Sustained inhibition of HBV replication and HBsAg levels after long-term treatment with CAM ABI-4334 in human hepatocytes

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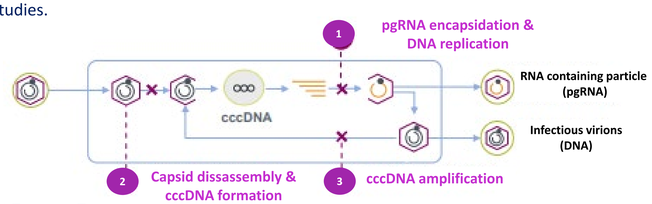
Introduction

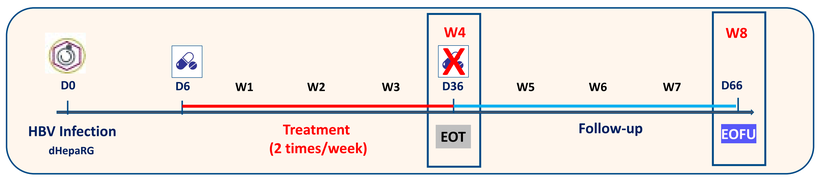
- **Chronic hepatitis B (CHB) infection is a significant global health problem**
  - Worldwide, an estimated 254 million people have CHB, resulting in about 820,000 deaths each year due to cirrhosis and hepatocellular carcinoma
- **Capsid assembly modulators (CAMs):**
  - **novel class of small molecules with the potential to improve cure rates in patients with CHB**
  - Inhibition of multiple steps in the HBV life cycle, including new capsid formation, pregenomic (pg) RNA encapsidation, formation of covalently closed circular (ccc)DNA from incoming HBV virions, and intracellular amplification of cccDNA
  - Demonstrated potent antiviral activity in Phase 1 studies and enhanced antiviral activity when combined with nucleos(t)ide reverse transcriptase inhibitors in Phase 2 studies.



ABI-4334 is a novel, next-generation CAM with improved *in vitro* potency against pgRNA encapsidation (MOA#1) and cccDNA formation (MOA#2) compared to first generation CAMs.

Methods

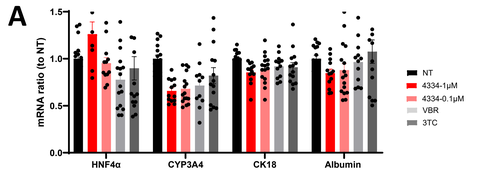
- **Cell Model:** differentiated HepaRG (dHepaRG)
- dHepaRG cells were treated from day 6 post-infection (pi) with 0.1 or 1µM ABI-4334, 1µM vebicorvir (VBR) or 1µM lamivudine (3TC) for 1 month, followed by 1 month off-treatment
- **Molecule concentration:** NT (Non Treated) / 4334 (1µM – 0.1µM) / VBR - 1µM / 3TC - 1µM



W (Week) – EOT (End of treatment) – EOFU (End Of Follow-Up)

Results

Results – Cell viability and differentiation at the EOFU



✓ ***ABI-4334 has no significant impact on common hepatocyte cell differentiation markers***

✓ ***ABI-4334: no impact on dHepaRG cell viability***

FIG.1 ***CAM ABI-4334: Cell viability / Hepatocyte markers / differentiation.*** *A) HNF4α (*Hepatocyte nuclear factor-4alpha) *- CYP (Cytochrome) 3A4, CK (Cytokeratin)18 and albumin mRNA expression by RT-qPCR (data expressed relative to NT after normalization on housekeeping GAPDH transcript levels - B) Toxicity test measuring the ATP production (CellTiter-Glo assay)*

Results – HBe and HBs levels and secretion

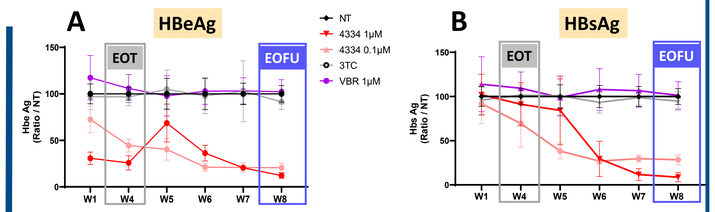
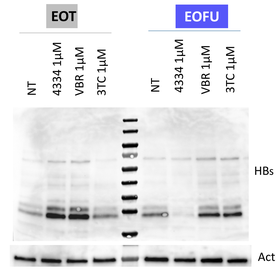
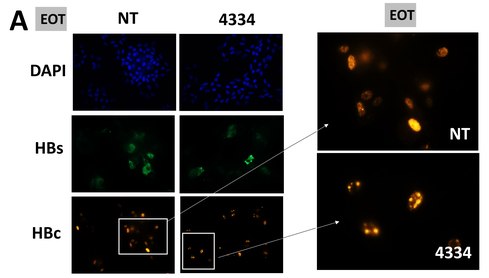


FIG.2 ***Measurement of HBe and HBs antigens levels.***
A) HBeAg secretion by ELISA - B) HBsAg secretion by ELISA - C) HBs Western Blot (H166 Ab)

ABI-4334 inhibits:
- **HBs Antigen secretion off-treatment**
- **Intracellular HBs protein levels at the EOFU**



Results – HBc and HBs immunofluorescence



CAM ABI-4334 induces the aggregation of HBc protein in the nucleus at the EOT

FIG.3
Immunofluorescence (IF) of HBc and HBs positive HepaRG cells at the EOT (panel A, 40X magnification) and at the EOFU (panel B, 10X magnification)
(HBs Ab: Novus NB100-62652
HBc Ab: Abcam Ab 18684)
CAM4334 – 1µM
VBR – 1µM

CAM ABI-4334 induces a decrease of HBc and HBs positive cells at the EOFU

Results – intracellular HBV replicative parameters

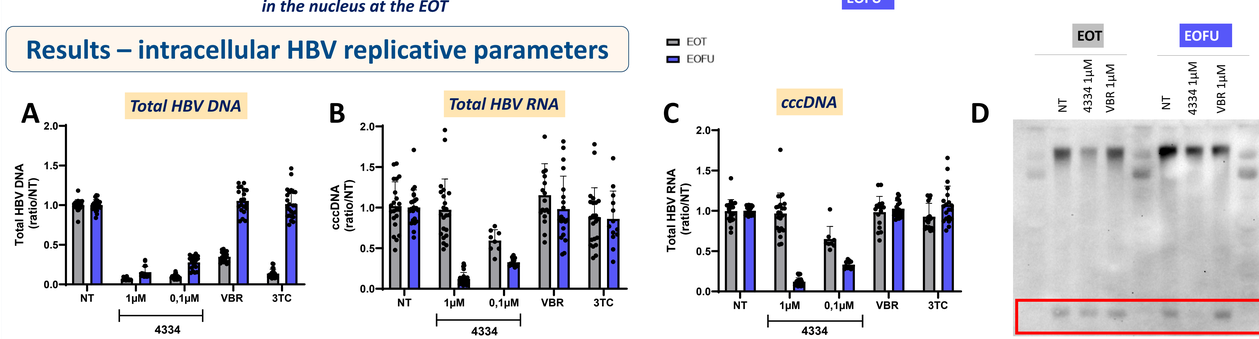


FIG.4. ***Intracellular HBV replicative parameters.*** *A) Total HBV DNA – (TaqMan qPCR) - B) total HBV RNA (RT-qPCR) - C) cccDNA (ExoI –III digestion / qPCR). For detailed protocol, see Allweiss et al., Gut 2023 – D) for Southern blot analysis, Hirt extraction was performed as described in Allweiss et al., Gut 2023. Mitochondrial DNA (ND2) was quantified by qPCR to normalize sample loading across the different conditions.*

Preliminary Results in Primary Human Hepatocytes (PHH)

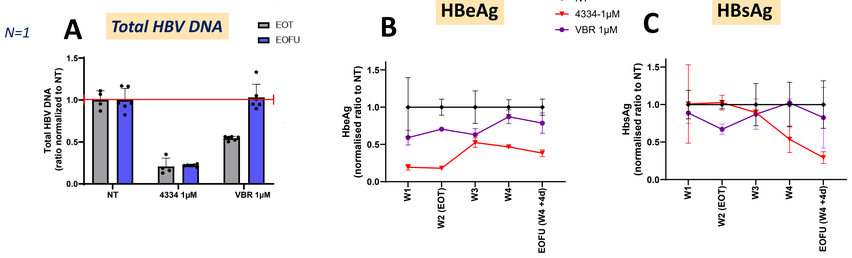


FIG.5. ***Total HBV DNA and HBe and HBs levels and secretion***
A) Total HBV DNA – (TaqMan qPCR)
B) HBeAg secretion by ELISA
C) HBsAg secretion by ELISA

Conclusions

Durable HBsAg decrease and lack of HBV rebound after stopping long-term ABI-4334 treatment of dHepaRG cells could be ascribed to the depletion of intracellular cccDNA pool in these experimental conditions.

References

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COI

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