OP-0682

Characterization of circulating Hepatitis B virus RNAs *in vitro* and chronic hepatitis B patients

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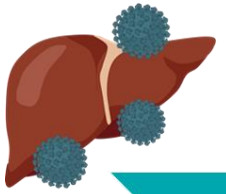
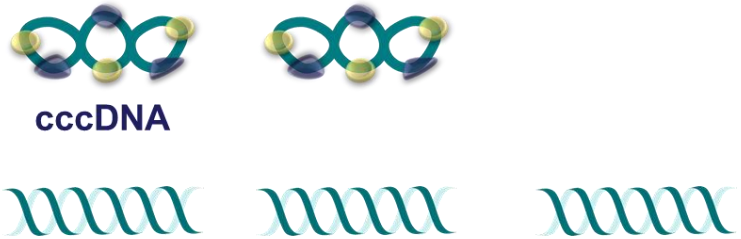
New biomarkers to HBV cure



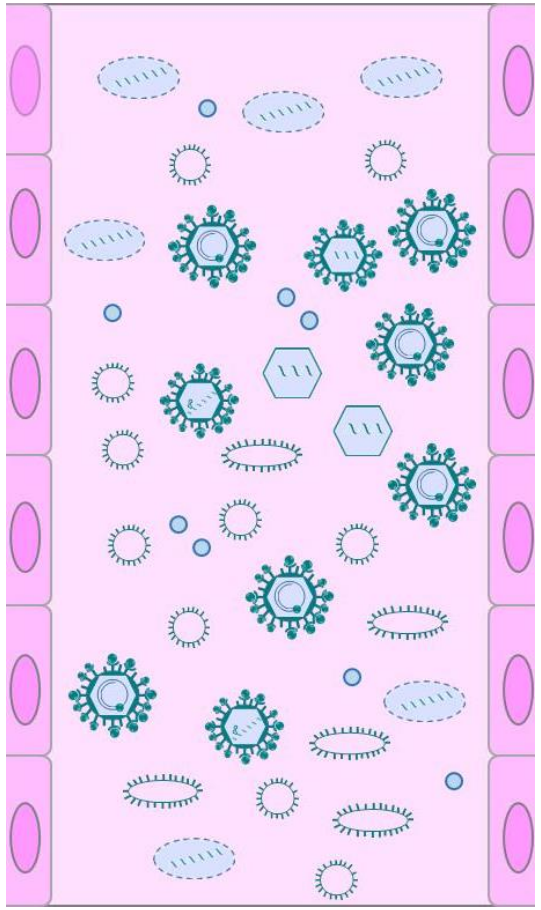
Therapy & Cure definition

Partial cure → Functional cure → Complete cure → Sterilizing cure



Ideal HBV biomarker?

- Non-invasive
- Reflects intrahepatic cccDNA pool and activity
- Predicts “HBV cure”



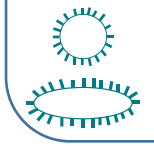
CHB patient
Bloodstream

Vehicle components in the bloodstream

Classical biomarker

HBV DNA

HBeAg



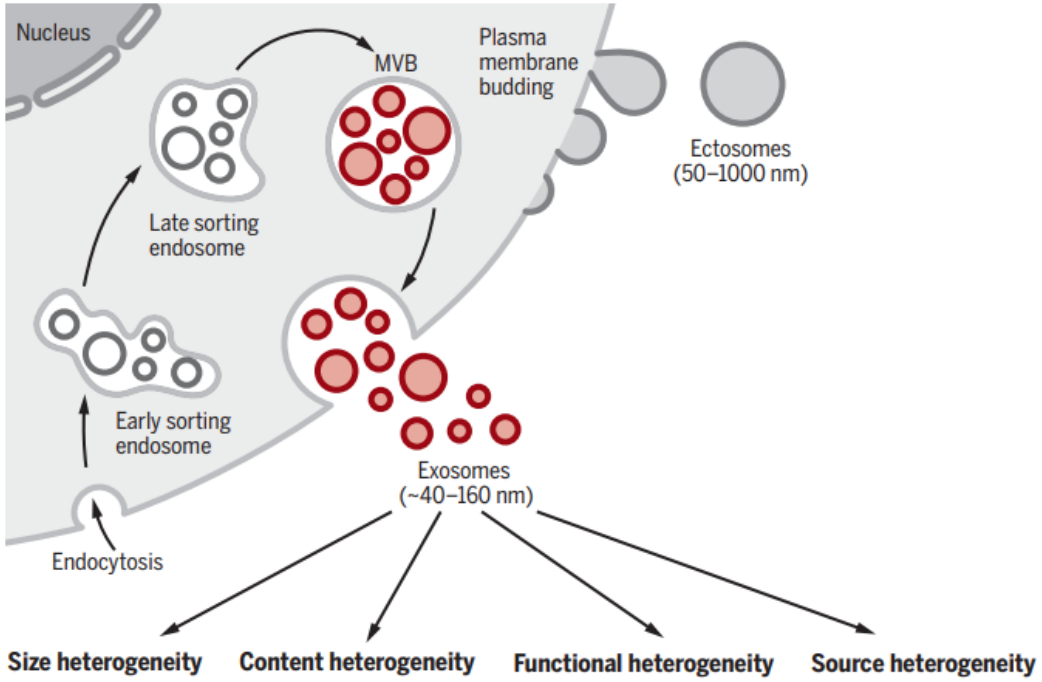
HBsAg

Circulating HBV RNA (CirB-RNAs)

Virion Like-Particles
(Jie W, J Hepatol, 2016)

Extracellular Vesicle

Extracellular Vesicles (EVs): Exosome

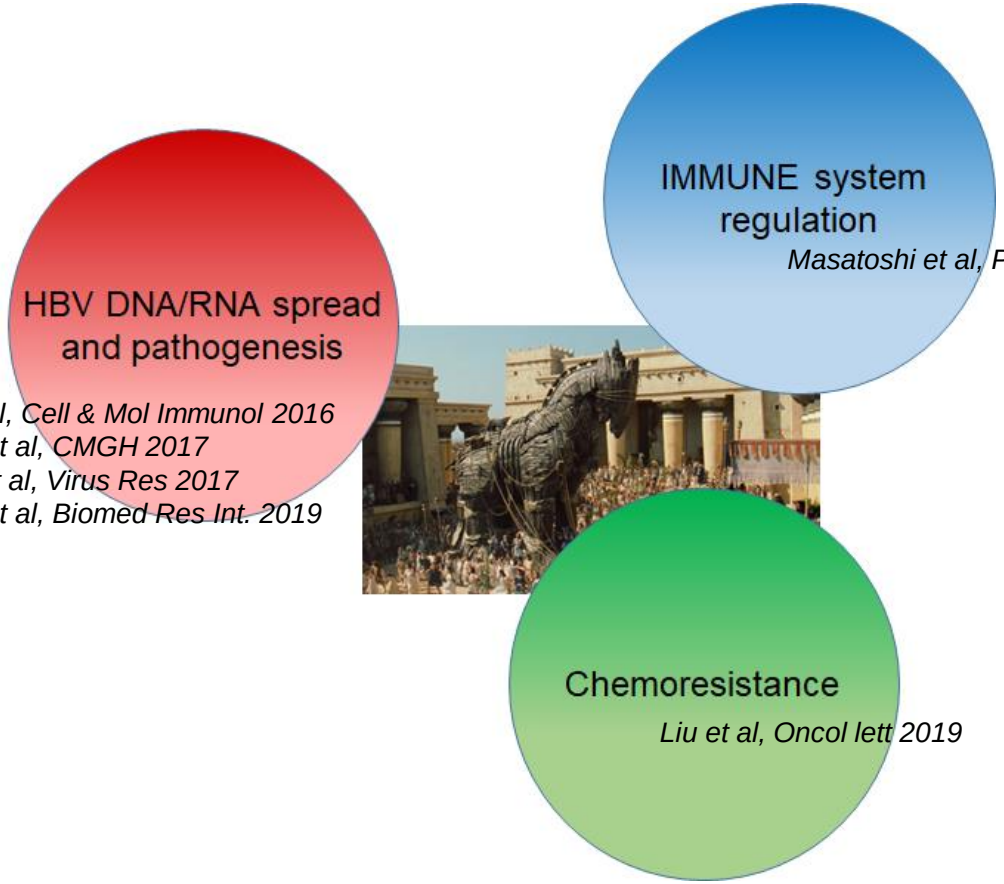


EVs

- Role: Cell to Cell communicator
- Cargo: DNA/RNA, protein, and miRNA
- Diameter: 40 - 160 nm
- Marker: CD9 and CD81

Kalluri et al, Science, 2020

Trojan EVs hypothesis

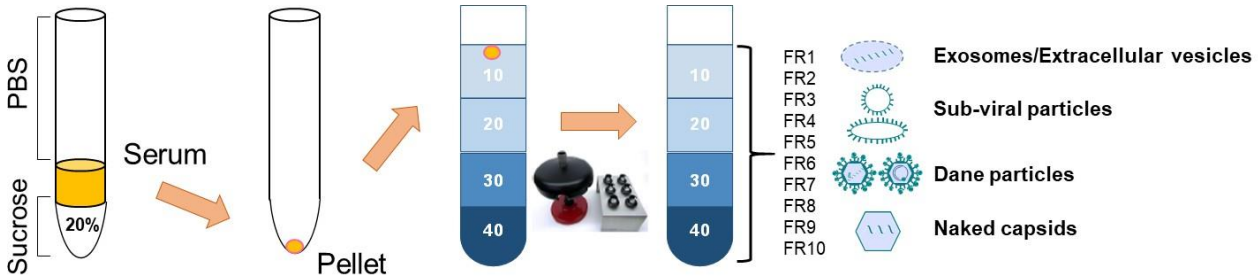


Yang et al, Cell & Mol Immunol 2016
Sanada et al, CMGH 2017
Kapoor et al, Virus Res 2017
Shuang et al, Biomed Res Int. 2019

Masatoshi et al, Plos One 2018

Liu et al, Oncol lett 2019

Method: Density gradient ultracentrifugation and Patients' Information



Experimental Method

1. Concentration: Ultracentrifugation for 5 hours at 35000rpm
2. Density gradient ultracentrifugation for 16h at 35000rpm
   -10-40% Iodixanol/Sucrose
3. Total of 10 fractions, 500ul each

Patients' Information

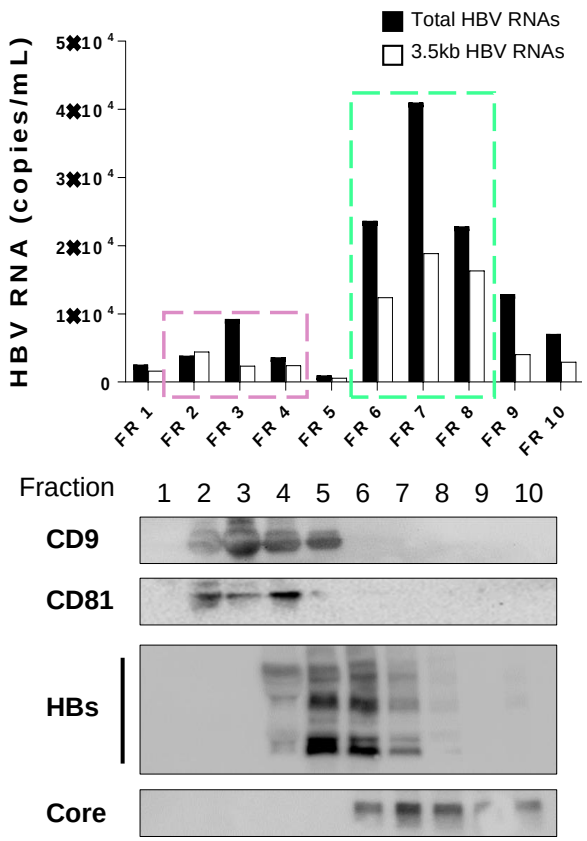
Sample Name	HBV load (log IU/ml)	HBeAg	HBsAg (log IU/ml)	ALT	Anti-HBV Treatment	CHB phase[1]
Patient 1	8.5	(+)	81000	36	NO	HBeAg+ CI
Patient 2	5.6	(-)	570	158	NO	HBeAg- CH
Patient 3 sample 1	8.3	(+)	37000	201	NO	HBeAg+ CH
Patient 3 sample 2	3.1	(+)	270	27	Tenofovir 6 months	
Patient 3 sample 3	<LLoQ	(+)	240	24	Tenofovir 10 months	

According to EASL CPG 2017

Where are CirB-RNAs distributed in CHB patients?

A. Patient 1

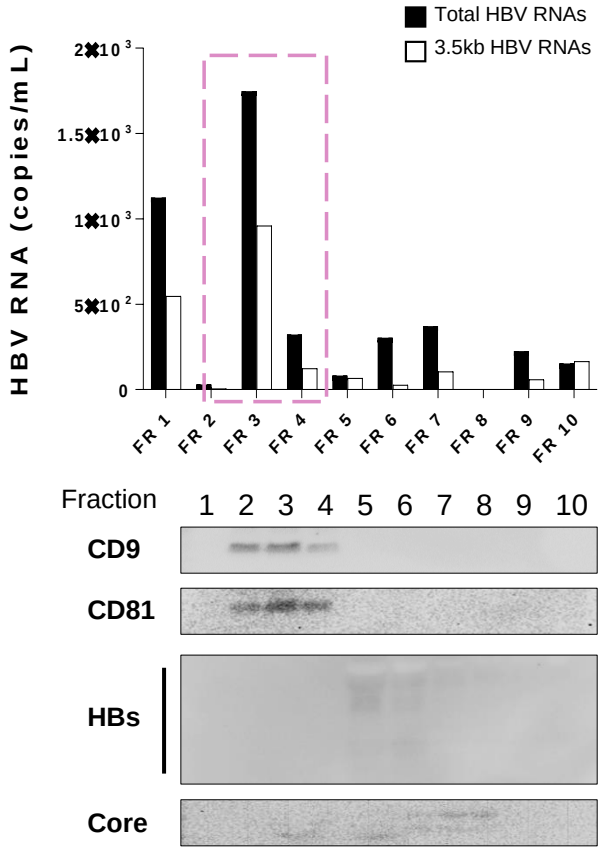
HBeAg(+), high viral load



CirB-RNAs are detected in EVs (FR2-4) and VLPs (FR6-8) fractions.

B. Patient 2

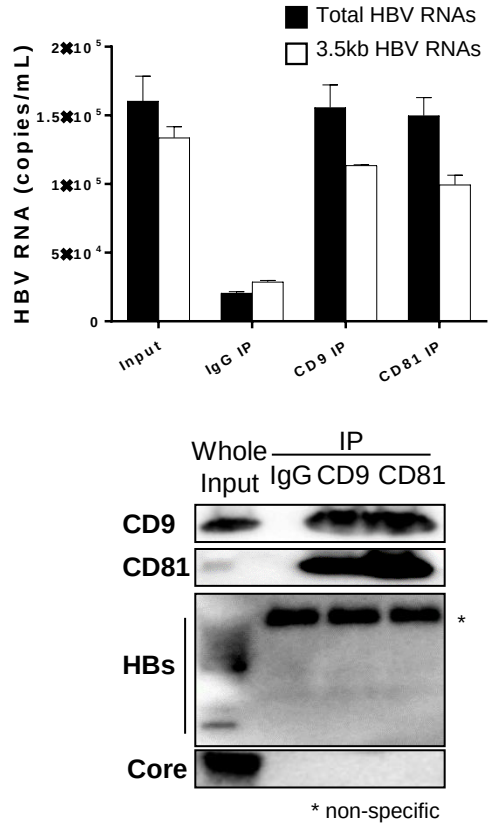
HBeAg(-), low viral load and HBsAg



CirB-RNAs are mostly detected in EVs (FR2-4) fractions.

C. Patient 1

IP assay

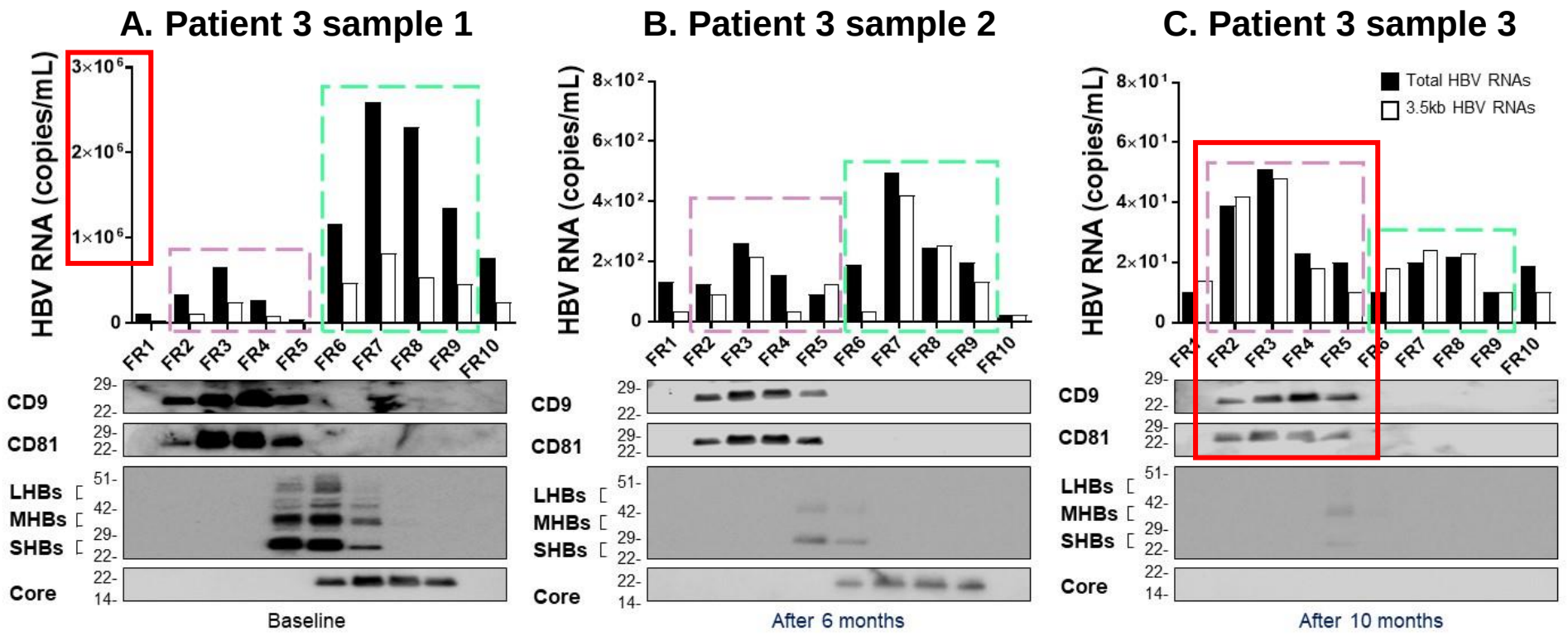


CirB-RNAs are detected in EVs.

Longitudinal study of CHB patient's treatment with TDF

Before treatment

During TDF treatment

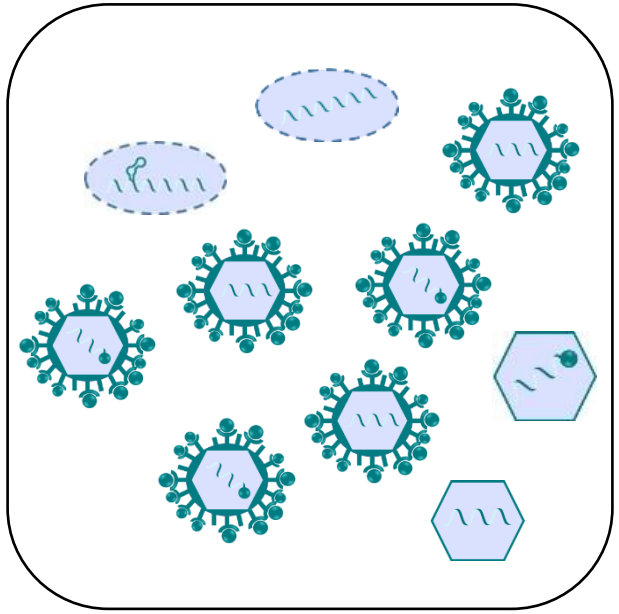


the distribution of cirB-RNAs changed from VLPs to EVs during TDF treatment

Conclusions

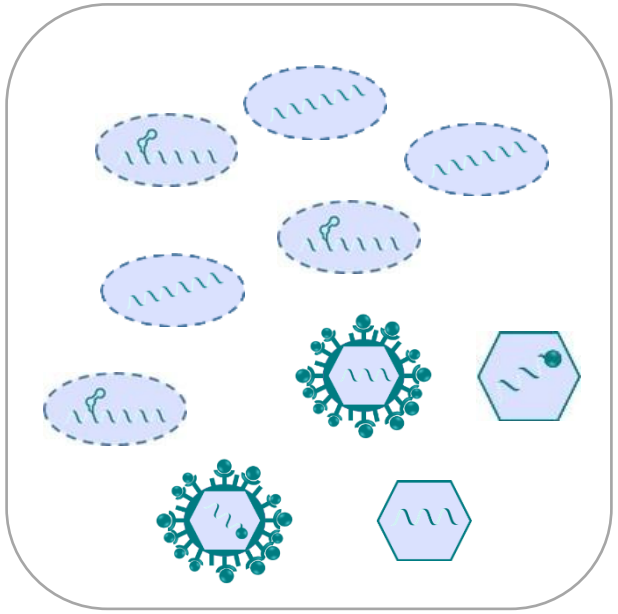
High viremia

HBeAg (+), High viral load,
High HBsAg



Low viremia

HBeAg (-), Low viral load,
Low HBsAg



Altogether, our results significantly contribute to the characterization of cirB-RNAs as new viral biomarker

ACKNOWLEDGEMENTS

INSERM U1052- RHU's Team

Pr. Fabien Zoulim
Barbara Testoni
Doohyun Kim
Hyoseon Tak
Xavier Grand
Delphine Bousquet

Pr. Massimo Levrero
Marie-Laure Plissonnier
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This work was supported by a public grant overseen by the **French National Research Agency (ANR)** as part of the second "Investissements d'Avenir" program (reference: ANR-17-RHUS-0003).