
Combination treatment of a TLR7 agonist RO7020531 and a core protein allosteric modulator RO7049389 achieved sustainable viral load suppression and HBsAg loss in an AAV-HBV mouse model

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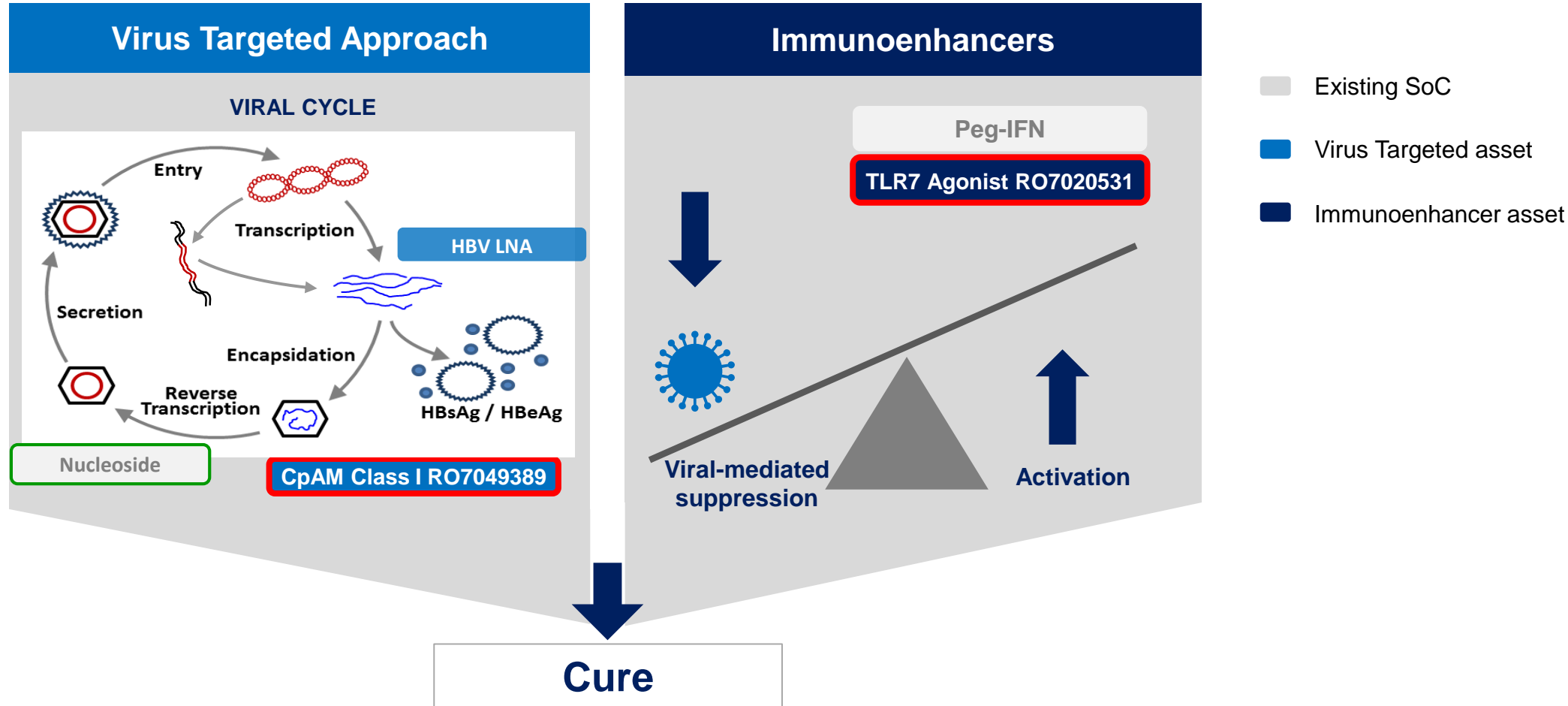
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Background

- 257 million people have chronic hepatitis B virus (HBV) infection which remains a leading cause of cirrhosis and hepatocellular carcinoma
- While current oral antiviral therapies can suppress HBV DNA levels and prevent disease progression and complications, most patients require life-long NUC therapy with issues of side effects, resistance and cost
- Development of a finite HBV cure will likely require combinations of novel compounds which inhibit HBV replication, reduce antigen production and enhance HBV-specific immune responses

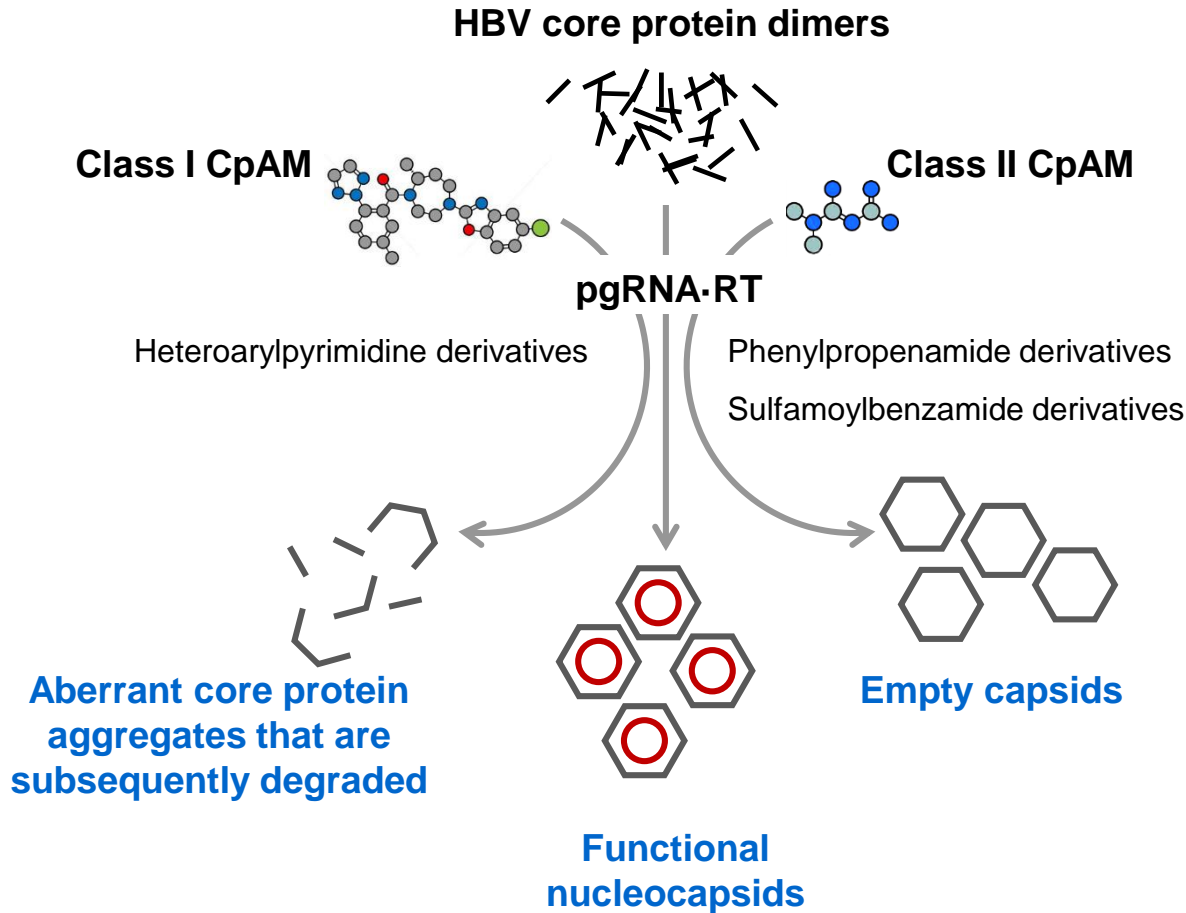
Roche approach toward combination therapy for HBV cure



Functional impairment of anti-HBV immune responses is a key feature of chronic HBV infection.

Development of a finite HBV cure will likely require combinations of novel compounds which inhibit HBV replication, reduce antigen production and enhance HBV-specific immune responses

RO7049389 is an orally administered Class I HBV CpAM



RO7049389 is highly potent and selective against HBV

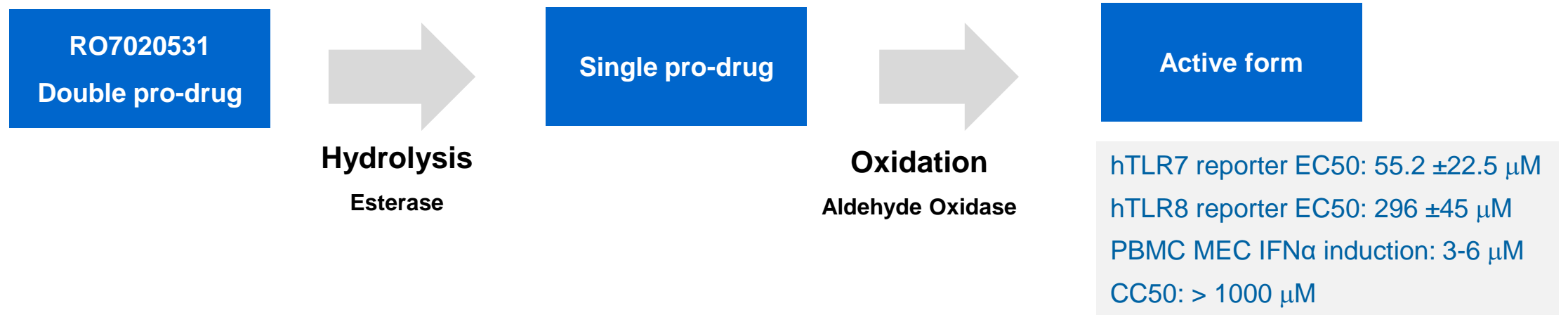
Antiviral activity and cytotoxicity HepG2.2.15 cells

RO7049389

EC ₅₀ (HBV DNA, n=3)	6.1 ± 0.9 nM
CC ₅₀ (n=3)	> 100 µM
Selectivity Index	> 10000

- Active against the most prevalent HBV genotypes (A-D)
- No cross-resistance with nucleos(t)ide analogue resistance variants

Roche TLR7 agonist is positioned to be differentiated as an oral double prodrug selectively converted in the liver



- TLR7 agonist induces broad immuno-modulatory effects including:
 - Activation of IRF7, NFκB, and AP-1 transcription factors
 - Differentiation of plasmacytoid dendritic cells (pDCs), and upregulation of co-stimulatory molecules and secretion of type I IFN and other cytokines/chemokines leading to activation of T-cells and NK cells
 - Differentiation of B-cells to antibody-producing plasma cells
- RO7020531 is an orally available double pro-drug of a TLR7 agonist which also activates TLR8 with lower potency
- It was safe and well tolerated in healthy volunteers with a favorable PK profile in single and multiple QOD doses up to 170 mg

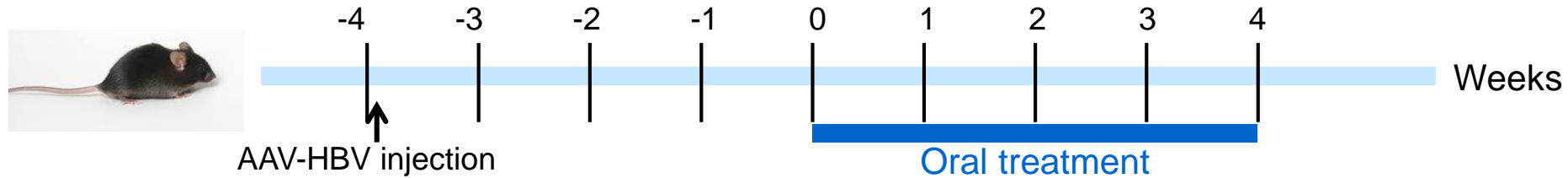
AAV-HBV mouse model is suitable to examine the anti-HBV activities of both immune modulators and direct antiviral agents

- rAAV8-1.3HBV infection/transduction through mouse tail vein injection
 - 1.3mer HBV genome
 - AAV2 ITR
 - AAV8 capsid (hepatotropic)
- Viral infection is fully established by ~ 4 weeks and can persist for more than five months
- Anti-HBV efficacy can be monitored by standard biomarkers
 - Serum HBV DNA, HBsAg, HBeAg, anti-HBs Ab
- The immune response can be monitored by:
 - HBV-specific T/B cell ELISpot (spleen)
 - Cytokine, interferon stimulated gene (ISG) mRNA expression
- Other parameters include the mouse body weight, serum ALT/AST and the pharmacokinetic profile of the investigational agent

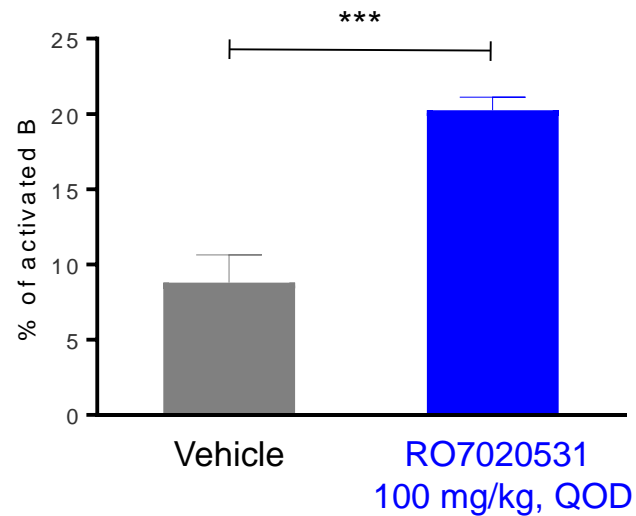
AAV vectors



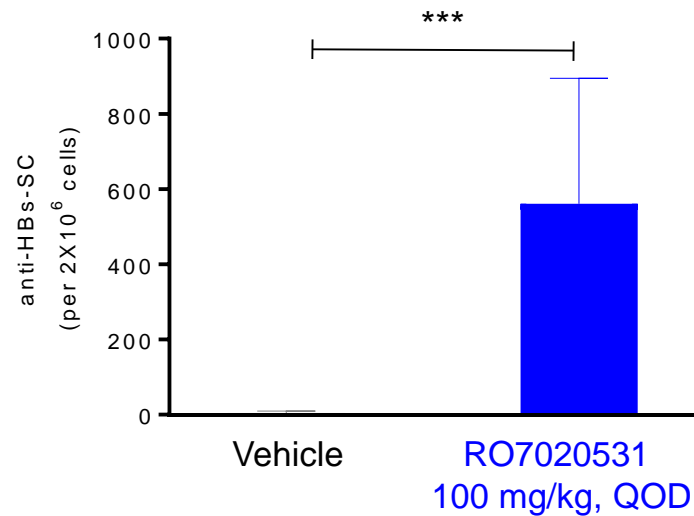
RO7020531 increases the number of germinal center B cells and HBsAg-specific B and T cells in the spleen of AAV-HBV infected mice



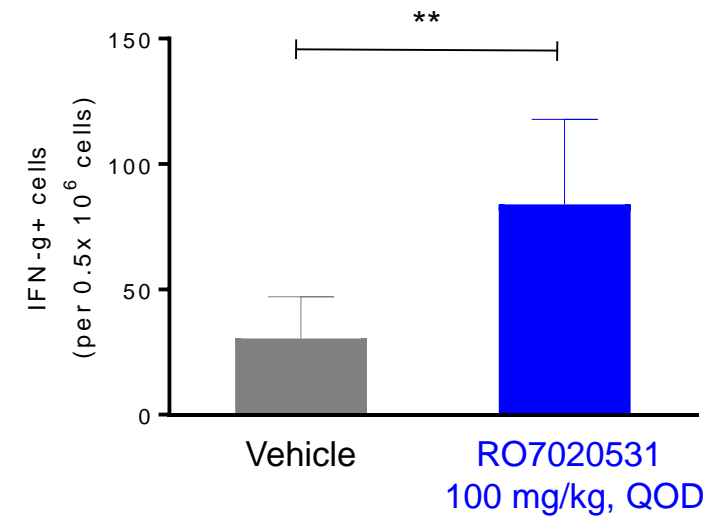
Germinal Center B cells



B ELISPOT (HBsAg-specific)



T ELISPOT (HBsAg-peptide stimulated)



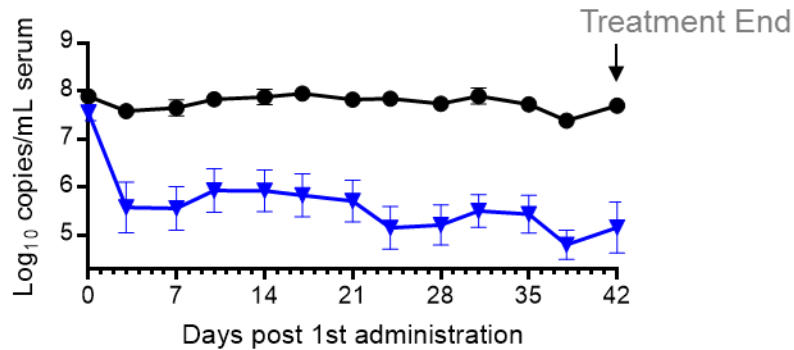
- HBsAg-specific B cells were captured and measured by ELISPOT with HBsAg-coated plate.
- HBsAg-specific T cells were measured by IFN- γ ELISPOT in the presence of HBsAg peptides.

The anti-HBV activity of RO7020531 in AAV-HBV infected mice relies on functional adaptive immune response (B- and T- cells)

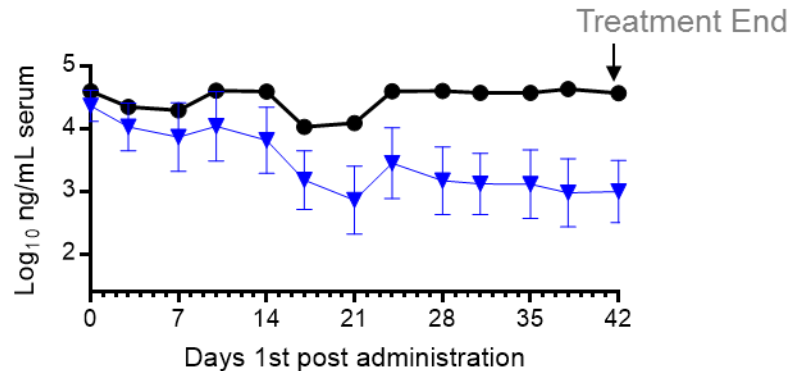


C57B/Bl6 (wild type mice)

HBV DNA

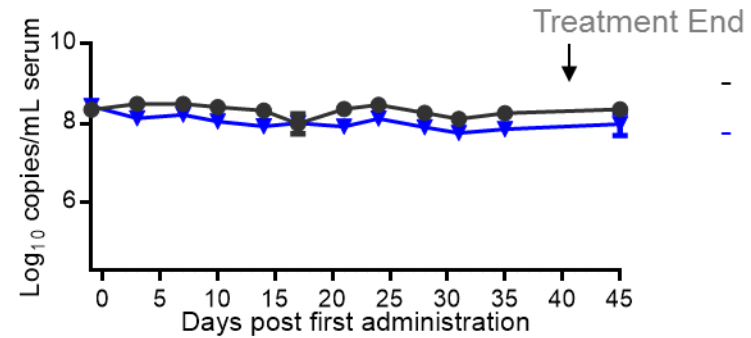


HBsAg



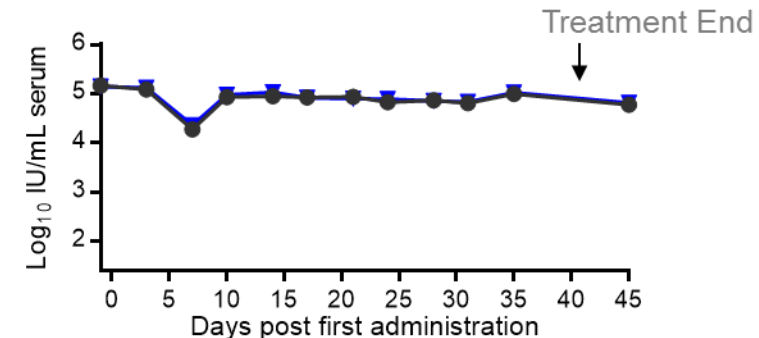
SCID (T- and B-cell deficient mice)

HBV DNA



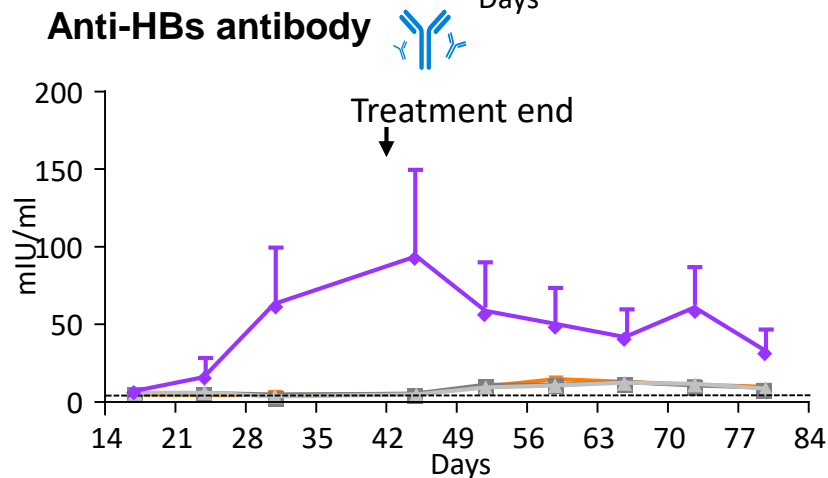
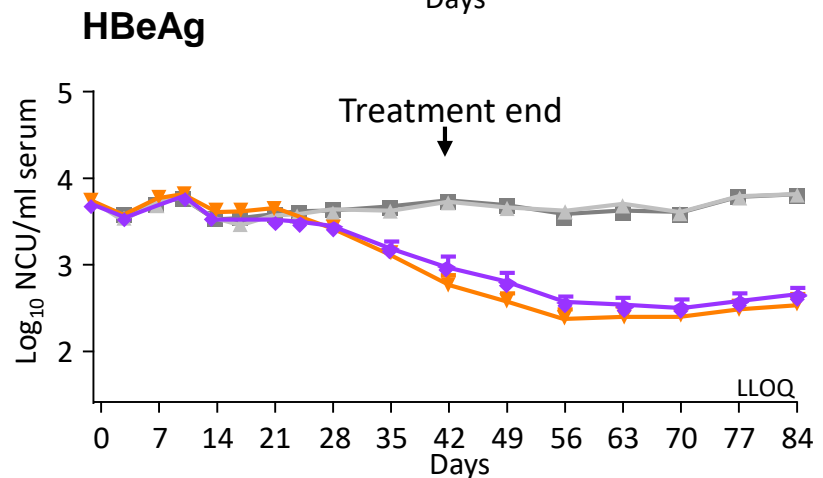
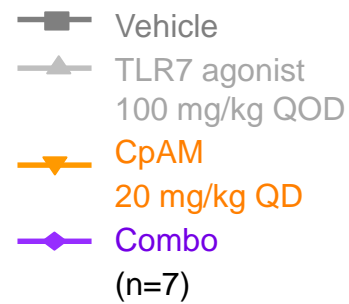
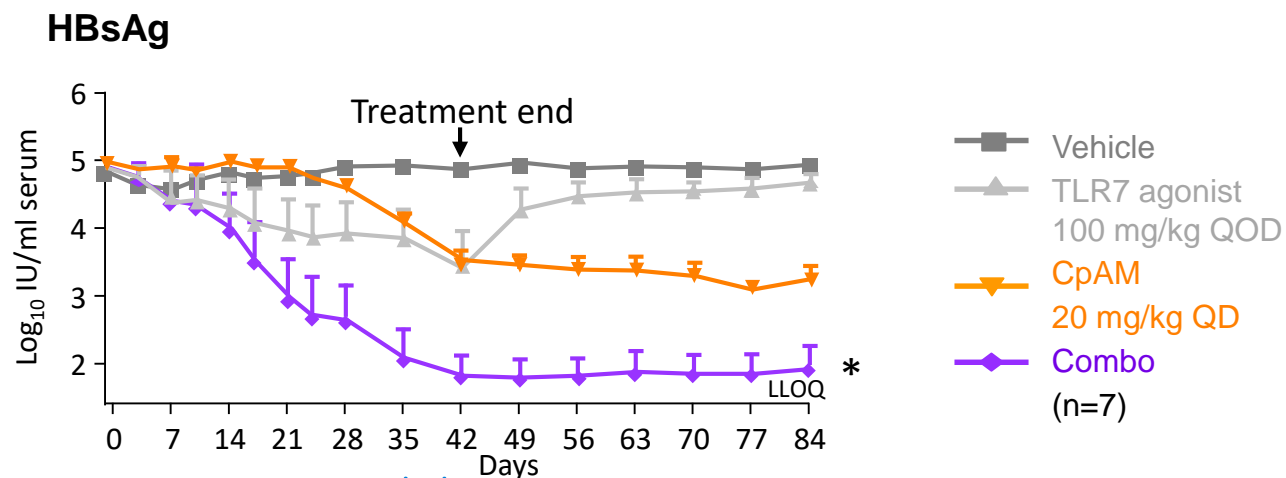
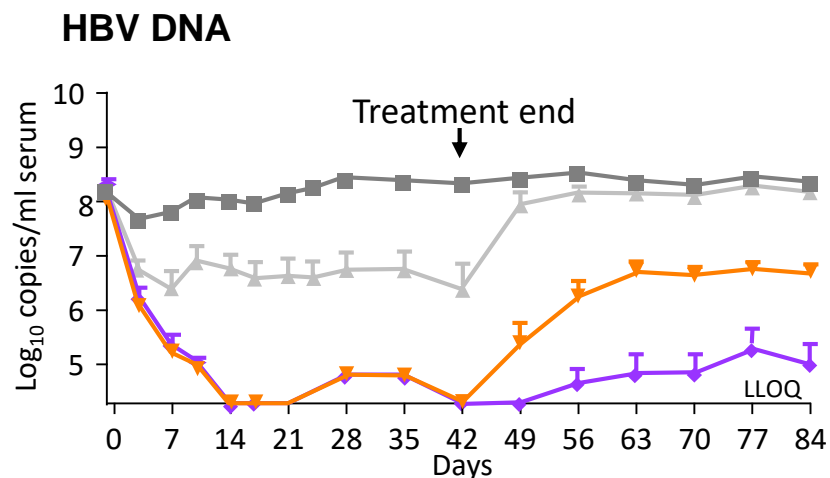
- Vehicle
- RO7020531
100 mg/kg, QOD

HBsAg



- Note: no change on HBeAg level was observed.
- Increasing dose levels of RO7020531 in SCID mice up to 300 mg/kg did not demonstrate anti-HBV activity.
- Plasma exposure of TLR7 agonist and innate immune responses (mRNA upregulation of interferon induced genes) in both SCID and C57B/Bl6 mice were comparable

In the AAV-HBV mouse model, oral combination treatment with the CpAM and TLR7 agonist leads to sustained viral load suppression and HBsAg loss

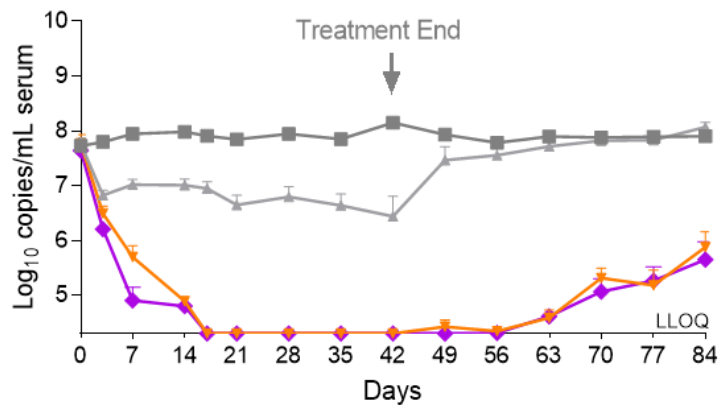


- The combination of RO7049389 and RO7020531 reduced HBsAg level to below LLOQ at the end of treatment in 5 of 7 animals, reduced HBV DNA level to below LLOQ in all animals, which sustained in 4 of 7 during 6-week off-treatment follow-up.

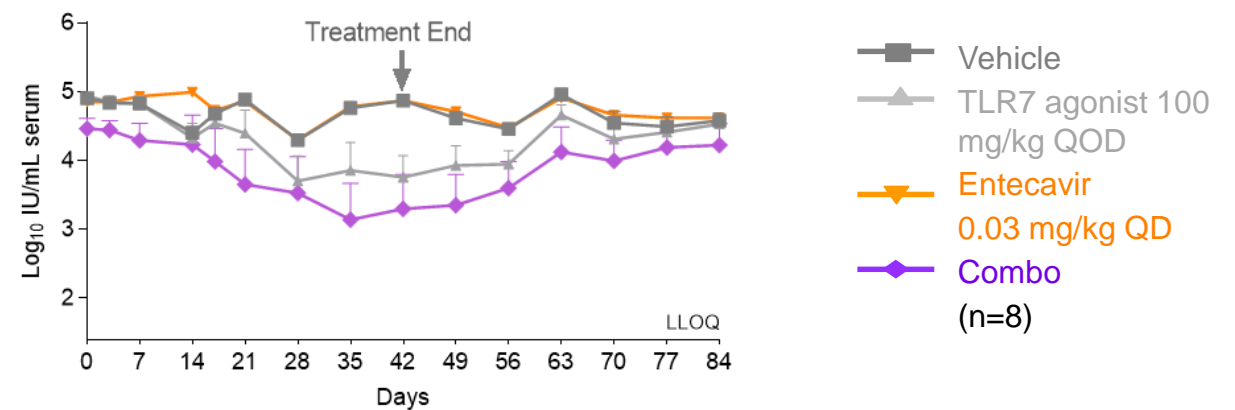
Results are presented as mean±SEM (n = 7). LLOQ = lower limit of quantification; QD = once a day; QOD = every other day

**In the AAV-HBV mouse model, combination treatment of the TLR7 agonist and entecavir demonstrates similar level of HBV DNA suppression as entecavir alone, similar level of HBsAg reduction as RO7020531 alone
No effect on HBeAg**

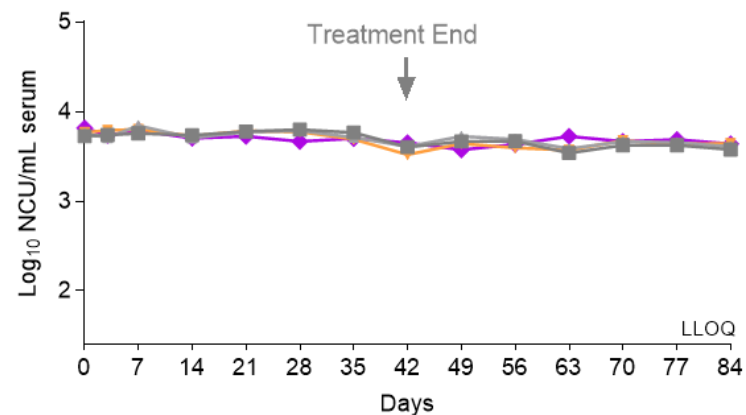
HBV DNA



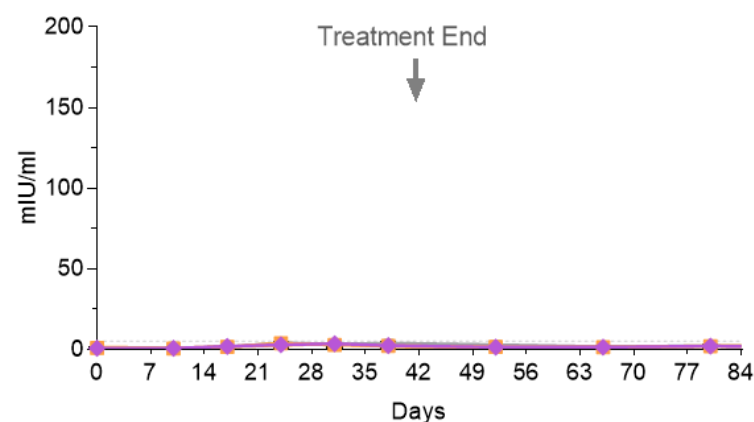
HBsAg



HBeAg

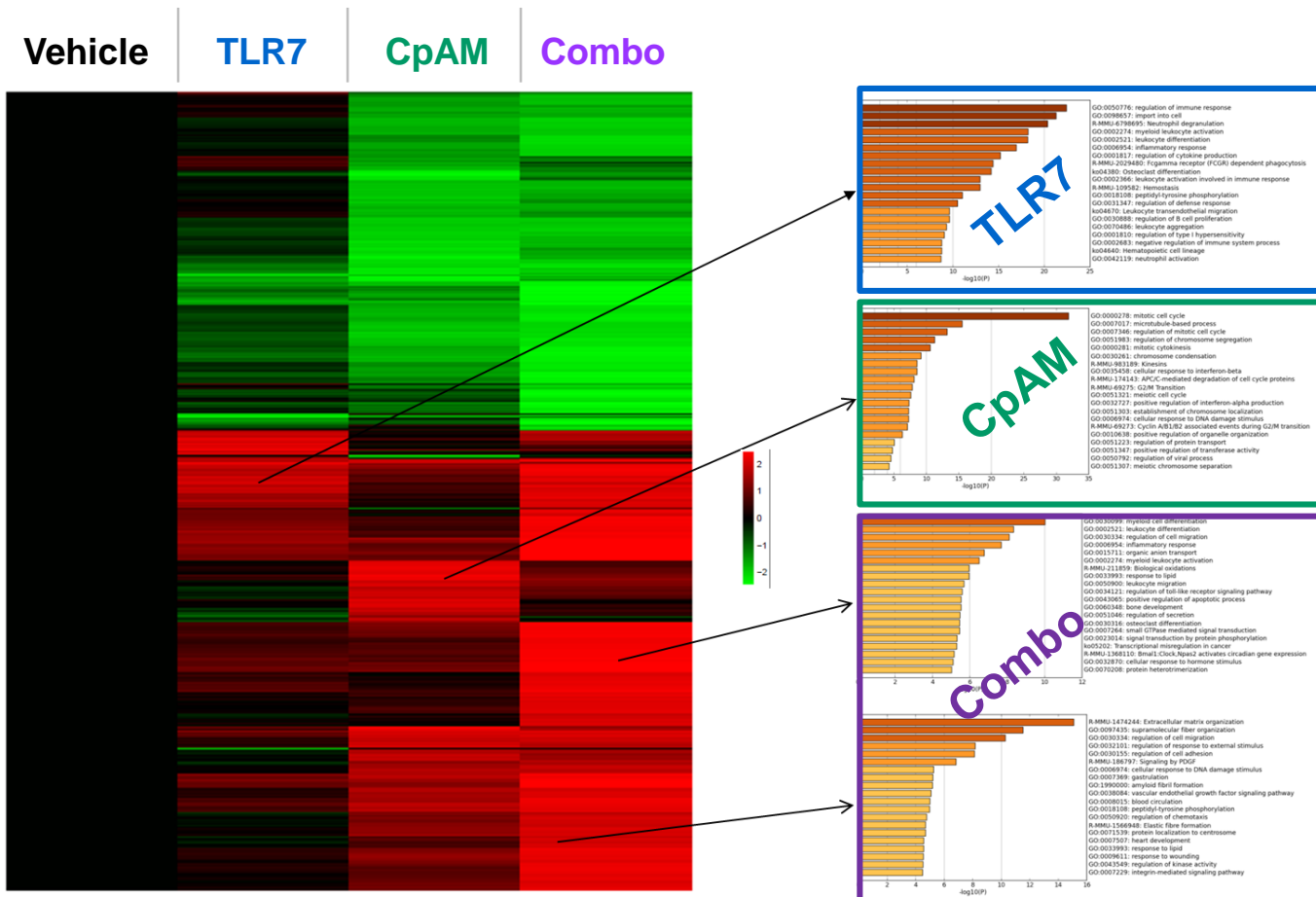


Anti-HBs antibody



- Note: on day 0, the HBsAg level in the combo group was already ~0.4-log lower than other groups.

Combo treatment is associated with an altered liver gene expression profile compared to the two monotherapy arms in the AAV-HBV mouse model



- TLR7 and CpAM induced distinct gene signatures.
- Additional or enhanced gene upregulation may be induced with the combination treatment.
- Gene enrichment analysis indicates broad activation of a connected immune network consisting of various immune cells and responses.
- Further analysis in gene expression between mono and combo therapies may reveal potential early efficacy biomarkers.

Summary

- RO7049389 is a Class I HBV core protein allosteric modulator (CpAM)
- RO7020531 is a double pro-drug of TLR7 agonist
- In the AAV-HBV mouse model, the oral combination of the CpAM and TLR7 agonist demonstrated robust suppression of both HBsAg and HBV DNA levels and with the additional emergence of anti-HBs antibodies in several animals
- Both compounds are currently in Phase I clinical trials with early data presented in conferences
- These promising preclinical results and Phase 1 clinical data provide encouragement for further exploring this combination drug therapy as a means to achieve a functional cure for CHB infection

Acknowledgements

- RO7049389 Project team
- RO7020531 Project team

Doing now what patients need next