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Phase I Trial of an Oral Immunomodulator and Interferon Inducer in Cancer Patients¹

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ABSTRACT

Imiquimod [1-(2-methylpropyl)-1*H*-imidazo[4,5*c*]quinolin-4-amine] is a compound of low molecular weight that, when administered p.o., induces interferon- α in several animal species and inhibits tumor growth in mice. To determine maximum tolerated dose, toxicity, and biological response in humans, a phase I clinical trial was conducted with 14 eligible cancer patients who received 100–500 mg imiquimod p.o. either once or twice weekly. Imiquimod induced interferon- α in serum in 10 of 19 doses of 200–300 mg. Interferon serum levels peaked 8–24 h after treatment and reached a maximum of 23,000 IU/ml in one patient. Significant mean increases ($P < 0.01$) in serum β_2 -microglobulin (1.5-fold), serum neopterin (3.5-fold), and 2-5A synthetase activity in peripheral blood mononuclear cells (7.9-fold) indicated that 200–300 mg imiquimod had biological and immunological activity in all evaluable patients. Increases in serum interferon, β_2 -microglobulin, and neopterin correlated significantly with dose ($P < 0.001$). No patient developed measurable antibody to interferon- α . Dose-limiting side effects included fatigue, malaise, fever, headache, and lymphocytopenia; no hepatic or renal toxicity or other hematological changes exceeded the normal range. Patients tolerated weekly doses of up to 500 mg, with the longest treatment lasting 4 weeks at 200 mg weekly. Twice-weekly doses up to 300 mg were tolerated, with the longest twice-weekly treatments being 200 mg for 9 weeks and 100 mg for 25 weeks. No clinical responses were observed. Imiquimod, as an oral inducer of interferon, may have therapeutic usefulness in human cancers, viral infections, and other diseases. However, before initiation of phase II trials, additional work will be required to establish a tolerated dose and schedule for continued administration.

INTRODUCTION

An orally active IFN³ inducer could be clinically advantageous in its convenience for long duration administration, especially in new clinical applications, such as prevention of neoplastic or viral diseases. Imiquimod [1-(2-methylpropyl)-1*H*-imidazo[4,5*c*]quinolin-4-amine; *M*, 240] given by mouth induces IFN- α in mice, guinea pigs, and monkeys (1, 2). It inhibited significantly the growth of 4 different transplantable murine tumors and as a single agent effected cures of the bladder transitional carcinomas FCB and MB49, and occasionally of colon carcinoma MC-26⁴ (3). Tumor inhibition was mediated to a substantial extent through IFN- α production, as demonstrated by the finding that tumor inhibition was abrogated when IFN was neutralized by anti-IFN antisera (3). Imiquimod also prevented primary and recurrent genital HSV infection in a guinea pig model (4). *In vitro*, imiquimod had no anti-HSV activity; however, it accelerated HSV-specific interleukin-2 production and PBM cell proliferation (5). In human PBM cells in culture, imiquimod (5 μ g/ml) also induced more

IFN than 50 μ g/ml polyriboinosinic:polyribocytidylic acid or 50 μ g/ml 2-amino-5-bromo-6-phenyl(3*H*)pyrimidinone (6).

Based upon these findings, a phase I clinical trial was initiated in cancer patients. Two schedules were evaluated: twice-weekly repetitive doses starting at 200 mg and weekly inpatient dose escalation starting at 100 mg. The objectives were to determine the maximally tolerated dose, define treatment-limiting toxicity, and assess biological and immunological response by measuring induction of IFN- α , TNF- α , IL-1 β , neopterin, and β_2 -microglobulin in serum, and 2-5A synthetase activity in PBM cells.

MATERIALS AND METHODS

Patient Selection

Eligibility requirements included a histologically confirmed diagnosis of cancer with locally incurable or metastatic disease refractory to standard treatment; performance status of 0 or 1 by Eastern Cooperative Oncology Group standards; age ≥ 18 years; adequate marrow function (WBC $\geq 3,500/\text{mm}^3$, platelet $\geq 125,000/\text{mm}^3$, hematocrit $\geq 27\%$, hemoglobin ≥ 10 g/dl); adequate renal function (creatinine ≤ 1.9 mg/dl or clearance ≥ 50 ml/min); and adequate liver function (bilirubin ≤ 1.7 mg/dl, serum glutamic-oxaloacetic transaminase no more than twice normal, and albumin ≥ 3.0 g/dl).

Exclusion criteria included: requirement for palliative radiation, chemotherapy, hormonal therapy, or concomitant barbiturates; aspirin, anticonvulsants, or nonsteroidal anti-inflammatory agents; medication for hypertension or chronic obstructive pulmonary disease; congestive heart disease; seizure disorders; central nervous system involvement with tumor; or clinically significant cardiovascular, hepatic, neurological, renal, endocrine, or gastrointestinal disease. Patients receiving these medications were excluded to ensure that patients with potentially severe underlying disease were not put at risk. Patients could not have had therapy with mitomycin-C or nitrosoureas within 6 weeks; therapy with immunomodulators, IFN, IFN inducer, or investigational drug within 4 weeks; cytotoxic chemotherapy within 3 weeks; hormones, radiation, or glucocorticosteroids within 2 weeks.

The twice-weekly dosing study was conducted at the Medical College of Wisconsin. Patients were domiciled for the first 24 h at the Clinical Research Center of Froedtert Memorial Lutheran Hospital, and follow-up visits took place at Milwaukee County Medical Complex's Hematology-Oncology clinic. The weekly inpatient dose escalation study was conducted at the Marshfield Clinic, Inc., Marshfield, WI. Both studies were conducted with Institutional Review Board approval under an approved Investigational New Drug plan from the Food and Drug Administration.

Treatment Plan

Imiquimod in 100-mg capsules was administered p.o. as a single dose in the morning with water. Doses twice-weekly were taken on the same 2 days of the week, whenever possible, or at least 3 days apart. Patients were treated early in the morning and then observed for 12 h. On the twice-weekly study, therapy was interrupted if grade 3 or 4 toxicity occurred, and when toxicity abated, the dose was reduced by 100 mg. On the weekly study, patients with grade 3 or 4 toxicity received a dose 100 mg less and continued at that weekly maintenance dose. In both studies, recurrence of grade 3 or 4 toxicity after resuming therapy required discontinuation of treatment and removal from study. One patient initially entered on the twice-weekly schedule was subsequently considered ineligible due to treatment with glucocorticoids, an exclusion criterion. A second patient was initially entered on the weekly schedule but was considered ineligible due to medication for hypertensive cardiovascular disease, an ex-

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³ The abbreviations used are: IFN, interferon; HSV, herpesvirus; PBM, peripheral blood mononuclear; TNF, tumor necrosis factor; IL, interleukin; polyICLC, polyI:C complexed with poly-L-lysine and carboxymethylcellulose.

⁴ E. C. Borden *et al.*, manuscript in preparation.

clusion criterion. These 2 ineligible patients were excluded from clinical and immunological analysis.

Schema

Twice-Weekly Schedule. Imiquimod was administered p.o. twice weekly. Patients were enrolled at a fixed dose beginning at 200 mg/dose and escalating by 100 mg.

Weekly Schedule. Imiquimod was administered once weekly with intrapatient dose escalation, beginning at 100 mg and escalating by 100 mg/week.

Clinical Parameters

Vital signs, performance status, toxicity, and tumor size were evaluated at pretreatment and monthly on study. An electrocardiogram was obtained at initial screening, before dose 1, and at weeks 4 and 16. Computed tomography or magnetic resonance imaging brain scans were done when clinically indicated. Clinical laboratory tests, including hematology, chemistry, and urinalysis, carcinoembryonic antigen, prothrombin time, and partial thromboplastin time were collected at numerous time points throughout the study. Modified WHO criteria were used to grade toxicity.

Biological and Immunological Measurements

Sample Preparation. Serum was obtained from fresh blood collected with no preservative, allowed to clot at room temperature for 30 min, and centrifuged at 1500 rpm for 10 min. Serum samples were frozen at -20°C , or for TNF and IL-1 assay, at -70°C . PBM cells were purified from heparinized blood on Ficoll-hypaque density gradients, washed in Dulbecco's phosphate-buffered saline, counted, pelleted, and frozen for assay of 2-5A synthetase enzyme activity, described below.

In the twice-weekly study, serum IFN levels were measured predose; at 4, 6, 8, 12, and 24 h after the first dose; and before and 8 h after the eighth dose (week 4). In the weekly study, serum IFN levels were measured predose, and 6, 8, 12, and 22 h postdose, each week. Serum IFN was also measured at therapy interruption and discontinuation. Serum samples for measurement of antibody to IFN were obtained pretreatment and at discontinuation.

Samples for measurement of biological response to IFN- α (neopterin, β_2 -microglobulin, and 2-5A synthetase activity) were collected before and 22–24 h after treatment at weeks 1 and 4. Two other cytokines, TNF- α and IL-1 β , were assayed in serum collected before dose 1 and 3 h after doses 1 and 3 from patients who received 200 and 300 mg doses of imiquimod.

Interferon. IFN in serum was quantitated by measuring its antiviral activity in a bioassay using human lung carcinoma cells (A549) and encephalomyocarditis virus, in comparison with International Reference standards for IFN- α , - β , and - γ (7). Serum IFN was confirmed to be IFN- α , and not IFN- β or IFN- γ , by neutralization. Values ≤ 10 IU/ml were taken as 5 IU/ml for statistical analysis.

Antibody to Interferon. Production of antibodies to IFN- α in patient serum was assessed by incubating dilutions of patient serum with a known amount of IFN- α and then assaying for IFN in the antiviral assay described above. A reportable antibody titer is defined as that dilution of antiserum that will neutralize 10 laboratory units/ml of IFN, as recommended by the WHO (8).

Interleukin-1 β . IL-1 β in serum was quantitated by enzyme-linked immunosorbent assay (sensitivity, 15 pg/ml; Cistron, Pine Brook, NJ). Patient samples were assayed in duplicate. Samples were read with a Bio-Rad microplate reader. A statistical program (Bio-Rad Microplate Manager 2.0) generated a standard curve with a 99% correlation coefficient, and determined the concentration of the patient samples.

Tumor Necrosis Factor- α . TNF- α in serum was measured by enzyme-linked immunosorbent assay (sensitivity, 20 pg/ml; Cistron). Duplicate samples were compared with a standard curve as for IL-1 β .

Serum β_2 -Microglobulin. Serum β_2 -microglobulin levels were measured in a quantitative competitive radioimmunoassay (Pharmacia Diagnostics, Piscataway, NJ).

Neopterin. Serum neopterin was assayed by radioimmunoassay (DRG International, Inc., Mountainside, NJ). All determinations were performed in duplicate with coefficients of variations less than 10%. The range was 3–800 nmol/liter.

2',5'-Oligoadenylate Synthetase. The activity of this enzyme was measured by incorporation of ^3H -labeled ATP into 2'5'-oligoadenylate (9, 10). Pelleted cell samples were lysed with Nonidet buffer and incubated with Poly(C)-agarose beads (Pharmacia), to which the 2-5A synthetase enzyme binds. The beads were washed and incubated with ATP and ^3H -labeled ATP (Amersham) in buffer, which is converted to the 2'5'-oligoadenylate product. Product is measured by scintillation counting, and specific activity units were expressed as pmol ATP converted/h/ 10^5 cells. Samples were assayed in duplicate, with a 7.3% coefficient of variation.

Statistical Analysis. All analyses were based on log-transformed data. Treatment effects over time were assessed via paired t tests. Spearman correlations were used to examine the effect of dose on changes in temperature, serum IFN, and IFN-induced proteins and metabolites. Changes in IFN concentration between the weekly and twice-weekly protocols were compared by two-sample t test. Correlation between the increase in IFN-induced proteins and the degree of severity of clinical symptoms was tested using Kendall or Spearman rank correlation.

RESULTS

Treatment Summary. Fourteen eligible patients (7 female, 7 male) with advanced cancer were treated with imiquimod on either a once- or twice-weekly schedule. The ages ranged from 34 to 80 with a median of 56 years. Cancers included lung (2 patients), colon (2 patients), and one each of ovarian, melanoma, carcinoid, renal cell, breast, mesothelioma, chondrosarcoma, rectal, pancreatic VIPoma, and pancreatic primary with secondary chronic lymphocytic leukemia. Prior therapy included chemotherapy (11 patients), surgery (10 patients), radiation (2 patients), and immunotherapies (2 patients); most patients had multiple treatments.

Of the 9 patients enrolled in the twice-weekly fixed dose schedule, 5 patients began at the 200-mg dose (Table 1). Two were discontinued for disease progression after 7 or 9 weeks of therapy. Two patients were discontinued at week 2 for dose-limiting toxicity (fatigue or dehydration and vomiting). One patient was interrupted at week 3 for grade 3 fatigue; this patient was dose-reduced to 100 mg and continued for 6 months. Four patients began at 300 mg and received between 1 and 10 doses (0.5–5 weeks) before dose reduction or discontinuation, three for grade 3 fatigue, and one for grade 3 hypoxemia. One patient declined to continue on study, and 3 were dose-reduced to 200 mg. Of these, one continued therapy for 3 weeks before progression of disease, one continued for 3 weeks before grade 3 side effects, and one discontinued therapy after one dose with grade 4 fatigue.

Five patients received weekly doses, beginning at 100 mg and escalating 100 mg weekly (Table 2). The 100- and 200-mg doses were tolerated by all patients. After the 300-mg dose, one patient exhibited

Table 1 Duration of twice-weekly p.o. administration of imiquimod and reason for discontinuation

Five patients received an initial 200-mg dose and 4 patients a 300-mg dose twice weekly. Treatment dose was reduced for first grade 3 or 4 toxicity; patients were discontinued at progressive disease or further grade 3 or 4 toxicity.

Dose (mg)	Total no. of patients	Duration of initial treatment		Duration at reduced dose			Reason for discontinuation		
		Median (wk)	Range (wk)	No. of patients	Dose (mg)	Wk	PD ^a	$\geq$ Grade 3 toxicity	None
200	5	3	2–9	1	100	25	2	2	1 ^b
300	4	1	0.5–5	3	200	0.5, 3, 3	1	3	0

^a PD, progressive disease.

^b Completed 6 months to end of study.

Table 2 Duration of weekly p.o. administration of imiquimod and reason for discontinuation

Five patients received weekly escalating doses beginning at 100 mg. Treatment dose was reduced for first grade 3 or 4 toxicity; patients were discontinued at progressive disease or further grade 3 or 4 toxicity.

Highest escalating dose received			Maintenance dose		
Highest dose (mg)	No. of patients	Reason reduced	Dose (mg)	Wks on maintenance	Reason discontinued
300	2	Grade 3 toxicity	200	4	1, PD ^a
500	3	2, Grade 3 toxicity 1, PD	400	2	1, declined PD

^a PD, progressive disease.

Table 3 Common side effects of p.o. imiquimod (moderate to severe, first 3 weeks of treatment)

Numbers of patients with documented or reported grade 3 (moderate) or grade 4 (severe) side effects were noted. Treatment dose was reduced for grade 3 or 4 side effects.

	Twice weekly study		Weekly study
	200 mg (n = 5)	300 mg (n = 4)	100/200/300 mg (n = 5)
Fatigue	3	2	
Vomiting	4	3	
Chills/rigor	4	1	1
Headache	2	1	1
Myalgia	2	0	
Diarrhea			1

grade 3 fever, and one grade 3 fever and rigors. These 2 patients then received 200 mg as a maintenance dose for 4 weeks. The other 3 patients continued to tolerate the drug up to 500 mg. One was discontinued due to disease progression after one dose of 500 mg. Two patients had grade 3 fatigue after 500 mg. They received a 400-mg maintenance dose for 2 weeks and were then discontinued for progressive disease.

Side Effects. On the twice-weekly schedule, moderate or severe fatigue occurred in 5 of 9 patients, resulting in dose reduction or discontinuation (Table 3). Within the first 3 weeks of treatment (6 doses) at 200 or 300 mg, 7 patients complained of nausea and vom-

iting, 5 of chills, and 3 of headache. On the weekly dose escalation schedule, major side effects were fatigue, fever, and headache. Anorexia with or without vomiting was common.

Physiological Changes. On either schedule, no patient experienced a clinically significant change in blood pressure, alkaline phosphatase, bone marrow compromise, or mental status as a result of imiquimod administration. One patient with marginal respiratory reserve became acutely dyspneic 7 h after imiquimod and required oxygen. This patient's clinical status improved within 24 h and although a cause and effect relationship with imiquimod could not be verified, she refused continued participation. No significant changes outside of the normal range in platelet count or hemoglobin were noted. Temperature increased after imiquimod, with a mean increase of 0.6°F at 12 h after the 100- and 200-mg doses and 3.4°F after 300 mg (Table 4). Fever was >102°F in 3 of 14 patients, including one of 105.5°F after a 300-mg weekly escalated dose. The increase in temperature was not dose-related (Spearman correlation analysis; $P = 0.24$ at 8 h and $P = 0.08$ at 12 h). In the twice-weekly schedule, grade 3 increases in serum glutamic pyruvic transaminase and in creatinine were each noted in one patient (Table 5). Absolute lymphocyte counts were below normal in some patients before treatment and were decreased by treatment (Table 5). The one statistically significant increase was in serum glutamic pyruvic transaminase on week 2 compared to pretreatment. In the weekly study, no grade 3 or 4 abnormalities in these parameters were observed (data not shown). No tumor responses were observed.

Induction of IFN- α . Within the first 24 h after imiquimod administration, all 4 patients receiving 300 mg as a first dose had detectable circulating IFN titers up to 8500 IU/ml and peaking at 12 or 24 h (Table 6). The induced IFN was confirmed to be IFN- α by neutralization with anti-IFN- α antibodies. One of the 5 patients treated with 200 mg imiquimod as a first dose had circulating IFN, with a peak of 400 IU/ml at 12 h (Table 6). At discontinuation of twice-weekly treatment, 2 additional patients at 200 mg and 2 of the patients at 300 mg also had detectable levels. Thus circulating IFN ≥ 10 IU/ml was

Table 4 Temperature after first administration of imiquimod

Temperatures were measured pretreatment (0 h) and at 8 and 12 h after the first dose. Increases within dose groups were not statistically significant; however, when all doses were taken together, the increase at 12 h was statistically significant ($P < 0.03$).

Dose (mg)	0 h			8 h			12 h		
	No. of patients	Mean (SE) °F	Range °F	No. of patients	Mean (SE) °F	Range °F	No. of patients	Mean (SE) °F	Range °F
100	5	97.4 (0.5)	96.0-99.4	5	97.3 (0.5)	96.0-98.8	5	98.0 (0.3)	97.6-99.2
200	5	98.9 (0.4)	98.4-100.2	5	99.8 (0.7)	98.4-102.8	5	99.5 (0.4)	98.6-100.4
300	4	98.1 (0.4)	97.2-99.0	4	100.1 (1.4)	96.8-104.8	3	101.5 (0.4)	100.8-102.4

Table 5 Organ toxicity after imiquimod administration: twice-weekly schedule

Numbers are mean $\pm$ SE (range) for the 9 patients who received initial 200- or 300-mg doses of imiquimod.

Test	Baseline	Day 2	Wk 2	Discontinuation
Hemoglobin (g/dl)	12.8 $\pm$ 0.5 (10.8-15.1)	11.4 $\pm$ 0.8 (9.6-15.1)	11.9 $\pm$ 0.5 (10.7-14.4)	12.0 $\pm$ 0.6 (9.2-14.3)
WBC count	6.6 $\pm$ 0.6 (4.9-10.6)	6.1 $\pm$ 1.2 (3.8-13.0)	6.2 $\pm$ 1.1 (2.3-17.3)	5.9 $\pm$ 0.8 (2.4-9.4)
ANC ^a	4,765 $\pm$ 539 (3,136-8,374)	5,208 $\pm$ 1,283 (2,795-12,740)	4,707 $\pm$ 1,062 (1,334-13,494)	4,750 $\pm$ 675 (2,405-8,648)
ALC	1,307 $\pm$ 189 (632-2,400)	783 $\pm$ 221 (50-1,680)	1,054 $\pm$ 160 (534-3,114)	1,008 $\pm$ 255 (188-1,103)
Platelet count	353 $\pm$ 36 (229-550)	289 $\pm$ 34 (219-474)	293 $\pm$ 53 (111-552)	277 $\pm$ 41 (137-495)
Creatinine (mg/dl)	1.3 $\pm$ 0.1 (0.7-1.9)	1.4 $\pm$ 0.2 (0.6-2.0)	1.3 $\pm$ 0.2 (0.6-2.1)	1.7 $\pm$ 0.5 (0.7-4.8)
SGPT ^b (units/liter)	12 $\pm$ 3 (11-78)	18 $\pm$ 5 (9-57)	31 $\pm$ 9 (7-71)	59 $\pm$ 25 (11-559)

^a ANC, absolute neutrophil count; ALC, absolute lymphocyte count; AMC, absolute monocyte count; SGPT, serum glutamine pyruvic transaminase.

^b A statistically significant increase in SGPT was observed on week 2 compared to pretreatment.

Table 6 Peak serum interferon first 24 h after imiquimod administration

≤10 IU/ml is the sensitivity of the bioassay. All pretreatment samples had ≤10 IU/ml IFN. On the twice-weekly schedule IFN was assayed pretreatment and at 4, 6, 8, 12, and 24 h after treatment; peak levels occurred at 12 or 24 h after treatment. On the weekly schedule IFN was assayed pretreatment and at 8, 12, or 22 h after each treatment; peak levels occurred at 8, 12, or 22 h after treatment.

Dose (mg)	Twice weekly schedule		Weekly schedule	
	No. of patients >10 units/ml	Range (units/ml)	No. of patients >10 units/ml	Range (units/ml)
100	0/5		0/5	
200	1/5	400	1/5	500
300	4/4	100–8,500	4/5	370–23,000
400			3/3	50–400
500			3/3	150–3,600
Maintenance ^a			3/4	50–4,700

^a Maintenance dose was 200 mg for 2 patients (4 and 8 wk) and 400 mg for 2 patients (both 6 wk).

Table 7 Increases in IFN-induced gene products 24 h after first imiquimod administration

β₂-Microglobulin and neopterin in serum and 2-5A synthetase activity in PBM cells were assayed before and 24 h after the first treatment. The -fold increase represents the mean of 24-h values over pretreatment values. Log-transformed values were compared by paired *t* test; *n* = the total number of patients receiving each dose.

	100 mg (n = 5)		200 mg (n = 5)		300 mg (n = 4)	
	-Fold increase	<i>P</i> value	-Fold increase	<i>P</i> value	-Fold increase	<i>P</i> value
β ₂ -Microglobulin	1.1	0.17	1.4	0.01	1.5	0.001
Neopterin	1.3	0.12	2.0	0.01	3.5	0.002
2-5AS	3.8	0.058	3.4	0.01	7.9	0.002

induced in 7 of 9 patients on the twice-weekly schedule. For the patients who received weekly escalating doses, serum IFN increased with increasing doses of imiquimod (Table 6). Circulating IFN was detected in 0 of 5 patients after the 100-mg dose, 1 of 5 after the 200-mg dose, 4 of 5 after the 300-mg dose, and 3 of 3 after the 400- and 500-mg doses. The highest serum concentration in the study, 23,000 IU/ml IFN, was measured 20 h after a 300-mg dose. The peak serum IFN increased significantly as dose increased between 100 and 300 mg (Spearman correlation, *r* = 0.80; *P* = 0.0005; *n* = 14). No antibodies to IFN-α were detected in post-study sera after up to 5 months (median 2 months) of weekly or twice-weekly treatment.

To examine whether accumulated doses of imiquimod led to greater biological response than the first dose, we compared the increases in serum IFN concentration after the first single dose of 300 mg and the 300 mg dose in the escalation schedule, *i.e.*, the third weekly dose. The increases in serum IFN after 300 mg by these 2 schedules were not different (*P* = 0.56), suggesting that there is not an accumulated effect of imiquimod over this time interval.

Induction of Other Cytokines. Changes in serum concentrations of TNF-α and IL-1 after imiquimod treatment were not detected. Samples were tested at 3 h after the first treatment and the week 2 treatment; all remained below the detectability limit of the assay.

2',5'-Oligoadenylate Synthetase, β₂-Microglobulin, and Neopterin. Neopterin and β₂-microglobulin in serum, and 2-5A synthetase activity in PBM cells were significantly increased at 24 h after 200 or 300 mg imiquimod (Table 7). After 300 mg, the mean increase in β₂-microglobulin was 1.5-fold over pretreatment, in neopterin 3.5-fold, and in 2-5A synthetase 7.9-fold. Over the range of 100–300 mg imiquimod, the increases were dose-related for β₂-microglobulin (*r* = 0.79; *P* < 0.001) and neopterin (*r* = 0.83; *P* = 0.0002) but not 2-5A synthetase (*r* = 0.444; *P* = 0.11) using Spearman correlation analysis.

Comparison of Protein Induction and Severity of Clinical Symptoms. No significant correlation was observed between increases in IFN-induced proteins and the degree of severity of clinical symptoms, for the 200- and 300-mg doses taken together or the

300-mg dose alone, using either Kendall or Spearman rank correlation tests (data not shown).

DISCUSSION

Imiquimod was administered at single doses of 100–500 mg *p.o.* with no hematological or biochemical toxicity. However, continuation of treatment was prohibited by marked fatigue, declines in performance status, and other side effects (fever, chills, headache, mild transaminase increase) consistent with the effects of IFN and/or cytokine induction. Dose reduction was required in 3 of 4 patients receiving 300 mg of imiquimod twice weekly. A dose of 200 mg of imiquimod twice weekly was tolerated for greater than 6 weeks in only 2 of 9 patients and a dose of 100 mg twice weekly for 25 wk in one patient. The induction of IFN up to 23,000 IU/ml IFN-α after ≥300 mg of imiquimod and the statistically significant increase in IFN-induced gene expression after ≥200 mg imiquimod in all patients indicated activation of the IFN system. Despite circulating IFN-α, no antibodies to IFN were detected in any patients during up to 5 months of either weekly or twice-weekly treatment. Although no changes in IL-1β or TNF-α in serum were detected, it is possible that biologically significant induction of these cytokines occurred that was either below the detectability of the assay method or at times not measured.

In several studies, intramuscular injection of 36–72 × 10⁶ units of IFN-α induced 50 to 400 units/ml IFN-α in serum (11–13). Therefore, the levels of serum IFN-α measured after 300–500 mg imiquimod, 100–23,000 U/ml, are comparable or higher than after direct injection of IFN-α. The serum IFN levels induced by imiquimod generally exceeded those reported following administration of other IFN inducers. Infusion *i.v.* of 100 μg/kg PolyICLC induced over 250 IU/ml in multiple sclerosis patients (14), and 3–12 mg/m²/day polyICLC induced over 100 U/ml IFN in children with acute lymphocytic leukemia, acute myelogenous leukemia, or neuroblastoma (15). In patients with advanced cancer, 0.01–12 mg/m² polyICLC induced up to 2000 U/ml IFN and increased natural killer cell activity (16). However, in another cancer study, 0.01 to 1.0 mg/m² PolyICLC induced neopterin and 2-5A synthetase activity but no IFN-α or IFN-γ in serum (17). PolyA:U (60 mg) increased both 2-5A synthetase activity and natural killer cell activity but not measurable serum levels of IFN in breast cancer patients (18). The induction of IFN by flavone acetic acid and xanthone-4-acetic acid derivatives correlated with their efficacy against murine renal cancer (19, 20), but these compounds induced little or no IFN and had no clinical efficacy in humans (21, 22).

Development of neutralizing antibodies to recombinant IFN has been shown to compromise response to treatment. Between 15 to 30% of patients treated with recombinant IFN developed neutralizing antibodies as early as 2 months after continuous therapy. Of these, 40–90% lacked clinical response or other biological response to IFN treatment, which was restored after treatment with natural IFN-α (23–26). In this study, antibodies to IFN-α were undetectable after up to 5 months of imiquimod treatment, the longest period tested, most likely because the IFN-α induced by the compound is endogenous. It is therefore possible that less resistance to long-term treatment may develop with the use of imiquimod than with exogenous IFN therapy, and further might be effective in those patients developing antibodies to recombinant IFNs.

The increases in neopterin, β₂-microglobulin and 2-5A synthetase activity in this study were comparable to those induced by exogenously administered IFN-α and IFN-β in patients (13, 27–29). Although it is not clear which IFN-induced gene products are most relevant to antineoplastic activity, the MHC Class I-related protein, β₂-microglobulin, is involved in antigen-specific activation of cytotoxic T lymphocytes (29), induction of the intracellular enzyme 2-5A

synthetase results in degradation of cellular and viral RNA (31), and neopterin reflects monocyte activation and is a secreted product of the IFN-induced protein GTP cyclohydrolase (32).

No antitumor responses were observed, as often occurs in phase I trials, since many patients entered were of histological types not often responsive to therapies. Given the high IFN levels induced by imiquimod, it is possible that establishing an acceptable schedule for prolonged administration will result in antitumor effects. Imiquimod may also have a role in maintenance of remission in malignant disease. In addition, a p.o. IFN inducer could be of value in chemoprophylaxis for neoplastic disease in high risk populations (33). The dose-schedules explored in this phase I evaluation exceeded patient tolerance due to profound constitutional symptoms. Lower or less frequent doses, which are being explored in other trials, may be more acceptable for longer term administration.

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