Phase 1B, Randomized, Double-Blind, Dose-Escalation Trial of CPG 10101 in Patients with Chronic Hepatitis C Virus

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CPG 10101, a synthetic oligodeoxynucleotide (ODN), is a toll-like receptor 9 (TLR9) agonist with antiviral and immunomodulatory properties that could potentially influence chronic infection with HCV. In this multicenter Phase 1b trial, 60 HCV-positive patients (50 genotype 1 HCV) were randomized and received either placebo or CPG 10101 at 0.25, 1, 4, 10, or 20 mg subcutaneously (SC) twice weekly for 4 weeks or at 0.5 or 0.75 mg/kg SC once weekly for 4 weeks. Dose-dependent cytokine induction was observed after administration of CPG 10101. At 24 hours after administering the highest dose of 0.75 mg/kg CPG 10101, interferon (IFN)-γ-inducible protein 10 (IP-10) had a mean increase over baseline levels (±SD) of 15,057 (±9769) pg/ml ($P < 0.01$, compared to placebo); IFN-α had a 106 (±63.3) pg/ml increase ($P < 0.01$); and 2′5′-oligoadenylate synthetase (OAS) had a 163 (±120.6) pmol/dl increase ($P < 0.01$). Decreases in HCV RNA also were dose-dependent, with the greatest group geometric mean maximum reduction of 1.69 ± 0.618 $\log_{10}$ ($P < 0.05$) observed in the 0.75 mg/kg dose group. Decreases ≥1 $\log_{10}$ were seen in 22 of 40 patients who received ≥1 mg CPG 10101, with 3 patients exceeding a 2.5-$\log_{10}$ reduction. CPG 10101 was well tolerated, and adverse events were consistent with CPG 10101's mechanism of action. *Conclusion:* In this Phase 1 study, CPG 10101 was associated with dose-dependent increases in markers of immune activation and decreases in HCV RNA levels. The data support further clinical studies of CPG 10101 for treating chronic HCV infection. (HEPATOLOGY 2007;46:1341-1349.)

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Abbreviations: CpG, region of DNA where cytosine and guanine nucleotides are separated by a phosphate; CTC, Common Toxicity Criteria; IFN, interferon; IP-10, interferon-γ-inducible protein 10; NK, natural killer; OAS, oligoadenylate synthetase; ODN, oligodeoxynucleotide; pDCs, plasmacytoid dendritic cells; PEG-IFN, pegylated interferon; SC, subcutaneously; TLR9, toll-like receptor 9.

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The hepatitis C virus (HCV) has infected approximately 170 million people worldwide and 4 million people within the United States.[1] When untreated, chronic HCV infection can lead to cirrhosis, hepatocellular carcinoma, and end-stage liver disease.[2-4] Currently, the most effective therapy for chronic HCV infection is the combination of weekly subcutaneous injections of pegylated interferon-α (PEG-IFN) and daily oral dosing of ribavirin. Although this regimen has resulted in substantial improvements in the response rates compared to the earlier use of nonpegylated IFN with or without ribavirin, current treatments are only effective in approximately 50% of infected patients and are not suitable for many patients; most failures occur in patients infected with HCV genotype 1 or 4.[5,6] In addition, HCV uses multiple mechanisms to render the immune system dysfunctional and to favor the development of chronic infection.[7]

CPG 10101 (Actilon; Coley Pharmaceutical Group, Inc., Wellesley, MA) is the first of a new class of investigational immunomodulatory antiviral drugs that stimulate the toll-like receptor 9 (TLR9). TLR9 is expressed in human B cells and plasmacytoid dendritic cells (pDCs) and detects regions of DNA where cytosine and guanine nucleotides are separated by a phosphate (CpG motifs) (unmethylated CpG dinucleotides within specific flanking base contexts) that are present frequently in bacterial and viral DNA.[8,9] CPG 10101 is a synthetic oligodeoxynucleotide (ODN) that contains immunostimulatory CpG motifs optimized for activation of human TLR9. It has been shown to activate B cells and pDCs in both healthy volunteers and HCV patients, and to stimulate production of cytokines, including those with known antiviral activity such as IFN-α.[10,11] Activation of pDCs is important for HCV treatment because these activated cells are not only the primary producer of the cytokine IFN-α, but are also involved in the induction of T cells that comprise an important part of the adaptive immune response. CPG 10101's ability to stimulate immune cells even in the presence of the immune dysfunction induced by chronic HCV infection makes it a candidate for development as a therapy for patients with chronic HCV infection.

In a previous Phase 1a, placebo-controlled, healthy volunteer trial, escalating subcutaneous (SC) doses (0.25 to 20 mg) of CPG 10101 were well-tolerated, and no serious adverse events or dose-limiting toxicities were reported.[10] The aim of this multicenter Phase 1b study was to assess the safety and efficacy of CPG 10101 in HCV-infected patients. We randomly assigned patients to receive SC doses of CPG 10101 or placebo in 5 sequential dose cohorts (0.25, 1, 4, 10, and 20 mg) given twice weekly for 4 weeks or in 2 sequential weight-based dose cohorts (0.5 or 0.75 mg/kg) given once weekly for 4 weeks. Tolerability, pharmacokinetics, pharmacodynamics, and HCV RNA levels were evaluated during and for 4 weeks after treatment.

Patients and Methods

Patients

Adult patients with hepatitis C infection were eligible for the study if they had detectable serum HCV RNA (HCV RNA $>$ 1000 IU/ml). In this study, we only enrolled individuals who had not previously received treatment for HCV if they were ineligible for, intolerant of, or unwilling to take standard therapy. We excluded patients who had received previous treatment if they received a prior IFN-based therapy (at least 4 weeks of $\geq$3 MU IFN administered 3 times per week or 180 μg or 1.5 μg/kg PEG-IFN weekly) that resulted in a reduction in HCV viral load less than 1 $\log_{10}$. We also excluded patients if they were seropositive for hepatitis B surface antigen or HIV-1, or if they had other serious medical or psychiatric conditions, or had a history of autoimmune disease, allogeneic transplant, or preexisting autoimmune or antibody-mediated disease. Women who were pregnant, lactating, or unable or unwilling to practice effective contraception were ineligible to participate. We obtained written informed consent from all patients, and the study protocol was approved by an institutional review board at each participating center in accordance with the Declaration of Helsinki.

Study Design

This was a randomized, double-blind, placebo-controlled trial of CPG 10101 performed at 8 sites throughout the United States. The first patient was screened on January 30, 2004, and the last patient completed the study on October 14, 2005. For randomization, we assigned all patients enrolled a number, and a randomization list was generated by a biostatistician at Coley Pharmaceutical Group, Inc., who provided the list to an unblinded pharmacist for dose preparation and assignment. We blinded patients and site personnel to treatment assignment. We screened a total of 61 patients and randomized them in a 3:1 ratio to receive either CPG 10101 or placebo in 5 sequential ascending dose cohorts (0.25, 1, 4, 10, and 20 mg SC) given twice weekly for 4 weeks or 2 sequential weight-based dose cohorts (0.5 or 0.75 mg/kg SC) given once weekly for 4 weeks. Five sequential escalating doses of 0.25 to 20 mg administered 2 weeks apart were previously well-tolerated in Phase 1a studies in normal volunteers, and increasing levels of biomarkers indicative of immune cellular activation were

shown.[10] Patients receiving the 0.5 mg/kg dose given weekly received approximately the same cumulative dose of CPG 10101 as those receiving 20 mg twice weekly. Dose escalation occurred only after a Safety Monitoring Committee had reviewed safety data in each cohort, and determined that it satisfied protocol-defined dose limiting toxicity criteria.

CPG 10101 is a 22-mer C-class CpG oligodeoxynucleotide with a wholly phosphorothioate backbone.[12] The drug was dissolved in preservative-free phosphate-buffered saline to final concentrations of 1 mg/ml (for the 0.25-mg/kg and 1-mg/kg dose groups), 10 mg/ml (4-mg/kg, 10-mg/kg, and 20-mg/kg dose groups), and 40 mg/ml (0.5-mg/kg and 0.75-mg/kg dose groups).

CPG 10101 Pharmacokinetic Assays

For pharmacokinetic analyses, we took blood samples from each dosed patient on Day 1 (day of first injection) and Day 22 (first injection of week 4) at 0, 0.5, 1, 3, and 24 hours after injection. We centrifuged blood samples, and we harvested plasma and stored it frozen.

We measured the concentrations of CPG 10101 in plasma using an optimized, sensitive, high-throughput, oligonucleotide hybridization assay. The hybridization assay used sequence-specific capture and detection ODN probes complementary to portions of the CPG 10101 sequence, similar to a previously published method for a different CpG oligodeoxynucleotide.[13] This method also detects biologically active 19-mer to 21-mer metabolites of CPG 10101 (Coley Pharmaceutical Group, unpublished results). We incorporated locked nucleic acids into the probe design, resulting in increased sensitivity and specificity. We biotin-labeled the detection probe at the 5′ end. We synthesized the capture probe with internucleotide phosphodiester except for the last 3′ internucleotide liaison (phosphorothioate), and contained an amino linker at the 3′ end (BioSpring, Frankfurt, Germany).

The linear range of the assay was 7.8 to 500 pg/ml with a 7.8 pg/ml lower limit of quantification and a detection limit of 2.3 pg/ml. We gave sample results less than the lower limit of quantification an arbitrary value of 7.8 pg/ml for the purpose of analyses.

Serum Cytokine Assays

We measured blood markers of immune response for Days 1 (predose), 2, 8 (predose), 15 (predose), 22 (predose), 23, 29, 36, and 50 [and Days 4 (predose), 5, 9, and 16 for the twice-weekly dose groups]. We used commercial assays for quantification of 2′5′-oligoadenylate synthetase (OAS; Eiken Chemical Company, Ltd., Tokyo, Japan), C-reactive protein (Alpco Diagnostics, Windham, NH), and interleukin-18 (Medical and Biological Laboratory, Piscataway, NJ). We developed enzyme-linked immunosorbent assays and optimized them for detecting IFN-γ, IFN-γ-inducible protein 10 (IP-10), and macrophage chemoattractant protein-1 (BD Bioscience Pharmingen, Toronto, ON, Canada), and macrophage inflammatory protein-3β (R&D Systems, Inc., Minneapolis, MN) using development reagents from each manufacturer. We performed tests according to the manufacturer's instructions, laboratory-specific protocols of Coley Pharmaceutical Group, Inc., and standard operating procedures. We tested at least duplicate in all standards and samples.

Measurement of HCV RNA

We measured serum HCV RNA levels for Days 1 (predose), 2, 8 (predose), 15 (predose), 22 (predose), 23, 29, 36, 50 [and Days 4 (predose), 5, 9, and 16 for the twice-weekly dose groups]. We performed quantitation of serum HCV RNA levels by a central laboratory using multicycle reverse-transcription (RT)-PCR (Super-Quant, National Genetics Institute [NGI], Culver City, CA) as described.[14] In the event that the viral RNA level was less than the lowest limit of quantification of this assay (40 IU/ml), we performed the NGI UltraQual assay on the sample. We identified HCV genotype by PCR and DNA sequencing at Day 1 (predose), Week 4 (predose), and at the End of Treatment visit (Day 50) (LabCorp, Raritan, NJ).[15]

Safety Evaluations

Safety data included adverse events graded according to the NCI Common Toxicity Criteria (CTC) version 2.0, clinical laboratory evaluations, vital signs, measurements, and physical exams.

Data Analysis and Statistics

The safety analysis included all randomized patients who received at least 1 dose of study medication. There were no discrepancies between randomized treatment assignments and treatments actually received. We analyzed cytokine and HCV RNA concentrations using the intent-to-treat population, which we defined as all patients who were randomized and received at least 1 treatment dose. We defined the pretreatment baseline to be the Day 1, 0 Hour (predose) value. We calculated the effect of CPG 10101 on serum HCV RNA levels for each patient by taking the difference in the $\log_{10}$ HCV RNA between the baseline and a given point in time. In some analyses of pharmacokinetic and pharmacodynamic data, we removed outliers that fell outside the 95% confidence limits for their group to allow clearer visualization of general trends for this data. When we removed outliers, it was

clearly stated which group(s) were affected and the rationale for this decision (see table footnotes and figure legends). We did not impute missing data. We performed statistical analyses using SAS, version 8.2.

Results

Study Patients

We randomized 60 patients and treated them with CPG 10101 or placebo. One randomized patient did not receive placebo because the patient had undetectable serum HCV RNA prior to the start of the study. Baseline characteristics were similar between treatment groups (Table 1). Patients were predominantly white (66%), male (58%), and infected with HCV genotype 1 (83% with 1a, 1a variant, or 1b). Although we did not collect information regarding prior therapy for HCV infection formally, retrospective telephone calls to study sites identified the 60 randomized and treated patients as 2 prior treatment-naive, 10 prior treatment-intolerant, 9 who did not clear virus on prior treatment, 30 treatment relapsers (cleared virus on previous treatment, but relapsed), and 9 incompletely characterized (decrease in viral titer reported on prior treatment, uncertain if they ever cleared virus, but viral-positive on screening prior to this study). Of the 60 randomized and treated patients, 51 patients received all scheduled doses of their assigned study treatment [13 (100%) placebo; 38 (81%) CPG 10101].

A total of 7 patients randomized to receive CPG 10101 withdrew from the study prior to Day 50. Three patients withdrew from the study because of an adverse event; 1 patient who received 4 mg CPG 10101 fell and fractured his pelvis and had to withdraw after his first dose; 1 patient who received 3 injections of 10 mg CPG 10101 experienced gastrointestinal side effects [Common Toxicity Criteria (CTC) grade 2 lower abdominal pain, diarrhea and vomiting, accompanied by grade 1 nausea, headache and pyrexia]; 1 patient who received 1 injection of 0.75 mg/kg CPG 10101 experienced CTC grade 1 injection site bruising that persisted for 13 days. Two patients (1 mg and 4 mg CPG 10101) discontinued early because they withdrew their consent. We withdrew 1 patient (0.5 mg/kg) because of protocol noncompliance. One patient randomized into the 0.75 mg/kg cohort was HCV RNA–negative at time of study entry and we withdrew the patient from the study on Day 2 after receipt of a single dose of CPG 10101. We included the data from this patient in safety summaries, but did not include it in the analyses of changes in HCV RNA levels.

Pharmacokinetics

Plasma CPG 10101 concentrations were dose-dependent, with the highest plasma concentrations seen in patients receiving 0.5 and 0.75 mg/kg SC injections (Table 2). Mean plasma concentrations peaked between 2 and 4 hours postdose, and plasma concentrations were greatly reduced (80% to 98%) by 24 hours. The mean time to maximum plasma concentration was independent of dose. Peak plasma concentration and area under the plasma concentration–time curve were similar following the first and seventh injections in HCV patients who were dosed twice weekly, and following the first and fourth dose in HCV patients who were dosed weekly, suggesting that CPG 10101 did not accumulate with repeated once-weekly or twice-weekly dosing for 4 weeks. The mean area

Table 1. Baseline Demographic and Disease Characteristics

	Twice-Weekly Dosing						Once-Weekly Dosing		
	CPG 10101						CPG 10101		
	Placebo	0.25 mg	1 mg	4 mg	10 mg	20 mg	Placebo	0.5 mg/kg	0.75 mg/kg
n	10	6	6	7	7	6	3	7	8
Age, mean (SD) (years)	52.7 (5.3)	49.3 (6.9)	48.5 (4.4)	54.6 (4.2)	50.0 (8.6)	53.5 (5.5)	54.3 (5.69)	50.3 (5.8)	54.4 (5.9)
Weight, mean (SD) (kg)	88.5 (12.1)	88.4 (10.5)	106.1 (26.6)	94.0 (14.2)	91.5 (35.0)	85.7 (9.7)	85.7 (14.4)	85.3 (19.0)	89.3 (21.6)
Female Sex (%)	5 (50)	0	3 (50)	2 (29)	4 (57)	2 (33)	0	5 (71)	4 (50)
Ethnicity									
White (%)	6 (60)	6 (100)	5 (83)	6 (86)	6 (86)	3 (50)	2 (67)	3 (43)	3 (38)
African-American (%)	4 (40)	0	1 (17)	1 (14)	0	2 (33)	0	1 (14)	1 (13)
Hispanic (%)	0	0	0	0	1 (14)	1 (17)	1 (33)	3 (43)	4 (50)
HCV Genotype									
1 (%)	8 (80)	6 (100)	5 (83)	6 (86)	6 (86)	4 (67)	3 (100)	6 (86)	6 (75)
2 (%)	1 (10)	0	0	0	1 (14)	0	0	0	1 (13)
4 (%)	0	0	1 (17)	1 (14)	0	1 (17)	0	0	0
Unknown (%)	1 (10)	0	0	0	0	1 (17)	0	1 (14)	1 (13)
ALT, mean (SD) (IU/l)	89.9 (58.1)	72.7 (43.8)	58.3 (35.9)	50.6 (19.4)	79.3 (37.7)	46.0 (7.1)	65.3 (16.6)	74.3 (49.7)	51.9 (31.7)
AST, mean (SD) (IU/l)	72.4 (46.5)	70.2 (40.1)	50.0 (25.2)	46.7 (25.9)	61.7 (24.2)	35.8 (7.9)	50.0 (29.5)	66.0 (46.7)	46.4 (31.7)
HCV RNA, mean (SD) (log_{10} IU/ml)	5.94 (0.97)	6.07 (0.42)	6.46 (0.45)	5.99 (0.66)	5.71 (0.86)	5.80 (0.73)	6.40 (0.36)	6.12 (0.76)	5.86 (1.76)

Table 2. Pharmacokinetic Parameters of CPG 10101

CPG 10101 Dose	Injection	n	C_{max} (μg/ml)	$AUC_{0\text{-last}}$ (μg-hour/ml)	T_{max} (hours)	$t_{½}$ (hours)
Twice-weekly injections for 4 weeks						
0.25 mg	1	6	0.002 (0.0009)	0.018 (0.0124)	2.6 (1.02)	3.5 (0.79)
	7	6	0.001 (0.0011)	0.015 (0.0143)	2.0 (1.09)	3.9 (1.03)
1 mg	1	6	0.007 (0.0040)	0.057 (0.0332)	2.3 (1.17)	3.5 (0.68)
	7	6	0.003 (0.0011)	0.035 (0.0222)	2.3 (1.03)	4.4 (1.59)
4 mg	1	7	0.031 (0.0187)	0.382 (0.2450)	2.7 (0.75)	3.5 (0.79)
	7	7	0.040 (0.0210)	0.495 (0.2536)	2.6 (0.89)	3.5 (0.42)
10 mg	1	7	0.069 (0.0394)	0.860 (0.4754)	3.0 (0.00)	3.5 (0.66)
	7	6	0.059 (0.0485)	0.696 (0.6636)	2.5 (1.22)	4.0 (0.69)
20 mg	1	5	0.086 (0.0179)	1.132 (0.2173)	3.0 (0.00)	5.3 (2.08)
	7	6	0.202 (0.1101)	2.606 (1.4167)	3.0 (0.00)	4.2 (0.86)
Once-weekly injections for 4 weeks						
0.5 mg/kg	1	6	0.354(0.1506)	4.456 (1.8370)	3.0 (0.00)	3.6 (0.66)
	4	6	0.346 (0.2046)	4.378 (2.6642)	2.7 (0.81)	5.3 (2.75)
0.75 mg/kg	1	7	0.518 (0.0821)	6.409 (0.9589)	2.8 (0.70)	5.3 (3.59)
	4	6	0.563 (0.2642)	7.098 (3.1850)	2.7 (0.81)	4.2 (2.07)

*Pharmacokinetic parameters are means (SD) and were determined from plasma concentration time curves. Data were available at 0, 0.5, 1, 3, and 24 hours for chronic hepatitis C patients that received once-weekly or twice-weekly injections. Two outliers (that gave C_{max} and $AUC_{0\text{-last}}$ values that fell outside the 95% confidence limits for their group; 1 in the 0.5 mg/kg, first injection group, and 1 in the 0.75 mg/kg, first injection group) are not included in this table. Abbreviations: $AUC_{0\text{-last}}$, area under plasma concentration-time curve; C_{max}, maximum concentration in plasma; T_{max}, time to maximum plasma concentration; $t_{1/2}$, termination half-life of CPG 10101 plus metabolites in plasma.

under the plasma concentration–time curve and peak plasma concentration increased with increasing dose; this was most clearly seen when we adjusted doses to the patient's weight (Supplementary Fig. 1)

Immune Marker Activity

Serum concentrations of IFN-α, IP-10, and 2′,5′-OAS increased in patients within 24 hours of receiving ≥1 mg CPG 10101. Serum concentrations of IFN-α and IP-10 increased during the first 24 hours after CPG 10101 administration and then returned to near baseline prior to the next administration, 3 to 4 days later (Fig. 1A-C). In contrast to IFN-α and IP-10, serum concentrations of 2′5′-OAS remained elevated and did not return to baseline prior to subsequent CPG 10101 injections. Serum concentrations of IFN-α, IP-10, and 2′5′-OAS did return to baseline following the end of treatment.

The increased serum concentrations of IFN-α, IP-10, and 2′5′-OAS were dose-dependent, with 0.75 mg/kg CPG 10101 producing the largest individual and group mean increases. At 24 hours following the first administration of 0.75 mg/kg CPG 10101, IP-10 had a mean increase over baseline values (SD) of 15,057 (9769) pg/ml ($P < 0.01$ compared to placebo); IFN-α had a 106 (63.3) pg/ml increase ($P < 0.01$); and 2′5′-OAS had a 163 (120.6) pmol/dL increase ($P < 0.01$).

Increases in markers such as C-reactive protein, IFN-γ, IL-18, macrophage chemoattractant protein-1, and macrophage inflammatory protein-3β were also noted (data not shown). Again, the largest increases were seen with 0.5 and 0.75 mg/kg CPG 10101.

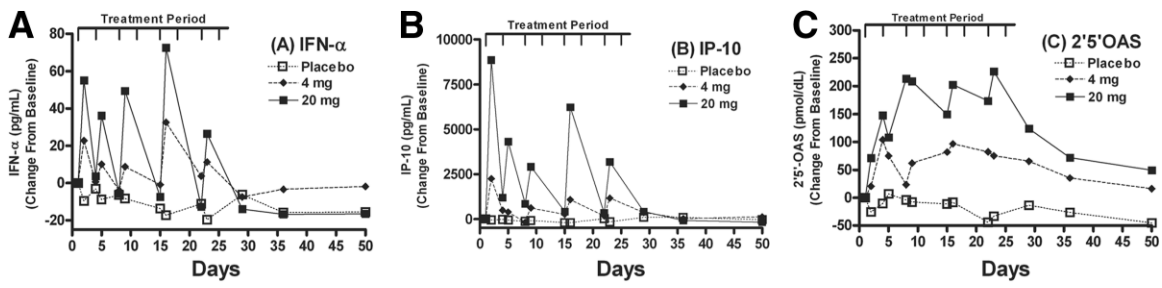

Fig. 1. Immune marker activity following CPG 10101 administration. Serum samples were obtained from HCV patients to measure levels of circulating cytokines during and after treatment. Group mean changes in (A) interferon (IFN)-α, (B) interferon-γ-inducible protein 10 (IP-10), and (C) 2′5′-oligoadenylate synthetase (OAS) are presented for patients receiving placebo, 4 mg CPG 10101, and 20 mg CPG 10101 twice weekly. The dash marks in (A–C) represent CPG 10101 administration on Days 1, 4, 8, 11, 15, 18, 22, and 25. One 20 mg outlier (that fell outside the upper 95% confidence limits) was removed at Day 22 in (C) to better visualize general trends.

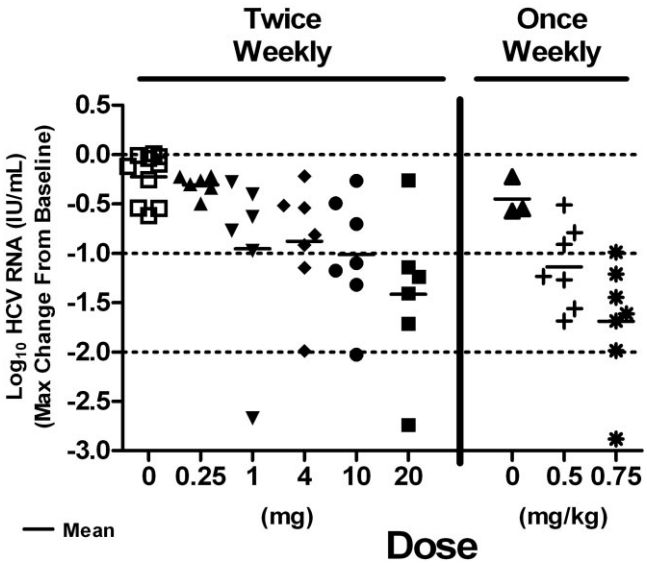

Fig. 2. Maximum reduction in HCV RNA. The maximum change from baseline in HCV RNA levels for each HCV patient during the first 12 weeks of treatment is shown. HCV patients were administered 0.25 to 20 mg CPG 10101 SC twice weekly for 4 weeks or 0.5 or 0.75 mg/kg CPG 10101 SC once weekly for 4 weeks. The bar represents the group geometric mean $\log_{10}$ maximum change (SD), for CPG 10101 treated groups as follows: 0.25 mg group, −0.31 (0.103); 1 mg group, −0.96 (0.877); 4 mg group, −0.88 (0.576); 10 mg group, −1.01 (0.589); 20 mg group, 1.42 (0.810); 0.5 mg/kg group, −1.14 (0.422); 0.75 mg/kg group, −1.69 (0.618). The values for the dose groups 20 mg, 0.5 mg/kg, and 0.75 mg/kg are significantly different from placebo values ($P < 0.05$).

Antiviral Effects

We calculated the maximal decrease from baseline in plasma HCV RNA levels obtained at any time during the study for each patient. Decreases in HCV RNA levels were dose-dependent (Fig. 2), with the largest geometric mean decrease of 1.69 $\log_{10}$ occurring after the 0.75-mg/kg CPG 10101 dose. HCV RNA decreases $\geq$1 $\log_{10}$ were seen in the 1 mg, 4 mg, 10 mg, 20 mg, 0.5 mg/kg, and 0.75 mg/kg dose groups, with 22 of those 40 patients achieving $\geq$1 $\log_{10}$ reduction. Six of the 7 HCV RNA-positive patients that received 0.75 mg/kg CPG 10101 experienced a $\geq$1 $\log_{10}$ decrease in plasma HCV RNA. Three patients experienced $\geq$2.5 $\log_{10}$ decreases in plasma HCV RNA levels; these patients were in the 1 mg, 20 mg, and 0.75 mg/kg dose groups, respectively. Group geometric mean maximum HCV RNA $\log_{10}$ decreases (SD) were 0.31 (0.10) for 0.25 mg, 0.95 (0.88) for 1 mg, 0.88 (0.58) for 4 mg, 1.01 (0.59) for 10 mg, 1.42 (0.81) for 20 mg, 1.14 (0.42) for 0.5 mg/kg, and 1.69 (0.62) for 0.75 mg/kg (Fig. 2). At 24 hours after the first dosing of CPG 10101, reductions in HCV RNA levels correlated significantly with increases in IFN-α, IP-10, and 2′5′-OAS (Fig. 3).

Of the 47 patients that received at least 1 dose of CPG 10101, we observed decrease in HCV RNA levels of $\geq$1 $\log_{10}$ in 2 out of 2 prior treatment-naive patients, 4 out of 9 prior treatment-intolerant patients, none of the 6 patients who did not clear virus on prior treatment, 12 out of 22 prior treatment relapsers, and 4 out of 8 incompletely characterized responders. Following termination of study treatment, HCV RNA levels increased toward the baseline in all patients.

Safety and Tolerability

CpG 10101 was generally well tolerated. The most common adverse events reported during treatment were local injection site reactions (erythema, pain, swelling, induration, pruritus, or warmth at the site of injection) and flu-like systemic reactions (fatigue, rigors, pyrexia, and malaise) (Table 3). Local injection reactions and flu-like systemic reactions were mild to moderate in intensity, and the incidence, severity, and duration appeared to be dose-related. Most patients who received 1 mg or more

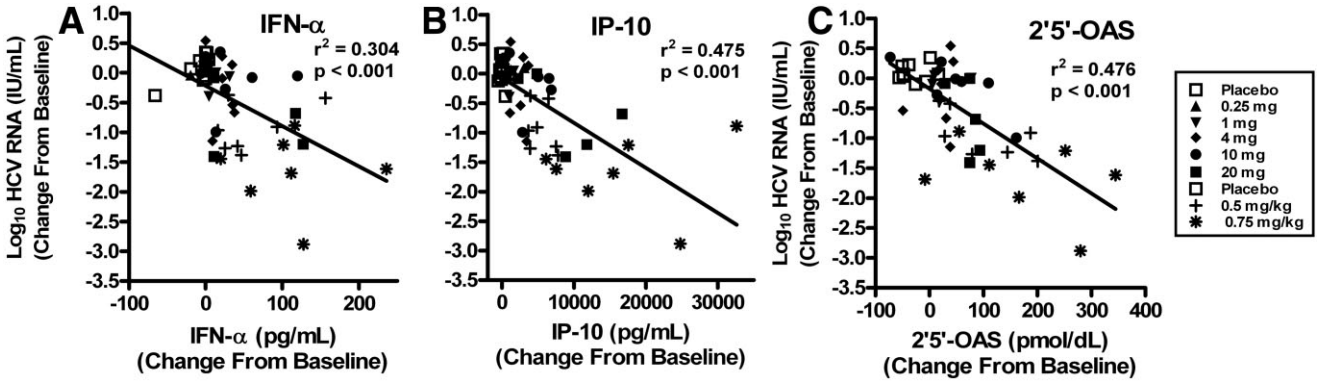

Fig. 3. Correlation of HCV RNA reduction with increases in IFN-α, IP-10, and 2′5′-OAS. The reductions in plasma $\log_{10}$ viral RNA versus the change from baseline of serum (A) IFN-α, (B) IP-10, and (C) 2′5′-OAS all measured 24 hours after the first dose are shown. All patients with very high baseline IFN-α levels [higher than 500 pg/ml, that fell outside the upper 95% confidence limits for baseline levels; placebo group (n = 1), 0.25 mg group (n = 3)] were removed from the analyses to better visualize general trends.

Table 3. Common Adverse Events by Categories of Interest

	Twice-Weekly Dosing						Once-Weekly Dosing		
	CPG 10101						CPG 10101		
	Placebo (n = 10)	0.25 mg (n = 6)	1 mg (n = 6)	4 mg (n = 7)	10 mg (n = 7)	20 mg (n = 6)	Placebo (n = 3)	0.5 mg/kg (n = 7)	0.75 mg/kg (n = 8)
Flu-like systemic reactions (%)*	4 (40)	4 (67)	4 (67)	4 (57)	6 (86)	6 (100)	0	6 (86)	8 (100)
Injection site reactions (%)†	2 (20)	3 (50)	5 (83)	5 (71)	7 (100)	6 (100)	0	5 (71)	4 (50)
Neutropenia (%)‡	0	1 (17)	0	3 (43)	1 (14)	1 (17)	0	0	0
Gastrointestinal disorders (%)§	6 (60)	1 (17)|	2 (33)	1 (14)	2(29)	2 (33)	0	5 (71)	7 (88)
Rash (%)	1 (10)	0	1 (17)	0	0	0	0	0	0
Leukopenia (%)	0	0	0	1 (14)	0	0	0	0	0

*Flu-like systemic reactions include fatigue, rigors, influenza-like illness, pyrexia, malaise, asthenia, and lethargy.

†Injection site reactions include injection site reaction, injection site erythema, injection site pruritus, injection site pain, injection site bruising, injection site dermatitis, injection site inflammation, injection site edema, injection site rash, injection site swelling, injection site urticaria, and injection site warmth.

‡Neutropenia includes neutropenia and decreased neutrophil count.

§Gastrointestinal disorders include nausea, upper abdominal pain, diarrhea, vomiting, abdominal pain, lower abdominal pain, and constipation.

| Does not include a patient with dyspepsia.

CPG 10101 experienced injection site reactions or systemic symptoms, which typically appeared within 12 to 24 hours of dosing. We also observed transient and reversible neutropenia during treatment with CPG 10101. Typically, white blood cell counts and absolute neutrophil counts increased within 24 hours of dosing, followed by a decline that reached a nadir at approximately 48 to 96 hours, depending on dose. Five patients (3 at 4 mg, 1 at 20 mg, and 1 at 0.5 mg/kg) had transient decreases in absolute neutrophil counts of CTC Grade 3. Recovery without intervention occurred 1 to 10 days after dosing. No clinical sequelae have ensued from these transient changes in hematologic parameters. No ALT flares (defined as increases in ALT levels 10× baseline, or 1000 IU/l sustained for 7 days or longer) were observed during this study.

We did not identify a maximum tolerated dose during this dose-escalation study. Two patients did experience serious adverse events. A 37-year-old HCV-positive female who received 10 mg CPG 10101 experienced urticaria and pruritus, delayed in onset, with the reported rash appearing on the last day of injection (injection 8), rash worsening 4 days later, and facial and labial swelling appearing 8 days after the last injection. The patient did not experience shortness of breath or wheezing. She was hospitalized for 1 day, receiving intravenous methylprednisolone. The rash resolved except for a small area; however, follow-up 1 week after discharge indicated that the rash had recurred, though the patient did not require rehospitalization. There were no sequelae. We assessed this event as possibly related to the study drug. Another HCV patient, who received 4 mg CPG 10101, fell and fractured his pelvis and thus required hospitalization. We considered the serious adverse event to be unrelated to CPG 10101 exposure.

Discussion

In this multicenter Phase 1b, randomized, placebo-controlled trial, escalating doses of CPG 10101 were well-tolerated in patients chronically infected with HCV. CPG 10101 treatment was associated with dose-dependent increases in IFN-α, IP-10, and 2′5′-OAS, as well as decreases in HCV RNA levels, and the changes correlated significantly.

The activity of CPG 10101 is consistent with the immunomodulatory activity of CpG ODNs. There are 3 major classes of CpG ODNs, all of which activate TLR9, but which have somewhat different effects.[12] The distinct biology of each class is thought to be related to their differential capacity to form secondary or higher-order structures and localize to discrete intracellular compartments.[16] The A-class have phosphodiester/phosphorothioate chimeric backbones, form complex tertiary structures, and are poor at activating B cells but potent for inducing IFN-α production from pDCs. B-class CpG ODNs have a whole phosphorothioate backbone, do not form secondary or tertiary structures, and are potent B cell activators but weak for inducing pDCs to produce IFN-α. However, they can induce a wide range of serum cytokines, including IP-10 and 2′5′-OAS in normal volunteers and in non-Hodgkin lymphoma patients taking rituximab,[17,18] as well as induce IP-10 production in human monocytes, pDCs, and B cells.[19] C-class CpG ODNs have a whole phosphorothioate backbone and a palindrome that permits formation of secondary structures and dimers, and can induce both B cell proliferation and IFN-α secretion from peripheral blood mononuclear cells of patients with chronic HCV infection.[20] In this study with C-class CPG 10101, serum levels of IFN-α, along with IP-10 and 2′5′-OAS, increased considerably

in a dose-dependent manner within the first 24 hours after administration. Levels of IFN-α and IP-10 typically returned to near baseline levels within 3 to 4 days after administration. Levels of 2′5′-OAS, which is induced by IFN, remained elevated throughout treatment.

Decreases in HCV RNA were dose-dependent, with the greatest group mean maximum reduction of 1.69 $\log_{10}$ observed in the 0.75 mg/kg dose group. Decreases $\geq$1 $\log_{10}$ measured as the maximum change from baseline at any time were seen in groups receiving $\geq$1 mg CPG 10101, with 3 patients exceeding a 2.5-$\log_{10}$ reduction (1 mg, 20 mg, and 0.75 mg/kg). Weekly and twice-weekly dosing regimens resulted in similar HCV responses when patients receiving similar cumulative amounts of CPG 10101 were compared. For example, patients receiving twice-weekly 20 mg doses and those receiving once-weekly 0.5 mg/kg doses received an average cumulative CPG 10101 exposure of 153.3 mg and 148.4 mg, respectively, and their group mean maximum HCV RNA reduction was 1.42 $\log_{10}$ and 1.14 $\log_{10}$, respectively.

We observed HCV RNA reductions with a range of doses, among different ethnic groups, and in genotype 1–infected patients. Although we did not prospectively collect information regarding prior therapy for HCV infection, retrospective telephone calls to study sites enabled patients to be characterized according to their prior treatment experience. Of the 47 patients that received at least 1 dose of CPG 10101, we observed a decrease in HCV RNA levels of $\geq$1 $\log_{10}$ in 2 out of 2 prior treatment-naive patients, 4 out of 9 prior treatment-intolerant patients, 0 out of 6 patients who did not clear the virus on prior treatment, 12 out of 22 prior treatment relapsers, and 4 out of 8 incompletely characterized patients.

Current approved therapies for the treatment of HCV-infected individuals are based upon the combination of IFN-α and ribavirin. IFN-α is an antiviral cytokine, inducing other downstream antiviral cytokines and chemokines (such as 2′5′OAS and IP-10), as well as activating natural killer (NK) cells that may play a role in the clearance of HCV-infected cells. In the present report, we show that CPG 10101 induced 2′5′OAS to levels comparable with those reported for PEG-IFN-α-2b at a dose of 1 μg/kg.[21,22] Additionally, CPG 10101 has been shown to induce NK cell activation to comparable levels as PEG-IFN, based upon the percentage of circulating NK cells expressing CD69.[11] Finally, results from this study suggest that the antiviral effects of CPG 10101 are similar to those induced with therapeutic doses of PEG-IFN in previously published studies involving similar patient populations. Following a single dose of CPG 10101 (0.75 mg/kg), the reduction in HCV RNA levels was 1.69 $\log_{10}$ IU/ml. In comparison, declines ranging from 0.96 $\log_{10}$ to 2.11 $\log_{10}$ have been reported with a single dose of PEG-IFN-α-2b (1 μg/kg and 1.5 μg/kg, respectively).[23,24] Similarly, declines ranging from 0.86 $\log_{10}$ to 1.08 $\log_{10}$ have been reported for HCV patients injected once with 180 μg pegylated IFN-α-2a.[24,25] Therefore, comparison of immune and antiviral activities induced by CPG 10101 and IFN suggests that CPG 10101 may provide similar antiviral effects and modulation of the innate immune response.

The control of either acute or chronic HCV infection, however, is thought to be dependent not only on the induction of innate immune responses, but also on the development of broad and sustained adaptive T cell responses directed to HCV antigenic determinants.[7] TLR9 agonist CpG ODNs have been shown to be very potent enhancers of adaptive immune responses both in animal models and humans.[26] We therefore hypothesize that CPG 10101 may have a broader spectrum of activities than IFN through better induction of HCV-specific immune responses. Interestingly, although we did not design the current study to properly address the issue of anti-HCV adaptive responses, we have observed that CPG 10101 was able to induce a robust polyclonal activation of T cells.[11]

In conclusion, our results indicate that CPG 10101 has HCV antiviral activity across a range of doses and is generally well-tolerated. The direct *in vivo* pharmacodynamic and antiviral activity of CPG 10101 appears to be similar to that seen with PEG-IFN, but the broader. mechanism of action of CPG 10101 may be especially important to consider in the context of emerging HCV-specific antiviral drugs such as protease and polymerase inhibitors. Recent reports of dramatic reduction in viral loads obtained with protease and polymerase inhibitors suggests that HCV-specific antiviral drugs will be incorporated in future therapies for chronic HCV infection.[27] Development of CPG 10101 as therapy for HCV in conjunction with PEG-IFN and/or ribavirin has been temporarily suspended to assess the impact of these new small molecules on HCV therapy. Although HCV-specific drugs may provide superior antiviral effect over the current therapies, they may not affect adaptive immune responses. In all likelihood, HCV therapy will continue to require the presence of an effective immunomodulatory agent, and CPG 10101 remains a possible candidate for this role.

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