LONG-ACTING ENTECAVIR PRODRUGS FOR HBV TREATMENT

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Calibr at Scripps Research

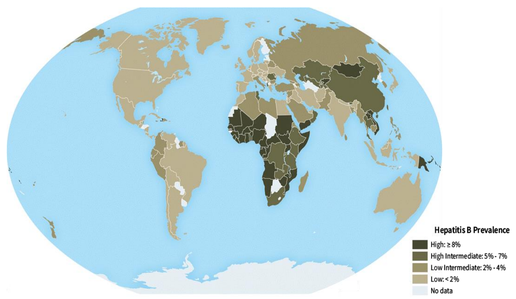
Background

- Hepatitis B virus (HBV) is a hepatotropic virus that can establish a persistent and chronic infection in humans through immune anergy,
- WHO estimates that 296 million people were living with chronic hepatitis B infection in 2019, with 1.5 million new infections each year
- Current HBV antiviral therapies aim to suppress HBV viral load to an undetectable level, reducing the risk of progressive liver disease and the development of HBV-related hepatocellular carcinoma
- As complete eradication of HBV infection is not possible with currently available therapies, the most realistic therapeutic goal is potent and durable suppression of viral replication**,** aiming to prohibit the progression and remission/regression of the underlying liver disease

Unmet Need

- Standard of care for chronic HBV treatment consists of interferon (IFN) and nucleoside analogue reverse transcriptase inhibitors (NARTI) (e.g. entecavir, tenofovir, adefovir) that interfere with viral replication mechanisms
- While current first-line therapies, such as entecavir, cause minimal risk of resistance, high viral suppression (>99%) and high rates of tolerability, these therapies require life-long, daily administration, making patients susceptible to missed treatments and disease relapse
- As adherence to therapy is imperative to achieve and maintain viral suppression, developing a therapy with minimal dosing requirements would significantly improve treatment outcomes

Geographic Distribution of HBV Infection (2020)



Currently Approved HBV Therapies
Require Daily Dosing

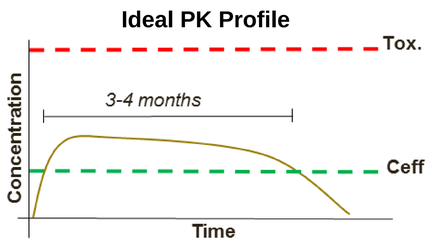
Approved Therapy	Company	Dosing
Entecavir (Baraclude)	BMS	0.5 mg daily
Adefovir Dipivoxil (Hepsera)	Gilead	10 mg daily
Tenofovir Alafenamide (Vemlidy)	Gilead	25 mg daily
Lamivudine (Epivir-HBV)	GSK	up to 100 mg daily
Tenofovir Disoproxil Fumarate (Viread)	Gilead	300 mg daily
Telbivudine (Tyzeka)	Novartis	up to 600 mg daily

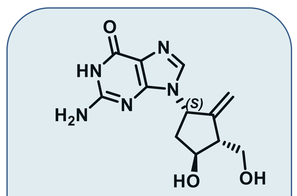
- Of the 369 HBV patients studied by K Xu et al., only 16.5% reported ”high adherence” to their treatment regimen, with 51.2% reporting “low adherence”

Xu, K. et.al *Global Health Action* **2018,** *11* (1), 1433987.

Long-Acting Injectable (LAI) Plan

Ideal PK Profile





- IC_{50} is 0.5 nM with low PPB (13%) and low clearance;100 x more potent > 3TC & ADV

Target Due Diligence
- ✓Unmet Medical Need
- ✓Clinical Efficacy and Safety
- ✓High Potency

Molecular Variant Exploration
- ✓Scaffold Selection
- ✓Prodrug Generation
- ✓Salt Generation
- ✓Characterize:
  - *Crystallinity*
  - *Polymorph*
  - *Particle size*
  - *Melting point*
  - *Solubility*

Formulation Exploration
- ✓Suspensions (e.g., aqueous/micro)
- ✓Solutions (e.g., oil)
- ✓PLGA

Candidate Selection
- ✓Performance characteristics:
  - *In vivo efficacy*
  - *PK/PD*
  - *Dosing regimen*
- ✓Chemical stability & polymorph changes
- ✓Flow properties & viscosity

Results

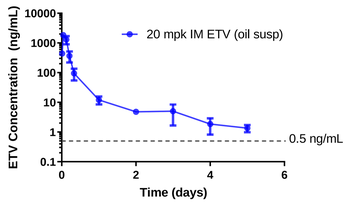
TPP (Target Product Profile)

A single IM/SC injection with low injection volume (< 1 mL), once every month to treat HBV infection (ideally once every 3 month)

A) Suspension based Depot Strategy for Parent ETV

- ETV has a high melt (~298 °C by DSC).
- 170 mg/mL susp. in sesame oil identified (easily passes 27G needle); aqueous-based vehicles were not as stable as oil-based suspensions.
- API formulation shows modest CL/F but good potency allow for low injection volumes; relatively high Cmax is an issue
- A desire to further reduce Cmax and shift the curve would be advantageous

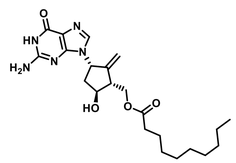
Parent ETV Oil Susp. Provides modest clearance



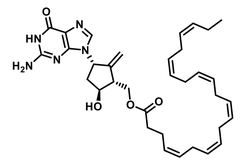
B) Development of LAI Prodrugs

- More than 30 ETV prodrugs (esters and carbonates) were synthesized with various physiochemical properties and unique polymorphs
- Subsequent solubility measurement, formulation development and follow up pharmacokinetic studies of selected prodrugs were done.
- Most esters still exhibited poor solubility in oil-based vehicles.

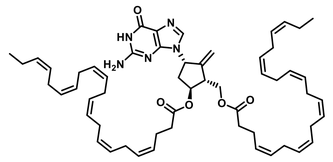
Prodrug	ALog P	Solubility
CBR109	2.9	1 mg/mL in SAIB (with 20% EtOH)
CBR261	5.7	<50 mg/mL (castor oil)
CBR540	12.7	250 mg/mL (castor oil)



CBD109



CBR261

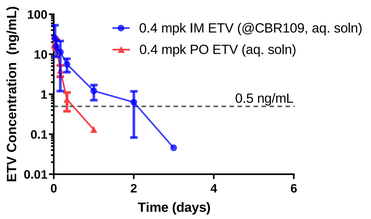


CBR540

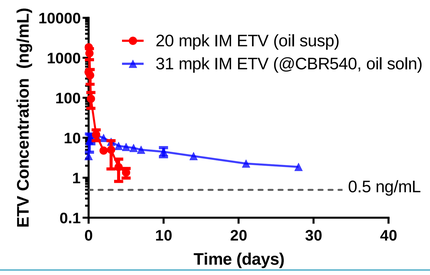
Solution based Depot Strategy for Prodrugs

- A pilot PK study was undertaken with CBR109 (in SAIB, sucrose acetate isobutyrate) and demonstrated that IM prodrug provided longer exposure of Entecavir (ETV) than dosed orally and comparable to IM ETV
- Mono-DHA CBR261 provided slightly improved oil solubility, even in castor oil – likely to be a good candidate for suspension
- Bis-DHA CBR540 provided good oil solubility in castor oil (150 mg/mL ETV corrected).
- While crystalline parent ETV oil suspension (100 mg/mL in sesame oil) rat PK shows high C_{max} with faster clearance, CBR540 (250 mg/mL in castor oil) provides flattened PK curve with plasma levels above therapeutic level for more than a month
- CBR540 has lower Cmax relative to oil suspension of parent drug, demonstrating prolonged exposure with low injection volumes
- **Next Steps**
  - Perform low dose PK in rodents and non-rodents
  - Complete detailed ISR studies and preclinical tox studies
  - Examine subcutaneous route of administration
  - Perform a detailed human dose projection

CBR109 Solution Provides 3x fold Improved Exposure in Rats vs. Oral Parent ETV



CBR540 Oil Solution Provides Extended Exposure in Rats vs. Oil Susp. Parent ETV



Summary and Conclusions

- Calibr-series of ETV prodrugs provide sustained release of ETV after a single intramuscular (IM) injection
- CBR540 provides long-acting “depot release” of Entecavir in rats
- Improvement in ~33x in mean residence time by administration of prodrug, delivers significantly “flattened” PK curve
- CBR540 has >150x lower C_{max} relative to oil suspension of parent drug, demonstrating prolonged exposure with low injection volumes
  - Predicted human dose volume < 500 µL for 1-month coverage
- Injection site reactions were not observed in rats

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