SELECTED OPPORTUNITIES IN INFECTIOUS DISEASES

Histone chaperone HIRA inhibitor for treating Hepatitis B Virus infection (BIO 17369)

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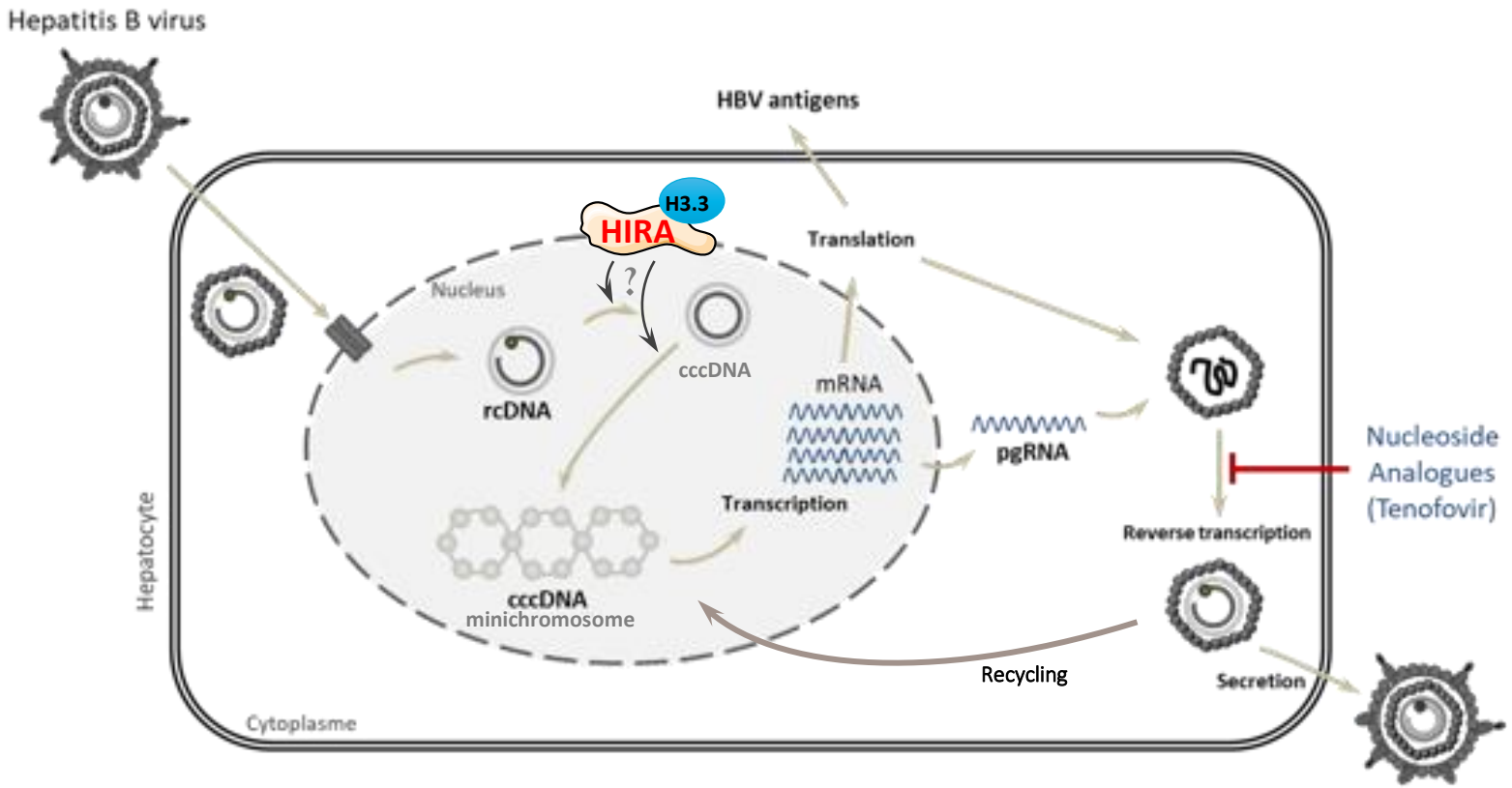
Product factsheet

In vitro

- **Target:**
  - Histone chaperone HIRA expression inhibition in HBV infected cells.
- **Product:**
  - Inhibitor of HIRA expression / siRNA
- **Application:**
  - Treatment for HBV infection
- **POC:**
  - Knock-down of HIRA before HBV inoculation led to an incomplete or delayed rcDNA to cccDNA, in HepG2-NTCP cell line. **Chromatinization of incoming viral DNA require the histone chaperone HIRA**
  - HIRA is able to interact with cccDNA and its recruitment is concomitant with deposition of histone variant H3.3 in HepG2-NTCP cell line**. HIRA interaction with cccDNA represents a new therapeutic target**
  - HIRA was able to interact with HBV capsid protein in infected hepatocytes and in an HepaRG cell line expressing HBc in an inducible manner. **The interaction between HIRA and HBc represents a new therapeutic target**.
- **Patent and publication:**
  - EP N°17306134.2 / Priority date 01 September 2017

Histone chaperone HIRA inhibitor for treating Hepatitis B Virus infection (BIO 17369)

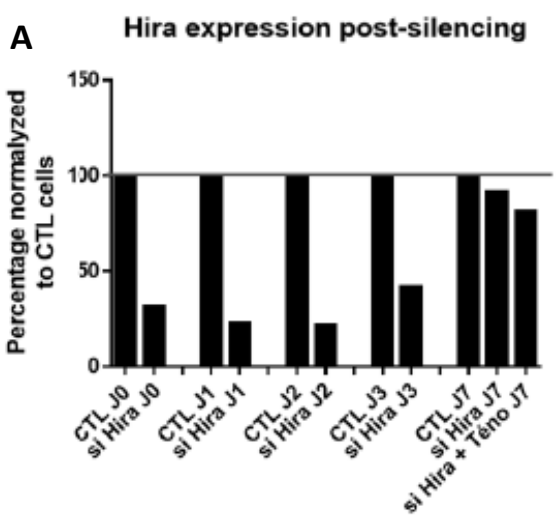


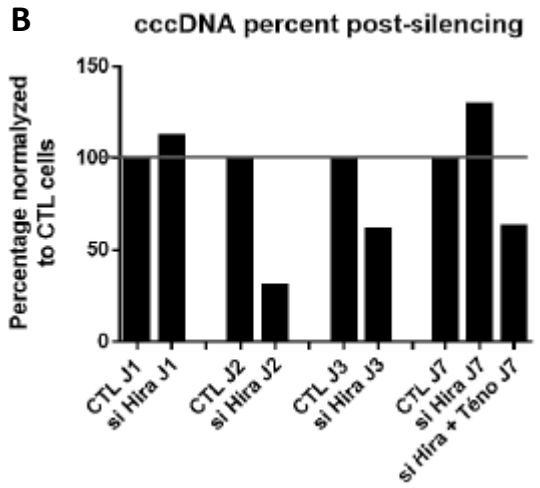
Histone chaperone HIRA inhibitor for treating Hepatitis B Virus infection (BIO 17369)

Proof of concept

Chromatinization of incoming viral DNA require the histone chaperone HIRA

- Transfection of HIRA siRNA reduced by nearly 70% endogenous level of HIRA mRNA
- HIRA extinction leads to a blockade of cccDNA formation that needs nucleocapsids recycling to be overcome





HepG2-NTCP cells were transfected twice with siHIRA within 4 days, and infected with HBV at day 4, followed by a tenofovir treatment (100µM) at day 3 and 5 post-infection. Cells were then harvested at day 1, 2, 3 and 7 post-infection.
(A) HIRA knockdown was determined by quantification of HIRA mRNA levels after transfection, using a Luciferase siRNA (CTL) as a control.
(B) Total intracellular HBV DNA was extracted and subjected to qPCR for cccDNA quantification.
After normalization to housekeeping genes, results were expressed as percentage of CTL transfected cells.

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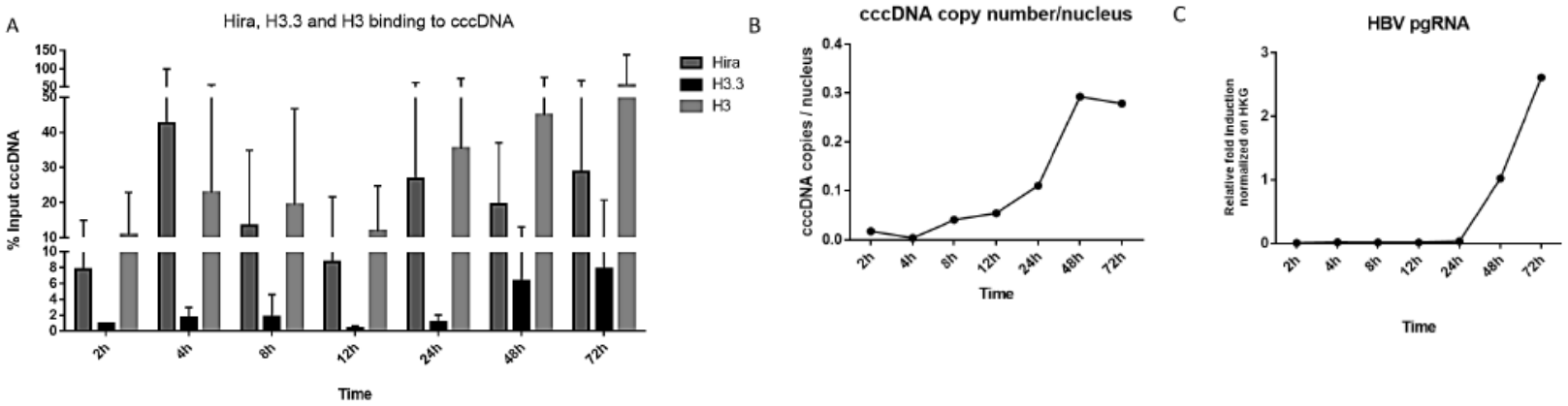
Proof of concept

Impact of the chaperone upon the cccDNA protein constitution

- Hira is recruited to cccDNA as early as 30 min after infection
- The binding of Hira is concomitant with the presence of H3.3 to cccDNA
- The presence of H3 on cccDNA seems to reflect the cccDNA pool formation, and the initiation of the transcription



Assessment of HIRA, histone H3 and variant H3.3 binding on cccDNA

(A) HepG2-NTCP cells were infected with of HBV from 2 hours to 3 days. After crosslinking and nuclear extraction, ChIP experiments were performed using antibodies against HIRA, panH3 and H3.3 histone variant.

(B) On those same cells, total intracellular HBV DNA was extracted, T5 digested and analyzed by cccDNA qPCR.

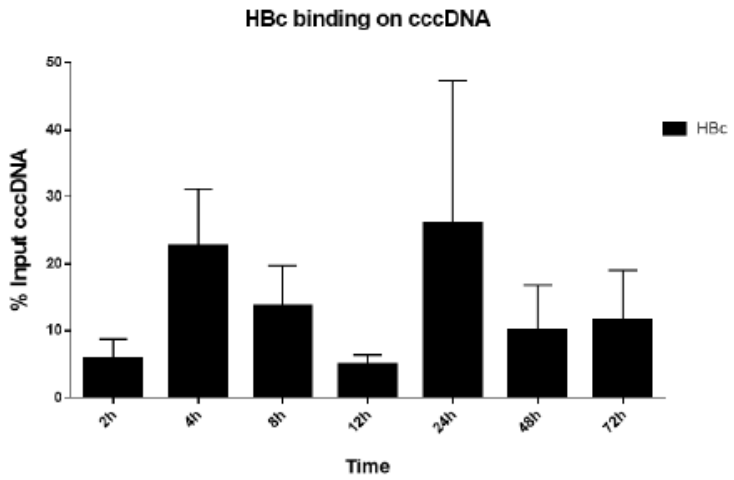
(C) Total HBV RNA was extracted too, retrotranscribed and pgRNA appearance was analysed by qPCR.

HISTONE CHAPERONE HIRA INHIBITOR FOR TREATING HEPATITIS B VIRUS INFECTION (BIO 17369)

Proof of concept

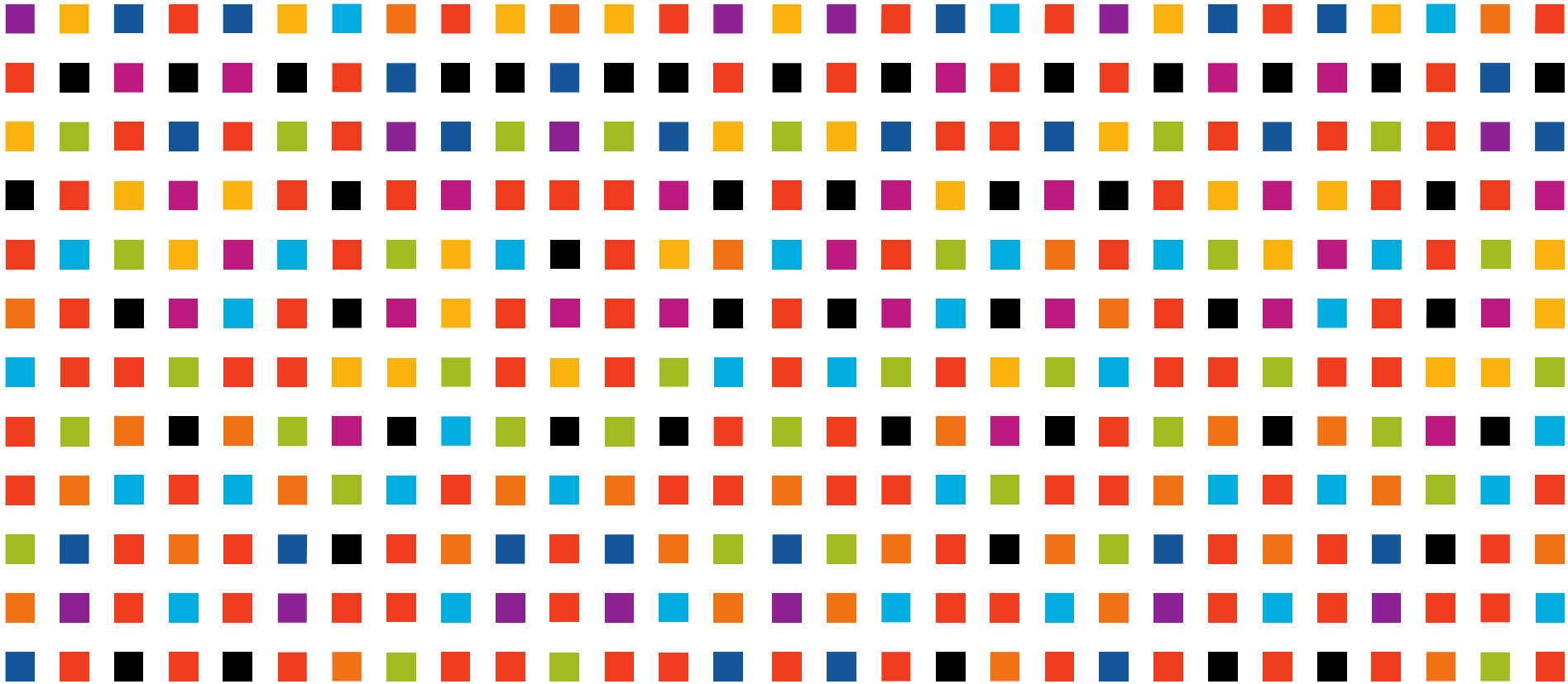
The interaction between HIRA and HBc could represent a new therapeutic target

- HBc binds to cccDNA as early as after 2 hours of infection
  - correlating with the recruitment of HIRA on the HBV minichromosome.



HepG2-NTCP cells were infected with 250 vge/cell of HBV from 2 hours to 3 days. After crosslinking and nuclear extraction, ChIP experiments were performed using antibodies against HBc protein.

- Hira interacts with HBc
  - HIRA was able to interact with HBc in an HepaRG cell line expressing HBc in an inducible manner (co-immunoprecipitation assay).
  - HBc and HIRA interact as soon as 24h post-infection in HBV infected hepatocytes (proximity ligation assay).



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