Discovery and preclinical profile of VNRX-9945, a potent, broadly active core protein inhibitor for the treatment of chronic hepatitis B virus (HBV) infection

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INTRODUCTION

- Individuals with chronic HBV infection (CHBV) are at risk for progressive liver diseases including fibrosis, cirrhosis and hepatocellular carcinoma (HCC)[1]
- Combinations of direct-acting antivirals and other agents that block expression of viral antigens or stimulate immune responses are likely required to achieve a functional cure for CHBV
  - ❖ Defined as a sustained loss of HBsAg
- Core protein allosteric modulators (CpAM) represent an attractive class of direct-acting antivirals that block the formation of new virus particles and cccDNA *in vitro*[2]
- Here we report on the discovery and preclinical profile of VNRX-9945, a potent and broadly active CpAM that has entered clinical development for the treatment of CHBV

AIM

- To investigate the preclinical antiviral, pharmacokinetic and safety profile of VNRX-9945 and position the compound for first-in-human testing in healthy volunteers and CHBV patients

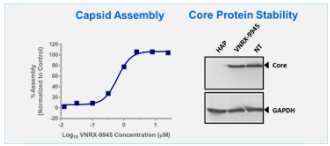
METHOD

- The *in vitro* antiviral properties of VNRX-9945 were determined in both HBV expressing human hepatoma cell lines or in transiently transfected HepG2 cells (genotypes and HBV variants) and in HBV infected primary human hepatocytes (PHHs)
  - ❖ Various concentrations of VNRX-9945 were prepared and evaluated for antiviral activity
  - ❖ HBV DNA and RNA in the cell culture medium was quantified using quantitative polymerase chain reaction (qPCR)
- The *in vivo* antiviral activity of VNRX-9945 was determined in the AAV-HBV mouse model of HBV infection following 8 weeks of continuous dosing
  - ❖ HBV DNA, pgRNA, HBeAg, HBsAg, ALT and body weights were monitored throughout the duration
- Safety and ADME properties of VNRX-9945 were extensively studied across multiple *in vitro* systems
- The pharmacokinetics, safety and tolerability of the compound were studied in rats and cynomolgus monkeys following single and repeated doses of VNRX-9945 for up to 28-days

RESULTS

Mechanism of action

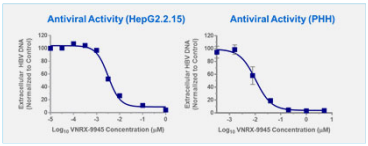
- VNRX-9945 induces the assembly of capsids from purified core protein dimers (EC_{50}= 0.75 µM)
- HBV core protein remains stable in the presence of VNRX-9945 suggesting that empty capsids are formed



HAP= Heteroaryl-pyrimidine; NT= Non-treated; GAPDH= Glyceradehyde 3-phosphate dehydrogenase

Antiviral activity

- VNRX-9945 exhibits potent antiviral activity in HepG2.2.15 cells: EC_{50}= 2.3 ±0.6 nM (EC_{90}= 21 nM)
- Addition of 40% human serum to the culture medium resulted in a 5-10-fold shift in EC_{50}
- VNRX-9945 exhibits potent antiviral activity in HBV infected primary human hepatocytes (PHH) EC_{50}= 10 nM



- Addition of VNRX-9945 to PHH at the time of infection blocks packaging of pgRNA into capsids EC_{50}= 16 nM
- VNRX-9945 blocks the formation cccDNA in naïve hepatocytes as determined by biomarker expression (i.e. HBsAg and HBeAg) and cccDNA levels (estimated EC_{50} ~100 nM)

Cmpd	Primary Human Hepatocytes				
	HBV DNA (EC_{50}; nM)	HBV pgRNA (EC_{50}; nM)	HBsAg (EC_{50}; nM)	HBeAg (EC_{50}; nM)	cccDNA (EC_{50}; nM)
VNRX-9945	10	16	90	74	100
ETV	12	>1000	>1000	>1000	>1000

Spectrum of antiviral activity

- VNRX-9945 exhibits broad antiviral activity against genotypes A-H (N=24 strains)

Cmpd	Genotypes (EC50; nM)							
	A	B	C	D	E	F	G	H
VNRX-9945	3 ± 2.3	3 ± 1.2	3 ± 0.7	10 ± 7.1	31 ± 27	7 ± 0.8	8 ± 2	14 ± 0.1
ETV	1 ± 0.9	1 ± 0.2	1 ± 0.2	2 ± 0.6	2 ± 0.4	2 ± 0.8	2 ± 0.6	2 ± 0.4

ETV= entecavir

- VNRX-9945 exhibits broad antiviral activity against HBV variants with amino acid substitutions in the CpAM binding site

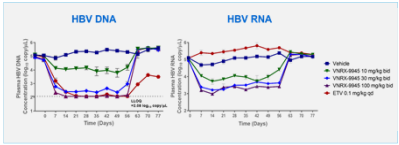
Change in Susceptibility to VNRX-9945	HBV Core Protein Variants (Genotype D Genetic Background)
Minimal (1-3-fold)	F23L, P25S, L30F, T33S, L37F, I105F/V/L, T109I/M/L, Y118F, L140I
Intermediate (10-25-fold)	F23Y, V124F
High (>50-fold)	T33N, F110Y

Combination with nucleos(t)ide reverse transcriptase inhibitors (NrtIs)

- VNRX-9945 demonstrated additive antiviral activity in HepAD38 cells when combined with tenofovir or entecavir[3]
- HBV variants that confer resistance to NrtIs remained fully susceptible to VNRX-9945
- HBV variants that confer reduced susceptibility to VNRX-9945 remained fully susceptible to ETV

Antiviral activity in the AAV-HBV mouse model

- AAV-HBV mice were administered oral doses of 10, 30, or 100 mg/kg/dose VNRX-9945 twice daily for 8 weeks
- Significant dose-dependent reductions in circulating HBV DNA and RNA from baseline were observed



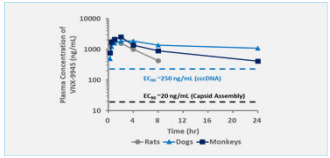
- No changes in HBsAg or HBeAg levels were noted

Dose (mg/kg)	C_{trough}/EC_{50}	HBV DNA from Baseline on Day 56 (log_{10} copies/µL)	HBV RNA from Baseline on Day 56 (log_{10} copies/µL)
10	2X	1.11 (p<0.01)	0.97 (p<0.01)
30	5X	2.32 (p<0.01)	1.75 (p<0.01)
100	15X	3.14 (p<0.01)	1.97 (p<0.01)

- Plasma trough concentrations of VNRX-9945 correlated with antiviral activity in the mouse model
- No changes in body weights or ALT levels were observed suggesting that VNRX-9945 was well tolerated in mice for up to 8 weeks

Pharmacokinetics in preclinical species

- PK was evaluated in rats, dogs and cynomolgus monkeys following administration of a single 10 mg/kg oral dose of VNRX-9945 formulated as a suspension
- Plasma concentrations (C_{24h}) maintained levels above the EC_{90} for both capsid assembly and cccDNA formation in dogs and monkeys



Species	IV (3mg/kg)					PO (10mg/kg)				
	$t_{1/2}$ (hr)	C_0 (ng/mL)	AUC_{inf} (hr*ng/mL)	Vss (L/kg)	CL (mL/min/kg)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{inf} (hr*ng/mL)	%F*
Rats	1.4	6403	5106	1.1	16	3.7	0.8	2043	11130	86
Dogs	11	4247	41623	0.9	1.2	11	3.3	1937	50404	37
Monkeys	3.9	13577	19041	0.4	2.6	11	1.7	2577	32732	36

Oral bioavailability (%F) of VNRX-9945 was greatly improved by formulating as a solution or spray dried dispersion

Non-clinical safety summary

- Doses of VNRX-9945 up to a maximum feasible dose of 600 mg/kg/day were evaluated in rats and monkeys for 28-days
- VNRX-9945 was well tolerated with no target organ toxicity identified in either rats or monkeys
- No off-target activity was identified against a diverse panel of enzymes and receptors
- VNRX-9945 was not mutagenic in the bacterial reverse mutation assay and did not generate micronuclei in mammalian lymphocytes either *in vitro* or *in vivo*
- Safety pharmacology studies did not reveal any meaningful changes to cardiovascular, respiratory or CNS parameters
- VNRX-9945 exhibited minimal inhibition of the hERG channel current with an IC_{50} > 100 µM
- No inhibition or induction of major CYP isoforms and minimal inhibition of human transporter assay suggest a low potential for drug-drug interactions

CONCLUSIONS

- VNRX-9945 blocks normal capsid assembly and exhibits potent antiviral activity against HBV both *in vitro* and *in vivo*
- Demonstrates broad activity against multiple genotypes and capsid polymorphisms *in vitro*
- Pharmacokinetics in non-rodent species are consistent with once daily dosing in humans
- Safe and well tolerated in 28-day GLP toxicology studies conducted in rats and cynomolgus monkeys
- Possesses a preclinical profile that complements NrtIs and other agents under development for CHBV
- VNRX-9945 has been advanced into Phase 1 first-in-human testing to evaluate safety and pharmacokinetics in healthy volunteers

ACKNOWLEDGEMENTS

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