Anti-Cancer Drugs 1999, **10**, pp. 837 844

Preclinical report

Antitumor activity of MGI 114 (6-hydroxymethylacylfulvene, HMAF), a semisynthetic derivative of illudin S, against adult and pediatric human tumor colony-forming units

Manuel Hidalgo, Elzbieta Izbicka, S Gail Eckhardt, John R MacDonald,[1] Cesario Cerna, Lionel Gomez, Eric K Rowinsky, Steven D Weitman and Daniel D Von Hoff

Institute for Drug Development, Cancer Therapy and Research Center, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA. [1]MGI Pharma, Inc, Bloomington, MN 55438-2318, USA.

MGI 114 (6-hydroxymethylacylfulvene, HMAF) is a novel semisynthetic antitumor compound derived from the sesquiterpene mushroom toxin illudin S. Although illudins did not demonstrate significant activity as antiproliferative agents in tumor-bearing animals, several properties including its potent inhibition of DNA synthesis and a unique interaction with DNA led to a structure–activity-based synthetic effort to obtain analogs with improved therapeutic potential. MGI 114 was selected for further development based on its antitumor activity in numerous preclinical tests. MGI 114 was evaluated against adult and pediatric human tumors taken directly from cancer patients and cultured in a human tumor colony-forming assay (HTCFA) to assess the antitumor spectra, concentration–response relationship, schedule dependence and activity of this agent against tumors considered resistant to conventional anticancer drugs. Human tumor colony-forming units were treated with HMAF at concentrations of 0.001, 0.01, 0.1 and 1 μg/ml, both as a 1 h exposure and as a continuous 14 day exposure. A response was scored if there was 50% or less colony survival. *In vitro* response rates in the range of 50–80% were observed against tumor colony-forming units originating from carcinomas of the colon, kidney, breast, lung cancer, ovary and melanoma. MGI 114 also demonstrated antitumor activity against neuroblastoma colony-forming units. Antitumor activity was not influenced by exposure time as demonstrated by the similar responses rates obtained with the 1 h and continuous exposure at all concentrations tested. However, there was a significant positive concentration–response relationship to both exposure duration with responses increasing from below 10% at the lowest concentration to over 70% at the highest concentration, except for the pediatric tumors on the 1 h exposure for which this relationship was less apparent. At the higher concentration tested, MGI 114 displayed substantial antiproliferative effects in the range of 70% against tumor specimens resistant to classic cytotoxic agents including irinotecan, paclitaxel, 5-fluorouracil, cisplatin, doxorubicin and cyclophosphamide. These results demonstrate that MGI 114 exhibits a broad spectrum of antitumor activity against both adult and pediatric primary tumor colony-forming units in a concentration-dependent manner both at short and prolonged exposure duration. The substantial *in vitro* activity of MGI 114 at concentrations achievable in clinical trials, together with its activity against tumors resistant to classic standard cytotoxic drugs, justifies the further clinical evaluation of this unique agent. [© 1999 Lippincott Williams & Wilkins.]

Key words: Adult, human tumor cloning assay, 6-hydroxymethylacylfulvene, MGI 114, pediatric.

Introduction

MGI 114 (6-hydroxymethylacylfulvene, HMAF, Figure 1) is a novel semisynthetic antitumor compound derived from the sesquiterpene mushroom toxin illudin S.[1,2] The illudins comprise a variety of naturally occurring compounds isolated from mushrooms of the genus *Omphalotus* (*O. illudens*) or the closely related *Lampteromyces* (*L. japonicus*). These compounds were tested as potential anticancer agents against a variety of murine solid tumors and leukemia. No increases in life span or tumor regression were observed in solid tumor models including lung carcinoma, sarcoma and melanoma, whereas the

MH is supported in part by the grant PF 97 52232879 from the Ministerio de Educacion y Cultura, Spain, and is the recipient of an EORTC/NCI Fellowship Award

Correspondence to E Izbicka, Nordan Colon Research Laboratory, Cancer Therapy and Research Center, 7979 Wurzbach Road, San Antonio, TX 78229, USA.
Tel: (+1) 210 616 5892; Fax: (+1) 210 616 5948;
E-mail: eizbicka@saci.org

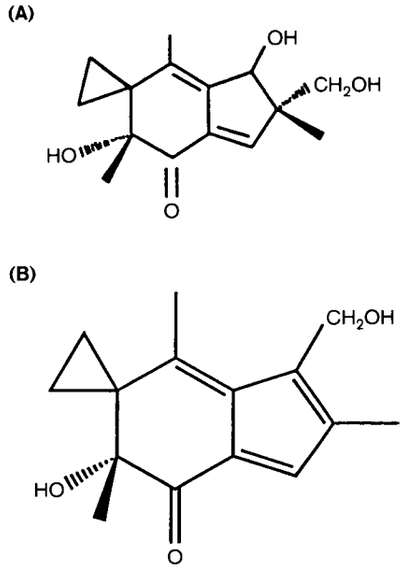

Figure 1. (A) Structure of illudin S. (B) Structure of MGI 114.

antitumor activity in leukemia models was limited by increased mortality in illudin S-treated animals.[3] Despite the limitations in preclinical testing, these compounds were found to possess several properties that make them unique as potential anticancer agents. In sensitive tumor cell types, illudins S is a potent inhibitor of DNA synthesis which results in cell cycle arrest in S phase. Illudin S also damages DNA in a unique manner that appears to require functional DNA helicase activity for DNA repair processes to occur.[3-5] Additionally, illudin S displayed significant antitumor activity in a variety of *in vitro* systems against a broad range of drug-resistant tumor cell lines.[3,6,7]

These unique properties of illudin S motivated a considerable structure-activity-based synthetic effort in an attempt to create chemical derivatives with improved therapeutic indices. MGI 114 was selected for clinical development based on its favorable *in vitro* and *in vivo* activity.[2] The agent demonstrated significant *in vitro* antitumor activity against a panel of murine and human tumor cell lines including B16 melanoma, P388 leukemia, HT-29 colon and MCF-7 breast carcinoma.[8] In these studies, MGI 114 IC_{50} values ranged from 160 nM in sensitive MCF-7 human tumor cell lines to 17 μM in relatively insensitive murine B16 melanoma cells. In *in vivo* experiments with human tumor xenograft models, MGI 114 was found to exhibit substantial antitumor activity against MX-1 breast carcinoma, MV522 lung carcinoma and HT-29 colon carcinoma models. Excellent responses were observed in animals bearing MX-1 tumors with HMAF administered i.v. or i.p. at doses of 3-7.5 mg/kg daily for 5 days, with a complete response rate of over 70%.[8] Administered i.p. daily $\times$ 5, the agent produced significant tumor growth inhibition in the range of 50-90% in the MV522 lung carcinoma and HT-29 colon carcinoma human xenograft models. Additionally, MGI 114 treatment resulted in complete tumor eradication in five of eight animals bearing the DU145 human prostate xenograft and in a 100% partial response rate against the PC-3 human prostate cancer model.[9] The antiproliferative properties of MGI 114 in combination with other cytotoxic agents have also been studied in preclinical experiments. MGI 114 demonstrated synergistic cytotoxic effects in combination with a variety of conventional anticancer agents including paclitaxel, topotecan and gemcitabine against pediatric tumor cell lines. Enhanced antitumor activity has been noted with this agent in combination with paclitaxel and topotecan against the MV522 human lung cancer model, and in combination with 5-fluorouracil (5-FU) and irinotecan against the HT29 human colon cancer model.[10,11] These findings, together with its tolerable toxicity profile in preclinical studies, have stimulated the initiation of phase I clinical trials with MGI 114.[12]

This study evaluated the *in vitro* activity of MGI 114 against a panel of human tumor colony-forming units taken directly from adult and pediatric patients. The objective of these experiments was to determine its spectrum of antitumor activity, concentration-response relationship and the relative antiproliferative effect of short-term versus prolonged exposure. In addition, this study analyzed the activity of MGI 114 against tumor specimens resistant to conventional cytotoxic agents.

Material and methods

Collection of tumor cells

Tumor specimens were obtained using standard sterile techniques from patients undergoing diagnostic or therapeutic procedures. Specimens included solid tumors, malignant pleural effusions, ascites and bone marrow aspirates. All patients gave written informed consent in accordance with federal and institutional guidelines. Solid tumors or lymph nodes were minced into 2-5 mm fragments in the operating room and immediately placed in McCoy's Medium 5A (Life Technologies, Grand Island, NY) containing 10% heat-inactivated newborn calf serum, 10 mM HEPES, 100 μg/ml penicillin and 90 μg/ml streptomycin (all Life Technologies) for transport to the laboratory. The

fluids and bone marrow aspirates were placed in sterile containers containing 10 U of preservative-free heparin (O'Neill, Johns and Feldman, St Louis, MO) per ml of malignant fluid or marrow to prevent coagulation. Within 4 h, solid specimens were minced and passed repeatedly through metal sieves with 40 μm mesh (EC Apparatus, St Petersburg, FL) to obtain single-cell suspensions. When necessary, effusions were centrifuged at 150 *g* and passed through 25-gauge needles to obtain single-cell suspensions. All specimens were washed twice in McCoy's Medium containing 5% horse serum (Sigma, St Louis, MO), 10% heat-inactivated fetal calf serum (Hyclone, Logan, UT), 2 mM sodium pyruvate, 2 mM glutamine, 90 U/ml penicillin, 90 μg/ml streptomycin and 35 μg/ml L-serine (all Life Technologies) as previously described.[14,15] The viability of cell suspensions was determined on a hemocytometer with Trypan blue.

Drugs

Purified MGI 114 was supplied by MGI Pharma (Minneapolis, MN). Stock solutions of MGI 114 were prepared in sterile, enriched CMRL Medium 1066 (Irvine Scientific, Irvine, CA). One-half milliliter aliquots of each stock solution were labeled and stored at −70°C. Aliquots were thawed for each new tumor sample tested. The final concentrations of MGI 114 tested were 0.001, 0.01, 0.1 and 1 μg/ml, both as a 1 h exposure and as a continuous exposure.

The cytotoxicity of 1 h and continuous exposure of MGI 114 at the 0.1 and 1 μg/ml concentration were also evaluated in tumor specimens resistant to a panel of conventional antitumor agents at the following concentrations and 1 h exposure: 6 μg/ml of 5-FU, 3.0 μg/ml of irinotecan, 2.5 μg/ml of paclitaxel, 0.04 μg/ml of doxorubicin and 3.0 μg/ml of cyclophosphamide.

Culture of cells

Tumor cells were suspended in 0.35% agar in enriched CMRL Medium 1066 (Irvine Scientific) supplemented with 15% heat-inactivated horse serum, penicillin (100 U/ml), streptomycin (2 mg/ml), glutamine (2 mM), insulin (3 U/ml), asparagine (0.6 mg/ml) and HEPES buffer (2 mM). Cells were plated in 35 mm Petri dishes in a top layer of agar and an underlayer of 0.3% agar to prevent growth of fibroblasts. Three plates were prepared for each data point. In the 1 h exposure studies, the cells were incubated with the drug in McCoy's medium and then washed. In the continuous exposure condition, cells were combined with the drugs being tested, plated and incubated for 14 days. The number of colonies (defined as greater than 50 cells) formed in the treated plates was compared to the number of colonies in the untreated control plates and the percentage of surviving colonies at each concentration was calculated.

Quality control

To assure the presence of an adequate single-cell suspension on the day of plating, positive controls were used. For each tumor tissue sample tested, three positive control plates were set up containing the cell poison orthosodium vanadate at 200 μg/ml. If there was no effect of the positive control on colony formation, then the single-cell suspension was deemed poor (due to cell clumping) and the sample tested was considered non-evaluable. An evaluable test had an average of 20 or more colonies present on day 14 in the untreated control plates and less than 30% survival in the positive (orthosodium vanadate) control compared to the untreated control plates.

Data analysis and statistics

Results were expressed as the survival of tumor colony-forming units for a particular drug relative to its control expressed as percentage of the total cells. Response was defined as growth inhibition of 50% or more in treated specimens compared to controls. The Mantel-Haenszel test for linear association was utilized to compare responses at each drug concentration for both schedules. The differences in responses at the same concentration for each schedule were analyzed with a two-sided Fisher exact test while non-parametric techniques for comparison of two related samples (McNemar test) were employed to assess differences in head to head comparisons.

Results

Antitumor effects against adult human tumor colony-units

A total of 205 and 279 fresh tumor specimens from adult patients tumor were exposed to MGI 114 in the 1 h and continuous exposure experiments, respectively, whereas 141 (68%) and 188 (67%) were evaluable according to the above specified criteria. Table 1 summarizes the *in vitro* response rates

Table 1. Concentration-dependent inhibition of colony formation by MGI 114 against adult patients tumor specimens[a]

Tumor type	1 h exposure (μg/ml)				Continuous exposure (μg/ml[b])			
	0.001	0.01	0.1	1	0.001	0.01	0.1	1
Brain	0/2	0/2	0/2	1/2	0/3	0/3	1/3	2/3
Breast	0/12	0/12	7/12	9/12	0/13	1/13	7/13	9/11
Cervix	—	—	—	—	0/2	2/2	2/2	—
Colon	0/9	0/9	0/10	7/10	1/11	0/11	7/12	9/10
Head and neck	2/3	2/3	2/3	3/3	0/3	1/3	2/3	2/2
Kidney	0/6	0/6	1/6	3/6	1/7	1/7	2/7	5/6
NSCLC	0/13	0/13	3/13	11/13	1/16	2/16	6/16	11/13
Melanoma	0/12	1/12	5/13	13/13	0/10	1/10	8/10	9/9
Mesothelioma	0/2	0/2	2/2	2/2	0/2	0/2	2/2	2/2
Ovary	2/16	3/15	15/23	21/23	2/18	4/20	18/27	17/10
Prostate	0/2	0/2	0/2	2/2	0/3	1/3	3/3	3/3
Sarcoma	0/2	0/2	1/2	2/2	0/2	0/2	0/2	1/2
Gastric	0/2	1/3	1/3	3/3	0/2	0/2	1/2	2/2
Unknown primary	0/3	0/3	1/3	3/3	0/3	1/3	3/3	3/3
Uterus	0/2	0/2	1/2	1/2	1/6	3/6	3/6	2/2
Miscellaneous[b]	0/4	1/4	2/4	3/3	0/6	2/6	4/6	3/3
Total	4/90 (4%)	8/90 (9%)	41/100 (41%)	83/100 (83%)	6/107 (7%)	19/109 (17%)	69/117 (59%)	80/91 (88%)

[a]Inhibition is given as the ratio of the number of specimens inhibited (colony formation less than 50% that of the control)/number of specimens evaluable.
[b]Fourteen days exposure.
[c]Included specimens of bladder cancer, peritoneal carcinomatosis, liver cancer, pancreatic cancer and small cell lung cancer.

observed in specific tumor types as a function of drug concentration and exposure. The major subgroups of tumors tested (and number of evaluable specimens exposed to 1 h and continuous exposure, respectively) were adenocarcinoma of the breast (12 and 13), adenocarcinoma of colon (nine and 11), renal cell carcinoma (six and seven), non-small cell lung (13 and 16), melanoma (13 and 10) and ovarian carcinoma (16 and 27). There was a significant increment in the overall antitumor activity with increasing drug concentrations at both exposure times. The *in vitro* responses rate augmented from 4–7% at the 0.001 μg/ml concentration (1 h and continuous exposure, respectively) to 83–88% at the 1 μg/ml concentration (1 h and continuous exposure, respectively) (p=0.0001, Mantel-Haenszel test for linear association) indicating that MGI 114 has a concentration-dependent antitumor activity in this *in vitro* assay. Although there was a trend towards a significant difference in the observed response rate at 0.1 μg/ml concentration with higher response rate (59%) at the continuous exposure as opposed to the 1 h exposure (41%, p=0.09, two-sided Fisher exact test), the response rate did not differ significantly between exposure type at any of the drug concentrations explored (Table 1, $p > 0.05$, two-sided Fisher exact test). This relatively similar activity between short and prolonged exposures was evident for all of the specific tumor types tested in these experiments with none of them being more sensitive to a particular schedule. These results suggest that, while there is a clear concentration-response relationship, MGI 114 antitumor activity in this assay is, at most concentrations, independent of exposure type.

At the highest concentration tested (1 μg/ml), significant antiproliferative effects with response rates over 70% were observed against adenocarcinoma of the breast, adenocarcinoma of the colon, NSCLC, melanoma and ovarian carcinoma colony-forming units with both exposure times. Significant activity was also detected against renal cell cancer specimens in the continuous exposure experiments. In addition, at both exposures there was evidence of antiproliferative activity against other types of tumor colony-forming units which included mesothelioma, gastric adenocarcinoma, soft tissue sarcoma and prostate carcinoma.

Antitumor effects against pediatric human tumor colony-forming units

The results obtained against tumor colony-forming units taken from pediatric patients and exposed to different concentrations of MGI 114 with either 1 h or continuous exposure are summarized in Table 2. A total of 37 specimens were tested in the 1 h experiment and 19 (51.3%) were evaluable. Comparable figures for the continuous exposure were 31 and

Table 2. Concentration-dependent inhibition of colony formation by MGI 114 against pediatric patients tumor specimens[a]

Tumor type	1 h exposure (μg/ml)				Continuous exposure (μg/ml[b])			
	0.001	0.01	0.1	1	0.001	0.01	0.1	1
Endocrine	1/1	1/1	1/1	1/1				
Kidney	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Lymphoma	–	–	–	–	0/1	–	0/1	–
Neuroblastoma	4/9	3/9	4/10	6/7	0/6	2/6	4/7	
Ovary	1/1	1/1	1/1	1/1	0/1	0/1	0/1	1/1
Sarcoma	–	–	–	–	0/1	1/1	1/1	–
Total	6/12 (50%)	5/12 (42%)	6/13 (46%)	8/10 (80%)	0/10 (0%)	2/9 (22%)	5/11 (45%)	4/6 (67%)

[a]Inhibition is given as the ratio of the number of specimens inhibited (colony formation less than 50% that of the control)/number of specimens evaluable.
[b]Fourteen days exposure.

16 (51.6%), respectively. With the 1 h exposure, although the *in vitro* response rate was higher at the maximum concentration of 1 μg/ml, it did not increase significantly at lower concentrations ranging from 0.001 to 0.1 μg/ml (p=0.17, Mantel-Haenszel test for linear association). However, the *in vitro* response rates increased significantly from 0% at the lowest (0.001 μg/ml) concentration to 67% at the highest (1 μg/ml) concentration in the continuous exposure (p=0.02, Mantel-Haenszel test for linear association). These data are slightly different from those observed in adult tumor specimens. However, considering the low number of specimens analyzed in these particular experiments, these observations should be interpreted with caution. The response rate observed at the 0.001 μg/ml concentration with the 1 h exposure (50%) was significantly higher than with the continuous exposure (0%, p=0.01, two-sided Fisher test). At higher concentrations these differences were narrower and non-statistically significant. Significant antitumor activity was identified against specimens of neuroblastoma at both exposure times with effects also noted against endocrine, ovarian and sarcoma specimens.

Activity against tumor specimens resistant to classical cytotoxic drugs

In an attempt to further delineate and characterize the cytotoxic effects of MGI 114 against human tumor colony-forming units, this study evaluated the activity of MGI 114 against a panel of tumor specimens from adult and pediatric patients selected based on their resistance (determined in the HTCFA) to clinically relevant concentrations of common cytotoxic agents including irinotecan, paclitaxel, 5-FU, cisplatin, doxorubicin and cyclophosphamide. The results of these studies, depicted in Tables 3 and 4, demonstrate that MGI 114 displayed substantial antiproliferative activity against tumors resistant to different classic cytotoxic agents with distinct mechanisms of action. At the 1 μg/ml concentration, MGI 114 treatment resulted in response rates in the range of 72-86% in the 1 h exposure experiment and 76-100% in the continuous exposure experiments against tumor specimens resistant to conventional agents. MGI 114 exhibited a positive concentration-response relationship against tumors resistant to the full spectrum of agents. The response rate increased from 25-44% at the 0.1 concentration to 72-86% at the 1 μg/ml in the 1 h exposure and from 45-67 to 76-100%, respectively, in the continuous exposure test. These differences were statistically significant, except for specimens resistant to paclitaxel (p=0.06) and 5-FU (p=0.08) in the continuous exposure, and for irinotecan (p=0.12 and p=0.2) in the 1 h and continuous exposures, respectively. Similar to the results obtained with adult tumor specimens, MGI 114 retained antitumor activity against pediatric tumor specimens resistant to common anticancer drugs (Table 4). While there was a clear trend for a concentration-response relationship in the 1 h exposure, the antitumor activity of MGI 114 was less concentration-dependent in the continuous exposure. Nevertheless, the limited number of specimens analyzed in these experiments precludes the application of formal statistical comparisons.

Discussion

This report demonstrates that the illudin analog MGI 114 exerts concentration-dependent cytotoxic effects against a variety of fresh human tumor colony-forming units taken directly from adult and pediatric patients, including specimens resistant to conventional antic-

Table 3. *In vitro* antitumor effects of MGI 114 against adult tumor specimens resistant to classical cytotoxic drugs[a]

Concentration and exposure time for classic agents	No. responses to HMAF/No. resistant specimens (% *in vitro* response)			
	1 h exposure		Continuous exposure	
	0.1 μg/ml	1 μg/ml	0.1 μg/ml	1 μg/ml
3 μg/ml irinotecan 1 h exposure	2/8 (25%)	6/8 (75%)	3/9 (33%)	7/7 (100%)
10 μg/ml paclitaxel 1 h exposure	6/18 (33%)	13/18 (72%)	13/29 (49%)	13/17 (76%)
6 μg/ml 5-FU 1 h exposure	11/25 (44%)	20/25 (80%)	21/35 (60%)	19/23 (83%)
0.2 μg/ml cisplatin 1 h exposure	15/43 (35%)	37/43 (86%)	34/57 (60%)	35/38 (92%)
0.04 μg/ml doxorubicin 1 h exposure	11/26 (42%)	20/26 (77%)	25/39 (64%)	20/25 (80%)
3 μg/ml cyclophosphamide 1 h exposure	7/18 (39%)	15/18 (83%)	20/30 (67%)	17/18 (94%)

[a]Inhibition is given as the ratio of the number of specimens inhibited (colony formation less than 50% that of the control)/number of specimens evaluable.

Table 4. *In vitro* antitumor effects of MGI 114 against pediatric adult tumor specimens resistant to classical cytotoxic drugs[a]

Concentration and exposure time for classic agents	No. responses to HMAF/No. resistant specimens (% *in vitro* response)			
	1 h exposure		Continuous exposure	
	0.1 μg/ml	1 μg/ml	0.1 μg/ml	1 μg/ml
3 μg/ml irinotecan 1 h exposure	0.4 (0%)	2.3 (67%)	2/3 (67%)	1/2 (50%)
10 μg/ml paclitaxel 1 h exposure	1/3 (33%)	1/1 (100%)	3/3 (100%)	1/1 (100%)
6 μg/ml 5-FU 1 h exposure	2/4 (50%)	3/4 (75%)	2/3 (67%)	2/3 (67%)
0.2 μg/ml cisplatin 1 h exposure	4/9 (44%)	3/4 (75%)	5/7 (71%)	2/3 (67%)
0.04 μg/ml doxorubicin 1 h exposure	3/7 (43%)	2/3 (67%)	3/3 (100%)	2/3 (67%)
3 μg/ml cyclophosphamide 1 h exposure	5/9 (55%)	4/5 (80%)	3/3 (100%)	4/5 (80%)

[a]Inhibition is given as the ratio of the number of specimens inhibited (colony formation less than 50% that of the control)/number of specimens evaluable.

ancer agents. The HTCFA was initially developed to select the most appropriated chemotherapy agents for an individual patient's tumor.[13–15] More recently, this system has been utilized in several areas of drug development including gauging the approximate drug plasma concentrations needed to achieve antitumor activity in patients, selecting the most appropriate schedule for clinical investigation and targeting tumor types for phase II clinical trials.[16–18] The implementation of appropriate positive control plates,[16] as well as the simulation of drug concentration versus time exposures *in vitro* similar to those obtained *in vivo*,[19] has made this system more attractive as a method to evaluate the potential of a new agent.

This study shows a clear positive relationship between *in vitro* response rate and drug concentration both at 1 h and continuous exposures. The response rates at concentrations over 0.1 μg/ml were about 2- to 3-fold higher than those at lower drug concentrations. Previous work has revealed that *in vivo* therapeutic effects in patients are observed only if drug concentrations associated with significant inhibition of colony

formation *in vitro* are obtained in the patients plasma.[20] The range of relevant concentrations with optimal antitumor activity in this *in vitro* study are indeed achievable in adult cancer patients as demonstrated by initial clinical pharmacologic studies conducted with this compound.[12] After 5 min rapid i.v. infusion daily for 5 consecutive days MGI 114 pharmacokinetics appeared linear and consistently resulted in peak plasma levels over the relevant *in vitro* concentration of 0.1 μg/ml at clinically tolerable dose levels. Non-compartmental pharmacokinetic analysis of the MGI 114 plasma disposition curve revealed an extremely short half-life of 4–6 min, indicating that exposure time at drug concentrations over the active range is notably short and certainly below the 1 h exposure time investigated in this study. However, the relevance of this observation in terms of predicting drug activity in clinical trials is limited and should be considered in conjunction with more comprehensive pharmacology evaluation of this compound, including drug distribution, metabolism and elimination processes, which are not available at this time. Additionally, based on the well-described differences in drug pharmacokinetics between adult and pediatric patients, these observations cannot be applied to pediatric patients for which there are no clinical data.

MGI 114 displayed significant *in vitro* antiproliferative effects against a wide range of common and difficult to treat adult malignancies including breast, colon, non-small cell lung, ovarian and kidney cancer in addition to melanoma. Activity was also observed against other tumor types which includes mesothelioma, sarcoma, gastric cancer, unknown primary tumors and prostate cancer. The anticancer activity of MGI 114 in these experiments is consistent with other preclinical evaluations of its antitumor activity in human tumor xenograft models. Impressive effectiveness has been reported against animals bearing MX-1 breast carcinomas and PC-3 prostate carcinoma with complete regressions in 29 of 30 animals treated at i.v. doses ranging from 3 to 7 mg/kg on a daily × 5 schedule of administration and five of nine animals treated i.p. at 7 mg/kg dose on the same schedule, respectively.[8,9] Extensive reduction in tumor size was observed in the MV522 lung cancer model and DU145 prostate cancer model, and significant tumor growth inhibition was obtained in the HT-29 colon cancer when animals received five daily i.p. doses ranging from 3.75 to 7.5 mg/kg.[8,9] These data indicate that MGI 114 possess substantial antitumor activity against a wide range of tumor types and suggests that it should undergo further development as a potential anticancer agent. However, the predictivity of this data with regards to the ultimate clinical efficacy of this agent remains speculative.

MGI 114 demonstrated activity against a variety of tumor specimens obtained from pediatric patients, particularly neuroblastoma. However, the lower number of specimens in the pediatric malignancy categories makes the observed results more uncertain. Considering the intrinsic difficulties in anticancer drug development in pediatric oncology and the paucity of relevant preclinical models to specifically explore cytotoxic agents against pediatric tumors, the observed activity of MGI 114 against tumor specimens from pediatric patients deserves particular attention and might indicate the appropriateness to conduct clinical trials in this population.

This study also evaluated the antiproliferative effects of MGI 114 against tumor specimens resistant to a number of commonly employed cytotoxics and demonstrated that, at concentrations within the range of attainable plasma levels in patients, MGI 114 retained substantial anticancer activity. These findings are in agreement with prior observations of the antitumor activity of illudin S and its derivatives acylfulvene and dehydroilludin M against a broad range of multidrug-resistant tumor cell phenotypes.[3,6,7] Additionally, a recent report indicates that MGI 114 has *in vivo* antitumor activity against a multidrug-resistant variant of the MV522 lung cancer xenograft generated by the transfection of a eukaryotic expressing vector containing the cDNA for the human gp170.[21]

MGI 114 has also demonstrated marked synergistic cytotoxic effects in combination with several agents which include paclitaxel, topotecan, irinotecan and 5-FU against a variety of human tumor xenograft models,[10,11] and resulted in synergistic cytotoxic effects when combined with a variety of anticancer drugs against neuroblastoma cell lines. MGI 114 has been administered at doses of 3.5 mg/kg daily × 5 in combination with irinotecan or 5-FU to mice bearing the HT-29 human tumor xenograft. The MGI 114–irinotecan regimen resulted in three complete responses and a mean tumor reduction greater than 75% in the remaining animals, while no complete regressions and a mean tumor reduction of 58% was observed with the MGI 114–5-FU combination. These results were superior to those observed with single-agent MGI 114 in the same experimental model. Similarly, the combination of MGI 114 with topotecan and paclitaxel resulted in enhanced antitumor effects with one and seven complete responses against MV522 human lung cancer xenograft, respectively.[10] These data reinforce the notion of the unique mechanism of cytotoxicity of this novel agent compared to conventional anticancer drugs with well-defined and well-characterized intracellular effects and

suggest the potential usefulness of MGI 114 as a cytotoxic drug both as a single agent and as a component of combination regimens. The observed activity of MGI 114 in chemotherapy resistant tumor types further augments the expectations in this agent MGI 114 as a valuable new anticancer compound. Particularly, the marked *in vivo* effects against the HT-29 human colon cancer and MV522 human lung cancer xenograft together with the pronounced *in vitro* activity against colon and lung cancer tumor specimens observed in the present study suggest that MGI 114 might be of potential utility in the treatment of these diseases.

In conclusion, this study demonstrated that MGI 114 exerts prominent *in vitro* concentration-dependent antitumor activity against common tumor types such as breast, colon, kidney, lung cancer and melanoma colony-forming units. There was also significant antitumor activity against pediatric tumor specimens and against tumor specimens resistant to conventional agents. Based on the results of this study, in conjunction with other relevant preclinical studies, MGI 114 may hold promise as an anticancer drug with potential for activity against a variety of common and refractory malignancies.

References

1. Kelner MJ, McMorris TC, Estes L, *et al.* Efficacy of HMAF (MGI-114) in the MV522 metastatic lung carcinoma model nonresponsive to traditional anticancer agents. *Invest New Drugs* 1996; **141**: 161–7.
2. McMorris TC, Kelner MJ, Wang W, *et al.* Hydroxymethylacylfulvene (HMAF), an illudin derivative with superior antitumor properties. *J Nat Prod* 1996; **59**: 896–99.
3. Kelner MJ, McMorris TC, Beck WT, *et al.* Preclinical evaluation of illudins as anticancer agents. *Cancer Res* 1987; **47**: 3186–89.
4. Kelner MJ, McMorris TC, Taetle R. Preclinical evaluation of illudins as anticancer agents: basis for selective toxicity. *J Natl Cancer Inst* 1990; **82**: 1562–5.
5. Kelner MJ, McMorris TC, Estes L, *et al.* Characterization of illudin S sensitivity in DNA repair deficient Chinese Hamster cells: unusually high sensitivity of ERCC2 and ERCC3 DNA helicase-deficient mutants in comparison to other chemotherapeutic agents. *Biochem Pharmacol* 1994; **48**: 403–9.
6. Kelner MJ, McMorris TC, Estes L, *et al.* 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 helicase-deficient cells. *Cancer Res* 1995; **55**: 4936–40.
7. Kelner MJ, McMorris TC, Taetle R. *In vitro* and *in vivo* studies on the anticancer activity of dehydroillidin M. *Anticancer Res* 1995; **15**: 873–8.
8. MacDonald JR, Muscoplat CC, Dexter DL, *et al.* Preclinical antitumor activity of 6-hydroxymethylacylfulvene, a semisynthetic derivative of the mushroom toxin illudin S. *Cancer Res* 1997; **57**: 279–83.
9. Mangold G, MacDonald J, Von Hoff DD, *et al.* Antitumor activity of MGI 114 against two human prostate tumor xenograft models. *Ann Oncol* 1998; **9**: 37 (abstr 142).
10. Mangold G, MacDonald J, Von Hoff DD, *et al.* Enhance antitumor activity of MGI 114 in combination with paclitaxel and topotecan against a human lung tumor xenograft model. *Ann Oncol* 1998; **9**: 37 (abstr 141).
11. Marty J, MacDonald J, Mangold G, *et al.* Evidence of synergistic antitumor activity with MGI 114 in combination with irinotecan (ITN) or 5-fluorouracil (5-FU) against a human colon tumor xenograft model. *Proc Am Ass Clin Oncol* 1998; **39**: 527.
12. Eckhardt SG, Baker SD, Moczygemba J, *et al.* A phase I and pharmacokinetic (PK) study of the novel mushroom-derived cytotoxin, MGI-114, in patients with advanced cancer. *Ann Oncol* 1998; **9**: 123 (abstr 471).
13. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. *J Clin Invest* 1977; **60**: 846–54.
14. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977; **197**: 471.
15. Von Hoff DD, Sandbach JF, Clark GM, *et al.* Selection of cancer chemotherapy for a patient by an *in vitro* assay versus a clinician. *J Natl Cancer Inst* 1990; **82**: 110–6.
16. Shoemaker RH, Wolpert-Defillpes MK, Kern DH, *et al.* Application of a human tumor colony-forming assay to new drug screening. *Cancer Res* 1985; **45**: 2145–53.
17. Hanauske AR, Degen D, Hilsenbeck SG, *et al.* Effects of Taxotere and taxol on *in vitro* colony formation of freshly explanted tumor cells. *Anti-Cancer Drugs* 1992; **3**: 121–4.
18. Raymond E, Izbicka E, Soda H, *et al.* Activity of temozolomide against human colony-forming units. *Clin Cancer Res* 1997; **3**: 1769–74.
19. Ali-Osman F, Giblin J, Dougherty D, *et al.* Application of *in vivo* and *in vitro* pharmacokinetics for physiologically relevant drug exposure in human tumor clonogenic cell assay. *Cancer Res* 1987; **47**: 3718–24.
20. Friedman HS, Schold SC, Muhlbaier LH, *et al. In vitro* versus *in vivo* correlations of chemosensitivity of human meduloblastoma. *Cancer Res* 1984; **44**: 5145–9.
21. Kelner MJ, McMorris TC, Estes L, *et al.* Efficacy of MGI 114 (6-hydroxymethylacylfulvene, HMAF) against the *mdr*/gp170 metastatic MV522 lung carcinoma xenograft. 1998; **34**: 908–13.

(Received 10 April 1999; revised form accepted 15 July 1999)