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Preclinical Characterization of GS-9620, A Potent and Selective Oral TLR7 Agonist

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Introduction

- Though antiviral treatments can suppress Hepatitis C virus (HCV) and Hepatitis B virus (HBV) viral load in chronic infection, novel strategies to enhance long term viral clearance and sustained immunological control represent a significant unmet need
- GS-9620 is a potent oral TLR7 agonist being developed for the treatment of chronic HBV and HCV
- The goal of GS-9620 treatment is to stimulate an innate antiviral response and enhance an antiviral adaptive immune response
- In vitro and in vivo studies were performed to characterize the selectivity, activity, pharmacodynamics (PD), pharmacokinetics (PK), and tolerability of GS-9620

Background

- Oral administration of GS-9620 caused lasting reduction in viral load and S-antigen and induction of anti-S antigen antibodies in the woodchuck model of HBV infection (Poster/Abstract 170)
- Oral administration of GS-9620 for 8 weeks reduced serum and liver viral DNA in HBV infected chimpanzees with a mean maximal reduction in serum viral load of 2.2 logs and induced dose dependent increases in serum IFN-α, ISGs in PBMCs and liver, and the activation of lymphocyte subsets (CD8+ T and NK cells) (Oral/Abstract 1771)
- GS-9620 was safe and well tolerated in oral single ascending doses up to 12 mg in healthy volunteers and had pharmacodynamic effects beginning at 2 mg. (Poster/Abstract 664)

Methods

- GS-9620 selectivity for TLR activation was investigated using a cell based reporter assay at Invivogen, Inc.. Agonist activity was measured by assessing the activation of an NF-kB responsive reporter gene (SEAP) in HEK293 cells expressing various human TLRs
- In vitro PBMCs stimulations utilized concentrations of GS-9620 from 100 pM to 30 μM for 24 hours (cytokine assays) and/or longer (FACS analyses)
- Serum and PBMC culture supernatants were analyzed for cytokine levels at Ricerca Biosciences by use of an ELISA (cynomolgus IFN-α) or by using multiplex beads (Panomics) specific for a panel of human (28-plex) or cynomolgus monkey cytokines (25-plex) with a Luminex instrument
- FACS analyses utilized human PBMCs from a total of 8 independent donors. PBMCs or isolated lymphocytes were treated with 0.64 to 10,000 nM GS-9620 and analyzed for CD69 on CD3+ CD4+ or CD3+ CD8+ T lymphocytes or for CD69, CD86 and HLA-DR on CD20+ B lymphocytes by flow cytometry
- GS-9620 serum concentrations were determined by a LC/MS/MS method. Pharmacokinetic parameters were estimated using WinNonlin (Pharsight, Mountain view, CA, USA) by non-compartment analyses.
- In vivo ISG induction was evaluated at Southwest Foundation of Biomedical Research (SFBR) by analyses of OAS-1 and MX-1 levels from total RNA isolated from whole blood samples as determined using specific primers/probes from ABI Assays on Demand™ (Applied Biosystems/Ambion, Austin, TX) and the RNA UltraSense™ One-Step Quantitative RT-PCR System (Invitrogen Corporation, Carlsbad, CA) qRT-PCR kit on an ABI 7500 TaqMan machine. Cross reactivity of the human ABI Assays-on-Demand™ premixed primer/probes for cynomolgus monkey OAS-1 and MX-1 RNA was verified prior to their use (Lanford, SFBR)

Table 1. Minimum Effective Concentration (MEC) for *In Vitro* Cytokine Induction in Human and Cynomolgus Monkey PBMCs

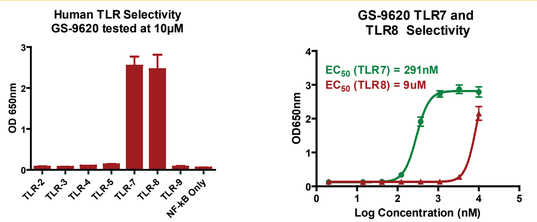
Cytokine Type	Cytokine	MEC Human (nM)	MEC Cynomolgus Monkey (nM)
Antiviral	IFN-α	66	308
Immunomodulatory	IFN-γ	131	3,334
	IL-2	108	11,000
	IL-10	263	> 30,000
	IL-12p40	256	91
Acute Phase Response	IL-1a	724	Not Done
	IL-1β	2,385	1,025
	IL-1ra	36	18
	IL-6	148	15
	TNF-α	3,650	285
Chemokines	IP-10	65	Not Done
	MCP-1	596	Not Done
	MIP-1α	2,385	35
	MIP-1β	407	773

Arithmetic means for n=10 human donors and n=4 cynomolgus monkeys

- GS-9620 selectively induces IFN-α, immmuno-modulatory cytokines and chemokines. The MEC for IFN-α induction was similar in pDCs (13.8 nM) and in PBMCs from HCV-positive donors (20.6 nM) (data not shown)

Results

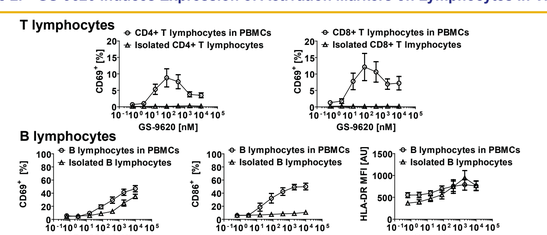
Figure 1. GS-9620 Human TLR Selectivity



Data represent the arithmetic mean of n = 2 and n = 3 independent experiments

- GS-9620 is specific for TLR7 and TLR8 at concentrations up to 10 μM
- GS-9620 demonstrates 30-fold selectivity for human TLR7 over TLR8

Figure 2. GS-9620 Induces Expression of Activation Markers on Lymphocytes In Vitro



PBMCs or isolated lymphocytes were treated with GS-9620 and analyzed for CD69 (day 1) on CD3+ CD4+ or CD3+ CD8+ T lymphocytes or for CD69 (day 1), CD86 (day 2) and HLA-DR (day 2) on CD20+ B lymphocytes by flow cytometry. Data is expressed as mean value ± SEM (n=8)

- GS-9620 directly activates B lymphocytes and indirectly activates T lymphocyte subsets in vitro

Table 2. *In Vitro* Physical Properties and Metabolism Profile

Kinetic Solubility in PBS (pH = 7.4, RT) (μM)	>100
logD (pH = 7.4)	1.3
$T_{1/2}$ in Mouse/Rat/Dog/Monkey/Human Hepatic Microsome (min)	4.9/3.7/23/5.9/31
Caco Permeability at 100 μM (cm/sec x 10^6) (A–B/B–A)	1.3/2.6
Plasma Protein Binding in Mouse/Rat/Dog/Monkey/Human (% Bound)	74/67/84/80/82
Major Metabolic Enzyme	CYP3A4
CYP Inhibition Potential	Low

Table 3. Key Pharmacokinetic Parameters in Preclinical Species

Parameter[a]	CD-1 Mice	SD Rats	Beagle Dogs	Cynomolgus Monkeys
CL (L/h/kg)[b]	5.8	4.7	2.7	0.9
Vss (L/kg)[b]	2.8	11.7	10.7	4.1
$t_{1/2}$ (h)[b]	1.9	2.3	6.8	7.3
F[c]	0.2%	20%	16%	1.2%

a The data are given as the mean of 3 animals. b Data from 30 min IV infusion. c Bioavailability from oral gavage

- The systemic clearance was moderate to high in all species tested
- The apparent volume of distribution was greater than the volume of total body water in all species tested

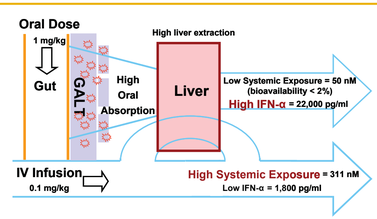
Table 4. Absorption in Portal Vein Cannulated Dogs

Parameter[a]	Sample Collection Site	
	Portal Vein[b]	Jugular Vein[b]
C_{max} (nM)	603	33
T_{max} (h)	0.83	0.92
AUC_{last} (nM•h)	736	171
AUC_{inf} (nM•h)	757	191
F[c]	82%	20%
Hepatic Extraction	76%	

a The data are given as the mean of 3 animals. b Oral gavage at 1 mg/kg. c Percent absorbed in portal vein or bioavailability in jugular vein

- GS-9620 demonstrated good oral absorption *in vivo*
- Estimated hepatic extraction was high which is consistent with hepatic extraction predicted *in vitro*

Figure 3. Pre-systemic Activity of GS-9620 in Cynomolgus Monkeys



- Oral administration induces a greater PD response with lower GS-9620 exposure than IV administration

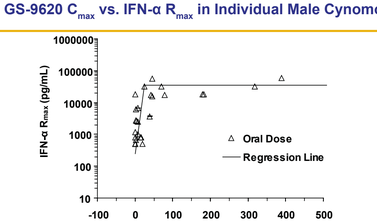
Table 5. GS-9620 Single Oral Dose Ranging PK-PD in Male Cynomolgus Monkeys

Dosage (mg/kg)	PK[a]	PD[a]
	C_{max} (nM)	IFN-α R_{max} (pg/mL)
0	BLQ[b]	≤130
0.05	0.3	≤130
0.15	0.9	≤130
0.30	11.3	763
0.50	15.6	8962
1.0	50.6	22367
1.5	105	14540

a The data are given as the mean of 3-5 animals. b GS-9620 limit of quantitation was 0.1 nM

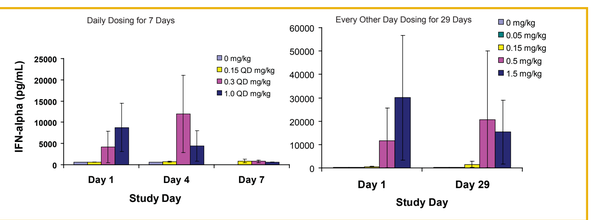
- Oral GS-9620 administration resulted in dose and exposure dependent induction of IFN-α *in vivo*

Figure 4. Serum GS-9620 C_{max} vs. IFN-α R_{max} in Individual Male Cynomolgus Monkeys



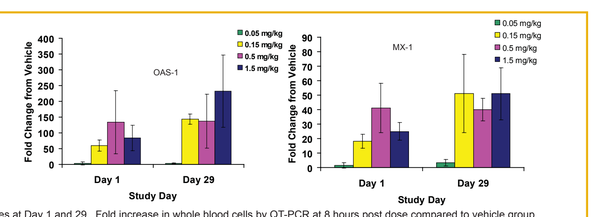
- The oral dose-response curve in cynomolgus monkeys was steep, consistent with in vitro assays
- IFN-α induction reached a plateau with increasing systemic exposure

Figure 5. Mean Maximal Serum IFN-α Induction with Repeated Daily versus Every Other Day Oral Administration of GS-9620 in Cynomolgus Monkeys



- Tachyphylaxis of the pharmacodynamic response occurred with every day dosing within 7 days
- No tachyphylaxis occurred with every other day dosing

Figure 6. ISG Induction in Cynomolgus Monkeys with Oral Every Other Day Administration of GS-9620 for 4 Weeks



Males at Day 1 and 29. Fold increase in whole blood cells by QT-PCR at 8 hours post dose compared to vehicle group

- Marked induction of ISGs occurred in vivo through 4 weeks of every other day dosing at ≥ 0.15 mg/kg
- No adverse effects were noted in 4-week studies at any dose evaluated (up to 1.5 mg/kg in cynomolgus monkeys)

Conclusions

- ***In vitro*** **GS-9620 has nanomolar potency for induction of IFN-α, immunomodulatory cytokines, chemokines and activation of lymphocyte subpopulations**
- **GS-9620 is 30-fold selective for TLR7 over TLR8 and has no cross-reactivity to other TLRs in a reporter assay**
- **In vivo GS-9620 has moderate to high clearance and high volume of distribution across preclinical species, demonstrated good oral absorption, and induces pre-systemic PD effects**
- **Low oral doses in cynomolgus monkeys induced serum IFN-α, immunomodulatory cytokines, chemokines and ISGs in blood cells**
- **No tachyphylaxis of the PD response was noted through 4 weeks of every other day dosing**
- **Repeated oral doses administered every other day for 4 weeks were well tolerated up to 1.5 mg/kg**