ANTICANCER RESEARCH *24*: 59-66 (2004)

Activity of Irofulven against Human Pancreatic Carcinoma Cell Lines *In Vitro* and *In Vivo*

EMILY S. VAN LAAR[1], STEPHANIE ROTH[2], STEVEN WEITMAN[2], JOHN R. MACDONALD[1] and STEPHEN J. WATERS[1]

[1]*MGI Pharma, Inc., 5775 West Old Shakopee Road, Suite 100, Bloomington, MN 55437-3174;*
[2]*Department of Preclinical Research, CTRC Research Foundation, Institute for Drug Development, 14960 Omicron Drive, San Antonio, Texas 78245, U.S.A.*

Abstract. *Background: Irofulven (MGI 114), a novel antitumor agent synthesized from the natural product illudin S, has a unique mechanism of action involving macromolecule adduct formation, S-phase arrest and induction of apoptosis. Materials and Methods: This study utilized MiaPaCa pancreatic xenografts to demonstrate irofulven antitumor activity using either a daily or intermittent dosing schedule. Additionally, irofulven and gemcitabine were tested in vitro and in vivo to assess the anticancer activity of the combination. Results: Both dosing regimens of irofulven demonstrated curative activity against the MiaPaCa xenografts. Similar activity of irofulven on the intermittent schedule was observed at lower total doses compared to the daily dosing schedule. Furthermore, enhanced antitumor activity was observed when irofulven and gemcitabine were combined compared to single agent activity. Conclusion: These results support further clinical characterization of intermittent irofulven dosing schedules and suggest that irofulven combined with gemcitabine may have activity in patients with pancreatic tumors.*

Irofulven (6-hydroxymethylacylfulvene, HMAF, MGI 114) is a unique cytotoxic agent derived from the sesquiterpene mushroom metabolite illudin S (1). A broad cytotoxic profile for irofulven has been demonstrated *in vitro* and *in vivo* against multiple tumor types (2-5). More importantly, objective responses have been reported after irofulven treatment in patients with a variety of solid tumors, including gemcitabine-refractory pancreatic cancer (6-8). Several aspects of the drug's mechanism of action have been characterized. Irofulven undergoes rapid cellular uptake, covalent binding to macromolecules, induces S-phase cell cycle arrest and preferentially induces apoptosis in tumor cell lines (9-11). In addition, apoptosis-dependent mitogen-activated protein kinase (MAPK) and caspase activation has been demonstrated in pancreatic cancer cell lines (12,13). Experiments characterizing the toxicity of irofulven in xeroderma pigmentosum cell lines, which lack certain nucleotide excision repair (NER) enzymes, suggest that a functional NER system is required for efficient repair of irofulven-induced DNA damage (14,15). All of these factors contribute to the broad antitumor activity of irofulven.

Pancreatic cancer is known to be highly resistant to conventional chemotherapy (16). Even gemcitabine, which is specifically approved for use in advanced and metastatic pancreatic cancer, produces a low rate of objective responses and limited improvement in survival rates in patients (17). Similar to the chemoresistance of pancreatic cancer observed clinically, preclinical models of pancreatic cancer, such as MiaPaCa human pancreatic tumor xenografts, show little response to conventional cytotoxics other than tumor growth delays (18,19). New agents capable of producing tumor shrinkage or complete regressions in preclinical models of pancreatic cancer are therefore of interest as potential novel therapies for the treatment of pancreatic cancer.

The current investigations characterize irofulven's potent antitumor activity against MiaPaCa pancreatic tumor xenografts using daily and intermittent dosing regimens of irofulven. In addition, it appears that the combination antitumor activity of irofulven and gemcitabine is at least additive against pancreatic tumor cell lines and tumors. This data supports the clinical investigation of irofulven as monotherapy or in combination with gemcitabine against pancreatic cancer.

Correspondence to: Emily S. Van Laar, MGI Pharma, Inc., 5775 West Old Shakopee Road, Suite 100, Bloomington, MN 55437-3174, U.S.A. e-mail: emily.vanlaar@mgipharma.com

Key Words: HMAF, MiaPaCa, cytotoxic.

Materials and Methods

Cell culture. Human MiaPaCa and Panc-1 (ATCC, Manassas, VA, USA) cell lines were grown in Dulbecco's Modified Eagle's Medium (DMEM) containing 4 mM L-glutamine, 4500 mg/L

glucose, 1 mM sodium pyruvate and 1500 mg/L sodium bicarbonate with 10% heat-inactivated fetal bovine serum. Cells were incubated at 37˚C with 5% CO_2, 95% air and 100% relative humidity.

Drugs. Irofulven was obtained from MGI Pharma, Inc. (Bloomington, MN, USA) and gemcitabine was obtained from Eli Lilly and Company (Indianapolis, IN, USA).

Cell viability assessment. Pancreatic cancer cell viability was tested by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Briefly, the MTT assay is a spectrophotometric assay based on the ability of viable cells to convert MTT to formazan. The number of viable cells was estimated by measuring absorbance at a test wavelength of 570 nm and a reference wavelength of 630 nm. Initially, irofulven and gemcitabine were examined separately over a range of concentrations. After the initial IC_{50} (concentration of drug producing a 50% inhibition of viability) analysis was complete, the combinations were tested over one-hour exposure times. The cells were then washed and allowed to incubate for five days at which time the MTT assay was performed. Three different exposure schedules were utilized for the sequence of administration testing: exposure to either irofulven or gemcitabine first for one hour then the alternate agent for one hour or concurrent administration.

Statistical analysis of in vitro combination studies. A model-free system was utilized to statistically test the variation of specific sample points along the expected additive e-isobole, allowing an assessment of cytotoxicity of the combined agents (20). Using single agent dose-response curves established by MTT assays for each cell line, five different dose combinations using various ratios of the IC_{50} of each agent were identified. The concentration (expressed as a percent of the individual agent's IC_{50}) schema used to characterize the type of interaction was based on a 1:0, 3:1, 1.5:1, or 1:1 ratio for each agent. Each combination was tested in triplicate. To interpret the combination curves, statistical comparisons were made between each combination and their respective endpoints (each standard agent alone at its IC_{50}) (21). A statistically significant observation, defined as p-values < 0.05, required that a difference existed between the combination absorbance value and both endpoint values and was determined by a one-sided t-test. If the majority (≥ 3 of 5) of the values were statistically above or below a line (endpoints), then synergy or antagonism is described, respectively. Otherwise, the pattern is more consistent with an additive interaction. Statistical analyses were performed using SAS (version 6:11; SAS Institute, Cary, NC, USA).

Animals. Female nude (nu/nu) mice 5-6 weeks of age (Harlan Sprague Dawley, Inc., Indianapolis, IN, USA) weighing approximately 20g were used for all *in vivo* studies.

Xenografts. Mice were implanted subcutaneously (*s.c.*) by trocar with fragments of MiaPaCa human pancreatic carcinomas harvested from *s.c.* growing tumors in nude mice hosts. When the tumors were approximately 60-110 mg in size (10-13 days following implantation), the animals were pair-matched into treatment and control groups. Each group contained 10 mice bearing tumors, each of which was ear-tagged and followed individually throughout the experiment.

Xenograft therapy. The administration of drugs or vehicle began on the day the animals were pair-matched (Day 1). The vehicle control (1% EtOH in D5W), gemcitabine and irofulven were administered *via i.p.* injection at a dose volume of approximately 0.2 ml/mouse. Four different irofulven doses were used for the *in vivo* studies: for the daily dosing studies (daily administration on Days 1 through 5), 7 and 3.5 mg/kg irofulven were used (35 and 17.5 mg/kg total dose, respectively) and for the intermittent regimen studies (dosing on days 1, 4, 7, 10), 5 and 3 mg/kg irofulven were tested (20 and 12 mg/kg total doses, respectively). Each study also included 40 and 80 mg/kg doses of gemcitabine (160 and 320 mg/kg total doses, respectively), which were dosed on the intermittent schedule. When the mean of the control tumors reached a size of approximately 1 g the experiment was terminated.

Tumor measurements. Mice were weighed twice weekly and tumor measurements were taken by calipers twice weekly, starting on Day 1. These tumor measurements were converted to mg tumor weight by the formula, Weight (mg) = (Width $(mm)^2$ x Length (mm))/2, and from these calculated tumor weights, the termination date was determined. Upon termination of the study, mice were sacrificed, and their tumors were excised.

Assessment of response. Excised tumors were weighed and the mean tumor weight per group was calculated. In this model, the change in mean treated tumor weight / the change in mean control weight x 100 ($\Delta T/\Delta C$) was subtracted from 100% to give the tumor growth inhibition (TGI) for each group. The change in mean tumor weights is calculated by subtracting the tumor weight on Day 1 from the final tumor weight. To calculate the percent shrinkage, the final weight of a given tumor was subtracted from its own weight at the start of treatment on Day 1 and this difference was divided by the initial tumor weight. Mean percent tumor shrinkage (%TS) was calculated from data from the mice in a group that experienced regressions. If the tumor completely disappeared in a mouse, it was considered to be a complete responses (CR) or complete tumor shrinkage. A partial response (PR) was defined as the number of mice that demonstrated tumor shrinkage but did not display a CR. Animals with a PR or CR were not included in the TGI calculation.

Statistics. Statistical analyses were performed on the actual tumor weights at the conclusion of the study using SAS version 8.2 software (SAS Institute, Cary, NC, USA). Independent two-sample t-tests (2-sided) were conducted for specific *ad hoc* hypotheses of interest. P-values were adjusted using the Bonferroni method for multiple comparisons. Statistical significance was concluded for p-values < 0.05.

Results

Irofulven demonstrates curative activity against MiaPaCa xenografts when administered on either a daily or intermittent dosing regimen. To investigate the activity of irofulven against MiaPaCa pancreatic xenografts, we assessed two dosing regimens: daily injection of irofulven over five days (dx5) and an intermittent administration over a ten-day period (q3dx4). Table I and Figure 1 display results of the dx5 dosing regimen where 9/10 CR or 1/10 CR with 37.5% TGI were observed in the mice treated with 7 (maximum

Table I. *Activity of irofulven on a daily dosing schedule against the MiaPaCa human pancreatic tumor xenograft.*

Group	N	Route/ Dose (mg/kg)	Total Dose (mg/kg)	Schedule	Maximum Mouse Wt Loss (Day)	Actual Tumor Wt (Mean±SEM)	% Tumor Growth Inhibition	Mice with Complete Shrinkage	# of Deaths
Control	10	Vehicle		*i.p.*; dx5	---	1133.4±128.1	---	---	---
Irofulven	10	3.5	17.5	*i.p.*; dx5	---	734.3±116.2	37.2%	1	---
Irofulven	10	7	35	*i.p.*; dx5	-21.4% (Day 8)	---*	---	9	1
Gemcitabine	10	40	160	*i.p.*; q3dx4	---	953.4±99.1	16.7%	---	---
Gemcitabine	10	80	320	*i.p.*; q3dx4	-7.8% (Day 26)	892.6±101.0	22.6%	---	---

N, number of mice per group; Wt, weight; *$p<0.0008$ compared with control and gemcitabine groups

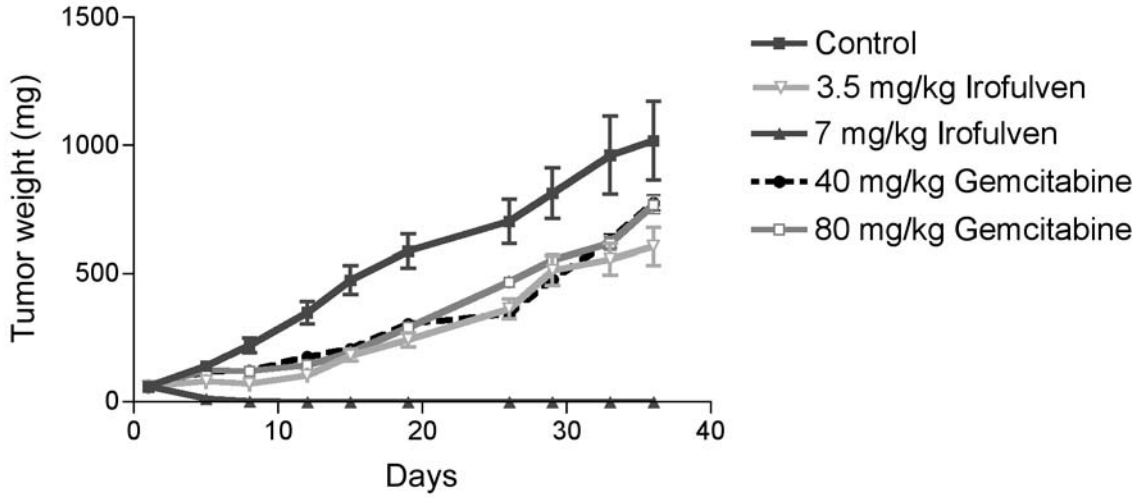


Figure 1. *Efficacy of irofulven against the MiaPaCa pancreatic xenograft model. Mice bearing MiaPaCa tumors received 1% EtOH as a control (solid rectangle), irofulven at 3.5 mg/kg (open triangle) and 7 mg/kg (solid triangle), or gemcitabine at 40 mg/kg (solid circle) or 80 mg/kg (open square). Approximately 10 days after tumor implantation, irofulven was administered daily over five days i.p; whereas gemcitabine was given i.p. on a q3dx4 schedule. There were ten animals per group. The data points indicate the mean for each group with bars representing the standard error.*

tolerated dose (MTD)) or 3.5 mg/kg of irofulven, respectively. In the 80 (MTD) and 40 mg/kg gemcitabine comparator groups, there were no animals with tumor shrinkage and 22.6 and 16.7% TGI, respectively, was observed. Statistically significant differences between the final tumor weights of the 7 mg/kg irofulven group and the control and gemcitabine groups were observed ($p<0.0008$). Marked weight loss (21.4% on day 8) and one toxic death were observed in the irofulven high-dose group.

Mice treated with 5 or 3 mg/kg irofulven given on a q3dx4 schedule demonstrated 10/10 CR or 2 PR with 70% tumor shrinkage, respectively (Table II). In the gemcitabine-treated groups, minimal tumor growth inhibition (13.2% TGI) was noted only in the 80 mg/kg-dose group. Statistically significant differences between final tumor weights were observed in the 5 mg/kg-irofulven group compared to the control and gemcitabine-dosed groups ($p<0.001$). Although tumor growth amongst the control group mice was similar, six mice were found dead on Days 12-16. Examination by a pathologist of the animals that died failed to show an obvious cause of death. Minimal body weight loss (< 5%) was observed in the mice treated with intermittent doses of irofulven compared to a 7.6% body weight loss in the gemcitabine high-dose group.

Irofulven in combination with gemcitabine demonstrates at least additive activity against pancreatic cell lines in vitro. For one-hour exposures, the irofulven IC_{50} was 0.012 μM and 3.04 μM

Table II. *Activity of irofulven on an intermittent dosing schedule in combination with gemcitabine against the MiaPaCa human pancreatic tumor xenograft.*

Group	N	Route/ Dose (mg/kg)	Total Dose (mg/kg)	Schedule	Maximum Mouse Wt Loss (Day)	Actual Tumor Wt (Mean±SEM)	% Tumor Growth Inhibition	Mice with Partial Shrinkage	Mean % Tumor Shrinkage	Mice with Complete Shrinkage	# of Deaths
Control	10	Vehicle		*i.p.*; q3dx4	-13.7% (Day 12)	579.3±165.5	---	---	---	---	6
Irofulven	10	3	12	*i.p.*; q3dx4	-1.8% (Day 12)	209.8±74.4	63.0%	2	69.6%	---	---
Irofulven	10	5	20	*i.p.*; q3dx4	-4.0% (Day 12)	---*	---	---	---	10	---
Gemcitabine	10	40	160	*i.p.*; q3dx4	-4.2% (Day 16)	668.4±82.7	---	---	---	---	---
Gemcitabine	10	80	320	*i.p.*; q3dx4	-7.6% (Day 12)	517.8±74.4	13.2%	---	---	---	---
Irofulven+ Gemcitabine	10	3 40	12 160	*i.p.*; q3dx4	-9.2% (Day 12)	9.7±6.1	---	5	67.0%	5	---
Irofulven+ Gemcitabine	10	3 80	12 320	*i.p.*; q3dx4	-14.4% (Day 12)	---	---	---	---	10	---

N, number of mice per group, Wt, weight; *$p<0.001$ compared to control and gemcitabine groups

for MiaPaCa and Panc-1, respectively, compared to respective gemcitabine IC_{50} values of 3.80 μM and 28.5 μM. In MiaPaCa cells, concurrent or sequential administration of irofulven and gemcitabine at fixed ratios for one-hour displayed decreased cell viability compared to each agent administered alone, indicating the combination of agents was at least additive (Figure 2A). In the Panc-1 cell line, one-hour simultaneous or sequential exposure of irofulven and gemcitabine was also considered additive (Figure 2B). These results suggest that the combination of irofulven and gemcitabine is at least additive in MiaPaCa and Panc-1 pancreatic cancer cells.

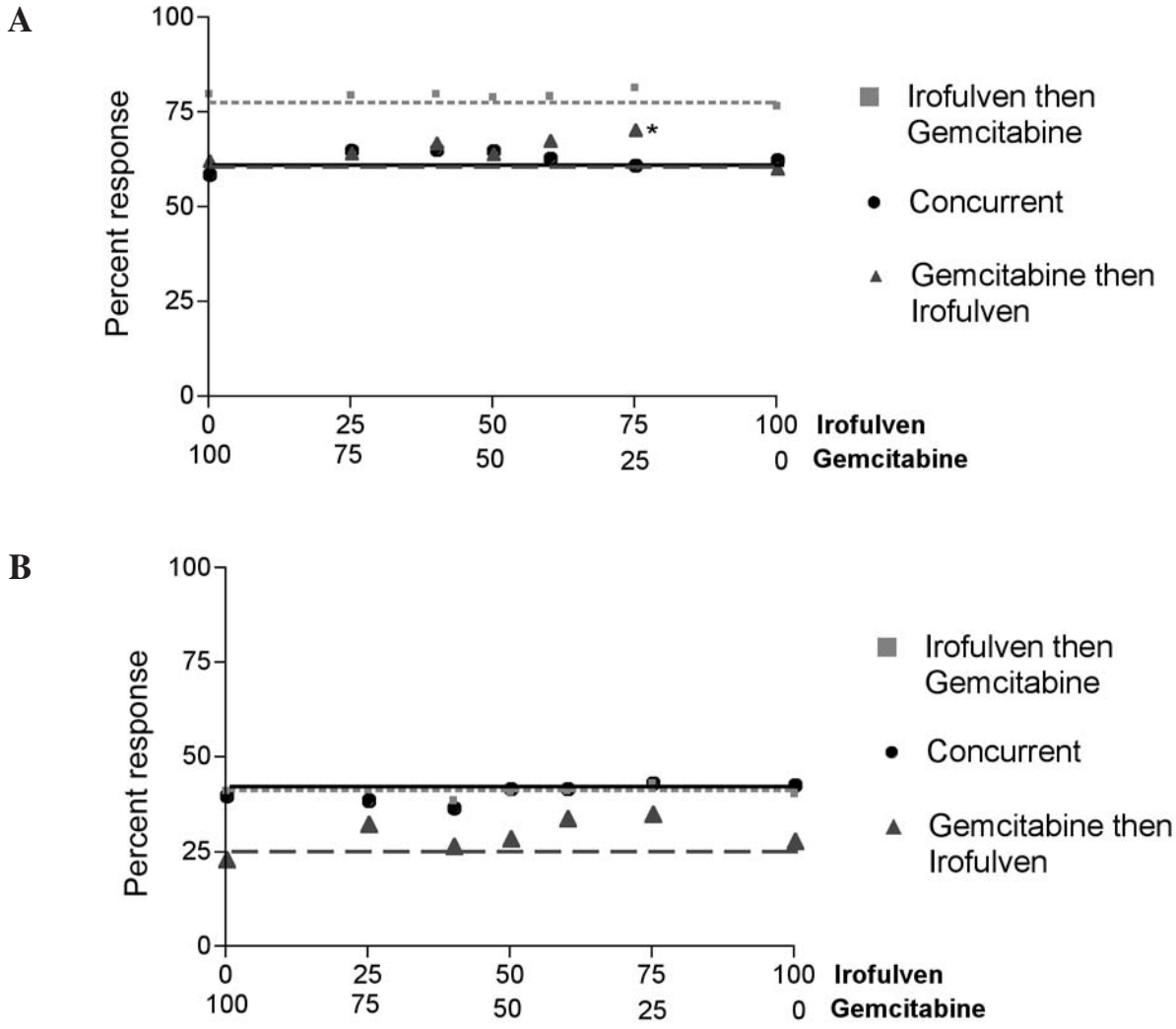
Irofulven in combination with gemcitabine demonstrates curative activity against MiaPaCa xenografts. To assess whether combining irofulven and gemcitabine at sub-MTD doses provides evidence of enhanced antitumor activity in comparison to each agent given as monotherapy, mice bearing MiaPaCa tumors were treated concurrently with the combination. Using the intermittent dosing regimen (q3dx4), 3 mg/kg irofulven was administered with either 40 or 80 mg/kg gemcitabine. Irofulven combined with the low dose of gemcitabine produced 5/10 CR with 5/10 PR (67% tumor shrinkage) (Table II). Furthermore, the combination at the higher gemcitabine dose produced 10/10 CR. In contrast, administration of irofulven or gemcitabine as monotherapy at these doses was unable to produce complete tumor regressions. Differences between the final tumor weights of the combination groups and their respective gemcitabine (but not irofulven) monotherapy groups were statistically significant ($p<0.001$).

Mean maximum weight loss was analyzed to assess the toxicological effects of the irofulven-gemcitabine combination. A -9.2% and -14.4% mean maximum mouse weight change on Day 12 was observed in the low and high dose gemcitabine combination groups, respectively; however, the body weights had recovered by Day 16 and no mortalities were observed.

Discussion

Pancreatic cancer is a tumor type where resistance to conventional cytotoxic therapies presents a significant clinical challenge. Although some chemotherapeutic agents have produced clinical responses in this patient population, the responses have not translated into a reliable or predictable survival benefit (16). Irofulven is a unique alkylating agent with potent activity against multiple tumor types as a single agent and in combination with various chemotherapies (22-24). Furthermore, potent growth inhibition and objective tumor regressions against pancreatic cancer in preclinical models and clinical activity in pancreatic tumors have also been demonstrated (6, 12, 13).

One of the objectives of this study was to investigate the activity of irofulven administered on a daily (dx5) and intermittent (q3dx4) schedule against MiaPaCa pancreatic xenografts. At total doses of 35 mg/kg, which corresponded to 7 mg/kg daily dosing, and 20 mg/kg, which corresponded to 5 mg/kg intermittent dosing, similar curative activity was demonstrated (10/10 CR); however, the maximum body weight loss in the daily-dosed irofulven group was significantly greater than the



Figsures 2. *Sequence administration effects on the combination of irofulven and gemcitabine against A) MiaPaCa or B) Panc-1 pancreatic cancer cells in vitro. The cells were exposed to irofulven in combination with gemcitabine in different sequences at various percentages of their individual IC_{50} to total 100%. Cytotoxicity greater than an additive effect of the two drugs is denoted by (*) and indicates the data point is significantly ($p<0.05$) above the isoeffect line. The isoeffect lines for the different sequence administrations are as follows: (- - -) irofulven then gemcitabine, (—) concurrent and (– –) gemcitabine then irofulven.*

intermittent-dosed group (-21.4% *vs.* -4.0%, respectively). This suggests that irofulven administered using an intermittent dosing regimen has an enhanced tolerance profile while retaining anticancer efficacy. Moreover, Phase I studies of irofulven intermittent schedules have demonstrated improved patient tolerability compared to consecutive daily dosing while maintaining dose intensity and evidence of antitumor activity (25).

The activity of irofulven against MiaPaCa xenografts is impressive considering that the conventional cytotoxic agents doxorubicin, cisplatin, 5-fluorouracil and gemcitabine are relatively inactive against this tumor model (18, 19, 26). The genetic alterations associated with pancreatic cancers, although not completely understood, include p53, $p16^{Ink4a}$ and K-ras mutations and abnormal bcl-2 expression (27,28). Previous studies have demonstrated that irofulven activity is independent of the expression of tumor suppressor genes p53 and $p21^{waf1/cip1}$, apoptosis genes bcl-2 and caspase-3, as well as drug resistance genes, P-gp and MDR (29-33). Additionally, transcription-coupled nucleotide excision repair enzymes have been shown to be required for irofulven-induced DNA repair (14, 15). Recently, analyses of human pancreatic tumor specimens have demonstrated a proportional lack of the NER repair enzyme ERCC3,

suggesting that these patients could be more susceptible to irofulven treatment (34). This data, taken together, suggests that the genetic modifications, which are intrinsic to pancreatic cancer, do not impede and in some cases may enhance the cytotoxicity of irofulven.

The second objective of this study was to test the activity of irofulven in combination with gemcitabine. *In vitro* studies in two different pancreatic cell lines, suggested that the two agents were at least additive when combined together. This *in vitro* combination experience was also seen *in vivo,* where marked activity was observed when irofulven and gemcitabine were tested in combination against MiaPaCa xenografts. Irofulven combined with low dose gemcitabine (40 mg/kg) produced 5/10 CR with 67% tumor shrinkage. When compared to the activity of each agent alone (no activity in the gemcitabine group and 2 PR with 70% tumor shrinkage in the irofulven group), the augmentation of antitumor activity is apparent. Similarly, irofulven combined with 80 mg/kg gemcitabine produced 10/10 CR; whereas minimal activity (13% TGI) was observed in the high dose gemcitabine monotherapy group. At a minimum, additive activity is observed with the combination of irofulven and gemcitabine against pancreatic cancer. The enhanced efficacy of these agents is further supported by previous preclinical irofulven and gemcitabine combination studies that showed greater than additive efficacy against the MV522 lung carcinoma xenograft model (35).

In conclusion, irofulven has demonstrated marked activity against pancreatic xenografts. Additionally, irofulven administration on an intermittent dosing regimen has been shown to be equally effective to consecutive daily dosing with lower toxicity. Further, the combination of irofulven and gemcitabine against the MiaPaCa xenograft model indicates at least an additive interaction between the two drugs. These conclusions support the further clinical investigation of irofulven as monotherapy and in combination with gemcitabine. Based on the preclinical activity of the irofulven and gemcitabine combination, a Phase I clinical study is currently underway (36).

Acknowledgements

The authors would like to thank Scott Lunos for his technical assistance with the statistical analyses.

References

1 McMorris TC, Kelner MJ, Wang W, Yu J, Estes LA and Taetle R: (Hydroxymethyl)acylfulvene: an illudin derivative with superior antitumor properties. J Nat Prod *59*: 896-899, 1996.

2 Kelner MJ, McMorris TC, Estes L, Wang W, Samson KM and Taetle R: Efficacy of HMAF (MGI-114) in the MV522 metastatic lung carcinoma xenograft model nonresponsive to traditional anticancer agents. Invest New Drugs *14*: 161-167, 1996.

3 MacDonald JR, Muscoplat CC, Dexter DL, Mangold GL, Chen SF, Kelner MJ, McMorris TC and Von Hoff DD: Preclinical antitumor activity of 6-hydroxymethylacylfulvene, a semisynthetic derivative of the mushroom toxin illudin S. Cancer Res *57*: 279-283, 1997.

4 Sato Y, Kashimoto S, MacDonald JR and Nakano K: *In vivo* antitumour efficacy of MGI-114 (6-hydroxymethylacylfulvene, HMAF) in various human tumour xenograft models including several lung and gastric tumours. Eur J Cancer *37*: 1419-28, 2001.

5 Hidalgo M, Izbicka E, Eckhardt SG, MacDonald JR, Cerna C, Gomez L, Rowinsky EK, Weitman SD and Von Hoff DD: Antitumor activity of MGI 114 (6-hydroxymethylacylfulvene, HMAF), a semisynthetic derivative of illudin S, against adult and pediatric human tumor colony-forming units. Anticancer Drugs *10*: 837-44, 1999.

6 Von Hoff DD, Cox JV, Eder VP, Tempero MA, Eckhardt SG, Rowinsky EK, Smith SL, Smith CL, Stuart KE, Proper JA, Campbell ER and MacDonald JR: Activity of irofulven (MGI 114) in patients with advanced pancreatic cancer refractory to gemcitabine. 11th NCI EORTC AACR Suppl. to Clin Cancer Res *6*: 103, #365, 2000.

7 Sarosy GA, Kotz H, Smith S, Murgo A, Minasian L, Dyer V, Kohler D, Kim S and Reed E: Phase II study of irofulven in platinum resistant recurrent ovarian cancer. Proc Am Soc Clin Oncol *20*: 218a, #868, 2001.

8 Culine S, Tombal B, Pozzo C, Tourani JM, Fizazi K, Merad Z, Benoit G, Eymard J C, Ould Kaci M, Weems G, Blakolmer K and Cvitkovic E: Randomized phase II trial of irofulven with or without prednisone in hormone-refractory prostate cancer (HRPC) patients (pts): preliminary results. Proc Am Soc Clin Oncol *21*: 161b, #2462, 2002.

9 Woynarowski JM, Napier C, Koester SK, Chen SF, Troyer D, Chapman W and MacDonald JR: Effects on DNA integrity and apoptosis induction by a novel antitumor sesquiterpene drug, 6 hydroxymethylacylfulvene (HMAF, MGI 114). Biochem Pharmacol *54*: 1181-1193, 1997.

10 Herzig MC, Arnett B, MacDonald JR and Woynarowski JM: Drug uptake and cellular targets of hydroxymethylacylfulvene (HMAF). Biochem Pharmacol *58*: 217-225, 1999.

11 Woynarowska BA, Woynarowski JM, Herzig MC, Roberts K, Higdon AL and MacDonald JR: Differential cytotoxicity and induction of apoptosis in tumor and normal cells by hydroxymethylacylfulvene (HMAF). Biochem Pharmacol *59*: 1217-26, 2000.

12 Wang W, Waters SJ, MacDonald JR, Von Hoff DD, Strodel WE and Miller AR: Irofulven (6-hydroxymethylacylfulvene, MGI 114) induces caspase 8 and 9-mediated apoptosis in human pancreatic adenocarcinoma cells. Anticancer Res *21*: 1789-94, 2001.

13 Wang W, Waters SJ, MacDonald JR, Roth C, Shentu S, Freeman J, Von Hoff DD and Miller AR: Irofulven (6-hydroxymethylacylfulvene, MGI 114)-induced apoptosis in human pancreatic cancer cells is mediated by ERK and JNK kinases. Anticancer Res *22*: 559-64, 2002.

14 Kelner MJ, McMorris TC, Estes L, Starr RJ, Rutherford M, Montoya M, Samson KM and Taetle R: Efficacy of acylfulvene illudin analogues against a metastatic lung carcinoma MV522 xenograft nonresponsive to traditional anticancer agents: retention of activity against various mdr phenotypes and unusual cytotoxicity against ERCC2 and ERCC3 DNA helicase-deficient cells. Cancer Res *55*: 4936-4940, 1995.

15 Jaspers NG, Raams A, Kelner MJ, Ng JM, Yamashita YM, Takeda S, McMorris TC and Hoeijmakers JH: Anti-tumour compounds illudin S and irofulven induce DNA lesions ignored by global repair and exclusively processed by transcription- and replication-coupled repair pathways. DNA Repair (Amst) *1*: 1027-1038, 2002.
16 Gunzburg WH, Lohr M and Salmons B: Novel treatments and therapies in development for pancreatic cancer. Expert Opin Investig Drugs *11*: 769-786, 2002.
17 Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD and Von Hoff DD: Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Onco *15*: 2403-2413, 1997.
18 Feng N, Jin H, Wang M, Du C, Wright JA and Young AH: Antitumor activity of virulizin, a novel biological response modifier (BRM) in a panel of human pancreatic cancer and melanoma xenografts. Cancer Chemother Pharmacol *51*: 247-255, 2003.
19 Schultz RM, Merriman RL, Toth JE, Zimmermann JE, Hertel LW, Andis SL, Dudley DE, Rutherford PG, Tanzer LR and Grindey GB: Evaluation of new anticancer agents against the MIA PaCa-2 and PANC-1 human pancreatic carcinoma xenografts. Oncol Res *5*: 223-228, 1993.
20 Laska EM, Meisner M and Siegel C: Simple designs and model-free tests for synergy. Biometrics *50*: 834-841, 1994.
21 Greco WR, Bravo G and Parsons JC. The search for synergy: a critical review from a response surface perspective. Pharmacol Rev *47*: 331-385, 2001.
22 Britten CD, Hilsenbeck SG, Eckhardt SG, Marty J, Mangold G, MacDonald JR,Rowinsky EK, Von Hoff DD and Weitman S: Enhanced antitumor activity of 6-hydroxymethylacylfulvene in combination with irinotecan and 5-fluorouracil in the HT29 human colon tumor xenograft model. Cancer Res *59*: 1049-1053, 1999.
23 Hammond LA, Hilsenbeck SG, Eckhardt SG, Marty J, Mangold G, MacDonald JR,Rowinsky EK, Von Hoff DD and Weitman S: Enhanced antitumour activity of 6-hydroxymethylacylfulvene in combination with topotecan or paclitaxel in the MV522 lung carcinoma xenograft model. Eur J Cancer *36*: 2430-2436, 2000.
24 Kelner MJ, McMorris TC, Rojas RJ, Trani NA and Estes L: Enhanced antitumor activity of irofulven in combination with thiotepa or mitomycin C. Cancer Chemother Pharmacol *49*: 412-418, 2002.
25 Alexandre J, Raymond E, Brain E, Ould Kaci M, Smith S and Misset J-L: Dose-intensity (DI) based escalation phase I study of MGI 114 (Irofulven) exploring 3 different *i.v.* schedules in advanced solid tumors. Ann Oncol *11(Supp. 4)*: 138, #632P, 2000.
26 Weitman S, Marty J, Jolivet J, Locas C and Von Hoff DD: The new dioxolane, (-)-2'-deoxy-3'-oxacytidine (BCH-4556, troxacitabine), has activity against pancreatic human tumor xenografts. Clin Cancer Res *6*: 1574-8, 2000.
27 Moore PS, Sipos B, Orlandini S, Sorio C, Real FX, Lemoine NR, Gress T, Bassi C, Kloppel G, Kalthoff H, Ungefroren H, Lohr M and Scarpa A: Genetic profile of 22 pancreatic carcinoma cell lines. Analysis of K-ras, p53, p16 and DPC4/Smad4. Virchows Arch *439*: 798-802, 2001.
28 Bold RJ, Virudachalam S and McConkey DJ: Bcl2 expression correlates with metastatic potential in pancreatic cancer cell lines. Cancer *92*: 1122-1129, 2001.
29 Izbicka E, Davidson K, Lawrence R, Cote R, MacDonald JR and Von Hoff DD: Cytotoxic effects of MGI 114 are independent of tumor p53 or p21 expression. Anticancer Res *19*: 1299-1307, 1999.
30 Herzig MC, Trevino AV, Liang H, Salinas R, Waters SJ, MacDonald JR, Woynarowska BA and Woynarowski JM: Apoptosis induction by the dual-action DNA- and protein-reactive antitumor drug irofulven is largely Bcl-2-independent. Biochem Pharmacol *65*: 503-513, 2003.
31 Herzig MC, Liang H, Johnson AE, Woynarowska B and Woynarowski JM: Irofulven induces apoptosis in breast cancer cells regardless of caspase-3 status. Breast Cancer Res Treat *71*: 133-143, 2002.
32 Kelner MJ, McMorris TC, Estes LA, Oval MY, Rojas RJ, Lynn JR, Lanham KA and Samson KM: Efficacy of MGI 114 (HMAF) against the MRP+ metastatic MV522 lung carcinoma xenograft. Anticancer Drugs *11*: 217-224, 2000.
33 Kelner MJ, McMorris TC, Estes L, Samson KM, Bagnell RD and Taetle R: Efficacy of MGI 114 (6-hydroxymethylacylfulvene, HMAF) against the mdr1/gp170 metastatic MV522 lung carcinoma xenograft. Eur J Cancer *34*: 908-913, 1998.
34 Von Hoff D, Han H, Nagle RB and Bearss DJ: ERCC3 expression-status in patients with pancreatic cancer. Proc AACR *44*: 1252, #5461, 2003.
35 Van Laar ES, Kelner MJ, Woldetsadik T, Hollister B, MacDonald JR and Waters SJ: Enhanced antitumor activity of irofulven in combination with gemcitabine against the MV522 human lung carcinoma xenograft. Eur J Cancer *38*: S30-31, 2002.
36 Witta SE, Eckhardt GS, Rothenberg M, Sosman J, Gustafson DL, O'Bryant CL, Weems G, Herdrich L, Cooper W and Berlin J: A phase I combination trial of irofulven and gemcitabine in patients with advanced solid malignancies. Proc ASCO *22*: 138, #552, 2003.

Received October 13, 2003
Accepted December 15, 2003