Novel hepatitis B virus gene expression inhibitor in clinical development

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Background and Aims

Currently used therapies for chronic hepatitis B virus (HBV) infection seldom achieve hepatitis B serum surface antigen (HBsAg) loss. However, HBsAg loss is considered one of the ultimate endpoints, leading to restoration of the immune function and achieving virus control. GST-HG131 is a small-molecule inhibitor of hepatitis B virus (HBV) gene expression that significantly reduces the levels of hepatitis B surface antigen (HBsAg) and HBV DNA in vitro and in HBV mouse models. Here we report preclinical characterization of GST-HG131, which is currently under phase I clinical evaluation.

Methods

Effects on HBsAg, hepatitis B e-antigen (HBeAg), HBV DNA and RNA were determined in HepG2.2.15 cells and in primary human hepatocytes (PHH), using ELISA, qPCR and northern blotting. Terminal nucleotidyl transferase 4B (TENT4B) binding and inhibition assays were done as previously described (Nagpal et al, Cell Stem Cell 2020, 26(6):896-909). The in vivo antiviral efficacy was assessed in the AAV-HBV mouse model.

Results

In HepG2 2.15 cells, stably replicating HBV, GST-HG131 potently inhibited secretion of HBsAg and HBV DNA in the absence of cytotoxicity (Table 1). The antiviral effects of GST-HG131 were not significantly affected (EC_{50} fold shift = 1.5) by up to 50% of human serum. Secretion of both HBsAg and HBeAg from primary human hepatocytes was also strongly inhibited, with the EC_{50} values of 18 and 19 nM, respectively). No significant cytotoxicity was observed in all nine tested human cell lines and primary cells (CC_{50}>10 μM).

Table 1. Antiviral activity and cytotoxicity of GST-HG131, follow-on compound GST-HG121 and reference drugs in HepG.2.2.15 cells.

Compound	Secreted HBV DNA EC_{50} (nM)	Secreted HBsAg EC_{50} (nM)	CC_{50} (μM)
GST-HG131	3.30	3.40	>100
GST-HG121	0.78	0.91	>100
Entecavir	0.10	>20	>1
Tenofovir	6.2	n/d	>10

The antiviral activity of GST-HG131 was HBV-specific, as other representative DNA and RNA (plus- or minus-strand) viruses tested were not inhibited in vitro (Table 2).

Table 2. Antiviral spectrum of GST-HG131. The mean values are shown.

Virus strain / Cells	GST-HG131		Control		
	EC_{50}, μM	CC_{50}, μM	Drug	EC_{50}, μM	CC_{50}, μM
HCV replicon GT1b / Huh7	>50	>50	GS-7977	0.050	>10
HSV-1 GHSV-UL46 / Vero	>50	>50	Acyclovir	1.28	>100
Flu A/PR/8/34 (H1N1) / MDCK	>23	23	VX-787	0.002	>0.1

Results (cont.)

In the drug combination studies with GST-HG131, GST-HG141 (novel HBV capsid assembly modulator, Abstract #1775) or Entecavir, additive antiviral effects were observed (Table 3 and Fig. 1). The effects on HBsAg and HBV DNA secretion by cultured HepG.2.215 cells was measured in these drug combination studies.

Table 3. Drug combination studies with GST-HG131, GST-HG121, GST-HG141 and Entecavir in HepG.2.2.15 cells. MacSynergyII indices are shown.

Compound A	Compound B	Synergy volume (95%)	Antagonism volume (95%)	Combination effect	Cell viability, %
GST-HG131	Entecavir	0	-7.85	Additive	~100%
GST-HG121	Entecavir	8.86	-20.39	Additive	~100%
GST-HG141	GST-HG121	2.77	-7.31	Additive	~100%
GST-HG141	GST-HG131	7.39	0	Additive	~100%

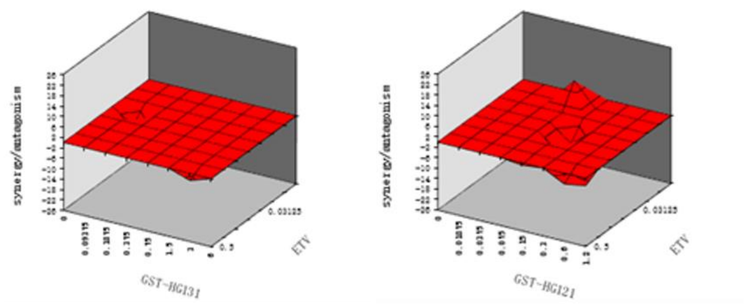

Fig. 1. Representative MacSynergyII plots of drug combination effects with GST-HG131, GST-HG121 and Entecavir on secretion of HBV DNA and HBsAg by cultured HepG.2.2.15 cells.

In cell culture experiments, GST-HG131 significantly reduced levels of HBV 2.4/2.1 kb RNAs, as well as 3.5 kb pre-genomic RNA, albeit to a lesser extent, in a concentration-dependent manner (Fig. 2).

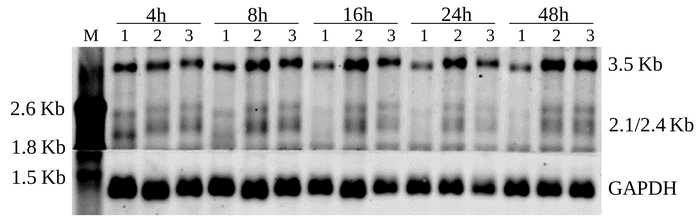

Fig. 2. Effect of 500 nM GST-HG131 (1), 50 nM Entecavir (2) or DMSO (3) on HBV RNAs in the HepG2.2.15 cells (Northern blot analyses).

Results (cont.)

GST-HG131 and GST-HG121, similarly to RG7834 (a known PAPD5 inhibitor), bind recombinant TENT4B (Table 4), and potently inhibit its enzymatic function *in vitro*, disrupting polyadenylation of an RNA oligonucleotide Fig. 3).

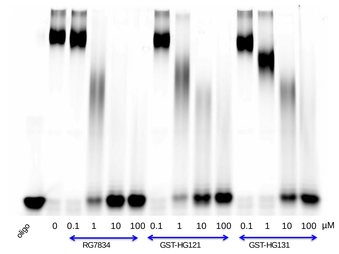

Table 4. Drug binding to rPAPD5 in vitro by the differential scanning fluorimetry (DSF) assay.

Compound	ΔTm (100 μM)
RG7834	23.5
GST-HG121	21.4
GST-HG131	20.8

Fig. 3. Inhibition of purified recombinant PAPD5 by GST-HG131, GST-HG121, and RG7834 *in vitro* (oligonucleotide polyadenylation assay).

In the AAV/HBV model, GST-HG131 demonstrated a strong dose-dependent reduction in serum HBsAg (~1 log_{10}), following 28 days of dosing (Fig. 4). GST-HG131 was well-tolerated, no significant effect on animal body weight was observed. No significant inhibition of major cytochrome P450 (CYP) isozymes (1A2/2C9/2C19/2D6/3A4) was observed *in vitro* (IC_{50} > 50 μM), suggesting a low GST-HG131 potential for drug-drug interactions.

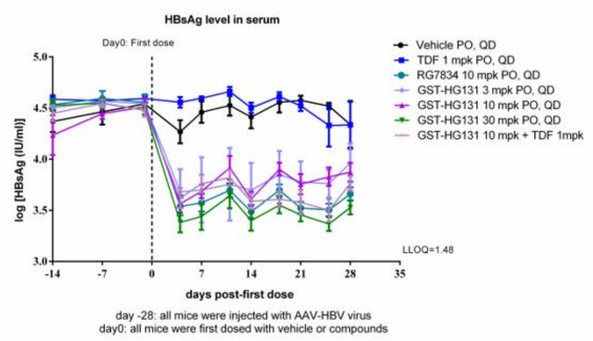

Fig. 4. Effect of GST-HG131 and Tenofovir Dipivoxil (TDF) on the serum HBsAg levels in the mouse AAV model.

Conclusions

GST-HG131 is a novel, orally-bioavailable inhibitor of HBV gene expression. It has an excellent antiviral potency in vitro, efficacy in vivo, and is well-tolerated in rodents. GST-HG131 is additive with nucleos(t)ide HBV inhibitors, as well as with HBV capsid assembly modulator, and has a low potential for drug-drug interactions. The molecular mechanism of GST-HG131 antiviral action, including strong inhibition of HBsAg secretion, involves binding to and inhibition of TENT4B, leading to shortening of poly-adenylation tail of HBV mRNAs and thus accelerating its degradation. Further development of GST-HG131 for chronic HBV infections is warranted. Currently GST-HG131 is undergoing phase I clinical evaluation.