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Preclinical Properties of the Novel HCV NS3 Protease Inhibitor GS-9451

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Introduction

- GS-9451 is a novel NS3 protease inhibitor in clinical development for chronic genotype 1 HCV infections
- Recent Phase 1 clinical studies indicated that GS-9451
  - has a long half-life in HCV patients (14 – 17 hours)[1]
  - elicits 3.2 – 3.6 $\log_{10}$ reductions in HCV viremia during 3 day monotherapy studies at doses of 200 – 400 mg QD[1]
  - is generally well-tolerated during short-term dosing[1]
- GS-9451 is currently in Phase 2 studies in combination with tegobuvir (GS-9190), pegIFN and ribavirin

Objectives

- To profile the in vitro antiviral properties of GS-9451 including potency, selectivity, cross-resistance and activity in drug combination assays
- To profile the metabolic and pharmacokinetic properties of GS-9451 in vitro and in vivo in preclinical species

Methods

- Recombinant NS3 protease activity (in the presence of synthetic NS4A peptide co-factor) was measured by monitoring cleavage of a fluorescent substrate
- Mammalian protease activity was determined using commercially available proteases and corresponding fluorescent substrates
- Antiviral potency and cytotoxicity were determined in a panel of replicon cells after three day treatments with compounds
- Cross-resistance was assessed in transient transfection assays using replicons encoding NS3, NS5A or NS5B inhibitor resistance mutations
- Antiviral combination assays were performed in 1b replicon cells and data were analyzed using MacSynergy II
- Metabolic stability was assessed in hepatic microsomes from rat, dog, cynomolgus monkey and human
- Plasma pharmacokinetics were assessed in Sprague Dawley rats, Beagle dogs and Cynomolgus monkeys after oral and intravenous (infusion) administration
- Excretion of GS-9451 was assessed in bile-duct cannulated rats by collecting bile and urine at various time points and measuring the amount of parent compound

Results

Table 1. GS-9451 is a Potent Inhibitor of Genotype 1 NS3/4A Protease in Biochemical Assays

Compound	K_i (nM)[a]	IC_{50} (nM)[b]			
	Genotype 1b	Genotype 1a	Genotype 1b	Genotype 2a	Genotype 3a
GS-9451	0.41 ± 0.12	2.1 ± 0.2	3.2 ± 0.7	118 ± 21	958 ± 73
VX-950	7.9 ± 3.1	99 ± 4.6	188 ± 80	50 ± 4.1	228 ± 76
BILN-2061	0.04 ± 0.02	0.5 ± 0.1	1.0 ± 0.0	10 ± 5.9	40 ± 20

[a] 50 pM of NS3 protease was pre-incubated with compounds for 1 hour before addition of substrate during the K_i assay
[b] 2 nM of NS3 protease was pre-incubated with compounds for 10 minutes before addition of substrate during the IC_{50} assay

Results

Table 2. GS-9451 is Highly Selective for HCV NS3 Protease Versus Mammalian Proteases

Compound	Selectivity (Ratio of Mammalian Protease K_i to 1b NS3 Protease K_i)				
	Porcine Pancreatic Elastase (serine)	Human Leukocyte Elastase (serine)	Human Proteinase 3 (serine)	Cathepsin D (aspartic)	Cathepsin L (cysteine)
GS-9451	510,000	50,000	320,000	90,000	400,000
BILN-2061	5,670,000	170,000	5,440,000	980,000	4,390,000
VX-950	7	88	43	3,132	1,491

Table 3. GS-9451 Exhibits Potent Antiviral Activity in Genotype 1 HCV Replicon Cell Lines

Compound	EC_{50} (nM)					CC_{50} (nM)
	GT 1b replicon cell lines			GT 1a replicon cell line	GT 2a replicon cell line	GT 1b replicon cell line
	Huh-luc	GFP1b-7	SL3	HSG-57	2aLuc-25	Huh-luc
GS-9451	7.5	7.2	7.2	22	316	>50,000
BILN-2061	0.7	0.5	0.9	13	116	>50,000
VX-950	388	177	86	737	346	>50,000

Figure 1. GS-9451 Shows Additive to Strongly Synergistic Antiviral Activity in Combination with Other HCV Antivirals

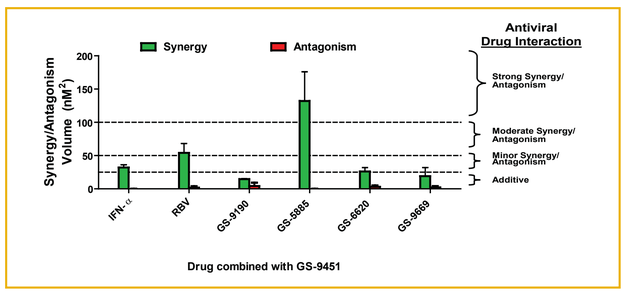

Figure 2. GS-9451 Retains Full Activity Against NS5B and NS5A Resistance Mutations

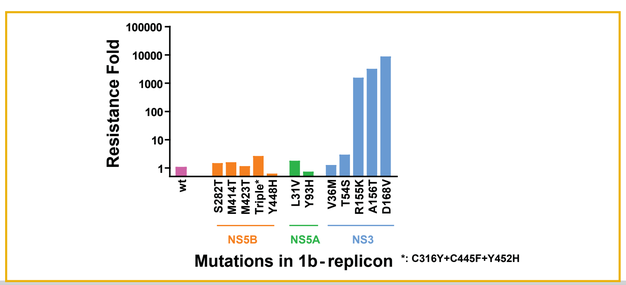

Table 4. Rate of Metabolism of GS-9451 in Hepatic Microsomes

Microsome Species	$T_{½}$ (min)	Predicted Hepatic Clearance (L/hr/kg)	Predicted Hepatic Extraction (%)
Rat	>395	<0.40	<9.4
Dog	>395	<0.12	<9.5
Monkey	79.1	0.59	37.2
Human	>395	<0.17	<12.7

Figure 3. IV PK Profile of GS-9451 in Rats, Dogs and Monkeys (30 minute infusion at 1 mg/kg)

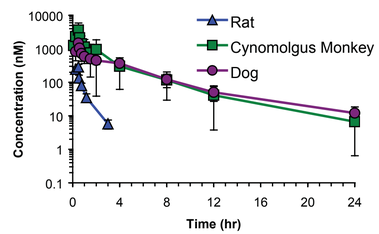

Table 5. Mean Plasma PK Parameters for GS-9451 Following IV Infusion into Rats, Dogs, or Monkeys at 1 mg/kg

Species	$AUC_{(0-\infty)}$ (nM•hr)	CL (L/hr/kg)	V_{ss} (L/kg)	$T_{½}$ (hr)	MRT (hr)
Rat	220 ± 44.3	4.04 ± 0.83	2.32 ± 0.30	0.62 ± 0.05	0.58 ± 0.05
Dog	3,845 ± 1055	0.26 ± 0.08	1.15 ± 0.16	4.2 ± 0.4	4.5 ± 0.7
Cynomolgus Monkey	5,536 ± 4107	0.29 ± 0.15	0.76 ± 0.36	3.9 ± 0.2	2.8 ± 0.4

Figure 4. Oral PK Profile of GS-9451 in Rats (10 mg/kg), Dogs (4 mg/kg) and Monkeys (5 mg/kg)

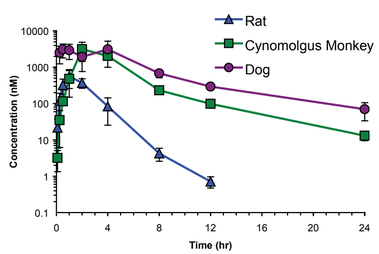

Table 6. Mean Plasma PK Parameters for GS-9451 Following Oral Administration to Rats (10 mg/kg), Dogs (4 mg/kg) or Monkeys (5 mg/kg)

Species	T_{max} (hr)	C_{max} (nM)	$T_{1/2}$ (hr)	AUC(0-∞) (nM•hr)	%F
Rat	1.3 ± 0.6	576 ± 278	1.2 ± 0.3	1,410 ± 185	61.9 ± 8.1
Dog	1.5 ± 2.2	3,937 ± 1,548	5.0 ± 1.1	22,304 ± 10,172	142 ± 65
Cynomolgus Monkey	2.7 ± 1.2	3,467 ± 1,208	3.9 ± 0.3	13,195 ± 2,070	49 ± 9

Table 7. Cumulative % of Total Dose Recovered as GS-9451 in Bile and Urine Following IV Infusion at 1 or 10 mg/kg in Bile-duct Cannulated Rats

Collection Period (hr)	1 mg/kg		10 mg/kg	
	Bile	Urine	Bile	Urine
0-3	57 ± 24%	-	45 ± 23%	-
0-6	59 ± 26%	-	51 ± 24%	-
0-12	61 ± 28%	<0.1%	52 ± 24%	<0.1%
0-24	61 ± 28%	<0.1%	53 ± 24%	<0.1%

Summary

- GS-9451 is highly potent against GT1 NS3 protease in biochemical assays (Table 1) with significant selectivity versus all tested mammalian proteases (Table 2)
- Similarly, GS-9451 is potent and selective in a panel of GT1 replicon cell lines (Table 3)
- GS-9451 demonstrated additive to strongly synergistic antiviral activity when combined with IFN-α, ribavirin, NS5A inhibitors or nuc and non-nuc NS5B inhibitors (Fig 1)
- The NS3 protease mutations R155K, A156T and D168V were cross-resistant to GS-9451. In contrast, GS-9451 retained activity against the NS3 mutations V36M and T54S and against a panel of NS5A and NS5B resistance mutations (Fig 2)
- GS-9451 was stable in rat, dog and human hepatic microsomes (Table 4)
- In preclinical species, GS-9451 had IV half-lives of 0.6 hours in rats and 4 hours in dogs and monkeys (Fig 3, Table 5). Bioavailability was high in all species (F = 49% to complete, Fig 4, Table 6)
- The primary mechanism of clearance appears to be through biliary secretion of unmodified GS-9451 (Table 7)

Conclusions

- **GS-9451 is a potent and selective inhibitor of GT1 NS3 protease with a favorable pharmocokinetic profile**
  - **these results are consistent with the potent antiviral activity observed in Phase 1 studies[1]**
- **GS-9451 showed additive to synergistic antiviral activity when combined with all other tested classes of HCV inhibitors; these results support**
  - **the ongoing Phase 2b investigation of GS-9451 with tegobuvir, pegIFN and ribavirin**
  - **future studies combining GS-9451 with GS-5885 (an NS5A inhibitor), GS-6620 (a nucleoside NS5B inhibitor) and GS-9669 (a site II non-nucleoside NS5B inhibitor)**

References

1. E. Lawitz; J. M. Hill; T. C. Marbury; M. Rodriguez-Torres; M. P. DeMicco; J. Quesada; P. Shaw; S. C. Gordon; M. J. Shelton; D. H. Coombs; J. Zong; A. Bae; K. A. Wong; H. Mo; E. Mondou; K. R. Hirsch; W. E. Delaney. Three-Day, Dose-Ranging Study of the HCV NS3 Protease Inhibitor GS-9451. Hepatology Vol 52, S1 p714A