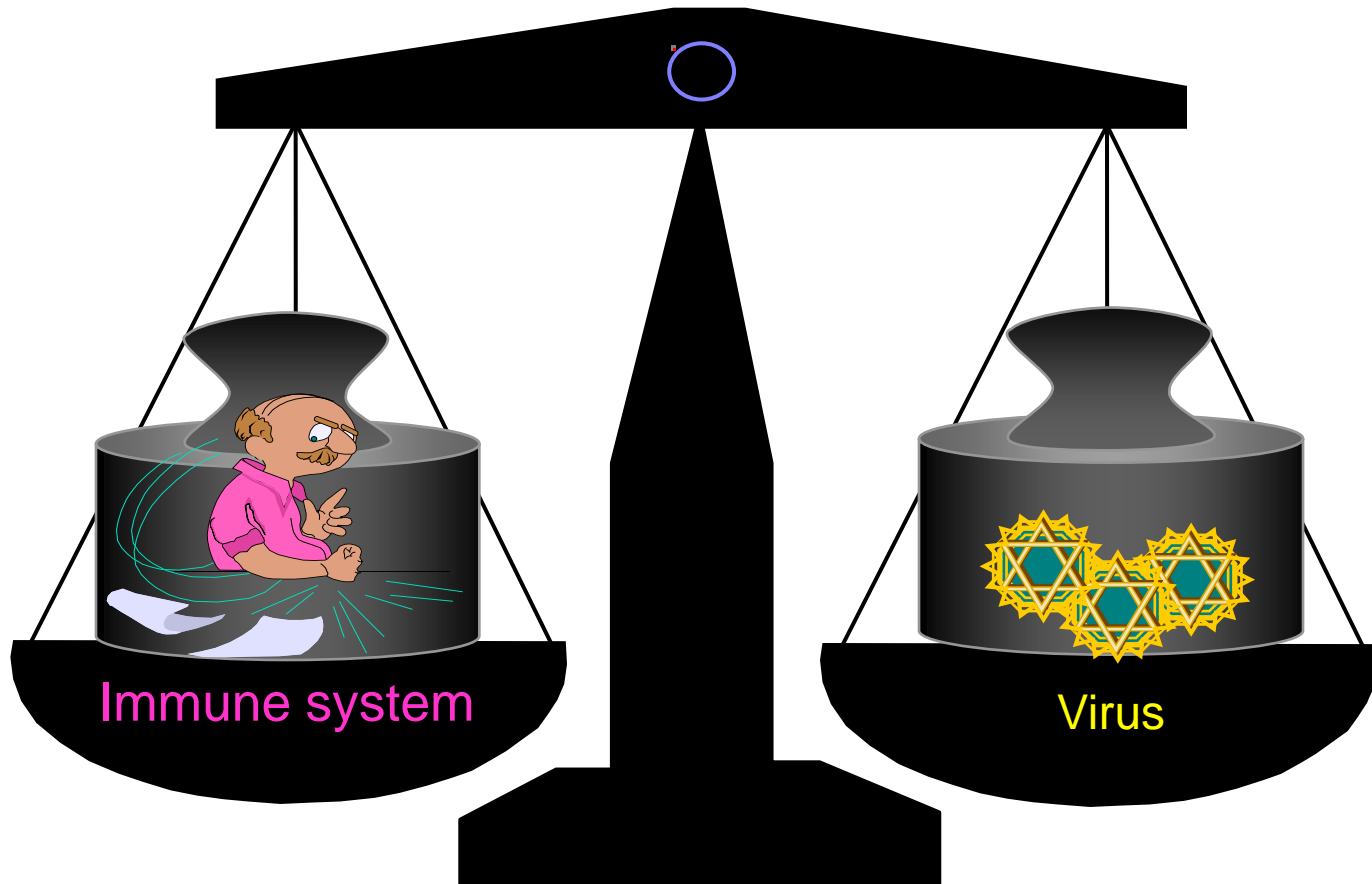
Epigenetics of liver diseases: potential clinical application of the study of serum miRNA profiling in chronic carriers of Hepatitis B Virus

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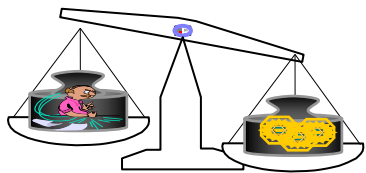
Outcome of HBV infection and liver disease



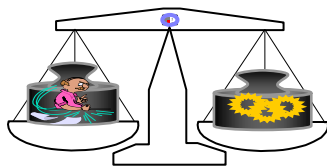
A coordinated humoral and cellular immune response may control HBV infection, allowing its persistence without liver damage

The unique HBV biology in the context of liver physiology, allows the virus to replicate without liver damage

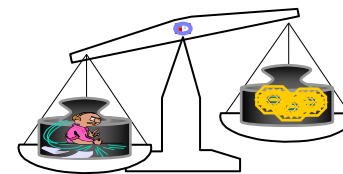
Natural history of Chronic Hepatitis B Virus Infection



Immune tolerance



Immune clearance



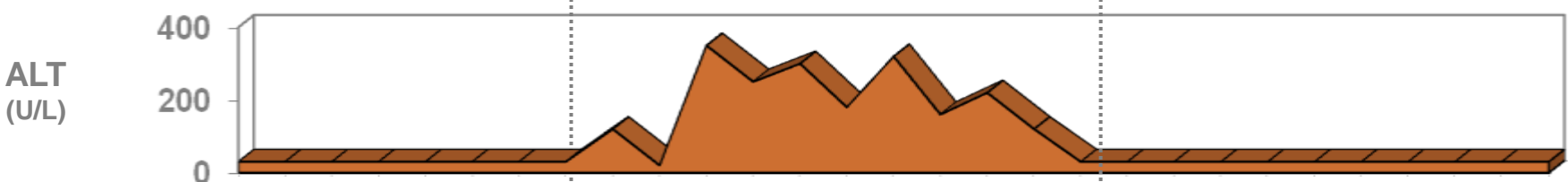
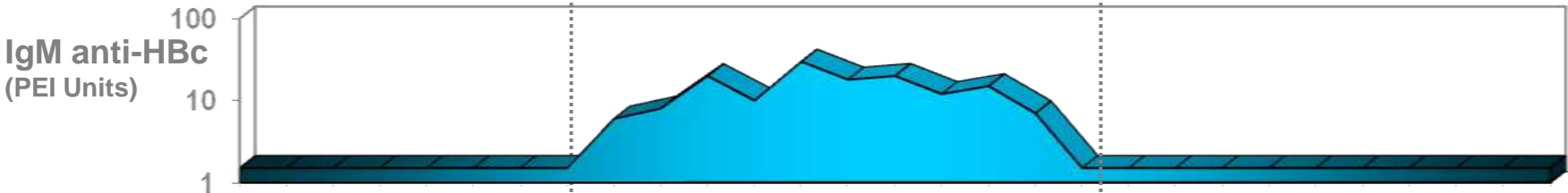
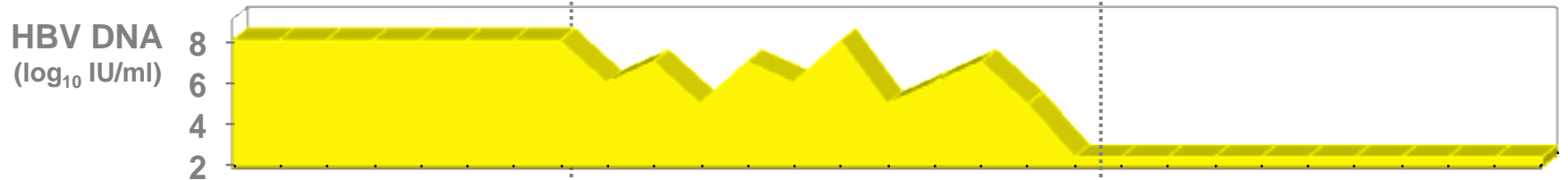
Immune control



HBeAg

HBeAg or anti-HBe

Anti-HBe



HBeAg Immune tolerant carriers

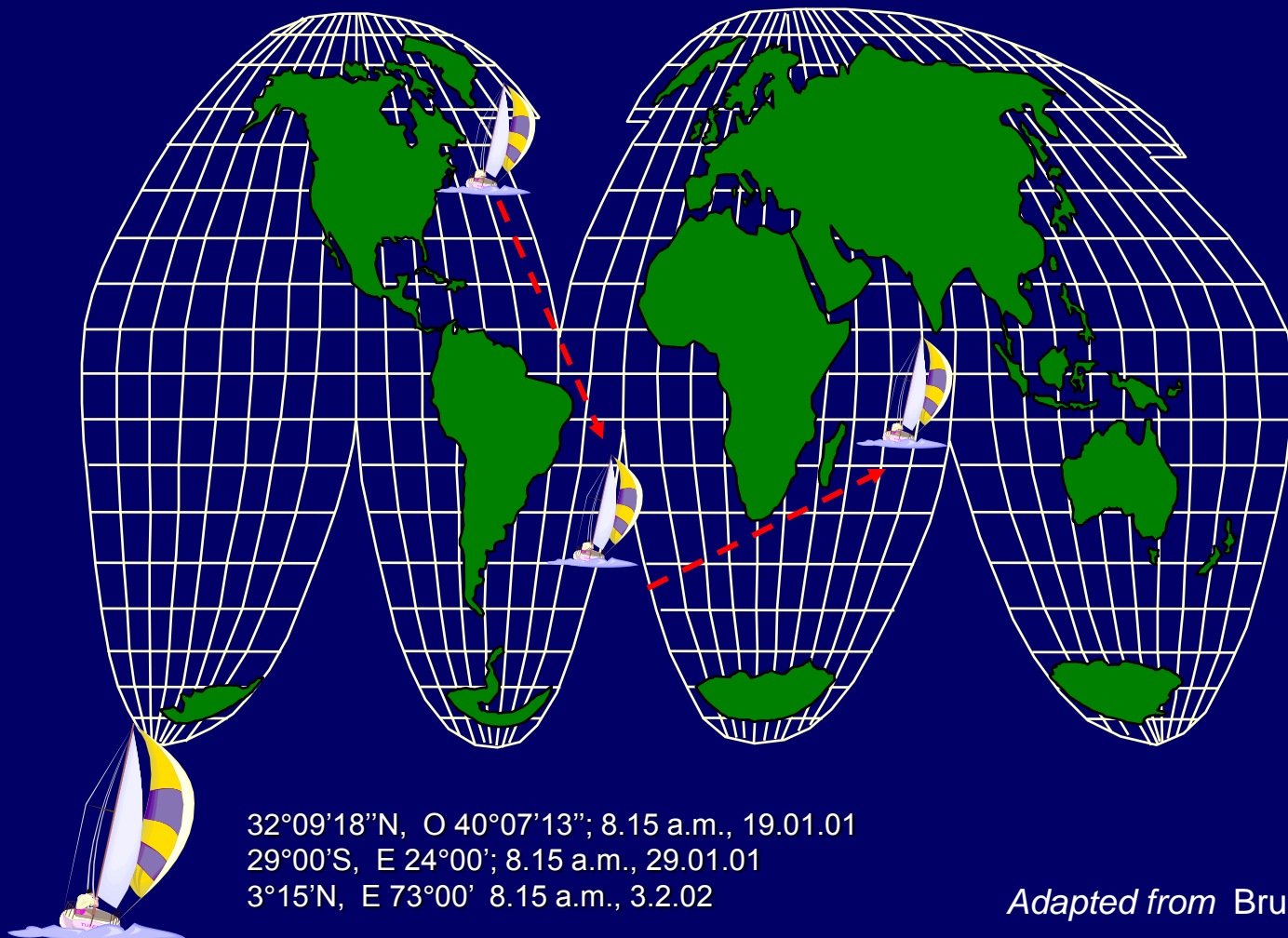
Patients with CHB HBeAg positive or negative

Inactive carriers

OBI carriers

Management of HBV carriers

Phases of infection and response to therapy are defined by **quantitative measurement of viral constituents** (nucleic acid, *HBV-DNA* and proteins, *HBeAg* and *HBsAg*) and **antiviral immune response** (antiviral antibody levels, IgM and Total anti-HBc)



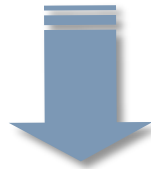
Latitude
Longitude
Time
Date

ALT
HBV-DNA
IgM anti-HBc
HBsAg

32°09'18"N, O 40°07'13"; 8.15 a.m., 19.01.01
29°00'S, E 24°00'; 8.15 a.m., 29.01.01
3°15'N, E 73°00' 8.15 a.m., 3.2.02

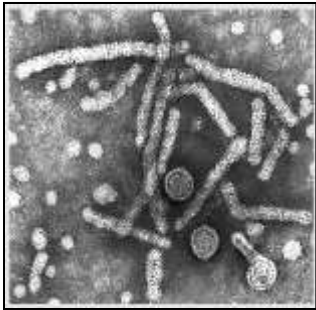
Adapted from Brunetto et al, J Hepatol 2010

However, serum biomarkers of the virus/host interplay are influenced by many interfering factors, such as host and virus heterogeneity and have to be repeatedly measured overtime to accurately define the extent of immune control or of the antiviral treatment effectiveness



Unmet needs are biomarkers of the achievement of a sustained control of HBV infection by the host's immune system

Serum HBsAg



Virions + defective particles
(exceeding virions by a factor of 10^2 - 10^5)



replication



cccDNA transcription/
mRNAs translation

HBsAg serum levels reflect:

- in HBeAg positive patients, the overall amount of cccDNA
- in HBeAg negative carriers, the transcriptionally active cccDNA

Brunetto et al, J Hepatol 2010

Circulating HBsAg particles carry human miRNAs

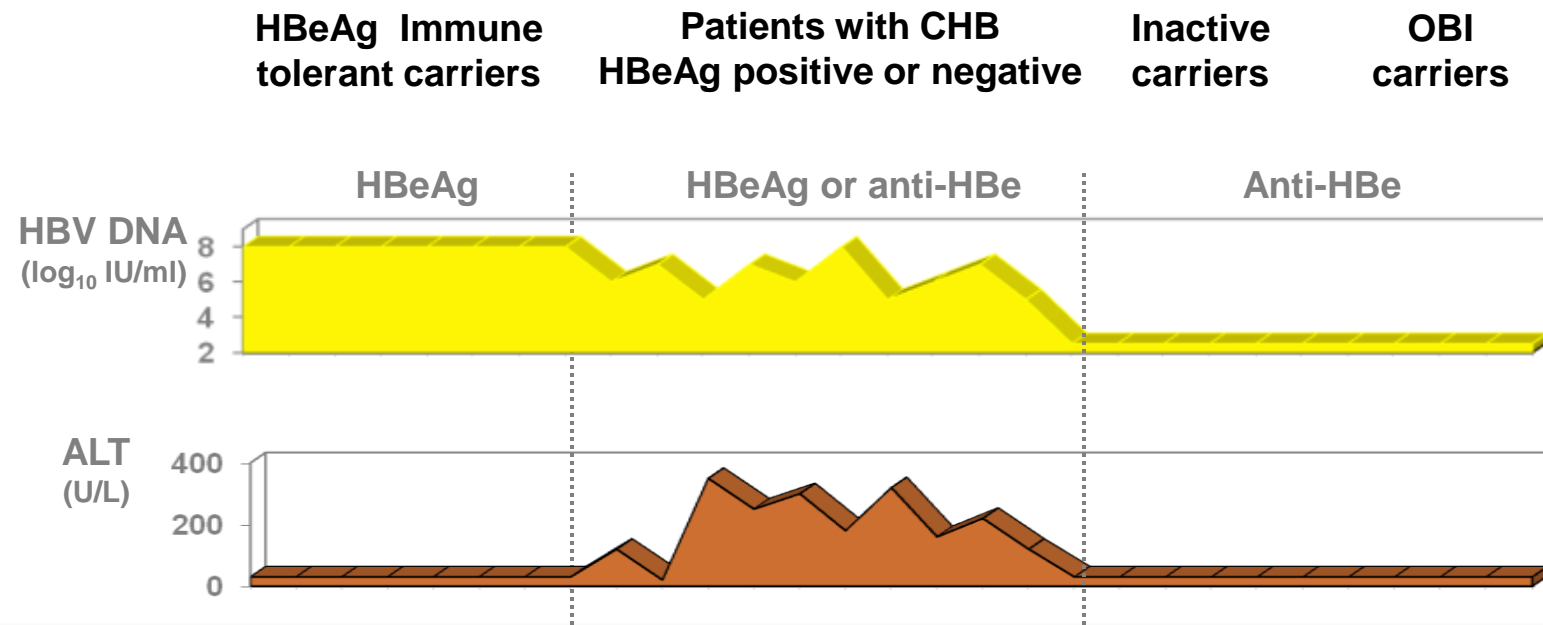
Liver Specific: miR-27a, miR-30b, miR-122, miR-126, miR-145

Immune regulatory: miR-106b, miR-223

Novellino L , Brunetto M et al PlosONE 2012

A serum microRNA signature is associated with the immune control of chronic hepatitis B virus infection

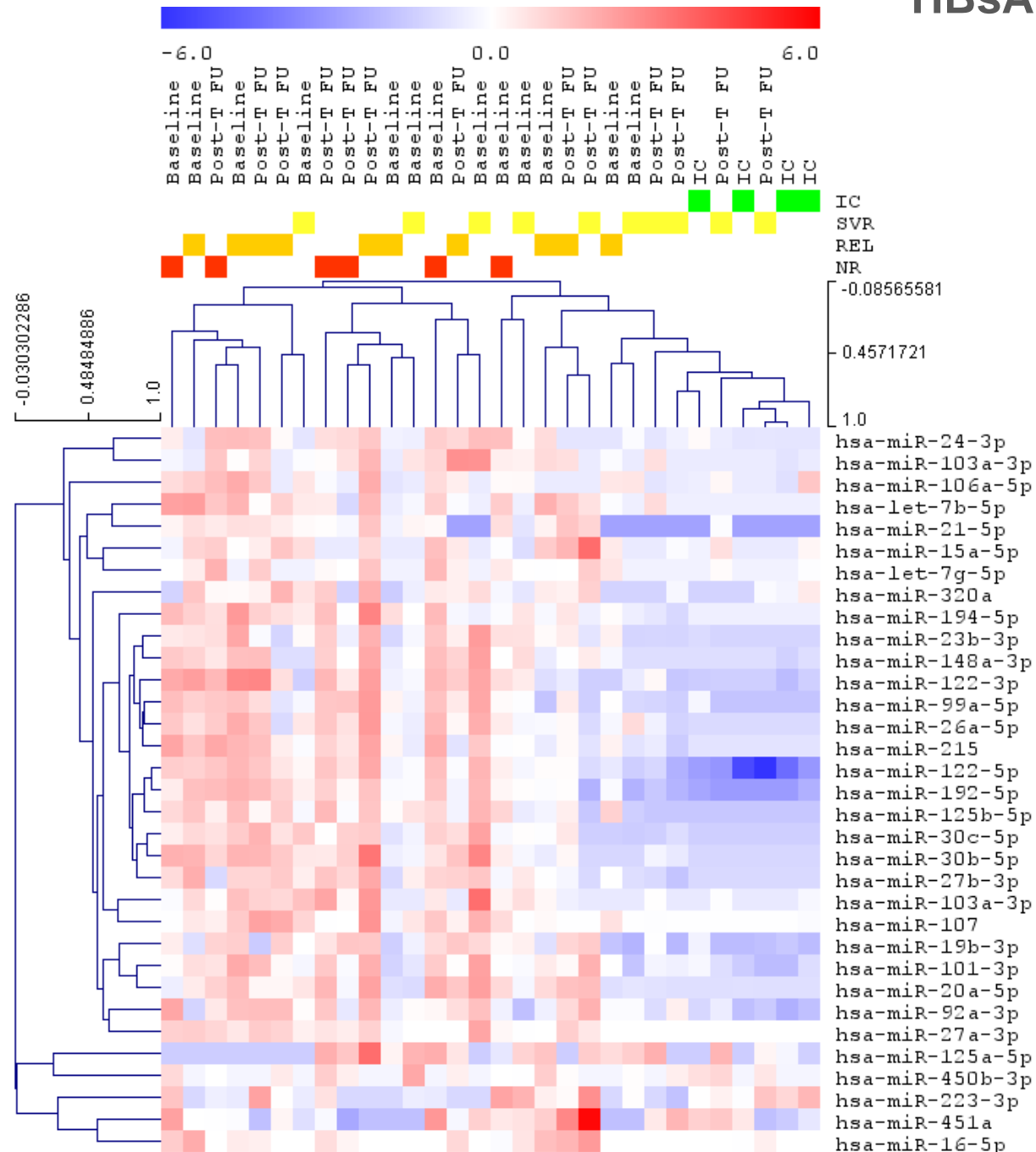
Dynamic variation of miRNA profiling was characterized in **141** sera and immunoprecipitated HBsAg-particles of **61** HBsAg-carriers during different HBV infection phases and according to treatment response in **32** patients



	Untreated	IFN Treated	NUCs Treated
HBsAg Immune tolerant carriers	1		
Patients with CHB HBeAg positive or negative	4	36	9
Inactive carriers		16	
OBI carriers		(13 SVR 5 Rel 3 NR)	(2 SVR)

(response to therapy; SVR= sustained virological responders)

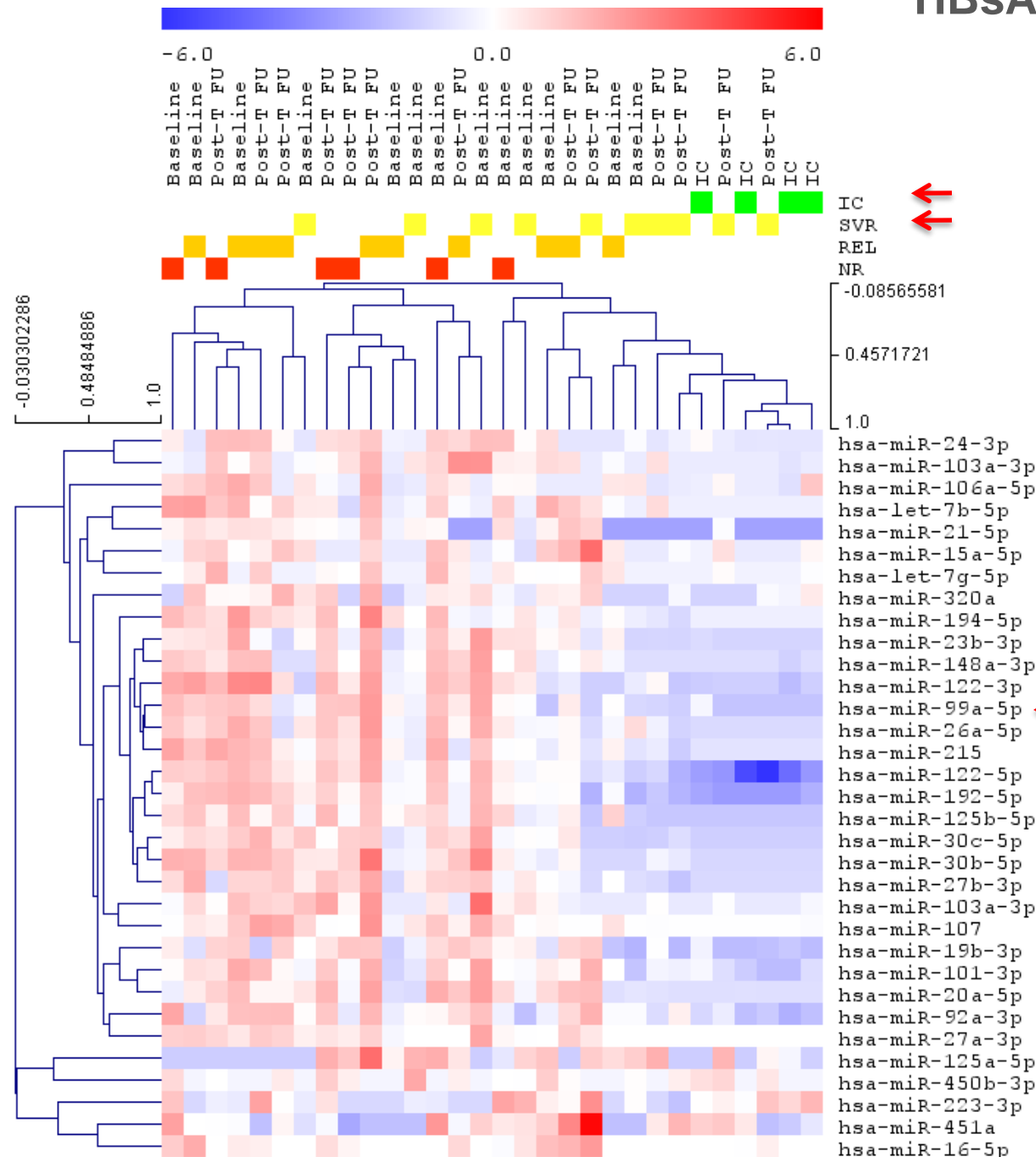
HBsAg particles miRNA profiling



Each miRNA was assayed once by qPCR on the Ready-to-use microRNA-PCR panels I/II containing 739-miRNA-assays

Differences were observed in the mean variation of the average signal (ΔCq) between Inactive Carriers (IC) and BL- Chronic Hepatitis B and post-treatment follow-up of NR and REL

HBsAg particles miRNA profiling



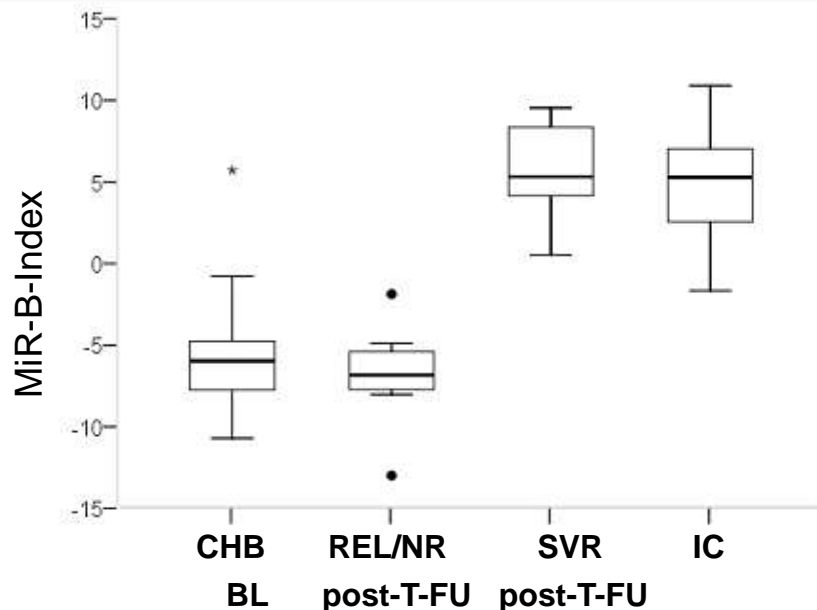
Unsupervised two-way hierarchical analysis of miRNA showed the **clustering of post-Treatment Follow-up of SVR with IC**

miRNA differentially expressed comparing BL-SVR-CHB to both post-Treatment-Follow-Up of SVR and IC

Serum miRNA profiling

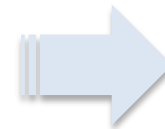
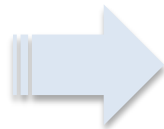
- ✓ 21 miRNAs were differentially expressed in Inactive Carriers (IC) and Chronic Hepatitis B (CHB) patients with the largest difference for **miR-122-5p**, **miR-99a-5p** and **miR-192-5p**.
- ✓ The **3 liver-miRNAs** were significantly **down-regulated** during and after end of **treatment in SVR**.
- ✓ MiRNA-profiles of IC and SVR at post-TFU clustered in the heatmap.

Liver-miRNAs were combined with 3 additional miRNA as internal controls to build a MiR-B-Index which showed 100%-sensitivity and 84.4%-specificity in identifying IC.



Mir-B-Index improved during therapy and post-treatment-follow-up reaching IC-like values in IFN/NUCs-SVR
(5.28, -1.65/10.91 vs 5.33, 0.54/9.53, $P=.553$)

- ★ Dynamic change of a miRNA signature may identify the natural occurring and therapy induced immune control of HBV infection.
- ★ The same signature qualifies as new diagnostic biomarker to satisfy the unmet need of the early identification of the sustained switch from chronic active hepatitis to the inactive HBV infection in patients treated with antivirals.
- ★ The *in vivo* study of circulating HBsAg-associated hepatocellular miRNAs provide a unique model to study the epigenetics of liver- physiopathology.

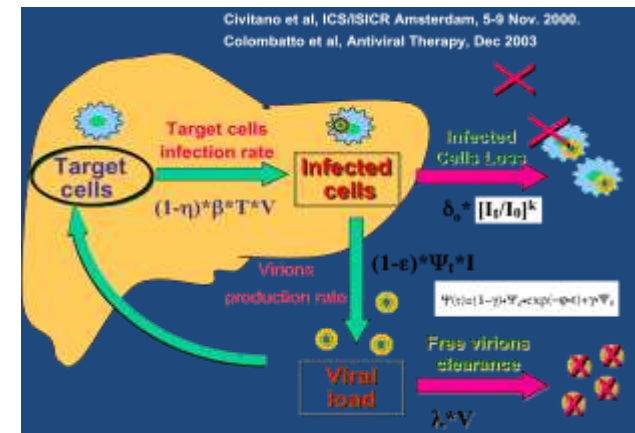




The implementation of new diagnostic tools warrant a more accurate management of chronic HBV carriers.

However, because of the highly dynamic course of both infection and disease, the appropriate therapeutic intervention requires an integration of all biological and medical data to accurately simulate the interplay between HBV and host's immune response during antiviral therapy, as we already done for chronic hepatitis C.

(Colombatto et al. Clin Phar & Ther 2008)

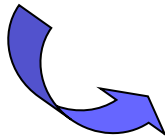


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at the moment missing...



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