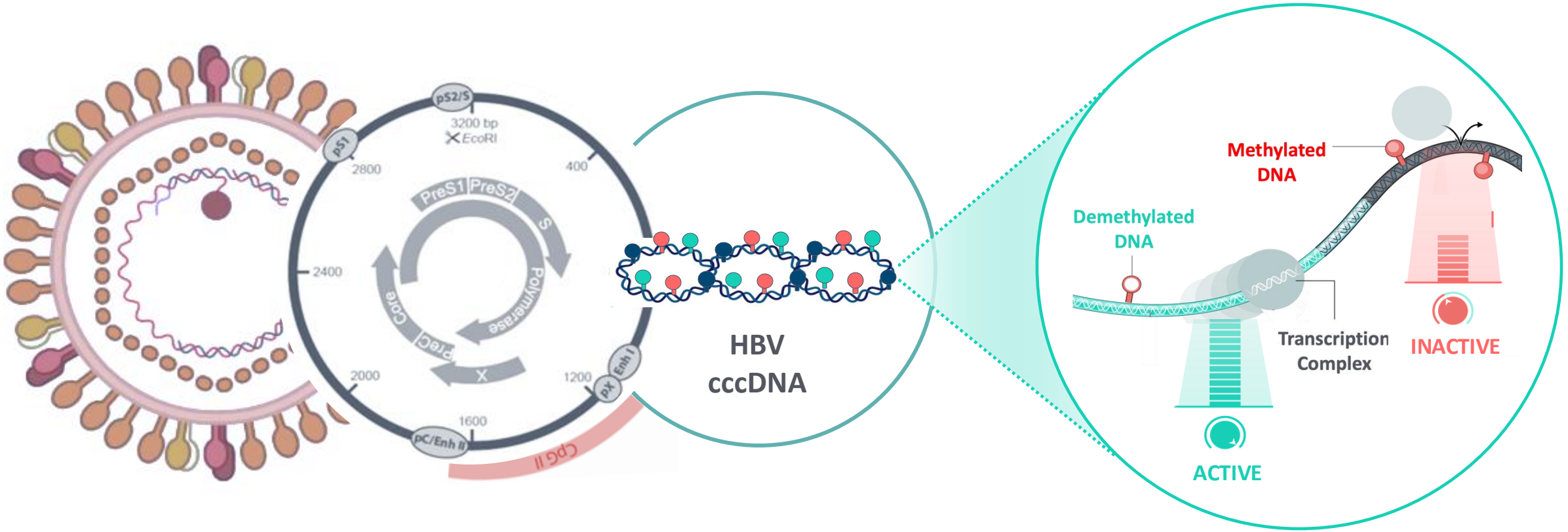
Tune-401:
a First-in-class Epigenetic Silencer Developed for the Treatment of Chronic Hepatitis B

TUNE THERAPEUTICS

AASLD 18th November 2024

TUNE THERAPEUTICS

Hepatitis B replication is controlled by epigenetics



Epigenetic silencing of HBV is observed in a subset of patients who **maintain stable viral control** off-treatment[1-3]

Can we precisely ***engineer*** this epigenetic silencing, across a **wide array** of patients, as a **therapeutic approach**?

[1] *Suslov et al., J Hepatology (2021)*

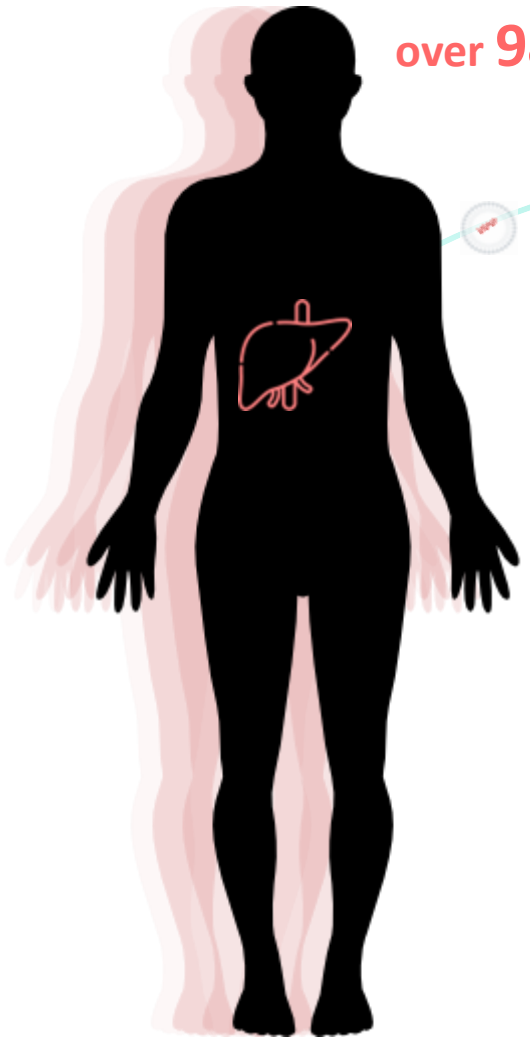
[2] *Pollicino et al., Gastroenterology (2006)*

[3] *Lebosse et al., Scientific Reports (2020)*

Tune-401: a precise and durable epigenetic silencer of HBV DNA

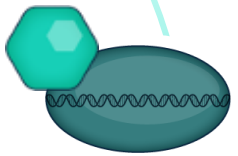
Tune-401 target site found in

over 98% of HBV genomes



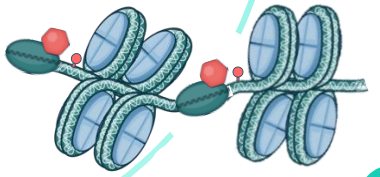
1

Tune-401 is a liver-targeted LNP encapsulating **mRNA** (encoding the epi-silencer) and **gRNA** (specifying the HBV target)



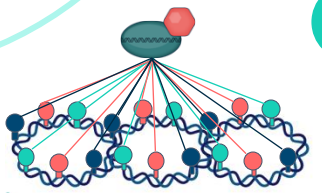
2

mRNA is **translated** into HBV epi-silencing **protein,** which complexes with gRNA to find HBV targets

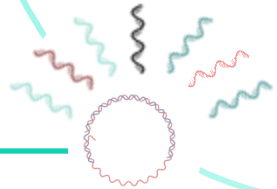


3

Methylation of **integrated HBV DNA**



Methylation of **HBV cccDNA**



4

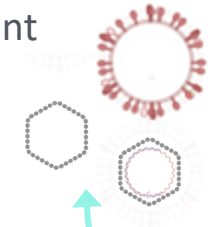
Corresponding **reduction** in **HBV RNA** (all sources)

5

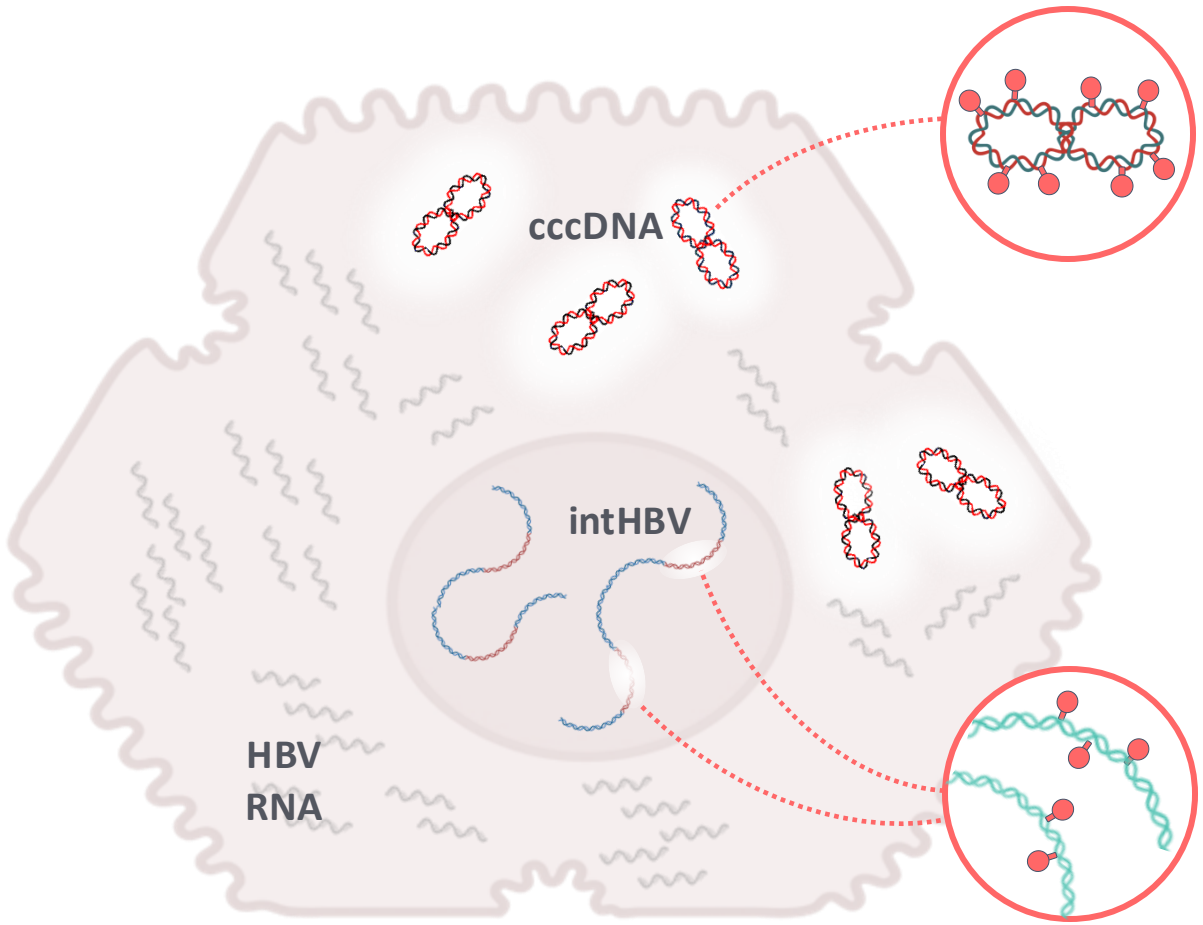
Durable off-treatment reductions in:

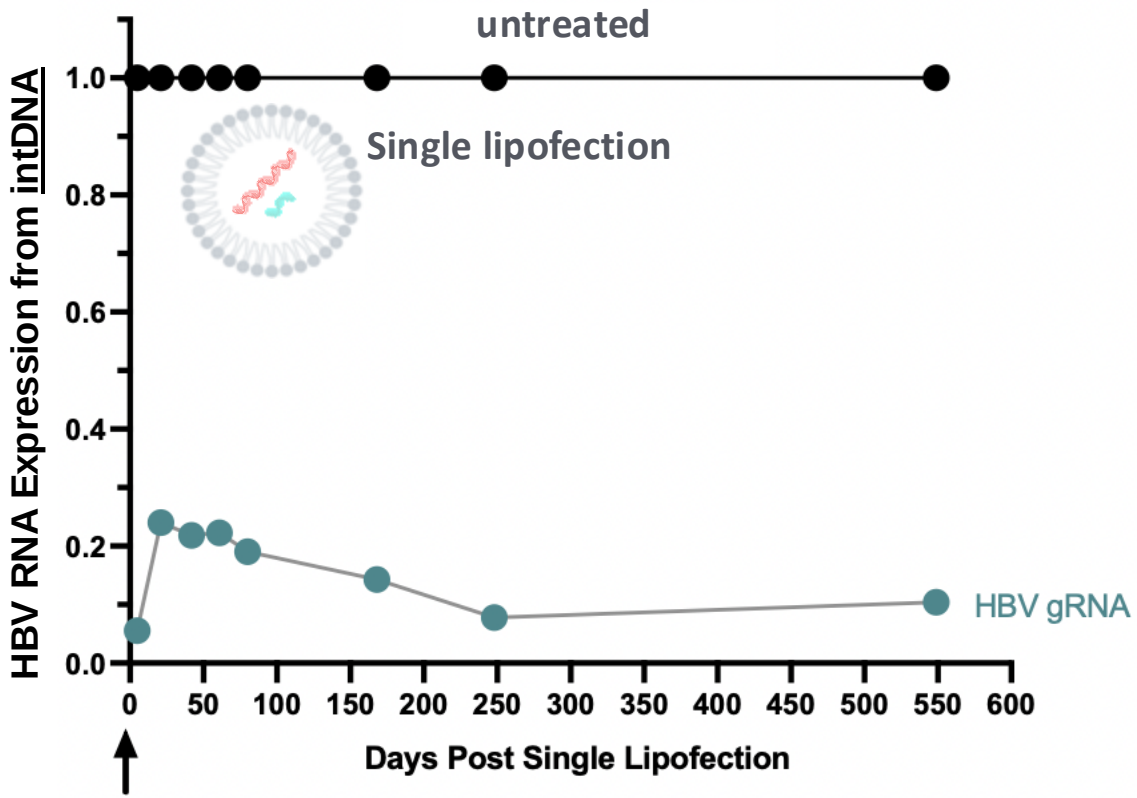
- **HBV proteins**
- **pgRNA**
- **viremia**

= core elements of **functional cure**



Why is Tune-401 different than other HBV therapies?



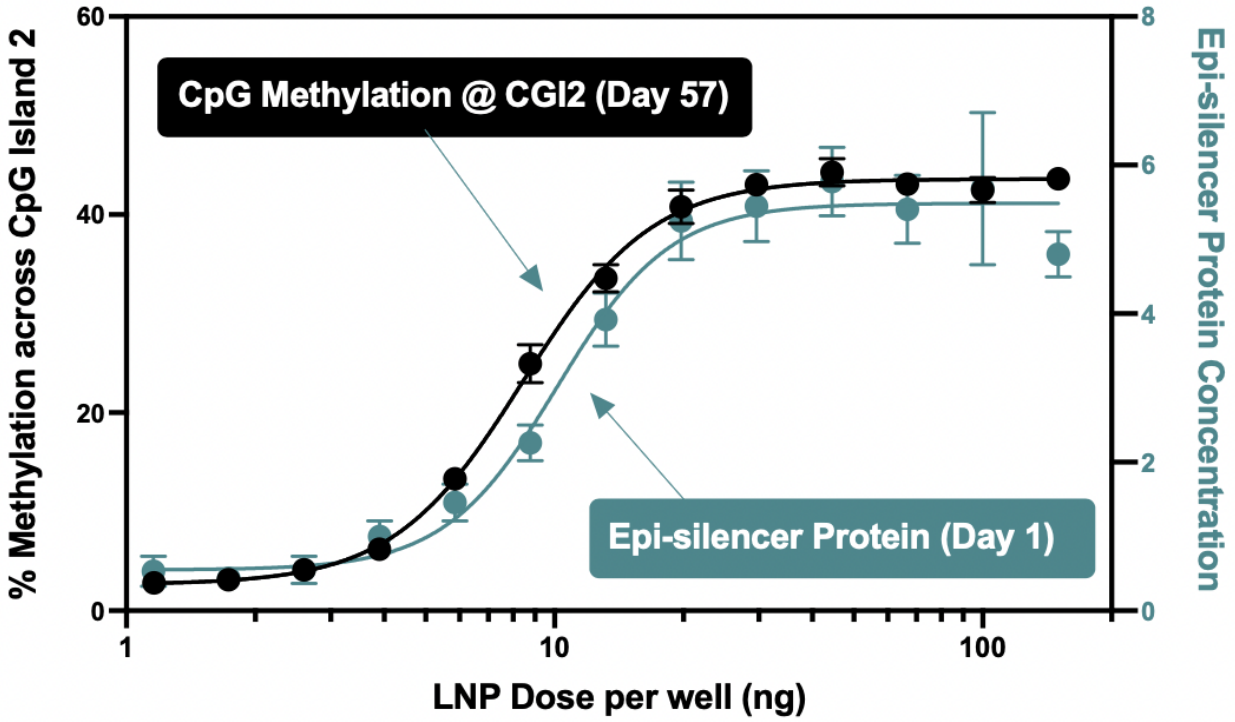


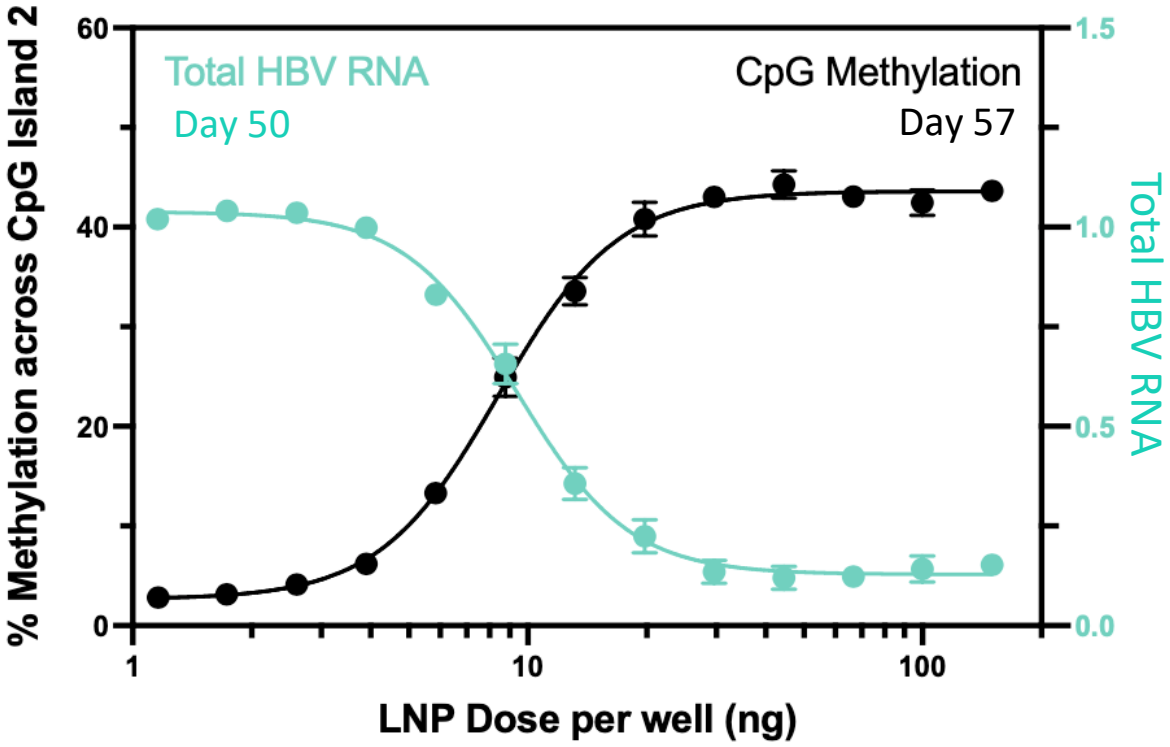
First therapy to silence transcription from intHBV + cccDNA without cutting genome

High durability following transient LNP delivery (without host immune clearance)

Strong, durable silencing of **integrated HBV DNA** in Hep3B cells

LNP dosing → Epi-silencer protein → HBV DNA methylation → HBV RNA & protein levels

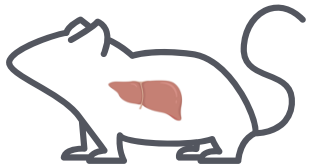




Near complete repression of 3.5kb HBV RNA transcription from **cccDNA** in human hepatocytes

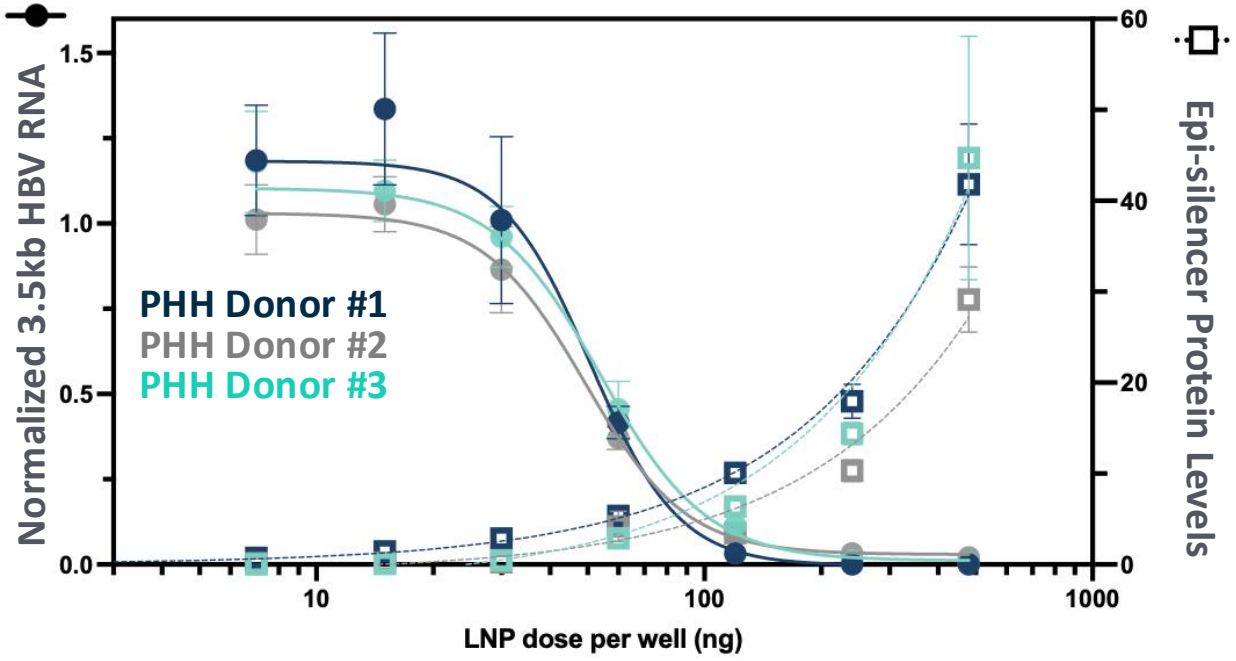
PHH + HBV

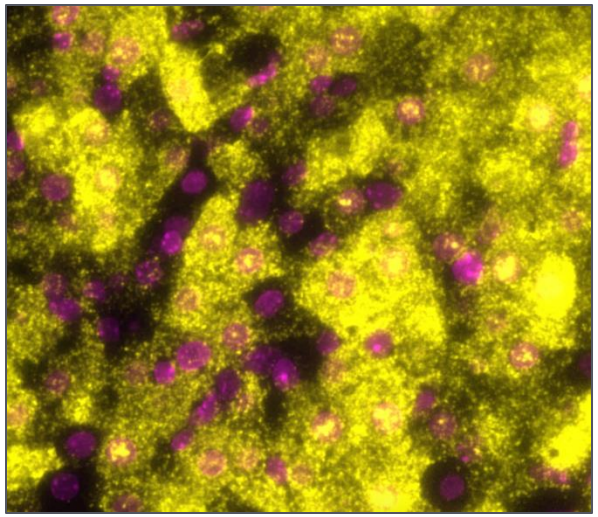
A **single dose** of Tune-401 repressed as much as **99.99%** of 3.5kb HBV RNA from physiologic ranges of cccDNA per cell



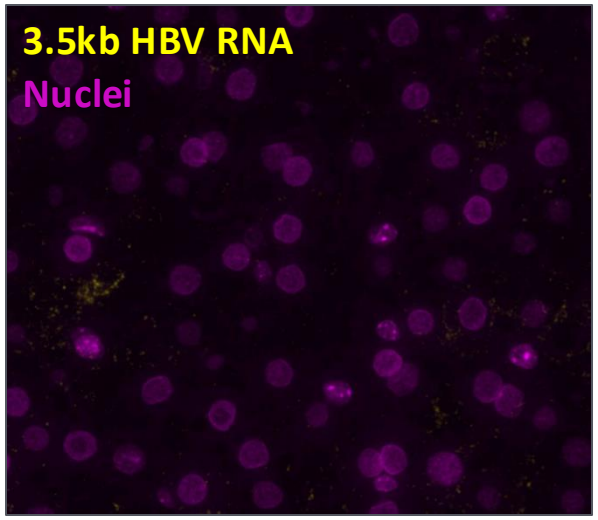
FRG Human Chimeric Liver Mouse + HBV

Near complete 3.5kb HBV RNA repression found in huHeps that received LNP. ISH quantification showed average population shift from 67 copies/cell at baseline to 0 copies/cell in cells that received Tune-401 (N= >17k huHeps/mouse)





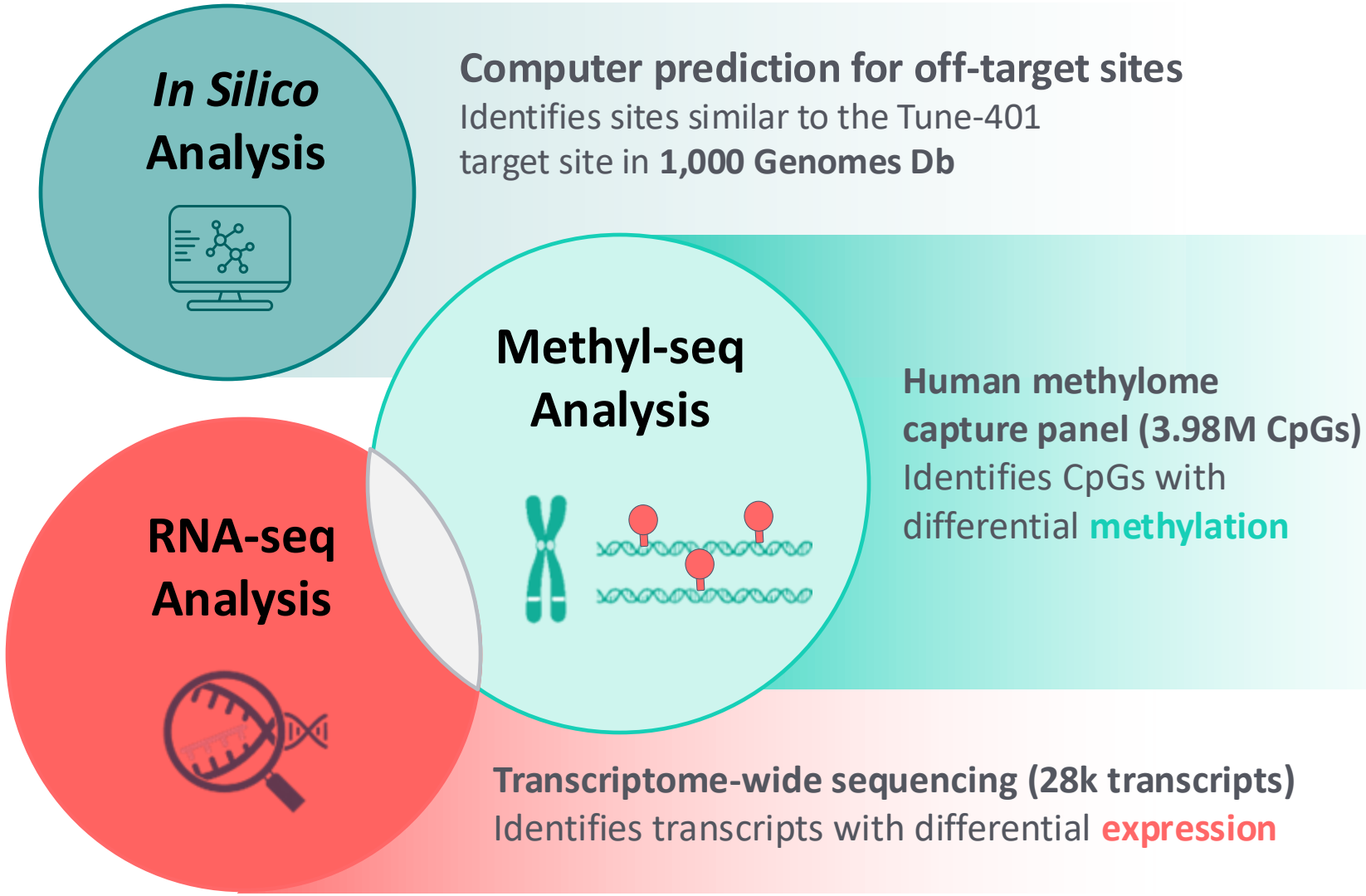
Non-Targeting Epi-silencer



Tune-401 Epi-silencer

Baseline values: 90% humanization, ~3 cccDNA/cell, 45k IU/mL HBsAg

Assessing the specificity of Tune-401 for the HBV genome



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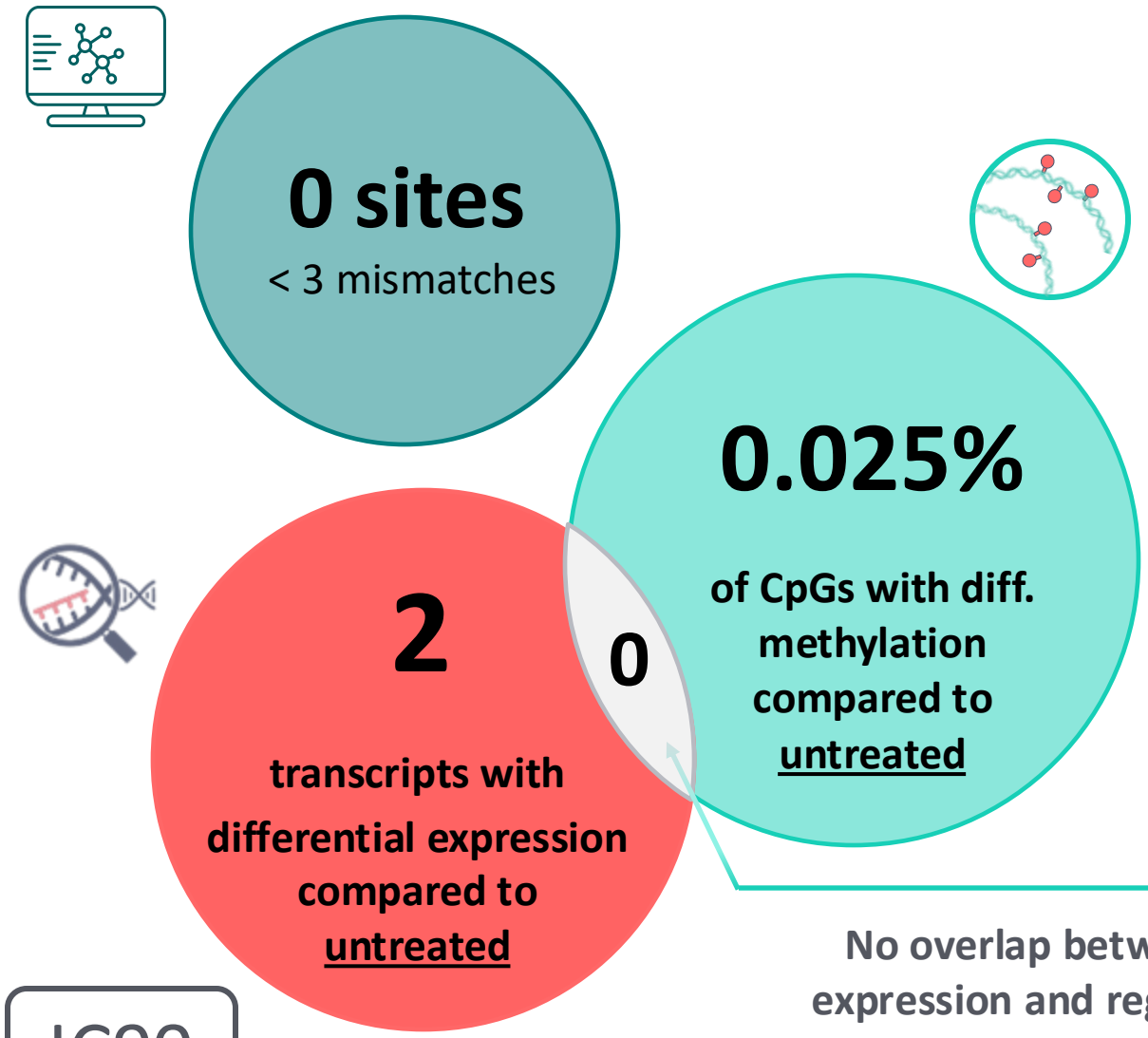


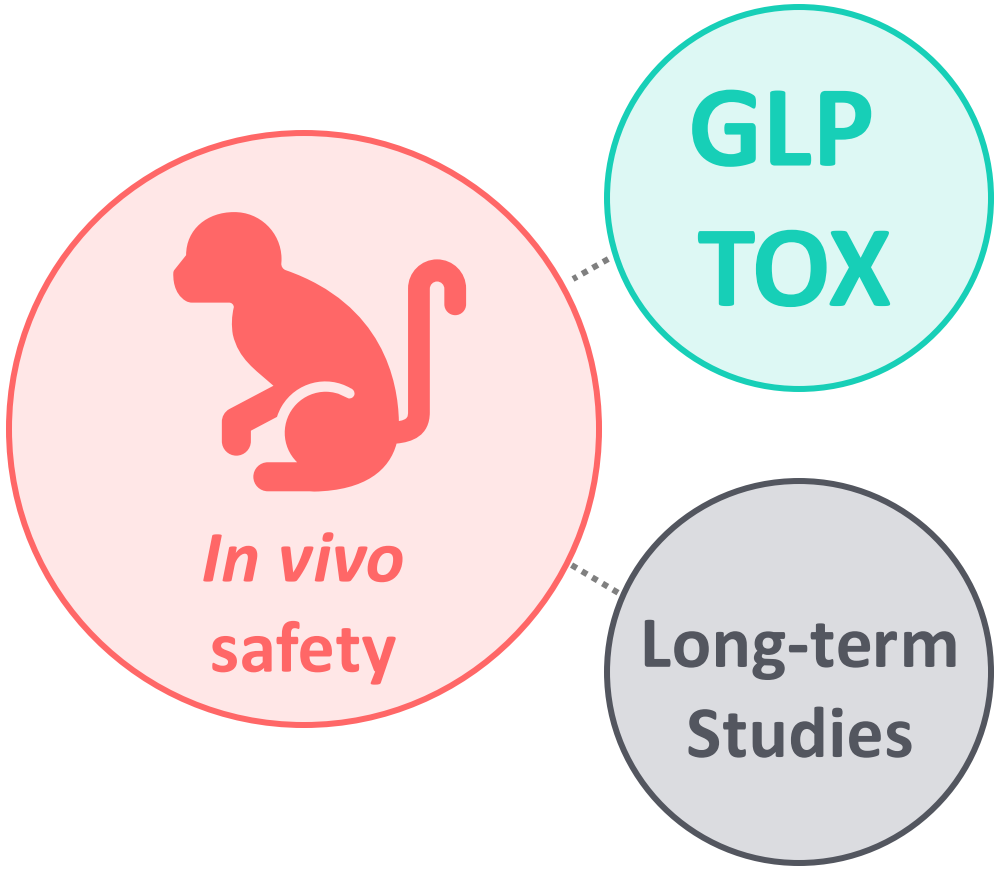
Sequencing Readouts

- Performed across 3 PHH donors
- 3 dose levels: IC50, IC90, 3xIC90
- Compared to untreated cells
- High degree of coverage: ~4,800 reads per unique transcript, 165 reads per unique CpG.
- Also performed sequencing analysis in primary cells from tissues with LNP biodistribution

No overlap between genes with differential gene expression and regions with differential methylation

Even at 3xIC90 dose, this overlap is 2 genes

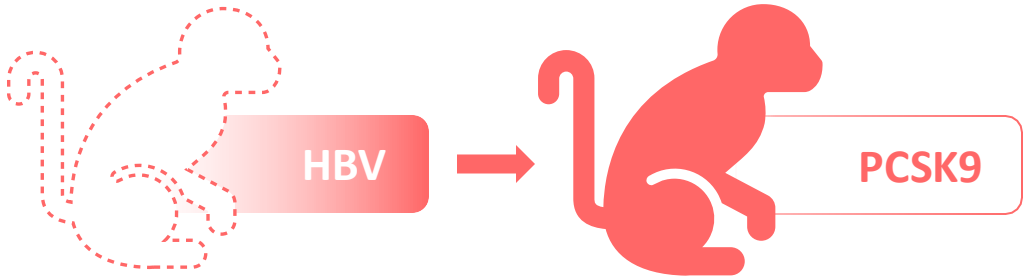
Assessing the safety of Tune-401 in large animal models



- NOAEL determined to be highest dose tested (3 mg/kg)
- Transient ALT/AST elevations that resolved by 14d post-dosing
- No cage-side observations
- No adverse histopathology findings
- No Tune-401 detected in germline cells (RNA and protein)

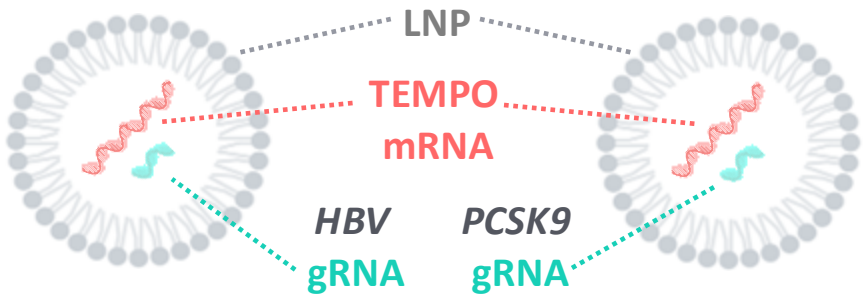
- No adverse clinical observations on NHPs dosed with surrogate research epi-editor (now at ~600d post-dosing)

Demonstrating the Tune-401 MoA in a large animal model



No large animal model available for HBV

Surrogate liver target in established NHP model



Tune-401

PCSK9 Surrogate

Same mode of epigenetic action

Methylation of CpG islands leads to repression of transcription initiation

Same delivery vehicle and effector protein

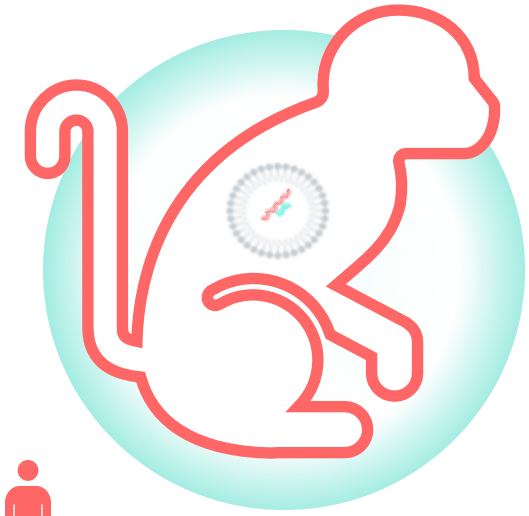
LNP and epi-silencing protein identical – only the targeting guide RNA differs

Demonstrating the Tune-401 MoA in a large animal model

TUNE THERAPEUTICS

Durability

How stable is repression following a single dosing?



What is the expected potency offset between species and target genes?

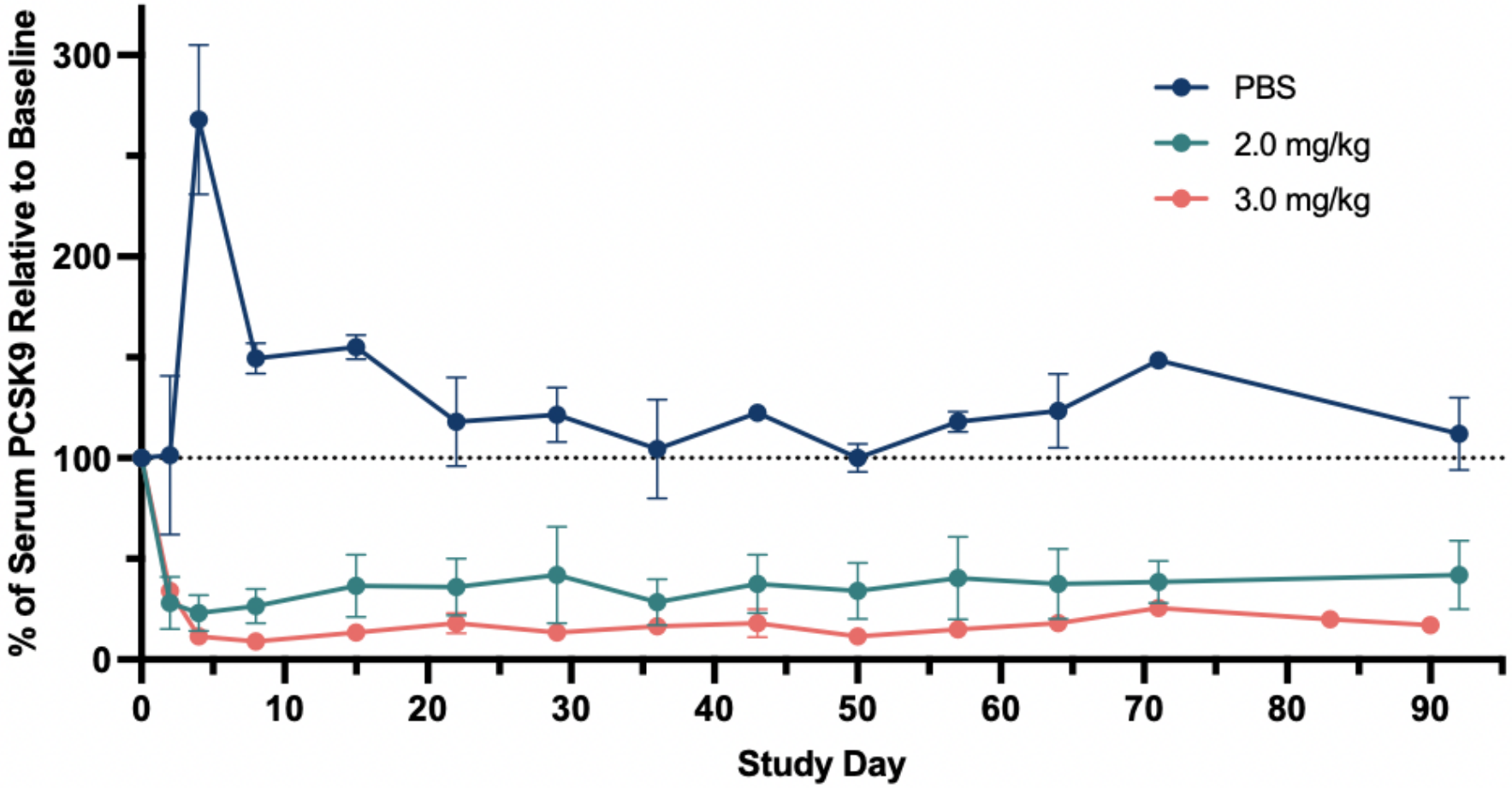
Potency Offset

Biodistribution

What % of hepatocytes can we deliver to?

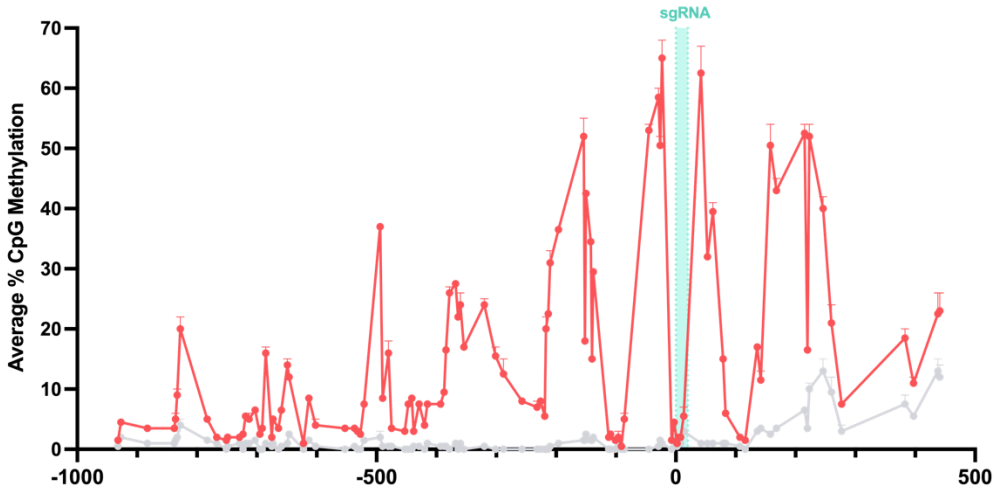
Surrogate PCSK9 epi-silencer shows strong and dose-dependent repression of PCSK9 in NHP

PCSK9

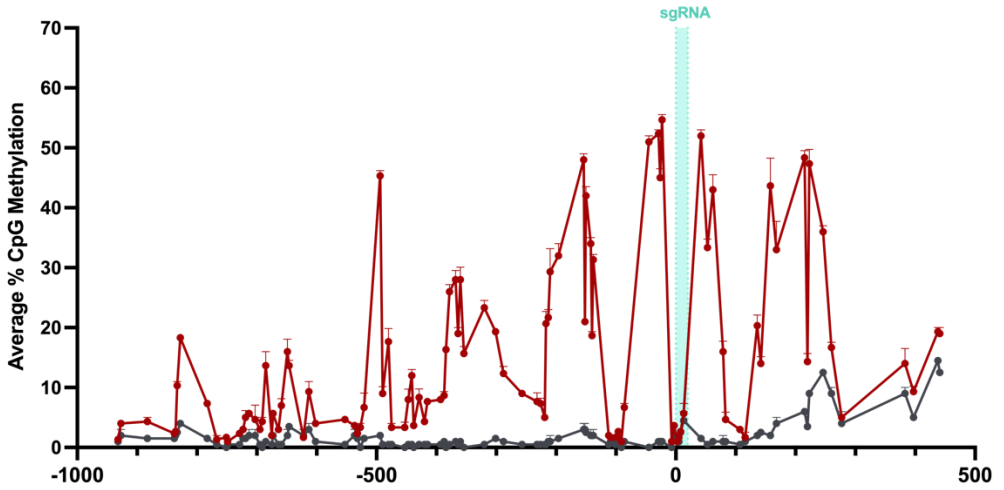


Strong durability of deposited CpG Methylation over time

Methylation @ 8 days post dose



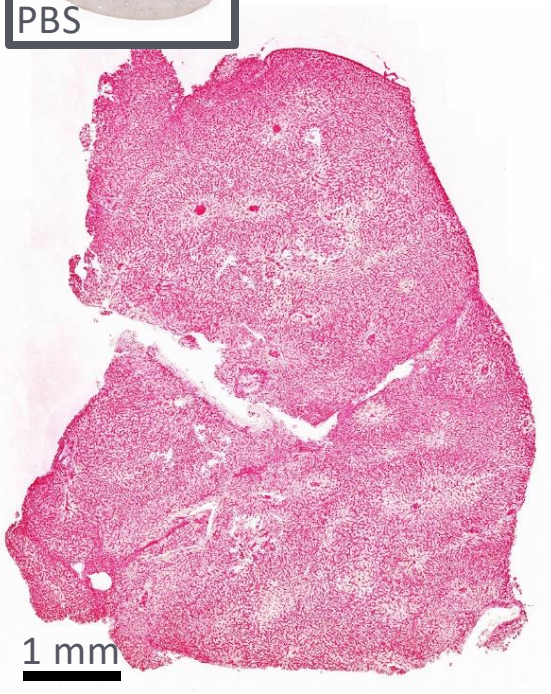
Methylation @ 365 days post dose



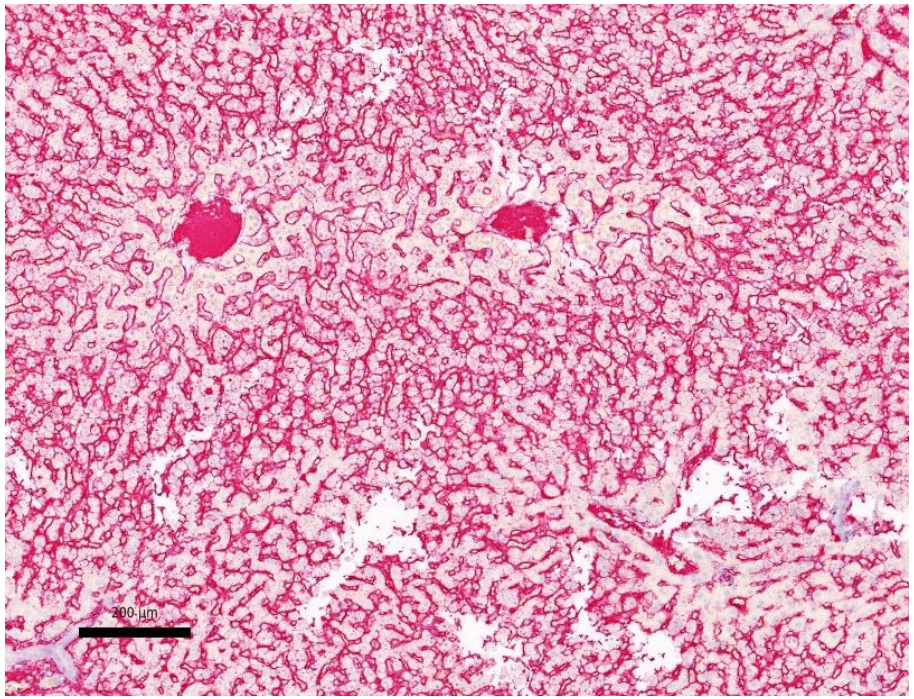
PCSK9

Deposited CpG Methylation around the PCSK9 proxy target locus was highly stable between **8d post-dose** and **365d post-dose**

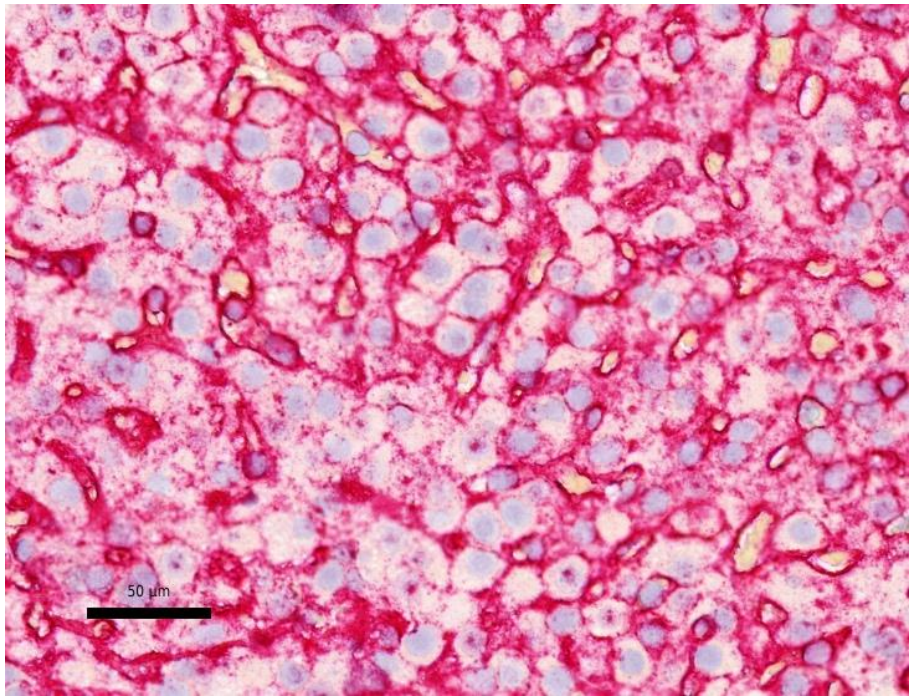
Strong biodistribution of PCSK9 epi-silencer in NHP livers shows functional delivery to nearly all hepatocytes



Epi-silencer mRNA ISH @ 6hr post-delivery



200 um



50 um

ISH, IHC, and serum PCSK9 repression all support that LNP delivery reaches nearly every hepatocyte in the NHP liver

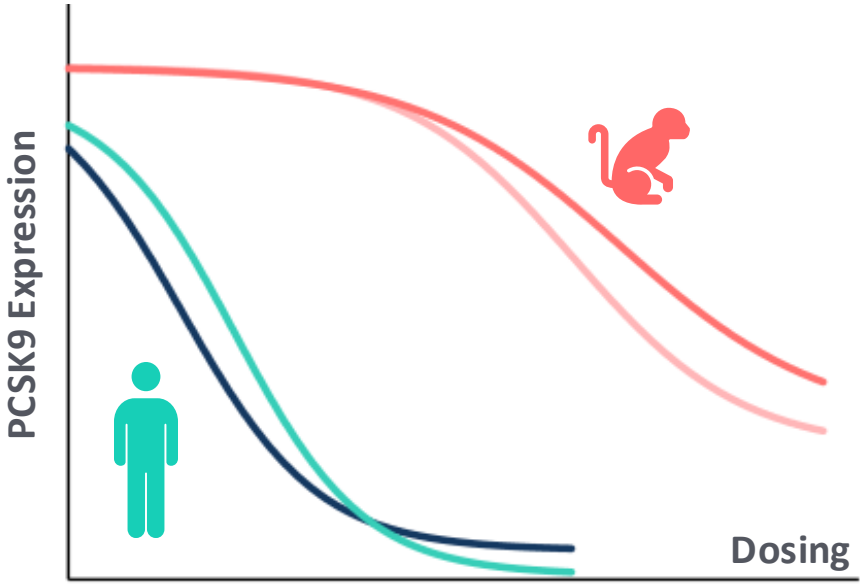
Estimation of HBV epi-silencer potency offset using data from the cyno PCSK9 epi-silencer

TUNE THERAPEUTICS

1 Species Bridge

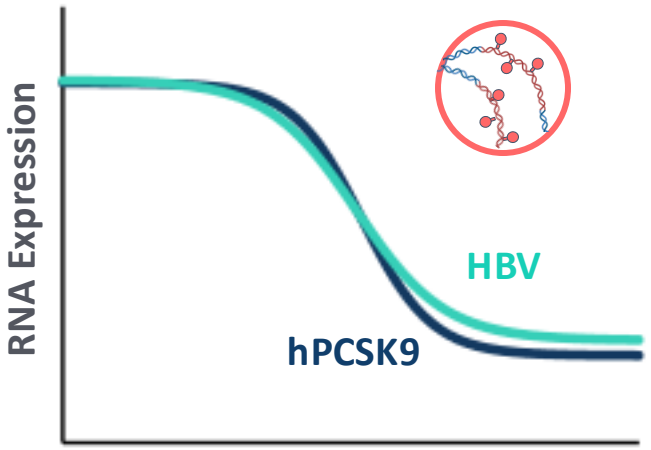
2 Target Bridge

PCSK9 in cyno NHP → PCSK9 in PCH → PCSK9 in PHH / Hep3B → HBV in PHH / Hep3B



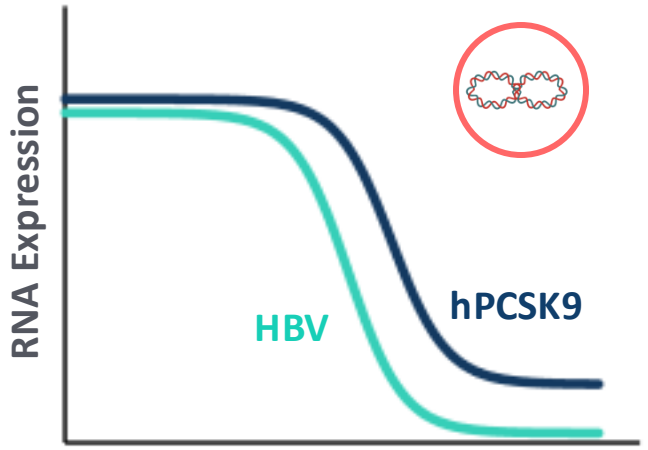
4.3x species potency offset

intDNA/Hep3B



0.9x potency offset

cccDNA/PHH



1.5x potency offset

Total Offset	3.87-fold ↗	6.45-fold ↗

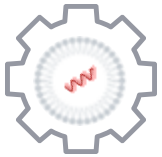
Tune-401 entering human clinical trials in 2024

Preclinical Research

- Protocol Optimization, CTA Enabling Studies
- Report Generation, Bioanalytics

Nonclinical

- Safety, Efficacy, Biodistribution
- NHP Toxicity, PK (GLP DP)



CMC /Manufacturing

- Analytical Readiness (DS & DP)
- GLP, GMP, DP Production, DP Release

Clinical / Regulatory

- Protocol Development & Clinical Readiness

Phase I

- First-in-human studies
- Safety assessment
- Dose ranging

New Zealand clinical trial **approved**

TUNE THERAPEUTICS

THANK YOU

LIPID NANOPARTICLE TECHNOLOGY PROVIDED BY ACUITAS THERAPEUTICS