Poster Number

664

46th Annual Meeting of the
European Association for the Study of the Liver
March 30 - April 3, 2011
Berlin, Germany

A Phase-I, Randomized, Double-Blind, Placebo-Controlled Study To Evaluate The Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Single Escalating Oral Doses of GS-9620 In Healthy Subjects

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Introduction

- HCV and HBV infections have been shown to be amenable to therapy with Interferon-α
  - Clinical "cure" rates with subcutaneous administration of Pegylated Interferons (PEG) are < 10% for HBV and only ~50% in HCV genotype 1 treated with PEG + Ribavirin (RBV)
  - Unfortunately, many patients who might benefit from interferon are unwilling or unable to tolerate its side effects.
- Toll Like Receptor (TLR)-7 has been shown to be a sensor of viral nucleic acid, primarily expressed by plasmacytoid dendritic cells (1,2,3)
- TLR-7 agonists induce anti-viral signaling pathways (including interferons) in multiple animal species.
- TLR-7 agonists may serve as a component of therapy for viral hepatitis (4, 5)

Background

- GS-9620 is an oral small-molecule TLR-7 agonist
- Preclinical studies have demonstrated that GS-9620 has potent pharmacologic activity in vitro and in vivo (Abstract 1176)
- GS-9620 was found to have lasting effects on both HBV viral loads and viral antigens (S and E) in pre-clinical studies in infected chimpanzees (Abstract 1771) and woodchucks (Abstract 1790)

Objective

- The objective of Study GS-US-243-0101 was to investigate the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of single ascending doses of GS-9620 in healthy volunteers

Methods/Study Design

- In this double-blind, placebo-controlled study of 75 healthy human volunteers, single ascending doses of 0.3, 1, 2, 4, 6, 8 and 12 mg GS-9620 were administered in seven fasted and three food effect cohorts in a 6:2 ratio, (GS-9620:placebo)
- Patients were sequestered for three days following study drug administration, and returned for follow up on Days 5 and 14
- Plasma PK profiles were evaluated, and PD were assessed by Luminex evaluation of 28 serum cytokines, quantitative evaluation of interferon-stimulated genes ISGs (MxA, OAS, ISG15) in PBMCs, and flow cytometry of circulating leukocytes (CD4, CD8 T cells, NK cells, and B cells)
- IL28B genotyping of the rs12979860 SNP was done using the ABI TaqMan allelic discrimination kit and the ABI7900HT Sequence Detection System (Applied Biosystems). A custom probe was designed by Applied Biosystems to genotype all samples

Table 1a. Demographics and Baseline Characteristics (Cohorts 1-7)

	Cohort 1 (0.3 mg) N=8	Cohort 2 (1 mg) N=8	Cohort 3 (2 mg) N=8	Cohort 4 (4 mg) N=8	Cohort 5 (6 mg) N=8	Cohort 6 (8 mg) N=8	Cohort 7 (12 mg) N=8
Age (SD)	32 (10.3)	33 (8.1)	32 (6.8)	30 (9.5)	31 (10.1)	34 (10.6)	27 (6.9)
Sex: Male	5 (62.5%)	6 (75.0%)	6 (75.0%)	8 (100.0%)	7 (87.5%)	6 (75.0%)	6 (75.0%)
Ethnicity: NH[a]	8 (100.0%)	8 (100.0%)	8 (100.0%)	8 (100.0%)	8 (100.0%)	8 (100.0%)	8 (100.0%)
Race : White Black	4 (50.0%) 4 (50.0%)	5 (62.5%) 3 (37.5%)	5 (62.5%) 3 (37.5%)	7 (87.5%) 1 (12.5%)	7 (87.5%) 1 (12.5%)	7 (87.5%) 1 (12.5%)	6 (75.0%) 2 (25.0%)
BMI (SD)	25.7 (2.5)	26.0 (2.4)	26.6 (1.8)	24.8 (2.7)	25.2 (2.8)	24.8 (3.1)	23.6 (1.9)
Cr_{Cl}[b](SD)	99.1 (13.8)	91.8 (9.1)	95.0 (12.8)	103 (15.1)	102 (17.9)	102 (15.9)	103 (14.6)
IL28B (n=6): CC Non-CC Missing	 2 (33.3%) 3 (50.0%) 1 (16.7%)	 2 (33.3%) 4 (66.7%)	 3 (50.0%) 3 (50.0%)	 2 (33.3%) 4 (66.7%)	 4 (66.7%) 2 (33.3%)	 2 (33.3%) 4 (66.7%)	 1 (16.7%) 5 (83.3%)

a. Non-Hispanic; b. CrCl: Creatinine clearance; SD: Standard Deviation

Table 1b. Demographics and Baseline Characteristics (Cohorts 6a, 8, 9)

	Cohort 6a (8 mg HFM) N=8	Cohort 8 (8 mg MFM) N=8	Cohort 9 (8 mg Post HFM) N=8
Age (SD)	30 (8.2)	26 (6.6)	27 (7.0)
Sex: Male	7 (87.55)	4 (50.0%)	5 (62.5%)
Ethnicity: NHa	8 (100.0%)	7 (87.5%)	6 (75.0%)
Race : White Black	7 (87.5%) 1 (12.5%)	8 (100.0%) 0	7 (87.5%) 1 (12.5%)
BMI (SD)	24.5 (2.7)	26.4 (2.3)	25.8 (3.2)
CrClb(SD)	105 (18.0)	102 (11.1)	99.7 (16.9)
IL28B (n=6): CC Non-CC	 2 (33.3%) 4 (66.7%)	 2 (33.3%) 4 (66.7%)	 3 (50.0%) 3 (50.0%)

MFM, moderate-fat meal with 27% of calories from fat

Results

Safety Results

- GS-9620 was safe and well tolerated with single ascending doses through 12 mg
- There were no serious adverse events (SAEs) or individual subject discontinuations due to AEs or laboratory abnormalities
- A total of 49 treatment-emergent AEs in 15 subjects were reported as study drug related by the investigator
- Treatment-emergent AEs increased from 1 per cohort within the 2-mg, 4-mg and 6-mg dose groups to 11 within the 8-mg fasted dose group and 31 within the 12-mg dose group, suggesting a dose effect. Each of these events resolved during follow up
- The most common AE preferred terms were: headache (9 GS-9620 subjects and 2 placebo subjects), chills (6 GS-9620 subjects and 0 placebo subjects), and pyrexia (5 GS-9620 subjects and 1 placebo subjects)
- The only Grade 3 treatment-emergent AE was pyrexia, reported in 2 patients at 12 mg of GS-9620
- 7 subjects reported Grade 3 and 4 treatment-emergent laboratory abnormalities (5 Grade 3 events of hematuria, 1 Grade 3 event of elevated creatine phosphokinase, and 2 Grade 4 events of lymphopenia). Both Grade 4 laboratory abnormalities resolved by Day 5
- Mild decreases in platelet counts occurred, however these changes were not notable enough to be considered AEs

Table 2a. Study Drug-Related Adverse Events > Grade 2, by Cohort

AEs by System Organ Class and Preferred Term	Cohort	Dose (fasted unless otherwise noted)	n[a]
Subjects with Any Grade ≥ 2 > AE	6 7 8	8 mg 12 mg 8 mg MFM	1/8 4/8 1/8
Nervous System Disorder Headache Paresthesia	 6 7 7	 8 mg 12 mg 12 mg	 1/8 1/8 1/8
General and Administration Site conditions Pyrexia Chills	 7 7	 12 mg 12 mg	 3/8 1/8
Musculoskeletal And Connective Tissue Disorders Myalgia	 6 7	 8 mg 12 mg	 1/8 1/8
Gastrointestinal Disorder Nausea	 7	 12 mg	 1/8

MFM, moderate-fat meal with 27% of calories from fat
a Denominator is 8 (6 active plus 2 placebo subjects per cohort)
Adverse events were coded using MedDRA version 12.

Table 2b. Distribution of Maximum Graded Adverse Events

Maximum Treatment Emergent AE Grade	GS 9620 (n=60)	Placebo (n=20)
≥ Grade 1	20	3
≥ Grade 2	6	0
≥ Grade 3	2	0

Pharmacokinetic Results

- Preliminary data for fasting PK parameters are provided in Table 2
- GS-9620 reached maximum plasma concentrations (Cmax) between 1.5 hours and 6.0 hours after dosing and declined with a terminal half-life (t1/2) between 15 and 25 hr
- GS-9620 exhibited approximately dose proportional kinetics with a trend towards great than proportional exposure at higher doses
- GS-9620 exhibited moderate intersubject variability in plasma PK
- GS-9620 administered with food (simultaneously w/ moderate- or a high-fat meals or 4 hours after a high-fat meal) resulted in ~ 30 to 50% lower drug exposures (data not presented)

Figure 1. Mean GS-9620 Plasma Levels in Healthy Volunteers (Fasted)

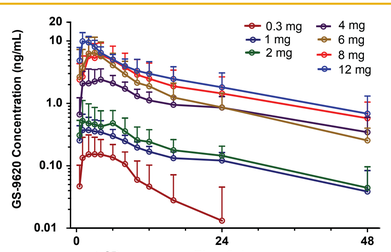

Table 3. Pharmacokinetic Data (Fasted)

Cohort (GS-9620 dose)	C_{max}[a] (ng/mL)	T_{max}[b] (hours)	AUC_{inf}[a] (ng•h/mL)	$T_{1/2}$[b,c] (hours)
Cohort 1 (0.3 mg) (n = 6)	0.184 (76)	3.00 (1.00, 6.00)	2.84[a] (66)	9.05[c] (6.92, 15.5)
Cohort 2 (1 mg) (n = 6)	0.440 (59)	3.00 (1.00, 8.00)	9.22 (32)	24.8 (14.2, 35.7)
Cohort 3 (2 mg) (n = 6)	0.635 (89)	6.00 (1.00, 6.00)	11.2 (48)	24.0 (14.7, 25.1)
Cohort 4 (4 mg) (n = 6)	2.93 (43)	4.00 (1.00, 6.00)	58.3 (48)	17.1 (13.3, 33.4)
Cohort 5 (6 mg) (n = 6)	7.26 (72)	2.50 (2.00, 6.00)	76.9 (64)	14.6 (12.1, 18.0)
Cohort 6 (8 mg) (n = 6)	8.34 (52)	2.50 (0.50, 6.00)	111 (74)	18.6 (14.4, 29.8)
Cohort 7 (12 mg) (n = 6)	12.0 (12)	1.50 (1.00, 2.00)	140 (54)	16.5 (14.5, 23.6)

a. AUC_{inf} and C_{max}: Mean (CV%); b. T_{max} and $T_{1/2}$: Median (min, max); n = 5 subjects;
c. $T_{1/2}$ not estimated in 1 subject

Pharmacodynamic Results

- GS-9620 treatment results in dose dependent increases in select cytokines, chemokines, and ISGs
- Dose related increases (> 3x Baseline) in ISGs were noted beginning at 2 mg
- No systemic interferon was noted until 12 mg
- No significant association was seen between PD results and IL28B status.
  - Sample size precludes ability to draw conclusions about the relevance of genetic polymorphisms at this locus for GS-9620
- Increases in % of T cells, B cells and NK cells expressing CD69 (an activation marker) were noted in subjects receiving GS-9620 at 8 and 12 mg doses

Table 4. Selected Cytokines: Peak Change: Baseline- 48

Selected Cytokine[a]	Cohorts 1-5 (0.3 mg – 6 mg)	Cohort 6 (8 mg Fasted)	Cohorts 6a, 8, 9 (8 mg Fed)	Cohort 7 (12 mg)	Pooled Placebo
IFNa	15 (7)	9 (3)	27 (2)	252 (112)	5 (1)
IL-6	16 (0)	40 (18)	23 (5)	447 (221)	5 (3)
MCP-1	31 (5)	93 (46)	53 (13)	1814 (811)	0 (5)
MIP-1B	38 (10)	71 (27)	27 (11)	415 (169)	7 (9)
MIG	65 (12)	60 (26)	43 (26)	597 (272)	10 (7)
IL-12p70	124 (22)	12 (9)	91 (20)	104 (67)	35 (12)
G-CSF	140 (13)	431 (192)	238 (49)	1664 (756)	40 (18)
I-TAC	176 (7)	607 (255)	490 (41)	2066 (956)	4 (2)
IL-5	323 (29)	37 (27)	160 (81)	16 (27)	66 (36)
IP-10	528 (20)	714 (332)	215 (59)	3475 (1375)	13 (12)
IL-1ra	569 (23)	1468 (726)	786 (25)	4745 (2158)	11 (7)

a. Mean(Standard Deviation)

Figure 2. Cytokine Induction: Mean Change by Cohort by Time-point (Placebo Adjusted)

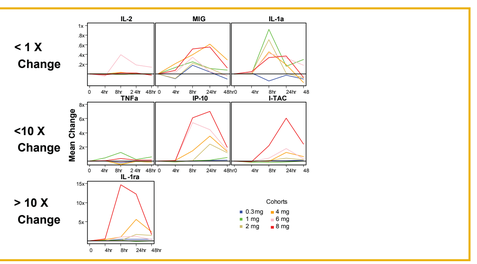

Figure 3a. Interferon Stimulated Genes: Mean Fold Change by Cohort by Time-point (Placebo Adjusted)

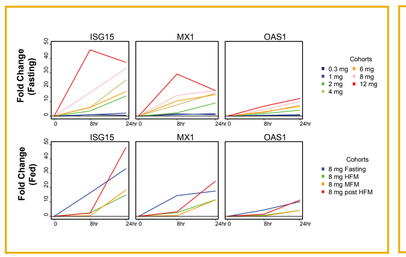

Figure 3b. Peak Fold Change by Median C_{max}

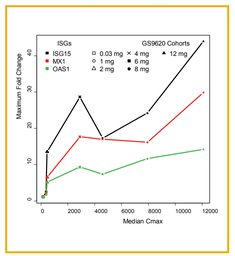

Figure 4. Median Lymphocyte Activation (%CD69+) by Cohort

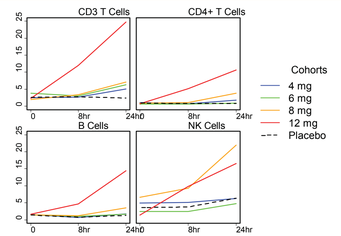

Table 5. Number Subjects with CD69+ Leukocytes > Placebo (at 8 and 24 hours)

	0.3 mg	1 mg	2 mg	4 mg	6 mg	8 mg	12 mg
T Cells CD3+CD69+	2/6	0	3/6	0	1/6	3/6	5/6
T Cells CD4+CD69+	1/6	0	2/6	0	0	1/6	4/6
B Cells CD20+CD69+	2/6	0	3/6	0	1/6	3/6	5/6
NK Cells CD3-CD56+ CD16+ CD69+	1/6	0	2/6	0	0	1/6	4/6

Conclusions

- GS-9620 is a potent, oral small molecule agonist of TLR-7, which was safe and well tolerated in single ascending doses up to 12 mg PO
- Preliminary PK data suggest GS-9620 reached C_{max} between 1.5 hours and 6.0 hours after dosing and had approximately dose-proportional trends in C_{max} and AUC_{inf}
- Pharmacodynamic effects are seen in ISG, cytokines, and hematologic responses, beginning at 2 mg PO
  - These findings confirm the preclinical data suggesting that GS-9620 induces multiple cytokines (including Interferon) pre-systemically, with the potential for decreased adverse events compared to systemic pegylated interferon
- GS-9620 is a promising, oral immunomodulatory agent with potency in the low milligram range and a therapeutic window which supports further evaluation in the therapy of viral hepatitis B and C

Acknowledgements & References

The authors would like to acknowledge Kevin Shianna, who supervised the IL28B genotyping at Duke University Genotyping Facility

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