

Oral resiquimod in chronic HCV infection: Safety and efficacy in 2 placebo-controlled, double-blind phase IIa studies ^{☆,☆☆}

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Background/Aims: To explore safety, pharmacokinetics, and pharmacodynamics of oral administration of resiquimod, a Toll-like receptor 7 and 8 agonist that induces endogenous interferon- α , in subjects with chronic hepatitis C virus infection.

Methods: Two randomized, double-blind phase IIa studies of resiquimod administered two times per week for 4 weeks. Multicenter study (U.S.): 12 subjects received resiquimod 0.01 mg/kg and 4 received placebo. Single center study (France): 6 subjects received 0.01 mg/kg, 11 received 0.02 mg/kg and 6 received placebo.

Results: Resiquimod 0.01 mg/kg was tolerated; two 0.2 mg/kg subjects discontinued treatment. More subjects reported severe grade adverse events at 0.02 mg/kg; events were consistent with systemic cytokine induction, including fever, headache, shivering, and lymphopenia. Mean maximum serum resiquimod concentrations were 3.82 ± 1.47 and 7.55 ± 4.17 ng/mL for 0.01 mg/kg and 0.02 mg/kg, respectively. At 0.02 mg/kg, two, three and one subjects had maximal reductions in viral levels of at least 1-, 2- and 3-logs, respectively; reductions were generally transient. Interferon- α levels appeared correlated with decreases in viral titer and lymphocyte counts, as well as increase in neutrophil counts.

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Conclusions: Oral administration of resiquimod 0.02 mg/kg transiently reduced viral levels but was associated with adverse effects similar to interferon-alpha.

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1. Introduction

An estimated 170 million people worldwide are infected with the hepatitis C virus (HCV) [1,2]. Continued inflammation and regeneration of the liver associated with chronic infection may result in cirrhosis and/or hepatocellular carcinoma. HCV infection is the most common cause for liver transplantation in the United States [1]. Interferon-alpha (IFN- α) has been the mainstay of treatment for chronic HCV, first as monotherapy and then in combination with ribavirin [3]. A substantial portion of patients with HCV genotype 1, the most prevalent genotype in the United States (U.S.) and Western Europe, however, still have an inadequate response.

The introduction of pegylated IFN- α in combination with ribavirin has reduced the frequency of subcutaneous administration from several times per week to once weekly [4–6]. An oral therapy with similar effects as parenterally administered IFN- α , however, may have advantages in terms of ease of usage and compliance. Resiquimod (S-28463, R-848, VML600) is related to imiquimod, a small molecule approved as a topical agent (imiquimod 5% cream) for the treatment of external anogenital warts, superficial basal cell carcinoma, and actinic keratoses [7]. In humans, resiquimod signals through both the Toll-like receptor (TLR) 7 and 8 pathways, inducing cytokines including IFN- α , interleukin (IL)-12 and tumor necrosis factor alpha (TNF- α) from innate immune cells [8–10]. Resiquimod also activates natural killer cells, upregulates antigen presentation, and indirectly induces IFN-gamma (IFN- γ), and therefore may promote the development of antigen-specific cell-mediated responses [11,12]. An effective HCV-specific CD4+ and CD8+ T lymphocyte response appears required for resolution of HCV infection [13–15]. In a placebo-controlled, single administration study in 48 healthy adults of up to 0.05 mg/kg, the maximum tolerated oral dose of resiquimod was 0.03 mg/kg; in a placebo-controlled, multiple administration study in 25 healthy adults, the maximum administered regimen of 0.2 mg/kg two times per week for 2 weeks followed by 0.03 mg/kg two times per week for 2 weeks was adequately tolerated (personal communication, T. Meng).

We report here the results of two phase IIa clinical studies, conducted concurrently, of oral administration of resiquimod in subjects with chronic HCV infection. The primary objective of each study was to assess the safety; secondary objectives were to evaluate pharmaco-

2. Patients and methods

2.1. Patient selection and randomization

The applicable study protocol was reviewed and approved by the Institutional Review Board (IRB) of each study center. Before study-specific procedures were performed, written informed consent was obtained. The studies were conducted in accordance with international guidelines and recommendations for clinical studies. Subjects were enrolled at three study centers in the United States (U.S. study) and at one in France (French study).

For both studies, major inclusion criteria were males or females 18–70 years of age who had evidence of chronic HCV infection with all of the following: positive HCV serology by enzyme-linked immunosorbent assay, serum HCV RNA >10,000 copies/mL, elevated serum alanine aminotransferase (ALT) level within 6 months, and a liver biopsy within 24 months demonstrating changes consistent with HCV infection. Exclusion criteria included clinically meaningful cirrhosis on prior liver biopsy (U.S. study), positive serology for possible autoimmune hepatitis (ANA \geq 1:640, ASMA > 1:320, ALKM antibody > 1:320), hepatocellular neoplasia, anemia (<12 g/dL for men, <11 for women), thrombocytopenia (<90,000/ μ L), leukopenia (<2500 cells/ μ L), neutropenia (<1500 cells/ μ L, U.S. study), ALT > 1000 U/L (French study) or aspartate aminotransferase (AST) or ALT > 500 U/L (U.S. study), bilirubin >1 mg/dL, decompensated liver disease, other liver diseases, positive serology for HIV, positive HBsAg, prior organ transplantation, significant psychiatric disease, alcohol or drug abuse within 12 months, systemic immunomodulatory or investigational therapy within 3 months, and significant cardiac, pulmonary, systemic inflammatory or thyroid disease.

For both studies, treatment assignment within each cohort (16 subjects U.S. study; 8 subjects French study) was determined via computer-generated randomization. In the U.S. study, subjects were assigned centrally across centers. Active to placebo assignment was 3:1 for each cohort. Sample sizes were not prospectively powered.

2.2. Study design

All subjects were to receive study drug two times per week for 4 weeks. Subjects self-administered study drug at home except on study visits with pharmacokinetic and pharmacodynamic sampling where it was administered in the clinic. Resiquimod or matching placebo was administered as oral capsules (3M Pharmaceuticals, Saint Paul, MN). In the U.S. study, subjects received 0.01 mg/kg of resiquimod. In the French study, sequential cohorts were to have received 0.01, 0.02 and 0.03 mg/kg (due to an adverse event, this dose level was not enrolled, see Results) of resiquimod per dose, respectively; a safety review was performed prior to escalation.

2.3. Safety

Safety was assessed based on adverse events, laboratory tests (hematology, biochemistry, urinalysis and where applicable, pregnancy tests), vital signs measurements, physical examination, and 12-lead electrocardiograms. Routine laboratory tests were performed at National Genetics Institute (NGI), Los Angeles, CA (U.S. study), or Centre de Lutte contre le cancer Eugene Marquis, Rennes, France (French study).

2.4. Pharmacokinetics

Pharmacokinetic samples were obtained at 0, 0.25, 0.5, 1, 1.5, 2, 4,

0.25, 0.5, 1, 1.5, 2, 4, 8 and 12 h after the second to last (day 22) or last dose (day 25) in the U.S. study, at 0 and 1 h after the last dose (day 25) in the French study. Serum levels of resiquimod were measured via liquid chromatography/mass spectrometry (Covance Laboratories Inc., Madison, WI). Pharmacokinetic analyses were performed by MedEval Ltd., Manchester, United Kingdom, using WinNonlin Professional (Pharsight Co., Mountain View, CA). No formal pharmacodynamic–pharmacokinetic analysis was performed. The relationships between cytokine/biomarker levels, viral load, and resiquimod concentrations were investigated by plotting the maximal response (R_{max}) of these variables against the maximum serum concentration (C_{max}) of resiquimod. Cytokine values below or above the limits of quantitation were set at those limits for calculations. R_{max} (HCV RNA decrease, other biomarker increase) was determined on log transformed values (\log_{10} HCV, natural log for others); negative values were set at 0. Simple linear regression was used to assess the relationship between resiquimod C_{max} and R_{max} (SAS, SAS Institute Inc., Cary, NC).

2.5. Pharmacodynamics

Serum HCV RNA was measured by quantitative polymerase chain reaction (NGI). Subjects were categorized as responders (reduction from baseline of ≥ 2 logs) or non-responders at the end-of-treatment visit (day 29), and at the last follow-up visit (day 113 U.S. study, and day 57 French study).

Samples for cytokines were obtained at 0, 2, 4, 6, 8, 12 and 24 h after the first dose, prior to dosing at days 8, 15, 22 or 25 (see above regarding pharmacokinetics) and day 29. Serum IL-6, IL-1RA,

TNF- α and IFN- γ were measured by enzyme-linked immunosorbent assay (Immunotech, Cedex, France) and neopterin by immunoenzymatic assay (Immunotech, Cedex, France). Serum type I IFN levels were determined by bioassay [16]. Serum 2',5' oligoadenylate synthetase (2'5' AS) was measured by radioimmunoassay (Eiken Chemical Co. Ltd., Tokyo, Japan). Serum IL-12 p40 was measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Immunophenotyping of T lymphocytes in the U.S. study was performed at NGI.

3. Results

3.1. Subjects

In the U.S. study, 27 subjects were screened, 16 were randomized to study drug (Table 1) and 15 completed the study; 1 subject discontinued (0.01 mg/kg) following withdrawal of consent. The first subject enrolled April 2000 and the last subject completed study in October 2000. Most subjects were male (81%) and Caucasians (81%). Compared with the resiquimod group, the placebo group had a slightly lower age, lower body weight, and higher alcohol consumption.

Table 1
Baseline characteristics of study populations, by study

Characteristic	US study		French study		
	0.01 mg/kg	Placebo	0.01 mg/kg	0.02 mg/kg	Placebo
Subject number	12	4	6	11	6
Gender ^a					
Male	11 (92%)	2 (50%)	6 (100%)	7 (64%)	2 (33%)
Female	1 (8%)	2 (50%)	0	4 (36%)	4 (67%)
Race ^a					
Caucasian	11 (92%)	2 (50%)	6 (100%)	11 (100%)	6 (100%)
Other	1 (8%)	2 (50%)			
Age ^b (years)	48.1 \pm 5.0	43.3 \pm 5.4	48.0 \pm 9.8	45.6 \pm 9.1	49.8 \pm 11.3
Range	40–58	38–50	36–60	34–64	36–64
Weight ^b (kg)	92.5 \pm 13.0	76.3 \pm 10.3	76 \pm 5.1	66.9 \pm 12.5	60.5 \pm 8.1
Alcohol intake (U/week)	0.1 \pm 0.29	0.8 \pm 0.5	0.7 \pm 1.6	2.6 \pm 4.6	0.8 \pm 1.3
HCV genotype ^a					
1a	6 (50%)	2 (50%)	1 (17%)	1 (9%)	2 (33%)
1b	5 (42%)	2 (50%)	4 (67%)	4 (36%)	4 (67%)
1/2	0	0	0	1 (9%)	0
2a	0	0	0	1 (9%)	0
3a	1 (8%)	0	1 (17%)	3 (27%)	0
4c/4d	0	0	0	1 (9%)	0
Prior treatment					
Yes ^c	11 (92%)	4 (100%)	5 (83%)	7 (64%)	4 (67%)
IFN ^d	8 (67%)	2 (50%)	5 (83%)	6 (55%)	4 (67%)
Responder ^e	0 (0%)	0 (0%)	0 (0%)	2 (18%)	1 (17%)
IFN + ribavirin ^f	4 (33%)	3 (75%)	3 (50%)	5 (45%)	3 (50%)
Responder ^e	0 (0%)	0 (0%)	2 (33%)	3 (27%)	2 (33%)
HCV load (log day 1)					
Median (range)	6.71 (6.36, 7.59)	7.05 (6.86, 7.26)	6.81 (5.63, 7.67)	6.48 (4.85, 6.79)	6.51 (4.81, 6.98)
Mean \pm SD	6.86 \pm 0.40	7.05 \pm 0.17	6.81 \pm 0.76	6.10 \pm 0.70	6.26 \pm 0.80

^a Results reported as subject number and percent of subjects (%).

^b Results reported as means \pm standard deviation.

^c Including interferon (IFN)- α , IFN- β , pegylated IFN- α or IFN- α con 1, ribavirin and/or IL-12.

^d Including IFN- α , IFN- β , pegylated IFN- α or IFN- α con 1 monotherapy.

^e If multiple treatment courses, counted as responder if responded and relapsed after any single treatment course.

^f Some subjects had prior IFN monotherapy as well as in combination with ribavirin.

In the French study severe grade lymphopenia in a subject in the 0.02 mg/kg cohort and higher than expected serum resiquimod C_{max} in some subjects resulted in cohort 3 receiving 0.02 mg/kg rather than 0.03 mg/kg. In total, 29 subjects screened, 23 were randomized to study drug (Table 1) and 21 completed the study. The first subject enrolled January 2000 and the last subject completed study in August 2000. Most subjects were male (65%) and all were Caucasian. The body weight for the combined placebo group was lower than those for the resiquimod cohorts.

3.2. Safety

There were no serious adverse events. No subject in the U.S. study discontinued treatment for adverse

events. In the French study, one subject (cohort 2) discontinued on day 4 because of severe grade lymphopenia and one subject (cohort 3) withdrew on day 19 after severe grade flu-like symptoms. One subject in cohort 2 was dose reduced to 0.01 mg/kg on day 11 due to lymphopenia, but resumed 0.02 mg/kg dosing on day 15.

In both studies, the proportions of subjects reporting adverse events considered to be treatment-related were higher in the resiquimod groups than in the placebo groups. Pyrexia, fatigue, headache, shivering and back pain were the adverse events, considered possibly or probably related to study drug, reported by the most resiquimod subjects (Table 2). In the U.S. study, most adverse events were mild in severity; only one severe grade adverse event was reported (fatigue, resiquimod).

Table 2
Possibly or probably related treatment-emergent adverse events reported by >20% of subjects^a, by treatment regimen, studies combined

System organ class preferred term	0.01 mg/kg		0.02 mg/kg		Placebo	
	All grades	Severe	All grades	Severe	All grades	Severe
Total subjects:	18		11		10	
Subjects:		4 22 (%)		8 73 (%)		1 10 (%)
General disorders and administration site conditions	16 89%	4 22	10 91%	8 73	4 40%	1 10
Fatigue	11 61%	2 11	8 73%	2 18	4 40%	1 10
Pain	7 39%	0 0	0 0%	0 0	0 0%	0 0
Pyrexia	13 72%	1 6	10 91%	8 73	0 0%	0 0
Shivering	4 22%	2 11	8 73%	4 36	0 0%	0 0
Blood and lymphatic system disorders	1 6%	0 0	10 91%	6 55	1 10%	0 0
Anemia	0 0%	0 0	2 18%	0 0	0 0%	0 0
Lymphopenia	1 6%	0 0	10 91%	6 55	1 10%	0 0
Monocytosis	0 0%	0 0	2 18%	0 0	0 0%	0 0
Neutropenia	1 6%	0 0	5 45%	0 0	1 10%	0 0
Eye disorders	0 0%	0 0	1 9%	0 0	0 0%	0 0
Gastrointestinal disorders	8 44%	0 0	8 73%	1 9	3 30%	0 0
Abdominal pain	2 11%	0 0	3 27%	0 0	1 10%	0 0
Diarrhea	0 0%	0 0	4 36%	0 0	2 20%	0 0
Nausea	5 28%	0 0	4 36%	1 9	1 10%	0 0
Vomiting	0 0%	0 0	3 27%	1 9	0 0%	0 0
Hepatobiliary disorders	0 0%	0 0	1 9%	0 0	0 0%	0 0
Infections and infestations	2 11%	0 0	3 27%	1 9	2 20%	0 0
Herpes simplex	1 6%	0 0	3 27%	1 9	0 0%	0 0
Investigations	0 0%	0 0	1 9%	1 9	0 0%	0 0
Metabolism and nutrition disorders	1 6%	0 0	4 36%	0 0	0 0%	0 0
Appetite decreased	1 6%	0 0	3 27%	0 0	0 0%	0 0
Musculoskeletal, connective tissue and bone disorders	9 50%	1 6	9 82%	4 36	6 60%	0 0
Arthralgia	4 22%	0 0	2 18%	1 9	0 0%	0 0
Back pain	4 22%	1 6	7 64%	3 27	1 10%	0 0
Neck stiffness	0 0%	0 0	3 27%	1 9	1 10%	0 0
Pain in limb	0 0%	0 0	2 18%	1 9	3 30%	0 0
Sensation of heaviness	0 0%	0 0	2 18%	0 0	0 0%	0 0
Nervous system disorders	11 61%	2 11	10 91%	4 36	6 60%	0 0
Headache	10 56%	2 11	9 82%	4 36	5 50%	1 10
Psychiatric disorders	2 11%	1 6	1 9%	0 0	1 10%	0 0
Respiratory, thoracic and mediastinal disorders	0 0%	0 0	4 36%	2 18	1 10%	0 0
Skin and subcutaneous tissue disorders	2 11%	0 0	1 9%	0 0	0 0%	0 0
Vascular disorders	1 6%	1 6	4 36%	1 9	1 10%	0 0

^a At the preferred term level for any regimen. System organ class total may exceed total for displayed preferred terms.

In the French study, all groups reported adverse events of severe intensity. More subjects receiving 0.02 mg/kg (73%) reported severe grade adverse events than those receiving 0.01 mg/kg (22%) or placebo (10%), especially with respect to pyrexia (Table 2).

In both studies, there were no clinically meaningful changes in physical examinations. A dose-dependent initial increase in absolute neutrophil count (ANC) and decrease in absolute lymphocyte count were observed post-dose (Table 3); ANC subsequently appeared decreased, overall, in the resiquimod groups (Table 3). Subjects in the 0.02 mg/kg group had greater maximum grade ANC and ALC toxicity (at any time during treatment period) by Common Terminology Criteria for Adverse Events (CTCAE) (Table 3). Of the 9 subjects with severe pyrexia, 6 had grade 3 and 2 had grade 4 ALC decrease, and 2 each had grade 2 and grade 3 ANC decrease.

Neither ALT nor AST levels appeared to be affected in the U.S. study (data not shown). In the French study, the proportion of subjects with AST and ALT elevations decreased slightly from day 1 to day 29 in the 0.02 mg/kg group, 55% (6/11) to 11% (1/9) and 82% (9/11) to 56% (5/9), respectively.

3.3. Pharmacokinetics

Resiquimod concentrations after single (Table 4) and multiple dosing rose rapidly, reaching C_{max} between 0.5 and 2.0 h post-dose. Thereafter, resiquimod concentrations appeared to decline in a biphasic manner, the terminal phase becoming apparent between 8 and 16 h post-dose. With dose doubling there was almost a 2-fold increase in both mean serum resiquimod C_{max} and area under the curve (AUC), suggesting linear kinetics within the dose range studied (Table 4). There was little or no evidence of drug accumulation on repeat dosing as determined by drug levels measured on day 22/25 for the U.S. study or day 15 for the French study (data not shown). Large inter-subject variability was observed, with day 1 coefficients of variance for C_{max} of 39% and 44% for 0.01 mg/kg (U.S. study and French study, respectively) and 55% for 0.02 mg/kg. Despite the large inter-subject variability, little intra-subject variability was observed for C_{max} or AUC; comparable values were obtained after single and repeated administration for a subject (data not shown). The two 0.02 mg/kg subjects who discontinued treatment for severe grade lymphopenia and for severe grade

Table 3
Change from baseline in absolute neutrophil and lymphocyte counts

	Change in absolute neutrophil count (cells/mm ³)			Change in absolute lymphocyte count (cells/mm ³)		
	Placebo	0.01 mg/kg	0.02 mg/kg	Placebo	0.01 mg/kg	0.02 mg/kg
Day 1 (8 h)						
<i>N</i>	10	18	11	10	18	11
Median (range)	480 (54, 3130)	677 (-970, 5200)	3170 (-2270, 6460)	174 (-600, 650)	-595 (-1595, 508)	-1720 (-3080, -50)
Mean ± SD	760 ± 881	804 ± 1312	2875 ± 2411	123 ± 364	-638 ± 641	-1638 ± 774
Day 1 (24 h)						
<i>N</i>	4 ^a	11 ^a	6 ^a	4 ^a	11 ^a	6 ^a
Median (range)	43 (-326, 788)	-533 (-1098, 1667)	-650 (-4530, 1700)	-232 (-416, -65)	-148 (-755, 656)	-520 (-2550, -250)
Mean ± SD	137 ± 546	-152 ± 781	-878 ± 2148	-236 ± 162	-156 ± 398	-965 ± 892
Day 29 end-of-treatment visit						
<i>N</i>	10	18	9 ^b	10	18	9 ^b
Median (range)	-166 (-1060, 980)	-65 (-3680, 491)	-470 (-3790, 1760)	-251 (-930, 340)	-153 (-1440, 969)	-295 (-2040, 250)
Mean ± SD	-54 ± 549	-452 ± 999	-727 ± 1647	271 ± 385	-166 ± 506	-478 ± 620
Maximum decrease during treatment period						
<i>N</i>	10	18	11	10	18	11
Median (range)	-328 (-1244, 300)	-738 (-4780, -15)	-730 (-4530, 360)	-550 (-1228, -10)	-865 (-1595, 0)	-1796 (-3080, -310)
Mean ± SD	-374 ± 463	-966 ± 1078	-1359 ± 1370	-530 ± 370	-889 ± 510	-1670 ± 723
Maximum toxicity						
	Neutropenia ^c (subject <i>N</i> , %)			Lymphopenia ^d (subject <i>N</i> , %)		
Grade 1	3 (30%)	5 (28%)	1 (9%)	1 (10%)	1 (6%)	2 (18%)
Grade 2	2 (20%)	5 (28%)	3 (27%)	0	1 (6%)	0
Grade 3	1 (10%)	0	2 (18%)	0	0	6 (55%)
Grade 4	0	0	0	0	0	2 (18%)

^a Not all subjects in French study had sampling at 24 h post dosing Day 1. One subject in U.S. study missing sample.

^b Two subjects discontinued treatment prior to day 29 end-of-treatment visit.

^c ANC grade 1 < lower limits of normal to 1500, grade 2 < 1500–1000, grade 3 < 1000–500, grade 4 < 500 cells/μL. Lower limits of normal 2250 cells/mm³ for U.S. and 1700 cells/mm³ for French study.

^d ALC grade 1 < lower limits of normal to 800, grade 2 < 800–500, grade 3 < 500–200, grade 4 < 200 cells/μL. Lower limits of normal 675 cells/mm³ for U.S. and 1200 cells/mm³ for French study.

Table 4
Pharmacokinetic parameters of resiquimod following oral administration, first dose, studies combined

	0.01 mg/kg	0.02 mg/kg
Total subjects	12	11
T_{\max}^a (h)	1.0	1.0
C_{\max}^b (ng/mL)	3.82 ± 1.47	7.55 ± 4.17
AUC ^c (ng h/mL)	20.97 ± 13.65	45.66 ± 43.98
$T_{1/2,z}$ (h)	6.77 ± 3.10	6.82 ± 3.51
CL/F (L/h/kg)	0.57 ± 0.42	1.11 ± 1.54
Vz/F (L/kg)	4.29 ± 1.84	6.58 ± 3.83

$T_{1/2,z}$: terminal phase half-life. Ln2 divided by apparent terminal phase rate constant estimated by log linear regression of at least three data concentration–time points after T_{\max} . Results reported as means ± standard deviation.

CL/F: apparent clearance. Results reported as means ± standard deviation.

Vz/F: apparent volume of distribution. Results reported as means ± standard deviation.

^a T_{\max} : time of maximum drug concentration, determined by direct inspection of the drug concentration versus time data point values. Results reported as median.

^b C_{\max} : maximum observed drug concentration, determined by direct inspection of the drug concentration versus time data point values. Results reported as means ± standard deviation.

^c AUC: area under the curve concentration versus time curve extrapolated to infinity, calculated by extrapolation of the elimination slope from t_z to infinity (t_z = time point for last sample on pharmacokinetic profile with quantifiable drug). Results reported as means ± standard deviation.

flu-like symptoms had resiquimod C_{\max} values of 12.7 and 10.8 ng/mL, respectively.

3.4. Pharmacodynamics

After the first dose, there appeared to be a dose-dependent decrease in serum HCV RNA levels peaking at about 24 h and trending toward baseline by 48 h (Fig. 1). One, five and six subjects had at least a 1-log reduction in HCV levels at any time during the study for the placebo, resiquimod 0.01 and 0.02 mg/kg groups, respectively (Fig. 2). Two, three and one subjects in the 0.02 mg/kg group had maximal decreases of at least 1-, 2- and 3-logs, respectively (Fig. 2). Of the 11 resiquimod subjects with at least a 1-log reduction at anytime during the study, the HCV R_{\max} occurred within 48 h after dose 1 in 6 subjects, and at day 29 or after in 5 subjects. At end-of-treatment visit only one subject (0.02 mg/kg) was considered a responder per protocol (≥ 2 log reduction); this was not sustained on follow-up.

There appeared to be a possible relationship between resiquimod C_{\max} and R_{\max} after dose 1 for HCV RNA (adjusted R^2 0.4833, Spearman correlation coefficient 0.51503, $p < 0.0008$), IFN- γ (0.5940, 0.6196, <0.0001), IL-1RA (0.6350, 0.7698, <0.0001), IFN- α (0.5118, 0.6354, <0.0001) and NPT (0.5301, 0.68610, <0.0001 ;

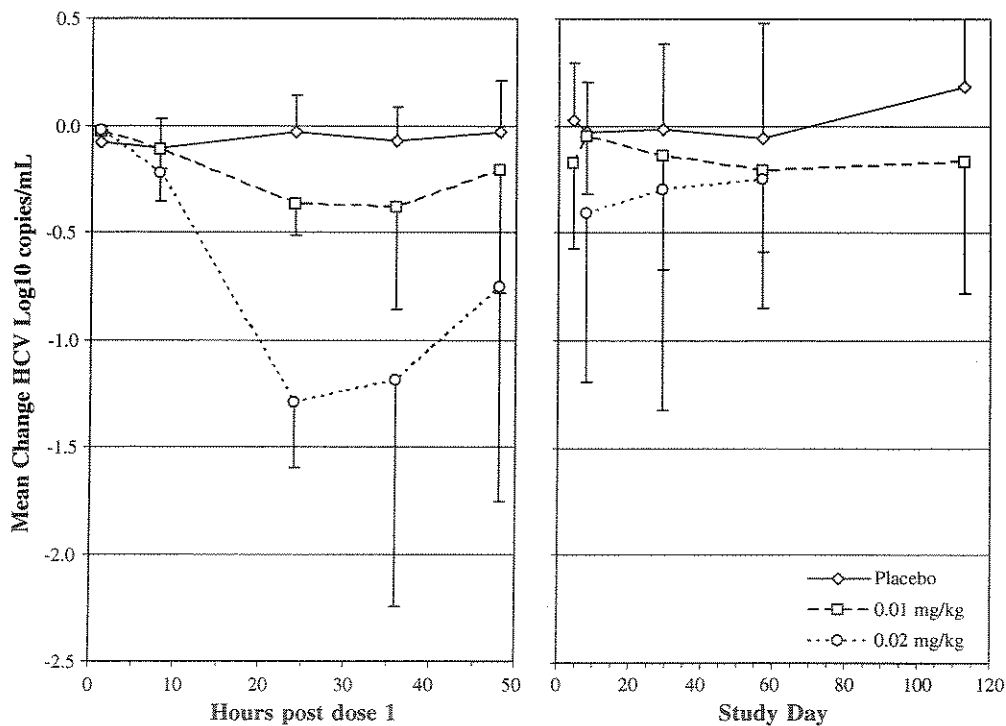


Fig. 1. Mean change in subject HCV log₁₀ copies/mL (± standard deviation) after initial dose (left) and during rest of study (right), by regimen. Study 1 and study 2 combined. Days 4 and 113, study 1 subjects only.

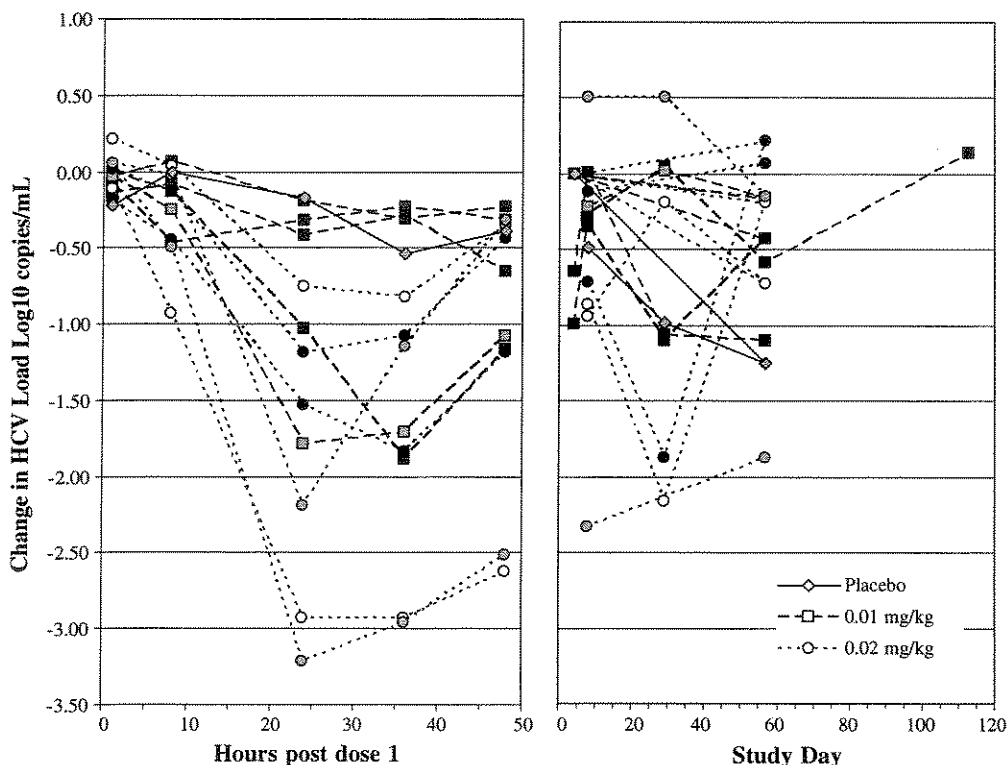


Fig. 2. Change in individual subject HCV log₁₀ copies/mL after initial dose (left) and during rest of study (right) for subjects that had at least 1 log decrease at anytime during study. Days 4 and 113, study 1 subjects only. Open symbol: naïve subject, gray symbol: prior treatment responder/relapser, black symbol: prior treatment non-responder.

Figs. 3a–c). IFN- α R_{\max} appeared to be associated with HCV R_{\max} (adjusted R^2 0.4944, Spearman correlation coefficient 0.6204, $p < 0.0001$; Fig. 3d) and 8 h change ALC (0.7369, -0.7495 , <0.0001) and possibly within 8 h change in ANC (0.3628, 0.4598, 0.0032; Fig. 3d) Median IFN- α R_{\max} after dose 1 appeared to be higher in those subjects who had a maximum CTCAE grade of 3 for ANC, 3 or 4 for ALC, and who had severe pyrexia (Fig. 3f). The two resiquimod 0.02 mg/kg subjects who discontinued treatment for severe grade lymphopenia and for severe grade flu-like symptoms had IFN- α R_{\max} post-dose 1 of 15,557 and 3946 IU/mL, respectively. There did not appear to be evidence of a relationship between resiquimod C_{\max} and the R_{\max} after the dose 1 with IL-6 (adjusted R^2 0.1280, Spearman correlation coefficient 0.4023, $p < 0.0111$), IL-12 p40 (0.0156, 0.2062, 0.2079), 2'5' AS (0.0194, 0.1945, 0.2354) and TNF- α (-0.0244 , 0.0989, 0.5491). Clinically relevant changes in CD4+ lymphocyte counts or CD4+/CD8+ lymphocyte ratios were not observed (data not shown).

4. Discussion

Parenteral IFN- α , including peg IFN- α , alone or in combination with ribavirin, remains the standard for the treatment of chronic HCV infection. Resiquimod appears to induce systemic IFN- α primarily from plas-

macytoid dendritic cells via the TLR7 pathway. TLR7 expression also has been reported to be expressed on hepatocytes, including in HCV-infected hepatocytes, as well as the Huh-7 cell line [17]. Supernatant from peripheral blood lymphocytes stimulated with resiquimod, as well as with the TLR7 agonist SM360320, significantly inhibited replication of HCV replicons in Huh-7 cells. The inhibition was associated with STAT-1 induction. SM360320 was also reported to have a TLR7-mediated, IFN- α independent, direct antiviral effect.

Oral administration of resiquimod 0.01 mg/kg two times per week for 4 weeks was adequately tolerated. With oral dosing of resiquimod 0.02 mg/kg, however, 2 of 11 subjects discontinued treatment with IFN-like side effects; this was in contrast to prior experience in healthy subjects. Although conclusions are limited by the number of subjects, there appeared to be greater systemic exposure with respect to mean serum resiquimod C_{\max} and AUC, as well as greater pharmacokinetic variability, in these HCV-infected subjects than previously observed in healthy subjects. Although these HCV-infected subjects did not have decompensated liver disease, it is possible that HCV-related liver disease may have contributed to these observed pharmacokinetic differences. In general, the adverse events were consistent with the effects of cytokines known to be induced by resiquimod, including IFN- α . In contrast to the acute ANC increase observed after oral resiquimod dosing in this

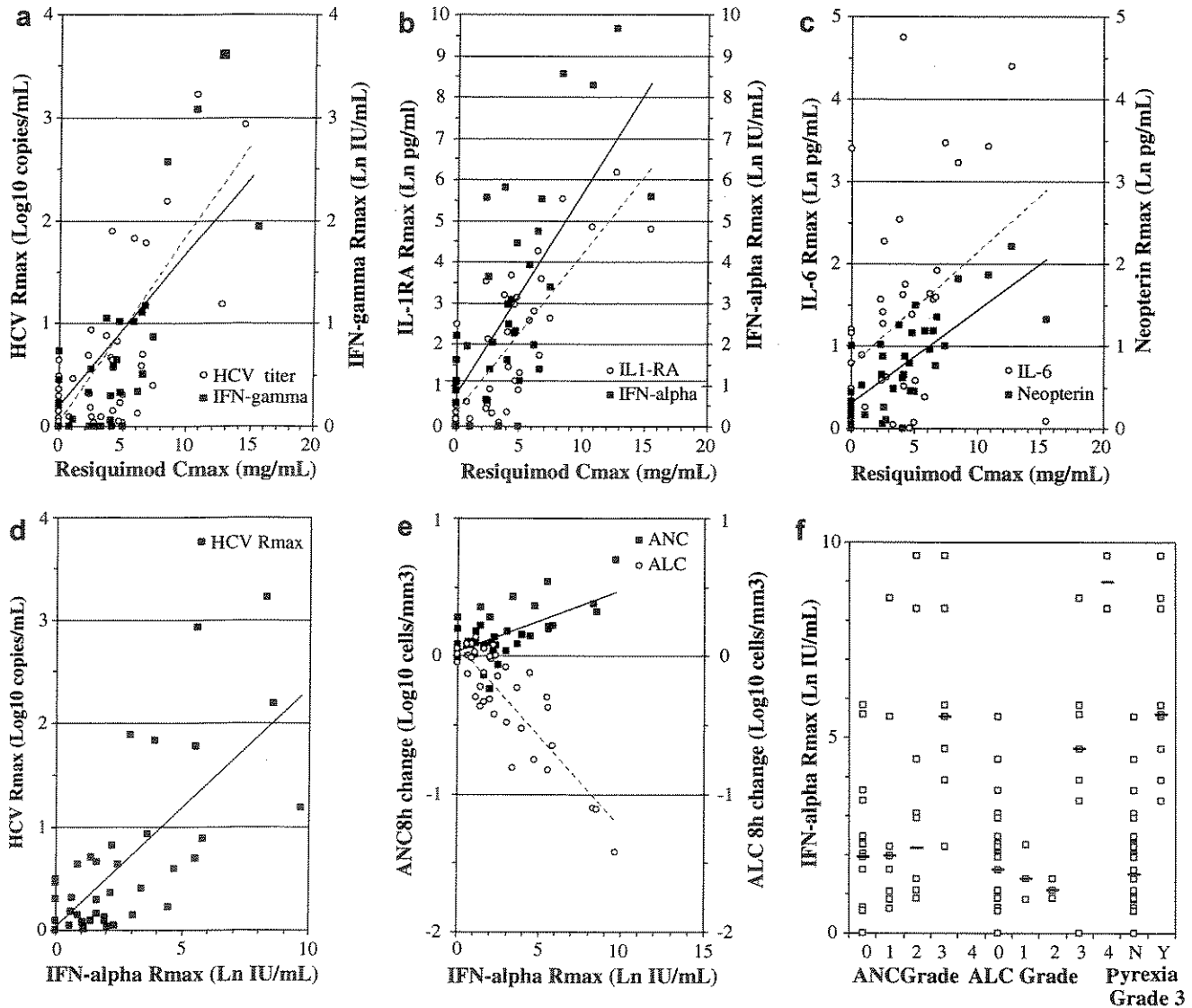


Fig. 3. By subject maximum biomarker response (R_{max}). (a) Post-dose 1 resiquimod C_{max} versus HCV RNA (\log_{10}) decrease and IFN- γ (Ln) increase, (b) resiquimod C_{max} versus IL-1 RA (Ln) and IFN- α (Ln) increase, (c) resiquimod C_{max} versus IL-6 (Ln) and neopterin (Ln) increase, (d) IFN- α R_{max} versus HCV RNA, (e) IFN- α R_{max} versus 8 h change in absolute neutrophil (Ln ANC) and absolute lymphocyte (Ln ALC) counts. Solid trendline for left variable, broken trendline for right variable. (f) Maximum ANC and ALC toxicity grade during treatment period and severe pyrexia versus IFN- α R_{max} . Bar indicates median for group.

study, transient decreases were observed after topical administration [18]. The changes in ANC and ALC likely reflect cellular trafficking; increases in vascular endothelial adhesiveness for leukocytes have been described in mice exposed to resiquimod [19].

Oral administration of resiquimod 0.01 mg/kg also appeared to have minimal effects with respect to cytokine induction or reduction in HCV RNA levels. Cytokine induction was more consistently observed at 0.02 mg/kg, and 5 of 11 subjects had at least a 1-log reduction in HCV RNA levels after the first dose. Treatment assignment was not stratified in either study based on prior HCV treatment history; however, of the 11 resiquimod subjects who had at least a 1-log reduction in

HCV RNA observed during the study, 6 and 3 were prior treatment non-responders and relapsers, respectively. There appeared to be a relationship between resiquimod C_{max} and R_{max} after dose 1 with HCV RNA decrease and IFN- α , IFN- γ , IL-1RA and IL-6 increases, as well as for IFN- α with HCV.

Despite the initial decreases in HCV RNA levels observed immediately after the first dose in the 0.02 mg/kg group, however, only one subject was considered a responder at the end of the 4 weeks of treatment. The immediate antiviral effect observed was likely a reflection of a short term cytokine-release from immune cells, rather than from augmentation of HCV-specific cell mediated immunity. There did not appear

to be further decreases in HCV RNA prior to dosing with continued dosing. Two times per week dosing was chosen because it was anticipated that the pharmacodynamic effects would reflect the kinetics of induced cytokines and IFN-stimulated gene products, rather than drug levels. Dosing appeared suboptimal to maintain an antiviral state, probably allowing viral rebound prior to the next dose. Achieving sustained 2-log reduction may have been overly ambitious for a treatment duration of 4 weeks, especially for monotherapy in the subject population, many of which had relatively high baseline HCV RNA levels, predominantly genotype 1 infection, and prior failure to respond to HCV therapy.

Other TLR agonists similar to resiquimod are being studied as therapy for chronic HCV infection [20]. Isatoribine, an injectable TLR7 agonist, reduced viral loads in patients with HCV; an oral prodrug of isatoribine, ANA975, is in early development and has been reported to have excellent bioavailability [21]. Other TLRs are also being targeted for the treatment of HCV, such as the TLR9 agonist CpG oligonucleotides; currently these require injection [22]. It remains unclear whether any of these compounds will have adequate and consistent oral pharmacokinetics in patients with HCV, as well as having efficacy comparable to parenteral IFN- α while improving tolerability. While the broadened profile cytokines induced by some of the TLR agonists may enhance efficacy compared to IFN- α , they may also result in additional side effects, either alone or when used in combination therapy.

In summary, oral administration of resiquimod at 0.01 mg/kg two times per week was adequately tolerated but had limited reduction in HCV levels. At 0.02 mg/kg, resiquimod more consistently induced several cytokines and transiently reduced HCV levels. However, these reductions were not sustained, and this dose was limited by IFN-like side effects. Further studies are necessary in order to determine the usefulness of this compound for treatment of HCV.

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