

Phase I and Pharmacokinetic Study of Irofulven Administered Weekly or Biweekly in Advanced Solid Tumor Patients

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ABSTRACT

Purpose: We performed a Phase I and pharmacokinetic study to determine the maximum tolerated dose of irofulven (6-hydroxymethylacylfulvene; MGI-114, MGI PHARMA, Inc.), administered in intermittent weekly schedules in patients with advanced solid tumors.

Experimental Design: Three schedules were tested: A, days 1, 8, and 15 every 4 weeks; B, days 1 and 8 every 3 weeks; C, days 1 and 15 every 4 weeks. Drugs were administered as 5- and 30-min (schedules B and C) infusions. Dose levels of 10, 12, and 14 mg/m²/week were explored.

Results: Ninety-nine patients received 256 cycles. Fifteen of 74 patients evaluable for maximum tolerated dose experienced 16 dose-limiting toxicities (5 of 17 patients on schedule A, 2 of 25 on schedule B, and 8 of 32 on schedule C), principally treatment delay for thrombocytopenia. Schedule A was considered unsuitable because of frequent thrombocytopenia and treatment discontinuations. Twenty-three percent of the overall population (22 patients with grade

1–2, and 1 patient with grade 3), including 37% of patients on dose level 3, experienced unexpected dose-limiting visual toxicity, which included color perception and visual field alterations linked to retinal cone cell toxicity; the visual toxicity had an early onset, was mostly reversible, and was related to higher dose per infusion. Safety profiles were similar for 5- and 30-min infusions. The relationships between dose and area under the plasma concentration–time curve and maximum plasma concentration were linear for both 5- and 30-min infusions in the 78 patients evaluated for pharmacokinetics. The area under the plasma concentration–time curve and clearance were comparable between infusion durations. Responses included one complete (ovarian), one partial (renal), and seven disease stabilizations lasting >4 months.

Conclusions: We recommend doses of 18 mg/m²/infusion for schedule B and 24 mg/m²/infusion for schedule C, limited to 0.55 mg/kg and a total dose of 50 mg/infusion, administered over 30-min.

INTRODUCTION

Irofulven (6-hydroxymethylacylfulvene; MGI-114; MGI PHARMA, Inc., Bloomington, MN) is a novel semisynthetic antitumor agent derived from the sesquiterpene mushroom toxin illudin S (1). Although illudins exhibited potent inhibition of DNA synthesis and a unique mode of interaction with DNA (2), their therapeutic index has limited further development and led to a synthesis effort aimed at identifying analogs with improved therapeutic potential. Irofulven demonstrated potent activity against a wide variety of human tumor xenograft models derived from breast, lung, ovary, and pancreas carcinomas and glioblastoma multiforme (3–5). In relatively chemoresistant tumors, such as HT 29 colon and MV 522 lung carcinoma, it demonstrated superior activity compared with a variety of cytotoxic agents (3), and antitumor activity was maintained in tumors overexpressing the multidrug resistant proteins MDR1/gp170 and MRP/gp180 (6, 7).

Although its molecular pharmacology and mechanism of action are not fully elucidated, the ability of irofulven to bind covalently to biological macromolecules, to inhibit DNA synthesis, and to induce apoptosis have characterized it as an alkylating agent (8, 9). The cytotoxic activity of irofulven seems to be at least in part related to its capacity to bind covalently to DNA; cells deficient for proteins involved in nucleotide excision repair showed marked sensitivity to irofulven (10). However, unlike other clinically active alkylating agents, irofulven is unable to form bifunctional adducts with DNA (8, 9), and its activity spectrum in a panel of cancer cell lines was clearly different from that observed for ET-743 (a DNA minor groove alkylator) or cisplatin (11). Moreover, irofulven-resistant cells showed only marginal cross-resistance to other alkylating agents (12). Finally, irofulven activity was independent of p53 and

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p21/WAF1 status and mismatch repair function (11, 13). In addition to DNA damage, action on cellular proteins might contribute to the proapoptotic effects of irofulven. It appears to interfere with redox homeostasis, leading to protein oxidation and mitochondrial dysfunction, which triggers a proapoptotic signal. The greater capacity of normal cells, compared with tumor cells, to maintain redox homeostasis could protect normal cells from the effects of irofulven (14).

In human tumor xenograft models, a daily times five (Dx5) schedule was associated with the highest antitumor activity among the schedules investigated (3, 15). An initial Phase I trial used a 5-min i.v. infusion administered daily for 5 days every 4 weeks, with the recommended dose (RD) being 11 mg/m²/day (16). Several Phase II studies using the Dx5 schedule have demonstrated promising antitumor activity for irofulven monotherapy, including in heavily pretreated ovarian cancer, androgen-independent prostate cancer, and gemcitabine-refractory pancreatic cancer (17–19). However, delayed thrombocytopenia and prevalent, severe emesis with anorexia and asthenia led to frequent treatment delays and/or discontinuations. Furthermore, the high rate of treatment delay and early discontinuation due to adverse events observed in all of these studies may have had a negative impact on assessing the true therapeutic potential of irofulven.

We decided to explore intermittent administration schedules that would be more suitable for use in combination therapy and would hopefully improve the safety and tolerance profile while preserving or increasing the delivered single-agent dose intensity. The primary objective of this study was to determine the maximum tolerated dose (MTD) of irofulven administered as a 5- or 30-min infusion weekly (days 1, 8, and 15 every 4 weeks or days 1 and 8 every 3 weeks) or every other week (days 1 and 15 every 4 weeks) in patients with advanced solid tumors. The secondary objectives were to determine the recommended dose for subsequent Phase II studies, to characterize the pharmacokinetic profile, and to undertake a preliminary assessment of the antitumor activity.

PATIENTS AND METHODS

Patient Selection. Patients with malignant solid tumors refractory to standard anticancer treatment or for whom no standard treatment existed were eligible for this study. Patients were to meet all of the following criteria: age \geq 18 years, Eastern Cooperative Oncology Group performance status \leq 2, life expectancy $>$ 3 months, discontinuation of all previous anticancer treatments for at least 4 weeks before first dose of study drug (6 weeks for mitomycin C), use of effective contraceptive methods, adequate bone marrow function (WBC count \geq $3.0 \times 10^3/\text{mm}^3$; absolute neutrophil count \geq $2.0 \times 10^3/\text{mm}^3$; platelet counts \geq $150 \times 10^3/\text{mm}^3$; hemoglobin \geq 9 g/dl), hepatic function (bilirubin within normal range of institutional value; aspartate aminotransferase and alanine aminotransferase \leq 1.5 times the upper limit of normal or \leq 2.5 times the upper limit of normal in cases of liver metastases), renal function (creatinine clearance, calculated according to the Cockcroft–Gault formula, \geq 60 ml/min), serum calcium $<$ 2.7 mmol/l, and a signed informed consent. Patients meeting any of the following criteria were prohibited from participating in this study: past

radiation therapy to $>$ 30% of total bone marrow; previous chemotherapy with nitrosoureas, high-dose carboplatin, or a previous mitomycin C cumulative dose \geq 25 mg/m²; previous bone marrow transplant or intensive chemotherapy with stem cell support; presence of any serious concomitant systemic disorders deemed incompatible with the study by the investigator; presence of any significant central nervous system or psychiatric disorder(s) that could hamper the patient's compliance; history of another malignancy; treatment with any other investigational agent or participation in another clinical trial within 30 days before study entry; and child-bearing or lactating women.

Drug Administration and Dose Escalation. This Phase I study used a planned dose-intensity-based dose escalation scheme. The starting dose intensity in each schedule was 10 mg/m²/week, \sim 75% of the RD determined for Dx5 administration (16). Patients who received less than two full cycles and did not experience dose-limiting toxicity (DLT) were not evaluable for determination of MTD. A minimum of two patients were to be enrolled at each dose level (DL), and two or four additional patients were to be included if one or two of the initial two patients experienced a DLT, respectively. The MTD was considered to have been attained if two or more of four patients or three or more of six patients experienced a DLT during the first two cycles. DLT was defined as grade 4 neutropenia lasting $>$ 5 days, grade 3/4 neutropenia with fever or infection, grade 4 thrombocytopenia (or grade 3 with bleeding), clinically significant grade 3/4 nonhematological toxicity, a delay due to toxicity of $>$ 1 week in the day 8 or 15 infusions, or a delay between cycles $>$ 2 weeks due to toxicity. The clinical relevance of grade 3/4 nonhematological toxicities was to be evaluated by the investigators and the sponsor before considering them DLTs. Toxicities were graded according to National Cancer Institute–Common Toxicity Criteria, version 2. The recommended dose was defined as the DL immediately below the MTD. A minimum of 20 cycles in six patients were to be given at the RD.

In the initial treatment schedule, irofulven was to be administered through a central venous access line in a 5-min infusion on days 1, 8, and 15 every 4 weeks (schedule A). According to the protocol, if a DLT for delay occurred in half of the patients, two alternative dosing schedules were to be investigated: days 1 and 8 every 21 days (schedule B), and days 1 and 15 every 28 days (schedule C). It was also anticipated by protocol that the length of the infusion could be prolonged from 5 to 30 min from DL 2 if the MTD was not reached. Patients

Table 1 Planned dose per infusion in each schedule and dose level^a

Planned dose levels	Planned dose intensity (mg/m ² /week)	Dose per infusion (mg/m ²)		
		Schedule A	Schedule B	Schedule C
1	10	13.3	15	20
2	12	16	18	24
3	14	18.6	21	28
4	16	21.3	24	32

^a All schedules were administered as 5-min infusions. Additionally, schedules B and C were administered as 30-min infusions. Schedule A, days 1, 8, and 15 every 4 weeks; schedule B, days 1 and 8 every 3 weeks; schedule C, days 1 and 15 every 4 weeks.

Table 2 Number of patients and cycles administered as a function of dose level and treatment regimen

		Dose level 1	Dose level 2	Dose level 3	Total
Schedule A					
5-min infusion	Patients (n)	6	9	6	21
	Cycles (n)	10	23	12	45
Schedule B					
5-min infusion	Patients (n)	3	9	7	19
	Cycles (n)	13	21	20	54
30-min infusion	Patients (n)		8	6	14
	Cycles (n)		30 ^a	14	44
Schedule C					
5-min infusion	Patients (n)	3	9	8	20
	Cycles (n)	11	23	16	50
30-min infusion	Patients (n)	1	16	8	25
	Cycles (n)	4	39 ^a	20	63
All schedules	Patients (n)	13	51	35	99
	Cycles (n)	38	136	82	256

^a Recommended dose and schedule.

were assigned to the schedule with an available slot in a non-randomized manner. The doses administered per infusion in each schedule and dose level are given in Table 1.

The dose for day 1 of cycles could be administered if patients had recovered to an absolute neutrophil count $\geq 1.5 \times 10^3/\text{mm}^3$ and platelet count $\geq 90 \times 10^3/\text{mm}^3$. An absolute neutrophil count $\geq 1.0 \times 10^3/\text{mm}^3$ and platelet count $\geq 75 \times 10^3/\text{mm}^3$ were required for dosing on day 8 and day 15. Patients were allowed to continue treatment in the absence of disease progression or DLT.

Before and after each irifolven infusion, 500 ml of normal saline or 5% dextrose solution was to be administered prophylactically over 1 h. Antiemetic therapy using a 5-hydroxytryptamine 3 receptor antagonist and steroids was required in all patients.

Clinical Assessments. Before inclusion and immediately before each treatment cycle, a medical history, evaluation of Eastern Cooperative Oncology Group performance status, physical examination, assessment of concomitant medications, and standard urinalysis were performed. Laboratory studies, including a complete blood count with differential and standard blood chemistry assessments, were performed weekly. Additionally, a 12-lead electrocardiogram was performed before the first infusion and at study termination. Late in the course of the study, an amendment was made to perform systematic ophthalmologic examinations, including funduscopy, formal visual field testing, an electroretinogram, and a color vision test, before the first infusion and at study termination.

Tumor assessment was to be performed every 8 weeks. Disease response was evaluated according to WHO criteria (20). If a response was observed, confirmatory assessments were to be undertaken no less than 4 weeks later.

Pharmacokinetics. A 24-h urine sample and ten 10-ml blood samples were collected for pharmacokinetic analysis on the day of the first three infusions of irifolven. After the 5-min infusion of irifolven, blood samples were taken at 0, 4, 10, 15, 25, 35, 45, 60, 120, and 240 min from the start of the infusion. After the 30-min infusion, blood samples were taken at 0, 25,

35, 40, 45, 50, 60, 70, 90, 150, and 270 min from the start of the infusion. Plasma and urine were assayed by an isocratic validated high-pressure liquid chromatography method with UV detection at 330 nm (21). The variables analyzed were maximum plasma concentration (C_{max}), area under the plasma concentration–time curve (AUC), terminal elimination half-life, clearance, and apparent distribution volume, calculated by a trapezoidal model for i.v. administration with constant infusion input and a one-compartment open model.

RESULTS

Patient Characteristics and Cohorts. Between December 1999 and September 2001, 99 patients were enrolled in the trial and received irifolven according to five treatment regimens (schedule A, 5-min infusion; schedule B, 5- and 30-min infusions; schedule C, 5- and 30-min infusions), and three DLs (dose intensities, 10, 12, and 14 mg/m²/week; Table 2). Patient characteristics (Table 3) were similar across the five regimens investigated and the three DLs. However, a lower proportion of patients with performance status 2 were entered in schedule C (7 versus 14 and 12% in schedules A and B, respectively).

Treatment. Two hundred fifty-six cycles were administered, for a median of 2 cycles/patient (range, 1–10). Thirty-four patients (34%) received three or more cycles. Median relative

Table 3 Patient characteristics

Patient characteristics	Patients	
	n	%
Sex		
Male	54	55
Female	45	45
Median (range) age (years)	53 (20–79)	
ECOG ^a performance status		
0	37	37
1	52	53
2	10	10
Primary tumor type		
Soft tissue sarcoma	30 ^b	31
Colorectal	15	15
Prostate	10	10
Breast	7	7
Ovary	6	6
Pancreas	5	5
Head and neck	5	5
Renal	5	5
Lung	4	4
Other	12 ^c	12
Extension at baseline		
Locally advanced	4	4
Metastatic	95	96
Median (range) number of organs involved	2 (1–6)	
Number of previous chemotherapy lines		
Median (range)	3 (0–9)	
0	7	7
1–2	29	29
≥ 3	63	64

^a ECOG, Eastern Cooperative Oncology Group.

^b Including one desmoplastic small round cell tumor.

^c Hepatocarcinoma, gastric carcinoma, osteosarcoma, abdominal wall adenocarcinoma, gastrointestinal stroma tumor, mesothelioma, cholangiocarcinoma, choroids melanoma, skin melanoma, uterine adenocarcinoma, unknown primary carcinoma.

Table 4 Dose-limiting toxicity during first two cycles as a function of dose level and treatment regimen

		DL ^a 1	DL 2	DL 3
Schedule A				
5-min infusion	Patients evaluable (n)	3	8	6
	Patients with DLT, n (%)	1 (33)	2 (25)	2 (33)
	Type of DLT	Treatment delay (1 patient) ^b	Treatment delay (2 patients)	Treatment delay (1 patient) ^b Grade 4 neutropenia (1 patient) ^b Grade 3 nausea/vomiting (1 patient)
Schedule B				
5-min infusion	Patients evaluable (n)	3	6	5
	Patients with DLT, n (%)			1 (20)
	Type of DLT			Treatment delay (1 patient)
30-min infusion	Patients evaluable (n)		7	4
	Patients with DLT, n (%)		1 (14)	
	Type of DLT		Treatment delay (1 patient)	
Schedule C				
5-min infusion	Patients evaluable (n)	2	8	4
	Patients with DLT, n (%)			2 (50)
	Type of DLT			Grade 3 nausea/vomiting (2 patients)
30-min infusion	Patients evaluable (n)		13	5
	Patients with DLT, n (%)		6 (46)	
	Type of DLT		Treatment delay (5 patients) Grade 3 nausea/vomiting (1 patient)	

^a DL, dose level; DLT, dose-limiting toxicity.

^b Patients experiencing two dose-limiting toxicities simultaneously.

dose intensity was at least 98% in all treatment regimens and DLs. Among the 95 patients who received more than one infusion, 27 (28%) experienced some type of treatment delay of at least 3 days; 17 had at least one cycle delayed because of toxicity. Delays due to toxicity occurred in 25 cycles (10%). Discontinuation due to toxicity was necessary in 14% of patients, affecting 33% of patients in schedule A *versus* <10% in schedules B and C.

Determination of MTD. Twenty-five patients were considered nonevaluable for adequate MTD determination: 23 patients received fewer than two full cycles without experiencing DLT; 1 patient, who experienced acute grade 4 thrombocytopenia, had early onset of extensive bone marrow involvement; and 1 patient underwent a treatment delay of 22 days to receive palliative radiotherapy. Among the 74 evaluable patients, 16 clinically relevant DLTs, occurring in 15 patients, were observed during the first two cycles (Table 4). Eleven DLTs were episodes of prolonged treatment delay, with 10 due to protracted thrombocytopenia without bleeding and 1 to delay for recovery from grade 2/3 neutropenia without fever. One DLT was grade 4 neutropenia lasting >5 days, and four DLTs consisted of grade 3 nausea/vomiting. In addition, seven patients experienced events considered to be DLTs during the third to eighth cycles: six patients experienced treatment delay due to thrombocytopenia, and one patient experienced grade 3 visual toxicity.

Initially, five patients each were treated in DL 1 and DL 2 of schedule A (5-min infusion). Three of eight evaluable patients experienced DLTs with treatment delays due to thrombo-

cytopenia, and another patient in DL 2 experienced a cycle delay of 13 days. Therefore, in accordance with the protocol, schedules B and C were opened for exploration. No further DLT occurred during the first two cycles in DLs 1 or 2 in schedules A, B, or C. However, at DL 3, DLTs occurred during the first two cycles in two of six, one of five, and two of four evaluable patients treated in schedules A, B, and C, respectively. At that time, it was decided to explore irofulven administration via 30-min infusion. Dose escalation started at DL 2 for the 30-min infusion because of the absence of DLT in DL 2 with schedules B and C. The longer infusion duration was not explored for schedule A because of the relatively high rate of DLTs due to treatment delays on this schedule.

At DL 2, one of seven evaluable patients in schedule B and three of eight in schedule C experienced DLTs during the first two cycles of 30-min infusion. No patients experienced DLT at DL 3 during the first two cycles. However, the unexpected rate of grade 1/2 visual toxicity observed in DL 3 and one episode of grade 3 visual toxicity occurring in DL 3 of schedule A led to closure of this level. Seven additional patients were included in DL 2 in schedule C (30-min infusion) to undertake a prospective evaluation of visual toxicity, three of whom experienced non-visual DLTs. The unexpected visual toxicity, regardless of grade, came to be considered the DLT of the intermittent weekly irofulven administration schedule (see below). DL 3 was considered to be the MTD because of visual toxicity. Further exploration in Phase II trials of schedules B and C using DL 2 (30-min infusion) was recommended.

Table 5 Frequency of treatment-related adverse events and laboratory abnormalities, worst grade per patient and per cycle for all treated patients (toxicity types occurring with severity grade 3/4 or in >10% of patients)

Event/Abnormality	NCI-CTC ^a grade							
	1		2		3		4	
	Patients <i>n</i> (%)	Cycles <i>n</i> (%)	Patients <i>n</i> (%)	Cycles <i>n</i> (%)	Patients <i>n</i> (%)	Cycles <i>n</i> (%)	Patients <i>n</i> (%)	Cycles <i>n</i> (%)
Gastrointestinal								
Anorexia	15 (15)	20 (8)	5 (5)	8 (3)				
Constipation	13 (13)	14 (5)	4 (4)	4 (2)				
Diarrhea	19 (19)	22 (9)	6 (6)	7 (3)	1 (1)	1 (<1)		
Stomatitis	7 (7)	7 (3)	1 (1)	1 (<1)	1 (1)	1 (<1)		
Nausea	35 (35)	81 (32)	40 (40)	57 (22)	6 (6)	7 (3)		
Vomiting	21 (21)	39 (15)	35 (35)	49 (19)	6 (6)	6 (2)		
Visual toxicity								
Blurred vision		2 (1)	7 (7)	10 (4)	1 (1)	1 (<1)		
Flashing lights/floater	9 (9)	17 (7)	7 (7)	13 (5)				
Photophobia			2 (2)	3 (1)	1 (1)	1 (<1)		
Other ^b	6 (7)	10 (4)	5 (6)	7 (3)				
Constitutional								
Fatigue	23 (23)	72 (28)	36 (36)	66 (26)	10 (10)	11 (4)		
Headache	11 (11)	13 (5)	2 (2)	3 (1)				
Hematology								
Anemia	23 (23)	63 (25)	32 (32)	68 (27)	14 (14)	23 (9)	6 (6)	7 (3)
Leukopenia	19 (19)	53 (21)	22 (22)	39 (15)	17 (17)	18 (7)	1 (1)	1 (<1)
Neutropenia	12 (12)	36 (14)	20 (20)	39 (15)	16 (16)	20 (8)	3 (3)	3 (1)
Thrombocytopenia	13 (13)	26 (10)	19 (19)	30 (12)	19 (19)	21 (8)	1 (1)	1 (<1)
Liver function tests								
AST increased	35 (35)	63 (26)	8 (8)	8 (3)	2 (2)	2 (1)		
ALT increased	29 (29)	62 (25)	10 (10)	15 (6)	5 (5)	6 (2)		
AP increased	38 (38)	91 (37)	14 (14)	25 (10)	7 (7)	16 (7)	2 (2)	2 (1)
Total bilirubin increased	10 (10)	21 (9)	9 (9)	10 (4)	4 (4)	4 (2)		
Renal function tests								
Serum creatinine increased	22 (22)	48 (19)	3 (3)	3 (1)				

^a NCI-CTC, National Cancer Institute-Common Toxicity Criteria; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase.

^b Darkening, color alteration, night blindness.

Visual Toxicity. Twenty-three of 99 patients experienced visual symptoms considered to be related to iriofulven treatment, including blurred vision, photosensitivity, photopsia/flashing lights, color perception alteration, floaters, and diminished night vision (Table 5). Seventeen of these patients (74%) experienced visual toxicity during the first cycle. Only 2 of 23 patients experienced a worsening of toxicity grade after receiving additional iriofulven treatment, and only 3 patients discontinued therapy because of visual toxicity. For 16 patients (70%), visual symptoms resolved completely after a median of 1.8 weeks (range, 0.1–20.7 weeks). Four patients (17%) had symptomatic improvement at the time of their deaths 4–27 weeks after onset of symptoms. Three patients (11%) had not improved in grade at 27, 37, and 41 weeks, respectively, after onset. Assessments of symptomatic patients indicated that this toxicity consists of an effect on retinal cells, with electroretinography investigations revealing changes in cone response. However, electroretinographic abnormalities did not appear to correlate well with the presence or severity of symptoms. Visual field tests typically revealed perimacular annular scotoma in patients with moderate or severe symptoms.

The incidence of visual toxicity did not appear to depend on duration of infusion but was higher in patients in schedule C and DL 3 (Table 6). These differences stem from a strong

association between dose per infusion and the occurrence of the toxicity: the mean dose per infusion in patients with visual toxicity was 23.6 ± 3.9 mg/m² compared with 20.2 ± 4.1 mg/m² in patients without visual toxicity. A higher dose per kg of body weight was also associated with increased incidence: mean doses/infusion were 0.65 ± 0.09 and 0.53 ± 0.12 mg/kg in patients with and without visual toxicity.

The risk of visual toxicity appeared to be more accurately predicted by dose per total body weight than dose per body surface area (Fig. 1). The observation that only 4 cases of visual toxicity occurred in the 52 patients (8%) treated below 0.55 mg/kg led to the adoption of a recommended doses of 18 and 24 mg/m²/infusion for schedules B and C (30-min infusions), respectively, with the dose limited to a maximum of 0.55 mg/kg and a total dose/infusion of 50 mg. All ongoing trials of iriofulven monotherapy were amended according to this recommendation, with prospective evaluation of visual events and ophthalmologic follow-up to assess the adequacy of this recommend dose.

Hematological Toxicity. All patients were evaluable for safety. Neither febrile neutropenia nor thrombocytopenia with bleeding was observed. Severe hematological toxicities and morbidities secondary to hematotoxicity were uncommon. Overall, half of the patients experienced some degree of neu-

Table 6 Visual toxicity and thrombocytopenia as function of schedule, dose level, and infusion duration

	Patients treated (<i>n</i>)	Patients with visual symptoms (<i>n</i> = 23)		Patients with thrombocytopenia (<i>n</i> = 52)	
		<i>n</i>	%	<i>n</i>	%
Schedule					
A	21	2	10	15	71
B	33	5	15	18	55
C	45	16	36	19	42
Dose level					
1	13	2	15	8	62
2	51	8	16	24	47
3	35	13	37	20	57
Infusion duration					
5 min	60	11	18	34	57
30 min	39	12	31	18	46
Recommended dose					
Schedule B, dose level 2	17	1	6	9	53
Schedule C, dose level 2	24	10	42	10	40

tropenia and/or thrombocytopenia (Table 5). Only one patient with extensive bone marrow involvement experienced grade 4 thrombocytopenia. Protracted grade 2/3 thrombocytopenia was the main cause of treatment delay (10% of cycles), and seven patients (7%) discontinued therapy as a result of nonrecovery from thrombocytopenia. Three patients experienced grade 4 neutropenia, with one episode lasting more than 5 days. Five patients received platelet transfusions, and 15 patients received RBC transfusions. The median days of nadir over the first two cycles for patients with grade 2–4 thrombocytopenia and grade 2–4 neutropenia were day 34 (range, 20–77 days) and day 41 (range, 4–70 days), respectively. The rates of thrombocytopenia

and of treatment delay due to thrombocytopenia were lower in schedule C than in schedule A or B (Table 6). In the dose range explored, hematological toxicity did not appear to be dose-intensity related, nor was it influenced by infusion duration. There was no evidence of cumulative hematological toxicity or increased hematological toxicity in heavily pretreated patients.

Other Toxicities. No grade 4 clinical toxicity or toxic deaths occurred. Most patients experienced some degree of nausea/vomiting during irofulven therapy despite standard antiemetic prophylaxis, but grade 3 toxicity was rare. Vomiting occurred more often in schedule C and was dose related. At the RD, grade 3 vomiting occurred in 0 and 8% of patients treated

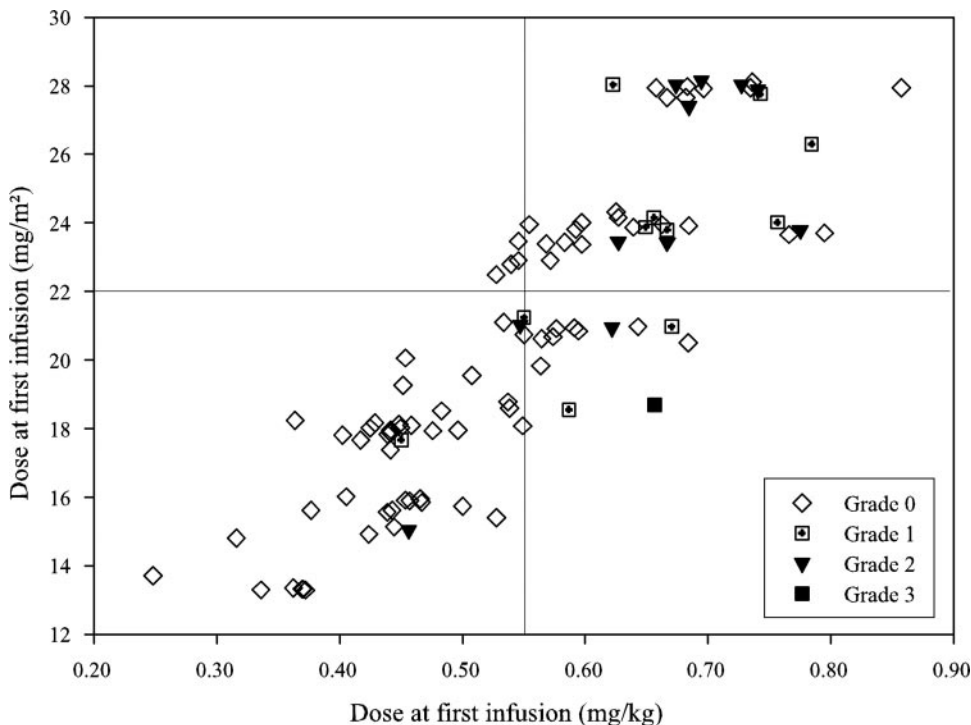


Fig. 1 Scatterplot of dose administered according to total body weight (mg/kg) and body surface area (mg/m²).

Table 7 Pharmacokinetic parameters (mean \pm SD), according to regimen

Planned dose intensity	Dose per infusion	Patients treated/evaluated	C_{\max}^a (ng/ml)	AUC (ng/ml·h)	Cl (l/h·m ²)	V_d (L/m ²)	$t_{1/2\beta}$ (min)
Schedule A							
5-min infusion							
10	13.3	6/5	190 \pm 99	25.1 \pm 12.7	654 \pm 320	28 \pm 8	6.1 \pm 1.4
12	16	9/7	300 \pm 307	38.9 \pm 38.4	855 \pm 750	22 \pm 5	4.5 \pm 1.0
14	18.7	6/6	413 \pm 335	45.8 \pm 27.2	509 \pm 240	50 \pm 53	3.7 \pm 1.4
Schedule B							
5-min infusion							
10	15	3/3	211 \pm 30	29.1 \pm 4.4	522 \pm 72	30 \pm 16	5.6 \pm 2.9
12	18.6	9/8	305 \pm 160	36.4 \pm 16.9	649 \pm 426	32 \pm 17	4.3 \pm 1.9
14	21	7/6	599 \pm 345	66.1 \pm 38.6	493 \pm 423	20 \pm 6	6.6 \pm 2.1
30-min infusion							
12	18	8/7	112 \pm 58	38.8 \pm 18.1	568 \pm 279	141 \pm 101	7.1 \pm 3.6
14	21	6/4	177 \pm 74	57.4 \pm 26.4	429 \pm 184	83 \pm 22	3.8 \pm 2.7
Schedule C							
5-min infusion							
10	20	3/2	334 \pm 74	60.0 \pm 10.3	339 \pm 59	32 \pm 43	4.5 \pm 1.5
12	24	9/6	586 \pm 562	78.5 \pm 68.6	431 \pm 186	29 \pm 26	4.2 \pm 2.0
14	28	8/7	754 \pm 268	92.2 \pm 48.9	380 \pm 174	19 \pm 9	6.0 \pm 1.2
30-min infusion							
12	24	15/11	165 \pm 64	54.8 \pm 18.5	501 \pm 216	100 \pm 57	6.4 \pm 6.0
14	28	8/6	201 \pm 38	70.3 \pm 14.8	418 \pm 114	89 \pm 34	8.0 \pm 2.6

^a C_{\max} , maximum plasma concentration; AUC, area under the plasma concentration–time curve; Cl, clearance; V_d , apparent volume of distribution; $t_{1/2\beta}$, terminal elimination half-life.

in schedules B and C, respectively. Asthenia (primarily grade 1 and 2) was observed in almost 70% of patients, but whether it was related to irifolven or the underlying disease was often difficult to determine. Liver function test abnormalities were common but were usually associated with liver disease and were never clinically significant. Of note, and despite many patients having borderline normal renal function, no patient experienced significant renal dysfunction attributable to irifolven, which was a dose-limiting event in the Dx5 Phase I trial before introduction of hydration prophylaxis (16).

Pharmacokinetics. In the 78 patients evaluated after the first infusion, the pharmacokinetics of irifolven were characterized by a very short terminal elimination half-life (\sim 6 min) and a high apparent distribution volume (80–140 L/m² for 30-min infusions; Table 7). A linear relationship was observed between administered dose and AUC and C_{\max} for both 5- and 30-min infusions. AUC and total body clearance did not differ according to infusion duration. The terminal elimination half-life appeared to be longer for the 30-min infusion. However, the difference may simply reflect small variations in sample timing for the 5-min administration, the small sample sizes, and/or large inpatient variability.

In this study, we observed a large interpatient variability in terms of C_{\max} , AUC, and clearance. Variability was not reduced by calculation of administered dose according to body weight or body surface area. In the total population, no correlation was detected between visual toxicity and the various pharmacokinetic parameters. A more reliable estimation of the terminal elimination half-life, C_{\max} , and AUC was possible in patients receiving 30-min infusions. Interestingly, in these patients visual toxicity was observed in 7 of 14 patients (50%) with AUC above the median value of 60 ng/ml·h compared with only 2 of 14 patients (14%) with AUC values below the median.

Antitumoral Activity. Two patients, both treated in schedule B, experienced an objective response. A 66-year-old woman with heavily pretreated peritoneal carcinomatosis (including cisplatin, paclitaxel, oxaliplatin, and 5-fluorouracil) was treated with six cycles in DL 3 (5-min infusion). She experienced a confirmed complete response in abdominal lymph nodes associated with normalization of CA125 (baseline of 1741 IU/ml) lasting 11 months. A 67-year-old man suffering from metachronous lung metastases of renal cell carcinoma was treated in DL 2 (30-min infusion). He experienced a confirmed partial response. After surgical resection of residual metastases, the patient was disease free at the last follow-up, 5 months later.

Seven patients (one patient in schedule A, two patients in schedule B, and four patients in schedule C) experienced stabilization of disease lasting at least 4 months, including two patients with soft tissue sarcoma (one with complete response of lung metastases and stable disease in liver metastases), and one patient each with prostate carcinoma, renal cell carcinoma, non-small cell lung cancer, mesothelioma, and head and neck carcinoma.

DISCUSSION

In this study, we evaluated three intermittent administration schedules of irifolven in a search for viable alternatives to the poorly tolerated Dx5 schedule and to enhance the combinability with other agents. On the basis of an acceptable safety profile and evidence of activity, we can recommend two schedules for further evaluation in Phase II trials: days 1 and 8 every 21 days (schedule B) and days 1 and 15 every 28 days (schedule C), at doses of 18 and 24 mg/m²/infusion, respectively, with the dose limited to \leq 0.55 mg/kg/infusion and $<$ 50 mg/infusion total

dose. These doses represent a common dose intensity of 12 mg/m²/week.

Under these schedules, thrombocytopenia without bleeding was frequent but rarely severe, with a delayed nadir. Treatment delay was the main consequence of irofulven-induced thrombocytopenia. Schedule C was associated with less profound thrombocytopenia and less frequent treatment delay. In contrast, the higher rate of treatment-related serious adverse events and treatment discontinuations rendered schedule A unsuitable for further evaluation. Nausea and vomiting were the principal clinical toxicities but were seldom severe when antiemetic prophylaxis was used.

The visual symptoms observed in 23% of patients during this study were an unexpected side effect of irofulven treatment. Typically they were reported by the patients during the first cycle, remained mild or moderate, and rapidly resolved in most but not all patients after the end of treatment. The clinical presentation and electroretinographic findings are consistent with retinal toxicity causing cone dysfunction, with patients typically displaying gradually reversible perimacular annular scotoma. Visual field tests appear to be the most appropriate method of evaluation. Although only one grade 3 event occurred and no decreases in visual acuity were observed, it was decided that visual toxicity was dose-limiting because visual disturbances, such as blurred vision or altered color perception, could affect patients' quality of life and their willingness to continue treatment.

Visual effects were more frequent in patients treated with schedule C and at DL 3. An increased rate of visual toxicity was observed for patients receiving higher individual doses of irofulven. Nevertheless, no clear relationship was found between visual toxicity and C_{max} . However, patients receiving an initial dose >0.55 mg/kg/infusion and with an AUC above the median value of 60 ng/ml·h had a higher likelihood of experiencing visual toxicity. Visual events occurred over the dose range 18–28 mg/m² in $>20\%$ of patients (including grade 2/3 in $>10\%$ of patients), a toxicity rate over such a range makes it difficult to recommend any dose. In contrast, the occurrence of visual events was $<20\%$ for doses <0.58 mg/kg (with $<10\%$ grade 2 and no grade 3 visual toxicity). We therefore propose to optimize the safety of irofulven administration by limiting the administered dose to 0.55 mg/kg/infusion. Forthcoming reports will describe in detail the ophthalmological and clinical characteristics of this toxicity on the basis of experience from Phase I and II trials of irofulven monotherapy.

The RD determined in this trial represents a dose intensity similar to that recommended for the Dx5 schedule (12 *versus* 13.5 mg/m²/week; Ref. 16). However, according to safety results reported for Phase I and II studies of Dx5 administration, overall tolerance of the intermittent weekly schedules appears to be superior, with improved treatment compliance and maintenance of delivered dose intensity. Treatment delay due to persistent thrombocytopenia was observed in 39–63% of subsequent cycles with the Dx5 schedule, leading to a dramatic decrease in observed dose intensity (16, 17, 22). In contrast, in the present trial prolonged thrombocytopenia led to delays in 10% of cycles. Grade 3 nausea/vomiting was observed in 22–40% of patients administered irofulven at the Dx5 schedule, compared with 6% of patients on weekly schedules (18, 22).

Twenty-five percent of patients discontinued therapy because of adverse events, mainly nausea/vomiting, asthenia, and persistent thrombocytopenia, in a Phase II study in pancreatic cancer patients (18), compared with 14% of patients in the present trial discontinuing because of adverse events, mainly thrombocytopenia. The renal toxicity observed in a Phase I trial of the Dx5 schedule at doses ≥ 14.15 mg/m²/day was not observed in the present study (16).

Prolongation of the length of the infusion from 5 to 30 min was implemented in the present study because the volume of infusion necessary at DL 2 and above was difficult to administer over 5 min. In addition, high interpatient variability in pharmacokinetic data observed with the 5-min infusion was believed to be an artifact of the short plasma half-life of irofulven and the difficulty in obtaining reliable end-of-infusion blood samples. It was also thought that a 30-min infusion would possibly result in higher activity, as has been suggested recently by the observation that *in vitro* cytotoxicity of irofulven in tumor cell lines increases with the duration of exposure (11). Preliminary results of ongoing Phase I and II trials of irofulven administered every other week (schedule C) show a good safety profile and promising activity, which supports further exploration (23–26).

Two objective responses and seven disease stabilizations lasting more than 4 months were observed in this heavily pretreated population, suggesting that the antitumor activity of irofulven is retained when it is administered on an intermittent schedule. These clinical results are supported by a preclinical study that compared the antitumor activity of irofulven given according to daily dosing or intermittent schedules in a MiaPaCa pancreatic tumor xenograft model (27). The intermittent schedule produced equivalent antitumor activity at a lower dose and resulted in reduced toxicity compared with equally efficacious daily dosing.

The potential of irofulven in combination therapy is promising. Supra-additive antitumor activity has been observed in human carcinoma xenograft models for the association with topoisomerase I inhibitors (topotecan and irinotecan), taxanes (paclitaxel and docetaxel), and cisplatin (12, 28–30). The improved safety profile of intermittent schedule should facilitate such combinations, as has been observed in preliminary results of a Phase I study of irofulven combined with irinotecan (31).

In summary, the results of this Phase I study demonstrate that the intermittent weekly administration of irofulven delivers an improved safety profile over that of daily dosing, allowing better compliance with planned dose intensity. We recommend two schedules: days 1 and 15 every 28 days and days 1 and 8 every 21 days, at doses of 24 and 18 mg/m²/day (30-min infusions; dose intensity, 12 mg/m²/week), respectively, limited to 0.55 mg/kg, and a total of 50 mg/infusion. Generally reversible visual toxicity was dose limiting but was severe in only one case. On the basis of the improved therapeutic index, intermittent schedules should be used for future clinical studies of irofulven alone or in combination.

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