Novel therapeutics for the treatment of acute and chronic hepatitis virus infections

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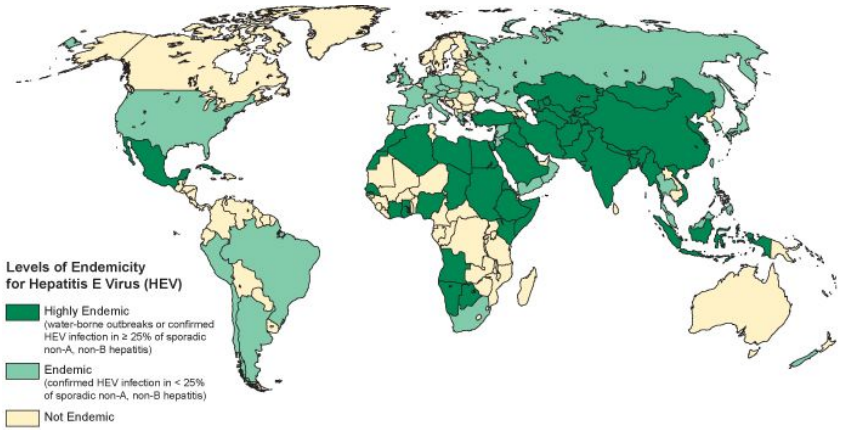
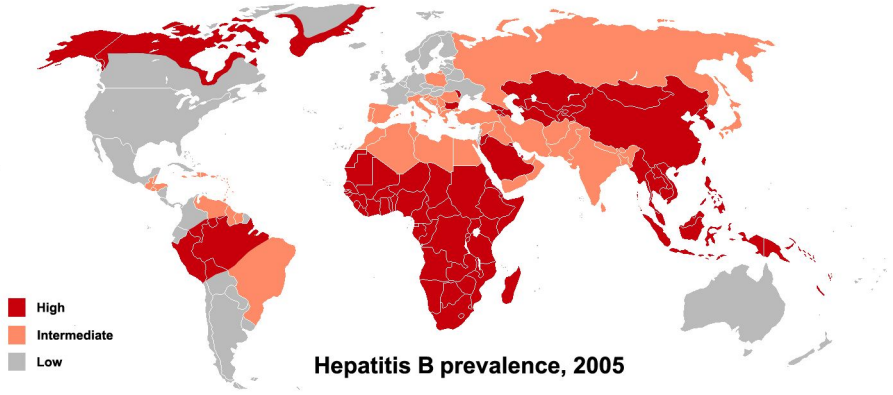
By 2040, deaths from chronic hepatitis are projected to exceed the combined mortality associated with HIV infection, tuberculosis, and malaria!

Hepatitis **E** virus



- #1 acute hepatitis cause in adults throughout Asia, some European countries[1]
- 2 billion estimated infections[2,3]
- 14 million symptomatic cases and 300,000 deaths per annum[2,3]
  - Persistent infections in immunocompromised patients
  - High fatality rate (25-40%) in pregnant women
- Vaccine: yes (but only licensed in China)
- Treatment: no specific treatments; ribavirin, RBV (teratogenic), peg-IFN (major side effects)

Hepatitis **B** virus



- 257 million chronic carriers world-wide
- Chronic HBV infections causes severe liver disease (80% of all hepatocellular carcinomas world-wide can be attributed to HBV)

- Vaccine: yes
- Treatment: yes but rarely achieves a cure

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Technology developed in the Ploss lab enables identification of small molecule inhibitors of HEV and HBV infections

target

goal

Candidate molecule(s) identified and validated

Patent filed

HEV

cure

HBV

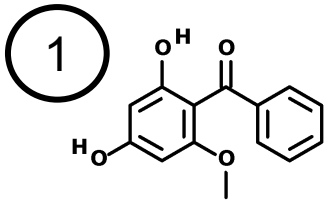
(functional) cure

cccDNA

2 transcriptional repression

3 formation

screen completed



Start-up (Acurasset Pharmaceuticals) was recently formed

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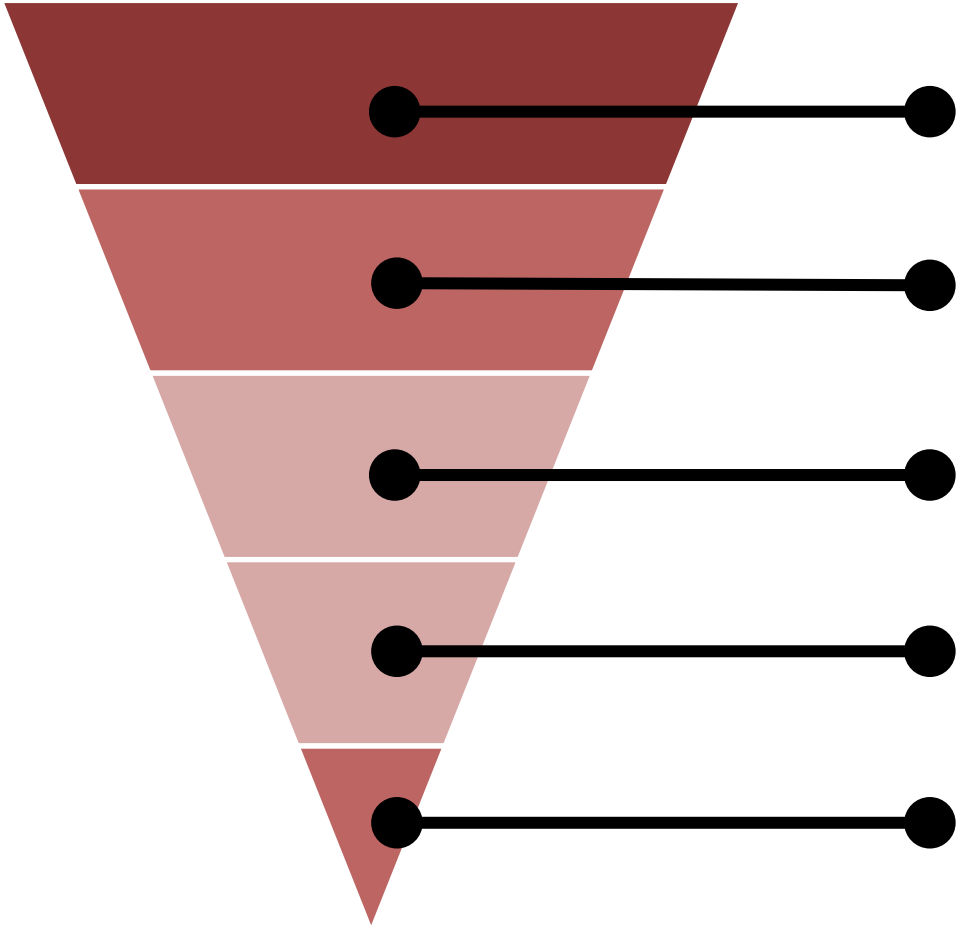
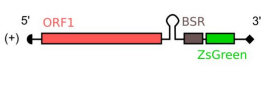
1

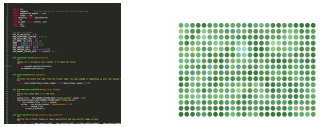
HTS identified isocotoin as a potent inhibitor of hepatitis **E** virus infection



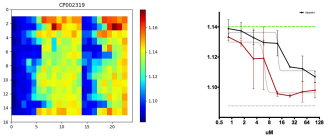
>60,000 compounds



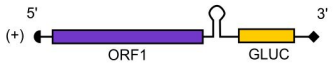
~800 hits, screened for cytotoxicity



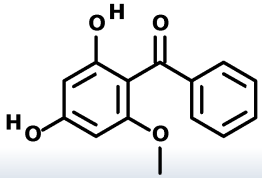
37 non-cytotoxic hits, dose titration



7 hits, lowest IC50. Gluc dose titration



Cytotoxicity assays, translational inhibitor assay



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1 Isocotoin holds promise as an effective treatment for hepatitis E virus infection

Current status

- Isocotoin* is a potent inhibitor of HEV replication
- No overt toxicity
- Activity against multiple HEV genotypes
- Inhibits HEV strains harboring mutations that associate with RBV-resistance
- Structure function analysis has revealed several related compounds with significantly greater potency
- Thermal shift analysis and genetic experiments define the MOA**

Ongoing/future work

- Structure function analysis
- Comprehensive DM-PK/Tox
- Efficacy testing in
  - Human liver chimeric mice (Ploss lab has pioneered this model)
  - Pigs (established collaboration with PI at VT Tech)
  - Rhesus monkeys (established collaboration with PI at Nationwide Children's Hospital)

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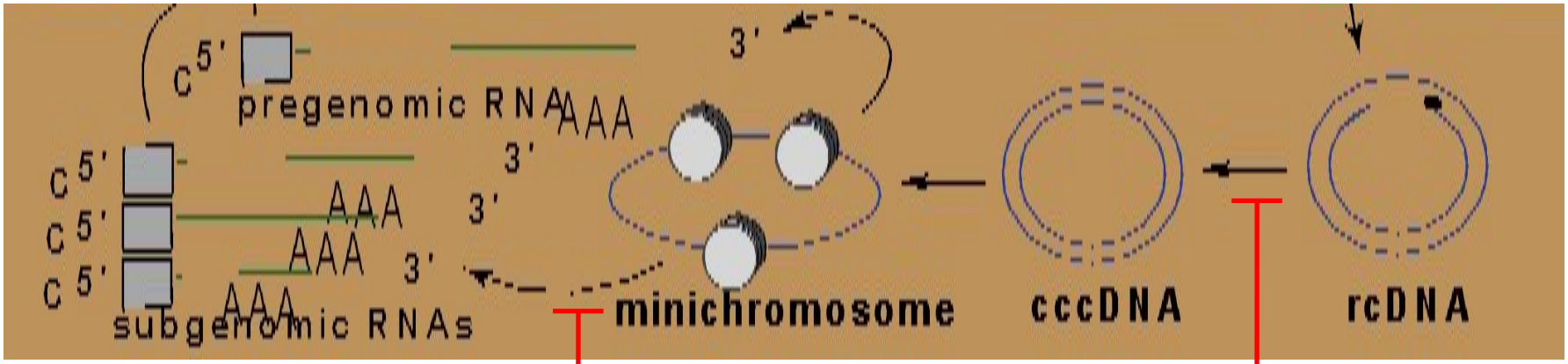
**patent filed on this and related compounds*
*** potential short-cut towards efficacy tests in humans*

2 3

Any attempts to **CURE** HBV will require elimination or permanent inactivation of cccDNA



Inhibitors of HBV gene transcription

2

Inhibitors of HBV cccDNA formation

3

NOTE: HBV encodes only 4 genes and thus provides for targets for antiviral therapy. Inhibitors for the HBV reverse transcriptase (the only virally encoded enzyme) can suppress but NOT cure the infection.

The use of HOST targeting antivirals will consequently be considered to achieve an HBV CURE

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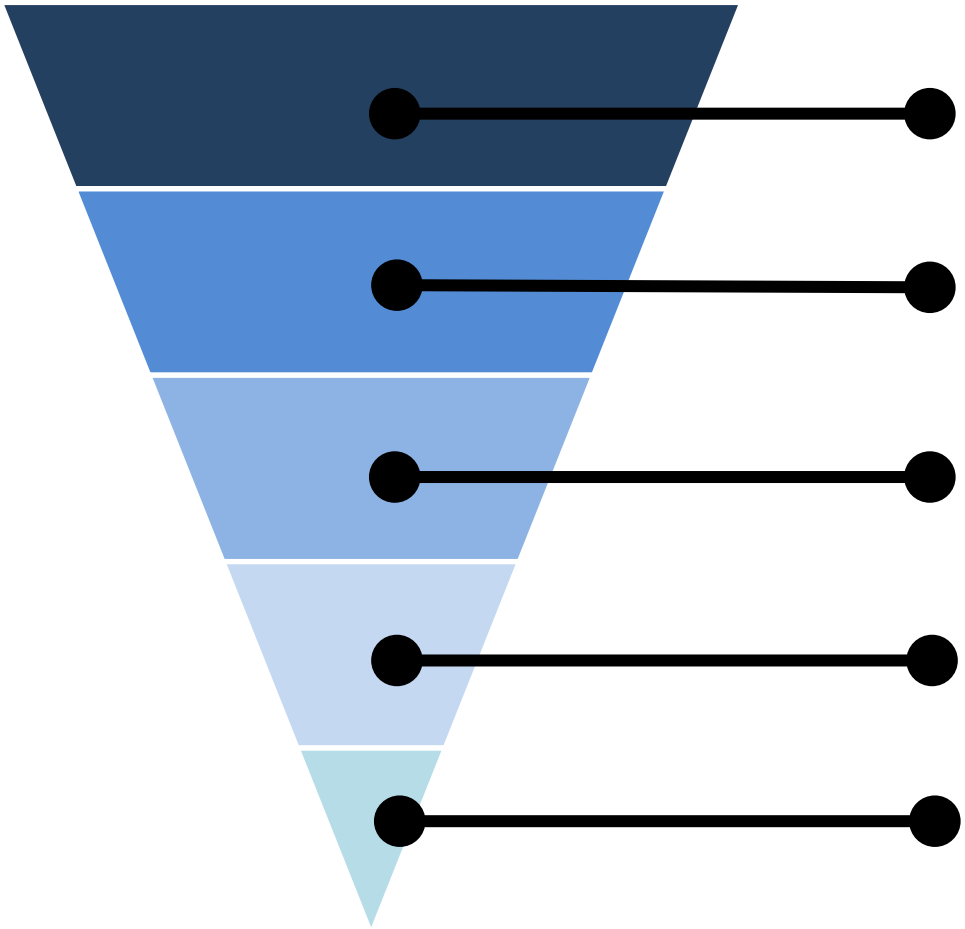
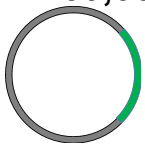
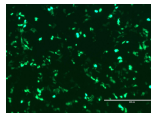
HBV transcriptional inhibitor screening Steps

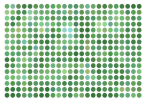


>60,000 compounds


~1000 hits, screened for cytotoxicity

72 non-cytotoxic hits



17 hits currently evaluated for efficacy

selective inhibition of
virus vs host gene transcription?

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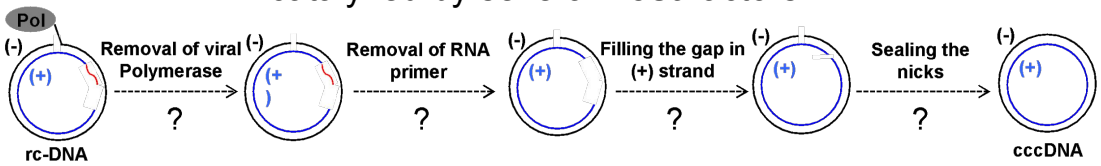
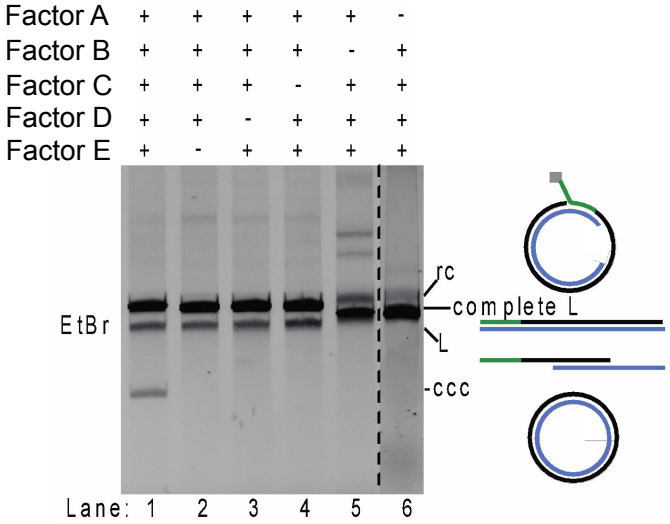
3 We have identified the minimal set of host factors that is necessary and sufficient for the formation of hepatitis B virus cccDNA

HBV cccDNA formation is a complex multistep process catalyzed by several **host** factors





- A combined genetic and biochemical loss of function screen yielded 5 host factors that are critical for HBV cccDNA formation
- Biochemical reconstitution of the rc- to cccDNA conversion proves that this set is necessary and sufficient for this process
- Omission of any one of these factors abrogates cccDNA formation
- Pharmacologic inhibition of one of these factors suppresses HBV infection in cell culture

We have further identified the **rate-limiting step** in the conversion of rc to cccDNA

Thus, we have (finite) specific target(s) for direct screening (e.g. through HT thermal shift or affinity-selection MS

Potential hits would interfere with a critical step in HBV persistence

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