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Anti-Viral Efficacy and Induction of an Antibody Response Against Surface Antigen with the TLR7 Agonist GS-9620 in the Woodchuck Model of Chronic HBV Infection

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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 medical need. Most HBV patients require chronic suppressive antiviral treatment for an indefinite period.
- GS-9620 is a potent oral TLR7 agonist being developed for the treatment of chronic Hepatitis B and C.
- The goal of GS-9620 treatment is to stimulate an innate antiviral response and enhance an antiviral adaptive immune response.
- Woodchucks chronically infected with woodchuck hepatitis virus (WHV), an animal model for chronic HBV, were treated with oral GS-9620 to investigate its efficacy.

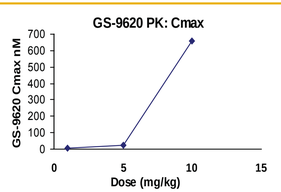
Background

- GS-9620 is an orally active, selective and potent TLR7 agonist that induced IFN-α and select cytokines in vivo with no reduction in pharmacodynamic response with every other day dosing for 4 weeks in cynomolgus monkeys (Poster/Abstract 1776).
- Oral GS-9620 treatment for 8 weeks in chimpanzees chronically infected with HBV reduced serum and liver viral DNA with a mean reduction in serum viral load of 2.2 logs. Treatment induced dose dependent increases in serum interferon-α (IFN-α), interferon-stimulated genes (ISGs) in PBMCs and liver, and activation of lymphocyte subsets (CD8+ T and NK cells) (Oral/Abstract 1771).
- GS-9620 was well tolerated in an oral single ascending dose study at doses up to 12 mg in healthy volunteers and had pharmacodynamic effects beginning at 2 mg. (Poster/Abstract 664).

Methods

- The study was conducted collaboratively between Cornell University (Ithaca, NY), Georgetown University Medical Center (Washington, DC), and Gilead Sciences, Foster City, CA.
- Single dose evaluation of pharmacokinetics (PK) and pharmacodynamics (PD) in uninfected animals was done to determine an active tolerated starting dose for the efficacy study.
- The efficacy study investigated 3 different dose regimens and used an infected and an uninfected control group. The experimental design is shown in Table 1.
- GS-9620 serum concentrations were determined by a LC/MS/MS method.
- Serum viral load was determined by slot blot hybridization and samples below the limit of detection were further evaluated by PCR (Menne et al, 2008).
- Serum WHsAg and antibodies to WHsAg (anti-WHs) were determined before treatment, during treatment, and during the post treatment follow-up period until Week 23. Serum WHsAg and anti-WHs antibody levels were assayed by ELISA (Cote et al, 1993).
- Induction of a PD response was determined by measuring RNA levels of IFN-α and interferon-stimulated genes, 2'5'-oligoadenylate synthetase (2'5'-OAS) and IFN-induced cellular resistance mediator protein (MxA), in whole blood samples collected at 24 hours post dose at different time points (pre-treatment, 1st dose, last dose). Total RNA was isolated, reverse transcribed to cDNA and evaluated by real time PCR using woodchuck-specific primers. Woodchuck β-actin mRNA expression was used to normalize target gene expression (Menne et al, 2007).
- Safety parameters included hematology, clinical chemistry, body weights, and observations.
- Animals were euthanized at 6 – 6 1/2 months after completion of treatment, necropsies were performed, and when present, the number and size of hepatocellular carcinomas (HCC) were determined.

Figure 1. Single Dose PK and PD Results in Uninfected Woodchucks



PK at 10 mg/kg PO was compromised due to a likely lowered metabolic state related to the entry into seasonal hibernation in these animals.

Mean fold increase compared to pre-treatment levels.

- GS-9620 induced a dose dependent increases in OAS and MxA RNA in blood cells.
- 5 mg/kg was well tolerated in uninfected animals, had demonstrable PD, and was chosen as the starting dose in infected animals.

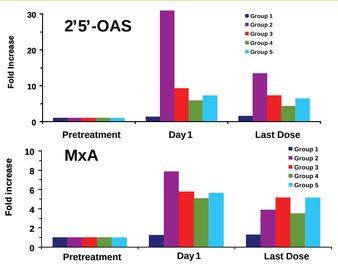
Results

Table 1. Experimental Design

Group (n=7/group)	Treatment and Intended Regimen	GS-9620 Dose (mg/kg)	Number of Doses	Study Endpoints
1	Placebo QOD x 4 weeks	0	14	• Serum viral load • Serum WHsAg • Serum anti-WHs antibody • PD markers: MxA and OAS RNA fold increase in whole blood cells • Safety parameters: observations, body weight, and clinical pathology • Incidence of hepatocellular carcinoma at study termination
2	GS-9620 QOD x 4 weeks	5 2.5	5-6 8-9	
3	GS-9620 QOD x 4 weeks	5 2.5	5-6 8-9	
4	GS-9620 QOD every other week x 8 weeks	5 2.5	4 12	
5	GS-9620 Weekly for 8 weeks	5	8	

Due to the occurrence of thrombocytopenia noted during the first 2 weeks of dosing in a few animals in each GS-9620 treatment group, dosing was halted for 9 to 10 days in Groups 2, 3 and 4. Upon re-initiation of dosing, dose levels were reduced to 2.5 mg/kg for all groups except for the once weekly treatment group (Group 5), which was maintained at 5 mg/kg. QOD = every other day.

Figure 2. PK and PD Group Mean Fold Increases in ISGs

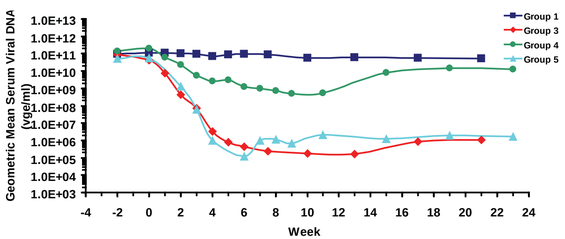


Group	GS-9620 Exposure (nM)[a]
1	0
2	6.2 ± 3.3
3	5.0 ± 2.4
4	7.4 ± 6.0
5	11.9 ± 9.7

[a] GS-9620 exposure was evaluated at 4 hours post dose at 2.5 mg/kg for Groups 2-4 and at 5 mg/kg for Group 5.
[b] Group 2 was uninfected animals.

Fold increases are compared to pretreatment levels

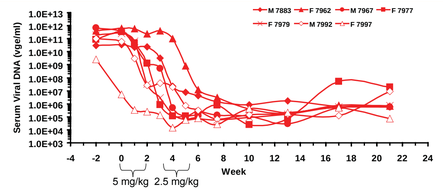
Figure 3. Viral Load



Duration of dosing was approximately 4 weeks for Group 3 and 8 weeks for Groups 4 and 5. The limit of detection for serum viral DNA levels 1 x 10^3 vge/ml.

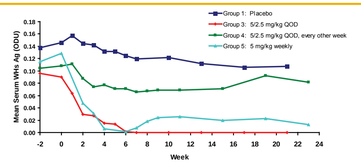
- GS-9620 reduced viral load in all GS-9620 treatment groups. The mean maximal log viral load reductions were 6.1, 2.9, and 5.8 for animals in Groups 3, 4 and 5, respectively.

Figure 4. Individual Animal Viral Load Group 3: Every Other Day Treatment for 4 Weeks



- All animals treated with GS-9620 every other day for 4 weeks (Group 3) had a maximal viral load reduction of 4.7 to 7.4 logs.
- Viral load reduction was sustained after cessation of treatment.

Figure 5. Serum WHsAg



Serum WHsAg levels were determined by ELISA. Limit of detection ~16 ng/mL.

- GS-9620 reduced serum WHsAg levels and this was sustained after cessation of treatment. Reduction to undetectable levels occurred in 100% of animals treated every other day for 4 weeks (Group 3).

Figure 6. Individual Animal Serum WHsAg Data for Placebo and Group 3: 5/2.5 mg/kg QOD x 4 weeks

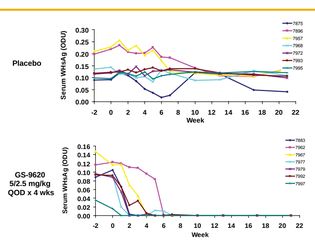
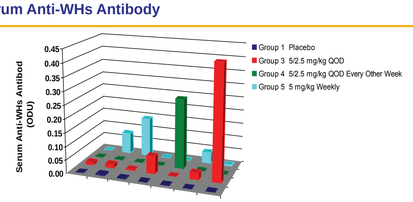


Figure 7. Serum Anti-WHs Antibody



Serum anti-WHs antibody levels were determined by ELISA. Data shown are from Week 23 (Groups 4 and 5) or Week 21 (Groups 1 and 3). All animals had no detectable anti-WHs antibody at pretreatment (ODU =0).

- GS-9620 induced an anti-WHs antibody response in a subset of animals in each treatment group.

Table 2. Summary of Efficacy Results

Group	Treatment and Intended Regimen	GS-9620 Dose (mg/kg x doses)	Mean Reduction in Viral Load Log Reduction	Mean Viral Load Log Reduction in Responders	Mean Percent Reduction of Serum WHsAg	Animals with Complete Loss of Serum WHsAg	Animals with Anti-WHs Antibody
1	Placebo QOD x 4 weeks	0 x 14 doses	0.3 ± 0.5	NA	20 ± 24%	0/7	0/7
3	GS-9620 QOD x 4 weeks	5 x 5–6 doses 2.5 x 8–9 doses	6.1 ± 0.9	6.1 ± 0.9 (7/7)	100 ± 0 %	7/7	2/7
4	GS-9620 QOD x 8 weeks	5 x 4 doses 2.5 x 12 doses	2.9 ± 2.8	5.9 ± 0.8 (3/7)	32 ± 47%	2/7	1/7
5	GS-9620 Once weekly for 8 weeks	5 x 8 doses	5.8 ± 1.7[a]	5.8 ± 1.7 (6/6)	91 ± 19 %	5/7	3/7

a Only 6 animals were evaluated in Group 5 due to loss of one animal at Week 3.

Table 3. Incidence of Hepatocellular Carcinoma at ≥ 6 Months after the End of Treatment

Group	Treatment and Intended Regimen	Incidence of HCC
1	Placebo QOD x 4 weeks	4/7
2 (Uninfected)	GS-9620 QOD x 4 weeks	0/7
3	GS-9620 QOD x 4 weeks	0/7
4	GS-9620 QOD x 8 weeks	1/6
5	GS-9620 Once weekly for 8 weeks	2/5

Evaluation by gross necropsy; animals included all surviving woodchucks at the study terminus (6 – 6 ½ months after the end of GS-9620 treatment) and 3 woodchucks that died which had a necropsy diagnosis of hepatocellular carcinoma.

- Treatment with GS-9620 every other day for 4 weeks markedly reduced the incidence of hepatocellular carcinoma at 6- 6 1/2 months after cessation of GS-9620 treatment.

Safety Results

Single dose PK and PD in uninfected woodchucks
- Mild transient thrombocytopenia, increased body temperature, and lymphopenia occurred at 5 mg/kg.
- 10 mg/kg was not tolerated.

Woodchucks chronically infected with WHV
- Adverse events included changes in clinical pathology parameters and mortality (3 animals).
- Clinical pathology changes included:
  - decrease in platelets primarily at the 5 mg/kg dose and sporadically at 2.5 mg/kg
  - anemia and increases in liver enzymes (ALT, AST, GGT and SDH) were observed at both doses in some animals in each group
  - mild transient increases (0.3 to 0.4 mg/dL) in serum bilirubin in a few animals treated with 5 mg/kg once weekly (Group 5)
- Drug-induced immune inflammatory processes directed at attempted clearance of the chronic WHV infection may have had a causal role for these changes.
- The every other day dose regimen for 4 weeks was the most tolerated.

Conclusions

- **Four weeks of oral treatment with the TLR7 agonist GS-9620 in woodchucks chronically infected with WHV resulted in a sustained, marked reduction in serum levels of viral DNA and WHsAg and in the induction of an anti-WHs antibody response.**
  - **14/21 animals treated with 9620 had sustained WHsAg loss compared to 0/7 animals treated with placebo**
  - **6/21 animals treated with 9620 had an anti-WHs antibody response compared to 0/7 animals treated with placebo**
- **The incidence of HCC was significantly reduced in GS-9620 treated woodchucks 6 - 6 1/2 months following the end of GS-9620 treatment.**
- **The results suggest that GS-9620 induces a protective anti-viral immune response during chronic active hepadnaviral infection and this approach presents the potential of a finite treatment duration for chronic Hepatitis B therapy using GS-9620.**

References & Acknowledgements

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