Capsid assembly modulators as backbone treatments for HBV functional cure

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Disclosures (equity) : Aligos, Cocrystal, Gilead, BMS, MDGL, Merck, Lilly, Roche, AbbVie, Pfizer, GSK, Vertex, Novo

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HBV cure Task Force

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ICE-HBV
International Coalition to Eliminate HBV

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LYON HEPATOLOGY INSTITUTE
COMPREHENSIVE LIVER CENTER

CAPSID ASSEMBLY MODULATORS AS BACKBONE TREATMENTS FOR HBV FUNCTIONAL CURE

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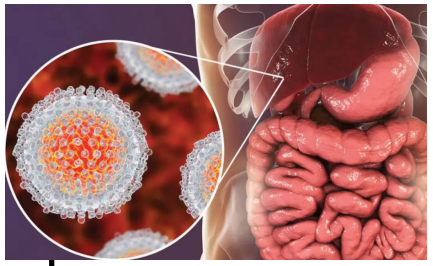
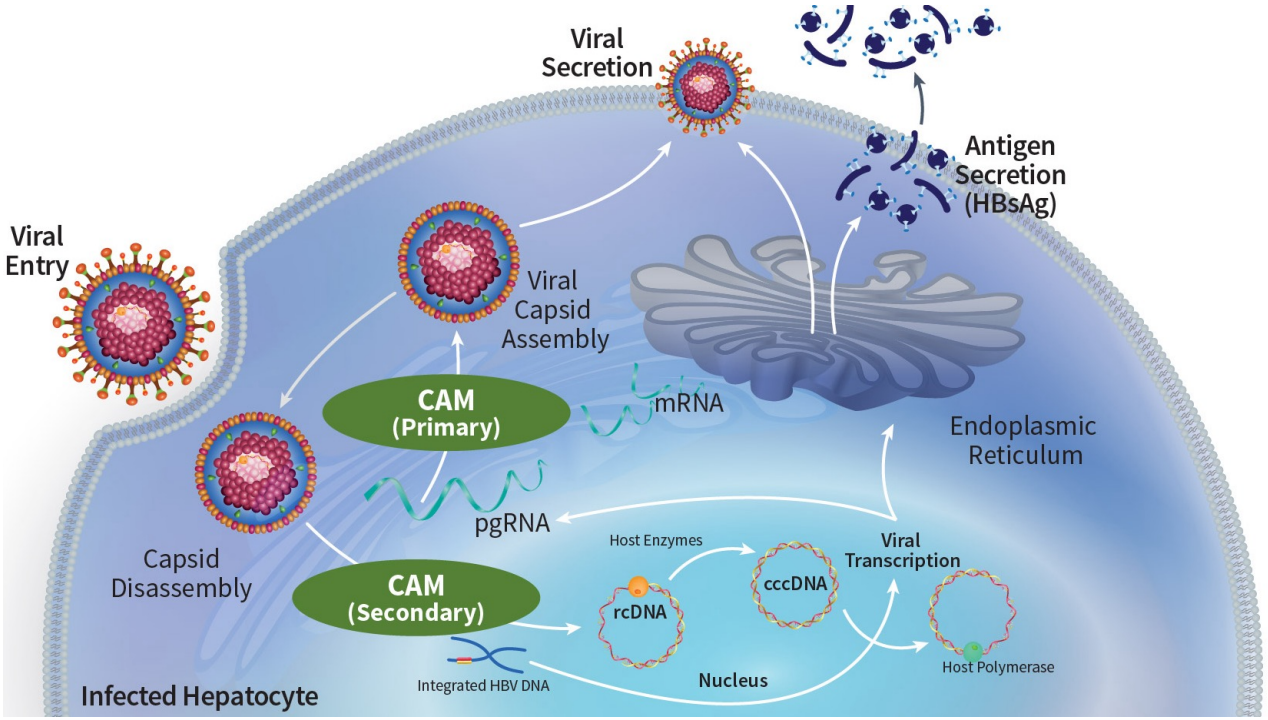
HEPATITIS B VIRUS

- ~ 1.5 million people become newly infected each year.
- ~ 300 million people are chronically infected.
- Only 10% of infected individuals are diagnosed.
- ~ 820,000 people die each year from hepatitis B and related complications (i.e.: liver cancer).
- The absence of symptoms for most infected or chronically infected patients leads to spread of the virus to others.
- Vaccines are available, but coverage among adults has been suboptimal.
- Existing drug therapies can suppress chronic hepatitis B infection but cannot cure HBV and *do not prevent HCC*.



HEPATITIS B VIRUS TREATMENT
DUAL ROLE OF CAMS

- Two mechanisms of action (MoA) can be demonstrated preclinically
  - Primary mechanism
    - Promotes the premature assembly of core protein, leading to the formation of empty capsids
    - Responsible for the deep reductions of HBV DNA and RNA observed clinically with CAMs
  - Secondary mechanism
    - Requires ~10-fold higher drug concentrations
    - Prevents the establishment / replenishment of cccDNA, which produces HBcrAg, HBeAg, and (some of) HBsAg
- 1st generation CAMs in development
  - Demonstrated DNA, RNA reductions (1st MoA)
  - No clear evidence of effects on cccDNA (2nd MoA)

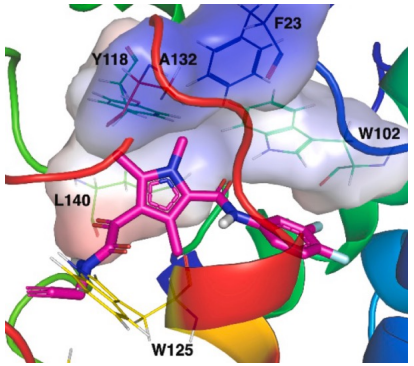


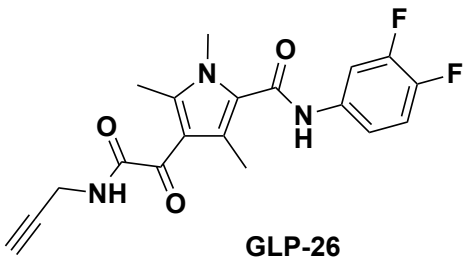
- Observing both mechanisms clinically requires potent compounds with excellent PK properties

Adapted from:: Aligos

EARLY CAM DISCOVERY: GLP-26

- ✓ Proprietary class II CAM
- ✓ Inhibits HBV DNA replication and HBeAg secretion/cccDNA amplification at low-nanomolar concentrations ($EC_{50/90}$ = 3 and 14 nM). No apparent cytotoxicity
- ✓ Long stability (> 24 h) in dog and human plasma
- ✓ Good human liver microsomal stability
- ✓ *Excellent oral bioavailability in mice*
- ✓ *Synergistic antiviral activity in culture with Entecavir (ETV)*
- ✓ In chimeric humanized liver mice:
  - ✓ no toxicity up to 30 mg/kg orally for 10 weeks
  - ✓ 4-log drop in HBV DNA
  - ✓ Decrease in HBsAg and HBeAg post treatment
- ✓ In an HBV nude mouse model bearing HBV transfected AD38 xenografts: 2.3-3 log drop in HBV DNA. Decrease in HBsAg was observed.
- ✓ Favorable PK profile in cynomolgus monkeys



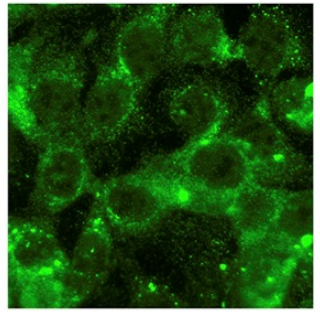


GLP-26

Amblard F, et al. *Antimicrob Agents Chemother*. 2020;64(2):e01701-19.
Hurwitz SJ, et al. *Viruses*. 2021;13(1):114.
Amblard F, et al. *Bioorg Chem*. 2023 Dec;141:106923.

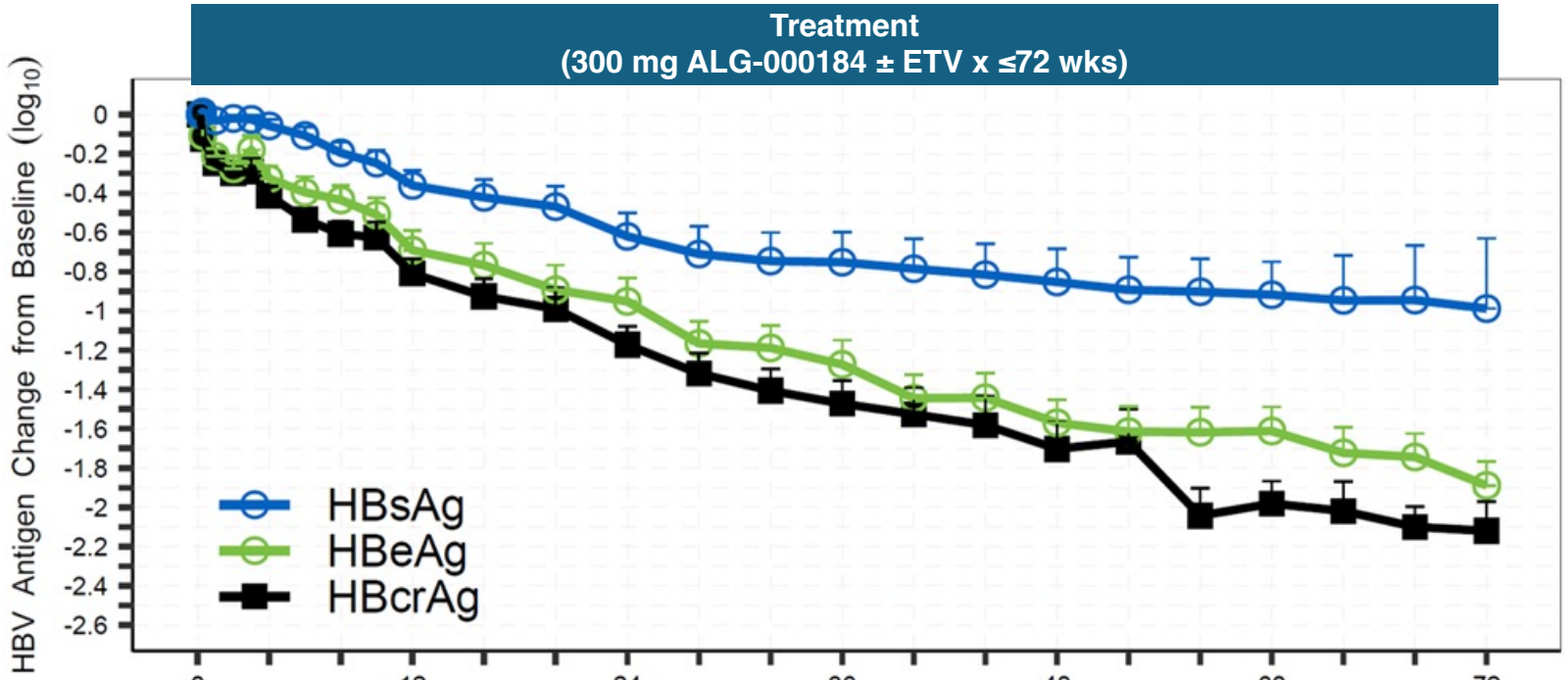
ALG-000184: A UNIQUE POWERFUL HBV CAM

- Design based on GLP-26 – sub-nM inhibition in culture
- Oral dosing with 300 mg ALG-000184 ± ETV x ≤72 weeks in untreated HBeAg+ CHB subjects results in:
  - A favorable safety profile
  - ALG-000184 ± ETV shows greater suppression of HBV DNA/RNA vs. ETV alone (1st MoA)
  - No viral breakthrough when ALG-000184 is given as monotherapy x ≤72 weeks
  - Multi-log reductions in HBsAg, HBeAg and HBcrAg, which may be mediated by ALG-000184 (2nd MoA)
- ALG-000184 may play an important role in enhancing rates of chronic suppression (superior/non-inferior to NAs) and functional cure
- Dosing is ongoing ≤96 week cohorts
- Phase 2 enabling activities planned for 1Q25



EASL 2024: ALG-000184-201

HBsAg, HBeAg and HBcrAg declines in HBeAg+ CHB subjects



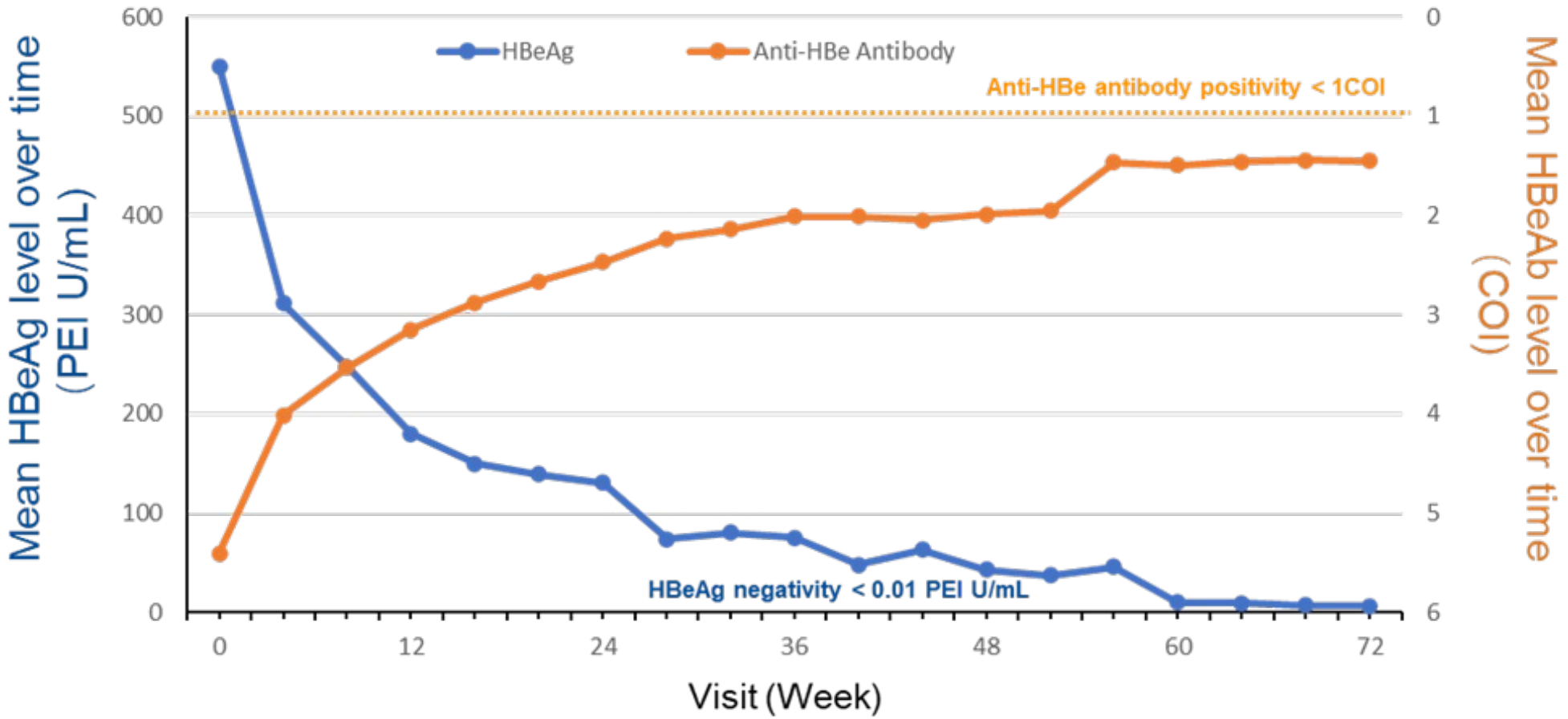
HBsAg	21	19	19	19	17	16	6
HBeAg	21	19	19	19	17	16	6
HBcrAg	21	19	19	19	17	9	5

Graph plots subjects initially randomized to: ALG-000184 + ETV and were compliant (confirmed by PK).
Yuen, M-F. et al., Late Breaker Poster #5028-C, AASLD (2023).
HBcrAg = HBV core Ag

Continued substantial HBsAg, HBeAg, and HBcrAg reductions noted with combo through W72
Max declines: 2.1, 2.6 and 2.7 log_{10} IU/mL, respectively

300 mg ALG-000184 ± ETV vs. ETV (New)

MEAN HBEAG AND ANTI-HBE ANTIBODY LEVEL OVER TIME

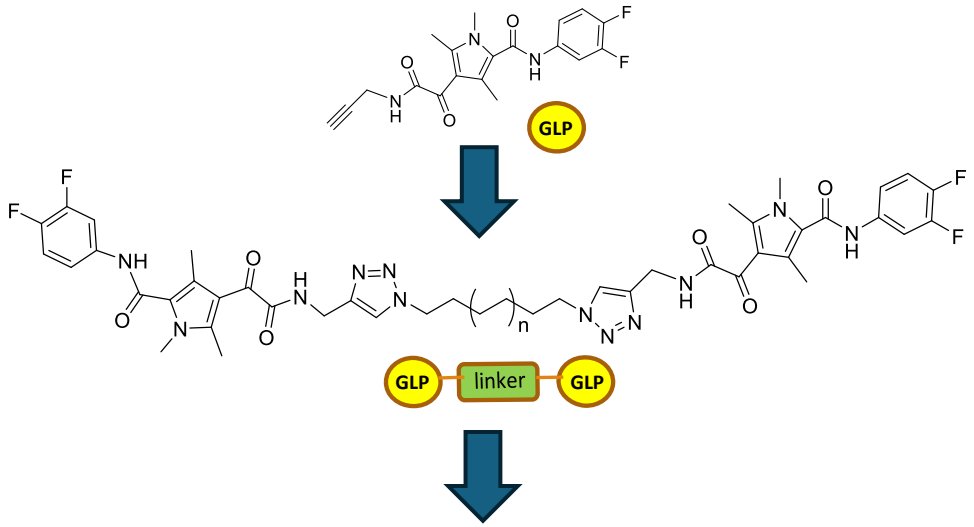


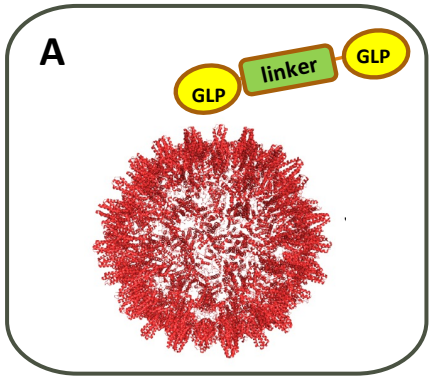
Anti-HBe antibody (HBeAb) level showed positive trend with decline of HBeAg

GLP-26 DIMERS

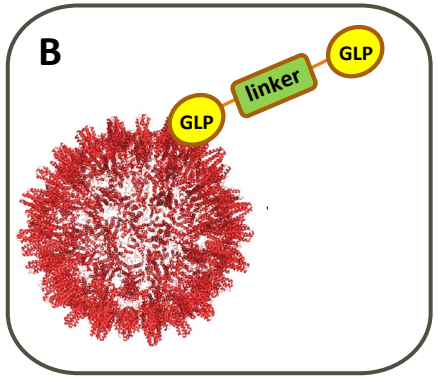
Toward the next generation of HBV CAMs?

GLP-26 DIMERS: HYPOTHESIS
COOPERATIVITY OR MULTIVALENT BINDING

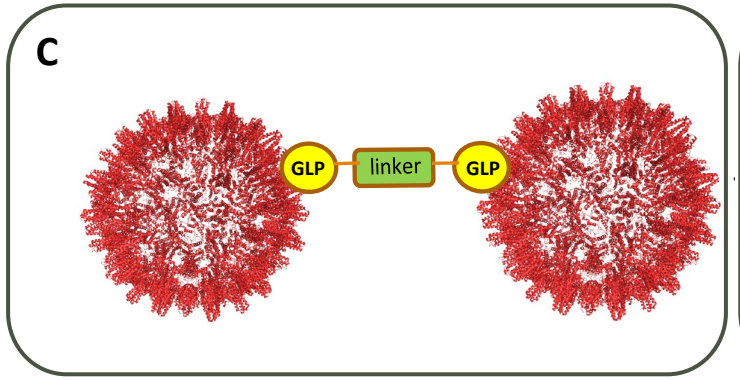




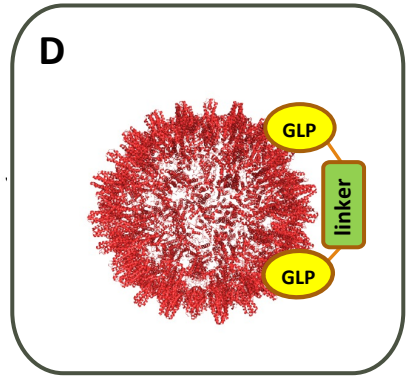
No binding/No activity



Binding similar to GLP-26

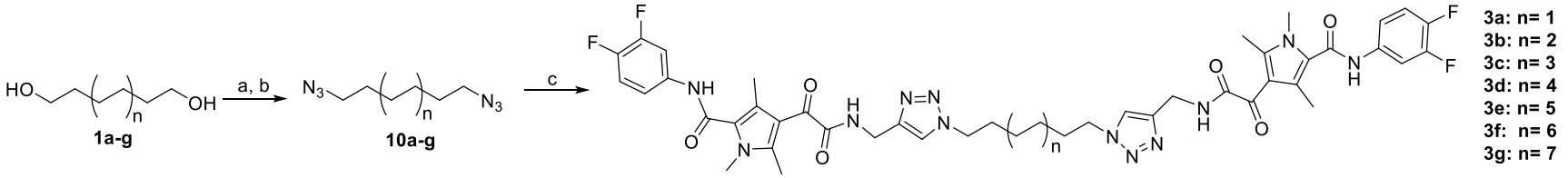


"Inter capsid staple"



"Intra capsid staple"

GLP26 dimers: Synthesis & anti-HBv activity



Synthesis. Reagents and conditions: (a) TSCl, Et_3N, $CHCl_3$, 8 h; (b) NaN_3, DMF, 90 °C-100 °C, overnight, 50%-70% over 2 steps. (c) GLP-26, $CuSO_4.5H_2O$, Na ascorbate, CH_3CN/H_2O, 100 °C, 8 h, 40-60%.

- Clear correlation between the length of the linker and the anti-HBV activity (bell curve)
- Dimers **3d** and **3e** (C_{12} and C_{14} linkers) were 10-80 times more potent than reference compound GLP-26
- Two "warheads" were essential for potent activity

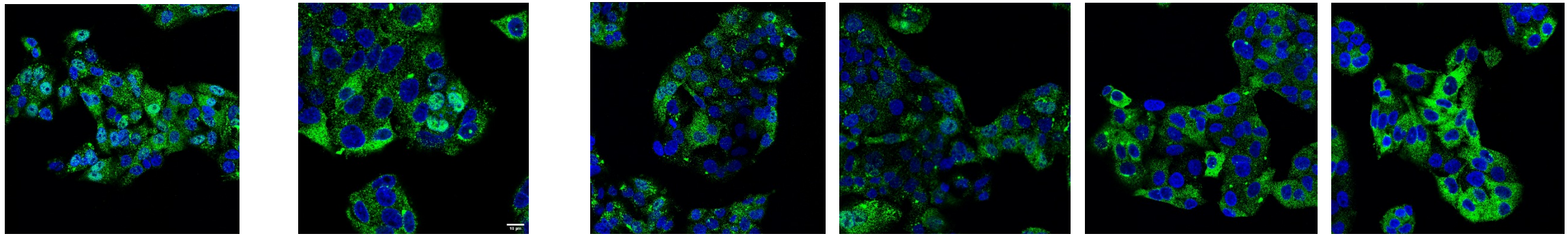
Compound	Anti-HBV activity In HepAD38 (nM)		Cytotoxicity CC_{50} (µM)			
	EC_{50}	EC_{90}	PBM	CEM	Vero	HepG2
3a	285 ± 21.2	>10,000	>100	ND	ND	>100
3b	438 ± 211	5,520 ± 1,520	26.4	>100	>100	>100
3c	4.0 ± 1.0	140 ± 110	>100	N/A	N/A	>100
3d	**0.1 ± 0.4**	**8 ± 1**	**63.4 ± 40.6**	**42.8 ± 0.6**	**>100**	**>100**
3e	**0.1 ± 0.5**	**9 ± 0.2**	**>100**	**ND**	**ND**	**>100**
3f	18 ± 11	130 ± 60	>100	ND	ND	>100
3g	25 ± 24	380 ± 10	>100	ND	ND	>100
GLP-26	8 ± 1.0	70 ± 20	>100	>100	25.0	>100

GLP-26 DIMERS: EFFECT ON CAPSIDS LOCATION (HEPAD-38)

Fluorescence microscopy

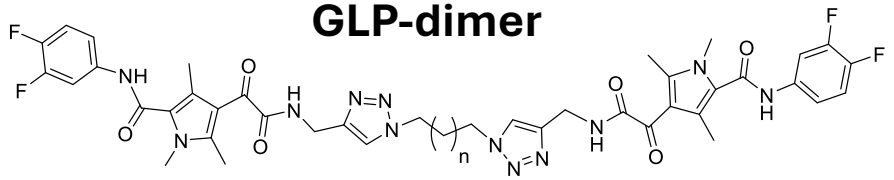
Concentration (μM)

5 | 0.001 | 0.01 | 0.1 | 1.0



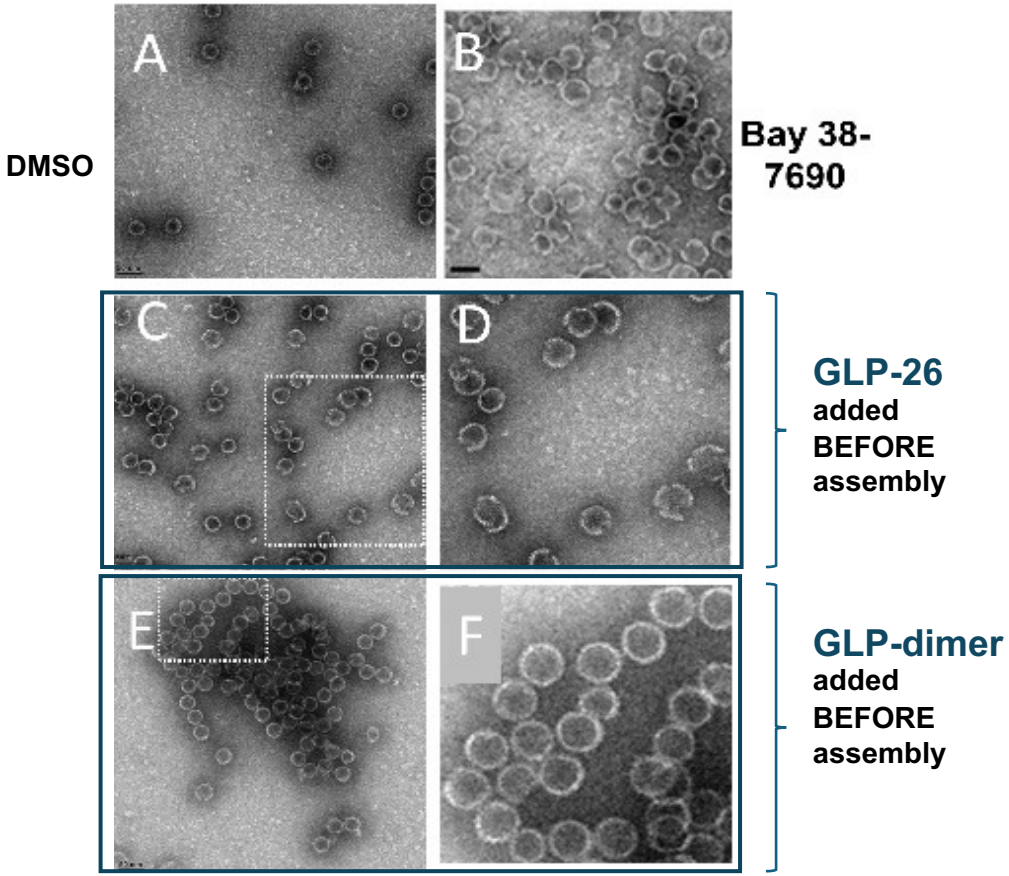
DMSO | GLP-26 | GLP-dimer

Nucleus
Capsid



Capsid accumulation outside the nucleus

GLP-26 DIMERS: EFFECT ON CAPSIDS MORPHOLOGY BEFORE ASSEMBLY



Differences between GLP-26 and GLP-dimer:

- No presence of aberrant structures
- Formation of aggregates

GLP-26 DIMERS: EFFECT ON CAPSIDS MORPHOLOGY AFTER ASSEMBLY

Determination of the effect of CAM type on the size of capsid aggregates using Dynamic light scattering (DLS)

DLS Experiments	HBV Capsid + DMSO	HBV Capsid + 20 µM GLP-26	HBV Capsid + 20 µM $(GLP\text{-}26)_2$
Radius (nm)	20.7 ± 1.2	28.4 ± 0.8	214 ± 12
% PD	28.5 ± 5.8	26.6 ± 5.9	Multimodal

Dynamic light scattering (DLS) experiments for HBV capsid/GLP-26 complexes. % PD = % Polydispersity.

Incubation of 10 µM **assembled** HBV capsids with 1% DMSO control, 20 µM GLP-26, or 20 µM GLP-dimer for 30 mins

GLP-dimer markedly increased the radius of HBV cores (>200 nm), consistent with the aggregation observed by EM

CONCLUSIONS:
CAMS AND GLP-26 DIMERS

Certain CAMs have sub-nM potency and high safety profiles in vitro and in humans.

Prevent the establishment / replenishment of cccDNA, which produces HBcrAg, HBeAg, and (some of) HBsAg

Super rapid declines of HBV DNA (> 6 logs) in humans as low as 10 mg/kg (po)

Significant reductions (> 2 logs) in HBs, HBe and HBcrAg. No seroconversion yet after 72 weeks

Effective in reducing or eliminating HBeAg - producing anti-HBeAg in humans (1st indication?)

GLP-26 dimers appear to have a different mechanism compared to certain monomers. Based on novel MoI, we can presume this is a new class of CAMs – named D-CAMs

Merci - Thank you – Happy 4th of July